SIV ISV

SOCIETA' ITALIANA DI VIROLOGIA

ITALIAN SOCIETY FOR VIROLOGY

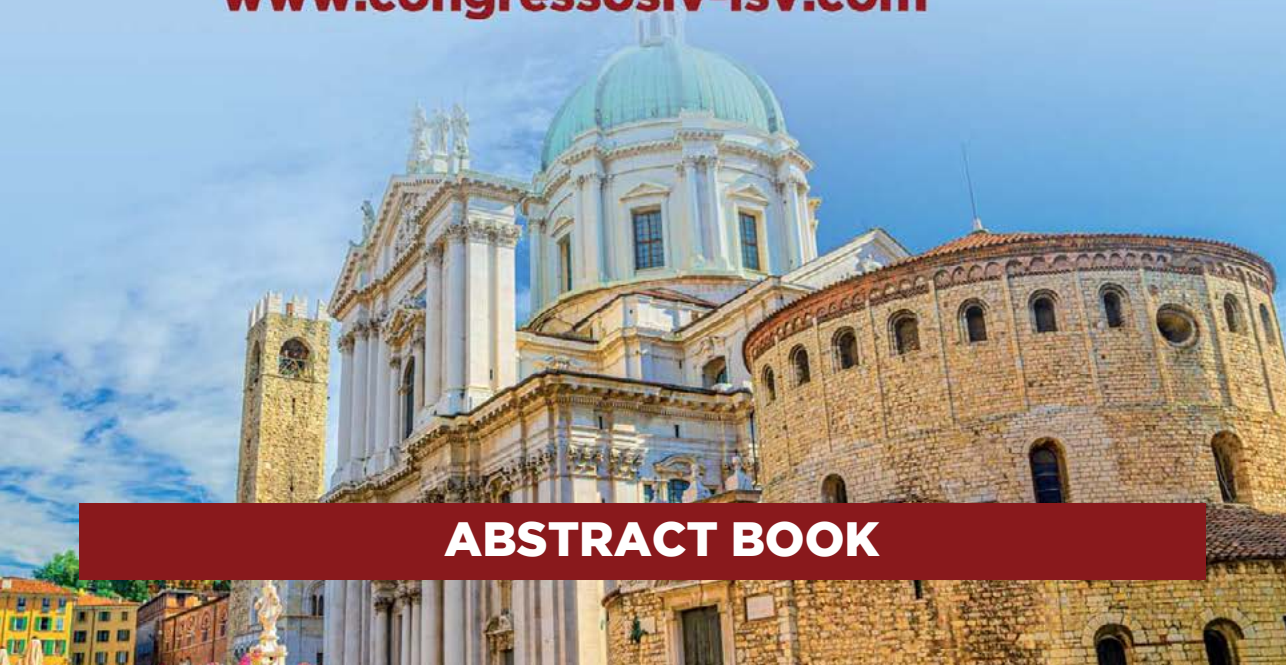
7th National Congress of the Italian Society for Virology

One Virology One Health

Brescia
June 25-27, 2023

Centro Congressi Paolo VI
Via Gezio Calini, 30

www.congressosiv-isv.com



ABSTRACT BOOK

Under the patronage of

Prefettura di Brescia

- **ISS**
  Istituto Superiore di Sanità

Consiglio Nazionale delle Ricerche

Università degli Studi di Brescia

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Istituto Nazionale Tumori IRCCS – Fondazione Pascale

AMCLI ETS
Associazione Microbiologi Clinici Italiani

ANIV
Associazione Nazionale Infettivologi Veterinari

Associazione MicrobiotaMI

SIDILV
Società Italiana di Diagnostica di Laboratorio Veterinaria

SIM
Società Italiana di Microbiologia

SIMIT
Società Italiana di Malattie Infettive e Tropicali

Rationale

The northern Italian cities of Bergamo and Brescia take up their joint status as Italy's Capital of Culture 2023. Bypassing the usual application process, the Italian parliament approved the candidature in "a symbol of hope and rebirth" for Bergamo and Brescia, both of which were devastated by the COVID-19 pandemic. The joint culture capital status "bears witness to a possible renaissance through the conscious choice of culture as a central element for civil education, creating skills, employment and social and economic resilience", reads a statement on the Bergamo Brescia 2023 website. In this context, it is a great honor that the city of Brescia will host the **7th National Congress of the Italian Society for Virology (SIV-ISV)**. The SIV-ISV National Congress represents the annual forum for the Italian scientists working in the different areas of virology (medical-clinical virology, molecular virology, veterinary virology, environmental and plant virology) involving basic, clinical and translational research and taking advantage of the most advanced technological platforms. In keeping with the tradition, the scientific structure of the Congress features different thematic sessions: Viral pathogenesis and disease, Virus-host interactions, Structural virology and biotechnology, Diagnostic virology, Antiviral therapy, Viral diseases and vaccines, Linking viruses to cancer, Emerging viruses, Zoonosis and viral infections in animals, plant and environmental virology, and for the first time, a Italian Society for Infectious and Tropical Diseases (SIMIT)/SIV-ISV joint session on HIV/AIDS. As usually, the Congress will be conceived to promote productive interactions between the different virological souls and we invite participants to explore synergies between different disciplines. Every session starts with Invited Lectures followed by oral communications selected from the "Call for Abstracts". Specific Symposia mainly dedicated to innovative methods of diagnosis, clinical aspects of virology, and tailored treatments, as well as Poster Exhibition and Discussion will complete the scientific structure of the Congress. Special attention has been given to Young Investigators whose participation has been encouraged by Scholarships.

The 7th National Congress of the Italian Society for Virology has been accredited to the Italian Ministry of health for CME Credits for the following professionals: Medical Doctor (all disciplines), Biologist, Biotechnologist, Medical laboratory technician, Nurse, Veterinary, Pharmacist, Physicist and Chemist.

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Antonella BUGATTI (Brescia)
Francesca CACCURI (Brescia)
Arnaldo CARUSO (Brescia)
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Simona FIORENTINI (Brescia)
Cinzia GIAGULLI (Brescia)

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Matteo UGGERI (*Brescia*)
Gabriele VACCARI (*Rome*)
Ilaria VICENTI (*Siena*)
Marco ZACCARONI (*Florence*)
Maurizio ZAZZI (*Siena*)

Sunday 25th June 2023

From 12.00 Registration of participants

13.15-14.00 Welcome addresses
Arnaldo CARUSO (President SIV-ISV)
Greetings from local Authorities

PLENARY SESSION
AULA MAGNA AND AULA FRANCESCHETTI

SESSION 1 **Antiviral therapy**
Chairs: **Enzo TRAMONTANO** (*Cagliari*), **Maurizio ZAZZI** (*Siena*)

14.00-14.30 Emerging antivirals for chronic hepatitis B infection
Raffaele DE FRANCESCO (*Milan*)

14.30-15.00 The rise and fall and the future of SARS-CoV-2 monoclonal antibodies
Rino RAPPUOLI (*Siena*)

15.00-15.15 **OC 1 -** Delivery of gold-nanoparticles functionalized with CRISPR-Cas13 as an antiviral strategy against emerging RNA viruses
Alessandro DE CARLI (*Siena*)

15.15-15.30 **OC 2** - Small-Molecule Inhibitor of Flaviviral NS3-NS5 Interaction with Broad-Spectrum Activity and Efficacy In Vivo
Beatrice MERCORELLI (*Padua*)

15.30-15.45 **OC 3** - Identification of novel antiviral compounds with nanomolar potency against influenza virus
Anna BONOMINI (*Padua*)

15.45-16.00 **OC 4** - DKA inhibitors of Nsp13 of SARS-CoV-2 block viral replication
Angela CORONA (*Cagliari*)

16.00-16.15 **OC 5** - A novel promising antiviral peptide: HS-1 and its ala-scanning analogs
Annalisa CHIANESE (*Naples*)

Sunday 25th June 2023

PARALLEL SESSIONS

AULA MAGNA

SESSION 2 **Viral pathogenesis and disease**
Chairs: **Massimo ANDREONI** (*Rome*), **Carlo Federico PERNO** (*Rome*)

16.30-17.00 From viral pathogenesis to clinical phenotype in viral diseases
Andrea ANTINORI (*Rome*)

17.00-17.30 RSV in immunocompromised patients: light and shadow
Raffaele BRUNO (*Pavia*)

17.30-17.45 **OC 6** - In vivo model of SARS-CoV-2 infection in K18-hACE2 mice
Paola QUARANTA (*Pisa*)

17.45-18.00 **OC 7** - Investigation of norovirus evolution using the cDNA of an archival collection of stools from children with acute gastroenteritis
Chiara FILIZZOLO (*Palermo*)

18.00-18.15 **OC 8** - The effects of COVID-19 on the epidemiology of common respiratory infections in children: a single-center analysis of the past 5 years
Luisa GIANNONE (*Brescia*)

18.15-18.30 **OC 9** - Coinfection of dermal fibroblasts by human cytomegalovirus and human herpesvirus 6 can boost the expression of fibrosis-associated micrornas
Irene SOFFRITTI (*Ferrara*)

18.30-19.30 **With the unrestricted grant of COPAN GROUP**
Chairs: **Clementina COCUZZA** (*Milan*), **Serena DELBUE** (*Milan*)

The role of saliva in virological diagnosis
Elisa BORGHI (*Milan*)

Monitoring viral zoonosis: the role of sample collection
Marco ZACCARONI (*Florence*)

End day 1

AULA FRANCESCHETTI

SESSION 3 **Diagnostics in human virology**
Chairs: **Rosanna CAVALLO** (*Turin*), **Tiziana LAZZAROTTO** (*Bologna*)

16.30-17.00 A glimpse into the future of diagnostic virology: what will it look like and what are the challenges?
Fabrizio MAGGI (*Rome*)

17.00-17.15 **OC 10** - Clinical utility of genotypic drug-resistance test in immunocompromised patients with cytomegalovirus refractory infection
Vincenzo MOTTA (*Bologna*)

17.15-17.30 **OC 11** - Analysis of human papillomavirus (HPV) genotypes detection in cervical, vaginal and urine samples
Marianna MARTINELLI (*Milan*)

17.30-17.45 **OC 12** - Detection of SARS-CoV-2 exploiting plasmonic plastic optical fiber probes combined with molecularly imprinted polymers
Raoul FIORAVANTI (*Rome*)

17.45-18.00 **OC 13** - HIV-1 DNA resistance assessment through NGS in highly treatment-experienced individuals under virological control enrolled in the prestigio registry
Maria M. SANTORO (*Rome*)

18.00-18.15 **OC 14** - Body fluids biomarkers for HPV-related head and neck cancer diagnosis
Luisa GALATI (*Milan*)

18.15-18.30 **OC 15** - HCV detection of active HCV infection with Elecsys HCV DUO test
Stefano BRUSA (*Naples*)

18.30-19.30 **With the unrestricted grant of ELITECH GROUP**
Chairs: **Giuseppe PORTELLA** (*Naples*), **Nicasio MANCINI** (*Varese*)

CMV-RNA detection as new marker in pediatric HSCT recipients
Tiziana LAZZAROTTO (*Bologna*)

CMV-RNAemia as new marker in pediatric HSCT recipients
Cristina RUSSO (*Rome*)

Universal CMV screening as a tool to manage congenital CMV infection
Simona FIORENTINI (*Brescia*)

Monday 26th June 2023

PLENARY SESSION
AULA MAGNA AND AULA FRANCESCHETTI

SESSION 4 HIV/AIDS SIMIT/SIV-ISV joint session
Chairs: **Guido ANTONELLI** (*Rome*), **Claudio MASTROIANNI** (*Rome*)

09.00-09.30 New targets in the HIV life cycle
Carlo Federico PERNO (*Rome*)

09.30-10.00 Clinical management of HIV multidrug resistance
Antonella CASTAGNA (*Milan*)

10.00-10.15 **OC 16** - A truncated isoform of Cyclin T1 could contribute to the non-permissive HIV-1 phenotype of U937 promonocytic Minus cells
Greta FORLANI (*Varese*)

10.15-10.30 **OC 17** - Extensive virologic characterization in an HIV-infected individual with apparent HIV remission for 2 years after allogeneic stem cell transplantation with CCR5 wild-type cells: a case study
Ilaria VICENTI (*Siena*)

10.30-10.45 **OC 18** - Identification of the minimal functional epitope of the HIV-1 matrix protein endowed with angiogenic activity via common beta chain receptor signaling
Matteo UGGERI (*Brescia*)

10.45-11.00 **OC 19** - Characterization and role of HIV-DNA minority mutations in paediatric patients cohort with a long history of infection
Luna COLAGROSSI (*Rome*)

11.00-11.15 **OC 20** - Characterization of type I/III interferon response to MPXV, HIV and HPV infections in anal mucosa of MSM
Matteo FRACELLA (*Rome*)

Monday 26th June 2023

PARALLEL SESSIONS

AULA MAGNA

SESSION 5 **Virus-host interactions**
Chairs: **Arianna CALISTRI** (*Padua*), **Francesca CACCURI** (*Brescia*)

11.30-12.00 Calcium-conducting viroporins: roles in viral replication and pathogenesis
Anna LUGANINI (*Turin*)

12.00-12.15 **OC 21** - NRF2 activators as innovative cell-targeted approaches against respiratory virus infections
Marta DE ANGELIS (*Rome*)

12.15-12.30 **OC 22** - Host and viral factors required for the biogenesis of positive-sense single-stranded RNA virus replication organelles
Mirko CORTESE (*Naples*)

12.30-12.45 **OC 23** - The N-acylethanolamine acid amidase is a SARS-CoV-2 host factor
Veronica LA ROCCA (*Pisa*)

12.45-13.00 **OC 24** - Adeno-associated virus type 2 (AAV2) uncoating is a stepwise process and is linked to structural reorganization of the nucleolus
Sereina O. SUTTER (*Zurich, Switzwerland*)

AULA FRANCESCHETTI

SESSION 6 **Zoonosis and viral infections in animals**
Chairs: **Gabriele VACCARI** (*Rome*), **Vito MARTELLA** (*Bari*)

11.15-11.45 Emerging zoonotic respiratory viruses: What can we expect?
Ana MORENO (*Brescia*)

11.45-12.15 African Swine Fever: exotic, epidemic or endemic disease? Each scenario requires an adapted strategy
Francesco FELIZIANI (*Perugia*)

12.15-12.30 **OC 25** - Hedgehogs (Erinaceus europaeus) as a new reservoir of Coronavirus
Giovanni IANIRO (*Rome*)

12.30-12.45 **OC 26** - Novel parvovirus associated with increased mortality in European hedgehogs (Erinaceus europaeus), in a wildlife rescue center, Italy, 2022
Gianvito LANAVE (*Bari*)

12.45-13.00 **OC 27** - Intra-farm circulation of swine influenza virus in North-East Italy between 2013 and 2022
Maria S. BEATO (*Perugia*)

PLENARY SESSION
AULA MAGNA AND AULA FRANCESCHETTI

13.00-13.30 **SIV-ISV GENERAL MEMBERS' MEETING**

13.30-14.30 **Lunch and Poster viewing**

Monday 26th June 2023

PARALLEL SESSIONS

AULA MAGNA

SESSION 7 **Viral diseases and vaccines**
Chairs: **Arnaldo CARUSO** (*Brescia*), **Franco Maria BUONAGURO** (*Naples*)

14.30-15.00 Immune-based approaches to cure HIV infection and AIDS
Guido SILVESTRI (*Atlanta, USA*)

15.00-15.30 Viral antigens as cancer vaccines
Luigi BUONAGURO (*Naples*)

15.30-15.45 **OC 28** - Virus-like particle–mediated delivery of the RIG-I agonist M8 triggers a robust prophylactic and therapeutic antiviral activity
Enrico PALERMO (*Rome*)

15.45-16.00 **OC 29** - Development and preclinical evaluation of virus-like particles as a vaccine platform against SARS-CoV-2
Alessandra GALLINARO (*Rome*)

16.00-16.15 **OC 30** - Assessment of BoHV-4-based vector vaccine intranasally administered in a hamster challenge model of lung disease
Valentina FRANCESCHI (*Parma*)

AULA FRANCESCHETTI

SESSION 8 **Plant and environmental virology**
Chairs: **Luisa RUBINO** (*Bari*), **Massimo TURINA** (*Brescia*)

14.30-15.00 Geminiviruses: master manipulators - and a threat to food security
Rosa LOZANO-DURAN (*Tübingen, Germany*)

15.00-15.15 **OC 31** - Plant viruses in motion: global movement and threats to plant health
Paolo MARGARIA (*Braunschweig, Germany*)

15.15-15.30 **OC 32** - Clinical and wastewater surveillance for SARS-CoV-2: the need for further studies for an integrated surveillance
Nebiyu Tariku ATOMSA (*Pisa*)

15.30-15.45 **OC 33** - Selection of naturally spread citrus tristeza virus isolates effective to prevent the infection of homologous VT- decline isolate predominant in Italy
Grazia LICCIARDELLO (*Catania*)

15.45-16.00 **OC 34** - The strange case of grapevine pinot gris virus and the grapevine leaf mottling and deformation syndrome: deep insights on virus-host interaction
Giulia TARQUINI (*Pisa*)

Monday 26th June 2023

PARALLEL SESSIONS

AULA MAGNA

SESSION 9 Linking viruses to cancer
Chairs: **Marisa GARIGLIO** (*Novara*), **Maria Lina TORNESELLO** (*Naples*)

16.15-16.45 HTLV-1 and its host: the "voices from inside" in the control or the development of oncogenicity
Roberto ACCOLLA *(Varese)*

16.45-17.00 **OC 35** - Antigenic molecular mimicry in viral-mediated protection from cancer: the HIV case
Maria TAGLIAMONTE (*Naples*)

17.00-17.15 **OC 36** - The cellular deacetylase SIRT1 contributes to HPV-Driven p53 inhibition and it is an actionable therapeutic target
Federica CALATI (*Novara*)

17.15-17.30 **OC 37** - Oral microbiome analysis in individuals with oral high-risk human papillomavirus infection
Eugenia GIULIANI (*Rome*)

17.30-17.45 **OC 38** - A genome-wide CRISPR-Cas9 loss-of-function screening to identify novel host restriction genes contributing to oncolytic virotherapy resistance in pancreatic ductal adenocarcinoma
Michela MUSCOLINI (*Rome*)

AULA FRANCESCHETTI

SESSION 10 Structural virology and biotechnology
Chairs: **Mauro PISTELLO** (*Pisa*), **Davide GIBELLINI** (*Verona*)

16.15-16.45 Enhancing genome-editing tools for treating viral infections
Michele LAI (*Pisa*)

16.45-17.00 **OC 39** - Functional and structural comparative analysis of nuclear localization signals on polyoma-viruses Large Tumor Antigens reveals crucial role of bipartite NLS in nuclear translocation
Gualtiero ALVISI (*Padua*)

17.00-17.15 **OC 40** - Oncolytic HSV-1 vectored by monocytes as a broad-spectrum anticancer therapy
Alberto REALE (*Padua*)

17.15-17.30 **OC 41** - Anti-cancer activity of the oncolytic adenovirus dl922-947 in triple-negative breast cancer cells
Fabiana NAPOLITANO (*Naples*)

17.30-17.45 **OC 42** - Gene editing of HIV-1 latently infected cells using an activation-inducible system
Gloria MAGRO (*Padua*)

PLENARY SESSION
AULA MAGNA AND AULA FRANCESCHETTI

SESSION 11 Plenary lecture
Chairs: **Arnaldo CARUSO** (*Brescia*), **Arianna CALISTRI** (*Padua*)

18.00-18.40 Planetary health: the threat of emerging viruses
Giorgio PALÙ (*Padua*)

18.40-19.00 **SIV-ISV Award 2023**

End day 2

20.30 Social Dinner

Tuesday 27th June 2023

PARALLEL SESSIONS

AULA MAGNA

SESSION 12 Viral immunology

Chairs: **Roberto BURIONI** (*Milan*), **Roberta RIZZO** (*Ferrara*)

09.00-09.30 Clash of the titans: RNA viruses and interferons
Nolwenn JOUVENET
(Paris, France)

09.30-09.45 **OC 43** - In cART-treated HIV-infected patients, the immunological failure is associated with a high myeloid derived suppressor cell frequency
Germana GRASSI (*Rome*)

09.45-10.00 **OC 44** - Efficacy of COVID-19 mRNA vaccination in patients with autoimmune disorders: humoral and cellular immune response
Federica FILIPPINI (*Brescia*)

10.00-10.15 **OC 45** - Neurodegerative role of west nile virus non-structural protein 1: effect on TLR3 and amyloid beta expression
Silvia BELTRAMI (*Ferrara*)

10.15-10.30 **OC 46** - Antibodies induced by smallpox vaccination after at least 45 years crossreact with and in vitro neutralize monkeypox virus
Roberto NISINI (*Rome*)

AULA FRANCESCHETTI

SESSION 13 Emerging viruses

Chairs: **Giovanni GIAMMANCO** (*Palermo*), **Massimo CICCOZZI** (*Rome*)

09.00-09.30 A reinassance for Poxviruses
Geoffrey SMITH (*Oxford, UK*)

09.30-10.00 Tracking the spread of emerging and re-emerging viral pathogens is key for predicting and preventing future epidemics
Marta GIOVANETTI
(*Rome/Rio de Janeiro, Brazil*)

10.00-10.15 **OC 47** - Exploring the neuro-pathogenicity of emerging strains of West Nile virus lineage 1 and 2
Alessandro SINIGAGLIA (*Padua*)

10.15-10.30 **OC 48** - Significant occurrence of rat hepatitis e in wastewater networks: surveillance of an emerging zoonotic virus
Andrea PALOMBIERI (*Teramo*)

10.30-10.45 **OC 49** - Response to monkeypox virus: serological evaluation of infected patients and naïve vaccinated healthcare workers
Josè Camilla SAMMARTINO
(*Pavia*)

Tuesday 27th June 2023

PLENARY SESSION
AULA MAGNA AND AULA FRANCESCHETTI

SESSION 14 The immunology and genetics of respiratory viruses
Chairs: **Anna Teresa PALAMARA** (*Rome*), **Massimiliano GALDIERO** (*Naples*)

10.45-11.15 Across the winter into the virus cloud
Fausto BALDANTI (*Pavia*)

11.15-11.45 Human respiratory viruses: immunity, circulation and clinical disease
Roberto BURIONI (*Milan*)

11.45-12.00 **OC 50** - Viral evolution surveillance: lessons learned from COVID-surveillance network on data quality
Laura SQUARZON (*Venice*)

12.00-12.15 **OC 51** - Epidemiology and molecular analyses of Respiratory syncytial virus in the season 2021-2022 in Northern Italy
Alessia LAI (*Milan*)

12.15-12.30 **OC 52** - Local inflammatory response during respiratory syncytial virus infection in infants
Valentina GENTILI (*Ferrara*)

12.30-12.45 **Luria Awards**

12.45-13.00 Closing remarks

13.00-13.30 Delivery and compilation CME questionnaire

Closing of the Congress

Poster & Oral Communications

PO 01 **OC 01** - DELIVERY OF GOLD-NANOPARTICLES FUNCTIONALIZED WITH CRISPR/Cas13 AS AN ANTIVIRAL STRATEGY AGAINST EMERGING RNA VIRUSES
Alessandro DE CARLI (*Siena*)

PO 02 **OC 02** - SMALL-MOLECULE INHIBITOR OF FLAVIVIRAL NS3-NS5 INTERACTION WITH BROAD-SPECTRUM ACTIVITY AND EFFICACY IN VIVO
Beatrice MERCORELLI (*Padua*)

PO 03 **OC 03** - IDENTIFICATION OF NOVEL ANTIVIRAL COMPOUNDS WITH NANOMOLAR POTENCY AGAINST INFLUENZA VIRUS - **Anna BONOMINI** (*Padua*)

PO 04 **OC 04** - DKA INHIBITORS OF Nsp13 OF SARS-CoV-2 BLOCK VIRAL REPLICATION
Angela CORONA (*Cagliari*)

PO 05 **OC 05** - A NOVEL PROMISING ANTIVIRAL PEPTIDE: HS-1 AND ITS ALA-SCANNING ANALOGS - **Annalisa CHIANESE** (*Naples*)

PO 06 NEUTRALIZING ACTIVITY AGAINST WILD-TYPE, DELTA PLUS AND OMICRON BA.2.1 VARIANTS OF SARS-CoV-2 ELICITED BY THE BNT162B2 COVID-19 VACCINE BOOSTER IN ELDERLY - **Kaleem SHAIK** (*Varese*)

PO 07 EFFECT OF ATMOSPHERIC COLD PLASMA TREATMENT ON MURINE NOROVIRUS AND HEPATITIS A VIRUS INFECTIVITY - **Teresa VICENZA** (*Rome*)

PO 08 ANTIVIRAL ACTIVITY OF RILK1 PEPTIDE ON MURINE NOROVIRUS AND HEPATITIS A VIRUS: STUDY ON THE ACTION MECHANISM - **Teresa VICENZA** (*Rome*)

PO 09 IDENTIFICATION OF OXYSTEROL SYNTHETIC ANALOGS AS A NOVEL CLASS OF ANTI-HERPES SIMPLEX VIRUS ANTIVIRALS - **Andrea CIVRA** (*Turin*)

PO 10 REPURPOSING ANTIVIRAL DRUGS AS INHIBITORS AGAINST SARS-CoV-2 AND HSV-2 VIRUSES - **Silvia NOTTOLI** (*Pisa*)

PO 11 DHFR INHIBITORS DISPLAY A PLEIOTROPIC ANTI-VIRAL ACTIVITY AGAINST SARS-CoV-2: INSIGHTS INTO THE MECHANISMS OF ACTION - **Alberto ZANI** (*Brescia*)

PO 12 STRIGOLACTONES AS BROAD-SPECTRUM ANTIVIRALS AGAINST β-CORONAVIRUSES THROUGH TARGETING THE MAIN PROTEASE MPRO
Greta BAJETTO (*Novara*)

PO 13 GENERATION OF A CELLULAR SCREENING PLATFORM TO IDENTIFY SMALL MOLECULES ACTIVE AT INHIBITING ANTI-TYPE-I INTERFERON (IFN-I) PROTEINS BELONGING TO PATHOGENIC VIRUSES - **Marco SGARBANTI** (*Rome*)

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Beatrice MOLA (*Bologna*)

Abstract

EMERGING ANTIVIRALS FOR CHRONIC HEPATITIS B INFECTION

R. De Francesco

INGM - Istituto Nazionale Genetica Molecolare INGM "Romeo ed Enrica Invernizzi", Milan, Italy;
Department of Pharmacological and Biomolecular Sciences (DiSFeB), University of Milan, Italy

Hepatitis B virus (HBV) is a major cause of liver disease, including chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). Current treatments can only suppress HBV replication, but they do not cure the infection. There is an urgent need for a cure for HBV, as it affects an estimated 240 million people worldwide.

The current standard of care for chronic HBV infection is pegylated interferon-alpha (pegIFNα) or nucleos(t)ide analog (NA). These therapies can suppress HBV replication and improve the patient's quality of life, but they do not completely cure the infection. This is because the virus can hide in the nucleus of infected cells in the form of stable covalently closed circular DNA (cccDNA). While a "sterilizing cure" that eliminates the viral genome from the body is difficult or even impossible to achieve in most patients, there have been major efforts to develop a "functional cure" for HBV. This would involve the sustained suppression of HBV replication and viral protein production, and possibly the restoration of the immune response to HBV.

To this end, a number of novel direct-acting antivirals (DAAs), such as those targeting virus entry or capsid assembly, are in clinical trials. In addition, viral gene silencing by RNA interference or antisense RNA oligonucleotides, as well as immune modulatory strategies to stimulate adaptive or innate immunity and/or to remove immune blockade, are being tested.

In this lecture, I will discuss the current state of the art in the search for a cure for hepatitis B. I will review the different approaches that are being explored, and I will discuss the challenges and potential benefits of each approach. I will also discuss the latest research findings in this area, and I will provide an overview of the future of HBV cure research.

THE RISE AND FALL AND THE FUTURE OF SARS-CoV-2 MONOCLONAL ANTIBODIES

R. Rappuoli
Fondazione Biotecnopolo di Siena, Italy

Human monoclonal antibodies are widely used for therapy in cancer, autoimmune, and inflammatory diseases. More than 100 have been licensed during the last 20 years and they represent a rapidly growing sector of the pharmaceutical market. They are safe and effective, but also quite expensive and require intravenous administration so that they have not been used much in the field of infectious diseases. During the last few years, several monoclonal antibodies have been developed against some infectious agents such as HIV, respiratory syncytial virus (RSV), Ebola virus, rabies, and malaria. They are usually isolated from memory B cells from convalescent or vaccinated people and in some cases, they are very potent, so that an effective dose, which provides protection for 6 months, can be delivered by intramuscular injection at a reasonable price. The COVID-19 pandemic provided an opportunity to develop and license several monoclonal antibodies, which proved to be safe and to protect from severe disease. However, by now all of them have been removed from the market because they are not able to neutralize the latest SARS-CoV-2 variants. The challenge for the future is to find a new generation of variant-resistant monoclonal antibodies and to expand their use also to bacteria, especially those resistant to antibiotics for which we have exhausted the conventional therapies.

RSV IN IMMUNOCOMPROMISED PATIENTS: LIGHT AND SHADOW

R. Bruno

Università di Pavia, SC Malattie Infettive, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

Respiratory syncytial virus (RSV) causes acute respiratory tract illness in persons of all ages. The clinical manifestations vary with age, health status, and whether the infection is primary or secondary. RSV has the potential to cause severe LRTI in certain high-risk groups, including young, premature infants with chronic lung disease, congenital heart disease, or Down syndrome; patients with persistent asthma; patients who are immunocompromised; and older adults. RSV should also be suspected in patients hospitalized with acute lower respiratory tract disease if they are immunocompromised or ≥50 years of age. Laboratory diagnosis of RSV should be pursued in if identification of RSV will affect clinical management. This is often the case for children hospitalized with acute lower respiratory tract disease, immunocompromised patients, immune-compete patients with recurrent respiratory illnesses, and immune competent older adults who are hospitalized with acute respiratory illness.

When confirmation of RSV infection is necessary, we prefer polymerase chain reaction (PCR)-based assays if they are available. PCR-based assays have high sensitivity and are not affected by passively administered antibody to RSV. If PCR-based assays are not available, rapid antigen detection tests are a reasonable alternative, although false negative results are common in adult patients. Decisions regarding treatment of RSV infection in immunocompromised patients should be individualized. The optimal treatment is uncertain. Interventions that have been associated with reduced rates of progression from upper to lower respiratory tract infection or decreased mortality in observational studies include single agent or combination therapy with ribavirin, intravenous immune globulin, palivizumab, and/or glucocorticoids.

THE ROLE OF SALIVA IN VIROLOGICAL DIAGNOSIS

E. Borghi
Department of Health Sciences, Università degli Studi di Milano, Italy

The COVID-19 pandemic has spurred the scientific community in implementing viral diagnosis for population-based surveillance, including asymptomatic carriers and close contact screening, and virological clearance monitoring in affected people.
The need for recurrent testing, in turn, highlighted the need for non-invasive and reliable sampling implementation. In this context, saliva gained popularity as it offers distinct advantages such as non-invasiveness, the possibility of self-sampling, and high sensitivity for detecting SARS-CoV-2 asymptomatic infections. Our group demonstrated a 0.96 concordance with nasopharyngeal swab (NPS) in asymptomatic people, and a 0.94 in children (both symptomatic and asymptomatic).
Besides SARS-CoV-2, saliva-based molecular tests revealed a good sensitivity for other viruses responsible for acute respiratory infections (ARI), such as influenza and respiratory syncytial virus. In the pediatric population, an elective reservoir and a recognized target for ARI, implementation strategies for non-invasive sampling could also reduce reported biases due to the NPS procedure. In a pilot study on influenza-like illnesses in preschools, instructed parents were able to self-sampled saliva for molecular testing, reducing the need for medical staff and unnecessary psychological stress for kids.
Less intuitively, saliva sampling is a reliable option for the detection of a variety of infectious diseases beyond the respiratory tract, with whom this fluid is in close contact.
Indeed, several salivary biomarkers, such as viral DNA and RNA, antigens and antibodies have been tested so far. The American FDA approved saliva HIV testing kits for public use in as early as in 2003, and Hepatitis A RNA as well as monkeypox DNA detection in saliva has been suggested as a useful marker for tracing and monitoring infection in community settings.
Despite convincing data from the literature, policymakers often consider saliva as an option for viral diseases when it is not possible to obtain nasopharyngeal swabs or blood/serum.
Hence, further efforts are needed for exploiting this biological fluid as a recognized and cost-effective tool in disease diagnosis and monitoring.

MONITORING VIRAL ZOONOSIS: THE ROLE OF SAMPLE COLLECTION

M. Zaccaroni[1], M. Delledonne[2], D. Lelli[3], C. Augugliaro[4], F. Gili[5], S. Bertolino[5], P. Agnelli[1], B. Nasanbat[6], G. Davaakhuu[6], M. Bazarragchaa[7], R. Fani[1], D. Cavalieri[1], A. Papini[1], G. Santini[1], L. Cecchi[1], M. Calzolari[3], E. Mori[8], L. Ancillotto[8], A. Andreani[1], M. Alfano[2], A. Montagna[9], F. Riga[10]

[1] *Università degli Studi di Firenze, Italy*
[2] *Università degli Studi di Verona, Italy*
[3] *Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy*
[4] *Wildlife Initiative NGO, Ulan Bator*
[5] *Università degli Studi di Torino, Italy*
[6] *Institute of Biology, Mongolian Academy of Sciences, Ulaanbaatar*
[7] *Mongolian National University of Medical Sciences, Ulaanbaatar*
[8] *Consiglio Nazionale delle Ricerche, Florence, Italy*
[9] *Freelance, Padua, Italy*
[10] *Istituto Superiore per la Protezione e la Ricerca Ambientale, Rome, Italy*

In May 2022, a 25-day scientific expedition took a group of 14 scientists and three support staff to one of the most remote and least densely populated regions on the planet: the Gobi desert of Mongolia. As a collaboration among the Institute of Biology of the Mongolian Academy of Sciences, the University of Verona, ISPRA (Italian Institute for Environmental Protection and Research), IZSLER (Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna) and the University of Florence, the mission was focused on the search for small mammals, such as hamsters, gerbils, bats or hedgehogs with the view of completing a checklist of the mammalian fauna of the Great Gobi. The animals have been studied from a systematic and conservation point of view and an epidemiological perspective by analyzing their microbiome (bacteria and viruses). These studies may lead to a better understanding of the microbial communities and therefore afford tools for potential cures, vaccines and anticipation of new diseases.
The collected specimens have been identified on-site through cutting-edge DNA technologies (DNA barcoding) in a fully portable molecular biology laboratory involving miniaturized DNA sequencing. The same approach has also been used to identify viruses and other pathogens in remote, isolated areas linked to mammals. In this scenario, the storage of DNA and RNA samples of good quality for lab analyses represented one of the biggest challenges we faced.

A GLIMPSE INTO THE FUTURE OF DIAGNOSTIC VIROLOGY: WHAT WILL IT LOOK LIKE AND WHAT ARE THE CHALLENGES?

F. Maggi

UOC Laboratory of Virology and Laboratories of Biosafety, National Institute for Infectious Diseases "Lazzaro Spallanzani", Rome, Italy

Diagnostic virology is crucial for detecting and controlling viral illnesses. Looking into the future, considerable innovations in genomics and diagnostic methods are expected to change virology, and new intriguing challenges lie on the horizon. Innovative technologies (such as NGS and metagenomics) offer the potential for sophisticated genomic analyses, allowing the detection and characterization of multiple viruses simultaneously. Artificial intelligence and machine learning algorithms are set to play an essential role. They can assist in big data analysis and development of predictive models, thus helping to identify complex viral signatures and enable personalized treatment strategies. These and other advancements (for example in POCT technology) promise greater accuracy, speed, and cost-effectiveness in viral diagnoses. However, challenges remain on the horizon. Establishing global, real-time surveillance systems as well as ensuring international collaboration, protocol standardization, accessibility and affordability of advanced diagnostic technologies will be essential for the rapid identification and characterization of viral outbreaks. Continuous research and development efforts will also be necessary to stay ahead of the challenge of evolving viral pathogens and ensure virological diagnostic methods remain effective. In conclusion, by embracing innovation while promoting inclusivity and collaboration diagnostic virology will be able to significantly contribute to the prevention, control, and management of viral diseases.

CMV-RNAemia AS ACCURATE MARKER OF ACTIVE VIRAL REPLICATION IN TRANSPLANT RECIPIENT

F. Lanna[1], G. Piccirilli[2], M. Franceschiello[2], V. Motta[1], M. Leone[1], A. Cantiani[1], A. Primavera[1], E.C. Borgatti[1], S. Felici[2], E. Petrisli[2], S. Venturoli[2], L. Gabrielli[2], T. Lazzarotto[1,2]

[1] Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Italy
[2] Microbiology Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Italy

Background: Cytomegalovirus (CMV) infection is an important cause of morbidity and mortality in transplant recipients. The study evaluated the clinical utility of CMV-RNAemia, combined with CMV-DNAemia, to detect active infection and guiding therapy (pre-emptive and prophylaxis strategies) during post transplant period.

Methods: A total of 217 plasma samples from 40 transplant recipients (32 hematopoietic stem cell transplant and 8 solid organ transplant) were retrospectively tested by CMV-RNA ELITe MGB® kit on ELITe InGenius® instrument (ELITechGroup) for the detection and quantification of CMV-RNAemia (target UL21.5 mRNA). Specifically: 99 samples were from 22 patients receiving letermovir (LTV) as prophylaxis and 118 samples from patients (n=18) treated with anti-CMV drugs as pre-emptive therapy. For all these cases, CMV-DNAemia on WB was positive.

Results: Among the patients receiving LTV, positive results of CMV-RNAemia were obtained in 4/22 (18.2%) cases, identifying active CMV infections that needed of pre-emptive treatment. In these cases, positive results were also obtained in at least one of the additional tests used in routine (CMV-shell vial and/or CMV-DNAemia post-DNase). In the remaining 18/22 (81.8%) cases, the CMV-RNAemia test was negative as well as the additional test used in routine. Among the patient receiving pre-emptive therapy, CMV-RNAemia positive results were also obtained in 17/18 (94.4%) cases. Analysing the active CMV infection episodes, the peaks of CMV-DNAemia on WB and CMV-RNAemia on plasma samples were reached simultaneously, with median levels equal to 13,799 copie/mL and 141 copie/mL, respectively. Interestingly, during the descending phase of infective episode, negative results were obtained earlier with RNAemia than DNAemia (mean time 10 days before), proving more rapidly an efficient viral clearance. This could be due to the detection of free viral DNA from abortive infection or released from infected cells.
Specificity of 100% was obtained analysing 100 plasma samples from patients without active CMV infection.

Conclusions: The results showed that the CMV-RNAemia used together with CMV-DNAemia in the viral surveillance of CMV infection provides accurate information on viral load kinetics, mostly in patients receiving LTV prophylaxis/therapy.

CMV-RNA DETECTION AS NEW MARKER IN PEDIATRIC HSCT RECIPIENTS

C. Russo

Responsabile Struttura Semplice, Virologia e Micobatteri, UOC Microbiologia e Diagnostica di Immunologia, Ospedale Pediatrico Bambino Gesù - IRCCS Roma Italy

CMV infection and disease can be life-threatening in immunocompromised patients and because common following allogenic HSCT, despite advances in diagnosis and pre-emptive therapy, it still causes significant morbidity and mortality. CMV viraemia occurs in approximately 25% of paediatric allogenic HSCT recipients, occurring mostly early post-transplant.
Effective lab tests for monitoring the drug efficiency are missing, mostly because the CMV-DNA is unable to properly evaluate antiviral efficacy. We present preliminary data on CMV monitoring by a new commercial assay for CMV-RNA detection and quantitation in a cohort of hematopoietic stem cell transplanted children after antiviral treatments.
Whole blood and plasma samples, referred to 30 patients (median age 10, IQR 1-21 years), were collected to perform simultaneously CMV-DNAemia and CMV-RNAemia.
CMV-DNA was persistently present in 88.5%, while only in 26.2% CMV-RNA was detected. Under pre-emptive therapy/treatment of infection, all patients achieved CMV-DNA negativization in blood within 20 days. Despite the presence of positive CMV-DNA, the clinical evolution was compatible with the absence of CMV activity thus suggesting that the undetectability of CMV-RNA is driven by the infection evolution and not by the lack in the method sensitivity.
The CMV-RNA may represent an accurate marker to monitor clinical antiviral efficacy, more than CMV-DNA. Moreover, CMV-RNA quantification could be used as pharmacodynamic read-outs to determine, in the future, the efficacy of vaccines activity against HCMV replication in this patient population.

UNIVERSAL CMV SCREENING AS A TOOL TO MANAGE CONGENITAL CMV INFECTION

S. Fiorentini
University of Brescia Medical School, Brescia, Italy

Early identification of congenital cytomegalovirus (cCMV) infection is crucial to optimize clinical management of symptomatic or asymptomatic newborns by means of therapy, intensified monitoring and/or early initiation of supportive care. To fullfill this goal two alternative strategies can be adopted:

a) a targeted screening, where testing is performed in newborns with a history of maternal primary infection during pregnancy, a failed hearing screening, or other symptoms suggestive of cCMV infection;
b) a universal cCMV screening where all newborns, independently from the presence of risk factors or cCMV-related clinical signs, are tested for the presence of CMV-DNA.

I will briefly summarize the state of the art on the topic describing the pros and cons of the two approaches. Further, data from our direct experience of a three-year-long screening will be discussed.

CALCIUM-CONDUCTING VIROPORINS: ROLES IN VIRAL REPLICATION AND PATHOGENESIS

A. Luganini

Laboratory of Microbiology and Virology, Department of Life Sciences and Systems Biology, University of Turin, Italy

Background: Viruses have acquired numerous cellular genes during the coevolution with their hosts. The captured homologs genes are then optimized to encode modern viral proteins that retain or inhibit the original cellular function, or, as in most cases, result in new functions that ensure viral persistence in the host. In this regard, the Human Cytomegalovirus (HCMV) has invested a large part of its protein coding potential to generate regulatory viral proteins able to interfere with many cell biochemical and physiological pathways. Of the numerous HCMV genes encoding proteins with signs of similarity to cellular proteins, the US21 viral protein provides an example of this evolutionary strategy: it is a homolog of cellular TMBIM proteins and an ER-resident virus-encoded calcium-[Ca^{2+}] permeable channel able to dysregulate intracellular Ca^{2+} homeostasis and inhibit apoptosis to confer edges to HCMV replication (Luganini et al., PNAS, 2018).

Aim of the study: Given the role of Ca^{2+} in controlling cell adhesion and motility, we also investigated whether the pUS21-mediated Ca^{2+} release from intracellular stores might influence these important cytobiological responses.

Methods: Tetracycline-Regulated Expression (T-REx) U2OS system was exploited to achieve tetracycline (Tet, 1 mg/ml) inducible expression of pUS21-HA in not infected cells. Intracellular Ca^{2+} homeostasis was determined by ratiometric cytosolic Ca^{2+} measurement with Fura-2 AM. Chemotactic and random migration assay were performed using a transwell system and time lapse microscopy, respectively. Calpain activity was evaluated by a fluorometric assay to measure the cleavage of calpain-substract. Adhesion assay was performed with counting cell nuclei stained with DAPI. Focal adesions was quantified by immunofluorescence staining, using a MAb against paxillin protein. A mass spectrometry study revealed an interaction between pUS21 expressed in Tet-inducible cells and cellular proteins. Such interaction was confirmed by both co-immunoprecipitation and immunofluorescence in infected cells.

Resuts and Conclusions: A chemotactic migration assay demonstrated that cells infected with TRUS21wt virus migrate faster than not-infected or TRDUS21-infected cells. Engineered human cell lines,to express pUS21 in an inducible manner, confirmed a significant increase in the migration rate of cells expressing pUS21wt, while its mutation in the critical D201 residue, that define the pUS21's Ca^{2+} channel leaking function, affected the ability to stimulate cell motility, thus suggesting an involvement of its ability to reduce the Ca^{2+} content in intracellular stores. To test this hypothesis, we performed migration assays in presence of an inhibitor of calpain 2, a Ca^{2+}-activated cysteine protease, that regulates the focal adhesion dinamycs promoting cell motility. The addition of the calpain 2-inhibitor abrogated the pUS21-mediated increase of migration in cells expressing pUS21. As a further confirmation, calpain 2's biochemical activity was measured in cell expressing either pUS21wt or its mutated forms in the two critical amino acid residues that define the TMBIM architecture of pUS21, D178 and D201. The expression of pUS21-D201N was unable to stimulate calpain 2 activity, thus confirming an involvement of pUS21 Ca^{2+} channel function in the control of calpain 2 activation. We also demonstrated that the US21-dependent Ca^{2+}-depletion from ER into cytoplasm activated the SOCE, that allows extracellular Ca^{2+} to enter cells and replenish the intracellular stores. Moreover, the functional relationship between pUS21 and calpain 2 was further suggested by the observation that talin 1, a well-known calpain proteolytic substrate, interact with pUS21 [Luganini et al., mBio 2023]. Together, these findings highlight a novel role of the pUS21 viroporin of HCMV as a regulator of cellular responses, such as cell migration and adhesion dynamics.

EMERGING ZOONOTIC RESPIRATORY VIRUSES: WHAT CAN WE EXPECT?

A. Moreno

Istituto Zooprofilattico della Lombardia e dell'Emilia-Romagna "B. Ubertini" (IZSLER), Brescia, Italy

Approximately 75% of the causative aetiological agents of disease reported in humans over the past 30 years are zoonotic agents that originated in animals, especially wildlife. This implies the occurrence of spillover events for which direct or indirect contact between humans and animals is essential and the subsequent efficient interspecific transmission necessary for the spread of disease in the human population. All these events are influenced by the interplay of complex and still poorly understood mechanisms. Although human-animal contact has existed for thousands of years, it is only in recent decades that various anthropogenic factors such as loss of biodiversity, human and agricultural intensification and increased movement of humans, animals and their products have led to an increase in human-animal interactions and thus to a potential increase in the risk of emergence and spread of new zoonotic agents. These factors can precipitate the emergence of new diseases, as they allow infectious agents to evolve in new ecological niches, to reach and adapt to new hosts and to spread more easily among new hosts. Consequently, the impact of an emerging disease is difficult to predict but could be significant, as humans may have little or no natural immunity to the disease. This report will describe both the general aspects of zoonotic spillover and the main factors involved such as inter-species interactions, phylogenetic distances between host species, environment and specific characteristics of pathogens, humans and animals. In particular, reference will be made to respiratory viruses, influenza A viruses and coronaviruses, which in recent years have been responsible for emerging zoonoses with epidemic and pandemic spread. Aspects of antigenic variability, characteristics and specific affinities between viral proteins and cellular receptors will also be explored, using as examples some subtypes of influenza A viruses involved in pathological processes in humans and the SARS- and MERS-like beta-coronaviruses identified in wildlife.

AFRICAN SWINE FEVER: EXOTIC, EPIDEMIC OR ENDEMIC DISEASE? EACH SCENARIO REQUIRES AN ADAPTED STRATEGY

F. Feliziani

Laboratorio Nazionale di Riferimento per lo Studio delle Malattie da Pestivirus e Asfivirus Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati" (IZSUM), Perugia, Italy

African Swine Fever (ASF) is an animal infectious disease with many original and unusual features. The disease agent is the only known DNA arbovirus; the cradle of origin is the African continent where the target hosts, in addition to ticks of the Ornithodoros genus, are wild suids (warthog, potamoceros), but certainly the greatest threat is the high mortality it causes in domestic and wild pigs. The disease does not affect humans, but has a strong economic and social impact and should therefore not be underestimated.

The first epidemic wave dates back in the late 1950s involving Europe and Latin America, whereas the last epidemic wave began in 2007 in Georgia and rapidly evolved into the current pandemic, spreading to several continents and currently hitting Europe, Italy included. Despite this prolonged circulation and therefore extensive experience gained on the disease, it remains difficult to identify a strategy to control and prevent ASF: in many scientific publications it is described as an exotic disease, several countries are concerned about the introduction of the virus and set up emergency plans, but its tendency to become endemicis becoming increasingly evident.

ASF lends itself to be a disease model in which interactions between pathogen, host and the environment, all together shape the epidemiological evolution. The virus is characterised by more than 20 different genotypes and some strains with low virulence are described, but, these are true exceptions. what makes the difference, therefore, are the conditions in which the virus finds susceptible hosts: the rearing conditions, if we are talking about domestic pigs, the habitat of wild suids, not forgetting the possible interactions between these populations, which are often intermingled.

Certainly, experts agree that the human factor is crucial in favouring both transmission and persistence of the infection.

ASF is defined as a trans-boundary disease, and indeed, it can be said that it recognised no borders, but is even capable of making real leaps and bounds and crossing oceans. In this view, ASF has now taken on an important political significance.

Currently, the strategy to eradicate infection in domestic farms is universally based on early detection of outbreaks and stamping out of live animals. These measures are unavoidable because no vaccine is available. In wild pigs it is much more complicated. The only successful models have been applied in Belgium and the Czech Republic: fences containment of the infected population and culling of wild boars is envisaged, but only after the epidemic peak. It is evident that such a model is applicable in restricted areas only.

In Europe, it is common to observe that wild boars have increased in numbers remaining in their natural habitat and occupying new ones such as the urban areas. This causes an increase in the risk of ASF spread.

However, ASF is now endemic in large areas in Africa, Asia and Europe. Despite the efforts of many researchers engaged in the development of an effective vaccine, this is not on the immediate objective. A strategy to control the disease must therefore be identified to avoid devastating economic impact. It is important to defend the pig industry, but also to take care of the many areas of the planet where pig farming is at subsistence levels and helps in the fight against poverty and hunger.

In conclusion, ASF is an infectious disease of veterinary interest, but it has a great impact on human health and the economy on a global scale. It can no longer be considered exotic and when introduced in epidemic form it tends to assume an endemic character that requires forms of control to limit its devastating effects on pig farming. It represents a challenge that needs cooperation and coordination of politicians, veterinarians, researchers, breeders, wildlife and stakeholders.

IMMUNE-BASED APPROACHES TO CURE HIV INFECTION AND AIDS

G. Silvestri
Emory University School of Medicine, Atlanta, GA, USA

While antiretroviral therapy (ART) is highly effective in suppressing active virus replication in HIV-infected individuals – and therefore has dramatically reduced the mortality and morbidity associated with this infection – we still do not have a reliable way of eliminating the persistent reservoir of latently infected cells harboring replication-competent virus, which are responsible for the rapid rebound of viremia if ART is interrupted.

In this presentation I will first briefly review the main approaches that are currently pursued in the field of HIV cure research to disrupt the reservoir establishment and persistence under ART, and ultimately to achieve either a cure or a long-term remission of this infection, These approaches include: (i) Limiting the reservoir establishment through early ART, associated with additional interventions; (ii) Direct elimination of the reservoir through interventional tools such as hematopoietic stem cell transplantation, gene therapy, gene editing, etc; (iii) Permanent silencing of the reservoir through interventions at the level of gene expression regulation ("block & lock" approach); (iv) Immunological control the reservoir through various type of immune-therapies and therapeutic vaccines); and (v) Reactivate the reservoir and then clear it via immune-mechanisms ("shock & kill" approach).

We will then focus on the recent important progress made in the area of "shock & kill" interventions that derived from studies conducted in our laboratory using the extensively validated model of SIV- or SHIV-infected, ART-treated rhesus macaques, by using various combinations of immune-based approaches and agents such as antibody-mediated CD8+ lymphocyte depletion (which allowed us to discover a previously unrecognized pro-latency activity of CD8+ T cells), Interleukin-15 (IL-15) super agonists such as N-803 and the heterodimeric IL-15, the SMAC mimetic/IAP inhibitor AZD-5582, the Bcl-2 inhibitor Venetoclax, Interleukin-10 inhibitors, PD-1 blockade, and a pool of SIV-Envelope-specific neutralizing antibodies targeting the CD4-binding site, the membrane external proximal region (MPER), and the V2-apex region. We will finally discuss the prospects for success of these interventions and the remaining challenges in our journey towards HIV eradication.

VIRAL ANTIGENS AS CANCER VACCINES

L. Buonaguro
Innovative Immunological Models Unit, Istituto Nazionale Tumori - IRCCS - "Fond G. Pascale", Naples, Italy

Background: The host's immune system develops in equilibrium with both cellular self-antigens and non-self antigens derived from microorganisms which enter the body during lifetime. In addition, during the years, a tumor may arise presenting to the immune system an additional pool of non-self antigens, namely tumor antigens (Tumor Associated Antigens, TAAs; Tumor Specific Antigens, TSAs).

Methods: In the present study we looked for homology between published TAAs and non-self viral-derived epitopes. Bioinformatics analyses and ex vivo immunological validations have been performed.

Results: Surprisingly, several of such homologies have been found. Moreover, structural similarities between paired TAAs and viral peptides as well as comparable patterns of contact with HLA and TCR α and β chains have been observed. Therefore, the two classes of non-self antigens (viral antigens and tumor antigens) may converge, eliciting cross-reacting CD8+ T cell responses which possibly drive the fate of cancer development and progression.

Conclusions: An established anti-viral T cell memory may turn out to be an anti-cancer T cell memory, able to control the growth of a cancer developed during the lifetime if the expressed TAA is similar to the viral epitope. This may ultimately represent a relevant selective advantage for cancer patients and may lead to a novel preventive anti-cancer vaccine strategy.

GEMINIVIRUSES: MASTER MANIPULATORS – AND A THREAT TO FOOD SECURITY

R. Lozano-Durán

Department of Plant Biochemistry, Centre for Plant Molecular Biology (ZMBP), University of Tübingen, D-72076 Tübingen, Germany

Viruses, as obligate intracellular parasites, need to subvert the host cell in order to enable their replication and efficient spread. Due to strict restrictions in genome size, viruses commonly produce a limited number of proteins; this is the case of geminiviruses, plant viruses with circular single-stranded (ss)DNA genomes that are believed to contain only 4-8 translated open reading frames. Strikingly, despite their limited armoury, geminiviruses are able to establish an infection, overcoming plant defence, dramatically altering plant development and physiology, and ultimately causing devastating diseases to crops worldwide. How these viruses successfully invade and manipulate their plant hosts by deploying only a handful of proteins is a long-standing, fascinating question.

In our group, we are interested in understanding how geminiviruses co-opt the plant cell and lead to disease, for which we use a combination of approaches, including molecular biology, cell biology, and genetics. Our results have shed light onto the molecular mechanisms underlying the replication of viral DNA, plant anti-viral defence and geminiviral counter-defence, and symptom development, and hint at novel virulence strategies potentially employed by geminiviruses to maximize their coding capacity and their impact on the host cell. We expect that our work will contribute to a deeper understanding of the infection process, which may in turn pave the way to the design of effective and sustainable anti-viral strategies and assist breeding programs to obtain virus-resistant plants.

HTLV-1 AND ITS HOST: THE "VOICES FROM INSIDE" IN THE CONTROL OR THE DEVELOPMENT OF ONCOGENICITY

R. Accolla

Laboratories of General Pathology and Immunology "Giovanna Tosi", Department of Medicine and Surgery, University of Insubria, Varese, Italy

Aim of the Study: Animals are indispensable in science to provide an empirical *bona fide* equivalent for pathophysiological processes in humans. Especially the investigation of infectious diseases requires models with a proper immune system to understand the spread and virulence of bacteria or viruses. In my talk, I will present results obtained from the rodent *Mastomys coucha*. This unique model was used to study the role of cutaneous papillomaviruses in non-melanoma skin cancer (NMSC) development, to test vaccines, to dissect the viral immune escape mechanisms and to understand tumor heterogeneity.

Methods: The *Mastomys coucha* breeding colony naturally infected by the cutaneous papilloma virus virus MnPV was maintained under SFP conditions. For all follow-up experiments, the animals were monitored for their whole lifetime until they had to be sacrificed due to tumor development. For serological analyses (GST-, VLP ELISAs, VLP, Pseudovirion productions and neutralization assays), blood was taken in intervals from 2-8 weeks, starting at the age of eight weeks. To understand tumor heterogeneity and the accompanied changes of intracellular networks, *in situ* proteomics of different tissue sections were performed.

Results and Conclusions: MnPV acts in combination with UV *via* a "hit-and-run" mechanism in the development of NMSC. Monitoring seroconversion during the complete course of viral infection, a novel mechanism was identified how these viruses escape from humoral immune surveillance. Using different isoforms of the major capsid protein L1, the virus first produces slightly longer versions of L1 that could not form a mature capsid, but evades the immune system by producing non-neutralizing antibodies (viral decoy). Consequently, viral amplification and spread can continue. Only after a delay of a couple of months the animals finally started making neutralizing antibodies, now directed against the shorter form of L1 that actually makes up the mature viral particles, encapsulating progeny viral DNA. The implications of these findings will be discussed in the light of our recent results where we showed that – in contrast to L1 short –the only 34 amino acid longer L1 isoform vaccine completely failed to prevent skin tumor formation after experimental infection. Moreover, subjecting big data after *in situ* proteomics to a principal component analysis (PCA), the individual skin samples cluster in strikingly distinct areas. This shows that the overall resemblance of the individual proteomes deriving from the same tissue type is principally higher than to those of other tissue origins. Spatial proximity even revealed that proteomes of well-differentiated tumor areas still preserved a higher similarity to morphologically normal epidermis, while proteomes obtained from MnPV-induced tumors and, most prominently, dedifferentiated areas of SCCs were more distinct and correlated with complete different intracellular networks. Hence, *Mastomys coucha* is a valuable preclinical model to answer a variety of questions in the context of papillomavirus-induced carcinogenesis. As an outlook, the generation of germ-free animals and their subsequent recolonization with defined microorganisms will also show the impact of both the microbiome and virome on the outcome of an infection with cutaneous PVs.

ENHANCING GENOME-EDITING TOOLS FOR TREATING VIRAL INFECTIONS

M. Lai[1], A. De Carli[1,2], D. Favaro[1], G. Freer[1], M. Pistello[1,3]

[1] *Retrovirus Center, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Italy*
[2] *Department of Medical Biotechnologies, University of Siena, Italy*
[3] *Virology Unit, Pisa University Hospital, Pisa, Italy*

Gene editing offers promising potential in the treatment of various human diseases, encompassing both infectious and non-infectious disorders. This includes conditions such as cancer, diabetes, heart failure, hematological diseases, and neurodegenerative ailments. Extensive clinical trials, numbering over 2000, have been conducted primarily focusing on genome editing to address immune system defects, viral infections, and cardiovascular diseases. However, only a limited number of gene therapeutic compounds have recently received approval, such as Gendicine in China for squamous cell carcinoma of the head and neck, and Cepero in Europe for brain cancers.
Among various gene-editing technologies, CRISPR/Cas9 has demonstrated new capabilities in combating human infectious viral diseases, which pose significant risks to human health and impose a socioeconomic burden on public health organizations worldwide. In this regard, several advances have proven the antiviral potential of CRISPR/Cas9 in managing severe human viruses, including human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2), human papillomaviruses (HPV), and other human herpesviruses.
While CRISPR has revolutionized the scientific and medical fields with its remarkable tools and strategies, concerns remain regarding off-target effects and delivery methods. Therefore, it is crucial to enhance the design of sgRNA and delivery vehicles to improve selectivity, efficacy, and minimize unintended consequences before implementing these techniques in clinical settings.

CLASH OF THE TITANS: RNA VIRUSES AND INTERFERONS

N. Jouvenet

Institut Pasteur, Université de Paris Cité, CNRS UMR3569, Virus Sensing and Signaling Unit, Paris, France

The interferon (IFN) response is a critical arm of the innate immune response and a major host defense mechanism against viral infections. Numerous genes that contribute to this antiviral state remain to be identified and characterized. Using large-scale loss-of-function strategies, we screened siRNAs or gRNAs libraries targeting hundreds of IFN-stimulated genes (ISGs) in IFN-treated cells infected with human RNA viruses, including SARS-CoV-2, Zika virus or tick-borne encephalitis virus. We recovered previously-unrecognized human genes able to modulate the replication these RNA viruses in an IFN-dependent manner. Mechanistic studies to decipher the molecular mechanisms by which these novel antiviral genes are functioning are on-going. We are also expanding our studies to the identification and characterization of ISGs in animal species that serve as viral reservoirs, such as bats and birds. Our studies should open new perspectives to target weakness points in the life cycle of these emerging RNA viruses.

TRACKING THE SPREAD OF EMERGING AND RE-EMERGING VIRAL PATHOGENS IS KEY FOR PREDICTING AND PREVENTING FUTURE EPIDEMICS

M. Giovanetti[1,2], V. Fonseca[3], T. de Oliveira[4], J. Lourenço[5], L.C.J. Alcantara[1]

[1] *Instituto Rene Rachou, Fundação Oswaldo Cruz, Belo Horizonte, Minas Gerais, Brazil*
[2] *Department of Science and Technology for Humans and the Environment, University of Campus Bio-Medico di Roma, Italy*
[3] *Organização Pan-Americana da Saúde/Organização Mundial da Saúde, Brasília, Distrito Federal, Brazil*
[4] *School for Data Science and Computational Thinking, Faculty of Science and Faculty of Medicine and Health Sciences, Stellenbosch University, South Africa*
[5] *BioISI (Biosystems and Integrative Sciences Institute), Faculdade de Ciências da Universidade de Lisboa, Portugal*

The economy and health of the world's population have been affected by infectious diseases whose main records since 1990 include diseases such as 'Spanish influenza', West Nile fever, Ebola haemorrhagic fever, HIV/AIDS, to name but a few. While dengue infection has affected several countries in the tropics and subtropics in the last 20 years, infections by zika and chikungunya viruses, which caused large outbreaks between 2014 and 2016, have threatened countries in the northern hemisphere that until then had no record of circulation of these viruses. Recently, the emergence and spread of SARS-CoV-2 virus has caused more than 460 million cases around the world and impacted people's livelihood. In Brazil, SARS-CoV-2 disruptions add to the burden of other infectious diseases caused by endemic viruses such as dengue, zika and chikungunya. Such an epidemiological context, where there is the chance of occurrence of co-infections, with possible implications for disease's severity, points to the challenge of correct identification of pathogenic agents that allows mitigating the underreporting of cases. The dramatic development of the COVID-19 pandemic has brought such attention to molecular methods for virus identification and data analysis on epidemiological and evolutionary aspects of pathogens. In this context, coupling genomic diagnostics and epidemiology to innovative digital disease detection platforms raises the possibility of an open, global, digital pathogen surveillance system. Real-time sequencing, bioinformatics tools and the combination of genomic and epidemiological data from viral infections can give essential information for understanding the past and the future of an epidemic, making possible to establish an effective surveillance framework on tracking the spread of infections to other geographic regions.

HUMAN RESPIRATORY VIRUSES: IMMUNITY, CIRCULATION AND CLINICAL DISEASE

R. Burioni
Università Vita-Salute San Raffaele, Milan, Italy

The circulation of a respiratory virus is an extremely complex phenomenon that depends on both the intrinsic characteristics of the infectious agent, the immunological status of the population, and the climatic conditions that influence contagion both directly with humidity and dominant temperature, and by inducing behaviors in the population. In temperate climates during the winter months, people tend to live indoors with closed windows, while in summer, they spend more time outdoors.
The situation becomes even more complicated when dealing with a virus that has just adapted to the human host, which rapidly evolves by generating variants while the host - initially little or not immune - modifies its immune status both through contact with the virus and thanks to vaccination. In particular, the unimaginable speed with which the COVID-19 vaccine was developed has allowed for the first time to modify the immunity of the population during the pandemic and change its course in real-time.

Poster & Oral Communications

PO 001 - OC 01

DELIVERY OF GOLD-NANOPARTICLES FUNCTIONALIZED WITH CRISPR/Cas13 AS AN ANTIVIRAL STRATEGY AGAINST EMERGING RNA VIRUSES

A. De Carli[1,5], D. Favaro[1], R. Fonnesu[1], P. Barski[4], V. Raffa[3], M. Pistello[1,2], M. Lai[1]

[1] *Department of Translational Research and New Technologies in Medicine and Surgery, Retrovirus Center and Virology Section, University of Pisa, Italy*
[2] *Laboratory of Virology Unit, Pisa University Hospital, Pisa, Italy*
[3] *Department of Biology, Nanomedicine and Molecular Biology Section, University of Pisa, Italy*
[4] *ProChimia Surfaces, Gdynia, Poland*
[5] *Department of Medical Biotechnologies, University of Siena, Italy*

Introduction: The SARS-CoV-2 pandemic highlighted the urgency of novel therapeutic approaches against emerging viruses, most of which lack of specific vaccines or therapies. Recent evidence shows that the enzyme CRISPR Associated Protein (Cas) 13 can destroy SARS-CoV-2 genomes in transfected cells. Unfortunately, there is currently no effective way to introduce this protein into virus-infected cells. The study was supported by the I-GENE project, which has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 862714.

Aim of the Study: The aim of the present work is to deliver Cas13 into infected cells using nanoformulated nanoparticles (GNPs) bypassing transfection protocols and to evaluate its antiviral efficacy against SARS-CoV-2 and an emerging virus with pandemic potential.

Materials and Methods: *Selection of gRNAs with antiviral activity:* Huh-7 lines stably expressing Cas13 and gRNAs targeting the genes of SARS-CoV-2 N/RdRP and ZIKV NSP3/C were infected with these two viruses. Viral production was determined by measuring intracellular and extracellular viral genome load by qRT-PCR and confirmed by Western blot (WB) and immunocytochemistry (IC).
Assessment of localization and antiviral efficacy of nanoformulated Cas13: GNPs functionalized with Cas13 and the most potent gRNAs (GNP-Cas13) were assessed for cellular localization using live confocal imaging assays. Reduction of SARS-CoV-2 and ZIKV replication was assessed in Huh-7 cells supplemented with GNP-Cas13 before or after viral infection by measuring the reduction of target gRNA proteins using IC assays.

Results: Among the designed gRNAs, we selected those that completely abrogated production of SARS-CoV-2 and ZIKV genomes and relative target proteins. GNP-Cas13 spontaneously enters into cells and localizes in cytoplasmic vesicles (autophagosomes, endosomes). The number of SARS-CoV-2 and ZIKV infected cells were reduced by 3 $\log_{10}$ when treated with gRNAs functionalized GNP-Cas13 before infection. When GNP-Cas13 are administered after infection we observed a 10-fold reduction of viral yield for both SARS-CoV-2 and ZIKV. These results are not surprising since the early disruption of viral genomes leads to inefficient viral replication.

Discussion: Our results highlight GNP-Cas13d as a novel approach against ss$^+$RNA viruses. Indeed, these nanoparticles are capable of entering cells without any transfection reagent, reach the very same organelles in which ZIKV and SARS-CoV-2 replicate, and cleave their genomes.

PO 002 - OC 02

A SMALL-MOLECULE INHIBITOR OF FLAVIVIRAL NS3-NS5 INTERACTION WITH BROAD-SPECTRUM ACTIVITY AND EFFICACY *IN VIVO*

M. Celegato[1], M. Sturlese[2], V. Vasconcelos Costa[3], M. Trevisan[1], A. Samer[1], L. Dias[4], I.B. Souza Passos[3], C.M. Queiroz-Junior[3], L. Messa[1], A. Favaro[2], S. Moro[2], M.M. Teixeira[4], A. Loregian[1], B. Mercorelli[1]

[1] *Department of Molecular Medicine, University of Padua, Italy*
[2] *Molecular Modeling Section (MMS), Department of Pharmaceutical and Pharmacological Sciences, University of Padua, Italy*
[3] *Departamento de Morfologia, ICB, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*
[4] *Departamento de Bioquímica e Imunologia, ICB, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*

Aim of the Study: Every year, Dengue virus (DENV) causes hundred million infections worldwide that can result in dengue disease and severe dengue. Two other mosquito-borne flaviviruses, i.e., Zika virus (ZIKV) and West Nile virus (WNV) are responsible of prolonged outbreaks and are associated with severe neurological diseases, congenital defects, and eventually death. All these three viruses, despite their importance for global public health still lack a specific drug treatment. Here, we describe the structure-guided discovery of small molecules with pan-flavivirus antiviral potential.

Methods: A virtual screening of ~1 million structures targeting the NS3-NS5 interaction surface of different flaviviruses was performed taking into account the crystal structures of NS5 of DENV, ZIKV, and WNV and predicting by molecular dynamic simulation the binding of a small molecule to a conserved site in flaviviral NS5. Thirty virtual hit compounds were then tested by an ELISA assay to investigate their ability to interfere with the NS3-NS5 interaction *in vitro* and in DENV-2 infected cells by plaque reduction assays to inhibit virus replication. Two nontoxic hit molecules were then subjected to further investigation by analyzing their pan-dengue antiviral activity, activity against other mosquito-borne flaviviruses and unrelated ssRNA viruses, the reduction of viral protein expression and the activity in disease-relevant ZIKV-infected neural stem cells. Morevoer, NMR studies were performed to validate NS5 as the target and *in vivo* studies in an animal model of DENV infection were also conducted.

Results and Conclusions: Two molecules inhibited the interaction between DENV NS3 and NS5 *in vitro*, the replication of all DENV serotypes as well as ZIKV and WNV in infected cells, while being inactive against other unrelated ssRNA viruses. Both hit compounds exhibited low propensity to select resistant viruses. By NMR studies we confirmed the interaction of the two hit compounds to DENV-2 NS5. Remarkably, one molecule demonstrated efficacy in a mouse model of dengue by reducing peak viremia, viral load in target organs, and associated tissue pathology. This study provides the proof-of-concept that targeting flaviviral NS3-NS5 interaction is an effective therapeutic strategy able to reduce virus replication *in vitro* and discloses new chemical scaffolds that could be further developed, thus providing a significant milestone in the development of much awaited broad- spectrum anti-flaviviral drugs.

PO 003 - OC 03

IDENTIFICATION OF NOVEL ANTIVIRAL COMPOUNDS WITH NANOMOLAR POTENCY AGAINST INFLUENZA VIRUS

A. Bonomini[1], C. Bertagnin[1], M. Pacetti[2], T. Felicetti[2], P. Zhan[3], S. Massari[2], O. Tabarrini[2], A. Loregian[1]

[1] *Department of Molecular Medicine, University of Padua, Italy*
[2] *Department of Pharmaceutical Sciences, University of Perugia, Italy*
[3] *Department of Pharmaceutical Chemistry, University of Shandong, China*

Aim of the Study: With the end of the SARS-CoV-2 emergency, influenza viruses have returned to draw attention as a major concern for public health. Indeed, many spillover events have already been registered worldwide due to the disruption of ecological niches and intensive animal farming. The currently available therapeutic and prophylactic approaches against the influenza virus present poor efficacy, mainly due to the continuous viral antigenic drift. Therefore, there is an urgent need for novel and potent anti-influenza drugs. Since many years, our research group has been focusing on two viral targets for the development of new anti-Flu antivirals: the viral neuraminidase (NA) and the interaction between the viral RNA polymerase subunits PA and PB1. NA is the target of the drug Oseltamivir (OST), against which many resistant variants have already emerged. With the aim of developing more effective compounds, we have designed many analogues of Oseltamivir that bind the 150-cavity next to the NA active site. The PA-PB1 interaction is highly conserved among influenza A and B viruses and presents considerable drug-resistance barrier. A previous *in silico* screening led to the identification of a chemical structure capable to accommodate very efficiently in the PA cavity, the cycloheptathiophene scaffold. Starting from this chemical scaffold, many derivatives have been designed and synthesized with the purpose to develop molecules with a higher binding affinity to the PA pocket.

Methods: MTT assays were first performed to exclude possible cytotoxicity of the compounds. Anti- Flu activity was then evaluated by plaque reduction assays (PRAs). In addition, the ability of anti- PA/PB1 compounds to inhibit the protein-protein interaction and the viral polymerase activity was assessed by an ELISA-based interaction assay and by a cell-based minireplicon reporter assay, respectively. Moreover, the anti-PA/PB1 mechanism of action of the compounds was further validated through a cellular PA/PB1 nuclear translocation assay. Preliminary *in vivo* studies were carried out in the chicken embryonated egg model infected with avian influenza virus H5N1 and H5N8 strains.

Results and Conclusions: Concerning the anti-NA compounds, many OST analogues showed an enhanced antiviral activity in PRA against influenza virus subtypes H1N1 and H3N2 with respect to the parental drug. Furthermore, some of these compounds exhibited *in vitro* a stronger ability to impair the activity of different NAs, including that of the H274Y resistant mutant, compared to OST. The most promising compounds were also tested *in vivo* in the chicken embryonated egg model in which they exhibited some efficacy against the avian influenza virus subtypes H5N1 and H5N8. Regarding the anti-PA/PB1 compounds, some cycloheptathiophene derivatives emerged which exhibited both potent anti-Flu activity in PRA and inhibitory activity in the ELISA PA-PB1 interaction assay. In particular, two compounds emerged as very promising since they exhibited broad-spectrum antiviral activity and the ability to inhibit the activity of the viral RNA polymerase in the minireplicon assay. In addition, their mechanism of action was validated by a PA/PB1 nuclear translocation assay.
Altogether, these results provide precious SAR information that will help us to further optimize our compounds, in order to obtain leads that could be promoted towards preclinical studies.

PO 004 - OC 04

DKA INHIBITORS OF Nsp13 OF SARS-CoV-2 BLOCK VIRAL REPLICATION

A. Corona[1], V.M. Madia[2], R. De Santis[3], C. Manelfi[4], R. Emmolo[1], D. Ialongo[2], E. Patacchini[2], A. Messore[2], D. Amatore[3], G. Faggioni[3], M. Artico[5], D. Iaconis[4], C. Talarico[4], R. Di Santo[2], F. Lista[3], R. Costi[2], E. Tramontano[1]

[1] *Dipartimento di Scienze della vita e dell'ambiente. Università degli Studi di Cagliari, Cittadella Universitaria di Monserrato, Cagliari, Italy*
[2] *Dipartimento di Chimica e Tecnologie del Farmaco, Istituto Pasteur-Fondazione Cenci-Bolognetti, "Sapienza" Università di Roma, Italy*
[3] *Dompé Farmaceutici SpA, L'Aquila, Italy*
[4] *Scientific Department, Army Medical Center, Rome, Italy*
[5] *Department of Sensory Organs, "Sapienza" Università di Roma, Italy*

For RNA viruses, RNA helicases have long been recognized to play critical roles during virus replication cycles, facilitating proper folding and replication of viral RNAs, therefore representing an ideal target for drug discovery. SARS-CoV-2 helicase, nsp13, unwind DNA or RNA in an NTP-dependent manner with a 5'-3' polarity. Nsp13 is a multidomain enzyme that couples two C-terminal RecA ATPase domains, characteristic of the 1B (SF1B) helicase superfamily, with other three domains: the N-terminal zinc-binding domain (ZBD), essential for the helicase activity, a stalk, and a 1B domain. Nsp13 is a highly conserved protein among all known coronaviruses, and, at the moment, is one of the most explored viral targets to identify new possible antiviral agents.
In the present study, we present six DKAs, as nsp13 inhibitors, investigated coupling molecular biology, in silico modelling and cell-based assays.
Among them, four compounds inhibit viral replication in the low micromolar range and block both nsp13 enzymatic functions. Mode-of-action studies revealed ATP-non-competitive kinetics of inhibition, not affected by substrate-displacement effect, suggesting an allosteric binding, further supported by molecular modelling calculations suggesting the binding into an allosteric conserved site located in the RecA2 domain.

PO 005 - OC 05

A NOVEL PROMISING ANTIVIRAL PEPTIDE: HS-1 AND ITS ALA-SCANNING ANALOGS

A. Chianese[1], C. Zannella[1], R. Giugliano[1], A. Ambrosino[1], P. More[1], G. Greco[1], A. Monti[2], C. Isernia[3], N. Doti[2], A. De Filippis[1], M. Galdiero[1]

[1] *Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy*
[2] *Institute of Biostructures and Bioimaging (IBB), National Research Council (CNR), Naples, Italy*
[3] *Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania "Luigi Vanvitelli", Caserta, Italy*

Aim of Study: Viral infections represent a serious threat to the world population and are becoming more frequent. However, most current antivirals are directed to inhibit specific viruses since these therapeutic molecules are designed to act on a specific viral target to interfere with a particular step in the replication cycle. Therefore, the search and identification of broad-spectrum antiviral molecules are necessary to ensure new therapeutic options. Recently, several studies on antimicrobial peptides (AMPs) identified them as promising antiviral agents. The AMPs, also known as host defense peptides (HDPs), represent an emerging class of therapeutic agents in several fields; they are used as antibacterial, antiviral, antifungal, antiparasitic, antioxidant, and anticancer agents (1). One of the natural sources of AMPs is represented by amphibian skin secretions. In this study, the antiviral effect of the peptide derived from the secretion of *Hypsiobas semilineatus*, i.e. HS-1, and its ala-scanning analogs was investigated.

Methods used: Peptides have been synthesized using the solid-phase Fmoc chemistry method, followed by purification by reversed-phase HPLC. In addition, HS-1 analogs have been synthesized performing ala-scanning mutagenesis, in which each residue was systematically replaced by alanine. Cytotoxic activity was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The antiviral activity was evaluated against a wide panel of viruses comprising enveloped, naked, DNA, and RNA viruses, using plaque assays and molecular test.

Results and Conclusions: Preincubation of HS-1 peptide with viruses has determined a significant antiviral activity, demonstrating that it could disrupt the viral envelope. Peptide interfered with the extracellular phases of the viral lifecycle, probably by blocking the viral attachment and entry phases. Ala-scanning mutagenesis demonstrated which residues were essential for the antiviral activity. In detail, all HS-1 analogs exhibited reduced toxicity and some of them an improved antiviral effect compared to the native peptide. Our results show possible novel applications of amphibian skin peptides in the field of antivirals. Further studies will focus on their specific mechanism of action to clarify the viral target on which the peptides act.

Reference

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PO 006

NEUTRALIZING ACTIVITY AGAINST WILD-TYPE, DELTA PLUS AND OMICRON BA.2.1 VARIANTS OF SARS-CoV-2 ELICITED BY THE BNT162B2 COVID-19 VACCINE BOOSTER IN ELDERLY

D. Dalla Gasperina[1], K. Shaik[2], G. Veronesi[3], C.M. Castelletti[4], G. Ferrari[4], F. Dentali[1], R. Accolla[2], G. Forlani[2]

[1] *Department of Medicine and Surgery, ASST Sette Laghi, University of Insubria, Varese, Italy*
[2] *Laboratories of General Pathology and Immunology "Giovanna Tosi", Department of Medicine and Surgery, University of Insubria, Varese, Italy*
[3] *Research Centre in Epidemiology and Preventive Medicine (EPIMED), Department of Medicine and Surgery, University of Insubria, Varese, Italy*
[4] *Molina's Foundation, Varese, Italy*

Background: The emerging variants of SARS-CoV-2 have raised serious concerns due to multiple mutations and reported significant immune escape. Although different studies describing the neutralization ability of different booster vaccination against different variants, especially against the Omicron variant and its subvariants such as BA.2-1, are emerging, little is known about the humoral immune response and the neutralizing activity against SARS-CoV-2 variants among long-term care facilities (LTCFs) elderly residents.

Methods: A single-center prospective cohort study was conducted to evaluate the humoral immune response elicited after BNT162b2 booster among vaccinated LTCF elderly residents. The anti-S1-protein IgG total antibodies titer and the presence of neutralizing antibodies against the Receptor Binding Domain of wild-type, Delta Plus and Omicron BA.2.1 were measured in sera by quantitative and by competitive ELISA, respectively, at 8 months after the second shot of the vaccine (T0) and 14 days after the booster (T1, 3rd dose). The median time between T0 and T1 was 56 days.

Results: Between October 2021 and January 2022, 49 LTCF residents (37 F, 12 M), median age of 84.8±10.6 years, were enrolled in the study. Previous COVID-19 infection (PI) was documented in 42.9% of subjects one year before the second shot of vaccine (T0). None developed reactions after the vaccine doses. After the booster, the IgG antibody concentration increased in all elderly subjects up to 10-fold (Geometric mean: T0: 360, T1: 4909). This ratio was lower in the subjects with previous COVID-19 infection (up to 4-fold) which presented higher level of IgG antibodies at T0 compared with subjects not previously infected ($T0_{PI}$: 1010 and T0: 166, respectively, $p<0.0001$). At T1, IgG levels were similar in both groups ($T1_{PI}$:4552 and T1: 4304, $p=0.64$). Before the booster, at least 86%, 79%, and 43% of enrolled subjects presented neutralizing antibodies in serum against wild-type and Delta plus and Omicron BA.2.1 variants, respectively. This percentage increased to 98% against wild type, 94% against Delta Plus, and 83% against Omicron BA.2 after the booster. Interestingly, the neutralizing activity against Omicron BA.2.1 was significantly lower (65%) than that measured against wild type and Delta Plus (90%). In the 6-month post-booster follow-up, nineteen subjects (38.7%) contracted the infection of SARS-CoV-2, all with mild symptoms.

Conclusions: Elderly patients boosted with mRNA vaccines exhibited potent neutralization against wild-type, Delta Plus SARS-CoV-2 variants. Neutralization of Omicron BA2.1 was significantly lower than wild type in serum, supporting the rapid spreading of the BA.2.1 variant in LTCFs.

PO 007

EFFECT OF ATMOSPHERIC COLD PLASMA TREATMENT ON MURINE NOROVIRUS AND HEPATITIS A VIRUS INFECTIVITY

L. Cozzi[1], T. Vicenza[1], S. Di Pasquale[1], S. Maccaroni[1], S. Tappi[2], F. Capelli[3], E. Suffredini[1]

[1] *Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, National Reference Laboratory for Foodborne Viruses, Rome, Italy*
[2] *Department of Agricultural and Food Sciences, University of Bologna, Italy*
[3] *Department of Industrial Engineering (DIN), University of Bologna, Italy*

Hepatitis A virus (HAV) and Norovirus (NoV) are the causative agents of a significant proportion of foodborne outbreaks worldwide. In recent years, new strategies for the prevention and control of HAV and NoV contamination in food and water sources were investigated. Among these, treatment with Cold Atmospheric Plasma (CAP) is gaining importance, given its proved efficacy in reducing other foodborne pathogens as bacteria and fungi. The aim of this study was to evaluate the impact of CAP on viral infectivity. The experiments were carried out on HAV and Murine Norovirus (MNV-1), a surrogate for human Norovirus, attached to the surfaces of inert material (borosilicate glass) and to the surface of soft fruits (strawberries), a food commodity repeatedly associated to HAV/NoV transmission.

Frp3 and RAW 264.7 cell lines were used to determine the viral infectivity of HAV and MNV-1 respectively. The samples were experimentally contaminated with 5 µl of viral suspensions (2×10^6 $TCID_{50}$/ml) and, after adhesion to the surface by drying, were treated with CAP. Two conditions of treatment and two different exposure times were tested:

1) Ozone-mode=33,3 W for 30 min and 60 min;
2) NOx-mode=332,9 W for 15 min and 30 min. Experimentally contaminated samples not subjected to the treatments and blank samples treated with CAP were used as controls.

After the treatments, viral particles were detached from glass surfaces and strawberries by repeated washing with cell culture medium and using the ISO 15216 method, respectively. The recovered viruses were then subjected to titration.

After 60 min, the ozone-mode treatment caused a reduction of viral infectivity below the detection limit of the method (20 $TCID_{50}$/ml) for both viral species and surface types (glasses and strawberries). The same reduction level was achieved after 15 min of NOx-mode treatment for HAV and after 30 min for MNV-1. Considering that natural decay of HAV and MNV-1 infectivity with viral particles attached to surfaces in environmental conditions, the reduction due to the two conditions of treatment is (1.2 log for MNV-1 regardless of the type of surface, and ranges from 1.7 log (glass) to 2.3 log (strawberries) for HAV.

In conclusion, the results of this study show that the application of CAP is effective for viral inactivation on food surfaces. The effect of the ozone-mode is depended on exposure time, which was related to the increase of ozone in the CAP chamber, while shorter times are needed using the NOx-mode possibly due to higher reactivity of nitrogen species. The CAP technology is a non-chemical and non-thermal promising tool in the food industry as an alternative to traditional food preservation methods.

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PO 008

ANTIVIRAL ACTIVITY OF RILK1 PEPTIDE ON MURINE NOROVIRUS AND HEPATITIS A VIRUS: STUDY ON THE ACTION MECHANISM

L. Cozzi[1], S. Maccaroni[1], T. Vicenza[1], Y.T.R. Proroga[2], M. Balestrieri[3], M. Gogliettino[3], B. Agrillo[3,4], A. Mancusi[2], S. Di Pasquale[1], E. Suffredini[1], G. Palmieri[3]

[1] *Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, National Reference Laboratory for Foodborne Viruses, Rome, Italy*
[2] *Department of Food Microbiology, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Naples, Italy*
[3] *Institute of Biosciences and BioResources, National Research Council (IBBR-CNR), Naples, Italy*
[4] *Materias s.r.l., Naples, Italy*

Antimicrobial peptides (AMPs) are amphiphilic agents with antimicrobial activity. In the last years, several studies on AMPs identified them as promising antiviral agents. In our previous study the peptide named RilK1 was tested on Hepatitis A Virus (HAV) and murine norovirus (MNV-1), a surrogate for human norovirus, to evaluate its virucidal potential, and a concentration of 80 µM was found to be effective (data not shown). The aim of this study was to investigate the action mechanism of Rilk1 peptide.

Four sets of experiments were performed to identify the step of viral infection in which Rilk1 exerted its activity:

1) *virus pre-treatment assay*, to assess the interaction between virus and peptide: incubation of Rilk1 (80 µM) with the virus (HAV 4.6×10^4 $TCID_{50}$/ml or MNV-1 3.2×10^4 $TCID_{50}$/ml) for 1 h at room temperature followed by cell infection (Frp3 for HAV and RAW 264.7 for MNV-1);
2) *cell pre-treatment assay*, to investigate peptide action on cells: incubation of Rilk1 with the cells lines for 1 h at 37°C followed by peptide removal and addition of the virus;
3) *attachment assay*, to evaluate peptide effect on viral attachment to cells: simultaneous addition of Rilk1 peptide and virus to the cells and incubation for 1 h at 4°C;
4) *entry assay*, to explore peptide activity on the internalization of viruses into cells: cells infection with the virus for 1 h at 4°C followed by treatment with Rilk1 for 1 h at 37°C. Untreated viral suspensions and Rilk1 solution, incubated at same conditions, were used as controls.

The most significant reduction of viral infectivity was obtained with the virus pre-treatment assay (91% for HAV and 93% for MNV-1) and with the attachment assay (91% for HAV and 95% for MNV-1). Lower reductions (<19% and <46% for HAV and MNV-1, respectively) were observed with the other assays.

This study demonstrated that Rilk1 exerts its action on HAV and MNV-1 viral particles and not on susceptible cells, likely by reducing their binding to cell receptors. Given its effectivenss at low concentrations and its action mechanism, Rilk1 peptide can be considered for a potential use in the control of contaminations by foodborne viruses.

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PO 009

IDENTIFICATION OF OXYSTEROL SYNTHETIC ANALOGS AS A NOVEL CLASS OF ANTI-HERPES SIMPLEX VIRUS ANTIVIRALS

A. Civra[1], M. Costantino[1], E. Carosati[2], G. Poli[1], F. Nigro[3], L. Pontini[3,4], M. Marinozzi[3], D. Lembo[1]

[1] *Department of Clinical and Biological Sciences; Laboratory of Molecular Virology and Antiviral Research, University of Turin, San Luigi Hospital, Orbassano, Turin, Italy*
[2] *Department of Chemical and Pharmaceutical Sciences, University of Trieste, Italy*
[3] *Department of Pharmaceutical Sciences, University of Perugia, Italy*

Aim of the Study: Genital herpes, most frequently caused by herpes simplex virus type 2 (HSV-2) infection, is one of the most prevalent sexually transmitted diseases. There is neither a cure nor a preventive treatment for genital herpes; the current rationale for the treatment of HSV-2 infection involves nucleoside analogs to suppress reactivation. Enzymatic oxysterols are endogenous 27-carbon molecule derived from cholesterol oxidation, and recently emerged as a broad-spectrum host targeting antivirals. In this study, we screened selected members of an in-house synthesized library of 27OHC analogues (named PFMs) for their activity against HSV-2.

Materials and Methods: In a first set of experiments, we tested the antiviral efficacy of a physiologic oxysterol (i.e. 27-hydroxycholesterol) and two libraries of oxysterol synthetic analogues by plaque reduction assay. Molecules with the highest selectivity indexes (SIs) were selected and their antiviral activity was confirmed by virus yield reduction assay. The step of viral replication inhibited and the putative cellular target of these molecules were investigated by transmission electron microscopy and indirect immunofluorescence.

Results and Conclusions: The screening of the panel against HSV-2 culminated in the identification of the two hit compounds, named PFM067 and PFM069, endowed 50% effective concentrations (EC_{50}) in the low micromolar range, and characterized by selectivity indexes ($SIs=CC_{50}/EC_{50}$) above 100. Moreover, the results obtained showed the interesting ability of the novel derivatives to inhibit cell-to-cell fusion induced by HSV-2, by sequestering viral glycoproteins in the Golgi compartment.
Taken together, these results point to PFM067 and PFM069 as promising chemical scaffolds for the development of novel antivirals.

PO 010

REPURPOSING ANTIVIRAL DRUGS AS INHIBITORS AGAINST SARS-CoV-2 AND HSV-2 VIRUSES

P. Quaranta[1-3], C.R. Piazza[1,4], S. Nottoli[2], A. Brai[5], P. Perrera[1], P. Mazzetti[3], G. Lottini[1,4], E. Plicanti[1,4], G. Freer[1], E. Dreassi[5], M. Pistello[1,2]

[1] *Retrovirus Centre, Department of Translational Research, University of Pisa, Italy*
[2] *Virology Operative Unit, Pisa University Hospital, Pisa, Italy*
[3] *Institute of Neuroscience, CNR, Pisa, Italy*
[4] *Department of Medical Biotechnologies, University of Siena, Italy*
[5] *Department of Biotechnology, Chemistry & Pharmacy, University of Siena, Italy*

Aim of the Study: This work was carried out under Tuscavir, an interdisciplinary consortium aimed at performing research and providing qualified services for the development of novel antiviral therapies. The project led to the development of several small molecules targeting different steps of HIV viral replication. These compounds are entry inhibitors, reverse transcriptase inhibitors, and kinase inhibitors. Since all compounds showed high activity against HIV, here we investigate the antiviral activity of these compounds against SARS-CoV-2 and HSV-2.

Methods: To determine the maximum concentration of compounds that can be used in the assays, we assessed cell cytotoxicity (CC) to Vero-TMPRSS2, VeroE6, and A549 cells using WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt]. The CC_{50} was calculated for each compound. The antiviral activity of selected molecules against SARS-CoV- 2 and HSV-2 was evaluated using a plaque assay or a limiting dilution assay. For the latter, inhibitory activity was determined by measuring the cytopathic effect (CPE) on the infected cell monolayer. Amount of viral genome production was assessed by real time PCR.

Results and Conclusions: The studied molecules showed no cytotoxic effects up to a concentration of 50 μM. Inhibition of SARS-CoV-2 and HSV-2 replication was assessed using different concentrations of the drugs that were administered according to the presumed mechanism of action. As judged by reduction of CPE and viral genome release in the supernatants of infected cells, some compounds exhibited antiviral activity against SARS-CoV-2 and HSV-2. The entry inhibitors Tus-1 and -7 were particularly effective against HSV-2 whereas the kinase inhibitors Tus-13 and -14 markedly reduced SARS-CoV- 2 with IC50 values >1 μM.

PO 011

"DHFR INHIBITORS DISPLAY A PLEIOTROPIC ANTI-VIRAL ACTIVITY AGAINST SARS-CoV-2: INSIGHTS INTO THE MECHANISMS OF ACTION"

A. Zani[1], D. Iaconis[2], F. Caccuri[1], C. Manelfi[2], C. Talarico[2], A. Bugatti[1], F. Filippini[1], R. Novelli[3], M. Kuzikov[4], B. Ellinger[4], P. Gribbon[4], K. Riecken[5], F. Esposito[6], A. Corona[6], E. Tramontano[6], A.R. Beccari[2], A. Caruso[1], M. Allegretti[3]

[1] *Section of Microbiology, Department of Molecular and Translational Medicine, University of Brescia, Italy*
[2] *EXSCALATE, Dompé farmaceutici SpA, Napoli, Italy*
[3] *Dompé Farmaceutici SpA, L'Aquila, Italy*
[4] *Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, 22525, Hamburg, Germany*
[5] *Research Department Cell and Gene Therapy, Department of Stem Cell Transplantation, University Medical Center Hamburg- Eppendorf, Hamburg, Germany*
[6] *Department of Life and Environmental Sciences, University of Cagliari, Monserrato, Cagliari, Italy*

Aim of the Study: Since the beginning of the COVID-19 pandemic, tremendous efforts have been made by the scientific community to find therapeutic approaches for the treatment of SARS-CoV-2-induced respiratory disease. In this study we evaluated the antiviral activity of several approved dihydrofolate reductase (DHFR) inhibitors, including methotrexate (MTX), pralatrexate (PTX), and tremietrexate (TMX), against SARS-CoV-2.

Methods: Infections were carried out using the SARS-CoV-2 B.1 lineage to infect Vero E6 and A549 ACE2+ cells at a multiplicity of infection of 0.05. Plaque assay, quantitative real-time RT-PCR and western blot analysis were performed to evaluate the antiviral efficacy of each compound. The inhibition of SARS-CoV-2 viral entry was evaluated infecting Caco-2 cells, previously treated or not with different doses of each selected compound, with SARS-CoV-2 pseudovirus which carries a luciferase reporter to monitor the infection. SARS-CoV-2 nsp13 unwinding associated activity and SARS-CoV-2 nsp12 RNA-dependent RNA polymerase (RdRp) activity were measured with Victor Nivo (Perkin) at 530/580 nm and 502/523, respectively.

Results and Conclusions: We assessed whether MTX could affect the replication of SARS-CoV-2. MTX reduced of nearly 70-fold at 25 μM and about 20-fold at 0.25 μM viral RNA copy number in the cell supernatants, as compared with untreated infected cells. In addition, MTX showed a dose-dependent inhibition of intracellular SARS-CoV-2 genome expression, ranging from 40% to 70% at the MTX tested concentrations (from 0.25 to 25 μM), and it showed approximately 60% inhibition of nucleoprotein (NP) accumulation in western blot analysis. Starting from these data on MTX, we evaluated the antiviral activity of several DHFR inhibitors. PTX exhibited a 90.4% to 96.5% inhibition of viral titer at 0.019 μM and 0.039 μM, respectively, with the IC50 value calculated to be 0.004 μM. At the same time, TMX significantly reduced the SARS-CoV-2 virus yield, with a 97.5% and 96.5% inhibition at 0.019 μM and 0.039 μM, respectively, with the IC50 value calculated to be 0.007 μM. We also tested the potential inhibitory effects of these compounds against 2 important enzymes in mediating SARS-CoV-2 replication: nsp13 helicase and RdRp. In agreement with data on antiviral activity, PTX and TMX were the most effective in inhibiting the unwinding activity of nsp13 helicase, displaying IC50 values of 0.14 and 1.56 μM, respectively, while MTX showed and IC50 of 2.03 μM. None of the tested compounds was able to inhibit RdRp. We finally investigated whether PTX and MTX were able to inhibit also viral entry by acting on virus-host interactions. Again, in agreement with the high antiviral activity of these compounds, PTX, TMX and MTX significantly inhibited viral entry, with IC50 values in the nanomolar range. Interestingly, only PTX displayed a significant inhibition of TMPRSS2, with an IC50 of 0.45 μM, while TMX and MTX did not display this activity. With this study, we confirm the importance of repurposing studies and of in silico/experimental synergy as very powerful methods to generate effective responses against diseases that are still untreatable. These compounds can thus potentially give a clinical advantage in the management of SARS-CoV-2 infection-associated complications in chronic diseases affected patients who are already treated with this class of drugs.

PO 012

STRIGOLACTONES AS BROAD-SPECTRUM ANTIVIRALS AGAINST ß-CORONAVIRUSES THROUGH TARGETING THE MAIN PROTEASE Mpro

G. Bajetto[4], M. Biolatti[1], M. Blangetti[2], M. Baggieri[3], A. Marchi[3], S. Gioacchini[3], D. Arnodo[2], P. Bucci[3], R. Fioravanti[3], M. Kojouri[3], M. Bersani[5], G. D'Arrigo[5], L. Siragusa[6,7], S. Ghinato[2], M. De Andrea[1,4], F. Gugliesi[1], C. Albano[1], S. Pasquero[1], I. Visentin[8], E. D'Ugo[3], F. Esposito[9], P. Malune[9], E. Tramontano[9], C. Prandi[2], F. Spyrakis[5], F. Magurano[3], V. Dell'Oste[1]

[1] Department of Public Health and Pediatric Sciences, University of Turin, Italy
[2] Departmentof Chemistry, University of Turin, Italy
[3] Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy
[4] Center for Translational Research on Autoimmune and Allergic Disease-CAAD, Novara, Italy
[5] Department of Drug Science and Technology, University of Turin, Italy
[6] Molecular Discovery Ltd., Kinetic Business Centre, Elstree, Borehamwood, Hertfordshire, United Kingdom
[7] Molecular Horizon s.r.l., Bettona, Perugia, Italy
[8] Department of Agricultural, Forestry, and Food Sciences, University of Turin, Grugliasco, Turin, Italy
[9] Department of Life and Environmental Sciences, University of Cagliari, Italy

Aim of the Study: The current SARS-CoV-2 pandemic and the likelihood that new coronavirus strains will emerge in the immediate future point out the urgent need to identify new pan-coronavirus inhibitors. Strigolactones (SLs) are a class of plant hormones with multifaceted activities whose role in plant-related fields has been extensively explored. Recently, we proved that SLs also exert an antiviral activity toward herpesviruses, such as human cytomegalovirus (HCMV). Based on this background, this work aimed to ascertain whether SLs might constitute a new class of broad-spectrum compounds against ß-coronaviruses (ß-CoVs).

Methods used: We employed two indole-based SL analogs, named TH-EGO and EDOT-EGO. We assessed the antiviral activity of the compounds against SARS-CoV-2 and the common cold human coronavirus HCoV-OC43, as prototypes of ß-CoVs by standard plaque assay on VERO-E6 and MRC- 5 cells, respectively. By using FLAP implemented in BiOGPS, we then investigate which might be the target of the analyzed SLs; we performed a docking simulation on all the available structures of SARS-CoV-2 proteins present in the Protein Data Bank, and among the highest-scored pockets we noticed the presence of the main protease (M^{pro}) orthosteric site. Therefore, with Gold, we performed a more accurate docking simulation between the compounds and the M^{pro} binding site to obtain ligand poses, and with Glide we simulated a covalent docking to obtain potential adducts. In the end, to strengthen the computational results, we tested the effect of EDOT-EGO and TH-EGO on SARS-CoV-2 M^{pro} activity using an *in-vitro* biochemical assay based on Fluorescence Resonance Energy Transfer (FRET).

Results and Conclusions: Here we show that the synthetic SLs TH-EGO and EDOT-EGO impair ß-CoVs replication, including SARS-CoV-2 and HCoV-OC43. Interestingly, in-silico simulations suggest the binding of SLs in the SARS-CoV-2 main protease (M^{pro}) active site, and this was further confirmed by an *in-vitro* activity assay. Overall, our results highlight the potential efficacy of SLs as broad-spectrum antivirals against ß-CoVs, which may provide the rationale for repurposing this class of hormones for the treatment of COVID-19 patients.

PO 013

GENERATION OF A CELLULAR SCREENING PLATFORM TO IDENTIFY SMALL MOLECULES ACTIVE AT INHIBITING ANTI-TYPE-I INTERFERON (IFN-I) PROTEINS BELONGING TO PATHOGENIC VIRUSES

C. Acchioni[1§], M. Acchioni[1§], F. Mancini[2], A. Amendola[1], G. Marsili[1], V. Tirelli[4], C.P. Gwee[5], K.W. Chan[5], S. Sandini[1], M.V. Chiantore[1], R. Orsatti[9], M. Bisbocci[3], M. Mysara[6], M. ElHefnawi[7], M. Sanchez[4], G. Venturi[1], M.L. Barreca[8], S. Valente[10], G. Manfroni[8], A. Mai[10], A. Bresciani[3], S.G. Vasudevan[5], M. Sgarbanti[1]

[1] *Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy*
[2] *National Center for Drug Research and Evaluation, Istituto Superiore di Sanità, Rome, Italy*
[3] *Department of Translational and Discovery Research, IRBM S.p.A., Pomezia, Roma, Italy*
[4] *Core Facility Service, Istituto Superiore di Sanità, Rome, Italy*
[5] *Program in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore*
[6] *Bioinformatics Group, Center for Informatics Sciences (CIS), School of Information Technology and Computer Science (ITCS), Nile University, Giza, Egypt*
[7] *Biomedical Informatics and Chemoinformatics Group, Informatics and Systems Department, National Research Centre, Cairo, Egypt*
[8] *Department of Pharmaceutical Sciences, Università degli Studi di Perugia, Italy*
[9] *DRUE -Ufficio Progetti e Convenzioni Istituto Superiore di Sanità, Rome, Italy*
[10] *Department of Drug Chemistry and Technologies, Sapienza University of Rome, Italy*
[§] *Equal contribution*

Aim of the Study: In the early phase of a viral infection, type I IFN (IFN–I) system, represents the most important effector mechanism activated by the host cell defenses. Pathogenic viruses evolved mechanisms to avoid detection or to express viral proteins endowed with anti-IFN-I activities. Herein, we report the development of a cellular platform based on the erythroleukemic K562 cell line, stably expressing the firefly luciferase reporter gene under the control of a repeated interferon-stimulated response element (ISRE), or the IFN-ß promoter sequence, and a RNA Pol III-driven non-targeting (nt), shRNA to mimic intermediates of RNA virus replication. Engineered cell lines also stably overexpress either the green fluorescent protein (GFP), as a control, or different anti IFN-I virus proteins, fused with GFP. The expression of Dengue virus NS5, and Ebola virus VP35, is described to exemplify the platform.

