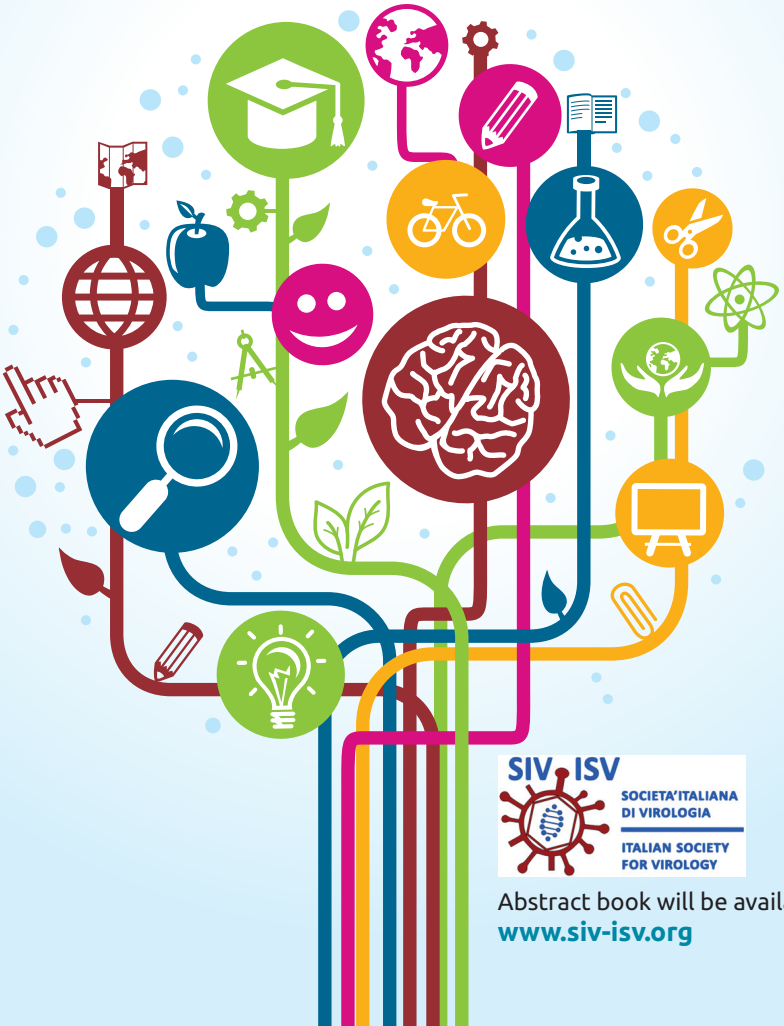
6th Italian experience in biomedical research: young minds at work

Desenzano del Garda (BS) • Park Hotel

12th-13th October, 2018



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Dear Friends,

We are very pleased to host you here in Desenzano del Garda at the "6th Annual Italian Experience in Biomedical Research: Young Minds at Work".

During the conference, you will be in touch with peers (PhD students, postgraduates, Post-Doctoral Scientists, etc.) from across all biomedical disciplines encompassing the latest and hottest frontiers of research and their innovative translational applications. We want you to take this opportunity and to make this conference very special. Be participative! Be curious! Ask! Be ready to share your knowledge! Even more importantly: Think! Think of possible new scenarios for your research! Think of how fascinating and intriguing is the work we are all involved in!

Your peers are not here to judge you, but to give you a fantastic opportunity to share your data in an extremely informal environment.

Your active participation is important to us and for the real effectiveness of the conference. We also hope it is important to you!

Francesca Caccuri
Nicasio Mancini
Marta Trevisan

Faculty

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MARILENA TRAVERSI
MARTA TREVISAN
STEFANIA VOGIATZIS
DANIELA ZAGO
ALBERTO ZANI

Friday, October 12th

PARTICIPANTS REGISTRATION
h 12.30-13.30

WELCOME AND INTRODUCTION TO THE MAIN LECTURE
Francesca Caccuri, Nicasio Mancini and Marta Trevisan

h 14.00-14.45 **Dario Bonanomi**, "*Vita-Salute San Raffaele" University, Milano*
Mechanisms of brain wiring: how neurons find their targets

ANTI-CANCER VIROLOGY AND ANTI-CANCER DRUGS

MICROBIOME

h 16.15-16.30 **Roberto Ferrarese**, *Microbiology and Virology Unit, "Vita-Salute San Raffaele" University, Milano*..... pag. 22
The microbiota of the prostate tumor microenvironment

BREAK
h 16.30-17.00

GENE THERAPY

Chairman: ***Alessandro Migliara***

h 17.00-17.15 **Alessandro Migliara**, *"Vita-Salute San Raffaele" University, Milano* ..... pag. 24
A high-throughput loss-of-function approach to uncover novel players involved in permanent epigenetic silencing

h 17.15-17.30 **Michela Milani**, *San Raffaele Telethon Institute for Gene Therapy, Milano*..... pag. 25
Liver-directed gene therapy for hemophilia B with immune stealth lentiviral vectors

h 17.30-17.45 **Diego Gilioli**, *San Raffaele Telethon Institute for Gene Therapy, Milano*..... pag. 26
Dissecting cell-autonomous and paracrine functions of cell senescence in AML response to therapy

NEUROPATHOLOGY AND NEUROPHARMACOLOGY

Chairman: ***Isabel Pagani***

h 17.45-18.00 **Isabel Pagani**, *Viral Pathogens and Biosafety Unit, Division of Immunology, Transplantation and Infectious Diseases, San Raffaele Scientific Institute, Milano*..... pag. 27
Zika virus replication in dorsal root ganglia explants from interferon receptor 1 (IFNR1) knockout mice causes myelin degeneration

h 18.00-18.15 **Francesca Aria**, *Department of Molecular and Translational Medicine, University of Brescia, Brescia*..... pag. 28
Chronic treatment of γ-oryzanol prevents neuroinflammation and cognitive impairment in LPS-injected adult mice

h 18.15-18.30 **Stefania Vogiatzis**, *Department of Molecular Medicine, University of Padova, Padova* ..... pag. 29
Chimeric NEDD4 ubiquitin ligases as an innovative approach for interfering with alpha-synuclein accumulation in Parkinson's disease

Saturday, October 13th

HIGHLIGHTS IN BACTERIOLOGY

ANTIMICROBIAL DRUGS

BREAK
h 10.45-11.15

INTRODUCTION TO THE MAIN LECTURE
Francesca Caccuri, Nicasio Mancini and Marta Trevisan

IMMUNE RESPONSE AND PATHOLOGY

LUNCH
h 13.00-14.00

VIRAL PATHOGENESIS

BREAK
h 16.00-16.15

AVIAN REOVIRUS P17 INHIBITS MOTILITY, MIGRATION AND PROLIFERATION OF HUMAN ENDOTHELIAL CELLS

E. Manocha, M. Comini, A. Caruso, F. Caccuri

Section of Microbiology, Molecular and Translational Medicine, University of Brescia, Italy

BACKGROUND. Avian Reovirus p17 is a 17-kDa non-structural protein encoded by the second open reading frame of S1 gene and contains 146 amino acids. The S1 genome segment of ARV contains three open reading frames that translates into p10, p17 and σC proteins, respectively. ARV p17 has been observed to induce cell growth retardation, cell cycle arrest and host cellular translation shutoff by suppression of CDK1 and PLK1 like signaling pathways and regulation of p53/PTEN/mTORC1 like pathways by directly binding to CDK, cyclin or CDK/cyclin complexes. ARVp17 is also known to induce autophagy and activate protein kinase RNA-activated signaling, thereby activating the innate immune system, which may induce the immune response against tumors.

METHODS. The full length of ARVp17 gene was cloned into pGEX-4T-1 prokaryotic expression vector and transformed into E. coli BL-21 competent cells. Human Vascular Endothelial Cells (HUVECs) were used to perform wound healing assay and Matrigel assay, whereas human breast cancer cells (MDA-MB 231) were used to perform soft agar assay after ARV p17 protein stimulation or gene nucleofection. The irrelevant protein Glutathione-S-transferase (GST) was used as a control.

RESULTS. ARVp17 is able to induce loss of the ability of endothelial cells to form a network of capillary-like structures when seeded on extracellular matrix (BME) coated plates. Moreover, migratory capacity of HUVECs treated with ARVp17 was inhibited, as demonstrated by "wound sealing" assays. Indeed, differently from cells treated with the GST protein, HUVECs treated with ARVp17 were incapable to repair a wound scratch. In addition, we have found that the expression of ARV p17 strongly decrease breast cancer cells proliferation in an anchorage independent microenvironment.

CONCLUSION. The ability of ARV p17 to suppress endothelial cells motility, impeding angiogenic processes and new vessels formation, may lead to the inhibition of tumor nutrition, growth and spreading.

GENERATION OF NEW HSV-1 BASED IMMUNO-ONCOLYTIC VIRUSES FOR THE TREATMENT OF SOLID TUMORS RESISTANT TO CURRENT IMMUNOTHERAPIES

A. Reale*, A. Vitiello*, V. Conciatori, C. Parolin, S. Piccolo, J. Von Einem[1], A. Calistri, G. Palù

Department of Molecular Medicine, University of Padova, Padova, Italy
1 Institute for Virology, University of Ulm
**These two Authors equally contributed to the work*

BACKGROUND. Curative pharmacological therapy of solid tumors is still an unmet clinical need. Immunotherapy by means of checkpoint inhibitors or chimeric antigen receptor (CAR) T cells is providing major breakthroughs in this field. However, many solid tumors surrounded by an immunosuppressive tumor microenvironment (TME), like triple negative breast carcinoma (TNBC), are usually resistant to immunotherapy. Oncolytic viruses (OVs) are defined as viruses able to selectively replicate in cancerous rather than healthy cells. OVs are very promising agents for the treatment of solid tumors as they provide powerful proinflammatory stimuli in the TME, thus subverting its immunosuppressive features. At the same time, they can be used as gene therapy vectors. Herpes simplex virus type 1 (HSV-1) proved to be a very good platform for the generation of OVs, due to its large dsDNA genome which allows extensive manipulation and insertion of therapeutic genes, viral lytic activity and a good safety profile. The HSV-1 based talimogene laherparepvec (Imlygic®, Amgen) is currently the only OV approved for clinical use in the EU and the US.
METHODS. HSV-1 genome was modified by bacterial artificial chromosome (BAC) mutagenesis in an appropriate strain of *Escherichia coli,* allowing lambda red recombineering, starting from an Δγ34.5 HSV-1 parental BAC. Viral stocks were then reconstituted by lipofectamine transfection of purified BAC DNA into 293T cells. We measured the replicative capacity of the recombinant viruses in mammary tumoral cell lines and in both tumoral and non-tumoral murine mammary organoids. The efficiency of expression of transgenes was also evaluated by different methods.
RESULTS AND CONCLUSIONS. The Us12 gene, encoding the ICP47 protein, was succesfully deleted from the parental BAC. This deletion shifts the expression kinetics of the Us11 gene from true late to immediate early, enhancing viral replication, without affecting safety. Two recombinant viral genomes were generated by the insertion within the UL55-UL56 intergenic region of the enhanced green fluorescent protein (EGFP) gene, or of a DNA sequence encoding a single chain antibody (scAb) targeting CCR4, that is expressed on regulatory T cells. All recombinant viruses efficiently replicated in the tested cell lines. Strikingly, replication of recombinant viruses was much more effi-

cient in tumoral murine mammary organoids than in non-tumoral organoids. Exogenous genes were also expressed. We are currently inserting further therapeutic genes including soluble Programmed cell Death-1 (sPD-1), human IL-12, and FMS-like tyrosine kinase 3 ligand (Flt3L) in the Δγ34.5/ΔUs12 BAC. Next steps will include full *in vitro* characterization of the new viruses and testing viral therapeutic efficacy on an appropriate mouse model.

CIITA-RELATED BLOCK OF HLA CLASS II EXPRESSION, UPREGULATION OF HLA CLASS I, AND HETEROGENEOUS EXPRESSION OF IMMUNE CHECKPOINTS IN HEPATOCARCINOMAS: IMPLICATIONS FOR NEW THERAPEUTIC APPROACHES

E. Ramia, A. M. Chiaravalli, F.B.N. Eddine, A. Tedeschi, F. Sessa, R.S. Accolla, G. Forlani

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

BACKGROUND. Hepatocellular carcinoma (HCC) is the second cause of death for cancer worldwide, justifying the urgent need for novel therapeutic approaches. Immunotherapeutic strategies based on triggering and/or rescuing tumor antigen-specific T cells may be promising particularly if combined together.

METHODS. As preliminary step toward this goal, we have investigated the expression of antigen presenting molecules (HLA class I and class II) and immune checkpoints (PD-1 and PD-L1) in 43 HCC samples from distinct patients by immunohistochemistry staining and in HCC cell lines using immunohistochemistry and flow cytometry. Moreover, DNA methylation status of CIITA promoter IV, the IFNγ-inducible promoter of the class II transactivator, was assessed by sequencing for analysis of the percentage of methylated CpGs.

RESULTS. While normal hepatocytes did not express HLA class I and II, HCC cells strongly upregulated HLA class I while remaining negative for HLA class II. The absence of HLA class II expression in HCC cell lines correlated with lack of expression of the HLA class II transactivator, CIITA, which could not be rescued even after interferon-gamma treatment. This was due to high methylation levels of interferon-gamma-sensitive CIITA promoter IV strongly suggesting a biologically relevant developmental silencing of HLA-II expression in liver cell lineage. HCC tumor tissues showed a variable degree of leukocyte infiltration. Infiltrating lymphocytes expressed PD-1, while PD-L1 was expressed in cells with monocyte-macrophage morphology mostly localized at the tumor margin, but not in tumor cells.

CONCLUSION. *de novo* expression of HLA class I, instrumental for presenting tumor antigens to cytotoxic T lymphocytes, and the correct characterization of the cells expressing checkpoint inhibitors in the tumor tissue should be the ground for setting novel strategies of combined approaches of immunotherapy in HCC based on tumor peptide vaccines and anti-checkpoint inhibitor antibodies.

