SIV ISV
SOCIETA' ITALIANA DI VIROLOGIA
ITALIAN SOCIETY FOR VIROLOGY
www.siv-isv.org

5th National Congress of the Italian Society for Virology

One Virology One Health

ABSTRACT BOOK

WEBINAR
5-6 JULY 2021

FAD

SEDE

Piattaforma FAD Nadirex
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Nadirex International S.r.l.
Via Riviera, 39 - 27100 Pavia
Tel. +39.0382.525714
Fax. +39.0382.525736

Contact: Gloria Molla
gloria.molla@nadirex.com
mob. +39 347 8589333

Contact: Francesca Granata
francesca.granata@nadirex.com

www.nadirex.com
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SCIENTIFIC PROGRAMME

MONDAY, 5th JULY 2021

OPENING OF POSTER SESSION AND SPONSOR EXHIBITION

08.00-08.30 Registration of participants (access and connection to web platform)

PLENARY SESSION

08.30-09.00 5th National SIV - ISV Congress - Welcome Address

SESSION 1: **COVID-19: EVOLUTION OF A VIRUS**
Chairs: **A. Caruso** (Brescia, Italy), **G. Palù** (Padua, Italy), **M. Tavio** (Ancona, Italy)

09.00-09.20 Lecture 1 - The crisis at the time of coronavirus: genomic evolution of SARS-CoV-2
M. Ciccozzi (Rome, Italy)

09.20-09.40 Lecture 2 - Intra-host evolution of SARS-CoV-2 during persistent infection
S. Fiorentini (Brescia, Italy)

09.40-10.00 Lecture 3 - Genetic variability of the SARS-CoV-2 genomes and its implications
F. Maggi (Varese, Italy)

ORAL COMMUNICATIONS

10.00-10.10 **OC1**: Early diffusion of SARS-CoV-2 infection in the inner area of the Italian Sardinia Island
Presenter: **N. Grandi** (Cagliari, Italy)

10.10-10.20 **OC2**: Identification of highly recurrent SARS-CoV-2 haplotypes circulating worldwide and in Veneto region
Presenter: **L. Manuto** (Padua, Italy)

10.20-10.30 **OC3**: Study of COVID-19 patients harboring variants of SARS-CoV-2: comparison with a population from the first pandemic wave
Presenter: **L. Mazzuti** (Rome, Italy)

10.30-10.40 **OC4**: Usefullness of rt-PCR in SARS-CoV-2 variant surveillance
Presenter: **A. Rizzo** (Milan, Italy)

10.40–10.50 **OC5**: Genomic characterization of the emergent SARS-CoV-2 lineage in two provinces of Campania (Italy): whole-genome sequencing study
Presenter: **M. Scrima** (Ariano Irpino, Italy)

10.50-11.00 Discussion

PARALLEL SESSION - ROOM A

SESSION 2: VIRAL DIAGNOSIS
Chairs: **R. Cavallo** (Turin, Italy), **S. Menzo** (Ancona, Italy)

11.00-11.20 Lecture 4 - A modern, integrated approach to molecular diagnostics of SARS-CoV-2
C.F. Perno (Rome, Italy)

11.20-11.40 Lecture 5 - Immunologic response to SARS-CoV2 infected patients and vaccinated subjects
F. Baldanti (Pavia, Italy)

11.40-12.00 Lecture 6 - Viral gastroenteritis: an overview of current diagnostic methods
G. Giammanco (Palermo, Italy)

ORAL COMMUNICATIONS

12.00-12.10 **OC6**: Colorimetric test for fast detection of SARS-CoV-2 in nasal and throat swabs
Presenter: **M. Cennamo** (Rome, Italy)

12.10-12.20 **OC7**: Analysis of anti-spike neutralizing antibodies titers and levels of circulating biomarkers in COVID-19 patients
Presenter: **F. Frasca** (Rome, Italy)

12.20-12.30 **OC8**: Vaginal self-collected vs cervical clinicians collected samples for cervical cancer screening
Presenter: **C. Sani** (Florence, Italy)

12.30-12.40 **OC9**: Exploratory analysis to identify the best antigen and the best immune biomarkers to study SARS-CoV-2 infection
Presenter: **S. Najafi Fard** (Rome, Italy)

12.40-12.50 **OC10**: Serosurvey in BNT162B2 vaccine-elicited neutralizing antibodies against authentic SARS-CoV-2 variants
Presenter: **A. Zani** (Brescia, Italy)

12.50-13.00 Discussion

PARALLEL SESSION - ROOM B

SESSION 3: **ENVIRONMENTAL AND PLANT VIROLOGY**
Chairs: **F. Di Serio** (Bari, Italy), **L. Rubino** (Bari, Italy)

11.00-11.20 Lecture 7 - Metagenomics of plant-associated viromes
T. Candresse (Bordeaux, France)

11.20-11.40 Lecture 8 - Environmental surveillance for early pathogen identification, epidemic trends and genomic variability: a focus on SARS-CoV-2
G. La Rosa (Rome, Italy)

ORAL COMMUNICATIONS

11.40-11.50 **OC11**: A tripartite narna-like mycovirus reveals the existence of a split RNA-dependent RNA polymerase palm domain hosted by two distinct proteins
Presenter: **M. Forgia** (Turin, Italy)

11.50-12.00 **OC12**: Transcriptomics to reveal the genetic basis of CMD2 resistance in cassava
Presenter: **A.V. Carluccio** (Bari, Italy)

SPONSORED SYMPOSIUM 1
with the unrestricted educational grant of QIAGEN SRL
Chair: **T. Lazzarotto** (Bologna, Italy)

12.00-12.20 A changing respiratory infection diagnostic paradigm amidst the COVID-19 pandemic
D. Manissero (London, UK)

12.20-12.30 Discussion

13.00-14.00 Break

PARALLEL SESSION - ROOM A

SESSION 4: **GENETICS, BIOTECHNOLOGY AND BIOINFORMATICS**
Chairs: **G. Portella** (Naples, Italy), **M.C. Parolin** (Padua, Italy)

14.00-14.20 Lecture 9 - Network medicine in infectious diseases
G. Ippolito (Rome, Italy)

14.20-14.40 Lecture 10 - CRISPR: diagnosis and treatment of viral infections
M. Pistello (Pisa, Italy)

14.40-15.00 Lecture 11 - Clinical applications of viral vectors for gene therapy
N. Brunetti Pierri (Naples, Italy)

ORAL COMMUNICATIONS

15.00-15.10 **OC13**: In silico and in vitro combined approaches identified promising candidates as SARS-CoV-2 and HCoV-OC43 inhibitors
Presenter: **I. Arduino** (Turin, Italy)

15.10-15.20 **OC14**: SARS-CoV-2 genomic characterization in Campania, Italy
Presenter: **A. Grimaldi** (Naples, Italy)

15.20-15.30 **OC15**: Plant-produced VP2-based particles provide protection against very virulent Infectious Bursal Disease Virus
Presenter: **C. Marusic** (Rome, Italy)

15.30-15.40 **OC16**: Identification of the nuclear proteome from all human viruses by a comprehensive analysis of classical nuclear localizations
Presenter: **H. Ghassabian** (Padua, Italy)

15.40-15.50 **OC17**: High-throughput platforms to assess neutralizing antibodies and antiviral molecules against SARS-CoV-2
Presenter: **D. Stelitano** (Naples, Italy)

15.50-16.00 Discussion

PARALLEL SESSION - ROOM B

SESSION 5: **FRONTIERS IN GENERAL VIROLOGY 1**
Chairs: **A. Calistri** (Padua, Italy), **G. Franci** (Salerno, Italy)

14.00-14.20 Lecture 12 - Viral respiratory pathogens: direct and indirect mechanisms of lung injury
N. Mancini (Milan, Italy)

ORAL COMMUNICATIONS

14.20-14.30 **OC18**: Molecular features of the measles fusion complex: infection and spread in the central nervous system
Presenter: **F.T. Bovier** (Naples, Italy)

14.30-14.40 **OC19**: Nrf2 and G6PD as key players in modulating cell antioxidant response and influenza virus replication
Presenter: **M. De Angelis** (Rome, Italy)

14.40-14.50 **OC20**: Analysis of adaptive immune response to SARS-CoV-2 infection and the acquired immune response after COVID-19 vaccination with different assays
Presenter: **F. Bossi** (Trieste, Italy)

14.50-15.00 **OC21**: The expression of the truncated ACE2 isoforms only is related to the interferon response in airway epithelial cells from young adults
Presenter: **G. Oliveto** (Rome, Italy)

SPONSORED SYMPOSIUM 2
with the unrestricted educational grant of ADA
Chairs: **G. Scalia** (Catania, Italy)

15.00-15.20 Active Surveillance of Asymptomatic SARS-CoV- Infection
E. Borghi (Milan, Italy)

15.20-15.40 The impact of automation in the COVID-19 pandemic management
Marc Lütgehetmann (Hamburg, Germany)

15.40-16.00 Discussion

PLENARY SESSION

SESSION 6: **VIRUS-HOST INTERACTION**
Chairs: **E. Affabris** (Rome, Italy), **G. Gribaudo** (Turin, Italy)

16.00-16.20 Lecture 13 - The SERINC enigma in viruses and cells
M. Pizzato (Trento, Italy)

16.20-16.40 Lecture 14 - ACE2: a key factor in regulating SARS-CoV-2 and influenza virus infection
L. Nencioni (Rome, Italy)

16.40-17.00 Lecture 15 - Identification of a novel gateway for SARS-CoV-2 entry into human endothelial cells
F. Caccuri (Brescia, Italy)

ORAL COMMUNICATIONS

17.00-17.10 **OC22**: The first high detailed human nuclear proteome and the HPV16 genome interaction
Presenter: **G. Franci** (Salerno, Italy)

17.10-17.20 **OC23**: Parallel G-quadruplexes recruit the HSV-1 transcription factor ICP4 to promote viral transcription in infected human cells
Presenter: **I. Frasson** (Padua, Italy)

17.20-17.30 **OC24**: Dissecting lyssavirus-host interaction in the syrian hamster model
Presenter: **M. Castellan** (Legnaro, Italy)

17.30-17.40 **OC25**: The US21 viroporin of human cytomegalovirus regulates cell adhesion and migration
Presenter: **A. Luganini** (Turin, Italy)

17.40-17.50 **OC26**: The innate immunological response mediated by PKR is counteracted by herpes virus tegument proteins
Presenter: **R. Pennisi** (Messina, Italy)

17.50-18.00 Discussion

18.00-19.00 **SIV-ISV GENERAL MEMBER MEETING**

18.00-20.00 POSTER SESSION AND SPONSOR EXHIBITION

TUESDAY, 6th JULY 2021

OPENING OF POSTER SESSION AND SPONSOR EXHIBITION

PLENARY SESSION

SESSION 7: VIRAL ONCOLOGY

Chairs: **F.M. Buonaguro** (Naples, Italy), **P. Marconi** (Ferrara, Italy)

09.00-09.20 Lecture 16 - The oncogenic role of HPV in the pathogenesis of orofaryngeal and genital cancers
M.L. Tornesello (Naples, Italy)

09.20-09.40 Lecture 17 - Oncolytic viruses for cancer therapy
P. Malatesta (Genoa, Italy)

09.40-10.00 Lecture 18 - Therapeutic vaccines for virus-associated tumors
A. Venuti (Rome, Italy)

ORAL COMMUNICATIONS

10.00-10.10 **OC27**: Immunotherapeutic efficacy of an oncolytic HSV retargeted to prostate specific membrane antigen
Presenter: **T. Gianni** (Bologna, Italy)

10.10-10.20 **OC28**: dl922-947 adenovirus and G-quadruplex binder combination against breast cancer
Presenter: **A.M. Malfitano** (Naples, Italy)

10.20-10.30 **OC29**: The cellular deacetylase SIRT1 contributes to p53 curbing by HPV16 and 18 and its targeting inhibits cancer cell proliferation
Presenter: **I. Lo Cigno** (Novara, Italy)

10.30-10.40 **OC30**: The unprecedented wide interaction of HTLV-1-encoded HBZ protein with the RNA splicing and stability machineries in leukemic cells
Presenter: **M. Shallak** (Varese, Italy)

10.40-10.50 **OC31**: Bovine Delta papillomavirus E5 oncoprotein interacts with TRIM25 and hampers antiviral innate immune response
Presenter: **S. Roperto** (Naples, Italy)

10.50-11.00 Discussion

PARALLEL SESSION - ROOM A

SESSION 8: **VETERINARY VIROLOGY**
Chairs: **C. Buonavoglia** (Bari, Italy), **G. Vaccari** (Rome, Italy)

11.00-11.20 Lecture 19 - SARS-CoV-2, a threat to marine mammals: One Health, One Ocean, One Virology
C. Casalone (Turin, Italy)

11.20-11.40 Lecture 20 - Hepadnavirus in cats: the discovery of a human hepatitis B-like virus
G. Lanave (Bari, Italy)

ORAL COMMUNICATIONS

11.40-11.50 **OC32**: Avian reovirus P17 suppresses angiogenesis by promoting DPP4 secretion
Presenter: **E. Manocha** (Brescia, Italy)

11.50-12.00 **OC33**: SARS-CoV-2 virus dynamic in a mink farm in Italy: lessons learned
Presenter: **A. Moreno** (Brescia, Italy)

12.00-12.10 **OC34**: Unrevealed genetic diversity of GII norovirus in the swine population of north east Italy
Presenter: **L. Cavicchio** (Padua, Italy)

12.10-12.20 **OC35**: Identification of different cress DNA viruses in lizards and geckos
Presenter: **P. Capozza** (Bari, Italy)

12.20-12.30 **OC36**: Isolation of HEV-3 strains from swine fecal samples on human A549 cell line
Presenter: **G. Ianiro** (Rome, Italy)

12.30-13.00 Discussion

PARALLEL SESSION - ROOM B

SESSION 9: **IMMUNITY AND VACCINES**
Chairs: **D. Gibellini** (Verona, Italy), **M.G. Cusi** (Siena, Italy)

11.00-11.20 Lecture 21 - Innate immune response: at the forefront of viral infections
R. Rizzo (Ferrara, Italy)

11.20-11.40 Lecture 22 - Caspase 8-virus interplay and innate immunity to HSV-1 infection
A. Mastino (Messina, Italy)

ORAL COMMUNICATIONS

11.40-11.50 **OC37**: Robust and persistent B and T-cell responses after COVID-19 in immunocompetent and transplanted patients
Presenter: **F. Zavaglio** (Pavia, Italy)

11.50-12.00 **OC38**: SARS-CoV-2 N protein target TRIM-25-mediated RIG-I activation to suppress innate immunity
Presenter: **G. Gori Savellini** (Siena, Italy)

SESSION 10: **UPDATES ON HIV**
Chair: **A. Manzin** (Cagliari, Italy)

12.00-12.30 Lecture 23 - New therapeutic strategies against HIV
A. Antinori (Rome, Italy)

ORAL COMMUNICATIONS

12.30-12.40 **OC39**: Binding to P1(4,5)P2 is indispensable for secretion of B cell clonogenic HIV-1 matrix protein p17 variants
Presenter: **A. Bugatti** (Brescia, Italy)

12.40-12.50 **OC40**: Potential of latency reversing agents in NK cell-mediated eradication of the HIV reservoir
Presenter: **D.A. Covino** (Rome, Italy)

12.50-13.00 Discussion

13.00-14.00 Break

PARALLEL SESSION - ROOM A

SESSION 11: ANTIVIRAL THERAPY
Chairs: **M. Galdiero** (Naples, Italy), **E. Tramontano** (Cagliari, Italy)

14.00-14.20 Lecture 24 - Oxysterols: a promising class of broad spectrum antivirals
D. Lembo (Turin, Italy)

14.20-14.40 Lecture 25 - Advanced development of filovirus medical countermeasures yields insights into viral pathogenesis
A. Griffiths (Boston, USA)

14.40-15.00 Lecture 26 - Intranasal fusion inhibitory lipopeptide prevents direct contact SARS-CoV-2 transmission in ferrets
M. Porotto (Naples, Italy)

ORAL COMMUNICATIONS

15.00-15.10 **OC41**: Antiviral therapeutics from amphibian skin peptides
Presenter: **C. Zannella** (Naples, Italy)

15.10-15.20 **OC42**: Identification of inhibitors of SARS-CoV-2 3CLpro enzymatic activity using a small molecule in vitro repurpusing screen
Presenter: **F. Esposito** (Cagliari, Italy)

15.20-15.30 **OC43**: In vitro investigation of the mechanism of action of two broadly neutralizing human monoclonal antibodies against rabies virus
Presenter: **M. Zorzan** (Padua, Italy)

15.30-15.40 **OC44**: Antiviral and antioxidant activities of *artemisia annua* against SARS-CoV-2
Presenter: **M. Baggieri** (Rome, Italy)

15.40-15.50 **OC45**: Antiviral activity against HSV-1 and SARS-CoV-2 of leaf extract derived from *vitis vinifera*
Presenter: **F. Dell'Annunziata** (Naples, Italy)

15.50-16.00 Discussion

PARALLEL SESSION - ROOM B

SESSION 12: **FRONTIERS IN GENERAL VIROLOGY 2**
Chairs: **E. Riva** (Rome, Italy), **V. Martella** (Bari, Italy)

14.00-14.20 Lecture 27 - MALDI-ToF mass spectrometry as innovative tool for viruses identification
A. Calderaro (Parma, Italy)

ORAL COMMUNICATIONS

14.20-14.30 **OC46**: Role of extracellular vesicles in propagating HSV-1 induced brain neurodegenerative damage
Presenter: **V. Protto** (Rome, Italy)

14.30-14.40 **OC47**: SARS-CoV-2 sensing by TLR3 enhances immune response and hACE2 expression
Presenter: **S. Rizzo** (Ferrara, Italy)

14.40-14.50 **OC48**: Molecular dynamics simulations to investigate the antiviral effect of heparin in SARS-CoV-2 spike infection
Presenter: **G. Paiardi** (Heidelberg, Germany)

14.50-15.00 **OC49**: Isolation and characterization of monoclonal antibodies specific for SARS-CoV-2 and its major variants useful for developing innovative diagnostic assays and immunotherapy
Presenter: **S. Mariotti** (Rome, Italy)

SPONSORED SYMPOSIUM 3
with the unrestricted educational grant of ABBOTT MOLECULAR
Chairs: **V. Svicher** (Rome, Italy)

15.00-15.20 Innovative approaches to control current and future viral diseases
J. Dhein (Wiesbaden, Germany)

15.20-15.30 Discussion

PLENARY SESSION

SESSION 13: **COVID-19: PATHOGENESIS, PREVENTION AND CONTROL**
Chairs: **G. Antonelli** (Rome, Italy), **M. Clementi** (Milan, Italy), **A. Lazzarin** (Milan, Italy)

16.00-16.20 Lecture 28 - Clinical Characteristics and Treatment of COVID-19 patients: an update
C.M. Mastroianni (Rome, Italy)

16.20-16.40 Lecture 29 - SARS-CoV-2 diagnostics: a critical review of the current assays
M.R. Capobianchi (Rome, Italy)

16.40-17.00 Lecture 30 - SARS-CoV-2 and antiviral interferon response: who wins at tug-of-war?
C. Scagnolari (Rome, Italy)

ORAL COMMUNICATIONS

17.00-17.10 **OC50**: Oral microbiome and local immune/inflammatory response in COVID-19 patients: a cross-sectional study
Presenter: **M. D'Accolti** (Ferrara, Italy)

17.10-17.20 **OC51**: The pathogenic HERV-W envelope protein is associated to the hyper-inflammation and lymphocytes exhaustion in COVID-19
Presenter: **S. Grelli** (Rome, Italy)

17.20-17.30 **OC52**: Study of entry checkpoints and kinetics of SARS-CoV-2 variants in VeroE6 cells
Presenter: **P. Quaranta** (Pisa, Italy)

17.30-17.40 **OC53**: A real-life use of remdesivir in patients with coronavirus disease 2019: a retrospective case-control study
Presenter: **F. Cogliati Dezza** (Rome, Italy)

17.40-17.50 **OC54**: Single-dose BNT 162B2 mRNA COVID-19 vaccine significantly boosts neutralizing antibody response in health care workers recovering from asymptomatic or mild natural SARS-CoV-2 infection
Presenter: **I. Vicenti** (Siena, Italy)

17.50-18.00 Discussion

CLOSING LECTURE

Introduction: **G. Antonelli** (Rome, Italy)

18.00-18.30 Closing Lecture 31 - SARS-CoV-2 and COVID-19: what has been done and what remains to do
Presenter: **G. Silvestri** (Atlanta, USA)

18.30-19.00 **LURIA AWARDS** - Chair: **A. Caruso** (Brescia, Italy)

19.00-19.15 Closing Remarks

Abstracts

THE VARIANTS QUESTION: WHAT IS THE PROBLEM?