Methods used: K562 cells were transduced with the *pSIREN RetroQ puro* retroviral vector expressing the luciferase reporter construct together with the nt shRNA expressing cassette, with the subsequent selection using puromycin. A second retroviral transduction of the engineered K562 cell population, was performed with two distinct *pMSCV neo* retroviral vector constructs to create a control cell line, expressing GFP, and test cell lines expressing fusion proteins (between GFP and Dengue virus NS5 protein or Ebola virus VP35 protein), with positive cell populations enriched by both, G418 selection, and fluorescent cell sorting. TPA-mediated cell differentiation was performed, with or without exogenous IFN-I stimulation, in miniaturized 384 well format before small molecule screenings.

Results and Conclusions: Our cellular platform demonstrated suitable for high throughput screening (HTS) campaigns. Positive hits were represented by compounds targeting STAT2 cellular protein in the NS5 system. Such compounds, targeting the host rather than the virus, would have a lower probability of being affected by viral escape mutations.

PO 014

A CELLULAR SCREENING PLATFORM, STABLY EXPRESSING DENV2 NS5, DEFINES A NOVEL ANTI-DENV MECHANISM OF ACTION OF APIGENIN BASED ON STAT2 ACTIVATION

C. Acchioni[1§], M. Acchioni[1§], F. Mancini[2], A. Amendola[1], G. Marsili[1], V. Tirelli[4], C.P. Gwee[5], K.W. Chan[5], S. Sandini[1], R. Orsatti[9], M. Bisbocci[3], M. Mysara[6], M. ElHefnawi[7], M. Sanchez[4], G. Venturi[1], M.L. Barreca[8], G. Manfroni[8], A. Bresciani[3], S.G. Vasudevan[5], M. Sgarbanti[1]

[1] *Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy*
[2] *National Center for Drug Research and Evaluation, Istituto Superiore di Sanità, Rome, Italy*
[3] *Department of Translational and Discovery Research, IRBM S.p.A., Pomezia, Roma, Italy*
[4] *Core Facility Service, Istituto Superiore di Sanità, Rome, Italy*
[5] *Program in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore*
[6] *Bioinformatics Group, Center for Informatics Sciences (CIS), School of Information Technology and Computer Science (ITCS), Nile University, Giza, Egypt*
[7] *Biomedical Informatics and Chemoinformatics Group, Informatics and Systems Department, National Research Centre, Cairo, Egypt*
[8] *Department of Pharmaceutical Sciences, Università degli Studi di Perugia, Italy*
[9] *DRUE - Ufficio Progetti e Convenzioni Istituto Superiore di Sanità, Rome, Italy*
[§] *Equal contribution*

Aim of the Study: No drugs are licensed yet to efficiently counteract the deleterious complications of Dengue virus (DENV) infection; at the same time, a vaccine against all DENV serotypes has been approved for people without any previous encounter with the virus only very recently in Indonesia and EU. The ability of the virus to evade innate immune responses in particular those triggered by interferon type I (IFN-I) represents one of the key event characterizing DENV pathogenesis. The non-structural protein 5 (NS5) is crucial for viral genome replication as well as to antagonize the IFN-I response by binding to and promoting the degradation of the signal transducer and activator of transcription 2 (STAT2). We developed a cellular platform for the high throughput screening of compounds that counteract the IFN-I antagonism by NS5.

Methods used: The platform is based on the erythroleukemic K562 cell line, engineered to stably express the firefly luciferase reporter gene under the transcriptional control of a repeated ISRE enhancer sequence, able to respond to IFN-I stimulation, and also stably overexpressing either the green fluorescent protein (GFP), or a GFP-DENV2 NS5 fusion protein.

Results and Conclusions: A screening with a library of 1220 approved drugs was carried out and 3 compounds previously linked to DENV inhibition (Apigenin, Chrysin, and Luteolin) were found to restore IFN-I signaling through STAT2 Tyr 689 phosphorylation upon IFN-I stimulation. Luteolin and Apigenin showed a significant inhibition of DENV2 replication in Huh7 cells, with an EC_{50} of 9.2±2.3 μM, and 29.7±6.6 μM, respectively. These compounds were able to restore STAT2 phosphorylation in our cellular platform and in the Huh7 infection model. Despite the "promiscuous" and "pan-assay-interfering" nature of Luteolin, Apigenin promotes STAT2 Tyr 689 phosphorylation and activation, even in the absence of DENV2 infection in Huh7 cells suggesting that its mechanism of action may not be a direct inhibition of NS5 but rather a STAT2 pre-activation capable of preventing NS5-mediated inhibition. Our finding highlights the importance of screening for antiviral compounds, targeting host factors that would have a lower probability of being affected by viral escape mutations.

PO 015

GENERATION OF A CELLULAR MODEL TO IDENTIFY SMALL MOLECULES ACTIVE AT INHIBITING THE ANTI-TYPE-I INTERFERON (IFN-I) FUNCTION OF THE ZAIRE EBOLA VIRUS PROTEIN VP35

M. Acchioni[1], C. Acchioni[1], F. Mancini[2], S. Sandini[1], M.V. Chiantore[1], R. Orsatti[3], S. Valente[4], A. Mai[4], M. Sgarbanti[1]

[1] *Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy*
[2] *National Center for Drug Research and Evaluation, Istituto Superiore di Sanità, Rome, Italy*
[3] *DRUE - Ufficio Progetti e Convenzioni Istituto Superiore di Sanità, Rome, Italy*
[4] *Department of Drug Chemistry and Technologies, Sapienza University of Rome, Italy*

Aim of the Study: Ebola virus (EBOV) is responsible for the Ebola virus disease (EVD), a severe hemorrhagic fever possessing one of the highest mortality rate (up to 90% in humans). To date, the infection is mainly prevalent in areas of Central and Western Africa. The periodic re-emergence of such epidemics depends on the presence of animal reservoirs of the virus such as fruit bats belonging to the Pteropodidae family. The 2014-2016 EBOV epidemic, has been the largest to date, forcing the World Health Organization (WHO) to declare a state of international emergency in 2014, being the global spreading of the infection a real possibility. Hence the need to find effective and specific drugs and vaccines to combat or prevent this disease. This work aimed to create a cellular biotechnological platform, based on the erythroleukemic K562 cell line, suitable for high throughput screening (HTS) of small molecules, useful to test compounds potentially capable of inhibiting the anti-IFN-I function of the VP35 protein of the EBOV. This protein is able to inhibit the production of IFN-I by the host cell, thus allowing the virus to evade the innate immune system.

Methods used: A first engineering of K562 cells was performed, by means of retrovirus vector transduction, to express a reporter construct that allows to indirectly measure, through the Luciferase assay, the production of IFN-ß stimulated by the constitutive expression of a dsRNA. A second retroviral engineering was then carried out, in order to generate two cell lines, one expressing the green fluorescent protein (GFP), as a control, and the other expressing VP35 from a Sierra Leone isolate of 2014, in-frame with GFP, followed by FACS-mediated cell sorting.

Results and Conclusions: Using this platform, a screening of 24 small molecules, selected on the basis of data in the literature that indicated the antiviral potential of molecules belonging to different classes of drugs, was carried out at two distinct concentrations (10 µM and 50 µM) and at two different times points (2h and 24h). The experiments revealed the efficacy of 6 compounds (named MC3249; MC3250; MC3251; MC3253; MC3256 and MC4202) at inhibiting the anti-IFN activity of the viral protein VP35, with MC3251 and MC3253 showing the best inhibitory activities (34% and 63%, respectively). K562 cells expressing VP35 have a reduced nuclear localization of the transcription factor IRF3, also in its hyperphosphorylated form, crucial for the induction of IFN-I production, compared to control cells. Five out of six identified compounds restored, at different extent, the nuclear levels of IRF3 and its hyperphosphorylated form, with MC3251 and MC3253 showing the best increase in overall IRF-3 nuclear localization.

PO 016

IS VITAMIN SUPPLEMENTATION EFFECTIVE IN THE PREVENTION AND MANAGEMENT OF COVID-19? A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CLINICAL TRIALS

A. Sinopoli

Department of Prevention, ASL Roma 1, Rome, Italy

Aim of the Study: The aim of our systematic review and meta-analysis was to identify the direct effects of vitamins in the prevention and management of COVID-19, including those on long-COVID, to provide a summary of the results and support healthcare professionals in evaluating alternative effective treatments against COVID-19.

Methods: PubMed, Scopus, and Web of Science were searched. Randomised controlled trials (RCTs) conducted in any country, published in English or Italian until August 2022, that compared data on the direct effects of vitamins administered in any form versus placebo in the prevention and/or management of SARS-CoV-2 infection and long-COVID were considered eligible. The random-effects model was used to conduct the meta-analysis and thus produce combined odds ratios (ORs) with their relative 95% confidence intervals (95% CIs).

Results: A total of 23 articles were included in the systematic review and, of these, 13 provided data for the meta- analysis. In two studies, the outcome considered was the prevention of COVID-19 and/or long-COVID, while the other 21 evaluated the effectiveness of vitamin supplementation in the management of COVID-19.

Prevention of COVID-19 and/or long-COVID: Of the two studies that investigated the effectiveness of vitamin supplementation for the prevention of COVID-19, only in one case was reported a significantly lower percentage of subjects who developed SARS-CoV-2 infection in the group treated with vitamin D. In contrast, any significant difference was shown in terms of either the development of long-COVID or mortality.

Management of COVID-19: Two studies investigated the effects of multivitamin supplements and in both cases any significant results were found on the immunological and hematological parameters investigated. Conversely, supplementation with vitamin A, D, E, C and B complexes significantly reduced the duration of hospitalization, while the combined intake of vitamin C and E, together with standard therapy, significantly reduced the respiratory rate of treated patients.

A total of 8 studies investigated the effects of vitamin C administration in the management of COVID-19. Any significant differences emerged in the immunological and hematological parameters investigated. By contrast, vitamin C intake, together with standard therapy, improved some respiratory parameters and led to a symptoms' resolution in 3 trials. Only in one study a reduction in length of stay in treated patients was observed. The meta-analysis showed that vitamin C does not appear to be effective in reducing in-hospital mortality in COVID-19 patients.

In total, 11 studies evaluated the effects of vitamin D in COVID-19 disease management. Any significant differences in immunological and hematological parameters were found. The clinical outcomes investigated showed great heterogeneity. An improvement in respiratory parameters and symptoms in patients who were treated with vitamin D in combination with standard therapy was described. Only one study reported a shorter length of stay in the same group of patients. The meta-analysis showed that vitamin D does not appear to be effective in reducing in-hospital mortality in COVID-19 patients.

Conclusions: Although the usefulness of vitamins in supporting innate and adaptive immunity is known, only weak results on the effectiveness of vitamin supplementation in preventing SARS-CoV-2 infection emerged from our study. About the management of COVID-19, an improvement in some clinical outcomes was observed, especially when vitamin supplementation was combined with standard therapy. These results suggest that vitamins may have an adjuvant role in facilitating the effectiveness of pharmacological therapy for COVID-19. However, further efforts are needed to clearly establish the role of vitamin supplements in clinical practice to fight COVID-19.

PO 017

IDENTIFICATION OF A NOVEL INTRA-GENOTYPE REASSORTANT G1P[8] ROTAVIRUS IN ITALY, 2021

S. De Grazia[1], C. Filizzolo[1], F. Bonura[1], M. Pizzo[1], F. Di Bernardo[2], A. Collura[2], F. Pellegrini[3], V. Martella[3], G.M. Giammanco[1]

[1] *Dipartimento di Promozione della Salute, Materno-Infantile, di Medicina Interna e Specialistica di Eccellenza "G. D'Alessandro", Università degli Studi di Palermo, Italy*
[2] *Unità Operativa di Microbiologia e Virologia, Ospedale Civico e Di Cristina, ARNAS, Palermo, Italy*
[3] *Dipartimento di Medicina Veterinaria, Università di Bari, Italy*

Aim of Study: Hospital-based surveillance conducted in Sicily, Italy, showed a progressive decline of rotavirus prevalence following the introduction of Rotarix vaccine in 2013 (from 24.2% of paediatric enteritis in 2014 to 7.9% in 2021) with a marked decrease in circulation of G1P[8] rotaviruses, the most epidemiologically relevant genotype targeted by the vaccine, as vaccine coverage increased (up to 63.8% in 2020). However, in 2021, despite the low prevalence of the infection, G1P[8] was detected in 90.5% (19/21) of rotavirus-positive samples. This study aimed to understand if the rise in activity of G1P[8] rotaviruses in 2021 was related to the introduction of a novel strain.

Methods used: In 2021, 266 patients <15 years of age were hospitalised with acute gastro-enteritis (AGE) and included in rotavirus surveillance. VP7/VP4 genotyping and sequence data were generated from all rotavirus-positive samples.

Results and Conclusion: A peculiar G1P[8] rotavirus strain, with both VP7 and VP4 genes belonging to novel sub-lineages, circulated in 2021 accounting for 76.2% (16/21) of all rotavirus infections. On full-genome analysis, the novel G1P[8] variant (herein termed Palermo 2021) displayed an intra-genotype (Wa-like) reassortant constellation, with a mix of genome segments previously detected in G12 and G1 strains, into a unique arrangement that had never been observed before. The G1P[8] variant Palermo 2021 differed in three and four aa residues in VP4 epitopes 8-1 and 8-3, respectively, from the Rotarix strain, whilst the VP7 epitopes were conserved. Prompt identification of virus variants circulating in the human population is pivotal to understand epidemiological trends and assess vaccines efficacy. VP4 mutations in selected epitopes might be crucial for escaping neutralizing antibodies induced by vaccines. However, only a minority (2/17) of the children affected by G1P[8] rotavirus infection in Palermo in 2021 had completed a full vaccination cycle. Care should be taken to monitor the emergence of the G1P[8] Palermo 2021 strain outside the geographical area where it apparently originated in order to evaluate its ability to cause severe infections and to escape immunity in the population.

PO 018

SYNERGISTIC EFFECTS OF ANTIVIRALS AND MONOCLONAL ANTIBODIES IN VITRO AGAINST SARS-CoV-2 WILD TYPE B.1 STRAIN AND BQ.1.1 OMICRON SUBLINEAGE

L. Fiaschi, C. Biba, I. Varasi, N. Bartolini, C. Paletti, F. Giammarino, F. Saladini, M. Zazzi, I. Vicenti
Department of Medical Biotechnologies, University of Siena, Italy

Background: Combination regimens can enhance antiviral potency, limit emergent drug resistance, and lower drug dosage. Aim of this work was to determine, in a live virus cell-based assay, the potential synergistic effects of

1) the combination of approved Directly Acting Antivirals (DAAs) against SARS-CoV-2 wild type strain B.1 (WT) and omicron BQ.1.1 and
2) the combination of remdesivir (RDV) with licensed monoclonal antibodies (mAbs) against WT.

Materials and Methods: The toxicity of the active form of molnupiravir (EIDD-1931), RDV, nirmatrelvir (NRM), cilgavimab (CIL), tixagevimab (TIX), bebtelovimab (BEB) and sotrovimab (SOT) was determined by luminescence in VERO-E6 treated with CP-100356 P-gp inhibitor. Scalar dilutions of each DAA were incubated in a 36 pairwise concentration matrix on VERO-E6 infected with WT and BQ.1.1 (MOI=0.01). After 72h cytopathic effect was quantified. The same experiments were performed with RDV plus each mAb. The synergistic score (SC) was calculated by SynergyFinder3.0 using Bliss/Loewe model and confirmed by Multi-dimensional Synergy of Combinations (MuSyC) post-analysis option. SC>10 was scored as synergistic, -10≥SC≤10 as additive, SC<-10 as antagonist.

Results: Half-maximal inhibitory concentrations (IC50) for EIDD-1931, RDV and NRM were 2.40±0.40/1.59±0.44, 0.06±0.03/0.03±0.01 and 0.10±0.03/0.10±0.01 μM against WT/BQ.1.1, respectively. CIL, TIX, BEB and SOT were active only against WT (IC50 of 0.20±0.13, 0.07±0.04, 0.03±0.01, 0.81±0.32 μg/ml, respectively). Global weighted SCs for all DAAs combinations showed additivity against WT (-0.96±1.69 EIDD-1931+RDV, -6.71±0.89 EIDD-1931+NRM and 0.02±0.33 RDV+NRM) and BQ.1.1 (-0.33±4.10 EIDD-1931+RDV, -0.47±0.37 EIDD-1931+NRM and -2.12±3.8 RDV+NRM). Additivity was also observed for all mAbs/RDV combos against WT (-5.9±4.4 SOT+RDV, -5.5±1.6 RDV+BEB, 0.88±2.1 RDV+CIL and −4.3±1.6 TIX/RDV). SC >10 was observed for a few DAAs combos at specific concentrations including EIDD-1931 0.05-0.19-0.75 μM + RDV 0.4 μM, EIDD-1931 0.075-1.5 μM + NRM 0.05 μM and RDV 0.02 μM + NRM 0.01 μM against both viral strains.

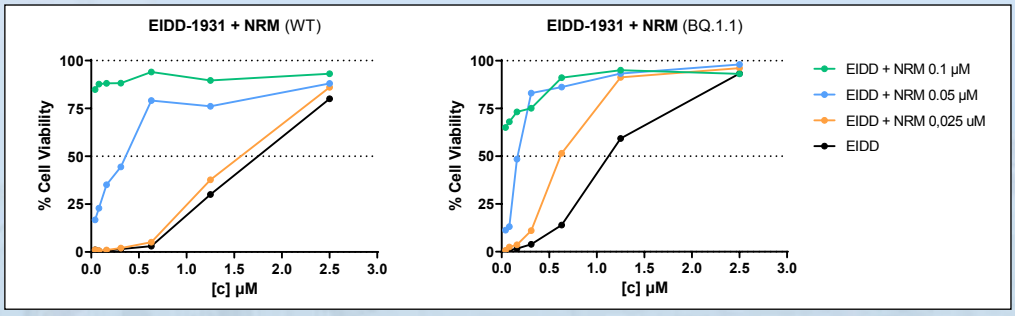

Figure 1 - Antiviral activity of EIDD-1931 + NRM against WT and BQ.1.1 strains in VERO-E6 cell line. IC_{50} shifts of EIDD-1931 were measured in infected cells treated with 2-fold serial dilutions of EIDD-1931 (5 to 0.04 μM) and 3 fixed NRM concentrations (0.1, 0.05, 0.025 μM). The micromolar drug concentration is indicated on the x-axis. The horizontal dashed line indicates the drug IC_{50} corresponding to 50% of cell viability generated by GraphPad PRISM software 6.01 (La Jolla, CA, USA).

Similarly, SC >10 was observed against WT for the following RDV+mAbs combinations: RDV 0.06 μM + SOT 0.09-0.04-0.1 μg/ml, RDV 0.016-0.06 μM + BEB 0.04 μg/ml, RDV 0.016-0.06 μM + TIX 0.04-0.009 μg/ml. As a proof of concept, IC50 shifts were measured in infected cells treated with 3 fixed NRM concentrations plus scalar EIDD-1931. The IC50 of EIDD-1931 with the addition of 0.1, 0.05, 0.025 μM NRM was reduced by >61-, 8- and 1-fold against WT and by >41-, 10- and 3-fold against BQ.1.1 (Figure 1).

Conclusions: Global weighted SCs indicated additive effects. However, each DAAs combination induced synergistic potency shifts against WT and BQ.1.1 at specific concentrations' combination. We observed the same effects for RDV in combination with SOT, BEB or TIX against WT, but it was not possible to evaluate these combos against the BQ.1.1 strain due to its resistance to all tested mAbs.

PO 019

A HEPARAN SULFATE PROTEOGLYCAN BINDING TETRAPEPTIDE SUCCESSFULLY INHIBITS SARS-COV-2 OMICRON REPLICATION

C. Biba, L. Depau, L. Fiaschi, J. Brunetti, I. Varasi, C. Falciani, M. Zazzi, I. Vicenti, L. Bracci
Department of Medical Biotechnologies, University of Siena, Italy

Background: The emergence of the SARS-CoV-2 Omicron variant has led to increased transmissibility and immune escape, due to the high number of mutations in the Spike (S) region, particularly in the receptor binding domain (RBD) responsible for the interaction with the Angiotensin Converting Enzyme 2 (ACE2). At the same time, the mechanism of Omicron entry has evolved into endocytic dependent internalization, as opposed to membrane fusion dependent internalization, which was predominant with previous variants. In addition, a possible interaction of membrane Heparan Sulfate Proteoglycans (HSPG) with SARS-CoV-2 during virus internalization has been recently suggested. The aim of this work was to determine the role of HSPG in the infectivity of the Omicron variant, and the efficacy of an HSPG binding tetrapeptide (LB-1) in inhibiting Omicron entry in a live virus cell-based model.

Materials and Methods: LB-1 was synthesized in a tetra-branched form on a multiple automated synthesizer by standard Fmoc chemistry. The infection model was set up with Omicron BA.1 and Delta SARS-CoV-2 strains in the adherent human intestinal Caco-2 cell line with 24h incubation followed by transfer of the virus supernatant to a highly permissive reporter cell line (VERO E6 monkey kidney cells). The half-maximal Tissue Culture Infectious Dose was determined after 24h in VERO E6 measuring the viral nucleocapsid expression by ELISA assay. The experiments were performed in quadruplicate, with and without treatment with heparinase to remove the HSPG chains. The half-maximal cytotoxic concentration of LB-1 was determined by luminescence. To determine the antiviral activity, serial dilutions of LB-1 starting from the not-toxic dose, were incubated with a fixed amount of BA.5, BA.1 and Delta strains (MOI=0.01) for 1h at 37°C on pre-seeded Caco-2 cells. After incubation, the virus-peptide mixture was removed, and fresh LB-1 was added. Viral supernatants were transferred in VERO E6 as previously described. The half-maximal inhibitory concentration of LB-1 (IC_{50}) was measured by SARS-CoV-2 nucleocapsid ELISA. Each experiment included a mock infection control, a virus control and two reference inhibitors with known IC_{50} (remdesivir and a previously tested human immune serum).

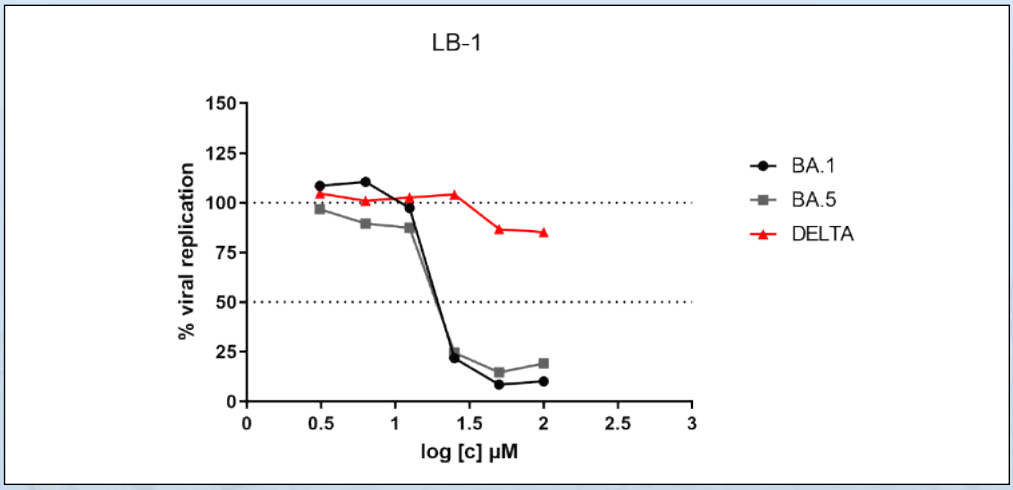

Figure 1 - Antiviral activity of LB-1,agoinst omicron sublineoges (BA.1, BA.5) and delta SARS-CoV-2 strains in Caco-2 cell line. On the x-axis is indicated the micromolar drug concentration in logarithmic scale. The horizontal dashed line indicates the drug IC_{50} corresponding to 50% of the viral replication generated by GraphPad PRISM software version 6.01 (La Jolla, CA, USA).

Results: LB-1 inhibited cell infection by BA.1 and BA.5 with 21.5±2.8 and 19.5±1.7 μM IC_{50}, respectively. By contrast, LB-1 had no effect against delta variant (Figure 1) indicating a crucial role for HSPG in cell infection by Omicron but not by Delta. Heparinase treated cells were not permissive to Omicron infection, confirming the role of HSPG, while a modest impact was measured on Delta (4.6-fold reduction).

Conclusions: The prototype HSPG binding tetrapeptide LB-1 selectively inhibited the replication of SARS-CoV-2 Omicron. The role of HSPG in Omicron infection and this proof-of-concept results support further development of HSPG targeting agents as a novel strategy to block SARS-CoV-2 infection.

PO 020

SURAMIN INHIBITS SARS-CoV-2 NUCLEOCAPSID PHOSPHOPROTEIN GENOME PACKAGING FUNCTION

A. Corona[1], I. Boniardi[2], J. Basquin[3], C. Basquin[3], J. Milia[1], I. Nagy[2], E. Tramontano[1], L. Zinzula[2]

[1] *University of Cagliari, Department of Life and Environmental Sciences, Monserrato, Italy*
[2] *Max Planck Institute of Biochemistry, Department of Molecular Structural Biology, Martinsried, Germany*
[3] *Max Planck Institute of Biochemistry, Department of Structural Cell Biology, Martinsried, Germany*

The coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to pose - despite the availability of licensed vaccines - a threat to global health, due to the potential emergence of vaccine-resistant SARS-CoV-2 variants. This makes the development of new drugs against COVID-19, and the repurposing of known ones, a persistent urgency, and sets as a research priority the validation of novel therapeutic targets within the SARS-CoV-2 proteome. Among these, a promising one is the SARS-CoV-2 nucleocapsid (N) phosphoprotein, a major structural component of the virion with an indispensable role in packaging the viral genome into a ribonucleoprotein (RNP) complex, which also contributes to SARS-CoV-2 innate immune evasion by inhibiting the host cell type-I interferon (IFN-I) production. By combining miniaturized differential scanning fluorimetry with microscale thermophoresis, we found that the 100-year-old drug Suramin interacts with SARS-CoV-2 N N-terminal domain (NTD) and C-terminal domain (CTD), thereby inhibiting their single-stranded RNA (ssRNA) binding function with low-micromolar K_d and IC_{50} values. Molecular docking suggests that Suramin interacts with the basic NTD cleft and CTD dimer interface groove, highlighting the presence of three potentially druggable ssRNA binding sites. Electron microscopy shows that Suramin inhibits the formation *in vitro* of RNP complex-like condensates by SARS-CoV-2 N with a synthetic ssRNA. In a dose-dependent manner, Suramin also reduced SARS-CoV-2-induced cytopathic effect on Vero E6 cells, and partially reverted the SARS-CoV-2 N-inhibited IFN-I production in 293T cells. Overall, our findings indicate that Suramin inhibits SARS-CoV-2 replication by hampering viral genome packaging.

PO 021

IMPORTIN α/ß-DEPENDENT NUCLEAR TRANSPORT OF HUMAN PARVOVIRUS B19 NONSTRUCTURAL PROTEIN 1 IS ESSENTIAL FOR VIRAL REPLICATION

G. Alvisi[1], E. Manaresi[2], E.M. Cross[3], M. Hoad[3], N. Akbari[1], S. Pavan[1], D. Ariawan[4], G. Bua[2], G.F. Petersen[3], J. Forwood[3], G. Gallinella[2]

[1]*Department of Molecular Medicine, University of Padua, Italy*
[2]*Department of Pharmacy and Biotechnology, University of Bologna, Italy*
[3]*School of Dentistry and Medical Sciences, Charles Sturt University, Wagga Wagga, NSW, 2650, Australia*
[4]*Dementia Research Centre, Macquarie University, Sydney, NSW, 2109, Australia*

Human parvovirus B19 (B19V) is a major human pathogen causing a variety of diseases, characterized by a selective tropism to human progenitor cells in bone marrow. In similar fashion to all *Parvoviridae* members, the B19V ssDNA genome is replicated within the nucleus of infected cells through a process which involves both cellular and viral proteins. Among the latter, a crucial role is played by non-structural protein (NS)1, a multifunctional protein involved in genome replication and transcription, as well as modulation of host gene expression and function. Despite the localization of NS1 within the host cell nucleus during infection, little is known regarding the mechanism of its nuclear transport pathway. In this study we undertake structural, biophysical, and cellular approaches to characterize this process. Quantitative confocal laser scanning microscopy (CLSM), gel mobility shift, fluorescence polarization and crystallographic analysis identified a short sequence of amino acids (GACHA**KK**P**R**IT-182) as the classical nuclear localization signal (cNLS) responsible for nuclear import, mediated in an energy and importin (IMP) α/β-dependent fashion. Structure-guided mutagenesis of key residue K177 strongly impaired IMPα binding, nuclear import, and viral gene expression in a minigenome system. Further, treatment with ivermectin, an antiparasitic drug interfering with the IMPα/β dependent nuclear import pathway, inhibited NS1 nuclear accumulation and viral replication in infected UT7/Epo-S1 cells. Thus, NS1 nuclear transport is a potential target of therapeutic intervention against B19V induced disease.

Keywords: B19V, nuclear transport, antiviral, cNLS, importins, ivermectin.

Abbreviations

B19V, Human parvovirus B19; NS, nonstructural protein; EPCs, erythroid progenitor cells; NE, nuclear envelope; NPC, nuclear pore complex; IMP, importin; NLS, nuclear localization signal; cNLS, classical NLS, MVM, minute virus of mice; PPV, porcine parvovirus; FBS, fetal bovine serum; DMEM, Dulbecco's Modified Eagle's Medium; IVM, ivermectin; PBS, phosphate buffered saline; wt, wildtype; CLSM, confocal laser scanning microscopy; RT, room temperature; Fn, nuclear fluorescence; Fc, cytoplasmic fluorescence; IBB, Importin-β binding; FITC, fluorescein isothiocyanate; SV40, Simian vacuolating virus 40; LTA, large tumor antigen

PO 022

REAL-LIFE DATA ON VIRAL VARIANTS AND ON ANTIVIRAL TREATMENT IN ADULT PATIENTS WITH MILD COVID-19 SYMPTOMS FROM DECEMBER 2021 TO JANUARY 2023

C. della Ventura[1], A. Lai[1], A. Bergna[1], M. Schiavini[2], L. Cordier[2], L. Galli[2], G. Scaglione[2], V. Colombo[2], D. Bernasconi[2], S. Landonio[2], S. Sollima[2], M. Corbellino[2], G. Casalini[2], F. Borgonovo[2], M. Gerbi[2], G. Rizzardini[2], A. Gori[2], S. Antinori[1,2], D. Visigalli[3], G. Zehender[1], A. Riva[1,2], S. Passerini

[1] *Department of Biomedical and Clinical Sciences, University of Milan, Italy*
[2] *Infectious Diseases Department, L. Sacco Hospital, Milan, Italy*
[3] *Pharmacy Unit, L. Sacco Hospital, Milan, Italy*

Background: In the outpatient setting, guidelines recommend treatment with COVID-19-specific therapy for symptomatic adults who have mild to moderate symptoms and are at increased risk for progression to severe disease based on older age, immune status, COVID-19 vaccination history, and comorbidities. The aim of the present study is to characterize non-hospitalized patients with mild/moderate COVID-19 who received different treatments between December 2021 and January 2023 and to find differences in the efficacy of treatments.

Methods: Data on 292 consecutive COVID-19 outpatients who received early treatment with antivirals (n=295) and/or monoclonal antibodies (n=103) at the Department of Infectious Disease at Sacco Hospital were collected. Follow-up was assessed by phone call after treatment. Viral load variation was indirectly assessed trough PCR cycle threshold (Ct) values in the nasopharyngeal swab. Differences between treatment groups were assessed by t test/chi-square test or Wilcoxon Mann-Whitney/Kruskal-Wallis for parametric and no parametric data, respectively. Lineage assignment was performed by real time PCR and/or whole genome sequencing (n=284).

Results: Males accounted for 52.7% (n=154) with a median age of 62 years (IQR:52-75). Among the 268 vaccinated subjects, 60.7% (n=156) had also received the 3rd dose. Median time from last vaccine dose to infection was 5 months (IQR: 2.3-6.2). Lineages and their descendants were distributed as follows: Delta 13%, BA.1 26.1%, BA.2 33.8%, BA.4 3.2% and BA.5 23.9%. No differences were observed among lineages distribution and patients age or vaccination status. Stratifying subject based on treatment, median age was significantly higher in subjects treated on molnupiravir (73.3 yrs SD 14, p<.001). Among patients >65 yo, a higher proportion was treated with mAbs (34.1%) or nirmatrelvir/ritonavir (31.9%) compared to molnupiravir (21.7%) or remdesivir (12.3%) (p=.002). A significant higher proportion of patients with cardiovascular disease (31.7%), COPD (34.9%) and primary or iatrogenic immunodeficiency (49.5%) received preferentially nirmatrelvir/ritonavir compared to other treatments (p<.0001, p<.02 and p<.0001, respectively). Globally, median duration of infection was 7 days (IQR:6-11), with no significant differences based on age or according to vaccination (and number of doses), lineage, therapy or related pathologies also considering a time of ≤ or >7 days. Median time of negativization was higher in males compared to females (9 vs. 7 days; p=.002). No differences were observed between negativization time and period from last vaccine dose and infection.

Conclusion: Our study provides an accurate overview of patients with mild/moderate COVID-19 by combining molecular and clinical data. Despite the large population on study, no significant differences were observed in the efficacy of different treatments. No long persistent viral shedding was observed even if several patients were immunodeficient confirming the efficacy of antiviral and mAbs therapies.

PO 023

IN VITRO ANTIVIRAL ACTIVITY OF EPIGALLOCATECHIN GALLATE AGAINST RABIES VIRUS

D. Pasqual, A. Loregian, C. Salata
Department of Molecular Medicine, University of Padua, Italy

Rabies is considered the most lethal zoonotic disease caused by lyssaviruses, especially Rabies virus (RABV). It is associated with the loss of 60,000 human lives each year thus representing a serious health issue. The high cost of immunoglobulin prophylaxis and the need to maintain the cold chain make it necessary to discover low-cost antivirals, especially, for developing countries. It has been reported that Epigallocatechin Gallate (EGCG), which represents the major polyphenolic compound extracted from the leaves of *Camellia sinensis*, inhibits in vitro the infection of several viruses, representing a potential broad-spectrum antiviral. Thus, we decided to test the antiviral activity of EGCG against RABV. Since RABV is a highly pathogenic virus that must be manipulated under BSL-3 conditions, a recombinant Vesicular Stomatitis Virus (pVSV) pseudotyped with RABV glycoprotein (pVSV-RABV) was employed as a safer surrogate. EGCG showed concentration-dependent inhibitory activity against pVSV-RABV infection with an EC_{50} of 0.14 µg/mL.
Next, for target identification, time-of-addition, viral attachment, and entry assays were performed in RABV-infected Vero CCL-81 in the presence of different concentrations of EGCG. Treating the pVSV-RABV with the compound before or during the infection resulted in a significant reduction of viral infectivity. In contrast, no inhibition was detected when cells were treated with the EGCG before or after virus internalization. Viral attachment and entry assays showed that virus infectivity is inhibited only at the stage of virus attachment. Overall, our data suggest a direct interaction of EGCG with virus particles affecting the ability of RABV to recognized target cells.
In conclusion, EGCG inhibits RABV infection acting at the early stages of viral replication cycle suggesting this polyphenolic compound as a promising antiviral candidate against RABV.

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PO 024

ACYCLOVIR AND VALACYCLOVIR TREATMENTS OF GENITAL HERPESVIRUS CURE ANOGENITAL CONDYLOMATOSIS AND INTRAEPITHELIAL NEOPLASIA: A CASE SERIES

M. Balestrieri[1], C. Carnovale-Scalzo[2], A.R. Garbuglia[3], M.V. Chiantore[4], L. Accardi[4], P. Di Bonito[4]

[1] *Gynaecology and Diagnostic Colposcopy Clinic, Rome, Italy*
[2] *Histopathology laboratory, Ospedale San Carlo di Nancy, GVM care and research, Rome, Italy*
[3] *Laboratory of Virology, National Institute for Infectious Diseases Lazzaro Spallanzani, IRCCS, Rome, Italy*
[4] *Department of Infectious Diseases, EVOR unit, Istituto Superiore di Sanità, Rome, Italy*

Aim: This report covers the case histories of several women affected by pathologies related to genital Herpesvirus (HHV) and Papillomavirus (HPV). They were referred to the gynaecology outpatient clinic for colposcopic examination, and received pharmacological antiviral treatment. The patients were affected by HHV-2 infections in the cervix and vulva. Cervical lesions and condylomatosis, which are characteristic of HPV infections, were also detected.

Method: Patients received conventional oral and topical treatment with Acyclovir (ACV) or oral treatment with Valacyclovir (VCV). Patients, when necessary, underwent cervical cancer screening with the established triage, which comprises a Pap-Test, HPV-DNA test and genotyping, biopsy and histology exam.

Results and Conclusions: In colposcopic examinations during follow-up visits, patients showed different HHV-2 remission times. During this antiviral treatment, there was also a complete resolution of vulvar and cervical HPV lesions with a *restitutio ad integrum* of the tissues, and no recurrence at follow-up visits. HHV and HPV infections are often associated in genital infections and, as sexually transmitted infections, share the same risk factors. In the cases presented here, the observed remission of HPV-related pathologies during ACV and VCV treatments may suggest that antivirals are also effective in the treatment of HPV lesions.

Hypothesis. ACV and VCV must be phosphorylated within infected cells by HHV-encoded tyrosine kinase before they can act as HHV-DNA polymerase inhibitors. However, in coinfections or possibly even in HPV infections only, ACV may be phosphorylated by the TK-1 cellular kinase before inhibiting cellular DNA Pol α. Both these enzymes are highly expressed in HPV-infected cells, but are silenced in fully differentiated and resting cells. This hypothesis, which is supported by the data presented in this study, could pave the way for future studies concerning the clinical efficacy of ACV and VCV in the treatment of HPV-related anogenital pathologies.

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PO 025

ANTIVIRAL ACTIVITY AND CONFORMATIONAL FEATURES OF NEW SHORT PEPTIDES DERIVED FROM THE INTERNAL FUSION PEPTIDE OF THE SARS-CoV-2 SPIKE GLYCOPROTEIN S2 SUBUNIT

M.A. Stincarelli[1], M. Quagliata[2], A. Di Santo[3], L. Pacini[2], F. Real Fernandez[4], R. Arvia[1], A.M. Papini[2], P. Rovero[3], S. Giannecchini[1]

[1] *Department of Experimental and Clinical Medicine, University of Florence, Italy*
[2] *Interdepartmental Research Unit of Peptide and Protein Chemistry and Biology, Department of Chemistry "Ugo Schiff", University of Florence, Sesto Fiorentino, Italy*
[3] *Interdepartmental Research Unit of Peptide and Protein Chemistry and Biology, Department of NeuroFarBa, University of Florence, Sesto Fiorentino, Italy*
[4] *CNR, Istituto di Chimica dei Composti Organometallici (CNR-ICCOM), Sesto Fiorentino, Italy*

Background: Emerging and re-emerging outbreaks of new coronaviruses as the recent severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a human health concern. To date, SARS-CoV-2 vaccines available exhibited a partial long-term efficacy and approved antivirals and other therapeutic monoclonal antibody treatment options have demonstrated limited efficacy, producing also virus resistance, against newly arisen variants.

Aim: The main objective is to develop new antivirals to fight the coronavirus emergence by using peptides deigned on the conserved amino acid region of the internal fusion peptide in the S2 subunit of the SARS-CoV-2 spike glycoprotein.

Methods: Peptides synthetized on conserved amino acid region of the internal fusion peptide in the S2 subunit of Spike glycoprotein of SARS-CoV-2. Antiviral activity against SARS-CoV-2 variants using plaque reduction assay and cytotoxicity was examined in Vero cells. Peptides structure was examined with Circular Dichroism (CD) spectra and secondary structure prediction. Peptides binding activity was examined with Surface plasmon resonance.

Results: Among the 11 overlapped peptides (9-23-mer), PN19 - a 19-mer peptide - revealed a powerful inhibitory activity against different SARS-CoV-2 clinical isolate variants, in absence of cytotoxicity. The PN19 antiviral activity was found to map in its 13 amino acid terminal segment and was dependent on the conservation of the central Phe and the terminal Tyr residues in the peptide sequence. Time of addition experiments proved that the activity was within 1 h post infection, presumably during the virus-cell fusion. Additionally, the PN19 peptide activity was reduced after adsorption with the virus during the fusion cell interaction and by adding S2 membrane proximal region derived peptides. Experiments to investigate the structural features of the active peptide and its target region on S2 subunit reported an alfa-helix CD spectrum and an ability to interact with the S2 membrane proximal region derived peptides.

Conclusions: Collectively, these results confirm that the internal fusion peptide region identified is a good candidate on which develop short peptidomimetic compounds as anti-SARS-CoV-2 antivirals for *in vivo* use.

PO 026

EXPLORING THE VIROME ASSOCIATED TO A COLLECTION OF *TRICHODERMA* ISOLATES FROM NATURAL ENVIRONMENTS IN SARDINIA

S. Pagnoni[1-3], S. Oufensou[2], V. Balmas[2], D. Bulgari[3], E. Gobbi[3], M. Forgia[4], Q. Migheli[2], M. Turina[4]

[1] *Department of Agricultural and Environmental Sciences – Production, Landscape, Agroenergy, University of Milan, Italy*
[2] *Department of Agricultural Sciences and NRD – Desertification Research Center, University of Sassari, Italy*
[3] *Department of Molecular and Translational Medicine, University of Brescia, Italy*
[4] *Institute for Sustainable Plant Protection, National Research Council of Italy, Turin, Italy*

Trichoderma genus comprises soil-inhabiting fungi that, while interacting with plants and other fungi, can provide valuable ecological services. Here we characterized the viral components associated with 113 selected *Trichoderma* spp. isolates, belonging to a previously described collection obtained from diverse soils from the Sardinian Island. After next-generation sequencing (NGS) on the ribosomal-depleted total RNA fraction, a specific bio-informatic pipeline allowed the identification of RNA-dependent RNA polymerases (RdRp) and other conserved protein sequences of viral origin. This method unveiled 17 viral RdRPs, two of which have already been detected in other regions of the world, while the remaining 15 represent new viral species. Among them, are eight negative-stranded RNA viruses, including a cogu-like virus very closely related to plant-infecting viruses, which represent the first report for negative-stranded viruses within the genus *Trichoderma*. With respect to positive-stranded viruses it is noteworthy the presence of an ormycovirus, belonging to a recently characterized group of bipartite ssRNA viruses but still lacking a reliable phylogenetic assignment in the Riboviria. Finally, we report the first bipartite *Mononegavirales* member infecting fungi. The second segment of the latter virus encodes for a protein product having a structurally conserved region with the nucleocapsid (NC) domain of rhabdoviruses, that could not be detected by similarity searches; moreover, these last findings allowed to retrieve RNA 2 associated to previously characterized members of the *Mononegavirales* in the *Penicillimonavirus* and *Plasmopamonavirus* genera, which we now show to be all bipartite.

PO 027

TARGETING INFLUENZA VIRUS USING A NOVEL *h*DHODH INHIBITOR

G. Sibille[1], A. Luganini[1], M. Pavan[1], S. Sainas[2], D. Boschi[2], M.L. Lolli[2], G. Gribaudo[1]

[1] *Department of Life Sciences and Systems Biology, University of Turin, Italy*
[2] *Department of Sciences and Drug Technology, University of Turin, Italy*

Aim of the Study: The pharmacological management of influenza virus (IV) infections still poses a series of challenges due to the limited anti-IV drug arsenal. Therefore, the development of new anti-influenza agents effective against antigenically different IVs is therefore an urgent priority. To meet this need, host-targeting antivirals (HTAs) can be evaluated as an alternative or complementary approach to current direct-acting agents (DAAs) for the therapy of IV infections. As a contribution to this antiviral strategy, in this study, we characterized the anti-IV activity of MEDS433, a new small molecule inhibitor of the human dihydroorotate dehydrogenase (*h*DHODH), a key cellular enzyme of the *de novo* pyrimidine biosynthesis pathway that catalyzes the oxidation of dihydroorotic acid (DHO) to orotic acid (ORO), a rate-limiting step of in the biosynthesis of uridine and cytidine required to fulfil the cell's pyrimidine nucleotide demand.

Methods: Antiviral assays were performed by VRA in A549 or Calu-3 cells infected with IVA or IVB. IVA and IVB were propagated and titrated in MDCK cells. To identify which stage MEDS433 affects the IV virus cycle, Time-of-Addition (TOA) assays were performed and the expression of representative IV proteins was assessed by immunoblotting.

Results and Conclusion: MEDS433 was observed to exert a potent antiviral activity against IAV and IBV replication in different cell types, with EC_{50} values ranging from 55 to 64 nM for IVA and from 52 to 65 nM for IVB. The anti-IV-activity of MED433 was observed to be reversed by the addition of exogenous uridine and cytidine or the hDHODH product orotate, thus indicating that MEDS433 targets specifically the *h*DHODH activity in IV-infected cells. When MEDS433 was then used in combination either with dipyridamole (DPY), an inhibitor of the pyrimidine salvage pathway, or with an anti-IV Direct-Acting Antiviral (DAA), such as N4-hydroxycytidine (NHC), synergistic anti-IV activities were measured.
These results suggest MEDS433 as an attractive promising HTA candidate, endowed with a potent antiviral activity against both IAV and IBV, and thus rapidly deployable against future novel emerging IVs. Moreover, MEDS433 could also be considered for combination drug treatments with both DAA nucleoside analogues and other anti-pyrimidines, such as NHC and DPY, to design new therapeutic strategies for the treatment of IV infections. Blocking *h*DHODH activity could therefore represent a strategy to inhibit the replication of different human respiratory viruses and to develop novel Broad-Spectrum HTA.

PO 028

SPIROKETALS DERIVED FROM PLAGIUS FLOSCULOSUS, A SARDINIAN ENDEMIC PLANT: AN OVERVIEW OF THEIR HIV-1 INTEGRASE INHIBITION ACTIVITY

F. Esposito[1], C. Sanna[1], B. D'Abrosca[2], A. Fiorentino[2], F. Giammarino[3], I. Vicenti[3], A. Corona[1], E. Tramontano[1]

[1] *Department of Life and Environmental Sciences, University of Cagliari, Italy*
[2] *Department of Environmental Biological and Pharmaceutical Sciences and Technologies, DiSTABiF University of Campania Luigi Vanvitelli, Naples, Italy*
[3] *Department of Medical Biotechnologies, University of Siena, Italy*

In this work we investigated, for the first time, the anti-HIV-1 activity of *Plagius flosculosus* (L.) Alavi & Heywood, a Sardinian endemic plant. The phytochemical research of the chloroform extract obtained from its leaves led us to isolate and characterize three diacetylenic spiroketals enol ether (SPK1- SPK3), a group of naturally occurring metabolites with interesting biological properties. Owing to their structural diversity, these cyclic ketals have attracted the interest of chemists and biologists. SPK1-SPK3 were here evaluated for their ability to inhibit HIV-1 integrase (IN) activity in biochemical assays. Results showed that all compounds inhibited HIV-1 IN activity. In particular, the most active one was SPK3 (IC50 of 1.46±0.16 μM), which interfered, in a low molecular range, with HIV-1 IN activity in the presence/absence of the LEDGF cellular cofactor. To investigate the mechanism of action, the three spiroketals were also tested on HIV-1 RT-associated Ribonuclease H (RNase H) activity, proving to be active in inhibiting this function. Although SPK3 was unable to inhibit viral replication in cell culture, it promoted the IN multimerization. We hypothesize that SPK3 inhibited HIV-1 IN through an allosteric mechanism of action.

PO 029

PREVALENCE AND PHENOTYPIC SUSCEPTIBILITY TO DORAVIRINE OF THE HIV-1 REVERSE TRANSCRIPTASE V106I POLYMORPHISM IN B AND NON-B SUBTYPES

F. Giammarino[1], A. de Salazar[2], A. Fuentes[2], L. Viñuela[2], F. Saladini[1], N. Bartolini[1], C. Charpentier[3], S. Lambert-Niclot[4], G. Sterrantino[5], G. Colao[6], V. Micheli[7], A. Bertoli[8], L. Fabeni[9], I. Malet[10], E. Teyssou[10], R. Delgado[11], I. Falces[12], A. Aguilera[13], P. Gomes[14], D. Paraskevis[15], M.M. Santoro[16], A.-G. Marcelin[10], F. Ceccherini-Silberstein[16], M. Zazzi[1], F. García[2]

[1] *University of Siena, Department of Medical Biotechnologies, Siena, Italy*
[2] *Hospital Universitario Clinico San Cecilio, Clinical Microbiology, Granada, Spain*
[3] *AP-HP, Hôpital Bichat-Claude Bernard, Laboratoire de Virologie, Paris, France*
[4] *AP-HP, Hôpital Saint-Antoine, Laboratoire de Virologie, Paris, France*
[5] *University of Florence, Department of Clinical and Experimental Medicine, Florence, Italy*
[6] *Careggi Hospital, Laboratory of Virology, Florence, Italy*
[7] *Sacco University Hospital, Department of Clinical Microbiology, Virology and Bioemergencies, Milan, Italy*
[8] *University Hospital "Tor Vergata", Laboratory of Virology, Rome, Italy*
[9] *"Lazzaro Spallanzani", IRCCS, National Institute for Infectious Diseases, Virology and Biosafety Laboratories Unit, Rome, Italy*
[10] *AP-HP, Hôpitaux Universitaires Pitié-Salpêtrière, Charles Foix, Laboratoire de Virologie, Paris, France*
11*Hospital 12 de Octubre, Clinical Microbiology Service, Madrid, Spain*
[12] *Hospital La Paz, Clinical Microbiology Service, Madrid, Spain*
[13] *Complejo Hospitalario Santiago, Clinical Microbiology Service, Santiago de Compostela, Spain*
[14] *CiiEM. IUEM. Almada, Portugal and Centro Hospitalar Lisboa Ocidental – HEM, Laboratório de Biología Molecular, LMCBM, SPC, Lisboa, Portugal*
[15] *National and Kapodistrian University of Athens, Department of Hygiene, Epidemiology and Medical Statistics, Athens, Greece*
[16] *University of Rome "Tor Vergata", Department of Experimental Medicine, Rome, Italy*

Background: This study aimed to evaluate prevalence and the *in vitro* susceptibility to doravirine of the HIV-1 RT V106I polymorphism detected in samples collected among the MeditRes HIV consortium.

Methods: MeditRes HIV includes ART naïve people living with HIV newly diagnosed in France, Greece, Italy, Portugal, and Spain during the years 2018-2021. We evaluated the impact of V106I on susceptibility to doravirine (a) in site directed mutants containing V106I, V106A, V106M & Y188L mutations in subtype B (NL4-3, HXB2) and CRF02_AG background and (b) in a subset of recombinant viruses with clinically derived RT-RNAseH coding region harboring V106I and no other major NNRTI RAMs. Phenotypic susceptibility to doravirine was determined through a TZM-bl cell-based assay and expressed as fold-change (FC) with respect to the reference wild type virus.

Results: MeditRes HIV includes 2705 patients. Viral subtypes were B in 1523 cases (56.3%), CRF02_AG 441 (16.3%), A 160 (5.9%), C 141 (5.2%), F 124 (4.6%), others 316 (11.7%). The prevalence of V106I was 2.9%, 3.2% and 2.5% in the overall dataset, in B and non-B subtypes, respectively. Among non-B subtypes, the prevalence of V106I was 3.1%, 0.7%, 8.1, 3.6%, 14.3%, 0.9%, and 3.1% in subtype A, C, F, G, D, CRF02_AG and CRF06_cpx, respectively. FC values for site directed mutants in the NL4-3, HXB2 and CRF02_AG background were 0.7, 2.0 and 2.5 with V106I, respectively; 3.4, 19.9 and na (not available) with V106A; 9.4, 27.3 and 13.5 with V106M; >100, na, and >100 with Y188L. The panel of clinically derived viruses tested includes 22 subtypes B and 28 non-B subtypes (2 A1, 2 CRF02_AG, 4 CRF06_cpx, 1 CRF44_BF, 3 D, 14 F1, 1 G and 1 URF). The median doravirine FC values were 1.3 (IQR 0.9-2.2) in the whole data set, while the susceptibility in B subtype is slightly lower than non-B subtypes (1.2 [IQR 0.9-1.6] vs. 1.8 [IQR 0.9-3.0]), and particularly than F1 subtype (2.6 [IQR 1.0-4.0]). Eight out of 50 (16%) viruses showed FC values equal or higher than the doravirine biological FC cutoff (3.0), one subtype B (FC 3.0) and seven non-B subtypes (A1, FC 5.5; CRF06_cpx, FC 3.7; F1, FC 7.9, 6.5, 3.1, 3.0, 3.0).

Conclusions: The prevalence of the HIV-1 RT V106I polymorphism in the MeditRes database remains low and comparable to previous studies. V106I appeared to minimally decrease the susceptibility to doravirine in site directed mutants and most of clinical isolates. Reduced susceptibility has been observed with increased frequency in non-B subtypes, especially subtype F1, however the clinical impact remains to be investigated.

PO 030

IDENTIFICATION OF ANTI-FLAVIVIRUS ACTIVITY OF APPROVED ANTIFUNGAL DRUGS BY A DRUG REPURPOSING APPROACH

S. Tuci, B. Mercorelli, A. Loregian
Department of Molecular Medicine, University of Padua, Italy

Aim of the Study: Arthropod-borne, single-stranded RNA flaviviruses are transmitted by mosquitos and ticks. These include human pathogens such as West Nile virus (WNV), dengue virus (DENV), Yellow fever virus (YFV), and Zika virus (ZIKV). These viruses are responsible for some of the most severe arbovirus infections affecting humans, posing a serious threat to global health, and potentially causing severe outbreaks. Unfortunately, there are neither effective antiviral treatments nor completely safe vaccines for all the circulating flavivirus infections. Therefore, there is an urgent need for novel antiviral compounds. In this regard, repurposing of drugs already licensed for the treatment of other unrelated diseases could be helpful to find new anti-flavivirus therapeutic options. Thus, the aim of this study is to identify and characterize the mechanism of action of some clinically approved antifungal drugs able to inhibit flavivirus replication.

Methods: Recently, some antifungal drugs have been reported to exhibit broad-spectrum antiviral activity by acting against host-related targets. Thus, based on those findings, antifungals belonging in particular to the class of azoles, which are classified in imidazoles and triazoles according to the number of nitrogen atoms in the azole ring, were tested *in vitro* to assess their ability to inhibit the replication of different flaviviruses. The antiviral activity was evaluated by plaque reduction assays in Vero cells and, in parallel, MTT assays were performed to determine the cytotoxicity of the compounds.

Results and Conclusion: Among the antifungals tested *in vitro* by plaque reduction assays, some were able to inhibit the replication of flaviviruses in a concentration-dependent manner, while others did not show activity in the range of the tested concentrations. Importantly, none of them demonstrated cytotoxicity in the same range of concentrations, excluding the possibility that the antiviral activity might be due to a decrease in cell viability. Starting from these preliminary results, further studies aimed at the elucidation of the mechanism of action of the active antifungal compounds and their cellular target are planned. The identification and characterization of the anti-flaviviral activity of some already approved drugs will be the starting point for the development of potential host-directed new antiviral strategies.

PO 031

EXPLORING THE ANTIVIRAL ACTIVITY OF QUERCETIN/FERULIC ACID CONJUGATES

V. Manca[1], G. Sanna[1], V. Palmas[1], G. Farina[1,2], D. Moi[2], F. Secci[2], V. Onnis[3], A. Manzin[1]

[1] *Department of Biomedical Sciences, University of Cagliari, Cittadella Universitaria Monserrato, Cagliari, Italy*
[2] *Department of Chemical and Geological Sciences, University of Cagliari, Cittadella Universitaria Monserrato, Cagliari, Italy*
[3] *Department of Life and Environmental Sciences, University of Cagliari, Cittadella Universitaria Monserrato, Cagliari, Italy*

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) has infected more than 762 million people to date and has caused 6.8 million deaths all around the world involving more than 187 countries. Despite currently available vaccines showing high efficacy in preventing severe respiratory complications in infected patients, the high number of mutations in the S proteins of the current variants is responsible for the high immune evasion and transmissibility and reduced effectiveness.

In this scenario, the development of secure and potent drugs to suppress viral replication and treat acute forms of COVID-19 remains a valid therapeutic challenge. Natural products, especially flavonoids, have been extensively studied in this field, showing great therapeutic potential: among them, Quercetin derivatives are currently considered appealing compounds as a complement to specific antiviral therapies. On the other hand, Ferulic Acid, a natural product with proven antiviral activity, has been recently studied for treating respiratory viruses, showing promising activity. Taken together these considerations, we developed Quercetin/Ferulic Acid conjugates to evaluate their in vitro activity against a broad spectrum of *Coronavirirus*.

PO 032

NEW STRATEGIES FOR INHIBITING HEPATITIS B VIRUS ENTRY USING CYCLOSPORINE A ANALOGUES

M. Longo Minnolo[1,2], L. Donnici[1], M. Conti[1], L.G. Guidotti[3,4], R. Di Fabio[4], R. De Francesco[1,2]

[1] *INGM, Istituto Nazionale Genetica Molecolare "Romeo ed Enrica Invernizzi", Milan, Italy*
[2] *Department of Pharmacological and Biomolecular Sciences (DiSFeB), University of Milan, Italy*
[3] *Division of Immunology, Transplantation, and Infectious Diseases, IRCCS San Raffaele Scientific Institute, Milan, Italy*
[4] *Vita-Salute San Raffaele University, Milan, Italy*

Chronic infection with hepatitis B virus (HBV) is a major cause of mortality worldwide and it is responsible for more than 50% of hepatocellular carcinoma cases. Despite the presence of antiviral drugs that effectively suppress viral replication, HBV eradication is difficult to achieve due to the persistence of viral genome in the nucleus of infected cells. A promising therapeutic approach to target the viral cycle is the use of entry inhibitors (EIs) that target the HBV receptor, i.e., the Na+-taurocholate cotransporting polypeptide termed NCTP.

Herein we studied a panel of new cyclosporine A (CsA) analogues as candidate EIs against HBV. CsA is an immunosuppressant calcineurin inhibitor that binds cyclophilin A (CyPA) and restrains the production of cytokines necessary for T cells activation and proliferation (Matsuda & Koyasu, 2000). CsA is also known to act as HBV EI (Watashi, Koichi et al., 2014) and to inhibit the replication of some viruses, like HCV (Glowacka, Paulina et al., 2020). Using different *in vitro* assays, we characterized the activity of a new series of modified CsA derivatives. We showed that most of these compounds are active against the infection of HBV without affecting its replication. These initial results indicated that the CsA analogues under investigation target an early step in the HBV life-cycle. Moreover, the lack of activity in replicon systems requiring CyPA binding to replicate HCV RNA, indirectly suggests that the CyPA-independent mechanism of action (MoA) of our CsA analogues should not induce immune suppression. Of note, the best performing CsA analogues were found to be about 100-fold more potent than CsA itself and 50-fold more potent than other published CsA derivatives (Shimura, Satomi et al., 2017).

In order to investigate whether our CsA analogues could prevent the interaction of HBV with the cognate NTCP receptor, we developed a competition receptor assay using MyrB-AF647, a fluorescently labelled analogue of Myrcludex B (MyrB). MyrB is a peptide drug derived from the region of the large HBV envelope protein known to interact with the receptor. Using this assay, we demonstrated that the CsA analogues are indeed able to inhibit the binding of MyrB-AF647 to NTCP, preventing NTCP-mediated viral attachment and entry. Finally, we used indirect immunofluorescence with anti-NTCP antibodies to demonstrate that our CsA analogues cause no reduction of NTCP expression and induced neither internalization nor degradation of this receptor.

In conclusion, we have identified a novel series of CsA analogues endowed with potent antiviral activity against HBV. Our data point to a MoA in which these compounds interact directly with NTCP, resulting in the inhibition of virus cell attachment and entry. Future *in vitro* and *in vivo* investigation will more directly confirm that these CsA analogues cause no immunosuppression and will evaluate the potential interference with the physiological bile acid transporter function of NTCP. We will also assess the antiviral potential of these compounds against hepatitis delta virus (HDV), a satellite virus of HBV that utilizes NTCP as entry receptor.

PO 033

IDENTIFICATION OF HIV-1 REVERSE TRANSCRIPTASE-ASSOCIATED RIBONUCLEASE H INHIBITORS BASED ON 2-HYDROXY-1,4-NAPHTHOQUINONE MANNICH BASES

A. Corona[1], K. Ahmedb[2], V.H. Masandc[3], R. Schobert[4], E. Tramontano[1,5], B. Biersack[4]

[1] *Laboratorio di Virologia Molecolare, Dipartimento di Scienze della Vita e Dell'Ambiente, Universitá degli Studi di Cagliari, Cittadella Universitaria di Monserrato, Cagliari, Italy*
[2] *Department of Chemistry, Abeda Inamdar Senior College, University of Pune, India*
[3] *Department of Chemistry, Vidyabharati Mahavidyalaya, Amravati, India*
[4] *Organische Chemie I, Universität Bayreuth, Bayreuth, Germany*
[5] *Istituto di Ricerca Genetica e Biomedica, Consiglio Nazionale delle Ricerche (CNR), Monserrato, Cagliari, Italy*

There is a strong demand for new and efficient antiviral compounds endowed with innovative mode-of-action. Ribonuclease H (RNase H) activity of HIV-1 reverse transcriptase (RT) is an essential viral function that catalyzes highly specific hydrolytic events on the RNA strand of the RNA/DNA replication intermediate that is critical to the synthesis of integration-competent double-stranded proviral DNA from the RNA viral genome. Since its peculiar role, RNase H is a promising target for drug development but, until now, no RNase H inhibitor is approved for treatment. One of the reasons for this is the difficulty to individuate a druggable pocket in the RNase H active site area given that it presents a morphology more open than the one of the, relatively similar, HIV-1 integrase (IN). Therefore developing allosteric inhibitors might represent a good strategy.
In the present work, a series of 2-hydroxy-1,4-naphthoquinone Mannich bases were screened for their inhibitory activity of HIV-1 RT-associated RNase H enzymatic function. Compounds showed to be active in the low micromolar range with compounds 1e and 2k showing distinctly higher HIV-1-RNase H inhibitory activity (IC_{50}=2.8-3.1 μM). Docking simulations were carried out to predict the binding of the most potent derivatives into HIV-1 RT. The interactions of 1e and 2k with the HIV-1-RNase H active site were predicted, also finding possible interaction with conserved residues in the area surrounding the RNase H active site and leading to the identification of salient and concealed pharmacophoric features of these molecules, leading bases for further scaffold optimization.

PO 034

N-PHENYL-1-(PHENYLSULFONYL)-1H-1,2,4-TRIAZOL-3-AMINE DERIVATIVES AS NEW INHIBITORS OF HIV-1 RT

G. Sanna[1], V. Palmas[1], V. Makarov[2], A. Manzin[1]

[1] *Department of Biomedical Sciences, Microbiology and Virology Unit, University of Cagliari, Cittadella Universitaria, Monserrato, Cagliari, Italy*

[2] *Federal Research Centre "Fundamentals of Biotechnology" of the Russian Academy of Sciences, Laboratory for Biomedicinal Chemistry, Moscow, Russia*

Human immunodeficiency virus (HIV) is one of the most important pathogens affecting mankind. Finding new treatments to cure HIV is, therefore, one of the most pressing challenges in contemporary virology and medicinal chemistry.

HIV-1 reverse transcriptase is a key enzyme in the HIV replication cycle and represents a privileged pharmacological target for anti-AIDS therapy. However, significant resistance has been developed against the current RTIs, and there is an urgent need to develop new anti-HIV agents that are effective against these resistant mutants. Here we report the anti-HIV-1 activity against wt and mutant strains of a novel N-phenyl-1-(phenylsulfonyl)-1H-1,2,4-triazol-3-amine derivatives. Several derivatives showed potent and selective anti-HIV-1 activity in the low micromolar range (EC_{50} value 0.03-0.4 μM range), with low cytotoxicity against the MT-4 cells (45-100 μM range). As a critical issue in the clinical management of HIV-1 disease is the development of drug-resistant strains, compound 1, identified as a lead, was further tested against a panel of viruses carrying mutations that confer resistance to NRTI and NNRTI inhibitors and that often appear during HAART therapy, reducing the effectiveness of these classes of compounds. Interestingly, the activity of these derivatives against AZTR and MDR strains is comparable with those of HIV-1 wild type while they resulted less active against NNRTI-resistant mutants.

Our lead compound was then tested in a colorimetric enzyme immunoassay to evaluate its ability to inhibit the reverse transcriptase of HIV-1. Results confirmed that the compound is a promising inhibitor with a 60% of inhibition at 10 μM and a 90% at 100 μM.

PO 035

IDENTIFICATION AND CHARACTERIZATION OF MAIN PROTEASE M^{PRO} INHIBITORS AND INDOMETHACIN-BASED PROTACs AS ANTI-SARS-CoV-2 ANTIVIRAL STRATEGIES

A. Bazzacco[1], B. Mercorelli[1], J. Desantis[2], S. Tuci[1], L. Siragusa[3], P. Benedetti[4], M. Eleuteri[2], F. Croci[2], G. Cruciani[2], L. Goracci[2], A. Loregian[1]

[1] *Department of Molecular Medicine, University of Padua, Italy*
[2] *Department of Chemistry, Biology, and Biotechnology, University of Perugia, Italy*
[3] *Molecular Horizon Srl, Bettona, Perugia, Italy*
[4] *Molecular Discovery Ltd, Centennial Park, Borehamwood, Hertfordshire, United Kingdom*

Aim of the Study: In this study, we aimed at identifying new strategies to inhibit the replication of SARS-CoV-2 and other human coronaviruses. We developed both inhibitors of the viral Main Protease (M^{Pro}) and PROTACs based on the anti-inflammatory drug indomethacin (PRO-INM), with the aim to identify potent antivirals endowed with a broad-spectrum activity against both pathogenic and endemic coronaviruses.

Methods: By exploiting three different virtual screening approaches, we identified 30 candidate M^{Pro} inhibitors. These hits were first tested *in vitro* for their ability to inhibit SARS-CoV-2 M^{Pro} catalytic activity; then, their antiviral activity was assessed by plaque reduction assays (PRAs) against both SARS-CoV-2 and the endemic β-coronavirus HCoV-OC43 and α-coronavirus HCoV-229E. In parallel, the cytotoxicity of the compounds was determined by MTT assay. One of the initial hits, compound *7*, which exhibited a weak anti-M^{Pro} inhibitory activity *in vitro*, was subjected to optimization by a fast-track rational design campaign. Its analogues were characterized with the assays described above, and their inhibitory activity was also assessed in a cell-based virus-free assay, which demonstrated the SARS-CoV-2 M^{Pro} inhibition also in a cellular context.

The activity of the indomethacin-based PROTACs was assessed by PRA and the absence of toxicity was determined by MTT assay. Next, to characterize their mode of action, we analysed the degradation of putative targets by Western blot in both infected and uninfected cells.

Results and Conclusions: Some of the hits identified by the *in silico* screenings (i.e., hit 3, 4, 5, 6, 25, and 29) inhibited SARS-CoV-2 M^{Pro} *in vitro* and were also able to inhibit the replication of both SARS-CoV-2 and HCoV-OC43 in a dose-dependent manner without displaying toxicity at the tested concentrations. This activity was maintained also towards HCoV-229E, even though with higher EC50 values, and with the exception of compound 25. The analogues of compound *7* resulted active against SARS-CoV-2 M^{Pro} both *in vitro* and in a cellular context, but not against the other coronaviruses. Finally, indomethacin-based PROTACs showed an improvement of the potency in PRA compared to the original compound INM, suggesting that the increase in antiviral activity could be due to the degradation of a protein required for viral replication. Further studies are ongoing to validate the putative target degradation.

PO 036

THE NAAA HYDROLASE IS A PAN-FLAVIVIRAL HOST FACTOR

E. Iacono[1,2], V. La Rocca[3], C. Filipponi[1], G. Sciandrone[1], A. De Carli[1,2], G. Freer[1], M. Pistello[1,4], M. Lai[1]

[1] *Retrovirus Center, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Italy*
[2] *Department of Medical Biotechnologies, University of Siena, Italy*
[3] *Institute of Life Sciences, Sant'Anna School of Advanced Studies, Pisa, Italy*
[4] *Department of Clinical and Experimental Medicine, University of Pisa, Italy*

Aim of the Study: Our study focused on the anti-viral potential of the N-Acylethanolamine acid amidase (NAAA) inhibition against flaviviruses. This enzyme hydrolyses palmitoylethanolamide (PEA), a bioactive lipid that mediates anti-inflammatory, neuroprotective and immunosuppressive activity through the activation of peroxisome proliferator receptor (PPAR-α).
Increasing levels of PEA could both protect the organism during viral infections, keeping the immune response under control, and exert a direct action towards viral replication, enhancing pathways, such as β-oxidation and changing the vesicular pH.
Previous results have supported our hypothesis by showing a strong reduction in the infectivity of Zika viral particles of about 3 log10 in neuroepithelial stem cells.
We aim to evaluate the possible correlation between the increase in intracellular PEA levels induced by NAAA suppression and the replication of another flavivirus, West Nile Virus (WNV) using the Syrian Golden Hamster, as *in vivo* model.

Methods used: Syrian Golden Hamsters were infected with 10^4 TCID50 of WNV. The NAAA inhibitor was administered the day before infection and every day until the eighth post-infection.

Blood, kidney, spleen and brain were collected at 3, 6 and 8 days post-infection to analyse the viral load and cytokines levels (IL-6, IL-1β, *IFN-γ*, *IFN-β*, *CXCL10*, *TNF-a*) by Real Time qPCR. The morphology of damaged tissues was evaluated using histological analysis.

Results and Conclusions: WNV genomes remained constant in kidney, spleen and blood 8 days p.i. However, NAAA inhibition decreased levels of WNV genomes by about 4-folds in the brain, the organ target of the virus, where we also observed a strong reduction of 3 folds of the inflammatory cytokines (IL-6, IL- β) confirming the major role of PEA as an anti-inflammatory drug. While the amount of viral genomes does not decrease significantly, previous results indicate that NAAA inhibition completely blocks the maturation of virions, but not their secretion. For this reason, we probed the histological samples for WNV infection to evaluate the effective number of infected cells. As expected we found that NAAA inhibition reduced the amount of infection by 3 log10 in the brain. In summary, these experiments indicate that NAAA inhibition can be considered as a pan anti-flaviviral treatment.

PO 037

ANTIVIRAL POTENTIAL OF TAURISOLO®, A GRAPE POMACE POLYPHENOL EXTRACT

A. De Filippis[1], A. Chianese[1], C. Zannella[1], G. Annunziata[2], F. Palma[1], F. Foglia[1], R. Della Marca[1], G.C. Tenore[2], M. Stornaiuolo[2], E. Novellino[3], M. Galdiero[1]

[1] Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy
[2] Department of Pharmacy, University of Naples Federico II, Naples, Italy
[3] Department of Medicine and Surgery, Università Cattolica del Sacro Cuore, Rome, Italy

Aim of the Study: Herpes simplex virus (HSV) is widespread in population and in most cases the infection is asymptomatic. Current available anti-HSV drugs are acyclovir and its derivatives, although long-term therapy with these agents can lead to drug resistance. Thus, the discovery of novel anti-herpetic compounds deserves additional studies. In recent decades, much scientific effort has been invested in the discovery of new synthetic or natural compounds with promising antiviral prop-erties. In our study, we tested the antiviral potential of a novel polyphenol-based nutraceutical formulation (named Taurisolo®) consisting of a water polyphenol extract of grape pomace (1, 2).

Methods used: The evaluation of the antiviral activity was carried out by using HSV-1 and HSV-2 in plaque assay experiments to understand the mechanism of action of the extract. Results were confirmed by Real-Time PCR, Trasmission Electron Microscope (TEM) and fluorescence microscope.

Results and Conclusions: Taurisolo® was able to block the viral infection by acting both when added together with the virus on cells and once virus was pre-treated with the extract, demonstrating an inhibitory activity directed to the early phases of HSV-1 and HSV-2 infection. Altogether these data evidenced for the first time the potential use of Taurisolo® as topical formulation for both preventing and healing herpes lesions.

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PO 038

INHIBITION OF ENTEROVIRUS A71 BY A NOVEL OXOCHROMANYL-DERIVATIVES

G. Sanna[1], F. Cacioppo[2], R. Piras[1,3], D. Moi[3], F. Secci[3], D. Ferraro[2], S. De Grazia[2], G. Giammanco[2], A. Manzin[1]

[1] *Department of Biomedical Sciences, Microbiology and Virology Unit, University of Cagliari, Cittadella Universitaria, Monserrato, Cagliari, Italy*
[2] *Department of Health Promotion, Mother and Child Care, Internal Medicine, and Medical Specialties, University of Palermo, Italy*
[3] *Department of Chemical and Geological Sciences, University of Cagliari, Cittadella Universitaria Monserrato, Cagliari, Italy*

Enterovirus A71 (EV-A71) infection has recently re-emerged globally, and its spread is a dynamic epidemiological phenomenon that deserves continuous surveillance.
In the past few years, widespread A71 infections occurred also in European countries. EV-A71 infection causes hand-foot-mouth disease (HFMD), herpangina, fever, but can sometimes induce a variety of neurological complications, including encephalitis, aseptic meningitis, pulmonary edema, and acute flaccid paralysis. Although three EV-A71 vaccines are available in China, they displayed reduced efficacy and limited protection against emerging strains. Therefore, an effectiveness antiviral therapy for EV-A71is advisable. With this purpose we identified new oxochromanyl derivatives and described their *in vitro* cytotoxicity and enterovirus broad-spectrum activity. In this context, derivative 4 showed a compelling anti-EV-A71 activity, and therefore it was selected for further investigations.

PO 039

DEFINITION OF THE FIRST INTACTION-MAP INVOLVING THE HUMAN PROTEOME AND THE HPV-16 VIRAL GENOME

V. Folliero[1], A. Chianese[1], G. Donadio[2], F. Mensitieri[3], A. Nebbioso[4], V. Sian[4], M.V. Morone[1], L. Altucci[4], V. Izzo[3], F. Dal Piaz[3], M. Galdiero[1], G. Franci[3]

[1] *Department of Experimental Medicine, University of Campania Luigi Vanvitelli, Naples, Italy*
[2] *Department of Pharmacy, University of Salerno, Italy*
[3] *Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Baronissi, Salerno, Italy*
[4] *Department of Precision Medicine, University of Campania Luigi Vanvitelli, Naples, Italy*

Aim of the Study: Human papillomavirus (HPV) infects actively proliferating cells, and itsreplication cycle is related to host cell differentiation (1). Thereby, it can cause diseases with asymptomatic or severe or mild clinical signs. HPV 16 and 18 genotypes are involved in 70% of cervical cancers worldwide (2). This study aims to identify human proteins that interact with the HPV-16 genome in infected cells, to research potential new therapies through an innovative approach, named "Long Regions of DNA Pull-down (LDP)".

Methods: The pHPV-16 *Escherichia coli* plasmid (ATCC 45113) was amplified, extracted, and used for the first amplification step by pull-down PCR. The 500 bp amplicons were used as templates in the second round of PCR to obtain fragments with biotinylated 5' ends. Thereafter, SiHa cells, containing integrated copies of HPV16, were cultured, and subjected to nuclear extraction. Subsequently, DNA pull-down was performed. Subsequently, DNA pull-down was allowed to perform. In detail, the biotinylated dsDNA fragment was allowed to interact with the streptavidin spheres; then, the SiHa nuclear extract was added to this complex and incubated to promote the interaction. The interactors were processed by mass spectrometry. Gene Ontology (GO) studies were performed to analyze the biological processes, the molecular functions in which the interactors are involved.

Results and Conclusions: Mass spectrometry identified approximately 4350 proteins, of which 310 interact with a single oligo. Some interactors were known to be implicated in HPV-associated diseases, while others had never been described yet. Indeed, chromodomain-helicase-DNA binding protein 4 (CHD4) is a component of the NuRD complex that participates in chromatin remodeling by deacetylating histones. These data could lay the groundwork for further studies to find new potential therapies to combat HPV16 infection, progression, and pathogenicity.

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PO 040

INTRA-HOST VARIABILITY OF MPXV GENOMES IN MULTIPLE BODY DISTRICTS OVER TIME

M. Rueca, E. Giombini, V. Mazzotta, G. Gramigna, C.E.M. Gruber, C. Pinnetti, L. Fabeni, F. Tucci, O. Butera, G. Matusali, A. Mariano, A. Mondi, E. Lalle, F. Forbici, E. Girardi, F. Vaia, E. Nicastri, A. Antinori, F. Maggi

National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Rome, Italy

Aim of the Study: During the last 2022 international outbreak of Mpox (Lineage B.1), the sexual contact was observed to be the principal transmission route for monkeypox virus (MPXV). Despite the fact that the genital tract represents the main target of viral lesions, other districts are permissive, and the virus can be found widespread in the body of an infected patient. Smallpox antiviral drugs such as cidofovir, brincidofovir, and tecovirimat are used in the treatment of mpox; the latter drug has been shown to be effective in vitro against the MPXV lineage B.1. Here, we investigated this wide-spectrum tropism of MPXV by studying the genetic intra-host variability of full-length genomes in multiple tissues from 5 patients.

Methods used: Two pools of primers for 163 amplicons with a medium length of 2000 bps were used to amplify MPXV genomes. Libraries were prepared starting from 10-100 ng of amplified DNA, and sequencing was performed on the Ion Torrent GSS5 Prime Sequencer to obtain 1 million reads/sample. Reads longer than 50 nucleotides and with a mean Phred score of at least 20 were mapped on the MPXV genome USA-2022-MA001 using the bwa-mem aligner software. A homemade script then reconstructed consensus sequences.

Results and Conclusions: Overall, a total of 43 (84%) full-length MPXV genomes were obtained: 7 genomes from pt 1(4 from skin lesions, 1 from Oropharyngeal swab - OPS, feces, and saliva), 2 from pt 2 (2 OPS), 2 from pt 3 (1 OPS, 1 ocular swab), 23 from pt 4 (8 skin lesions, 4 anal swabs, 3 plasma, 4 OPS, 1 saliva, 1 semen, 1 BAL, 1 trachea biopsy), and 9 from pt 5 (4 anal swabs, 3 OPS, 1 from plasma, 1 saliva). Pt 1 to 3 were immunocompetent and cleared the infection rapidly, while pt 4 and 5 were immunocompromised due to HIV-1 advanced infection (CD4+ T cell count < 200) and present prolonged MPXV shedding (up to more than 141 and 33 days, respectively for pt 4 and 5). All MPXV genomes belong to the B.1 clade, and the intra-patient analysis revealed high intra-host variability among the MPXV genomes in pt 4 and 5: overall, 20 nucleotide mutations were identified in the 32 genomes from HIV patients. Fifteen of these genetic mutations are distributed in different samples at different times and the presence of tissue-specific mutations that remained stable over time was revealed. Moreover, the gp045 gene encoding for F13L (target of tecovirimat) harbours more substitutions than the other genes (6; 37,5% vs. 1; 7% for the other genes) in genomes obtained from pt 4. Mutations in the gp045 gene were discovered in pt 4 samples from day 94 post symptoms onset (pso) (OPS, tongue lesion swabs, anal swabs, and trachea biopsy). Since pt 4 was treated from days 65 to 79 pso, this evidence could lead to the hypothesis that the selective pressure induced by treatment drives the emergence of such substitutions. Little is known to date about tecovirimat's mode of action and interaction with the F13L protein. Even though, recently FDA raised the concern that small changes to the F13L could have a large impact on the antiviral activity of tecovirimat suggesting for this drug a low genetic barrier to the development of viral resistance.

Although more research is needed, these findings show that prolonged viral shedding in immunocompromised patients can result in different MPXV evolution in different body districts, contributing to replication compartmentalization and led to the hypothesis that tecovirimat treatment could have a role in MPXV variability. Further studies are ongoing to investigate possible role of tecovirimat treatment in emergence of resistance variants and its pathogenetic implications.

PO 041

EMERGENT CILGAVIMAB RESISTANCE MUTATIONS IN OMICRON BA.4/5 DURING EVUSHELD TREATMENT

C.E.M. Gruber[1], F.G. Tucci[1], V. Mazzotta[1], M. Rueca[1], G. Gramigna[1], A. Vergori[1], L. Fabeni[1], E. Giombini[1], O. Butera[1], D. Focosi[2], E. Nicastri[1], E. Girardi[1], F. Vaia[1], A. Antinori[1], F. Maggi[1]

[1] *National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Rome, Italy*
[2] *North-Western Tuscany Blood Bank, Pisa University Hospital, Pisa, Italy*

Aim of the Study: The long-acting anti-Spike monoclonal antibody (mAb) cocktail tixagevimab/cilgavimab has been authorized by European Medical Agency (EMA) for both pre-exposure prophylaxis (PrEP) and for early treatment of COVID-19. The Spike (S) aminoacidic substitution that confer resistance to each of the components have been recently identified. Mutation causing increase in neutralization titers are R346X, K444X, and V445X for cilgavimab, F486X for tixagevimab and R346X, K444X, and L452R for both mAbs. In vitro, cilgavimab selected variants that expressed Spike protein amino acid substitutions R346G/I, K444E/N/Q/R/T, or N450D, each associated with a reduction in susceptibility. Resistance mutations in immunocompromised BA.2 infected patients have been previously reported. Our hypothesis is to assess if the observation of increasing treatment-emergent resistance could be described in immunocompetent patients as well.

Methods used: We retrospectively reviewed 22 consecutive outpatients at high risk for disease progression who received intramuscular tixagevimab/cilgavimab 300/300 mg as early treatment for COVID-19 at the National Institute for Infectious Diseases "L. Spallanzani" (INMI) in Rome from April to December 2022. Drug choice prescription was based on clinical judgment. None of them had received tixagevimab/cilgavimab for PreEP, nor had received previous or concurrent direct acting antivirals. By protocol, nasopharyngeal swabs (NPS) were collected at baseline and at day 7 after treatment with tixagevimab/cilgavimab. Whole viral genome sequencing was performed using the Ion Torrent Gene Studio S5 Prime (GSS5 Prime) platform and assembled with ESCA software[4].

Results and Conclusions: All genomes obtained belong to BA.4.*/5.* lineages: three cases were excluded from the 7-day sequencing because of the baseline occurrence of the cilgavimab-resisting mutations R346T or K444N/T. None of the 19 patients was negative at day 7, while 3 out of 5 patients with a NPS available at day 30 were negative.

Only 1 out of 19 cases (5.3%), was unvaccinated and showed evidence of treatment-emergent virological resistance to cilgavimab: one case showed *de novo* S:K444N mutation (due to G22894T) at day 7 (53% prevalence of the quasispecies), associated to an increase in the RT-PCR cycle threshold from 16 to 22.

Our data, compared with previous findings, demonstrate that anti-Spike mAb immune escape is more commonly detected in severely immunocompromised than in our cohort in which immunocompetent and vaccinated patients were prevalent.

Anti-Spike mAb resistance has been previously described for all authorized mAbs, including in patients treated with fully susceptible mAbs at baseline. Unfortunately, the R346 and K444 residues have been described as object of convergent evolution since summer 2022, with a widespread baseline *in vitro* resistance to cilgavimab. Based on baseline immune evasion of newly emerging SARS-CoV-2 Omicron subvariants, on January 26th, 2023, FDA revoked the emergency authorization for PrEP use of tixagevimab/cilgavimab mAbs. Nonetheless, if new sub-lineages fully susceptible to tixagevimab/cilgavimab could return to be prevalent, virological, or clinical failures in both cohorts of immune compromised or competent patients, should prompt early virological investigations to rule out treatment-emergent resistance.

Moreover, further studies are ongoing on samples of patients administered with other medical treatments in order to better characterize SARS-CoV-2 mutational evolution for the early identification of newly drug-induced variants.

PO 042

ANTIVIRAL POTENTIAL OF PEA AGAINST IAV AND ZIKV INFECTION

G. Sciandrone[4], E. Iacono[1], V. La Rocca[2], C. Filipponi[1], G. Freer[1], M. Pistello[1-3], M. Lai[1]

[1] *Retrovirus Center, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Italy*
[2] *Department of Medical Biotechnologies, University of Siena, Italy*
[3] *Institute of Life Sciences, Sant'Anna School of Advanced Studies, Pisa, Italy*
[4] *Department of Clinical and Experimental Medicine, University of Pisa, Italy*

Aims: Palmitoylethanolamide (PEA) is a bioactive lipid that mediates anti-inflammatory and immunosuppressive activity. In 1970s, PEA was found to reduce the severity and duration of symptoms caused by the influenza virus in miners. Since 2008 PEA-containing products have been approved for use in human as a nutraceutical, a food supplement, or a food for medical purposes to contrast inflammation-associated pain. PEA cellular target is the peroxisome proliferator receptor (PPAR α), a transcription factor mainly involved in the regulation of β-oxidation of fatty acids and inflammation. Since many viruses induce a strong inflammatory response, increasing level of PEA in cell cytoplasm might keep the immune response under control, reducing symptoms associated with immune system overactivation. Additionally, PEA exerts a direct action against viral replication interfering with lipid metabolism. Our aim is to investigate the antiviral role of PEA against viruses that differ in genome composition, replication or entry mechanism.

Methods: To study the effect of PEA administration on virus replication we infected A549 cells with 1 M.O.I. of Herpes Simplex Virus 1 (HSV1), Influenza A Virus (IAV) and ZIKA Virus (ZIKV). PEA was administered prior, during or post infection to cells at different concentrations (1 μM -10 μM). We performed Real-Time qPCR, viral yield assays and immunostainings to test infection levels and inflammation.

Results and Conclusions: We observed a 2-fold reduction in the number of viral genomes released from IAV-infected cells, when PEA is administered with the virus. Similar results were obtained by counting the number of infected cells using immunofluorescence stainings. Moreover, similar antiviral activity was detected on ZIKV, where we observed a 3-fold reduction in the number of viral genomes when PEA is administrated to cells before infection. Oppositely, PEA does not inhibit HSV-1 replication, evaluated as number of virions released and immunostainings.
We suppose that PEA exerts a virus-specific antiviral effect by affecting different phases of the infection included early (for IAV) or late/egress (for ZIKV). Moreover, PEA antiviral mechanism does not affect HSV-1 replication. In conclusion PEA administration might hinder viral infectivity of IAV and interfere with ZIKV replication while maintaining an anti-inflammatory environment that mitigates infection-related symptoms.

PO 043

DDX3 INHIBITORS AS ANTIVIRAL AGENTS AGAINST CHIKUNGUNYA VIRUS

C.R. Piazza[1,2], G. Lottini[1,2], P. Perrera[2,3], C. Di Primio[4], M. Mignanelli[5], P.G. Spezia[2,3], M. Lai[2], G. Freer[2], P. Quaranta[2], M. Pistello[2,6]

[1] *Department of Medical Biotechnologies, University of Siena, Italy*
[2] *Centro Retrovirus, University of Pisa, Italy*
[3] *Department of Traslational Research, Retrovirus Center and Virology Section, University of Pisa, Italy*
[4] *Institute of Neuroscience, CNR of Pisa, Italy*
[5] *Laboratorio di Biologia Bio@SNS, Scuola Normale Superiore, Pisa, Italy*
[6] *Virology Operative Unit, University Hospital of Pisa, Italy*

Aim of the Study: The human protein DDX3 is a DEAD box ATP-dependent RNA helicase that regulates transcription, mRNA maturation and translation. DDX3 is also involved in replication of several RNA viruses. Recently, we have shown that some DDX3 inhibitors are effective against several viruses with positive sense single-stranded RNA (ssRNA+) genome such as Coxsackie B (CV-B), whereas they are not effective against viruses with negative sense single-stranded RNA (ssRNA-) genome. Moreover, inhibition of DDX3 also appears to affect DNA virus replication. To further elucidate the role of DDX3 protein in viral infection, we are investigating the effect of DDX3 inhibitors in chikungunya virus (CHIKV) infection. CHIKV is a mosquito-borne virus that belongs to the Togaviridae family. As an emerging alphavirus, it caused massive outbreaks of Chikungunya fever in several countries and regions in Africa, Asia and, more recently, in Central and South America. The compounds we tested specifically target the DDX3 RNA binding domain and leave the ATP-binding domain unchanged.

Materials and Methods: Huh-7 cells were infected with 33 MOI of CHIKV in the presence of DDX3 inhibitors and monitored up to 2 days post infection (DPI). Antiviral activities of these compounds were determined *in vitro* by plaque assay and the half maximal inhibitory concentration (IC_{50}), and selectivity index (SI) were calculated. To evaluate the production of DDX3 and viral proteins, Western blotting was performed by infecting Huh-7 cells with 15 MOI of CHIKV and doing protein extraction at different hour post infection (PI). Moreover, Huh-7 cells were infected with 15 MOI of CHIKV in presence of DDX3 inhibitors and western blotting was carried out 3 days PI. Finally, distribution and localization of DDX3 during CHIKV infection were visualized by immunofluorescence.

Results and Conclusions: During the infection of cells with CHIKV, the level of DDX3 protein is significantly reduced. In the presence of DDX3 inhibitors, there is an antiviral activity against CHIKV, but DDX3 protein levels are no longer comparable to those of uninfected cells. Moreover, the cytoplasmatic distribution of DDX3 is altered and there is a dramatic decrease in DDX3-related fluorescence levels, which appear to take on an aggregated form. DDX3 appears to be involved in CHIKV infection. For this reason, it may be an excellent target to develop antiviral agents clinically relevant against CHIKV infection.

PO 044

PRELIMINARY EVIDENCE OF HEPATITIS E VIRUS CIRCULATION IN PIGS AND SHEEP IN SARDINIA

A. Oggiano[1], M.L. Sanna[1], A. Ladu[1], R. Bazzardi[1], S. Dei Giudici[1], M. Fiori[1], C. Ligios[1], B. Tanda[1], L. De Sabato[2], G. Ianiro[2], G. Vaccari[2], M. Monini[2], I. Di Bartolo[2]

[1] Istituto Zooprofilattico Sperimentale della Sardegna, Department of Animal Health, Sassari, Italy
[2] Istituto Superiore di Sanità, Department of Food Safety Nutrition and Veterinary public Health Rome, Italy

Hepatitis E is an emerging disease causing an increasing number of autochthonous cases in Europe. The disease is generally self-limited but can become chronic in immunocompromised patients. The aetiological agent is a small RNA virus, named hepatitis E virus (HEV), characterized by a high heterogeneity, and classified into 8 genotypes, 4 of which cause most of human cases. HEV-1 and HEV-2 only infect humans and circulate in low-income countries; HEV-3 and HEV-4 are zoonotic. Domestic and wild pigs are the main reservoir. Recently, the HEV-3 has also been detected in novel hosts such as sheep and goat. The role of these animals in the virus transmission is still unknown. The HEV-3 is the most common genotype in Europe in both humans and pigs. The virus is widespread and a very high number of farms are HEV-positive. The frequent exposure of pigs to the virus is confirmed by the detection of high seroprevalence, which can be up to 90-100% in adult animals. Pigs become susceptible to the infection when they lost maternal immunity. After a stage of viremia, fecal viral shedding follows replication in liver. Meanwhile, the IgM anti-HEV antibodies are produced early after the viremia stage and after a few weeks anti-HEV IgG appears. It is unknown if the immune response is protective during the whole life of the animals since also adult pigs can result infected, even at low percentage. AIM: This study aimed to evaluate the circulation of HEV in domestic pigs and sheep in Sardinia. METHODS: Three-hundred thirty-six and two-hundred one sera were collected from sheep and pigs, respectively during 2022. One serum was sampled from each farm, located in eight local health units over the region. All animals were housed in indoor farms. The presence of antibodies against HEV were tested by multi-species commercial ELISA test (ID Screen Hepatitis E indirect multi-species, ID.Vet) for the detection of antibodies against HEV. RESULTS: In pigs, 42.2% of sera were positive for anti-HEV antibodies, indicating a wide circulation of the virus. Positive sera were detected in pigs from the 8 local health units, in all but one a prevalence of positive anti-HEV >35% was retrieved. In sheep, 4 sera out of 336 tested were anti-HEV positive (1.2%). The four positive sera were sampled from animals housed in 4 local health units. None of the investigated sheep could have interact with pigs, neither directly through close contact nor by grazing in the same area. This study revealed a wide exposure to HEV of pigs in Sardinia and showed a limited exposure of sheep to the virus. Virological studies will be conducted to investigate the circulation of the virus and its similarity with human strains.

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PO 045 - OC 06

IN VIVO MODEL OF SARS-CoV-2 INFECTION IN K18-hACE2 MICE

P. Quaranta[1-3], P. Perrera[1], C.R. Piazza[1,4], P.G. Spezia[1], G. Lottini[1,4], E. Plicanti[1,4], G. Freer[1], M. Pistello[1,2]

[1] *Retrovirus Centre, Department of Translational Research, University of Pisa, Italy*
[2] *Virology Operative Unit, Pisa University Hospital, Pisa, Italy*
[3] *Institute of Neuroscience - CNR, Pisa, Italy*
[4] *Department of Biotechnology, Chemistry & Pharmacy, University of Siena, Italy*

Aim of the Study: An *in vivo* model of acute infection with SARS-CoV -2 was developed in transgenic mice to evaluate the safety and efficacy of new antiviral drugs and perform studies of the airborne spread of the virus and the efficacy of sterilization technologies.

Methods: K18-hACE2 transgenic mice (Jackson Laboratories) expressing the human angiotensin I- converting enzyme (peptidyl dipeptidase A) 2 (hACE2) were used. hACE2 is expressed in the epithelia of the upper respiratory tract and in the epithelia of other internal organs, including the liver, kidney, and gastrointestinal tract of K18-hACE2 transgenic mice, making these animals susceptible to SARS-CoV -2 infection. Animals were infected under anesthesia by intranasal administration with 10^2, 10^3, and 10^4 $TCID_{50}$ of SARS-CoV-2, strain B.1. After infection, animals were observed for ten days to assess weight loss and the appearance of clinical signs. A Kaplan-Mayer curve was plotted for each dose. At the end of the follow-up, the animals were sacrificed and all organs were harvested for histological examination and real-time PCR.

Results and Conclusions: Animals developed self-limited infection following intranasal infection with 10^2 $TCID_{50}$, mild disease with 10^3 $TCID_{50}$ and fatal disease with 10^4 $TCID_{50}$. Using the latter dose, animals showed weight loss and rapidly developed pulmonary symptoms. In animals receiving 10^3 $TCID_{50}$ of virus, the brain was the most infected organ and the virus was present disseminated in all organs including the adipose tissue. The healthy animals exposed via airborne to the infected animals developed a self-limited infection. In summary, we have created an *in vivo* model of SARS-CoV-2 infection that mimics COVID-19 pathology. This model is useful for studying airborne transmission of the virus and pathology of chronic and acute infections, and for testing antiviral drugs *in vivo*.

PO 046 - OC 07

INVESTIGATION OF NOROVIRUS EVOLUTION USING THE cDNA OF AN ARCHIVAL COLLECTION OF STOOLS FROM CHILDREN WITH ACUTE GASTRO-ENTERITIS

F. Bonura[1], C. Filizzolo[1], M. Pizzo[1], G.L. Sanfilippo[1], F. Cacioppo[1], E. Palazzotto[1], F. Di Bernardo[2], A. Collura[2], V. Martella[3], S. De Grazia[1], G.M. Giammanco[1]

[1] Dipartimento di Scienze per la Promozione della Salute e Materno infantile "G. D'Alessandro", Università di Palermo, Italy
[2] Unità Operativa di Microbiologia e Virologia, Ospedale Civico e Di Cristina, ARNAS, Palermo, Italy
[3] Dipartimento di Sanità Pubblica e Zootecnia, Università Aldo Moro di Bari, Valenzano, Bari, Italy

Aim of Study: Norovirus (NoV) are recognised as a major cause of epidemic and sporadic acute gastroenteritis (AGE) in all age groups and surveillance activities for monitoring their epidemiology and genetic diversity have been enacted globally in the last decades. However, information on the genetic diversity of the NoVs circulating in the 1980s and 1990s, before the development and adoption of molecular assays, is limited. From 1986 to 2020, uninterrupted surveillance of viral AGE was conducted providing a unique observatory for exploring the epidemiological and evolutionary dynamics of NoVs.

Material used: Over 35 consecutive years, a total of 8433 stool samples were collected from paediatric patients (<5 years old) hospitalized with AGE at the ''G. Di Cristina'' Children's Hospital of Palermo, Italy. NoVs were detected by (RT)-PCR able to differentiate GI and GII NoVs and the polymerase (pol) and capsid (cap) genotypes were determined by a multi-typing diagnostic approach (Kojima et al. 2002, Kageyama et al. 2003, Vinje et al. 2015). The hypervariable P2 domain was also analysed on a selection of 40 NoV strains (Lindesmith et al., 2008).

Results and Conclusions: In this retrospective study, NoV infection was detected in 17% of patients. NoVs belonging to GII genogroup were detected starting from 1989 and represented the prevalent NoVs over the whole study period (15.6%), whilst GI NoVs were detected in 1.4% of cases since 1996.
Overall, the predominant NoV strain was GII.4 (60.8%), with temporal replacement of the GII.4 cap variants and associated pol types. Temporal patterns were also observed for GII.3 and GII.2 NoVs, with circulation of different strains, differing in either the cap or pol genes or in both.
This long-term study allowed to observe the NoV genetic diversity and evolution. Multiple NoV genotypes simultaneously co-circulated over time, showing continuous and rapid ability to modify their genomic makeup. This results also underline the contribution that long-term surveillance data can provide for devising vaccine strategies, such as vaccine design and updated.

PO 047 - OC 08

THE EFFECTS OF COVID-19 ON THE EPIDEMIOLOGY OF COMMON RESPIRATORY INFECTIONS IN CHILDREN: A SINGLE-CENTER ANALYSIS OF THE PAST 5 YEARS

L. Giannone[1], S. Roversi[2], P. Saini[1], V. Pintabona[3], A. Caruso[2], R.F. Schumacher[4], L. Dotta[5], R. Badolato[5]

[1] *Department of Pediatrics, University of Brescia and ASST Spedali Civili di Brescia, Italy*
[2] *Department of Molecular and Translational Medicine, Section of Microbiology, University of Brescia, Italy*
[3] *Department of Pediatrics, ASST Spedali Civili di Brescia, Italy*
[4] *Hematology-Oncology Department, ASST Spedali Civili of Brescia, Italy*
[5] *Department of Clinical and Experimental Sciences, Department of Pediatrics, University of Brescia and ASST Spedali Civili of Brescia, Italy*

Introduction: Recent scientific literature has shown that the preventive and control measures used during the COVID-19 Pandemic impacted heavily on the spread of other pathogens causing acute respiratory tract infection in children.

Methods: We retrospectively analyzed data from all patients admitted to the University Children's Hospital in Brescia between January 2018 and December 2022, who had at least one respiratory virus identified by PCR on nasopharyngeal aspirate.

Results: 1921 patients were included in the study. We recorded an increasing number of viral infections in hospitalized patients: 389 cases in 2018, 432 cases in 2019, 312 cases in 2020, 384 in 2021 and 507 in 2022. No differences were observed in gender distribution; the majority of viral infections occurred in children aged 6 months-3 years.
However we showed that the median age raised: in 2018 and 2019 it was 1.8 years, in 2020 it was 2.5 years, in 2021 it was 1.8 years, and in 2022 it reached 3 years of age, with an increase of infections among older children (3-6 years and >6 years).
We also observed a slight increase (38,1% in 2022, in contrast to 30,5% in 2021, 31,5% in 2020, 33,3% in 2019, and 37,4% in 2018) in the admittance to hospital of patients with comorbidities (i.e genetic diseases, chronic lung diseases, heart disease, neuromuscular diseases, and immunodeficiencies). We evaluated patients who had severe infection considered as respiratory failure, localized infectious disease to two major districts, sepsis/septicemia and/or death, and we reported a rate of 13% in 2018 and 14.5% in 2022, in contrast to 6.5% in 2019, 8% in 2020, and 7.2% in 2021.
A detailed analysis of causing pathogens in 2022 showed that severe illness occurred in Parainfluenza Virus infection (6,1% of patients), Rhinovirus (33,3%), SARS-CoV2 infection (6,1%), and Adenovirus (3%). Conversely, in 2022, Influenza Virus (both A or B) demonstrated a decline of severe illness compared to previous years (6,1% of patients with Influenza in contrast to 11% in 2018) while Respiratory Syncitial Virus (RSV) showed a serious illness rate per year of 22.2% in 2018, 23.3% in 2019, 22.7% in 2021, and in 2022 18.2%, with the exception of 2020 when it was 9.1%.
We observed interesting variation in the epidemiological pattern over the study period: Influenza A or B peaked in November-December 2023, while no significant peaks were observed in the previous seasons in 2021 and 2022; RSV peaked between October and December 2021 reaching a significantly higher incidence compared to 2019 and 2018 (95 cases in 2021, compared to 69 cases in 2018, 79 cases in 2019, and 45 cases in 2022) with a shorter and earlier peak compared to the pre-pandemic years; Parainfluenza Virus was the second most frequent pathogen in 2022, with a peak in June and July, and was responsible for 11% of all infections, whereas in previous years it accounted only for 2.5% in 2018, 4.0% in 2019, 0.3% in 2020, and 1.6% infections in 2021, respectively. Rhinovirus also gradually increased from 2020 (13,4%) reaching an incidence of 30% of infections in 2022, with maximum circulation between September and November; Bocavirus represented an emerging pathogen in 2021 (3,5%) and 2022 (4%), while in previous years it accounted for only 1%, with a maximum spread

in April and June 2022. In children of our cohort, SARS-CoV2 infection peaked between September and October 2022.

Conclusion: The post-pandemic era associated with significant changes in the circulation of respiratory viruses, particularly in the early peak of Influenza at the end of 2022, and the increase of RSV, Bocavirus, Parainfluenza Virus and Rhinovirus infections, the latter responsible for the majority of severe infections. The analysis of the epidemiological and clinical variation of viral respiratory infections may explain community transmission and allow timely and effective preventive measures.