DOWN-REGULATION OF THE ANDROGEN RECEPTOR BY G-QUADRUPLEX LIGANDS SENSITIZES CASTRATION-RESISTANT PROSTATE CANCER CELLS TO ENZALUTAMIDE

M. Tassinari[1], G. Cimino-Reale[2], M. Nadai[1], F. Doria[3], E. Butovskaya[1], M. Recagni[2], M. Freccero[3], N. Zaffaroni[2], S.N. Richter[1], M. Folini[2]

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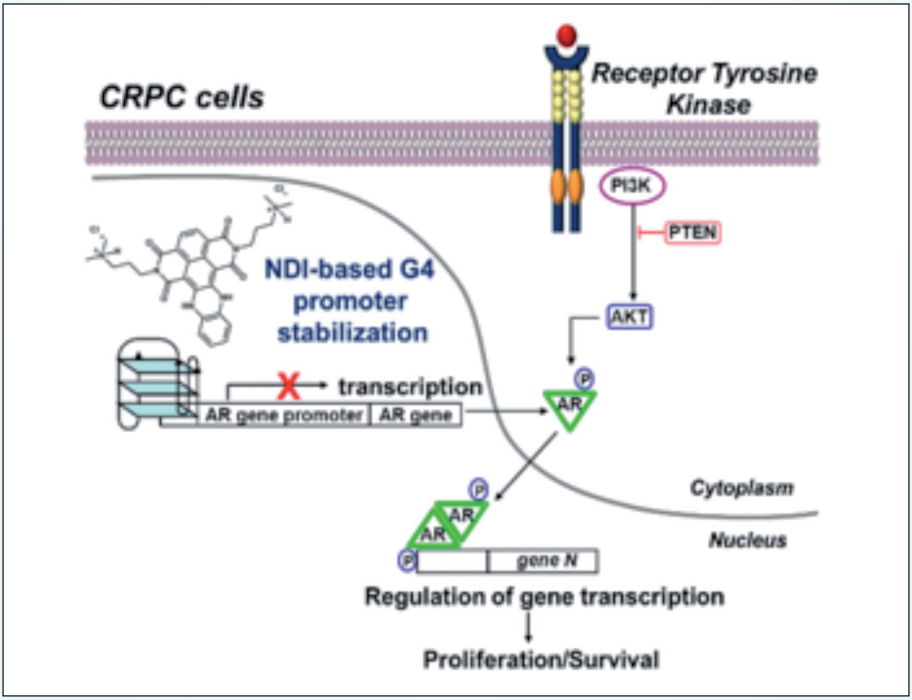
3 Department of Chemistry, University of Pavia, Pavia, Italy

BACKGROUND. Prostate cancer (PCa) is the second leading cause of cancer-related death[1]; despite the current therapy provides good disease control, some patients develop metastatic castration resistant phenotype (CRPC)[2]. Given that i) the interference with androgen receptor (AR) gene transcription represents a suitable approach to overcome the resistant mechanisms[3] and ii) a G-quadruplex (G4) forming sequence has been characterized within the AR gene promoter[3], here, we have explored the possibility to block AR transcription by stabilizing the AR-G4s.



METHODS. Fluorescence resonance energy transfer (FRET), circular dichroisms (CD), Taq polymerase stop assay and surface plasmon resonance (SPR) were employed to evaluate the stabilizing activity and affinity of an in-house library of differently functionalized naphtalene diimide (NDI) derivatives as AR-G4 ligands. MTS assay and confocal microscopy were used to investigate the cytotoxicity and cellular uptake of NDIs on a panel of CRPC and normal prostatic epithelial cells. Real-time RTPCR, Western Blot, telomeric-repeat amplification protocol (TRAP) assay were performed to assess the effect of the most promising ligand on gene expression and protein amount of AR and other 92 genes associated with the molecular mechanisms of cancer, and telomerase activity, respectively.
RESULTS. Through biochemical and biophysical methods, the core-extended NDI 7 stood out as the most promising AR-G4 ligand. AR-positive cells were remarkably sensitive to 7 in comparison to AR-negative CRPC or normal prostate epithelial cells. 7 induced a significant impairment of AR mRNA and protein amount, as well as perturbation in the expression levels of KLK3 and of genes involved in the activation of AR program via feedback mechanisms. Moreover, 7 synergistically interacted with enzalutamide, an inhibitor of AR-signaling used in second-line therapies.
CONCLUSIONS. Our data provided the rationale for the future development of alternative treatment for CRPC and other malignancies relying on aberrant androgen signaling, based on AR-G4 recognition and stabilization.

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CLONAL DIFFUSION OF EXTENSIVELY DRUG-RESISTANT ACINETOBACTER BAUMANNII STRAINS (XDR) ISOLATED FROM INTENSIVE CARE UNITS

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2 School of Specialization in Microbiology and Virology, Università degli Studi di Milano, Milano, Italy

BACKGROUND. *Acinetobacter baumannii* is a ubiquitous pathogen resistant to detergents and drying. This bacterium is responsible for severe nosocomial infections especially in intensive care units (ICU), where it is often isolated from patients developing pneumonia after intubation (VAP). The aim of this study was to evaluate the phenotypic antimicrobial resistance profiles and to characterize the molecular mechanisms of carbapenemase resistance. Furthermore, it was evaluated the clonal correlation of these *Acinetobacter baumannii* isolates from the intensive care units of the Spedali Civili of Brescia, a hospital with a low endemicity for this pathogen.
METHODS. In the present study, 17 clinical isolates of *Acinetobacter baumannii* were obtained from patients admitted to the intensive care units of the Spedali Civili of Brescia in the period between January 2015 and March 2018. The identification was performed by mass spectrometry (MALDI -TOF, Biomerieux). The antibiograms were performed using the VITEK2 automatic system. MICs for colistin were determined by microdilution in broth according to EUCAST recommendations. The genes coding for carbapenemases (*bla*OXA-51-like, *bla*OXA-23-like, *bla*OXA-24-like, *bla*OXA-48-like, *bla*OXA-58-like, *bla*OXA-143-like and *bla*KPC) have been detected by PCR. The clonal correlation of these isolates was examined by MLST (multilocus sequence typing) based on the Pasteur scheme of seven constitutive genes (*cpn60, fusA, gltA, pyrG, recA, rplB, rpo*).
RESULTS. All isolates were resistant to most common antibiotics including imipenem (MIC≥ 16µg / mL). Fifteen isolates (88%) were sensitive to colistin (MIC <0.5µg / mL) and had an XDR phenotype, while two were also resistant to colistin with a totally drug-resistant phenotype (PDR). All isolates were positive for the blaOXA-51-like chromosomal / constitutive gene, 15 (83%) had the blaOXA-23-like gene. The MLST analysis identified several STs (ST2, ST19, ST195, ST439, ST577 and ST632). The various STs were grouped into two major clonal complexes: CC2 (11 isolates, 64%) and CC1 (3 isolates, 17%), a clonal complex CC215 comprising the ST577 isolate and two STs not belonging to any known clonal complex.
CONCLUSIONS. Isolates of *Acinetobacter baumannii* with XDR phenotype and

having the blaOXA-23-like gene are the isolates most frequently circulating in these intensive care units, greatly reducing the therapeutic options for these patients often with infaust clinical outcome. The MLST has highlighted the prevalent circulation of the clonal complex 2 and to a lesser extent of the clonal complex 1, similarly to what is the European prevalence for this microorganism.

DECODING THE CENTRAL REPRESENTATIONS OF VISCERAL GASTROENTERIC INPUTS

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AIM. Understanding the complex interplay between gastroenteric information and brain functioning requires a deep analysis of the neuronal substrate where gut-derived information is processed in the brain. In this work we mapped responses from cortical and subcortical areas of the mouse brain to glucose, water and LPS from *E. coli* administration in the lumen of the jejunum.
METHOD. We mapped cortical and subcortical responses to intraluminal stimulation in C57BL/6 mice by functional ultrasound imaging, performed through a cranial window exposing the whole brain in the medio-lateral direction, and spanning a length of ±2 mm around bregma in the rostrocaudal direction. To distinguish responses related to the injection procedure (e.g. piercing of the gut wall) from the ones linked with the administration of the various compounds, we separated the two events in time (10 seconds apart).
RESULTS. We found that the three molecules induces different activations in a same complex intercoceptive network, spanning both cortical and subcortical areas in the brain. Such a network comprises a number of visceral sensory areas, e.g. subregions of the *insular cortex,* multisensory integration areas, such as the *olfactory tuberculum,* known as integrator of olfactory and gustatory information with motivation/reward inputs to drive behavior, reward/pleasure areas, such as the *Lateral Septal Nucleus*, and a large number of limbic areas, such as different nuclei of the *Amygdala* and the *Bed Nucleus of the Stria Terminalis*. This work demonstrated the existence of a complex and widespread interoceptive network in the basal brain, which provides different responses to nutritive, homeostatic or microbial information.

THE VAGINAL AND SEMINAL MICROBIOME IN COUPLES WITH IDIOPATHIC INFERTILITY

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BACKGROUND. The genital tract microbiome has gained increasing interest for its role in reproductive health and fertility. Although the vaginal microbiome is overall well described, there is a considering lack of knowledge about the seminal counterpart, as well as complementary studies on infertile couples.

The vaginal microbiota plays a pivotal role in maintaining physiological homeostasis of the environment and protects from the colonization by opportunistic pathogens. A healthy vaginal ecosystem is characterized by a predominance of *Lactobacillus species*.[1] Lactobacilli actively contribute to lower vaginal pH (< 4.5) through lactic acid production, thereby creating a hostile environment for the growth of most pathogenic bacteria.[2] A marked depletion in lactobacilli causes a perturbation in the vaginal microbial composition, which is typically associated with an overgrowth of anaerobic bacteria (i.e. *Gardnerella, Atopobium, Prevotella*) and an increase in vaginal pH (> 4.5). Vaginal dysbiosis can lead to unfavourable conditions, including bacterial vaginosis (BV)[3] and increased susceptibility to adverse pregnancy outcomes and infertility.

Differently from the vaginal ecosystem, seminal microbiota is characterized by a high species richness, low biomass and high variability, as well as a lack of predominant microorganisms.[4] The polymicrobial community found in semen has diverse origins, since it is related to urethral, cutaneous and vaginal flora.[5] According to current knowledge, the most prevalent *phylum* in semen is Firmicutes, followed by Bacteroidetes, Proteobacteria and Actinobacteria.[6] An increase in Proteobacteria has been associated to infective conditions, like prostatitis.6 However, a descriptive pattern in microbiota composition that could allow discriminating between a fertile and infertile condition is still lacking.

Therefore, in this study we aimed to characterize vaginal and seminal microbiome in infertile couples in order to assess a potential association to infertility.

PATIENTS AND METHODS. The study includes 23 idiopathic infertile couples undergoing intrauterine insemination (IUI) treatment at Fertility center of the IRCCS San Raffaele Hospital, Milan. Vaginal swabs and seminal fluids were collected from 23 women and 23 men, with a mean age of 33 years. Metagenomic sequencing was performed on the V3-V4 region of the 16S rRNA gene using

the MiSeq Illumina platform. Sequencing data were processed with QIIME (Quantitative Insights Into Microbial Ecology v1.9.0) software for the taxonomic analysis.

RESULTS. We performed taxonomic analysis of both vaginal and seminal samples at *phylum*, class, order, family, and genus level. As expected, we observed a different composition between vaginal and seminal microbiome in terms of alpha and beta diversity. In vaginal samples, at *phylum* and class levels we observed the same average distribution of microbial taxa as the one observed in a previous study on idiopathic infertile women[7]. We confirmed as well that seminal fluids contain a great variety of bacteria, with a lack of predominant taxa. Within couples, several microorganisms were found in both partners, suggesting a *continuum* in the genital tract microbiome. Furthermore, in both women and men we observed a few cases of dysbiosis, which deserve additional investigations.

CONCLUSIONS. We believe that this is the first study to investigate vaginal and seminal microbiome in infertile couples. Our results confirm the complementarity between partners' genital tract microbiota and support the hypothesis that infertility condition is associated to a perturbation of the vaginal flora. Deeper taxonomic analysis at species level will permit a better understanding and, hopefully, reveal the functional meaning of the observed alterations in both vaginal and seminal microbiome. Further investigations are needed to assess the impact of our results on reproductive failure.

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FMT AND ANALYSIS APPROACHES

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Clostridium difficile infections are the main cause of nosocomial acquired diarrhea, because of prolonged antibiotic regimens. In the last years, mortality has increased due to recurrent infections caused by metronidazole and vancomicin resistant hypervirulent C. difficile strain 027. Faecal Microbiota Transplantation (FMT) is an infusion of faecal material obtained from healthy donors. Promising findings suggest that FMT may play a role also in the management of other disorders associated with the alteration of gut microbiota. Although the health community is assessing FMT with renewed interest and patients are becoming more aware, there are technical and logistical issues in establishing such a non-standardised treatment into the clinical practice with safety and proper governance. For the analysis of gut microbiota-host interections there are many approaches. In particular, the use of metagenomics has revealed the diversity of the gut microbiota, but it has also highlighted that the majority of bacteria in the gut remain uncultured. Culturomics was developed to culture and identify unknown bacteria that inhabit the human gut as a part of the rebirth of culture techniques in microbiology. Consisting of multiple culture conditions combined with the rapid identification of bacteria, the culturomic approach has enabled the culture of hundreds of new microorganisms that are associated with humans, providing exciting new perspectives on host-bacteria relationships.