D. Zella[1], M. Giovanetti[2,3], F. Benedetti[1], F. Unali[4], S. [5], M. Ciccozzi[6]

[1] Institute of Human Virology and Global Virus Network Center, Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, MD 21201, USA; [2] Laboratório de Flavivírus, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil; [3] Laboratório de Genética Celular e Molecular, ICB, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil; [4] Area Comunicazione e Brand Management, University Campus Bio-Medico of Rome, Rome, Italy; [5] Unit of Clinical Laboratory Science, University Campus Bio-Medico of Rome, Rome, Italy; [6] Medical Statistic and Molecular Epidemiology Unit, University of Biomedical Campus, Rome, Italy

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) originated in Wuhan, China in early December 2019 has rapidly widespread worldwide becoming one of the major global public health issues of the last centuries. Over the course of the pandemic, due to the advanced whole genome sequencing technologies, an unprecedent number of genomes have been generated, providing invaluable insights into the ongoing evolution and epidemiology of the virus allowing the identification of hundreds of circulating genetic variants during the pandemic. In recent months variants of SARS-CoV-2 that have more mutations on the Spike protein has brought concern all over the world. These have been called 'variants of concern' (VOC) as it has been suggested that their genome mutations might impact transmission, immune control, and virulence. Tracking the spread of SARS-CoV-2 variants of concern is crucial to inform public health efforts and control the ongoing pandemic. In this review, a concise characterization of the SARS-CoV-2 mutational patterns and variants of concern circulating and co-circulating across the world has been presented in order to determine the magnitude of the SARS-CoV-2 threat and to better understand the virus genetic diversity and its dispersion dynamics.

INTRA-HOST EVOLUTION OF SARS-COV-2 DURING PERSISTENT INFECTION

S. Fiorentini

Section of Microbiology, University of Brescia, Brescia (Italy)

Duration of SARS-CoV-2 shedding through respiratory secretions is highly variable among individuals and is independent from the presence of symptoms. In general, viral transmission posed by post-convalescent COVID-19 patients may be negligible, however these results cannot be applied to special populations such as immunocompromised patients. In fact, accumulating data indicate that, in the absence of adequate immune response, a prolonged SARS-CoV-2 replication occurs and individuals release infectious viral particles for months. Immunocompromised individuals, being capable to spread a contagious virus for a long time, are now considered the main reservoir where new SARS-CoV-2 variants could emerge and spread. Viral persistence is a prerequisite for intra-host virus evolution and particular attention should be paid to the within-host mutations occurring in immunocompromised patients. In this population the immunological pressure restricting mutations is strongly reduced therefore the persistent replication of SARS-CoV-2 leads to the accumulation of amino acid (aa) changes, especially within the Spike protein. These mutations often occur early during infection and, in the absence of a competent host immunity, are maintained, stabilized and may become predominant.
Due to the fact that within-host evolved infectious viral variants have the potential to escape the vaccine-induced immunity and to potentially expand the viral host range, it is of paramount importance to avoid their spread in the general population.

GENETIC VARIABILITY OF THE SARS COV-2 GENOMES AND ITS IMPLICATIONS

F. Maggi

Department of Medicine and Surgery, University of Insubria, Varese

SARS CoV-2 makes, such as natural products of viral replication, fewer mutations than most viruses with an RNA genome. However, several mutations have been revealed at a significant level during the pandemic, some of which able to spread to global dominance in a very short time. These mutations can increase the viral fitness conferring a competitive advantage for SARS CoV-2 replication, transmission, and/or escape from immunity. Virus genomes can acquire one mutation or many and genomes that differ in sequence are generally called variants. The SARS CoV-2 variants surveillance is largely focused on mutations in the spike protein, which is involved in the attachment to the cell receptor and it's the most important target of neutralizing antibodies. Thus, the focus of SARS CoV-2 interest is investigating if a variant with changes at specific sites in spike has acquired a demonstrably different phenotype, replacing other circulating variants because more transmissible, and/or influencing the rate of reinfections or the efficacy of current vaccines because more able to escape from host antibodies. Variants with increased risk have been recently labeled by WHO using letters of the Greek alphabet (i.e. Alpha, Beta, Gamma, etc…), and classified as Variants of Concern (VOC), those with the highest global public health significance, and Variants of Interest (VOI), those with established or suspected phenotypic implications.

IMMUNOLOGIC RESPONSE TO SARS-COV-2 IN INFECTED PATIENTS AND VACCINATED SUBJECTS

F. Baldanti

Dpt. Clinical, Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Molecular Virology Unit, Fondazione IRCCS Policlinico San Matteo, 27100 Pavia, Italy

A strong and persistent humoral and T-cellular response is elicited following SARS-CoV-2 infection as well as following vaccination. In particular, total IgM and IgG can be detected in patients after a couple of weeks from SARS-CoV-2 infection and last for a prolonged time span. In addition, higher neutralization titers are detected in patients recovering from severe than from mild syndromes. Anti-S antibodies, and in particular anti-RBD, dominate the neutralizing activity against SARS-CoV-2. No significant cross-reactivity with other coronavirus is detectable. Specific T-cell response to SARS-CoV-2 peptides can also be determined. Both responses grossly correlate, but maturation of T-cell immunity take longer. Following vaccination both B-and T-cell response are detectable. While following the first vaccine dose the level of immunity is suboptimal, it increases to maximum levels following the second dose. B- and T-cell responses are higher in individuals vaccinated following natural infection, documenting the increased protection of a triple exposure to SARS-CoV-2 antigens against the major circulating virus variants.

A MODERN, INTEGRATED APPROACH TO MOLECULAR DIAGNOSTICS OF SARS-COV-2

C.F. Perno

Microbiology and Diagnostic Immunology Unit, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

VIRAL GASTROENTERITIS: AN OVERVIEW OF CURRENT DIAGNOSTIC METHODS

G.M. Giammanco, F. Bonura, C. Filizzolo, L. Mangiaracina, G. Sanfilippo, G. Sciortino, S. De Grazia

Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (PROMISE), University of Palermo, Palermo

Acute gastroenteritis (AGE) is defined as a diarrheal disease (loose or watery stools at least three times in 24-hours) lasting less than 14 days, frequently accompanied by nausea, vomiting, fever, or abdominal pain. The impact of AGE on public health is underestimated due to several factors: most cases are mild and the patient does not consult a doctor; a coprological examination is not always prescribed; the diagnostic protocols used in the laboratories are not uniform; the notification is strongly disregarded. Globally, diarrhea is identified as the second leading cause of death among children under 5 years, causing an estimated 800,000 deaths annually, mostly in developing countries. Although in industrialized countries deaths from AGE are rarely seen, gastrointestinal diseases remain an important cause of morbidity in younger children resulting in medical and healthcare expenses, loss of productivity, and other costs to society and families. Rotavirus was regarded as the most frequent cause of AGE in infants and children before rotavirus vaccines were introduced in many countries. Norovirus, *Campylobacter* and non-typhoid *Salmonellae* are the most common causes of AGE in adults, but Norovirus alone is responsible of almost half of gastroenteritis epidemics. Norovirus infection can be easily transmitted either directly or indirectly with contaminated water and food, generating sporadic cases, epidemic clusters or large outbreaks. Although 90% of viral AGEs are due to group A Rotavirus (RVA) and Norovirus (NoV), also other viruses, such as Sapovirus (SaV), Adenovirus 40/41 (AdV) and Astrovirus (AstV), are being considered as clinically relevant. Historically, the diagnosis of viral AGE has progressively evolved from electron microscopy and culture on cell lines, to viral antigens detection and nucleic acids amplification (PCR or RT-PCR). More recently, several commercial multiplex molecular assays have become available for comprehensive (syndromic) detection of gastrointestinal pathogens, including many enteric viruses. Multiplex molecular assays have the potential to consolidate laboratory workflow reducing the time to result, improving diagnostic accuracy and allowing to simultaneously detect different pathogens. Widespread adoption of multiplex PCR diagnostic methods could help to adequately define the prevalence of the different enteric viruses and their etiologic role in AGE in both single and co-infections. However, the high genetic and antigenic variability of enteric viruses, due to evolutionary mechanisms such as point mutations accumulation, recombination, and cross-species transmission and reassortment, represents an unceasing challenge for both antigenic and biomolecular diagnostic tools.

METAGENOMICS OF PLANT-ASSOCIATED VIROMES

T. Candresse

Univ. Bordeaux, INRAE, UMR BFP, F33140 Villenave d'Ornon, France

In the past few years, the rapid development of high-throughput sequencing (HTS) technologies has opened a new era of unbiased discovery and genomic characterization of viruses in plant and environmental samples. Protocols for the efficient analysis of a variety of nucleic acid templates, including virion-associated nucleic acids (VANA) and double-stranded RNAs (dsRNAs), are now available, together with efficient pipelines for the bioinformatics analysis of the huge volumes of sequence data involved. As a consequence, virus metagenomics have already dramatically challenged the limits of our knowledge of (plant) virus biodiversity. Whereas few studies have focused so far on plant viral metagenomics in natural or in agro-ecosystems, large-scale environmental metagenomics studies have revealed that phages and eukaryotic viruses are the most abundant biological entities on earth with an estimated number of $10^{30\text{-}31}$ species. Even if the study of phytoviromes is still very recent, a first glimpse at their structure and diversity and at the functioning of viral populations at the ecosystem level has been achieved, revealing as for other viruses the extremely partial and fragmentary nature of our previous knowldege. Yet, the very nature and diversity of viruses impose methodological limitations or questions on such efforts, in particular when it comes to the reproducible description of viromes, and to the precise estimation of their richness and diversity. Similarly, the ability to unambiguously separate between the plant-infecting and the plant-associated (i.e. viruses infecting plant-associated hosts but not the plants themselves) components of the virome has proven a very significant challenge. Taking examples from our recent metagenomics efforts, possible methodological approaches will be critically presented, together with some of the recently discovered characteristics of plant-associated viromes. Some standing questions calling for further research will also be discussed.

ENVIRONMENTAL SURVEILLANCE FOR EARLY PATHOGEN IDENTIFICATION, EPIDEMIC TRENDS AND GENOMIC VARIABILITY: A FOCUS ON SARS-COV-2

G. La Rosa

Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy

Wastewater surveillance offers a practical approach to the identification of viruses excreted in feces, in the catchment area of any given wastewater treatment plant. This type of surveillance strategy can be useful as an "early warning" system to detect the introduction/re-introduction of SARS-CoV-2 in a community, or to identify increasing/decreasing trends in the prevalence of infections following public health interventions etc. What is more, sequence analysis of SARS-CoV-2 found in wastewater opens up the possibility of detecting variants circulating in a community, information that is essential for the planning of adequate preventive measures for potentially affected areas.
To date, over 50 studies have been published worldwide, reporting the molecular detection of SARS-CoV-2 in wastewater, with viral concentrations of up to 106 copies per liter. In Italy, the first study on SARS-CoV-2 found positive influent sewage samples collected between February and April 2020 from wastewater treatment plants in Milan and Rome, a few days after the first notified Italian case of autochthonous SARS-CoV-2. Another study, subsequently performed on archival samples collected between October 2019 and February 2020, demonstrated that SARS-CoV-2 had already been circulating in northern Italy at the end of 2019. Interestingly, it had been circulating in different geographic regions simultaneously, which changed our previous understanding of the geographical circulation of the virus in Italy. Recently, mutations characteristic of variants of concern 20I/501Y.V1 (UK variant) and 20J/501Y.V3 (Brazilian variant) and 20E.EU1 (Spanish variant) were detected in sewage samples collected in central Italy, where outbreaks of the UK and Brazilian variants had been documented by public health authorities, illustrating the potential contribution of wastewater surveillance to explore SARS-CoV-2 diversity.
Surveillance of SARS-CoV-2 in wastewater can provide valuable complementary and independent information to public health decision-makers by monitoring the levels of virus circulating in the population and exploring SARS-CoV-2 diversity. Commission Recommendation (EU) 2021/472 of 17 March 2021 on a Common Approach to Establish a Systematic Surveillance of SARS-CoV-2 and its Variants in Wastewaters in the EU supports Member States in establishing wastewater surveillance systems across the Union as a complementary data collection and management tool of the COVID-19 pandemic, focusing on the emergence and spread of SARS-CoV-2 variants.

NETWORK MEDICINE IN INFECTIOUS DISEASES

G. Ippolito

Scientific Direction, National Institute for Infectious Diseases Lazzaro Spallanzani-IRCCS, Rome, Italy

CRISPR IN THE DIAGNOSIS AND TREATMENT OF VIRAL INFECTIONS

M. Pistello

Department of Translational Research University of Pisa and Pisa University Hospital

Aim of the study. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) gene editing is an invaluable asset for gene therapy. Since its discovery, the gene therapy trials increased significantly as well as aim and expectancies of the studies. Tweaking nucleic acids with CRISPRS now promises to advance the microbiology field further and faster. Researchers have used CRISPR gene-editing technology to modify eukaryotic and prokaryotic species, as a tool for molecular testing, and to develop models to study disease. Most recently, researchers carried out studies aimed to develop faster, more accurate COVID-19 and other viral screening methods as well as cell and gene therapies, the latter with the aim to cure infected cells and animals.
Methods used. CRISPR coupled with different nucleases can target DNA (e.g. Cas9 and Cas 12) or RNA (Cas13), and precisely edit the nucleic acid strands with the help of programmable guide RNAs.
Results and conclusions. CRISPR/Cas9 has been used to cure humanized mice from HIV infection, CRISPR/Cas13a proved effective to halt replication of flu and SARS-CoV-2 viruses in murine lung tissue and diagnostic tests using Cas12 and Cas13 enzymes have already been developed for detection of SARS-CoV-2 and other viruses. Additionally, CRISPR diagnostic tests can be performed using simple reagents and paper-based lateral flow assays, which can potentially reduce laboratory and patient costs significantly.
The classification of CRISPR-Cas systems as well as the basis of the CRISPR/Cas mechanisms of action will be presented. The application of these systems in medical diagnostics and treatment with emphasis on the diagnosis and therapy of COVID-19 will be discussed.

CLINICAL APPLICATIONS OF VIRAL VECTORS FOR GENE THERAPY

N. Brunetti-Pierri[1,2]

[1] Department of Translational Medicine, "Federico II" University, 80131 Naples, Italy and [2] Telethon Institute of Genetics and Medicine, 80078 Pozzuoli (Naples), Italy

Gene therapy has the potential to provide effective therapies or even a definitive cure for several genetic diseases. The delivery of therapeutic genes is largely performed with virus-based vectors that retain the natural capacity of viruses to deliver DNA or RNA to infected cells but are devoid of harmful viral coding sequences. Among several viral vectors, lentiviral and adeno-associated virus (AAV) vectors have shown the greatest clinical success in ex vivo and in vivo gene therapy, respectively. Inborn errors of metabolism (IEM) are genetic disorders affecting human biochemical pathways that are attractive targets for gene therapy because of their severity, high overall prevalence, lack of effective treatments, and possibility of early diagnosis through newborn screening. Gene replacement strategies for IEM are aimed either at correcting the liver disease or providing a source for production and secretion of the lacking enzyme for cross-correction of other tissues. Several preclinical studies have been conducted in the last years and, for several diseases, gene therapy has reached the clinical stage, with a growing number of ongoing clinical trials.
I will present the status of current applications of viral vectors for gene therapy of inherited diseases, highlighting the most recent data from a phase I/II open label, dose escalation gene therapy clinical trial that we are conducting for Mucopolysaccharidosis type VI (MPS VI), a multi-system and severe IEM caused by deficiency of arylsulfatase B (ARSB) (Clinicaltrials.gov Identifier: NCT03173521). In this clinical trial, nine MPS VI subjects of 4 years of age have been enrolled to receive an intravenous injection of an AAV2/8 vector expressing the ARSB gene under the control of a liver-specific promoter. Although further clinical follow up is needed, AAV2/8-mediated, liver-directed gene therapy in MPS VI was found to be safe and resulted in sustained expression of ARSB with preliminary evidence of disease stabilization.