PO 048 - OC 09

COINFECTION OF DERMAL FIBROBLASTS BY HUMAN CYTOMEGALOVIRUS AND HUMAN HERPESVIRUS 6 CAN BOOST THE EXPRESSION OF FIBROSIS-ASSOCIATED MICRORNAS

F. Bini[1], I. Soffritti[1], M. D'Accolti[1], C. Maccari[2], E. Mazziga[1], C. Arcangeletti[2], E. Caselli[1]

[1] *Department of Chemical, Pharmaceutical and Agricultural Sciences, Section of Microbiology, CIAS research Center and LTTA, University of Ferrara, Italy*

[2] *Department of Medicine and Surgery, University of Parma, Italy*

Aim of the Study: Human cytomegalovirus (HCMV) and Human herpesvirus type-6A (HHV-6A) have been reportedly suggested as triggers of the onset and/or progression of many autoimmune diseases, including systemic sclerosis (SSc), a severe autoimmune disease with still unclarified etiology, causing progressive fibrosis of skin and internal organs (1). Reactivation of such viruses and (specific antiviral immune responses have been detected in SSc patients, and infection by HCMV or HHV-6A was shown to induce the expression of fibrosis-associated transcriptional factors and miRNAs in human dermal fibroblasts (2, 3). However, it is unlikely that such viruses have separated effects on infected cells since both viruses are ubiquitously present in the human population and can mutually boost each other. Consistently, we recently reported that the simultaneous presence of HCMV and HHV-6 induced a higher expression of fibrosis-associated factors associated, compared to what observed in single infected cells (4). Based on these observations, this study aimed to investigate in primary human dermal fibroblasts (the primary targets of SSc), the effect HCMV/HHV-6A coinfection, focusing on the expression of miRNAs associated with pro-fibrotic pathway.

Methods: Human primary dermal fibroblasts were infected *in vitro* with cell-free inocula of HCMV (T40E) and HHV-6A (U1102), and samples were collected at different times post infection (0, 1, 2, 4, 7 and 10 d.p.i.). Total nucleic acids were extracted from collected cells and analyzed by virus- specific real time quantitative PCR (qPCR), and by qPCR microarrays simultaneously detecting and quantifying 84 human microRNAs associated with cell fibrosis.

Results and Conclusions: The results evidenced increased HCMV and HHV-6A replication in coinfected cells, accompanied by increased induction of fibrosis-miRNAs in coinfected compared to single-infected cells, thus supporting the hypothesis that HCMV and HHV-6 can enhance each other and may cooperate at inducing enhanced miRNA driven fibrosis. These data also suggest the use of virus-induced miRNAs as novel diagnostic or prognostic biomarkers for SSc and its clinical treatment.

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PO 049

miRNAs AS POTENTIAL BIOMARKERS IN MYALGIC ENCEPHALOMYELITIS/ CHRONIC FATIGUE SYNDROME

I. Soffritti[1], S. Gravelsina[2], M. D'Accolti[1], F. Bini[1], E. Mazziga[1], A. Vilmane[2], S. Rasa-Dzelzkaleja[2], Z. Nora-Krukle[2], A. Krumina[3], M. Murovska[2], E. Caselli[1]

[1] *Department of Chemical, Pharmaceutical and Agricultural Sciences, Section of Microbiology, CIAS research Center and LTTA, University of Ferrara, Italy*
[2] *Institute of Microbiology and Virology, Rīga Stradiņš University, Riga, Latvia*
[3] *Faculty of Medicine, Department of Infectology, Rīga Stradiņš University, Riga, Latvia*

Aim of the Study: Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a complex multifactorial disease that causes increasing morbidity worldwide, and many individuals with ME/CFS symptoms remain undiagnosed due to the lack of diagnostic biomarkers. Its aetiology is still unknown, but increasing evidence support a role of Herpesviruses (including Human Herpesvirus 6 species, HHV-6A and HHV-6B) as potential causative agents (1). Interestingly, the infection by these viruses has been frequently reported to profoundly impact the expression of microRNAs (2, 3), short sequences (18-23 nucleotides) of non-coding RNA, which have an important role in regulating gene expression at the post-transcriptional level, and that have been suggested also as epigenetic factors modulating ME/CFS pathogenic mechanisms. In addition, the recent discovery of miRNAs in plasma has raised the possibility that circulating miRNAs may serve as valuable biomarkers for autoimmune diseases (4). The objective of this study was to determine the potential role of specific circulating miRNAs as biomarkers of ME/CFS disease, and investigate the correlation between miRNAs expression, disease severity, inflammatory status, and HHV-6 infection/reactivation in ME/CFS patients, compared to healthy subjects.

Methods: Peripheral blood specimens were collected from 40 patients that received ME/CFS clinical diagnosis and from 20 healthy controls, recruited at the Rīga Stradiņš University Ambulance outpatient clinic. Total RNA including miRNA fraction was extracted from plasma samples, and the expression of eight CFS-associated circulating miRNAs, together with exogenous and endogenous controls, was evaluated by TaqMan Advanced miRNA assays (ThermoFisher Scientific).

Results and Conclusions: Six miRNAs resulted statistically significant up-regulated in the serum of CFS/ME patients (miR-127-3p, miR-140-5p, miR-142-5p, miR-143-3p, miR-150-5p and miR-448), evidencing a direct correlation between miRNAs levels and disease severity. Target genes of altered miRNAs were involved in pathways related to transcriptional control of herpesvirus reactivation, immune response to viral infection, extracellular matrix remodeling, inflammation, cell viability and immune cell death. The results may open the way for further validation of miRNA as new potential biomarkers in ME/CFS and increase the knowledge of the complex pathways involved in the ME/CFS.

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PO 050

DROPLET DIGITAL PCR (ddPCR) FOR THE ABSOLUTE QUANTIFICATION OF DIFFERENT VIRUSES INFECTING HONEY BEES (*APIS MELLIFERA* L.)

R.A. Kubaa[1], S.A.E. Zaidat[2,3], S. Sanzani[2], K. Djelouah[3], M. Saponari[1]

[1] *Consiglio Nazionale Delle Ricerche, Istituto per la Protezione Sostenibile delle Piante, Sede Secondaria di Bari, Italy*
[2] *Department of Soil, Plant, and Food Sciences University of Bari, Aldo Moro, Bari, Italy*
[3] *Mediterranean Agronomic Institute, CIHEAM Bari, Valenzano, Bari, Italy*

The western honey bee (*Apis mellifera* L.) is an important component of agricultural systems and play a crucial role in food production via crop pollination. It is commonly known that many factors threaten and have a role in pollinators' decline and induce colony losses of managed honey bees, of which pathogens and parasites are the main ones. Therefore, the early detection of these diseases could help in the management of the apiaries and prevent or limit the spread of these pathogens among colonies. On the other hand, although there are several different methods of detection based on PCR, the sensitivity of droplet digital PCR (ddPCR), a new generation of the traditional quantitative (qPCR), in measuring nucleic acids in a certain tissue has made it a technology of high interest. Unlike qPCR, which needs a standard calibration curve to determine different concentrations from the amplification rates, ddPCR yields absolute concentrations after cycling the tested sample to the endpoint, and thus it counts target nucleic acid molecules by enumerating positive droplets, allowing the determination of template copies/µl in each sample, making the process more accurate, faster and reproducible. Moreover, ddPCR is now used in many applications to detect pathogens such as bacteria, viruses, fungi and other causal agents of various diseases. Since few reports are available on the use of ddPCR in detecting viruses in honey bees. In the present work, we describe two-step RT-ddPCR for detecting and quantifying different viruses in honey bee samples collected in recent years from the Apulia region, South Italy. ddPCR was performed using the QX200 Droplet Digital PCR System (Bio-Rad, Hercules, CA). Reactions included 10 µL1× ddPCR Evagreen Supermix, 2 µL of cDNA, 0.6 µL forward and reverse primers of selected viruses in a final volume of 20 µL. Tested samples were then partitioned into thousands of droplets using the droplet generation oil and the droplet generator (BioRad). After performing PCR of each virus in 96-well plates, the fluorescence of the reaction was read (QX200 droplet reader, BioRad), and raw data were analysed with QuantaSoft Analysis Pro Software (v.1.0.596) to assign positive and negative droplets and provide absolute quantification of the target molecules. Results of ddPCR were compared with those generated from qPCR and thus allowed us to set up a qPCR cycle threshold (Ct), which could exclude false positives or questionable results. Even both methods are sensitive and give comparable results for identical samples, ddPCR proved to be an advanced technology, able to offer highly sensitive and absolute nucleic acid detection and quantification and can interpret qPCR values to diagnose viruses in honey bees.

PO 051

SARS-CoV-2 DIFFERENTIALLY DEREGULATES SERUM CYTO-/CHEMOKINES WITH SPECIFIC FUNCTIONS AT DIFFERENT TIME INTERVALS IN COVID-19 PATIENTS

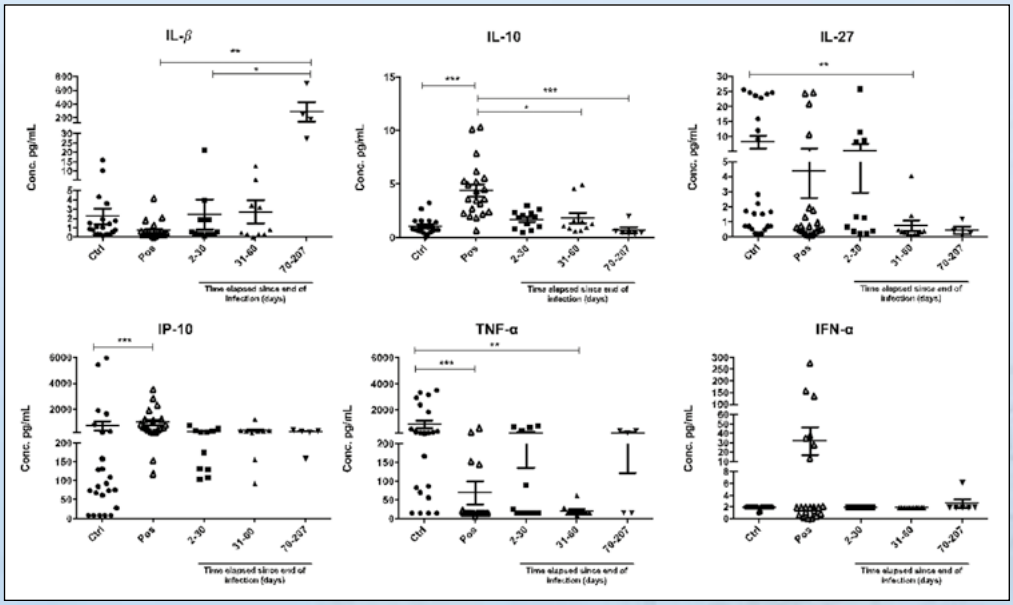
M. Iuliano[1], R. Mongiovì[1], A. Parente[2], L. Grimaldi[1], B. Kertusha[2], A. Carraro[2], R. Marocco[2], G. Mancarella[2], C. Del Borgo[2], M. Lichtner[2], G. Mangino[1], G. Romeo[1]

[1] *Dept. of Medico-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Italy*
[2] *Dept. of Public Health and Infectious Disease, S. Maria Goretti Hospital, Sapienza University of Rome, Italy*

Background: SARS-CoV-2 is the causative agent of Coronavirus disease 2019 (COVID-19), an inflammatory disorder that mainly affects the respiratory tract, also characterized by the dysregulation of cytokine levels (cytokine storm), both locally and systemically. Cytokine storm induces hyperinflammation by causing dysregulation of various inflammatory mediators such as interleukins (ILs), chemokines and factors regulating growth and differentiation. The cytokine storm constitutes also one of the main reasons behind the severe consequences of COVID-19, causing long-term effects on patient and tissue health, mostly due to the injuries elicited by the immune cells. Measuring serum levels of pro/anti-inflammatory cytokines could represent a viable resource in the management of COVID-19, ranging from risk assessment, monitoring of the disease progression and determination of the prognosis, to better select therapy and predict the response to the treatment. The aim of this study was to follow the level of a cluster of cyto-/chemokine in serum samples from recovered subjects, possibly predictive of the severity and/or the duration of the disease.

Methods: Serum samples from healthy (HD, n=10), positive (T0, n=29) and recovered subjects (T1, 31-60 or 70-200 days post negativization, n=18 and n=6, respectively), were collected and tested for 11 cyto-/chemokines through Super-X Cytokine Assay. Altered levels of selected cyto-/chemokines were, then, confirmed by ELISA on a group of 58 subjects.

Results: IL-10 is statistically increased in the positive group compared to the control group (pos: 4,40 pg/ml ±2,57 vs CTR: 1,06 pg/ml ± 0,77; p<0.001), as well as compared to groups 31-60 days (pos: 4,40 pg/ml ±2,57 vs 31-60 pg/ml days: 1,80±1,60; p <0.001) and 70-200 days from the end of the infection (pos: 4,40 pg/ml ±2,57 vs 70-200 days: 0,69 pg/



ml ± 0,65; p <0.05). IL-27 shows elevated concentrations in CTR which significantly decrease in 31-60 days (CTR: 8,62 pg/ml ±10,30 vs 31-60 days: 0,74 pg/ml ±1,16; p<0.01). IL-1β has a particular trend as it seems to maintain rather low concentrations in all the examined groups, significantly increasing in 70-200 days group (70-200 days: 289,08 pg/ml ± 290,58 vs 2-30 days: 2,45 pg/ml ±5,66; p<0.05) (70-200 days: 289,08 pg/ml ±290,58 vs pos: 0,73±1,00; p<0.01). TNF-α significantly decreased in the positive group compared to control (pos: 69,99 pg/ml ±141,77 vs CTR: 905,18 pg/ml ± 1276,56; p<0.001) as well as in 31-60 days group versus CTR (pos: 69,99 pg/ml ±141,77 vs 31-60 days: 21,05 pg/ml ±15,31; p<0.01). Finally, IP-10 significantly increased in the positive group compared to control (pos: 1022,06 pg/ml ±946,40 vs CTR: 762,42 pg/ml ±1641,71; <0.001).

Conclusion: Univariate and multivariate Cox regression analyses performed on all the obtained data indicated that IP-10 concentration can be associated to the severity of the COVID-19 disease. In addition, univariate regression reveals that both TNF-alfa increased release and IL-10 decrease secretion depend on disease severity. Interestingly, while the IP-10 increased serum levels in severe COVID-19 patients could represent an early marker of clinical worsening, the high IL-10 levels at the follow-up in patients with pneumonia, might be explained by the persistence of post-COVID19 long syndrome.

PO 052

PRELIMINARY SURVEY OF COMMON HONEY BEE (*APIS MELLIFERA* L.) VIRUSES IN APIARIES LOCATED IN APULIA REGION (SOUTHERN ITALY)

S.A.E. Zaidat[1,3], R.A. Kubaa[2], S. Sanzani[1], M. Saponari[2], K. Djelouah[3]

[1] *Department of Soil, Plant, and Food Sciences University of Bari, Aldo Moro, Bari, Italy*
[2] *Consiglio Nazionale elle Ricerche, Istituto per la Protezione Sostenibile Delle Piante, Sede Secondaria di Bari, Italy*
[3] *Mediterranean Agronomic Institute, CIHEAM Bari, Italy*

In recent decades, Colony Collapse Disorder (CCD) has been affecting bee colonies worldwide, posing a serious threat to Honey bee populations. This phenomenon is particularly concerning because of the crucial role of honey bee in pollinating crops and maintaining the balance of ecosystems. Several factors have been associated with CCD, including pesticide use (such as neonicotinoids), habitat destruction (the loss of wildflowers and other flowering plants), climate change, and spreading diseases and parasites. The most common parasites and diseases are the Varroa mite (*Varroa destructor*), *Nosema* fungi, and viruses which can weaken the bees' immune system, making them more susceptible to CCD. In addition, honey bee viruses, often vectored by the Varroa mite, are a significant threat to worldwide honey bee populations. In response to this problem, a preliminary study was conducted in Apulia region (southeast of Italy) to assess the presence of common honey bee pathogens in 30 apiaries during spring-summer 2022. The study aimed to monitor the presence of black queen cell virus (BQCV), deformed wing virus (DWV), *Nosema ceranae*, and less known or emerging pathogens in honey bees such as bee macula-like virus (BeeMLV), slow bee paralysis virus (SBPV), *Aspergillus flavus* and *Peanibacillus larvae*. About 300 samples were collected from 90 hives (3 samples/each to ensure a comprehensive assessment of the bee population), in which 10-15 adult bees were collected from young bees and foraging bees inside and outside the hive, respectively. In general, most colonies in the visited apiaries were asymptomatic; whereas in some cases few showed symptoms of paralysis, tumbling bees with weak movement. Other hives had dead bees in front of them hives, which could signify disease or other problems. The extraction of total nucleic acid (TNA) was performed and developed using different methods such as TRIZOL, CTAB, and Maxwell instruments (Promega). The quality and quantity of these methods were later compared and evaluated. All collected samples were assessed by reverse transcription (RT)-PCR and RT-qPCR using available universal primers for each selected virus. Our study revealed that BQCV, DWV, BeeMLV, and *N. ceranae* are widespread in the Apulia region, while *P. larvae* and *A. flavus* were not detected. Interestingly, many of the investigated hives had different degrees of co-infection, despite being asymptomatic. Furthermore, this study allowed for the first detection of SBPV in the Apulia region. This study provides valuable information on the prevalence of honey bee pathogens in the Apulia region and highlights the importance of monitoring and managing these diseases.

PO 053

A CASE OF ADULT-ONSET STILL'S DISEASE WITH PROBABLE COXSACKIE VIRUS INFECTION TRIGGER

A. Gallicchio[1], N.S. Moriello[1], G. Calabria[1], M. Catalano[1], G. Liorre[1], N. Farella[1], A. Franco[1], P. Bellopede[1], A. Giaccone[1], L. Maddaloni[1], M. Talamo[1], I. Gentile[2]

[1] Division of Infectious Diseases, Cotugno Hospital, AORN Ospedali dei Colli, Naples, Italy
[2] Division of Infectious Diseases, University of Naples Federico II, Naples, Italy

Introduction: Adult-onset Still's disease (AOSD) is a multisystem inflammatory disease of unknown etiology. The diagnosis of AOSD is a great challenge for clinicians. The Yamaguchi criteria are the most widely used diagnostic criteria and the suspicion of an infectious trigger is highly debated. We present a case of a patient diagnosed with Coxsackie virus infection and concomitant appearance of AOSD.

Clinical case: A 44-year-old previously healthy man presented with fever, chills, scratchy cough, relative bradycardia and unilateral eyelid ptosis. The inflammation indexes were increased with neutrophilic leukocytosis and hepatic cytolysis indexes altered. The autoimmune investigations were negative, haematological pathologies were excluded and there was no benefit to different empirical antibiotic therapies. The microbiological investigations were negative, only Coxsackie virus serology was positive, with demonstrated seroconversion of IgM and IgG. The clinical condition was suggestive for AOSD as he satisfied two major and three minor of Yamaguchi criteria, so a presumptive diagnosis was made. He then started corticosteroids with clinical improvement and was discharged on oral steroid therapy.

Discussion: The true etiopathogenesis of the AOSD is still unclear. In our case the high index of inflammation, the negativity of most laboratory tests and the response to steroid therapy supported an immunopathogenic mechanism. Yamauchi's diagnostic criteria sustained the diagnosis. Nevertheless there was evidence of Coxsackievirus infection. Although the temporal correlation between Coxsackie virus infection and the onset of AOSD could be casual, as well it has been already hypothesized, an infectious etiology could initiate a cascade of immunological insults which results in the clinical syndrome of AOSD.

Conclusion: Coxsackie virus infection could be a potential trigger for the development of AOSD. So it would be appropriate in cases of diagnosis of AOSD to investigate and research potential infectious causes/triggers. These data should be investigated with more diagnostic attention especially for infections in patients with AOSD and with further studies.

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PO 055

THE ENVELOPE PROTEIN OF USUTU VIRUS ATTENUATES WNV VIRULENCE IN MICE

L. Jurisic, G. Zaccaria, D. Malatesta, G. Di Teodoro, A. Lorusso
Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise, Teramo, Italy

Aim of the Study: Recent data indicate that West Nile virus (WNV) outbreaks have increased in number in Europe and that clinical neuroinvasive USUV infections in humans may have been underestimated. Virulence factors of USUV, unlike WNV, are still poorly investigated. In study we attempted to rescue by reverse genetics WNV, USUV and chimeric WNV/USUV viruses and to evaluate the virulence in mice.

Methods: We used the ISA (infectious-subgenomic-amplicons) reverse genetics method to rescue, from transfected BSR cells, the following viruses: recombinant wild type (r-wt) WNV and USUV viruses, and two chimeric viruses in which the E protein (r-$WNV_{E\text{-}USUV}$) of USUV and the 5'UTR of WNV (r-$USUV_{5'\ UTR\text{-}WNV}$) replaced those of WNV and USUV, respectively. Rescued viruses were tested by neutralization using anti-WNV and anti-USUV hyperimmune sera. Rescued viruses were administered intraperitoneally to 21 day-old competent CD1 mice in order to evaluate survival and quantitate viral RNA in the internal organs.

Results and Conclusions: r-wt WNV was successful rescued from transfected BSR cells as well as two chimeric viruses including r-WNVE-USUV and r-$USUV_{5'\ UTR\text{-}WNV}$; r-wt USUV was not rescued. r-$WNV_{E\text{-}USUV}$ was neutralized only by the USUV antiserum whereas r-wt WNV and r-$USUV_{5'\ UTR\text{-}WNV}$ were neutralized, as expected, by WNV and USUV antisera, respectively. wt USUV and r-$USUV_{5'\ UTR\text{-}WNV}$ did not cause clinical signs in infected mice; also, viral RNA was absent in the internal organs. On the other hand, wt and r-wt WNV caused severe and fatal disease (up to 100%) in mice starting from 6 dpi; high titres of viral RNA were present in all organs. Instead, r-$WNV_{E\text{-}USUV}$ had intermediate characteristics between WNV and USUV as only the 50% of mice died and lower viral RNA titres, with respect those observed in wt and r-wt WNV infected mice, were evidenced in the internal organs. The ISA reverse genetics system was successfully validated and the E protein of USUV was shown to attenuate virulence of WNV in mice.

PO 056

RNAemia IN PATIENTS DIAGNOSED WITH MILD-MODERATE COVID-19 IN COURSE OF TREATMENT WITH ANTI-SARS-CoV-2 MONOCLONAL ANTIBODIES

M.D. Marsiglia[1], F. Bai[2], E. Ottaviano[1], S. Ancona[1], T. Beringheli[2], F. Triva[1], M.S.F. Campagnoli[1], P. Pasutto[1], G. Marchetti[2], S. Bianchi[1], E. Borghi[1]

[1] Microbiology and Clinical Microbiology, Department of Health Sciences, ASST Santi Paolo e Carlo, University of Milan, Italy

[2] Clinic of Infectious Diseases, Department of Health Sciences, ASST Santi Paolo e Carlo, University of Milan, Italy

Aim of the Study: Several anti-SARS-CoV-2 neutralizing monoclonal antibodies (mAbs) have been developed. They have shown efficacy and good tolerability in non-hospitalized patients with mild-to-moderate COVID-19. Notably, different studies demonstrated that mAbs treatment reduces COVID-19-related hospitalizations and deaths and promotes virological clearance. SARS-CoV-2 RNA in blood, also known as RNAemia, has been reported but data about its clinical implications are scarce, although its presence is usually associated with severe disease. We aimed at understanding the kinetics of the nasopharyngeal viral load and SARS-CoV-2 RNAemia in relation with clinical outcomes in patients treated with mAbs.

Methods: From March 2021 to May 2022, high-risk patients with mild-moderate COVID-19 were recruited from the Clinic of Infectious Diseases of the San Paolo Hospital (Milan). High-risk patients were defined according to AIFA indications for treatment with mAbs, i.e. advanced age, obesity, chronic diseases, and immunodeficiencies. Patients at an early stage of illness (≤10 days from Symptoms onset) were treated with bamlanivimab, bamlanivimab/etesevimab, casirivimab/imdevimab or sotrovimab depending on the current variant circulation. We collected nasopharyngeal swabs (NPS) and plasma samples from enrolled patients at t0 (mAbs infusion) and at t1 (7 days after the treatment). SARS CoV-2 viral load was measured on NPS and plasma by quantitative RT-PCR. Mann-Whitney and Wilcoxon tests were used for statistical analyses.

Results and Conclusions: 174 patients were enrolled in the study period. 137 out of 163 patients (84.0%) reached clinical recovery in a median time of 12 days (IQR 10-20). Twenty patients (12.3%) were hospitalized and 6 (3.7%) died. 11 patients were lost at the follow-up. Viral load significantly decreased from t0 to t1 in both NPS ($p<0.0001$) and plasma ($p<0.0001$). A higher viral load in NPS at t0 was observed in patients presenting RNAemia (62.6%) compared with patients without RNAemia (37.4%) ($p=0.0016$). After the treatment (t1), viral load in NPS decreased in both groups ($p<0.0001$) without any statistical difference ($p=0.1029$). Patients presenting RNAemia (t0) registered a median time of clinical recovery of 12 days (IQR 10-20) while the other group registered a time of 14 days (IQR 10-20) ($p=0.5511$).
Our real-life data confirmed that anti-SARS-CoV-2 mAbs can be safely administered in outpatient settings and are effective in reducing viral load in nasopharyngeal swabs and plasma. Despite the presence of RNAemia, early treatment with mAbs slowed down the disease progression and avoided a severe outcome in most subjects.

PO 057

FIFTY YEARS AFTER THE FIRST IDENTIFICATION OF TOSCANA VIRUS IN ITALY: GENOMIC CHARACTERIZATION OF VIRAL ISOLATES WITHIN A GENOTYPE AND AMINOACIDIC MARKERS OF EVOLUTION

G. Marsili[1], C. Pallotto[2,3], C. Fortuna[1], A. Amendola[1], C. Fiorentini[1], S. Esperti[2,4], P. Blanc[2,5], L.R. Suardi[2,6], G. Venturi[1], C. Argentini[1]

[1] *Dipartimento di Malattie Infettive, Istituto Superiore di Sanità, Rome, Italy*
[2] *SOC Malattie Infettive 1, Azienda USL Toscana Centro, Bagno a Ripoli, Florence, Italy*
[3] *Clinica delle Malattie Infettive, Azienda Ospedaliera Santa Maria della Misericordia, Università di Perugia, Italy*
[4] *Dipartimento di Malattie Infettive, Azienda Ospedaliero-Universitaria di Modena, Policlinico di Modena, Università di Modena e Reggio Emilia, Modena, Italy*
[5] *SOC Malattie Infettive 2, Azienda USL Toscana Centro, Pistoia, Italy*
[6] *UO Malattie Infettive, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy*

Aim of the Study: Toscana Virus was firstly described in our laboratory of Istituto Superiore di Sanità by Paola Verani and her group fifty years ago (10.1016/j.cmi.2019.12.015 The aim of the study was the characterisation of 3 new viral isolates from cerebrospinal fluid samples of patients diagnosed with TOSV infection in July 2020 in Tuscany region. We also aimed to preliminarly assess bioinformatic tools for the investigation for markers potentially involved in new host adaptation or pathogensis.

Methods: An amplicon of 839 nt of the G1 gene in M segment was obtained by RT-PCR plus nested PCR from the three viral isolates and sequenced by Sanger method. Sequences were compared with all M segment sequences available till december 2022. We have further analysed the aminoacidic sequences translated from nucleotide sequences. An analysis by mean of the bioinformatic package Gemme (https://doi.org/10.1093/molbev/msz179) was performed, to investigate the effects of mutations on protein functions by means of a reiterate structure based phytogenic analyses.

Results: The 3 isolates belong to genotype A. The phylogenesis shows tree main clusters. The 2020 isolate ISS-2020-L1507/V2 clusters together with clinical isolates from Tuscany and Umbria regions characterised between 1983 and 2007. The isolates ISS-2020-L1509/V2 and ISS-2020-L1503/V2 cluster together with older clinical isolates from Tuscany region (1962-1984), with Phlebotomine vectors- derived isolates and a bat-derived isolate from Tuscany region, and with some clinical isolates derived from Sicily, Emilia Romagna, Marche and Tuscany regions between 1993 and 2013. The third cluster contains the prototype virus ISS-Phl3 (X89628) and isolates mainly from human specimens from Tuscany, and Phlebotomine vectors-derived isolates from Tunisia between 1971 and 2014. A putative forth cluster contains only an isolate collected in Algeria in 2013. We have identified aminoacids which we hypothesize have been conserved during the evolution and that are able to be identified 9 diverse strains among all TOS A sequences analysed. Furthermore, the analysis by Gemme has identified 27 aminoacidic residues in this protein fragment with a potential role in adaptation and pathogenesis.The possible implications of this results will be discussed.

PO 058

ZIKA VIRUS NS4A ABOLISHES CYTOPATHIC EFFECT IN OVEREXPRESSING CELLS AND INCREASES COXSACKIE VIRUS REPLICATION

S. Nottoli[1,2], G. Lottini[1,3], M. Sidoti[1,2], E. Plicanti[1,3], M. Matteucci[1], M. Pistello[1,2], G. Freer[1]

[1] *CentroRetrovirus, Department of Translational Research, University of Pisa, Italy*
[2] *Virology Operative Unit, Pisa University Hospital, Pisa, Italy*
[3] *Department of Medical Biotechnologies, University of Siena, Italy*

Aim of the Study: Zika virus (ZIKV) is a member of *Flavivirus* genus of the *Flaviviridae* family. It is a positive sense single-stranded RNA virus. ZIKV is transmitted by Aedes mosquito bites, sexually among humans or vertically from mother to fetus. It was first isolated in 1957 in Uganda from a primate in Zika forest but in 2015, during a ZIKV epidemic in Brazil, it came to public attention because of its association to significant increase in microcephaly cases. Despite considerable efforts to understand the impact of ZIKV on fetal brain development, the mechanism that correlates infection and subsequent congenital cortical abnormalities are still unknown.
ZIKV RNA genome encodes three structural and seven nonstructural proteins that are involved in viral replication, organization and evasion from host immunity. Among them, it was described that NS4A is integrated into the endoplasmic reticulum membrane and is a component of the viral replication complex. The aim of this study is to investigate the role of this protein, infecting A549 and HuH7 cells stably expressing NS4A with ZIKV and evaluate its role in infection.

Methods used: A549 and HuH7 cells were transduced with pseudotyped lentiviral vectors carrying *NS4A-GFP*, and, as control *GFP* genes, to evaluate the activity of these proteins separately from the others expressed during natural infection with ZIKV. Stably expressing NS4A cell lines were clonally selected. To assess the effect of ZIKV, Usutu virus (USUV) and Coxsackie B5 (CoxB5) infection on these cells, have been performed both a direct assay to the cells themselves and a measurement of viral yield in the supernatant of different cell lines following infection.

Results and Conclusions: Because, after ZIKV infection, NS4A-expressing stable cell lines did not show a clear cytopathic effect, that instead was seen in wild type and GFP expressing cell lines, we hypothesize that there could be a sort of protective effect following infection in cells expressing the ZIKV protein. The viral yield, although slightly reduced in recombinant lines, was comparable. Next, we investigated whether the protective effect observed also occurred during infection with USUV, that belongs to *Flaviviridae* family too. In this case cells overexpressing NS4A were not protected from the lytic effect induced by USUV. Instead, CoxB5 replication was increased. Further experiments were performed to shed light into these differences.

PO 059 - OC 10

CLINICAL UTILITY OF GENOTYPIC DRUG-RESISTANCE TEST IN IMMUNOCOMPROMIZED PATIENTS WITH CYTOMEGALOVIRUS REFRACTORY INFECTION

V. Motta[1], G. Piccirilli[2], E.C. Borgatti[1], L. Gabrielli[2], S. Venturoli[2], E. Petrisli[2], S. Felici[2], M. Leone[1], M. Franceschiello[2], M.C. Morelli[3], F. Bonifazi[4], G. Comai[5], L. Potena[6], T. Lazzarotto[1,2]

[1] *Microbiology Unit, DIMEC, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Italy*
[2] *Microbiology Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Italy*
[3] *Internal Medicine Unit for the treatment of Severe Organ Failure, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Italy*
[4] *Istituto di Ematologia "Seràgnoli" IRCCS Azienda Ospedaliero-Universitaria di Bologna, Italy*
[5] *Nephrology, Dialysis and Renal Transplant Unit, IRCCS-Azienda Ospedaliero-Universitaria di Bologna, Italy*
[6] *Heart failure and Ttransplant Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Italy*

Aim: The study evaluates the clinical utility of genotypic test for cytomegalovirus (CMV) drug resistance (DR) in immunocompromised patients with CMV refractory infection.

Methods: From 2013 to date, 79 samples (77 whole blood-WB, 1 cerebrospinal fluid-CSF, 1 bronchial aspirate-BAS) were tested. The samples were related to 78 episodes of refractory CMV infection occurred in 55 patients: 31 recipients of hematopoietic stem cell transplant (HSCT), 22 solid organ transplant (SOT), 1 with autoimmune disease (AD) and 1 with acquired immune deficiency syndrome (AIDS). The UL97, UL54, UL56, UL89 and UL51 viral genes were analyzed by Sanger sequencing to identify valganciclovir/ganciclovir (GCV), foscarnet (FOS), cidofovir (CDV), maribavir (MBV) and letermovir (LTV) resistance-associated mutations (r).

Results: CMV-DR mutations were detected in 29/78 (37.2%) episodes (21 patients: 11 CSE, 10 SOT, 1 AIDS). For 22/29 (75.8%) cases, single or multiple DR mutations on UL97 gene were found: 21 associated with GCVr (n=9 L595S, n=7 A594V, n=2 M460V, n=1 K359E, n=1 M460V with C603R and n=1 M460I and L595S) and 1 associated with MBVr (T409M). Therapy was switched to FOS (n=19), CDV (n=1) or LTV (n=1). Only one patient developed CMV myocarditis. Mutations associated to multidrug resistance (MDR) were detected in 4/29 (13.8%) episodes: A594V or C603W (UL97) with P522S (UL54) associated with GCVr and CDVr, A594P (UL97) with V715M (UL54) associated with GCVr and FOSr and M460V (UL97) with Q578H (UL54) associated with GCVr, CDVr and FOSr. Treatment was shifted to FOS (n=2), MBV (n=1) and anti-CMV Ig with leflunomide (n=1). One case developed CMV retinitis. Finally, single mutations (C325Y, R369S and C325F) in UL56 gene associated with LTVr were detected in 3/29 (10.3%) cases receiving LTV off-label treatment, that was switched to GCV. Of note, among these 29 episodes, two cases of compartmentalized CMV-DR infection were documented: 1 AIDS patient with CMV-encephalitis and 1 lung transplant recipient with respiratory signs. The first patient presented a viral load (VL) of 132,975 and 400,044 copies/ml on WB and CSF, respectively, when GCV treatment was started. After 20 days, a viral clearance was reached only on WB. CMV-DR test performed on CSF (VL=186,406 copies/ml) identified mutations associated with GCVr (M460V and C603R, UL97). The second case presented a primary CMV-infection (232,938 copies/ml on WB and 204,211 on BAS) when started GCV treatment. After 62 days, M460I and L595S mutations (UL97) associated with GCVr were found on BAS and only L595S mutation was found on WB. In both cases the treatment was switched to FOS. In the remaining 49/78 (62.8%) episodes (33 patients: 20 HSCT, 12 SOT, 1 with GPA) no CMV-DR mutations (clinical resistance) were identified and in 48.1% of cases the therapy was remodulated without the need for second line therapy. Among them, 2 (6%) developed CMV-related symptoms: chorioretinitis, gastroenteritis.

Conclusions: The results showed *i)* the clinical utility of CMV-DR genotypic in the choice of appropriate antiviral therapy limiting the use of drugs with high toxicity *ii)* the relevance to test representative samples in cases of compartmentalized CMV drug resistant strains.

PO 060 - OC 11

ANALYSIS OF HUMAN PAPILLOMAVIRUS (HPV) GENOTYPES DETECTION IN CERVICAL, VAGINAL AND URINE SAMPLES

M. Martinelli[1], C. Giubbi[1], M.L. Di Meo[2], R.C. Njoku[1,3], F. Perdoni[1], R. Musumeci[1], F. Landoni[1,2], R. Fruscio[1,2], C.E. Cocuzza[1]

[1] *Department of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy*
[2] *San Gerardo Hospital, ASST Monza, Monza, Italy*
[3] *Department of Biomedical Science, University of Sassari, Italy*

Aim of the Study: Human Papillomavirus (HPV) testing offers the possibility to introduce self-sampling as a valid well-accepted alternative to improve screening coverage rates. This study investigated the accuracy of HPV testing on urine and vaginal self-samples as compared to physician-collected cervical swabs (gold standard) and evaluated the HPV genotypes distributions in different sample types.

Methods used: Clinician administered cervical, self-collected vaginal (FloqSwab, Copan) and urine (Collipee, Novosanis) samples, were collected from women referred to colposcopy for a recent cervical dysplasia. All samples were tested using Anyplex™II HPV28 (Seegene) able to identify 14 hr-HPV (hrHPV, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) and 14 low-risk HPV types (lrHPV, 6, 11, 26, 40, 42, 43, 44, 53, 54, 61, 69, 70, 73, and 82) with two different Real-Time PCR reactions performed on CFX96 (Bio-Rad). Agreement of HPV typing results between different sample types was evaluated with the Cohen's kappa (κ).

Results and Conclusions: Self-collected vaginal, urine and clinician administered cervical samples, were presently collected from 245 women referred to colposcopy. 65.7% (161/ 245), 69.4% (170/245) and 77.1% (189/245) of women's cervical, vaginal and urine samples resulted hrHPV positive, respectively. HPV16 and HPV31 were found to be the most frequently detected hrHPV types, while HPV53 and HPV42 were the most commonly detected lrHPV types (Figure 1). Good overall agreement for hrHPV detection between cervical and self-taken samples was found (k=0.898 and k=0.792 for vaginal and urine specimens, respectively). A slightly inferior agreement was found for lrHPV detection (vaginal self-sample: 88.16%, k=0.762; urine: 85.71%, k=0.715).

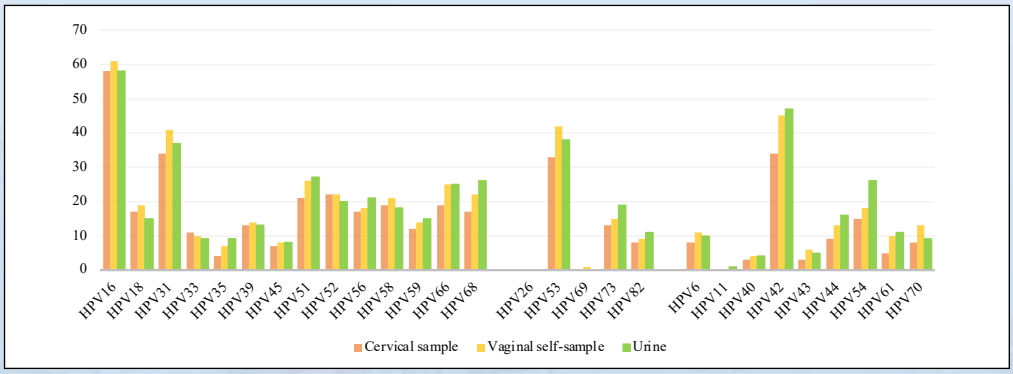


Figure 1

Self-collected specimens were demonstrated as accurate as clinician collected swabs for the detection of hrHPV types. The slightly inferior agreement between cervical and self-collected samples for lrHPV detection may reflect a different distribution and/or prevalence of infection of lrHPV genotypes within different sites of the urogenital tract. These data support the introduction of self-sampling in cervical cancer screening programs to improve women's participation and to achieve by 2030 the goal proposed by Word Health Organization (WHO) to eradicate cervical cancer as public health problem.

PO 061 - OC 12

DETECTION OF SARS-CoV-2 EXPLOITING PLASMONIC PLASTIC OPTICAL FIBER PROBES COMBINED WITH MOLECULARLY IMPRINTED POLYMERS

R. Fioravanti[1], S. Gioacchini[1], G. D'Agostino[2], C. Perri[2], F. Arcadio[3], M. Baggieri[1], A. Marchi[1], P. Bucci[1], R. Giuseppetti[1], E. D'Ugo[1], G. Porto[2], L. Zeni[3], N. Cennamo[3], F. Magurano[1]

[1] *Department of Infectious Diseases, Istituto Superiore di Sanità (ISS), Rome, Italy*
[2] *Moresense Srl, Filarete Foundation, Milan, Italy*
[3] *Department of Engineering, University of Campania Luigi Vanvitelli, Aversa, Caserta, Italy*

Aims of the Study: Since its outbreak in 2019, SARS CoV-2 has spread through the world's population, causing a pandemic, focusing the scientific society on its biology and pathogenesis to stem the virus propagation. Meanwhile, the same efforts have been spent in developing rapid detection methods to limit virus circulation among the population. Because of its pathogenesis, it is possible to detect SARS-CoV-2 markers in biological fluids such as *sputum*, bronchial lavage, and saliva, thus increasing the chance of its detection by less invasive methods than other viruses. This feature, coupled with the requirement to test large amounts of samples rapidly, has prompted scientific research to develop several devices to support or replace standard assays (i.e., RT-PCR, ELISA), which require expensive equipment and infrastructures, systematic quality control and experienced personnel, in circumstances where a point-of-care test is demanded. The most widely used point-of- care device so far, the lateral flow test, is based on antigenic recognition in a manner like ELISA but with the edge of portability, user-friendly and low cost. On the other hand, the main drawbacks are moderate sensibility and the lack of response quantification, leading to the frequent occurrence of false responses. Therefore, the development of fast and cheap point-of-care tests (POCT) with high sensibility, fast responses, and the ability to provide quantitative analysis focusing on the detection method is still in progress.

Methods used: One of the methods is to employ the surface plasmon resonance (SPR) of gold nanofilm combined with plastic optical fibers (POFs) to obtain ultra-high sensitivity, useful to realize different biosensors by a wide range of SPR-chip functionalization processes. Here, we present experimental results carried out exploiting an SPR-POF platform combined with a molecularly imprinted polymer specific for the Sars-Cov-2 spike protein [Cennamo N. et al. Proof of Concept for a Quick and Highly Sensitive On-Site Detection of SARS-CoV-2 by Plasmonic Optical Fibers and Molecularly Imprinted Polymers. Sensors 2021, 21, 1681]. Tests were carried on biological fluids and commonly used transport and storage media to develop a robust analysis protocol.

Results and Conclusions: Besides the protocol implementation, experimental tests are focused on the recognition of SARS-CoV-2 strains with their limit of detections compared to RT-PCR viral titration. The obtained results show that the device could be a powerful POCT for the detection of SARS-CoV-2 in a real scenario.

PO 062 - OC 13

HIV-1 DNA RESISTANCE ASSESSMENT THROUGH NGS IN HIGHLY TREATMENT-EXPERIENCED INDIVIDUALS UNDER VIROLOGICAL CONTROL ENROLLED IN THE PRESTIGIO REGISTRY

D. Armenia[1], V. Spagnuolo[2], M.C. Bellocchi[3], L. Galli[2], L. Duca[3], G. Marchegiani[3], T. Clemente[2], L. Carioti[3], R. Lolatto[2], L. Calza[4], B.M. Celesia[5], A. Cascio[6], D. Francisci[7], A. Saracino[8], C. Torti[9], M. Zazzi[10], A. Castagna[2], M.M. Santoro[3], on behalf of the PRESTIGIO Registry

[1] *Saint Camillus International University of Health Sciences, Rome, Italy*
[2] *Clinic of Infectious Diseases, Istituto Scientifico San Raffaele, Milan, Italy*
[3] *Department of Experimental Medicine, University of Rome "Tor Vergata", Rome, Italy*
[4] *Policlinico Sant'Orsola-Malpighi, Bologna, Italy*
[5] *Unit of Infectious Diseases, Garibaldi Hospital, Catania, Italy*
[6] *Infectious and Tropical Diseases Unit - Department of Health Promotion, Maternal and Infant Care, Internal Medicine and Medical Specialties, University of Palermo, Italy*
[7] *Clinic of Infectious Diseases, Department of Medicine and Surgery, University of Perugia, Italy*
[8] *Clinic of Infectious Diseases, University of Bari, Italy*
[9] *Department of Medical and Surgical Sciences, University "Magna Graecia", Catanzaro, Italy*
[10] *Department of Medical Biotechnologies, University of Siena, Italy*

Aim: This study aimed to clarify whether NGS might be useful for resistance assessment in virologically suppressed highly treatment-experienced (HTE) individuals with multidrug resistance (MDR).

Methods: Ninety-one HTE MDR individuals from the PRESTIGIO registry were analysed. HIV-1 DNA PR/RT/IN were obtained through NGS on MiSeq platform. Major resistance mutations (MRM) and APOBEC editing estimation (APOBEC mutations [APO-M]; stop codons) were evaluated through HIVdb algorithm. NGS cut-offs at ≥1%, ≥5% and ≥20% were tested. Minority MRM with frequency ranging 1-5% (mV1%) and 5-20% (mV5%) and majority MRM (frequency ≥20%, mV20%) were compared to historical-GRT. Variants distribution was compared between individuals who experienced virological rebound after NGS-GRT and those who maintained virological control.

Results: At NGS-GRT, individuals had been virologically suppressed since 3 (2-5) years and had a total HIV-DNA of 2,377 (1,274-4,949) copies/10^6 CD4+ cells. A total of 1,772 MRM were detected, of whom 361 (20.4%) exclusively by historical-GRT, 875 (49.4%) by both NGS- and historical-GRT, and 536 (30.2%) exclusively by NGS. The detection rate of historical MRM by NGS was 70.8%, 67.2% and 59.3% at 1%, 5% and 20% NGS cut-off, respectively. NGS set at 1% showed poor reliability, although associated with the highest detection rate of historical MRM. In fact, mV1% (N=337) were frequently detected in samples with stop codons (94.4%) or APO-M (97.4%) providing potential misleading resistance assessment. Differently, among mV5% (N=370), a substantial proportion of cases was not affected by APOBEC editing and contributed in expanding detection of historical MRM (25.9%) or detecting new MRM (18.6%). Regarding majority variants, mV20% (N=704) were marginally detected in samples with stop codons (2.9%) or APO-M (5.3%), and mostly contributed to detect (69.4%) historical MRM or detect new MRM (25.4%).
After NGS-GRT, 21 individuals underwent virological rebound with a median (IQR) viremia of 365 (98-7,840) copies/mL. Among them, the median (IQR) number of mV5% detected exclusively by NGS-GRT was higher (2 [1-3]) compared to those who maintained virological control (1 [0-2], p=0.030 by Mann-Whitney test). No significant differences in the number of mV1% and MV20% were observed. The number of mV5% newly detected by NGS in failing individuals positively correlated with plasma HIV-RNA levels detected at virological rebound (Spearman test, Rho=0.474, P=0.030).

Conclusions: In HTE MDR virologically suppressed individuals, NGS-GRT on HIV-1 DNA allows detection of around 60-70% historical MRM and detects considerable new resistance. Our results confirm that setting NGS at 5% might be a good choice to obtain reliable sequence data. At this setting, an increased number of minority species correlates with loss of virological control and with viremia levels at virological rebound.

PO 063 - OC 14

BODY FLUIDS BIOMARKERS FOR HPV-RELATED HEAD AND NECK CANCER DIAGNOSIS

L. Galati[1], M. Tagliabue[2], T. Gheit[3], S. Mackay-Chopin[3], R. De Bernardis[2], M.L. Tornesello[4], G. Blandino[5], G. De Palma[6], S. Vecchio[7], F. Maffini[1], A.V. Paradiso[6], M. Ansarin[2], M. Tommasino[6]*, S. Chiocca[1]

[1] *Department of Experimental Oncology, IEO, European Institute of Oncology IRCCS, Milan, Italy*
[2] *Division of Otolaryngology and Head and Neck Surgery, IEO, European Institute of Oncology IRCCS, Milan, Italy*
[3] *International Agency for Research on Cancer (IARC), Lyon, France*
[4] *Molecular Biology and Viral Oncology Unit, IRCCS Istituto Nazionale dei Tumori "Fondazione G. Pascale", Naples, Italy*
[5] *UOSD Oncogenomica ed Epigenetica, IRCCS Istituti Fisioterapici Ospitalieri, Rome, Italy*
[6] *IRCCS "Giovanni Paolo II" Cancer Institute, Bari, Italy*
[7] *IRCCS Ospedale Policlinico San Martino, Genova, Italy*
* *Deceased*

Aim of the Study: Head and neck cancer (HNC) groups several malignancies, mostly head and neck squamous cell carcinomas (HNSCCs), arising in the oral cavity, oropharynx, larynx, and hypopharynx. HNCs have been clearly associated with risk factors such as tobacco, alcohol and human papillomavirus (HPV) infections. Differently from cervical cancer, no screening programs and diagnostic algorithms have been identified yet for HPV-related HNCs. Thus, identification of HPV biomarkers that could improve early diagnosis for HPV-driven HNCs are needed. This study is a proof of concept for a non-invasive or minimally-invasive molecular-based tool for early diagnosis of HPV-related HNCs. We aimed to evaluate whether the presence of HPV biomarkers (e.g. ctHPV DNA, oral HPV DNA) in body fluids (e.g. saliva/plasma) could mirror HPV positivity in HNC tissues.

Methods: For each patient we analyzed
1) plasma,
2) oral exfoliated cells by oral rinse and gargling and
3) Formalin-fixed paraffin-embedded (FFPE) tissue sections.
To determine the presence of viral DNA we used a well validated HPV genotyping assay (IARC-Luminex platform) able to detect 21 HPV genotypes. HPV DNA prevalence was estimated as the proportion of samples that tested positive for any HPV DNA genotype with corresponding binomial 95% confidence intervals (CIs). The Cohen's kappa coefficient was calculated to estimate the HPV16 concordance between body fluids biomarkers.

Results and Conclusion: HPV16 DNA was the prevalent genotype in all analyzed samples, being prevalent in oral gargles (13.6%), ctHPV DNA (11.3%) and oral swabs (5%), respectively. The agreement between both oral gargle and plasma biomarkers for HPV16 DNA detection was 94.6% (k=0.77; 95% CI, 60.0-93.3) which corresponds to a substantial agreement. By anatomical site, HPV16 DNA was mainly detected in oral gargles (10.7%) and plasma (9.9%) then in oral swabs (4.3%) from oropharyngeal squamous cell carcinomas (OPSCCs).
In conclusion, HPV16 DNA detection in body fluids resulted as a good biomarker in OPSCCs and more sensitive for oral gargles than oral swabs. Importantly, it showed improved sensitivity in early T stage HPV16-related OPSCCs when used in combination with ctHPV16 DNA, underscoring its potential as an early diagnostic tool.

PO 064 - OC 15

HCV DETECTION OF ACTIVE HCV INFECTION WITH ELECSYS HCV DUO TEST (ROCHE DIAGNOSTICS)

S. Brusa, F. Passeggio, M. Baldi, G. D'ambrosio, L. Stanziola, C. Polito, A. Saviano, D. Terracciano, G. Portella

Department of Translational Medical Sciences, University of Naples Federico II, Naples, Italy

Aim: Hepatitis C virus (HCV) is a major cause of chronic liver disease. The morbidity of HCV infection remains a serious global health issue with about 130-150 million people suffering from chronic HCV. The availability of effective therapies has significantly reduced the number of infected subjects, but many active infections are still not properly detected and treated. For this reason, campaigns have been launched in many Italian regions for the emergence of these cases. As part of the campaign promoted by the Campania Region at the AOU Federico II was activated a project for the emergence of active cases and their subsequent framing and treatment.

Methods: From 01/11/22 to 31/12/22 patients positive for total anti HCV antibodies with an s/co ratio >11 to the ADVIA Centaur HCV test (Siemens Healthneers) were selected and further analyzed with an Elecsys HCV DUO test (Roche Diagnostics), which simultaneously detects both HCV antigens and anti-HCV antibodies. 86 patients were selected because they had an s/c ratio of >11 and tested with Elecsys HCV DUO tests. 58 patients tested positive for IgTotali-anti HCV with a ratio <11 were also included.

Results and Conclusions: The 86 patients confirmed positive antibody with Elecsys HCV DUO and 17 samples (19.77%) were positive for HCV antigens, with a peak in the over 80 age group of 38.89. Of the 58 patients positive for Total Ig -anti HCV with s/co <11 none tested positive for HCV antigens.

The use of a test for HCV antigens in patients with a high antibody titre is useful to discriminate against active HCV infection from a past history and is a valuable support in detecting submerged cases and for proper treatment.

PO 065

USE OF EASYSEQ™ SARS-CoV-2 WGS LIBRARY PREP KIT AS A RAPID TOOL FOR VARIANTS CALLING: APPLICATION IN COVID PATIENS FROM VALLE D'AOSTA

D. Mugetti[1], A. Arillo[1], M.G. Maniaci[1], S. Nodari[1], M. Di Benedetto[2], P. Falcone[2], P.L. Acutis[1], E. Bozzetta[1], S. Peletto[1]

[1] *Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy*
[2] *Ospedale Umberto Parini, Aosta, Italy*

Information regarding the SARS-CoV-2 variants were of fundamental importance during the pandemic to understand the virus transmission and for the development of vaccines. In this perspective, Next Generation Sequencing (NGS) technologies are the only tools available for Whole Genome Sequencing (WGS) analysis. Several generic or specific commercial kits have been developed for SARS-CoV-2 virus variants calling. Here we describe the application in COVID patiens from the Umberto Parini Hospital of Aosta (Italy) of the EasySeq™ SARS-CoV-2 WGS Library Prep Kit (Nimagen) for diagnostics and research purposes.

We started from nasopharyngeal swabs positive to a commercial real-time rt-PCR with target E-gene (Sarbecovirus), N-gene (SARS-CoV-2) and RdRp-gene (SARS-CoV-2). Samples with ct<30 were sent to the Istituto Zooprofilattico Sperimentale di Piemonte, Liguria e Valle d'Aosta for WGS analysis, which was performed with a 2x250 paired-end sequencing on a MiSeq™ System (Illumina) with MiSeq Reagent Kit v3 (Illumina). Analysis of fastq were performed with CLC Genomics Workbench (QIAGEN).

A total of 141 samples were analysed in the period 2021-2022: specifically, 96 patients were sampled in 2021, while the remaining 45 in 2022. Sequence analysis and variant calling generated the following results: Omicron (samples: 49), Delta (38), B.1.1.7 (36), B.1.160 (8), B.1.177 (4), P.1 (2), B.1.1.8 (1), B.1.525 (1) and Q.4 (1). Specifically considering the Omicron variant, the presence of different lineages was highlighted, in particular B.1.1.529 (12), BA.2 (7), BA.1.1 (5), BA.5.1 (5), BA.5.2.1 (3), BA.2.3 (2), BA.5 (2), BF.5 (2), BA.1 (1), BA.2.9 (1), BA.5.1.10 (1), BA.5.1.18 (1), BA.5.1.23 (1), BA.5.2 (1), BA.5.3.1 (1), BE.1.1 (1), BF.7 (1), BQ.1.1.1 (1), BQ.1.13 (1), B.1.528.3 (1). Also, for the Delta variant it was possible to highlight the presence of different lineages: B.1.617.2 (16), AY.43 (10), AY.5 (6), AY.42 (3), AY.4.2.1 (2), AY.121 (1).

Considering the variants circulating in Valle d'Aosta in relation to the study period, it was possible to observe that until June 2021 the most circulating variant was the Alpha variant B.1.1.7 (32, p=65,3%), followed by B.1.160 (8, p=16,4%), B.1.177 (4, p=8,2%), Gamma P.1 (2, p=4,1%), Eta B.1.525 (1, p=2,0%), B.1.1.8 (1, p=2,0%) and B.1.258.3 (1, p=2,0%). With the onset of the Delta variant starting from July 2021 there has been a progressive decline in cases of the Alpha variant, with the last case found in December 2021. Concurrently with this, the first cases of the Omicron variant were encountered. Therefore, variants prevalence in detail for this period (July-December 2021) were Delta B.1.617.2 (16, p=34,0%), Delta AY.43 (10, p=21,3%), Delta AY.5 (6, p=12,8%), Omicron (6, p=12,8%), Alpha B.1.1.7 (4, p=8,5%), Delta AY.42 (3, p=6,4%), Delta AY.4.2.1 (1, p=2,1%), Alpha Q.4 (1, p=2,1%). As regards the year 2022, the progressive disappearance of all the variants has been witnessed in favour of the Omicron variant with its different lineages. In fact, the last cases of the Delta variant were found in January 2022, specifically with lineages AY.4.2.1 and AY.121 with one case each.

No sampling was carried out for the year 2023 as no cases were found during the flash surveys organized by the Istituto Superiore di Sanità (ISS). Despite this, national data confirm the trend found in the analysed territory, only with the Omicron variant currently circulating.

PO 066

SARS-CoV-2, INFLUENZA A/B AND RESPIRATORY SYNCYTIAL VIRUSES RAPID DIFFERENTIAL DIAGNOSIS IN PEDIATRIC PATIENTS

M.T. Della Rocca, V. Panetta, A. Durante, F. Merola, G. Tucci, S. Labella, A. Marino, R. Greco
UOSD Microbiology - AORN Sant 'Anna and San Sebastiano, Caserta, Italy

Aim of the Study: Respiratory viral infections are a leading cause of pediatric disease. The rapid identification of pathogens, epidemiologic tracing, description of symptoms, and development of preventive and therapeutic measures are crucial to limiting the spread of these viruses. Some pathogens have a geographic focus that providers be aware of all emerging diseases in order to recognize outbreaks and diagnose and treat patients. This study aimed to compare two molecular rapid tests, M10 Flu/RSV/SARS-CoV-2 and BioFire Resiratory Panel in upper respiratory specimens' pediatric population.

Methods: M10 is an all-in-one cartridge-based ready-to-use multiplex RT-PCR kit intended for the qualitative detection of influenza A, influenza B, RSV and SARS-CoV-2 RNAs. The time-to-result is 60 min. Testing was performed on the STANDARD M10 analyzer (SD Biosensor Inc., Seoul, Korea) and BioFire Respiratory Panel 2.1 plus (RP2) (BioFire, Salt Lake City, Utah,USA) according to the manufacturer's instructions. RP2 panel included respiratory pathogens: Adenovirus, Coronaviruses 229E, HKU1, 299 NL63, OC43, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), Severe Acute 300 Respiratory Coronavirus 2 (SARS-CoV-2), Human Metapneumovirus, Human 301 Rhinovirus/Enterovirus, Influenza A, Influenza B, Respiratory syncytial virus (RSV), parainfluenza virus 1, 2, 3, 4. Bacterial detection revealed the eventual presence of *Bordetella parapertussis*, *Bordetella pertussis*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*.

Results and Conclusions: 50 samples were tested in both M10 and RP2. Of these, 32 samples had negative results. The overall agreement between the M10 and RP2 test was 98.0% (49/50). Discordant results were observed for the remaining 1 sample, which were then tested with SARS-CoV-2 specific PCR kit.
For what concerns final reconciled diagnoses, 16 (32%) samples were positive for at least one of four viruses and presented a wide range of Ct values (22.19-25.73). In particular, positive samples were distributed as follows: influenza A (n = 4), RSV (n = 4), SARS-CoV-2 (n = 2), influenza A+RSV (n = 2) and rhinovirus/enterovirus (n = 4). The remaining 32 (68%) specimens were negative for all these viruses. In the M10 test, 97% of positive samples were displayed as such in <60 minutes than RP2 revealed positive or negative results in 80 minutes. In our study population RSV, influenza A and rhinovirus/enterovirus were the most commonly detected pathogens. RSV season last from January to March in Italy. Shortening the duration/severity of respiratory viral illness through initiation of effective antiviral therapy may reduce the incidence of secondary bacterial infection, thereby reducing the number of antibiotic courses prescribed to pediatric patients. A specific, ready-to-use all-in-one cartridge like M10, in line with seasonal virus circulation and epidemiology, could potentially reduce the risk of antibacterial co-infections with more effective treatment and improvement of *cost-benefit ratio*. In conclusion, this study demonstrates that M10 assay reliably detects influenza A/B, RSV and SARS-CoV-2 in less than one hour and is therefore suitable for a rapid differential diagnosis of the pediatric common respiratory viruses. We therefore suggest that M10 assay may be best allocated to the emergency and critical pediatric care departments, where an urgent differential diagnosis is required and secondly, in case of negativity with respiratory illness, use a multitarget test like RP2. In this way, in pediatric population we could reduce hospital admissions, avoid further unnecessary procedures and promote the use of specific antibiotic and antiviral treatments to improve clinical outcomes.

PO 067

HIV-2 RNA PLASMA QUANTIFICATION: PERFORMANCE EVALUTATION OF A COMMERCIAL RUO RT-PCR ASSAY

F. Salari, A. Rizzo, F. Bracchitta, L. Morelli, A. Tamoni, F. De Poli, M. Cuomo, M.R. Gismondo, V. Micheli

Laboratory of Clinical Microbiology, Virology and Bioemergencies. ASST Fatebenefratelli Sacco, "L. Sacco" University Hospital, Milan, Italy

Aim of the Study: HIV-2 infection is characterized by a low viral titer, slow disease progression, and poor response to standard antiretroviral therapies. The availability of a sensitive and consistent assay for the quantification of HIV-2 RNA is crucial for supporting laboratory HIV-2 infection diagnosis, as well as for managing antiretroviral decisions and for monitoring infected patients over time.

The aim of this study was to assess the performance of a commercial RUO RT-PCR based assay for the quantification of HIV-2 RNA using plasma from suspected HIV-2 positive patients and an HIV-2 external quantification standard.

Methods used: RealStar® HIV-2 RT-PCR kit 1.0 (RUO) on AltoStar® AM16 platform (altona Diagnostics, Germany) was used on 13 plasma samples from 11 HIV-2 suspected patients and on dilution series of the external International Standard (IS) P0318 ViraQ HIV-2 Check 125 (genotype A). The IS (concentration: 161.25 UI/mL) was diluted using a 65 mL human plasma pool from HIV-1/2 negative patients to prepare ten replicates of IS at different concentrations (30, 20, 15, 10, 7.5 and 5 UI/mL). HIV-2 RNA was extracted from 1 mL of plasma and eluted in 80 µL using the AltoStar® purification kit 1.5 (altona Diagnostics, Germany). The 96-well plate was automatically set up as follows: 4 RT-PCR standards (1x10^4, 1x10^3, 1x10^2, 1x10^1 UI/µL) and the negative control of the assay, 15 plasma samples for diagnostic purposes, 10 replicates of each IS concentration, 2 replicates of IS at 40 UI/mL (1 in plasma and 1 in PBS), 1 undiluted IS (161.25 UI/mL) and a negative sample (plasma pool).

Results and Conclusions: The standard curve showed the following parameters: E=99.1%; R^2=1.000; slope=-3.344; y int=32.752. Up to the concentration of 7.5 UI/mL all the 10 IS replicates of each concentration were detected and showed a mean (SD; CV) Cycle threshold (Ct) value of 33.87 (0.70; 0.02), 34.44 (0.48; 0.01), 34.83 (0.75; 0.02), 35.96 (0.67; 0.02) and 35.85 (1.44; 0.04), corresponding to 40.98 (19.75; 0.48), 26.28 (8.75; 0.33), 21.61 (11.13; 0.52), 9.61 (4.14; 0.43) and 7.50 (3.65; 0.49) UI/mL, respectively. For the concentration of 5 UI/mL, 7 out of 10 IS replicates were detected, showing a mean (SD; CV) Ct value of 36.41 (0.60; 0.02) that corresponded to 5.92 (2.18; 0.37) UI/mL.

The undiluted IS sample resulted 126.8 UI/mL, while there were no differences comparing plasma vs PBS diluted IS sample at 40 UI/mL.

All but one of the routine plasma samples resulted undetectable: the only positive sample showed a viral load corresponding to 126.800 UI/mL, consistently with the untreated status of the patient.

The assay showed a good performance, especially at the concentrations of 20 and 10 UI/mL (lower CVs compared to the others); 7.5 UI/mL would represent the lowest reliable concentration for HIV-2 detection. The sensitivity of the assay coupled with the detection of HIV-2 RNA in plasma sample from an untreated patient confirms its usefulness in the clinical practice. Further analyses on a larger number of replicates are needed to better investigate the sensitivity below 7.5 UI/mL.

PO 068

A COMPARATIVE STUDY OF ALLPLEX® (SEEGENE) AND SARS-CoV-2 PLUS ELITE MGB® KIT (ELITECHGROUP) FOR DIAGNOSIS OF FLUA, FLUB, RSV, AND SARS-CoV-2 INFECTION

M. Zoppelletto, S. Bernardi, M. Dorigo, F. Merola

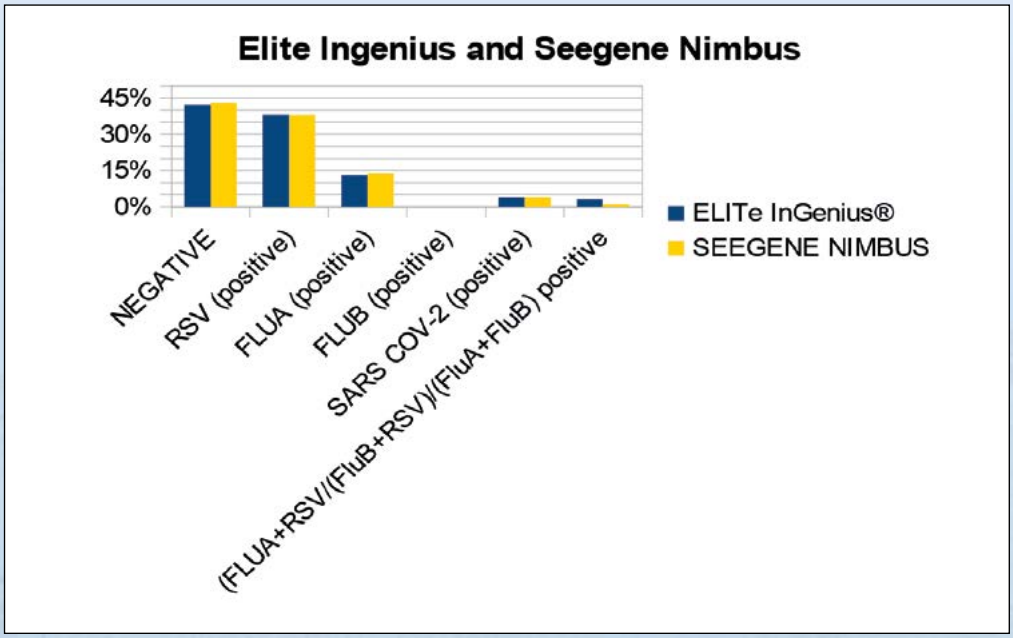
UOC Medicina di Laboratorio, Ospedale San Bassiano, Bassano del Grappa, Vicenza, Italy

Aim: The aim of this work is to compare two molecular platforms, SEEGENE Nimbus and ELITe InGenius®,in the molecular analysis of viral pneumotropic genes, mostly found in the seasonal infections of the respiratory system.

Methods: Samples (114) were enrolled: 42% were from pediatric and 20% from adult patients from different medical wards of San Bassiano Hospital, while 38% were from subjects under home health care. All patients had a medical prescription for a molecular test of FluA, FluB, RSV, SARS-CoV-2, or RSV.

A nasopharyngeal swab was collected from each patient and stored in 3mL of standard virus transport media (VTM) at 4°C until processing. The samples were selected to be representative of all the viruses of interest, based on the routine method (BINAXNOW™ RSV CARD system and/or). A group of frank negative samples was used as a control. The parallel testing with the systems SARS-CoV-2 plus ELITe MGB® kit - Elite InGenius® (ELITechGroup) and Allplex™ SARS-CoV-2/FluA/FluB/RSV assay – Nimbus (Seegene Inc.) was performed at the Microbiology Laboratory of San Bassiano Hospital.

Results: Between SARS-CoV-2 plus ELITe MGB® kit on Elite InGenius® and Allplex™ SARS-CoV-2/FluA/FluB/RSV the overall inter rank agreement was high (k= 0.83; SE=0.04; 95% CI 0.75-0.91). When compared to the routine method, the overall agreement of both systems was high (k= 0.87 for InGenius® and k=0.95 for Nimbus). However, with Elite InGenius® a higher number of coinfections including Sars CoV2 was observed (n=6). This allows to identify positive patients early and suggests a robust sensitivity of the system in detecting different viruses simultaneously.



Analisi inter class correlation

Observer A	ING
Observer B	SEG

	INGENIUS								
SEEGENE	NEG	SCoV2	FLUA	RSV	FLUB+RSV	SCov2+FLUA	SCov2+RSV	FLUA+RSV	
NEG	43	3	1	2	0	0	0	0	49 (43.0%)
SARSCoV2	0	5	0	0	0	0	0	0	5 (4.4%)
FLUA	1	0	14	0	0	1	0	0	16 (14.0%)
RSV	0	0	0	37	1	0	5	0	43 (37.7%)
FLUB+RSV	0	0	0	0	0	0	0	0	0 (0.0%)
SCov2+FLUA	0	0	0	0	0	0	0	0	0 (0.0%)
SCov2+RSV	0	0	0	0	0	0	0	0	0 (0.0%)
FLUA+RSV		0	0	0	0	0	0	1	1 (0.9%)
	44 (38.6%)	8 (7.0%)	15 (13.2%)	39 (34.2%)	1 (0.9%)	1 (0.9%)	5 (4.4%)	1 (0.9%)	114

Weighted Kappa[a]	0.83355
Standard error	0.04132
95% CI	0.75256 to 0.91453

[a] Linear weights

yellow= single target infections detected only by InGenius
light blue= coinfections detected only by InGenius
green= cocordant

Conclusions: In patients with flu-like symptoms a differential diagnosis of viral infection or coinfection is essential for the correct patient management.
Multi-target molecular assays and fully automated medium throughput systems allow the laboratory to process a larger number of samples (up to 72 per session) in a short time, guaranteeing adequate information to the clinicians, for a correct and timely diagnosis. In our study the SARS-CoV-2 plus ELITe MGB® kit with Elite InGenius® and Allplex™ SARS-CoV-2/FluA/FluB/RSV showed verry good agreement in the differential detection of multiple respiratory viruses and a higher processivity than the composite routine method (BINAXNOW™ RSV CARD system and RELAb real time PCR). This different numerical loading capacity allows to formulate a laboratory workflow, with continuous loading of samples with requests for pneumotropic viruses.

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PO 069

HIGH RESOLUTION MELTING ANALYSIS (HRMA) AS TOOL TO DETECT MOLECULAR MARKERS ASSOCIATED TO BALOXAVIR MARBOXIL RESISTANCE IN INFLUENZA VIRUSES

R. Arvia, A. Rocca, B. Casciato, M.A. Stincarelli, S. Giannecchini
Department Experimental and clinical medicine, University of Florence, Italy

Background: Historically, an important limitation in the use of adamantane derivatives and neuraminidase inhibitors in the clinic is the development of resistance which reduces their efficacy. To date, a new anti-flu drug, Baloxavir marboxil (BXM), inhibitor of the endonuclease activity of the protein PA, has been approved. However, there is a significant concern regarding the emergence of resistant strains. The most common mutation associated with BXM resistance in influenza viruses is the I38T.

Aim: The objective of this study was to develop three rapid and sensitive assays for the detection and monitoring of the known molecular markers associated with resistance to BXM in A(H1N1), A(H3N2) and B influenza viruses.

Methods: Three RT real-time PCR-HRM were developed for rapid detection of antiviral resistance markers by using oligonucleotides with the substitution of interest and on PA/I38T laboratory strains. The target of these assay was the mutation I38T (T113C) in PA gene of A(H1N1), A(H3N2) and B influenza viruses. HRM assays were applied to 17 clinical samples from 14 patients A(H1N1) virus positives, to 47 clinical samples from 45 patients A(H3N2) virus positives, and to 23 clinical samples from 23 patients B virus positives. Moreover, serial passages of the viruses in the presence of Baloxavir acid (BXA, the active molecular form of Baloxavir marboxil drug) were carried out to select PA/I38T mutation and assayed with HRM. All HRM data obtained were confirmed by sequencing.

Results and Conclusions. HRM analysis detected PA/I38T mutation and it was able to characterize oligonucleotides wild type from oligonucleotides mutated. The I38T mutation was not shown in all our clinical samples examined. In order to validate the results of HRMA, the PCR products were analyzed by dideoxy Sanger sequencing. The selection of mutation induced by BXA was carried out on influenza virus H1N1pdm09 clinical isolates in MDCK cell substrate with serial passages in presence or absence of different inhibitory concentration (from 0.5 μM to 20 μM of BXA). Selected variants were assayed for the sensibility of BXA in a plaque reduction assay. The BXA selected variants were analyzed with HRM and confirmed by sequencing.
Collectively these results suggest that our HRM-based assay is a powerful tool for rapidly monitoring antiviral resistance to BXM antiviral among influenza viruses during seasonal circulation.

PO 070

INFLUENZA-LIKE-ILLNESS IN PATIENTS ACCESSING THE PEDIATRIC EMERGENCY ROOM DURING WINTER SEASON 2022/2023

S. Vituliano[2], A. Liberatore[2], A. Primavera[2], I. Banchini[2], T. Ferniani[2], M. Bitelli[1], I. Corsini[1], I. Finguerra[2], L. Sili[2], A. Cantiani[2], S. Venturoli[3], G. Piccirilli[3], E. Petrisli[3], L. Gabrielli[3], M. Lanari[1], T. Lazzarotto[2]

[1]Pediatric Emergency Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Italy
[2]Microbiology Unit, DIMEC, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Italy
[3]Microbiology Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Italy

Introduction: During the last two winter seasons, a drastic reduction in cases of influenza-like-illness (ILI) has been observed worldwide.
Our aim is to evaluate, in the current winter season, the spread of the main viruses responsible for ILI in pediatric patients, with the exclusion of SARS-CoV-2.

Methods: Between 12 October 2022 and 31 March 2023, in patients with respiratory symptoms (cough, persistent fever and rhinitis) accessing the Pediatric Emergency Room (ER) at the Sant'Orsola Polyclinic of Bologna, naso-pharyngeal (NP) swabs were collected for the detection of: Influenza A (FLU-A), Influenza B (FLU-B), Respiratory Syncytial Virus (RSV), Rhinovirus (HRN), Parainfluenza Virus (PIV), Adenovirus (ADV) and Metapneumovirus (MPV). The samples were processed using the AllplexTM RV Essential Assay (Seegene MuDTTM technology, South Korea) multiparameter molecular panel and the results were obtained within few hours.

Results: During the study period, 1414 NP swabs were analysed. A positive result, for at least one of the viruses responsible for ILI, has been found in 1190 samples (84%). In particular, positivity was observed as follows: 386 (32%) FLU-A, 88 (7%) FLU-B, 283 (24%) ADV, 276 (23%) RSV, 330 (28%) HRN, 105 (9%) MPV, 54 (5%) PIV. A coinfection was found in 301 cases (25%).
The first FLU-A positive sample was detected in the 41st week of 2022 and the peak was reached in the 48th. At the end of the 13th week of 2023, the number of positive samples was almost zero.
The first FLU-B positive sample was detected later than the one for FLU-A, in particular in the 46th week of 2022. The peak was reached between the 8th and the 10th week of 2023 and by the 13th week an important reduction of positive samples was observed.
The first RSV positive sample was detected in the 46th week of 2022, while the peak was in the 49th week; by the 13th week of 2023 there are no cases of infection.
Between the 12th and the 13th week of 2023, a peak of ADV has occurred and it has not been decreased yet.
The 92% of RSV positive children was less than two years old, in comparison to the 53% of ADV positive children and to the 50% of FLU-A positive children.

Conclusions: In the current influenza season, cases of ILI, with the exclusion of SARS-CoV-2, were mainly caused by FLU-A, RSV and later also ADV, especially in children under 2 years of age.
The use of the multiparameter molecular test with results available in few hours, allowed to simplify the diagnostic path used in children with ILI accessing the ER. If the patient has a clinical suspicion of viral respiratory infection, no further diagnostic tests is performed. Only in case of negative result with the persistence of symptoms, further diagnostic investigation should be carried out.

PO 071

VIRAL CENTRAL NERVOUS SYSTEM INFECTIONS: 10 YEARS OF DIAGNOSTIC EXPERTISE

A. Cantiani[1], I. Banchini[1], A. Liberatore[1], A. Primavera[1], T. Ferniani[1], A. Balboni[1], E.C. Borgatti[1], F. Lanna[1], S. Venturoli[2], G. Piccirilli[2], E. Petrisli[2], L. Gabrielli[2], T. Lazzarotto[1]

[1]Microbiology Unit, DIMEC, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Italy
[2]Microbiology Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Italy

Introduction: Central nervous system (CNS) infections are severe clinical conditions associated with high rates of morbidity and mortality. Early identification of causative pathogens can reduce mortality and permanent neurological damage.
The aim of this study is to evaluate the positivity on cerebrospinal fluid (CSF) samples for: Herpes Simplex Virus 1 (HSV-1), Herpes Simplex Virus 2 (HSV-2), Varicella Zoster Virus (VZV), Enterovirus (EV), Cytomegalovirus (CMV) and Human Herpesvirus 6 (HHV-6), when a clinical suspicion of acute CNS infection occurs.

Methods: Results obtained from the analysis of CSF samples performed over the past 10 years at Microbiology Unit of Sant'Orsola Polyclinic were retrospectively evaluated.
From 2013 to 2017, extraction was performed with the QIAsymphony SP instrument (Qiagen, Hilden, Germany) and the ELITe MGB® kits (ELITechGroup, Italy) were used for real-time PCR amplification. From 2018 to 2022, the ELITeInGenius SP 200 and ELITe MGB® kits (ELITechGroup, Italy) were used on the ELITeInGenius® instrument for both nucleic acid extraction and real-time PCR amplification.

Results: From January 2013 to December 2022, virological examinations were performed in 4026 CSF and a positive result was observed in 291 samples (7%).
In particular, 81 (28%) samples were positive for VZV, 63 (22%) for EV, 58 (20%) for HHV-6, 47 (16%) for HSV-1, 24 (8%) for CMV and 18 (6%) for HSV-2.
No cases of EV meningitis were observed in the years 2020-2021 probably due to the hygiene measures implemented to prevent SARS-CoV-2 infection.
Focusing our attention to the 58 patients with HHV-6 positivity, the determination of blood and hair follicle viral load permitted the identification of 26 cases of HHV-6 integration, 26 of HHV-6 latency, and only in 6 pediatric cases an acute HHV-6 infection associated with CNS infection.
Considering the patient's immune status, in immunocompetent individuals CNS infections were mostly caused by VZV (35%), followed by EV (31%), while in immunocompromised individuals were prevalently caused by CMV (63%).
In relation to the patient's age it was observed that CNS infections in pediatric patients were mainly caused by EV (55%) and VZV (6%), while in adult patients VZV was prevalent (38) followed by EV (22%).

Conclusions: In CNS infections, the viral etiological agent is related to the patient's age and immunological status. A positive HHV-6 result on CSF has to be interpreted with caution and is not by itself exhaustive for patients' final diagnosis, always requiring further diagnostic investigation on blood and eventually on hair follicle.

PO 072

TARGETED SEQUENCING AS A TOOL FOR RESPIRATORY PATHOGENS IDENTIFICATION AND DIAGNOSIS

A. Grimaldi[1], T. Fusco[1], C. Tiberio[2], P. Annunziata[3], F. Panariello[1], D. Cacchiarelli[1,3-5]

[1] *Telethon Institute of Genetics and Medicine (TIGEM), Armenise/Harvard Laboratory of Integrative Genomics, Pozzuoli, Italy*
[2] *UOC Microbiologia e Virologia, P.O. Cotugno A.O. dei Colli, Naples, Italy*
[3] *Next Generation Diagnostic srl, Pozzuoli, Italy*
[4] *Department of Translational Medicine, University of Naples Federico II, Naples, Italy*
[5] *SSM School for Advanced Studies, University of Naples Federico II, Naples, Italy*

Aim: Respiratory pathogens are considered a leading threat to human health worldwide. They account for 17.2 billion cases and over 2.5 million fatalities yearly. In particular, lower tract respiratory infections are the primary underlying cause of sepsis in patients, responsible for an additional 11 million lives lost annually. Most respiratory infections are due to bacteria, and while several antimicrobial treatments are available, antimicrobial-resistant (AMR) have generated concern in the last few years. Viruses, especially RNA viruses, are the second significant cause of respiratory infections but have greater spreading potential, as shown by the *Influenza* and *SARS-CoV-2* pandemics of the last century. Here we propose a collection of technologies for diagnosing and characterizing respiratory pathogens to tackle the growing threat they represent to human health.

Methods: We have matured multiple approaches for pathogens identification based on targeted Next Generation Sequencing (NGS). Specifically, we developed and optimized an amplicon sequencing-based strategy for the rapid and cost-effective sequencing of the SARS-CoV-2 genome from unquantified micro volumes of RNA extracted from swabs. In addition, we optimized a probe capture-based strategy to identify thousands of microbiological with a single NGS analysis. Furthermore, such a strategy also allows sequencing of the whole genome of the main Influenza viruses, unlocking the full diagnostic potential of NGS directed toward respiratory infections. Finally, to identify and prioritize samples from patients infected by SARS-CoV-2 and Influenza virus, we developed a strategy based on Reverse transcription and Loop Amplification (RT-LAMP), which allows the cost-effective and rapid identification of the samples of interest.

Results and Conclusions: Our amplicon-based approach targeting the SARS-CoV-2 genome was exploited for Campania's COVID-19 pandemic genome surveillance. Thanks to this approach, we sequenced over 50,000 viral genomes in 2 years and a half, producing the highest number of sequences in Italy. The data we produced allowed us to reconstruct the pandemic dynamics in the regional territory and identify multiple new SARS-CoV-2 variants. Furthermore, optimizing reaction conditions and sequencing parameters has reduced costs by over 80%. On the other hand, our capture-based sequencing strategy for respiratory pathogens proved its capability to identify over 40 different viruses, 200 bacteria, and over 2,000 AMR markers. We are currently working to optimize the strategy with the final goal of reducing costs and turn-around time.
To further exploit the power of this strategy, we will leverage an RT-LAMP methodology to identify the *Influenza* genome in SARS-CoV-2 positive swabs. Therefore, such samples will be sequenced to reconstruct both viruses' genomes and identify genetic signatures associated with this coinfection. As already proved by our genomic surveillance activity on SARS-CoV-2, we anticipate that the results gained with this project will represent a key added value in the routine implementation of NGS technologies in diagnostic settings.

PO 073

TOTAL HDV AB REFLEX TEST ENABLES IDENTIFICATION OF SUBMERGED CASES

S. Brusa[1], V. Cossiga[2], F. Passeggio[1], M. Baldi[1], G. D'ambrosio[1], L. Stanziola[1], G. Felicelli[1], F. Morisco[2], D. Terracciano[1], G. Portella[1]

[1] Department of Translational Medical Sciences, University of Naples "Federico II", Naples, Italy
[2] Department of Clinical Medicine and Surgery, Diseases of the liver and biliary system Unit, University of Naples "Federico II", Naples, Italy

Aim: The prevalence in the Campania region of HDV infection in patients with chronic hepatitis B is estimated to be around 6.4% in native subjects. However the actual prevalence is underestimated as the test is not performed in all subjects with chronic hepatitis B but it is usually performed upon a clinical order. To assess the true prevalence of HBV HDV, total anti-HDV antibodies were tested in all HBsAg positive subjects in the period from 02/21 to 12/22.

Methods: 439 patients of A.O.U. Federico II routinely tested for HBV markers with chronic hepatitis B were enrolled and a reflex test for the detection of total anti-HDV antibodies was carried out. Sera were evaluated with ADVIA Centaur HBsAgII Qualitative, Liaison Murex HBsAg Quantitative and Liaison Murex Anti-HDV Qualitative.

Results and Conclusion: We found 390 HDV negative subjects and 49 HDV positive. 30 positive were already diagnosed as HBV-HDV infected, whereas in 19 patients HDV infection was detected for the first time. Our data confirm that a relevant percentage of HBV-HDV infections are unnoticed. Due the forthcoming approval of specific anti-HDV drugs, our approach could be useful to identify patients that could benefit of anti HDV treatments.

PO 074

RAPID DIFFERENTIAL DIAGNOSIS OF SARS-CoV-2, INFLUENZA A/B AND RESPIRATORY SYNCYTIAL VIRUSES WITH A MULTIPLEX PCR ASSAY AND ITS IMPACT ON AN ITALIAN EMERGENCY DEPARTMENT

C. Daleno[1], M. Calabrò[1], E. Cavedoni[1], D. Cirasola[1], F. Lattanzi[1], R. Renzulli[1], C. Scuderi[1], A. Voza[2], V. Cento[1,3], E. Casari[1]

[1] *Microbiology and Virology, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy*
[2] *Emergency Department, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy*
[3] *Department of Biomedical Sciences, Humanitas University, Pieve Emanuele, Milan, Italy*

Background: Respiratory signs and symptoms for Influenza viruses A/B (Flu-A/B), Respiratory Syncytial Virus (RSV) and SARS-CoV-2 infections are similar, and, therefore, a clinical differential diagnosis is difficult. During seasonal epidemics these viruses can co-circulate and a rapid differential test may help physicians in treatment decisions, while improving patient management by preventing nosocomial infections transmission. Several molecular tests have been developed for simultaneous detection of SARS-CoV-2, Flu-A/B and RSV. We report our recent experience with a rapid and easy to use, fourplex Real Time PCR assay in order to evaluate the laboratory turnaround time (TaT) test in a level-2 Emergency Department (ED).

Methods: Nasopharyngeal swabs collected in ED of Humanitas Research Hospital from symptomatic patients between December 2022 and March 2023 were tested with STANDARD M10 Flu/RSV/SARS-CoV-2 (SD Biosensor Inc., Seoul, Korea) in the central laboratory. TaT was calculated on the bases of elapsed time between arrival of the sample in the laboratory and the release of the result.

Results: A total of 104 nasopharyngeal swabs were processed. Of these, 22/104 (21.1%) were positive for at least one virus. SARS-CoV2 was the most frequently detected, in 9/22 swabs (40.9%), followed by Flu-A (n=8, 36.4%), Flu-B (n=4, 18.2%) and RSV (n=2, 9%). Viral co- infections were found in one sample (Flu-A plus RSV). Swabs arrived in laboratory between 7.30 am and 8.00 pm had a mean Tat of 65 minutes. Following laboratory data, the majority of positive patients (n=17, 72%) were hospitalized but nobody was admitted to the Intensive Care Unit. All patients with SARS-CoV-2 were rapidly isolated from negative patients, and those with Flu-A/B infections (n=7; 58.4%), received oseltamivir treatment directly in ED; all the remaining Flu-A/B infected patients (n=5; 41.6%) were discharged within 1 day, with at-home therapy prescription.

Conclusions: STANDARD M10 Flu/RSV/SARS-CoV-2 is a rapid RT-PCR assay able to detect influenza A, influenza B, RSV and SARS-CoV-2 and allowed us to communicate almost all results within 1 h in order to help patients management. Additional studies are needed to evaluate the benefits of this approach on clinical impact, cost-effectiveness and management in our ED setting.

PO 075

FOLLOW UP CMV INFECTION AFTER HEMATOPOIETIC STEM CELL TRANSPLANTION IN PEDIATRIC PATIENTS BY THE NEW MOLECULAR TARGET FOR CMV-RNA

C. Russo[1], L. Gentile[1], M. Rossi[1], S. Landi[1], P. Merli[2], F. Quagliarella[2], F. Galaverna[2], F. Locatelli[2], C.F. Perno[3]

[1] Virology and Mycobacteria Unit - [1]Microbiology and Diagnostic Immunology Unit, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy
[2] Department of Pediatric Hematology and Oncology and Cell and Gene Therapy, IRCCS Bambino Gesù Children's Hospital, Rome, Italy
[3] Unit of Diagnostic Microbiology and Immunology and Multimodal Medicine Area, IRCCS, Rome, Italy

Introduction: Letermovir blocks CMV infection by inhibiting the viral terminase and the virion maturation, without blocking viral-DNA production. Approved in adults, it represents an attractive alternative for CMV-treatment also in children. Effective lab tests for monitoring the drug efficiency are missing, mostly because the CMV-DNA is unable to properly evaluate antiviral efficacy. We present preliminary data on CMV monitoring by a new commercial assay for CMV-RNA detection and quantitation in a cohort of hematopoietic stem cell transplanted children after antiviral treatments.

Patients and Methods: Between July-to-November 2022, 53 children were treated with Letermovir after HSCT at the Bambino Gesù Children's Hospital. Thirty-six received primary or secondary prophylaxis, 17 pre-emptive or treatment therapy (median dosage 240 mg/mq, range 120-480). CMV-DNAemia was evaluated twice a week (artus® CMV-TM-PCR_Qiagen_ Hilden, Germany). In 30/53, CMV-RNA was also investigated in plasma samples using CMV-RNA ELITe MGB® (Kit-ELITe_InGenius System: ELITechGroup).

Results: 61 samples, referred to 30 patients (median age 10, IQR 1-21 years), were collected to perform simultaneously CMV-DNAemia and CMV-RNAemia. In 54/61 samples (88.5%) CMV-DNA was persistently present (median 848 copies/mL, IQR 314-3995), while only in 16/61 (26.2%) CMV-RNA was detected (median 23 copies/mL, IQR 6-150). When CMV-DNAemia was quantified <1000 copies/mL (26/54 samples including 11 samples pre-treated with DNA-si), RNA-CMV resulted not detected or below 30 copies/mL. Under pre-emptive therapy/treatment of infection, all patients achieved CMV-DNA negativization in blood within 20 days.

Conclusions: Despite the presence of positive CMV-DNA, the clinical evolution was compatible with the absence of CMV activity thus suggesting that the undetectability of CMV-RNA is driven by the infection evolution and not by the lack in the method sensitivity.
The CMV-RNA may represent an accurate marker to monitor clinical antiviral efficacy, more than CMV-DNA.

PO 076

PREVALENCE OF RESPIRATORY VIRUS CIRCULATION AND IN OUTPATIENT CHILDREN DURING THE WINTER 2022-2023 IN ROME

G. Linardos[1], S. Ranno[1], L. Coltella[1], E. Pandolfi[2], I. Croci[2], D.P. Ristagno[1], K. Yu La Rosa[1], G. Costantini[1], C.F. Perno[1], C. Russo[1], A.E. Tozzi[2]

[1] Microbiology and Immunological Diagnostics Unit, Bambino Gesù Children Hospital-IRCCS, Rome, Italy
[2] Multifactorial and Complex Diseases Unit, Bambino Gesù Children Hospital-IRCCS, Rome, Italy

Aim of the Study: Respiratory viruses are a common cause of respiratory tract infection, particularly in neonates and children, and represent one of the leading causes of morbidity and mortality worldwide. Preschool children may have up to 6-10 viral colds a year due to the fact that humoral and phagocytic immunity reach their best efficacy during the fifth or sixth years of age and also due to their behaviour and close relation with other kids, that may be virus carriers. It is now known how the SARS-CoV-2 pandemic has had an impact on the circulation of respiratory pathogens, due to the containment measures applied to prevent the spread of COVID. Most of the existing studies refer to the prevalence of viral infections in hospitalized children; few studies have focused on the impact of these diseases in the primary care setting. The aim of this project is to prospectively study the trends of some of the most frequent respiratory infections in children (RSV, Influenza and SARS-CoV-2) in the community (not hospitalized) thanks to the development of a laboratory surveillance system based on a network of family pediatricians.

Methods used: The project involves the enrollment of children aged ≤ 5 yrs with ARI who present in the outpatient clinics of the pediatricians participating in the programme. The study takes place during the season 2022/2023, (from the 47th-2022 to the 17th-2023). All patients enrolled by the network's pediatricians are given a nasopharyngeal swab in UTM® (Copan _Brescia). The swab is sent to the laboratory of the UOS Virology and Mycobacteria of Bambino Gesù Children Hospital and tested with an all-in-one RT-PCR multiplex molecular assay, M10 Flu/RSV/SARS-CoV-2 (SD BIOSENSOR_Korea) for the search of 4 targets: RSV, SARS-CoV-2, Influenza-A and Influenza-B.

Results: To date (week 16th of 2023), 122 samples (related to 122 patients) have been collected. Among those, 2/122 were excluded retrospectively because they did not meet age requirements. Out of 120 children (60 F and 60 M, median age 1.10 yrs, IQR 0.62- 2.05),67.5% (N=81) were positive for at least one respiratory virus target and 32.5% (N=39) were negative for all4. In detail, the results showed positivity rate to RSV for 57.5% (N=69 median age 1.04 yrs), while InfluenzaA resulted present in 8.3% (N=10 median age 1.66 yrs), followed by detection of InfluenzaB and SARS-CoV-2 in 3.3% (N=4; median age 0.91 and 0.83 yrs respectively). Co-infections were detected in6 patients:RSV+FLUA (4/122; 3.3 %) and RSV+SARS-CoV-2 (2; 1.6%). We evaluated also the cycle threshold as a proxy of Viral load obtaining a mean value for RSV of 24.06 and for InfluenzaA of 27.59.

Conclusions: The collected data show a significant clinical impact of ARIs in the primary care setting. RSV was the predominant pathogen in our cohort as already shown in the pre-pandemic era and was shown to be the leading cause of respiratory illness in children < 1 yrs. In the season under review 65.8%of the patients referred to the pediatrician tested positive for RSV and/or InfluenzaA showing how these are the predominant viruses in the community during the epidemic season. The illness reflects moderate to severe disease with symptoms such as shortness of breath (76%) and wheezing (10%). As described in the literature, our data show a lower mean CT for RSV than the other viruses evaluated, likely indicating the severity of the infection and the need to seek medical attention.

PO 077

NON-SARS-CoV-2 RESPIRATORY VIRUSES CO-DETECTION IN SPECIMENS COLLECTED FROM CHILDREN ADMITTED TO THE BAMBINO GESÙ CHILDREN'S HOSPITAL IN ROME OVER A PERIOD OF 1 YEAR

V.C. Di Maio[1], V. Costabile[2], L. Coltella[1], S. Ranno[1], G. Linardos[1], L. Colagrossi[1], L. Gentile[1], C. Russo[1], C.F. Perno[1]

[1] *Microbiology and Diagnostic Immunology Unit, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy*
[2] *Multimodal Research Area, Microbiology and Diagnostics of Immunology Unit, Bambino Gesù Children Hospital IRCCS, Rome, Italy*

Background: Respiratory viruses other than severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continue to represent a significant burden of disease. Although respiratory virus co-detections are common, analysis of combinations of co-detected viruses is not fully investigated. The aim of this study is to analyse the epidemiology of non-SARS-CoV-2 respiratory viruses and the most frequently occurring viral pairings in clinical specimens collected from children admitted to the Bambino Gesù Children's Hospital in Rome over a period of 1 year.

Methods: Between January 2022 and January 2023, a total of 6969 specimens (3086 nasopharyngeal swabs, 3739 nasopharyngeal aspirates and 144 Broncho-Alveolar Lavage) were screened for respiratory viruses using a multiplex PCR respiratory panel assay which simultaneously detects 16 pathogens. Respiratory samples were collected and retrospectively analysed from a total of 3704 children with a median age of 2.4 years (interquartile range [IQR]: 0.5-6.8).

Results: Of 6969 respiratory samples, 58.3% (N=4062) were positive for at least one virus. Of these, viral mono-infection was found in 71.0% (N=2886/4062) while co-infection with more than one pathogen was found in 28.9% (N=1176/4062) specimens. In particular, co-detection mainly involved two viral species (71.5%, N=841/1176) while the detection of three or more viruses was less frequent 21.2% (N=250/1176) and 7.2% (N=85/1176), respectively. Overall, in samples with viral mono-infection, rhinovirus was the most frequently detected pathogen 47.4% (N=1387/2886), followed by respiratory syncytial virus B 10.0% (N=289/2886) and influenza virus A 6.9% (N=198/2886). Interestingly, by analyzing combinations of co-detected respiratory viruses the most frequently observed pairs involved mainly rhinovirus detected with adenovirus (7.6%, N=89/1176), respiratory syncytial virus B (6.8%, N=80/1176), human enterovirus (6.0%, N=71/1176) and human bocavirus (5.7%, N=67/1176). However, although rhinovirus was identified as a co-pathogen in viral pairings at high prevalence, the viruses mainly detected in viral co-infections were enterovirus (74.1%, N=240/324), bocavirus (73.5%, N=311/423) and adenovirus (71.8%, N=275/383).

Conclusion: The use of multiplex PCR respiratory panels, which allow simultaneous detection of different pathogens in a single test is useful to better understand the role of co-infections. Further studies are required to confirm the significance of the viral pairings observed in this study and to elucidate the virologic mechanisms underlying the associations that may lead to cooperation or competition between co-detected viruses.

PO 078

IMPLEMENTATION OF A PROTOCOL FOR THE DIFFERENTIAL DIAGNOSIS OF SARS-CoV-2, FLU A, FLU B AND RSV AT ASST BERGAMO EST DURING FLU SEASON 2022-2023

A.M. Palladino[1,4], M. Airoldi[2], B. Brugnetti[1], E. Bombana[2], F. Mallus[1], D. Nagel[1], F. Manelli[3], A. Callegaro[1]

[1] *Labortory Medicine Department, ASST Bergamo EST Seriate, Bergamo, Italy*
[2] *Infectious Disease and Vaccination Unity, ASST Bergamo EST Seriate, Bergamo, Italy*
[3] *Emergency Department, ASST Bergamo EST Seriate, Bergamo, Italy*
[4] *Medicine and Surgery, University of Milano Bicocca, Milan, Italy*

Aim of the Study: Influenza and respiratory syncytial (RSV) viruses are expected to co-circulate with SARS-CoV-2 and clinical differential diagnosis between them is difficult. During the Flu season 2022-2023 the protocol "*Managment and execution of rapid test for Flu A, Flu B , SARS-CoV-2 and RSV*, have been implemented in emergency room and inpatient units at ASST Bergamo Est in order to identify which patients should undergo nasopharyngeal swabs for the identification of FLU A and B, SARS-CoV-2 and RSV. The aim of the study was to analyze the result from the protocol and evaluate the chance of its clinical aid to choose the appropriate intervention.

Methods: The protocol calls for the execution of multiplex swab in patients presenting flu-like illnesses, pneumonia or non-specific respiratory diseases, and who are hospitalized due to severity and high risk of complications, including:

– Adults aged >65.
– Patients with chronic lung diseases, cardiovascular diseases, chronic diseases, renal diseases, liver diseases, hematological diseases, metabolic diseases (including type II diabetes), neurological diseases.
– Patients with primary or secondary immunosuppression.
– Pregnant or post-partum women.
– Obese patients (BMI >40).
– Patients from health care residence (RSA).

Detection of Sars-CoV-2, Flu A and Flu B using Multiplex PCR is carried out at the Laboratory Medicine Department from nasopharyngeal swabs using the Ce IVD Standard TM M10 modular system (SD Biosensor, Suwon, South Korea). 300 ul of sample (swab transport medium) were processed in 60 minutes using integrated extraction and RT-PCR. The demographic and clinical data were collected from the patients records and descriptive analysis was performed. A binomial probit model have been used for the multivariate analysis.

Results and Conclusions: Of the 271 individuals included, 183 were males (67,5%) with a mean age of 66. The patients positive for at least one of the four viruses were 91 (33,6%) distributed as follows: 41 RSV, 21 SARS-CoV-2, 35 Flu A and 1 Flu B. 7 cases of double positivity were also reported (4 SARS-Cov-2 + Flu A, 3 Flu A + RSV) Of the positive patients, 18 (19.8%) were treated with specific antiviral therapy, 87 survived the infection and 4 died. Although several baseline characteristics were significantly linked to the positivity of the swab for any of the tested viruses (e.g. age, presence of comorbidities, location of admittance) when entered into the multivariate model only an older age of patients retained statistical significance ($P<0.01$) for the prediction of a positive swab. Respiratory infections are characterized by overlapping clinical pictures and the use of a rapid RT-PCR assay allows differential diagnosis of common respiratory viruses. In high risk patients for complications the early identification of the responsible pathogen allows a rapid intervention. As the age was determinant in viruses positivity, it could be useful to extend the rapid molecolar identification of common respiratory viruses in patients over 65 years of age.

PO 079

BENCHMARKING NEXT-GENERATION SEQUENCING METHODS TO INVESTIGATE TTV SPECIES COMPOSITION AND ESTIMATE PREVALENCE

P.G. Spezia[1,2], F. Filippini[1], A. Baj[3,4], F. Novazzi[3,4], A. Genoni[3,4], F.D. Ferrante[3,4], M. Pistello[1,2], F. Maggi[5]

[1] *Department of Translational Research, Retrovirus Center and Virology Section, University of Pisa, Italy*
[2] *Virology Division, Pisa University Hospital, Pisa, Italy*
[3] *Department of Medicine and Surgery, University of Insubria, Varese, Italy*
[4] *Laboratory of Microbiology, ASST dei Sette Laghi, Varese, Italy*
[5] *Laboratory of Virology, Spallanzani National Institute for Infectious Diseases, Rome, Italy*

Aim of the Study: In recent years, there has been a growing interest in the use of Torquetenovirus (TTV), the major component of the human virome, for the prediction of post-transplant complications such as severe infections or graft rejection. The complete genetic characterization of TTV is poorly understood and constantly evolving due to its high degree of diversity. To overcome this challenge, the aim of the present study is to characterize the TTV species profiles in transplanted and healthy individuals by comparing the gold standard method of metagenomic next- generation sequencing (mNGS) with a newly developed TTV amplicon-based NGS (TTV-NGS).
The final objective is to assess the complexity and diversity of the TTV virome in the study population.

Methods used: To compare two different NGS approaches, the gold standard mNGS and our in- house TTV-amplicon based approach, 34 plasma samples (10 liver transplant, 10 kidney transplant, 14 healthy) were analyzed. Bioinformatic analysis of sequences was performed using an in-house bioinformatics pipeline.