THE MICROBIOTA OF THE PROSTATE TUMOR MICROENVIRONMENT

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2 Division of Experimental Oncology/Unit of Urology, URI, IRCCS Ospedale San Raffaele, Milano, Italy
3 Microbiology Unit, IRCCS Ospedale San Raffaele, Milano, Italy

INTRODUCTION. Prostate cancer (PCa) remains among the most relevant tumors in men[1]. While age, ethnicity, and family history maintain their etiologic role as risk factors, throughout the last decades, viral and bacterial infections, inflammatory stimuli, and environmental factors have gained attention for their recognized involvement in terms of prostate biology[2,3]. The advent of new molecular-based methods of identification and characterization of complex microbial populations (microbiota analysis) has led to a new era of microbial discovery, thus unveiling important associations between the microbiome composition and several pathological conditions[4,5]. According to previous demonstrations in different inflammatory and tumor conditions, it is conceivable that modifications in terms of bacterial populations could be also observed in PCa lesions and partly contribute to cancer development[6]. Despite the growing body of literature reporting the composition of the microbiome in various body niches and clinical conditions, a detailed and comprehensive analysis of the microbial ecosystem of the pathologic and healthy prostate tissues has not been reported yet. For this reason, we decided to characterize the microbiome associated with the non.tumor, peri-tumor, and tumor tissue of the human prostate by using massive ultra-deep pyrosequencing in order to assess its relevance upon the pathogenesis of PCa.
MATERIALS AND METHODS. Prostate specimens from 16 White Caucasian, nondiabetic, non-obese PCa patients submitted to radical prostatectomy were considered for the analyses. Specimens were postoperatively staged according to the TNM staging system and American Joint Committee on Cancer stage groupings (I–IV). Matched tumoral (T), peri-tumoral (PT), and non-tumoral (NT) areas were isolated from each FFPE peripheral zone of prostate tissue samples. Total DNA was purified from the samples and the V3-V5 region of the 16s rRNA gene was amplified using a nested PCR protocol. Amplicons were then purified and used for emulsion-PCR and ultra-deep pyrosequencing, following 454 GS Junior manufacturer's instruction (Roche). Sequences with high quality score and length >250bp were used for the taxonomic analysis with QIIME (Quantitative Insights Into Microbial Ecology v1.6.0) software.
RESULTS. We analyzed three distinct areas of each prostate (T, PT, and NT) and performed analysis at *phylum*, class, order, family, and *genus* levels. At *phylum* level, we observed an enrichment in *Actinobacteria*, as the dominant *phylum* in all types of samples, followed by *Firmicutes* and *Proteobacteria* and at *genus*

level *Propionibacterium spp*. was the most abundant. We observed significant differences in specific microbial populations among the different areas. In particular, *Staphylococcus* and *Streptococcus spp.* were more represented, respectively, in the tumour/peri-tumour tissues and in the non-tumor tissues ($p<0.05$). To assess potentiality of specific bacteria to be exploited as new biomarkers, we also characterized the urine microbiome from the same PCa-patients in comparison to urine from men with benign prostate hyperplasia, as control. As expected, alpha-diversity demonstrated a richer microbiome in urine samples when compared to the prostate ($p<0.05$). The beta-diversity could also distinguish urinary samples from prostate samples but not differentiate among the three different areas of the prostate.

CONCLUSIONS. Prostate tissue contains a great variety of bacteria that reside within the gland with a distribution dependent on the nature of the tissue (non-tumoral, peritumoral, tumoral). This suggests a possible pathophysiological correlation between the composition of the local microbial niche and the presence of the tumor itself. Future studies will help to clarify the role of these specific bacteria and their potential to be exploited as new biomarkers.

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A HIGH-THROUGHPUT LOSS-OF-FUNCTION APPROACH TO UNCOVER NOVEL PLAYERS INVOLVED IN PERMANENT EPIGENETIC SILENCING

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BACKGROUND. Transposable elements (TEs) are able to move throughout the genome, representing both drivers of evolution and mutagenic agents. The host genome has evolved powerful mechanisms to permanently block TEs mobilization, including heterochromatin deposition at the regulatory sequences of the TEs by epigenetic repressors. We have exploited the functional domain of these repressors to generate Engineered Transcriptional Repressors (ETRs) recognizing the promoter of human genes of therapeutic relevance. When transiently delivered into the cells, these ETRs were able to instruct repressive chromatin, ensuring effective (up to 90%) and long-term (up to 180 cell generations) silencing of multiple endogenous genes. Importantly, silencing was relieved only by targeted DNA demethylation (Amabile*, Migliara* et al., *Cell*, 2016).

METHODS. To identify the endogenous players involved in the processes of permanent silencing induced by the ETRs, we performed genome-wide loss-of-function (LOF) studies in the ETR-silenced cells. To this end, we have generated drug-inducible Cas9-expressing cell lines, and transduced these cells with CRISPR pooled gRNA libraries against genes with either unknown function or encoding for nuclear proteins.

RESULTS. Upon Cas9 induction, a fraction of the transduced cells reactivated the expression of the ETR-silenced genes. We then purified the reactivated cells and exploited Next Generation Sequencing to identify the integrated gRNAs, thus retrieving a list of candidate genes involved in epigenetic silencing. Importantly, further experiments confirmed that cells reactivate the ETRs-target gene upon knock-out of some of the candidates. Interestingly, beyond the expected DNMT1 gene, most of the validated hits have not been linked before to epigenetic silencing.

CONCLUSIONS. By a high-throughput LOF approach we are uncovering novel players involved in epigenetic silencing. We are now starting to both assess the relevance of these players in physiologic processes of epigenetic regulation, such as multipotent cells differentiation, and identify their molecular mechanism of action.

LIVER-DIRECTED GENE THERAPY FOR HEMOPHILIA B WITH IMMUNE STEALTH LENTIVIRAL VECTORS

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BACKGROUND. Gene therapy is rapidly gaining renewed interest and is emerging as a realistic treatment option for several genetic and acquired diseases. Underlying this success is the development of improved gene transfer vectors. Among them, lentiviral vectors (LV) are emerging as versatile vehicles of relatively large capacity for stable transgene integration in the genome of target cells.
METHODS. We developed LV that achieve stable transgene expression in the liver and establish correction of hemophilia, in mouse and dog models of the disease, upon systemic administration. These LV stringently target transgene expression to hepatocytes through transcriptional and microRNA-mediated regulation. We modified the vector envelope by changing the protein composition of the producer cell plasma membrane and obtained novel LV that are more resistant to phagocytosis and innate immune sensing because of increased surface display of CD47 (CD47hi LV), a natural phagocytosis inhibitor, and the lack of polymorphic major histocompatibility molecules (MHC-free LV), that may trigger allogeneic immune responses.
RESULTS. We have now administered these MHC-free or MHC-free/CD47hi-LV to 6 non-human primates (NHP). Administration was well tolerated, without significant elevation of serum aminotransferases or increase in body temperature and only caused a transient self-limiting leukopenia. Human-specific FIX activity reached up to 300% of normal and was nearly 3-fold higher in the CD47hi-LV treated animals. Upon necropsy, we measured vector copies in liver, spleen and major organs of treated animals and found between 0.5 and 1.5 LV copies in the liver accounting for 80-90% of all the retrieved LV copies, showing selective targeting and efficient gene transfer to the liver by LV in NHP.
CONCLUSION. Overall, our studies show a favorable efficacy and safety profile of these LV in NHP and position them to address some of the outstanding challenges in liver-directed gene therapy for hemophilia and conceivably other diseases.

DISSECTING CELL-AUTONOMOUS AND PARACRINE FUNCTIONS OF CELL SENESCENCE IN AML RESPONSE TO THERAPY

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Acute myeloid leukaemia (AML) is a common and aggressive blood cancer, associated with dismal prognosis. First-line treatment of AML relies on cycles of intensive chemotherapy that by triggering DNA Damage Response (DDR) activation may in turn lead to senescence. The establishment of therapy-induced senescence (TIS) represents a tumour suppressive mechanism, recently exploited as a powerful anti-cancer strategy in solid tumours. Cellular senescence is characterized by cell cycle arrest, DDR induction and by the activation of the so-called Senescence Associated Secretory Phenotype (SASP) that can promote either immune-surveillance. Because of SASP paracrine effects, senescent cancer cells, if not eradicated, may contribute to cancer relapse. Therefore, it is important to unveil the contribution of TIS in AML response to therapy.

In this study, we characterized induction of senescence in AML cell lines following different genotoxic stressors. We found that irradiation and hydroxyurea treatment were both able to induce senescence in different AML cell lines that were characterized by cell cycle arrest and by a strong induction of SASP. To prove the paracrine detrimental effect of SASP, we used senescence-derived conditioned medium (CM) to culture untreated AML cells. Interestingly, we observed a proliferative boost in AML cells cultured in CM derived from senescent cells. This suggests the presence of pro-tumorigenic factors released by senescent cells. Importantly, we discovered that Anakinra, the recombinant antagonist of IL1R, rescues the proliferative advantage of AML cells cultured in the presence of senescence-secreted factors, thus suggesting that IL1α and/or IL1β are involved in the process. Overall, in this study we present SASP as possible target to improve AML response to therapies.

This scenario emphasized the need to better understand the molecular events underlying leukemic cells response to chemotherapy with the final long-term goal to identify predictors of AML outcome and develop hypothesis-driven strategies for AML treatment.

ZIKA VIRUS REPLICATION IN DORSAL ROOT GANGLIA EXPLANTS FROM INTERFERON RECEPTOR1 KNOCKOUT (IFNR1) MICE CAUSES MYELIN DEGENERATION

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BACKGROUND. Zika virus (ZIKV) is a neurotropic agent that has been recently link to acute peripheral neuropathies in adults, such as Guillain-Barre Syndrome (GBS). Although ZIKV tropism for the central nervous system has been extensively studied, its capacity of infecting the PNS has been poorly explored. Two recent study show that ZIKV can directly infect Schwann cells (SCs) and peripheral neurons, however, whether ZIKV can directly infect mature SCs, which have already formed myelin, and cause demyelination is unknown. For this reason, we tested whether ZIKV productively infects myelinating SC and, eventually, altered myelin stability.

METHODS. Dorsal root ganglia (DRG) explants are isolated from *Ifnar1*-KO mice at embryonic day 13.5 and cultured for 14 days to undergo myelination. Then, the DRG explants are infected with two different ZIKV strains: the historical MR766 and the 2015 Puerto Rican PRVABC59. The kinetics of ZIKV replication is determined both by immunofluorescence and by plaque forming assay. Peripheral DRG neurons are visualized by neurofilament-H staining, whereas myelin protein zero is used to identify myelinating SC and ZIKV infection is revealed by anti-viral envelope protein monoclonal Ab.

RESULTS. We show that both ZIKV strains establish a productive infection in DRG explants. Both neurons and SCs are productively infected by ZIKV, although the MR766 infection is more efficient than that of the PRVABC59 isolate. Nevertheless, both viruses are cytopathic as measured by both the cleavage of Caspase-3 and the release of Adenynate kinase in the supernatant as an index of cell death. Moreover, a stress response is triggered by ZIKV infection of SCs as observed in immunofluorescence by expression of CHOP in the nuclei of infected cells. This sustained ZIKV-induced stress leads to a progressive myelin disruption.

CONCLUSION. Thus, our results confirm and extend previous observations supporting the hypothesis of a direct role of ZIKV replication in causing PNS pathology that may eventually lead to GBS.

CHRONIC TREATMENT OF Γ-ORYZANOL PREVENTS NEUROINFLAMMATION AND COGNITIVE IMPAIRMENT IN LPS-INJECTED ADULT MICE

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BACKGROUND. γ-Oryzanol is a mixture of phytosteryl ferulates containing ferulic acid esters of phytosterolsis, presents in high amount in the rice (Oryza sativa L.). Its antioxidant and anti-inflammatory properties have been well documented in both in vitro and in vivo models. The fact that it has lipophilic characteristics, allows it to pass the blood brain barrier and potentially exert effects at the CNS. However until now, its central effects are still poor investigated.

METHODS. Mice received 100 mg/kg γ-oryzanol (ORY) or vehicle once a day for 21 consecutive days, followed by an acute inflammatory stimulus elicited by10 µg/mouse i.p. lipopolysaccharide (LPS). Behavioural test were performed 48 h after LPS injection. Successively mice were scarified, and brain area were collected for mRNA expression measures.

RESULTS. LPS alone induced the mRNA expression of the majority of inflammatory markers, including IL1b, IL6, INOS, COX2 in mice hippocampus. In addition, LPS treated mice showed a lower performance in the Novel Object recognition test. ORY pre-treatment prevented LPS-induced neuroinflammatory response and cognitive impairment. Interestingly this rice compound was able to upregulation the phase II antioxidant enzymes, heme oxygenase-1 (HO-1) and NADPH-dehydrogenase-quinone-1 (NQO1), in hippocampus of ORY and ORY+LPS treated mice.

CONCLUSIONS. Altogether these results suggest that a chronic consumption of γ-oryzanol have positive effects on cognitive performance, even in presence of neuroinflammation, through hippocampal antioxidant and antinflammatory molecular responses.

CHIMERIC NEDD4 UBIQUITIN LIGASES AS AN INNOVATIVE APPROACH FOR INTERFERING WITH ALPHA-SYNUCLEIN ACCUMULATION IN PARKINSON'S DISEASE

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BACKGROUND. The main pathological features of Parkinson's disease are the death of dopaminergic neurons and the diffuse accumulation of alpha-synuclein (aS) aggregates in neurons. The NEDD4 E3 Ub ligase has been shown to promote aS degradation by the endosomal/lysosomal route. Interestingly, NEDD4 is protective against human aS toxicity in evolutionary distant models. Furthermore, a small molecule able to activate NEDD4 functions, was neuroprotective in evolutionary distant models of aS toxicity. While activation of E3s cannot be easily obtained pharmacologically, their flexibility and the lack of "consensus" motifs for Ub-conjugation allow the development of engineered Ub-ligases able to target proteins of interest. The aim of our study is to exploit chimeric Ub-ligases specifically targeting aS to prove the protective role of aS degradation pathway towards the development of innovative strategies.
METHODS. To this end, we have developed lentiviral vectors encoding well characterized human aS scFvs fused to the NEDD4 catalytic domain, in order to obtain enzymes that specifically ubiquitinate aS either in its monomeric and/or oligomeric form.
RESULTS AND CONCLUSIONS. The recombinant proteins are expressed in human embryonic 293T cells, as well as in the human dopaminergic neuroblastoma SH-SY5Y cell line. We also generated two lentiviral vectors expressing wild type aS and A53T aS either alone or fused in frame with the reporter protein EGFP. We were able to demonstrate that recombinant lentiviral particles transduce not only 293T and SH-SY5Y cells, but also human iPSCs, neural stem cells (NSCs) and NSC-derived dopaminergic neurons. Finally, a preliminary experiment, showed that a specific degradation of aS is obtained in 293T cells transduced with the chimeric Ub-ligases Nac32HectWT. The results achieved so far represent a starting point strongly supporting the validity of the strategy we intend to adopt in order to obtain a specific degradation of aS.

FULMINANT SEPTIC SHOCK CAUSED BY CAPNOCYTOPHAGA CANIMORSUS IN ITALY: CASE REPORT

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BACKGROUND. Capnocytophaga canimorsus infection was recently recognized as a zoonosis. We report the first case of fulminant septic shock in Italy caused by this pathogen. The patient, with a history of splenectomy, died at the main hospital in Brescia with a presumptive diagnosis of sepsis.
METHODS. PCR and sequencing on post mortem samples confirmed C. canimorsus as a causative organism.
CONCLUSIONS. Our purpose is to alert medical professionals to the virulence of C. canimorsus in asplenic and immunocompromised patients.