VIRAL RESPIRATORY PATHOGENS: DIRECT AND INDIRECT MECHANISMS OF LUNG INJURY

N. Mancini

Laboratorio di Microbiologia e Virologia, Università Vita-Salute San Raffaele, IRCCS Ospedale San Raffaele, Milano

Viral respiratory infections are characterized by a highly diversified plethora of clinical manifestations, spanning from mild upper airway involvement to life-threatening lower respiratory tract involvement. The underlying pathophysiological mechanisms are a mix of direct viral and indirect "patient-specific" immune-mediated damage. In this communication, I will make a quick overview of some of these mechanisms trying to evidence the thin line existing between a protective immune response, potentially limiting viral replication, and an unbalanced, dysregulated immune activation possibly leading to the most severe complication. The comprehension of these mechanisms is increasing and could pave the way for the development of new tailored immune-based antiviral strategies.

THE SERINC ENIGMA IN VIRUSES AND CELLS

M. Pizzato

Department of Cellular, Computational and Integrative Biology, University of Trento, 38123 Trento, Italy

The SERINC family of genes encode multipass transmembrane proteins present in all eukaryotes, including yeast. During evolution, the family expanded to include five paralogs in higher eukaryotes. While the core cellular function of these proteins remains enigmatic, SERINC5 was found to potently inhibit retrovirus infectivity, and to be counteracted by Nef of primate lentiviruses, by glycoGag of gammaretroviruses and by S2 of equine infectious anemia virus. As well as inhibiting infectivity, SERINC5 alters also retroviral sensitivity to neutralizing antibodies (nAbs).

Our studies show that, SERINC3 and SERINC1 also exert a modest activity against HIV-1. In contrast, SERINC4 inhibits the virus potently even at low expression levels. To gain understanding of the molecular activity of these proteins, we investigated their molecular structure by cryo-EM, to discover an unprecedented bipartite transmembrane topology and identify subdomains critical for HIV-1 restriction activity. The ability of inhibiting HIV-1 infectivity mirrors the effect of the SERINC mutants and paralogs on HIV-1 susceptibility to neutralizing antibodies, indicating that inhibition of infectivity is functionally related to the effect on neutralization and supporting the notion that SERINCs target the envelope glycoprotein.

We also investigated the counteracting abilities of the known retroviral antagonists against the different human paralogs. HIV-1 Nef, MLV glycoGag and EIAV S2 appear to be maximally active against SERINC5, while their ability to downregulate other human SERINC paralogs varies, with S2 being the most versatile towards different SERINCs. This indication suggests that the three retroviral antagonists target SERINC proteins with diverse mechanisms. Supporting this possibility, we identified SERINC regions that are differentially required for downregulation by the three antagonists. As a result, we could design a SERINC5 gene that is no longer targeted by Nef and is no longer be counteracted by HIV-1.

Altogether, our data show that SERINC proteins are unusual restriction factors and provide evidence that they pose a strong selective pressure which has driven the independent emergence of counteracting factors in different retroviral genera.

ACE2: A KEY FACTOR IN REGULATING SARS-COV-2 AND INFLUENZA VIRUS INFECTION

M. De Angelis[1], D. Amatore[2], A. Di Martino[3], F. Pacifici[4], P. Checconi[5], A. Fraternale[6], R. De Santis[2], M. Magnani[6], P. Stefanelli[3], D. Della Morte[4], F.R. Lista[2], L. Nencioni[1], A.T. Palamara[1,3]

[1] Dept. Public Health and Infectious Disease, Sapienza University, Rome, Italy; [2] Scientific Department, Army Medical Center, Rome, Italy; [3] Dept. Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy; [4] Dept. Systems Medicine, University Tor Vergata, Rome, Italy; [5] Dept. Human Sciences and Promotion of the Quality of Life, Telematic University, Rome, Italy; [6] Dept. Biomolecular Sciences, University Carlo Bo, Urbino (PU), Italy

Multiple host factors, including the number and distribution of receptors for viral entry, the redox balance and the activation of related pathways, play a pivotal role in determining the rate of viral yield and the pathogenesis of infections. Among them, the expression of Angiotensin Converting Enzyme 2 (ACE2) on different cell types is a key factor, not only in favouring virus entry, but also in regulating the cell response to infection, thanks to its ability to modulate antioxidant and anti-inflammatory responses. Indeed, ACE2 downregulation mediated by respiratory viruses including SARS-CoV-2 and influenza virus, has been associated with tissue damage and ARDS development supporting the ACE2 anti-inflammatory and antioxidant role. These properties may be mediated by ACE2 regulation of nuclear factor erythroid 2-related factor 2 (Nrf2), an important regulator of the antioxidant response involved in the expression of genes related to GSH synthesis, ROS elimination and NADPH production. Furthermore, the hyperactivation of ACE2/Ang 1-7/Mas axis has been associated to protection against lung fibrosis by attenuating the ROS production mediated by the isoform of NADPH oxidase (NOX4), the main source of ROS during influenza virus infection. The generation of ROS is also important for virus/host cell interaction, since it can oxidize the cysteine residues on the peptidase domain of ACE2 and RBD of the Spike protein, keeping them in oxidized form that increases the affinity for ACE2 receptor and favors the viral entry.

On the basis of these evidence, we aimed at exploring the efficacy of redox-modulating compounds, against SARS-CoV-2 and influenza virus infection, by interfering with the ACE2 and Spike binding affinity, as well as on virus-modulated ACE2 expression and its related pathways.

SARS-CoV-2 was pre-incubated for 1 h at 37° C with different thiol compounds: a GSH-precursor, a conjugate of N-acetylcysteine and s-acetyl-β-mercaptoethylamine, able to increase GSH content; its dithiol derivative; or the N-butanoyl GSH derivative (GSH-C4). The mixture was used to infect ACE2-expressing cell lines. In parallel, stilbene derivatives or a mixture of polyphenols, known to regulate ACE2 expression through sirtuin pathways, were added at different steps of SARS-CoV-2 or influenza virus replication. Viral titration was performed by TCID50, hemagglutination and plaque assays. ACE2 and its related pathways as well as viral proteins expression was analyzed by western blot and in cell western assays.

The results showed that all the reducing agents were able to impair the infectivity of SARS-

CoV-2. In particular, the GSH-C4 molecule decreased the viral titer by 40%, while the GSH-precursor and its metabolite showed a higher efficacy in reducing plaque formation (84 and 70% respectively) compared to untreated conditions.
The use of polyphenols decreased the expression of influenza virus proteins (Nucleoprotein and Hemagglutinin) and consequently the viral titre, when they were added after infection for the following 24 h. In addition, they were able to counteract the influenza virus-induced decrease of ACE2, by up-regulating the protein expression at the levels of mock-infected cells. In the same conditions, the pro-inflammatory cytokine IL-6 levels were strongly reduced suggesting a potential use of redox-modulating compounds in attenuating virus-induced inflammation. In SARS-CoV-2 model the use of mixture of polyphenols reduced the viral titre of 2 log as well as viral proteins expression, in particular when the compound was added at all the steps of viral replication. Accordingly, ACE2 expression increased in these treated cells.
In conclusion, redox-modulating compounds can act by a dual mechanism: preventing the binding between ACE2 and Spike protein or increasing ACE2 expression in cells already infected by respiratory viruses and suggest that reducing conditions in host cells may decrease the severity of infection and its outcome.

IDENTIFICATION OF A NOVEL GATEWAY FOR SARS-COV-2 ENTRY INTO HUMAN ENDOTHELIAL CELLS

F. Caccuri

Section of Microbiology, Department of Molecular and Translational Medicine, University of Brescia Medical School, 25123 Brescia, ItalySection of Microbiology, Department of Molecular and Translational Medicine, University of Brescia Medical School, 25123 Brescia, Italy

SARS-CoV-2-associated acute respiratory distress syndrome (ARDS) and acute lung injury are life-threatening manifestations of severe viral infection. The pathogenic mechanisms that lead to respiratory complications, such as endothelialitis, intussusceptive angiogenesis, and vascular leakage remain unclear. In this study, by using an immunofluorescence assay and in situ RNA-hybridization, we demonstrate the capability of SARS-CoV-2 to infect human primary lung microvascular endothelial cells (HL-mECs) in the absence of cytopathic effects and release of infectious particles. Our data point to the role of integrins in SARS-CoV-2 entry into HL-mECs in the absence of detectable ACE2 expression. Following infection, HL-mECs were found to release a plethora of pro-inflammatory and pro-angiogenic molecules, as assessed by microarray analyses. This conditioned microenvironment stimulated HL-mECs to acquire an angiogenic phenotype. Proteome analysis confirmed a remodeling of SARS-CoV-2-infected HL-mECs to inflammatory and angiogenic responses and highlighted the expression of antiviral molecules as annexin A6 and MX1. These results support the hypothesis of a direct role of SARS-CoV-2-infected HL-mECs in sustaining vascular dysfunction during the early phases of infection. The construction of virus-host interactomes will be instrumental to identify potential therapeutic targets for COVID-19 aimed to inhibit HL-mEC-sustained inflammation and angiogenesis upon SARS-CoV-2 infection.

THE ONCOGENIC ROLE OF HPV IN THE PATHOGENESIS OF OROFARYNGEAL AND GENITAL CANCERS

M.L. Tornesello

Molecular Biology and Viral Oncology Unit. Istituto Nazionale Tumori IRCSS Fond. Pascale, Napoli, Italy

The persistent infection with high risk human papillomaviruses (HPVs) represents the necessary cause of nearly all cases of invasive cervical carcinoma and a variable proportion of other anogenital cancers (anus, vagina, vulva and penis) as well as of oropharyngeal cancer. The identification of HPV DNA combined with markers of viral activity, such as E6* mRNA or p16INK4a expression, provided an accurate assessment of the burden of HPV-related cancers at specific sites. The results showed active HPV (6, 11, 16, 18, 31, 33, 45, 52, 58) in 84% cervical, 82.6% anal, 71% vaginal, 27.7% penile, 24.6% vulvar and 22.4% oropharyngeal cancers (de Sanjosè et al., *JNCI Cancer Spectrum*, 2019).

The oncogenic activity of high risk HPVs differs in each anatomical site reflecting the viral tropism, the natural history of infection, the expression of HPV mRNA isoforms and the activation of different pathways of carcinogenesis. Moreover, cell transformation is sustained by the accumulation of genetic alterations, including APOBEC-associated mutagenesis, cancer driver mutations in protein-coding genes (i.e. PIK3CA, KRAS, and EGFR), as well as a large number of non-coding RNA alterations and other epigenetic changes. Telomerase reverse transcriptase (TERT) overexpression is a hallmarks of many cancers including HPV-related types and its activation is caused either by HPV E6 transactivation or by activating mutations in the TERT promoter (TERTp) region.

We have identified by Sanger sequencing and droplet digital PCR the TERTp mutations, C228T and C250T, in 17% of cervical squamous cell carcinoma (SCC), 54% vulvar and 37% penile cancers as well as in 60% of oral carcinoma. TERTp mutations are more frequent in HPV-negative cancers of different sites. Moreover, expression of TERT gene is significantly higher in TERTp mutated compared to not mutated tumors irrespective of HPV16 E6 and E7 levels. Therefore, TERT promoter mutations play a relevant role in oral SCC as well as in cervical SCC, in addition to the already known effect of HPV16 E6 protein on TERT expression. Expression profile of p53-related genes showed a significant de-regulation of those associated with cell proliferation and apoptosis, suggesting the activation of non-canonical pathways in cancer cases overexpressing telomerases.

In conclusion, HPV-related tumors are characterized by distinctive genetic changes compared with HPV-negative tumors resulting in distinct biological and clinical entities. The HPV status, cancer driver mutations in coding and non-coding regions of human genome may define new biomarkers to guide for cancer diagnosis, prognosis and targeted therapy in anogenital and oropharyngeal cancers.

ONCOLYTIC VIRUSES FOR CANCER THERAPY

F. Alessandrini [1], F. Piaggio[1,2], D. Ceresa[2], I. Appolloni[1], L.Menotti[3], G. Campadelli-Fiume[4], P. Malatesta[1,2]

[1] Department of Experimental Medicine, University of Genoa, Genoa, Italy. [2] Ospedale Policlinico San Martino IRCCS per l'Oncologia, Genoa, Italy. [3] Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy. [4] Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Bologna, Italy

Oncolytic HSVs (oHSVs) are emerging as one of the efficient therapies to fight malignant neoplasms lacking effective therapeutic alternatives. The high safety profile of the oHSVs currently in clinical trials or practice was achieved at the expense of virulence. These viruses are attenuated to varying degrees. To overcome these limitations without hamper the safety of the treatment, an appealing alternative to attenuation is virus retargeting against relevant tumor-specific receptors. Retarget virus can be used to specifically infect and kill tumor cells and to stimulate the immune system to react against consequently released (neo-)antigen. This effect can be potentially enhanced by arming o-HSV with immunostimulatory cytokines, as Il-12 or by employing them together immune-checkpoint inhibitors. These approaches may represent an opportunity for high grade gliomas that are both highly lethal and immune-evasive tumors.

Aim of the study: We evaluated for the first time, the effects of an o-HSV retargeted to human HER2, fully virulent in its target cells and armed with mIL-12 (R-115) on a murine model of glioblastoma induced by overexpression of PDGF-B and engineered to express human-HER2 (mHGG^{pdgf}-Her2). The model faithfully recapitulates key features of the human glioblastoma as its peculiar immune-evasivity and leads invariably to the death mice harboring them. The aim of the study was to determine the safety of the virus and its performance, in term of survival time of treated mice, compared to the non-armed version (R-LM113). In addition, we assayed the combination of R-115 with immune-checkpoint inhibitors.

Methods: Balb/c mices were orthotopically transplanted with mHGG^{pdgf}-Her2 and treated with intra-tumor injection of R-115 or R-LM113, 8 or 21 days after transplant. Survival time of treated mice were then recorded. Long survivors were rechallenged with an additional transplant of mHGG^{pdgf}-Her2 or the non HER2-expressing mHGG^{pdgf} parental cells.

Results and conclusion: The injection of R-115 and R-LM113 in established tumors resulted in an almost doubling of survival time, but, only with R-115 leaded to the complete eradication of hHER2-positive tumor, in about 40% of animals. The treatment with R-115 induced the acquisition of a resistance to recurrence from the same neoplasia as well. Administration of immune-checkpoint inhibitors, however, was not associated with any further improvement. The treatment with R-115 was effective also in tumors heterogeneous for hHER2 expression, resulting in a significant improvement in the median survival time of mice and in about 15% of completely cured animals. Overall, such a high degree of protection was unprecedented. Our study constitutes a proof-of-concept evidence of the benefits offered by a fully virulent, retargeted and armed oHSV in the treatment of glioblastoma and paves the way for clinical translation.

THERAPEUTIC VACCINES FOR VIRUS-ASSOCIATED TUMORS

A. Venuti

HPV-Unit IRCCS Regina Elena National Cancer Institute – Rome

Almost one in every five malignancies can be attributed to infectious agents. Among infection-related neoplasms, cancers of the stomach, liver and cervix uteri are largely attributable to Helicobacter pylori, hepatitis B and C viruses, and human papilloma virus, respectively. Other infectious organisms can also cause cancer; these include the Epstein-Barr virus (nasopharyngeal carcinoma, and different types of lymphoma), Merkel cell Polyomavirus (Merkel carcinoma), Human herpes virus-8 (Kaposi's Sarcoma), human T-cell leukemia virus type I (leukaemia, lymphoma), liver flukes (cholangiocarcinoma) and schistosomiasis (bladder cancer). Infection with human immunodeficiency virus, although strongly associated with an excess of cancer incidence at many cancer sites, is probably not carcinogenic per se, but acts mainly via immunodeficiency. Finally, CMV is suspected to be involved in glioblastoma development. However, associations with new infectious agents remain yet to be explored. As the large majority of infection linked tumors are associated with viruses, the idea of developing vaccines against these viruses was a logical/scientific consequence. Indeed preventive vaccine were developed and proved efficacious against hepatitis B virus and HPV. However, a vaccine can be also utilized in a therapeutic setting in order to eliminate cells already infected/transformed by viruses. The concept that a vaccine could be useful in the treatment of cancer diseases is a long-held hope coming from the observation that patients with cancer who developed bacterial infections experienced remission of their malignancies. The earliest mention of cancer-fighting infections dates to a citation from Ebers papyrus (1550 B.C.) attributed to great Egyptian physician Imhotep (2600 B.C.), who recommended to treat tumors (swellings) with a poultice followed by an incision. In 1896, New York surgeon William Coley locally injected streptococcal broth cultures to induce erysipelas in an Italian patient (Mr. Zola) with an inoperable neck sarcoma, obtaining a tumor regression. Virus-associated tumors offer the unique opportunity to develop vaccine against true tumor associate antigen (TAA). In particular HPV viral oncoproteins are ideal TAA because they are always expressed by tumor cells. A number of technologies were develop as anti-HPV therapeutic vaccines. Live vector vaccines employing viruses (Ankara modified vaccine virus, TG4001 Transgene Inc. France) or bacteria (Listeria monocytogenes, ADXS11-001 Advaxis Inc, Princeton, NJ, USA) are in clinical trials with promising results (NCT03260023 and NCT02853604, respectively). Protein- or peptide-based vaccines have been also evaluated and tested in clinical trials with some interesting outcomes for synthetic long peptide-vaccine in early stages of HPV carcinogenesis. More challenging approaches such as vaccines based on dendritic cell (DC), tumor cells or adoptive T-cell therapy (ACT) have been developed but they cannot be easily performed and require specialized clinical centers. On the contrary, technologies utilizing DNA or RNA vaccines can be easily performed and are in advanced clinical trials. Our group has developed innovative technologies that utilize green world to enhance activity of such DNA vaccines. Many other different HPV DNA vaccines have been constructed and proven to be active in pre-clinical models and few of them are in clinical trials. In particular, VGX3100 (Inovio Pharmaceuticals Inc., USA)

is a plasmid DNA-based immunotherapy delivered by electroporation, under investigation for the treatment of HPV 16 and HPV 18 infection and pre-cancerous lesions of the cervix, vulva, and anus (Phase II/III) (NCT01304524 and NCT03603808). Two studies that are currently in phase III (NCT03721978 and NCT03185013) using VGX-3100 against cervical cancer show promising results. Another DNA vaccine with potential clinical use is the GX-188E (Genexine, Inc., Korea) that recently, showed to be highly efficacious in patients with grade 3 cervical intraepithelial neoplasia (CIN3) (NCT02139267). This vaccine consists of shuffled E6 and E7 genes of HPV type 16/18. VGX-3100 has the potential to be the first approved treatment for HPV infection of the cervix and the first non-surgical treatment for precancerous cervical lesions. Finally, very preliminary evidences suggest that CMV antigens could be utilized to set-up vaccines against glioblastoma.