Results and Conclusions: In healthy and transplanted individuals, a high degree of complexity in TTV species was identified. Compared with mNGS, the TTV-NGS method showed good performance in terms of accuracy and reproducibility. However, the TTV-NGS method showed higher sensitivity than mNGS. It was able to improve TTV classification, especially in samples with low viral load. Finally, the prevalence of other anelloviruses was also evaluated. TTMV (genus Betatorquevirus) was present in most patients. TTMDV (genus Gammatorquevirus) was found only in a few cases. This study represents the first comparative study between different molecular approaches to characterize the TTV-virome present in solid organ transplant recipients and healthy subjects. This novel in-house method TTV-NGS based on amplicon sequencing is very specific for the detection of TTV species and may represent an important and simple tool to study the dynamic viral composition of this so widespread but still poorly understood virus in its enormous genetic variability.

PO 080

MOLECULAR GENOTYPING OF CIRCULATING ENTEROVIRUS IN LAZIO REGION FROM 2017 TO 2022

M. Rueca, C.E.M. Gruber, F. Vairo, F. Forbici, L. Fabeni, E. Girardi, F. Vaia, F. Maggi, M.B. Valli
National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Rome, Italy

Aim of the Study: Enteroviruses (EVs) are the more frequent etiological agent of aseptic meningitis in developed countries. Infection is usually sporadic, but it can cause outbreaks and epidemic events that typically occur during the summer and fall. The genus Enterovirus includes more than 100 genotypes, and occasionally some of them upsurge as emerging pathogens, like EV-71 and EV-D68, which were recently associated with a poliomyelitis-like disease called acute flaccid paralysis/myelitis (AFP/AFM). The EV genome is characterized by a high degree of variability, and little is known about the viral determinants underlying the acquisition of increased virulence and high epidemic potential. In this study, we present genotyping data from EV circulating strains of clinical settings in Lazio region and collected at National Institute for Infectious Diseases "L. Spallanzani" (INMI) from 2017 to 2022.

Methods used: Enterovirus RNA from collected samples (cerebrospinal fluid (CSF) and/or stool specimens) was detected by commercial methods (One-Step qRT-PCR). The EVs species and genotype identification were determined by analyzing the nucleotide partial lengths of the 5'UTR and VP1 respectively, with the web-based, open-access Enterovirus Genotyping Tool Version 0.1.

Results and Conclusion: Over the five years, 1259 specimens from 985 patients were tested at INMI for the diagnosis of EV. Overall, a marked decrease in the number of requests for EV diagnosis was observed in the three-year period 2020-2022 compared to the period 2017-2019, probably due to the effect of the COVID-19 pandemic which involved Italy and the Lazio Region from early 2020. From 63 cases of EV infection (6.4% of those analyzed), 95 samples (7.5% of those tested) were positive. Infections diagnosed at INMI in 2017, 2018, 2019, and 2022 followed a seasonal trend, with a peak in the period between spring and autumn. On the contrary, in the pandemic period (2020-2021), a different distribution of EV cases over the year was noted, concentrated more in the autumn and winter periods. The species was assigned to 52 cases: more than 50% of patients were infected with the B species, approximately 48% were infected with the A species, and only one patient was infected with an EV of the D species. Moreover, for 10 of the strains belonging to B species (67%), the analysis of the partial sequence of the VP1 gene allowed the characterization of the genotype: 20% of the strains were Echovirus 2 (E-2), E-30, and Coxsackievirus B3 (CV-B3), with a percentage of 10% for CV-A9, E-21, and E-13. Instead, with regard to the species A, for nine strains (73%) the partial sequence of the VP1 gene allowed the assignment of the genotype: seven samples belonged to the CV-A6 genotype (78%) and two samples to the EV-A71 genotype (22%). It was not possible to assign the genotype to the EV sample belonging to species D, due to the low viral load.
In conclusion, the epidemiological trend of EV infection in the Lazio region has undergone changes due to the SARS-CoV-2 pandemic in the period 2020-2021, which resulted in a modification of its trend in terms of the number of diagnosed cases and seasonality. In 2022, because of the reduction of social distancing measures, the circulation of EVs showed a behavior typical of the pre-pandemic period. EVs belonging to B species are the most prevalent circulating strains, followed by those belonging to A species, among them, two cases belonged to EV-A71.
Further analyses are ongoing in order to better characterize any strain with higher potential pathogenic power and to identify possible recombinant strains by whole genome sequencing.

PO 081

SARS-CoV-2 VARIANTS DETECTION: A MULTI-ISTITUTIONAL EXPERIENCE

E. Serretiello[1], E. Cuscianna[2], A. Smimmo[1], S. Cantore[1], U. Trama[3], A. Ballini[1,4], L. Santacroce[5], M. Di Domenico[1]

[1] *Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy*
[2] *Coordinator, USCA ASM Matera, Italy*
[3] *General Direction for Health Protection and Coordination of the Campania Regional Health System, Naples, Italy*
[4] *Department of Clinical and experimental Medicine of Foggia, Italy*
[5] *Interdisciplinary Department of Medicine, Microbiology and Virology Unit, School of Medicine, University of Bari, Italy*

Aim of the Study: During the global spread of the COVID-19, genetic variants of the SARS-CoV-2 virus have emerged. Several variants have increased transmissibility or could exhibit an increased propensity for escape from host immunity, and consequently cause an increased risk to global public health (ref(s). *CDC-. COVID-19: SARS-CoV-2 variant classifications and definitions-2023; Happi et al. 2021*). In the present study, in population of 137 positive samples the molecular epidemiology of SARS-CoV-2 was monitored in order to track the circulation of dominant variants (June-September 2021), providing data on its breakthrough and rapid spread, in the province of Matera (Italian Region of Basilicata), with the collaboration of the University Campania "Luigi Vanvitelli" - Laboratory Medicine Unit.

Methods used: Total RNAs were extracted from nasopharyngeal swabs by using MagMAX Viral RNA Isolation Kit (Applied biosystems by life technologies), followed by Real-Time Polimerase Chain Reaction (qPCR). The qPCR was perfomed by GenFinder COVID-19 Plus RealAmpKit (by GeneFinder "SMART Solution for Better Life") and cDNA synthesis with SuperScript Vilo cDNA synthesis kit (Invitrogen by Thermo Fisher Scientific). Lineages were assigned from alignment file using Ion Chef System Ion Torrent™ (Life technologies) an authomatic library, and sequencing by Ion GeneStudio S5 S5-0465 Semiconductor Sequencer (Thermo Fisher Scientific), according to the manufacturer's protocol. Individual patient consent was not required by the committee for the use of these samples for sequencing as a part of the public health emergency response.

Results and Conclusions: By analyzing samples, were derived some relevant observations concerning the lineages of SARS-CoV-2 in infected subjects. SARS-CoV-2 lineages belong to percentage (%) different variants, as B. 1.1.7 alpha (English) in the 35,7 %, and to P.1 Gamma (Brazilian) for 1,5%, B.1.617.2 (Delta) for 57,6%, AY7.2 (Delta-Like) 2,2% and B1.177 Spanish (3%) of the investigated sample (137 subjects).
Novel SARS-CoV-2 variants will emerge through the time (ref. Charitos et al. 2020). Specific mutation profiles deserve further investigations to clarify potential effects on vaccination efficacy. The epidemiological profile of the variants we provided here is valuable for the comparative study on the infectivity of other emerging variants and then evaluating their epidemic risks in the future.

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PO 082

IMPLEMENTATION AND DIAGNOSTIC PERFORMANCE OF A RAPID SARS-CoV-2 AND FLU A/B ANTIGENIC SCREENING AT EMERGENCY DEPARTMENT ADMISSION BY A POINT-OF-CARE ASSAY

F. Panvini[1], G. Mammoliti[2], A. Vagnini[1], L. Ferretti[1], V. Preti[2], A. Voza[2,3], G. Da Rin[1], V. Cento[1,3]

[1] *Clinical Analysis Laboratory, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy*
[2] *Emergency Department, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy*
[3] *Department of Biomedical Sciences, Humanitas University, Pieve Emanuele, Milan, Italy*

Aim: A rapid and accurate discrimination of SARS-CoV-2 and Flu A/B positive patients at Emergency Department (ED) admittance is critical to provide safe and effective patient's management, and optimize triage procedures. We analyzed the diagnostic performance of a point-of-care rapid antigenic assay used as ED entry screening for symptomatic patients.

Methods: Nasal swabs from consecutive patients presenting at the ED of IRCCS Humanitas Research Hospital from December 2022 to March 2023 with respiratory symptoms were prospectively processed with STANDARD F COVID-19 FLU Ag FIA (SD Biosensor). Diagnostic performance of the point-of-care test (POCT) was assessed by comparison with the IV generation LIAISON® SARS-CoV-2 Ag test (Diasorin) as reference test in a subset of patients. POCT test was performed by ED triage personnel, and results were validated by the Central Laboratory staff.

Results: From December 12th, 2022 to March 30th, 2023 a total of 527 swabs were processed by triage personnel at ED access. Of these, 110 (20.9%) resulted positive for at least one targeted virus, with individual prevalence at 10.1% (n=53) for SARS-CoV-2, 5.9% (n=31) for Flu-A, and 6.3% (n=33) for Flu-B; 21 swabs (4.0%) gave an invalid result on the first attempt, but were all successfully resolved upon the second. Overall, 86.1% (n=454) symptomatic patients included in the study avoided entrance in the COVID-19 triage area by rapid assessment of negativity by the POCT assay, compatible with triage workflow. Indeed, the time-to-result for the POCT test was of 20 minutes since sampling (with 15 minutes of instrumental processing). This was significantly shorter than the time-to-result for the laboratory-based assay, that needed a median (IQR) of 1.8 (1.4-2.2) hours for the 157 samples analyzed with both assays ($p<0.001$), thereby requiring symptomatic patients to access the COVID-19 triage area while waiting for laboratory-based antigen test result.
Sensitivity of POCT for the 23 lab-based antigen-positive cases was 81.0% (95% CI: 73.8-86.6). Among the 136 lab-based antigen-negative samples, targeted viral infections were excluded by the triage POCT with 97.1% (95% CI: 92.7-99) specificity. Positive Predictive Value and Negative Predictive Value were 81.0% and 97.1% respectively.

Conclusions: Rapid SARS-CoV-2 and Flu A/B antigen screening at ED access by a laboratory-monitored POCT can significantly shorten triage waiting time for symptomatic patients, optimizing resources and improving patients' safety by avoiding access to COVID-19 waiting-areas in 86.1% of cases.

PO 083

DETECTION OF CIRCULATING HUMAN PAPILLOMAVIRUS (HPV) DNA IN PLASMA AND SALIVA OF PATIENTS WITH HEAD AND NECK CANCER

R.C. Njoku[1,2], M. Martinelli[1], C. Giubbi[1], F. Perdoni[1], C. Gurizzan[3], C. Paduano[3], L. Lorini[3], D. Smussi[3], C. Assoni[3], P. Bossi[3], C.E. Cocuzza[1]

[1] *Department of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy*
[2] *Department of Biomedical Science, University of Sassari, Italy*
[3] *Division of Medical Oncology, Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, University of Brescia, ASST Spedali Civili, Brescia, Italy*

Aim: This study aims to investigate the presence of circulating Human Papillomavirus (HPV) DNA in liquid biopsies (plasma and saliva) and its correlation with tumor relapse in patients with a recent histological diagnosis of HPV-positive oropharyngeal squamous cell carcinoma (OPC).

Methods: Blood and saliva samples were collected from patients with locally advanced and histologically confirmed HPV-associated OPC at diagnosis and at 3, 6, 12, and 18 months post-treatment follow-ups, as part of an ongoing longitudinal study (Identify Study). Blood samples were collected using Cell-Free DNA BCT® tubes (Streck) and saliva samples were collected using FLOQSwab resuspended in 1 mL eNAT (Copan). Nucleic acid extraction was performed using NucliSENS easyMAG (bioMérieux) and HPV detection using OncoPredict HPV assay (Hiantis), allowing viral load determination of 12 high-risk HPV genotypes.

Results and Conclusions: Samples collected at baseline from 18 patients with OPC (13 p16-Pos; 5 -Neg) have presently been analyzed. Data from p16-Pos patients showed HPV DNA positivity in 77% (10/13) of plasma and saliva samples, respectively with 7 (7/13; 54%) being positive in both sample types. One (1/5) p16-Neg patient (BS019) was HPV positive in plasma and showed a co-infection in saliva. HPV16 was the most prevalent genotype and multiple high-risk HPV infections were detected only in three saliva samples. Circulating HPV viral loads ranged from 7.77 to 6,200 viral copies/mL in positive patients.
In conclusion, preliminary results reported that HPV DNA has been detected in 92.3% of plasma and/or saliva samples of patients with p16-Pos OPC. HPV positivity in both plasma and saliva was found in one p16 negative patient. Analysis of samples collected at follow-up visits will allow to better understand the role of HPV DNA detection as a possible biomarker for recurrent disease.

Patient ID	Tissue	Plasma (Molecular method)		Saliva (Molecular method)
	Immunohistochemical detection (p16)	HPV genotype	Copies/mL	HPV genotype
BS008	POS	NEG	NEG	HPV16
BS013	POS	NEG	NEG	HPV16, HPV45, HPV35
BS018	POS	HPV16	5,13E+03	NEG
BS019	NEG	HPV16	7,77E+00	HPV16, HPV51
BS020	POS	NEG	NEG	HPV16
BS023	POS	HPV16	8,60E+00	HPV16
BS024	POS	HPV16	5,51E+01	NE
BS025	NEG	NEG	NEG	NE
BS026	NEG	NEG	NEG	NE
BS031	POS	HPV16	1,31E+03	HPV16
BS038	NEG	NEG	NEG	NE
BS039	POS	HPV16	3,45E+01	HPV16
BS043	POS	HPV16	1,52E+03	HPV16
BS044	POS	HPV33	6,32E+01	NE
BS052	NEG	NEG	NEG	NE
BS053	POS	HPV16	6,20E+03	HPV16, HPV58
BS058	POS	HPV33	1,28E+02	HPV 33
BS062	POS	NEG	NEG	NEG

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PO 084 - OC 16

A TRUNCATED ISOFORM OF CYCLIN T1 COULD CONTRIBUTE TO THE NON-PERMISSIVE HIV-1 PHENOTYPE OF U937 PROMONOCYTIC *MINUS* CELLS

G. Forlani[1], T. Alberio[2], M. Shallak[1], K. Shaik[1], M. Fasano[2], R. Accolla[1]

[1] *Laboratories of General Pathology and Immunology "Giovanna Tosi", Department of Medicine and Surgery, University of Insubria, Varese, Italy*

[2] *Laboratory of Biochemistry and Functional Proteomics, Department of Science and High Technology, University of Insubria, Busto Arsizio, Varese, Italy*

Background: Previous studies identified clones of the U937 promonocytic cell line that were either permissive *(Plus)* or nonpermissive (*Minus*) for human immunodeficiency virus type 1 (HIV-1) replication. This different phenotype has been associated with the expression of both CIITA and TRIM22 restriction factors. CIITA was expressed only in HLA-II-positive U937 *Minus* cells, and this was strictly correlated with inhibition of Tat-dependent HIV-1 LTR transactivation in *Minus* but not in *Plus* cells. Overexpression of CIITA in *Plus* cells (*Plus-CIITA*) restored the suppression of Tat transactivation, confirming the inhibitory role of CIITA. By contrast TRIM22 is induced by interferon gamma in U937 *Minus* cells and significantly impairs HIV-1 replication, by interfering with Tat- and NF-κB-independent Long Terminal Repeats (LTR)-driven transcription. Moreover, TRIM22 interacted with CIITA and promoted recruitment of the complex into nuclear bodies containing also PML and Cyclin T1, a crucial component with CDK9 of the PTEFb elongation factor, necessary to elongate HIV-1 primary transcripts.

Methods: Expression pattern of Cyclin T1 proteoforms in U937 *Minus*, U937 *Plus, U937 Plus-CIITA* cells and Jurkat T cells was assessed by western blotting with a rabbit polyclonal anti- Cyclin T1 antibody. Cyclin T1 was immunoprecitated from *U937 Minus* and *Plus* cells, then resolved by SDS-PAGE finally stained with Coomassie Brilliant Blue. Each band was subjected to an *in situ* trypsin digestion and the peptide mixtures were analyzed by nano-Liquid Chromatography-tandem Mass Spectrometry (nano-LC-MS/MS). Protein identification was obtained by Mascot query. Intracellular distribution of Cyclin T1 was investigated by immunofluorescence and confocal microscopy analysis in the two U937 clones. Gene expression pattern of Cyclin T1 in U937 *Plus* and *Minus* cells was assessed by RT-PCR using specific primers. Both U937 *Minus* and *Plus* cells were treated with MG132 or chloroquine or Interferon gamma at different time points and the protein pattern of Cyclin T1 was analysed by western blotting.

Results: A 50 kDa truncated isoform of Cyclin T1 was expressed in *Minus* but not in *Plus* cells, in addition to the canonical 80 kDa isoform. Gene expression analysis of Cyclin T1 mRNA demonstrated that the 50 kDa Cyclin T1 isoform did not result from a specific splicing event. Mass spectrometry analysis after Cyclin T1 immunoprecipitation demonstrated that the protein band of 50 kDa is enriched in N- terminal peptides of Cyclin T1 (to aminoacid 481). Treatment of U937 *Minus* cells with proteasome inhibitor MG132 or lysosome inhibitor chloroquine did not affect the expression level of 50 kDa protein suggesting that this Cyclin T1 isoform is not a product of protein degradation but of a specific peptidase cleavage. Interestingly treatment of U937 *Minus* cells with interferon gamma (IFNγ) significantly reduced the amount of 50 kDa Cyclin T1 proteoform.

Conclusions: The truncated Cyclin T1 proteoform, specifically expressed in U937 *Minus* cell clones, might contribute to the non-permissive HIV-1 phenotype of *Minus* cells by acting as dominant negative factor of the canonical 80 kDa proteoform, thus affecting the formation and the activity of PTEF-b complex. The observation that IFNγ treatment significantly reduced the 50 kDa Cyclin T1 proteoform might indicate the existence of a regulatory loop whereby the dominant negative function of the 50 kDa Cyclin T1 is now taken over by the excess of CIITA induced by IFNγ. CIITA, by binding to the canonical form of Cyclin T1, may displace it from the PTEF-b complex resulting again in stronger inhibition of HIV-1 replication.

PO 085 - OC 17

EXTENSIVE VIROLOGIC CHARACTERIZATION IN AN HIV-INFECTED INDIVIDUAL WITH APPARENT HIV REMISSION FOR 2 YEARS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION WITH CCR5 WILD-TYPE CELLS: A CASE STUDY

I. Vicenti[1], S. Piemontese[2], L. Fiaschi[1], C. Biba[1], A. Cervo[3], I. Varasi[1], F. Saladini[1], G. Leonardi[4], C.F. Perno[5], C. Mussini[6], M. Zazzi[1], V. Borghi[6]

[1] *Department of Medical Biotechnologies, University of Siena, Italy*
[2] *Hematology and Bone Marrow Transplantation Unit, IRCCS San Raffaele Hospital, Milan, Italy*
[3] *Infectious Diseases Unit, Department of Health Promotion Sciences and Mother and Child Care "Giuseppe D'Alessandro" (PROMISE), University of Palermo, Italy*
[4] *Hematology Unit and Chair, Department of Medical and Surgical Sciences, Azienda Ospedaliera Universitaria di Modena, University of Modena and Reggio Emilia, Modena, Italy*
[5] *Multimodal Research Area, Unit of Microbiology and Diagnostics in Immunology, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy*
[6] *U.O. of infectious Diseases, University of Modena and Reggio Emilia, Modena, Italy*

Background and Aim: Allogenic hematopoietic stem cell transplantation (allo-HSCT) with homozygous CCR5Δ32 donor cells is the only potentially HIV-1 curative intervention. However, allo-HSCT has been associated with a drastic reduction in the HIV reservoir independently of engraftment with CCR5Δ32 or CCR5 *wild-type* cells. In this study, we investigated *ex-vivo* the reservoir of an HIV-1 infected individual with no evidence of infection at 2 years after allo-HSCT with wild-type CCR5 genotype.

Case Report: The study participant was an adult male, HIV-1 infected since 1994 with 5 antiretroviral class multidrug resistance strain and under treatment with ART. On January 2020, at the age of 58, he received an allo-HSCT from an HLA-identical sibling donor for a Hodgkin lymphoma in second complete remission after 24 cycles of therapy with the anti- PD1 pembrolizumab. The patient received a reduced toxicity conditioning regimen, based on fludarabine and treosulfan at myeloablative dosage, and was discharge on day 47 after transplant without major complications. He never developed graft-versus-host disease, neither acute nor chronic. Despite positive HIV-1 serology, he maintained undetectable viremia and he was negative for HIV-1 DNA by routine diagnostic analysis. In March 2022 CD4+ T cells were isolated after leukapheresis and maintained in culture to determine:

1) the CCR5 genotype by Sanger sequencing,
2) the amount of cell-associated HIV-1 DNA (CAD) and cell-associated HIV-1 RNA (CAR) by digital droplet PCR at baseline and after ex-vivo stimulation with ionomycin (ION, 1 μg/ml) plus phorbol-myristate-acetate (PMA, 50 ng/ml) using a protocol previously published. Briefly, 2-days post induction (dpi) 4 x 10^7 patient derived CD4+ were co-cultivated with MOLT-4 CCR5, a cell line permissive for HIV-1 infection. CAR and CAD were quantified at baseline (T_0) and 2-7-14-21 dpi (T_2, T_7, T_{14}, T_{21}). At T_7, T_{14} and T_{21}, the infectivity of the CD4+T/MOLT-4 CCR5 co-cultures was evaluated using a modified TZM-bl based assay (TZA) protocol. In addition, a positive control of 1.2 x 10^6 CD4+ T cells, deriving from an HIV-1 positive, virologically suppressed patient was analysed in parallel.

Results: DNA sequencing confirmed a wild type homozygous CCR5. CAR and CAD were negative at baseline and at all time points analysed. The CD4+T/MOLT-4 CCR5 co-cultures were not infectious in TZA at T_7, T_{14} and T_{21}. The HIV-1 positive control was quantifiable in CAD at T_7, T_{14} and T_{21} with 931.2±400.1, 68.9±10.1, 128.6±15.3 HIV-1 copies per 10^6 CD4+ T cells respectively and in TZA at T_{21} with 16.1 [5.4-47.8] IUPM/cells.

Conclusions: Two years after *wild-type* CCR5 allo-HSCT, the patient remained without measurable or inducible HIV-1 DNA and RNA in the blood compartment by multiple ultrasensitive assays on a total of 40 million CD4+ T cells. However, the presence of the virus in other tissues was unexplored. Ultimately, only analytical treatment interruption may reveal whether full remission has been achieved.

PO 086 - OC 18

IDENTIFICATION OF THE MINIMAL FUNCTIONAL EPITOPE OF THE HIV-1 MATRIX PROTEIN ENDOWED WITH ANGIOGENIC ACTIVITY VIA COMMON BETA CHAIN RECEPTOR SIGNALING

M. Uggeri[1], F. Caccuri[1], P. D'Ursi[2], A. Bugatti[1], A. Zani[1], F. Filippini[1], M. Salmona[3], D. Ribatti[4], M. Slevin[5], A. Orro[2], W. Lu[6], P. Liò[7], R.C. Gallo[6], A. Caruso[1]

[1] *University of Brescia Medical School, Brescia, Italy*
[2] *National Research Council, Segrate, Milan, Italy*
[3] *Istituti di Ricovero e Cura a Carattere Assistenziale Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy*
[4] *University of Bari Medical School, Bari, Italy*
[5] *Manchester Metropolitan University, M15GD Manchester, United Kingdom*
[6] *University of Maryland, Baltimore, MD 21201, USA*
[7] *University of Cambridge, CB3 0FD Cambridge, United Kingdom*

Aim of the Study: Human Immunodeficiency Virus type 1 (HIV-1) is derived from Simian Immunodeficiency Virus (SIV) of chimpanzee (SIVcpz) and gorilla (SIVgor), while HIV-2, a less pandemic variant, derived from SIV of the sooty mangabey (SIVsmm). All HIVs and SIVs translate for a conserved matrix protein called p17. It is one of the earliest proteins synthesized during the virus replication cycle and it plays a significant role in virus assembly. Moreover, p17 is also endowed with proangiogenic activity (F. Caccuri *et al.* 2012) and it is a promising target for the development of antiviral drugs. This study aimed to identify the minimal functional epitope promoting angiogenesis in the p17 HIV-1 protein and to study its evolution starting from monkeys and apes to humans.

Methods: Eight partially overlapping p17-derived peptides (F1-F8) were produced and tested *in vitro* on endothelial cells (ECs) for their angiogenic activity. Then, to determine the phylogenetic relationship among p17 sequences of humans, apes, and monkeys, *in silico* analyses were performed on a dataset of 33220.

Results and Conclusions: *In vitro* data highlighted a potent angiogenic activity of the p17-derived peptide F3. Interestingly, it is homologous to the N-terminal region of the human erythropoietin (EPO), a well-known proangiogenic protein that activates the beta common chain receptor (βCR) signaling (D. Ribatti *et al.* 1999). Based on this homology, two additional peptides were designed: 8-18 EPO and 37-52 F3 p17 (F3S). Both peptides were found to promote angiogenesis via βCR. It is known that HIV-1 and HIV-2 share an epitope endowed with CXCRs-mediated angiogenic activity (F. Caccuri et al. 2014), thus F3Ss peptides derived from HIV-2, SIVcpz, SIVgor, and SIVsmm were designed based on the most representative amino acidic sequence of each virus and tested. Interestingly, we found that, other than the HIV-1 F3S, only the SIVcpz and SIVgor F3Ss induce angiogenesis on ECs. So, to follow the evolution of the F3S peptide, the p17 protein sequences derived from HIVs and SIVs from cpz, gor, smm, and other Old-World Monkey (OWM) were retrieved from the Los Alamos HIV database (HIV.lanl.gov) and the Uniprot database (https://www.uniprot.org/), aligned, and clustered focusing on the F3S peptide, obtaining 608 sequences. These were used to implement a phylogenic tree that showed a well-defined grouping in three branches:

1) HIV-1/SIVcpz-gor
2) HIV-2/SIVsmm
3) OWM SIV. Interestingly, one SIVcpz sequence clustered with the OWM SIV branch, evolutionarily connecting the HIV-1 and the OWM SIV.

Overall, results highlighted that the active F3S fragment is unique and specific for HIV-1, according to biological data. Moreover, the minimal functional epitope of F3S was identified by dividing the peptide into three specifically designed subfragments (S1, S2, and S3). The phylogenetical analysis identified S2 as the evolutionary switch on/off component that drives the angiogenic activity of the F3S fragment, and it was confirmed by *in vitro* assays.

In conclusion, the present study allowed us to discover the pathway of the βCR as the one responsible for F3S peptide-induced angiogenic activity and to identify the F3S minimal active moiety.

PO 087 - OC 19

CHARACTERIZATION AND ROLE OF HIV-DNA MINORITY MUTATIONS IN PAEDIATRIC PATIENTS COHORT WITH A LONG HISTORY OF INFECTION

L. Colagrossi[1], S. Bernardi[2], R. Scutari[3,4], V. Fini[1], K. Yu La Rosa[1], A. Granaglia[1], L. Forqué Rodriguez[1], L. Coltella[1], S. Ranno[1], G. Linardos[1], V.C. Di Maio[1], L. Gentile[1], M. Di Giuseppe[2], F. Leone[2], C. Russo[1], C.F. Perno[1]

[1] *Microbiology and Diagnostic Immunology Unit, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy*
[2] *Unit of Immune and Infectious Disease, University Department of Pediatrics DPUO, Bambino Gesù Children's Hospital, Rome, Italy*
[3] *Multimodal Research Area, Microbiology and Diagnostics of Immunology Unit, Bambino Gesù Children Hospital IRCCS, Rome, Italy*
[4] *Department of Oncology and Hemato-Oncology, University of Milan, Italy*

Background and Aim: Next Generation Sequencing (NGS) has changed the paradigm of HIV-1 drug resistance monitoring mostly in individuals with a long history of treatment, such as paediatric patients (pts). The aim of the study was to better understand the impact of HIV-1 minority mutations to guide the best personalize antiretroviral treatment (ART).

Materials and Methods: An observational study was carried out during the period from August 2022 to March 2023 at IRCCS Bambino Gesù Children's Hospital among 18 paediatric/young adults pts with HIV-1 diagnosis, of them three received a first HIV diagnosis within this period. The HIV-RNA and total HIV-DNA were determined by Xpert HIV-1 (Cepheid) and droplet digital PCR (ddPCR) technique, respectively. NGS was performed on HIV-1 positive samples using DeepChek Assay (ABL diagnostics). The presence of minority resistance mutations (mRMs) (frequency of 5-20%) and signature APOBEC-related mutations (APO-Ms) was evaluated through the DeepChek and HIVdb Stanford tools.

Results: The enrolled pts were mainly female (10,55.6%) with a median (IQR) age of 18 (11-24) years. All pts were followed in our hospital since the first diagnosis, three of them received a HIV-1 diagnosis during the analysis period and thus were naïve-treated at the enrolment. Fourteen (77.8%) pts had an undetectable or <200 cp/mL of HIV-RNA, the remaining 4 pts, had a median (IQR) HIV-RNA of 43272(16373-488497) cp/mL. The HIV-DNA load was detectable for all tested pts with a median (IQR) of 1435(553-3503) copies/10^6 CD4+T-cells.
Nine pts (50%) received an early ART with a median duration of 10 years (IQR 6-18). The majority of pts (16,88.9%) had received Nucleoside Reverse Transcriptase inhibitors (NRTi) and Integrase inhibitor (INI) regimen, 16.7% (N=3) Protease Inhibitors (PI) regimen and 11.1% (N=2) Non-Nucleoside Reverse Transcriptase inhibitors (NNRTi) regimen.
The strains obtained were B (6, 33.3%), A (4, 22.2%), CRF02_AG (4, 22.2%), C (3, 16.7%) and CRF02_AE (1, 5.6%).
Looking at drug resistance mutation in HIV-DNA, 10 (55.6%) pts had at least one HIV-1 major RMs, in particular the NRTI, NNRTI and PI resistance were present in 27.8%, 22.2% and 5.6% of cases, respectively. Eleven (61.1%) pts were detected to harbour at least one HIV-1 mRMs, mainly localized in RT region followed by Integrase and Protease regions. Half of patients showed at least one APO-Mutation and only one APO-related stop codon at a frequency ranging 3-56%.

Conclusions: This study demonstrated that despite the prolonged and in some cases early treatment the HIV-1 reservoir results in minority and APOBEC-related mutations. However, the role of minority variants in the context of personalized therapy remains unclear, also considering the historical HIV resistance test. Further analysis is needed to understand whether these mutations are the evolutionary adaptation of the virus under pharmacological pressure that could affect viral fitness.

PO 088 - OC 20

CHARACTERIZATION OF TYPE I/III INTERFERON RESPONSE TO MPXV, HIV AND HPV INFECTIONS IN ANAL MUCOSA OF MSM

M. Fracella[1], L. Maddaloni[2], A. D'Auria[1], F. Frasca[1], L. Santinelli[2], C. Bitossi[1], N. Cavallari[2], P. Mazzetti[3], A. Pierangeli[1], C.M. Mastroianni[2], M. Pistello[3], G. Antonelli[1], G. D'Ettorre[2], C. Scagnolari[1]

[1] Virology Laboratory, Department of Molecular Medicine, "Sapienza" University of Rome, Italy
[2] Department of Public Health and Infectious Diseases, "Sapienza" University of Rome, Italy
[3] Virology Section and Retrovirus Centre, Department of Biomedicine, University of Pisa, Italy

Background: The recent outbreak of monkeypox virus (MPXV) in non-endemic countries and its biological implications on human health have aroused wide concern. The possibility of MPXV sexual transmission is being investigated, as transmission rate appears to be elevated in men who have sex with men (MSM) and has been associated with unexpected anal and genital lesions. Previous studies have reported that poxviruses use a multitude of strategies to disable host innate immunity, including Interferon (IFN) response. It is well documented that sexually transmitted infections (STIs) modify the mucosal environment; notably, HIV may alter the epithelial integrity, favoring HPV infection on basal cells, where it dysregulates cell division and local immunity. To gain insight into the mucosal immunity to MPXV, in comparison with HIV and HPV infections, we investigated the expression levels of type I and III IFNs related genes in anal cells from MSM.

Material and Methods: Forty-five anal canal brushing samples were collected from MSM patients attending a proctology clinic. Detection of HPV and MPXV DNA was performed by RT-PCR. From purified cellular RNA, transcripts of genes coding for type I IFNs (α2A, β) and their receptor subunits (IFNAR1/-2), for type III IFNs (λ1 to 3) and their receptor subunit (IL28R1) and for ISGs (ISG15 and ISG56) were measured by quantitative RT-PCR assays and normalized to the housekeeping GUS gene (the 2^−ΔCt method).

Results and Conclusion: Five MPXV(+) men (that were HIV and HPV negative), 10 HIV(+)/HPV(-) men, 10 HPV(+)/HIV(-) men, 10 HIV(+)/HPV(+) men and 10 healthy controls (HC) were enrolled in this study. Comparing groups, IFN-AR1, IFN-AR2, ISG15 and ISG56 [$p<0.05$; *Kruskal-Wallis* (KW) test)] were highly expressed in MPXV(+) patients, while IFN-λ3 ($p< 0.001$ KW test) was drastically down-regulated. MPXV group also showed lower levels of IFN-λ1 mRNA [$p<0.05$; *Mann-Whitney* (MW) test] compared to HIV(+)/HPV(-) and HIV(+)/HPV(+) men. Moreover, IFN-α2A and IFN-β ($p<0.05$; MW test) decreased in MPXV(+) patients compared to HC. Comparing HIV(+)/HPV(+) men and HC, IFN-β and IFN-λ2 ($p<0.05$; MW test) were less expressed in co-infected patients. In addition, HPV(+)/HIV(-) patients had lower levels of IFN-α2A and IFN-β mRNA as opposed to other groups ($p<0.05$; KW test). Our investigations indicated a differential and dysregulated expression of IFNs and ISGs genes in anal cells of MPXV(+) patients, supporting the notion that down-regulation of type III IFNs with the activation of heterogeneous patterns of IFN expression, including ISG15 and ISG56, may be associated to disease severity. Also, our investigations on HPV(+)/HIV(+) co-infected men reported a significant downregulation of IFN genes that may facilitate both HIV spread to adjacent cells and HPV persistence. Hence, clarifying IFN pathway dysregulation in MSM during STIs may help in devising immunotherapeutic strategies to limit the risk of HPV/HIV-related anal cancer and MPXV disease severity.

PO 089

ACUTE ENCEPHALITIS IN PRIMARY HIV INFECTION: A CASE REPORT

D. Basile[1], A. Zignoli[2], M. Fezzi[3], I. Cerbaro[1], F. Onelia[1], M. Pascarella[1], G. Battagin[3], V. Manfrin[3], M. Rassu[1]

[1]U.O.C. Microbiologia Ospedale San Bortolo Vicenza, Italy
[2]Dipartimento di Medicina Molecolare, Università degli Studi di Padova, Italy
[3]U.O.C. Malattie Infettive Ospedale San Bortolo Vicenza, Italy

Aim of the Sudy: HIV invasion of the central nervous system (CNS) occurs early, in the 'window-phase' when serological assays are still negative (1). Following infection with HIV, monocytes migrate to the brain, enabling HIV to gain access to the CNS. Direct damage of CNS tissue due to HIV and active immune response to infection have been implicated (2). Acute Encephalitis in primary HIV infection is a rare but life-threatening condition.
We report a case of Acute Encephalitis that occurred in a newly diagnosed HIV-infected patient hospitalized at "San Bortolo Hospital - ULSS8 Berica" in Vicenza, Italy.

Methods: Elecsys HIV Duo Cobas® (ECLIA) detects the presence of antibodies against HIV-1/2 and the p24-Antigen of HIV-1. Positive results are confirmed on the VIDAS® HIV (4th generation ELFA). Bio-Rad Geenius™ HIV 1/2 Confirmatory Assay is a second level test (immunochromatographic) that detects antibodies against p31, gp160, p24, gp41 from HIV-1 and gp36, gp140 from HIV-2. Molecular diagnosis is performed using the Cobas® 6800 system and the one-step method HIV1 ELITe MGB® on ELITe® InGenius system. Cerebral spinal fluid (CSF) was tested with Biofire® Filmarray® Meningitis Panel, bacterial culture and also HIV1 ELITe MGB® on ELITe® InGenius.

Results and Conclusions: A 37 years old man came on the 19th of March at our hospital (San Bortolo-ULSS8) in Vicenza and was hospitalized for fever, dyspnea, desaturation, confusion and cognitive-motor slowing. He had been living in prison since the beginning of March with a history of cocaine and injection drug use. He was first tested in prison (14th of March) for Quantiferon (positive) and HIV serology ("reactive serum"). No more tests were done for confirmation until hospitalization. At hospital admission drug intoxication and Acute Renal Failure were suspected. Biochemical parameters were compatible with acute infection (6,2x10⁹/L Leucocytes, 2,95 mg/dL creatinine, hyponatremia, PCR 83 mg/dL and PCT 2 ng/ml): 2 sets of blood culture were collected (with negative result). He was also retested for HIV serology (Cobas®) which resulted positive also with the confirmatory assay (VIDAS®). The second level test (Bio-Rad Geenius™) was negative. On the 20th of March a HIV-RNA PCR was conducted on a blood sample: it detected over 10 million c.v./mL. This result was confirmed on the same sample using HIV1 ELITe MGB® performed on ELITe® InGenius system (12,291,536 c.v./mL). The patient underwent lumbar puncture which showed no cytochemical alterations. Biofire Filmarray® Meningitis Panel and bacterial culture resulted in negative. The CSF was tested for HIV-RNA, with a detectable result (184,168 c.v./mL). The patient was diagnosed with primary HIV-related encephalitis.
He received early antiretroviral therapy: bictegravir, emtricitabine and tenofovir, with a quick improvement of symptoms.
Primary infection from HIV can rarely present as an acute encephalitis. Health professionals should be prepared to recognize it and to take quick care of the patient, in order to reduce viremia.
It is important to get a correct diagnosis: PCR for HIV RNA is fundamental to confirm the diagnostic suspect when the infection is in the "window phase". It is also important to consider HIV as a cause of encephalitis and for the Laboratory to be able to test the CSF for this pathogen.

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PO 090 - OC 21

Nrf2 ACTIVATORS AS INNOVATIVE CELL-TARGETED APPROACHES AGAINST RESPIRATORY VIRUS INFECTIONS

M. De Angelis[1,2], G. Gori Savellini[3], A. Fraternale[4], A. Mai[5], A.T. Palamara[2,6], L. Nencioni[2]

[1] *Dept. of Molecular Medicine, Sapienza University, Rome, Italy*
[2] *Dept. of Public Health and Infectious Diseases, Sapienza University, Rome, Italy*
[3] *Dept. of Medical Biotechnologies, University of Siena, Italy*
[4] *Dept. of Biomolecular Sciences, University Carlo Bo, Urbino, Italy*
[5] *Dept. of Chemistry and Technologies of Drugs, Sapienza University, Rome, Italy*
[6] *Dept. of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy*

Nuclear factor erythroid 2-related factor 2 (Nrf2) is the transcriptional master regulator of antioxidant responses. In presence of oxidative stress, Nrf2 translocates to the nucleus where it binds to the antioxidant-response element (ARE) and initiates the expression of a variety of cytoprotective genes. In addition to antioxidant responses, Nrf2 is involved in the regulation of many other cellular processes, such as metabolism and inflammation. Most respiratory viruses, including influenza virus and coronaviruses, cause oxidative stress in host cells by producing reactive oxygen species (ROS) to maintain a cell microenvironment useful for their replication. Furthermore, several evidence including ours, reported the inhibition of Nrf2 expression upon viral infections to maintain pro-oxidant conditions.
The aim of this study was to evaluate different approaches to activate Nrf2-mediated antioxidant response in respiratory virus infection models and their potential antiviral effects.

Methods: Permissive cell lines (Human epithelial lung cells, A549 and Madin-Darby canine kidney cells, MDCK) were infected with influenza A/Puerto Rico/8/34 H1N1 virus; Human lung fibroblast cells (MRC-5) were infected with seasonal coronavirus (HCoV 229E) and African Monkey Epithelial cells (Vero E6) were infected with SARS-CoV-2. Different compounds able to restore the expression and activation of Nrf2 were used: low molecular weight monothiol (I-152) or dithiols (I-152SdAc); epigenetic modulators, including inhibitors of histone acetyltransferase (HAT) P300 (C-646 and A-485), histone deacetylase (HDAC) 6 (tubastatin), HDAC class I (MS275) and DNA methyltransferase (DMT). Each compound was added at different times post-infection. Viral titration was performed by plaque assay, hemagglutination assay (HAU) or TCID50. Western blot and Real Time PCR were performed to analyse the modulation of antioxidant proteins and the expression of Nrf2-target genes. Glutathione (GSH) production was evaluated by using a colorimetric assay kit.

Results and Conclusions: The results showed that both thiol agents were able to impair the infectivity of influenza virus when added for 24 hours post-infection. The analysis of redox parameters showed a rescue of the antioxidant response in terms of Nrf2-related antioxidant genes expression as well as GSH production in infected treated cells. The same compounds were also able to impair SARS-CoV-2 infection by inhibiting the interaction with ACE2 receptor and to decrease viral replication of both pandemic and seasonal coronaviruses when added post-infection for 24 hours.
The use of epigenetic modulators decreased influenza virus titre and viral protein synthesis at different times post-infection indicating a possible role of iHDAC and iHAT to restore gene expression of intracellular factors modulated by the virus and useful for its replication. Indeed, in infected cells, C-646 treatment restored the acetylation of SIRT2, a down-regulated deacetylase during influenza virus infection, and it rescued the expression and activity of glucose-6-phosphate dehydrogenase (G6PD), a protein involved in GSH restoration. Nrf2 mRNA levels and the expression of its target genes were increased in infected cells treated with both HDAC and HAT inhibitors. The use of iP300 and iHDAC was also able to reduce HCoV 229E replication during a time course of infection.
In conclusion, our data indicate that the activation of Nrf2 pathway leads to restoration of intracellular reducing conditions, thus counteracting respiratory virus replication. This approach may open to innovative cell-targeted strategies characterized by broad-spectrum activity against different viruses.

PO 091 - OC 22

HOST AND VIRAL FACTORS REQUIRED FOR THE BIOGENESIS OF POSITIVE-SENSE SINGLE-STRANDED RNA VIRUS REPLICATION ORGANELLES

D. Stelitano[1], V. Marano[1], S. Tiano[1], G. Bianco[1], Š. Vlachová[1], M. Cortese[1,2]

[1] Telethon Institute of Genetics and Medicine, Pozzuoli, Italy
[2] University of Campania "Luigi Vanvitelli", Caserta, Italy

Upon infection, positive-sense single-stranded RNA (+RNA) viruses remodel the host endomembrane system to create an environment conducive to viral replication. The most striking among the alterations induced by the infection of +RNA viruses is the formation of the viral replication organelle (RO), a specialized membrane-delimited organelle where the viral genome replication takes place. For SARS-CoV-2 the etiological agent of the COVID-19 disease, the viral ROs are composed of double membrane vesicles that originate from and are in contact with the endoplasmic reticulum. Additionally, SARS-CoV-2 infection induces a strong reshaping of all cellular organelles, such as the fragmentation of the Golgi apparatus and the relocalization of the peroxisomes and of the cytoskeleton. The remodeling of the cellular landscape might contribute to the cytopathogenic effect observed during SARS-CoV-2 infection. To date, the host and viral factors and the molecular mechanisms that bring to the formation of the viral ROs and are responsible for the cellular alterations induced by SARS-CoV-2 are still unknown. By integrating imaging and -omics approaches, we have identified potential factors co-opted required for SARS-CoV-2 replication that can provide insights into ROs biogenesis and their role in coordinating the different steps of SARS-CoV-2 replication.

PO 092 - OC 23

THE N-ACYLETHANOLAMINE ACID AMIDASE IS A SARS-CoV-2 HOST FACTOR

V. La Rocca[1,2], R. Fonnesu[2], C. Filipponi[2], E. Iacono[2], V. Diesendorf[3], K. Sewald[4], H. Obernolte[4], G. Freer[2], J. Bodem[3], M. Pistello[2,5], M. Lai[2]

[1] *Institute of Life Sciences, Sant'Anna School of Advanced Studies, Pisa, Italy*
[2] *Retrovirus Center, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Italy*
[3] *Institute of Virology and Immunobiology, Julius-Maximilians-Universität Würzburg, Germany*
[4] *Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, Member of Fraunhofer international Consortium for Anti-Infective Research (iCAIR), Member of the German Center for Lung Research (DZL), Biomedical Research in Endstage and Obstructive Lung Disease (BREATH), Hannover, Germany*
[5] *Virology Unit, Pisa University Hospital, Pisa, Italy*

Introduction: The over-production of pro-inflammatory factors contributes to the high mortality of COVID-19 patients caused by the severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2). As for other positive-stranded-RNA viruses, SARS-CoV-2 modifies the lipid host metabolism to support its replication by boosting lipogenesis and lipid droplet synthesis. In addition, SARS-CoV-2 infection alters bioactive lipids implicated in the inflammatory process. Nuclear factor-κB (NF-κB) is a well-characterized transcription factor that regulates the production of pro-inflammatory mediators. Upon activation, NF-κB translocate to the nucleus and activates the expression of downstream target genes. In SARS-CoV-2 infection, it has been shown that NF-κB drives robust cytokine production and is also required for viral replication.

Aim of the Study: Here, we investigate how the enzyme N-Acylethanolamine acid amidase (NAAA) contributes to SARS-CoV-2 replication. NAAA is a lysosome enzyme that catalyzes the hydrolysis of the anti-inflammatory lipid Palmitoylethanolamide (PEA) into ethanolamine and palmitic acid. PEA exerts its anti-inflammatory action by activating Peroxisome Proliferator-Activated Receptor-α (PPAR-α), leading to the downregulation of NF-κB. In summary, we hypothesize that inhibiting NAAA will lead to NF-κB suppression that ultimately hinders SARS-CoV-2 replication.

Methods: We used genetic and pharmacological approaches to suppress NAAA expression in Huh-7 cells, using CRISPR/Cas9 system and selective inhibitors, respectively. SARS-CoV-2 replication was analysed by qRT-PCR and confocal imaging. Then, data were confirmed by pharmacological inhibit NAAA during SARS-CoV-2 replication *ex-vivo*, using human precision-cut lung slides (PCLS) derived from three donors. To test the activation of the NF-κB pathway, we used the NF-κB Luciferase reporter construct in HEK-293T cells treated or not with NAAA inhibitor.

Results and Conclusions: NAAA genetic ablation suppress SARS-CoV-2 replication by 2 log10 compared to their wild-type counterparts. Moreover, the NAAA inhibitor reduced SARS-CoV-2 replication in cell culture when administered above 30 μM and up to 3 log10 in PCLS. We next tested whether the inhibition of NAAA could affect NF-κB activation through the activation of the transcription factor PPARα. We found that NAAA-/- cells increase PPARα expression by 1.5 folds, oppositely, PPARα expression is kept low in parental-infected cells. As expected, the increased PPARα expression was accompanied by a 1.6-fold reduction in NF-κB activation in Huh-7 NAAA$^{-/-}$ cells and HEK-293T cells treated with NAAA inhibitor at 30 μM. These results show that NAAA is a host factor for SARS-CoV-2 replication and highlights a possible mechanism of action based on the reduction of NF-κB activation during viral replication.

PO 093 - OC 24

ADENO-ASSOCIATED VIRUS TYPE 2 (AAV2) UNCOATING IS A STEPWISE PROCESS AND IS LINKED TO STRUCTURAL REORGANIZATION OF THE NUCLEOLUS

S.O. Sutter[1], A. Lkharrazi[1], E.M. Schraner[1], A.F. Meier[1], K. Michaelson[1], B. Vogt[1], J. Marx[2], H. Büning[2], C. Fraefel[1]

[1] *Institute of Virology, University of Zurich, Switzerland*
[2] *Institute of Experimental Hematology, Hannover Medical School, Hannover, Germany*

Adeno-associated virus type 2 (AAV2) is a small, non-pathogenic, helper virus-dependent parvovirus with a single-stranded DNA genome of approximately 4.7 kb. In absence of a helper virus, AAV2 can integrate its genome site-preferentially into the adeno-associated virus preintegration site (AAVS1) on human chromosome 19 or persist in an episomal form in the nucleus.
While AAV2 capsids have previously been reported to enter the host cell nucleus and accumulate in the nucleolus, both the role of the nucleolus in AAV2 infection, and the viral uncoating mechanism remain elusive. Nucleoli are membrane-less nuclear structures formed by liquid-liquid phase separation and are known to be involved in many cellular functions, such as ribosomal RNA (rRNA) synthesis, ribosome biogenesis, stress response and cell cycle regulation. Many viruses can employ the nucleolus or nucleolar proteins to promote different steps of their life cycle including replication, transcription and assembly. In order to elucidate the properties of the nucleolus during AAV2 infection and to assess viral uncoating on a single cell level, we combined immunofluorescence analysis for detection of intact AAV2 capsids and capsid proteins with fluorescence *in situ* hybridization for detection of AAV2 genomes. The results of our experiments provide evidence that uncoating of AAV2 particles occurs in a stepwise process that is completed in the nucleolus and supported by alteration of the nucleolar structure.

PO 094

THE D405N MUTATION IN THE SPIKE PROTEIN OF SARS-CoV-2 OMICRON BA.5 INHIBITS SPIKE/INTEGRINS INTERACTION AND VIRAL INFECTION OF ACE2-NEGATIVE ENDOTHELIAL CELLS

A. Bugatti[1], F. Filippini[1], S. Messali[1], M. Giovanetti[2,3], C. Ravelli[4], A. Zani[1], M. Bertelli[1], M. Duheric[1], M. Ciccozzi[5], A. Caruso[1], F. Caccuri[1]

[1] *Section of Microbiology, Department of Molecular and Translational Medicine, University of Brescia, Italy*
[2] *Laboratório de Flavivirus, Instituto Oswaldo Cruz, Rio de Janeiro, Rio de Janeiro, Brazil*
[3] *Department of Science and Technology for Humans and the Environment, University of Campus Bio-Medico of Rome, Italy*
[4] *Section of Experimental Oncology and Immunology, Department of Molecular and Translational Medicine, University of Brescia, Italy*
[5] *Unit of Medical Statistics and Molecular Epidemiology, University Campus Bio-Medico of Rome, Italy*

Aim of the Study: SARS-CoV-2 infects ACE2-negative human endothelial cells (ECs) through the interaction between Spike protein and $\alpha v\beta 3$ integrin. This binding occurs through a conserved Arg-Gly-Asp (RGD) motif expressed in the Receptor Binding Domain (RBD) at amino acid positions 403 to 405. After its entry, SARS-CoV-2 promotes a potent pro-angiogenic and inflammatory microenvironment without releasing virions. The RGD motif is peculiar to all pre-Omicron SARS-CoV-2 variants. Interestingly, Omicron BA.2, BA.4 and BA.5 have a dominant D405N mutation in their Spike protein. Here we aimed to understand whether this mutation inhibits the ability of SARS-CoV-2 to interact with integrins and, consequently, the inability of a primary isolate of Omicron BA.5 to infect human pulmonary endothelial cells (HL-mECs), thus preventing virus-induced endothelial cell dysfunction.

Methods used: To determinate the percentage of SARS-CoV-2 isolates in the world that carry the D405N mutation, 6.910.627 million full-length SARS-CoV-2 sequences were aligned using the ViralMSA tool, and IQ-TREE2 was used for phylogenetic analysis following the maximum likelihood (ML) approach. The Surface Plasmon Resonance (SPR) assay was used to evaluate the direct binding of Omicron BA.5 RBD to the $\alpha v\beta 3$ integrin. SARS-CoV-2 entry into HL-mECs was scrutinized by immunofluorescence and qRT-PCR and tube formation assays were performed to analyze infected-HL-mECs pro-angiogenic functions.

Results and Conclusions: In this study, we show how the RGD motif was well conserved in all pre-Omicron SARS-CoV-2 lineages, while the D405N mutation occurred and became stable in May 2021, with the emergence of the Omicron BA.2 lineage. SPR analysis showed that Omicron BA.5 RBD, which carries the D405N mutation, completely lost the ability to bind the $\alpha v\beta 3$ integrin. The lack of interaction with integrins prevents Omicron BA.5 isolate entry into HL-mECs cells by blocking their pro-angiogenic functions. In conclusion, SARS-CoV-2 variants with the D405N mutation lost the ability to infect ACE-2-negative ECs, thus suggesting how emerging variants of SARS-CoV-2 could represent an evolution of the virus capable of giving rise to less severe pathological manifestations than those observed with previous variants.

PO 095

BREAST MILK FROM COVID-19 NEGATIVE LACTATING MOTHERS SHOWS NEUTRALIZING ACTIVITY AGAINST SARS-CoV-2

L. Signorini[1], D. Morniroli[2], M. Dolci[1], G. Vizzari[3], A. Ronchi[3], C. Pietrasanta[2], L. Pugni[3], F. Mosca[2], S. Delbue[1], M.L. Gianni[2]

[1] *Department of Biomedical, Surgical and Dental Sciences, University of Milan, Italy*
[2] *Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy and Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy*
[3] *Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, NICU, Milan, Italy*

Introduction: Immunity transfer through human milk is a key element in the infant's developing immunity. Recent studies have demonstrated the presence of antibodies against SARS-CoV-2 in human breast milk (HBM). Furthermore, it is known that aspecific antiviral activity not related to the presence of antibodies, is reasonably present in the HBM of all lactating women. The aim of the study was to detect the presence and magnitude of the neutralizing activity against SARS-CoV-2 of HBM from infected and previously infected lactating mothers (group A and B) and from mothers who never experienced SARS-CoV-2 infection (group C).

Materials and Methods: Forty-two women were enrolled, 11 in group A, 5 in group B and 26 in group C. Milk samples were collected 2 days (T0 - colostrum), 7 days (T1 - transition milk), 20 days (T2 - mature milk) and 30 days (T3) after the delivery. The SARS-CoV-2 50% neutralizing endpoint (NT_{50}) titers were determined at each time point, based on a microneutralization assay results. A semiquantitative enzyme-linked immunosorbent assay (ELISA) was performed to investigate the presence of anti-SARS-CoV-2 neutralizing antibodies.

Results: Milk samples from group A showed antiviral activity with median NT50 titers of 1:32.0 (T0, range 1:16.0 - 1:512.0), 1:55.2 (T1, range 1:19.8 - 1:90.5), 1:2.6 (T2). No neutralizing activity was observed at T3. Median NT50 titers in group B were 1:15.7 (T1, range 1:4.0 – 1:256.0) and 1:6.7 (T2, range 1:2.3 – 1:20.7). Milk samples from group C presented neutralizing activity with median NT50 titers of 1:32.0 (T0, range 1:2.8 - 1:81.7), 1:4.0 and 1:3.6 (T1 and T2, range 1:2.3 - 1:6.3). NT50 titers were confirmed by positivity in the ELISA assay.

Discussion ad Conclusions: Persistency of anti-SARS-CoV-2 activity was observed up to twenty days after delivery in groups A and B, probably due to the presence of specific neutralizing antibodies in the milk. Interestingly, 63.6% of milk samples collected from group C showed neutralizing activity against the virus and a borderline positivity in the ELISA test, suggesting the possible presence of aspecific antiviral activity. Our study confirms that HBM from infected mothers favors specific immunity in breastfed infants and HBM from non-infected mothers might contain aspecific antiviral compounds, serving as initial protection against SARS-CoV-2 infection.

PO 096

EXPLOITING LIPID METABOLISM BY HSV-1: A CHALLENGE TO RETHINK NEW THERAPIES FOR ALZHEIMER'S DISEASE

L. Trifirò[1], C. Albano[1], S. Pasquero[1], F. Gugliesi[1], G. Bajetto[2], W. Hewelt-Belka[3], E. Mina[4], P. Porporato[4], D. Cairns[5], D. Kaplan[5], A. Mullis[5], S. Landolfo[1], M. De Andrea[1,2], V. Dell'Oste[1], M. Biolatti[1]

[1] Department of Public Health and Pediatric Sciences, University of Turin, Italy
[2] CAAD Center for Translational Research on Autoimmune and Allergic Disease, Novara, Italy
[3] Department of Analytical Chemistry, Gdańsk University of Technology, Gdańsk, Poland
[4] Department of Molecular Biotechnology and Health Sciences, University of Turin, Italy
[5] Department of Immunobiology, University of Arizona, Tucson, Arizona, USA

Introduction: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive decline in cognitive functions leading to memory loss and dementia. Only a minority of AD cases have a genetic inheritance and are referred to as familial AD, most (90%) of them are sporadic. Among the environmental risk factors that may promote the development of AD, persistent brain infections, particularly those induced by herpes simplex virus-1 (HSV-1), seem to play a key role in AD pathogenesis although the underlying mechanisms have not been fully elucidated yet. Tightly connected with AD etiopathogenesis is an alteration of lipid metabolism. Recently, CMS121 was shown to protect transgenic AD mice by reducing cognitive loss and inflammation. Interestingly, fatty acid synthase (FAS), a key enzyme in the synthesis of lipids that is increased in AD patients, was identified as a target of CMS121. Considering that cellular lipid metabolism plays a pivotal role in viral infections and that the mechanisms for the metabolic reprogramming by HSV-1 are still poorly understood, we aim at dissecting the host metabolic pathways modulated by HSV-1 in a neuronal-like cell line to identify new targets to prevent AD.

Methods: The experiments were performed in SH-SY5Y neuronal-like cells. The cells were successfully infected with HSV-1, thus representing a suitable *in vitro* model of HSV-1-associated neuronal pathologies. Next, cells were treated with different compounds (*i.e.*, CMS121, C75, SSO) targeting lipid metabolism to test the antiviral activity. Moreover, to understand the link between FAS and HSV-1 infectivity and rule out any off-target effects of the inhibitors, FAS gene expression was silenced by specific short hairpin RNA (shRNA).

Results and Conclusion: We demonstrated the capability of HSV-1 to upregulate the expression of the main lipogenesis enzymes. Accordingly, lipidomic analyses revealed an increase in both *de novo* synthesis and lipid storage following HSV-1 infection, confirming the role of HSV-1 in the deregulation of lipogenesis. In addition, we showed that the virus was still able to replicate upon treatment with compounds, but with reduced infectivity. Thus, we may speculate an antiviral activity of the drugs during virion assembly, leading to a block of virus production.
Overall, our data unveil new aspects of HSV-1-AD interplay and uncover new potential targets to rethink new possible therapies for AD.

PO 097

NOVEL ROLE OF DNMT3L AS A KEY EPIGENETIC MODULATOR UPON MOUSE MAMMARY TUMOR VIRUS INFECTION OF MAMMARY EPITHELIAL CELLS

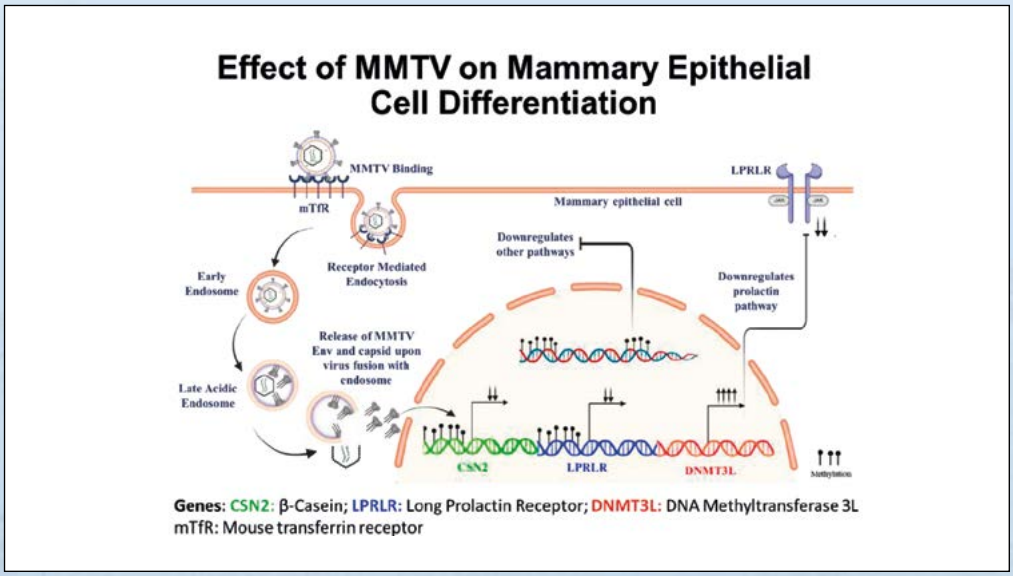
N.G. Panicker[1], W. Ahmad[1], T.A. Khader[1], S. Akhlaq[1], T.A. Rizvi[2], F. Mustafa[1]

[1] *Department of Biochemistry & Molecular Biology, United Arab Emirates (UAE) University, Al Ain, UAE*
[2] *Department of Microbiology & Immunology, College of Medicine & Health Sciences, United Arab Emirates (UAE) University, Al Ain, UAE*

Introduction: Mouse mammary tumor virus (MMTV) is the etiological agent of mammary epithelial cell tumors in mice unlike other related retroviruses which cause hematopoietic cancers. Since its discovery in the 1930s as a milk-transmitted cancerous-causing agent, MMTV has been the focus of much study to get a deeper understanding of its role in the progression of tumors originating in the mammary gland. Lactating mammary glands have the highest expression of MMTV since the MMTV promoter has hormone-inducible elements, leading to release of high titers of virus during pregnancy and lactation. This makes mammary epithelial cells central to MMTV life cycle. Not much is known about the interplay between MMTV and mammary epithelial cell differentiation.

Aim: Investigate how MMTV expression affects mammary epithelial cell differentiation upon prolactin induction using the normal mouse mammary epithelial cell line, HC11.

Methods and Results: TaqMan real time RT-qPCR assays revealed that MMTV disrupted expression of β-casein as well as whey acidic protein (WAP), important markers of differentiation. mRNAseq analysis revealed that MMTV in fact triggered a global downregulation of several critical molecular pathways, including the prolactin signaling pathway which controls mammary epithelial cell differentiation. To explain the global downregulation of gene expression observed in our data, a drastic upregulation of the *de novo* methyltransferase, DNMT3L, was observed in our mRNAseq data that was verified by western blot analysis. Interestingly, expression of prolactin receptor gene was affected by MMTV irrespective of the differentiation state of the cells. Investigation of the prolactin receptor promoters cloned upstream of the *luciferase* reporter gene revealed that MMTV expression resulted in their downregulation. Not only that, we were able to restore *luciferase* gene expression by the prolactin receptor promoter when DNMT3L was inhibited, suggesting that this methyl transferase was responsible for the suppression of the prolactin receptor promoter.



To determine how MMTV was inducing DNMT3L expression, test of individual genes of the virus revealed that MMTV env was directly responsible for this downregulation.

Conclusions: These data suggest that MMTV acts at the promotor level and regulates expression of the prolactin receptor (and many other genes) by epigenetic reprogramming caused by DNMT3L, providing new mechanistic insights into the molecular pathways perturbed by MMTV in normal mammary epithelial cells. Considering that a similar activation of DNMT1 has been observed in the MMTV-promoter driven-polyoma middle T (PyMT) oncoprotein model of mouse mammary tumorigenesis that induces global downregulation of gene expression during four sequential stages of mammary tumorigenesis (Cai et al., *BMC Genomics*, 18:185. 2017), our results have direct relevance to the ability of MMTV to replicate and transform mammary epithelial cells in vivo. While MMTV does not encode an oncogene, our data also points to the env gene of MMTV as a possible oncogene involved in this process as has been suggested earlier (Ross et al., *J. Virol.*, 80, 9000-9008. 2006).

PO 098

HIV-1 TAT AND HTLV-1 TAX PROTEINS CAN TRANSACTIVATE THE NOVEL 5' *CIS*-ACTING ELEMENT IN MMTV

T.A. Khader[1], W. Ahmad[1], S. Akhlaq[1], N.G. Panicker[1], T.A. Rizvi[2], F. Mustafa[1]

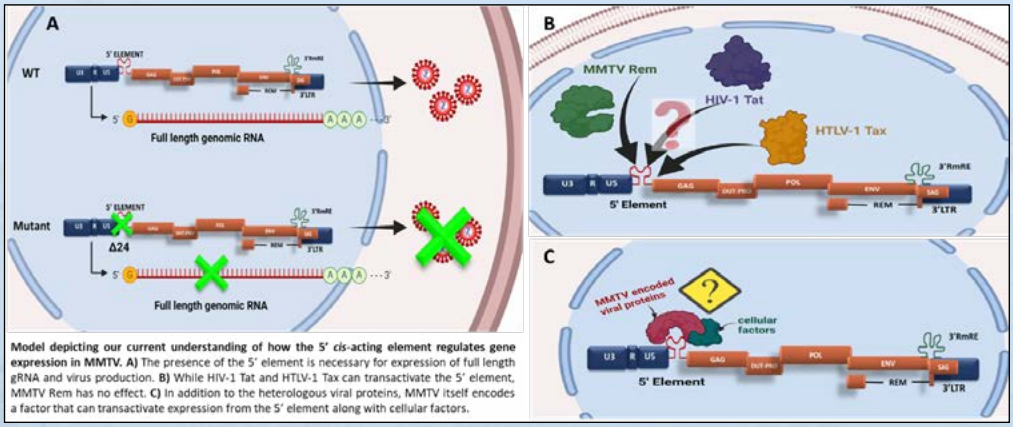
[1] *Department of Biochemistry & Molecular Biology, United Arab Emirates (UAE) University, Al Ain, UAE*
[2] *Department of Microbiology & Immunology, College of Medicine and Health Sciences, United Arab Emirates (UAE) University, Al Ain, UAE*

Introduction: The mouse mammary tumor virus (MMTV) is a prototypic type B *betaretrovirus*, known to cause breast cancer and sporadically lymphomas in mice. Similar to other retroviruses, MMTV too must export its full-length genomic RNA (gRNA) to the cytoplasm without getting spliced. The interaction of virally-encoded Rem protein with Rem responsive element facilitates the nucleocytoplasmic transport of genomic RNA. Previously, we have identified another *cis*-acting element at the 5' end of MMTV that facilitates the transcription, stability, and elongation of the gRNA (Akhlaq et al., *J. Mol. Biol.*, 430:4307-4324, 2018). However, whether this element is Rem-responsive or has any functional interaction with RmRE to facilitate MMTV gene expression is unclear, as has been observed for human immunodeficiency virus (HIV).

Aim: The aim of our study was to investigate the role of the 5' *cis*-acting element in transcriptional regulation of MMTV. We hypothesised that either a transcriptional factor involved in transcript initiation/elongation and/or an RNA stability protein binds to the 5' region to enhance expression of the full-length gRNA, while Rem/RmRE ensures its safe export to the cytoplasm.

Methods and Results: Quantitative real time RT-PCRs on Actinomycin D-treated cells revealed significant reduction in efficiency of transcription initiation with truncated transcripts from the 5' element mutants, while nuclear export of RNA was primarily affected in the RmRE mutants. Reporter gene assays revealed the lack of Rem responsiveness of the 5' element cloned downstream of the *luciferase* (LUC) gene flanked by retroviral splice sites. Furthermore, luciferase-based assays expressing the LUC gene from the MMTV or CMV promoters in the presence of wild type or mutated 5' element revealed that the 5' element could be transactivated with heterologous retroviral proteins like HIV-1 Tat or human T cell leukemia virus (HTLV-1) Tax. Additional experiments revealed that an unknown factor from the MMTV genome itself could transactivate gene expression from the 5' element in a dose responsive manner. In a reciprocal experiment, replacement of the 5' element with the HIV-1 TAR region in the reporter construct led to transactivation of gene expression by not only HTLV-1 Tax protein, as reported earlier, but also by the unknown factor from the MMTV genome.

Conclusion: MMTV contains a gene regulatory system similar to HIV-1 Tat/TAR and HTLV-1 Tax/TRE. This makes MMTV the first among *betaretrovirus* that is truly complex with the ability to not only encode a Rem/RmRE nuclear export system for the safe transport of the gRNA, but also a separate Tat/TAR-Tax/TRE transactivation system present in prototypic complex retroviruses.



Model depicting our current understanding of how the 5' *cis*-acting element regulates gene expression in MMTV. A) The presence of the 5' element is necessary for expression of full length gRNA and virus production. **B)** While HIV-1 Tat and HTLV-1 Tax can transactivate the 5' element, MMTV Rem has no effect. **C)** In addition to the heterologous viral proteins, MMTV itself encodes a factor that can transactivate expression from the 5' element along with cellular factors.

PO 099

THE EXPRESSION OF HUMAN ENDOGENOUS RETROVIRUSES IN PBMC IS MODULATED BY SARS-CoV-2 ACUTE INFECTION AND SHOWS A SPECIFIC TRANSCRIPTIONAL PATTERN AS COMPARED TO OTHER COVID-19 CLINICAL STAGES

N. Grandi[1], M.M. Cara[1], S. Piras[1], D. Carta[1], J. Milia[2], R. Cusano[2], M.I. Monne[3], G. Piras[3], M. Fiamma[3], A.D. Palmas[3], E. Tramontano[1]

[1] *Lab. of Molecular Virology, Dept. of Life and Environmental Sciences, University of Cagliari, Italy*
[2] *CRS4 - Center for Advanced Studies, Research and Development in Sardinia, Science and Technology Park Polaris, Cagliari, Italy*
[3] *ASL Nuoro, Ospedale San Francesco, Nuoro, Italy*

It is known that SARS-CoV-2 infection stimulate an important inflammatory response, which has a major role in COVID-19 pathogenesis (1). Similarly, the transcriptional modulation of Human Endogenous Retroviruses (HERV) has been broadly reported in various infectious and inflammatory contexts, being able to further sustain innate immune activation due to the expression of immunogenic viral transcripts and, eventually, immunogenic proteins (2). Despite such potential interplay, the impact of SARS-CoV-2 infection and the related immune trigger on the individual HERV loci expression remains poorly characterized.
To gain insights on this, we performed the high-throughput sequencing and differential expression analysis of ~3300 HERV loci in the peripheral blood mononuclear cells (PBMC) of 37 individu-

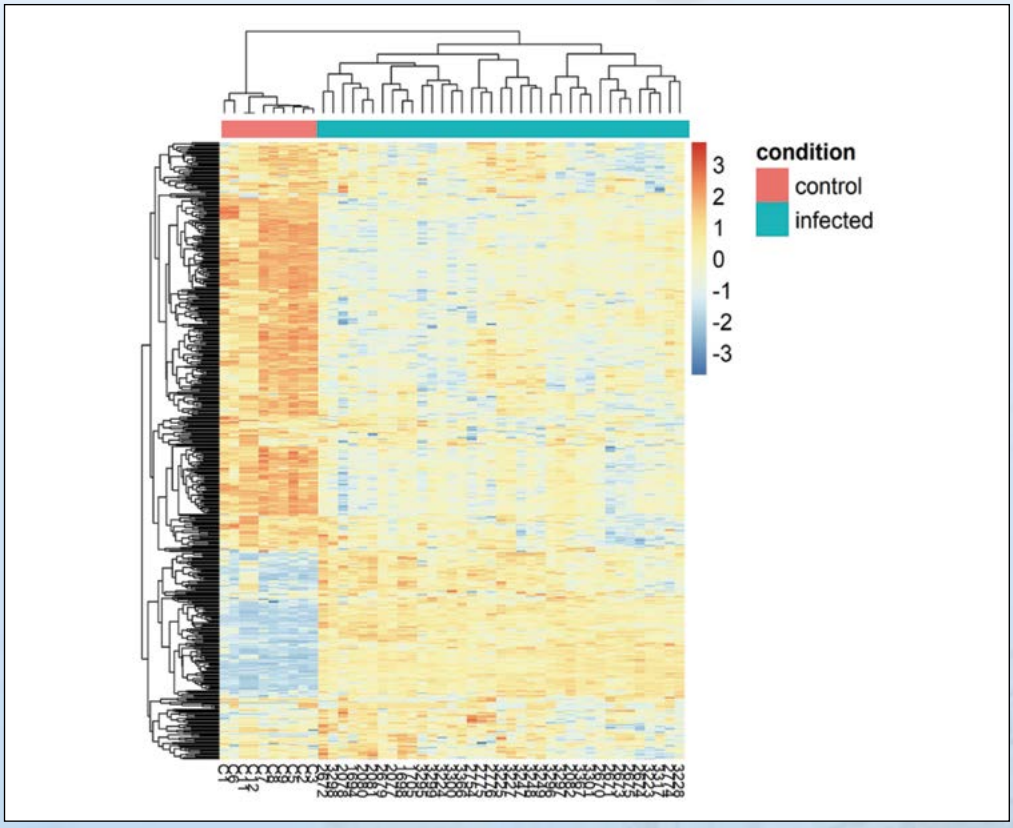


Figure 1 - Heatmap of the 400 HERV loci showing the highest mean of expression, which allow to cluster samples according to the presence (blue) or absence (red) of SARS-CoV-2 infection.

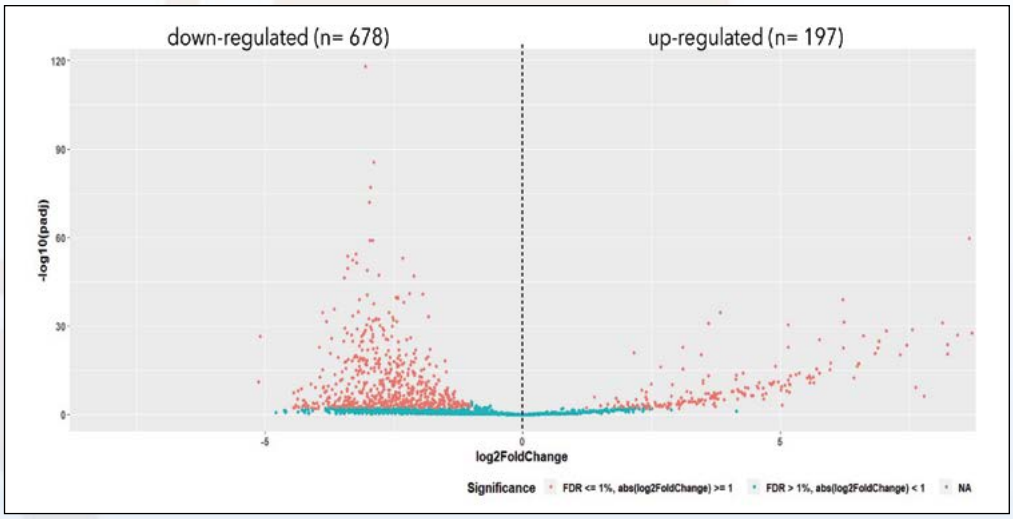


Figure 2 - Volcano plot of HERV differential expression analysis in the PBMC of SARS-CoV-2 infected individuals as compared to healthy controls. HERVs that are significantly modulated are represented in red, being either downregulated (left) or upregulated (right).

als with acute SARS-CoV-2 infection and 10 healthy controls (HC). Particularly, we have chosen PBMC as these cells are not directly infected by the virus but have a crucial role in the plethora of inflammatory and immune events that constitute a major hallmark of COVID-19 pathogenesis. Results showed that the exposure to SARS-CoV-2 has a deep impact on HERV expression, accounting for 40% of its variance and allowing to clearly divide infected individuals from HC in unsupervised clustering analyses (Figure 1). Differential expression analyses confirmed that a total of 875 HERV loci (~27%) were significantly modulated in the presence of SARS-CoV-2 infection (Figure 2). The identified HERV loci belong to 55 different HERV groups from all three classes and included members of the same group with opposite modulation, in contrast with the idea of a general modulation of whole HERV families. Interestingly, the majority of these HERVs was downregulated (678), also indicating that the presence of infectious agents is not necessarily increasing HERV expression, as commonly thought. The genomic context of integration of differentially modulated HERV loci has been characterized, with particular attention to the co-localization with cellular genes involved in innate immune responses to the infection. Finally, the obtained transcriptional signature during SARS-CoV-2 acute infection has been compared with previous results as obtained in the PBMC from in convalescent and retesting positive patients (GSE166253) (3), revealing a specific pattern of HERV modulation but also a subset of HERV loci that remains significantly modulated in all COVID-19 clinical stages.

Overall, the present study provides an exhaustive picture of HERV transcriptome in PBMC during SARS-CoV-2 acute infection, revealing specific modulation patterns as compared to HC but also to different COVID-19 clinical stages, which can be relevant to the disease manifestation and outcome.

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PO 100

ASSESSMENT OF THE IMPACT OF A TOLL-LIKE RECEPTOR 2 AGONIST SYNTHETIC LIPOPEPTIDE ON MACROPHAGE SUSCEPTIBILITY AND RESPONSES TO AFRICAN SWINE FEVER VIRUS INFECTION

G. Franzoni[1], S. Zinellu[1], E. Razzuoli[2], L. Mura[1], C.G. De Ciucis[2], L. De Paolis[2], T. Carta[3], B. Chessa[3], S.P. Graham[4], S. Dei Giudici[1], A. Oggiano[1], M.L. Sanna[1]

[1] Department of Animal Health, Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy
[2] National Reference Center of Veterinary and Comparative Oncology (CEROVEC), Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Genoa, Italy
[3] Department of Veterinary Medicine, University of Sassari, Italy
[4] The Pirbright Institute, Ash Road, Pirbright, Woking GU24 ONF, UK

Toll-like receptor 2 (TLR2) ligands are attracting attention as prophylactic and immunopotentiator agents against pathogens, including viruses [Luchner et al., 2021, Misfud et al., 2014]. We previously reported that a synthetic diacylated lipopeptide (Mag-Pam2Cys_P48) polarized porcine macrophages towards a proinflammatory antimicrobial phenotype [Franzoni et al., 2021]. Here, we investigated its role in modulating monocyte-derived macrophage (moMΦ) responses against African swine fever virus (ASFV), the etiological agent of one of the greatest threats to the global pig industry. Six healthy pigs were used as blood donors and moMΦ were generated in vitro using human M-CSF. Cells were left untreated or stimulated with Mag-Pam2Cys_P48 (100 ng/mL) for 24 h, then moMΦ were infected with ASFV, alongside mock-infected control. Two ASFV strains were compared: the attenuated NH/P68 and the virulent 26544/OG10. ASFV-moMΦ interaction was investigated using flow cytometry, multiplex and singleplex ELISA, and titration assays. No effect on virus infection nor the modulation of surface marker expression (MHC I, MHC II DR, CD14, CD16, and CD163) were observed when MagPam2Cys_P48 treated moMΦ were infected using a multiplicity of infection (MOI) of 1. MagPam2Cys_P48 treated moMΦ released higher levels of IL-1α, IL-1β, IL-1Ra, and IL-18 in response to infection with NH/P68 ASFV compared to 26544/OG10-infected and mock-infected controls. When infected using a MOI of 0.01, the virulent ASFV 26544/OG10 isolate replicated slightly more efficiently in Mag-Pam2Cys_P48 treated moMΦ. These effects also extended to the treatment of moMΦ with two other lipopeptides: Mag-Pam2Cys_P80 and Mag-Pam2Cys_Mag1000 [Franzoni et al., 2022]. Our data suggested limited applicability of TLR2 agonists as prophylactic or immunopotentiator agents against virulent ASFV and highlighted the ability of the virulent 26544/OG10 to impair macrophage defenses.

Keywords: Pig; macrophages; TLR2 agonist; cytokines; surface markers.

PO 101

GESTATIONAL COVID-19: MORPHOLOGICAL ALTERATIONS AND DECREASED HLA-G EXPRESSION CAUSED BY SARS-CoV-2 INFECTION

S. Beltrami[1], G. Schiuma[1], S. Rizzo[1], R. Rizzo[1], A. Passaro[1], P. Greco[1], R. Gafà[1], S. Skuja[2], D. Bortolotti[1], V. Groma[2]

[1] *University of Ferrara, Italy*
[2] *Riga Stradiņš University, Riga, Latvia*

Background: The evaluation of the effect of SARS-CoV-2 infection during pregnancy has raised interest. Even if virus vertical transmission is still controversial, several researches have focused on the possible distinctive markers associated with different susceptibility to SARS-CoV-2 infection during pregnancy.

Aim: Evaluate the effect of SARS-CoV-2 infection at tissue level in gestational COVID-19

Methods: Morphological alterations were assessed in the placental/chorionic villi, chorionic plate, basal plate, and umbilical cord tissues obtained from 7 subjects with symptomatic respiratory SARS-CoV-2 infection and compared with those in 7 non-COVID control subjects. The expression of SARS-CoV-2 Nucleoprotein (NP) and Human Leukocyte Antigen-G (HLA-G) was estimated by the use of immunohistochemistry.

Results: The 57%, 42,8%, and 28,6% of placental/chorionic villi, chorionic plate, and basal plate, respectively, were found positive for NP antigen ($p<0.01$), while none of the umbilical cords stained for NP. Placental/chorionic villi samples showed the highest positivity for NP. The presence of NP positivity correlated with high levels of the fibrinoid component in placental/chorionic villi samples and leukocyte infiltration in basal plate. All placental/chorionic villi samples were found positive for HLA-G, independently from NP staining. All the NP positive chorionic plate and half of the NP positive basal plate samples expressed HLA-G. On the contrary, the placental/chorionic villi, chorionic plate, and basal plate of all non-COVID subjects were positive for HLA-G, with a higher H-score in comparison to pathological samples-($p<0.05$).

Conclusions: The presence of SARS-CoV-2 NP expression in gestational tissues correlates with morphological alterations and a decreased HLA-G expression compared to the control group. These data suggest a possible implication of SARS-CoV-2 infection in morphological and protein expression modification during pregnancy, which might impact infection susceptibility, pregnancy complications, and vertical transmission.

Keywords: COVID-19; pregnancy; HLA-G; immunohistochemistry.

PO 102

SARS-CoV-2 nsp3, nsp4 AND nsp6 EXPRESSION IN *SACCHAROMYCES CEREVISIAE*

L. Rubino[1], A. De Stradis[1], C. Telegrafo[2], A. Antonacci[1], F. Mastrorocco[2], S. Giannattasio[2]

[1] *Institute for Sustainable Plant Protection, National Research Council, Bari, Italy*
[2] *Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies, National Research Council, Bari, Italy*

Background and Aims: Positive-strand RNA [(+)RNA] viruses are agents of important diseases in humans, animals and plants, including COVID-19. Regardless of the host, all (+) RNA viruses share common replication mechanism, including the formation of the virus replication complex and RNA replication in close association with the host endomembrane system. We took advantage of our experience in the study of the replication-associated proteins of (+RNA) plant viruses to express three replication-associated proteins of the (+)RNA severe acute respiratory syndome coronavirus 2 (SARS-CoV-2) in the surrogate host *Saccharomyces cerevisiae* to study virus-cell membrane interactions and to identify host factors co-opted for viral replication.

Methods: SARS-CoV-2 non-structural proteins nsp3, nsp4 and nsp6 were selected for their role in the formation of the double-membrane vesicles, likely representing the virus replication site. The corresponding sequences were cloned under the control of the inducible *GAL1* promoter and fused to the V5 epitope at the 3' end. Cell growth was determined spectrophotometrically as a function of time. Cell viability was determined by measuring colony-forming units (cfu) after 2 days of growth on YPD plates at 3°C. SARS-CoV-2 nsp3, nsp4 and nsp6 expression and targeting were monitored by Western blot and immunofluorescence analyses using anti-V5 monoclonal antibodies.

Results and Conclusions: SARS-CoV-2 non-structural proteins nsp3, nsp4 and nsp6 were expressed in *S. cerevisiae* strain YPH499, as demonstrated by Western blot analysis. It was shown that nsp3 and nsp4, but not nsp6 expression significantly reduced cell growth. Viability was significantly reduced in nsp4-expressing cells at all time points, however at 48 h the viability of all cell types was virtually the same. Accordingly, nsp4 expression was reduced after 48 h growth. nsp3, nsp4 and nsp6 were shown to sediment in a membrane-enriched fraction, thus indicating the association of SARS-CoV-2 nonstructural proteins with cell membranes. The intracellular localization of the expressed proteins was determined by immunofluorescence analysis. It was shown that nsp3, nsp4 and nsp6 localized to the endoplasmic reticulum in *S. cerevisiae* cells, and not to mitochondria or Golgi apparatus. Targeting properties of SARS-CoV-2 non-structural proteins expressed in yeast are maintained and mirror those in their natural hosts. *S. cerevisiae* is confirmed as amenable and safe surrogate model host to study (+)RNA virus replication and possibly identify novel druggable targets to develop antiviral strategies.

PO 103

NEURAL PROGENITOR CELLS AND BRAIN ORGANOIDS TO STUDY THE VIRAL PATHOGENESIS AND POTENTIAL THERAPEUTIC INTERVENTIONS FOR HUMAN CYTOMEGALOVIRUS CONGENITAL INFECTION

M. Trevisan[1], A. Pianezzola[1], F. Tommasi[1], S. Motolese[1], M. Onorati[2], M. Pistello[3], B. Mercorelli[1], A. Loregian[1]

[1] *Department of Molecular Medicine, University of Padua, Italy*
[2] *Unit of Cell and Developmental Biology, Department of Biology, University of Pisa, Italy*
[3] *Centro Retrovirus, Department of Translational Research, University of Pisa, Italy*

Background and Aim: Human cytomegalovirus (HCMV) congenital infection (cCMV) is the most common cause of congenital infection worldwide and is frequently associated with neurodevelopmental defects. Little is known about the mechanisms underlying the pathogenesis of HCMV during the infection of the developing brain and no effective drug or vaccine have been approved yet for cCMV. This is in part due to the narrow species-specificity of the virus, and the difficulties in recapitulating the developing brain *in vitro*. The aim of this study was to characterize HCMV infection in 2D systems of neural progenitor cells, i.e. human embryonic stem cells (hESCs)- derived neural stem cells (NSCs), foetus-derived neural epithelial stem cells (NESCs), differentiating neural progenitor cells and neurons, and then to validate the results obtained in an *in vitro* 3D model of brain organoids.

Methods: hESCs, NSCs, NESCs, differentiating neural progenitors and neurons were all infected, at different MOIs, with a clinical isolate of HCMV or HCMV TB40/E-pp65-YFP strain. Infection of cells was monitored by observing cytopathic effects, by detecting viral proteins by immunofluorescence and western blot, and by measuring viral transcripts by quantitative Real-Time RT-PCR (qRT-PCR). RNA isolated from infected and mock-infected cells was employed to analyse the relative expression of key cellular genes of neurogenesis and neural differentiation by qRT-PCR. Brain organoids were generated from hESCs and, at day 30 of maturation, were infected with HCMV TB40/E-pp65-YFP strain. The extent of the infection was evaluated by analysing pp65+ cells with a live confocal microscope. Infected organoids were then embedded in OCT and cryosections were stained for stage-specific cell markers. Finally, infected cells and brain organoids were treated with different approved or candidate anti-HCMV drugs to evaluate their efficacy in blocking viral replication and their potential neuroprotective effects on virus-induced neuropathogenesis.

Results: All tested 2D neural cell lines and brain organoids showed to be susceptible and permissive to HCMV infection, with NSCs and NESCs, which represent the natural target of the infection *in vivo*, showing the highest rate of productive infection. All infected progenitor cells showed a virus- induced cytopathic effect and expression of both viral transcripts and proteins and revealed a dysregulation of key markers of neurogenesis (e.g., *Pax6*, *SOX-2*, *Nestin*, *Doublecortin* (*DCX*), *MAP2*, *S100B* and *PPAR-γ*). Infected brain organoids showed typical signs of HCMV infection revealed by the presence of large syncytial cells containing multiple nuclei and the disruption of the infected tissues. Expression analysis of infected organoids confirmed a significant dysregulation of markers of neurogenesis, e.g., downregulation of *DCX*. Finally, treatment of infected progenitor cells and brain organoids with antiviral drugs, *i.e.*, ganciclovir, letermovir, and nitazoxanide, was able to block viral replication, reduce viral DNA synthesis and, in neural progenitor cells, to partially restore the dysregulation, caused by the infection, of the neural stem cell specific stage markers *SOX2* and *Nestin*.

Conclusions: 2D and 3D models of neural cells represent a useful platform to recapitulate and deepen the mechanisms of neuropathogenesis triggered by HCMV infection in the developing brain, and in the future might represent an invaluable tool to test antiviral and neuroprotective drugs for cCMV therapy.

PO 104

PAN PRIMATE SCREENING OF ENVELOPE GENES UNVEILING RECOMBINANT DIVERSITY PATTERNS OF ENDOGENOUS RETROVIRUSES

S. Shriwardhan Chabukswar, N. Grandi, E. Tramontano

Laboratory of Molecular Virology, Department of Life and Environmental Sciences, University of Cagliari, Italy

Endogenous Retroviruses (ERVs) are integrated into the host DNA as result of ancient germ line infections, majorly by extinct exogenous retroviruses. In fact, vertebrates' genomes contain thousands of ERV copies, providing a "fossil" record for the ancestral retroviral diversity and its evolution within the host. Like other retroviruses, ERV proviral sequence also consists of *gag, pro, pol,* and *env* genes flanked by long terminal repeats (LTRs). Particularly, the retroviral envelope protein (Env) plays a significant role in the initiating the infection process by binding to the host cellular receptor(s). The characterization of *env* gene changes over time allows both to understand ERVs evolutionary trajectory and possible physiological and pathological domestication. However, so far, only the latter have been explored to study their "actual" role, while few studies were focused on how ERV genes were once acquired and modified within the host. Therefore, one of the factors relevant in understanding the evolutionary dynamics of human ERVs is studying the genetic diversification of the *env* genes in primate genomes. Thus, by combining various approaches of similarity search, phylogenetic analysis, and recombination events analysis we screened around 45 primate species present in the *Catarrhini* and *Platyrrhini* parvorders. While most of the studies commonly use *pol* gene for phylogenetic analysis, we wanted to analyze *env* genes and, through our comprehensive pipeline, we observed a phylogenetic distribution of ERV based specifically on the *env* genes. Importantly, we detected several notable interclass and intra-class *env* recombination events. As a result, we can hypothesize the retroviral evolutionary history of ERVs in *Catarrhini* and *Platyrrhini* parvorders by tracing the diversity patterns of ERVs' *env*. Overall, our findings uncover the major contribution of *env* recombination in the diversification of ERVs further expanding the understanding of retroviral evolution.

PO 105

HUMAN CYTOMEGALOVIRUS INFECTION TRIGGERS A PARACRINE SENESCENCE LOOP IN RENAL EPITHELIAL CELLS

S. Raviola[1], A. Iannucci[1], V. Caneparo[1], D. Lacarbonara[1], G. Bajetto[1,5], I. Lo Cigno[2], F. Favero[4], D. Corà[4], V. Cantaluppi[3], S. Landolfo[5], M. Gariglio[1,2], M. De Andrea[1,5]

[1] *CAAD - Center for Translational Research on Autoimmune and Allergic Disease, Intrinsic Immunity Unit, University of Piemonte Orientale, Novara, Italy*
[2] *Department of Translational Medicine, Virology Unit, University of Piemonte Orientale, Novara, Italy*
[3] *Department of Translational Medicine, Nephrology and Transplantation Unit, University of Piemonte Orientale, Novara, Italy*
[4] *CAAD - Center for Translational Research on Autoimmune and Allergic Disease, Bioinformatics Unit, University of Piemonte Orientale, Novara, Italy*
[5] *Department of Public Health and Pediatric Sciences, Viral Pathogenesis Unit, University of Turin, Italy*

Introduction and Aim: Human cytomegalovirus (HCMV), a member of the *Betaherpesvirinae* subfamily, is one of the most frequent viral pathogens associated with acute kidney injury and kidney rejection. Recently, it was found that SARS-CoV-2 and other viruses can evoke a form of cellular senescence defined virus-induced senescence (VIS). VIS appears to be a universal stress response in host cells and can be induced by many different virus species through multiple mechanisms. In addition, senescent cells produce and secrete a complex combination of factors, collectively referred as the senescence-associated secretory phenotype (SASP), that mediate most of their immune-modulatory effects along with the induction of paracrine senescence. When present in a transitory state, senescent cells can exert beneficial effects on tissue homeostasis, whereas their aberrant accumulation and amplification through paracrine loops are associated with organ-specific disease progression or deterioration. Renal proximal tubular epithelial cells (RPTECs) are natural site of infection and virus-related disease in vivo, thus we asked whether HCMV infection of RPTECs would promote cytopathic changes conducive to VIS and ensuing organ damage.

Material and Methods: RPTECs, human foreskin fibroblasts (HFFs) and the retina-derived epithelial cell line ARPE-19 were infected with HCMV strain TR. The outcome of the infection was evaluated through time course morphological analysis, western blotting for viral protein expression and immunofluorescence staining. Moreover, transcriptome analysis using SenMayo, a universal signature for the identification of senescent cells across tissues and cells, was performed to determine the presence of senescence induction. Then the senescent profile was assessed in RPTECs, HFFs and ARPE-19 *in* vitro using a panel of senescent markers. Finally, using tissue sections derived from a full-blown HCMV infection in a preterm child, we showed a large number of tubular epithelial cells in kidney expressing both HCMV proteins and the senescence marker lipofuscin.

Results: We demonstrate that RPTECs fully supports HCMV replication and undergoes a senescence program upon infection that triggers a harmful secretory phenotype with the ensuing induction of paracrine senescence in uninfected surrounding cells. Specifically, the transcriptome comparison between HCMV-infected *vs.* mock-infected RPTECs showed the enrichment of "hallmark of IL-6/Jack-STAT3 signaling" and "IL-6/STAT3 signaling" which are the pathways predominantly associated with paracrine senescence induction, that were not observed in HFFs nor in ARPE-19. Accordingly we have observed that induction of paracrine senescence in target RPTECs was significantly reduced when the anti-IL-6 receptor antibody Tocilizumab was added to UVB-inactivated conditioned medium from infected RPTECs. Altogether, our findings strongly suggest that the observed HCMV-evoked paracrine senescence may contribute to disease pathogenesis, at least with regard to renal tubular cells which are a natural site of HCMV replication.

Conclusion: Altogether, we define novel pathogenetic mechanisms of renal injury upon HCMV infection that involve induction of a senescence program which in turn may pave the way for novel intervention strategy to counteract HCMV-related kidney disease.

PO 106

PERSISTENT AND TRANSIENT OLFACTORY DEFICITS IN COVID-19 ARE ASSOCIATED TO NEUTROPHIL-MEDIATED IMMUNE RESPONSE AND ZINC HOMEOSTASIS

L. Lupi[1,2], A. Bordin[3], G. Sales[2], D. Colaianni[2], A. Vitiello[1], A. Reale[1], A. Biscontin[2], A. Garzino-Demo[1,4], A. Antonini[5,6], G. Ottaviano[3], C. Mucignat[1], C. Parolin[1], A. Calistri[1], C. De Pittà[2]

[1] *Department of Molecular Medicine, School of Medicine and Surgery, University of Padua, Italy*
[2] *Department of Biology, School of Sciences, University of Padua, Italy*
[3] *Department of Neurosciences, Otolaryngology Section, University of Padua, Italy*
[4] *Department of Microbiology and Immunology, School of Medicine, University of Maryland, United States*
[5] *Department of Neurosciences, University of Padua, Italy*
[6] *Parkinson and Movement Disorders Unit, Study Center for Neurodegeneration (CESNE), Department of Neurosciences, University of Padua, Italy*

The Coronavirus Disease 2019 (COVID-19) is a respiratory syndrome caused by *Severe Acute Respiratory Syndrome Coronavirus-2* (*SARS-CoV-2*). Because of viral tropism, the disease can affect multiple organ systems, causing a variety of symptoms. Among the most salient symptoms are deficits in smell and taste perception, which may last for weeks/months after COVID-19 diagnosis, owing to mechanisms that are not fully elucidated. In order to identify the determinants of olfactory symptom persistence, we obtained olfactory mucosa from 21 subjects, grouped according to clinical criteria: i) with persistent olfactory symptoms; ii) with transient olfactory symptoms; iii) without olfactory symptoms; and iv) non-COVID-19 controls. Cells from the olfactory mucosa were harvested for transcriptome analyses. RNA-Seq assays showed that gene expression levels are altered up to 14 months after infection. The expression profile of micro RNAs appeared significantly altered after infection, but no relationship with olfactory symptoms was found. On the other hand, patients with persistent olfactory deficits displayed increased levels of expression of genes involved in the neutrophil responses and zinc homeostasis, suggesting an association with persistent or transient olfactory deficits in individuals who experienced *SARS-CoV-2* infection. The long-term duration of the effects that we measured shows that *SARS-CoV-2* infection alters gene expression for periods that are well beyond the duration of nucleic acid signal detection in the olfactory epithelium, possibly contributing to post-acute sequelae of infection, often referred to as "long COVID". Our data thus identifies neutrophils and zinc homeostasis as potential therapeutic target to mitigate the long term effects of *SARS-CoV-2*.

PO 107

CFTR INHIBITORS INTERFERES WITH SARS-CoV-2 REPLICATION IN BRONCHIAL EPITHELIAL CELLS

A. Lagni[1], V. Lotti[1], E. Diani[1], C. Sorio[2], D. Gibellini[1]

[1] Microbiology Section, Department of Diagnostic and Public Health, University of Verona, Italy
[2] General Pathology Section, Department of Medicine, University of Verona, Italy

Background: People with cystic fibrosis (pwCF) are characterized by a severe impairment of lung function, so CF could be considered an unfavorable comorbidity in case of SARS-CoV-2 infection, as the lungs have difficulty eliminating pathogens, resulting in inflammation and persistent infection (1with highest prevalence in Europe, North America, and Australia. The disease is caused by mutation of a gene that encodes a chloride-conducting transmembrane channel called the cystic fibrosis transmembrane conductance regulator (CFTR). However, according to several findings, SARS-CoV-2 infection has a remarkably mild clinical impact on pwCF (2, 3). In particular a recent study pointed an important role of CFTR protein in the regulation of SARS-CoV-2 replication and suggested CFTR as a potential novel molecular target for innovative antiviral treatment (4).

Aim: These observations indicate an important role of CFTR protein in the regulation of SARS-CoV-2 replication; thus, CFTR was suggested as a potential novel molecular target for innovative antiviral treatment. In this study, we evaluated the effect of short-term (up to 48 hpi) incubation with the specific pharmacological CFTR inhibitors (IOWH-032 and PPQ-102), against both B.1 and Omicron SARS-CoV-2 variant, in bronchial epithelial cells expressing native CFTR. Therefore we investigate the potential link between CFTR activity and SARS-CoV-2 replication assessing CFTR inhibitors IOWH-032 and PPQ-102 antiviral activity.

Methods: Firstly we investigated the CFTR inhibitors cytotoxicity in Wild Type WT-CFTR bronchial cells according to CellTiter 96® AQueous One Solution Cell 109 Proliferation Assay (Promega) at 1h, 24h and 48h post-treatment. Once we assessed cytotoxicity, we investigated the antiviral activity; real-time RT-PCR was used to evaluate supernatant and cell extracts viral load 48h post treatment and infection. Three different CFTR inhibitors treatment protocols were used to assess the replication cycle phase targeted by CFTR inhibitors.

Results: The analysis of cytotoxicity reported a IOWH-032 CC_{50} >50 μM at 48h post-treatment, while PPQ-102 does not reached a CC_{50} at the tested concentrations. Regarding the CFTR inhibitors antiviral effects, IOWH-032 had an IC_{50} of 4.52 μM, while PPQ-102 had an IC_{50} of 15.92 μM, both of which reduced SARS-CoV-2 replication. We confirmed a significant antiviral effect also on primary cells (MucilAir™ wt-CFTR). Regarding the viral inhibition stages, CFTR inhibitors did not significantly impact SARS-CoV-2 cell entry; however, they were significantly more effective in the post-entry phase. Importantly, CFTR inhibitors antiviral activity was confirmed against two viral strains: B.1 and Omicron SARS-CoV-2 variants.

Conclusions: According to our findings, CFTR suppression can successfully tackle SARS-CoV-2 infection, implying that CFTR expression and function may play an essential role in SARS-CoV-2 replication, opening up new insights on the processes governing SARS-CoV-2 infection in both healthy and CF patients, and providing novel possible treatments.

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PO 108

THE ROLE OF THE ICP27 PROTEIN OF HSV-1 ON INFLAMMASOME ACTIVATION

A. Caproni, C. Nordi, C. Coccomini, R. Fontana, M. Buratto, M. Pappadà, P. Marconi
Department of Chemical, Pharmaceutical and Agricultural Sciences (DOCPAS), University of Ferrara, Italy

Introduction: Infection of a host cell by a viral pathogen triggers a multilevel antiviral response. Viruses have co- evolved by triggering several mechanisms that make them capable of counteracting various pathways of the innate and, subsequently, adaptive immune response. One of the systems involved in the innate signaling of infected cells is the formation of inflammasomes, multiprotein cytoplasmic complexes that promote inflammation through caspase-1 activation and subsequent activation of the pro- inflammatory cytokines IL-1β and IL-18. (Gram, Frenkel, & Ressing, 2012)
Herpes simplex 1 (HSV-1) is known to use strategies to counteract the induction of pro-inflammatory cytokines. Indeed, HSV-1 is a virus with a broad cellular tropism that, in addition to infecting epithelial cells and neurons, can infect macrophages, which are crucial in the activation of the immune response in the host. (Mogensen, Melchjorsen, Malmgaard, Casola, & Paludan, 2004)

Aim of the Study: The aim of the study is to investigate the possible role of HSV-1 immediate early protein ICP27 in the inflammasome pathway in different cell types, such as macrophages and HRPE cells (Human Retinal Pigment Epithelial cells). In fact, the release of pro-inflammatory cytokines by macrophages is important in the prevention of ocular disease by HSV-1, as they are one of the predominant cell types that infiltrate the eye after corneal infection and are crucial in preventing herpetic keratitis. (Lee & Ghiasi, 2017)

Materials and Methods: To assess the possible role of ICP27 in inflammasome signaling, both resting (M0), proinflammatory (M1) macrophages and HRPE cells are used.
THP-1 human monocytic cells (ATCC TIB-202) are differentiated into resting adherent macrophage cells (M0) by treating them with PMA (phorbol 12-myristate 13 acetate, 50 nM/ml) for 24h or into proinflammatory macrophages (M1) through the addition of IFNγ (25 nM) for the 24h following the treatment with PMA. Resting (M0) or proinflammatory (M1) macrophages and HRPE cells are subsequently infected at 3 MOI with HSV-1 wild type strain HSV-1 (F) or with the mutant virus HSV-1ΔICP27 in which the immediate early ICP27 gene is deleted. Uninfected cells are used as negative control and cells treated with nigericin and LPS are used as positive control.
To investigate the role of ICP27 in inflammasome activation, the level of active caspase-1 is closely monitored (2, 4, 8 and 24 hours) by performing an assay based on a coupled enzyme system that generates a stable luminescent signal proportional to active caspase-1 activity. To verify specificity, the assay is repeated by using a selective caspase-1 inhibitor (AcYVAD-CHO) which determines the levels of cross-reacting caspases 4, 5, 7 and 11, also known as non-canonical inflammasomes. ELISA to detect the release of IL-18 and Western Blots assays are both conducted to confirm the results.