ASSESSING THE ROLE OF RSEA AS ANTI-SIGMA FACTOR OF THE *MYCOBACTERIUM TUBERCULOSIS* EXTRACITOPLASMIC SIGMA FACTOR SigE

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BACKGROUND. Rv1222 is a *Mycobacterium tuberculosis* (Mtb) protein which was reported to function as an anti-sigma factor of σ^E by inhibiting the σ^E-RNAP holoenzyme transcription initiation complex formation.
Recently it was proposed a different mechanism according to which Rv1222 inhibits aspecifically DNA transcription and leads to a decrease in bacterial growth rate.
METHODS. A series of Mtb mutants were constructed and characterized in order to evaluate whether Rv1222 could affect the bacteria growth rate and characterize its role in the regulation of σ^E activity through real-time PCR experiments.
RESULTS. Neither deletion nor overexpression of *rv1222* had any direct effect on Mtb growth rate. Real time RT_PCR experiments showed that when Rv1222 was absent the expression of *sigB* (whose expression is regulated by σ^E) was strongly upregulated. However, when Rv1222 was overexpressed we observed a reduction of the *sigB* mRNA explicable with a reduced amount of active σ^E. Finally, the activity of Rv1222 was demonstrated to be σ^E specific, as the expression level of different genes whose expression is not σ^E-dependant did not change in response of *rv1222* deletion or overexpression.
CONCLUSION. In this work, we challenged the recently published hypothesis about Rv1222 function, clearly showing that its deletion or overexpression do not have any pleyotropic effects on growth rate or RNA transcription, but specifically affect σ^E-dependant genes further reinforcing the idea of its role as σ^E-specific anti-sigma factor.

RAPID DIAGNOSTIC FOR A REEMERGENT PATHOLOGY: PERTUSSIS

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BACKGROUND. *Bordetella pertussis* is responsible of a high contagious disease which targets mainly newborns and teenagers.
In the last 10 years resurgences in pertussis infections are attributed to bacterial mutations, capable to elude vaccines (PtxP3, PRN-), and waning immunity; indeed protective immunization after vaccination decreases after 5 years.
In the past cultural and serological methods were standard procedure for pertussis diagnosis in
"U.O.C. di Microbiologia e Virologia della Fondazione IRCCS Policlinico San Matteo di Pavia". Since 2017 LAMP (Loop-Meediated Isothermal Amplification) technique was introduced for a faster (results in less than 1 hour) and more accurate diagnosis.
Objective of this study is the description of samples analyzed in our laboratory with LAMP technology from 2017 to August 2018.
METHODS. LAMP technology is a polymerase chain reaction (PCR) method that detects the target IS481 of *B. pertussis/holmesii/parapertussis* from nasopharyngeal swab and nasopharyngeal aspirate.
RESULTS. A total of 50 sample were analyzed and 10 (20%) were positive for *B. pertussis*. Cases of clinical interest concern two newborns. The first arrived at the Pediatric Emergency Room (ER) with symptoms characterized by cough, food and catarrhal vomiting and an episode of apnea; he was apyretic throughout the hospitalization with an improvement following aerosol therapy with bronchodilator and steroid. The second was admitted to ER following a pertussoid cough aggravated by an episode of apnea. In addition to oxygen therapy and aerosol steroids, clarithromycin was added once the positivity for B. pertussis from pharyngeal swab was confirmed.
CONCLUSION. LAMP technology proved to be a simple and prompt method of pertussis diagnosis, providing a great support in clinic and therapeutic management. LAMP technology will also help in prevent spread thanks to a more efficient surveillance of the disease.

CARBAPENEMASES PRODUCING KLEBSIELLA PNEUMONIAE: AN EPIDEMIOLOGICAL SURVEY AND A LONGITUDINAL STUDY OF THE SWITCH FROM COLISTIN SENSITIVE TO COLISTIN RESISTANT IN AN ITALIAN TEACHING HOSPITAL

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BACKGROUND. The aim of this project was to analyze the spread of carbapenemase-producing Klebsiella pneumoniae (CPKP) in the clinical samples (CS) and in the surveillance rectal swabs (SRS) of patients in a highly accessed tertiary level hospital. Furthermore, this study characterized the evolution of CPKP strains from Colistin-sensitive (CoS) to Colistin-resistant (CoR). Since the '70s the selective pressure exerted by antibiotics has resulted in bacteria that are increasingly resistant and the last 20 years have seen a dramatic rise in the number of MDR strains. Carbapenem and colistin are now the most reliable last resort treatment for Klebsiella pneumoniae (KP) infections but after the spread of strains resistant to β-lactams, it is increased the diffusion of isolates of KP carbapenem and colistin resistant, with an important reduction of treatment options. CPKP is endemic in Italy since 2010 and to date the percentage of resistance is equal to 34%, one of the highest rates in Europe. In Padova hospital a CPKP survey is conducted from January 2012 and it is still ongoing.
MATERIAL AND METHODS. To describe CPKP spread in Padova hospital, the first positive SRS or CS isolated in the medical department, surgical department and intensive care department from January 1, 2012 to December 31, 2017 were included in the study. To characterize the evolution of CPKP from CoS to CoR we enrolled patients admitted to the Padova hospital from January 1, 2014 to March 31, 2016 selected for the detection of a switch from CPKP CoS strain to CoR strain, collected in any material (CS or SRS), during the same admission, or in a subsequent hospitalization. Clinical, laboratoristic and instrumental data were retrospectively collected, as well as data on therapy, outcome and on the role of the infectious disease specialist, when he was involved in the management of the patient. Phenotypic tests for the production of carbapenemases were performed in the swabs and clinical samples. Genotypic tests were also performed using in-house PCR to detect carbapenemases (KPC, VIM, NDM, OXA-48) and multilocus sequence typing (MLST) for further characterization of the strains in order to investigate possible cases of intra-hospital transmission and clusters.
RESULTS. One thousand and thirty CPKP strains were isolated in the 2012 – 2017 study period: 149 strains in 2012 (39 [26.2%] from SRS), 133 in 2013 (70

[52.6%] from SRS), 214 strains in 2014 (164 [76.6%] from SRS), 216 strains in 2015 (143 [66.2%] from SRS), 121 strains in 2016 (83 [68.6%] from SRS), 197 strains in 2017 (138 [70.1%] from SRS). We observed a significant increase in the percentage of positive SRS in 2014 relative to 2013 and 2012 (p=0.001 and p=0.0172, respectively). This trend was not confirmed in 2016 when the number of CPKP decreased. Moreover in 2017 the 33.3% of patients with a positive SRS at the first detection showed a subsequent CPKP detection in other sites: urine (30.4%), respiratory tract (43.5%), skin (10.9%) and blood (15.2%).
We enrolled in the study 48 patients with a CoS strain and subsequently a CoR isolate. The known risk factors for the acquisition of MDR were confirmed: advanced age (median age 63.5), admission in the previous year (66.7%), admission in an Intensive Care Unit for at least one day (81.3%), previous use of antibiotics (72.9%). SRS were obtained within the fourth day of admission in 54.2% of cases. In most cases in Intensive Care Units and in Surgical department the SRS were performed at the entrance or 1-3 days after admission, following the guidelines of the hospital. Among CoS isolated 35 were collected from SRS, 13 from CS; 29 out of 48 had a nosocomial acquisition, 13 were community-acquired and 6 had an unknown origin. Among CoR samples, isolated after the switch of CPKP from CoS, 12 were isolated from SRS, 36 from CS; the median interval from the detection of the first CoS to the first CoR in the same patient was 27 days (range 3- >100 days). 229 infectious disease consultations were required, 127 for the management of CPKP, in 75% of patients after 4 days of CoS isolation. Most patients were treated with the association of colistin + meropenem + tigecycline; 9 subjects received colistin monotherapy with therapeutic failure and resistance development. MLST analysis was performed in 58.3% of samples: in all but 3 CoS-CoR belong to the same ST.
Conclusion: The study underlined the need to implement and improve adherence to active surveillance protocols and to promote phenotypic and molecular analysis to drive to more effective infection control strategies. The data highlighted the important role of the infectious disease consultant, in the correct use of antibiotics, particularly in the management of the colistin therapy.

ESTABLISHMENT OF A MODEL FOR THE IDENTIFICATION OF TREATED SURFACES WITH ANTIBACTERIAL PROPERTIES AGAINST *LEGIONELLA PNEUMOPHILA*

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BACKGROUND. *Legionella pneumophila* is an ubiquitous pathogenic microrganism that is usually found in aquatic environments. Human infection leads to a life-threatening disease mainly occurring through inhalation of contaminated vaporized water.
So far, there are no definitive and standardized solutions to prevent bacterial entry into man-made water systems so the interest in identifying materials that possess antibacterial properties and are, at the same time, safe for health is increasing.
METHODS. Samples of tubes made of a polymeric substrate (MDPE) treated with different antibacterial compounds containing Silver, Zinc, Graphene and Usnic acid were seeded with *Legionella pneumophila* ATCC 33152 strain previously grown in Buffer Charcoal Yeast Exstract (BCYE). Viability analyses upon contact of bacteria with treated surfaces were performed by flow cytometry using the LIVE/DEAD *Bac*Light Kit and a standard plate count assay according to the ISO 22196:2007. We also evaluated the transcriptional profile of stress genes *Legionella pneumophila* after contact with the treated pipes and the inhibition of biofilm formation by quantification of absorbance using the Crystal-Violet assay.
Citotoxicity of different treatments were evaluated by ATP quantification, cell count and microscopic analysis.
RESULTS. Legionella growth was reduced by Silver-based masters (56%) and treatment with Usnic Acid (48%) whereas Zinc and Graphene additivation didn't show antimicrobial properties. Compared to bacterial seeding on MDPE, treatment of Silver and Usnic Acid induced an up-regulation of AhpC2, AhpC1 and AhpD which are redundant genes required for bacterial viability. Moreover, Usnic Acid was also able to partially inhibit biofilm (62%) formation. Unfortunately, Usnic acid showed cytotoxicity, as assessed in mammalian cell culture.
CONCLUSION. Despite we did not identify an optimal treatment for the complete containment of Legionella, our experimental strategy is feasible for the identification of anti-legionella surfaces. A set of reproducible assays is essential to validation of manufacts useful for Legionella containment.

WGS-BASED RETROSPECTIVE ANALYSIS FOR SURVEILLANCE OF *L. MONOCYTOGENES* INFECTIONS IN EMILIA-ROMAGNA (ITALY)

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BACKGROUND. Whole Genome Sequencing (WGS) of clinical, food and environmental isolates is often performed for detecting and tracing of *L. monocytogenes* outbreaks. Nevertheless, due to the long incubation of listeriosis, most outbreak sources remain undetected with the consequence that clusters of genomically-similar isolates remain only presumptive outbreaks. In this condition of missing epidemiological confirmation, the identification of a cut-off of genomic similarity leading to a confident definition of the outbreak borders is crucial for the attribution of isolates to the outbreaks. In this retrospective study, we analyzed human isolates collected in Emilia-Romagna region of Italy from 2012 to 2017 to look for possible outbreaks.
METHODS. A total of 119 *L. monocytogenes* isolates belonging to serogroup IIa were subjected to WGS with 250x2bp paired-end runs on a Miseq Platform. Genomes were de-novo assembled and cgMLST was performed using the BigsDB allele scheme. Genomic clusters were identified according to the cut-off proposed by ECDC. For long-lasting clusters, phylogenetic analysis based on SNPs was performed through molecular clock models in BEAST. Statistical analyses were performed to detect the presence of within-cluster evolutionary signals against date-randomized datasets.
RESULTS. The analysis performed with cgMLST highlighted the presence of 13 clusters, which included between 2 and 21 cases and lasted between 3 months and 5 years. We found that the larger cluster detected showed enough evolutionary signal to allow phylogenetic inference. We estimated a very small effective population size confirming the clonal origin of the infection. We also found that including late isolates did not change the size of the population, suggesting a common origin. The presence within the cluster of isolates with identical sequences did not allow defining a fully resolved tree. By comparing the trees produced by the Bayesian simulations with date-randomized trees, we found a high level of population drift, suggesting that the source of infection is under high selective pressure.
CONCLUSIONS. Our study suggests that allele thresholds should not be used as the only parameter to define *L. monocytogenes* clusters. It is critical to complement allele counts with clustering generated by phylogenetically meaningful algorithms. This approach can help retrospectively evaluate cluster membership and identify persistent strains in food facilities.

OPTIMIZE THE USE OF BLOOD CULTURES FOR THE DIAGNOSIS OF BLOOD STREAM INFECTIONS: THE BACT/ALERT VIRTUO EXPERIENCE

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BACKGROUND. Blood culture (BC) remains the gold standard for diagnosing bacteremia. Over the decades, improvements in culture media and the availability of software-assisted, automated growth detectors have enhanced the recovery of bloodstream pathogens and decreased the time to detection (TTD) of microbial growth. The BacT/Alert VIRTUO Microbial Detection System is a recently developed automated BC instrument which uses a new algorithm for the colorimetric detection of microbial growth. Limitations, however, persist. One of the most important limit involves the diagnostic performance of BCs collected from patients who are already on antimicrobial therapy. In up to 87% of patients with severe sepsis, empirical antimicrobial therapy is started before blood samples for cultures are drawn, and this practice can reduce or delay pathogen recovery. Increasing the diagnostic yield of positive BCs in this setting would allow more effective patient management.
The aims of our study were, first, to compare the VIRTUO, Bact/Alert 3D and Bactec FX automated BC systems in terms of TTD of the most common bacteria and yeasts responsible for Blood Stream Infections under simulated conditions. In the second part of the study we compared microbial recovery and TTD of the VIRTUO and the Bactec systems, using simulated BCs containing therapeutic levels of antibiotics.
MATERIALS/METHODS. I part: we used 330 clinical blood isolates, including 14 species of Gram-negative bacteria, 14 species of Gram-positive organisms and 5 species of yeasts. For each species, 10 strains were tested in duplicate. The media used with the BACTEC were the Plus Aerobic/F, Plus Anaerobic/F, and Peds Plus/F (pediatric), while BacT/ALERT FA Plus (aerobic), FN Plus (anaerobic) and PF Plus (pediatric) were used with the BacT/ALERT and the VIRTUO systems. Each bottle was inoculated with 8 ml (4 ml for pediatric bottle) of fresh, whole blood and a bacterial suspension of 30-50 CFU for bottle and immediately incubated in the instruments.
II part: Overall 48 organism-antimicrobial combinations were evaluated. BacT/ALERT FA Plus and FN Plus bottles and BACTEC Plus Aerobic/F and Anaerobic/F vials, both containing antimicrobial-binding resins, were inoculated with 10 ml of whole blood, 0.5 ml of bacterial suspensions containing 50 to 100 CFU and 0.5 ml of each antibiotic at 3 different concentrations.