SARS-COV-2, A THREAT TO MARINE MAMMALS: ONE HEALTH, ONE OCEAN, ONE VIROLOGY

C. Casalone

Turin, Italy

Zoonotically transmitted coronaviruses were responsible for three disease outbreaks since 2002, with the "Severe Acute Respiratory Syndrome Coronavirus-2" (SARS-CoV-2) causing the dramatic "Coronavirus Disease-2019" (CoViD-19) pandemic, which affected public health, economy, and society on a global scale. The impacts of the SARS-CoV-2 pandemic permeate into our environment and wildlife as well; in particular, concern has been raised about the viral occurrence and persistence in aquatic and marine ecosystems. The discharge of untreated wastewaters carrying infectious SARS-CoV-2 into natural water systems that are home of sea mammals may have dramatic consequences on vulnerable species.

The efficient transmission of coronaviruses raise questions regarding the contributions of virus-receptors interactions. The main receptor of SARS-CoV-2 is Angiotensin Converting Enzyme-2 (ACE-2), serving as a functional receptor for the viral spike (S) protein. This study was aimed, through the comparative analysis of the ACE-2 receptor with the human one, at assessing the susceptibility to SARS-CoV-2 of the different species of marine mammals living in Italian waters . It was determined, by means of immunohistochemistry, the ACE-2 receptor localization in the lung tissue from different cetacean species, in order to provide a preliminary characterization of ACE-2 expression in the marine mammals' respiratory tract.

Furthermore, in order to evaluate if and how wastewater management in Italy may lead to susceptible marine mammal populations being exposed to the virus, geo-mapping data of wastewater plants, associated to the identification of specific stretches of coast more exposed to extreme weather events, overlapped to marine mammal population data, were carried out. The results of our research showed the SARS-CoV-2 exposure for marine mammals inhabiting Italian coastal waters. Thus, we highlight the potential hazard of reverse zoonotic transmission of SARS-CoV-2 infection, along with its impact on marine mammals regularly inhabiting the Mediterranean Sea, whilst also stressing the need of appropriate action to prevent further damage to specific vulnerable populations.

HEPADNAVIRUS IN CATS: THE DISCOVERY OF A HUMAN HEPATITIS B-LIKE VIRUS

G. Lanave

Department of Veterinary Medicine, University of Bari

Hepadnaviruses are enveloped spherical viruses of 42–50 nm in diameter with an icosahedral capsid. The viral genome comprises a circular, partially double-stranded DNA molecule. Hepadnaviruses can be classified into five genera. Orthohepadnavirus infects mammals, Avihepadnavirus infects birds, Herpetohepadnavirus infects amphibians and reptiles, whilst Metahepadnavirus and Parahepadnavirus infect fish. The prototype species, hepatitis B virus (HBV), increases the risk of chronic liver diseases, cirrhosis and hepatocellular carcinoma (HCC) in humans. Approximately 257 million people worldwide are HBV carriers, and HBV causes 887,000 deaths annually (https://www.who.int/news-room/fact-sheets/detail/hepatitis-b).

In 2018, a feline homolog of HBV was detected in a domestic cat (*Felis catus*) diagnosed with multicentric large B-cell lymphoma and co-infected with feline immunodeficiency retrovirus (FIV) (Aghazadeh et al., 2018). By screening of an age-stratified population the virus, Domestic Cat Hepadnavirus (DCH), was detected in cats, with a significantly higher prevalence in the sera of animals with a clinical suspicion of infectious disease, suggesting a possible association with FIV and feline leukaemia virus (FeLV) infections (Lanave et al., 2019). Virus load found in majority of the animals with suspected hepatic disease was $>10^4$ genome copies per mL, which is above the threshold for the risk of active hepatitis and liver damage from HBV infection in humans (Lanave et al., 2019). A multicentre study in USA, UK, Australia and New Zealand has revealed an association between DCH infection and chronic hepatitis and HCC in cats (Pesavento et al., 2019). A Thailandese study identified DCH in cat sera and in necropsied animals, with the highest prevalence in FIV-infected animals (Piewbang C, et al., 2020). Immunohistochemistry in Thai DCH-positive cats revealed chronic active hepatitis and hepatic fibrosis and cytoplasmic expression of the DCH-viral C protein in hepatocytes, using a commercial serum containing antibodies specific for HBV, thus indicating antigenic cross-reactivity between DCH and HBV.

A cross-sectional study in Malaysia revealed the presence of DCH in liver tissue samples and in blood samples from domestic cats (Anpuanandam et al., 2021). Interestingly, pet cats or cats with elevated ALT were significantly more likely to be DCH positive. Cats with positive DCH detection from liver tissues may not necessarily have viraemia.

Overall, the pathogenic potential of DCH in cats is still uncertain, although hepadnaviruses are generally considered hepatotropic viruses. The discovery of novel viruses has been increasing in recent years, due to the large adoption of new sequencing technologies. Understanding if these viruses play a role as pathogens poses several challenges and opens interesting perspectives for the research.

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INNATE IMMUNE RESPONSE: AT THE FOREFRONT OF VIRAL INFECTIONS

R. Rizzo

Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Italy

The human innate immune response, particularly Natural killer cell response, is highly robust and effective first line of defense against virus invasion. NK cells are a rapidly expanding family of innate lymphoid cells and represent 5–20% of all circulating lymphocytes in humans. The role of NK cells is analogous to that of cytotoxic T cells in the vertebrate adaptive immune response. NK cells provide rapid responses to virus-infected cell and other intracellular pathogens and respond to tumor formation. They act with signaling/communication molecules to activate an antiviral response in neighboring cells so that those cells become active against the infection. Previous experimental studies have identified some of the molecular mechanisms for NK cell response. However, the principles underlying how NK cells might differ in their ability to counteract the infection is not understood (Eliassen E, et al, Rizzo R, et al. The Interplay between Natural Killer Cells and Human Herpesvirus-6.Viruses. 2017;9(12):367.).

We take a network modeling approach to provide a theoretical framework to identify key factors that determine the effectiveness of the NK cell response against virus infection of a host. In this approach, we consider the virus spread among host cells and the NK cell activation when infection of cells is mostly well mixed (e.g., in the blood) and when infection is spatially segregated (e.g., in tissues). We show that in general, NK cell response is controlled, when virus infection spreads spatially, by KIR (killer-cell immunoglobuline-like receptor) receptors, which regulate the killing function of NK cells by interacting with major histocompatibility (MHC) class I molecule (Rizzo R, et al. KIR2DS2/KIR2DL2/HLA-C1 Haplotype Is Associated with Alzheimer's Disease: Implication for the Role of Herpesvirus Infections. J Alzheimers Dis. 2019; 67(4):1379-1389. Rizzo R, et al. Implication of HLA-C and KIR alleles in human papillomavirus infection and associated cervical lesions. Viral Immunol. 2014; 27(9):468-70. Caselli E, Rizzo R, et al. High prevalence of HHV8 infection and specific killer cell immunoglobulin-like receptors allotypes in Sardinian patients with type 2 diabetes mellitus. J Med Virol. 2014; 86(10):1745-51. Rizzo R, et al. Altered natural killer cells' response to herpes virus infection in multiple sclerosis involves KIR2DL2 expression. J Neuroimmunol. 2012;251(1-2):55-64. Rizzo R, et al. J Neuroimmunol. KIR2DL2 inhibitory pathway enhances Th17 cytokine secretion by NK cells in response to herpesvirus infection in multiple sclerosis patients. 2016;294:1-5. Rizzo R, et al. Increase in Peripheral CD3-CD56brightCD16- Natural Killer Cells in Hashimoto's Thyroiditis Associated with HHV-6 Infection. Adv Exp Med Biol. 2016;897:113-20. Caselli E, et al, Rizzo R, et al. Virologic and immunologic evidence supporting an association between HHV-6 and Hashimoto's thyroiditis. PLoS Pathog. 2012;8(10):e1002951).

Pattern recognition receptors (PRRs), the key components of antiviral immunity that detect conserved molecular features of viral pathogens and initiate signalling that results in the expression of antiviral genes, are more effective in tissue infections (Bortolotti D, et al., Rizzo R. SARS-CoV-2 Spike 1 Protein Controls Natural Killer Cell Activation via the HLA-E/NKG2A Pathway. Cells. 2020; 9(9):1975. Bortolotti D, et al., Rizzo R. DNA Sensors' Signaling in NK Cells During HHV-6A, HHV-6B and HHV-7 Infection. Front Microbiol.

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Thus, our work provides an explanation of why the NK cell response can serve an effective and robust response in different tissue types to a wide range of viral infections of a host.

CASPASE 8/VIRUS INTERPLAY AND INNATE IMMUNITY TO HSV-1 INFECTION

F. Marino-Merlo[1], A. Serafino[2], C. Borner[3], A. Mastino[2]

[1] Dept. of Chemical, Biological, Pharmaceutical, and Environmental Sciences, University of Messina, Messina, Italy; [2] Institute of Translational Pharmacology, CNR, Rome, Italy; [3] Institute of Molecular Medicine and Spemann Graduate School of Biology and Medicine, University of Freiburg, Freiburg, Germany

Aim of the study. Different laboratories demonstrated that regulated cell death (RCD) by apoptosis plays an important role in the progression of viral replication in cells infected by herpes simplex viruses (HSV). Moreover, it is now clear that not all cell types undergo the same fate in terms of cytopathic effect in response to HSV infection and that the absolute paradigm "more apoptosis/less viral replication" is a reductive explanation of complex mechanisms controlling RCD during infection by HSV. Thus, to get further information on cellular proteins controlling both induction of apoptosis and virus replication in cells infected by HSV-1, attention of the present study was focused on caspase-8. In fact, data from studies of other authors indicated that this caspase was implicated in processes occurring during RCD caused by HSV infection, while results of our studies excluded a role for death receptors in HSV-1 induced RCD.
Methods used. Human monocytic U937 cells and their stable transfectants carrying a dominant negative murine IκBα were used as suspension cells prone to RCD in response to HSV-1 infection. Mouse embryonic fibroblasts (MEFs), wt or knock-out/knock-down for caspase-8, FADD, Bax/Bak, or HEp-2 human epithelial cells were used as adherent cells for HSV-1 infection. Vero kidney epithelial cells of African green monkey, were used for virus production. For all experiments the "F" strain of HSV-1 was used. Caspase-3 and caspase-8 activities were measured by fluorogenic assays or by Western blot analysis. The later technique was utilized also for Beclin-1 or LC3-I/LC3-II analysis. Immunofluorescence by optical or confocal microscopy and transmission electron microscopy were used to detect HSV-1 infection and RCD- or autophagic-like-related cell features. Cell viability was detected by classical assays.
Results and conclusions. HSV-1 induced caspase-8 activity in apoptosis-prone infected cells. HSV-1-induced cell death was hindered in C8-KO-MEFs. Interestingly, caspase-8 functionality was required not only for apoptotic RCD induction but also for infectious virus release in HSV-1 infected MEFs. However, HSV-1 did not utilize the FADD adaptor protein for inducing RCD in target cells and recruitment of caspase-8 in response to HSV-1 infection in MEFs occurred downstream of the mitochondrial signalling, thus suggesting a non-canonical role of caspase-8 in modulation of RCD following HSV-1 infection. Considering that caspase-8 was shown to target Beclin-1 in different experimental conditions we then analysed Beclin-1 expression following HSV-1 infection. In fact, Beclin-1 cleavage was clearly detected in wt MEFs, but not in caspase-8 KO MEFs infected by HSV-1. Moreover, LC3-I/LC3-II conversion in response to HSV-1 infection remarkably occurred only in MEFs in which caspase-8 cleavage was not achieved. Actually, experiments carried out by confocal and electron microscopy clearly showed that in caspase-8 KO cells infection by HSV-1 stimulated a strong perinuclear vesicular response, resembling autophagy, capable of trapping virions in cellular endosomes and thus containing the spread of the virus.

In this context, the production of p18 in caspase-8 functional cells would act as a negative inhibitor of the autophagic-like response, indirectly strengthening the mechanisms of apoptotic RCD. Finally, pharmacological inhibition of PI3-kinase restored the ability of HSV-1 to induce apoptotic RCD in C8-KO-MEFs. All together our results provide support for a non-canonical role of caspase-8 in HSV-1 infection as the possible regulator for the switching of autophagic-like and apoptotic response in cells prone to apoptosis following infection.

NEW THERAPEUTIC STRATEGIES AGAINST HIV

A. Antinori

Clinical and Research Department of Infectious Diseases, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Roma, Italy

The availability of new antiretroviral drugs and new drug classes has strongly modified the approach to initial and long term strategies in antiretroviral setting. Integrase strand transfer inhibitors (INSTI) are now the new standard for ARV initiation and switching in patients with virologic suppression. The 2nd generation INSTIs, characterized by high potency and elevated genetic barrier, admit high rapid decay, elevated efficacy, prolonged persistence and long term suppression with low risk of viral rebound and selecting resistance. Two drug regimens are now feasible, combining a 2nd generation INSTI and a reverse transcriptase inhibitor or a protease inhibitor, both for ART initiation and for switching during viral suppression. The elevate protection from viral resistance conferred by new high-genetic barrier regimens, allows new initiation strategies, as rapid od same-day treatment initiation, in order to improve retention to care and adherence to ART program. New insight for ART starting in naïve population come from the emerging frontiers of starting ART in pre-exposure prophylaxis (PrEP) failure, ART initiation in late HIV presenter and in advanced HIV disease (AIDS presenters). The availability of new drug class (reverse transcriptase/translocation inhibitors, maturation inhibitors, attachment inhibitors, new entry inhibitors, capsid inhibitors), admits new effective approach both in early than in the late lines rescue of therapy. Most of the new ARV drugs are developed by nanoformulated, long-acting injectable formulation, and this emerging scenario represents the main issue and challenge in ARV setting, for optimizing viral suppression and long term control of viral replication and HIV disease.

OXYSTEROLS: A PROMISING CLASS OF BROAD SPECTRUM ANTIVIRALS

D. Lembo

Turin, Italy

Oxysterols are a family of cholesterol oxidation derivatives that contain an additional hydroxyl, epoxide or ketone group in the sterol nucleus and/or a hydroxyl group in the side chain. The majority of oxysterols in the blood are of endogenous origin, derived from cholesterol via either enzymatic or non-enzymatic mechanisms. An increasing number of reports demonstrate the role of specific oxysterols as effectors of the host antiviral response. Here we review the recent findings about the broad antiviral activity of oxysterols against enveloped and non-enveloped human viral pathogens (including SARS-CoV-2), and provide an overview of their putative antiviral mechanism(s).

ADVANCED DEVELOPMENT OF FILOVIRUS MEDICAL COUNTERMEASURES YIELDS INSIGHTS INTO VIRAL PATHOGENESIS

A. Griffiths

Boston, USA

Ebola virus (EBOV), of the family Filoviridae, is an RNA virus that can cause hemorrhagic fever with high lethality. Defective viral genomes (DVGs) are truncated genomes that have been observed during multiple RNA virus infections and have been associated with viral persistence and immunostimulatory activity. As DVGs have been detected in cells persistently infected with EBOV, we hypothesized that DVGs may also accumulate during viral replication in filovirus-infected hosts. Therefore, we interrogated sequence data from serum and tissues using a bioinformatics tool in order to identify the presence of DVGs in nonhuman primates (NHPs) infected with EBOV, and the related viruses Sudan virus (SUDV) and Marburg virus (MARV). Serum collected during acute EBOV and SUDV infections, but not MARV infection, contained a higher proportion of short trailer sequence cbDVGs than the challenge stock. This indicated an accumulation of these DVGs throughout infection by EBOV and SUDV. Using RT-PCR and deep sequencing, we also confirmed the presence of 5' cbDVGs in EBOV-infected NHP testes, which is of interest due to EBOV persistence in semen of male survivors of infection. This work suggests that DVGs play a role in EBOV infection in vivo and further study will lead to a better understanding of EBOV pathogenesis.

INTRANASAL FUSION INHIBITORY LIPOPEPTIDE PREVENTS DIRECT CONTACT SARS-COV-2 TRANSMISSION IN FERRETS

M. Porotto

Naples, Italy

Containment of the COVID-19 pandemic requires reducing viral transmission. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is initiated by membrane fusion between the viral and host cell membranes, which is mediated by the viral spike protein. We have designed lipopeptide fusion inhibitors that block this critical first step of infection and, on the basis of in vitro efficacy and in vivo biodistribution, selected a dimeric form for evaluation in an animal model. Daily intranasal administration to ferrets completely prevented SARS-CoV-2 direct-contact transmission during 24-hour cohousing with infected animals, under stringent conditions that resulted in infection of 100% of untreated animals. These lipopeptides are highly stable and thus may readily translate into safe and effective intranasal prophylaxis to reduce transmission of SARS-CoV-2.

APPLICATION OF MALDI-TOF MS AS INNOVATIVE TOOL IN CLINICAL VIROLOGY

A. Calderaro

Department of Medicine and Surgery, University of Parma, Parma, Italy

Introduction. Virus detection and/or identification is traditionally performed using methods based on cell culture, electron microscopy and antigen or nucleic acid detection. In this study, Matrix- Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS), commonly used in clinical microbiology, was evaluated as an innovative tool to be applied to virus identification by using two different approaches.
Materials and Methods. In the first approach, human polioviruses were selected as a model to evaluate the ability of MALDI-TOF MS to identify specific viral protein as biomarkers of purified virus particles, followed by the serotypes identification. To this aim the Sabin reference strains were firstly analysed and, subsequently, the results were corroborated by a blind application of the assay to clinically isolated strains.
In the second approach, a protein profiles library was newly create to discriminate between uninfected and respiratory virus infected cell cultures after a viral proteins enrichment method. The library was built using different reference strains after an extensive modification of the MALDI-TOF MS pre- processing, MSP creation, subtyping MSP creation and identification default parameters setting.
Results. The very efficient technique adopted to obtain highly purified polioviruses allowed us to discriminate viral protein peaks from uninfected cells peaks and to detect specific poliovirus protein biomarkers. Moreover, MALDI-TOF MS analysis applied to the three Sabin poliovirus serotypes revealed characteristic peak profiles for each of them, as demonstrated by statistical analysis, showing three independent clusters for the three serotypes. After a statistical investigation, the VP4 was recognized as a potential biomarker to identify poliovirus strains at the serotype level. On the bases of VP4 all clinical isolates were identified at the serotype level.
In the second approach, the spectra generated from virus infected cell cultures, analysed in the molecular weight range 2000–20,000 Da, revealed the presence of some different peaks not overlap- ping those of uninfected cell cultures for all the reference virus infected cell cultures, with a frequency of 100% in all the replicates. The parameters for the creation of the Main Spectrum Profile (MSP) for each of the reference virus infected cell cultures were set on the basis of these peaks and the obtained MSP spectra were used to create a new respiratory viruses library in our Bruker Daltonics database in order to blindly identify viruses isolated from biological samples after a cell culture step. The spectra obtained by 58 additional cultured strains correctly matches with the new database demonstrating its reliability.
Discussion and Conclusions. In conclusion, this study could be considered a starting point for further evolutions of the developed system, since the differences observed comparing the virus infected cell
culture spectra suggest the possibility to apply these method to the identification of other viruses responsible for respiratory tract infections, as well as to viral agents causing infections of other body sites.