Results: In resting and proinflammatory macrophages, the ICP27 gene of HSV-1 play a role in the reduction of the active caspase-1 and thus the release of pro-inflammatory cytokines, suggesting that ICP27 is involved in evading the immune response in the first hours of infection. ICP27 also appears to have an effect in the caspase-1 activity in HRPE cells, although the overall expression of this enzyme is markedly lower than in macrophages.
Further experiments need to be done to understand the mechanism by which ICP27 counteract the inflammasome pathway of the innate immune system.

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EFFECT OF CCR5-REACTIVE ANTIBODIES FROM HIV-1-EXPOSED SERONEGATIVE INDIVIDUALS AND LONG-TERM NON-PROGRESSORS ON CCR5 EXPRESSION AND R5 HIV-1 INFECTIVITY IN CD4+ T LYMPHOCYTES AND PRIMARY MACROPHAGES

I. Farina[1], C. Pastori[2], R. Bona[1], C.M. Galluzzo[1], R. Amici[1], C. Purificato[1], M.C. Gauzzi[1], M. Andreotti[1], L. Lopalco[2], L. Fantuzzi[1]

[1] *National Center for Global Health, Istituto Superiore di Sanità, Rome, Italy*
[2] *Division of Immunology, Transplantation and Infectious Diseases, San Raffaele Scientific Institute, Milan, Italy*

Aim of the Study: Natural antibodies (Abs) against the first extracellular loop of CCR5 were found in some HIV-1-exposed seronegative (ESN) individuals and long-term non-progressors (LTNP), suggesting a role for such Abs in controlling viral replication *in vivo*. These Abs do not interfere with virus binding but determine sustained CCR5 internalization and inhibit HIV-1 infection in CD4+ T lymphocytes. The aim of this study was to assess the effect of sera from ESN, LTNP, HIV-1+ and HIV-1- individuals, characterized for the presence or absence of natural anti-CCR5 Abs, on CCR5 membrane expression and R5 HIV-1 infectivity in monocyte-derived macrophages (MDMs) compared to $CD4^+$ T lymphocytes.

Methods: Serum samples were obtained from 3 ESN subjects and 2 LTNP with anti-CCR5 Abs, and from 3 ESN, 2 HIV-1 positive and 3 HIV-1 negative individuals without anti-CCR5 Abs as control. CCR5 membrane expression was measured in subject's autologous $CD4^+$ T lymphocytes. The effect of sera on CCL4 binding and CCR5 internalization as well as the neutralizing activity of isolated immunoglobulins (Ig) against an R5 primary isolate were respectively evaluated in $CD4^+$ T lymphocytes and PBMCs from healthy donors. MDMs were obtained from $CD14^+$ monocytes isolated from the peripheral blood of 12 healthy donors not carrying the CCR5-Δ32 allele, as assessed by PCR. MDMs were exposed to different sera dilutions for 48 h and then infected with HIV-1_{BaL}. Membrane CCR5 expression was evaluated by flow cytometry at the time of infection. HIV-1 *Gag* and *Env* genes expression was assessed 72 h post infection by qPCR.

Results and Conclusions: CCR5 was expressed by most control subject's autologous $CD4^+$ T lymphocytes (mean value 97.8 %, range 92-100%), but only in a low percentage of lymphocytes from subjects with anti-CCR5 Abs (mean value 15.4 %, range 10-25 %). Sera containing anti-CCR5 Abs caused a partial internalization of CCR5 and inhibition of CCL4 binding, and Ig isolated from these sera neutralized the infectivity of R5 HIV-1 primary isolate in PBMCs. Unlike lymphocytes, a variable, low percentage of MDMs expressed CCR5 on the membrane (mean value 10.5 %, range 3.8-22 %), and this percentage did not correlate with HIV-1 DNA copy number. Exposure to sera from ESN, LTNP, and HIV-1 positive subjects, but not those from HIV-1 negative individuals, caused a variable reduction of CCR5 expression in almost all donors MDMs, with a trend dependent on the donor but independent of the serum type. Furthermore, exposure to sera from all the study subjects caused a variable reduction of viral DNA copies in nearly all donors MDMs tested, showing again a trend independent of the serum type but donor-dependent. No correlation was observed between reduction of membrane CCR5 expression and HIV-1 DNA copies decrease. These results suggest that CCR5 membrane expression is not a major determinant of MDMs infection and that the modulation of CCR5 and HIV-1 infectivity observed in these cells might depend on the interaction with factors other than CCR5-reactive Abs present in sera and/or intrinsic to the donors on which sera were tested. This work was supported by RF-2018-12365208 to LF.

PO 110

HSV-1-INDUCED HOST PROTEIN CITRULLINATION REVEALS A NEW TARGET FOR ANTIVIRAL THERAPY

S. Pasquero[1], F. Gugliesi[1], Q. Yang[1,2], C. Albano[1], G. Bajetto[1,3], F. Caffaro[1], B. Brugo[1], S. Sudeshna[4], L. Barasa[4], H. Haniff[4], V. Dell'Oste[1], M. Biolatti[1], P.R. Thompson[4], S. Landolfo[1], M. De Andrea[1,3]

[1] *Department of Public Health and Pediatric Sciences, University of Turin, Medical School, Turin, Italy*
[2] *Avian Disease Research Center, College of Veterinary Medicine, Sichuan Agricultural University, Wenjiang, Chengdu City, P.R. China*
[3] *CAAD Center for Translational Research on Autoimmune and Allergic Disease, Novara Medical School, Novara Italy*
[4] *Department of Biochemistry and Molecular Pharmacology, UMass Medical School, Worcester, Massachusetts, United States*

Aim of the Study: Herpes simplex virus type 1 (HSV-1) is a neurotropic virus that remain latent in neuronal cell bodies but reactivates due to a variety of stresses throughout an individual's Slife. In some cases, individuals can develop adverse reactions such as herpes simplex encephalitis (HSE) and recent evidence suggests the involvement of HSV-1 in the etiology of Alzheimer's disease (AD). The absence of an effective vaccine and the emergence of numerous drug resistant variants led the need to develop new antiviral agent able to tackle HSV-1 infections. In this scenario, host-targeting antivirals (HTAs), which act on host-cell factors essential for viral replication, are emerging as a promising class of antiviral compounds. Based on our previous evidence that human cytomegalovirus infection of primary human fibroblasts triggers PAD-mediated citrullination of several host proteins, and that this activity promotes viral fitness (Griffante *et al.*, Nat Commun 2021), we asked whether a similar scenario is also true for HSV-1.

Methods used: Human foreskin fibroblasts (HFFs), the human neuroblastoma cell line SHSY-5Y and retinal pigment epithelial cell (ARPE-19) were infected with a clinical isolate of HSV-1 (MOI 1) for 6, 12, 24 and 48 hours, and left untreated or treated with different pan-PAD (Cl-amidine or BB-Cl-amidine) and PAD-specific inhibitors. Then, total mRNAs or protein extracts were prepared to analyze the expression of PAD isoforms and viral proteins. Cells and supernatants were also harvested, pooled, and then lysed by two freeze-thaw cycles, and the extent of virus replication assessed by titrating the infectivity of sample by standard plaque assay on Vero cells, while to determine the number of viral DNA genomes, viral DNA levels were measured by qPCR. Moreover, total proteins from HSV-1-infected cells, either untreated or treated with different PAD inhibitors, were also labeled with a citrulline-specific rhodamine phenylglyoxal (Rh-PG) probe that made citrullinated proteins detectable both on blots and by LC-MS/MS for a complete citrullinome analysis.

Results and Conclusions: Here we show that a new class of HTAs targeting peptidylarginine deiminases (PADs), a family of calcium-dependent enzymes catalyzing protein citrullination, is endowed with a potent inhibitory activity against HSV-1 *in vitro*. Specifically, we show that inhibition of PADs-mediated citrullination significantly suppresses HSV-1 replication in primary foreskin fibroblasts, ARPE-19 and SHSY-5Y cells. Furthermore, we show that HSV-1 infection leads to enhanced protein citrullination through transcriptional activation of tree PAD isoforms: PAD2, PAD3 and PAD4. Interestingly only PAD3 specific inhibitors, CAY10727 or HF4, or siRNA dramatically curbs HSV-1 replication. Finally, an analysis of the citrullinome reveals significant changes in the deimination levels of both cellular and viral proteins, with interferon (IFN)-inducible proteins IFIT1 and IFIT2 being among the most heavily deiminated ones. As genetic depletion of IFIT1 and IFIT2 strongly enhances HSV-1 growth, we propose that viral-induced IFIT1 and 2 citrullination is a mechanism of HSV-1 evasion from host antiviral resistance. Overall, our findings point to a crucial role of citrullination in subverting cellular responses to viral infection and demonstrate that PAD inhibitors efficiently suppress HSV-1 infection *in vitro* which may provide the rational for their repurposing as antiviral drugs.

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UNVEILING THE ROLE OF NAAA ENZYME IN HSV-1 INFECTION AND NEURODEGENERATION

C. Filipponi[1], E. Iacono[1-4], V. La Rocca[1,2], A. De Carli[1-4], G. Sciandrone[1], G. Freer[1], M. Pistello[1-3], M. Lai[1]

[1] *Retrovirus Center, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Italy*
[2] *Institute of Life Sciences, Sant'Anna School of Advanced Studies, Pisa, Italy*
[3] *Department of Clinical and Experimental Medicine, University of Pisa, Italy*
[4] *Department of Medical Biotechnologies, University of Siena, Italy*

Aim of the Study: The exact causes of Alzheimer Disease (AD) are currently unknown, but several studies highlight how Herpes Simplex Virus type 1 (HSV-1) might be involved in the progression of AD. Indeed, HSV-1 interferes with amyloid beta precursor protein (AβPP) distribution and alters its intracellular kinetics, inducing its amyloid processing and accumulation of amyloid β (Aβ) plaques. Additionally, HSV-1 alters the phosphorylation of tau proteins, which is a hallmark of neurodegenerations. In this context, HSV-1, not only regulates the production of Aβ and the tau phosphorylation, but also decreases the autophagic degradation of Aβ resulting in intracellular aggregation. In fact, HSV-1 neurovirulence factor infected cell protein 34.5 (ICP34.5) and viral protein Us11 subvert the autophagic pathway, enabling viral replication. As the main catabolic pathway for aggregates in AD, investigating autophagy role in HSV-1 infection and its interaction with viral protein might help analyzing the discussed link between AD and HSV-1 infection. For this reason, the aim of the study is to evaluate the role of the main autophagic genes in HSV-1 viral infection and understand novel pathways to counteract the autophagy deregulation found in AD patients.

Methods: To deep study the role of autophagy in the replication of HSV-1, we used a siRNA library specifically designed to silence a pool of 80 genes involved in autophagy onset and progression. The siRNA library silences the major genes involved in the autophagosome formation and in the load of autophagosome cargoes, together with the genes involved in the fusion between autophagosomes and lysosomes. We tested these genes in the progression of HSV-1 infection by analyzing whether gene silencing negatively or positively affects viral replication. The analyses were performed using the high content confocal microscopy Operetta CLS, evaluating the number of infected cells, total cell number after infection, and the effect of infection on cell morphology.

Result and Conclusions: We observed that HSV1 replication depends on a controlled regulation of autophagic flux. Indeed, we found that optineurin (OPTN) silencing supports rapid spread and replication of HSV1. We observed the same results by using the siRNA against ULK1 gene, and the overall results suggest that autophagy inhibition favors HSV-1 viral replication by 3-fold compared to the untreated counterparts. The deregulation of these genes might be related to AD development and progression. In addition, OPTN mutations have been related with neurodegenerative disease as AD and amyotrophic lateral sclerosis. We aim to further analyze the role of these genes in neuron degeneration, finding whether the HSV1 autophagy deregulation will affect the disruption of neuronal viability in AD.

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INFLUENCE OF HLA-C GENOTYPE ON HIV-1 PROGRESSION

C. Stefani[1], A. Sangalli[1], E. Locatelli[1], T. Federico[1], G. Malerba[1], M.G. Romanelli[1], G.A. Argañaraz[2], B.C.M. Da Silva[3], A.J. Duarte Da Silva[3], J. Casseb[4], E.R. Argañaraz[2], A. Ruggiero[1], D. Zipeto[1]

[1] *Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Italy*
[2] *Lab of Molecular NeuroVirology, Faculty of Health Science, University of Brasília, DF, Brazil*
[3] *Medical Investigation Laboratory Unit 56 (LIM/56), Faculdade de Medicina FMUSP, Universidade de São Paulo, SP, Brazil*
[4] *Faculty of Medicine, Institute of Tropical Medicine, University of São Paulo, Brazil*

As demonstrated by previous studies HLA-C variants which are less expressed and less stably bound to β2microglobluin/peptide complex, are associated with increased HIV-1 infectivity and scarce HIV-1 infection control. To understand the impact of HLA-C in HIV-1 progression we investigated whether there is a correlation between different stages of HIV-1 progression and the presence of specific HLA-C allotypes in a multicentric cohort (Brazil, Canada and USA) of 96 treatment-naïve patients. HLA-C genotyping was performed by an allele-specific PCR (ASPCR) approach. If the genotype could not be determined by ASPCR, Sanger sequencing was applied. The Chi-square statistical analysis has been used to evaluate the association between HLA-C and HIV-1 progression. HIV-1 positive subjects were classified as Progressors (n = 48), Long-Term Non-Progressors (n=37) and Elite Controllers (n=11) according to their disease progression status. Previously published papers ranked HLA-C variants as stable or unstable based on their binding stability to the β2-microglobulin/peptide complex. Our work showed a statistically significant association between faster HIV-1 progression and the presence of two or one HLA-C unstable variants (p-value: 0.0078 and p-value: 0.0143, respectively) (Figure 1). These data indicate a connection between faster HIV-1 progression and unstable HLA-C variants both at genotype and at alleles levels and provides further insights on how host genetic factors can influence HIV-1 progression.

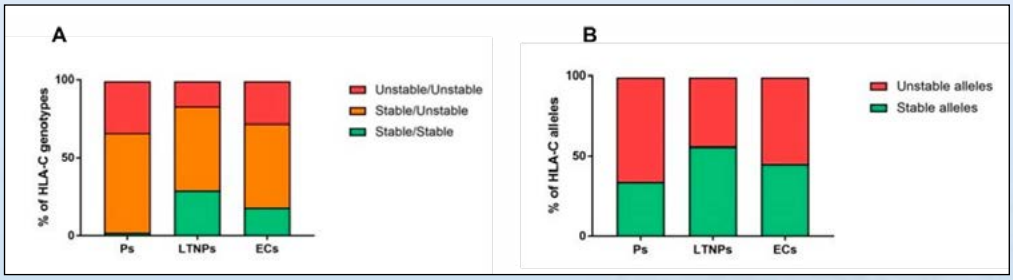

Figure 1 - (A) Percentage distribution of HLA-C genotypes and (B) Percentage distribution of HLA-C alleles for each category of the studied cohort: Progressors (Ps); Long-Term Non-Progressors (LTNPs) and Elite Controllers (ECs).

PO 113

DYNAMICS OF NASOPHARYNGEAL TRACT PHAGEOME AND ASSOCIATION WITH COVID-19

V. Folliero[1], C. Ferravante[2], F. Dell'Annunziata[1], D. Palumbo[2], J. Lamberti[2], E. Alexandrova[2], D. Di Rosa[2], O. Strianese[3], A. Giordano[2], L. Palo[2], G. Giurato[2,3], F.A. Salzano[4], M. Galdiero[1], A. Weisz[2,3,5], G. Franci[4], F. Rizzo[2,3]

[1] *Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy*
[2] *Department of Medicine, Surgery and Dentistry 'Scuola Medica Salernitana', Laboratory of Molecular Medicine and Genomics, University of Salerno, Baronissi, Salerno, Italy*
[3] *Genome Research Center for Health - CRGS, Campus of Medicine - University of Salerno, Baronissi, Salerno, Italy*
[4] *Department of Medicine, Surgery and Dentistry, "Scuola Medica Salernitana", University of Salerno, Baronissi, Salerno, Italy*
[5] *Medical Genomics Program, Clinical Microbiology Program, Infectivology and NAT Molecular Biology Units, AOU "S. Giovanni di Dio e Ruggi d'Aragonà" Università di Salerno, Italy*

Aim of the Study: COVID-19 is a highly infectious respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Numerous studies demonstrate variation of the host microbiota depending on the severity of the disease, age groups of patients and the temporal evolution of the disease (1). One of the more under-appreciated aspects of microbiota is bacteriophages. The aim of this work is the evaluation of the phagome profiles in the three conditions mentioned above.

Methods: Fifty-five SARS-CoV-2 patients from the Campania Region were included in the analysis. Nasopharyngeal swab samples were collected during the three main SARS-CoV-2 outbreaks in Italy: March-May 2020 (n. 25); September-November 2020 (n. 25); January-February 2021 (n. 5) (2, 3). RNA extraction was performed through the ELITeInGenius system. RNA samples were sequenced using the NextSeq 500 and data analyzed using HOME-BIO 15 software.

Results and Conclusions: A total of 6 phage families were detected in nasopharyngeal swabs of COVID-19 patients. Siphoviridae was the most frequently encountered family, following Myoviridae. Regarding the temporal evolution of the pandemic, Peduovirinae, Autographiviridae and Microviridae were more abundant in the first wave than in the second wave. Otherwise, Peduovirinae, Autographiviridae and Microviridae were more frequent in the first period than in the third. As for the disease severity, phage populations were more prominent in patients with severe clinical signs. Indeed, Siphoviridae, Myoviridae and Microviridae were less abundant in the symptom-free patients than in the severe group. Based on age ranges, Autographiviridae and Siphoviridae were significantly less abundant in patients aged 41 to 59 years than in all other groups. In conclusion, significant variations in phage composition in the nasopharyngeal tract of SARS-CoV-2 patients in relation to infection severity and positivization times. This evidence could be used to improve the management of COVID-19.

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PO 114

A DELETION IN THE SPIKE PROTEIN SPANNING THE FURIN CLEAVAGE SITE REDUCES SARS-CoV-2 VIRULENCE IN K18-ACE2 MICE

F. Valleriani, C. Di Pancrazio, G. Di Teodoro, M. Marcacci, M. Luciani, T. Petrova, F. Bonfante, A. Lorusso

Istituto Zooprofilattico Sperimentale Dell'Abruzzo e Molise, Teramo, Italy

Aim of the Study: SARS-CoV-2 uses the S glycoprotein to bind to its receptor and to induce host membrane cell fusion and virus entry. In this study, we compared the virulence properties of a SARS-CoV-2 FCS 10 amino acid-deletion spike mutant (Δ680SPRAARSVAS689; Δ680-689, ΔFCS) in K18-ACE2 mice to those of the parental viral isolate and of two major Delta variant of concern (VOC) isolates.

Methods: Mice were intranasally inoculated with four SARS-CoV-2 isolates: two early B.1 lineage isolates including wild type (wt)-B.1 and B.1-ΔFCS, and two Delta isolates. Mice were monitored for 14 days for weight change, lethality, and clinical score. Oral swabs were collected daily and tested for SARS-CoV-2-RNA. From succumbed mice and mice sacrificed on day 3 and 7 post-inoculation (dpi) organs were collected for molecular and histopathological investigations. Quantification of cytokine and chemokine was conducted on sera and lung homogenates of mice at 3- and 7dpi.

Results and Discussion: Both Delta VOC isolates showed high rates of lethality, clinical score, and severe body weight loss. As opposite, B.1-ΔFCS exhibited significant lower virulence properties than its parental wt-B.1strain. Oral swabs tested positive from 1 to 9 dpi, with B.1-ΔFCS demonstrating the lowest RNA loads. Delta VOC isolates showed the highest RNA copy numbers in all tested organs. In this regard, significant differences were also recorded between the two B.1 isolates, mainly in brain and lungs. Severe interstitial pneumonia was observed in all Delta VOC infected mice, while both B.1 strains caused mild pulmonary histological lesions. Cytokines levels significantly increased in lung tissues of both Delta VOC infected mice. The B.1-ΔFCS isolate was obtained by natural deletion through serial passages on VERO E6 monolayers. In our experimental setting, the B.1-ΔFCS showed reduced shedding and lower virulence in the K18-hACE2 mice compared to wt-B.1 and Delta isolates. Similar results were also previously observed in mice and hamsters infected with a recombinant SARS-CoV-2 without the four amino acid motifs (PRRA) in the S protein. Overall, our work highlights the critical nature of the FCS in understanding SARS-CoV-2 infection and pathogenesis.

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INTRA-HOST SARS-CoV-2 EVOLUTION IN IMMUNOCOMPROMISED AND IMMUNOCOMPETENT SUBJECTS

L. Manuto[1], E. Vanzo[1], M. Antonello[1], M. Cola[1], M. Pacenti[2], E. Franchin[1,2], L. Sasset[2], A.M. Cattelan[1,2], S. Toppo[1], E. Lavezzo[1]

[1] *University of Padua, Italy*
[2] *University Hospital of Padua, Italy*

At the beginning of 2020, the world was swept over by the circulation of a new virus, subsequently named SARS-CoV-2, that led to the COVID-19 pandemic. To date, more than 762 million cases have been registered globally, while the deaths have reached almost 7 million (1). Over the last three years, the virus accumulated several mutations in its genome, giving rise to different variants which strongly challenged the prevention and containment measures, as well as some therapeutic treatments. To monitor the emergence of novel and potentially more dangerous variants, it is crucial to track viral mutations through the sequencing of viral genomes. Moreover, identifying particular host conditions, where viral evolution occurs more rapidly, is fundamental for surveillance purposes. Many studies hypothesised that some variants emerged due to the intra-host environment specific to the immunocompromised patients (2, 3), characterised by a prolonged viral infection associated with the accumulation of several mutations over time (3-7).
In this work, the intra-host evolution of SARS-CoV-2 was investigated in a group of ten immunocompromised patients and compared with a group of twelve immunocompetent individuals, with the aim of assessing whether an impaired immunity could lead to an increase in the acquisition of novel viral mutations. The viral genomes of longitudinal SARS-CoV-2 samples were sequenced and the viral haplotypes variation over time was compared between the two groups.
Although immunocompromised patients had significantly longer infections, no significant differences in the acquisition of novel intra-host viral mutations were observed between the two groups when considering comparable infection windows. Thus, this study suggests that the compromised immune system alone does not affect SARS-CoV-2 evolution. Nonetheless, given that immunocompromised patients often face chronic infections, the accumulation of mutations reported in such subjects might be dependent on the length of the infection, or on some specific treatments, rather than on an immunocompromised system environment.

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SARS-CoV-2 LIFE CYCLE: VIRAL MECHANISM OF INFECTION IN VERO E6 CELLS

S. Gioacchini[1], A. Pietrantoni[2], F. Spadaro[2], L. Bertuccini[2], M. Baggieri[1], A. Marchi[1], P. Bucci[1], R. Fioravanti[1], R. Giuseppetti[1], E. D'Ugo[1], F. Magurano[1]

[1] *Dipartimento di Malattie Infettive, Istituto Superiore di Sanità, Rome, Italy*
[2] *Servizio tecnico scientifico grandi strumentazioni e core facilities, Istituto Superiore di Sanità, Rome, Italy*

Aim of the Study: The COVID-19 pandemic caused by Severe Acute Respiratory Coronavirus 2 (SARS-CoV-2) remains a global emergency due to the rapid spread of its variants. SARS-CoV-2 continued to evolve under immune selective pressure, and while transmission levels remain high, there is an increased likelihood of vaccine escape variants evolving. Currently we can rely on the knowledge of different biological features of this virus but it is also crucial to link those findings to the disease induced by SARS-CoV-2 and its variants infection. The aim of this study was to identify the crucial steps involved in the SARS-CoV-2 infection in VERO E6 cell model and to evaluate the replicative fitness of the different viral variants. The study wanted to describe an approach to better understand the mechanism used by SARS-CoV-2 for replication and the role of the main protein actors involved in its infection.

Methods used: Viral isolate BetaCov/Italy/CDG1/2020|EPI ISL 412973|2020-02-20 obtained from a COVID-19 patient, was propagated by inoculation of Vero E6 cells. Stocks of SARS-CoV-2 Wuhan strain were harvested, and supernatants collected, clarified, stocked, and stored at -80°C (1). VERO E6 cells were plated on Labteck chamber slides and on 6-well plates and then infected with this virus isolate. These procedures were performed in the certificate BSL-3 Laboratory at Istituto Superiore di Sanità. A set of preliminary Indirect Fluorescent antibody assay (IFA) experiments were performed to track the progression of viral protein synthesis during virus life cycle. To better investigate IFA results at ultrastructural level, Transmission electron microscopes (TEM) studies of SARS-CoV-2 morphogenesis were performed at different time points.

Results: Vero E6 cells were tested in Immunofluorescence assays (IFAs) to track the progression of viral proteins during virus cycle by labelling them with specific antibodies. The preliminary results show that the Endoplasmic Reticulum and Golgi Apparatus are the cytoplasmic compartments involved in the accumulation of viral structural proteins S, M and N. SARS-CoV-2 infection on Vero E6 cells observed by Transmission Electron Microscopy analysis (TEM) showed very interesting ultrastructural modifications of the infected cells and confirmed the host compartment involved starting from the early steps of infection. Given these promising results, we are going to perform co-localization IFAs of the viral genome with the viral proteins produced and with the cellular host machinery.

Conclusions: The data of the study describe the biological importance of the viral proteins and cellular structures, involved in the different stages of viral cycle of SARS-CoV-2.

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TRACEABILITY OF SARS-CoV-2 TRANSMISSION THROUGH QUASISPECIES ANALYSIS

S. Messali[1], A. Rondina[1], M. Bertelli[1], M. Duheric[1], M. Giovanetti[2], C. Bonfanti[1], M. Ciccozzi[3], A. Caruso[1], F. Caccuri[1]

[1] *Section of Microbiology Department of Molecular and Translational Medicine, University of Brescia, Italy*
[2] *Department of Science and Technology for Humans and the Environment, University Campus Bio-Medico of Rome, Italy*
[3] *Unit of Medical Statistics and Molecular Epidemiology, University Campus Bio-Medico of Rome, Italy*

Aim of the Study: Understanding SARS-CoV-2 transmission in the presence of a high proportion of asymptomatic infections has been crucial for planning efficient prevention measures. Throughout the pandemic, public health authorities have employed many different strategies to observe transmission links. In particular, consensus genomic sequences were used to rapidly monitor the spread of the virus worldwide. However, SARS-CoV-2 has been reported with limited and, in most cases, identical consensus sequences emerging from transmission pairs (donor and recipient), thus precluding a complete reconstruction of specific transmission events. Analysis of quasispecies data already provided valuable information to manage molecular contact tracing for other RNA viruses, such as human immunodeficiency virus (HIV) and human hepatitis C virus (HCV). In addition, several studies have shown that intra-host genomic variation is a common trait of SARS-CoV-2 infection. Here we evaluated whether a stringent approach based on intra-host single nucleotide variants (iSNVs), which was obtained by next generation sequencing (NGS), may prove crucial to trace SARS-CoV-2 transmission dynamics.

Methods: From January to July 2022, we performed a longitudinal follow-up on 85 healthcare workers employed in the Microbiology unit of the Brescia Civic Hospital (Italy). Twenty-nine nasopharyngeal swabs were collected from 18 subjects undergoing symptomatic SARS-CoV-2 infection. Total RNA was extracted using the QIAamp DSP Virus kit. The Paragon Genomics' CleanPlex multiplex PCR Research and Surveillance Panel was used to obtain the full-length SARS-CoV-2 genome. The created libraries were sequenced on an Illumina MiSeq platform. Raw data were checked for quality and analyzed with the software SOPHiA GENETICS' SARS-CoV-2 Panel. Lineage assessment was conducted using the Phylogenetic Assignment of Named Global Outbreak LINeages tool and intra-host single nucleotide variant (iSNVs) were carried out by the Variant Finder Tool (Geneious). The beta-binomial method was used on iSNVs data to infer the transmission bottleneck size and then individuals were clustered based on Euclidean metric using Pearson correlation coefficients in order to perform genomic tracing.

Results and Conclusions: Most of the identified iSNVs were substitutions (86%), including 29% synonymous and 57% missense mutations, and it has been observed that the frequency of iSNVs was correlated with the length of the gene (R^2 = 0.7818). In addition, the greater number of the shared iSNVs was found in the ORF1ab and S genes. An accurate quantification of the bottleneck size is essential to understand whether iSNVs can be transmitted during acute SARS-CoV-2 infection. To this end, we evaluated the linked pairs, observing a wider transmission bottleneck (range 37-200) as compared to the random pairs. Therefore, when the bottleneck size is wide enough, epidemiologically related subjects share more iSNVs than not related ones. Thus, this evidence suggests that, when the consensus sequences are identical, it is possible to reconstruct the transmission chains by limiting genomic investigations analysis to low frequency iSNVs. Specifically, we found that iSNVs in the nsp2, ORF3 and ORF7 genes are sufficient to correctly identify direct transmission infections. In consideration of the increasing costs of the NGS approach and the infectious diseases' relevance, this method has several advantages, such as getting results quickly and the easy implementation of other pathogens besides SARS-CoV-2.

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EMERGENCE OF S GENE-BASED QUASISPECIES EXPLAINS AN OPTIMAL ADAPTATION OF OMICRON BA.5 SUBVARIANT IN THE IMMUNOCOMPETENT VACCINATED HUMAN HOST

S. Messali, M. Duheric, M. Bertelli, A. Bugatti, F. Filippini, A. Caruso, F. Caccuri

Section of Microbiology Department of Molecular and Translational Medicine, University of Brescia, Italy

Aim of the Study: RNA virus in a single host consists of a heterogeneous population of replicating viruses designated as quasispecies. Quasispecies dynamics during infection represent an adaptive strategy of the virus to evade immune responses. Thus, in vaccinated immunocompetent individuals, appearance of quasispecies is expected to inversely correlate with level of master sequence adaptation to a hostile microenvironment. Here, we considered the intra-host dynamics of SARS-CoV-2 quasispecies in 2 subsequent nasopharyngeal swabs obtained at 4-7 days' interval from 7 up-to-date vaccinated health care workers at the Brescia Civic Hospital (Italy) who experienced mild COVID- 19 symptoms.

Methods: Seven health care workers (A, B, C, D, E, F, G) at the Brescia Civic Hospital (Italy) were enrolled in the study. Nasopharyngeal swabs (NPS) and serum samples were obtained from each study participant. All the individuals received three doses of BNT162b2 vaccine from Pfizer and reported no history of precedent SARS-CoV-2 infection. Serological tests were performed by Electro- chemiluminescence immunoassay (ECLIA) for detection of antibodies against the Spike glycoprotein and the Nucleocapsid protein. Total RNA was extracted from 200 µl of NPS using the QIAamp DSP Virus kit. The Paragon Genomics' CleanPlex multiplex PCR Research and Surveillance Panel was used to obtain the full-length SARS-CoV-2 genome. The created libraries were sequenced on an Illumina MiSeq platform. Raw data were checked for quality and analyzed with the software SOPHiA GENETICS' SARS-CoV-2 Panel. Lineage assessment was conducted using the Phylogenetic Assignment of Named Global Outbreak LINeages tool and the variant calling was carried out by the Variant Finder Tool (Geneious) using a minimum variant frequency of 0 and default parameters for maximum variant P-value. To minimize false discoveries, an intra-host single nucleotide variant (iSNV) in the S gene was identified by the following criteria: (i) sequencing coverage of paired-end mapped reads >100, (ii) at least four reads supporting the nucleotide substitution, (iii) minor allele frequency ≥1%.

Results and Conclusions: Whole Genome Sequencing revealed that patients were infected with the following omicron sub-lineages: BA.1 (subject A), BA.2 (B), BA.2.3 (C and D), and BA.5 (E, F, and G). Comparison of iSNVs frequency in the S gene, obtained by subtracting the frequency value of iSNVs found in the second sample by those observed in the first one, revealed a genetic differentiation between intra-host populations in patients infected with BA.1 and BA.2 compared to those infected with BA.2.3 and BA.5. In particular, with a cut-off of 1% minor allele frequency, patients infected with BA.1 and BA.2 harbored 40 and 44 quasispecies, respectively. On the other hand, patients infected with BA.2.3 and BA.5 harbored a mean number of 15 and 9.6 quasispecies, respectively. Additionally, our results show that frequency of iSNVs in BA.1 and BA.2 viral populations displays a broader range (from 0.30 to 58.10% for patient A and from 0.60 to 64.9% for patient B) than in BA.2.3 and BA.5 (from 0.10 to 6% in C and D patients and from 0.10 to 3.70% in E, F and G patients). Minor mutants may obtain a fitness advantage to become the master one under high selective pressure. Whereas, an already adapted virus to a specific high selective pressure does not foster minor quasispecies to evolve. Collectively, our data show that complex dynamics of viral quasispecies occur over a short period of time in patients infected with BA.1 and BA.2. as compared to patients infected with BA.2.3 and BA.5. The low frequency of quasispecies in BA.2.3- and BA.5-infected patients supports the hypothesis that these omicron sub-lineages are adapted to vaccine-elicited immune responses.

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INVESTIGATING THE INTERPLAY BETWEEN PEROXISOMES AND VIRUSES

D. Stelitano[1], F. Camerota[1], V. Marano[1], S. Tiano[1], G. Bianco[1], Š. Vlachová[1], M. Cortese[1,2]

[1] *Telethon Institute of Genetics and Medicine, Pozzuoli, Italy*
[2] *University of Campania "Luigi Vanvitelli", Caserta, Italy*

Peroxisomes are ubiquitous cellular organelles that represent a central hub in cell metabolism. They act as key players in a variety of biological processes including β-oxidation of fatty acids, biosynthesis of bile salts, ether glycerophospholipids and polyunsaturated fatty acids (i.e. docosahexaenoic acids), and ROS metabolism. Additionally, peroxisomes have emerged as crucial modulators of the innate immune response. During viral infection, these organelles act as a platform for the signaling of pattern recognition receptors (PRRs) such as RIG-I and MDA5 blocking the spread of the virus to the neighbor cells. SARS-CoV-2 infection causes a deep reshaping of cellular organelles, including peroxisomes. Upon infection, peroxisomes undergo a profound remodeling increasing in number and translocating in the perinuclear region near the double membrane vesicles (DMVs), replication organelles in which the replication of the viral genome takes place. However, the biological significance of this peroxisomal reshaping has yet to be clarified. Here we combined high-throughput screening, with virological assays to elucidate the role of peroxisomes in SARS-CoV-2 infection and spread.

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DISTINCT HIV LATENCY ESTABLISHMENT IN HUMAN CD4+ T CELL SUBSETS STIMULATED WITH IL-15

G.M. Butta[1,3], G. Bozzi[1], C. Cordiglieri[3], M. Crosti[3], D. Prati[4], V. Simon[5-7], A. Gori[1,8], A. Bandera[1,8], R. De Francesco[2,3], L. Manganaro[2,3]

[1] *Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Infectious Diseases Unit, Italy*
[2] *Università degli Studi di Milano, Department of Pharmacological and Biomolecular Sciences, Milan, Italy*
[3] *INGM-Istituto Nazionale Genetica Molecolare, Virology, Milan, Italy*
[4] *Department of Transfusion Medicine and Hematology, Fondazione IRCCS Cà Granda, Ospedale Maggiore Policlinico, Milan, Italy*
[5] *Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, USA*
[6] *Global Health Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, USA*
[7] *Division of Infectious Diseases, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, USA*
[8] *Università degli Studi di Milano, Department of Pathophysiology and Transplantation, Centre for Multidisciplinary Research in Health Science, (MACH), Milan, Italy*

HIV persists in a small pool of long-lived resting CD4+ memory T cells harboring integrated and transcriptionally silent virus despite anti-retroviral therapy (ART). This latent reservoir is highly heterogeneous and the mechanisms of latency establishment in the different subsets are still unclear. We analyzed HIV latency establishment in different CD4+ T cell subsets stimulated with IL-15, an upregulated cytokine during acute infection when the reservoir is seeded. Using a single-round dual reporter virus that allows discrimination between latent and productive infection (HIV-GKO), we found that IL-15 treated primary human CD4+ T naïve and CD4+ T_{SCM} are less susceptible to HIV infection compared to CD4+ T_{CM}, T_{EM} and T_{TM}, but are also more likely to harbor transcriptionally silent provirus. The propensity of these subsets to harbor latent provirus compared to the more differentiated memory subsets was independent of the differential expression of pTEFb components. Microscopy analysis of NF-κB suggested that CD4+ T naïve express lower amounts of nuclear NF-κB compared to the other subsets, partially explaining the inefficient LTR-driven transcription. On the other hand, CD4+ T_{SCM} display similar levels of nuclear NF-κB compared to the other more differentiated memory subsets.

T_{SCM} are capable of reconstituting the full diversity of memory and effector T lymphocytes and may thus represent a core element of the HIV reservoir. Both the inhibition of mTOR and the activation of Wnt/β-catenin pathways concur with the generation and homeostatic maintenance of T_{SCM}. The role of these molecular pathways in HIV latency in T_{SCM} remains unclear. To address this gap, we infected with HIV-GKO CD4+ memory T cells from the peripheral blood of healthy donors cultured with IL-7 and IL-15 and treated with TWS119 (Wnt/β-catenin activator) or IWR-1 (Wnt/β-catenin inhibitor). In CD4+ cells treated with TWS119 we specifically observed higher percentages of latently infected T_{SCM} compared to the same subset in CD4+ cells treated with the IWR-1, suggesting that the Wnt/β-catenin pathway modulates latency establishment.

Taken together, our data suggest that each subset has a different tendency to harbor transcriptionally silent provirus and that molecular pathways involved in the maintenance of stem-like features in T_{SCM} are involved in latency establishment.

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HIV-1 DNA QUANTIFICATION IN CD4+ T-CELL SUBSETS OF ART-NAÏVE PEOPLE WITH HIV HIGHLIGHTS INCREASED SUSCEPTIBILITY OF TRANSITIONAL MEMORY T CELLS TO INFECTION

M. Centazzo[1,2*], G. Bozzi[1*], G. Butta[1,2], P. Saltini[1], A. Gori[1,3], L. Porretti[4], E. Trombetta[4], R. De Francesco[2,5], A. Bandera[1,3], L. Manganaro[1,5]

[1] *Infectious Diseases Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy*
[2] *Istituto Nazionale di Genetica Molecolare (INGM) "Romeo ed Enrica Invernizzi", Milan, Italy*
[3] *Department of Pathophysiology and Transplantation, University of Milan, Italy*
[4] *Clinical Pathology Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy*
[5] *Department of Pharmacological and Biomolecular Sciences, University of Milan, Italy*
* *Shared first authorship*

Background and Aims: Total HIV-1 DNA measurement in people with HIV-1 (PWH) has been proven to hold great prognostic value and is the most used measurement to estimate the viral reservoir, few use it to describe the ongoing infection in different CD4$^+$ T cell subsets of ART-Naïve individuals. To better understand HIV-1 infection dynamics in CD4$^+$ T cells, we enrolled 42 people newly diagnosed with an HIV-1 infection either during primary infection (AI, less than 6 months after infection) or chronic infection (CI, more than 6 months after infection) and measured the amount of total HIV-1 DNA in the sorted CD4 T cell subsets before the initiation of therapy.

Methods: HIV-1 DNA was quantified using a digital droplet PCR (ddPCR, BioRad QX200™) with primers directed toward the LTRs and CCR5 for reference. CD4+ T cells from PWH were isolated from peripheral blood via density gradient separation and sorted into five subsets (BD FACSAria™), specifically: Naïve, stem cell memory (SCM), central memory (CM), effector memory (EM) and transitional memory (TM).

Results and Discussion: No significant difference in either CD4+ immune phenotype composition or HIV-1 DNA levels was observed between the two groups and no correlation was observed between viremia and HIV-1 DNA levels in any of the subsets measured. Interestingly, in people with primary infection, the levels of HIV-1 DNA in T_{TM} were significantly higher than the ones of T_{Naive} and T_{CM} reflecting also in vitro infection experiments. Notably, in people with chronic infection, these differences were lost, probably due to the progressive loss of infected CD4 T cells. T_{TM} constitute approximately 20% of total memory subsets in peripheral blood and based on phenotype and functional capacities, they are believed to be an intermediate step of differentiation between T_{CM} and T_{EM}. Of note, previous studies, have shown that T_{TM} together with T_{EM} have high levels of genetically-intact HIV-1 provirus highlighting the importance of this subset in reservoir establishment and viremia rebound after ART is discontinued. In order to determine whether initiation of ART during primary or chronic infection impacts immune phenotype or HIV-1 DNA content in the different CD4 T cell subsets, our cohort will be followed up after one year of ART.

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STRUCTURAL AND FUNCTIONAL CHARACTERISATION OF NUCLEAR LOCALISATION SIGNAL OF FAdV pVII PROTEIN DEMONSTRATES AN INDEPENDENT NUCLEAR TRANSPORT

S. Nematollahzadeh[1], A. Athukorala[2], C.M. Donnelly[3], S. Pavan[1], V. Atelie-Djossou[1], B. Nath[3], K.J. Helbig[2], B.P. McSharry[3], G. Alvisi[1], J.K. Forwood[3], S. Sarker[2]

[1] *Department of Microbiology, Anatomy, Physiology, and Pharmacology, School of Agriculture, Biomedicine and Environment, La Trobe University, Melbourne, VIC 3086, Australia*
[2] *School of Dentistry and Medical Sciences Biomedical Sciences, Charles Sturt University, Wagga Wagga, New South Wales, Australia*
[3] *Department of Molecular Medicine, University of Padua, Italy*

Adenovirus pVII plays a crucial role in facilitating the nuclear localisation of viral DNA following viral infection and has signal sequences for the nuclear import pathway. However, precise mechanisms underlying the functional analysis of pVII in adenoviruses have not fully elucidated. Here we studied the potential of pVII from frog siadenovirus (FAdV), to be involved in adenovirus nuclear localisation. We identified four putative nuclear localisation signals (NLSs) within FAdV pVII and characterized them by a wide range of techniques. Those include electrophoretic mobility shift and fluorescence polarization assays between FITC-labelled NLS peptides and members of the importin (IMP) superfamily. Additionally, we conducted crystallisation experiments with IMPα2, as well as quantitative confocal laser scanning microscopy (CLSM) in cells expressing peptide inhibitors of different nuclear import pathways to validate our findings in a cellular context. In analogous fashion to what reported for human adenovirus 5, we found that FAdV pVII possess multiple NLSs and nucleolar localisation signals (NoLSs), able to directly interact with different members of the IMP superfamily. Consistent with this idea, quantitative CLSM analysis of full length FAdV pVII revealed strong nucleolar and nuclear localization upon transient expression in the absence of other adenoviral proteins. Such localisation was only partially inhibited by expression of the RanQ69L transdominant negative mutant, and completely unaffected by the IMPα/β inhibitor Bimax2, confirming transport through multiple pathways. By systematically mutating key residues within the identified NLSs, we could dissect the individual contributions of each NLS to the nuclear and nucleolar targeting of pVII. Therefore, our results pave the way for an evolutionary comparison of the interaction of different AdVs with the host cell nuclear transport machinery.

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IDENTIFICATION OF SARS-CoV-2 OMICRON BA.5 STRAINS WITH DELETIONS OF THE ORF8 GENE IN NASOPHARYNGEAL SWABS OF COVID-19 PATIENTS

S. Delbue[1], M. Dolci[1], C. Galli[2], L. Signorini[1], R. Ticozzi[1], K. Maina[1], L. Pellegrinelli[2], E. Pariani[2]

[1] *Laboratory of Molecular Virology, Department of Biomedical, Surgical and Dental Sciences, University of Milan, Italy*

[2] *Department of Biomedical Sciences for Health, University of Milan, Italy*

Background-Aim: Epidemiological surveillance of SARS-CoV-2 infection is useful to identify new variants and mutations which might modify the viral fitness. SARS-CoV-2 ORF 8 is an accessory protein involved in the host's immune system modulation.

Methods: Nasopahryngeal (NSP) swabs were collected from patients subjected to SARS-CoV-2 epidemiological surveillance in Lombardy, Italy, and tested for the presence of SARS-CoV-2 genome. The viral genome, when present, was subjected to Next Generation Sequencing (NGS), and the presence of the more frequent mutations was confirmed by specific PCR and subsequent sequencing by Sanger method. SARS-CoV-2 strains were isolated from NSP, on VERO E6 cells, titrated by plaque assay, and the kinetic of their replications was defined in VERO E6 and LLC cell models, in vitro, by measuring the viral load in cells supernatants, at different times, up to 7 days post infection.

Results: In the period between 11 August and 4 November 2022, 170 NSP were collected. A deletion of SARS-CoV-2 ORF8 gene was found in 14/170 (8.24%) NSP, by NGS analysis. In all samples, this deletion was confirmed by PCR of ORF8 and by subsequent analysis of sequence, by Sanger method: 1/14 sample (7.15%) had a partial deletion of 226 nucleotides, while 12/14 (85.7%) had a complete deletion of 417 or 418 nucleotides and 1/14 (7.15%) had a complete deletion of 727 nucleotides. The deletion-free virus and the one with the complete ORF8 deletion were successfully isolated from NSP, titrated and used to perform in vitro infection kinetic analysis in the VERO E6 and LLC cell models. The kinetics, followed over time, up to 7 days post infection, showed no differences in terms of trend and viral load between the two variants of SARS-CoV-2, in both the cell lines.

Conclusions: Presence of ORF8 gene deletions was found in NSP collected in a limited time range (about three months) and had never been found again until now. The deletions did not modify the kinetic of viral replication in vitro. Probably, such deletions are negatively selected, since they do not they represented an evolutionary advantage for the virus itself. However, analysis of cytokines production are ongoing to verify the role of ORF8 in the host's immune system modulation.

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HEDGEHOGS *(ERINACEUS EUROPAEUS) AS A NEW RESERVOIR OF* CORONAVIRUS

G. Vaccari[1], L. De Sabato[1], G. Ianiro[1], F. Manzia[2], M. Monini[1], B. Chiappini[1], I. Di Bartolo[1]

[1] *Istituto Superiore di Sanità, Department of Food Safety, Nutrition and Veterinary public Health, Rome, Italy*
[2] *Center for the Recovery of Wild Fauna in Rome, LIPU, Rome, Italy*

Aim of the Study: During the last twenty years, novel zoonotic CoV strains have emerged (SARS-CoV, MERS-CoV and SARS-CoV2), and due to increase surveillance novel species have been reported in animals. In Europe, the Erinaceus coronavirus (EriCoV) species have recently been described in *Erinaceus europaeus*. However, information on prevalence and duration of viral shedding are unknown.
The aim of this study was to assess the presence of EriCoV in hedgehogs living in the ecosystems of the urban area of Rome, or in the nearby rural areas, and to establish the persistence of infection in this animal host in a non-invasive, *in vivo*, experimental setting.

Methods used: One-hundred-two injured hedgehogs (*Erinaceus europaeus*) hosted in the Center for the Recovery of Wild Fauna in Rome (LIPU), between February and June 2021 (n=37) and between September 2021 and April 2022 (n=65), were enrolled in this study. Fecal samples from 58 out of 102 animals were repeatedly sampled, approximately every two days until complete recovery or death. Total RNA was extracted using the QIAmp Viral Mini kit (Qiagen, Milan, Italy) from 10% (w/v) fecal suspensions and tested for the presence of EriCoV species using a RT-PCR, designed on the EriCoV genome. The Maximum Likelihood tree was built using IQ-TREE Ver. 2 with 1000 bootstrap replicates.

Results and Conclusions: Forty-five animals (44.1%) resulted positive for EriCoV at the first sampling, and 63 (61.7%) animals, were positive in the follow up carried out from 3 to 86 days. The virus shedding period showed a mean duration of 20.8 days, lasted up to 76 days and 18 hedgehogs showed intermitted viral shedding.
Phylogenetic analysis showed correlation with EriCoV strains reported in Germany, United Kingdom, and Northern Italy. None of the EriCoV sequences reported the CD200 ortholog insertion previously observed in animals from Northern Italy. Interestingly, all but one animal revealed the presence in their feces of the same EriCoV sequences (100% nt.id.) in both first and follow up samples, suggesting the infection with the same strain during the permanence in the center.
Our findings confirm that EriCoV can persist in hedgehogs for a long time, underlining that hedgehogs are important reservoir of *Merbecovirus*, highlighting a prolonged and intermittent viral shedding, increasing the spreading of the virus in the environment.
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NOVEL PARVOVIRUS ASSOCIATED WITH INCREASED MORTALITY IN EUROPEAN HEDGEHOGS *(ERINACEUS EUROPAEUS)*, IN A WILDLIFE RESCUE CENTER, ITALY, 2022

G. Diakoudi[1], G. Lanave[1], F. Pellegrini[1], R. Lombardi[1], A.M. Prioletti[1], E. Circella[1], A. Camarda[1], B. Di Martino[2], M. Camero[1], N. Decaro[1], C. Buonavoglia[1], V. Martella[1]

[1] *Università degli Studi di Bari, Dipartimento di Medicina Veterinaria, Valenzano, Bari, Italy*
[2] *Università di Teramo, Facoltà di Medicina Veterinaria, Teramo, Italy*

Aim of the Study: European hedgehogs (*Erinaceus europaeus*) can be found in a variety of habitats. Although possessing European hedgehogs is illegal in most Western countries, often juvenile or injured individuals can be retrieved and temporarily or permanently kept in household as pets. This has generated concerns about the potential of zoonotic diseases posed by hedgehogs to humans. European hedgehogs are known to harbor a variety of bacterial, fungal and viral pathogens (1, 2) with zoonotic potential. In this work, we describe an enteric disease associated with increased mortality in immature weaned hedgehogs housed in a rescue center in Southern Italy in the 2022 breeding season.

Materials and Methods: In the period between 1 June and 15 July 2022, increased mortality occurred in orphaned weaned European hedgehogs (Erinaceus europaeus) rescued at the regional wildlife observatory (CRAS) of Bitetto, Bari, Italy. In total 13 animals, aged between 1 and 5 months, died in the rescue center. Death occurred within 4-6 days of the onset of enteric signs in all the animals, with a case fatality rate of 100%. Overall, on a year-to-year basis mortality in immature hedgehogs increased from 42% (36/84) in 2021 to 64% (38/59) in 2022. Necropsy was carried out on the carcasses revealing inflammation of the small intestine with thickening of the walls and congestion of the mucosa. All the intestinal loops were overextended. Parasitological and bacteriological examinations of the intestinal samples were inconclusive. Tissue samples from hedgehogs were subjected to a sequence-independent enrichment protocol and sequenced using Oxford Nanopore Technology (ONT™) platform.

Results and Conclusions: Parvovirus-related contigs were generated and the genome of strain ITA/2022/265 was reconstructed by combining 5' RACE (rapid amplification of cDNA ends) protocols. The genome sequence of the virus was 4.4 kb in length and showed the highest nucleotide identity (67.5%) to a common vampire bat (*Desmodus rotundus)* parvovirus, strain DRA25, identified in Brazil 2017. To detect and quantify parvovirus load in samples from 9 animals, we used a quantitative PCR (qPCR). The virus was detected in the stools, liver, kidney and spleen from 9/9 tested animals. The viral loads ranged from CT=23,37 to 39,66. On electron microscopy, parvovirus-like particles were observed in the stools although the virus was not aggregated with a serum specific for canine parvovirus and with a serum obtained from an adult hedgehog. In summary, we identified a virus with genetic features resembling members of the *Chaphamaparvovirus* genus, subfamily *Hamaparvovirinae*.
Exploring the virome of wildlife animals is now recognized as a priority in terms of animal conservation and in the perspective of One Health postulates.

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INTRA-FARM CIRCULATION OF SWINE INFLUENZA VIRUS IN NORTH-EAST ITALY BETWEEN 2013 AND 2022

L. Cavicchio[1], A. Pastori[1], L. Tassoni[1], A. Fusaro[1], M. Mion[1], L. Gagliazzo[1], M.S. Beato[2]

[1] *Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Legnaro, Padua, Italy*
[2] *Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati" (IZSUM), Perugia, Italy*

Influenza A virus (IAV) is a common virus in humans and in several animal species, including pigs, that are susceptible to human and avian IAV. Genetic reassortment of IAV of swine, human or avian origin resulted in the human pandemic H1N1 ($H1_{pdm}N1_{pdm}$) in 2009. Influenza remains a common infection of pigs worldwide, causing mild to severe respiratory disease. Swine influenza virus (SIV) circulating in Europe are classified based on haemagglutinin (HA) protein phylogeny and on the whole genome constellation. According to the HA, the European SIV are characterized as human-like H1N2 (huH1N2), avian-like H1N1 (avH1N1) and H3N2, and pandemic H1N1 ($H1_{pdm}N1_{pdm}$), with avH1N1 lineage being the dominant subtype. Recently, SIV of the H1 subtype were classified based on HA global phylogeny in three clades: 1A, 1B and 1C corresponding to $H1_{pdm}$, huH1 and avH1, respectively (Anderson et al., 2016). According to the gene constellation, the first classification available in Europe (Watson et al., 2015) grouped SIV into 23 different genotypes (A-W), which was subsequently updated in 2020 with the description of additional eleven genotypes (Henritzi et al., 2020). However, between 2016 and 2017, in Italy, other undescribed genotypes by the two classifications were detected namely: 1-4, 6-12, 26, 27, previously published as Novel, (Beato et al., 2016), 29-32 (Chiapponi et al., 2018). The aim of the present study was to investigate the genetic diversity of SIV strains circulating in a densely populated pig area of Italy (North East) collected between 2013 and 2022, identifying pig farms with multiple introductions or by persistent virus circulation. Nasal swabs, lungs, oral fluids and bronchial swabs were collected through passive surveillance in pig farms with respiratory signs. Samples were screened by a Real time RT PCR targeting the M gene. The HA and NA genes of all M gene positive samples were characterized by multiplex Real time RT PCRs. A selection of 83 virus isolates were fully sequenced by NGS. The HA clade and genotype were inferred by the phylogenetic analysis. The sequenced SIVs belonged to the H1N1 and H1N2 and H3N2 subtypes and phylogenetic analysis revealed the circulation of thirteen different genotypes of which two described herein for the first time (A, B, D, H, F, M, P, T, U, Novel (27), AH, Novel1 and Novel2). Ten swine farms tested SIV positive twice over a six months period between 2013 and 2022. In the ten farms, eleven genotypes were detected including the two novel ones: B, D, F, M, P, T, U, Novel, AH, Novel1 and Novel2. Eight out of ten farms were characterized by the circulation of different genotypes, probably resulting from distinct introduction events. The highest number of different genotypes characterized (n=4) was observed in a single farm with the highest number of samples submitted in the study period. Two of these farms showed the circulation of multiple genotypes in the same year. In five farms, we identified ten strains belonging to the P genotype ($H1_{pdm}N1_{pdm}$) isolated between 2016 and 2020 that could represent recent spillover events from humans. Only one out of ten farms displayed the circulation of an H3 subtype. Our results highlight the importance of continuous monitoring of SIV circulation in swine farms allows the detection of introductions and re-occurrence of new and multiple genotypes and therefore reassortment events. Such an intra-farm longitudinal monitoring approach coupled with proper collection of epidemiological data, may aid in identifying risk factors linked to new virus introductions or persistence of the virus within each farm. Ultimately, data generated may help improve farm management to reduce the risk of multiple introductions and, consequently, reassortment events, which lead to the emergence of new viruses with zoonotic potential.

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MONITORING OF SALMONELLA, HEPATITIS E VIRUS (HEV) AND VIRAL INDICATORS OF FECAL CONTAMINATION IN FOUR ITALIAN PIG SLAUGHTERHOUSES, 2021-2022

G. Ianiro[1], E. Pavoni[2], E. Delibato[1], L. De Sabato[1], M. Monini[1], G.L. Alborali[2], F. Guadagno[2], V. Perrone[3], F. Ostanello[4], I. Di Bartolo[1]

[1] Istituto Superiore di Sanità, Dept. Food Safety Nutrition and Veterinary Public Health, Rome, Italy
[2] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Brescia Italy
[3] Veterinary Service, Department of prevention, Local Health Unit Roma 2, Rome Italy
[4] Dept. of Veterinary medical sciences, University of Bologna, Italy

Food safety is one of the most important topics of the European politics, guaranteed by strict set of official controls aimed to verify compliance with EU regulations and directives. The safety of food of animal origin is supported by survey of the whole food chain from farm to table. In the pork production chain, the control at slaughterhouse aims to ensure safe food thanks to proper hygienic conditions during all steps of the slaughtering. Salmonella is one of the main foodborne pathogens in the EU causing a great number of human cases, and pigs also contribute to its spreading. Another important and still poorly know foodborne pathogen is the zoonotic hepatitis E virus (HEV). Pig is the main reservoir and HEV can be present in liver, bile, feces and even rarely in blood and muscle. The presence of the virus can determine direct contamination of food and indirect cross-contamination due to improper handlings procedures. Salmonella is present in pork gut and lymph nodes and can easily spread through feces, for improper handling and, during transport and lairage, can be transmitted among pigs.

Aim: The aim of this study was to assess the presence of both Salmonella and HEV in several points of the slaughtering chain including transport, lairage, carcasses and utensils by collection of swab samples. Other viruses hosted in the gut flora of pigs and shed in feces were also assayed (porcine adenovirus PAdV, rotavirus, norovirus, and mammalian orthoreovirus MRV). Torque teno sus virus (TTSuV) present in both feces, liver and blood was also considered.

Methods: Four Italian pig abattoirs were sampled in 12 critical points, five of which were the outer surface of carcasses before processing. Viruses were detected by target-specific Real-Time PCRs on nucleic acids isolated from swabs by Nuclisens Magnetic Extraction System (BioMerieux, Marcy-l'Étoile, France). For Salmonella, sponges were sampled separately analysed by real-time PCR after pre-enrichment. Positive samples were confirmed according to ISO 6579-1:2017/Amd 1:2020.

Results: HEV and rotavirus were not detected. Norovirus was detected once. Salmonella was detected in two of the 4 abattoirs: in the two lairage pens, in the site of evisceration and on one carcass, indicating the presence of Salmonella in carcasses if improper handled. The sites positive for Salmonella were also positive for PAdV. The latter was present in all abattoirs, in 10 sampled points including 5 outer surfaces of carcasses. MRV was detected in 10 swabs, from only two abattoirs, mainly in outer surface of carcasses (7). TTSuV was also detected in all abattoirs, being widely spread among both utensils and carcasses surfaces, highlighting possible cross contamination with blood of infected pigs.

Conclusions: This pilot study provides the evidence of fecal contaminations during slaughtering by using viruses hosted in gut of asymptomatic pigs as indicators. Salmonella can be present and PAdV could be used as fecal indicator, being frequently associated with the presence of Salmonella. In addition to the process hygiene criteria defined in Commission Regulation (EC) 2073/2005, it would be useful to evaluate the role of certain viruses, which could reflect presence of both viral and bacterial pathogen for humans.

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WIDE EXPOSURE OF ADULT PIGS TO HEPATITIS E VIRUS BUT LACK OF EVIDENCE OF ACTIVE INFECTION IN PIGS AT SLAUGHTERHOUSE

M. Monini[1], L. De Sabato[1], F. Agostinelli[1], G. Ianiro[1], F. Ostanello[2], I. Di Bartolo[1]

[1] Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy
[2] Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Bologna, Italy

Hepatitis E virus is definitively considered an emerging treat in Europe for the increased number of humans cases reported and for the wide presence of the virus in pigs at farms. Most of human cases in industrialized countries are caused by the zoonotic HEV-3 genotype that is largely detected in both domestic and wild pigs. The main route of transmission in EU of the HEV-3 is foodborne, trough consumption of raw or undercooked pork and wild boar containing liver. Pigs still positive for HEV have been detected at the slaughterhouse in feces, bile and liver samples. The liver is the main organ of HEV replication, resulting frequently positive, but the probability of HEV liver positive decreases with the age of animals. At slaughterhouse, viremia has been rarely investigated and only sporadically detected.

Aim: The present study aimed to investigate the prevalence of HEV positive batches of pigs being slaughtered evaluating the presence of animals still shedding HEV in feces shortly before being slaughtered. The detection of anti-HEV IgM antibodies and anti-HEV total antibodies was also evaluated as evidence of both recent and past infection.

Methods: 255 fecal pools picked randomly from the floor of the transport trucks were analysed by Real-Time PCR to detect the presence of HEV RNA. The seroprevalence was determined for 260 serum samples using a commercial ELISA kit (HEV ELISA 4.0 kits, MP Biomedicals), specific for the detection of total antibodies in animal sera. For the detection of HEV-IgM antibodies in sera positives in the first ELISA test, a commercial ELISA kit (HEV ELISA 3.0 kits, MP Biomedicals) was used with some modifications. Afterward, presence of viremic animals was also assessed, performing a Real-Time PCR analysis on 80 serum samples including all those resulted positive for anti-HEV IgM antibodies.

Results: A high seroprevalence was observed in the 260 analysed sera, 232 samples (89.23%) resulted positives for anti-HEV total antibodies. Data obtained showed the absence of viremic pigs, even among animals positive for anti-HEV IgM (28/260 10.77%). No fecal shedding of HEV in pigs was detected.

Conclusion: Seroprevalence results confirmed a wide exposure of the animals to HEV, as revealed by the high number of anti-HEV seropositive pigs in the 6 batches investigated.

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SARS-CoV-2 AND OTHER CORONAVIRUSES IN WILD ANIMALS FROM THE EMILIA-ROMAGNA REGION, NORTHERN ITALY

A. Reggiani, E. Carra, L. Gelmini, C. Garbarino, F. Barsi, A. Prosperi, S. Bariselli, P. Bassi, R. Taddei, L. Fiorentini, A. Di Donato, M. Frasnelli, S. Rubini, A. Moreno, P. Bonilauri, G. Rugna

Istituto Zooprofilattico della Lombardia e dell'Emilia-Romagna "B. Ubertini" (IZSLER), Brescia, Italy

Introduction: The *Coronaviridae* family is a well-known and studied family of viruses, whose members are able to infect a wide number of farmed, companion, and wild animal species, as well as humans. In the last two decades, three serious epidemics or even pandemics (SARS, MERS, and COVID-19) have been caused by members of this family (SARS-CoV-1, MERS-CoV, and SARS-CoV-2 respectively). Despite the fact that SARS-CoV-2 spreading has been sustained mostly by human-to-human transmission, several animal species have been proven to be susceptible to infection and able to shed the virus, with the risk of the emergence of novel variants (Reggiani et al., 2022).

Aim of the Study: This project focuses on the surveillance of *Coronaviridae* infections in wild animals from the Emilia-Romagna region (RER). Wild mammals' carcasses (with the exception of Suidae) being conferred to our institution for public veterinary health purposes (passive surveillance) were tested for the presence of both the SARS-CoV-2 genome and anti-SARS-CoV-2 antibodies, as well as for the presence of other *Coronaviridae* genomes. The goal was to identify potential reservoir species for SARS-CoV-2 and monitor whether other coronaviruses are able to cross the species barrier.

Methods: From each animal, several samples were taken (intestine, lung, retro-mandibular lymph node, tracheal, nasal and rectal swabs, serum from cardiac clot), and 1 g of each was homogenised in PBS in a 10% W/V ratio. Homogenised samples were then pooled (1 pool per animal), and RNA was extracted using an automated nucleic acid extractor. RNA was then used to perform RT-qPCRs on SARS-CoV-2 genes E and N and a Pan-Coronavirus nested RT-PCR on the RdRp gene with degenerate primers, as described in Drzewnioková et al. (2021). The organs of positive-testing animals were re-analysed separately to identify the infected organ, and then sequenced to confirm the presence of SARS-CoV-2 or another coronavirus. Serum samples were tested with a double-antigen sandwich ELISA targeting anti-SARS-CoV-2 N- antigen antibodies, in order to identify potential past infections. Positive samples were analysed using commercial surrogate virus neutralization test (VNT) antibody ELISA (sELISA) testing the presence of specific antibodies for the S antigen of different variants, and if suitable, with the VNT.

Results and Conclusions: During 2022, 691 mammals were sampled in the IZSLER diagnostic branches located in RER. None of them tested positive for SARS-CoV-2, while 27 tested positive for the Pan-Coronavirus analysis. Of these, 18 hedgehogs (*Erinaceaus europaeus*) were infected by the Erinaceus coronavirus 1, 1 red fox (*Vulpes vulpes*) was infected by the Canine alphacoronavirus, while the nucleotide sequences obtained from 2 foxes, 4 roe deer (*Capreolus capreolus*), 1 hare (*Lepus europaeus*), and 1 porcupine (*Hystrix cristata*) seem to align with hedgehog or bovine coronaviruses and deserve further studies. Two hundred and forty samples were analysed serologically, with four testing positive for the anti-N ELISA but just one (hedgehog) being confirmed by two different anti-Omicron-S sELISA. Even though results are so far not conclusive regarding SARS-CoV-2 circulation, this work supports the global need to better manage risks from emerging diseases at the interface of human-animal ecosystems, while protecting wildlife health (WOAH, 2023).

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GENETIC VARIABILITY OF GENOTYPE 3 HEPATITIS E VIRUS IN WILD BOAR IN UMBRIA, CENTRAL ITALY: TIME TO TACKLE THIS PUBLIC HEALTH ISSUE

M. Borghi[1], F. Beikpour[2,3], E. Scoccia[1], T. Vicenza[3], A. Valiani[1], S. Di Pasquale[3], S. Bozza[4], B. Camilloni[4], L. Cozzi[3], P. Macellari[5], V. Martella[2], S. Farneti[1], E. Suffredini[3]

[1] *Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy*
[2] *Department of Veterinary Medicine, University of Bari Aldo Moro, Bari, Italy*
[3] *Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy*
[4] *Microbiology and Clinical Microbiology Section, Department of Medicine and Surgery, University of Perugia, Italy*
[5] *Regione Umbria, Direzione Salute e Welfare - Servizio Prevenzione, Sanità Veterinaria e Sicurezza Alimentare, Sezione Sanità Pubblica Veterinaria, Perugia, Italy*

Aim of the Study: Hepatitis E virus (HEV) is the etiological agent of human hepatitis E, a zoonotic disease in which wild and domestic suids serve as the main reservoir hosts for the virus. In Central Italy where the main source of HEV transmission is the consumption of contaminated food, mostly raw or undercooked pork or wild boar meat (Montone, A.M.I., *et al.*, 2019), the number of confirmed HEV infection in human has increase over the last years (Mauceri, C., *et al.*, 2018). Genotype 3 viruses, with the most common subtypes 3c, 3e, 3f, and 3g, appear to represent the main responsible for hepatitis E in Europe so far, but several clades not assigned to known HEV subtypes have been emerging recently (De Sabato, L., *et al.*, 2020). In this study we surveyed HEV circulation in wild boar in Umbria, a region of the Central Italy, during the 2021-2022 hunting seasons.

Material and Methods: Liver samples from 179 wild boar were collected with a stratified sampling plan from different districts of the region of Umbria. Tissue preparation, viral RNA extraction with the NucliSENS miniMag extraction system (bioMérieux, France) and HEV detection by real-time RT-qPCR were performed as previously described (Di Pasquale, S., *et al.*, 2019). Samples that were positive for HEV by RT-qPCR were then genotyped and subtyped with a RT-nested-PCR in the ORF2 region of the HEV genome. Consensus sequences were submitted to BLAST analysis, to the HEV Typing Tool (http://www.rivm.nl/mpf/typingtool/hev/), and to were analysed for phylogenetic relationship (MEGAX software).

Results and Conclusions: Of the 179 wild boar liver samples tested in the study, 78 (43.6%) were positive for HEV and this result represent one of the highest prevalence values reported for HEV in wild boar, in Italy. Sequence characterization was obtained for 41 samples, all of which belonging to genotype 3. Five different subtypes were detected: 3c (*n*=4), 3f (*n*=11), 3e (*n*=1), the putative subtype 3w (*n*=1), and the putative subtype 3t (*n*=12). An additional 12 sequences could not be assigned to any subtype and on phylogenetic analysis clustered into two distinct groups, the first one in closest phylogenetic relation with the 3i subtype, and the second one with the 3h subtype. Full genome analysis will be undertaken to characterize these clusters.
The high prevalence of HEV in wild boar and the wide heterogeneity of subtypes detected in our study provide evidence that foodborne exposure to HEV in Italy - and especially in Central Italy - should be considered a public health issue and that surveillance programs for wild animals should be implemented to ensure food safety and to reduce the risk for the consumers.

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SEROLOGICAL EVIDENCE OF INFLUENZA D VIRUS IN OVINE POPULATION

G. Lanave[1], C.M. Trombetta[2], C. Coppola[2], S. Marchi[2], A.E. Odigie[1], G. Cascone[3], M. Coltraro[3], V. Martella[1], C. Buonavoglia[1], M. Camero[1]

[1] Department of Veterinary Medicine, University Aldo Moro of Bari, Valenzano, Bari, Italy
[2] Department of Molecular and Developmental Medicine, University of Siena, Italy
[3] Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Palermo, Italy

Aims of the Study: The family *Orthomyxoviridae* includes four types of influenza viruses, A, B, C, and D (IAV to IDV). IAV, IBV and ICV are known to cause human respiratory disease and have also been described in several animal hosts (1). IDV was initially isolated from pigs with severe respiratory signs in Oklahoma in 2011 (2). To date, IDV has been detected globally in livestock animals (cattle and swine) but also in wild boar, camelids, small ruminants and horses, with cattle being regarded as the main host (3). Even more interestingly, a possible zoonotic role has been hypothesized, chiefly in operators of the livestock industry (4, 5). This study was designed to assess the prevalence of antibodies against IDV in ovine serum samples collected in Sicily, 2022.

Methods: A total of 600 serum samples were collected from 15 dairy sheep herds located in two prefectures (Ragusa and Siracusa) of Sicily from March to May 2022. Serum samples were tested by haemagglutination inhibition (HI) and virus neutralization (VN) assays using two IDV strains, D/bovine/Oklahoma/660/2013 (D/660) and D/swine/Italy/199724/2015 (D/OK), representative of distinct lineages. An antibody (Ab) titre ≥1:20 was considered as positive. Each assay was carried out in duplicate. Statistical analyses were performed by using the online software package EZR version 1.40 (Saitama Medical Centre, Jichi Medical University) $P<0.05$ was considered as statistically significant.

Results and Conclusions: Abs were detected by HI assay in 74 sera (12.3%, 95% CI:9.8-15.2) for D/660 and in 9 samples (1.5%, 95% CI: 0.7-2.8) for D/OK. HI Abs for D/660 were detected in 9/9 herds from Ragusa prefecture and in 2/6 dairy sheep herds from Siracusa prefecture. HI Abs for D/OK were detected in 2/9 herds from Ragusa prefecture and in 2/6 herds from Siracusa prefecture. The HI titers reached 1:160 for D/660 and 1:80 for D/OK. The sera were also tested by VN assay. VN Abs were detected in 304 (50.7%, 95% CI: 46.6-54.7) sera for D/660 and in 105 samples (17.5%, 95% CI: 14.5-20.8) for D/OK. VN Abs for D/660 and D/OK were detected in 9/9 herds from Ragusa prefecture and in 6/6 and 5/6 herds from Siracusa prefecture, respectively. The VN titers reached 1:1280 for D/660 and 1:905 for D/OK. Serological investigations in European countries (3) have revealed markedly lower prevalence rates, ranging from 0.4 to 6.3% in sheep and from 1.3 to 5.8% in goats. However, in all the above studies only HI Abs were assessed. In addition, we tested the sera using two isolates, each representative of a distinct clade, observing marked variations in terms of positivity. Accordingly, the antigenic differences between the circulating strains and the laboratory isolates could also account for the different prevalence rates observed in the various studies. Overall, our findings demonstrate that IDV circulates in ovine dairy herds in Sicily. Since IDV seems to have a broad host range and it has a zoonotic potential, it is important to collect epidemiological information in susceptible species.

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GENERATION OF EFFICIENT TOOLS FOR DEVELOPING SAFE AND ROBUST SEROLOGIC PLATFORM FOR THE DIAGNOSIS OF RIFT VALLEY FEVER VIRUS INFECTION

A. Capocefalo[1], A. Gallinaro[2], A. Iacobino[3], M.V. Chiantore[3], A. Canitano[2], C. Falce[2], R. Teloni[3], S. Arancia[1], L. Zirpolo[3], M. Borghi[3], A. Zappitelli[3], A. Facchiano[4], F. Facchiano[5], M.R. Castrucci[1], A. Cara[2], D. Negri[3], R. Nisini[3], S. Mariotti[3]

[1] *Dept Veterinary Public Health & Food Safety, Istituto Superiore di Sanità, Rome, Italy*
[2] *National Center for Global Health, Istituto Superiore di Sanità, Rome, Italy*
[3] *Dept Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy*
[4] *Institute Food Sciences, National Research Council, Avellino, Italy*
[5] *Dept Oncology and Molecular Medicine, Istituto Superiore di Sanità, Rome, Italy*

Aim: Rift Valley Fever Virus (RVFV) is a zoonotic pathogen that causes serious outbreaks among livestock and severe symptoms and mortality in humans. Although viremia in infected subjects reaches high titers, it is of short duration, thus limiting the use of antigenic assays and molecular detection systems. Moreover, collection of diagnostic specimens after viral clearance and inappropriate transportation and storage conditions may negatively affect molecular assays, making serology testing an important diagnostic tool in remote locations where limited resources are available. Various serological ELISA tests have been developed and validated using purified viral antigens derived from infected tissue cultures that require high bio-containment facilities. Virus neutralization test is considered the gold standard for indirect diagnosis of infection, but it can be performed only in dedicated laboratories with high containment level and trained staff, which precludes its use in many endemic regions and leads to delays in outbreak detection. To address these problems, we carried out the generation of efficient tools for developing safe and robust serologic platform for the diagnosis of RVFV infection.

Methods: Structural proteins were expressed transiently in HEK293T cells and purified by affinity chromatography from the cell culture medium. Recombinant proteins were quantified by fluorescent krypton protein staining and used to coat ELISA plates. Luciferase-expressing lentiviral vector (LV) pseudotyped with GnGc (LV-Luc/GG) were produced transiently in HEK293T cells co-transfected with the LV-Luc plasmid, the packaging plasmid and the GnGc plasmid. Serial dilutions of neutralizing antibodies (nAbs) or sera were incubated with LV-Luc/GG, added to HEK293T cells and neutralization activity was evaluated by measuring luciferase activity.

Results: RVFV structural protein NP, Gn and Gc ORFs were re-designed to allow protein secretion into the medium of transfected cells in a mammalian cell expression system. Full-length sequence of nucleoprotein NP and the glycoprotein Gc coding region lacking the transmembrane domain (691-1119) were well secreted and successfully purified. The construct expressing the Gn ectodomain (154-560) was well expressed in the cell fraction and was efficiently secreted. The recombinant purified NP, Gc and Gn proteins were quantitatively and qualitatively checked, showing the absence of contaminants, and were used to set up serologic indirect assays.
An efficient production system of RVFV-pseudotyped LV expressing luciferase (LV-Luc/GG) as reporter gene has been developed. LV-Luc/GG infectivity was evaluated by measuring Luc activity in Vero, Vero E6 and HEK293T cells; the latter were selected as target cells for the neutralization test. RVFV-pseudotyped LV expressing luciferase was exploited to set up a safe and highly performing neutralization assay using serial dilutions of nAbs and the results expressed as the inhibitory concentration providing 50% inhibition of infection compared to the virus control wells (ID50).

Conclusion: In response to the increasing demand for safe and less expensive mass-serological diagnosis to accelerate and improve RVFV surveillance and disease control measures, we
1) assessed the suitability of a mammalian expression system to produce high-quality soluble recombinant RVFV proteins for use as serodiagnostic antigens in a robust indirect ELISA and
2) evaluated the performance of a high-throughput and safe neutralization assay, based on pseudovirus technology, that represents an elegant solution especially in geographical region where high biosafety level containment laboratories are unavailable.

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SMALL RUMINANT LENTIVIRUSES: GENETIC CHARACTERIZATION OF STRAINS CIRCULATING IN ITALY BETWEEN 2019 AND 2022

P. Gobbi, S. Pirani, M.S. Beato, M. Giammarioli, F. Feliziani

National Reference Laboratory for Ruminant Retroviruses, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche (IZSUM), Perugia, Italy

Small Ruminant Lentiviruses (SRLVs) are retroviruses that include VISNA-MAEDI and CAEV, which cause disease of different severity in sheep and goats. Since control and eradication plans are not mandatory in several countries, including Italy, SRLVs are distributed worldwide. SRLVs are characterized by a high genetic variability, with five genotypes (A-E) and several sub-genotypes (A1-24; B1-5; E1-2). In Italy three genotypes and fourteen sub-genotypes were identified so far, two of which have been recently described, through a retrospective study conducted on positive cases diagnosed from 1998 to September 2019. This highlights that molecular characterization of positive samples is pivotal to understand and the SRLV evolution in Italy. The present study phylogenetically characterized SRLV diagnosed during the syndromic surveillance conducted from October 2019 to May 2022 in Italy. During this period, 182 sheep and goat farms distributed in fifteen Italian regions and 31 provinces were subjected to SRLV diagnosis, with 630 samples tested. Samples were analyzed by nested PCR targeting the gag-pol conserved region, generating an amplicon of about 800 bp. Extraction of proviral DNA or RNA was performed from different matrices (somatic cells, buffy coat, joint fluids, organ homogenates) Positive samples were Sanger sequenced and sequences generated aligned with reference strains for genotyping. Maximum Likelihood (ML) phylogenetic analysis was performed with 10,000 replicates and boostrap values>70%. During the study period, 59 farms out of 182 tested (30.84%) were positive, distributed in ten regions and nineteen Italian provinces. In 2019, 27/74 farms resulted positive, in 2020 16/51, in 2021 8/42 and in 2022 8/22. Eighty-nine out of 630 samples analysed resulted positive (14.12%), of which 40 were successfully sequenced. Three genotypes (A, B, E) and seven sub-genotypes (A9, A19, A24, B1, B2, B3 and E2) distributed in nine regions were identified. The presents study identified the circulation of A9 sub-genotype in Northern Italy (Veneto) in 2021 for the first time, previously reported solely in central (2010-2018) and south (2017-2019) Italy. In addition, the phylogenetic analysis conducted revealed the circulation of the A24 sub-genotype in South Italy for the first time. As regards the B genotype (1-3), this is widely distributed across the in peninsular Italy, with the B1 being the most widespread. The genetic characterization of SRLVs is an essential tool for mapping the circulation and distribution of the sub-genotypes in sheep and goat farms in Italy, the effectiveness of which would be enhanced by continuous and constant monitoring of the infection in the field.

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RECOMBINATION BETWEEN VIRAL GENOMES AS A POTENTIAL MECHANISM FOR LAGOVIRUSES EVOLUTION

P. Cavadini, G. Campisi, A. Vismara, V. Di Giovanni, T. Trogu, A. Lavazza, L. Capucci
Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy

Lagoviruses are a group of riboviruses from the *Caliciviridae* family whose host spectrum is represented by lagomorphs. While some lagoviruses are hepatotropic and cause acute fulminant viral hepatitis, others are enterotropic and benign. These viruses are Rabbit Haemorrhagic Disease Virus (RHDV) and Rabbit Calicivirus (RCV). RHDV emerged in China in the 80s and Europe in 1986, whereas a new RHDV-related virus, called RHDV2, appeared in Europe in 2010 and rapidly spread worldwide. Among the differential characteristics of RHDV2 indicating that this is an emerging lagovirus, there is an extended host range, being able to infect also hares and cottontails.
Thanks to its specific antigenic profile, allowing it to escape the herd immunity previously generated by RHDV, it became prevalent in the field, causing extended epidemics in wild and domestic rabbits and affecting other lagomorph species. Indeed, since the first identification, RHDV2 has increased its virulence and has shown the tendency to undergo recombination events frequently. However, its occurrence, likely linked to the complex RHD epidemiology contemporarily involving domestic, farmed and wild rabbits, could have been underestimated in the past. Furthermore, another lagovirus highly related to RHDV is the causative agent of European Brown Hare Syndrome (EBHS), mainly observed in European brown hares (*Lepus europeaus*), characterised by severe necrotic hepatitis. Finally, RCV was first detected in domestic and wild rabbits in Italy in 1996 and then in other European countries as well as in 2000 in Australia (RCV-A1). More recently, a non-pathogenic virus (Hare Calicivirus, HaCV) has also been identified in hares from Italy, France and Australia. These viruses cause a silent intestinal tract infection without inducing clinical signs and relevant pathological lesions. Because several recombinants have been described so far, comprising the non-structural (NS) and structural genes of all lagoviruses (e.g., RCV/RHDV2, EBHSV/RHDV2 etc.), this work aimed to check the presence of recombinants strains in Italy, including the already described or even new recombinants. To reach the aim, we sequenced the vp60 capsid gene plus an 800bp upstream of the start codon of the vp60 of approximately 170 RHDV2 strains identified in rabbits and hares from 2012 until today. As a result, we detected in rabbits 17 recombinant strains that show the breakpoint of recombination located in a region close to the vp60 initiation codon, where the parental strains are RHDV2 as a donor for the structural portion of the genome, and RHDV, RCV-E1 and RCV-X as a donor for the NS of the genome. In addition, we found that the first RHDV2 identified in one hare in Italy in Bergamo province in 2012 (RHDV2_Bg1) was a recombinant between an "EBHSV-like" virus whose origin is not yet recognised, as a donor of the NS portion of the genome and RHDV2 as a donor for the structural part of the genome. Moreover, this recombinant is not epidemiologically competitive since we have no longer found this strain.
In conclusion, while the case of RHDV2_Bg12 had no practical implications, it prompts more speculations on the scientific side, i.e., the host spectrum of lagoviruses is determined not only by the sequence of the vp60 gene, but it likely also depends on specific NS genes involved in the intracellular replication of the virus.

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PO 135

EXPLORING THE GENETIC DIVERSITY OF HEPATITIS E VIRUS IN FOOD PRODUCTS AND WILD BOAR FROM ABRUZZO AND MOLISE, ITALY

F. Beikpour[1,5], T. Vicenza[1], G. Aprea[2], N. Barile[3], S. Scattolini[2], E. Nerone[3], I. Del Matto[2], A. Boni[1], D. D'Angelantonio[2], S. Di Pasquale[1], L. Cozzi[1], B. Di Martino[4], V. Martella[5], G. La Rosa[6], E. Suffredini[1]

[1] Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy
[2] Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy
[3] Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Termoli, Campobasso, Italy
[4] Department of Veterinary Medicine, University of Teramo, Teramo, Italy
[5] Department of Veterinary Medicine, University of Bari 'Aldo Moro', Bari, Italy
[6] Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy

Hepatitis E virus (HEV) is a zoonotic virus responsible for large waterborne outbreaks in low-inco me countries and small foodborne outbreaks or sporadic cases in developed countries. The transmiss ion dynamics and the respective role of food, water and animal reservoirs in HEV transmission in Italy have not been completely elucidated yet. To contribute to this topic, a multidisciplinary project focusing on transmission routes in Abruzzo, a 'hot-spot' region for HEV, was carried out. This article focuses on the detection and genetic characterization of HEV in food products and wild boar.

Overall, 356 food samples (including 159 ready-to-eat meat products with and without swine liver, 126 fruits/vegetables and 71 bivalve shellfish) were collected at primary production sites or at retail in the Regions of Abruzzo and Molise, between January 2020 and December 2022 (AQ=117, PE=20, TE=75, CH=119, IS=1, CB=2, unk=22). Furthermore, 1018 wild boar liver samples were taken within the population control programs (AQ=121, PE=36, TE=136, IS=671, CB=54). Sample preparation of food products and livers was carried out with matrix-specific protocols and HEV detection was performed by a quantitative reverse transcription PCR (RT-qPCR). Quality control procedures were applied for viral recovery and PCR inhibition. Positive samples were typed through amplification and sequencing of the ORF2 partial region.

None of the food products tested positive for HEV RNA, therefore the prevalence in food was 0%, with 95%CI 0–1.3% (overall), 0-2.8% (meat products), 0-3.5% (fruits/vegetables), 0-6.2% (bivalves). In contrast, HEV RNA was detected in 94 liver samples (9.2%; 95%CI 7.6-11.2%), of which 34 were successfully sequenced. In detail, 33 sequences were identified as genotype 3 and the following subtypes were detected: 3a (n=2), 3c (n=18), 3e (n=3), 3f (n=9, divided in two clusters), the putative subtype 3t (n=1). One sequence clustered in a distinct group (bootstrap 99%) with GenBank KJ508209 and with two swine slurries collected in the same period in Abruzzo. These sequences could not be assigned to established subtypes, suggesting the presence of a novel subgroup, although this should be confirmed by generating full genome sequence data.

In conclusion, the prevalence of HEV in food products was low, also in the HEV 'hot-spot' region of Abruzzo and in the nearby region of Molise. However, HEV was common in wildlife and displayed a high genetic diversity within the surveyed geographic areas.

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THE NAGOYA PROTOCOL ON ACCESS AND BENEFIT SHARING: THE NEGLECTED ISSUE OF ANIMAL HEALTH

M.S. Beato[1], V. Veneroso[2]

[1] *National Reference Laboratory for African Swine Fever, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati" (IZSUM), Perugia, Italy*
[2] *Lawyer - Independent researcher, Udine, Italy*

More than eight years have passed since the entry into force of the Nagoya Protocol (NP), which sets an international legally binding framework for the access to genetic resources (GRs) and the fair and equitable sharing of benefits arising from their utilization, conceived as the third objective of the Convention on Biological Diversity (CBD), and known as access and benefit sharing (ABS). The NP ABS mechanism relies on a bilateral system, where the users shall seek an express authorization for access, Prior Informed Consent (PIC), and shall share the benefits arising from their utilization with that provider country "in a fair and equitable way," under the conditions of a binding agreement: Mutually Agreed Terms (MATs). The implementation of the Nagoya Protocol is still posing many challenges, which arise from the extraordinary breadth of its scope and the lack of clarity and common understanding of some key concepts that underpin the treaty (i.e. "genetic resource", "utilisation", "research" ecc) and from its multilevel governance framework.
The extension of the "sovereignty principle" to pathogens affirmed by the CBD and NP brought up some concern: preparedness and response actions to emerging or re-emerging global public health threats rely on prompt access to such GRs and case-by-case ABS negotiations with national competent authorities could hamper this goal. Moreover, many conflicting incidents based on sovereignty on viruses claims occurred in recent years (i.e., 2007 avian influenza H5N1 Indonesia; 2012 - MERS-CoV - Saudi Arabia; 2014-2016 Ebola Crisis - West Africa). Acknowledging the necessity to safeguard prompt access to pathogens, article 8.(b) of the NP claims "special consideration" for these GRs and requires parties to pay "due regard to cases of present or imminent emergencies that threaten or damage human, animal or plant health, as determined nationally or internationally". This provision appears as a weak and perhaps hasty solution, rather than an appropriate regulation of such a sensitive issue. Although the scope of article 8(b) is reasonably broad, covering all kinds of health emergencies (humans, animals, and plants and all related GRs), substantial discretion is left to each country on how to implement such measures at a national level potentially jeopardizing the laudable ratio of this article.
While the debate on how NP and its "quid pro quo" bilateral ABS mechanism may hinder the fast response to global public health threats, the impact of NP on the animal health sector still appears as a rather neglected issue.
Though, the WHO stated that, over the last decade, microorganisms from animals or animal products have caused approximately 75% of the new diseases in humans. The COVID pandemic highlighted the importance of the research in veterinary microbiology, investigating, with a preventive approach, the role of the animal reservoir in maintaining and spreading microorganisms that may or may not cause disease in humans. In this view, a multilateral approach to ABS for animal GRs, namely, animal microorganisms, might have potential advantages over the bilateral system of NP. The recent decision of the Conference of the Parties (COP) to the CBD (CBD-COP15, Montreal 18 December 2022) on the need of a multilateral ABS mechanism for digital sequence information, opens up a reflection on the potential to extend the multilateral mechanism to "genetic resources" too. In this perspective, animal health should definitely be an issue to address.

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NOVEL PAPILLOMAVIRUS IDENTIFIED FROM AURAL PLAQUES OF A HORSE

P. Capozza[1], A.H. Omar[1], G. Lanave[1], V.I. Vasinioti[1], C.M. Trombetta[2], L. Occhiogrosso[1], G. Patruno[1], F. Pellegrini[1], G. Diakoudi[1], C. Buonavoglia[1], V. Martella[1]

[1] *Department of Veterinary Medicine, University of Bari, Italy*
[2] *Department of Molecular Medicine and Development, University of Siena, Italy*

Aim of the Study: Equine aural plaques, also known as papillary acanthoma or ear papillomas, are caused by papillomavirus (PV). Clinically, the lesions are characterized by depigmented, hyperkeratotic, coalescing papules and plaques localized to the concave part of the pinna. Lesions usually do not regress spontaneously (3). Classification of PV is based on the highly conserved L1 gene. Presently, at least ten types of *Equus caballus* papillomavirus (EcPV 1-10) have been completely sequenced and classified (2). PVs belonging to the same genus share at least 60% nt sequence similarity in the L1 gene, with coverage of >90% between any two members (1). Herewith we describe the identification of a novel horse PV species.

Materials and Methods: A surveillance study for PV in horses with aural plaques was conducted. In the frame of this study, a 4-year-old bay mare (#405/22) was enrolled after developing multiple lesions involving the left pinna. Multifocal, up to 1 cm in diameter, coalescing papules and plaques were visible in the concave part of the left pinna. A tissue sample was obtained with a skin punch biopsy procedure. The biopsy was tested for PV DNA using three different pan-PV consensus PCR assays, as described elsewhere (4- 6). The sample tested positive in all the three PCR assays but on direct Sanger sequencing, two different types of PV sequences were obseerved. The complete genome of the two viruses was obtained after enrichment of the circular DNA template with rolling circle amplification (RCA). The enriched DNA was sequenced with Oxford Nanopore Technologies (ONT™) platform.

Results and Discussions: The complete genome sequence (7.5 kb in lenght) of a EcPV-4 strain (99.9% nt identity to the reference sequence NC_020085) was assembled. Also, the complete 7.5 kb-long genome sequence of an unclassified PV was assembled. In the L1 gene, the novel PV had the highest identity to EcPV- 1 (64% nt identity), genus *Zetapapillomavirus*. Based on the ICTV classification criteria, the virus was classified as a different PV species. The early genes E1, E2, E4, E6 and E7 were mapped, along with the late structural genes L1 and L2. To date, 15 species of PVs have been reported to infect equines, including three bovine PVs (BPV1, BPV2 and BPV13), two donkey PVs (EaPV1 and EaPV2), and ten horse PVs (EcPV1-10) (2). Genotyping investigations with specific primers/probes are required to assess the epidemiological relevance of this novel EcPV.

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PO 138

OREOCH-1: A PISCIDIN-LIKE PEPTIDE AGAINST ANIMAL VIRUSES

C. Zannella[1], A. Chianese[1], B. Nastri[1] , C. Capasso[1], MV. Morone[1], V. Iovane[2], S. Montagnaro[2], U. Pagnini[2], A. De Filippis[1], M. Galdiero[1]

[1] *Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy*
[2] *Department of Veterinary Medicine and Animal Productions, University of Naples "Federico II", Naples, Italy*

Aim of the Study: Fish continually fight against pathogens by secreting a wide range of antimicrobial peptides (AMPs) as an innate defense mechanism. AMPs, also known as host defense peptides, play major roles in the innate immune system, and protect against bacterial, fungal, viral, and other pathogenic infections. Acosta et al. identified three piscidin-like peptides from the Teloest fish tilapia gills called *Oreochromis niloticus* (1). Among them, oreoch-1 (single-letter primary sequence: FIHHIIGGLFSVGKHIHGLIHGH) showed activity against Gram-positive and Gram-negative bacteria, as well as antifungal activity against *Candida albicans*. On the contrary, its exploitation as potential antiviral agent have yet to be fully investigated.

Methods used: Oreoch-1 was synthesized using oxyma/DIC as coupling agents. The crude peptide was purified by High Performance Liquid Chromatography (HPLC). Cytotoxicity was evaluated on Vero/hSLAM, A72 and MDBK cell lines at different concentrations via the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Then cells were simultaneously treated with peptide and infected by Canine distemper virus (CDV), Schmallenberg virus (SBV), Caprine herpesvirus (CpHV) and Bovine herpesvirus (BHV) gently provided by University Federico II of Naples. After 1 hour of incubation, cells were washed to remove all the unattached virus, and then were overlaid with a viscous medium. To better understand how peptide could interfere with the viral life cycle, different time of addition assays were performed at scalar concentrations.

Results and Conclusions: Emerging and re-emerging virus outbreaks remind us of the urgent need for broad-spectrum antivirals. Oreoch-1 inhibited the early phases of the infection by acting directly on the viral particles. In detail the peptide showed a huge activity against all the viruses tested and could represent an ideal candidate that can act on multiple viruses by intercepting some common steps of their life cycle.

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PO 139

STUDY OF IRIDOVIRUS PREVALENCE WITHIN IN STURGEON FARMS IN NORTHERN ITALY IN THE YEARS 2021-2023

F. Bondavalli[1], D. Schleicherova[1,2], D. Mugetti[1], P. Pastorino[1], A. Zanoli[1,2], T. Bezzo Llufrio[1,2], A. Dondo[1], M. Prearo[1]

[1] Fish Diseases Laboratory, Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy
[2] DBIOS, University of Turin, Italy

Aim of the Study: Sturgeon fishing has all but disappeared and the sale of caviar from caught fish is now prohibited due to the depletion of wild populations. This is why aquaculture has taken the place of fishing. Here, Italy and France are the largest producers in the European Union. The main threat to farmed sturgeon is infection with Iridovirus, a virus belonging to the superfamily or Nucleocytoplasmic Large DNA Viruses (NCLDV), which mainly affects fry and juveniles.

Methods used: In this study, 303 sturgeons from a farms in Northern Italy, belonging to different species of the genus *Acipenser* (*A. transmontanus*, *A. naccarii*, *A. stellatus*, *A. gueldenstaedtlii* and *A. baerii*) and to the species *Huso huso* and aged 6 months or less, were tested between January 2021 and January 2023. The test fish were killed with lethal doses of the anaesthetic tricaine methanesulphonate (MS-222), according to current regulations. Necroscopic, bacteriological, parasitological and virological control was subsequently carried out on each specimen to certify its state of health.
To perform the virological analysis, the gills were taken, as they are the organ in which the highest viral concentration is found. Once the extract was obtained, it was subjected to a PCR-real time specific for the major protein of the iridovirus viral capsid, according to the protocol described by Bigarrè et al., 2017.

Results and Conclusions: Once the molecular analyses were performed, 116 samples tested positive, i.e. 38.2% of the total samples. Almost all the fish that tested positive in the real time PCR showed a tense and swollen abdomen, hepatic steatosis, splenomegaly and increased gill volume on an anatopathological level.
No statistically significant differences in the number of positives from the different species could be found, suggesting possible horizontal transmission within the tanks. On the other hand, it was possible to find a statistically significant difference between infections in the different seasons of the year. In fact, in the winter months the positivity rate stands at 57%, whereas it does not exceed 37% in the other months of the year.
This type of breeding has become increasingly important over the years, which is why it is extremely important to constantly monitor incoming batches and breeding stock, as well as batches already on the farm even if they are older than the most at risk, in order to prevent the introduction of the virus and to avoid losses in such a significant sector.

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HEPADNAVIRUS INFECTION IN A CAT WITH CHRONIC LIVER DISEASE

P. Capozza[1], F. Pellegrini[1], M. Camero[1], N. Decaro[1], G. Elia[1], L. Catucci[2], B. Di Martino[3], P. Fruci[3], G. Lanave[1], V. Martella[1]

[1]Department of Veterinary Medicine, University of Bari, Italy
[2]ACV Triggiano srl, Triggiano, Bari, Italy
[3]Department of Veterinary Medicine, University of Teramo, Italy

Aim of the Study: Domestic cat hepadnavirus (DCH) is an emerging virus related to human hepatitis-B virus (HBV). On molecular inestigation worlwide, DCH prevalence in feline blood/serum ranges from 0.78 to 18% (1-3). A potential role for DCH in feline liver hepatocellular carcinoma or hepatitis is under investigation (1-4). Alteration of serological markers of liver disease (ALT, AST, ALP, GGT and total bilirubin) and coinfection with feline retroviruses (FIV, FeLV) have been identified as major risk factors for DCH DNA positivity (1-3). We describe below a case of DCH infection in a cat with evidence of liver injury.

Methods used: A 3-year-old female stray, domestic shorthair cat was referred to a private veterinary clinic in March 2021 for lethargy, anorexia, and weakness. On physical examination, the cat showed pale mucous membranes and weight loss. A complete blood count revealed leukocytosis associated with anemia, while abnormalities in serum chemistry included elevations of ALP, GGT, total bilirubin, globulins, γ-globulin and amylase, with cholesterol, total iron and saturation being decreased. The cat tested negative for FIV and FeLV. Hematological parameters were monitored repeatedly over four additional time points (April 2021, October 2021, November 2021, and February 2022), evidencing a persistent elevation in ALP, GGT, total bilirubin, along with leukocytosis and anemia. Abdominal ultrasonographic examinations performed in March 2021 and repeated in April and November 2021, showed chronic liver disease and chronic gall-bladder disease, with mild cholestasis. Histological examination carried out on the liver biopsy specimen (collected in November 2021) revealed mild chronic cholangiohepatitis with periportal fibrosis. No bacteria or fungal elements were evident. Upon virological investigations performed on the liver biopsy sample and on serum specimens collected respectively in November 2021 and January 2022, DCH DNA was detected at low viral loads. Viral DNA was enriched by using a rolling circle amplification protocol and sequenced with MinION (Oxford Nanopore Technology™). The serological status of the cat against DCH was evaluated by using two in-house ELISA assays based on the recombinant core (DCHc) (4) and surface (DCHs) antigens (Ag). Only IgG anti-DCHcAg was detected, whilst both sera resulted negative for IgM anti-DCHcAg and IgG anti-DCHsAg. In May 2022, the animal was reportedly found dead by the caretaker, but autopsy could not be performed.

Results and Conclusions: This report describes the case of a cat with clinical signs and serum chemistry markers indicative of hepatic disease. DCH infection was detected and, coupling molecular and serological data, the infection was seemingly contextualized into a chronic phase, since IgM anti-core, a marker indicative of early stage of HBV infection, were not detected. However, the cat possessed IgG anti-DCHcAg, a common indicator of chronic infection in HBV-infected human patients, and did not show seroconversion to anti-DCHsAg, considered protective during HBV infection, and associated with long-term protective immunity (4). On genome-wise sequence analysis, the DCH strain showed 97.2% nucleotide identity to strains previously identified in Italy (2,3). Information on DCH is pivotal to understanding its pathobiological role and developing specific treatment and control strategies.

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PO 141

CARP EDEMA VIRUS AND THE IMPORTANCE OF MONITORING

F. Bondavalli[1], D. Shleicherova[1,2], P. Pastorino[1], G. Esposito[1], A. Gabetti[1,2], A. Maganza[1,2], M. Abbà[1,2], A. Dondo[1], M. Prearo[1]

[1] Fish Diseases Laboratory, Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy
[2] DBIOS, University of Turin, Italy

Aim of Study: Carp edema virus (CEV) a Poxvirus that causes Koi sleepy Disease, it can affect various cyprinids, but especially *Cyprinus carpio* and its varieties, causes a systemic infection with a mortality rate that varies from 10 to 100% depending on age and environmental conditions. In particular, the virus replicates in the gills and causes tissue alterations that are at the basis of impaired gas exchange. Contagion occurs horizontally between diseased and healthy fish. The disease course may vary depending on water temperature, population density and other stress factors.
The CEV epidemiological data concerning the national territory are fragmentary, as is the real susceptibility to the virus of the various species of cyprinids that could act as a reservoir.

Method used: The most easily detected lesions are swelling and gill hypermucosity, accompanied by oedema, lethargy, anorexia and rarely by skin erosions.
During 2022 nine Koi carp (*Cyprinus carpio* var. koi) and twentytwo common carp (*Cyprinus carpio*) from 3 fishfarms and 4 private ponds presenting symptoms compatible with CEV, were analysed anatomopathologically, bacteriologically, parasitologically and virologically.

Results and Conclusions: On necropsy, the fish examined showed gill hypermucosity and skin erosions, while in two koi carp bilateral enophthalmos was also present.
In order to perform molecular analysis, gill lamellae were taken from which DNA was subsequently extracted. This was followed by a nested PCR, according to the protocol of Matras et al., 2016, to confirm the diagnostic suspicion, resulting a positive CEV in 90,32% of the samples tested (28 out of 31 subjects tested, including 8 koi carp and 20 common carp). All the positive samples were confirmed by sequencing the amplicons obtained from the second reaction of the nested PCR. All the farms tested were positive, as well as the private ponds.
Although the number of samples is limited, such a high percentage of positivity confirms the importance of continuous monitoring of this virus, especially with regard to release into natural watersheds, in order to avoid the spread of the disease and the large die-offs that this viral pathology can bring.