RESULTS. I part: Out of the 2850 BC bottles used in this study, 2820 (98.9%) had bacterial growth detected by the automated BC systems. The bacterial growth was detected earlier in VIRTUO instrument than in BacT/Alert 3D and BactecFX systems (p<0.001) except for *S. maltophilia*. Mean TTD differences were 2 and 1.89 h, for aerobic and anaerobic bottles respectively and 1.85 and 1.42 h respectively for pediatric vials. VIRTUO and BACTEC displayed similar performances for detecting growth of *C. albicans, C. krusei* and *C. parapsilosis* while *C. glabrata* detection was faster in the BactecFx system and *C. tropicalis* in the VIRTUO instrument.
II part: Analyzing the 2280 BC bottles inoculated, for all drug/organism combinations, the FA and FN Plus bottles/VIRTUO system showed a better overall recovery rate compared to Plus/F AE and AN/ BACTEC (980/1104, 88.8% e 891/1104,80.7%, respectively, p <0,001). The average TTDs were 12.95 h and 14.07 h for the VIRTUO system and the BACTEC FX system, respectively.
CONCLUSIONS. This initial evaluation of the VIRTUO system in a simulated BC setting suggests enhanced performance of the former in terms of recovery rate and TTD, with greater ability to neutralize the effects of antimicrobials.

NEUROINVASIVE *STREPTOCOCCOCUS GROUP A* IN PAEDIATRIC PATIENTS: THREE NON-CLUSTERED SUBSEQUENT CASES

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BACKGROUND. *Group A streptococcus* (GAS) is responsible for a wide spectre of human pathologies. Since 1980s a great increase in the incidence of GAS-related invasive diseases has been observed but GAS neuroinvasion remains a rare event. Here we report a 3-cases serie occurred in Brescia from Febuary to April 2018.
METHODS. A total of 4 GAS strains, isolated from nasal swab (SP1), CSFs (SP2 and SP3) and abscess (SP4), were subjected to Whole Genome Sequencing (WGS) with 250x2bp paired-end runs on a Miseq. Genomes were de-novo assembled and compared to GAS genomes downloaded from international databases using kSNP3. Phylogenetic distances were inferred by Maximum Likelihood (ML) algorithms using PhyML. Virulence factors were detected by mapping reads against Virulence Factor (VFDB).
RESULTS. GAS neuroinvasion occurred in a two-years-old female with meningitis (sample SP1 and SP2), a six-years-old male with meningitis and post-infection sequelae (sample SP3) and, a four-years-old female presenting crebellar abscess that leaded to exitus (sample SP4).
Among virulence factors (VF), the M protein is crucial and its gene is used as the basis for GAS typing. Strains SP1 and 2 were found to be close related to MGAS 15252 and no exclusive VF were found comparing these isolates with others in this study (SP3 and 4). SP3 resulted to be an M6 type GAS similar to MGAS10394 with an exclusive VF (exoenzyme mf4). SP4 strain, resulted to be a Manfredo M5 strain, carrying several exclusive VF: exoenzyme sda and exotoxins speA e speI.
CONCLUSIONS. Genomic investigation elucidated that neuroinvasive GAS isolates belong to different known virulent type (e.g. M5 and M6 type) and highlighted that they possess several significant VFs. According to the detected genes, SP4, isolated from the patient with the worst outcome, appeared as the most virulent among the analyzed strains.

BIOACTIVE COMPOUNDS FROM GRAPE CANE EXTRACTS: AN ANTIMICROBIAL EVALUATION

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BACKGROUND. Antimicrobial drugs is lacking of innovative therapies leading the possibility to develop resistance mechanism in the major part of microorganism. In this scenario the World Health Organization (WHO) defines as priority the discovery of new drugs able to fight the superbugs that are emerging. For instance the Herpes Simplex Virus type 1 strain resistant to acyclovir and its modifications are nowadays a reality. In this scenario the characterization and the development of innovative antimicrobial therapies represent a mission for the researcher. In this direction in the last years there was a growing interest toward the exploitation of agro-industrial wastes as natural sources for the production of high added-value compounds. Usually, these residues are burned or used for composting, although they still contain valuable bioactive molecules that can be used for several purposes in many sectors. Even though the early research on active molecules was mainly directed towards pure compounds, an alternative trend focuses on mixtures of natural molecules; indeed, it has been established that natural compounds' blends are more active than the isolated molecules thanks to their synergic activity. Based on this class of natural compounds we developed a new treatment against Herpes simplex virus type 1 and 2 (HSV-1 and 2) infection through the exploitation of waste in the agro-industrial chain.

MATERIALS AND METHODS. The antiviral activity was evaluated against HSV-1 and HSV-2 through co-treatment, cell pre-treatment, virus pre-treatment and post-treatment assays in a range of concentrations between 0,01microg/ml of solution and 100 ~g/ml. The toxicity of natural extract was evaluated via MTT assay at 24h and 48h post stimulation. The Chemical composition was evaluated via filtration, HPLC and Mass Spectrometry.

RESULTS. The natural extract was not toxic on the cellular system used even at maximum concentration of 1 mg/ml. The antiviral activity achieved was in the range of 2-10 ~g/ml in the virus pre-treatment assay for both HSV-1 and HSV-2. This widespread therapeutic window leads the possibility, *bonafide*, to translate the extract in a commercial product.

CONCLUSIONS. We developed a natural treatment for the HSV-1 and 2 infections that could represent an alternative route compared to the acyclovir.

HELIX ASPERSA MULLER MUCUS (HELIXCOMPLEX ®) P41 PROTEIN PREVENTS *PSEUDOMONAS AERUGINOSA* GROWTH AND PROMOTES MAMMALIAN BRONCHIAL EPITHELIAL CELL PROLIFERATION

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BACKGROUND. Snail mucus is a mixture of active substances commonly thought to have healthy properties for the treatment of skin disorders. Although snail mucus is an ingredient of various cosmetic and para-pharmaceutic products, the chemical composition and the biological effects are still unknown.

MATERIALS AND METHODS. Crude extract from *Helix aspersa muller* mucus (HelixComplex®) was obtained (Patent N:102017000117547) and, after chemical and molecular characterization, tested on in vitro experimental models of microbial infections and bronchial epithelial cell proliferation.

RESULTS. Differently from what expected, HelixComplex® was characterized by the presence of small amounts of glycolic acid and allantoin. In size separation experiments, we observed a peculiar protein band at 30-40 kDa. Protein band was extracted and analyzed identifying a single protein of 41kDa (p41). The product obtained from size exclusion centrifugation at 41 kDa showed anti-bacterial activities. In particular, it presents a strong bactericidal effect on *P. aeruginosa*, with an IC50 dose of 16ug/ul, as early as 15 minutes after treatment, on both laboratory strain PAO1 and *P. aeruginosa* clinical strains. This bactericidal effect was evident on both planktonic and biofilm-forming cultures of clinical *P. aeuruginosa* strains. By using different *in vitro* assays on epithelial bronchial cell cultures, we found that HelixComplex® lacked of cytotoxicity, and, importantly, that it was able to significantly induce cell proliferation.

CONCLUSIONS. These results identify the *H. aspersa* snail mucus p41 as a suitable antimicrobial compound for the treatment of *P. aeruginosa* associated infections, where we need to: i) block *P. aeruginosa* proliferation and biofilm formation; ii) ensure a regenerative efficacy on bronchial epithelial cells. Interestingly, we obtained efficacy results only in the presence of a protein complex purified by size exclusion centrifugation. This procedure guarantees the presence of the p41, but also of other actives present in the *H. aspersa* mucus, confirming the power and uniqueness of the actives present inside this natural product.

BACTERIAL QUORUM SENSING SYSTEM: A TARGET FOR TREATMENT OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* INFECTIONS

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BACKGROUND. *Staphylococcus aureus* is involved in a large variety of disease. Due to the invasive nature of infections and the spreading of antibiotic resistance, the development of new interventions without the selective pressure of antibiotics is mandatory. Quorum sensing (QS) is a bacterial signalling mechanism regulating invasion and virulence. Recent report highlighted Diflunisal as inhibitor of QS in *S. aureus* methicillin-resistant (MRSA) but since it is insoluble a systemic administration is not feasible. In this study we investigated the anti-virulence properties of aza-analogs of Diflunisal acting on the *agr* operon.
METHODS. The cytotoxic activity of aza-analogs was tested by MTT assay on cell lines and primary macrophages. MRSA cultures were incubated with 10 μM aza-analogs for 16 hrs and we evaluated: a) the antibacterial effects by the microplate method; b) the regulation of QS operon by qRT-PCR; c) the hemolysis by incubating rabbit erythrocytes with the MRSA conditioned medium. Finally, RAW264 cells were incubated for 1 or 5 hrs with MRSA treated with aza-analogs. The macrophage killing was assessed by plating macrophage lysates on LB agar plates and counting the survived bacterial colonies.
RESULTS. All the tested aza-analogs reported no cytotoxicity towards mammalian cells and no bactericidal activity against MRSA. On the contrary, aza-analogs downregulated the expression of genes involved in Agr-mediated QS system and showed reduced hemolysis. In particular, we observed that two of the tested aza-analogs increased killing capability in macrophages.
CONCLUSION. The tested aza-analogs inhibited the QS system and reduced virulence factors in MRSA without effects on bacterial survival. Therefore, these new compounds can be proposed as new agents for MRSA infections.

STUDY OF THE ANTI-HSV HUMAN HUMORAL RESPONSE

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BACKGROUND. HSV-1 and -2 infections are characterized by high incidence worldwide. FDA approved systemic drugs, can improve clinical outcome. However, these drugs can have side effects when high doses and high frequency of administration are required. Moreover, drug resistant HSV isolates have been so far described. Among the novel anti-HSV compounds under development, human monoclonal antibodies (mAbs) are a promising therapeutic option. The synergy between a neutralizing human mAb protecting mice from lethal HSV challenge, named IgG#33, and standard therapy was demonstrated. Moreover, for better dissecting the biological activity of such mAb, its capability to block HSV infection was tested in combination with human sera collected from subjects infected by HSV-1 or -2.

MATERIAL AND METHOD. Vero E6 cells and the laboratory strains of HSV-1and HSV-2 were used. ELISA assays were performed to analyze binding activity while plaque assay, microneutralization and post-entry assay were used to verify the capability of both mAb and sera to neutralize and block HSV infection.

RESULT. The IgG#33 showed greater ability, especially in post-entry assays to block the cell-to-cell transmission mechanism compared to human sera. Post entry assay allows to better mimic the physiological conditions in which the drug is administered after virus infection. Importantly, none of the tested sera interfered with both neutralizing activity of the mAb and its capability to inhibit HSV replication after virus entry.

CONCLUSION. The monoclonal antibody IgG#33 is endowed with high neutralizing activity and great capability to block virus infection even after virus entry into target cells. By the contrary, the human sera, positive for antibodies directed against HSV tested so far, despite able to neutralize the tested viruses, do not show any capability to inhibit virus replication after virus entry at the tested concentrations. Moreover, the presence of synergy between IgG#33 and anti-HSV drugs, the lack of competition observed between serum IgGs and IgG#33 pave the way to a possible systemic use of such IgG for the treatment of HSV recurrences caused by viruses less susceptible to anti-HSV therapy.

WHOLE BLOOD TRANSCRIPTOMIC ANALYSIS OF IMMUNE RESPONSE TO THE RVSV-ZEBOV EBOLA VACCINE

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INTRODUCTION. rVSV-ZEBOV is a live-attenuated recombinant vesicular stomatitis virus vaccine expressing the Ebolavirus glycoprotein and is the only Ebola vaccine with demonstrated clinical efficacy. Goal of this work was to characterize the blood transcriptomics response upon a single injection of the vaccine and identify a predictive signature of efficacy.

METHODS. Whole blood RNA from 64 healthy volunteers, 51 injected rVSV-ZEBOV and 13 with placebo, collected at days 0, 1, 3, 7, 14, and 28 after vaccination, was analyzed by targeted transcriptome sequencing. To characterize the blood transcriptomic response, differential gene expression analysis was performed with edgeR package to identify differentially expressed genes (DEGs) between each time point and baseline, while a time course analysis was performed in python fitting an impulse model, to identify activation and deactivation times (T_a and T_d, respectively) for each gene. Enrichment in blood transcription modules (BTMs) was assessed with tmod package. Spearman's correlation coefficient was calculated to assay correlation between gene expression and antibody titer.

RESULTS. A peak of 5,469 DEGs (FDR<0.05) was detected at day 1 after vaccination. This number decreased over time: no DEGs were found at day 28. Impulse model could well fit 472 genes (R2>0.6) that showed a T_a <30 hours, while only 30 of these genes had T_d>7 days. Functional analysis identified activated innate immunity BTMs from day 1 to day 14 and a plasma cells BTM activated at day 14. Correlation analysis of gene expression with anti-glycoprotein antibody titers identified 15 strongly correlated genes at day 14 after vaccination.

CONCLUSIONS. Vaccination with rVSV-ZEBOV induced a rapid and strong modulation of genes associated with innate response that decreased until day 28 after vaccination. At day 14, a signature of 15 genes was identified, that had a strong correlation with antibody titers measured one year after vaccination.

PREDICTIVE ROLE OF NADIR CD4+ T-CELL COUNT IN THE RISK STRATIFICATION OF OPPORTUNISTIC INFECTIONS IN KIDNEY TRANSPLANT RECIPIENTS

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BACKGROUND. In this prospective longitudinal study, the predictive role of nadir CD4+ T-cell count in the stratification of infectious risk among kidney transplant recipients (KTR) was investigated.

PATIENTS AND METHODS. In 110 KTR, lymphocytes T-cell count (enumeration of T CD3+, T CD4+, T CD8+, CD4+/CD8+ ratio) during the first year post-transplant, as well as viral and fungal infections were strictly monitored.