CLINICAL CHARACTERISTICS AND TREATMENT OF COVID-19 PATIENTS: AN UPDATE

C.M. Mastroianni

Department of Public Health and Infectious Diseases, Sapienza University, Rome, Italy

The manifestations of COVID-19 are characterized by an heterogeneous spectrum of clinical severity ranging from a mild, self-limiting, upper respiratory tract infections with minimal symptoms to a diffuse viral pneumonia causing significant hypoxia with ARDS, multiorgan failure and death. Beside dyspnea, hypoxemia and acute respiratory distress, lymphopenia and cytokine release syndrome are also important clinical features in patients with severe COVID-19 pneumonia suggesting that homeostasis of the immune system plays an important role in the development of COVID-19 pneumonia. In addition to lung disease, clinical features of SARS-CoV-2 include myocardial damage and ischemia-related vascular disease, which are associated with a hypercoagulable state predisposing to thrombotic-related complications and eventually death. Most SARS-CoV-2 infected persons are not hospitalized, and relatively little is known about the progression of symptoms, clinical outcomes, and severity predictors among outpatients

At present only limited therapeutic options have been approved for the treatment of COVID- 19 by regulatory agencies on the basis of randomized controlled trials (RCTs). Moreover, clinicians who at present are facing either the first or the beginning of the second epidemic wave are influenced in their everyday clinical practice, not only by new published studies but also by press releases concerning RCTs.Recently a promising approach in the control of COVID19 is the isolation of human SARS CoV- 2-neutralizing monoclonal antibodies (MoAbs) that could be used as therapeutic or prophylactic agents. Monoclonal antibodies could be also useful in high risk patients with severe immunodepression who do not respond to vaccine.

SARS-COV-2 DIAGNOSTICS: A CRITICAL REVIEW OF THE CURRENT ASSAYS

M.R. Capobianchi

Virology and Biosecurity Laboratories, National Institute for Infectious Diseases "Lazzaro Spallanzani" IRCCS, Rome, Italy

SARS-COV-2 AND ANTIVIRAL INTERFERON RESPONSE: WHO WINS AT TUG-OF-WAR?

C. Scagnolari

Virology Laboratory, Department of Molecular Medicine, Sapienza University, Rome Italy

COVID-19 can result in severe disease characterized by significant immunopathology that is spurred by an exuberant, yet dysregulated, innate immune response with a poor adaptive response. A limited and delayed interferon I (IFN-I) and IFN-III response has been shown to result in exacerbated proinflammatory cytokine production and in extensive cellular infiltrates in the respiratory tract, resulting in lung pathology. COVID-19 patients have highly heterogeneous patterns of IFN-I/III response both in the respiratory tract. In particular, type I/III IFNs, IFN-regulatory factor 7 (IRF7), and IFN stimulated genes (ISGs), appear to be highly expressed in the oropharyngeal cells of SARS-CoV-2 positive patients compared to healthy controls but the critically-ill patients that required invasive mechanical ventilation had a general decrease in expression of IFN/ISG genes. Notably, several SARS-CoV-2 proteins, including open reading frame 3b (ORF3b), ORF6, ORF7, ORF8, and the nucleocapsid (N) protein, can inhibit IFN type I and II (IFN-I and -II) production. However, a number of likely pathogenic autoreactive antibodies including anti IFN neutralizing antibodies have been associated with life-threatening SARS-CoV-2 infection. Moreover, at least 3.5% of patients with life-threatening COVID-19 pneumonia had known or new genetic defects at eight of the 13 candidate loci involved in the TLR3- and IRF7-dependent induction and amplification of type I IFNs. Thus, the lack of IFN responses against SARS-CoV-2 early on during infection in combination with hyperinflammation, makes the patients succumb rapidly to COVID-19. The development of effective therapeutics for patients with severe COVID-19 depends on our understanding of the pathological elements of this unbalanced innate immune response.

COVID-19: WHAT HAS BEEN DONE, AND WHAT REMAINS TO DO

G. Silvestri

Emory University School of Medicine, Yerkes National Primate Research Center, Atlanta, USA

Eighteen months after the beginning of the COVID-19 pandemic and the discovery of its causative agent, the human beta-coronavirus SARS-CoV-2, much progress has been made in several research areas related to this novel disease. However, much work remains to be done even in those areas of COVID-19 research that have seen the most significant advances. In this presentation, I will briefly review the state-of-the-art of our knowledge of this novel viral infection, with focus on the following areas: (i) the origin of SARS-CoV-2; (ii) the main mechanisms involved in its transmission and the key aspects of COVID-19 pathogenesis; (iii) the innate and adaptive immune responses to SARS-CoV-2; (iv) the efficacy (or lack thereof) of the so-called non-pharmacological interventions such as lockdowns and other restrictions to people movement, social distancing, and universal face-masking; (v) the main therapeutic approaches that are effective in the initial stages of COVID-19, including small molecule antiviral and monoclonal antibodies; (vi) the key therapeutic approaches to severe COVID-19; (vii) the characteristics, safety, and efficacy of the main COVID-19 vaccines. I will conclude this lecture by providing the audience with a brief perspective of some specific scenarios that might characterize the future of the pandemic.

Poster & Oral Communications

P01

Circulation of new SARS-CoV-2 variants in central Italy: characterization by deep -sequencing of the full-lenght spike

M.C. Bellocchi[1], R. Scutari[1], L. Carioti[1], M. Iannetta[2], L. Piermatteo[1], M. Botticelli[1], M. Alkhatib[1], S. Tedde [2], L. Duca [1], V. Malagnino [2], A. Crea [2], L. Ansaldo [2], S. D'Anna [1], A. Bertoli[1,2], P. Paba[2], A. Di Lorenzo[2], R. Salpini[1], V. Svicher[1], L. Sarmati[2], M. Andreoni[2], F. Ceccherini-Silberstein[1] for the PTV-ID-COVID Group

[1] University of Rome Tor Vergata, Rome, Italy - [2] University Hospital of Rome Tor Vergata, Rome, Italy

Aim. The spread of SARS-CoV-2 variants of concern (VOC) has led to increased attention on the spike protein analysis. Our aim was an in-deep characterization of the spike variants circulating in Central Italy by next generation sequencing (NGS) of the S gene.
Methods: Nasopharyngeal-swabs (NS) of SARS-CoV-2 infected individuals were collected between June 2020 to April 2021. NGS full-length S gene was performed using MiSeq platform (Illumina-Inc) with home-made protocols. Mutations were defined according to the frequency prevalence as: *major* (>99%), *minor* (2-20%), *intermediate* (>20-70%). All individuals had NS RT-PCR positive for 3 genes: *envelope* (E), *nucleocapsid* (N) and *RNA-dependent-RNA-polymerase* (RdRp) with Cycle-Threshold (Ct) values <35. Mann-Whitney and Kruskal-Wallis tests were used for statistical analyses.
Results. Full-length spike sequences were characterized in 93 individuals: 63 (67.7%) males, with median(IQR) age of 62 (49-71) years; 80 (86.0%) with Italian ethnicity and 13 (14.0%) non-Italian [5(5.4%) African, 2(2.2%) Asiatic, 6(6.4 %) Hispanic]. Seventy-three (78.5%) were hospitalized patients, of whom 62 (84.9%) with reported diagnosis of pneumonia. Median (IQR) NS Ct of E-N-RpRd was 25(21-28), 24(20-27) and 25(21-28), respectively. The median (IQR) reads for each individual sample was 167580 (127213-225050). Overall, 51 (54.8%) patients carried VOC, with the UK B.1.1.7_501Y.V1_20I and Brazilian P.1_501Y.V3_20J, starting from February and March 2021, respectively. Thirty-nine (41.9%) patients were characterized by the UK VOC B.1.1.7 and 11 of them (28.2%) carried also one *additional major* spike mutation. Brazilian VOC P.1 was observed in 12 (12.9%) patients and of these, 4 (33.3%) had one *additional major* mutation. As expected, 8 individuals with a suspect of transmission cluster, showed an identical pattern of P.1 Brazilian VOC, without any *additional major* or *minor* mutations. Interestingly, in April 2021, 2 patients (2.2%) showed the Nigerian-variant B.1.525, one with *additional major* mutation. Moreover, 26 (28.0%) patients showed EU1 variant characterized by A222V+D614G, 69.2% with 2 *additional major* mutations (A262S+P272L); 3 (3.2%) showed the Scottish-variant S:N439K characterized by N439K+D614G, 1 patient (1.1%) showed the Portuguese-variant 20A.EU2 characterized by S477N+D614G, all others (10, 10.7%) showed variants with the D614G.
Overall, *minor* mutations were observed in 45 patients [48.4%, median (IQR) 2 (1-3) mutations]; while only 2 patients showed 3 mutations with intermediate prevalence.
Stratifying individuals according to type of unknown *additional* mutations [only *major additional (Ma, N=23)*, *minor +/- major (mMa*, N=45), and *without additional* mutations (*Wa*, N=25)] we observed that individuals with *mMa* mutations had higher Ct-values respect to

all other groups: [E/N/RdRp Ct median(IQR) *mMa*: 27(24-29)/26(24-29)/28(25-31) *vs Ma*: 24(21-27)/23(20-26)/25(22-28) *vs Wa*: 21(17-24)/19(16-25)/21(16-26), all p<0.001]. Interestingly, days from first COVID19 symptoms to NS sampling was significant longer in hospitalized patients with *mMa* respect to others [median (IQR) *mMa*: 9 (7-11) days vs *Ma*: 5 (4-10) days vs *Wa:* 9 (5-11) days, p=0.02].
Finally, regarding patients infected by VOC, significant lower Ct-values were observed in patients carrying the Brazilian P.1compared to the UK B.1.1.7 [E/N/RdRp Ct median(IQR): 16(15-25)/15(13-24)/15(14-25) *vs* 24(21-27)/24(20-26)/25(22-28), p=0.011/p=0.009/p=0.007, respectively].
Conclusion. We confirm a spread of VOC in our Center, starting from February 2021. The presence of *minor additional mutations*, associated with higher Ct-values, seems to correlate with a longer duration of infection and lower viral-load, suggesting an evolution with increased variability regardless of the type of variant and/or VOC.

P02
Lineage B.1.525 SARS-CoV-2 infection in vaccinated patients

L. Cardillo[1], L. Cozzolino[1], M. Viscardi[1], E. De Carlo[2], C. de Martinis[1], G. Fusco[1]

[1] Istituto Zooprofilattico Sperimentale del Mezzogiorno - Portici, Naples- Italy - Department of Animal Health-Unit of Virology - [2] Istituto Zooprofilattico Sperimentale del Mezzogiorno - Portici, Naples- Italy - Scientific Director

Aim of the study. During March 2021, nasopharyngeal swabs were performed on eleven regularly vaccinated with Pfizer–BioNTech COVID-19 vaccine patients, belonging to a hospital in Napoli province, and submitted to SARS-CoV-2 qRT-PCR.
All samples were tested positive and subsequently nested-PCR was conducted in order to better clarify the Lineage involved against vaccinated subjects.
Methods used. Nested-PCR reactions were obtained by modifying the ThermoFisher Scientific's protocol for sequencing the SARS-CoV-2 S gene(5) that gave rise to 669 bp amplicons.
Tested positive samples, that gave rise to the correct target size, were purified, followed by Sanger sequencing. Finally, the sequencing reactions were applied to a 3500 Genetic Analyzer capillary electrophoresis system (Applied Biosystems).
Results and conclusions. Sequencing results revealed that 3 of 11 patients had a particular variant G/484K.V3 (Lineage B.1.525), with E484K, del69-70 among other defining mutations, belonging from several countries. Regarding the remaining 8 patients, further investigations using the Next-Generation Sequencing (NGS) method are still ongoing.
First identified as a geographically dispersed cluster in UK on the 2 February 2021. This variant was designated VUI-21FEB-03 (B.1.525) on 12 February 2021. The earliest sample date for VUI-21FEB-03 (B.1.525) in England was 15 December 2020. As of 31 March 2021, cases have been reported in 39 countries or territories(4).
Other characteristic mutations of this variant are basically Spike A67V, Spike D614G, Spike E484K, Spike F888L, Spike H69del, Spike Q677H, Spike V70del, Spike Y144del, E L21F, M I82T, N A12G, N T205I, NSP3 T1189I, NSP6 F108del, NSP6 G107del, NSP6 L260F, NSP6 S106del, NSP12 P323F.
Among all the mentioned mutation E484K being the most concerning as it aids in immune evasion and drastically causes the efficacy of current vaccines to be reduced by large margins(1). Protective mRNA- and vector-based vaccines encoding the SARS-CoV-2 S protein have been recently largely used worldwide and are considered a key point for the containment of the COVID-19 pandemic. The most worrisome variant is the South African or B.1.351 which harbors the above mutations E484K found in our Lineage B.1.525.
It is evident that the SARS-CoV-2 variants represent an international health risk, the E484K and N501Y mutations being the two most implicated mutations(2). In fact some authors suggest that this combined mutation may affect the neutralizing antibody response. It also seems that the replacement of asparagine (N) with tyrosine (Y) in position 501 (N501Y), present in the variants belonging to lineages B.1.1.7, B.1.351 and P.1, does not appear to affect in vitro sera neutralization. However, additional substitutions, such as Spike E484K present in lines B.1.351 and P.1, could allow evasion of neutralizing antibodies (3). Lineage B.1.525 contains numerous mutations together with the Spike E484K

and this suggests dutifully to investigate the involvement of this mutation in the lack of efficacy of currently available vaccines.
It is a crucial key point that targeted vaccines are synthesized to ensure that immunized individuals gain effective protection against these upcoming variants.

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P03

Metatranscriptomic characterization of COVID-19 to identify co-infections, altered upper respiratory tract microbiome and host functional responses

C. Ferravante[1,2], E. Alexandrova[1], Y. D'Agostino[1], J. Lamberti[1], O. Strianese[3], D. Memoli[1,4], T. Rocco[4], A. Sellitto[1], I. Terenzi[1], D. Palumbo[1,3], V. Mirici Cappa[1], G. Nassa[1,3], R. Tarallo[1,3], G. Franci[1,5], P. Pagliano[1,6], M. Cennamo[7], G. Portella[7], G. Fenza[8], A. Saccomanno[9], M. Galdiero[10,11], F. Rizzo[1,3], A. Weisz[1,3,4], G. Giurato[1,3]

[1] Department of Medicine, Surgery and Dentistry 'Scuola Medica Salernitana' and Laboratory of Molecular Medicine and Genomics, University of Salerno, Baronissi (SA), Italy - [2] Department of Veterinary Medicine and Animal Production, University of Napoli 'Federico II', Napoli, Italy - [3] Genome Research Center for Health, Campus of Medicine, Baronissi (SA), Italy - [4] Program of Medical Genomics, AOU 'SS Giovanni di Dio e Ruggi d'Aragona' University of Salerno, Italy - [5] Program of Clinical Microbiology, AOU 'SS Giovanni di Dio e Ruggi d'Aragona' University of Salerno, Italy - [6] Unit of Infectious Diseases, , AOU 'SS Giovanni di Dio e Ruggi d'Aragona' University of Salerno, Italy - [7] Dept of Medical Translational Sciences, University of Napoli 'Federico II', Napoli (Italy) - [8] Department of Management & Innovation Systems, University of Salerno, Fisciano (SA), Italy - [9] Centre for Research in Pure and Applied Mathematics, University of Salerno, Fisciano (SA), Italy - [10] Department of Experimental Medicine, University of Campania 'L. Vanvitelli', Napoli, Italy - [11] Unit of Virology and Microbiology, AOU University of Campania 'L. Vanvitelli', Napoli, Italy

Objectives. The last 16months have been characterized by a global outbreak of respiratory illnesses caused by the SARS-CoV-2 coronavirus. Due to virus high transmissibility, Coronavirus Disease 2019 (COVID-19) rapidly become a pandemic. To elucidate the pathogenesis of the variable clinical expression of the disease, information concerning co-infections with bacterial or viral pathogens in COVID-19 patients are needed. This investigation, performed mainly on patients from Campania region, focused on three core elements inferable from metatranscriptomics analysis of RNA form nasopharyngeal swabs: SARS-CoV-2 genome analysis, nasal microbiome composition and host cell responses.
Methods. Whole RNA sequencing (metatranscriptomics) was applied to nuclei acids extracted from nasopharyngeal swabs from 91 patients infected by SARS-CoV-2, selected based on different criteria, including age and sex, sampling time and clinical information. Samples were gathered between February 2020 and February 2021. Libraries were prepared using the Illumina TruSeq stranded total RNA protocol and sequenced on Illumina platforms. Sequence reads mapping on human genome were filtered out and analysis focused first on SARS-CoV-2, by genome assembly and variant identification. Unmapped fragments were then aligned to all microbial reference genomes available, to identify the taxonomic composition and functional pathways of active microbiota. Finally, host transcriptome analysis was performed on the human sequence reads.
Results and Conclusion. SARS-CoV-2 analysis revealed the presence of 9 different clades (20B, 19A, 20A, 20C, 20D, 20E (EU1), 20A.EU2, 19B, 20I/501Y.V1). Analysis on microbial composition of COVID-19 samples showed so far an altered airway microbiome, with co-detection of other human respiratory viruses (including human alphaherpesvirus 1, rhinovirus B, and human orthopneumovirus) in severely ill patients. Notably, the predominant respiratory microbial taxa were Burkholderia cepacia complex (BCC), Staphylococcus epidermidis, or Mycoplasma spp. (including M. hominis and M. orale). Preliminary analysis on host expressed genes revealed a transcriptional signature of several RNAs associated with

immune pathway activation, such as cytokine signaling. The profiling data relative to the host transcriptome collected here represents a starting point to fully elucidate the involvement of SARS-CoV-2 in COVID-19 disease. In this context, the immune-associated host transcriptional signatures of COVID-19 could represent a tool for improving COVID-19 diagnosis and indicating disease severity.