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VIROLOGICAL AND SEROLOGICAL DETECTION OF CORONAVIRUSES IN MARMOTS IN THE ALPINE REGION: PRELIMINARY RESULTS

A. Moreno[1], T. Trogu[1], S. Canziani[1], S. Toce[1], D. Lelli[1], S. Giacomelli[2], A. Campagnoli[2], A. Cordedda[1], A. Nicoloso[3], I. Bertoletti[1], A. Bianchi[1]

[1] Istituto Zooprofilattico della Lombardia e dell'Emilia-Romagna "B. Ubertini" (IZSLER), Brescia, Italy
[2] ATS della Montagna, Sondrio, Italy
[3] Dream Italia Soc. Coop., Pistoia, Italy

Introduction: Over the past two decades, three CoVs responsible for serious diseases in humans have been identified. The zoonotic origin of these viruses confirms that coronaviruses have the potential to move from natural hosts to different species and cause serious diseases with fatal implications. Surveillance of coronaviruses in wild hosts is essential to better understand viral diversity and may also provide useful indications to reduce the risk of future spillover events from animals to humans. In this study, coronavirus surveillance was conducted in marmots in the Alpine region in 2022, captured as part of a population control programme in the Livigno area. The large increase in these animals led to the animals being captured and transported to other Alpine areas with lower animal densities.

Methods: During the provincial relocation plan implemented in 2022, 60 alpine marmots were captured in the municipality of Livigno in the province of Sondrio (northern Italy) and, after a quarantine period, were released in other alpine locations. From each animal, faecal samples (n. 60) were collected at the time of capture (1st sampling) and faecal (n. 35) and blood samples (n. 35) were collected at the time of release (2nd sampling) in the Brescia area 15 days later.
Faecal samples were tested for Coronavirus using a nested Pan-Coronavirus RT-PCR and real time RT-PCR for SARS-CoV-2. PCR-positive samples for Pan-CoV were then sequenced by sanger sequencing. Serum samples were analysed with a Mab-based competitive ELISA for the detection of antibodies against Bovine Coronavirus (Bov-CoV).

Results: Six samples from the first sampling were positive by Pan-CoV RT-PCR. Unexpectedly, the CoV sequences obtained showed the highest homology (98.41%) with Bovine Coronavirus detected in France. All samples analysed for SARS-CoV-2 were negative.
Analysis of sera collected during the second sampling revealed that 9/35 sera were positive for antibodies against Bov-Cov in a competitive Mab-based ELISA.

Conclusions: These results, although preliminary, showed a possible infection of marmots with bovine CoV. The presence of Ab against Bov-CoV in the marmots' sera indeed supported the possible Bov-CoV infection and possible spillover phenomenon. However, in order to confirm these results, further analysis is necessary, such as the complete genome analysis of pan-CoV positive samples and serological analysis of positive sera with the serum neutralisation test using Bov-CoV as antigen. Marmots share alpine pastures with cattle species in the Livigno area; in fact, the capture area in 2022 represented areas of pasture and forage production and this could explain these results.
These results highlight the need to investigate wild animals and marmots in particular more deeply for Coronavirus surveillance, in order to provide more information on the circulation and diversity of Coronavirus in wildlife.

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VIRUS-LIKE PARTICLE–MEDIATED DELIVERY OF THE RIG-I AGONIST M8 TRIGGERS A ROBUST PROPHYLACTIC AND THERAPEUTIC ANTIVIRAL ACTIVITY

E. Palermo, M. Alexandridi, D. Di Carlo, M. Muscolini, J. Hiscott

Pasteur Laboratories, Istituto Pasteur Italia - Fondazione Cenci Bolognetti, Rome, Italy

SARS-CoV-2 pandemic has highlighted weaknesses in preparedness and response to global public health emergencies and emphasized the crucial need for timely and efficient interventions to control future outbreaks. The rapid development of SARS-CoV-2 vaccines is a clear demonstration of the extraordinary efforts made during the last years, with the release of several approved vaccines and others currently undergoing clinical trials. However, commercially available vaccines failed to induce a long-lasting protection against the virus due to the emergence of several variants of concern (VOCs). Therefore, a host-targeted therapy with a broad spectrum of activity represents an important therapeutic strategy.

Virus-Like Particles (VLPs) are nanostructures that share conformation and self-assembly properties with viruses but lack a viral genome and therefore the infectious capacity. In this study, we describe the production of VLPs *in vitro* by co-expression of Vesicular Stomatitis Virus (VSV) glycoprotein (VSV-G) and HIV structural proteins (Gag), incorporating a strong sequence-optimized 5'ppp-RNA RIG-I agonist, termed M8. Besides, we evaluated the ability of VLPs to release M8 into cells and to trigger a type I interferon antiviral response.

Treatment of target cells with VLPs-M8 generated an antiviral state that conferred resistance against multiple viruses, including VSV, Dengue virus, hCoV-229E and SARS-CoV-2 Spike pseudotyped VSV (VSV-Spike). Interestingly, administration of VLPs-M8 also elicited a therapeutic effect by inhibiting ongoing viral replication in previously infected cells. Finally, the presence of SARS-CoV-2 Spike glycoprotein on the VLP surface retargeted VLPs to A549 cells expressing the ACE2 receptor (A549-ACE2), thus selectively delivering M8 and blocking VSV infection in susceptible cells but not in wild-type A549. These results highlight the potential of VLPs-M8 as a therapeutic and prophylactic vaccine platform (1).

Overall, these observations demonstrate that the modification of VLP surface glycoproteins and the incorporation of nucleic acids or therapeutic drugs, can re-program VLPs cell tropism, direct specific innate and adaptive immune responses in target tissues, and boost immunogenicity while minimizing off-target effects.

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PO 144 - OC 29

DEVELOPMENT AND PRECLINICAL EVALUATION OF VIRUS-LIKE PARTICLES AS A VACCINE PLATFORM AGAINST SARS-CoV-2

A. Gallinaro[1], C. Falce[1], A. Canitano[1], M.F. Pirillo[1], M. Borghi[2], Z. Michelini[1], A. Zappitelli[2], F. Grasso[2], S. Cecchetti[3], A. Di Virgilio[4], D. Negri[2], A. Cara[1]

[1] *National Center for Global Health, Istituto Superiore di Sanità, Rome, Italy*
[2] *Dept Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy*
[3] *Confocal Microscopy Unit NMR, Confocal Microscopy Area Core Facilities, Istituto Superiore di Sanità, Rome, Italy*
[4] *Center for Animal Research and Welfare, Istituto Superiore di Sanità, Rome, Italy*

Aim: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the agent causative of coronavirus disease 2019 (COVID-19). COVID-19 pandemic posed an extraordinary global health emergency leading to the rapid development of preventive vaccines against the transmembrane homotrimeric Spike (S) glycoprotein that SARS-CoV-2 uses to enter into target cells via the angiotensin-converting enzyme 2 (ACE2) receptor. Although these vaccine strategies reduced effectively hospitalization and death caused by COVID-19, virus escape mutants have occurred extensively throughout the pandemic, causing the insurgence of SARS-CoV-2 Variants of Concern (VoC) less susceptible to the protective effect of vaccines. Therefore, new generations of vaccines based on different delivery approaches are in continuous development. In this context, Virus-like particles (VLPs) are an attractive platform for delivering optimized membrane-tethered Spike protein. Indeed, VLPs are self-assembling nanostructures incorporating key viral structural proteins with a high biosafety profile, since they are non-infectious and non-replicating. VLPs are also potent immune-stimulatory particles, eliciting strong T and B cell immune responses. Therefore, we developed and validated a new vaccine platform against SARS-CoV-2 based on VLP derived from Simian Immunodeficiency Virus (SIV) and displaying S protein from B.1.617.2 (Delta) VoC (VLP/S-Delta).

Materials and Methods: *Preparation of VLP/S-Delta:* 293T cells were co-transfected with pSIV-Gag-GFP plasmid, necessary for VLP assembly, a plasmid coding the Delta S protein with a 19 amino acid deletion at the cytoplasmic tail (CT), to improve membrane tethering and pseudotyping, and the VSV.G-expressing plasmid, to increase immunogenicity. The obtained VLPs were characterized *in vitro* by flow cytometry, Western Blot (WB) and confocal microscopy (CLSM).

Immunization Study: BALB/c mice were immunized intramuscularly with VLP/S-Delta following a prime-boost regimen; mice inoculated with non-pseudotyped VLP (VLP/mock) were used as negative control. Immune response was assessed at 4, 8, 12 and 24 weeks after inoculation by ELISA, neutralization assays, and INFγ ELISPOT.

Results: WB and CLSM on VLP/S-Delta showed that the CT-truncated S-Delta was incorporated on VLP particles at high density. Analysis of immune response in vaccinated BALB/c mice showed that a single immunization elicited specific anti-RBD IgG Abs, which significantly increased after the boost. All animals developed homologous neutralizing antibodies (NAbs), measured by a pseudovirus-based NAb assay developed in our laboratory. Kinetics of NAbs mimicked the trend of binding Abs, showing an approximately 20-fold increase two weeks after the boost. Importantly, prime/boost vaccination elicited cross-NAbs against B.1.1.7 (Alpha) and B.1.1.529 (BA.1) VoC, although at lower levels compared to anti-Delta S NAbs. The reduction in NAbs titers was particularly evident for the BA.1 VoC, probably due to the higher genetic distance between BA.1 and the other VoC. Moreover, S-specific IFNγ producing T cells were detected in all VLP/S-Delta vaccinated mice at 6 months after the prime, suggesting that VLP-based immunization can elicit efficient and durable T cell immunity.

Conclusions: These data provided evidence that SIVGag-based VLPs were able to induce specific and persistent cellular and humoral immune responses in the mouse preclinical animal model, representing an effective candidate for the development of safe SARS-CoV-2 vaccines. Further improvements in the design of the immunogen aimed at further increasing the incorporation of Spike and improving the exposure of epitopes targeted by NAbs could enhance the quality and breadth of the immune responses.

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ASSESSMENT OF BoHV-4-BASED VECTOR VACCINE INTRANASALLY ADMINISTERED IN A HAMSTER CHALLENGE MODEL OF LUNG DISEASE

S. Park[3]*, L. Conti[2]*, V. Franceschi[1], B. Oh[3], M. Yang[3], G. Ham[3], A. Di Lorenzo[2], E. Bolli[2], F. Cavallo[2], B. Kim[3], G. Donofrio[2]

[1] *Department of Medical Veterinary Sciences, University of Parma, Italy*
[2] *Department of Molecular Biotechnology and Health Sciences, University of Turin, Italy*
[3] *Research Institute and Laboratory of Veterinary Pathology, College of Veterinary Medicine, Jeonbuk National University, Iksan 54596, Korea*
* *These authors contributed equally to this work and share first authorship*

Bovine Herpesvirus 4 (BoHV-4) is a bovine Rhadinovirus not associated to a specific pathological lesion or disease and experimentally employed as viral vector vaccine. BoHV-4-BV (BoHV-4-BV) has been shown to be effective in immunizing and protecting several animal species when systemically administrated through the intramuscular, subcutaneous, intravenous, or intraperitoneal rout. However, BoHV-4-BV has never been intranasally tested for respiratory disease protection. In the present study, a recombinant BoHV-4, BoHV-4-A-S-ΔRS-HA-ΔTK, delivering an expression cassette for SARS-CoV-2 Spike glycoprotein, was constructed and its immunogenicity, as well as its capability to transduce cells of the respiratory tract, was tested in mice. Next, to test the intranasal administration of BoHV-4-A-S-ΔRS-HA-ΔTK efficacy in terms of protection toward a SARS-CoV-2 challenge, the well-established COVID-19/Syrian Hamster model was adopted. Intranasal administration of BoHV-4-A-S-ΔRS-HA-ΔTK elicited protection against SARS-CoV-2, with improved clinical signs, including significant reduction of body weight loss, significant reduction of viral load in trachea or lung and significant reduction of histopathologic lung lesions if compared with intramuscular administered BoHV-4-A-S-ΔRS-HA-ΔTK. These results suggested that intranasal immunization with BoHV-4-BV induced a protective immunity and BoHV-4-BV could be a vaccine platform potentially appliable for the protection of other animal species toward respiratory diseases.

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AGE DIFFERENTIALLY AFFECTS THE MAINTENANCE OF MEMORY HUMORAL AND CELLULAR RESPONSES INDUCED BY MRNA AND VECTOR COVID-19 VACCINES

D. Proietto[1]*, B. Dallan[1]*, M. De Laurentis[1], E. Gallerani[1], M. Martino[1], S. Ghisellini[2], A. Zurlo[3], S. Volpato[3], B. Govoni[3], V. Albanese[4], V. Appay[5], S. Llewellyn-Lacey[6], S. Pacifico[1], D.A. Price[6,7], A. Caputo[1], R. Gavioli[1], F. Nicoli[1]

[1] *Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Italy*
[2] *Laboratory of Clinical Pathology, St. Anna Hospital Ferrara, Italy*
[3] *Department of Medical Sciences University of Ferrara, Geriatrics Unit, Azienda Ospedaliero-Universitaria di Ferrara, Italy*
[4] *Department of Environmental and Prevention Sciences, University of Ferrara, Italy*
[5] *Université de Bordeaux, CNRS UMR 5164, INSERM ERL 1303, ImmunoConcEpT, Bordeaux, France*
[6] *Division of Infection and Immunity, Cardiff University School of Medicine, Cardiff, UK*
[7] *Systems Immunity Research Institute, Cardiff University School of Medicine, Cardiff, UK*
* *These authors contributed equally to this work*

Aim of the Study: Despite older subjects are more at risk of severe COVID-19, especially few months after vaccination, at which extent age and the type of vaccine affect the maintenance of antibody and cellular responses has not been addressed. Through this study, we characterized immune responses induced by two different vaccine types (BNT162b2 and ChAdOx1) 6 months after the primary vaccination course and 6 months after a mRNA booster dose in subjects of different age.

Materials and Method: Donors with no known history of SARS-CoV-2 infection (n=244) were enrolled at 6 months on average after the second dose of homologous immunization (two doses of BNT162b2 or of ChAdOx1 vaccines). Ninety-three participants were followed up, and 45 newly enrolled volunteers were recruited, at 6 months on average after a mRNA booster dose.
We evaluated the titers of SARS-CoV-2- spike RBD-specific IgG and we analyzed cellular responses by IFNγ ELISpot and intracellular cytokine staining. Moreover, the expansion, functionality, and cross-recognition capacity of spike-specific $CD8^+$ T cells was measured after expansion with cognate peptide epitopes.

Results: Primary vaccination with BNT162b2 ensured better maintenance of spike-specific antibodies than ChAdOx1 vaccination, while both vaccines induced similar memory T cell responses. The booster dose markedly improved the maintenance of both humoral and cellular responses, especially in subjects previously vaccinated with ChAdOx1. However, spike-specific memory $CD8^+$ T cells displayed a reduced capacity in recognizing mutated epitopes.
Aging profoundly affected long-term humoral responses, while the effects on cellular responses were less pronounced, mainly affecting the $CD4^+$ compartment in BNT162b2-recipients.

Conclusions: Our data suggest that spike-specific cellular responses induced by different vaccine types last up to 6 months upon the last vaccination but fail in cross-recognizing mutated epitopes and undergo a deeper reduction in older subjects primed with mRNA, rather than vectored, vaccines.

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SARS-CoV-2 VACCINEES ELICITING AN UNCONVENTIONAL IgM/IgG RESPONSE SHOW A LESS DURABLE HUMORAL IMMUNITY AND AN INCREASED RISK OF INFECTION

C. Piubelli[1], A. Ruggiero[2], L. Calciano[2], C. Mazzi[1], C. Castilletti[1], N. Tiberti[1], S. Caldrer[1], M. Verzè[1], S.S. Longoni[1], S. Accordini[2], Z. Bisoffi[1], D. Zipeto[2]

[1] IRCCS Sacro Cuore Don Calabria Hospital, Negrar, Verona, Italy
[2] University of Verona, Italy

Introduction: In a previous study (eBiomedicine 2022; 77: 103888), we described 3 different response patterns after SARS-CoV-2 vaccination: subjects developing a) IgG but not IgM; b) IgM and IgG at the same time; c) IgM appearing after IgG. We observed that coordinated expression of IgG and IgM was associated with a higher response in both IgG antibody levels and their neutralizing activity. The unconventional IgG-positive/IgM-negative response was suggestive of an immune imprinting due to previous encounters with seasonal human coronaviruses (hCoV), an observation that needed further investigation. The aim of this study was to evaluate the specific antibody response after BNT162b2 SARS-CoV-2 vaccination and boosts over a long follow-up period.

Methods: We longitudinally analyzed anti-SARS-CoV-2 IgG for the Spike protein (S) receptor binding domain RBD (IgG-S) and anti-S IgM (IgM-S) in 1872 health care workers vaccinees at the first, at the second dose, and at 3 and 23 weeks after the second dose; 109 subjects agreed to be followed up at the booster dose and at 3 weeks and 6 months after. The study population included subjects with a) documented history of SARS-CoV-2 infection before the 1st vaccine dose (infected before vaccination, IBV); b) no evidence of a previous infection (non-infected, NI), and c) infected during or after vaccinations. Two-level linear regression models were used to assess the differences in IgG-S concentration.

Results and Conclusions: In NI subjects, the development of IgM-S after the first and/or second dose was associated with higher levels of IgG-S at short and long follow-up compared to subjects who only developed IgG-S. A similar trend of higher IgG-S concentrations was observed in IBV subjects who developed IgM-S. Furthermore, most subjects who developed IgM-S at the time of vaccination did not become infected during the study, and, alternatively, most subjects who did not become infected were more likely to have developed IgM-S in response to vaccination (eBiomedicine 2023; 89: 104471).
In conclusion, the development of anti-SARS-CoV-2 IgM after the first two doses of vaccine is predictive of higher levels of IgG-S. The majority of individuals who developed IgM-S never became infected, suggesting that IgM-S elicitation may be associated with a lower risk of infection. This protection is not mediated by the persistence of IgM-S, but rather by higher IgG-S levels.
These results suggest an important role of IgM-S development in the maintenance of the anti-SARS-CoV-2 humoral response and in protection against infection, mediated by a higher and more sustained IgG-S response. These data draw attention on the so-called "original antigenic sin", also known as "immune imprinting", whereby an immune response conditioned by previous immunity against other hCoVs may result in less-specific immunity against SARS-CoV-2 after vaccination, reducing the duration and the strength of immune protection.

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THE IMPACT OF INSULARITY ON SARS-CoV-2 DIFFUSION: GENETIC SNAPSHOT OF TWO YEARS OF COVID-19 PANDEMIC IN SARDINIA

N. Grandi[1], J. Milia[2], R. Cusano[2], S. Rubino[3], C. Serra[3], S. Uzzau[3], G. Orrù[4], S. Orrù[4], M. Fiamma[5], G. Piras[5], M.I. Monne[5], T. Fancello[5], G. Mameli[5], E. Tramontano[1]

[1] *Lab. of Molecular Virology, Dept. of Life and Environmental Sciences, University of Cagliari, Italy*
[2] *CRS4 - Center for Advanced Studies, Research and Development in Sardinia, Science and Technology Park Polaris, Cagliari, Italy*
[3] *Dept. of Biomedical Sciences, University of Sassari, Italy*
[4] *Dept. of Surgical Sciences, University of Cagliari, Italy*
[5] *ASL Nuoro, Ospedale San Francesco, Nuoro, Italy*

In the last 3 years, COVID-19 pandemics has triggered intensive research efforts on SARS-CoV-2, including an imponent global epidemiological surveillance. In Europe, Italy has been the first Country dealing with SARS-CoV-2 diffusion, whose geographical distribution on the territory has not been homogeneous. In fact, some administrative regions have been severely affected by the infection, while others showed a remarkably lower number of cases. Among the latter, Sardinia represented one of the lowest incidence areas, likely due to its insular nature. Despite such potential epidemiological impact, the genetic diversity and dynamics of diffusion of Sardinian isolates has not been described yet, nor compared to the national scenario.
To this purpose, in collaboration with the three main clinical centers of the Island (located in Cagliari, Sassari, and Nuoro), we performed the high throughput sequencing of ~900 SARS-CoV-2 genomes circulating in Sardinia from February 2020 to December 2021, selecting them to represent the different clusters of infection across the territory. Such dataset is hence representative of the first 23 months of COVID-19 pandemics in the Island. Using bioinformatic analyses, we characterized the nucleotide variations and phylogenetic relations among our SARS-CoV-2 genomes and compared the epidemiology of COVID-19 with respect to the regional and national ones.
Based on our dataset, in the considered period Sardinia has been interested by the circulation of at least 28 SARS-CoV-2 lineages, as characterized through phylogenetic analyses of Spike

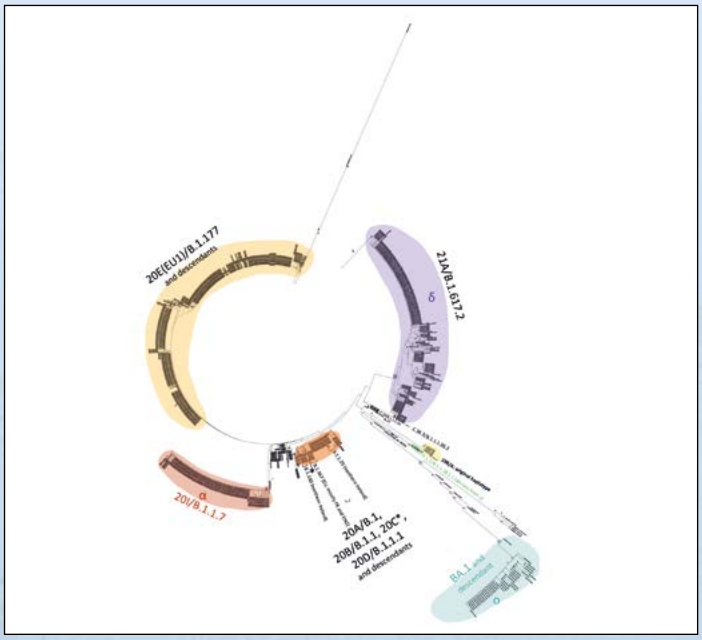

Figure 1
Neighbor Joining phylogenetic tree of S protein from our dataset (888 SARS-CoV-2 genomes).

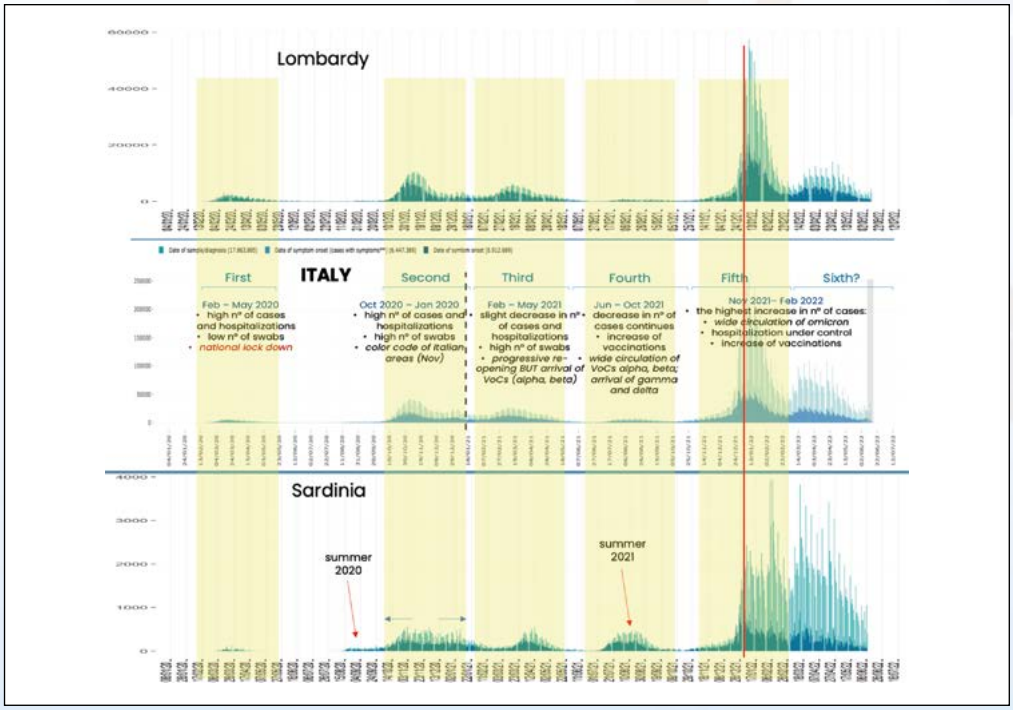

Figure 2 - Comparison of the different pandemic waves as reported in Sardinia (bottom) with the national scenario (middle) and the one of a highly affected administrative area (Lombardy, top).

protein (S) (Figure 1) and of the whole genomic nucleotide sequence. Lineages found in Sardinia included the ones known to be widely spread in Italy as well as some others that are instead poorly represented at national and/or European level, likely linked to multiple importation events. Interestingly, results also revealed that the course of the pandemic in Sardinia showed substantial differences with respect to both the overall national panorama and the one of highly affected areas, such as Lombardy (Figure 2). Especially in the early pandemic phases, Sardinia resulted lowly reached by SARS-CoV-2 diffusion as compared to the rest of the Country, in line with a decreasing North-to-South gradient of incidence. On the counterpart, the Island epidemiology showed some additional peaks of infections that are absent in the national epidemic curve, which reflects the national and regional policies of reopening and the subsequent touristic arrivals. To better characterize our sequences in the regional and national context, the phylogenetic characterization has been extended including all SARS-CoV-2 sequences reported in Sardinia until now (~1300) and in Italy during the same period (~70000), respectively, as included in GISAID repository. Finally, the presence and relative frequency of SARS-CoV-2 lineages in Sardinia have been compared to those from other Islands in the Mediterranean area.

Overall, our results provide a detailed picture of the epidemiological and phylogenetic distribution of Sardinian SARS-CoV-2 genomes, describing the impact of insular geography on viral genetic variability and epidemiological dynamics.

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NATURAL KILLER CELLS IN SARS-CoV-2-VACCINATED SUBJECTS WITH INCREASED EFFECTOR CYTOTOXIC CD56dim CELLS AND MEMORY-LIKE CD57+NKG2C+CD56dim CELLS

S. Rizzo[1], D. Bortolotti[1], L. Morandi[1], V. Gentili[1], G. Schiuma[1], S. Beltrami[1], F. Casciano[1], A. Papi[1], M. Contoli[1], G. Zauli[2], R. Rizzo[1]

[1] *University of Ferrara, Italy*
[2] *King Khaled Eye Specialistic Hospital, Riyadh, Saudi Arabia*

Background: As other European countries, Italy launched its SARS-CoV-2 vaccination campaign on 27 December 2020. The analysis of SARS-CoV-2 vaccination impact on host immune system of healthy subjects might elucidate the potential impact on COVID-19 outcomes. We evaluated whether mRNA-based anti-SARS-CoV-2 vaccination (Comirnaty) elicited a robust protective innate immune response.

Aim: to evaluate the relevance of innate immune response to SARS-CoV-2 vaccines.

Methods: PBMC were obtained form whole blood obtained by donors who received three doses of mRNA-based anti-SARS-CoV-2 vaccination (Comirnaty). NK (Natural Killer) cells immunophenotype and cytotoxicity have been tested after stimulation with SARS-CoV-2 spike antigen (Wuhan, Alpha B.1.1.7, Delta B.1.617.2, Omicron B1.1.529 variants) by FACS assay and related with the anti-SARS-CoV-2 antibody production.

Results: We reported the presence of specific effector cytotoxic CD56dim, characterized by high levels of CD107a and granzyme production, and memory-like CD57+NKG2C+CD56dim phenotype of NK cells exposed to SARS-CoV-2 spike antigen (Wuhan, Alpha B.1.1.7, Delta B.1.617.2, Omicron B1.1.529 variants), in association with specific anti-SARS-CoV-2 antibody production, especially after the booster dose.

Conclusions: We found that the booster dose caused early NK CD56dim subset activation and memory-like phenotype, confirming the relevance of innate immune response in the efficacy of SARS-CoV-2 vaccination.

Keywords: SARS-CoV-2; vaccination; NK cells.

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DID SARS-CoV-2 PANDEMIC REALLY CHANGE THE INFLUENZA EPIDEMIOLOGY?

S. Ranno, L. Coltella, L. Colagrossi

Unit of Microbiology and Diagnostic Immunology, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

Aim of the Study: Before the SARS-CoV-2 pandemia, InfluNet Epidemiological Report showed the Influence Like Illness (ILI) peak during the first weeks of the year. Through 42th 2020 to 17th 2021 week, no peak was reported, while in the 2021-2022 season, a slight peak was referred to the week 2022, 13.

Here we report our experience of Influenza (Flu) viruses detection during the last year (2022), in comparison with the previous five years (2017-2021).

Materials and Methods: This retrospective study involved a total of 38556 respiratory samples referred to 22772 patients (pts), admitted with respiratory symptoms at Bambino Gesù Children's Hospital in Rome from 1st January 2017 to 31st December 2022. Samples were collected and immediately processed for respiratory viruses detection by molecular AllplexTM Respiratory Panel Assays on All-in-One Platform (Seegene, Korea).

Results and Conclusions: Distribution of the study population over the 6 years is reported in table 1.

Year	*2017*	*2018*	*2019*	*2020*	*2021*	*2022*
Requests for year	5386	6698	7442	6095	6558	6377
Flu positive for year	279	325	296	389	4	241
Requests weeks 1-17 (%)	2212 (41%)	2563 (38.2%)	3013 (40.5%)	3282 (53.8%)	1641 (25%)	1890 (29.6%)
Flu positive weeks 1-17 (%)	159 (57%)	309 (95.1%)	269 (90.9%)	389 (100%)	0	37 (15.4%)
Requests weeks 18-41 (%)	1785 (33%)	2603 (38.9%)	2519 (33.8%)	1811 (29.6%)	2553 (38.9%)	2508 (39.3%)
Flu positive weeks 18-41 (%)	2 (0.7%)	5 (1.5%)	5 (1.7%)	0	0	30 (12.4%)
Requests weeks 42-52 (%)	1389 (26%)	1532 (22.9%)	1910 (25.7%)	1002 (16.4%)	2364 (36.1%)	1979 (31.1%)
Flu positive weeks 42-52 (%)	118 (42.3%)	11 (3.4%)	22 (7.4%)	0	4 (100%)	174 (72.2%)

Regarding the pre pandemic years, 2017-2019, distribution of Flu positive patients focused in the first weeks of the year (weeks 1-17). During the pandemic period, Flu was not detected. In 2022, 241 respiratory samples resulted positive for Flu viruses: 37 FluA were detected in the weeks 2022, 1-17, and 169 FluA and 5 FluB were detected in the weeks 2022, 42-52.

During the non epidemic period (weeks 2022, 18-41) 30 FluA virus were detected (12.4% of total Flu positive patients detected). In the same period, for the years 2017-2021, the number of requests for suspected ILI ranged from 30.1% to 38.9%, and Flu circulation was nearly absent (0-1.7% of Flu positive patients).

Our data are globally consistent with those of the influent Epidemiological Report.

Aim of this study was to evaluate what happened in the period out of influenza virus circulation (weeks 18-41), normally not investigated by InfluNet. For the pre SARS-CoV-2 pandemic years, in this period, we can appreciate a significant number of requests for suspected ILI (33-39%) and, as expected, a clear reduction of Flu circulation. The few positives patients (3-6 pts) were detected close to the end or the beginning of the previous or following flu epidemic season, respectively. Unexpectedly this was not observed for the year 2022, in which, in spite of a comparable number of requests (39%), the rate of influenza diagnosis was 12.4% (30 pts) of total Flu positive patients detected (241 pts) over the year. Surprisingly, just weeks 2022, 26-30, showed to be influenza virus free, revealing an unexpected circulation of influenza virus in the summer period of the year 2022.

The question is: Did SARS-CoV-2 pandemic really change the influenza epidemiology, or this is just a transitional phase after the absence of circulation in which viruses are regaining their usual place?

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INDUCTION OF SARS-CoV-2 SPIKE SPECIFIC CD4 T MEMORY STEM CELLS (TSCM) AFTER VACCINATION IS IMPAIRED IN PEOPLE WITH HIV WITH RESIDUAL IMMUNE DYSFUNCTION

S. Scaglioni[1,2*], A. Lombardi[3,4*], G. Butta[1,2], G. Bozzi[4], M. Centazzo[2,4], A. Muscatello[4], S. Ludovisi[4], C. Azzarà[4], R. Grifantini[2], S. Abrignani[2], A. Gori[3,4], R. De Francesco[1,2], A. Bandera[3,4#], L. Manganaro[1,4#]

[1] *Department of Pharmacological and Biomolecular Sciences, University of Milan, Italy*
[2] *Istituto Nazionale di Genetica Molecolare (INGM) "Romeo ed Enrica Invernizzi", Milan, Italy*
[3] *Department of Pathophysiology and Transplantation, University of Milan, Italy*
[4] *Infectious Diseases Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy*
* *Shared first authorship*
\# *Shared last authorship*

Aim: Despite the glaring results of the mass vaccination campaign activated in response to the COVID19 pandemic, it is still unclear whether SARS-CoV-19 vaccines can evoke long-term immunity in immune compromised individuals, including people with HIV (PWH) that fail to reconstitute a functional immune response while on antiretroviral therapy (ART). We characterized the humoral and cellular immune response elicited by mRNA-1273 vaccine in a cohort of PWH receiving ART (20 individuals) that were characterized by a CD4 T cell count >500 or <500, and healthy donors (HDs, 13 individuals).

Methods: Serum and PBMC were collected before the first vaccine dose (T0), 28 days after the second dose (T1b), 6 months after the first dose (T6), 28 days after the third dose (T1c) and 6 months after the first dose (T6c). We determined the neutralizing activity of sera against different VoCs (ND50) with a system based on VSV-pseudoparticles bearing SARS-CoV-2 spike glycoproteins. Cellular immune response and immunophenotype were determined through flow cytometry analysis of surface markers and cytokines after stimulation of PBMC with pools of Spike peptide.

Results and Conclusions: We observed a similar neutralizing activity of sera against SARS-CoV-2 variants in PWH and HD after the third dose, indicating the importance of the booster.
When we analyzed the cellular immune response elicited by mRNA1273 vaccine we observed that the percentage of both CD4 and CD8 T Spike reactive cells was similar in PWH and HD regardless CD4 T cell count. However, the response towards SARS-CoV2 Spike of CD4 T memory stem cells (TSCM) isolated form PWH with a CD4 T cell count <500 was impaired at T1c and T6c. In addition, we observed that the percentage of both CD4 TSCM and Central Memory (CM) expressing markers of exhaustion was significantly higher in PWH with a CD4 T cell count <500 compared with HD, suggesting the possibility of an impairment in long-term immunity in PWH with a CD4 T cell count <500. Of note, expression of exhaustion markers in TSCM from PWH with CD4 T cell count<500 can be reduced by blocking GSK3B (Glycogen Synthase Kinase 3 Beta).

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PLANT VIRUSES IN MOTION: GLOBAL MOVEMENT AND THREATS TO PLANT HEALTH

P. Margaria, S. Winter

Leibniz Institute DSMZ, German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany

Pests and diseases pose a significant threat to availability and safety of food for human and animal consumption. Global yield losses of important staple crops can range up to 30%, and viruses play a major role in emerging and re-emerging plant disease epidemics worldwide with devastating impact. Transboundary and transcontinental movement of plant propagules, seeds and plants for planting is the driver of introduction and spread into new regions. The outbreak of ToLCNDV in Mediterranean regions of Europe, the recent, almost concomitant outbreak of ToBRV in tomato production across Europe and the outbreak of Sri Lankan cassava mosaic virus in Cambodia, Vietnam and Laos almost destroying the cassava industry, provide ample evidence of the constant threats from new virus introductions with severe impact to crops worldwide. Indexing plant materials in transit prior to transcontinental movement and introduction into new areas is a key activity of the DSMZ Plant Virus Department to ensure that plant materials and germplasm are free from pathogens including viruses. Vegetative planting materials present a particular threat of inadvertent movement of known and enigmatic viruses. We therefore have established a competent high throughput sequencing and bioinformatics workflow for pathogen discovery and subject the suspect new virus(es) to biological assays to assess infectivity and potential impact on economically important crops. Exemplary cases in vegetable and ornamental crops will be presented and discussed.

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CLINICAL AND WASTEWATER SURVEILLANCE FOR SARS-CoV-2: THE NEED FOR FURTHER STUDIES FOR AN INTEGRATED SURVEILLANCE

N.T. Atomsa, G. Lauretani, M. Verani, I. Federigi, A. Pagani, A. Carducci
Laboratory of Hygiene and Environmental Virology, Department of Biology, University of Pisa, Italy

Introduction: During the current pandemic, clinical surveillance of COVID-19 cases has been used to assess public health status and implement containment and prevention measures. However, many studies have focused on the use of Wastewater Based Epidemiology (WBE) as an additional analysis tool for the circulation of SARS-CoV-2 in the community. The data from these two types of approaches are quite different and can both be strongly influenced by some variables, such as the concentration of wastewater, different detection methods, population fluctuations, and regulatory changes. It is therefore very important to be aware of these uncertainties when interpreting COVID-19 clinical case data and wastewater analysis and to use caution when making inferences about the epidemiology and impact of the disease in the community.

Objectives: This study seeks to reduce some of the possible distortions of data resulting from WBE and clinical data by studying in depth the evolution of the association of data based on policy and regulatory changes.

Methods: From October 2021 to January 2023, 390 wastewater samples were collected at the inlet of four wastewater treatment plants in the northwest area of Tuscany, on a weekly basis. The viral quantitative data, obtained by RT-qPCR, was normalized taking into account the daily inflow rate and the population served by each treatment plant. Clinical data on the incidence of COVID-19 cases, obtained from the Tuscany Regional Health Agency, were weighted based on the percentages of the population of each municipality belonging to the catchment area of the four treatment plants. The two years of monitoring were divided into 3 phases, depending on some significant policy changes regarding containment measures for COVID-19, and 2 periods following a change in the method for identifying positive COVID-19 cases. The association between clinical data and environmental data was verified using Spearman's rank correlation.

Results: The presence of the SARS-CoV-2 genome was detected in 54.6% (213/390) of wastewater samples. Notably, significant correlations were observed between clinical and environmental data throughout the entire study period. Moreover, the statistical association varied depending on the method used to identify positive COVID-19 cases, as well as the different phases of the study based on policy regulations.

Conclusions: Although both wastewater analysis and clinical cases can be used independently to estimate SARS-CoV-2 infection, the combination of these two approaches can be crucial in providing clear and comparable data to policymakers.

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SELECTION OF NATURALLY SPREAD *CITRUS TRISTEZA VIRUS* ISOLATES EFFECTIVE TO PREVENT THE INFECTION OF HOMOLOGOUS VT- DECLINE ISOLATE PREDOMINANT IN ITALY

G. Licciardello[1], M. Russo[2], M. Bazzano[2], G. Scuderi[2], A. Catara[3]

[1] *Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria-Centro di Ricerca Olivicoltura, Frutticoltura e Agrumicoltura (CREA), Acireale Catania, Italy*
[2] *Agrobiotech soc. coop. ZI Blocco Palma I, Catania, Italy*
[3] *Formerly, Department of Phytosanitary Science and Technologies, University of Catania, Italy*

Citrus tristeza virus (CTV) is a flexuous filamentous closterovirus transmitted in a semi-persistent manner by a few aphid species. Its +ssRNA genome (approximately 19.5 kb in length), includes twelve open reading frames (ORFs) flanked by 5'- and 3'- untranslated regions (UTRs). CTV causes quick decline (QD) of citrus grafted on sour orange (SO) rootstock, stem pitting (SP) in grapefruit and sweet orange and seedling yellow (SY) of sour orange, lemon and grapefruit. Mild strain cross-protection (CP), also known as "pre-immunization", has been shown to prevent stem pitting expression through the mechanism of superinfection exclusion that is potentially regulated by host species and CTV isolate (Folimonova 2013; Harper et al., 2017).

On the contrary, the several experiments conducted worldwide to find cross protective isolates efficient to inhibit quick decline, have been unsuccessful (Lee et al., 2013). Therefore, to prevent the losses caused by QD, alternative scion/rootstock combinations are used to counteract the disease symptoms, but the plants remain an inoculation source of severe CTV strains that are readily spread by aphids. The alternative rootstocks used for the management of QD, do not perform as well as the SO rootstock in calcareous and heavy soils. To continue the use of SO as a rootstock in areas with these soil types and where severe VT strains are present, there is a significant interest to find cross protective isolates suitable to prevent QD.

From this perspective, we have examined the genetic makeup of CTV VT isolates, which are responsible for prevalent decline in Sicily. To achieve this, we sequenced several field isolates using high throughput sequencing (HTS), conducted molecular assays, and carried out extensive bioindexing.

In this investigation five CTV VT field isolates were evaluated for their potential as CP sources. They are SY asymptomatic on SO and on sweet orange grafted on SO but induce slight stem pitting on Citrus macrophylla and grapefruit. These field sources were shown to provide CP against the aggressive VT isolate SG29 (Genbank KC748392) which is prevalent in Sicily in sweet orange/SO trials. The genomes of the field sources were determined by HTS and found to be sequence variants of the QD SG29 isolate. Polymorphisms are mostly associated with ORFs 1a (REP), 2 (p33), 4 (p65) and 11 (p23). Comparative genome analysis revealed that some polymorphisms are conserved between the variants and could be linked to the different phenotype while retaining its ability to repress superinfection from a declining isolate.

When challenged with two declining isolates in different combinations, these field variants are effective to prevent symptoms on sour orange seedlings and sweet orange grafted on sour orange plants. HTS sequencing of total RNAs from challenged sour oranges failed to recover the genome of the declining isolate, allowing to identify the CP isolate. Back inoculation on sour orange of tissues from different hosts cross protected and challenged several years before did not reveal SY reaction typical of SY isolates, suggesting that pre-immunized plants inhibited the SG29 infection.

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THE STRANGE CASE OF GRAPEVINE PINOT GRIS VIRUS AND THE GRAPEVINE LEAF MOTTLING AND DEFORMATION SYNDROME: DEEP INSIGHTS ON VIRUS-HOST INTERACTION

G. Tarquini, P. Ermacora
Department of Agri-food, Environmental and Animal Sciences, University of Udine, Italy

In the early 2000s, a new syndrome characterized by symptoms typical of a virus disease was observed in grapevine (*Vitis vinifera*) in several vineyards in north Italy, and in western Slovenia. The syndrome, later named grapevine leaf mottling and deformation (GLMD), was initially reported on cv. Pinot gris and afterwards on several white berry cultivars. In 2012, a novel virus named grapevine Pinot gris virus (GPGV) was discovered by deep sequencing of the small RNA population of Pinot gris grapevines (1)ampelo- and vitiviruses. An analysis of small RNA populations from two PG grapevines showing or not symptoms was carried out by Illumina high throughput sequencing. The study disclosed the virus and viroids contents of the two vines that was composed by Grapevine rupestris stem pitting-associated virus (GRSPaV. Although, the great variation of symptom severity and the frequent detection of the virus in asymptomatic grapevines did not allow to unambiguously associate the presence of GPGV with the GLMD syndrome, giving rise to a yet unsolved controversy (2). In order to elucidate the relationship between the presence of GPGV and the GLMD syndrome, several ultrastructural and molecular studies have been carried out. Relevant breakthroughs was achieved through the development of two infectious clones of the virus, named pRI::GPGV-vir and pRI::GPGV-lat, which were generated from total RNA collected from a symptomatic and an asymptomatic Pinot gris grapevine, respectively (3). The agroinoculation of the clones in both natural (*V. vinifera*) and model (*Nicotiana benthamiana*) host of the virus allowed to confirm the role of GPGV as causative agent of GLMD syndrome, also demonstrating the existence of virus variants with different patterns of symptom progress (3, 4). In this work, the virulent clone of GPGV (pRI::GPGV-vir) was exploited to induce an antiviral response in N. benthamiana plants. In silico analyses of small RNA (sRNA) libraries of V. vinifera have revealed the abundance of 21-nt long, 5'- terminal cytosine- and uridine-enriched GPGV-derived small interfering RNAs (siRNAs), suggesting the participation of Dicer-like 4 (DCL4), Argonaute 1 (AGO1), and/or AGO5 genes in plant antiviral defense (5). The predicted enzymes, together with RNA-dependent RNA polymerase 6 (RDR6), represent the biochemical core of the post-transcriptional gene-silencing (PTGS) mechanism (6), which was assumed to be the silencing pathway that mediates plant responses to GPGV infection. These assumptions were experimentally validated through gene expression assays, which revealed a significant upregulation of the NbDCL4, NbAGO5, and NbRDR6 genes in N. benthamiana plants inoculated with the GPGV-vir clone, as compared to healthy plants (5). The ability of N. benthamiana plants to spontaneously silence exogenous green fluorescent protein (GFP) transgene offers a straightforward method to evaluate the inhibition of RNA silencing in the presence of viral suppressors (VSRs) (7). Thus, to test the putative ability of one/more GPGV protein(s) to suppress antiviral silencing by encoding VSRs, GPGV proteins were individually co-inoculated with p35S-GFP construct into GFP transgenic N. benthamiana plants (line 16c). 16c plants co-inoculated with p35S-GFP and GPGV-CP showed high expression level of GFP mRNA and low accumulation of GFP-derived siRNAs, similarly to plants co-inoculated with p35S-GFP and the well-known P19 suppressor, demonstrating that GPGV CP may function as VSR (5). Considering the significant contribution of the PTGS in symptom recovery (8), as well as the crucial role of VSR in restoration of symptoms (9), the intriguing GPGV- host interaction may provide the initial clues for a molecular interpretation of the puzzling field observations on GLMD symptomatology. A deeper knowledge of the molecular context of the disease is key to establishing whether the combination with the impressive epidemic potential of GPGV makes it a candidate as an emerging threat for the grapevine industry.

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TOMATO YELLOW LEAF CURL SARDINIA VIRUS AND ITS C4 PROTEIN PRIME TOMATO PLANTS AGAINST DROUGHT STRESS AND FUNGAL INFECTIONS

S. Matić[1], C. Pagliarani[1], A. Moine[1], C. Sacco Botto[1], C. D'Errico[1], W. Chitarra[2], L. Nerva[2], M. Forgia[1], M. Vallino[1], R. Tavazza[3], E. Noris[1]

[1] *Istituto per la Protezione Sostenibile delle Piante, Consiglio Nazionale delle Ricerche (IPSP-CNR), Turin, Italy*
[2] *Centro di Ricerca in Viticoltura ed Enologia, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA-VE), Conegliano, Treviso, Italy*
[3] *Agenzia Nazionale per le Nuove Tecnologie, L'energia e lo Sviluppo Economico Sostenibile (ENEA), Centro Ricerche Casaccia, Rome, Italy*

Plants can be subjected simultaneously to abiotic and biotic stresses, such as drought and viral infections. The possibility that viruses repay host plants for the damages caused by the infection, helping them to endure abiotic stresses has been described, underpinning the existence of common molecular networks that regulate the plant responses towards the stimuli provoked by biotic and abiotic stresses. Recently, begomoviruses causing the tomato yellow leaf curl disease in tomatoes were shown to increase heat and drought tolerance, possibly through the intervention of the viral C4 protein. Here, we elucidated how the begomovirus tomato yellow leaf curl Sardinia virus (TYLCSV) and its C4 protein overexpressed in transgenic tomato plants modulate physiological and molecular events in tomato plants. Combining morphometric and physiological parameters and hormone content with transcriptional analysis of genes involved in water-stress response and hormone metabolism, we assessed that TYLCSV infection and the overexpressed C4 protein delay the onset of stress-related features, improve the water use efficiency, and facilitate a rapid post- rehydration recovery of plants. Specific anatomical and hydraulic traits, rather than biochemical signals, support such increased drought stress resilience. Moreover, we observed that plants overexpressing C4 induced tolerance to *Oidium neolycopersici*, the agent of powdery mildew, not only counteracting symptoms, conidia adhesion and secondary hyphae elongation, but also changing the expression of the pathogenesis-related genes and phytohormone biosynthesis. Overall, the priming role exerted by TYLCSV and its C4 protein in the adaptation to abiotic and biotic stresses opens new perspectives in the management of the effects of climate change in horticultural crops.

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NANOPORE-BASED DETECTION OF PLANT VIRUS: CHALLENGES AND OPPORTUNITIES

S.S. Amoia[1,2], R.A. Kubaa[1], F. Cillo[1], G. Loconsole[1], A. Minafra[1], M. Chiumenti[1]

[1] *Institute for the Sustainable Plant Protection, National Research Council (CNR), Bari, Italy*
[2] *Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy*

Plant viruses pose a threat to sustainable agriculture worldwide causing considerable economic losses. The factors driving virus emergence rely mainly on the worldwide trade of plant propagation materials (budwoods, rootstocks, seeds), which behave as reservoir sources and move viruses, hosts, and vectors to new areas. Furthermore, the climate change affects the distribution area of hosts and vectors and the ability of viruses for evolution and adaptation. Since chemicals able to heal virus- infected plants are not available, disease management is based on preventing virus spread and obtaining plants resistant to viral infections. Currently, virus surveillance methods involve the collection of samples and laboratory assays, which is time-consuming. Additionally, this approach is inadequate in an outbreak situation, when quick response is critical, and delays ease the virus spreading. The advent of high-throughput sequencing (HTS) technologies has led to a revolution in plant virus diagnosis, as they do not require any previous knowledge of viral targets and could sequence millions of nucleic acid molecules simultaneously, enabling the detection of all virus-like agents infecting a plant, including the latent and/or still unknown ones. Among the HTS technologies, single molecule sequencing produces reads with a length exceeding 10 kb. Nanopore sequencers (i.e MinION, Oxford Nanopore technology) measure the ionic current fluctuations when single-stranded nucleic acids pass through protein nanopores; then, convert the raw data into nucleic acid sequences. In this frame, we applied a nanopore sequencing for the characterization of the virome of three plant samples. A cDNA-PCR barcoding kit (SQK-PCB109) was used to sequence dsRNA or rRNA- depleted total RNA extracted from leaf tissues of ornamental (*Jasminum officinale* L.), woody (*Ficus carica* L.) and herbaceous (*Solanum lycopersicum* L.) plants. This technique allowed the first identification in Europe of the carlavirus jasmine virus C in *J. officinale*, the two closteroviruses (fig virus A and fig virus B), recently described in Argentina and Korea, and, in tomato, mixed infections of tomato brown rugose fruit virus (ToBRFV) and pepino mosaic virus (PepMV). Although the intrinsic potential of the technology, which allows a fast, low cost and portable sequencing of samples, and makes it ideal for the prompt identification of plant pathogens, its application for the viral metagenomics has still several drawbacks. In our experience, nanopore sequencing is affected by the viral target concentration in the sample but also by any secondary structures that could characterize viral RNAs. This in turn influences the proportion of sequenced viral genomes, which in most cases are difficult to be assembled, due to insufficient coverage and/or lack of bioinformatic tools adequate for the reconstruction of viral contigs/genomes. In contrast, by using targeted approaches (i.e primer-specific cDNA synthesis of ToBRFV) in tomato, the coverage problems were overcome although off-target viral (PepMV) and host (tomato genome) sequences were also retrieved. In conclusion, the absence of standardized sequence-independent laboratory protocols together with the lack of specific bioinformatic tools currently represent the main bottleneck for a repeatable and large-scale application of nanopore technology in plant virus diagnostics.

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FIRST CHARACTERIZATION OF TWO VIROID-LIKE RNAs IN THE ORCHID-ASSOCIATED FUNGUS *TULASNELLA sp.*

M. Forgia[1], S. Daghino[1], B. Navarro[2], M. de la Peña[3], F. Di Serio[2], M. Turina[4]

[1] *Institute for Sustainable Plant Protection, National Research Council of Italy, Turin, Italy*
[2] *Institute for Sustainable Plant Protection, National Research Council of Italy, Bari, Italy*
[3] *Instituto de Biología Molecular y Celular de Plantas, Universidad Politécnica de Valencia-CSIC, Valencia, Spain*
[4] *Institute for Sustainable Plant Protection, National Research Council of Italy, Brescia, Italy*

Introduction: Fungi have been a great source of new viral diversity through the recent past, but only few information is available on viroid and viroid-like RNAs associated to fungal hosts. Recent studies on public metatranscriptomes showed more than 20,000 new circular elements containing ribozymes. Among these elements, it was possible to characterize, specifically from fungal samples, RdRp encoding RNA viruses with circular genomes also encoding ribozymes (named ambiviruses). With the same bioinformatics approach, we could detect two contigs (called contig_11108 and contig_6686) corresponding to two viroid-like RNAs containing one ribozyme in each polarity strand and infecting *Tulasnella sp.* isolates from a collection of orchid roots-associated fungi previously investigated for the viral presence.

Methods: The identification of the ribozyme-encoding circular elements was obtained through a public pipeline (1). qRT-PCR was used to detect the contigs in the infected isolates and to confirm the absence of DNA counterparts. The *in silico* obtained sequence was validated and completed through inverse RT-PCR. Northern blot analysis after polyacrylamide gel electrophoresis was used to confirm the circularity of the contigs and *in vitro* assays were performed to confirm the activity of the ribozymes.

Results and Conclusions: Contig_6686 corresponds to a circular RNA sequence of 1575 nt named Tulasnella viroid-like RNA 1 (TulVd-LR1) found in the *Tulasnella* isolate O10, while contig_11108 corresponds to a circular RNA sequence of 1212 nt long named Tulasnella viroid-like RNA 2 (TulVd-LR2) found in *Tulasnella* isolate O9. A small portion of TVd-LR1 and TVd-LR2 sequence shows a high identity with Tulasnella ambivirus 1 and Tulasnella ambivirus 4, respectively, which are two ambiviruses detected in the same fungal isolates (2). Both viroid-like RNAs contain two different ribozymes, one in each polarity strand (a hammerhead ribozyme, HHRz, in the plus polarity and a hairpin ribozyme, HPRz, in the minus polarity). The viroid-like RNAs were confirmed to be present in the infected fungi as RNA only, thus excluding a genomic origin. The circularity of the two viroid-like RNAs was demonstrated by Northern blot analysis in denaturing conditions, showing the presence of both circular and linear forms, and the activity of the ribozymes was demonstrated *in vitro*. All the results collected so far confirm the hypothesis that TVd-LR1 and TVd-LR2 are indeed viroid-like RNAs replicating through rolling cycle amplification in *Tulasnella sp.*, suggesting that they could be viroids or viroid-like satellite RNAs.

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IMPLICATIONS OF MIXED VIRUS INFECTIONS FOR THEIR PERSISTENCE AND SPREAD

K. Zhao[1], N. Johnson[2], M. Islam[1], M. Axtell[3], C. Rosa[1,2], A. Taylor[4]

[1] *Department of Plant Pathology and Environmental Microbiology, The Pennsylvania State University, USA*
[2] *Huck Institutes of the Life Sciences, The Pennsylvania State University, USA, Millennium Science Initiative-Millennium Institute for Integrative Biology (iBio), Santiago, Chile, Centro de Genómica y Bioinformática, Facultad de Ciencias, Universidad Mayor, Santiago, Chile*
[3] *Department of Biology, The Pennsylvania State University, USA, Huck Institutes of the Life Sciences, The Pennsylvania State University, USA*
[4] *Huck Institutes of the Life Sciences, The Pennsylvania State University, USA*

Mixed infections of plant viruses are commonly found in natural patho-systems and present a valuable opportunity to understand how multiple viruses can co-infect the same host. Tomato spotted wilt orthotospovirus (TSWV) and impatiens necrotic spot orthotospovirus (INSV) are present in the same geographic areas and are closely related. More mixed infections of TSWV and INSV have been reported in recent years, and the INSV host range has been reported to be increasing. The aim of this study was to investigate how mixed INSV and TSWV infections drive their persistence and spread in the agricultural landscape. The methods used consisted of a series of TSWV and INSV serial and co-inoculations followed by real time virus quantification, vector oviposition preferences tests, virus transmission, plant volatile collection and analysis and small RNA profiling. Our results showed that INSV and TSWV have an antagonistic relationship in their vectors, and that INSV alters plant responses and the processing of TSWV. In conclusion, determining if the host defense system (i.e., RNAi) responds similarly to TSWV and INSV in single or mixed infection could explain the ecological interactions that led to an increase in INSV prevalence and could provide novel orthotospovirus species or genus specific targets to use for disease management.

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GRAFTING TO MANAGE *TOMATO LEAF CURL NEW DELHI VIRUS* IN CUCURBITS

M. Mastrochirico, R. Spanò, T. Mascia

Università degli Studi di Bari "Aldo Moro", Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Bari, Italy

Tomato leaf curl New Delhi virus (ToLCNDV) is an emerging begomovirus listed in the EPPO Alert-list 2 and reported in 2015 for the first time in southern Italy on Sicilian courgette. The virus is transmitted in a persistent manner by *Bemisia tabaci*, an aleurodide very diffuse in the Mediterranean area, and is particularly harmful in cucurbits where it causes 100% production losses. For this reason, a sustainable and environmentally friendly approach must be adopted to counteract the virus. Genetic resistances have been identified in *Cucurbita moschata* and *Luffa cylindrica*, but the graft could provide a faster and more flexible solution inducing tolerance rather than resistance, as shown for susceptible commercial tomato varieties grafted onto a tomato wild ecotype tolerant to virus infections.

Twenty-one local cucurbit ecotypes were screened to assess tolerance levels against mechanical transmission of an Apulian isolate of ToLCNDV (ToLCNDV-Le). Disease symptoms development and viral DNA accumulation were assessed by quantitative dot-blot assays at 14 and 28 days post inoculation (dpi).

C. melo var. Retato standard (F1 commercial hybrid) and *C. pepo* var. Scuro di Milano proved the most susceptible, whereas *C. melo* var. Barattiere and *C. pepo* accession 5 the most tolerant. In particular, *C. melo* cv. Barattiere did not show any detectable disease symptoms and very low levels of viral DNA accumulation was recorded. Moreover, this variety was most suitable to be employed as rootstock for its morphological characters; thus, it was used as potential rootstocks to achieve adequate levels of tolerance against the virus in commercial cucurbit varieties.

Different graft combinations of susceptible and moderately susceptible cucurbit genotypes onto *C. melo* cv. Barattiere plants showed a generalized delay in viral symptom appearance and a less severe disease than those observed in non-grafted counterparts. Moreover, the severity of symptoms observed in grafted plants was congruent with the viral DNA accumulation estimated in all grafted combinations, which showed a significant reduction between 14 and 28 dpi.

On the whole, the results of this study suggest that the grafting of commercial cucurbit varieties onto *C. melo* cv. Barattiere used as rootstock could provide interest levels of tolerance against ToLCNDV-Le disease.

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MYCOVIRUS HOMOLOGUES OF PLANT VIRUS MOVEMENT PROTEINS: ARE THEY FUNCTIONAL?

I. Martinelli[1], F. Bono[1], S. Pagnoni[1], E. Gobbi[1], M. Turina[2]

[1] *Department of Molecular and Translational Medicine, University of Brescia, Italy*
[2] *Institute for Sustainable Plant Protection, National Research Council of Italy, Turin, Italy*

Viral movement in plants requires one or more specific proteins defined movement proteins (MP) that facilitate the passage of virus components (virus genomic RNA or whole virions) through plasmodesmata, the plant-specific connections between cells. MPs are specific of plant viruses. Their origin is still an unsolved mystery, even though some plant-encoded paralogues have been characterized and were also shown to assist cell to cell and long-distance movement of macromolecules. Here we investigate the recent discovery that a subset of *bona fide* fungal viruses (mycoviruses) having negative strand RNA genomes encode proteins that share some degree of similarity to plant virus MPs. One of such mycovirus, named Trichoderma gamsii cogu-like virus 1 (TgClV1) is a negative strand RNA virus detected in a recent work we carried out to characterize the virome associated to a wide collection of *Trichoderma* spp. from Sardinia island. After aligning multiple 30 kDa superfamily MP members and different MP-like representatives of cogu-like fungal viruses we could confirm the presence of the seven beta-strands structural module in the latter, which is the characteristic domain of the 30 kDa superfamily. Such structurally conserved module is also predicted by the artificial intelligence for in silico protein structural analysis alphafold. Given these premises we proceeded to test *in vivo* if fungal homologues of MP can indeed functionally complement movement deficient plant viruses: we used a Tomato apex necrosis virus (ToANV) MP deficient virus vector (expressing GFP in single isolated cells) and we showed that its cell-to-cell movement could be complemented providing tobacco mosaic virus-MP in trans, but not the two cogu-like MP-like proteins we are currently testing. We then exchanged the MP from ourmia melon virus with MP from TMV and MP-like from coguvirus, but also in this case, with the latter, we failed to complement movement. Finally, we checked if any MP-fusion to GFP would localize to plasmodesmata, one of the main features of virus MP: in the case of TMV-MP and other plant viral MP we observed the expected localization, while for cogu-like MPs plasmodesmata were not specifically targeted. In conclusion we could not provide evidence that fungal MP-like proteins are indeed functional plant MPs. This raises questions about the original functional role of the seven beta-strand module characteristic of MP like proteins.

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ELECTRONIC NOSE APPLICATION FOR THE EARLY IDENTIFICATION OF TOMATO SPOTTED WILT VIRUS IN TOMATO PLANTS

G. Zambotti[1,2], E. Gobbi[3,1], M. Turina[4], A. Torri[3], A. Ponzoni[1,2]

[1] *National Research Council, National Institute of Optics (CNR-INO), Unit of Brescia, Italy*
[2] *University of Brescia, Department of Information Engineering, Brescia, Italy*
[3] *University of Brescia, Department of Molecular and Translational Medicine, AgriFood and Environmental Microbiology Platform (Pi.Mi.A.A.), Brescia, Italy*
[4] *Institute for Sustainable Plant Protection, National Research Council of Italy, Turin, Italy*

Tomato spotted wilt virus (TSWV) (genus *Orthotospovirus*, family *Tospoviridae*) is globally recognized as an emerging threat to agricultural crops. In particular, this virus and its thrips species vectors pose a major problem for tomato, *Solanum lycopersicum*, causing systemic infection in susceptible tomato cultivars (1), and greatly hampering their production. Studies have revealed that VOCs emitted by plant tissues are closely associated with the metabolic or pathologic processes in the live plant system and reflect the physiological state of the individual plant (2); thus, different VOC patterns could characterize healthy and diseased plants, possibly before the onset of visible symptoms. Given these premises, the primary objective of this work was to verify the feasibility of the electronic nose (EN) technology as a non-invasive tool for the early detection of TSWV in tomato plants. Tomato plants, (cultivar Marmande) were grown at 24°C in a growth chamber with a 14 hrs artificial light supply. Plants were mechanically inoculated following standard protocols with the TSWV strain I244, originally isolated from endive lettuce in Brescia province (Italy) and maintained in *Nicotiana benthamiana* and tomato plants.
The electronic nose was based on a portable, commercial control unit (JLM Gigamostic from JLM innovation Gmbh, Tübingen, Germany) equipped by a metal oxide gas sensor working in temperature modulation mode (3). After plant inoculation, both the tomato plants and the sensor were enclosed within plastic bags, positioning the EN near the leaves. In parallel, mock-inoculated plants were tested as a control sample. The analyses were conducted for 10 days since inoculation and the infections were confirmed by enzyme-linked immunosorbent assay (ELISA) technique at the end of the monitoring timeframe. EN signals were analysed by means of statistical methods, including Principal Component Analysis.
Distinction between TSWV-infected tomato plants and non-infected plants began to emerge around day five from inoculation, while, by naked eye, that symptoms began to appear only after the seventh day. These preliminary results suggest that the EN is a promising technology for the non-invasive monitoring of tomato plants against TSWV infection, and may be considered a candidate for the development of technology based strategies to mitigate and control the spread of the disease.

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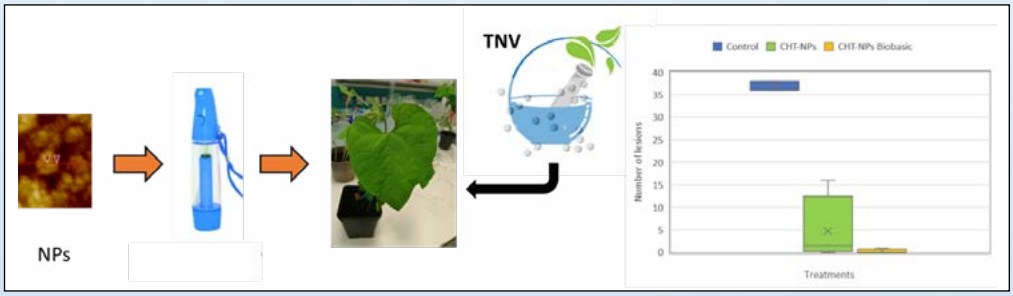
PO 163

ANTIVIRAL ACTIVITY OF GREEN NANOPARTICLES FROM SHRIMP PROCESSING WASTE

D. Bulgari[1], M. Maddaloni[2], I. Vassalini[2], I. Alessandri[2], M. Turina[3], E. Gobbi[1]

[1] *Department of Molecular and Translational Medicine, University of Brescia, Italy*
[2] *Department of Information Engineering, University of Brescia, Italy*
[3] *Institute for Sustainable Plant Protection, National Research Council of Italy, Turin, Italy*

Chitosan (CHT) is a natural biopolymer widely used in food and bioengineering industries, enzyme immobilization, and as a carrier for controlled drug delivery. CHT is a biodegradable and non-toxic compound derived from chitin deacetylation that exerts its effects both as a plant resistance activator and as a fungitoxic compound. The former property is due to the induction of localized micro-oxidative bursts in treated plants, and fungal toxicity is possibly the result of increased membrane permeability, together with chelation of essential nutrients and binding to DNA. The properties of CHT biopolymer can be further improved in the form of nanoparticles. CHT nanoparticles were effective in enhancing plant productivity in inducing expression of pathogenesis-related proteins and thus improved resistance to fungi (i.e. *Fusarium* sp., *Botrytis cinerea).* Even if chitosan is mainly extracted from crustacean's exoskeletons different new technology are emerging to produce CHT from waste. Crustacean's farming is one of the most widespread fish farms all over the world generating a considerable amount of wastes (heads and shells of crustaceans). Extracting chitosan represents an efficient use of these materials, not only to boost the economic revenues but also to solve the problem of a surplus of waste that has a slow degradation rate and contributes significantly to greenhouse gas emissions.
In this work shrimp waste was valorised for producing chitosan nanoparticles as a source for eco-friendly nano-antiviral technology.



Tobacco necrosis virus (TNV) - *Phaseolus vulgaris* L. system was used to assess the antiviral activity of chitosan nanoparticle (CHT-NPs). CHT extracted from shrimp wastes was characterized for the deacetylation degree that was 79%. CHT- NPs prepared with the ionotropic gelation method were analysed in terms of shape and particle size. AFM analyses highlighted a spherical shape and a mean diameter size of 1-5 nm. The CHT-NPs were sprayed on bean leaves 24 hours before TNV inoculation to test phytotoxicity and to elicit plant defence response. The protective effect of CHT-NPs obtained from extracted chitosan was 87% compared to the untreated plants. These nanoparticles are less effective compared to those obtained from a commercial chitosan that showed a higher protective effect maybe due to the higher deacetylation degree.
This study presents a sustainable approach to obtain antiviral compounds from waste management in a circular economy view.

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EXPLORING THE ROLE OF PROPHAGES IN INTERFERING WITH THE MICROBIOME OF A PHYTOPLASMA INSECT VECTOR

M. Vallino, M. Rossi, L. Galetto, C. Marzachì, S. Abbà

Institute for Sustainable Plant Protection of the National Research Council of Italy, Turin, Italy

Euscelidius variegatus (family Cicadellidae) is an insect vector of phytoplasmas, wall-less plant-pathogenic bacteria that cause yield losses in many crops worldwide. Phytoplasmas are obligate parasites colonizing the phloem of the host plant and the body of the insect vector. The main control strategies to limit these pathogens are insecticide treatments against the vector species. *E. variegatus* is a natural vector of chrysanthemum yellows phytoplasma, which is associated with a disease in ornamental plants in Northern Italy, and a laboratory vector of Flavescence dorée phytoplasma, which represents one of the major threats to grapevines, especially in Southern Europe. *E. variegatus* harbors an heritable bacterial endosymbiont, originally called BEV (Bacterial parasite of *E. variegatus*), which was recently assigned to the genus *Symbiopectobacterium* (family *Enterobacteriaceae*). BEV can be cultured in axenic conditions, but its growth is slow and its survival is limited in time.

Some phage-like sequences were found in the metatranscriptome of *E. variegatus* and their association with BEV genome was assessed by PCR on BEV isolates growing in axenic cultures. Moreover, transmission electron microscopic (TEM) observations of phage particles, both in BEV cultures and in a viral purification obtained from *E. variegatus,* confirmed that some prophages are actively replicating.

The objectives of this work are:

1) to identify and characterize the prophages sequences harbored in the BEV genome;
2) to study prophages activation in different conditions;
3) to investigate the role of lysogenic phages in the survival of the phytoplasma vector *E. variegatus*.

BEV genome was sequenced using Illumina and Nanopore technologies, assembled with Flye and polished with several rounds of Racon, Medaka and Pilon. Phaster program predicted 25 intact phage-like sequences in the 5 million bp-long BEV genome. All the predictions were manually checked for the presence of at least two structural proteins and eight bacteriophages belonging to the class *Caudoviricetes* were retained for further analyses. Preliminary results obtained with specific primers showed that five of them were active both in the insect and in the bacterial culture, one was active only in the bacterial culture and two appeared to be inactive in both conditions so far. Observations under a Philips CM10 TEM showed at least five different phage particle morphologies in both BEV cultures and insect body.

After three days of growth in the dark at 26°C in Tryptic Soy Broth, BEV showed a slow linear growth. Such growth might be explained by a continuous, basal production of phage particles without any phage bursts. This phenomenon suggests that phages harboured in BEV genome are not true temperate phages and may have one of the alternative infection strategies (such as pseudo-lysogeny, carrier state, chronic infection) that are recently emerging as ubiquitous and potentially decisive in the ecology and evolution of their bacterial hosts.

A BEV suspension was microinjected into an *E. variegatus* population lacking this endosymbiont. The survival of the injected insects reared on chrysanthemum decreased and the development of the new generation was delayed with respect to control. Our hypothesis is that in *E. variegatus* prophages could play a key role in maintaining the bacterial growth within levels tolerated by the insect host, whereas in non-host species/populations the lytic phase of prophages might not be triggered, thus causing an uncontrolled growth of the bacterium and the death of the insect host.

PO 165

THE BINOMIAL NOMENCLATURE FOR VIRUS SPECIES: AN UPDATE ON PLANT VIRUS TAXONOMY

L. Rubino

Istituto per la Protezione Sostenibile delle Piante, Consiglio Nazionale delle Ricerche, Bari, Italy

The International Committee on Taxonomy of Viruses (ICTV) is responsible for developing and maintaining an internationally agreed system of hierarchical classification of viruses and naming of taxa. The advancements in virus taxonomy are made available to the scientific community through a website (https://ictv.global/) and published reports and papers to keep virologists informed about the decisions on virus classification and nomenclature.

To reflect current knowledge on the evolution of global virosphere, taxonomic ranks have been expanded beyond orders up to realms, provided that taxa in this 15-rank taxonomy are monophyletic. The only mandatory taxa are genus and species. At present, virus taxonomy consists of 6 realms, 10 kingdoms, 17 phyla, 2 subphyla, 40 classes, 72 orders, 8 suborders, 264 families, 182 subfamilies, 2818 genera, 84 subgenera and 11273 species.

The expansion to 15 ranks aligns virus taxonomy to other biological taxonomies, which adopt a Linnaean Latinized binomial format (i.e., binomial nomenclature), consisting of two italicized words indicating the genus ("genus name") and the species ("specific name/species epithet"), respectively. In contrast, viral species names did not follow a uniform format, except for a requirement to be italicized and to have the first letter of the first word capitalized. To reach a standardized nomenclature, in 2021 the ICTV ratified the adoption of a binomial nomenclature for virus species. Based on this, a virus species name will be composed by two italicized words, the first one being the genus name and the second one consisting of a "free-form" species epithet. In this framework, Linnaean-style, Latinized virus species are permitted, but not mandated. This change in nomenclature must be completed by 2023 and it will apply to virus species only. Virus common names will remain unchanged.

Following open debates, the Plant Viruses Subcommittee Study Groups are developing a binomial nomenclature format for virus species. Most of the Study Groups have adopted a Latinized binomial format, while discussions are still going on within others and a variety of epithets is being proposed. The most recent update will be reported in the corresponding poster.

PO 166

THE MYCOVIROME OF THE GREY MOULD FUNGUS BOTRYTIS CINEREA

R.M. De Miccolis Angelini, C. Rotolo, S. Distante, P.R. Rotondo, S. Pollastro, F. Faretra

Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy

Botrytis cinerea is the fungus responsible for grey mould on a wide range of plant species, both in the field and postharvest, resulting in heavy yield losses. Several mycoviruses are known to infect fungal plant pathogens and they might represent a source of variation in the fungal populations. In this study, a metagenomic approach was applied to obtain a comprehensive characterization of the mycovirome in a collection of 60 *B. cinerea* isolates. High-throughput Illumina sequencing of double-stranded (ds)RNAs extracted from 12 pools of isolates generated about 64 million of high-quality short sequence single reads (50 bp). Contigs obtained through de novo assembly were analysed by sequence similarity to identify putative mycoviral sequences. A great abundance and diversity of mycoviruses was found. A total of 22 different viruses were identified, at different frequencies, in one or more of the analysed pools. These mostly included dsRNA and positive-sense (+) single-stranded (ss) RNA viruses. Among dsRNA viruses, two viruses in the family *Partitiviridae*, a victorivirus in the family *Totiviridae*, a virus in the family *Quadriviridae*, and three unclassified viruses were identified. (+)ssRNA viruses included seven mitoviruses in the family *Mitoviridae*, three hypoviruses in the family *Hypoviridae*, a fusarivirus in the proposed family *Fusariviridae*, Botrytis virus F in the family *Gammaflexiviridae*, a virus in the family *Endornaviridae*, and an umbra-like mycovirus in the family *Tombusviridae*. Botrytis cinerea mymonavirus 1 in the family *Mymonaviridae* was the only negative-sense (-)ssRNA virus found in the analysed isolates. For eight mycoviruses selected as putatively associated to host hypovirulence, the presence of the viral sequences was checked by RT-PCR in 28 individual *B. cinerea* isolates, revealing single or multiple viral infections. These findings will serve as a basis for further studies aimed at investigating the complex virus - host and virus - virus interactions and at exploring the potential use of mycoviruses as biocontrol agents against grey mould.

PO 167

A NOVEL AND UNCOMMON GENOTYPE 4 OF HEPATITIS E VIRUS DISCOVERED IN URBAN WASTEWATER IN ITALY

C. Veneri[1], G. Bonanno Ferraro[1], P. Mancini[1], C. Del Giudice[1], E. Suffredini[2], M. Iaconelli[1], D. Brandtner[3], A.R. Ciccaglione[4], R. Bruni[4], M. Equestre[5], C. Marcantonio[4], G. La Rosa[1]

[1] *Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy*
[2] *Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy*
[3] *Independent Researcher, Rome, Italy*
[4] *Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy*
[5] *Department of Neuroscience, Istituto Superiore di Sanità, Rome, Italy*

Aim of the Study: Hepatitis E is a viral infection that affects the liver and is caused by the hepatitis E virus (HEV). It is usually spread through contaminated water or food. There are four main genotypes of the virus in humans, with genotypes 1 and 2 being the most common. Genotypes 3 and 4 are mainly found in animals but can also infect humans. Genotype 4 is mainly found in Asia, particularly China, but has also been identified in other parts of the world, including Africa, Europe and North America. Like genotype 3, genotype 4 has been shown to be zoonotic. There are several subtypes and variants within genotype 4 of the hepatitis E virus and their prevalence can vary according to geographical region. In Italy, HEV genotype 4 is rare, with only one documented outbreak occurring in 2011 involving five patients. However, in 2013, genotype 4 hepatitis E virus (HEV) was first discovered in a pig herd in north-eastern Italy. Subsequently, no additional evidence of HEV genotype 4 has been found in the country. Here we describe the first detection of an HEV genotype 4, subtype-d strain in a wastewater sample collected in central Italy in the absence of reported clinical cases in the area.

Methods and Results: The sample was collected on 19th April 2022 in the wastewater treatment plant of San Salvo (Chieti, Abruzzo). The concentration was carried on through PEG precipitation and the extraction of nucleic acids was completed using magnetic silica beads. The HEV strain present in the sample was initially identified by amplification and sequencing of partial ORF1 as genotype 4. Further subtyping was performed by sequencing a region of ORF2 (450 bp), which confirmed the sample as HEV 4d. A phylogenetic tree generated using all available GenBank HEV-4d sequences revealed that the wastewater sample was most closely related to strains a.n. JX401928 and KF939867, detected in Italy in human and swine samples on March 2011 and June 2013, respectively. To fully characterize the genome of this environmental 4d strain, 20 partially overlapping PCR assays were designed, each spanning 450 bp, using a closely related genotype 4d sequence retrieved from GeneBank. We then used Illumina NGS platform to sequence the PCR products. Approximately 4000 bp of the genome were obtained, confirming the assignation to HEV subtype 4d. Further efforts are ongoing to complete the missing sections of the genome.

Conclusion: In summary, despite the rarity of genotype 4 HEV infections in Italy, the recent discovery of HEV genotype 4d in a wastewater sample collected in central Italy highlights the importance of continuous monitoring and detecting infectious agents in wastewater as a method for supporting public health surveillance.

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RF-2018-12365399 "Improving understanding of autochthonous Hepatitis E transmission routes: a focus on foodborne and waterborne pathways".

PO 168

INVESTIGATING THE ECOLOGY AND GENETIC DIVERSITY OF HEPATITIS E VIRUS IN ITALY USING WATER MATRICES AND SWINE FARMING SLURRY

G. Bonanno Ferraro[1], P. Mancini[1], C. Veneri[1], C. Del Giudice[1], M. Iaconelli[1], M. Brambilla[2], C. Bisaglia[2], A. Filisetti[2], S. Giovinazzo[2], B. Di Martino[3], V. Martella[4], E. Suffredini[5], G. La Rosa[1]

[1] Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy
[2] CREA- Council for Agricultural Research and Economics, Research Centre for Engineering and Agro-Food Processing, Treviglio, Bergamo, Italy
[2] Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy
[3] Department of Veterinary Medicine, University of Teramo, Teramo, Italy
[4] Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy
[5] Department of Veterinary Medicine, University of Bari "Aldo Moro", Bari, Italy

Aim of the Study: Hepatitis E virus (HEV) is the most common cause of acute viral hepatitis worldwide. In non-endemic countries, the presence of HEV in water environments has been demonstrated. In Italy, the transmission pathways for autochthonous HEV have not been clearly defined, but there is evidence of virus occurrence in food and the aquatic environment, as well as in animals, including pig farms. Therefore, a multidisciplinary project in the Abruzzo region, considered a "hot spot" for HEV, has been funded, to enhance knowledge of the transmission pathways. This study reports the results of investigations exploring the ecology and genetic diversity of HEV in water environments and pig farming activities.

Methods: A total of 295 water samples were collected between 2019 and 2023, including untreated wastewater collected from 22 wastewater treatment plants, treated wastewater, river water, marine water in bathing and non-bathing areas, and water used for irrigation purposes. In addition, 164 raw slurry samples were collected from 19 intensive swine farming units located across the Abruzzo region during four different sampling campaigns (July and November 2021, May and November 2022). Both real-time PCR and nested PCR techniques were used for the detection and molecular characterisation of HEV. The typing/subtyping of the samples was performed using the Hepatitis E Virus Genotyping Tool (Hepatitis E Virus Genotyping Tool).

Results and Conclusions: Twenty-eight samples of raw wastewater (12.1%) were found to be positive for HEV, while treated wastewater and river, marine, or irrigation waters did not result in any positivity for the virus. According to the HEV typing tool, 27 wastewater samples were identified as HEV genotype 3, while one sample belonged to the HEV-4 genotype. Subtype genotype 3 analysis showed that six samples were classified as subtypes 3e, six samples 3c, three samples 3f1, one sample 3f2; in addition, five samples clustered near subtype 3e, but with a low bootstrap value in the phylogenetic tree.
HEV was detected in 18.9% of swine slurry samples. Subtypes 3e, 3c, 3f1 and 3f2 were detected in these samples. In addition, two sequences clustered in a distinct group (bootstrap 100%) along with a wild boar liver sample taken in the same period in Abruzzo. No established subtypes could be assigned to these sequences, suggesting the presence of a new subgroup. In conclusion, phylogenetic analysis revealed diverse HEV genotypes and subtypes circulating in the region, including novel lineages found in both human and animal populations. These findings highlight the importance of continued surveillance and an improved understanding of transmission pathways to control HEV infections.

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RF-2018-12365399 "Improving understanding of autochthonous Hepatitis E transmission routes: a focus on foodborne and waterborne pathways".

PO 169

THE USE OF NANOPORE SEQUENCING TECHNOLOGY PROVIDES NEW INSIGHTS INTO THE WIDE RANGE OF HUMAN ADENOVIRUSES PRESENT IN ASTEWATER

G. Bonanno Ferraro[1], P. Mancini[1], C. Veneri[1], C. Del Giudice[1], M. Iaconelli[1], D. Brandtner[2], the SARI network[4], E. Suffredini[3], G. La Rosa[1]

[1] *Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy*
[2] *Independent Researcher, Rome, Italy*
[3] *Members are listed in 10.5281/zenodo.7836308*
[4] *Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy*

Aim of the Study: Wastewater-based epidemiology is a powerful tool for monitoring the circulation of pathogens in the population, including both asymptomatic and symptomatic infections. Human adenovirus (HAdV) is an enteric virus, responsible for various clinical manifestations, including upper respiratory tract infections, conjunctivitis, gastroenteritis, bronchitis and pneumonia, urinary tract infections, and skin infections. Occasionally, adenoviruses can cause more severe illnesses, including meningitis, encephalitis, and myocarditis. Adenovirus can be spread through water, making it a potential waterborne pathogen. Human HAdVs are classified into seven species (A-G) and more than 110 types. Species A and B are responsible for common respiratory infections, while species F includes serotypes 40 and 41, which are recognised as some of the most common causes of gastroenteritis.

Methods: From 9 to 13 of January 2023, a total of 170 sewage samples were collected in 165 wastewater treatment plants (WTPs) in 18 regions and 2 autonomous provinces of Italy. HAdV was detected by amplification of a fragment of the HAdV hexon gene (714 to 855 bp long, depending on the HAdV type). Amplicons were sequenced by both Sanger (individual samples) and next-generation sequencing (in a pool of all PCR amplicons), using the Oxford Nanopore Technology (MinION platform). The long reads obtained by NGS were compared with 110 reference prototypes belonging to the Species HadV-A (n°=4), HadV-B (n°=17), HAdV-C (n°=7), HAdV-D (n°=78), HAdV-E (n°=1), HAdV-F (n°=2), and HAdV-G (n.=1).

Results and Conclusions: The results showed a wide diversity of HAdVs in wastewater, indicating genetic diversity within the population at a national level. The nested PCR results showed the presence of HAdVs in 118 out of 170 samples (69.4%). Seventy-six samples were successfully sequenced and characterised by conventional Sanger sequencing as belonging to 5 HAdV Species and 7 types: Species A (HAdV-12, 2 samples), Species B (HAdV-3, 33 samples), Species C (HAdV-1, 2 and 89, 6 samples), Species D (HAdV-45, 1 sample), and Species F (HAdV-41, 34 samples). NGS analysis confirmed the results obtained by Sanger sequencing but was able to detect additional less represented types: HAdV-B21, HAdV-C5, HAdV-C89, HAdV-D23, HAdV-D46, HAdV-D49, HAdV-D83, and HAdV-F40. Other sequences could not be definitively assigned because they shared the same % nt identity with more than one HAdV prototype: HAdV-B7 and B66; HAdV- C1 and C104; HAdV- D45 and D73; HAdV- D48 and D81.
Our findings highlight the diversity of HAdV species and types present in raw sewage, with the potential to impact surface waters and pose a significant threat to human health. By using next-generation sequencing, we were able to identify a broad range of "less prevalent" types in addition to those identified by Sanger sequencing. The results show that although the exon region is recognised as being able to discriminate among different types of human adenovirus, it is not actually able to identify certain types that have a high degree of nucleotide identity, particularly those belonging to the D Species.

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PO 170

ENHANCING UNDERSTANDING OF SARS-CoV-2 VARIANTS USING WASTEWATER ANALYSIS: AN In-DEPTH 18-MONTH INVESTIGATION

P. Mancini[1], E. Suffredini[2], G. Bonanno Ferraro[1], C. Veneri[1], C. Del Giudice[1], M. Iaconelli[1], D. Brandtner[3], L. Lucentini[1], the SARI network[4], G. La Rosa[1]

[1] Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy
[2] Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy
[3] Independent Researcher, Rome, Italy
[4] Members are listed in 10.5281/zenodo.7836308

Aim of the Study: Since its onset in 2020, the COVID-19 pandemic has rapidly spread across the world, resulting in a significant increase in morbidity and mortality rates. The emergence of several SARS-CoV-2 variants has added to the concern of global health authorities. These variants contain unique sets of mutations that provide higher transmissibility or, in some cases, more severe symptoms in infected individuals. Wastewater-Based Epidemiology (WBE) has proved to be an effective tool for understanding and monitoring the COVID-19 pandemic. The analysis of wastewater provides valuable insights into the extent and progression of a disease outbreak within a specific area. Additionally, this method enables tracking the emergence and spread of new viral variants. Recognizing the potential of WBE technology, the European Commission recommended establishing systematic surveillance of SARS-CoV-2 and its variants in wastewater across all EU regions. Since October 2021, Italy has been conducting monthly "flash surveys" in all regions to monitor the SARS-CoV-2 variant's spread in the community. In this study, we present the results of 18 months of wastewater monitoring in Italy.

Methods: During the period spanning October 2021 to March 2023, a total of 2731 samples of wastewater were collected from 167 water treatment plants located in 18 regions and 2 autonomous provinces across Italy. These samples underwent analysis to detect the presence of SARS-CoV-2 variants. Specifically, the samples underwent amplification using previously described nested RT-PCR (1), able to amplify a long fragment (1592 bps) of the spike region to detect distinctive mutations of SARS-CoV-2 variants. Positive PCR amplicons were sequenced by both Sanger and Next-Generation (NGS) techniques.

Results and Conclusions: Overall, 654 samples were successfully sequenced. The study found shifts in the predominant viral variants in wastewater over the 18-month monitoring period, in line with the epidemiological conditions. In fact, from October 2021 to December 2021, the only variant identified was the Delta. Starting January 2022, the Omicron variant was dominant, with several sublineages emerging periodically: BA.1, BA.1.1, BA.2, BA.4, BA.5, BQ1, BQ.1.1, BA.2.75 (including BN.1* and CH.1.1), XBB.1, XBB.1.6, XBB.1.5, and CM*. The NGS approach enabled the identification of less prevalent viral variants that might otherwise be missed by Sanger sequencing. These findings demonstrate that WBE can be used to monitor the evolution and spread of SARS-CoV-2 at the population level. The results of this study emphasize the importance of wastewater surveillance for SARS-CoV-2 control strategies and the need for continued monitoring of viral strains in both clinical and environmental settings.

Acknowledgements

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EXPLORING THE FEASIBILITY OF WHOLE-GENOME SEQUENCING OF MULTIPLE VIRUSES FOR WASTEWATER MONITORING: PRELIMINARY FINDINGS

P. Mancini[1], C. Veneri[1], G. Bonanno Ferraro[1], C. Del Giudice[1], T. Vicenza[2], M. Iaconelli[1], A.R. Ciccaglione[3], R. Bruni[3], M. Equestre[4], C. Marcantonio[3], E. Suffredini[2], G. La Rosa[1]

[1] *Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy*
[2] *Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy*
[3] *Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy*
[4] *Department of Neuroscience Istituto Superiore di Sanità, Rome, Italy*

Aim of the Study: Wastewater surveillance is the practice of monitoring wastewater samples to detect the presence of viruses and other pathogens in a community. This approach has become increasingly important, particularly during the COVID-19 pandemic, as it provides a non-invasive way to monitor the spread of viruses in a population. To make this surveillance method even more effective, it would be useful to be able to detect multiple viruses at once, rather than targeting them individually. Whole-genome sequencing of multiple viruses in wastewater can provide important information about the prevalence and diversity of viral pathogens in a community.

Materials and Methods: In an effort to test this approach, a commercial viral surveillance panel (Illumina) designed to detect 66 (both DNA & RNA) different viral genomes, including those identified as important risks to public health by the World Health Organization, was tested. Overall, 21 wastewater samples collected in Rome, Italy in the period between January and November 2022 were analysed. The panel uses a hybrid-capture target enrichment workflow that allows for sequencing of various sample types. Wastewater samples were collected at the inlet of the Wastewater Treatment Plant and subjected to viral concentration (50 ml, PEG 8000/NaCl precipitation) and RNA extraction (fully automated system using patented magnetic bead purification technology). The comprehensive hybrid capture-NGS workflow integrates library preparation, target enrichment, sequencing, and data analysis to identify the viral species present in the sample. One control sample was constructed containing nucleic acids obtained from viral stocks including: Human Adenovirus, Rotavirus, Rhinovirus, Monkeypox virus, Coxsackievirus A and B, Echovirus, Enterovirus 68 and 70, Norovirus genotype II, Hepatitis A and E virus, SARS-CoV and SARS-CoV-2, MERS-CoV, HCoV-OC43, HCoV-NL-63 and HCoV-229E.

Results and Conclusions: In the control sample, the vast majority of the spiked viruses were detected. Viral genome sequences were obtained in all the wastewater samples tested. Specifically, viruses belonging to different families and genera were detected and fully sequenced: SARS-CoV-2, Norovirus GI and GII, polyomavirus JC and BK, Merkel cell Polyomavirus, Astrovirus, Coxsackievirus A and B, Enterovirus 68 and 70, Salivirus, human Papillomavirus HPV18, Rhinovirus, Rotavirus, and Hepatitis A virus. Up to 7 virus groups could be detected in one sample.
Despite the preliminary nature of this experiment, conducted with a limited number of samples and without controls designed to properly assess sensitivity, the potential of this multi-target approach for a complex matrix such as wastewater is encouraging and can provide valuable insights into the viral diversity and prevalence in a community.

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THE VECTOR *POLYMYXA BETAE* AFFECTS THE ACCUMULATION OF MULTIPARTITE BNYVV GENOMIC RNAs

Y. Guo[1], M. Dall'Ara[1], D. Gilmer[2], C. Ratti[1]

[1] *University of Bologna, Department of Agricultural and Food Sciences, Bologna, Italy*
[2] *Institut de Biologie Moléculaire des Plantes (IBMP), Strasbourg, France*

Soil-borne *beet necrotic yellow vein virus* (BNYVV) is the causal agent of the *Rizomania* disease, transmitted by the protozoan *Polymyxa betae*. BNYVV is a multipartite virus containing 4 or 5 genomic RNAs which are encapsidated into individual rod-shaped viral particles. A potential benefit for multipartitism is the regulation of gene expression through gene (or segment) copy number variations. Here, we analyzed the relative frequencies of the four genomic RNAs of BNYVV in its host *Beta vulgaris* at different times after inoculation by a validated protocol of dual step reverse transcriptase droplet digital PCR (RT - ddPCR). The relative frequencies of BNYVV RNA1 and RNA4 show significant differences at different times post-inoculation. We also investigated the genome formula (GF) of BNYVV in the host B. vulgaris when its vector *P. betae* is present and absent demonstrating that the viral segments copy number is changing according to the presence of the vector. Moreover, the BNYVV GF was characterized in the two forms of *P. betae* life cycle, zoospores and resting spores. Results show the virus reaches a dedicated set-point genome formula in each of the two forms of the protozoan life cycle. Hence, our results indicate that the vector, *P. betae*, affects the accumulation of BNYVV genomic RNAs in its hosts suggesting that the virus may differentially control the copy number of its segments.

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DETECTION OF CRYPTIC VIRUSES AND CHARACTERIZATION OF ANTIVIRAL DEFENSE IN *CANNABIS SATIVA* THROUGH SMALL RNA SEQUENCING

N. Miotti[1,2], N. Sukhikh[2], N. Laboureau[2], P. Casati[1], M.M. Pooggin[2]

[1] *Department of Agricultural and Food Sciences, Production, Landscape, Agroenergy, University of Milan, Italy*
[2] *PHIM Plant Health Institute, Univ Montpellier, INRAE, CIRAD, IRD, Institut Agro, Montpellier, France*

Cannabis sativa (hemp) is currently undergoing a rediscovery in different economically relevant fields, from industrial to medical, leading to an increase of cultivated areas. Virome of this plant has been only recently explored through NGS technologies, leading to detection of known and unknown viruses (1). Cryptic viruses, characterized by persistent infection without symptoms, have been isolated from many plants including hemp. Cannabis cryptic virus (CanCV, family *Partitiviridae*) and Cannabis sativa mitovirus 1 (CasaMV1, family *Mitoviridae*) have been reported as widely distributed in *C. sativa* (2,3). In this work, we performed an Illumina small RNA (sRNA) sequencing analysis of different varieties of *C. sativa* to reconstruct their virome components and characterize antiviral defenses based on RNA interference (RNAi). Total RNA was extracted from 15 dioecious plants cultivated for cannabinoid production (CBD or CBG) and 4 monoecious plants cultivated for seeds and fiber production. *De novo* and reference-based assembly of sRNA reads allowed us to detect both CanCV and CasaMV1 and reconstruct their complete genome sequences. Incidence of CanCV was lower than expected (2 of 19 plants) and this virus was targeted by antiviral RNAi generating predominantly 21 and 22 nt small interfering RNA (vsiRNAs) from both strands of the viral genome, suggesting that their biogenesis is mediated by Dicer-like (DCL) 4 and DCL2 enzymes, respectively. CasaMV1 had a higher incidence (42.1% plants) and its vsiRNAs had a wider size range and a positive strand bias, with 21 nt vsiRNAs being the most represented on both strands. Analysis of 5'-terminal nucleotide identity of sRNAs indicated that the major size classes of vsiRNAs derived from both viruses are mainly associated with Argonaute (AGO) 1-like (5'U) and AGO5-like (5'C) enzymes that can catalyze cleavage and degradation of viral RNA.

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BIOBANKS AND COLLECTIONS: A FOCUS ON PLANT VIRUSES

L. Rubino[1], M. Ciuffo[2], M. Vallino[2], A.M. Vaira[2], P. Mussano[2], M. Marra[2,3], R. Pierro[2,4], G.P. Accotto[2]

[1] *Institute for Sustainable Plant Protection, National Research Council, Bari, Italy*
[2] *Institute for Sustainable Plant Protection, National Research Council, Turin, Italy*
[3] *Department of Soil, Plant and Food Science, University of Bari Aldo Moro, Bari, Italy*
[4] *Department of Pharmacy, University of Salerno, Fisciano, Salerno, Italy*

Microorganisms are ubiquitous in the environment and represent the major form of life on Earth. They play fundamental roles in ecosystems, contributing to their functioning, and cover the surface of other organisms, interacting with their physiology. Therefore, microorganisms are key to environmental and human health, to science as well as to bioeconomy and biotechnology applications. Most of the microbial biodiversity is still unveiled, prompting for the need to expand our knowledge on yet uncharacterized microorganisms. At the same time, it is essential to preserve and maintain the currently known microbial biodiversity. In this view, collections and biobanks play a crucial role. The European Virus Archive - Global (EVA-Global; https://www.european-virus-archive.com/) is a non-profit organization involving a global network with expertise in animal, human and, starting from 2020, plant virology. It consists of more than 40 partner laboratories both based in EU member states and abroad. EVA-Global as part of the European Union's Horizon 2020 research and innovation programme's funding is conceived to be an open access entity aiming at facilitating and developing synergies between scientific and industrial community. Its web-based catalogue (https://www.european-virus-archive.com/evag-portal), provides easy access for the end user to the collections including viruses, derived materials (nucleic acids, antisera, controls, infectious cDNA clones etc...) and services. Whereas EVA-Global is focused on viruses, SUS-MIRRI.IT (Strengthening the MIRRI Italian Research Infrastructure for Sustainable Bioscience and Bioeconomy) is a comprehensive PNRR project aimed at improving and reinforcing the Italian microbial collections, including those of fungi, yeasts, bacteria and viruses, as well as derived products (https://www.sus-mirri.it/it/). Both consortia share the highest scientific standards in terms of safety, quality and characterization through the implementation by partners of a quality management system. Besides fundamental studies and applied uses of microorganisms, a major final perspective is the preparedness of the scientific community, which is essential to face future challenges, as amply demonstrated during the current SARS-CoV-2 pandemic.

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NEW FEATURES IN THE VIROME OF FREESIA HYB. SHOWING LEAF NECROSIS SYNDROME IDENTIFIED BY AN NGS-BASED OMICS APPROACH

S. Rotunno[1], R. Pierro[1,2], J. Hammond[3], M. Marra[1], F. Frascati[1], A.M. Vaira[1], L. Miozzi[1]

[1] *Institute of Sustainable Plant Protection, CNR, Turin, Italy*
[2] *Department of Pharmacy, University of Salerno, Fisciano, Salerno, Italy*
[3] *FNPRU USDA-ARS USNA, 10300 Baltimore Avenue, Beltsville MD, United States of America*

The necrosis syndrome of freesia was first described before 1970 in Northern Europe, and it is still jeopardizing freesia cultivation all over the world, producing severe symptoms. Different viruses have been suggested as possible causal agent, but, until now, none of them have been convincingly associated with the disease. The ophiovirus freesia sneak virus (FreSV) has been proposed as the causal agent of the necrotic disease in Europe, USA, New Zealand, and South Korea, but the correlation between symptoms and the presence of FreSV was not clearly shown. Here we applied a next generation sequencing (NGS) based approach to investigate the virome associated to the necrosis syndrome of freesia and the possible involvement of other viral agents in the etiology of this disease. Freesia plants showing strong necrotic symptoms were collected in different years in Northern Italy and associated virome was reconstructed using an *ad hoc* bioinformatics pipeline. Results, validated by independent molecular methods, enlarged our knowledge of the virome of freesia, highlighting new features for known and new viral entities; among them a new genome component of FreSV, and an uncharacterized virus, likely belonging to a new family in the Bunyavirales order, as suggested by the Serratus-mediated comparison with sequencing data available at the NCBI Sequence Read Archive database.

Keywords: Virome, next-generation sequencing, NGS, freesia, leaf necrosis, Bunyavirales.

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VIROME ANALYSIS OF *SILENE ALBA* PLANTS REVEALS A NEW POTYVIRUS AND A NEW ALPHAPARTITIVIRUS

G. Parrella[1], E. Troiano[1], A. De Stradis[2], A. Marais[3], C. Faure[3], T. Candresse[3], T. Elbeaino[4]

[1] *Institute for Sustainable Plant Protection, National Research Council (CNR), Portici, Naples, Italy*
[2] *Institute for Sustainable Plant Protection, National Research Council (CNR), Bari, Italy*
[3] *Univ. Bordeaux, INRAE, UMR 1332 Biologie du Fruit et Pathologie, 33882 Villenave d'Ornon Cedex, France*
[4] *Mediterranean Agronomic Institute of Bari (CIHEAM-IAMB), Valenzano, Bari, Italy*

Silene bianca (*Silene alba* (Miller) Krause, family Caryophyllaceae) plants displaying mottling mosaic symptoms were found in the Campania region (Southern Italy). Purified dsRNAs were extracted from symptomatic leaves, randomly reverse-transcribed and submitted to Illumina high throughput sequencing (HTS) to determine the virome associated with symptomatic plants. Two viral agents were identified: the first had a positive-sense and single-stranded RNA genome of 9681 nucleotides (nts) excluding a poly(A) tail. The complete viral genome encodes a large open reading frame, encoding a polyprotein of 3069 amino acids (aa). Sequence comparisons and phylogenetic analyses demonstrated that the viral agent belonged to the genus *Potyvirus* in the family *Potyviridae* and is most closely related to carnation vein mottle virus, sharing with it 66% nt and 59% aa sequence identities. In addition, HTS analysis revealed also mixed infections with a new alphapartitivirus, for which a near complete RNA1 sequence (1896 nt) and a partial RNA2 sequence of (1491 nts) were determined. The RNA1 is most closely related to Vicia faba partitivirus 1, with 74.6% of nucleotide identity while the corresponding RNA-dependent RNA polymerases share 82% aa identity. These two new viral agents were tentatively named silene mottle mosaic virus (SiMMV) and silene cryptic virus (SiCV).

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MITOVIRUS-INFECTED AND NON INFECTED *CHENOPODIUM QUINOA* PLANTS SHOW DIFFERENT RESPONSES TO DROUGHT

M. Ciuffo[1], G. Vigani[2], M. Turina[1]

[1] *Institute for Sustainable Plant Protection, CNR, Turin, Italy*
[2] *Plant Physiology Unit, Dept Life Science and Systems Biology, University of Turin, Italy*

The FAO policy strongly promotes quinoa as viable alternative crop, for its high nutritional properties, rich in protein, lipids, minerals and tocopherols, and for the adaptability to a wide range of marginal agricultural soils, including those with high salinity and those tending to drought. Recently, we provided evidence that the biodiversity of some *C. quinoa* lines is associated to the presence of a cryptic mitochondrial virus (CqMV1), giving them higher tolerance to virus infection and hydric stress.

For better understanding of the role of CqMV, we compared two mitovirus-infected lines (Regalona and IPSP1) and two mitovirus-free lines (BO78 and BO25).

We looked at the effect of some physiological parameters in abiotic stress condition (water stress) and some biotic stress (virus infections). We looked at the differential mitochondrially-enriched proteome of mitovirus-infected and mitovirus-free leaf extracts, showing some specific virus-caused down-regulation or up-regulation of a number of proteins. We discovered that quinoa hosting mitovirus activate some metabolic process that might help them to face drought. For example some proteins belonging to branched amino acid catabolism such as BCE2 and MCCB, and DELTA-OAT, reported to enhance multiple abiotic stress. Finally, we began the process of obtaining mitovirus-infected and mitovirus-free quasi-isogenic lines, focusing on reciprocal crosses between Regaloma and BO78, and evaluating the different expression of these genes in water stress condition.