RESULTS. Twenty-nine patients out of 110 (26,4%) showed nadir CD4+ T-cell count <200 cells/µl while in the other 81 (73,6%) nadir CD4+ T-cell count was ≥200 cells/µl. In the first group a significantly lower and slower CD4+ and CD8+ T-cell reconstitution was observed. Moreover, twenty out of 29 (69%) patients with nadir CD4+ T-cell count <200 cells/µl showed multiple infections (p=0,0170) and the cumulative incidence of opportunistic infections was higher in this group (85,2% vs 67%, p<0,0001). The occurrence of CMV infection was higher in the group of patients with nadir CD4+ T-cell count <200 cells/µl (29/29; 100% vs 67/81; 83%, p=0,0191) as well as the number of treated patients (18/29; 62,1% vs 23/67; 34,3%, p= 0,0143). The occurrence of EBV reactivations was also higher in this group (16/23; 70% vs 21/53; 40%, p=0,0242) as well as the number of treated patients (18/29; 62,1% vs 11/81; 13,6%, p=0,0012). Finally, number of patients with fungal infection was higher in the group of patients with nadir CD4+ T-cell count <200 cells/µl (13/29; 45% vs 20/81; 25%, p=0,0585).

CONCLUSION. Stratification of patients according to nadir CD4+ T-cell count represents a preliminary approach for early identification of transplanted patients with high risk of opportunistic infections.

VEGF-A/NOTCH SIGNALING IN THE REGULATION OF VEGFR2 EXPRESSION IN BILIARY CELLS

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BACKGROUND. Cholangiocytes are a unique example of epithelial cells able to generate and respond to angiogenic factors. In fact, we have previously demonstrated the expression of VEGF and its receptor VEGFR2 in cholangiocytes during liver repair, and more so in cholangiocytes from polycystic liver diseases (PLD). Furthermore, we have shown that the VEGF/VEGFR2 axis sustains the pericystic vasculogenesis, stimulates the proliferation of cholangiocytes, and in cooperation with Notch signaling is involved in tubulization during development or after liver damage. However, it is still unclear how the expression of VEGFR2 and its downstream signaling are regulated in cholangiocytes and this will be the aim of this project.
MATERIAL AND METHODS. To study the mechanisms regulating VEGFR2 expression, we used cholangiocytes isolated from normal and Pkd2KO mice, a model of PLD. Normal and Pkd2KO cholangiocytes cultured as monolayers and organoids were exposed to VEGF-A(25ng/ml), IL1ß (20 U/ml), TNFα (500 U/ml), IFNγ (100 U/ml) alone or in combination with inhibitors of VEGFR2, Notch, ERK, and PIK pathways. VEGFR2, VEGFR2 expression in the nuclear fraction and Src tyrosine kinase activation were assessed by Western Blot (WB). Organoids growth was assessed by measuring their volume overtime for 9 days.
RESULTS. Pkd2KO cholangiocytes showed increased expression of VEGFR2 in the nuclear fraction and enhanced VEGFR2 phosphorylation at Y949, a site that is able to induce Src phosphorylation. In fact, Src phosphorylation was significantly increased after treatment with VEGF-A. Furthermore, VEGFR2 protein levels increased upon administration of pro-inflammatory cytokines ((IL1ß (20 U/ml), TNFα (500 U/ml), IFNγ (100 U/ml)). Noteworthy, exposure to pro-inflammatory cytokines also increased the growth of WT and Pkd2KO organoids. Growth of biliary organoids was significantly reduced by the VEGFR2 inhibitor SU-5416 and by the Notch inhibitor DAPT. WB analysis also revealed that exposure to immobilized-Dll4 inhibited the expression of VEGFR2 while exposure to immobilized Jagged-1 increased VEGFR2 expression. This result was confirmed by silencing of Dll4 or Jagged-1 in Pkd2KO cholangiocytes. Finally, we found that the expression of VEGFR2 induced by VEGF was blocked by both ERK1/2 (UO106) and PI3K (LY294002) inhibitors in Pkd2KO cholangiocytes.
CONCLUSIONS. These findings suggest that VEGF-A may promote the expres-

sion of its own receptor and activation of Src, which may also act as a downstream mediator of VEGFR2 activation. VEGFR2 also cross-talks with Notch signalling during biliary morphogenesis and in response to biliary damage. Our future studies will clarify which member of the Src kinases family is expressed in cholangiocytes and mediates the effects of VEGF, and the effect on VEGFR2 of pro-inflammatory cytokines on VEGFR2 and Notch signalling.

HCV/E2 HYPERVARIABLE REGION 1 PLASTICITY DRIVES ISOLATE-SPECIFIC EPITOPES PROTECTION

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Hepatitis C virus evades the host humoral immune response exploiting several molecular processes that involve a high degree of flexibility and glycosylation, a remarkable structural plasticity of the neutralizing epitopes and the presence of immunodominant hypervariable regions. Glycoprotein E2 hypervariable region 1 (HVR1) has been long thought to act as an immune decoy that directly shields the CD81 binding site on E2. However, by comparing monoclonal antibodies (mAbs) neutralization profile against HCV isolates in their full-length and HVR1-deleted forms, it was recently highlighted how it has a much broader protective role, as it shields several non-overlapping neutralizing epitopes on both E1 and E2 by directly impairing the binding of specific mAbs.
To understand whether HVR1 shielding varies among HCV strains, we swapped HVR1 sequences between different HCVcc isolates and compared mAbs inhibition against chimeras and parental strains. The results herein presented suggest that the HVR1 sequences are sufficient to determine HCVcc sensitivity to neutralization, as the HVR1 sequences of resistant strains confer protection to sensitive strains and *vice versa*.
In absence of extensive direct structural data for HCV E1 and E2 glycoproteins to explain these drastic differences, we modeled HVR1 structures from the tested strains and studied their properties in molecular dynamics simulations. The output highlights drastic differences between HVR1 sequences in terms of flexibility, stability of secondary and tertiary structures and globularity that can be related to the differences in their protective role.
The presented results remark the protective role of HVR1 and how it can drastically vary in response to few mutations, stressing the extremely elevated potential of HCV to evade the host immune response. On the other hand, these results might help the identification of the ideal E1E2 sequence and construct to be used in a vaccinal strategy.

MIXED LINEAGE KINASE 3 CONTROLS PARACRINE INTERACTIONS IN INFLAMMATORY CELL ADHESION AND MIGRATION

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BACKGROUND. Leukocyte extravasation during the inflammatory response is crucial to recruit immune cells from the blood to the inflamed tissue. While extravasating, leukocytes are exposed to a plethora of microenvironmental cues that are relayed intracellularly to promote adhesion, migration and coordinate gene expression. We hypothesize that pro-adhesive and pro-migratory cues could also cooperatively control downstream signals leading to gene expression regulation. We and others have shown that chemokine receptor-coupled β-arrestin2 and stress-activated protein kinases (SAPKs) such as p38 MAPK, play a key role in the process (Molteni et al., 2009; Eichel et al., 2016).
METHODS. We performed protein-protein interaction screening to identify β-arrestin2 interactors. We characterized the proteome and secretome associated to MLK3, an interactor of β-arrestin 2 we found, by mass spectrometry analysis.
Finally we generated knockout cell lines by targeting Osteopontin gene using the CRISPR/Cas9 gene editing system to investigate the role of this MLK3-overexpressing neutrophils secreted protein in inflammatory response amplification.
RESULTS. we identified MLK3, a well-known p38 activator (Gallo and Johnson, 2002), as a novel interactor of β-arrestin2. We found that inducible overexpression of MLK3 in primary neutrophils coordinately controls spreading, adhesion and induction/secretion of paracrine factors promoting the recruitment and swarming behaviour of other neutrophils.
CONCLUSION. MLK3 interacts with proteins involved in cell adhesion, vescicle-mediated transport and secretion of proteins, including the matricellular proteins osteopontin, potentially playing a role in inflammatory response amplification. Knockout cells are being used in functional *in vitro* neutrophils adhesion assays.

IN VITRO MODELING OF PATIENT-SPECIFIC SUSCEPTIBILITY TO FLAVIVIRUSES INFECTION BY USING INDUCED PLURIPOTENT STEM CELLS

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BACKGROUND. West Nile virus (WNV), Zika virus (ZIKV) and Usutu virus (USUV) are mosquito-borne Flaviviruses that generally cause mild or asymptomatic disease in humans. However, WNV may be responsible for severe neuroinvasive diseases in the elderly and immunocompromised subjects, ZIKV may cause fetal microcephaly or Guillain-Barré syndrome in adults, while only a few cases of USUV infection in humans have been described so far, including one recent case of encephalitis in an healthy individual in Veneto region. Aim of this study was to evaluate and compare the susceptibility of human neural cells to WNV, ZIKV and USUV infection and the mechanism of neural injury and immune-inflammatory response.
MATERIAL AND METHODS. Induced pluripotent stem cells (hiPSCs) were generated from individuals with asymptomatic WNV infection and from patients with WNV-dependent CNS disorders (WNND). Patient-specific iPSCs were than differentiated into neural stem cells (NSCs) and infected with contemporary ZIKV Asian lineage (KU853013), WNV lineage 2 (KF179640) and USUV lineage Europe 1 (AY453411). Time course experiments were performed to evaluate viral replication kinetics, cytokine expression, neural cell differentiation markers and cell viability in these patient-specific systems.
RESULTS. Preliminary data showed that USUV and WNV at MOI 0.01 and 0.1 and 1 replicated more efficiently in NSCs derived from symptomatic patients than in those derived from healthy subjects. These evidence were confirmed through evaluation of viral titre by TCID50 which showed higher viral yield in cells derived from WNND patients than in cells derived from asymptomatic subjects, at 72h post infection. Analysis of the effect of flavivirus infection on patient-specific NSCs viability showed that WNV and USUV but not ZIKV induced a marked cytopathic effect on NSCs derived from patient with neuroinvasive disease. Several genes were significantly upregulated in both cell lines after USUV infection, such as the PRRs genes MDA5 and RIG-1, the ISGs IFIT1 and IFIT2 and Caspase1, the protease which cleaves the precursor of inflammatory cytokine IL-1β. Two genes, IRF7 and Casp1, appeared to be upregulated in asymptomatic patient compared to WNND patient-derived NSCs. However, the most notable inflammatory response following USUV infection in both cell line was the strong induction of IFN beta and type3 IFNs, indicating a general activation of the innate antiviral response. WNND-derived NSC infected with

WNV specifically upregulated the expression of the endosomal TLR8 gene, the ISG Viperin and IL-1β.

CONCLUSIONS. Preliminary data showed that WNV and USUV replicated more efficiently and induced cell death in NSCs derived from patients with neuroinvasive disease than in NSCs derived from subjects with asymptomatic WNV infection. Subtle changes in innate antiviral response gene expression were also detected, including decreased caspase-1 levels in NSCs derived from patients with WNND.

HUMAN LUNG EPITHELIAL CELLS SUPPORT HUMAN METAPNEUMOVIRUS PERSISTENCE BY OVERCOMING APOPTOSIS

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BACKGROUND. Human metapneumovirus (hMPV) has been identified as a major cause of lower respiratory tract infection in children. Epidemiological and molecular evidence has highlighted an association between severe childhood respiratory viral infection and chronic lung diseases, such as asthma and chronic obstructive pulmonary disease. Currently, animal models have demonstrated the ability of hMPV to persist *in vivo* suggesting a role of the virus in asthma development in children.
However, mechanisms involved in hMPV persistence in the respiratory tract are not yet understood.
METHODS. For all the experiments A549 cells were infected with MPV at MOI 0.25 and the infection of the cells was assessed by detecting the expression of the hMPV protein F on the cells. Detection of apoptotic DNA fragmentation was performed using the APO-brdUTM TUNEL assay kit and caspase activity was evaluated by using the Caspase-Glo 3/7 assay kit, which measures the combined activity of caspases 3 and 7. Western blot analyses were performed to evaluate the PARP-1 cleavage, phosphorylation of Wee1 and the levels of p53 and Bcl-2.
RESULTS AND CONCLUSIONS. In the present study we monitored hMPV infection in human alveolar epithelial A549 cells in order to understand if the virus is able to persist in these cells upon acute infection. Our data show that hMPV initially induces an apoptotic process in A549 cells through poly (ADP-ribose) polymerase 1 cleavage, caspase-3/7 activation and Wee1 activity. The hMPV-infected cells were then able to overcome the apoptotic pathway and cell cycle arrest in G2/M by expressing B-cell lymphoma 2 and to acquire a reservoir cell phenotype with constant production of infectious virus. These findings provide evidence of the ability of hMPV to persist in alveolar epithelial cells and help in understanding the mechanisms responsible for hMPV persistence in the human respiratory tract.

HHV-6A INFECTION OF NK CELLS MODIFIES THEIR CHEMO-ATTRACTIVE RESPONSE

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BACKGROUND. Human Herpesvirus 6 (HHV-6) is a set of closely related herpesviruses known as HHV-6A and HHV-6B. HHV-6B is the cause of roseola or exanthem subitum. Little is known about the prevalence of HHV-6A or how it is acquired. We have recently observed that HHV-6A and HHV-6B can interfere with NK cell-mediated anti-viral responses, altering miRNAs and transcriptional factors (Rizzo R. et al., Front Microbiol, 2017). Here we propose to investigate if HHV-6A and HHV-6B infection of NK cells alters their chemo-attractive response.

METHODS. NK cells were promptly infected with HHV-6A and HHV-6B, as shown by analysis of virus presence (DNA) and transcription (RNA). NK cell culture supernatants were collected and analyzed for cytokine/chemokine levels by ELISA and for the ability to induce/inhibit cell migration by migration assay.