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P04

SARS-CoV-2 complete genome sequencing from the Italian Campania Region using a highly automated next generation sequencing system

M. Fiorenza[1], M. Cennamo[1], E. La Civita[1], A. Liotti[1], A. Furno[1], L. Conte[1], S. Di Somma[1], F. Napolitano[1], L. Vallefuoco[1], R. Sorrentino[1], E. A. Molinari[1], N. Normanno[2], D. Terracciano[1], G. Portella[1]

[1] Department of Translational Medical Sciences, University of Naples "Federico II", 80131 Naples, Italy - [2] Cell Biology and Biotherapy Unit, Istituto Nazionale Tumori-IRCCS-"Fondazione G. Pascale", Naples, Italy

Aim of the study: Since the first complete genome sequencing of SARS-CoV-2 in December 2019, more than 550,000 genomes have been submitted into the GISAID database. Sequencing of the SARS-CoV-2 genome might allow identification of variants with increased contagiousness, different clinical patterns and/or different response to vaccines. A highly automated next generation sequencing (NGS)-based method might facilitate an active genomic surveillance of the virus.
Methods used: RNA was extracted from 27 nasopharyngeal swabs obtained from citizens of the Italian Campania region in March-April 2020 tested positive for SARS-CoV-2. Following viral RNA quantification, sequencing was performed using the Ion AmpliSeq SARS-CoV-2 Research Panel on the Genexus Integrated Sequencer, an automated technology for library preparation and NGS. The SARS-CoV-2 complete genomes were built using the pipeline SARS-CoV-2 RECoVERY (REconstruction of COronaVirus gEnomes & Rapid analYsis) and analysed by IQ-TREE software.
Results and conclusions: The complete genome (100%) of SARS-CoV-2 was successfully obtained for 21/27 samples. In particular, the complete genome was fully sequenced for all 15 samples with high viral titer (>200 copies/µl), for the two samples with low (<200) copies of the viral genome and for 4/10 samples with a viral load below the limit of detection (LOD) of 20 viral copies. The complete genome sequences classified into the B.1 and B.1.1 SARS-CoV-2 lineages. In comparison to the reference strain Wuhan-Hu-1, 51 total nucleotide variants were observed with 29 non-synonymous substitutions, 18 synonymous and 4 reported in untranslated regions (UTRs). Thirteen of the 29 non-synonymous variants were observed in ORF1ab, 7 in S, 1 in ORF3a, 2 in M and 6 in N genes. The Genexus system resulted successful for SARS-CoV-2 complete genome sequencing, also in cases with low viral copies. The use of this highly automated system might facilitate the standardization of SARS-CoV-2 sequencing protocols and make faster the identification of novel variants during the pandemic.

P05

A platform for spatial and temporal monitoring of COVID-19 genetic evolution in Campania

G. Fenza[1], A. Saccomanno[2], O. Strianese[3], V. Mirici Cappa[4], D. Memoli[4], C. Ferravante[5], E. Alexandrova[4], Y. D'Agostino[4], J. Lamberti[4], G. Pecoraro[4], V. Melone[4], V. D'Amore[4], G. Nassa[3,4], R. Tarallo[3,4], G. Franci[4,6], P. Pagliano[4,7], A. Weisz[3,4], F. Rizzo[3,4], G. Giurato[3,4]

[1] Department of Management & Innovation Systems, University of Salerno, Fisciano (SA), Italy - [2] Centre for Research in Pure and Applied Mathematics, University of Salerno, Fisciano (SA), Italy - [3] Genome Research Center for Health, Campus of Medicine, Baronissi (SA), Italy - [4] Department of Medicine, Surgery and Dentistry 'Scuola Medica Salernitana' and Laboratory of Molecular Medicine and Genomics, University of Salerno, Baronissi (SA), Italy - [5] Department of Veterinary Medicine and Animal Production, University of Napoli 'Federico II', Napoli (Italy) - [6] Program of Clinical Microbiology, AOU 'SS Giovanni di Dio e Ruggi d'Aragona' University of Salerno, Italy - [7] Unit of Infectious Diseases, AOU 'SS Giovanni di Dio e Ruggi d'Aragona' University of Salerno, Italy

Objective: The novel coronavirus named SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), responsible for COVID-19 disease, has spread in a worldwide pandemic since its first appearence in late 2019 in Wuhan (China) end constantly changed it genome leading to the appearance and diffusion of viral variants with changing biological and epidemiological behaviours. The continuous appearance and quick diffusion of mutated viruses obliged the decision-makers to make fast and reliable choices about a number of activities (free circulation of individuals, education, work, etc.). The continuous evolving situation and the lack of an overall data hub providing an exhaustive awareness of this phenomenon to decision-makers made the decisions hard and often riskful. This work focused on collection and visualization of epidemiological data concerning presence of COVID-19 variants in the 5 provinces of the Campania region. The use of NGS technology allowed to obtain the unique identification of any variant of the viral genome in each sample analyzed. To provide support to decision-making bodies an interactive valuable dashboard was created which provides the situation awareness with timely data and emerging territorial conditions.
Methods: We used state of the art paradigm and big data-enabled technologies to build a platform which adopts opensource solutions to index epidemiological metadata, like ELK stack. We integrated several third-party libraries to annotate and classify input genomic sequence of the virus, such as NextClade and Pangolin (Phylogenetic Assignment of Named Global Outbreak LINeages). These modules are used to extract Clade and Lineage corresponding to the input samples. Additionally, geographically and demographic annotations are ingested into the platform whenever available, to enable the monitoring of virus and variant evolutions along the time and space dimensions.
Results and conclusions: The results led to creation of TREVOM (Temporal and Regional Evolution of Virus Omics Monitoring), a platform for monitoring COVID-19 variant spreading in Campania. At present, the largest sets of data it collects are relative to samples collected in two distinct periods: February-April 2020 and September 2020-March 2021. The system used a multi-dimensional classification mechanism that allows to filter the search space of the collected samples by cross-relating collected samples through practical di-

mensions. The final result is an interactive dashboard that can select the data through the geographic filter (by province and municipality), time slider (date of the collected sample), Clade and Lineage of interest, demographic information (gender, age), types mutations found (proteins, nucleotides, and type of mutation). Visualization, temporal and spatial data aggregation functions have been made available to decision-makers. Mainly three temporal patterns in reported cases, characterized by the prevalence of specific clades in Campania: 20A, between March and June 2020, 20E (EU1), between September and December 2020 and a rapid raising of the 20C and 19B in January to March 2021.

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P06 - OC 1

Early diffusion of SARS-CoV-2 infection in the inner area of the Italian Sardinia Island

N. Grandi[1*], G. Piras[2*], M. Monne[2], R. Asproni[2] , T. Fancello[3], M. Fiamma[4], G. Mameli[4], G. Casu[3], I. lo Maglio[4], A.D. Palmas[2] and E. Tramontano[1,5]

[1] Laboratory of Molecular Virology, Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy - [2] UOC Ematologia, P.O. "San Francesco", Azienda Tutela Salute, ASSL Nuoro, Italy - [3] UOC Cardiologia, P.O. "San Francesco", Azienda Tutela Salute, ASSL Nuoro, Italy - [4] UOC Laboratorio Analisi clinico-chimiche e Microbiologia, P.O. "San Francesco", Azienda Tutela Salute, ASSL Nuoro, Italy - [5] Istituto di Ricerca Genetica e Biomedica, Consiglio Nazionale delle Ricerche, Cagliari, Italy - * = *equally contributors*

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been responsible for coronavirus disease 2019 (COVID-19) pandemic, which started as a severe pneumonia outbreak in Wuhan, China, in December 2019. Italy has been the first European country affected by SARS-CoV-2 diffusion, registering more than 1.650.000 cases and 57.600 deaths until the end of 2020. The geographical distribution of SARS-CoV-2 in Italy during early phases of 2020 pandemics has not been homogeneous, including severely affected regions as well as administrative areas being only slightly interested by the infection. Among the latter, Sardinia represented one of the lowest incidence areas, likely due to its insular nature. The present study investigated the degree of diversity of SARS- CoV-2 genomes circulating in Sardinia at the beginning of the pandemics, to assess whether the limited diffusion of the infection in the island led to a lower diversity in local viral genomes with respect to the ones circulating in highly exposed parts of the country.

To this aim, we performed next generation sequencing of a small number of complete SARS-CoV-2 genomes from clinical samples obtained at "San Francesco" Hospital laboratory for COVID-19 between the end of March and the end of May 2020. The obtained viral genomes have then been characterized in terms of structure and phylogeny and compared to national and international SARS- CoV-2 variants.

Overall, results provided a snapshot of the initial phases of the SARS-CoV-2 infection in inner area of the Sardinia island, showing an unexpected genomic diversity and confirming the relevance of superspreading events in shaping the early phase of the pandemic. In fact, our analysis revealed a remarkable genetic diversity in local SARS-CoV-2 viral genomes, showing the presence of at least 4 different clusters that can be distinguished by specific amino acid substitutions. Based on epidemiological information, these sequences can be linked to at least 8 different clusters of infection, 4 of which likely originated from imported cases that were mainly represented by professionals returning from scientific congresses. In addition, the presence of amino acid substitutions which were not previously reported in Italian patients has been observed, asking for further investigations in a wider population to assess their prevalence and dynamics of emergence during the pandemic.

P07
Impact of PD-L1 checkpoint in COVID-19 patients

G. Greco[1], F. Sabbatino[2], V. Conti[2], G. Franci[2], P. Pagliano[2], C. Zannella[1], C. Vecchione[2], M. Ciccarelli[2], A. Filippelli[2], S. Pepe[2]

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

Aim of study. The outbreak of severe acute respiratory syndrome 2 (SARS-CoV-2) as the fifth pandemic in the 21st has raised major concerns. SARS-CoV-2, the aetiological pathogen of coronavirus disease 2019 (COVID-19), causes acute respiratory infections and gastroenteritis, inducing critical conditions such as sepsis, dyspnoea, hypoxemia, acute respiratory syndrome, cytokine secretion syndrome, and advanced T-cell lymphopenia has been [1]. During SARS-CoV-2 infection, there is a positive relationship between the destruction of T lymphocytes and the increased expression of inhibitory immune system molecules on their surface (aDiao, 2020). Indeed, it has been shown that serum levels of the programmed cell death ligand (PD-L1 or CD274) become significantly elevated in patients with COVID-19. Furthermore, in a 2012 study on the PD-1 / PD-L1 axis in acute viral infections affecting the lower respiratory tract by Erickson (John J. Erickson), it was shown that this pathway alters the activity of CD8+ T lymphocytes in the human respiratory system, and in addition, dendritic cells prevent T lymphocyte activity in acute viral infections by expressing high levels of PD-L1 on their surfaces [2]. The aim of this work is to elucidate the functions of these high levels of PD-L1 and their effects on T lymphocyte dysfunction and depletion, rendering them ineffective in individuals with COVID-19.
Methods used. RNAseq profiles used to assess PD-L1 gene profiles in human cells were collected by GEO (http://www.ncbi.nlm.nih.gov/geo/, s.d.), accession GSE147507 and GSE148729. In addition, RNAseq profiles of immune cells were collected by the Immunological Genome project (ImmGen, s.d.) using the Skyline RNA-seq tool. Human expression levels are the counts of reads aligned to the genome and expressed as counts per million. Values were extracted from supplemental data from GSE147507 and GSE148729. Gene counts were normalised using the EdgeR package, Bioconductor, (Mark D. Robinson D. J., 2010) which considers that most genes are invariant between experiments.
Results and conclusions. In order to understand the molecular basis of COVID-19 disease, we characterised PD-L1 transcript levels in a variety of model systems infected with SARS-CoV-2, including in vitro tissue cultures, ex-vivo infections of primary cells, samples derived from COVID-19 patients and immune cells. In infections with low multiplicity of infection (MOI 0.2), exogenous expression of ACE2 allowed SARS-CoV-2 to replicate in cell lines. Differential analysis of these cells showed that PD-L1 levels were similar to the control. Interestingly, a dramatic increase in viral load (MOI 2) was associated with an increase in PD-L1 RNAseq levels in the cells. Furthermore, transcriptional levels of CD274 were abolished after ruxolitinib treatment, indicating that IFN-I was involved in its expression. At this point we correlated these results with human infections by comparing post-mortem lung samples from COVID-19 positive patients with healthy lung tissue biopsied from uninfected individuals. The transcriptional profile of these samples demonstrated increased PD-L1 transcript levels compared to healthy subjects. In addition, to profile PD-L1 expres-

sion in immune system cells, data on PD-L1 transcript levels were collected from the Immunological Genome database of 8 peripheral blood samples. The PD-L1 gene was highly reduced in interferon-stimulated T4 cells in COVID-19 patients. In contrast, in neutrophils and T gamma delta cells, CD274 expression levels were dramatically increased. Several innate immune cell subsets were depleted in COVID-19 patients, including γδ T cells, plasmacytoid dendritic cells (pDCs), conventional dendritic cells (DCs), CD16+ monocytes and NK cells. In addition, elevated IL-10 levels observed in COVID-19 patients may also indicate the role of the PD-1 / PD-L1 axis in the development of acute viral infections and monocyte rearrangement. Patients with more severe states had higher expressions of PD-L1 on both monocytes and DCs. Thus, PD-1 / PD-L1 pathways play a crucial role in COVID-19 infection and increased expression of PD-L1, in γδ T cells, monocytes and neutrophils may serve as a biomarker for the assessment of SARS-CoV-2 infection.

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P08 - OC 2

Identification of highly recurrent SARS-CoV-2 haplotypes circulating worldwide and in Veneto region

L. Manuto, M. Bado, G. Mazzotti, A. Crisanti, E. Lavezzo, S. Toppo

University of Padova, Department of Molecular Medicine (DMM)

Various severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants have emerged since the start of the pandemic, declared by the World Health Organization (WHO) on 11 March
2020. The prompt identification of novel viral strains, or genetic mutations promoting viral fitness or immune escape is pivotal to control the spread of the virus, especially as vaccines are currently administered globally. The vast availability of genomic data related to SARS-CoV-2 facilitates immune surveillance. However, the available datasets are heterogeneous and the contribute of different countries is unbalanced, thus an ad-hoc pipeline is crucial to filter out low quality data and to extrapolate unbiased information from the publicly available datasets. In this study, a pipeline was defined to accurately filter and analyze publicly available SARS-CoV-2 sequencing data and to further identify and monitor over time and space highly recurrent viral mutations circulating globally and in Veneto region. Accordingly, all SARS-CoV-2 high quality and complete genome sequences available on GISAID were downloaded and further processed. Sequences collected in Veneto region were included in the study to compare the global evolution of the virus to the local one. To follow viral evolution overtime, the sequences were grouped according to their collection date and each group was individually analysed to identify genomic positions with a remarkable frequency of mutation. Due to a strong imbalance in the contribution of the different countries to the GISAID dataset, a normalization method was implemented in the mutation frequency calculations, to prevent an over representation of only a restricted group of nations. As a result, 129 hotspots were identified within the SARS-CoV-2 genome, with ORF8 and N gene having the highest mutation rate. An overall accumulation of mutations with respect to the reference genome from Wuhan was observed, with the emergence and spread of novel variants becoming prevalent worldwide and in Veneto region with a similar trend. Interestingly, the spatiotemporal analysis revealed groups of mutations that increased in frequency over time in different countries but disappeared later on, without being defined as a novel variant, while others emerged as low frequency mutations and subsequently took over as novel viral strains. As it can be easily updated, our pipeline is a useful tool to monitor the global and local emergence of novel viral variants or genetic mutations across space and time, based on public and private data.

P09

Evaluation of SARS-CoV-2 infection on a sample of population of the Campania Region

C. Maiello, M. Ferrigno*, R. Angrisani**, A. Rainone *, M. A. Sorrentino*, N. Occhiati*, L. Di Maio*, L. Lovriso*, E. Policano*, L. Folliero*, M. Aloisio*, P. Petrosino*, E. Mocerino**, M. D'Orazio*, M. Ametrano**, C. Furfaro***

(*) Azienda Aanitaria Locale Napoli 3 sud Presidio Ospedaliero "Cav. R. Apicella" Pollena Trocchia (NA) Laboratorio Di Patologia Clinica - (**) Azienda Sanitaria Locale Salerno Presidio Ospedaliero Di Agropoli (Sa) Laboratorio Di Patologia Clinica

Introduction. The SARS CoV 2 pandemic, which affected the entire world population, took the scientific community by surprise, unleashing countless and useless, if not deleterious, controversies. In addition to catching us unprepared to face the complications of virosis, the new coronavirus has found us ignorant about its gene structure, in part about the mode of transmission, its incubation, the course of the disease it generates and the immune response of the human body.

Epidemiological studies are still underway aimed at the mode of onset and stability of antibodies to SARS CoV 2, as well as their neutralizing power. In our facilities we have dealt with the molecular diagnostics of SARS CoV 2 and the monitoring of the samples that were positive, as well as the antibody assay as soon as it was available.

The RT - PCR real time methods validated at international level provide for the detection, in addition to the internal control, of various genes: the RdRp gene as a stable marker for the diagnosis of SARS CoV2 and those related to the genes common to the Coronavirus.

Materials and methods: Approximately 31.000 rhino-pharyngeal swabs were tested.

The tests were performed with PANA 9600 extraction and amplification workstations using reagents Tianlong - Viral DNA and RNA Extraction kit and KHB - Diagnostic kit for SARS CoV 2 - Nucleic Acid Real Time PCR.

The rhino - pharyngeal swabs, both of patients hospitalized in the ward and of those in quarantine at their home, persistent in area, were taken in charge within a few hours of collection and processed within 24 hours.

Results.

N° Samples	Negative	Positive	2nd Positive on a sample taken 10 days after the 1st positive result
31.000	24.746	2.821 of which 138 low charge	2.356

Of the 31.000 samples, 1.077 were unsuitable due to the absence of biological material.

Conclusions. From November 2020 to date, a number of results equal to about 31.000 have been reported and this observational study will continue in order to evaluate the incidence of SARS CoV 2 positive patients, monitor the course and evolution towards the disease.