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WASTEWATER MONITORING AS PUBLIC HEALTH TOOL TO STUDY EPIDEMIC AND ENDEMIC VIRUSES DISTRIBUTION TRENDS AMONG THE POPULATION

A. Pagani, M. Verani, I. Federigi, L. Viviani, N.T. Atomsa, G. Lauretani, A. Carducci
Laboratory of Hygiene and Environmental Virology, Department of Biology, University of Pisa, Italy

Introduction: Viral agents excreted in feces and urine of infected individuals can be found in wastewater, that, for this reason, can be used to study their spread among the population. In particular, wastewater monitoring, also called *Wastewater based epidemiology* (WBE), has the potentiality to describe viral spatial-temporal distribution, potential circulation of variants and the possible relation with clinical data. These data could be very useful both during viral pandemics (e.g., SARS-CoV-2) than for the surveillance of endemic pathogens (e.g., Human Adenovirus).

Objectives: The purpose of this study is to determine the concentration of SARS-CoV-2 and Human Adenovirus (HAdV) in wastewater samples of urban plants (WWTP) in Tuscany to investigate the spatial and temporal distribution and the relationship with the clinical data, available only for COVID-19.

Methods: Samples were weekly collected at the entrance of the treatment plants, and after thermal inactivation, were concentrated through precipitation with PEG/NaCl. Viral detection was performed by molecular methods: nucleic acids, extracted by a magnetic silica-based kit and treated to remove inhibitors, were amplified using specific real-time (RT-)qPCR and the genomic copies per liter (GC/L) were normalized basing on daily inflow rate and served population. The processing methods used were those indicated by the ISS (*Istituto Superiore di Sanità*) in SARI project (*Sorveglianza Ambientale di SARS-CoV-2 in Reflui in Italia*). Available clinical data were obtained by Health Authority Database.

Results: Starting from October 2021, 302 samples were analysed to evaluate the presence of SARS-CoV-2 and 301 were analysed to evaluate the presence of HAdV. The results showed 196 positive SARS-CoV-2 samples (64,9%), with geometric mean (±SD) of 6.26E+08±3.84 GC/l, and 232 positive HAdV samples (77%), with geometric mean (±SD) of 4.43E+10±4.46 GC/l. The obtained data revealed that the viral concentration is influenced by the season and the location of sampling throughout the monitoring period (2wayANOVA of both SARS-CoV-2 and HAdV showed P_{value} WWTP and P_{value} seasons <0.05). Moreover, the trend of SARS-CoV-2, especially in the last period, was similar to common endemic viruses' trend, such as Human Adenovirus. Furthermore, in some period, clinical COVID-19 data were correlated to those obtained from wastewater (Spearman, $p<0.05$).

Conclusion: Our results highlight that WBE could be used as a relevant tool to study the spread of viral pathogens, both in pandemic and in endemic scenarios, and that it could be integrated to clinical data.

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ARTIFICIAL CHIMERIC DEFECTIVE INTERFERING RNA DERIVED FROM RNA2 AND -5 OF BEET NECROTIC YELLOW VEIN VIRUS

M. Dall'Ara[1], S. Baisotti[1], D. Gilmer[2], C. Ratti[1]

[1] *University of Bologna, Department of Agricultural and Food Sciences, Bologna, Italy*
[2] *Institut de Ciologie Moléculaire des Plantes (IBMP), Strasbourg, France*

A defective RNA (D-RNA) is a deleted or rearranged genome variant of an RNA virus created by polymerase template switching or reassortment events during replication. Like satellite RNAs, D-RNAs are replication-dependent on their helper virus, from which they were generated. By retaining parental replication promoters, most D-RNAs act as defective interfering RNAs (DI-RNAs) affecting viral accumulation and, therefore, attenuating symptoms in the infected plants.
Here we present the construction of a chimeric DI-RNA derived from the fusion of RNA2 and RNA5 portions of beet necrotic yellow vein virus (BNYVV), the etiological agent of sugar beet rhizomania. The pentapartite virus holds in RNA1 and -2 all the functions required for infection, and their combination serves as helper viruses for RNA3 -4 and -5, which are ancillary and can be transformed into viral replicons after remotion of their coding sequences. Therefore, we inserted the first 400 nucleotides of RNA2 into the replicon derived from RNA5, obtaining the rep5DIRNA2 construct aiming to have multiple replication promoters in the same construct to exclusively recruit viral replicase at the expense of the genomic RNAs. We then demonstrated its interfering activity on viral replication by co-transfecting or co-inoculating it together with RNA1 and -2 in *Chenopodium quinoa* protoplasts and leaves. Transient expression of rep5DIRNA2 in *Nicotiana benthamiana* was carried out to test its ability to prevent infection by BNYVV and, thus, to consider the production of virus-resistant transgenic sugar beets.

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GENOME WIDE ANALYSIS OF CYTOSINE AND ADENINE METHYLATION CHANGES INDUCED BY VIROID INFECTIONS IN TOMATO

B. Navarro[1], G. Marconi[2], V.M. Nicoloso[1], M. Chiumenti[1], M. Bocchini[2], E. Albertini[2], F. Di Serio[1]

[1] *Istituto per la Protezione Sostenibile delle Piante, Consiglio Nazionale delle Ricerche, Bari, Italy*
[2] *Department of Agricultural, Food and Environmental Sciences, University of Perugia, Italy*

DNA methylation is an epigenetic mark involved in the regulation of multiple processes including gene expression, genome stability and gene imprinting that can be induced by environmental changes and biotic stresses. Viroids are infectious agents of plants composed of a non-coding small circular RNA than can cause diseases. Pioneering studies demonstrated that potato spindle tuber viroid (PSTVd), a nuclear-replicating viroid, was able to induce DNA methylation in a PSTVd-cDNA transgene, leading to the discovering of the afterwards called RNA-directed DNA methylation and suggesting that viroids could interfere with host DNA methylation. With the objective to assess the effects of viroid infections in the methylation status of the host genome, we studied PSTVd infecting its natural host, tomato, by genome-wide analyses based on MCSeEd (Methylation Context Sensitive Enzyme ddRAD) method. MCSeEd allows comparative analysis of methylation levels of cytosine (5-methylcytosine, 5mC) in all three contexts (CG, CHG, CHH) and of adenosine (N^6-methyladenine, 6mA). To this aim, libraries generated by double restriction–adapter ligations using a methylation insensitive endonuclease (*Mse*I) in combination with one methylation-sensitive enzyme (*Aci*I, *Pst*I, *Eco*T22I, and *Dpn*II, for the CG, CHG, and CHH and 6mA, respectively) were sequenced by high-throughput techniques. A total of twenty-four libraries, corresponding to each of the four methylation contexts for three biological replicates of PSTVd-infected and mock-inoculated tomato plants, were generated, pooled in four indexed libraries and Illumina-sequenced using 150 pair-end chemistry. The relative methylation levels at each site, the Differentially Methylated Positions (DMPs) and the Differentially Methylated Regions (DMRs) that showed significant differences in the methylation levels between PSTVd-infected and mock were identified. Moreover DMRs were mapped in the gene bodies (5'UTR, CDS and 3'UTR) and in their regulatory sequences to identify the differentially methylated genes (DMGs). This study reveals that early PSTVd-infections induce a global hypomethylation in the CG, CHH and m6A contexts and a global hypermethylation in the CHG context. DMRs mapping in genes as well as in lncRNAs were preferentially identified in all methylation contexts except for CHH, where significative mapping of DMRs in intergenic regions was observed. Global transcriptomic analysis of PSTVd-infected versus mock-inoculated tomato plants will be performed to correlate viroid-induced differential methylation with host gene expression and further dissect the role of epigenetic changes in PSTVd/tomato interactions.

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A YEAR-LONG ENVIRONMENTAL SURVEILLANCE OF SARS-CoV-2: ESTIMATING THE EFFECTIVE REPRODUCTION NUMBER OF SARS-CoV-2 INFECTIONS USING WASTEWATER DATA (*RWASTEWATER*)

G. La Rosa[1], C. Veneri[1], P. Poletti[2], M. Manica[2], G. Bonanno Ferraro[1], P. Mancini[1], C. Del Giudice[1], M. Iaconelli[1], L. Lucentini[1], M. Cerroni[1], D. Brandtner[3], M. Rossi[4], M. Grigioni[4], G. D'Avenio[4], M. Del Manso[5], F. Riccardo[5], A. Bella[5], P. Pezzotti[5], A.T. Palamara[5], U. Agrimi[6], M. Martuzzi[1], the SARI network[7], S. Merler[2], E. Suffredini[6]

[1] *Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy*
[2] *Bruno Kessler Foundation, Trento, Italy*
[3] *Independent Researcher, Rome, Italy*
[4] *National Center for Innovative Technologies in Public Health, Istituto Superiore di Sanità, Rome, Italy*
[5] *Department of Infectious Disease, Istituto Superiore di Sanità, Rome, Italy*
[6] *Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy*
[7] *Members are listed in 10.5281/zenodo.7836308*

Aim of the Study: The shedding of viral particles in wastewater allows for the application of wastewater-based epidemiology as a complementary approach to clinical testing, enabling monitoring of SARS-CoV-2 transmission in the community. Several studies have successfully measured the concentration of SARS-CoV-2 RNA in wastewater and established a correlation between viral loads and clinically confirmed cases of COVID-19 in the corresponding catchment areas. In Italy, environmental surveillance of SARS-CoV-2 began in October 2021. Here we present the results of a year-long wastewater surveillance of SARS-CoV-2, and the development of a COVID-19 transmissibility indicator using wastewater data, called Rwastewater (Rw).

Methods and Results: Overall, 9,389 wastewater samples (24h composite) were collected from 167 wastewater treatment plants across 18 regions and 2 autonomous provinces in the period October 2021- September 2022. A real-time RT-qPCR assay targeting the ORF1b region was used for SARS-CoV-2 quantification in wastewater. Quantification data were expressed as genome copies (g.c.)/Litre of sewage. For data normalization, SARS-CoV-2 concentration was multiplied by the daily flow rate of the WTP and then divided by the WTP equivalent inhabitants to account for the catchment area population. The collected samples were used to track the virus's RNA concentrations during the rise and fall of clinical COVID-19 cases. Three waves were observed in wastewaters in the study period, corresponding to the spread of three different SARS-CoV-2 Omicron subvariants (BA.1, BA.2, and BA.5). To measure SARS-CoV-2 effective reproduction number in the population, a COVID-19 transmissibility indicator derived from wastewater data, called Rw, was developed by assuming that changes in the viral concentration in sewage reflect changes in the COVID-19 incidence in the population. The national level analysis of Rw demonstrated a similar temporal pattern as the net reproduction number derived from human surveillance data (Rt) in the same period. When compared to Rt, Rw estimates correctly indicated either an expanding or a contracting epidemic in about 75% of wastewater sampling dates at national level. Results at regional level were more heterogeneous when compared with human surveillance.

Conclusion: These findings suggest that estimating SARS-CoV-2 transmission from wastewater surveillance data offers an independent, cost-effective, and fast approach for monitoring the infection's spread. The information obtained from wastewater surveillance can complement that derived from the surveillance of human cases.

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DUAL TRANSMISSION OF THE CRINIVIRUS TOMATO CHLOROSIS VIRUS BY THE WHITEFLIES *BEMISIA TABACI* AND *TRIALEURODES VAPORARIORUM*: REVEALING THE MOLECULAR DETERMINANTS

A.C. García-Merenciano, E. Navas-Hermosilla, E. Fiallo-Olivé, J. Navas-Castillo

Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC), Consejo Superior de Investigaciones Científicas, Algarrobo-Costa, Málaga, Spain

Tomato chlorosis virus (ToCV, genus *Crinivirus*, family *Closteroviridae*) is an emerging plant virus present in at least 40 countries and territories around the world. ToCV prevalence in tomato has been reported to frequently reach 100%, with a consequent loss in production. Although ToCV infects mainly tomato, it has been reported to infect a wide range of plants, both cultivated and wild, including many economically important crops, such as pepper and potato. Symptoms caused by ToCV in tomato include interveinal yellowing and thickening of lower leaves that advance towards the upper part of the plant. ToCV has a bipartite genome of positive-sense single-stranded RNA. RNA1 contains four open reading frames (ORFs) which encode proteins related to virus replication, and RNA2 contains nine ORFs encoding proteins associated with virus encapsidation, movement and whitefly transmission. Both RNAs are encapsidated separately in flexuous virions with the typical body-tail (rattlesnake) structure of closterovirids. The body is composed of a single coat protein (CP) whereas the tail is putatively composed of at least four proteins including the minor coat protein (CPm), suggested to be involved in transmission by insect vectors. ToCV is transmitted in a semipersistent manner by whiteflies belonging to two genera: *Bemisia tabaci*, *Trialeurodes vaporariorum* and *T. abutilonea*. In order to know the molecular basis involved in the atypical dual transmission of ToCV, nine partial deletion mutants for the CPm gene were constructed using an infectious RNA2 clone. The viral progeny of the mutant clones was assayed for infectivity in *Nicotiana benthamiana* and tomato plants as well as for transmission by *B. tabaci* and *T. vaporariorum* between tomato plants. Results have revealed a candidate region in the CPm protein that could be involved in the specific transmission of ToCV by *T. vaporariorum*, in agreement with predictions based on *in silico* analysis of the CPm proteins of criniviruses.

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THE BEGOMOVIRUS TOMATO LEAF CURL NEW DELHI VIRUS-SPAIN STRAIN IS NOT TRANSMITTED BY *TRIALEURODES VAPORARIORUM* AND IS POORLY TRANSMITTED BY *BEMISIA TABACI*-MEDITERRANEAN BETWEEN ZUCCHINI AND *ECBALLIUM ELATERIUM*

A. Farina[1,2], C. Rapisarda[2], J. Navas-Castillo[1], E. Fiallo-Olivé[1]

[1] *Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC), Consejo Superior de Investigaciones Científicas, Algarrobo-Costa, Málaga, Spain*

[2] *Applied Entomology Section, Department of Agriculture, Food and Environment (Di3A), University of Catania, Italy*

Tomato leaf curl New Delhi virus (ToLCNDV) is a bipartite begomovirus (genus *Begomovirus*, family *Geminiviridae*) persistently transmitted, as with all other begomoviruses, by whiteflies (Hemiptera: Aleyrodidae) of the *Bemisia tabaci* (Gennadius) cryptic species complex. The virus, originally from the Indian subcontinent, was recently detected in the Mediterranean basin (in Spain in 2012 and in Italy in 2015), where it is currently a major concern for protected and open-field horticulture. The Mediterranean ToLCNDV isolates were shown to belong to a novel strain named "Spain strain" (ToLCNDV-ES), which infects zucchini (*Cucurbita pepo* L.) and other cucurbit crops but is poorly adapted to tomato. Recently, it has been reported that the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), is able to transmit an isolate of ToLCNDV from India which infects chayote [*Sechium edule* (Jacq.) Sw.], a cucurbit. In this work we aimed to clarify some aspects of whitefly transmission of ToLCNDV-ES. Thus, it was shown that *T. vaporariorum* is not able to transmit ToLCNDV-ES between zucchini plants. In addition, *Ecballium elaterium* (L.) A. Rich., a wild cucurbit usually found infected by ToLCNDV-ES in Spain, may not act as a relevant reservoir for this virus strain as *B. tabaci* Mediterranean, the most prevalent species of the complex in the region, is not an efficient vector of this begomovirus between cultivated zucchini and wild *E. elaterium* plants. In summary, our results do not support ToLCNDV transmission by whiteflies other than members of the *B. tabaci* complex and question the role of *E. elaterium* in ToLCNDV epidemics in the Mediterranean basin. These findings are a good starting point to advance our understanding of ToLCNDV epidemiology, thus helping to develop control strategies to fight the harmful disease that this virus causes to important vegetable crops

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DETECTION OF ANTIBODY RESPONSES AGAINST PARIETARIA MOTTLE VIRUS IN PLASMA OF INDIVIDUALS WITH PARIETARIA POLLINOSIS

G. Parrella[1], S. Vatrella[2], F. Perna[3], R. Parrella[4]

[1] *Institute for Sustainable Plant Protection, National Research Council (CNR), Portici, Naples, Italy*
[2] *Department of Medicine, Surgey and Dentistry, "Scuola Medica Salernitana", University of Salerno, Baronissi Campus, Baronissi, Salerno, Italy*
[3] *Department of Clinical Medicine and Surgery, Section of Respiratory Diseases, Monaldi Hospital, University of Naples Federico II, Naples, Italy*
[4] *Department of infectious Disease, Cotugno Hospital, A.O.R.N. dei Colli, Naples, Italy*

Plant viruses are known to be abundant and ubiquitous in nature and animals, including humans, are frequently exposed to these viruses. Tobamoviruses in the *Virgaviridae* family were by far the most frequently detected in humans being of dietary origin or introduced through infected cigarettes in the smokers. In the present study we have detected for the first time by molecular tools the parietaria mottle virus (PMoV, genus *Ilarvirus*, family *Bromoviridae*) in the induced sputum of subjects affected by parietaria pollinosis. The virus was also isolated from the expectorates on *Nicotiana* plants through mechanical inoculation. Using different serological assays, we also detected anti-PMoV antibodies (IgG class) in the serum of most of the subjects enrolled in the study. PMoV is a pollen-borne virus, transmitted by seed and pollen in *Parietaria officinalis* and *P. judaica* and therefore probably it was introduced through the respiratory track in the subjects with anti-PMoV antibodies, who inhaled infected pollen. These findings highlight a new way of nearly continuous exposure for humans of all ages to a plant virus. Whether the common presence of this plant viruses can affect the human healthy and immune system, either directly or through interaction with other known parietaria allergens, remains to be investigated.

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NEW MYCOVIRUSES IN YEASTS OF OENOLOGICAL INTEREST

D. Crucitti[1], M. Forgia[2], M. Chiapello[2], D. Oliva[3], M. Turina[2], F. Carimi[1], F. La Bella[1], D. Pacifico[1]

[1] *Dipartimento di Scienze Bio-Agroalimentari, Istituto di Bioscienze e BioRisorse (IBBR), C.N.R., Palermo, Italy*
[2] *Dipartimento di Scienze Bio-Agroalimentari, Istituto per la Protezione Sostenibile delle Piante (IPSP), C.N.R., Turin, Italy*
[3] *Istituto Regionale del Vino e dell'Olio (IRVO), Palermo, Italy*

Aim of the Study: Yeasts can be natural hosts for mycoviruses with dsRNA or ssRNA genomes as well as retrotransposon elements. We applied the NGS approach combined to bioinformatic analysis to verify the presence of new mycoviruses in *Saccharomyces* and non-*Saccharomyces* isolates of oenological interest. Our aim was to lay the bases for future studies assessing the effect of virus/yeast interaction with possible new virus types, as well as to identify viruses in new yeast isolates with technological characteristics useful for the production of high-quality wines.

Methods used: Total RNA was extracted from 24 yeast isolates of oenological interest. RNAs were pooled and sequenced by an Illumina HiSeq4000 system (Macrogen). De novo assembly was made with Trinity, and assembled contigs were aligned with BLAST. Bowtie2 and SAMtools were used to map reads on assembled contigs and visualized with IGV. Open reading frames were searched with ORF finder, while ORFan contigs were identified by blasting the assembled contigs against the NCBI non-redundant whole database. The distribution of the in silico-assembled viruses among the yeast isolate was verified by reverse transcription followed by real-time PCR. RACE analysis was used to confirm the conservation of the 5- and 3- termini of ORFan sequences. ORFan mycovirus RdRPs protein sequences were aligned using MAFFT.

Results and Conclusions: We found eight viral species included in four different families, two of which - *Partitiviridae* and *Mitoviridae* - not reported before in yeasts. Four new putative mycovirus species distributed in *Totivirus*, *Cryspovirus*, and *Mitovirus* genera were identified. *S. cerevisiae* strains hosted helper L-A totiviruses and satellite M dsRNAs associated with the killer phenotype, both in single and mixed infections with L-BC viruses and viral sequences belonging to a new cryspovirus putative species. Single infection by a narnavirus 20S-related sequence was also found in *S. cerevisiae*. Among the non-*Saccharomyces* yeasts, *Starmerella bacillaris* hosted four viral RNAs, two included in *Totivirus* and *Mitovirus* genera, and two ORFans. The ORFan segments found corresponded to a new putative species included in a new Ormycovirus (ORFan mycovirus) phylogenetic clade, characterised by viruses with an SDD triplet in their RdRp catalytic site.
This work confirmed the infection of oenological yeasts by viruses associated with useful technological features and highlighted the presence of new viral species, in mixed and complex infections with unknown effects for yeast biology and ecology.

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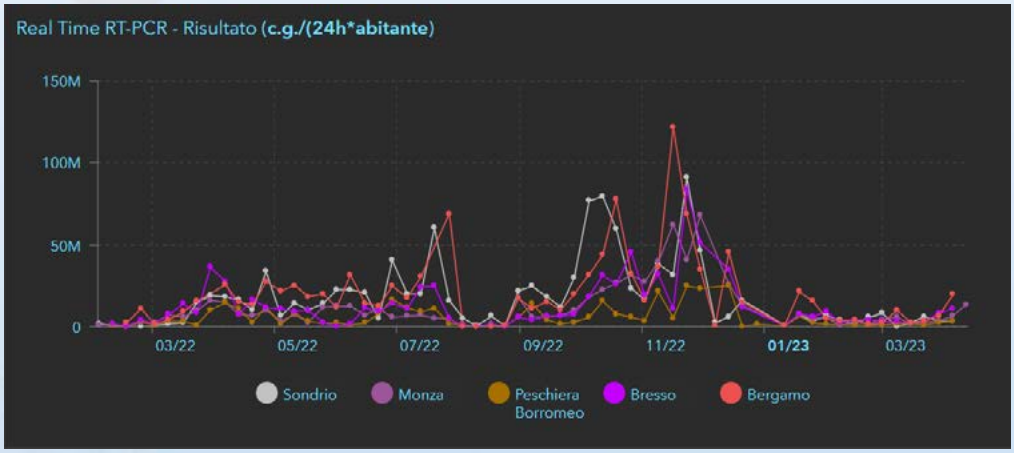
ENVIRONMENTAL SURVEILLANCE OF SARS-CoV-2 IN URBAN WASTEWATER IN LOMBARDY

E. Bruno[1,2], M. Martinelli[1], C. Giubbi[1], F. Perdoni[1], A. Franzetti[2], R. Musumeci[1], G. La Rosa[3], E. Suffredini[3], C.E. Cocuzza[1]

[1] *University of Milano-Bicocca, Department of Medicine and Surgery, Monza, Italy*
[2] *University of Milano-Bicocca, Department of Earth and Environmental Sciences, Milano, Italy*
[3] *Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy*

Aim: The aim of this study was to investigate the presence of SARS-CoV-2 RNA in five Wastewater Treatment Plants (WWTPs), located in the Lombardy region of Italy (Bergamo, Bresso, Monza, Peschiera Borromeo and Sondrio). This is a part of a national on-going surveillance study entitled "SARI Project - Sorveglianza Ambientale di SARS-CoV-2 attraverso i Reflui urbani in Italia", coordinated by the Italian National Institute of Health (Istituto Superiore di Sanità - ISS).

Methods: Composite 24h raw wastewater samples (500 mL) were collected weekly from the five WWTPs from February 2022 to March 2023. According to the ISS protocol, all samples were concentrated by centrifugation for 2h at 12000xg and 4°C using PEG 8000. Nucleic acid extraction was performed using the NucliSens EasyMag system (bioMeriéux). SARS-CoV-2 ORF1ab (nsp14) gene, together with two other biomarkers (N1 and N3; data not shown), were detected by one-step real time RT-PCR for viral quantification. SARS-CoV-2 viral load was expressed as viral genome copies/equivalent inhabitant per day, normalized according to flow rate and number of inhabitants.



Results and Conclusions: SARS-CoV-2 RNA was detected in 284 out of 296 (95.9) wastewater samples collected. The highest percentage of positive samples was observed in Bergamo (98.25%; 56/57), while Peschiera Borromeo had the lowest (93; 55/59). Bresso, Monza, and Sondrio showed a similar percentage of positivity, 96.6% (57/59), 95% (57/60) and 96.7% (59/61), respectively. The highest SARS-CoV-2 concentrations were obtained from samples collected between October and December 2022 with the following peaks reached for the WWTPs under investigation: Bergamo, 4.09E+05 g.c./L; Sondrio, 3.45E+05 g.c./L; Monza, 2.31E+05 g.c./L; Bresso, 2.18E+05 g.c./L and Peschiera Borromeo, 1.89E+05 g.c./L. During this period, sequence analysis carried out by ISS confirmed the sole presence of SARS-CoV-2 Omicron variant in Italy, with sublineage BA.5 being the most prevalent.
The monitoring of SARS-CoV-2 in wastewater represents an important tool for the surveillance of COVID-19 in the population that can be used both as an early predictor of new infection waves of SARS-CoV-2 as well as monitoring for the emergence of novel viral variants.

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ANTIGENIC MOLECULAR MIMICRY IN VIRAL-MEDIATED PROTECTION FROM CANCER: THE HIV CASE

M. Tagliamonte[1], C. Manolio[1], C. Ragone[1], B. Cavalluzzo[1], A. Mauriello[1], M.L. Tornesello[2], F.M. Buonaguro[2], A. Salomone Megna[3], G. D'Alessio[3], R. Penta[4], L. Buonaguro[1]

[1] Innovative Immunological Models Unit, Istituto Nazionale Tumori - IRCCS "Fond G. Pascale", Naples, Italy
[2] Molecular Biology and Viral Oncogenesis Unit, Istituto Nazionale Tumori - IRCCS "Fond G. Pascale", Naples, Italy
[3] Division of Infectious Diseases, AORN San Pio Hospital, Benevento, Italy
[4] Cellular Manipulation and Immunogenetics, Oncology Dep, Ba.S.C.O. Unit, AORN Santobono-Pausilipon, Naples, Italy

Background: People living with HIV/AIDS (PLWHA) show a reduced incidence for three cancer types, namely breast, prostate and colon cancers. In the present study, we assessed whether a molecular mimicry between HIV epitopes and tumor associated antigens and, consequently, a T cell cross-reactivity could provide an explanation for such an epidemiological evidence.

Methods: Homology between published TAAs and non-self HIV-derived epitopes have been assessed by BLAST homology. Structural analyses have been performed by bioinformatics tools. Immunological validation of CD8+ T cell cross-reactivity has been evaluated ex vivo by tetramer staining.

Results: Sequence homologies between multiple TAAs and HIV epitopes have been found. High structural similarities between the paired TAAs and HIV epitopes as well as comparable patterns of contact with HLA and TCR α and β chains have been observed. Furthermore, cross-reacting CD8+ T cells have been identified.

Conclusions: This is the first study showing a molecular mimicry between HIV antigens an TAAs identified in breast, prostate and colon cancers. Therefore, it is highly reasonable that memory CD8+ T cells elicited during the HIV infection may play a key role in controlling development and progression of such cancers in the PLWHA lifetime. This represents the first demonstration ever that a viral infection may induce a natural "preventive" anti-cancer memory T cells, with highly relevant implications beyond the HIV infection.

Keywords: Tumor-associated antigens, Viral antigens, Molecular mimicry, Cross-reactive T cells, HIV-1, Colon cancer, Breast cancer.

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THE CELLULAR DEACETYLASE SIRT1 CONTRIBUTES TO HPV-DRIVEN p53 INHIBITION AND IT IS AN ACTIONABLE THERAPEUTIC TARGET

I. Lo Cigno[1], F. Calati[1], C. Girone[1], C. Borgogna[1], A. Venuti[2], R. Boldorini[3], M. Gariglio[1]

[1] *Virology Unit, Department of Translational Medicine, Eastern Piedmont University, Novara, Italy*
[2] *HPV Unit, UOSD Tumor Immunology and Immunotherapy, IRCCS Regina Elena National Cancer Institute, Rome, Italy*
[3] *Pathology Unit, Department of Health Sciences, Eastern Piedmont University, Novara, Italy*

Human papillomavirus (HPV)-driven cancer relies on viral E6/E7-mediated inactivation of both p53 and pRb, which results in the unique maintenance of these tumor suppressor genes in a wild-type conformation. Thus, the possibility of pharmacologically targeting the cellular circuit that maintains E6-mediated permanent inactivation of p53 activity represents a promising therapeutic option against HPV-associated cancer. SIRT1, the principal NAD^+-dependent deacetylase in mammalian cells, catalyzes the deacetylation of its substrates, which include histone and non-histone targets, such as p53. Although SIRT1-mediated regulation of p53 stability is well known - p53 acetylation at K382 competes with ubiquitination and promotes p53 stabilization and activation - its engagement in E6/E7-driven oncogenesis has never been explored. Here, we reveal the existence of a novel SIRT1-dependent circuit whose disruption leads to restoration of a functional p53 in HPV-transformed cells. In addition to the cervical carcinoma-derived HeLa and CaSki cell lines, harboring integrated HPV18 and HPV16 genome respectively, a newly established model of HPV-induced head and neck carcinogenesis (NOKsHPV16E6/E7), and a recently generated HPV16-positive hypopharyngeal squamous cell carcinoma cell line (UPCI:SCC152) have also been included. Specifically, we show that SIRT1 pharmacological or genetic inhibition restores a transcriptionally active K382-acetylated p53 in HPV^+ but not HPV^- cell lines. Furthermore, SIRT1 inhibition by the specific inhibitor EX527 (Selisistat) promotes G_0/G_1 cell cycle arrest and reduces cell viability and clonogenicity of HPV^+ *vs* HPV^- cells. Lastly, EX527 treatment increases the sensitivity of HPV^+ cells to sublethal doses of standard genotoxic agents, such as doxorubicin and cisplatin. Enhanced sensitivity to the anticancer activity of cisplatin also occurs in an *in vivo* tumorigenicity assay based on subcutaneous injection of syngeneic C3.43 cells, harboring an integrated HPV16 genome, in C57BL/6J mice. This sensitization is likely due to restoration of a functional p53 as shown by immunohistochemistry of tumors from EX527-treated mice. Altogether, these findings uncover an essential role of SIRT1 in HPV-driven oncogenesis, which may have direct translational implications for the treatment of HPV-associated cancers.

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ORAL MICROBIOME ANALYSIS IN INDIVIDUALS WITH ORAL HIGH-RISK HUMAN PAPILLOMAVIRUS INFECTION

D.I. Escobar Marcillo[1], F. Rollo[2], G.F. Privitera[3], A. Latini[4], E. Giuliani[5], B. Pichi[6], M. Benevolo[2], R. Pellini[6], M.G. Donà[4]

[1] *Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy*
[2] *Pathology Department, Regina Elena National Cancer Institute IRCCS, Rome, Italy*
[3] *Department of Clinical and Experimental Medicine, Bioinformatics Unit, University of Catania, Italy*
[4] *STI/HIV Unit, San Gallicano Dermatological Institute IRCCS, Rome, Italy*
[5] *Scientific Direction, San Gallicano Dermatological Institute IRCCS, Rome, Italy*
[6] *Otolaryngology Head Neck Surgery Department, Regina Elena National Cancer Institute IRCCS, Rome, Italy*

Aim of the Study: Oral HPV infections have become increasingly interesting because of the rising incidence of oropharyngeal squamous cell carcinoma (OPC) in several countries, along with the increase in the proportion of HPV-driven OPCs. Although a persistent infection by High-risk HPVs (HrHPVs) is the primary cause for cancer development, only a small portion of HrHPV-infected individuals will ultimately develop malignant lesions, suggesting that other factors play a role in progression from infection to cancer. Microbiome gained attention as a predicting biomarker of infection as well as an important cofactor predisposing to cancer. We aimed to investigate oral microbiome in HIV-infected (HIV+) and HIV-uninfected (HIV-) cancer-free subjects harboring prevalent oral infection by HrHPVs.

Methods: Study subjects were selected among male participants of the OHMAR longitudinal study carried out at the San Gallicano Dermatological Institute (Rome, Italy). Based on the Linear Array HPV test results on oral rinse-and-gargles, oral DNA samples were selected from:

1) HrHPV+ subjects (at least one of the followings: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68);
2) HPV-negative subjects (at least for 3 consecutive 6-month-apart tests).

HrHPV+ individuals and HPV- controls were matched for HIV status, age, smoking, alcohol, oral hygiene, sexual habits and HIV-related parameters (for HIV+). Bacterial 16S rRNA gene sequencing (V3-V4 region) was performed using the Quick-16S™ NGS Library Prep Kit (Zymo Research). QIIME2 (v. qiime2-2022.11) was used to process the trimmed FASTQ files. Comparisons of alpha-diversity were performed using Kruskall-Wallis and Wilcoxon tests. Beta-diversity significance was calculated with a PERMANOVA analysis. Taxonomy was assigned to the ASV using the expanded Human Oral Microbiome Database (eHOMD, v.15.22).

Results and Conclusions: A total of 63 samples were analyzed (22 from HIV+ and 41 from HIV- men). Among HIV+ subjects, alpha diversity did not significantly differ between HPV- and HrHPV+ individuals (Shannon: p=0.35; Chao1: p=0.55). Among HIV- subjects, alpha diversity was higher in those with HrHPV infection (Shannon: p=0.055; Chao1: p=0.033). Jaccard and weighted UniFrac beta diversity did not show significant differences between the study groups, either among HIV+ or HIV- subjects. Differently, a significant difference in unweighted UniFrac distance was observed between HrHPV+ and HPV- subjects, but only for the HIV- group (p=0.003). Firmicutes represented the most abundant phylum, followed by Bacteroidetes and Protecobacteria in all study groups, irrespectively of HPV and HIV status. Among HIV- individuals, Gracilibacteria were only found in those HrHPV+ (0.09% vs. 0%). Among HIV+ individuals, Saccharibacteria were significantly less abundant in HrHPV+ subjects (0.79% vs. 1.36%). At the genus level, a significant differential abundance ($p<0.05$) was observed for *Alloscardovia*, *Sneathia* and *Parvimonas* in HIV-, *Bulleidia*, *Simonsiella* and *Klebsiella* in HIV+ subjects. Composition of oral microbiome may vary in subjects harboring HrHPV infection, but the observed variations also depend on the HIV status of the study subjects. Investigating the composition of oral microbiome in individuals with oral HrHPV may help understand its role in HPV infections that may lead to OPC development.

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A GENOME-WIDE CRISPR-CAS9 LOSS-OF-FUNCTION SCREENING TO IDENTIFY NOVEL HOST RESTRICTION GENES CONTRIBUTING TO ONCOLYTIC VIROTHERAPY RESISTANCE IN PANCREATIC DUCTAL ADENOCARCINOMA

M. Muscolini[1], E. Tassone[1], D. Di Carlo[1], D. Olagnier[2], P. Kinderman[3], N. van Montfoort[3], J. Hiscott[1]

[1] *Istituto Pasteur Italia, Rome, Italy*
[2] *Dept. of Biomedicine, Aarhus University, Denmark*
[3] *Dept. of Gastroenterology & Hepatology, LUMC, The Netherlands*

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest forms of cancer and largely resistant to multiple forms of therapy, including oncolytic virotherapy. Due to the absence of specific symptoms and the lack of early detection assays, PDAC is usually diagnosed only at advanced stages and survival is extremely poor. A major hurdle to oncolytic virotherapy, especially when used as single treatment, is the resistance to oncolytic viruses (OVs) infection, due at least in part to the residual antiviral immunity of some cancer cell lines and primary tumors. The identification of host factors that determine the resistance to OVs by limiting viral entry, replication and oncolysis is crucial to extend the efficacy of OV-mediated therapy. To identify loss-of-function mutations that alter PDAC cell sensitivity to OV infection and to develop novel treatments for PDAC, we performed an unbiased genome-wide CRISPR-Cas9 knockout screen in a human PDAC cell line that emerged from our analyses as highly resistant to VSVΔM51. Cas9-expressing cells were transduced with a library of pooled single guide RNA (sgRNA)-expressing lentiviruses that target all human genes to obtain a population where each cell is knocked out for a single gene. Upon VSVΔM51 infection, due to the virus selective pressure there was an enrichment of resistant clones, while sensitive cells died. The variation in the relative abundance of each genome-integrated sgRNA was measured by NGS. NGS data analyses has uncovered more than 12 target genes that were missed by previous studies, including genes involved in transcriptional control, apoptosis regulation, oxidative metabolism, and tumor cell migration. The detailed protocol was described in *Muscolini, Hiscott and Tassone,* Methods Mol Biol. 2023, doi: 10.1007/978-1-0716-2788-4_25. To validate the role of the top gene as a host resistance candidate (HRC), single-knockout (KO) cell lines were generated in different PDAC models. Upon VSVΔM51 challenge, the number of KO-infected cells was at least 3-fold higher compared to control cells. The augmented sensitivity of HRC-KO cells to VSVΔM51 was accompanied by a strong increase of cell death induction. Further, the treatment of resistant PDAC cells with specific HRC-inhibitors led to an increased percentage of VSVΔM51-infected cells compared to untreated cells. Conversely, the overexpression of the HRC of interest in two VSVΔM51-sensitive PDAC models, with a low basal HRC protein level, was found to correlate with a significant reduction of infected cells. *In vitro* results confirmed a prominent role of the top HRC gene in PDAC intrinsic resistance to VSVΔM51 infection and replication. Ongoing *in vivo* analyses and studies to elucidate the mechanisms responsible for the enhanced permissiveness to virotherapy in knockout cells will be discussed. Our findings provide a resource amenable to uncover host factors involved in the resistance to different OVs in multiple tumor models and highlight the potential to understand and reverse host resistance to oncolytic virotherapy.

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EXPRESSION OF HUMAN ENDOGENOUS RETROVIRUSES (HERV) IN CLINICAL SPECIMENS OF PATIENTS WITH COLON CANCER

M. Dolci[1], L. Signorini[1], P. Bagnoli[2], V. Edefonti[3], F. Ambrogi[3], K. Maina[1], R. Ticozzi[1], P. Ferrante[4], S. Delbue[1]

[1] *Department of Biomedical, Surgical and Dental Sciences, University of Milan, Italy*
[2] *General Surgery Unit, Istituto Clinico Città Studi, Milan, Italy*
[3] *Department of Clinical Sciences and Community Health, University of Milan, Italy*
[4] *Istituto Clinico Città Studi, Milan, Italy*

Background: Human endogenous retroviruses (HERV) are relicts of exogenous retroviral infections, constituting 8% of the human genome. The genomic structure of HERV is composed of four main genes: group-specific antigen (*gag*), protease (*pro*), polymerase (*pol*) and envelope (*env*). Alteration of HERV expression has been related to several cancers, but researches regarding HERV gene expression in colon cancer are still sporadic. Very few reports investigated the presence of HERV transcripts in plasmatic extracellular vesicles (EV). The aim of the study was to analyze the role of HERV in colon cancer pathogenesis.

Materials and Methods: Fifty-seven Italian patients with advanced-stage colon cancer were enrolled. The expression of HERV-H, -K, -P *env* gene, and HERV-K *pol* gene was analysed in the tumor tissues and negative surgical margins and, when possible, in the peripheral blood. The presence of HERV transcripts was evaluated in the EV, isolated from the plasma of 42 patients. The expression of HERV-K Env protein was evaluated in tumor tissue and in negative surgical margins by Western Blot. Associations among clinical characteristics, and HERV gene expression levels were analysed.

Results: HERV-P *env* was more expressed in blood compared to tumor tissue ($p<0.05$), while HERV-H, -K *env*, and HERV-K *pol* expression levels were similar in the clinical specimens. HERV genes were expressed, at low levels, in the plasmatic EV of 19% (-H *env*), 38% (-K *env*), 24% (-K *pol*), and 17% (-P *env*) tested patients. Preliminary results showed higher expression of Env protein in the tumor tissue than in the negative margins.

Conclusions: Differentially expression of HERV-P *env* may play a role in colon cancer. As known, cancer-secreted EV influence the tumor microenvironment and support cancer growth and metastasis: HERV transcripts may be carried in the circulating plasma EV and transferred from one cell to another, favouring cellular transforming mechanisms. On the other hand, in tumor cells, Env protein might promote cell to cell fusion.

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HUMAN POLYOMAVIRUS JC (JCPyV) IN ONCOGENESIS OF PEDIATRIC GLIOMAS

S. Passerini[1], G. Babini[1], C. Prezioso[2], L. Bargiacchi[3], D. Bartolini[4], M. Antonelli[3], U. Moens[5], V. Pietropaolo[1]

[1] *Departmen of Public Health and Infectious Diseases, "Sapienza" University of Rome, Italy*
[2] *Laboratory of Microbiology of Chronic-Neurodegenerative Diseases, IRCCS San Raffaele Rome, Italy*
[3] *Department of Radiological, Oncological and Anatomo-Pathological Sciences, "Sapienza" University of Rome, Italy*
[4] *Pathology Unit, Bufalini Hospital, Cesena, Italy*
[5] *Department of Medical Biology, Faculty of Health Sciences, University of Tromsø - The Arctic University of Norway, Tromsø, Norway*

Since gliomas are primary neoplasms with poorly understood etiology, viral infections have been proposed as a possible risk factor in the development of this kind of tumor (Bondy et al. 2008). JC polyomavirus (JCPyV) is a small non-enveloped DNA virus, well known to be the etiological agent of progressive multifocal leukoencephalopathy (PML) (Ferenczy et al. 2012). Moreover, several studies demonstrated its potential role in cancer development (Ahye et al. 2020). Specifically, JCPyV Large T Antigen (LTAg), thanks to its capability to bind and inactivate tumor suppressor proteins as Retinoblastoma (Rb) and p53, by the N-terminal and C-terminal region respectively, is considered the principal actor in JCPyV mediated oncogenesis (Zheng et al. 2022). Therefore, in order to better understand JCPyV's involvement in tumor development, the presence of JCPyV has been examined in 56 pediatric patients affected by gliomas, by quantitative polymerase chain reaction (qPCR). Then nested polymerase chain reactions (nPCRs) were performed in order to investigate LTAg N- and C-terminal regions, the Non-Coding Control Region (NCCR) and the Viral Protein 1 (VP1). In addition, after total RNA extraction, LTAg and VP1 gene and miRNA expressions were evaluated by reverse transcription PCR (RT-PCR). JCPyV DNA was detected in 18/56 (32%) tumors with a viral load mean value of $6x10^3$ gEq/mL. nPCR could amplify sequences corresponding to the LTAg N-terminal region, but not the C-terminal domain. Although previous studies reported a rearranged form of the NCCR in human brain tumors, the 18 samples showed an archetype structure. The analysis of transcripts showed that the N-terminal portion of LTAg gene but not the C-terminal one was expressed in all the JCPyV positive samples; the VP1 sequence instead, was detected both at DNA and RNA level in 13/18 (72%) of the JCPyV positive samples. In addition, JCPyV-encoded miRNAs expression was observed in the same 13 samples positive for VP1 detection. This study confirms the presence of JCPyV in human brain tumors. Specifically, in 5/18 JCPyV positive tumors only early genes are expressed concomitant to no detection of late genes suggesting that hampering of viral replication could be a crucial event for tumor development. In addition, since only LTAg N-terminal region was found both at DNA and RNA level, we can suppose that, as in MCPyV positive MCC, a C-terminal truncated LTAg is expressed and consequently oncogenesis could depend on Rb sequestration. On the other hand, in 13/18 JCPyV positive tumors, VP1 DNA and transcripts are found together with miRNA expression. Further studies are warranted to elucidate how JCPyV infection can contribute to gliomagenesis.

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DETECTION OF MERKEL CELL POLYOMAVIRUS (MCPyV) DNA AND TRANSCRIPTS IN MERKEL CELL CARCINOMA (MCC)

S. Passerini[1], C. Prezioso[2], G. Babini[1], A. Ferlosio[3], T. Cosio[4,5], E. Campione[5], U. Moens[6], M. Ciotti[7], V. Pietropaolo[1]

[1] *Department of Public Health and Infectious Diseases, "Sapienza" University of Rome, Italy*
[2] *Laboratory of Microbiology of Chronic-Neurodegenerative Diseases, IRCCS San Raffaele Rome, Italy*
[3] *Anatomic Pathology, Department of Biomedicine and Prevention, Tor Vergata University of Rome, Italy*
[4] *Department of Experimental Medicine, University of Rome "Tor Vergata", Rome, Italy*
[5] *Dermatologic Unit, Department of Systems Medicine, University of Rome "Tor Vergata", Rome, Italy*
[6] *Department of Medical Biology, Faculty of Health Sciences, University of Tromsø - The Arctic University of Norway, Tromsø, Norway*
[7] *Virology Unit, Polyclinic Tor Vergata Foundation, Rome, Italy*

Merkel Cell Polyomavirus (MCPyV) is a non-enveloped double stranded DNA virus well known to be the causative agent of Merkel Cell Carcinoma (MCC), a rare but aggressive neuroendocrine carcinoma of the skin (Feng *et al.* 2008; Pietropaolo 2020). In order to better understand the MCPyV role in MCCs, detection and analysis of MCPyV DNA and transcripts were performed on primary tumors and corresponding lymph nodes from two MCC patients, one of them with diagnosis of metastasis. Following DNA extraction, the presence and quantity of MCPyV DNA were evaluated by quantitative polymerase chain reaction (qPCR) using primer and probe targeting small T *gene* (sT) (Rodig et al. 2012). Nested polymerase chain reactions (nPCRs) were then carried out in order to amplify MCPyV Large T Antigen (LTAg) and Viral Protein 1 (VP1) DNA sequences, using three set of primers (LT1, LT3 and VP1). Samples were further analyzed for Non-Coding Control Region (NCCR) and sequenced. In parallel, following total RNA extraction, LTAg and VP1 *gene* expression was investigated by reverse-transcription PCR (RT-PCR). MCPyV DNA was detected in the primary tumor of both patients and in the lymph node, belonging to the one with diagnosis of metastasis, with an average value of viral DNA of 7.0×10^2 copies/µg supporting the association between MCPyV and MCC. Moreover, in the same samples, MCPyV DNA sequences, corresponding to LTAg and VP1, were amplified. Analysis of NCCR revealed a canonical structure, compared to the MCC350 (Feng et al. 2008), in all analyzed sequences.

As shown in most MCPyV positive MCCs (Feng et al. 2008), at RNA level, only LTAg *gene* expression was detected, whereas no VP1 transcripts were found, suggesting that viral replication could be hampered, as observed in virus mediated oncogenesis (Zur Hausen 2008). The obtained results confirm the association between MCPyV and MCC, assuming LTAg as a key player in MCPyV mediated oncogenesis.

PO 195

A SPECTRUM OF PAPILLOMAVIRUSES AND POLYOMAVIRUSES DETECTED IN MATCHED HEALTHY AND LESIONAL SKIN SCRAPINGS OF PATIENTS AFFECTED BY ACTINIC KERATOSIS, A PRECURSOR OF KERATINOCYTE CARCINOMA

M.V. Chiantore[1], T. Gheit[2], M.F. Vescio[1], F. Luzi[3], F. Rollo[4], L. Accardi[1], L. Galati[2], G. Romeo[5], M. Giuliani[3], P. Di Bonito[1], M. Tommasino[2§], M.G. Donà[3]

[1] *Dept Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy*
[2] *International Agency for Research on Cancer (IARC), Lione, France*
[3] *San Gallicano Dermatological Institute IRCC, Rome, Italy*
[4] *Pathology Department, Regina Elena NCI-IRCCS, Rome, Italy*
[5] *DSBMC La Sapienza Polo Pontino, Rome, Italy*
[§] *We dedicate this abstract to the memory of Massimo Tommasino passed away on 2022*

Aim: Actinic Keratosis (AK) is currently recognized as a precursor lesion of cutaneous squamous cell carcinoma (cuSCC), and can regress or evolve into invasive cuSCC. Ultraviolet (UV) radiation exposure along with genetic factors and immunosuppression are the main risk factors in cuSCC development. Additionally, certain beta-HPVs in cooperation with UV-radiation are also suggested to be involved. Together with HPVs, HPyVs are also present in the normal skin. A cross-sectional study to assess HPVs and PyVs prevalence was performed on AK patients comparing matched healthy-looking skin (HS) and lesional (AK) scrapings.

Methods: DNA was extracted from the AK and HS scrapings of 244 patients. Prevalence of cutaneous HPVs and PyVs was determined through a semi-quantitative type-specific PCR assay, which detects 46 beta- and 52 gamma-HPVs, and 13 of the 15 known PyVs detected in human tissues. Statistical analyses were performed using MedCalc Statistical Software.

Result: The majority of analysed–scraping were positive for beta-HPVs (HS: 98.3% and AK: 85.8%), gamma-HPVs (HS: 87.1% and AK: 77.6%) and PyVs (HS: 89.7% and AK: 94.6%). HPV-prevalence was significantly higher in HS than in AK for beta and gamma-HPV types (except for HPV4 from species gamma-1), while PyV-prevalence was higher in AK than HS specimens. HPV38 from species beta-2 and HPV5 from species beta-1 were the most frequent beta-types, while HPV-SD2 was the most prevalent gamma-type detected in AK and HS samples. MCPyV, HPyV6 and JCPyV (HS: 87.2%, 58.7%, 6.6%; AK: 88.8%, 51.2%, 9.9%) were the most frequent detected PyVs. Semi-quantitative genome copy number of PyVs were lower in AK compared to HS, except for JCPyV. In the majority of cases, matched HS and AK scrapings were both positive (MCPyV: 78.1%, HPyV6: 41.7%), or both negative for the individual PyVs.

Conclusion: A large spectrum of HPVs and PyVs was detected in HS and AK scrapings. The detection of HPVs and HPyVs exclusively in AK was rare. In matched HS-AK positive cases, semi-quantitative viral genome copy number was higher in HS than in AK samples both for HPVs and PyVs.

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PO 196

INTRABODIES AGAINST E6 AND E7 ONCOPROTEINS: A TARGETED THERAPEUTIC APPROACH FOR THE TREATMENT OF HPV16-ASSOCIATED LESIONS

S. Falcucci[1], F. Paolini[2], M.G. Donà[3], M.V. Chiantore[1], A. Venuti[2], P. Di Bonito[1], L. Accardi[1]

[1] Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy
[2] HPV Unit, UOSD Tumor Immunology and Immunotherapy, IRCCS Regina Elena National Cancer Institute, Rome, Italy
[3] STI/HIV Unit, IRCCS Istituto Dermatologico San Gallicano, Rome, Italy

Aim: HPV is the cause of almost all cervical cancer and is responsible for a substantial fraction of other anogenital and oropharyngeal cancers. It is necessary to develop new intervention strategies for the treatment of the HPV16-associated lesions, currently treated surgically and/or by chemotherapy/radiotherapy, to solve the problems associated with the toxicity and inappropriate targeting of non-infected tissue.
The E6 and E7 viral oncoproteins, expressed only in the infected cells, are major players in tumor development and progression and are therefore recognized targets for therapy.
The aim of this work was the development and validation of single-chain variable fragments (scFvs) as a safe and targeted therapy for the treatment of HPV-associated lesions.

Methods: ScFv recombinant antibodies targeting the HPV16 E6 (16E6) and E7 (16E7) oncoproteins were selected, characterised and expressed as intrabodies in HPV16+ cells. The anti-E7 antibodies were selected from the ETH-2 library of human recombinant antibodies. With the aim of interfering with the oncogenic activity of the E7 protein, scFv43M2 was provided with SEKDEL signal for localization in endoplasmic reticulum (43M2SD), expressed in HPV16+ SiHa cells by retroviral system and tested for its ability to inhibit cell proliferation. 43M2 was also tested for its anti-proliferative activity when loaded onto engineered exosomes as a fusion with the Nef^{mut} gene.
The anti-E6 scFvI7 was selected from the SPLINT Library of Intracellular Antibodies by Intracellular Antibody Capture Technology, provided with a nuclear localization signal (scFvI7nuc) and expressed in SiHa cells by retroviral system. I7nuc ability to hamper cell proliferation, favour apoptosis and inhibit degradation of p53, the main target of E6, was also tested.
In order to investigate their antitumor activity, scFvI7nuc and scFv43M2SD were tested in preclinical models for HPV tumors based on C57/BL6 mice. The effect on tumor growth and development was studied.

Results and Conclusions: Intrabodies targeting the HPV16 oncoproteins were able to specifically inhibit cell proliferation. Epitope mapping showed that scFv43M2 binds to the pRb-binding region on E7. Therapeutic Antibody Profiling analysis of scFv43M2 showed a good score agreement with antibodies in the post-Phase I clinical phase. ScFvI7nuc was able to partially inhibit the degradation of the E6 main target p53. Both intrabodies induced necrosis and cell death by apoptosis in tumor mass and were able to delay tumor onset and development thus showing specific anti-tumor activity.
These results could pave the way for the development of a safe and specific antibody-based therapy, particularly for early HPV16-associated lesions.

PO 197

ROLE OF HUMAN SPLICING FACTORS IN HPV16 E6*I PRODUCTION IN HEAD AND NECK CANCERS

A. Cerasuolo[1], N. Starita[1], A.L. Tornesello[1], F. Longo[2], F. Ionna[2], R. Rega[3], R. De Cecio[3], G. Ferrara[3], F.M. Buonaguro[1], M.L. Tornesello[1]

[1] *Molecular Biology and Viral Oncology Unit, Istituto Nazionale Tumori IRCCS Fond. Pascale, Naples, Italy*
[2] *Maxillofacial and ENT Surgery Unit, Istituto Nazionale Tumori IRCCS Fond. Pascale, Naples, Italy*
[3] *Pathology Unit, Istituto Nazionale Tumori IRCCS Fond. Pascale, Naples, Italy*

Background: Human papillomavirus (HPV) has emerged as a novel etiological agent for a subgroup of head and neck squamous cell carcinomas (HNSC). The oncogenic activity of high-risk HPVs relies on the constitutive expression of E6 and E7 oncogenes. The differential production of HPV E6, E6*I isoform and E7 transcripts during cancer progression is modulated by host cell splicing factors. In particular, different studies showed deregulation of HNRNPA1, HNRNPA2B1, SRSF1, SRSF2, SRSF3, BRM and SAM68 expression in cervical squamous cell carcinoma (CSC) causing increased E6*I production. However, their function in HPV-related HNSC has been poorly characterized. The aim of the study was to analyse the expression profile of splicing factors and their role in the production of HPV16 E6*I isoform in HNSC.

Materials and Methods: HPV DNA was searched by broad spectrum PCR in 31 HNSC cases, including 14 oropharynx (OPC), 9 oral cavity (OC); 5 larynx (LC), 1 nasopharynx (NPC), 1 hypopharynx (HPC), 1 salivary gland (SGC) carcinomas and 9 head and neck dysplasia (HND). HPV viral load was measured by performing quantitative PCR (qPCR) and copy number estimated by calculating the E6/TP53 ratio. The expression levels of E6 and E6*I as well as of HNRNA1, HNRNPA2B1, SRSF1, SRSF2, SRSF3, BRM and SAM68 transcripts were analysed by quantitative reverse transcriptase-PCR (RT-qPCR) in HPV16-positive HNSCs and compared to previously analysed HPV16-positive CSC samples. The E6 and E6*I transcript levels was validated by absolute quantification with droplet digital PCR (ddPCR). The correlation between the viral load, E6 and E6*I mRNA levels was evaluated by Fisher's exact test.

Results: HPV DNA was detected in 45% of HNSC (36% of OPC, 37% of OC, 20% of LC, 100% of NPC and SGC) and 33% of HND. HPV16 was the most frequent viral genotype, being found in 86% of HPV-positive samples. HPV16 viral load was much higher in HNSC (<1 to 115 copies/genome equivalent) than in HND (<1 copy/genome equivalent). Both E6 and E6*I transcripts were concordantly expressed in 40% of HNSC. In particular, the expression of E6*I was between 1.7 and 17 folds greater than E6 levels. Neither E6 nor E6*I transcripts were detected in the majority of OC, LC and SGC as well as in HND positive for HPV16 DNA. Concordant results were obtained by analysing E6 and E6*I expression with RT-qPCR and ddPCR. A statistically significant correlation was found between the viral copy number and E6*I mRNA levels (p=0.04). Notably, HNRNPA1, HNRNPA2B1, SRSF1, SRSF2, SRSF3, BRM and SAM68 expression profile was similar between HNSC and CSC, while HNRNPA1, SRSF2 and SAM68 were more expressed in HNSC than in CSC.

Conclusions: In conclusion, HPV16 was confirmed to be the most frequent genotype in HPV-related HNSC and E6*I was shown to be a sensitive molecular marker of HPV oncogenic infection in HNSC. Moreover, deregulation of splicing factors involved in HPV16 E6*I production was found in HNSC, with HNRNPA1 and SRSF2 possibly playing a major role in E6 RNA splicing specifically in HNSC.

PO 198

INHIBITION OF ROCK KINASE ENHANCES ANTI-CANCER ACTIVITY OF THE ONCOLYTIC ADENOVIRUS dl922947 IN ANAPLASTIC THYROID CANCER CELLS

G. Castellano, F. Napolitano, C. Maglio, A.M. Malfitano, G. Portella

Department of Translational Medical Sciences, University of Naples Federico II, Naples, Italy

Aim of the Study: Anaplastic thyroid carcinoma (ATC) is a rare and undifferentiated form of thyroid cancer with poor prognosis. The median overall survival varies from 4 to 10 months after diagnosis. Among new therapeutic strategies, the oncolytic viral therapy might represent an alternative opportunity. We have previously described that the oncolytic adenovirus dl922-947 showed anti-tumor efficacy in ATC cells alone and in combinatory treatments. dl922-947 is a replicating oncolytic adenovirus bearing a 24bp deletion in E1A-Conserved Region 2, by deleting this domain viral replication can only proceed in cells with defective pRb/G1-S cell cycle checkpoint, an abnormality in the majority of human cancer cells. In this study, we investigated in ATC cells the anticancer efficacy of dl922-947 in combination with Y27632 an inhibitor of ROCK, a kinase involved in the regulation of cell migration, microtubule dynamics and β-catenin turnover

Methods used: We used as ATC cells 8505c to evaluate the anticancer effects of *dl*922-947 in combination with Y27632. Cell cytotoxicity was determined by sulforhodamine B assay. Viral entry was determined by the evaluation of AdGFP positivity by flow cytometry. Viral replication was evaluated in the intracellular and extracellular fraction by qPCR.

Results and Conclusions: We observed that the combinatory treatment of *dl*922-947 with Y27632 enhanced the cytotoxic effect and viral entry. Additionally, the replicative potential of dl922-947 was increased by the combination. Overall, our data suggest that inhibition of ROCK signaling might potentiate oncolytic viral therapy.

PO 199

HOPE5 PROJECT: HIGH-RISK HPV INFECTION IN HEALTHY INDIVIDUALS AND HPV16E5 IN HPV-DRIVEN OROPHARYNGEAL CARCINOMAS

E. Giuliani[1], F. Paolini[2], M.G. Donà[3], F. Rollo[4], C. Bonomo[4], F. Campo[5], S. Falcucci[2], R. Covello[4], E. Merenda[4], M. Benevolo[4], B. Pichi[5], A. Venuti[2], LILT Working Group*

[1] *Scientific Direction, San Gallicano Dermatological Institute IRCCS, Rome, Italy*
[2] *HPV-Unit, Unità Operativa Semplice Dipartimentale (UOSD) Tumor Immunology and immunotherapy, IRCCS Regina Elena National Cancer Institute, Rome, Italy*
[3] *STI/HIV Unit, San Gallicano Dermatological Institute IRCCS, Rome, Italy*
[4] *Pathology Department, Regina Elena National Cancer Institute IRCCS, Rome, Italy*
[5] *Otolaryngology Head Neck Surgery Department, Regina Elena National Cancer Institute IRCCS, Rome, Italy*
**M. Meleti, R. Antonelli, F. Cecconi, L. Davì, F. Lorusso, A. Maniaci, A. Scalisi, G. D'ermo*

Aim of the Study: The Italian multicenter project "HPV in OroPharyngeal carcinoma: presence of E5 specific transcripts" (HOPE5) aimed:

1) to investigate the presence of HPV16E5 specific transcripts in oropharyngeal carcinomas (OPCs) together with EGFR and HLA I expression;
2) to evaluate oral rinse and gargles (ORG) of healthy subjects for the presence of high-risk HPV infection (HR-HPV).

Methods: ORG were collected with 15 ml of Listerine® mouthwash from healthy (oral lesion-free) men enrolled at the Regina Elena National Cancer Institute (IRE, Rome, Italy) and ENT outpatient clinics of five different LILT Provincial Committees. The Xpert® HPV assay (Cepheid, Inc) was used to detect HR-HPV DNA. A retrospective series of formalin-fixed paraffin-embedded (FFPE) tissue blocks corresponding to a cohort of HPV16-driven OPC patients with at least a 2-year follow-up was retrieved from the archives of the IRE Pathology Department. After total RNA extraction, HPV16E5 mRNA and E6/E7 mRNA expression was investigated by RT-qPCR with specific primers for E5, E6 and E7 transcripts. Expression of EGFR (3C6, Roche Diagnostics) and class I HLA (D-2, HLA-A/B/C, Santa Cruz Biotechnology, Inc.) was evaluated by IHC staining.

Results and Conclusions: Overall, 105 healthy men were enrolled (median age: 32 years, IQR: 28-38). HR-HPV DNA was detected in 2 samples that tested positive for HPV16 (1.9%). E6/E7 polycistronic transcripts containing E5 coding sequence, as well as E5-specific transcripts, were not detected in any of these samples. For the retrospective analysis, 71 HPV16-driven OPCs were included in the study (median age: 61 years, IQR: 54-69; 73.3% men). The E6/E7 polycistronic transcripts containing E5 coding sequence were identified in all cases. E5-specific transcripts were detected in 7 cases (9.8%). Thirty-eight OPCs (53.5%) showed a positive staining for EGFR, with 10% to 90% of tumor cells positive for this biomarker. Of the 7 OPCs with E5-specific transcripts, 5 (71.5%) showed a negative/low staining for class I HLA and 2 (28.5%) displayed a moderate/high staining. In conclusion, HR-HPV prevalence found in ORG of healthy individuals is in line with the literature. The absence of E5 and E6/E7 transcripts suggests that HPV is not transcriptionally active in these lesion-free subjects, but more samples need to be analyzed to confirm these data. E5 specific transcripts were detected only in a minor fraction of HPV16-driven OPCs. However, EGFR expression does not seem to be affected by the presence of E5 transcripts, confirming previous data. The majority of OPCs harboring E5 specific transcripts showed no/poor staining for class I HLA, in line with one of the main biological activity of E5, i.e., MHC I down regulation. Survival analysis will be conducted in order to investigate the potential role of E5 specific transcripts, also together with EGFR and HLA I expression, as a prognostic marker in patients with HPV16-driven OPCs.

PO 200

KAPOSI'S SARCOMA IN PLWH: A PILOT STUDY ON HHV8 AND HIV-RELATED CLINICAL, IMMUNOLOGICAL AND VIROLOGICAL PARAMETERS

E. Giuliani[1], E. Trento[2], C. Stingone[3], L. Gianserra[3], I. Lesnoni La Parola[3], F. Pimpinelli[2], M.G. Donà[3], A. Latini[3]

[1] *Scientific Direction, San Gallicano Dermatological Institute IRCCS, Rome, Italy*
[2] *Microbiology and Virology, San Gallicano Dermatological Institute IRCCS, Rome, Italy*
[3] *STI/HIV Unit, San Gallicano Dermatological Institute IRCCS, Rome, Italy*

Aims of the Study: Kaposi's sarcoma (KS), an HHV8-induced neoplasm, is one of the most common cancers in PLWH. Previous studies have suggested a correlation between CD4+/CD8+ ratio and KS development in HIV-infected subjects. The aim of the present study was to investigate CD4+/CD8+ ratio, along with CD4+, CD8+, CD19+ and NK cell counts, in HIV-infected patients diagnosed with KS in comparison with HIV-infected patients with no history of KS.

Methods: Three groups of HIV-infected patients, attending the HIV Unit of the San Gallicano Dermatological Institute IRCCS (Rome, Italy), were included: 1) individuals diagnosed with KS; 2) individuals with detectable HHV8-DNA and HIV-RNA (HHV8+/KS-); 3) individuals always negative for HHV8-DNA and with detectable HIV-RNA (HHV8-). Clinical, laboratory and follow-up data were retrieved from the medical records. Independent and paired groups were compared by Mann-Whitney and Wilcoxon test, respectively. A p-value <0.05 was considered as significant.

Results and Conclusions: Thirty-nine patients were included: 8 with KS (median age: 36 ys; IQR; 29-47), 9 HHV8+/KS- (median age: 42 ys; IQR; 40-51), and 22 HHV8- (median age: 37 ys; IQR; 33-44). At diagnosis, KS patients had a median HIV-RNA load of 290,634 copies/ml. Median counts of CD4+ and CD8+ cells were 195 (IQR: 126-294) and 799 cells/mm3 (IQR: 687-1130), respectively. Median CD4+/CD8+ ratio was 0.19 (IQR: 0.17-0.26). Two patients were treated with antiretroviral therapy only, whereas the remaining 6 subjects were treated also with chemotherapy. Therapy was successful in all but one patient, who always maintained a detectable HHV8-DNA, with a fatal outcome.
A significant difference in the median count of CD4+ was observed when comparing KS patients with the two control groups (195 vs. 399 cells/mm3 of HHV8+/KS-, p=0.027; 195 vs. 444 cells/mm3 of HHV8-, p=0.003). CD4+/CD8+ ratio was also significantly lower in KS patients compared to the control subjects (0.19 vs. 0.50 of HHV8+/KS-, p=0.005; 0.19 vs. 0.70 of HHV8-, p=0.0001). A significantly decreased number of NK cells was also observed in KS patients compared to the control groups. After therapy, CD4+ and NK cell counts, as well as CD4+/CD8+ ratio, significantly increased in KS patients. In conclusion, KS patients showed a low CD4+/CD8+ ratio at KS diagnosis. Further studies are needed to investigate whether a low CD4+/CD8+ ratio may serve as a biomarker of KS risk, and how the different antiretroviral regimens may affect restoration of CD4+/CD8+ ratio.

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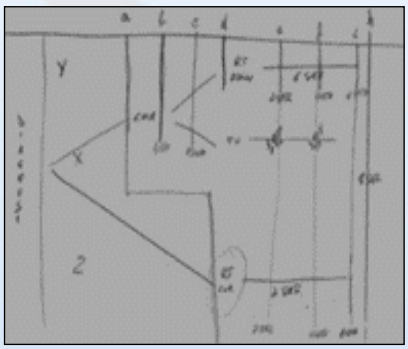
PLASMA ctHPV16-DNA IN HPV+ OROPHARYNGEAL SQUAMOUS CELL CARCINOMA: EARLY RESULTS FROM BIOTOTOP STUDY

F. Paolini[1], F. Campo[1], V. De Pascale[1], M. Allegretti[1], A. Cutarelli[2], F. De Falco[3], S. Falcucci[1], F. Ganci[1], A. Palcau[1], M.G. Donà[1], F. Rollo[1], V. Manciocco[1], M. Benevolo[1], G. Blandino[1], R.L. Pellini[1], A. Venuti[1]

[1] *Istituti Fisioterapici Ospitalieri, IRCCS Regina Elena National Cancer Institute and San Gallicano Dermatologic Institute Rome, Italy*
[2] *Zooprophylactic Institute of Southern Italy, Portici, Naples, Italy*
[3] *University of Naples Federico II, Naples, Italy*

Increased incidence of Human Papillomavirus (HPV)-associated Oropharyngeal Squamous Cell Carcinoma (OPSCC) and carcinoma of unknown primary (CUP) in the head and neck area have been reported. Currently, diagnostic value and accuracy of clinical evaluation and imaging remain doubtful, exposing patients to unnecessary surgical approach. BIOTOTOP (viral and microRNA **BIO**markers for **T**umors of **O**ropharynx and **T**umors of **O**ccult origin positive to **P**apillomaviruses) study utilizes liquid biopsy for circulating HPV16-DNA (ctHPV-DNA) and specific microRNAs (miRNA) as biomarkers. Their expression levels are also correlated with radiomics imaging. Briefly:



Drawing idea of study design by R. Pellini's note

- or OPSSC treated with TransORal Surgery (TORS), blood and saliva samples are taken at various times: before surgery; day 1 post-surgery; at the time of the patient's discharge; iv) during follow-up every 3 months (first 2 yrs) and every 6 months (following 3 yrs).
- for OPSCC patients treated with upfront RT or RT-CT, collection of liquid/blood are at: i) day before treatment; ii) every 2 weeks during treatment; iii) during follow-up as above.
- for OPSCC patients treated with immunotherapy, collection of liquid/blood and saliva samples are at: i) first day of treatment; ii) before administering immunotherapy doses.

Primary aim of whole project: ctHPV-DNA and specific microRNAs kinetic in liquid biopsies of OPC patients.

Methods: Since the study is actively enrolling patients (>150 at the end of enrolment), present results become from an initial number of patients and are related only to ctHPV DNA detection. Collected serial blood drawings were analysed with standard protocols by three different platforms, QuantStudio 3D Digital, QuantStudio Absolute Q Digital PCR and QX200 Droplet Digital PCR.

Results: Preliminary data showed that all utilized methods had a good specificity and sensitivity, but concordance among methods was moderate indicating that further analyses are necessary to asses this point. Until now (more data are expected for Congress presentation), 83 consecutive cases were enrolled; and 72 pts were analysed: 47 pts were HNSCC p16 IHC positive and 25 HNSCC p16 IHC negative. Mean ctHPV-DNA at presentation was 1200 copies/ml, the range was 0-7100 copies/ml. T stage and N stage were not correlated with ctHPV-DNA as well as tumor burden. ctHPVDNA kinetics after TORS (16 pts) showed a reduction of ctHPV-DNA copies/ml in 13 pts. Three pts had ctHPV-DNA levels that remained markedly elevated; 2 of these pts had gross extra-nodal extension (ENE) and 1 pt positive margins. Representative cases of longitudinal monitoring will be shown.

Conclusions: These preliminary data suggest that these innovative and promising approaches may improve the diagnostic work-up, decreasing the time needed for the first diagnosis, identifying microscopic residual disease (data post-surgery) and early relapse (longitudinal data). Overall, our results confirm that ctHPV-DNA analysis is clinically meaningful, showing potential to guide personalized treatment decisions in HPV+ OPSCCs.

PO 202

EVALUATION OF CO-INFECTIONS BETWEEN HUMAN PAPILLOMAVIRUS AND OTHER SEXUALLY TRANSMITTED PATHOGENS IN WOMEN REFERRED FOR COLPOSCOPY

C. Giubbi[1], M. Martinelli[1], M.L. Di Meo[2], R.C. Njoku[1], F. Perdoni[1], E. Bruno[1], R. Musumeci[1], F. Landoni[1,2], R. Fruscio[1,2], C.E. Cocuzza[1]

[1] Department of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy
[2] San Gerardo Hospital, ASST Monza, Italy

Aim: Human Papillomavirus (HPV) persistent infection is the necessary cause for the onset of cervical cancer responsible worldwide for 234.000 deaths in 2020. Additional risk factors, such as the presence of co-infections with other sexually transmitted agents (STIs), may influence the development of high-grade cervical lesions and the persistence of HPV infection. This study aims to assess the prevalence of 7 STIs in women referred to colposcopy and to evaluate their association with HPV infection, its persistence and the grade of cervical lesion.

Methods: Cervical samples were collected from 315 women undergoing colposcopy at San Gerardo Hospital, Monza following a recent abnormal cervical cytology result. Some women were treated with conization, based on clinical judgment, and followed-up after 6-12 months. Microlab Nimbus platform was used for nucleic acid extraction and PCR plate set-up for the detection of 14 high-risk HPV (hrHPV) genotypes and 7 other STIs using, respectively, Anyplex™II HPV and Anyplex™II STI-7e (Seegene).

Results and Conclusions: 66.7% (210/315) and 47.3% (149/315) of women were respectively hrHPV and STIs positive at the time of colposcopy (T0). 119 out of 149 (79.9%) with STIs showed a coinfection with hrHPV, with a positive association of the two variables (odds ratio 3.3333; p<0.0001). Figure 1 shows the different distribution of each sexually transmitted pathogen in HPV- positive and negative women. Co-infections of hrHPV and STIs were more frequent in women with high-grade (28/63; 44.4%) than in those with low-grade (87/231; 37.7%) cervical lesions or with negative cytology (4/21; 19.0%). At the follow-up visit, 44.8% (48/107) of women were hrHPV positive. As expected, a higher prevalence of persistent infections was detected in women not treated at T0 (36/48; 75.0%). hrHPV persistent infections were not associated with coinfection with other STIs at T0 (STIs positive: 25/48; 52% vs STIs negative: 23/48; 48%). These preliminary results underline a higher percentage of STIs among HPV-positive women. Further analysis of a larger number of women is necessary to clarify the role of each STI as a co-factor of cervical cancer lesion progression.

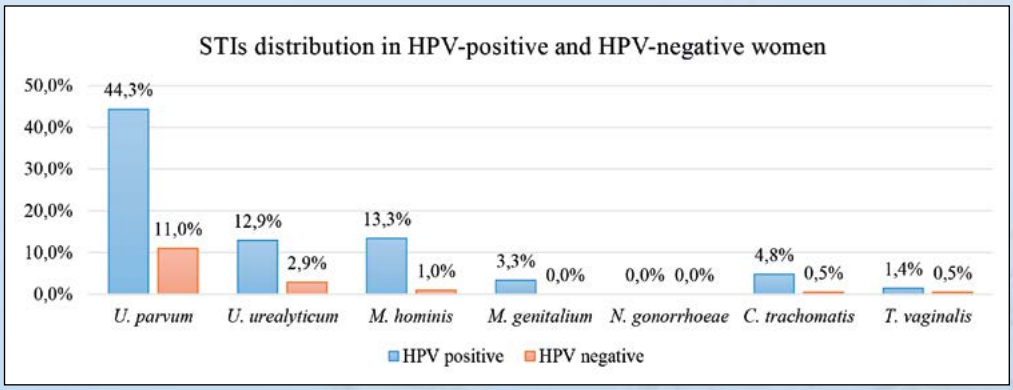


Figure 1 - Different distribution of 7 sexually transmitted pathogens in HPV-positive and HPV- negative women.

PO 203

HUMAN PAPILLOMAVIRUS (HPV) E6 mRNA POSITIVITY IN WOMEN REFERRED TO COLPOSCOPY

C. Giubbi[1], M. Martinelli[1], M.L. Di Meo[2], R.C. Njoku[1], F. Perdoni[1], E. Bruno[1], R. Musumeci[1], F. Landoni[1,2], R. Fruscio[1,2], C.E. Cocuzza[1]

[1] Department of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy
[2] San Gerardo Hospital, ASST Monza, Italy

Aim: Persistent Human Papillomavirus (HPV) infection is well known to be a necessary cause for the onset of cervical cancer. Since most HPV infections are transient and clinical lesions undergo spontaneous regression, it is important to investigate new molecular biomarkers associated with a higher risk of disease progression. The persistence of HPV infection has been proposed to be related to viral genome integration, loss of function of E2 and overexpression of E6 and E7 oncoproteins. The aim of this study was to investigate the presence of E6 mRNA in cervical samples of women with cervical dysplasia.

Methods: 328 women referred to colposcopy at San Gerardo Hospital (Monza, Italy) with a recent diagnosis of cervical dysplasia were enrolled in the study and managed according to clinical judgment and local protocols. A cervical sample was collected from each woman and nucleic acids extraction performed using MicroLab Nimbus platform (Seegene). Type-specific HPV DNA was detected using Anyplex™II HPV HR (Seegene), while the presence of HPV RNA was evaluated by "in-house" assays targeting the E6*1 spliced mRNA of HPV16 and HPV18.

Results and Conclusions: According to clinical data, 53 women were diagnosed with high-grade cervical lesions (CIN2+), while the remaining with low-grade cervical lesions (CIN2-). 217/328 (66.1%) women were HPV-positive when tested with Anyplex™II HPV HR. In particular, 73/328 (22.2%) resulted HPV16-positive, while 20/328 (6.1%) were HPV18-positive. Among those women, respectively 26/73 (35.6%) and 9/20 (45.0%) were found to have CIN2+ lesions. In the group of 73 women positive for HPV16 DNA, 61 (83.6%) were positive for the presence of viral RNA. All women positive for HPV18 showed the presence of viral transcripts. For both HPV genotypes, the mean Ct value in women with high-grade cervical dysplasia was lower than in women with low-grade dysplasia (HPV16: CIN2+: 28.92; CIN2-: 30.98; HPV18: CIN2+:31.80; CIN2-: 33.73). Furthermore, for HPV16 a different prevalence of viral RNA was detected in the group of CIN2+ (92.3%) and CIN2- (78.7%) women.
In order to better establish the possible role of viral mRNA as a biomarker of oncogenic progression, these results need to be confirmed on a larger set of HPV-positive women undergoing cytological triage as part of organized cervical cancer screening programs.

PO 204 - OC 39

FUNCTIONAL AND STRUCTURAL COMPARATIVE ANALYSIS OF NUCLEAR LOCALIZATION SIGNALS ON POLYOMAVIRUSES LARGE TUMOR ANTIGENS REVEALS CRUCIAL ROLE OF BIPARTITE NLS IN NUCLEAR TRANSLOCATION

E.M. Cross[1,2], N. Akbari[3,4], H. Ghassabian[3], M. Hoad[1], S. Pavan[3], D. Ariawan[5], C. Donnelly[1], E. Lavezzo[3], G.F. Petersen[1], J. Forwood[1], G. Alvisi[3], S. Nematollahzadeh[6]

[1] *School of Dentistry and Medical Sciences, Charles Sturt University, Wagga Wagga, NSW, 2678, Australia*
[2] *Diamond Light Source, Harwell Science and Innovation Campus, Didcot, OX11 0DE, United Kingdom*
[3] *Department of Molecular Medicine, University of Padua, Italy*
[4] *College of Medicine, University of Saskatchewan, Saskatoon SK, Canada*
[5] *Dementia Research Centre, Macquarie University, Sydney, NSW, 2109, Australia*
[6] *Department of Microbiology, Anatomy, Physiology, and Pharmacology, School of Agriculture, Biomedicine and Environment, La Trobe University, Melbourne, VIC 3086, Australia*

Polyomaviruses (PyVs) include a rapidly growing number of small non enveloped viruses causing a variety of diseases in mammals, birds and fishes. Expression and replication of their DNA genome occur in the nuclei of infected cells and require the concerted action of cellular and viral proteins, in a highly coordinated manner as regulated by the viral-encoded large tumor antigen (LTA). By combining bioinformatics, confocal laser scanning microscopy, biochemical and crystallographic approaches we demonstrate that LTAs from all human (H)PyVs possess functional classical NLSs, responsible for their nuclear transport through the importin (IMP)α/β pathway. Intriguingly, such NLSs are highly heterogenous in number, structural organization, IMPα binding preferences and nuclear targeting abilities. Several LTAs possess more than one closely located stretch of basic amino acids, thus forming bipartite cNLS. Bipartite cNLSs are absolutely required for nuclear import of Karolinska Institutet (KI) and Sant Louis (STL) PyV, which do not possess a functional monopartite cNLS. Finally, a bioinformatics analysis of LTAs from all known PyVs revealed that cNLSs are highly conserved across mammal - but not bird or fishes - infecting viruses. Given the fact that LTA nuclear accumulation is absolutely required for viral replication and impacts cell transformation, our results have important implications for a better understating of PyVs and nuclear transport receptors evolution as well as of the molecular mechanisms of PyV pathogenesis.

PO 205 - OC 40

ONCOLYTIC HSV-1 VECTORED BY MONOCYTES AS A BROAD-SPECTRUM ANTICANCER THERAPY

A. Reale[1], L. Krutzke[2], M.V. Fornaini[1], M. Cadamuro[1], A. Calistri[1]

[1] Department of Molecular Medicine, University of Padua, Italy
[2] Department of Gene Therapy, University of Ulm, Germany

Oncolytic viruses (OVs) are therapeutics which combine cancer cell-killing activity, immunotherapy and gene therapy, if the viral genome is modified to encode therapeutic genes. This is possible because cancer cells are more susceptible than healthy cells to viral replication, thus OVs can be obtained by attenuation of different human viruses, including herpes simplex virus type 1 (HSV-1). Oncolytic HSV-1 (oHSV1) talimogene laherparepvec was approved for the treatment of unresectable melanoma in the USA and the EU. The most employed delivery method is intratumoral administration, both in preclinical and clinical studies. In fact, following intravenous injection, the host's immune system can clear the attenuated viruses before they can reach the target. Regardless of the value of intratumoral injection, a strategy to achieve systemic delivery and targeting of metastases involves using carrier cells which can be infected *ex vivo*, shielding the OV from the immune system. We proposed a novel approach, i.e the use of monocytes as carrier cells for oHSV1. Human monocytes can be infected by HSV-1 but are relatively resistant to infection. At the same time, in many tumors, circulating monocytes are the source of tumor-associated macrophages (TAMs), and are actively recruited by cancer cells. Human monocytic THP-1 cells were susceptible to infection with an EGFP-expressing oHSV1 based on a backbone similar to talimogene. THP-1 cells could also transfer infection to human breast cancer MDA-MB-231 cells in coculture and transwell migration experiments. Primary human monocytes were also susceptible when infected with a higher viral load and could migrate towards and transfer infection to human tumor cells of different origins, including breast cancer (MDA-MB-231) squamous head-and-neck cancer cells (UM-SCC-11B) and glioblastoma cells (U87-MG and LN-229). To confirm the migration of infected monocytic cells towards human tumor cells in a more relevant setting, we injected THP-1 cells in chicken embryos with UM-SCC-11B cells growing on the chorioallantoic membrane (CAM). Furthermore, mouse WEHI274.1 monocytic cells are also permissive to oHSV-1 infection. Viral infection was assessed by fluorescence microscopy for expression of EGFP or immunofluorescence to detect viral proteins, while replication was assessed by plaque titration assay and the effects of infection on cancer cell viability by counting vital cells after Trypan blue staining and/or using commercial cytotoxicity assays. As a result, we conclude that oHSV1 delivered by monocytes is a promising broad-spectrum antitumoral therapy which deserves further investigation.

PO 206 - OC 41

ANTI-CANCER ACTIVITY OF THE ONCOLYTIC ADENOVIRUS *dl*922-947 IN TRIPLE-NEGATIVE BREAST CANCER CELLS

F. Napolitano, G. Castellano, C. Maglio, A.M. Malfitano, G. Portella

Department of Translational Medical Sciences, University of Naples Federico II, Naples, Italy

Aim of the Study: Triple-negative breast cancer (TNBC) is the most common female malignancy, with poor prognosis and limited treatment options. Novel therapeutic strategies are needed and among new treatments, oncolytic viral therapy might represent an alternative opportunity. *dl*922-947 is a replicating oncolytic adenovirus bearing a 24bp deletion in E1A-Conserved Region 2, by deleting this domain viral replication can only proceed in cells with defective pRb/G1-S cell cycle checkpoint, an abnormality in the majority of human cancer cells. In this study, we investigated the anticancer efficacy and cell death mechanism of *dl*922-947 in TNBC cell models.

Methods used: We used a panel of TNBC cells: MDA-MB-231, DU-4475 and MDA-MB-468 to evaluate the anticancer effects of *dl*922-947. Cell cytotoxicity was determined by sulforhodamine B assay. To characterize the mechanism underlying *dl*922-947 effects on cancer cell viability, we assessed apoptosis induction and Immunogenic cell death (ICD) markers (Calreticulin, High mobility group box protein 1 HMGB1 and ATP). Apoptosis, calreticulin expression and HMGB1 secretion were determined by flow cytometry, whereas a luminescence-based bioassay assessed ATP content. Furthermore, we investigated the modulation of cGAS-STING-STAT3 pathway by Western blot.

Results and Conclusions: We compared the sensitivity of different cancer cell lines to *dl*922-947-induced cytotoxicity and we observed higher efficacy in MDA-MB-231 cells that we used for further assays. We found that *dl*922-947 infection induces apoptosis and modulates the main hallmarks of ICD-inducing calreticulin surface expression, HMGB1 and ATP release. We observed modulation by *dl*922-947 of cGAS-STING-STAT3 pathway that is highly activated in TNBC cells. Our data show that *dl*922-947 induces anticancer effects in TNBC cells likely associated with anti-tumor immune response. Overall, oncolytic viral therapy might represent a possible therapeutic strategy against TNBC.

PO 207 - OC 42

GENE EDITING OF HIV-1 LATENTLY INFECTED CELLS USING AN ACTIVATION-INDUCIBLE SYSTEM

G. Magro[1], V. Poletti[2,3], A. Garzino-Demo[1,4], A. Calistri[1], C. Parolin[1]

[1] *Department of Molecular Medicine, University of Padua, Italy*
[2] *Woman and Child Health Department, University of Padua, Italy*
[3] *Harvard Medical School, Harvard University, Boston, MA, USA*
[4] *School of Medicine, University of Maryland, Baltimore, Maryland, USA*

Background: HIV-1 integrates in the host cell genome and its persistence as latent reservoir in a small subset of infected cells represents one of the main barriers to find a functional cure. Recently, CRISPR-Cas9-based gene editing approaches have been tested in *in vitro* and *in vivo* models to disrupt the provirus both in productively and latently infected cells, leading to efficient inhibition of viral replication. However, major challenges remain, for the successful translation of these technologies to the clinic, including efficient delivery in patients and safety issues correlated to undesired off-target effects and nuclease persistence within the cells.

Aim: We hypothesized that constraining Cas9 expression in latently infected cells upon their activation, that is necessary for HIV-1 replication, could reduce the likelihood of off-target effects while leading to an effective disruption of the viral reservoirs.

Methods: We designed an all-in-one lentiviral vector, expressing an array of gRNAs targeting the host *CCR5* co-receptor and HIV-1-specific genes (*gag*, *tat/rev*), along with the SpCas9 nuclease under a cell-activation inducible promoter (NFAT-regulated). To evaluate the inducible expression of the nuclease, T-lymphoblastoid cells were stably transduced and single cell clones were generated. The vector copy number (VCN) was determined using ddPCR. Next, Cas9 expression and activity in stimulated and un-stimulated cells was assessed using qPCR, western blot, and a fluorescent reporter assay. The system's efficacy to inhibit viral replication was then evaluated in cellular models of HIV latency. Transduced cell clones were selected and p24 levels in the culture supernatant were tested by ELISA. Finally, disruption of the provirus sequence was determined by PCR amplification of the target regions and Sanger sequencing.

Results and Conclusions: We confirmed activation-induced Cas9 expression in stably transduced T cell line derived clones. The activity of the nuclease was proven in the fluorescent-based assay, with one of the clones with the highest VCN (=2) showing a fluorescence signal decrease of more than half with respect to the control. The system's efficiency against viral replication was then demonstrated in a monocyte-derived latently infected cell line, exhibiting an up to 4-fold decrease in viral p24 antigen. These results were supported by mutations detected in the provirus target regions. These data will further endorse our hypothesis of adopting a cell-activation-inducible Cas9-based approach for the potential functional disruption of the HIV-1 latent reservoir.

PO 208

ONCOLYTIC HSV-1 EXHIBITS POTENT REPLICATION AND LYTIC ACTIVITY IN HUMAN MULTIPLE MYELOMA CELLS

A. Reale, M.V. Fornaini, A. Calistri

Department of Molecular Medicine, University of Padua, Italy

Multiple myeloma (MM) is a neoplasm originating from differentiated B lymphocytes (plasma cells) which proliferate in the bone marrow, with pathology ensuing from bone destruction, immunosuppression and paraproteinemia. In spite of several therapeutic options available, MM remains incurable and its prognosis is inferior in patients who are unable to tolerate more aggressive treatments such as bone marrow transplantation. Cancer cells are more susceptible than healthy cells to viral replication, therefore oncolytic viruses (OVs) can be obtained by attenuation of different human viruses, including herpes simplex virus type 1 (HSV-1). Oncolytic HSV-1 (oHSV1) talimogene laherparepvec was approved for the treatment of unresectable melanoma in the USA and the EU. So far, few studies have investigated the effect of oHSV1 on MM cells, using highly attenuated viral strains which reportedly did not replicate, although they caused cancer cell death (Oku et al., 2021) (Ghose et al., 2021). Another significant barrier to the use of oHSV1 for the treatment of MM is the elaboration of an effective way to achieve systemic delivery. In fact, following intravenous injection, the host's immune system can clear the attenuated viruses before they can reach the target. Furthermore, in the case of MM, it is not possible to circumvent this problem by intratumoral injection of the OV as it is often done in solid tumors.

The aim of this study is to evaluate the oncolytic potential of the clinically approved backbone (Δγ34.5/ΔUs12) in MM cells.

Two human MM cell lines, SKO-007 and MM.1R, were infected with a Δγ34.5/ΔUs12 oHSV1 expressing the reporter gene enhanced green fluorescent protein (EGFP). EGFP expression was evaluated by fluorescence microscopy at several time points, as well as cell viability by Trypan blue staining and viral replication by plaque titration assay. Thereby we were able to show that Δγ34.5/ΔUs12 oHSV1 does not only infect MM cells and induce cell death, but actively replicates in these cells.

As a result, we conclude that Δγ34.5/ΔUs12 oHSV1 is very effective against MM cells *in vitro* and that its delivery by monocytes is a promising therapeutic strategy that will be further investigated. To solve the problem of systemic delivery we proposed a novel approach, i.e the use of monocytes as carrier cells for oHSV1. We showed that human monocytes can be infected by oncolytic HSV-1. At the same time circulating monocytes are the main source of tumor-associated macrophages (TAMs), and are actively recruited in the tumor microenvironment of many malignancies including MM. This system of delivery was already validated in vitro using cells from different tumors, including breast cancer, head-and-neck squamous carcinoma and glioblastoma. As a next step, we will investigate carrier monocytes for the delivery of oHSV-1 to MM cells.

PO 209

SIMULTANEOUS EXPRESSION OF DIFFERENT THERAPEUTIC GENES AFTER INFECTION WITH MULTIPLE ONCOLYTIC HSV-1 VECTORS

A. Vitiello[1], A. Reale[1], V. Conciatori[1], A. Vicco[1], A. Garzino Demo[1], J. Von Einem[2], C. Parolin[1], A. Calistri[1]

[1] *Department of Molecular Medicine, University of Padua, Italy*
[2] *Institute for Virology, University of Ulm, Ulm, Germany*

Oncolytic viruses (OVs) are emerging therapeutics that selectively replicate in cancer cells, either naturally or following genetic engineering. OVs also elicit an immune response against cancer and are therefore an immunotherapeutic tool. Furthermore, OVs can be modified to express therapeutic genes. For our studies, we engineered an OV analogous to talimogene laherparepvec (T-VEC). T- VEC is an attenuated herpes simplex virus type 1 (HSV-1) with deletions in the neurovirulence γ34.5 gene and Us12 gene (Δγ34.5/ΔUs12) and is endowed with human granulocyte-monocyte colony stimulating factor (hGM-CSF) gene, and it has been approved for use in humans by the US FDA in 2015 and by the EMA in 2016 for the treatment of advanced-stage malignant melanoma. Using BAC mutagenesis, we deleted Us12 in the 17+ Δγ34.5 HSV-1 strain, which contains a firefly luciferase (FLuc)-expressing cassette in the UL55-UL56 intergenic region. Subsequently, we generated several oncolytic HSV-1 (oHSV1s) with our Δγ34.5/ΔUs12 backbone containing different foreign genes including immunotherapeutic genes other than GM-CSF. We then addressed the question of which strategy should be employed to express multiple therapeutic genes using oncolytic viruses. While inserting different genes in a single vector is feasible with large viruses such as HSV-1 and minimizes the number of viruses that must be produced, it is technically demanding. On the other hand, the use of multiple vectors potentially allows a flexible and rapid combination of therapeutic genes. Our data show that coinfection of human breast cancer cells with two different oncolytic HSV-1 vectors with a Δγ34.5/ΔUs12 backbone, each expressing an individual foreign gene (enhanced green fluorescent protein, firefly luciferase, or human interleukin 12) leads to high-level expression of both transgenes, suggesting that vector combination is a flexible strategy which allows to modulate the expression of multiple therapeutic genes depending on the patient characteristics.

PO 210

QUANTIFICATION OF TOTAL BACTERIAL 16S rDNA IN SWAB SAMPLES CHECKED FOR THE PRESENCE OF SARS-CoV-2 RNA

C. Orlandi[1,2], M. Magnani[1,2], A. Casabianca[1,2]

[1] *Department of Biomolecular Sciences, Section of Biochemistry and Biotechnology, University of Urbino Carlo Bo, Fano, Pesaro-Urbino, Italy*

[2] *Laboratorio Covid, University of Urbino Carlo Bo, Fano, Pesaro-Urbino, Italy*

Aim of the Study: Increased systemic microbial translocation (MT) contributes to the pathogenesis of various infectious diseases. The magnitude of MT may be measured by total bacterial 16S ribosomal DNA (rDNA) in plasma using quantitative PCR (qPCR). Recently, we have developed a method for bacterial 16s rDNA quantification in both plasma and blood in HIV-1 infected patients to assess MT. The method involved the simultaneous quantification, starting from a single sample, of HIV DNA (marker of reservoir size), 16S rDNA (marker of MT) and a single copy gene (internal control, IC) to produce accurate quantification of HIV DNA and 16S rDNA (data partially submitted to ICAR 2020 Congress).
In this pilot study we investigated the use of this method to quantify 16S rDNA in different type of samples and different microbial biomass samples, such as the nasal microbial community in 100 positive and 50 negative swab samples tested for the SARS-CoV-2 RNA.

Methods: Total nucleic acid (RNA, DNA and microbial DNA) from nasopharyngeal swab (NPS) was extracted using Total RNA Purification Kit (Norgen) starting from 250 µl of sample and following the manufacturer's instructions. For the detection of the SARS-CoV-2 RNA, a one-step real-time RT-PCR multiplex (COVID-19 PCR DIATHEVA Detection kit, CE-IVD certified) was used. For the quantifications of the 16S rDNA and the IC, a Sybr-Green qPCR based assay was performed using a single standard curves for both targets. The results were expressed as 16S rDNA copies per 10^6 cells.

Results: All samples had a valid result with a mean (SD) RNAseP ct value of 23.42 (2.43). All SARS CoV-2 RNA positive samples had a mean (SD) ORF1b/N ct value of 25.21 (7.43) [range 15.90-37.39]. The median [IQR] 16S rDNA copies per 10^6 cells were 2.17×10^6 [6.13×10^5 - 5.16×10^6] and 5.12×10^6 [8.64×10^5 - 1.15×10^7] in SARS-CoV-2 positive and SARS-CoV-2 negative NPS, respectively (p=0.0933, Mann Whitney test).

Conclusions: Our previously developed qPCR format, was suitable for the accurate measurements of total bacterial 16S rDNA in the swab samples, thus providing information on microbial community content in SARS-CoV-2 RNA positive and negative samples. Although in this study, no significant difference was noted between both groups, this observation deserves further investigations using a larger number of samples.

PO 211

FROM COMPUTATIONAL ANALYSIS TO EXPERIMENTAL VALIDATION: UNDERSTANDING THE MECHANISM OF p17 VARIANTS AND IMPLICATIONS FOR DRUG REPOSITIONING

A. Rondina[1], S. Messali[1], A. Zani[1], P. D'Ursi[2], F. Caccuri[1], A. Caruso[1]

[1] *Section of Microbiology Department of Molecular and Translational Medicine, University of Brescia, Italy*
[2] *Institute for Biomedical Technologies, National Research Council (ITB-CNR), Segrate, Milan, Italy*

Aim of the Study: This work has been focused on understanding the relationship between the molecular structure of p17 variants (vp17s) and their biological activity. HIV-1 Matrix Protein (MA) p17 is a regulatory protein that undergoes mutations and insertions (positions 114-115 and 117-118 mainly covered in this study) along its sequence (Dolcetti R et al., 2015). vp17s are known to promote B-cell clonogenicity through activation of the EGFR/PI3K/Akt signaling pathway (Giagulli C. et al., 2017). The functional epitope responsible for the clonogenic activity of vp17s is located in their N-terminal region, at residues Arg15, Lys18, and Arg20. Computational studies carried out by our research group made it possible to identify the key mechanism related to vp17s clonogenic activity. In particular, we observed that the stability of their functional epitope may depend on two fundamental residues: Trp16 and Tyr29.

Methods: To validate the computational data *in vitro*, we produced the recombinant p17 proteins carrying mutation at positions Trp16 and Tyr29, alone or in combination. To this end, the QuikChange Lightning Site-Directed kit (Stratagene) was used to insert the two desired mutations, W16A and Y29A, in the protein backbone. We obtained three recombinant proteins, two with the single amino acid mutations (W16A or Y29A), and one with the double mutation (W16A and Y29A). The absence of endotoxin contamination (<0.25 endotoxin units/ml) in protein preparations was assessed by Limulus amoebocyte assay. Each protein was tested for its ability to promote clonogenic activity by performing the B-cell colony formation assay. With regard to computational studies, drug repositioning analyses were performed using Autodock Vina software.

Results and Conclusions: Data obtained demonstrated a significant increase of B-cell proliferation by all mutated p17 proteins compared to untreated cells or cells treated with p17. We have thus been able to demonstrate that by introducing specific mutations into the backbone of p17, it could induce clonogenic activity like vp17s, and therefore validate the conformational mechanism hypothesized by us. To assess the specificity of these results, we performed an assay for B-cell growth-promoting activity in the presence of the p17 neutralizing monoclonal antibody MBS-3, with an unrelated antibody (anti-p24) as a control. This assay confirmed that the mutated p17 proteins as well as the vp17s were neutralized by the MBS-3 antibody. We further tested sera collected from vaccinated patients, specifically using two types of serum: serum 0, containing non-neutralizing antibodies against p17, and serum 8, containing neutralizing antibodies. The tests on the mutated protein p17W16A-Y29A revealed that serum 8 neutralized the protein only at high concentrations, whereas serum 0 was incapable of neutralizing p17. Analysis of the mutated protein W16A was found to be not significant. Therefore, the results suggest the need to focus on potential binding pockets in proximity of residues 10 to 20, and to proceed with drug repositioning studies in order to inhibit the clonogenic activity of vp17s. Binding pockets analysis was performed on more representative cluster conformations, followed by drug repositioning using a home-made ligand library. The analysis is ongoing.

PO 212 - OC 43

IN cART-TREATED HIV-INFECTED PATIENTS, THE IMMUNOLOGICAL FAILURE IS ASSOCIATED WITH A HIGH MYELOID DERIVED SUPPRESSOR CELL FREQUENCY

G. Grassi[1], S. Notari[1], S. Cicalini[2], R. Casetti[1], E. Cimini[1], V. Bordoni[3], R. Gagliardini[2], V. Mazzotta[2], A. Antinori[2], C. Agrati[3], A. Sacchi[4]

[1] *Cellular Immunology and Pharmacology Laboratory, National Institute for Infectious Diseases "Lazzaro Spallanzani", Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Rome, Italy*
[2] *Clinical Division, National Institute for Infectious Diseases "Lazzaro Spallanzani", Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Rome, Italy*
[3] *Oncoematologia e Officina Farmaceutica, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Bambino Gesù Children's Hospital, Rome, Italy*
[4] *Molecular Virology and Antimicrobial Immunity Laboratory, Department of Science, Roma Tre University, Rome, Italy*

Background and Aim: During HIV infection, effective combined antiretroviral therapy (cART) suppresses viral replication and restores the number of circulating CD4+ T cells. However, 15–30% of treated patients show a discordant response to cART. Myeloid-derived suppressor cells (MDSC) are expanded in HIV+ patients, suppress T cell functions and impair the expansion of CD34+CD38- hematopoietic early progenitors *in vitro*. To better understand the role of MDSC on CD4 T cell recovery, we evaluated the frequency of MDSC in HIV+ patients under cART and its association with immunological response.

Methods: We enrolled sixty HIV+ patients under cART from at least one year, including complete Responders (R, n=44), Virological Non-Responder (VNR, n=5) and Immunological Non-Responder (INR, n=11). The frequency of circulating MDSC and the percentage of activated and naïve CD4 T cells were evaluated by flow cytometry. Plasmatic cytokines levels were analyzed by automated ELISA.

Results: As previously observed, polymorphonuclear MDSC (PMN-MDSC) frequency was higher in HIV+ patients compared to healthy donors (5.35% vs 1%, $p<0.0001$). Furthermore, PMN-MDSC percentage was higher in INR than R patients (16% vs 3.7%, $p=0.04$), and a significative association between MDSC frequency and immunological failure was confirmed by a ROC analysis (Area=0.82, SD=0.068, $p=0.001$). Accordingly, an inverse correlation was found between the percentages of PMN-MDSC and naïve CD4 T cells ($r=-0.33$, $p=0.012$). A positive correlation was seen between MDSC frequency and the percentage of HLA-DR+ CD4 T cells ($r=0.36$, $p=0.005$) and the plasmatic level of IL-1β and IL-8 ($r=0.38$, $p=0.04$ and $r=0.4$, $p=0.04$, respectively).

Conclusion: Our data indicate that, once HIV replication is suppressed by cART, inflammation and persistent T-cell activation, possibly induced by the exposure to translocated microbial products, could sustain PMN-MDSC differentiation and in turn CD4 T cell recovery inhibition. These findings confirm the detrimental role of MDSC during HIV infection, and open to a new scenario, where new therapeutic options could be evaluated to deplete/inhibit MDSC and improve CD4 T cell recovery and HIV-specific immune response.

PO 213 - OC 44

EFFICACY OF COVID-19 mRNA VACCINATION IN PATIENTS WITH AUTOIMMUNE DISORDERS: HUMORAL AND CELLULAR IMMUNE RESPONSE

F. Filippini[1], M. Giacomelli[2], C. Bazzani[3], M. Fredi[4], P. Semeraro[4], C. Tomasi[4], F. Franceschini[4], A. Caruso[1], I. Cavazzana[3], C. Giagulli[1]

[1] Section of Microbiology, Department of Molecular and Translational Medicine, University of Brescia, Italy
[2] Section of Microbiology, ASST Spedali Civili of Brescia, Italy
[3] Rheumatology and Clinical Immunology, ASST Spedali Civili of Brescia, Italy
[4] Rheumatology and Clinical Immunology, ASST Spedali Civili of Brescia and Department of Clinical and Experimental Sciences, University of Brescia, Italy

Aim of the Study: The impact of immunosuppressive therapies on the efficacy of vaccines to SARS-CoV-2 is not completely clarified. We analyzed humoral and T cell-mediated response after COVID-19 mRNA vaccine in immunosuppressed patients and patients with common variable immunodeficiency disease (CVID).