RESULTS. The infection of NK cells with HHV-6A induced a significant increase in IL-5 and IL-13 cytokines (150 pg/ml; 210 pg/ml respectively) ($p<0.001$; Student T test) and in CCL20 and CCL2 chemokines secretion (876pg/ml; 2000 pg/ml respectively). HHV-6B infection induced prevalently the secretion of interferons (INF-β and –γ, 460 pg/ml and 360 pg/ml respectively). The ex vivo migration assay on purified primary immune cells, showed an effect on T and B lymphocytes and monocytes cell migration only in the presence of the culture supernatants of NK cells infected with HHV-6A ($p<0.05$ Student T test) compared to control. On the contrary, the culture supernatants of NK cells infected with HHV-6B inhibited the migration of monocytes ($p<0.05$ Student T test) Since HHV-6A and HHV-6B infection of NK cells differed for the induction of CCL20 and CCL2 chemokines, we evaluated their implication in the different chemo-attractive response towards immune cells. We performed an inhibitory assay with the addition of anti-CCR6 and anti-CCR2, the receptors for CCL20 and CCL2 chemokines, to the ex vivo migration assay on purified primary immune cells treated with the culture supernatants of NK cells infected with HHV-6A. These treatments drastically reduced cell migration, suggesting that these chemokines, are involved in the chemo-attractive response induced in NK cells by HHV-6A infection.

CONCLUSIONS. In conclusion, we demonstrate, for the first time, the engagement of a peculiar cytokine/chemokine pattern of secretion in HHV-6A-infected NK cells, characterized by a unique chemo-attractive response that

influence immune cell migration and consequently the ability of the host to counteract HHV-6A infection.

This study was supported by HHV-6 Foundation grant and FISM - Fondazione Italiana Sclerosi Multipla - cod. 2015/R/20.

IS THE TIME NOW FOR POSTNATAL CYTOMEGALOVIRUS UNIVERSAL SCREENING? REPORT OF THE BRESCIA CHILDREN HOSPITAL EXPERIENCE

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BACKGROUND. Congenital Cytomegalovirus (cCMV) infection is a main cause of neurodevelopmental disabilities. Most infants (85-90%) do not manifest clinical abnormalities at birth however as much as 10-15 % of cCMV children develop sensorineural hearing loss. A substantial portion of disabilities are not diagnosed by means of newborn hearing screening, therefore a strategy of universal CMV screening at birth could help to improve clinical outcome. Goal of this study was to better define the clinical relevance of performing postnatal universal screening for CMV infection.
METHODS. Saliva samples were obtained by placing a foam swab on the inside of infant cheek. Swabs were immediately eluted in 500 µl of transport medium and stored at -20°C. A 2-µl aliquot of the eluate was amplified without undergoing nucleic acid extraction. Screening was performed using an in house CMV-DNA rt-PCR and positivity were confirmed using CMV ELITe MGB kit. cCMV infection was definitively assessed by quantitation of urine DNA load.
RESULTS. Between October 2015 and September 2017 saliva samples were collected from 6268 neonates 12-24 hs after birth. Twentysix infants (0.41%) were found CMV-positive both at screening and confirmation tests. All babies had high viral load both in saliva (median: 698745 gEq/ml eluate; range 37150-6125482) and urine (median: 942887 gEq/ml eluate; range 154961-7856477). Interestingly, as much as 15 infants (58%) had not cCMV risk factors. Clinical follow-up reveals that 5 out of 15 (33%) not-expected cCMV babies were symptomatic at birth and were therefore enrolled for strict follow-up/therapy. Clinical monitoring of every cCMV child is still ongoing.
CONCLUSION. Saliva PCR assay is a feasible method to identify unexpected cCMV-infected infants who might benefit from early clinical care and/or antiviral treatment.

THE MEASURE OF TORQUETENOVIRUS LOAD AS PREDICTIVE BIOMARKER IN SOLID ORGAN TRANSPLANT RECIPIENTS AND ELDERLY SUBJECTS

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BACKGROUND. Torquetenovirus (TTV) is the most representative and abundant component of human virome. The interplay of TTV with the immune system of the infected host is still poorly understood. However, increasing evidence exists that the immune system plays a role in controlling TTV replication and that the size of TTV replica might be a good marker of dysregulation of immune response.
MATERIALS AND METHODS. TTV loads were studied by in-house single step real-time PCR in a total of 659 samples: 280 plasma samples obtained from liver or kidney transplant recipients during one-year post-transplant follow-up, who underwent different drug regimens to maintain immunosuppression; 379 polymorphonuclear leukocytes from elderly subjects who were followed up for 3 years. Association of TTV load was investigated with a number of parameters (age, gender, type of transplanted organ, immunosuppressive drugs, CMV reactivation, inflammatory/immune markers, and mortality).
RESULTS. With regard to transplant patients, TTV viremia fluctuated irrespective of transplanted organ type but consistent with the immunosuppression regimen. TTV kinetics in patients who manifested CMV reactivation within the first four months post-transplant differed from that observed in patients who did not experience CMV complications. Importantly, TTV viremia above 3.45 log DNA copies/ml within the first 10 days post-transplant correlates with higher propensity to CMV reactivation following transplantation. With regard to elderly subjects, TTV load was positively correlated with age and a value of TTV DNA copies ≥ 4.0 log represented a strong predictor of mortality.
CONCLUSION. Overall, these findings suggest a role of TTV in the prediction of risk factors bound to imbalance of immune system, and provide evidence for the potential use of TTV load as innovative, simple and rapid biomarker in several cohorts of subjects.

G-QUADRUPLEX AND I-MOTIF STRUCTURES IN THE HIV-1 LTR PROMOTER

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BACKGROUND. The LTR promoter within the HIV-1 integrated genome is a key regulatory region for viral transcription. It is highly enriched in GC content (70% in the U3 region) and thus the G-rich strand is able to fold into multiple G-quadruplexes (G4), which modulate viral transcription through binding with different cellular proteins.[1] Conversely, the C-rich strand can potentially fold into i-motifs (iM). These structures are characterized by intercalated H-bonds between hemiprotonated C-C^+ base pairs and have been visualized in regulatory regions of the human genome where they are involved in the regulation of transcription.[2,3] However, nothing has been reported about their presence in non-human genomes.
With the ultimate goal of a better understanding of the the HIV-1 viral pathogenesis, here we present for the first time the investigation of iMs in the HIV-1 LTR promoter.
METHODS. Circular dichroism spectroscopy was employed to assess LTR iMs folding and the stability. Br2-footprinting protection assay indicated the possible structure adopted by LTR iMs. A combined pull-down/mass spectrometry approach allowed the identification of cellular proteins bound by these structures. FRET-based techniques assessed the effect of cellular proteins on the LTR iMs.
RESULTS. We demonstrated formation of a major iM, the LTR-IIIc, the folding of which was maintained in the full-length sequence. The footprinting assay provided the LTR-IIIc folding pattern, revealing an unprecedented 4:2:11 loop composition and demonstrating a highly dynamic behaviour for the LTR. Finally, we demonstrated that the LTR iM is recognized by the cellular protein hnRNPK, which strongly stabilizes the LTR-IIIc at physiological conditions.
CONCLUSIONS. Our data clearly show that the HIV-1 integrated genome contains a stable iM, which can be individually recognized by cellular proteins. These outcomes provide new insights into the investigation of HIV regulatory mechanisms, paving the way to innovative possible targets for antiretroviral therapy.

** This work was supported by the European Research Council (grant ERC Consolidator No. 615879 to S.N.R.).*

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THE HIV-1 MATRIX PROTEIN P17 INDUCES A PROCOAGULANT STATE AND PROMOTES THROMBOSIS

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BACKGROUND. HIV infection has been recognized as a prothrombotic condition. HIV infected (HIV^+) patients show a two to ten-fold increased risk of venous thrombosis in comparison with a general population of the same age. The precise mechanisms driving a state of activation of coagulation in HIV+ patients are not entirely clear, even if appears to involve systemic inflammation and endothelial cell (EC) activation. In combined antiretroviral therapy (cART)-treated patients, in the absence of any viral replication, a possible direct role of HIV proteins in sustaining inflammation and immune cell activation has not yet been considered in detail until now. The matrix protein p17 (p17) is continuously released into the extracellular space from HIV infected cells. Extracellularly, p17 activates ECs and promotes a potent pro-angiogenic and lymphangiogenic activity. We tested the prothrombotic activity of this viral protein.
METHODS. Human primary umbilical vein endothelial cells were nucleofected with a mCherry-vWF-expressing plasmid, 24 h after nucleofection, ECs were used to perform fluorescence and ELISA assays after stimulation with or without p17.
RESULT. In this study we provide evidence that the p17-driven activation of human ECs is associated with an increased expression and production of a critical factor of coagulation, von Willebrand Factor (vWF). We used a mCherry-vWF expressing plasmid to transfect HUVECs and monitor vWF accumulation in WPBs by the classical red bright cigar-shaped appearance in the cytoplasm under normal or serum-deprived conditions. P17 induces cytoplasmic vWF accumulation activating a cell stress pathway. Furthermore, we tested the ability of p17 to promote vWF secretion from endothelium. Our data support and demonstrate the key role of autophagy-based pathway in sustaining vWF secretion triggered by p17.
CONCLUSION. In this study, we provide evidence of a direct role of p17 in increasing the risk for coagulopathy in HIV^+ patients, fostering intracellular vWF accumulation and secretion.

EVALUATION OF HAZARA VIRUS INFECTION IN TICK CELL LINES AS A MODEL TO STUDY THE PERSISTENT INFECTION OF CRIMEAN-CONGO HAEMORRHAGIC FEVER VIRUS IN TICKS

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BACKGROUND. Crimean-Congo haemorrhagic fever (CCHF) is a severe tick-borne disease caused by CCHF virus (CCHFV). Ticks of the genus Hyalomma are the principal vectors and they play an important role as natural reservoir. CCHFV establishes a persistent infection in ticks and they can transmit the virus to their hosts during blood feeding. CCHFV infection is asymptomatic in animals while in humans, that are not part of normal zoonotic cycle of this virus, it can causes a haemorrhagic disease.
To date, the mechanism allowing the persistent viral infection in tick are fully unknown. The aim of this study is to evaluate the virus/vector interaction that allows the establishment of the persistent infection in ticks using Hazara virus (HAZV) as a model. HAZV is an apathogenic virus closely related to CCHFV and can be handled in BSL2 instead of BSL4 containment that is required for CCHFV.
METHODS. It has been shown that the presence of viral-derived DNA sequences (vDNA) induces persistent infection without showing any cytopathic effects for some viruses that infect mosquitos or fruit flies, thus promoting host survival and viral persistence. In this study we evaluated if this mechanism can explain the establishment of persistent infections also in ticks. To this end, two Hyalomma anatolicum-derived cell lines, Hae/CTVM8 and Hae/CTVM9, were infected with HAZV at the MOI of 1 and 0.1 FFU/cell. At different time-points post infection, DNA was extracted from infected tick cells and analysed by PCR, using 9 pairs of primers mapping within the S segment of the HAZV ssRNA genome, to investigate the presence of vDNA.
RESULTS AND CONCLUSIONS. Overall our results indicate that HAZV infection induces vDNAs in tick cells, already at 24 hours post-infection and independently from the employed MOI. Experiments with the retrotranscriptase inhibitor AZT suggest that the synthesis of vDNA molecules maybe dependent on reverse transcriptase activity, likely codified by host-specific endogenous retrotransposons.
In conclusion, vDNA synthesis seems to represent a common strategy to control the replication of RNA viruses both in insect than in ticks.

INCREASED SAMHD1 CORRELATES WITH INTERFERON-STIMULATED GENES IN HIV-1-POSITIVE PATIENTS

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BACKGROUND. SAMHD1 is an inducible host innate immunity restriction factor that inhibits HIV-1 replication. The underlying mechanisms of SAMHD1 transcriptional regulation remains elusive and considerable controversy exists over whether type I IFN can support SAMHD1 production. In order to gain new insights into the role played by SAMHD1 in regulating the natural course of HIV-1 infection, we evaluated SAMHD1 expression and its relationship with the IFN response *in vivo*.

METHODS. Peripheral blood mononuclear cells (PBMC) from 388 HIV-1-infected patients, both therapy naïve (n=92) and long-term HAART-treated (n=296), and from 100 gender and age-matched healthy individuals were examined. Treated HIV-1 patients were also divided into two groups based on achieving (n=243) or not (n=53) virological suppression (defined as persistent undetectable viral load) in response to HAART. CD4+ T cells, CD14+ monocytes and gut biopsies were also analysed in HIV-1-infected subjects on suppressive antiretroviral therapy. Gene expression levels of SAMDH1 and ISGs (MxA, MxB, HERC5, IRF7) were evaluated by real-time RT-PCR assays.

RESULTS. SAMHD1 levels in HIV-1-positive patients were significantly increased compared to those in healthy donors. SAMHD1 expression was enhanced in treated patients compared to naïve patients ($p<0.0001$) and healthy donors ($p=0.0038$). Virologically suppressed treated patients exhibited higher SAMHD1 levels than healthy donors ($p=0.0008$), viraemic patients ($p=0.0001$) and naïve patients ($p<0.0001$). SAMHD1 levels were also increased in CD4+ T cells compared to those in CD14+ monocytes ($p=0.038$), and in PBMC compared to those of GALT ($p=0.04$). Moreover, SAMHD1 was expressed more strongly than the ISGs in HIV-1-infected patients ($p<0.0001$ for all the analyses), and positive correlations were found between SAMHD1 and ISGs levels.

CONCLUSIONS. Taken together these findings indicate that SAMHD1 is more strongly expressed than the classical IFN-stimulated genes, increased during antiretroviral therapy and correlated with several ISGs in HIV-1-infected patients.

THE DIMERIC FORM OF HIGH-RISK HPV E6 ONCOPROTEIN SUSTAINS THE TRANSFORMATION PROCESS THROUGH A P53-INDEPENDENT MECHANISM

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BACKGROUND. High-risk human papillomaviruses (HR-HPV) are still the cause of several epithelial cancers worldwide. HR-HPV E6 is the driving oncogene responsible for malignant cell transformation and acts by inducing the proteasome-mediated degradation of many cellular proteins. Recent structural and mutational studies revealed the importance of a conserved alpha-helix (α2) in the N-terminal domain of E6 for its transforming activities. Indeed, a few key residues of the α2-helix were shown to be crucially involved in the interaction with p53. In addition, the α2-helix was previously characterized to be important also for E6 self-association, an event still poorly understood that involves the same amino acids important for the binding to p53.
METHODS. Protein-protein interactions involving the N-terminal α2-helix of HPV16 E6 were studied in living HEK 293T cells with a Bioluminescence Resonance Energy Transfer (BRET) assay and in H1299 cells with a Bimolecular Fluorescence Complementation (BiFC) technique. Functional correlations were investigated through western blotting following the transient transfection of wild-type or dimerization-defective HPV16 E6 into HPV-negative cells.
RESULTS. Our data demonstrate that the hydrophobic residues of the α2-helix of HPV16 E6 are crucial either for the binding of E6 to p53 and for E6 homodimerization. Additionally, our results indicate that E6 self-association and the interaction of E6 with p53 occur independently of each other and, most importantly, E6 homodimerization is a cytoplasmic event. Strikingly, we observed that the dimeric form of E6 is crucially involved in transforming activities which are unrelated to the E6-mediated degradation of p53.
CONCLUSIONS. Taken together, our results suggest that E6 self-association and the interaction of E6 with p53 are two separated processes occurring in different cellular compartments. Thus, here we propose a model in which nuclear E6 binds to p53 as a monomer, while cytoplasmic E6 can dimerize and interact with other cellular targets.