P10

Genomic analysis of SARS-CoV-2 variants in Lombardy: a single-center perspective

A. Mancon[1], F. Bracchitta[1], L. Rizzuto[1], A. Rizzo[1], D. Mileto[1], S. Giubileo[1], A. Romeo[1], A. Tamoni[1], L. Fiori[1], C. Tonielli[1], C. Bossi[1], S. Grosso[1], A. Lombardi[1], M.R. Gismondo[1], V. Micheli[1]

[1] Laboratory of Clinical Microbiology, Virology and Bioemergencies, ASST Fatebenefratelli Sacco, University Hospital L. Sacco, Milan

Aim: SARS-CoV-2 evolved in multiple lineages and variants, that spread around the world during the pandemic. The aim of this work was to evaluate the changes in viral strains epidemiology during the three Italian epidemic waves using whole genome sequencing.
Methods: Samples were selected according to RT-PCR Ct values (<30), both clinical and Istituto Superiore di Sanità indications (i.e.: random sampling, travellers, cluster, re-infection, post-vaccine infection and other) and positivity for E484K by RT-PCR. The epidemic waves were defined as follows: 1st=20/02/2020–30/08/2020, 2nd=01/09/2020–31/01/2021, 3rd=01/02/2021–present. Viral genome was extracted using QIAamp DSP Virus Mini kit and sequenced by means of CleanPlex® SARS-CoV-2 Panel kit (Paragon Genomics, Inc., USA) on MiSEQ instrument (Illumina, Inc., USA); the FASTQ files were processed on SOPHiA™ platform (SOPHiA GENETICS SA, CH). Viral lineage was attributed using Pangolin COVID-19 Lineage Assigner software.
Results and conclusions: A total of 302 whole genome sequences were obtained from swabs collected in different Lombard centers (lineages in Table 1). An increase in viral heterogeneity was evident, probably due to travels and local evolution. In particular, B.1.177 viral strain became dominant during the second epidemic wave, while UK variant (B.1.1.7) entered in January, paving the way to extended diffusion in February 2021; low prevalence of both Brazil (P.1) and South Africa (B.1.351) variants was found, suggesting minor transmission success. These data are confirmed by RT-PCR variants detection, which estimates a current prevalence of 90% for B.1.1.7 in our centre; RT-PCR can not however detect all strains, especially new ones, thus making sequencing essential for a proper surveillance.

Table 1. Distribution of viral strains in the three epidemic waves

FIRST WAVE		SECOND WAVE		THIRD WAVE	
LINEAGE	N (%)	LINEAGE	N (%)	LINEAGE	N (%)
B.1	17 (100.0)	A.19	1 (1.2)	A	1 (0.5)
		AD.2	3 (3.5)	A.23.1	1 (0.5)
		B.1.1	1 (1.2)	B.1	2 (1.0)
		B.1.1.7	20 (23.5)	B.1.1.318	2 (1.0)
		B.1.160	3 (3.5)	B.1.1.355	3 (1.5)
		B.1.177	49 (57.6)	B.1.1.7	86 (43.0)
		B.1.221	1 (1.2)	B.1.160	1 (0.5)
		B.1.235	1 (1.2)	B.1.177	77 (38.5)
		B.1.241	3 (3.5)	B.1.221	2 (1.0)
		B.1.258	1 (1.2)	B.1.258	3 (1.5)
		B.1.416	2 (2.4)	B.1.351	1 (0.5)
				B.1.525	7 (3.5)
				B.1.575.1	1 (0.5)
				C.36	3 (1.5)
				P.1	10 (5.0)
TOT	17	TOT	85	TOT	200

P11 - OC 3

Study of COVID-19 patients harboring variants of SARS-COV-2: comparison with a population from the first pandemic wave

L. Mazzuti[1], M.A. Zingaropoli[2], P. Pasculli[2], G.M. Masci[3], R. Campagna[1], G. Oliveto[1], F. Iafrate[3], C. Catalano[3], P. Ricci[3], M.R. Ciardi[2], C.M. Mastroianni[2], A. Pierangeli[1], G. Antonelli[1], O. Turriziani[1]

[1] Department of Molecular Medicine, Sapienza University of Rome, Italy - [2] Department of Public Health and Infectious Diseases, Sapienza, University of Rome, Italy - [3] Department of Radiological, Oncological and Pathological Sciences, Sapienza University of Rome, Italy

Since severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) virus initially emerged in China, it has rapidly spread to other countries worldwide as a global threat until causing "the first pandemic of the 21st century" as defined by the WHO on March 11, 2020.

In this last year across the world, several variants of SARS-CoV-2 emerged. Among these, three new variants have rapidly become dominant, spreading also in Italy and arousing concerns: B.1.1.7 (UK), B.1.351 (South Africa), and P.1 (Brazil). The recently emerged SARS-CoV-2 variants harbor several mutations at the receptor binding domain in the spike (S) glycoprotein that may alter virus-host cell interactions, contributing to immune escape, increased transmissibility and conferring resistance to inhibitors and antibodies.

Aim of the study was to investigate differences in clinical, laboratory and radiological findings in patients with coronavirus disease 2019 (COVID-19) comparing patients harboring a wild-type SARS-CoV-2 and patients with new emerged variants.

We included 62 nasopharyngeal swabs from COVID-19 patients admitted to University Hospital "Policlinico Umberto I" in Rome. To define the SARS-CoV-2 genotype, S gene was sequenced using Sanger method. Patients were stratified into two groups: wild-type group, patients harboring wild-type SARS-CoV-2 hospitalized from March 2020 since April 2020, and variant group, patients infected with new emerged variants enrolled from March 2021 since April 2021. Laboratory and clinical data and imaging findings from chest computed tomography (CT), were also collected and evaluated.

Thirty-nine patients (17 females, 22 males) with a median age of 56 (IQR: 50-62) were included in the wild-type group and 23 patients (17 females, 6 males) with a median age of 46 (IQR: 37-57) were included in the variant group. According to clinical outcome, a significant high percentage of patients with Acute respiratory distress syndrome in the variant group compared to the wild-type group was observed (56.5% vs 25.6%, respectively; p= 0.0282).

At admission, the absolute number of white blood cells (WBC), neutrophils and the neutrophil/lymphocyte ratio (NLR) were significantly higher in the variant group than in the wild-type group. [WBC: 6.4 (IQR: 5.1-9.4) vs 4.8 (IQR: 3.8-5.6), p=0.0002; neutrophils 5.7 (IQR:3.8-7.1) vs 2.8, (IQR: 2.2-3.8), p<0.0001; NLR: 6.3 (IQR: 3.2-14.7) vs 2.4 (IQR: 1.6-4.3), p<0.0001). Conversely, a significantly lower number of lymphocytes was found

in the variant group compared to the wild-type one [0.9 (IQR:0.5-1.1) vs 1.2 (IQR:0.8-1.7), p=0.0139].
Finally, in the variant group, chest CT score was higher compared to the wild-type one [15 (IQR:11-21) vs 12 (IQR:8-15), p=0.0399]. Otherwise, lower Ct values in the variant group was observed compared to the wild-type group [26 (IQR:21-28) vs 30 (IQR:25-32), p=0.0014].
Our preliminary data showed higher chest CT score, absolute count of WBC and neutrophils, and NRL in the variant group compared to the wild-type one suggesting that the high chest CT score observed in variant group may be due to an increased neutrophils mediated organ damage.

P12
SARS-CoV-2 B.1.1.7 reinfection after previous COVID-19

F. Novazzi[1], A. Baj[1,2], A. Genoni[2], PG. Spezia[3], A. Colombo[1], G. Cassani[1], C. Zago[1], R. Pasciuta[1], R. Capuano[2], M. Prestia[2], D. Dalla Gasperina [4,2], W. Ageno[5,2], P. Severgnini[6], F. Dentali[4,2], D. Focosi[7], F. Maggi[1,2]

[1] Laboratory of Microbiology, ASST Sette Laghi, Varese, Italy - [2] Department of Medicine and Surgery, University of Insubria, Varese, Italy - [3] Department of Translational Research, University of Pisa, Pisa, Italy - [4] Unit of Internal Medicine, ASST dei Sette Laghi, Varese, Italy - [5] Department of Emergency, ASST dei Sette Laghi, Varese, Italy - [6] Anesthesia and Intensive Care, ASST Sette Laghi, and Biotechnology and Life Sciences Department, Varese, Italy - [7] North-Western Tuscany Blood Bank, Pisa University Hospital, Pisa, Italy

We report here two SARS-COV-2 B.1.1.7 reinfection cases in Lombardy residents.
Case 1 was a 56-years old immunocompetent male, admitted to the hospital on January 3, when interstitial pneumonia was diagnosed and treatment with continuous positive airway pressure ventilation was initiated. Tested positive for SARS-CoV-2 RNA, he was then moved to the intensive care unit, and intubated. After a gradual improvement, NPSs tested negative for SARS-CoV-2 RNA on Jan 23, Jan 31, and Feb 2, while anti-SARS-CoV-2 IgG was 194 AU/ml. On Feb 4, a new NPS tested positive for SARS-CoV-2 RNA, without clinical worsening, and additional NPSs tested negative for SARS-CoV-2 RNA on Feb 5, Feb 6, and Feb 11. Sequences of the RBD fragment of the spike gene from the Jan 4 sample showed no mutations when compared with SARS CoV-2 isolate Wuhan-Hu-1, whereas the Feb 4 strain resulted with all the barcoding mutations described above (deposited in GenBank as MW599860).
Case 2 was a 58-years old immunocompetent male who tested positive for SARS-CoV-2 RNA on NPS on Jan 7, 2021. He was moved to the COVID ward for oxygen support, progressing to CPAP ventilation on Jan 21. CPAP was discontinued on Jan 26, and two follow-up NPSs on Jan 31, and Feb 2 were negative for SARS-CoV-2 RNA. New NPSs on Feb 4 and 6 were positive for SARS-CoV-2 RNA, with anti-RBD IgG >400 AU/ml. On Feb 10 the patient was moved to subacute medical unit. At that point, we decided to sequence the RBD from NPS samples dated Jan 19 and Feb 6: the Jan 19 RBD sequence showed absence of mutation if compared with SARS CoV-2 isolate Wuhan-Hu-1, while the Feb 6 strain resulted in B.1.1.7 (deposited in GenBank as MW599954).
According to Facebook mobility data (COVID-19 Mobility Data Network. Available from: https://visualization.covid19mobility.org/?date=2021-02-09&dates=2020-11-09_2021-02-09&region=WORLD.), in 16 out of 19 countries analyzed, there is at least a 50% chance the variant was already imported by travelers from the UK by December 7th (Du Z, Wang L, Yang B, Ali ST, Yang B, K. Tsang T, et al. International risk of the new variant COVID-19 importations originating in the United Kingdom. 2021:2021.01.09.21249384.), with Italy being the country with the highest risk. Accordingly, many cases have been reported in Lombardy. Theoretical models have estimated the reinfection rate at 0.7%, similar to older strains (Graham MS, Sudre CH, May A, Antonelli M, Murray B, Varsavsky T, et al. The effect of SARS-CoV-2 variant B.1.1.7 on symptomatology, re-infection and transmissibility. 2021:2021.01.28.21250680), so that many more cases are likely undetected.

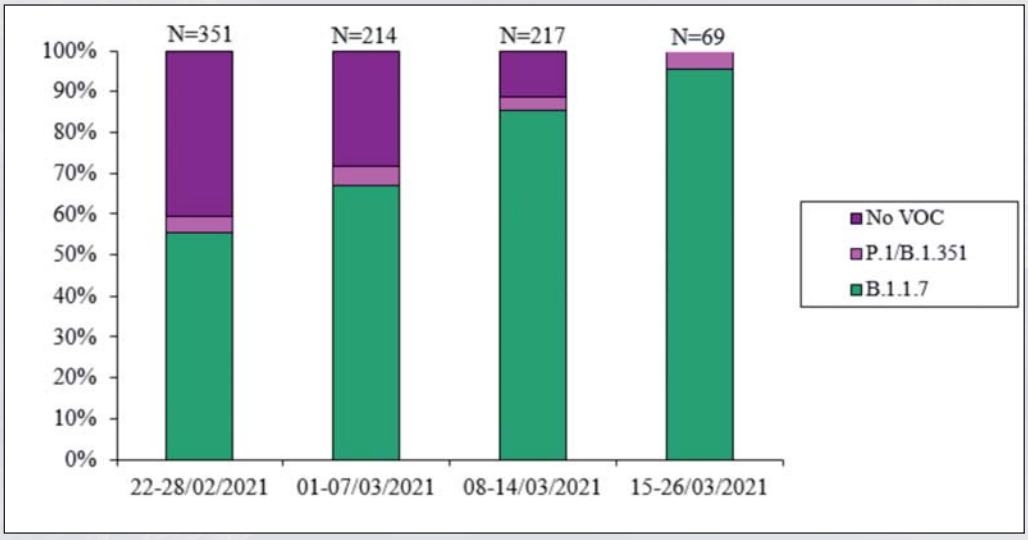
P13 - OC 4

Usefullness of RT-PCR in SARS-CoV-2 variant surveillance

A. Rizzo, F. Bracchitta, G. Gagliardi, L. Rizzuto, A. Mancon, C. Pagani, S.G. Rimoldi, A. Gigantiello, D. Mileto, D. Curreli A. Lombardi, M.R. Gismondo, V. Micheli

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

Aim of the study: The recent emergence of SARS-CoV-2 variants worldwide with the potential for increased transmission, disease severity and resistance to current vaccine requires a real-time strain surveillance. Being the gold standard approach by viral full genome sequencing expensive and time-consuming, RT-PCR could be a good alternative tool to rapidly track the major Variant of Concern circulation (VOC). The aim of this study was to evaluate the local circulation of SARS-CoV-2 variants in patients addressing to ASST-Fatebenefratelli-Sacco Hospital in Milan.
Methods used: A total of 973 molecular nasopharyngeal swabs resulted positive with RT-PCR Ct mean value < 35 was selected in the period 22 February-26 March. For the detection of the SARS-CoV-2 major VOC B.1.1.7, P.1 and B.1.351 two commercial multiplex RT-PCRs specific for crucial aminoacid positions (RIDA GENE SARS-CoV-2 Lineage kit - BioPharm and COVID-19 Variant Catcher - Clonit) were performed on in- and out-patients from 22 to 28 February (n=351) and subsequently only on hospitalized patients until 26 March (n=622). The patients were classified according to sex, age (11 groups: 0-1, 2-6, 7-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70-79, 80-89 and ≥90 years old) and clinical setting hospitalisation.
Results and conclusions: A total of 851 out 973 samples provided a reliable result, while 122 were not evaluable due to low viral load. In details, 69% (589/851) of the cases were



classified as B.1.1.7, 4% (34/851) as P.1/B.1.351 and 27% (228/851) harbouring a no VOC virus. B.1.1.7 and P.1/B.1.351 variants resulted most frequently in the age groups 50-59 (19%, 109/589) and 60-69 (21%, 7/34) years old, respectively; according to gender, males harbouring more frequently P.1/B.1.351 versus females (71%, 24/34). When considering the clinical conditions, no significantly differences in VOC distribution between in- and out-patients were found in the subgroup analyzed (89/161 vs 120/190; p=0.16). VOC B.1.1.7 became quickly the dominant strain, spanning from 56% to 96% in just over a month (chi-square test for trend p<0.0001), confirming the national trend observed in the same period. In conclusion, RT-PCR assays represent a valid alternative tool in detection and surveillance for SARS-CoV-2 VOCs, especially in those laboratories where sequencing technology is not available. Genomic analysis is, however, essential for a definitive lineage assignment and an accurate characterization of new emerging viral variants.
Graph 1. Distribution of B.1.1.7, P.1/B.1.351 and no VOC

P14 - OC 5

Genomic characterization of the emergent SARS-CoV-2 lineage in two provinces of Campania (Italy): whole-genome sequencing study

M. Scrima[1,3], A.M. Cossu[1,3], E.L. D'Andrea[1,2], Y. Abruzzese[1], C. Iannarone[1], C. Miarelli[1], T.M.R. Noviello[4], M. Ceccarelli[4], A. Fucci[1,2] and M. Caraglia[1,3,10]

[1] COVID Lab; Biogem scarl Ariano Irpino, Italy - [2] Protein Factory, Biogem Scarl, Ariano Irpino, Italy - [3] Molecular Oncology and Precision Medicine Laboratory, Ariano Irpino, Italy - [4] Bioinformatics Core, BIOGEM s.c.a.r.l, Italy - [5] Department of Precision Medicine, University of Campania Luigi Vanvitelli, Naples, Italy

Aim of the study. Coronavirus disease 2019 (COVID-19) has emerged in December 2019 when the first case was reported in Wuhan, China and turned into a pandemic with 27 million cases.
Nevertheless, there is still much to learn about the viral population variation when the virus is evolving as it continues to spread.
In the past few months, we have seen emergence of clinically important mutations that alter infectivity, severity, or immune susceptibility of SARS-CoV-2.
Methods. We have performed a Whole-Genome Sequencing (Ion Torrent System, Thermo Fisher Scientific) for 62 SARS-CoV-2 strains obtained from individuals who live in Campania (Italy).
We focused our attention on 2 provinces of Campania (Benevento and Avellino) in a time span of three months from January to March 2021.
Results. The sequence data were subjected to phylogenetic analysis using Nextclade, identifying six types of clades: 20A (Lineage B.1.258.14) (n = 6; 9.7%), 20B (Lineage B.1.1.351) (n = 5; 8%), 20A.EU2 (Lineage B.1.160) (n=2; 3%), 20E (EU1) (Lineage B.1.177.75) (n=5; 8%), 20I/501Y.V1 (B.1.1.7) (n=40; 65%), 20 J/501Y.V3 (Lineage P.1) (n = 4; 6%).
The five of lineages B.1.258.14, B.1.1.351, B.1.160, B.1.177.75 and P.1 were identified first in Benevento and after in Avellino respectively in early and in the second half of March but B.1.1.7 (Variant UK) was identified in two province from early January to end of March.
Conclusions. We have identified several missense mutation of Spike gene in all lineage identified whose co-occurrence not previously observed but we have to investigate the role and the effect of each mutations through model conformational state transition analysis.
Prominent mutations in the Spike protein have emerged independently in many global strains, possibly driving resurgence of the pandemic when it appeared to be coming under control.
In conclusion our initial study have detected five different lineage of SARS-CoV-2 in a small part of Campania region and the presence of new virus variants in each lineage whose role we must investigate in future studies.