Methods used: We enrolled 38 patients, from the Unit of Rheumatology and Clinical Immunology, of the A.O. Spedali Civili of Brescia, Italy, and 11 healthy sex- and age- matched controls (HC). Four patients were affected by CVID and 34 by chronic rheumatic diseases (RDs). All patients with RDs were treated by corticosteroid therapy and/or immunosuppressive treatment and/or biological drugs: 14 patients were treated with abatacept, 10 with rituximab and 10 with tocilizumab. Total antibody titer to SARS-CoV-2 spike protein was assessed by electrochemiluminescence immunoassay, $CD4^+$ and $CD4^+$-$CD8^+$ T cell-mediated immune response was analyzed by interferon-γ (IFN-γ) release assay, the production of IFN-γ-inducible (CXCL9 and CXCL10) and innate-immunity chemokines (CCL2, CXCL8 and CCL5) by cytometric bead array after stimulation with different spike peptides. The expression of CD40L, CD137, IL-2, IFN-γ and IL-17 on $CD4^+$ and $CD8^+$ T cells, evaluating their activation status, after SARS-CoV-2 spike peptides stimulation, was analyzed by intracellular flow cytometry staining.

Results and Conclusions: The main findings were

1) a reduced anti-S response in ABA-treated group, restored after the third dose of vaccine;
2) an impaired T cell activation, represented by a reduction of IFN-γ and related chemokines;
3) a reduction of effector memory $CD8^+$ T cells in ABA-treated group;
4) a significant ability of ABA treated group to mount a $CD4^+$ T cell response, when stimulated with spike derived antigens.

Our work was limited by the low number of patients enrolled but performing extended cellular assessments, contributed to explain which kind of immune response patients chronically exposed to different immunosuppressive regimen are able to generate in response to COVID-19 vaccination. The preserved ability to generate clones of $CD4^+$ T lymphocytes specific for SARS-CoV-2 spike proteins represents the assurance of an effective protection of vaccination to SARS-CoV-2. Moreover, after the third dose of COVID-19 mRNA vaccine, ABA-treated patients acquired the capability to produce a strong antibody response, despite they maintained a significant reduction of $CD8^+$ T response. All these data represent a critical message from laboratory research bench to clinical patients' side, suggesting that repeated vaccine doses may be necessary to optimize the immunological response and to induce more robust serological responses in these high-risk vulnerable patients.

PO 214 - OC 45

NEURODEGERATIVE ROLE OF WEST NILE VIRUS NON-STRUCTURAL PROTEIN 1: EFFECT ON TLR3 AND AMYLOID BETA EXPRESSION

S. Beltrami, S. Rizzo, V. Gentili, G. Schiuma, R. Rizzo, D. Bortolotti
University of Ferrara, Italy

Introduction: In the last years, the North-Est region of Italy, in particular Veneto and Emilia-Romagna (Riccò M. et al., 2022), has been characterized by a significant increase of West Nile Virus (WNV) infection rate. Neuroinvasive WNV viral infection may be linked epidemiologically and mechanistically to neurodegeneration, which have been associated with a significant prevalence of sequelae such as memory loss, confusion, and fatigue years later.
Non-structural protein 1 (NS1) is a highly conserved protein among Flaviviruses, which is actively secreted by infected cells and detected in the serum between days 3 and 8 post-infection, peaking on day 5, the day prior to the onset of clinical disease. Extracellular forms of NS1 are implicated in immune modulation and in promoting endothelial dysfunction at blood-tissue barriers, facilitating WNV dissemination to the brain and affecting disease outcomes.

Aim: Focusing on the recently discovered antimicrobial roles of amyloid beta (Bortolotti et al., 2019), we connected WNV late pathology to overlapping features encountered in neurodegenerative diseases such as Alzheimer's disease. We aimed to investigate the possible effect of soluble NS1 on neurodegenerative and dysfunctional biomarkers (e.g. amyloid beta (Aβ), total and phosphorylated tau protein (p-tau), alpha-synuclein (α-syn), transactive response (TAR) DNA-binding protein 43 (TDP-43), prion protein (PrPSc), neurofilament light chains (NFL)) expression in neuronal cells (neurons, oligodendrocytes, and microglia), to clarify the mechanism underlying the CNS sequelae associated to WNV infection.

Methods: 2D cultures and 3D neuronal model were obtained with iPS (Induced Pluripotent Stem) cells and treated with purified WNV NS1. The mRNA and proteomic profiles were evaluated.

Results: We observed the ability of soluble NS1 to affect the expression of neurodegenerative and dysfunctional biomarkers. In particular, NS1 induced Aβ altered expression via TLR3, an endosomal Pathogen Pattern Receptors (PPRs) involved in RNA viruses sensing (Wang T. et al., 2004).

Conclusion: Our preliminary results suggest a possible role of soluble NS1 on CNS damage associated to WNV infection. Interestingly, TLR3 increased expression has been found associated to Aβ plaque in AD brains (Walker et al., 2018) and Aβ itself stimulates TLRs expression, prompting the neurodegeneration (Caldeira et al, 2017). NS1 released by WNV infected cells might participate in CNS neurodegenerative process by altering TLR3 signaling and Aβ expression, suggesting a novel pathogenetic role.

PO 215 - OC 46

ANTIBODIES INDUCED BY SMALLPOX VACCINATION AFTER AT LEAST 45 YEARS CROSSREACT WITH AND *IN VITRO* NEUTRALIZE MONKEYPOX VIRUS

R. Nisini[1], G. Venturi[1], M.V. Chiantore[1], P. Di Bonito[1], R. Teloni[1], A. Amendola[1], C. Fortuna[1], G. Marsili[1], R. De Santis[2], G. Grilli[2], M.S. Lia[2], A. Iacobino[1], S.T. Kiros[3], F. Lagi[4], A. Bartoloni[3,4], R. Biselli[5], F. Lista[2], S. Mariotti[1]

[1] *Dipartimento di Malattie Infettive, Istituto Superiore di Sanità, Rome, Italy*
[2] *Dipartimento Scientifico, Centro Medico dell'Esercito Italiano, Rome, Italy*
[3] *Unità di Malattie Infettive e Tropicali, Ospedale Careggi, Florence, Italy*
[4] *Dipartimento di Medicina Clinica e Sperimentale, Università di Florence, Italy*
[5] *Ispettorato Generale Sanità Militare, Rome, Italy*

Aim: To set up methods for quantifying of the antibody response to smallpox-vaccination and to evaluate the humoral immune response to monkeypox virus (MpoxV) after infection. To evaluate whether antibodies specific for vaccinia virus (VV) are still detectable after at least 45 years from immunization. To confirm that VV-specific antibodies are endowed with the capacity to neutralize MpoxV *in vitro*.

Methods: Tested sera were collected from the donors that gave their informed consent to participate in the study: 49 smallpox-vaccinated individuals (age range 46-64 years), 8 non-vaccinated donors (age range 32-44 years), and 8 convalescent individuals after MpoxV infection (7 non-smallpox vaccinated with an age range of 25-43, and one, 53 years old, probably vaccinated). VV and MpoxV were grown in Vero cells. Supernatant of infected cells containing the viruses were inactivated and used as coating antigens in ELISA or for Western Blot (WB) analyses. ELISA results were expressed as natural logarithm (LN) of the reciprocal of the last positive dilution of sera. A MpoxV plaque reduction neutralization test (PRNT) was optimized and performed on study samples. Statistical analyses were performed using Stat view Statistical Software.

Results: The measure of IgG anti-VV or MpoxV by ELISA test resulted tightly correlated (R=0.8098). A correlation (R=0.4952; p<0,0001) between the result of ELISA and the neutralization test was shown. None of the smallpox non-vaccinated donors resulted positive in ELISA or WB analysis and their sera were unable to neutralize MpoxV in *vitro*. Sera from all the convalescent individuals from MpoxV infection resulted positive for anti-VV or MpoxV IgG with titres ranging from 1:640 (LN=6.461) to 1:5120 (LN=8.541) and showed MpoxV *in vitro* neutralization capacity with PRNT50 ranging from 1:40 (LN=3.689) to 1: 320 (LN=5.768). ELISA performed on sera from smallpox-vaccinated individuals showed that 5 donors were negative for IgG anti-VV and anti-MpoxV and 7 showed low titres ranging from 1:10 (LN=2.303) to 1:80 (LN=4.382), while the other donors showed high titres against both antigens. WB analyses showed that positive sera from vaccinated or convalescent individuals recognized the same antigens in VV or MpoxV supernatants.

Conclusion: ELISA and WB performed using supernatant of cells infected with VV or MpoxV are suitable to identify individuals vaccinated against smallpox at more than 45 years from immunization and convalescent individuals from recent MpoxV infection. ELISA and WB results show a good correlation with *in vitro* neutralization test. Data confirm that smallpox vaccination induces a long-lasting memory in terms of specific IgG and that antibodies raised against VV may neutralize MpoxV *in vitro*. Finally, results confirm previous data from the literature indicating that smallpox-vaccination confer protection against MpoxV.

PO 216

PMN-MDSC FROM SEVERE COVID-19 PATIENTS PRODUCE EXTRACELLULAR TRAPS AND CONTRIBUTE TO ENDOTHELIAL CELL DYSFUNCTION

G. Grassi[1], S. Gili[1], S. Notari[1], R. Casetti[1], Z. Percari[3], E. Affabris[3], V. Bordoni[4], E. Cimini[1], D. Rubino[2], F. Nonini[2], L. Marchion[2], C. Agrati[4], A. Sacchi[3]

[1] *Cellular Immunology and Pharmacology Laboratory, National Institute for Infectious Diseases "Lazzaro Spallanzani", IRCCS, Rome, Italy*
[2] *Clinical Division, National Institute for Infectious Diseases Lazzaro Spallanzani, IRCCS, Rome, Italy*
[3] *Molecular Virology and Antimicrobial Immunity Laboratory, Department of Science, Roma Tre University, Rome, Italy*
[4] *Oncoematologia e Officina Farmaceutica, IRCCS, Bambino Gesù Children's Hospital, Rome, Italy*

Background and Aim: The immunological mechanisms underlying the clinical presentation of SARS-CoV-2 infection and those influencing the disease outcome remain to be defined. Polymorphonuclear Myeloid-derived suppressor cells (PMN-MDSC) have been described to be highly increased during COVID-19, they are involved in the inhibition of SARS-CoV-2 specific T-cell response, platelet activation, and have been proposed as an early marker of COVID-19 fatal outcome. In this work, we evaluated the role of PMN-MDSC from COVID-19 patients in the formation of extracellular traps (ET).

Methods: COVID-19 patients were enrolled at the National Institute for Infectious Diseases (INMI) "Lazzaro Spallanzani" (Rome, Italy) between September 2021 and December 2022. Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation and the frequency of MDSC was analyzed by flow-cytometry. PMN-MDSC were purified by immunomagnetic sorting. Suppression capacity of PMN-MDSC was evaluated by T cell proliferation assay by flow-cytometry. Platelets depleted and platelets rich plasma (PL and PRP respectively) samples were obtained from peripheral blood. ET formation was evaluated by confocal microscopy by using Syto Green dye and quantified by pico488 fluorescence. PMN-MDSC-induced primary endothelial cell apoptosis was evaluated by flow-cytometry by annexin V/PI staining.

Results: Confocal microscopy experiments showed that PRP from COVID-19 patients were able to induce ET formation by purified PMN-MDSC. The extrusion of DNA fibers was confirmed by treating with DNase. Of note, PMN-MDSC from some patients were able to spontaneously produce ET. The quantification of ET formation indicated a significant extrusion of ET upon PRP stimulation (median 59.3 pg/ml *vs* 110.9 pg/ml p=0.03). Differently, PRP from healthy donors was not able to induce ET. We wondered whether platelets play a role in inducing ET by PMN-MDSC. To this aim, we treated PMN-MDSC from COVID-19 patients with PL and found a decrease of ET release compared with PRP that did not reach statistical significance, suggesting that also other factors are involved in ET extrusion. PMN-MDSC treatment with SARSCoV-2 Spike protein induced ET by PMN-MDSC (median ctr 140 pg/ml *vs* spike 400pg/ml, p=0.06), indicating that the virus itself can contribute to ET formation. It has been demonstrated that neutrophils extracellular traps (NET) can induce endothelial cells apoptosis. We found that PMN-MDSC induced endothelial cell apoptosis (10.4% *vs* 33.4%, p=0.009). PMN-MDSC stimulation with PRP or spike protein had not impact on the apoptosis of endothelial cells, suggesting that PMN-MDSC can induce endothelial cell apoptosis in an ET-independent manner. Further, we confirm that the phenotypically identified PMN-MDSC had suppressive capacity, indeed, they were able to inhibit T cell proliferation.

Conclusions: We demonstrated that PMN-MDSC can extrude ET and contribute to endothelial cell apoptosis/permeability. These data highlight a new role of PMN-MDSC that, together with neutrophils and platelets, might participate to thrombotic events during SARSCoV-2 infection.

PO 217

DEVELOPMENT OF ORAL IgA RESPONSE AGAINST SARS-CoV-2 FOLLOWING COVID-19 VACCINATION

M. D'Accolti[1], I. Soffritti[1], F. Bini[1], E. Mazziga[1], F. Nicoli[2], R. Gavioli[2], A. Caputo[2], E. Caselli[1]

[1] *Department of Chemical, Pharmaceutical and Agricultural Sciences, Section of Microbiology, CIAS research Center and LTTA, University of Ferrara, Italy*

[2] *Department of Chemical, Pharmaceutical and Agricultural Sciences, Laboratory of Biochemistry, Immunology and Microbiology (BIM), University of Ferrara, Italy*

Aim of the Study: Local immune response at oral level has been suggested to have an important role in the early control of SARS-CoV-2 infection at the primary site of infection and viral replication, as local secretory IgA (sIgA) were reported to inversely correlate with patients' symptom severity and oral inflammation (1). Moreover, sIgA were also detected in the conjunctival fluid of COVID-19 patients, and SARS-CoV-2 vaccines were shown to elicit specific sIgA response at ocular level (2, 3). Despite the protective role of mucosal immunity, only few studies report data on the development of oral mucosal response following SARS-CoV-2 vaccination. Thus, the aim of this study was to characterize sIgA mucosal immunity in saliva samples collected from vaccinated subjects.

Methods: A total of 112 subjects vaccinated with booster dose were recruited at the University Hospital of Ferrara (Italy) and the presence of anti-SARS-CoV-2 oral sIgA was evaluated in their saliva by quantitative ELISA assay targeting human IgA directed against the receptor-binding domain of the viral spike protein S1-RBD (RayBiotech Life, Georgia, United States).

Results and Conclusions: Results showed that 94.6% of vaccinated developed sIgA upon vaccination. No statistically significant differences were observed in sub-groups based on the combination of vaccines received, Comirnaty (Pfizer-BioNTech), Spikevax (Moderna), and Vaxzevria vaccine (AstraZeneca). However, higher sIgA response was observed in subjects receiving at least one dose of Moderna vaccine, compared to those receiving only combinations of Pfizer and AstraZeneca vaccines (mean IgA titer 555.9 U/ml vs. 326.4 U/ml, respectively), confirming previous results obtained at the eye mucosa. Monitoring the development of mucosal response in the oral cavity may drive forward vaccination design and surveillance strategies, potentially leading to novel therapeutic approaches and new routes of vaccine administration and boosting.

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PO 218

EVALUATION OF ANTI-SARS-CoV-2 IgA RESPONSE IN TEARS OF VACCINATED COVID-SUBJECTS

E. Mazziga[1], M. D'Accolti[1], I. Soffritti[1], M. Maritati[2], F. Bini[1], C. Contini[2], E. Caselli[1]

[1] *Department of Chemical, Pharmaceutical, and Agricultural Sciences, and LTTA, Section of Microbiology, University of Ferrara, Italy*

[2] *Infectious Diseases Unit, Department of Medical Sciences, University of Ferrara, Italy*

Aim of the Study: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), responsible for the COVID-19 pandemic, is primarily spread by airborne transmission, and several evidence highlights the role of the conjunctiva in virus entry and transmission (1). Besides findings regarding the viral presence and replication in the eye (2), relatively little is known about the acquisition and development of mucosal immunity specifically directed against SARS-CoV-2 at the ocular surface. Secretory IgA (sIgA), which may play an important role in the early defence against SARS-CoV-2 infection, were detected in the eye of COVID-19 patients (3). Since there were no available results regarding the state of the local mucosal response at the ocular level in vaccinated individuals who subsequently developed COVID-19, the aim of this study was to analyze the presence and amount of sIgA in the conjunctival fluid of COVID-19 patients by comparing SARS-CoV-2 vaccinated and non- vaccinated subjects.

Methods: An observational study was conducted at the COVID Unit of the University Hospital of Ferrara (Italy). Tear samples from 77 COVID-19 patients, including 63 vaccinated and 14 non-vaccinated subjects, were collected and the evaluation of anti-SARS-CoV-2 IgA levels was performed by the quantitative ELISA assay RayBio COVID-19 S1RBD protein Human IgA ELISA Kit.

Results and Conclusions: Unless the groups showed superimposable epidemiological features, differences were observed in the percentage of asymptomatic/pauci-symptomatic subjects in the vaccinated vs. non-vaccinated cohort, as expected (46% and 29% of the total, respectively). Both frequency and titer of ocular sIgA were remarkably different in vaccinate vs non-vaccinated group (69.8% vs. 57.1%; 1372.3 U/mL vs. 143.7 U/mL, respectively; p=0.01). sIgA titer significantly depended on the type of administered vaccine, evidencing important differences in the vaccines' ability of eliciting a sIgA response in the eye. Data suggest that quantitative tear-based sIgA tests may potentially serve as a rapid and easily accessible biomarker for the assessment of the development of a protective mucosal immunity toward SARS-CoV-2.

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PO 219

PERFORMANCE OF WHOLE BLOOD ASSAY FOR THE QUANTIFICATION OF T-CD4-MEDIATED CMV-SPECIFIC RESPONSE IN HEALTHY SUBJECTS AND TRANSPLANTED PATIENTS

F. Bergami[1], F. Zavaglio[1], G. Comolli[1], D. Lillei[1], I. Cassaniti[1], F. Baldanti[1,2]

[1] Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
[2] Department of Clinical, Surgical, Diagnostics and Pediatric Sciences, University of Pavia, Italy

Aim: Human Citomegalovirus (HCMV) is one of the most common viral infections in transplanted patients. HCMV specific T-cell response plays an important role during infections and the role of $CD4^+IFNg^+$ have been demonstrate. The large majority of WB assay for CMV T-cell response are mainly restricted to CD8 T-cell response. Thus, the aim of our study was to set-up a "simple in house" method for the quantification of pp65-specific $CD4^+IFNg^+$ T-cell response using whole blood sample.

Methods: Whole blood and peripheral blood mononuclear cells (PBMC) were collected from 35 subjects (21immunocompetent and 14 immunocompromised). Patients were screened for CMV IgG. PBMC were isolated from heparin-treated blood by standard density gradient centrifugation and used for ELISpot assay (netspots/million PBMC) against pp65 peptide pools (pp65_pep) and pp65 recombinant protein (pp65_rec). "In house" whole blood interferon-gamma release assay (HM-WB IGRA; pg/mL) against the same antigens was performed. As a gold standard, flocytometry (%$CD4^+IFNg^+$ and %$CD8^+IFNg^+$) was used.

Results and Conclusions: The cut-off for positive response in Elispot and HM-WB IGRA was calculated as mean of pp65_ pep and pp65_rec – specific T cell response of seronegative controls plus two standard deviations. Values higher than 10 and 8 netspots/million respectively, were considered positive for ELIspot assay. Similarly in HM-WB IGRA, 0.32 and 5 pg/ml for pp65 peptide pools and recombinant protein respectively were given as cut-off.
35 subjects were tested in ELISpot assay and flow cytometry (%$CD4^+IFNg^+$ and %$CD8^+IFNg^+$). As regards pp65_pep, a good correlation between $CD4^+IFNg^+$ was detected ($r=0.7937$, $p<0.001$), but the best correlation was detected between $CD4^+IFNg$ and pp65_rec ($r=0.8263$, $p<0.0001$). In details, 15/35 subjects were positive in ELISpot and $CD4^+IFNg^+$, and 9/35 were negative for both assays. No subjects results positive for $CD4^+IFNg^+$ and negative in ELISpot. Finally, 11/35 results positive in ELISpot but negative for $CD4^+IFNg^+$.No correlation between pp65 recombinant protein and $CD8^+IFNg^+$ were detected ($r=0.4246$, $p=0.0110$).
As regards whole blood stimulation 34 subjects were tested and a good correlation between $CD4^+IFNg^+$ and pp65_rec were detected ($r=0.7051$, $p<0.0001$), but no correlation were detected with pp65_pep ($r=0.5549$, $p=0.007$). Finally, no correlation between $CD8^+IFNg^+$ and pp65 peptide pool ($r=0.4571$ $p=0.0066$) or pp65 recombinant protein ($r=0.3446$, $p=0.0459$) were detected.
In conclusion, a good correlation between our "in house" HM-WB IGRA and %$CD4^+IFNg^+$ using the recombinant protein was detected, suggesting that it could be a good method for evaluating $CD4^+IFNg^+$response. Our result must be confirmed on other subjects, both healthy and transplanted patients.

PO 220

EVALUATION OF HUMORAL CROSS-IMMUNITY TO MPOX VIRUS (MPXV) DUE TO SMALLPOX HISTORIC VACCINATION

G. Matusali, F. Colavita, A. Bettini, S. Meschi, D. Lapa, M. Francalancia, L. Bordi, V. Mazzotta, K. Mizzoni, A. Beccacece, S. Coen, E. Nicastri, A. Antinori, E. Girardi, F. Vaia, V. Puro, F. Maggi
National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Rome, Italy

The success of the smallpox vaccination campaign resided on the variola virus's biological properties, such as the lack of an animal reservoir and re-infection events, and on the availability of an effective vaccinia virus-based vaccine that led to cross-protective immunity. Clinical experience and animal model studies have shown that humoral immunity plays a critical role in protecting against variola and other orthopox viruses, including the Mpox virus (MPXV). When the MPXV began spreading globally last spring, causing the 2022 multi-country outbreak, it became critical to determine whether previous smallpox vaccination conferred MPXV cross-protection.
Using a viral stock derived from a skin lesion of an MPXV-infected patient hospitalized during the 2022 outbreak, we set up immunofluorescence (IF) and plaque reduction neutralization (PRNT50) assays to investigate for the presence of anti-MPXV IgG and neutralizing antibodies (nAbs) in serum samples from 67 smallpox-vaccinated (born before 1974) and 29 unvaccinated (born after 1979) individuals.
In the smallpox vaccinated cohort, anti-MPXV IgG was found in 89.6% of individuals, and 70.1% displayed nAbs. The geometric mean titre was 75.2 (95% CI 56.7-99.7) for IgG and 17.5 (95% CI 13.4-22.8) for nAbs. In the non-vaccinated cohort, IgG level**s** were always below the detection limit, while one individual showed weak levels of nAbs (1:10). In the vaccinees, no significant correlation was observed between age and either anti-MPXV IgG or nAbs. To further investigate the effect of time from vaccination, we analyzed the MPXV antibody titres in three groups of vaccinees: those born from 1950 to 1957 (n=22); 1958 to 1965 (n 21); and 1966 to 1973 (n=24). Even if higher antibody levels and positivity rates were observed in individuals born between 1966 and 1973, no statistically significant differences were obtained when comparing the three groups for either anti-MPXV IgG or nAbs.
Our findings show that humoral cross-immunity against MPXV exists in a high percentage of smallpox vaccinated subjects and that humoral immunity is maintained for more than 60 years after vaccination. Together with information related to the immunity conferred by natural infection and the administration of the currently available licensed vaccines, this study will contribute to the understanding of antibody-driven protection against MPXV, an emerging virus with epidemic and pandemic potential.

PO 221

NEUTRALIZING ANTIBODIES RESPONSE TO NOVEL SARS-CoV-2 OMICRON SUBLINEAGES IN LONG-TERM CARE FACILITY RESIDENTS AFTER THE FOURTH DOSE OF MONOVALENT BNT162b2 COVID-19 VACCINATION

I. Varasi[1], A. Lai[2], A. Bergna[2], L. Fiaschi[1], A. Gatti[3], B. Caimi[3], C. Balotta[3], C. Biba[1], A. Riva[2], G. Zehender[2], M. Zazzi[1], I. Vicenti[1]

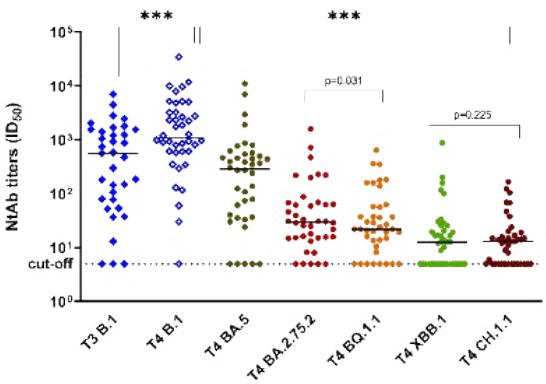
[1] *Department of Medical Biotechnologies, University of Siena, Italy*
[2] *Department of Biomedical and Clinical Sciences, University of Milan, Italy*
[3] *Azienda di Servizi alla Persona, Istituto Milanese Martinit e Stelline e Pio Albergo Trivulzio, Milan, Italy*

Background: The dynamics of SARS-CoV-2 variants has transitioned from long-term dominance of a successful variant to a dynamic swarm of genetically related sublineages, carrying convergent aminoacidic mutations in the spike region. Aim of this work was to quantify the neutralizing antibodies (NtAb) titre against the recently predominant omicron sublineages BA.2.75.2, BQ.1.1, XBB.1 and CH.1.1 in a fragile population naïve for SARS-CoV-2 infection and vaccinated with four doses of monovalent BNT162b2 COVID-19 mRNA vaccine.

Materials and Methods: Plasma samples were collected from 40 residents at Pio Albergo Trivulzio, the largest Italian long-term care facility, 71 [68-75] and 89 [80-91] median [IQR] days after the third (T3) and fourth (T4) BNT162b2 vaccine dose. The study group (91 [84-94] years, 5 males) had negative anti-nucleocapsid serology at first vaccine dose and was weekly screened by swab analysis to exclude subsequently SARS-CoV-2 infection. NtAb titers were measured at T3 and T4 against the wild type strain B.1 and at T4 against five Omicron sublineages (BA.5/BQ.1.1/BA.2.75.2/XBB.1/CH.1.1). Live virus microneutralization was performed in VERO E6

Figure 1

Neutralizing antibodies (NtAbs) titers to SARS-CoV-2 variants: B.1, BA.5, BA.2.75.2, BQ.1.1, XBB.1 and CH.1.1 in 40 fragile long-term care facility residents after third (T3) and fourth (T4) BNT162b2 COVID-19 mRNA monovalent vaccine dose. Antibodies with ID_{50} titers < 10 were defined as negative and scored as 5 for statistical analysis. Asterisks indicate significance levels: *** correspond to p<0.001



VIRUS	T3	T4
B.1	518 (78-1515)	1093 (612-3252)
BA.5	NP	287 (38-533)
BA.2.75.2	NP	30 (15-67)
BQ.1.1	NP	22 (11-58)
XBB.1	NP	13 (5-24)
CH.1.1	NP	13 (5-19)

NP: Not performed

cells quantifying the cell viability by luminescence. The NtAb titer was defined as the reciprocal value of the sample dilution showing 50% protection of virus-induced cytopathic effect (ID_{50}). SARS-CoV-2 IgG II Quant assay (Abbott) was used to quantify the anti-spike protein Ab at T3, T4 and after the first and the second vaccine dose (T1, T2).

Results: Prevalent comorbidities were dementia (59%), diabetes (31%), history of ictus (26%) or ischemic heart disease (23%). Thirty-four and 13 patients had at least 1 and 3 comorbidities respectively, and polypharmacy was common. NtAb titers to B.1 variant significantly increased ($p < 0.001$) at T4 (1094 [612-3252] ID_{50}) with respect to T3 (518 [60-1515] ID_{50}). A significant increase was also observed when comparing the anti-spike Ab median titers at T3 and T4 (9939 [4168-12,500] vs. 12,500 [6413-12,500], $p<0.001$). One patient never responded to the full vaccination cycle showing negative anti-spike Ab and NtAb titers at each time point analyzed. Overall, at T4 median NtAb titers to the B.1 strain correlated with those to each omicron variant ($p<0.001$ for all comparisons) but absolute values expressed as ID_{50} were significantly lower (B.1>BA.5>BA.2.75.2>BQ.1.1>XBB.1=CH.1.1; Figure 1). At each time point analyzed the anti-spike Ab and NtAb titers against B.1 and different variants, were not correlated to the different comorbidities when evaluated individually or stratified for increasing number ($p>0.05$).

Conclusions: In this elderly fragile population, circulating omicron sublineages BQ.1.1, BA.2.75.2, XBB.1 and CH.1.1 showed greater escape from monovalent BNT162b2 COVID-19 mRNA vaccine than the previously dominant BA.5 variant. It remains to be established whether the reduced NtAb titers still protect from incident infection with these and future variants.

PO 222

ADENOVIRAL DELIVERY OF CIITA REDUCES TUMOR GROWTH IN ESTABLISHED MURINE TS/A ADENOCARCINOMA

G. Forlani[1], F. Celesti[1], M. Shallak[1], A. Gatta[1], I. Salvato[2,3], A. Marchini[2], R. Accolla[1]

[1] *Laboratories of General Pathology and Immunology "Giovanna Tosi", Department of Medicine and Surgery, University of Insubria, Varese, Italy*

[2] *Laboratory of Oncolytic-Virus-Immuno-Therapeutics (LOVIT), Department of Cancer Research, Luxembourg Institute of Health (LIH), Luxembourg*

[3] *NORLUX Neuro-Oncology Laboratory, Department of Cancer Research, Luxembourg Institute of Health (LIH), Luxembourg*

Introduction: Immunotherapy has been shown to have a significantly impact on the fight against cancer, particularly by the use of immune checkpoint inhibitors and, to lesser extent, terapeutic vaccines. Both approaches, however, have limitations. In particular, vaccination strategies using MHC class I-bound tumor-specific peptides have encountered critical difficulties due to the limited effect of these vaccines in stimulating and maintaining MHC class-I restricted tumor specific CD8+ effector cells (CTL). Our vaccination approach is based on the triggering of tumor-specific MHC class II (MHC-II)-restricted CD4+ T helper cells, as these cells, hierarchically, are fundamental to both initiate all adaptive immune responses and maintain the proliferation and cytolytic activity of CTL, the terminal effectors of anti-tumor immunity. We have previously demonstrated that CIITA-induced MHC-II expression in tumor cells, promoted CD4+ T helper protective immune response against tumors of different histotype. In the present study, we investigated the possibility to use adenovirus vectors (AdVs) to deliver CIITA into established tumors.

Methods: AdVs containing wild type CIITA (Ad-CIITA) were generated using a replication-defective serotype5 adenoviral backbone. AdVs containing a mutated, non-functional version of CIITA (Ad-CIITA mut) was used as a control. AdV-mediated MHC-II expression was monitored at cell surface level. Murine mammary adenocarcinoma TS/A cells infected with Ad-CIITA or Ad-CIITA mut were injected sub-cutaneously into syngeneic BALB/c mice and the tumor growth was monitored over time. Ad-CIITA or Ad-RFP control vectors were intratumorally injected in TS/A established tumors and the tumor growth was monitored over time.

Results: Ad-CIITA infection induced the expression of MHC-II molecules on the cell surface of TS/A tumor cells up to day 10. TS/A cells infected with Ad-CIITA vector were significantly retarded in their *in vivo* growth with respect to TS/A cell infected with Ad-CIITAmut or their parental tumor counterpart (TS/A-pc). To assess the feasibility of Ad-CIITA as therapeutic agent, mice bearing TS/A tumors were injected intratumorally with Ad-CIITA or Ad-RFP control vectors. Intratumoral injection of Ad-CIITA significantly reduced tumorigenicity and growth of established tumors with respect to intratumor injection of control Ad-RFP.

Conclusion: Adenoviral vectors expressing CIITA induce a specific antitumoral immune response and may represent an effective therapeutic approach to treat poorly immunogenic tumors.

PO 223

MOLECULAR MAPPING OF ANTIGENIC DETERMINANTS OF RHDV2

V. Di Giovanni[1], G. Pezzoni[1], R. Benevenia[1], A. Lavazza[1], L. Capucci[1], P. Cavadini[1]

Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy

Introduction and Aim of the Study: Rabbit Haemorrhagic Disease (RHD) is a severe and fatal hepatitis caused by a very virulent RNA virus of the genus *Lagovirus* emerged in China for the first time in the 80s, reaching Europe in 1986. This disease affects European rabbit (*Oryctolagus cuniculus*), with very high level of morbidity (100%) and mortality (80-95%). In 2010 a new RHDV-related virus, called RHDV2, rapidly spread worldwide becoming prevalent and affecting also other lagomorph species and young rabbits (few weeks old), its success was due to a specific antigenic profile that eluded the heard immunity previously generated by RHDV.
Since it is an RNA virus, mutations and recombinant events underlie its evolution but the relation between mutants and host it's difficult to prove because lagovirus do not replicate *in vitro*.
To map the major antigenic determinants on the viral surface, in a previous study, we used a typing ELISA test based on a panel of 22 RHDV2-specific MAbs to characterize more than 300 Italian isolates collected from 2011 to date. Thereafter, by comparing the reactivity of single MAbs with the VP60 aminoacidic sequence of the isolates, we preliminary mapped 3 putative epitopes on the capsid protein, located on the external VP60 loops that constitute the immunogenic surface of the virion. Aim of this work was to confirm that the different amino acid mutations identified in the analysis of RHDV2 isolates with the MAbs panel, actually constitute part of the epitope recognized by the corresponding MAb.

Material and Methods: by site-specific mutagenesis performed on an expression plasmid containing the vp60 gene of RHDV2, we induced some mutation at aminoacidic position 299 and 347 and we expressed the mutated VP60 viral capsid via baculovirus system infecting *Spodoptera frugiperda* (Sf9) cells. The mutated virus like particles (VLPs) produced were analyzed in ELISA, using a panel of monoclonal antibodies.

Results and Discussion: with this strategy, we confirmed that the change of a single amino acid at position 347 is responsible for the loss or gain binding specificity with some monoclonal antibodies. This amino acid is located on the loop L2 of the P2 subdomain of the VP60 and exhibits high variability within RHDV2 strains.
The second epitope, more difficult to map on the basis of the available VP60 sequences, involves the amino acid at position 299 located on the beta strand of the P2 subdomain. Based on our results, the presence of lysine at position 299 (mutation found in few isolates), seems to have a negative effect on the binding with MAb 4H12, MAb which it is normally used at IZSLER lab for diagnostic purposes.
With this strategy, we confirmed that the change of a single amino acid is responsible for the loss or gain binding specificity with some MAbs. Because baculovirus expression system proved to be a good tool for expressing capsid protein, it could be used to verify other potential epitopes. Finally, to map the main antigenic determinants of RHDV2 will be an important tool for the design and production of effective vaccines against RHD.

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PO 224

EFFECTS OF TNF-a INHIBITORS ON CIRCULATING Th-17 CELLS AND VZV CELL-MEDIATED IMMUNITY IN PATIENTS AFFECTED BY SEVERE PSORIASIS

A. Saldan[1] Ph., D.C. Mengoli[1] MD, E. Sandini[2] MD, L. Barzon[1] MD, S. Piaserico[2] MD, D. Abate[1] MD, Ph.D.

[1] *Department of Molecular Medicine, University of Padua, Italy*
[2] *Department of Medicine, University of Padua, Italy*

Background: Psoriasis is an inflammatory, erythemato-squamous dermatosis affecting 2/3% of the whole human population. For long time psoriasis has been considered a Th1 cell-related disorder, but recently Th17 cell has emerged as a main determinant in the psoriatic pathogenesis. Anti-TNF-α biological agonists were recently introduced into clinical practice as a therapeutic option for psoriasis, however a major concern for the clinical usage of these drugs is the increased risk of VZV reactivation, potentially leading to life-threatening VZV encephalitis. In this study we assessed the effect of etanercept (50 mg twice per week followed by 50 mg/week) and adalimumab (80 mg the first week followed by 40 mg/week) on Th17 and VZV immunity

Methods: 48 patients were enrolled at the Padova dermatology clinic. Treatment group included patients who received etanercept or adalimumab. Control group received standard Cyclosporine, or Methotrexate, or Acitretin therapy. Th-17 immunity was determined by flow cytometry and VZV immunity by ELISPOT assays at baseline and at 3 months after treatment. Psoriasis area and severity index (PASI) was used to determine the clinical outcome. PASI90 response represents the 90% improvement from baseline PASI score.

Results: As expected the median PASI decreased sharply after the biological therapies (from 11 to 2, P=0.001) The average initial PASI was lower in females than in male, with an average difference of 5.65 (P=0.0000). The reduction of plasma Th17 cell count from baseline was significantly greater in patients who reached PASI90 than in those who did not (P<0.05 with Mann–Whitney test). The anti-TNF-a therapies did not significantly affect VZV immunity.

Conclusions: anti-TNF-α treatment has a limited impact on VZV immunity however anti-TNF-α therapies achieve a substantial decrease of Th17 cell count, suggesting a crucial role of Th-17 producing cells in psoriasis pathogenesis.

PO 225

EVALUATION OF THE IN VITRO CAPACITY OF ANTI-HCMV ANTIBODIES TO INITIATE ADCC

P. D'Angelo[1,2], E. Gabanti[1], C. Fornara[1], P. Zelini[1], F. Zavaglio[1], S. Bernuzzi[3], D. Lilleri[1], F. Baldanti[1,4]

[1] *Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy*
[2] *Department of Molecular Medicine, University of Pavia, Italy*
[3] *Department of immunohematology and transfusion medicine, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy*
[4] *Department of Clinical, Surgical, Diagnostics and Pediatric Sciences, University of Pavia, Italy*

Aim of the Study: Studying the activation of NK cells in the presence of HCMV antibodies in vitro and its role in the control of HCMV infection through ADCC.

Methods: ARPE-19 cells, infected for 120 hours with VR1814 strain at a MOI of 10, were co-cultured with PBMCs in the presence of serum collected from 23 HCMV-seropositive and 9 HCMV-seronegative donors. Moreover, 13 pregnant women with primary HCMV infection followed for 6 months after the infection, and 25 pregnant women with pre-conception immunity have been tested and compared.
Pool of seropositive HCMV sera and pool of seronegative HCMV sera have been used as positive and negative controls.
After 4 hours of co-culture, we determined the percentage of activated NK cells by analysis of CD107a expression as a marker of degranulation.

Results and Conclusion: In seropositive subjects, the median percentages of CD107a-positive NK cells in the presence of autologous serum and pooled HCMV positive serum were similar (14.03% [range 0.00-48.93] and 12.42% [range 1.01-46.00], respectively), while NK activation was negligible (1.16% [0.00 -14.75]) using an HCMV negative serum pool. In HCMV seronegative subjects, the median percentage of NK cells activation with autologous serum was 0.90% [range 0.00-3.92], in the presence of HCMV-negative sera pool was 2.07% [0.00-4.79] and with the pool of HCMV positive sera was 8.97% [0.00-26.49].
Serum of primary HCMV infection shows a low capacity of activation of the NK until 6 months after infection (median: 12.45; range 5.91-36.06). Comparison in the degranulation of NK cells between primary infection at 1, 2, 3 and 6 months post infection and HCMV-seropositive women (median: 24.07; range 9.13-56.68) showed a significant difference ($p<0.021$).
The method developed shows that the NK activation against HCMV-infected epithelial cells is dependent on the presence of HCMV-specific antibodies. This serum activity increases with time after the onset of the HCMV infection, and even at 6 months post infection it is not comparable with that observed in subjects with remote infection. The protective role of NK activation by HCMV-specific serum antibodies should be verified in clinical settings.

PO 226

EXHAUSTION AND REPROGRAMMING OF CD8+ T MEMORY CELLS IN HIV CHRONICALLY INFECTED PATIENTS

S. Scaglioni[1,2], G.M. Butta[2,3], M. Centazzo[2,3], A. Lombardi[3], G. Bozzi[3], A. Gori[3], A. Bandera[3], R. Grifantini[2], R. De Francesco[1,2], L. Manganaro[1,2]

[1] Università degli studi di Milano - Dept. of Pharmacological and Biomolecular Sciences (DiSFeB), Milan, Italy
[2] Fondazione Istituto Nazionale di Genetica Molecolare (INGM) "Romeo ed Enrica Invernizzi", Milan, Italy
[3] Policlinico di Milano Ospedale Maggiore - Fondazione IRCCS Ca' Granda - Malattie Infettive, Milan, Italy

Although antiretroviral therapy (ART) successfully suppresses HIV viral load and prevents AIDS development, it does not eradicate HIV infection and treatment is lifelong. Moreover, residual inflammation persist despite ART treatment, especially in individuals who fail to achieve a normal CD4+ T cells count during therapy, leading to immunosenescence and the early onset of age-associated diseases. In this state, the immune system exhibits a less functional and exhausted profile (Deeks SG. Annu Rev Med. 2011).

Aim of this study is to analyze the exhaustion state of CD8+ T cell subpopulations (Naïve, T stem cell memory, central memory and effector memory) in a group of ART-treated people with HIV (PWHIV) stratified according to CD4+ T cells count. As the inhibitory receptors PD-1, TIGIT and TIM-3 have been reported to be over-expressed in exhausted T cells (Chew GM et al. PLoS Pathog. 2016; Jin HT et al. Proc Natl Acad Sci USA. 2010) we used a panel of fluorophore-conjugated antibodies to quantify their expression in the CD8+ T cell subsets through flow cytometry. We observed an increased frequency of TIGIT+ PD-1+ population and a higher expression of TIM-3 in each CD8+ T cell subset isolated from PHWIV characterized by a CD4+ T cells count <500 compared to PWHIV with a higher CD4+ T cells count and HDs. We further investigated the expression of TCF-1, a main transcription factor within the WNT signaling pathway, that has been associated with the stemness and immunological memory of CD8+ T cells (Kared, H. et al. Nat Commun 11, 821 (2020); Jeannet G et al. Proc Natl Acad Sci U S A. 2010). As expected, we observed a higher expression of TCF-1 in the less differentiated CD8+ T memory subsets. In addition, we observed that all the analyzed subsets, isolated from PWHIV with low CD4+ T cells count exhibited a lower expression of TCF-1 compared to HDs, suggesting a possible impairment in longevity, self- renewal and memory profile of CD8+ T cells in PWHIV with low CD4+ T cells count. Therefore, we tried to boost CD8+ T cells stemness features in PWHIV characterized by a low CD4+ T cells count, through a treatment with TWS119, a GSK3β inhibitor that activates WNT/β-Catenin pathway and inhibits mTOR pathway. The treatment increased TCF-1 expression, especially in the more undifferentiated memory subsets. Consistently, after CD3/CD28 stimulation TWS119-treated CD8+ T cells showed a higher prevalence of CCR7+CD45RO- cells compared to untreated control, confirming a switch to a more undifferentiated state even after T cell stimulation.

In conclusion, PWHIV who failed to achieve a normal CD4+ T cells count despite ART treatment exhibit a CD8+ T memory pool skewed toward an exhausted and differentiated profile, however, novel strategies could reverse such exhausted CD8+ T cells toward a more stem-like profile. Future investigations will be aimed to determine whether reprogrammed CD8+ T cells exhibit improved functionality.

PO 227 - OC 47

EXPLORING THE NEUROPATHOGENICITY OF EMERGING STRAINS OF WEST NILE VIRUS LINEAGE 1 AND 2

A. Sinigaglia[1], M. Pacenti[2], A. Giannella[3], E. Dal Molin[1], S. Vogiatzis[1], C. Lucca[1], A. Volpe[1], S. Riccetti[1], F. Bonfante[4], I. Monne[4], A. Fusaro[4], E. Quaranta[4], C. Terregino[4], G. Capelli[4], G. Ceolotto[3], M. Trevisan[1], L. Barzon[1,2]

[1] *Department of Molecular Medicine, University of Padua, Italy*
[2] *Microbiology and Virology Unit, Padova University Hospital, Padua, Italy*
[3] *Department of Medicine, University of Padua, Italy*
[4] *Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padua, Italy*

Background: West Nile virus (WNV) is a neurotropic mosquito-borne flavivirus, which is endemic in several European countries, including Italy, where the virus is expanding its geographic range and causing an increasing number of outbreaks of neuroinvasive disease (WNND) among humans and equids.

Aim: In 2022, the largest human outbreak of WNV infection ever recorded in Italy occurred in the Veneto Region, where a newly introduced strain of WNV lineage 1 (WNV-1) co-circulated with the endemic WNV-2 strain. In this context, aim of the study was to compare the neuropathogenicity of the two WNV lineages by clinical investigations and *in vitro* infection experiments in human neurons and brain organoids.

Methods: We analyzed demographic, clinical, and virological data of human cases of WNV infection occurring in the Veneto Region in 2022 and confirmed by the Regional Reference Laboratory (RRL). Sequencing of the WNV genome was done by amplicon sequencing of viral RNA purified from biological specimens on an Illumina MiSeq platform. WNV-1 and WNV-2 strains isolated during the 2022 outbreak, as well as WNV-1 Ita09, WNV-2 AUT/08, and Usutu virus Europe 1 (USUV-E1) strains were used in *in vitro* infection experiments. The human H9-NGN2 cell line was used to generate cortical neurons grown in 2D and brain organoids. Neuronal identity was verified by IF of stage-specific markers. WNV infection and replication in neurons and organoids was evaluated by IF, qPCR and infectious virus titration. Viral modulation of gene expression in neurons was analyzed by RNA sequencing on an Illumina NextSeq550 platform.

Results: In 2022, 438 human cases of WNV infection were confirmed by the RRL, including 186 with WNND, 210 with fever (WNF), and 42 WNV NAT-positive blood donors. Viral lineage was determined in 181 cases with WNV-1 infection and 82 with WNV-2. WNV-1 infection was significantly associated with increased risk of WNND ($p<0.05$, Chi square test for trend), which presented with an unusual high incidence as acute flaccid paralysis or Guillain-Barré syndrome. Phylogenetic tree analysis demonstrated that WNV-1 genomes formed a monophyletic cluster with minimal genetic diversity, suggesting a recent introduction, while WNV-2 genomes showed geographical custering and suggested evolution from locally circulating strains. *In vitro* experiments demonstrated that both WNV-1 and WNV-2 strains efficiently infected and replicated in human cortical neurons and brain organoids *in vitro*, and up-regulated expression of genes involved in ER stress response, innate antiviral response, apoptosis induction, and neurodegeneration. At variance, USUV-E1, used as control, infected and replicated in neurons with low efficiency, in agreement with the low pathogenicity observed in humans.

Conclusions: A new WNV-1 strain associated with increased risk of neuroinvasive disease in humans emerged in the Veneto Region in 2022. Preliminary experiments characterized its replication kinetics in human cortical neurons and brain organoids and the impact on host gene expression in comparison with other epidemic strains. Monitoring WNV genetic evolution and phenotypic characterization of new variants is crucial to early detect the emergence of new strains with epidemic potential.

PO 228 - OC 48

SIGNIFICANT OCCURRENCE OF RAT HEPATITIS E IN WASTEWATER NETWORKS: SURVEILLANCE OF AN EMERGING ZOONOTIC VIRUS

A. Palombieri[1], F. Di Profio[1], V. Sarchese[1], P. Fruci[1], E. Suffredini[2], V. Martella[3], B. Di Martino[1], C. Veneri[4], G. Bonanno Ferraro[4], P. Mancini[4], G. La Rosa[4]

[1] *Department of Veterinary Sciences, University of Teramo, Italy*
[2] *Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy*
[3] *Department of Veterinary Medicine, University of Bari Aldo Moro, Valenzano, Bari, Italy*
[4] *Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy*

Aim of the Stud: Hepatitis E virus (HEV) is the most common etiological agent of viral acute hepatitis worldwide, primarily caused by strains belonging to genotypes HEV1-HEV4 of the species *Paslahepevirus balayani* (formerly classified as *Orthohepevirus A*), with HEV3 and HEV4 infections mainly acquired through zoonotic pathways. In recent years, hepeviruses highly divergent from HEV- A genotypes, commonly found in rats, and classified in the species *Rocahepevirus ratti* genotype C1 (Rat HEV: RHEV), have been also detected in patients with acute or chronic hepatitis. Up to now, 20 cases of RHEV infected humans, including 16 in Hong Kong (1), 3 in Spain (2) and 1 in Canada (3) have been reported. Overall, RHEV is currently considered an underrated cause of hepatitis infection, since it is overlooked by the diagnostic systems for hepatitis E. Wastewater surveillance is often used to gather information on the health status of the served population. In the case of RHEV, wastewater collects viruses of both human and murine origin, hence providing an exhaustive picture of the viral strains circulating in the area under observation. In this study, to collect data on the epidemiology of RHEV in Italy, a pilot study was conducted by testing urban wastewater samples collected in Abruzzo region (Italy), that is already as a hot spot area for HEV infection in humans.

Materials and Methods: A total of 158 raw sewage samples were included in the study. The specimens were collected from 18 wastewater treatment plants (WTPs) between October 2019 and March 2022 in 12 different localities in Abruzzo region, Italy, including sites from all the 4 provinces (Chieti, L'Aquila, Pescara and Teramo). Total RNA was extracted from each sample and tested by heminested RT-PCR using a pan-hepeviridae strategy.

Results and Conclusions: *Hepeviridae* RNA was found in 68/158 (43.0%) of the specimens collected and in 15 out of 18 WTPs investigated, with an overall prevalence of 65.4% (17/26) in Pescara, 53.8% (7/13) in Teramo, 43.1% (38/88) in Chieti and 19.3% (6/31) in L'Aquila. Upon sequence analysis, the strains shared 82.5-95.8% nucleotide (nt) identity to each other and displayed the highest nt identities (79.0–91.6%) to viruses belonging to the species *Rocahepevirus ratti*, genotype C1 of human and rodent origin. In the tree, the Italian RHEV strains grouped in several different genetic clusters with a clear geographic pattern. In conclusion, our results provided evidence for a wide distribution of RHEV in the geographical setting investigated and for a high genetic diversity of the circulating strains. Prospective surveys aimed to evaluate whether RHEV could represent a public health problem in local population are warranted. Furthermore, a national environmental surveillance could be useful to provide a more complete picture on the epidemiology of the RHEV strains circulating in Italy.

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PO 229 - OC 49

RESPONSE TO MONKEYPOX VIRUS: SEROLOGICAL EVALUATION OF INFECTED PATIENTS AND NAÏVE VACCIANTED HEALTHCARE WORKERS

J.C. Sammartino[1], I. Cassaniti[2], A. Ferrari[2], F. Bergami[2], F.A. Arena[2], S. Paolucci[2], F. Rovida[1,2], D. Lilleri[2], A. Piralla[2], E. Percivalle[2], F. Baldanti[1,2]

[1] *Department of Clinical, Surgical, Diagnostics and Pediatric Sciences, University of Pavia, Italy*
[2] *Cellular Virology Unit, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy*

Aim: The Monkeypox Virus (MPXV) 2022 outbreak is the first multi-country spread of MPXV outside Africa. MPXV is related to Variola virus, the causative agent of smallpox. Eradication of smallpox was achieved with a global vaccination programme that averted millions of deaths. Due to antigenic similarity, smallpox vaccination gives approximately 85% protection against MPXV. However, this vaccination was suspended following the eradication of smallpox in 1980 leading to increased human- to-human transmission with 70% of people living today unprotected. Knowledge about viral shedding kinetics and host serological responsiveness need to be improved. Here we describe the immune response to MPXV in infected patients of the ongoing epidemic in comparison with historic (Spox) and newly vaccinated healthcare workers (HCW).

Methods: Real-time PCR was performed in patients with suspected MPXV infection. The positive samples were used for MPXV isolation. Study populations: 43 MPXV patients were enrolled in the study, for 11 we had follow-up samples at 1-week (T0) and 1-month (T1) post onset; eight newly vaccinated naïve HCW (New-HCW), 23 historically vaccinated HCW (Spox-HCW) and 19 naïve negative controls were also included. VERO cells were used for the isolation, titration and serological assays. An *in-house* immunofluorescence assay was set up to evaluate the anti-MPXV specific IgM and IgG antibodies. Neutralizing antibodies (NT-Abs) against MPXV were defined by microneutralization assay. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized whole blood samples to perform ELISpot assay using and *in-house* MPXV viral lysate.

Results and Conclusions: The rate of isolation was inversely correlated with the days from symptoms' onset (r=-0.58, p:<0.0001). There is a 50% probability of positive isolation at 2 weeks post onset that drops to 20% at 3 weeks. The majority of patients were positive for IgG/NT-Abs or IgM/IgG/NT. In MPXV patients' follow-up samples, the full positive were 50% at T0, and 73% at T1. In the New-HCW the IgG and NT-Abs titres have a significant increase at 1-month post second dose (respective p: 0.0002 and 0.0073), while the IgM titres do not (p: 0.0504). Comparing MPXV patients to New-HCW serological results, we observed no difference for IgM, either after first or second dose. New-HCW had significantly lower IgG and NT-Abs titres after one dose (p:<0.0001), but NT-Abs were comparable to the patients' levels after second dose. We also assessed T-cell response in New- and Spox-HCW, but not in MPXV patients, as the amount of PBMC from the residual samples was not sufficient. T- cell response increases in all New-HCW. Cross-reactive immune response against MPXV was detectable in 60% of the Spox-HCW. We also observed that the 48% of SPOX-HCW still presented circulating IgG, but no IgM or NT-Abs. In conclusion, in the natural infection, the humoral response is stronger and faster for the majority of patients, while in the naïve subjects takes more time to develop after vaccination. A second dose is necessary to boost the immune system and produce similar levels of NT-Abs to the patients. T-cellular memory in New-HCW is comparable to the Spox-HCW. This proves that the T-cellular response is maintained for decades after vaccination and at levels comparable to the recently vaccinated naïve subjects.

PO 230

MUTATIONAL PROFILE OF FOUR MONKEYPOX ITALIAN CASES TROUGH WHOLE GENOME SEQUENCING

M. Deiana, D. Lavezzari, A. Mori, S. Accordini, A. Donini, C. Piubelli, M.R. Capobianchi, C. Castilletti

Department of Infectious-Tropical Diseases and Microbiology, IRCCS Sacro Cuore Don Calabria Hospital, Negrar di Valpolicella, Verona, Italy

In 2022, an unprecedented outbreak of human monkeypox virus (hMPXV) infection occurred across multiple countries. The first cases of hMPXV infection in Europe have been reported in mid-May 2022 and apparently, they did not have connection or link to MPXV-endemic countries. The ongoing outbreak is caused by MPXV clade IIb, lineages linked to but different from viruses transmitted within Nigeria. Genetic analysis pinpoint that APOBEC-mediated editing might be responsible for the huge number of mutations observed in hMPXV genomes. Here, we provided the analysis of four cases detected in Verona (Italy), including three imported cases and one with no travel history. Through next generation sequencing we isolated and characterized the genome those samples. Using the public available sequences, we compared our data in order to identify the mutational profile of our sample, investigate the presence of new mutation and the phylogenetic diversity of B.1 sublineages. Our analysis indicated that the known and described above mutation that characterized the sublineage B.1 and as described elsewhere, samples presents unique mutations in several genes involved in virulence, host immune evasion as well as mutation APOBEC3 derived, that, overtime, might contribute to hMPX lineages diversification. In conclusion, our studies pointed out novel hMPXV mutations that should be monitored overtime used and can be used for future studies.

PO 231

CLIMATE CHANGE AND WEST NILE VIRUS: AUTOCTONE CASE IN NORTHEN ITALY

E. Bombana[1], M. Airoldi[1], P. Fusetti[2], N. Ghidelli[2], G. Bertulezzi[2], A.P. Callegaro[3], P. Imbrogno[4], F. Locati[5]

[1] *SC Vaccinazioni e Sorveglianza Malattie Infettive, ASST Bergamo Est, Bergamo, Italy*
[2] *SC Medicina, Ospedale di Alzano Lombardo, ASST Bergamo Est, Bergamo, Italy*
[3] *SC Medicina di Laboratorio, ASST Bergamo Est, Bergamo, Italy*
[4] *Direttore Sanitario, ASST Bergamo Est, Bergamo, Italy*
[5] *Direttore Generale, ASST Bergamo Est, Bergamo, Italy*

Introduction: West Nile virus (WNV) is a mosquito-borne *Flavivirus* maintained in an enzootic cycle between *Culex* mosquitoes and birds. Human WNV infections usually follow the bite of infected mosquitos, exhibiting a distinctive seasonal trend from April to November, which mirrors the ecology of hosts and vectors, but the variability of climate conditions during the last decade have influenced the ecology of both vectors and reservoirs. Infection in humans is usually asymptomatic, but a mild influenza-like syndrome (West Nile fever, WNF) may be observed in around 20% of all cases. Less than 1% of infected subjects develop a West Nile neuro-invasive disease (WNND), encephalitis, meningitis or acute flaccid paralysis.

Case Report: At the beginning of September 2022, a 97-year-old Italian woman presented to our Institution with history of fever, cognitive status change, confusion, aphasia, lethargy and dysphagia. The patient had hypertension, breast cancer, with no history of travelling abroad. Vital signs were normal. On neurological examination, the patient was aphasic, hemiplegic lower limb, lethargic and with no sign of meningeal irritation (GCS 10). Blood tests showed neutrophilic leucocytosis (WB 12.500/mmc, neutrophiles 70%), mildly elevated PCR (9.89 mg/dl) and she tested negative for SARS-CoV-2. CT pulmonary scan indicated interstitial pneumonia. Brain CT scan was negative for haemorrhage and focal lesions. A lumbar puncture yielded clear, colourless CSF, showing pleocytosis (72 cells/mL) and high protein levels (156 mg/dL) consistent with aseptic meningitis. Microbiological cultures on blood and urine resulted negative as well as the CSF search for the genome of the most common virus and bacteria. In the view of the CT scan consistent with pneumonia and suspecting meningitis with colourless CSF, she started empirical antibiotic therapy with ceftriaxone and ampicillin. In the suspicion of WNND, blood, CSF and urine samples were sent to Virology Laboratory, Policlinico San Matteo (PV) for testing. On day 5, WNV infection was diagnosed by positive RT-PCR on serum and urine (23820 and 144.585 copies/mL, respectively), while CSF tested negative. WNV IgM serology on serum and CSF was positive, with IgG negative.
Empirical antimicrobial treatment was stopped, the patient's Glasgow Coma Score improved GCS 13, with residual mild cognitive impairment and slow improvement of hyposthenia. Inflammatory markers and all other blood parameters were normalized. On day 22, she was transferred to a rehabilitation facility. At follow-up 10 weeks after presentation, she showed residual mild motor deficit, with normal level of consciousness.

Conclusion: Climate change affects physiology, behaviour, life cycle and geographical distribution of vectors and could influence the emergence of epidemics of various arboviruses. Prevention and control of arbovirus infections requires surveillance to determine virus activity combined with health education and vector control strategies (One Health approach).
Emerging of these infectious diseases constitutes a significant public health problem, representing not only loss of health but substantial health care cost.
WNND is associated with significant morbidity and mortality in older and compromised individuals, while a high degree of suspicion is required outside the epidemic areas.

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PO 232

EVALUATION OF TWO COMMERCIAL REAL TIME PCR ASSAYS FOR DETECTION OF MONKEYPOX VIRUS IN DIFFERENT CLINICAL SAMPLES

A. Mori[1], E. Pomari[1], S. Accordini[1], A. Donini[1], D. Treggiari[1], F. Perandin[1], M. Cordioli[2], E. Tacconelli[2], C. Castilletti[1]

[1] *Dept. of Infectious, Tropical Diseases and Microbiology, IRCCS Sacro Cuore Don Calabria Hospital, Verona, Italy*

[2] *Division of Infectious Diseases, Dept. of Diagnostic and Public Health, University of Verona, Italy*

Aim: The 2022 outbreak of human monkeypox in multiple non-endemic countries was caused by lineage B.1 Monkeypox virus (MPXV), belonging to Clade IIb. MPXV is a double-stranded DNA virus included into the *Poxviridae* family (*Orthopoxvirus* genus) that causes a diseases with symptoms similar, but less severe, to smallpox. To face to outbreak, increasing the capacity of laboratory molecular diagnostics have become fundamental and different commercial kits were developed and released to the market as for research use only (RUO). The aim of our study was to evaluate the performance of two RUO commercial assays (kit A: Bioperfectus Technologies, China; and kit B: Clonit, Italy) for the detection of MPXV in clinical samples from different body sites.

Methods: A total of 114 clinical samples, collected at diagnosis or during follow up, was tested: 92 positive (skin lesion n=35, pharyngeal swab n=16, rectal swab=19, whole blood n=22) and 22 negative (n=5 for skin lesion, pharyngeal swab and rectal swab, and n=7 whole blood) at the routine molecular diagnostics "RealStar Orthopoxvirus PCR Kit 1.0" (Altona diagnostics GmbH, Germany) (followed by confirmation with Sanger sequencing for *crmB*.

Results: The performance evaluation of the two assays in comparison to the routine molecular diagnostics showed a specificity of 100% for all biological matrices, while a sensitivity (kit A vs kit.B) of: 81.25% (95% CI: 53.35%-95.95%) vs 100% (95% CI: 79.41%-100%) for pharyngeal swab, 84.21% (95% CI: 60.42%-96.62%) vs 89.47% (95% CI: 66.86%-98.70%) for rectal swab, and 54.55% (95% CI: 32.21%-75.61%) vs 77.27% (54.63%-92.18%) for whole blood. About skin lesion, both the kits reported the same sensitivity (94.29; 95% CI: 80.84%-99.30%).

Conclusions: WHO guidelines on laboratory diagnosis of MPXV infection indicate nucleic acid amplification testing (NAAT), as the gold standard test. Since most of the commercial kits for the molecular testing are for research use only (RUO), it is essential to evaluate their clinical performance in order to identify the most accurate kits for really good monkeypox diagnosis. This evaluation performed on our clinical samples from different biological matrices showed a better sensitivity of kit A compared to kit.B, especially for whole blood samples. While on skin lesion both assays showed the same sensitivity. In conclusion the two kits were suitable to identify MPXV DNA for diagnosis using properly sampled skin lesions.

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OCCURRENCE OF PROTOPARVOVIRUSES IN BIVALVE MOLLUSKS: A PRELIMINARY STUDY

A. Mancusi[2], T. Vicenza[1], O. Dimaro[2], A. Boni[1], E. Suffredini[1], Y.T.R. Proroga[2]

[1] *Department of Food Microbiology, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Naples, Italy*
[2] *Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, National Reference Laboratory for Foodborne Viruses, Rome, Italy*

Protoparviruses are small, non-enveloped, ssDNA viruses within the family Parvoviridae. The constant progress in metagenomic sequencing has led, in the last decade, to the discovery of three members of the Protoparvovirus genus infecting humans:

1) bufavirus (BuV), first detected in 2012 in the diarrhoeic faeces of children from Burkina Faso;
2) tusavirus (TuV), discovered in 2014 in the faeces of a Tunisian child with diarrhea of unknown origin;
3) cutavirus (CuV), reported in 2016 in faecal and cutaneous specimens and since then associated with cutaneous T-cell lymphoma.

Studies on the occurrence of these protoparvoviruses has been undertaken in some EU countries, but data on their circulation in the Italian population are scarce. Bivalve mollusks are filter feeder animals which may accumulate to significant levels microorganisms present in their seawater growing areas. Due to this, they are often used for indirect monitoring of environmental contaminants and/or of microorganisms originating by the discharge of wastewater into the sea.

Aim of this study was a preliminary investigation of the occurrence of protoparvoviruses in bivalve mollusks, as a marker of their circulation in the population.

A total of 43 bivalve bivalve shellfish samples (mussels, *Mytilus galloprovincialis*), collected between January and April 2022 in 15 production areas of the Region of Campania, were tested. Samples were prepared by dissection and lysis of the digestive tissue, according to ISO 15216. Detection of BuV, TuV and CuV was performed by real-time PCR using previously described primers/probes (Väisänen et al., 2014; Väisänen et al., 2019). The three protoparvoviruses were detected in 4 samples (9%). In detail, BuV and CuV occurred in 2 samples, respectively, while TuV was detect in one of the samples already positive for BuV. Positive samples were all characterized by high *Cq* values (>38) and had been all collected between the end of February and first part of March. Interestingly, both CuV-positive samples had been taken in concecutive samplings from the same shellfish growing area, indicating either a steady or a recurring contamination. Further and more extensive studies are required to assess the circulation of these parvoviruses at national level.

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ANTIBODY RESPONSE AGAINST SIX HUMAN CORONAVIRUSES AND SARS-CoV-2 IN CHILDREN AND ADULTS

A.L. Tornesello[1], C. Botti[2], A. Micillo[2], F. Labonia[3], S. Arpino[3], S. Meola[3], M.A. Isgrò[3], L. Russo[3], E. Cavalcanti[3], S. Sale[4], C. Nicastro[4], L. Atripaldi[4], N. Starita[1], A. Cerasuolo[1], U. Reimer[5], P. Holenya[5], L. Buonaguro[6], F.M. Buonaguro[1], M.L. Tornesello[1]

[1] Molecular Biology and Viral Oncology Unit, Istituto Nazionale Tumori IRCCS "Fondazione G. Pascale", Naples, Italy
[2] Clinical Pathology Unit, AORN Santobono-Pausilipon, Naples, Italy
[3] Laboratory Medicine Unit, Istituto Nazionale Tumori IRCCS "Fondazione G. Pascale", Naples, Italy
[4] Clinical Pathology Unit, AORN Ospedale dei Colli P.O. Monaldi, Naples, Italy
[5] JPT Peptide Technologies GmbH, Berlin, Germany
[6] Innovative Immunological Models Unit, Istituto Nazionale Tumori IRCCS "Fondazione G. Pascale", Naples, Italy

Aim: Seven human coronaviruses have been identified in humans so far and found to cause a spectrum of clinical manifestations ranging from asymptomatic infections to severe disease and death. In particular, HCoV-OC43, HCoV-HKU1, HCoV-229E and HCoV-NL63 commonly cause mild- to-moderate disease, SARS-CoV-1 and MERS-CoV are responsible for severe acute respiratory syndromes and SARS-CoV-2, responsible for the coronavirus disease 2019 (COVID-19). In this study, we aimed to evaluate the immune response against the full SARS-CoV-2 proteome and selected epitopes of the other six coronaviruses, in order to identify immunological determinants of disease outcome in paediatric and adult patients infected with SARS-CoV-2.

Methods: Serum samples were collected from 49 SARS-CoV-2 infected subjects, including 19 children and 30 adults, with increasing COVID-19 severity from mild to critical illness. In addition, sera samples obtained from 30 uninfected subjects who were vaccinated with one dose of SARS-CoV-2 spike mRNA BNT162b2 vaccine were used as control. Serum samples were probed against a peptide microarray comprising 5828 overlapping 15-mer synthetic peptides, covering the full SARS-CoV-2 proteome and selected peptides of the spike (S), nucleoprotein (N) and membrane (M) proteins of common-cold coronaviruses 229E, OC43, NL63 and HKU1 (isolates 1,2 and 5) as well as SARS- CoV-1 and MERS.

Results and Conclusions: All paediatric and adults patients exhibited high IgG reactivity against the central region and C-terminus peptides of N and S proteins of SARS-CoV-2. The signal intensities were variable between and within patient groups and were significantly higher among adult patients with severe symptoms. By setting the threshold above 25'000 units, 100% and 81% of patients with severe disease, 36% and 29% of subjects with mild symptoms, and 8% and 17% of children younger than 8-years reacted against N and S proteins, respectively. Of note, the antibody response to SARS-CoV-2 M peptides was dissimilar between adults, mainly reacting against C-terminus M peptides, and children, which were highly responsive to N-terminus M epitopes. In addition, IgG signals against NS7B, NS8 and ORF10 peptides were found to be primarily elevated among adults with mild (63%) symptoms. Antibodies towards S and N proteins of other coronaviruses (SARS-CoV, MERS, 229E, OC43, NL63 and HKU1) were detected in all groups without a significant correlation with SARS-CoV-2 antibody levels. Overall, our results showed a cross-reaction of antibodies to epitopes of other human coronaviruses in all patients with distinct profiles between children and adults. Antibodies against linear epitopes of SARS-CoV-2 S and N proteins, particularly N aa 157-175, N aa 221-235 and S aa 785-799, are age dependent and correlate with clinical severity. These epitopes are of particular interest for the development of diagnostic tests to predict Covid-19 clinical outcome (Tornesello et al. J Transl Med. 2023).

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DETECTION OF DIFFERENT CIRCOVIRUSES IN DOMESTIC AND WILD CARNIVORES, ITALY

F. Alfano[1], M.G. Lucibelli[1], N. D'Alessio[1], C. Desario[2], A. Gallo[1], C. Auriemma[1], M.G. Amoroso[1], E. De Carlo[1], A. Salvaggiulo[2], N. Decaro[2], V. Martella[2], G. Fusco[1]

[1] *Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Naples, Italy*
[2] *Dipartimento di Medicina Veterinaria, Università degli Studi di Bari, Valenzano, Bari, Italy*

Circoviruses (CVs) infect both domestic and wild mammals, sporadically leading to onset of severe clinical signs. In addition to domestic carnivores and swine, CVs have been detected in several other species, which could have an impact on the emergence of new variants. In recent years, non- porcine strains have attracted more attention of researchers. Information on their circulation in wild animals is scarce and these viruses can also represent a potential threat to the conservation of endangered species.

The aim of our work was to evaluate the CV circulation in domestic and wild carnivores in Italy. In the period 2017-2019, 1300 samples from 214 domestic and wild animals (104 dogs, 80 cats, 15 wolves, 15 foxes), from all over Italy and abroad, were analyzed. The nucleic acid extraction was done by means of an automatic extractor (QIAsymphony, Qiagen, Hilden, Germany), using the "Virus/Pathogen" kit (Qiagen). The extracts were analyzed for canine (Li et al 2013) and porcine circovirus (Song 2019). Only positive animals were analyzed for the following emerging viruses: aichiviruses (Kitajima et al. 2013), bufaviruses (Martella et al. 2018), sapoviruses (Varela et al. 2016), astroviruses (Le Cann et al. 2004), hepatitis E virus (Jothikumar et al. 2006), hepatitis A virus (ISO 15216), noroviruses GI and GII (ISO 15216), rotaviruses (Zeng et al. 2008). The animals positive to CVs were also analyzed for the most common viruses infecting carnivores, including for canids canine coronavirus, canine parvovirus, canine distemper virus, canine adenovirus type 1 and type 2, canine herpesvirus type 1, and for felids feline coronavirus, feline panleukopenia virus, feline herpesvirus type 1.

Of the 214 tested animals, 9 (3,7%) were positive to canine circovirus (8 dogs, 1 cat) and 5 (2%) were positive to porcine circovirus (2 dogs, 1 cat, 1 red fox, 1 wolf). Only one dog displayed a single canine circovirus infection. Four dogs were co-infected with canine circovirus, canine bufavirus and parvovirus. In addition, two of them also show strains of astrovirus and herpesvirus. Coinfection with canine circovirus, porcine circovirus, and parvovirus was observed in two dogs. Another dog was coinfected with canine circovirus and astrovirus. In one cat a coinfection with canine circovirus, astrovirus and feline parvovirus was found. A coinfection with porcine circovirus and feline parvovirus was evident in another cat. Single porcine circovirus infection was observed in a fox and a wolf.

Noteworthy, canine circovirus was present only in domestic carnivores, while porcine circovirus was detected in both domestic and wild carnivores.

The results of the present study help expand the scarce knowledge currently available about the epidemiology of circoviruses in domestic and wild carnivores and highlight the need to monitor the circovirus circulation in these animals.

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WEST NILE VIRUS HEPATITIS: CASE REPORT

G. Mori[1], M. Strano[1], M. Chiurlo[1], S. Bossolasco[2], M. Cernuschi[2], A. Castagna[2]

[1] Vita-Salute San Raffaele University, Milan, Italy
[2] San Raffaele Hospital (IRCCS), Milan, Italy

Aim of the Study: 1145 West Nile Virus (WNV) infections were notified in Italy between 2012 and 2020, including 487 West Nile Neuroinvasive Diseases and 60 related deaths (1). In the last year 588 WNV infections were reported in our country (2). It is generally estimated that about 80% of persons infected with WNV remain asymptomatic and approximately 20% develop an acute, systemic febrile illness. In fewer than 1% of symptomatic individuals, virus entry into the central nervous system results in neuroinvasive manifestations (3, 4). We present a case of probable WNV-isolated non-fulminant hepatitis, to shed light on a possibly underestimated clinical picture.

Case Report: A 39-year-old man presented to the ER of IRCCS San Raffaele, Milan, for persisting fever and asthenia without any other more specific symptom/sign. Blood exams showed lymphocytosis and elevated transaminases, with slightly elevated C-reactive protein, normal cholestasis indices, albuminemia, and INR. Protein electrophoresis and immunofixation showed a monoclonal component in the gamma globulin area. Physical examination, chest X-ray, abdomen ultrasonography, blood cultures, serologies for HAV, HCV, HBV, CMV, and EBV acute infections were negative. The patient was referred to our Infectious Diseases Day Hospital service. We started daily administration of 5% glucose solution. We re-collected blood culture and performed a plasma dosage of copper, anti-LKM Ab, ANA, SMA, anti-SLA/LP, and PCR for HEV, all resulting negative. The serology (CLIA alifax) for WNV demonstrated positive IgM and negative IgG. This result was confirmed in the convalescent plasma two weeks later, with index reduction, but without IgG seroconversion. We were not able to isolate the virus through PCR nor were we able to prove seroconversion of IgG or confirm IgM positivity with a PRNT. Therefore, WNV infection should only be considered probable (5). However, considering that the patient did not report vaccination for JEV and YFV nor travel to endemic areas for DENV, ZIKV, SPOV, MVEV or SLEV, the likelihood of possible serological cross-reactivity is low. Moreover, the patient presented to our department after a 17-days course of symptoms, and it is well known that WNV's viremia is of short duration. In the following weeks resolution of symptoms, normalization of blood counts and liver indices were obtained.

Conclusions: The occurrence of visceral disease and hepatitis in flaviviruses infection is well known (eg. Hepatic failure as an aspect of the viral hemorrhagic fever syndrome caused by both YFV and DENV) (6). However, a Medline search on PubMed using the words "West Nile virus," "hepatitis," and "liver failure" yielded only 5 total descriptions of WNV-induced hepatic injury. WNV's liver tropism has probably been underestimated to date and all acute hepatitis occurring during periods of high WNV activity and with compatible epidemiology should be screened for this virus.

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DETECTION OF BOVIN MEAT AND MILK FACTORS (BMMFs) IN CATTLE FAECES: A PRELIMINARY STUDY

T. Vicenza[1], A. Boni[1], L. Cozzi[1], S. Di Pasquale[1], V. Martella[2], E. Suffredini[1]

[1] *Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy*
[2] *Department of Veterinary Medicine, University of Bari 'Aldo Moro', Bari, Italy*

In recent years, a variety of circular Rep-encoding ssDNA (CRESS) viruses were isolated from commercial milk samples, from meat from healthy cattle, from bovine serum and brain tissue of patients with multiple sclerosis. These infectious agents, later named Bovine Meat and Milk Factors (BMMFs) appear to be zoonotic agents, suspected to act as co-factors in the development of colon and breast cancer and neurodegenerative diseases. BMMFs, present in cattle, infect humans through the diet. The infection occurs in the cells of the lamina propria of the colon, with local spread, expression of the Rep protein and prolonged activation of the macrophage population, resulting in chronic inflammation, increased oxidative stress and reactive nitrogen and oxygen species. Inducing, in the end, an increase in cell mutation rate (de Villiers, et al., 2021), a process that is thought to play a role in the pathogenesis of breast and colon cancer, and in neurodegenerative diseases, such as Alzheimer's and multiple sclerosis. BMMFs are divided into 4 groups: Group 1 presents high homology with Sphinx 1.76 and Sphinx 2.36 episomes, isolated from brain tissue of patients with Creutzfeldt-Jakob; Group 2, 3 and 4 show homology with *Acinetobacter baumanii*'s plasmids, Gemycircularvirus and *Psychrobacter*'s plasmids, respectively (Pohl, et al., 2022). Since BMMFs were identified in cattle, we conducted a preliminary study on faecal samples collected from dairy cattle (Friesian breed), with the aim of investigating the presence of these agents.

The nucleic acid of 12 faecal samples was extracted using commercial kits and assayed by Rolling Circle Amplification (RCA), to selectively enrich circular DNA sequences, followed by PCR amplification of target regions, using specific primers. Amplicons were then confirmed by Sanger sequencing. Results showed the presence of Group 1 BMMF-like sequences in 8 samples, and Group 2 sequences in 6 samples. Interestingly, 4 samples were negative for both groups. Further analysis, including full sequencing of the circular DNAs detected in the study are needed to better characterize these genetic elements.

Despite the preliminary nature of this study, the results of this research demonstrate the presence of circular DNA sequences with high identity to BMMFs in cattle faeces and highlight to need to further investigate the occurrence of these biological agents in livestock and their role in triggering degenerative diseases.

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CRYO-EM STRUCTURE OF MARBURG VIRUS SINGLE-LAYERED NUCLEOPROTEIN-RNA COMPLEX

L. Zinzula[1,2], F. Beck[3], M. Camasta[1], D. Morado[4], C. Liu[1], A. Bracher[5], J.M. Plitzko[1,3], W. Baumeister[1]

[1] *Max Planck Institute of Biochemistry, Department of Molecular Structural Biology, 82152 Martinsried, Germany*
[2] *iHuman Institute, ShanghaiTech University, 201210 Shanghai, China*
[3] *Max Planck Institute of Biochemistry, CryoEM Technology, 82152 Martinsried, Germany*
[4] *Max Planck Institute of Biochemistry, Department of Cell and Virus Structure, 82152 Martinsried, Germany*
[5] *Max Planck Institute of Biochemistry, Department of Cellular Biochemistry, 82152 Martinsried, Germany*

Marburg virus (MARV) is a highly pathogenic filovirus endemic to Sub-Saharan African countries, which causes severe hemorrhagic fever in humans and non-human primates. The lack of licensed vaccines and antivirals against MARV disease, as well as the risk for international spread of MARV outbreaks, pose a threat to global health and urge for structural investigations to unveil antiviral targets within the MARV proteome. MARV nucleoprotein (NP) encapsidates viral single-stranded RNA (ssRNA) into a helical ribonucleoprotein (RNP) complex, which protects the MARV genome and provides the scaffold for viral transcription and replication. Thus far, there has been a lack of consensus on the structural aspects of these processes, since the high-resolution information available of a double-layered helical RNP complex, recently determined by cryogenic electron microscopy (cryo-EM), is not consistent with molecular architectures of the same complex reconstructed with cryo-electron tomography (cryo-ET) from MARV infected cells and intact virions, which both show a single-layered helical assembly instead. To fill this knowledge gap, we reconstituted the MARV RNP complex *in vitro* by assembling purified protomers of recombinant MARV NP with synthetic ssRNA of viral sequence, and determined by cryo-EM its structure, in single-layered helical conformation, to 3.4 angstrom resolution. The molecular details provided by this structural framework expand current knowledge on MARV genome packaging and nucleocapsid assembly, illuminating on diverse NP-NP and NP-ssRNA interfaces, and fully reconciling previous cryo-ET observations of native MARV RNP complexes. Furthermore, these findings substantiate the rationale for targeting the RNP complex with antiviral candidates to counter MARV infection.

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GENOMIC EPIDEMIOLOGY OF THE MAIN SARS-CoV-2 VARIANTS CIRCULATING IN ITALY IN 2020 AND 2021 PERIOD

A. Bergna[1], A. Lai[1], C. Della Ventura[1], A. Weisz[2], B. Bruzzone[3], F. Sagradi[4], G. Brindicci[5], D. Francisci[6], N. Clementi[7], I. Vicenti[8], A. Callegaro[9], S. Menzo[10], E. Venanzi Rullo[11], G. Giurato[2], F. Rizzo[2], T. Rocco[2], V. De Pace[3], C. Fraccalvieri[3], S. Testa[4], L. Lanfranchi[4], A. Saracino[5], S. Caucci[10], G. Nunnari[11], M. Clementi[7], M. Galli[1], A. Riva[1], S. Antinori[1], G. Zehender[1], SARS-CoV-2 Italian Research Enterprise - (SCIRE) collaborative Group

[1] *Department of Biomedical and Clinical Sciences, University of Milan, Italy*
[2] *Laboratory of Molecular Medicine and Genomics, Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Italy*
[3] *Hygiene Unit, IRCCS AOU San Martino-IST, Genoa, Italy*
[4] *Unit of Infectious Diseases, Azienda Socio Sanitaria Territoriale Cremona, Italy*
[5] *Infectious Diseases Unit, University of Bari, Italy*
[6] *Department of Medicine and Surgery, Clinic of Infectious Diseases, "Santa Maria della Misericordia" Hospital, University of Perugia, Italy*
[7] *Laboratory of Microbiology and Virology, Università "Vita-Salute" San Raffaele, Milan, Italy*
[8] *Department of Medical Biotechnologies, University of Siena, Italy*
[9] *Microbiology and Virology Laboratory, ASST Papa Giovanni XXIII, Bergamo, Italy*
[10] *Department of Biomedical Sciences and Public Health, Virology Unit, Polytechnic University of Marche, Ancona, Italy*
[11] *Unit of Infectious Diseases, Department of Experimental and Clinical Medicine, University of Messina, Italy*

Introduction: Since the beginning of the pandemic, SARS-CoV-2 has shown a great genomic variability, due in part to a high mutation rate and driven by the widespread and rapid circulation in the human population. The continuous emergence of SARS-CoV-2 new variants has made their global monitoring and the study of their characteristics a priority. Aim of this work was to study the genomic heterogeneity, the temporal origin, the rate of viral evolution and the population dynamics of the main circulating variants (20E.EU1, Alpha, Delta and Omicron BA.1) in Italy, in the period August 2020-January 2022.

Methods: 861 Whole Genome sequences of SARS-CoV-2 have been collected at the centres of the collaborative group SCIRE (SARS-CoV-2 Italian Research Enterprise). For each variant, two datasets were analyzed, the former including international genomes and the latter focusing on Italian sequences. Phylogenetic trees were estimated using IQ-TREE v.1.6.12. The Italian clusters were analyzed using BEAST v.2 to estimate tMRCA (time of the Most Recent Common Ancestor) and main epidemiological parameters.

Results: Compared to the other variants in the study, Delta variant sequences showed a high number of additional mutations, especially in the ORF1a region.
International clusters, including more than 70% of Italian genomes (11, 17 and 8 for 20E.EU1, Alpha and Delta, respectively), presented a tMRCAs between 13/06/2020-28/09/2020 (95%HPD:19/05/2020-05/11/2020), 10/11/2020-20/02/2021(95%HPD:11/10/2020-07/03/2021) and 13/03/2021-27/07/2021 (95%HPD:11/02/2021-24/08/2021) for 20E.EU1 clade, Alpha and Delta variants, respectively. Preliminary analysis of Italian clusters of BA.1 Omicron variant (n=14) presented a tMRCAs between 10/08/2021-28/10/2021 (95%HPD: 22/05/2021-16/12/2021). R_e values showed the highest level between May and June 2020 in 20E.EU1 clade until autumn 2020. Starting from January 2021, it was observed a reduction of R_e around the unit and the joining of the plateau. R_e of Alpha variant was estimated above 1 since October 2020 when the highest mean value was estimated (1.18), remaining above 1 until March 2021, when it started to decrease until June 2021. For Delta variant, we observed two peaks: the first in March-May 2021 (1.34), and the second in June-July 2021 (1.14), while the decrease of R_e to 1 matched with the achievement of the plateau in August remaining stable until 2022. Omicron BA.1 showed growth in R_e values between October and November 2021 (1.4) until January 2022 when it decreased reaching value around 1 and then stabilizing.

Conclusions: Our work highlighted a different evolutionary dynamic of studied lineages. A high concordance was observed between epidemiological parameters estimation and phylodinamic trends. When the Skyline Plot displayed an exponential increase in the viral population (indicating an increase in transmission events) the birth-death analysis showed R_e values above 1, while R_e periods below 1 corresponded to a decrease in the size of the epidemic.

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EXPLORING THE CIRCULATION OF PROTOPARVOVIRUS THROUGH ENVIRONMENTAL SURVEILLANCE

T. Vicenza[1], C. Veneri[2], P. Mancini[2], G. Bonanno Ferraro[2], C. Del Giudice[2], M. Iaconelli[2], L. Orlandi[1], A. Boni[1], S. Di Pasquale[1], L. Cozzi[1], Y.T.R. Proroga[3], E. Suffredini[1], G. La Rosa[2]

[1] Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, National Reference Laboratory for Foodborne Viruses, Rome, Italy
[2] Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy
[3] Department of Food Microbiology, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Naples, Italy

Protoparvovirus is a genus of non-enveloped, ssDNA viruses within the Parvoviridae family. Two species, bufavirus (BuV) and tusavirus (TuV), described in 2012 and 2014 respectively, have been reported in association with gastroenteritis in children, while a third species, cutavirus (CuV), has been detected in both faecal specimens and skin biopsies, and has been later associated with the development of cutaneous T-cell lymphoma. Although BuV has been previously investigated in a group of children displaying symptoms of gastroenteritis, data on the prevalence and distribution of protoparvoviruses are currently unavailable in Italy. Aim of this preliminary study was exploring the circulation of BuV, TuV and CuV in the population through environmental surveillance.
A total of 51 samples (24h composite) were collected monthly between February 2021 and March 2023 from three wastewater treatment plants (WTPs) in Rome. Samples were concentrated by PEG precipitation and nucleic acids were extracted according to a standardized protocol and analysis was undertaken by real-time qPCR using previously described primers/probes. For target quantification, an in vitro synthetized template was used, and the quality of the analysis was ensured using recovery and inhibition controls.
Thirty samples (59%) tested positive for at least one of the viruses. Specifically, BuV was detected in 11 samples (22%), TuV in 22 (43%) and CuV in 15 (29%). Viral concentrations in positive samples were generally low and, considering WTP daily flow rates and contributing population, the median excretion was estimated to be 2.2×10^{2} (range 5.5×10^{1} - 4.3×10^{2}) genome copies/inhabitant* die for BuV, 4.5×10^{2} (range 5.2×10^{1} - 2.6×10^{3}) for TuV, and 3.5×10^{2} (range 1.2×10^{2} - 5.6×10^{2}) for CuV. Although no clear seasonal trend was observed in viral occurrence, most of the positive samples and higher TuV concentrations were obtained in samples collected beween late winter and early summer (February to July).
The results of this waterwater surveillance study highlights a significant ciruclation of BuV, TuV and CuV within the population. However, further studies are needed to define the potential impact of these viruses on human health.

PO 241 - OC 50

VIRAL EVOLUTION SURVEILLANCE: LESSONS LEARNED FROM COVID-SURVEILLANCE NETWORK ON DATA QUALITY

L. Squarzon[1], M. Favarato[1], V. Cristofori[2], V. Selle[2], A. Fusaro[3], I. Monne[3], C. Terregino[3], C. Berti[1], D. Gibellini[4]

[1] *Azienda ULSS3 Serenissima, Ospedale dell'Angelo, UOSD Genetica e Citogenetica, Mestre, Venice, Italy*
[2] *Azienda ULSS3 Serenissima, Servizio Igiene e Sanità Pubblica, Mestre, Venice, Italy*
[3] *Istituto Zooprofilattico delle Venezie, Legnaro, Padua, Italy*
[4] *Dipartimento di Diagnostica e Sanità Pubblica, Università di Verona, Italy*

Aim of the Study: Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) since 2019, has been one of the greatest opportunity for scientific community to share data, protocols and suggestions to reach results that even scientists had never imagined to achieve in such a sort time. Starting from establishing networks, including healthcare professionals, bioinfomatics and vets, surveillance of the virus has been promptly followed in its evolution and variants' onset, from Alpha to Omicron, in Veneto Region. Step by step, sharping communication between institutions, providing detailed reports, all professionals, regardless of their roles, contributed to improve data quality of the sequences.

Methods used: Recurring external quality assessments have been carried out, constantly comparingextraction, amplification methods, sequencing data on nucleotides and aminoacid changes in SARS-CoV-2 genome. Allelic dropout, sensitivity, specificity, accuracy, reproducibility and repeteability have been investigated and aligned between different platforms and labs, showing the enriching power of data sharing.

Results and Conclusion: Results have been produced by following same steps protocols, starting from pre-analytics to post-analytics, embracing strict quality parameters, such as standard deviation and linearity for RT-Real Time PCR, as well as cluster density, cluster passing filter and Q30 for next- generation sequencing (NGS) data. Home made and commercial data interpretation pipelines have been compared head to head, in order to achieve the highest reliability of sequences for acceptability submission in Italian (I-Co-Gen) and worldwide (GISAID) databases. Here we present the experience and the impact of three years of well structured Coronavirus surveillance on diagnosis, prevention and new waves forecast with the aim to exploit this network for other possible emerging infections, with the unique effective perspective of one health, about environment, animals and humans.

PO 242 - OC 51

EPIDEMIOLOGY AND MOLECULAR ANALYSES OF RESPIRATORY SYNCYTIAL VIRUS IN THE SEASON 2021-2022 IN NORTHERN ITALY

A. Lai[1], A. Bergna[1], V. Fabiano[2], C. della Ventura[1], A. Mari[2], M. Loiodice[2], G.V. Zuccotti[1,2], G. Zehender[1]

[1] *Department of Biomedical and Clinical Sciences, University of Milan, Italy*
[2] *Pediatric Department, "Vittore Buzzi" Children's Hospital, Milan, Italy*

Background: Human respiratory syncytial virus (RSV) is the leading cause of acute lower respiratory tract infection among infants and young children worldwide. Most infections occur seasonally during the winter months in temperate regions, with seasonal peaks in January/February. HRSV has a single-stranded negative-sense RNA genome, encoding for 10 viral proteins; two of them (G and F) playing a significant role in viral pathogenicity and immune evasion. Based on the genetic variability of the G gene, RSV strains are classified into subtype A or B and further characterized into different genotypes. HRSV-A infection progress to more severe clinical manifestations than HRSV-B, despite the association between HRSV genotypes and disease severity remains unclear. Aim of this study was to characterize the HRSV in the 2021-2022 season in a paediatric cohort.

Methods: 104 samples were collected between November 2021-January 2022, from paediatric patients attending the "Vittore Buzzi" Children's Hospital in Milan. A and B subtypes were discriminated by RT-PCR. Whole genomes (n=88, 49 HRSV-A and 39 HRSV-B) were obtained through NGS procedures applying subtype specific protocols. Maximum-Likelihood and Bayesian phylogenetic methods were used to analysed Italian sequences in the European contest and dated Italian clusters.

Results: Males accounted for 58.7% and the median age was 87 days. 76.8% of subjects required hospitalization, with a median stay of 8 days. Stratifying subjects by age, a significant proportion of hospitalized subjects was observed in patients aged <3 months (*p=.037*).
An equal proportion of subtypes A and B was found. Subtype A patients were significantly older than that of B subtype, (109 *vs.* 66 days, *p=.007*). Significant differences were found in length of hospitalization (8 for HRSV A *vs.* 6 days for HRSV B, *p=.05*), days of supplemental oxygen treatment (6 *vs.* 4 days, *p=.004*) and intravenous hydration duration (4 *vs.* 2 days, *p=018*).
All A strains belonged to genotype GA2.3.5 while all B strains to genotype GB5.0.5a. By analysing number of substitutions, a higher heterogeneity was found among HRSV-A sequences compared to B. Phylogeny highlighted the presence of 20 clusters containing quite the totally of Italian sequences and 35 clusters involving 60% of Italian strains for HRSV-A and B, respectively. Clusters presented a tMRCA between 01/2012-09/2017 for A subtype, and 12/2013-02/2019 for B subtype. No differences were observed between sequences inside or outside clusters. Italian sequences showed a peculiar mutational patter compared to European sequences. Some mutations such as V279A in G gene, N117K in M2-1 portion, I7V and L422M in L gene for HRSV A and N5K in NS2 portion, V90A in N gene, T198A in G gene and A675V, UI677V, I1588, K1589, Y1590S, V1592L and T1596I for HRSV B, were present only in Italian strains.

Conclusions: These data confirmed a more severe clinical course of HRSV-A, in particular in young children. Our study permitted the characterization of recent HRSV whole genomes of Italian strains highlighting the peculiar pattern of mutations, compared to available European strains, that needs to be more investigated and monitored.

PO 243 - OC 53

LOCAL INFLAMMATORY RESPONSE DURING RESPIRATORY SYNCYTIAL VIRUS INFECTION IN INFANTS

V. Gentili, D. Bortolotti, S. Beltrami, S. Rizzo, G. Schiuma, G. Strazzabosco, R. Rizzo
Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Italy

Aim of the Study: Respiratory syncytial virus (RSV) is the major cause of lower respiratory tract illness (LRTI) in infants and young children. Although most cases result in mild disease, the host immune response against RSV infection can affect the clinical outcome and the possibility to develop chronic inflammatory diseases. Acute RSV infection is often accompanied by transient elevated C-reactive protein (CRP), significant for inflammation and immune dysregulation.
We evaluated the local inflammation in RSV-infected infants, without a familiar history of asthma, analyzing the airway levels of cytokines and of human leukocyte antigen G (HLA-G). HLA-G is a non-classical major histocompatibility complex class I molecule with a tolerogenic function due to its ability to interact with immune inhibitory receptors (ILT2, ILT4, KIR2DL4), and it is suggested to be implicated in airway immune system regulation. Moreover, HLA-G interacts with several regulatory mediators such as pro- and anti-inflammatory cytokines, modulating the inflammatory response during viral infection.

Methods used: Nasal washes were collected from 52 infants (age: 0-36 months), prospectively enrolled at admission for acute bronchiolitis in a RSV standard care, and at the remission of clinical symptoms during the follow-up visit. The subjects were at their first episode of severe RSV bronchiolitis (mean O(2) saturation: 91.6+/-1.3%; length of stay: 2.5+/-2.5 days; presence of radiographic opacities: 75%). Patients were excluded for a variety of reasons including previous wheezing, regular use of bronchodilator or anti-inflammatory medications, any preexisting lung disease including asthma, chronic lung disease of prematurity/bronchopulmonary dysplasia, or cystic fibrosis; gastroesophageal reflux disease on medical therapy; or congenital anomalies of the chest or lung. Nasal washes were analyzed for the presence of RSV-RNA by nested rt-PCR (reverse transcriptase-polymerase chain reaction). Enzyme-linked immunosorbent assay (ELISA) measured soluble HLA-G, CRP and cytokines levels in nasal washes.

Results and Conclusions: At admission, 28 subjects showed nasal washes positive for the presence of RSV RNA, while all subjects became negative at the follow-up visit. At admission, the levels of CRP were 11.1±2.3 mg/L in RSV-positive subjects in comparison with a CRP of 2.6±4.2 mg/L in RSV-negative subjects ($p<0.001$; Student t test). At admission, pro-inflammatory cytokines in nasal washes (IL-1, IL-6, IL-8) were higher in RSV-positive subjects in comparison with RSV-negative subjects ($p<0.05$; Student t test). The mean concentration of sHLA-G in nasal washes at admission was significantly lower (2.8±5.2 ng/ml) in RSV-positive infants compared to RSV-negative subjects (7.6±3.5 ng/ml) ($p=0.01$; Student t test). HLA-G nasal washes levels were comparable between RSV-positive and -negative patients at the follow-up visit (8.0±2.3 ng/ml).
Our data show an increased inflammatory status in RSV-positive subjects at admission, which was restored at remission. The downregulation of HLA-G expression during RSV infection might contribute to increased pro-inflammatory response and viral spread. Although further confirmation is needed, we suggest that HLA-G could influence the dynamics of the local immune response to RSV infection, supporting viral infection.

PO 244

MOLECULAR AND CLINICAL ANALYSIS OF RSV-POSITIVE BRONCHIOLITIS CASES BEFORE AND AFTER PANDEMIC RESTRICTIONS, IN ROME

M. Fracella[1], C. Scagnolari[1], G. Oliveto[1], L. Sorrentino[1], M. Ardone[1], F. Frasca[1], L. Matera[2], M.G. Conti[2], L. Petrarca[2], R. Nenna[2], F. Midulla[2], P. Papoff[2], O. Turriziani[1], G. Antonelli[1], A. Pierangeli[1]

[1] Virology Laboratory, Department of Molecular Medicine, "Sapienza" University of Rome, Italy
[2] Department of Pediatrics and Infantile Neuropsychiatry ,"Sapienza" University of Rome, Italy

In the first pandemic period, measures adopted to reduce SARS-CoV-2 transmission also prevented the spread of other respiratory viruses. After restrictions were lifted, there was a surge of respiratory syncytial virus (RSV) hospitalization in early autumn 2021, and a large number of cases also in winter season 2022/2023. Bronchiolitis cases caused by RSV subtype A are generally more severe than those caused by RSV-B; nonetheless, several reports showed no differences between subtypes. In this study, we aimed to characterize the molecular epidemiology of RSV-A and -B causing hospitalization for bronchiolitis in Rome, also comparing clinical data, before and after the COVID-19 pandemic.

Methods: Infants hospitalized for bronchiolitis in the Pediatric Emergency Department, and in the pediatric intensive care unit (PICU) of the "Sapienza" University of Rome Hospital, were prospectively enrolled, during the epidemic seasons from 2017-2018 to 2022-2023, after the informed consent was obtained from infants' parents. Demographic and clinical data were obtained from medical files; a Clinical Respiratory Score (CRS) was assigned at Hospital admission. Nasopharyngeal washings (NPW) or bronchoalveolar lavages (BAL) were tested with molecular methods for 14 respiratory viruses and RSV-positive residual samples were sequenced in the second-half of the G gene. RSV-A and -B phylogenetic trees were constructed using the Maximum Likelihood method based on the Tamura-Nei model and a discrete Gamma distribution with 5 categories (+G) to model evolutionary rate differences among sites, with bootstrap values of 1,000.

Results and Conclusions: RSV positivity rates in bronchiolitis cases significantly differed among seasons, with higher percentages in the post-pandemic period ($p<0.001$). Contrasting with PICU admission rates that were similar, CRS values and need of O_2 therapy were lower during the 2021-2022 season with respect to all other seasons. RSV-A dominated in three epidemic seasons: 2017-2018 (69%), 2019-2020 (89%) and 2021-2022 (74%) whereas RSV-B was predominant in 2018-2019 (77%) and 2022-2023 (72%). RSV-A cases did not differ in PICU admission among seasons whereas more infants needed O_2 therapy and had worse CRS in the pre-pandemic than in the post-pandemic period. Contrastingly, RSV-B infected infants were more frequently admitted to PICU and needed O_2 therapy more in the post-pandemic period. In the phylogenetic analysis, RSV-A sequences were ON1 genotype but evolutionary distant from the reference strain; strains within an epidemic season grouped in different clusters together with sequences from other seasons, both in the pre- and post-pandemic periods. Nearly all RM RSV-B strains were BA10 genotype; interestingly, a divergent and well-supported clade was formed only by sequences of 2021-2022 and 2022-2023 seasons, pointing for higher evolutionary divergence of RSV-B strains in the post-pandemic period.
These data show that the increased number of total RSV cases observed in 2021-2022 is not driven by a single more virulent RSV strain but more hospitalizations were likely due to a waning population immunity; however, a divergent RSV-B variant could be responsible of subtype specific higher bronchiolitis severity in the post-pandemic seasons. The national RSV surveillance could be improved to monitor variants more virulent and/or resistant to monoclonal antibodies.

PO 245

GENOMIC CHARACTERIZATION AND INTRA-HOST VARIATION OF SARS-CoV- 2 IN IMMUNOCOMPROMISED PATIENTS WITH PROLONGED INFECTION

B. Mola[2], S. Silenzi[1], L. Ruotolo[2], T. Lazzarotto[1,2], G. Rossini[1]

[1] *Microbiology Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Italy*
[2] *Section of Microbiology, Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Italy*

Background and Aim: The chronic infection hypothesis is proposed to be one of the most accredited mechanisms for the emergence of genetically divergent SARS-CoV-2 variants (1). In immunocompromised subjects the virus persists for a long time due to low immunization coverage and could act as a "reservoir" for the emergence of new mutations. The purpose of this study is to monitor intra-host SARS-CoV-2 genomic evolution in immunocompromised patients with prolonged viral shedding to provide further insight about viral evolutionary dynamic in chronic infection compared to the general population mutational pattern.

Materials and Methods: Between 22 May and 18 August 2022, sequential nasopharyngeal swabs (NPS) from two severely immunosuppressed cases (case A and B) with a history of onco-hematological disease and prolonged SARS-CoV-2 shedding were collected. For early SARS-CoV-2 detection, all collected samples were analyzed with RT-PCR-based assay performed by *Allplex™ SARS-CoV-2 Variants I Assay* (Seegene). All samples eligible (Ct<27) were sequenced by Next Generation Sequencing (NGS) technology (Illumina COVIDSeq Assay). Finally, data were analyzed by Illumina DRAGeN™ COVID Lineage Application and Stanford SARS-CoV-2 software.

Results: For case A, sequencing of NPS at three different time points showed that all sequences matched the BA.2 Omicron lineage. For case B, the analysis of two NPS at different time points revealed the presence of BA.2 and BA.2.1 Omicron lineages, indicating the occurrence of an intra-host viral evolution. In both cases whole-genome sequencing showed an extensive viral genomic evolutionary dynamic within-patient with the emergence of some amino acid mutations (A685S, M749I in RdRp gene; V642G, R408S in S gene and T30I in E gene) and disappearance of others. Interesting, T30I mutation, considered a sensitive marker mutation for persistent infection (2), was observed only in case B whereas N440K substitution, fixed in Omicron lineages, was found in both cases but with an opposite trend: disappearing in case A and appearing in B.

Conclusions: This study supports the hypothesis that immunocompromised patients may be one of the driving forces leading to the generation of new viral variants (3). The detected mutations may be the result of an intra-host evolution followed by a fixation event over time.

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