CONTROL OF HPV16 E6* ISOFORMS EXPRESSION BY CELL SPLICING FACTORS IN OROPHARYNGEAL AND CERVICAL CANCERS

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BACKGROUND. Human papillomavirus type 16 (HPV16) is the main causative agent of cervical carcinoma (CSCC) and a sub-group of oropharyngeal carcinoma (OPSCC). Isoforms of HPV16 E6 oncogene such as E6 full, translated in the E6 protein, and E6*, coding for short E6 proteins and favoring E7 translation, are differentially expressed in OPSCC and CSCC. We aimed to study seven human splicing factors potentially involved in HPV16 E6 mRNA alternative splicing.
METHODS. Ten OPSCC, 28 CSCC and 12 cervical intraepithelial neoplasia (CIN) biopsies have been evaluated by RT-PCR for HPV16 full E6 and E6* isoforms as well as for the splicing factors HNRNPA1, HNRNPA2B1, SRSF1, SRSF2, SRSF3, SAM68 and BRM mRNA expression levels.
RESULTS. HPV16 E6, E6*I and E6*II mRNAs were detected in 14.3% of OPSCC, 100% of CSCC and 25% of CIN. The levels of E6 and E6* isoforms were 2-fold higher in CSCC than in OPSCC and CIN ($p \leq 0.007$). The full E6 was less expressed than E6*I in OPSCC and CSCC, while it was higher expressed than E6*I in CIN. The levels of E6*I isoform were higher than E6*II in all the samples and significantly higher in CSCC ($p = 0.002$). The expression of HNRNPA1 and SRSF2 was 2-fold higher in OPSCC and CIN than in CSCC ($p \leq 0.04$) while SAM68 was 2-fold more expressed in CSCC than in CIN ($p = 0.01$). The levels of the other splicing factors were comparable in the different samples.
CONCLUSION. Among the seven cell splicing factors the HNRNPA1 and SRSF2 may play a major role for E6*I production in OPSCC, while the higher expression of SAM68 in CSCC may be related to the abundance of E6*I in cervical cancer. These results suggest that the SAM68-related E6*I mRNA may concur to the E7 oncoprotein accumulation in CSCC.

THE HPV E6 PDZ BINDING MOTIF LINKS DNA DAMAGE RESPONSE SIGNALING TO E6 INHIBITION OF P53 TRANSCRIPTIONAL ACTIVITY

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BACKGROUND. The presence of a PDZ binding motif (PBM) in the HPV E6 oncoprotein appears to be a characteristic marker of high oncogenic potential and confers interaction with a number of different cellular PDZ domain-containing substrates. The E6 PBM is also subject to phosphorylation, resulting in an inhibition of E6 PDZ binding activity and instead allowing E6 to associate with 14-3-3 proteins.
METHODS. In this study, we have analyzed the conditions under which the E6 PBM is phosphorylated. To understand the biological relevance of these phospho-modifications of E6, we analysed their effects upon the ability of E6 to inhibit p53 transcriptional activity.
RESULTS. We demonstrate that in normal cycling cells the levels of E6 phosphorylation are very low. However, following exposure of cells to oxidative stress or the induction of DNA damage, there is a striking increase in the levels of E6 phosphorylation. Depending on the specific stimulus, this phosphorylation of E6 can involve the ATM/ATR pathway and is performed primarily through Chk1, although the Chk2 pathway is also involved indirectly through activation of PKA. We show that an intact E6 phospho-acceptor site plays an essential role in the ability of E6 to inhibit p53 transcriptional activity on a subset of p53-responsive promoters, in a manner that is independent from E6's ability to direct p53 degradation.
CONCLUSION. These results are, to our knowledge, the first example of a DNA damage response controlling PBM-PDZ recognition. This study also provides links between the DNA damage response, the regulation of E6 PBM function, and the inhibition of p53 activity, and begins to explain how HPV-infected cells remain within the cell cycle, despite activation of DNA damage response pathways during productive virus infections.

CHRONIC AND ACUTE HEV INFECTION IN ONE YEAR OF OBSERVATION AT ASST GOM NIGUARDA

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BACKGROUND. Hepatitis E virus (HEV) can lead to acute and chronic hepatitis and it is an emerging cause of liver disease also in high-income countries where estimated prevalence is 2 million of new infections/year. At our knowledge no data from our country are available. HEV has been considered a virus transmitted via faecal-oral route, nevertheless cases of transfusion-transmitted hepatitis E infection were recently described. Its prevention and early diagnosis seems to be a crucial medical and public health concern.

AIM. The aim of our study was to investigate the rate of HEV infection expressed by plasma RNA presence in our hospital "ASST GOM Niguarda".

METHODS. Between September 2017 and September 2018, 420 pts were tested for anti-HEV antibodies with ELISA test (DIA.PRO), among them 167 were also tested for HEV-RNA with Fast-Track Diagnostics kit.

RESULTS. Overall seroprevalence of HEV-IgG was 11.2% (47/420). Eight (4.8%, 8/167) new HEV infection were found in our hospital, they were mainly male (87.5%, 7/8), 50% Italian, with a median(IQR) age of 57(51-6), median follow-up was six months. Among the selected cases, we detected 3 (37.5%) chronic infection, all in immunosuppressed pts for solid organ transplant, they received a specific anti-viral treatment that lead to HEV eradication. The remaining 5 cases showed a symptomatic acute infection, one of them had a concomitant solid neoplasm under treatment. Only 2 pts of this group were treated with Ribavirine. Overall 80% (4/5) recovered HEV infection, whereas one pt died for multi-organ failure.

CONCLUSIONS. HEV is potential under-diagnosed infection, but it is an emerging cause of acute hepatitis and can lead to potential severe liver diseases in immunosuppressed patients. More efforts are needed for increasing diagnosis rate, special in patients without an acute presentation, in order to correctly establish the real weight of infection, to prevent and treat hepatitis E.

HUMAN DDX3 HELICASE INHIBITORS INHIBIT COXSACKIE B5 VIRUS REPLICATION

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INTRODUCTION. Coxsackie B (COXB) viruses are common pathogens belonging to *Picornaviridae*, genus Enterovirus and clustering in serotypes B1 to B6. COXB are small, naked icosahedral viruses with a positive single-stranded RNA genome (7,4 Kb in size) and transmitted by respiratory route. Infection is usually subclinical but, especially in neonates and children, can manifests with variety of syndromes such as myocarditis, pericarditis, aseptic meningitis, encephalitis, and respiratory distress. Neither vaccines nor specific antiviral drugs are available for clinical use. DDX3 is a human RNA helicase playing a key role in cell gene expression and cycle regulation. Since DDX3 has been shown to be involved in replication of HIV, HCV, Dengue, and other viruses, it is considered a potential antiviral target. Indeed, some DDX3 inhibitors have proved broad-spectrum antivirals, particularly against HCV and drug-resistant HIV. From this ground, and as part of UNAVIR, we demonstrated that some DDX3 inhibitors are also effective against the prototype strain COXB serotype 5 (COXB5).
MATERIALS AND METHODS. COXB5 was expanded *in vitro* on KB cells and titrated by limited dilution and plaque assay. Compounds UVR01, 02, 03, 05, 06, 08, 10, 11, 12 and 21 were tested for cytotoxicity on KB cells and antiviral activity using plaque inhibition assay; subsequently, inhibition concentration 50% (IC50) was calculated. Western blot and molecular analyses were used to investigate viral protein and genome production.
RESULTS. All compounds showed no overt cytotoxicity up to 50 μM, although UVR03 and UVR12 slowed down cell proliferation. Except UVR02 and 06 that showed no activity, all compounds reduced COXB5 replication at IC50 values from 0.35 to 12.50 microM.
CONCLUSION. The DDX3 inhibitors tested herein showed potent antiviral activity against COXB5 and low cytotoxicity effect. Further studies are warranted but the results accrued insofar indicate that human DDX3 are promising drug candidates against these viruses.
This work was funded by "UNAVIR malattie virali rare: una strategia innovative per combatterle con un unico agente antivirale" PAR FAS 2007-2013 Bando FAS Salute 2014, Regione Toscana.

NEW ANTIVIRAL STRATEGIES AGAINST INFLUENZA VIRUSES

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BACKGROUND. Influenza viruses cause a highly contagious respiratory disease in humans. In particular, influenza A virus is responsible for periodic widespread epidemics and also for pandemics that can have high mortality rates. Currently available drugs against influenza viruses are M2 blockers and neuraminidase inhibitors, but all of them have several drawbacks, including development of drug resistance, poor efficacy, and toxic side effects. On the other hand, vaccines provide good protection against the infection, but they must be reformulated each year according to the circulating strains.
Thus, there is still an urgent need for new anti-influenza compounds with a broad spectrum of activity and a novel mechanism of action.
Recently, the viral RNA polymerase complex emerged as an interesting target for drug development.
METHODS. The disruption of the interaction between the PA and PB1 subunits of the viral RNA polymerase was assessed through ELISA-based assays. Antiviral activity was assessed by means of plaque reduction assays.
The second part of this project regards the characterization of some analogs of Oseltamivir, a neuraminidase inhibitor. These compounds were tested for their ability to inhibit viral replication of different influenza virus subtypes. Compounds cytotoxicity was assessed through MTT assay.
RESULTS. Some of the anti PA-PB1 compounds were able to inhibit viral replication at low concentrations, although their inhibitory concentrations in the ELISA assays were higher. Unfortunately, some compounds also resulted to be toxic.
Two out of five Oseltamivir analogs resulted effective against different influenza virus subtypes at low concentrations, and none of them was toxic.
CONCLUSIONS. SAR analysis will allow the determination of chemical groups responsible for the disruption of PA-PB1 interaction. Further characterization is required to investigate whether the new Oseltamivir analogs do not elicit the emergence of resistance, differently from Oseltamivir.

QUINDOLINE-DERIVED G4 LIGANDS TARGETING HSV-1 G-QUADRUPLEXES

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BACKGROUND. The herpes simplex virus-1 (HSV-1) genome is distinguished by an unusually high GC nucleotide content (68%) and we identified multiple clusters of repeated sequences that form very stable G4s[1]. The characterized G4s have been shown to be significant elements both for their massive presence during the viral cycle, suggesting a key role in the HSV-1 biology[2], and for the antiviral effects resulting after G4 ligand treatment[1,3]. Considering that the vast majority of G4 binders show poor druggability due to their typical large aromatic surface, the aim of this study was to test the anti-HSV-1 activity of Quindoline derivatives presenting more drug-like features.
METHODS. Circular dichroism (CD), Taq polymerase stop assays and mass spectrometry (MS) competition assays were performed to evaluate the stabilizing activity of the Quindoline derivatives for viral over cellular G4s. Antiviral activity of the compounds was assessed in HSV-1-infected cells and their viral target step was identified by time of addition (TOA) experiment.
RESULTS. CD demonstrated that compounds were able to stabilize the HSV-1 G4s and, moreover, Taq polymerase stop assay proved their capacity to inhibit the polymerase processing at the HSV-1 G4 forming sequences. Through MS competition assays we validated Quindoline derivatives as ligands that preferentially recognize HSV-1 G4s over the cellular telomeric G4, the most represented within cells. Treatment of HSV-1 infected cells with nanomolar concentrations of compounds induced inhibition of virus production. In particular, some compounds showed a very pronounced antiviral activity paralleled by low cytotoxicity, thus presenting promising selectivity indexes (SI). Furthermore, we demonstrated that the last step targeted by the compounds was viral DNA replication, thus we postulated that the Quindoline derivatives inhibit HSV-1 replication by a G4-mediated mechanism.
CONCLUSION. Our data demonstrate that the tested compounds display considerable anti-herpetic activity, thus confirming the importance of G4s as innovative anti-HSV-1 targets.

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IDENTIFICATION, CHARACTERIZATION AND BIOLOGICAL SIGNIFICANCE OF A HIGHLY STABLE G-QUADRUPLEX IN *MDM2* PROTO-ONCOGENE INDUCIBLE PROMOTER

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BACKGROUND. Human cancers are heterogeneous and complex malignancies which remain one of the main causes of death in the developed world. A connection between G-quadruplexes (G4s) and cancer was observed since the six hallmarks were defined[1].
Liposarcoma (LPS) is a rare soft tissue sarcoma arising from mesenchymal cells: it causes high mortality rates when occurring in the retroperitoneal cavity. Treatment of LPS is challenging due to the inefficacy of the conventional chemotherapies[2]. A diagnostic factor for LPS is the genomic amplification of a portion of chromosome 12, containing the proto-oncogene *MDM2*.
METHODS. The computational tool QGRS was used to identify putative G4 sequences in the *MDM2* P2 promoter, Circular Dichroism (CD) and DMS footprinting were used to assess the G4 folding and conformation of a key *MDM2* G4 region, a pull-down assay followed by mass spectrometry allowed the identification *MDM2*-G4 interacting proteins.
RESULTS. In the present project, we identified a highly G-rich region in the inducible promoter P2, main responsible of *MDM2* transcription in the tumoral environment[3]. CD and DMS footprinting confrim the formation of a highly stable, antiparallel G4 in a key region of P2. The ability of the G-quadruplex ligand BRACO-19 to stabilize the identified G4 has been verified and cytotoxicity tested through MTT assay on a liposarcoma cell line (CRL-3043 ATCC). A protein pull down revealed the specific interaction of several helicases with the folded G-quadruplex.
CONCLUSION. The unique and rare conformation of *MDM2*-P2 G4, its vicinity to one transcription factor binding site (e.g. ETS/AP1) and the recruitment of helicaes at its level suggest an involvement of the identified G4 in the regulation of gene expression. Moreover, our observations make *MDM2* G4 an interesting and previously unexplored target for antitumoral therapy.

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