P15
Genomic monitoring of SARS-CoV-2 variants in Campania

Y. D'Agostino[1], V. Mirici Cappa[1], E. Alexandrova[1], O. Strianese[2], T. Rocco[3], D. Memoli[3], J. Lamberti[1], G. Pecoraro[1,2], V. Melone[1], V. D'Amore[1], I. Terenzi[1], G. Nassa[1,2], R. Tarallo[1,2], P. Pagliano[1,4], G. Franci[1,5], E. Vaccaro[6], M. Cennamo[7], G. Portella[7], G. Fenza[8], A. Saccomanno[9], M. Galdiero[10,11], G. Giurato[1,2], A. Weisz[1,2,3], F. Rizzo[1,2]

[1] Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana' and Laboratory of Molecular Medicine and Genomics, University of Salerno, Baronissi (SA), Italy - [2] Genome Research Center for Health, Campus of Medicine, Baronissi (SA), Italy - [3] Program of Medical Genomics, AOU 'SS Giovanni di Dio e Ruggi d'Aragona' University of Salerno, Italy - [4] Unit of Infectious Diseases, AOU 'SS Giovanni di Dio e Ruggi d'Aragona' University of Salerno, Italy - [5] Program of Clinical Microbiology, AOU 'SS Giovanni di Dio e Ruggi d'Aragona' University of Salerno, Italy - [6] Unit of Transfusion Medicine, Molecular Biology Section, AOU 'SS Giovanni di Dio e Ruggi d'Aragona' University of Salerno, Italy - [7] Department of Medical Translational Sciences, University of Napoli 'Federico II', Napoli (Italy) - [8] Department of Management & Innovation Systems, University of Salerno, Fisciano (SA), Italy - [9] Centre for Research in Pure and Applied Mathematics, University of Salerno, Fisciano (SA), Italy - [10] Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Napoli, Italy - [11] Unit of Virology and Microbiology, AOU University of Campania 'L. Vanvitelli', Napoli, Italy

Objective: The novel coronavirus named SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) and responsible for COVID-19 disease, has spread in a worldwide pandemic since its first outbreak in late 2019 in Wuhan (China). The infection in humans is generally associated with a mild to severe respiratory disease, with a mortality rate of almost 2%. As many RNA viruses, SARS-CoV-2 evolves rapidly and accumulates genetic diversity in relatively short periods. Indeed, starting from mid-2020, new viral mutants and variant strains have been detected, in some cases associated with higher transmissibility and severity of symptoms, highlighting the need to monitor the genetic diversity and epidemiology of SARS-CoV-2 throughout the pandemic. Currently, next-generation sequencing (NGS) is the gold standard method used for the effective unbiased identification and classification of new and existing variants that are circulating in the population.
Methods: We used a targeted amplicon-based NGS panel protocol for high-throughput sequencing of SARS-CoV-2 whole-genome on Illumina platform. Bioinformatics analysis was performed to assembly the genome, identify InDel and SNV and classify clades/lineages.
Results and conclusions: Starting from March 2020, we collected mainly in Campania region, about 2.000 RNA samples, extracted from oropharyngeal swab, from COVID-19 positive patients exhibiting differential disease severity and we investigated the phylogenetic evolution of viral mutations.
The complete genome sequencing was been obtained for 924 samples (all deposited in the GISAID portal) allowing the identification of viruses belonging to 11 different clades, according to the Nextstrain nomenclature system, or 55 lineages, according to the Pango system. Globally we identified 18.555 SNV and 64 InDel, of which 2.302 inside the Spike protein.
Moreover, we observed in Campania a three waves temporal pattern in reported cases, characterized by the prevalence of specific clades, the 20A between March-June 2020,

the 20E (EU1) between September-December 2020, and a rapid raising of the 20I/501Y.V1 in January-March 2021, which tend replaced now those previously circulating.
Moreover, based on the collected NGS data, we developed a highly sensitive real-time-PCR based assay that allows rapid classification of variants/clades even with a relatively low limit of detection (LOD), and which could be adaptable in the future to virus evolution.
We expect that the extensive application of genomic surveillance, by NGS and/or variant-specific PCR, will provide powerful support for the fight against this public health emergency.

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P16

Spike is the most recognized antigen in the whole-blood platform in both acute and convalescent COVID-19 patients

A. Aiello[a], S. Najafi Fard[a], E. Petruccioli[a], L. Petrone[a], V. Vanini[a,b], C. Farroni[a], G. Cuzzi[a], A. Navarra[c], G. Gualano[d], S. Mosti[d], L. Pierelli[e], E. Nicastri[f] and D. Goletti[a]

[a] Translational Research Unit, National Institute for Infectious Diseases Lazzaro Spallanzani-IRCCS, Rome, Italy - [b] UOS Professioni Sanitarie Tecniche, National Institute for Infectious Diseases Lazzaro Spallanzani-IRCCS, Rome, Italy - [c] Clinical Epidemiology Unit, National Institute for Infectious Diseases Lazzaro Spallanzani-IRCCS, Rome, Italy - [d] Clinical Division of Respiratory Infectious Diseases, National Institute for Infectious Diseases Lazzaro Spallanzani-IRCCS, Rome, Italy - [e] UOC Transfusion Medicine and Stem Cell Unit, San Camillo Forlanini Hospital, Rome, Italy - [f] Clinical Division of Infectious Diseases, National Institute for Infectious Diseases Lazzaro Spallanzani-IRCCS, Rome, Italy

Aims: To identify the best experimental approach to detect a SARS-CoV-2-specific T cell response using a whole-blood platform.
Methods: Whole-blood from 56 COVID-19 and 23 "NO-COVID-19" individuals were overnight stimulated with different concentrations (0.1 or 1 µg/mL) of SARS-CoV-2 PepTivator® Peptide Pools, including spike (pool S), nucleocapsid (pool N), membrane (pool M), and a MegaPool (MP) of these three peptide pools. ELISA was used to analyse IFN-γ levels.
Results: IFN-γ-response to every SARS-CoV-2 peptide pool was significantly increased in COVID-19 patients compared with "NO-COVID-19" individuals. Pool S and MegaPool were the most potent immunogenic stimuli (median: 0.51, IQR: 0.14-2.17; and median: 1.18, IQR: 0.27-4.72, respectively) compared to pools N and M (median: 0.22, IQR: 0.032-1.26; and median: 0.22, IQR: 0.01-0.71, respectively). Whole-blood test based on pool S and MegaPool showed a good sensitivity of 77% and a high specificity of 96%. IFN-γ-response was mediated by both CD4+ and CD8+ T cells, and detected independently of clinical parameters in both hospitalized and recovered patients.
Conclusions: This easy-to-use assay for detecting SARS-CoV-2-specific T cell response may be implemented in clinical laboratories as a powerful diagnostic tool.

P17

Hematological parameters as indicators of disease severity in COVID-19 patients; Pakistan's experience

M. Ali[1,2*], A. Waris[1]

[1] Department of Biotechnology, Quaid-i-Azam University Islamabad, Pakistan-45320 - [2] Pakistan Academy of Sciences, Islamabad, Pakistan

Aim of the study. The progression of COVID-19 could be identified by various factors, including biochemical and hematological parameters.
Methods. Various hematological parameters were examined and evaluated for their association with the severity and mortality of COVID-19 disease. In this study, a total of 101 COVID-19 positive patients were examined, in which 52 were mild, 24 were moderate, 09 were severe, and 16 were critical diseased patients.
Results and conclusion. A significant association between the disease severity and elevation in blood parameters, including white blood cells, platelets, lymphocytes, and granulocytes were observed. The white blood cells, minimal infectious dose, and granulocytes were significantly elevated (p-value = <0.001), while the platelet count was decreased in severe and critical patients compared to other groups. We also recorded 16 deaths associated with the critical diseased group. From these findings, it can be concluded that these parameters could be used as a predictor for the prognosis and severity of COVID-19.

P18

Symptomatic SARS-CoV-2 infections after full schedule BNT162b2 vaccination in seropositive healthcare workers

A. Baj[1], F. Novazzi[2], A. Genoni[3], F. Drago Ferrante[2], S. Taborelli[4], B. Pini[5], M. Partenope[5], M. Valli[5], R. Pasciuta[2], A. Colombo[2], C. Zago[2], G. Cassani[2], D. Dalla Gasperina[1], R. Capuano[2], M. Prestia[2], P. G. Spezia[6], L. Azzi[7], D. Focosi[8], F. Maggi[1,2]

[1] Department of Medicine and Surgery, University of Insubria, Varese, Italy - [2] Laboratory of Microbiology, ASST dei Sette Laghi, Varese, Italy - [3] Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy - [4] Unit of Occupational Medicine, ASST dei Sette Laghi, Varese, Italy - [5] Clinical Pathology Unit, St. Anna Hospital, Como, Italy - [6] Department of Translational Research, University of Pisa, Pisa, Italy - [7] Unit of Oral Medicine and Pathology, ASST dei Sette Laghi, Varese, Italy - [8] North-Western Tuscany Blood Bank, Pisa University Hospital, Pisa, Italy

In Italy and many EU countries, vaccination campaigns have been started in December 2020 with the BNT162b2 mRNA vaccine Comirnaty® (Pfizer/BioNtech), prioritizing healthcare workers (HCW). The susceptibility of vaccinated HCW to active, albeit asymptomatic, infection is of high interest given the risk of transmitting the virus to frail hospitalized patients. Additionally, the asymptomatic status in the vaccinated HCW could delay recognition of the index case, favoring nosocomial outbreaks, with obvious implications for the final reach of herd immunity.

We report here 11 cases of SARS-CoV-2 infection in seropositive healthcare workers (HCW) vaccinated with BNT162b2 mRNA vaccine. All of them had successfully mounted anti-Spike serum IgG after full schedule vaccination: while most cases were asymptomatic for the entire time between the first positive and the first negative NPS, 5 reported fever and arthralgia. Among them, two cases are notable because of positivity in a rectal swab.

Given the ECDC and Italian Ministry of Health recommendation to sequence SARS-CoV-2 isolated from vaccinees to detect immune-escaping variants of concern (European Centre for Disease Prevention and Control. Sequencing of SARS-CoV-2: first update. 18 January 2021. ECDC: Stockholm; 2021), we sequenced the entire Spike gene in all 11 cases. The clades were characterized using NextClade (https://clades.nextstrain.org/), and showed high heterogeneity, confirming the synchronous circulation of many different SARS-CoV-2 strains in the Lombardy region.

Given the risk for SARS-CoV-2 introduction from asymptomatic vaccinees, this case series suggests the need to continue nasopharyngeal screening program.

At least one case of asymptomatic infection in a vaccinated HCW has been recently reported (Schiavone M, Gasperetti A, Mitacchione G, Viecca M, Forleo GB. Response to: COVID-19 re-infection. Vaccinated individuals as a potential source of transmission. European Journal of Clinical Investigation. 2021 Mar:e13544), but since the biological samples were limited to NPS, it could not be concluded whether the infection had been contained on the nasopharynx or not. Our series is the first to demonstrate that in fully vaccinated HCW, SARS-CoV-2 is not only able to colonize the nasopharynx, but also to in-

fect cells in distant tissues and give clinical symptoms. No case of nosocomial transmission from the HCW described in this series to inpatients has been documented to date. While we cannot conclude whether the detected viral RNA was immunocomplexed or not, it is well known that sterilizing immunity against respiratory viruses largely depends on neutralizing IgA levels in secretions. Unfortunately, the intramuscular route of the currently approved vaccines only induces low-level IgA in secretions in a minority of recipients.

P19
SARS-CoV-2 wastewater surveillance in Sicily

F. Bonura[a], C. Filizzolo[a], L. Mangiaracina[a], G. Sanfilippo[a], G. Sciortino[a], P. Gervaso[b], G. Caruso[c], D. Mirabile[d], G. Ferrera[e], V. Ingallinella[f], C. Buonora[f], V. Cammarata[g], M. Palermo[h], C. Maida[a], D. Pistoia[a], S. De Grazia[a], G.M. Giammanco[a]

[a] Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (PROMISE), University of Palermo, Palermo - [b] UOC Servizio Igiene degli Ambienti di Vita (SIAV), Dipartimento prevenzione medico, ASP2, Caltanissetta, Italy - [c] UOC Servizio di Igiene Ambienti di Vita (SIAV), Dipartimento Strutturale di Prevenzione – Area Igiene Sanità Pubblica, ASP5, Messina, Italy - [d] UOC Igiene degli Ambienti di Vita (SIAV), Dipartimento di Prevenzione Medico, ASP6, Palermo, Italy - [e] Servizio Epidemiologia e Profilassi, Dipartimento di Prevenzione, ASP7, Ragusa, Italy - [f] UOC Servizio di Igiene Ambienti di Vita (SIAV), ASP8, Siracusa, Italy - [g] UOC Servizio di Igiene Ambienti di Vita (SIAV), ASP9, Trapani, Italy - [h] DASOE - Public Health and Environmental Risk, Sicilian Region, Department of Public Health, Palermo, Italy

Introduction: Wastewater surveillance of the circulation of the novel SARS-CoV-2 virus has been suggested as a tool to support public health decisions. Monitoring environmental contamination through the detection of SARS-CoV-2 RNA in wastewater may also help to determine the potential of environmental transmission in areas highly exposed to SARS-CoV-2. Italy is one of the European countries most affected by the COVID-19 pandemic. To monitor the presence of SARS-CoV-2 genome in wastewater in Italy, in June 2020 a surveillance project was proposed (SARI, Sorveglianza Acque Reflue in Italia) adapting the standard WHO procedure for Poliovirus environmental surveillance, under the technical-scientific coordination of the Istituto Superiore di Sanità (ISS). The SARI project consists of a network of regional structures were the Subnational Reference Laboratory (SNRL) for Polio surveillance at the PROMISE Department of the University of Palermo was also included.
Materials and methods: From July 2020 to January 2021, a total of 86 wastewater samples were collected from the Sicilian environmental sampling network that includes 6 Wastewater Treatment Plants: Piana degli Albanesi (PA), Catania (Pantano D'Arci), Messina (Mili), Siracusa, Augusta (SR) and Trapani; 2 Hotspots for migrants: Rosolini (SR) and Pozzallo (RG); 2 Reception Centers for Asylum Seekers (CARA): Milo (TP) and Pian del Lago (CL); and 4 touristic areas: Balestrate (PA), Selinunte (TP), San Vito Lo Capo (TP), and Avola (SR). All samples were analysed for virological investigations according to standard protocols approved by the WHO, including clarification and concentration of samples (centrifugation and sedimentation in dextran/PEG gradient) and molecular analysis performed by Real Time RT-PCR. All PCR tests were performed in triplicate and a cycle threshold (Ct) value less than 40 cycles was defined as a positive test. All specimens were also tested by nested RT-PCR with primer sets specific for SARS-CoV-2 and positive samples were genotyped by sequence analyses in the diagnostic region included in ORF-1ab genome portion.
Results: Overall, 10.5% (9/86) of the wastewater samples collected in Sicily showed positive results for SARS-CoV-2 RNA by Real Time RT-PCR, with Ct values ranging from 33.88 to 38.87, and the temporal distribution of positive samples was correlated to the swabs

surveillance results. Among the positive samples, 44.4% came from Catania, 33.3% from Messina, 8.3% from Siracusa and 20% from the touristic area of Balestrate (PA). Nested RT-PCR showed lower sensitivity, being able to detect only the samples with lower Ct values in RT-PCR. Sequence analysis was performed to define the viral genotype.
Discussions and conclusions: Sewage surveillance provides early detection of SARS-CoV-2 circulation, possibly revealing also mild, subclinical, or asymptomatic infections in the population. Biomolecular analyses could be able to define the genotype of a SARS-CoV-2 through peculiar aminoacidic substitutions, allowing timely discovery of emerging of new variants of SARS-CoV-2.

P20

Bowel ischemia in otherwise asymptomatic patients: a possible COVID-19 onset

D. Bortolotti[1], P. Zamboni[2,3], S. Occhionorelli[2,3], L. Traina[3], L. M. Neri[2], R. Gafà[2,4], A. Passaro[2], R. Rizzo[1]

[1] Department of Chemical and Pharmaceutical Sciences, University of Ferrara, Ferrara, Italy - Via Luigi Borsari, 46 - 44121 Ferrara - [2] Department of Translational Medicine, University of Ferrara, Ferrara, Italy - Via Luigi Borsari, 46 - 44121 Ferrara - [3] Department of Surgery, University Hospital of Ferrara Arcispedale Sant'Anna, Ferrara, Italy – Via Aldo Moro, 8 Cona – 44124 Ferrara - [4] Oncological and Medical Department, University Hospital of Ferrara Arcispedale Sant'Anna, Ferrara, Italy – Via Aldo Moro, 8 Cona – 44124 Ferrara

Background: Even if the majority of SARS-CoV-2 infected subjects have no or very mild symptoms, little is know about the clinical course of COVID-19 in asymptomatic patients. In particular, It seems that asymptomatic patients with SARS-CoV-2 can become hypercoagulable and develop acute vascular events.
Cases description: In this study, three consecutive patients hospitalized during the first COVID-19 wave in Italy, presenting severe abdominal symptoms due to extensive ischemia and necrosis of the bowel, with co-existent thrombosis of abdominal blood vessels. None had the usual manifestations of COVID-19, in particular chest computed tomography (CT) showed no signs of interstitial pneumonia and repeated pharyngeal swabs tested persistently negative by RT-PCR. By contrast, blood tests and abdominal CT suggested intestinal ischemia, which was confirmed by laparotomy and histology of resected specimens. Patients underwent emergency surgery with intestinal resection.
Aim and methods: Immunohistochemical testing for SARS-CoV-2 on resected tissue were performed with the aim to elucidate whether asymptomatic patients with SARS-CoV-2 can become hypercoagulable and develop acute vascular events.
Results: Histological examination of all samples showed extensive intestinal and gallbladder wall ischemia and necrosis, with thrombosis of intramural blood vessels. Blood vessel endotheliitis and neo-angiogenesis were also observed. Interestingly, all tested samples were strongly positive for SARS-CoV-2, as shown in Figure 1a. In particular, we found the highest extent of viral presence in the gallbladder sample, in terms of H-Score (Figure 1b). This acute and severe intestinal onset has, to our knowledge, not been reported previously, although intestinal infarct may be a complication of interstitial pneumonia in patients testing positive for SARS-CoV-2, as supported by our previous results (PMID:33465499).
Conclusions: our results reported that the three patients enrolled for this study had been infected with SARS-CoV-2 some months before coming to our attention. Their disease was probably asymptomatic, but the virus persisted in the gut, as shown by IHC analysis.
This evidence suggests that the resected samples of recent emergency cases of intestinal ischemia should be tested for SARS-CoV-2, perhaps to reveal considerably more victims of the pandemic than currently suspected and to better elucidate SARS-CoV-2 pathogenesis process.

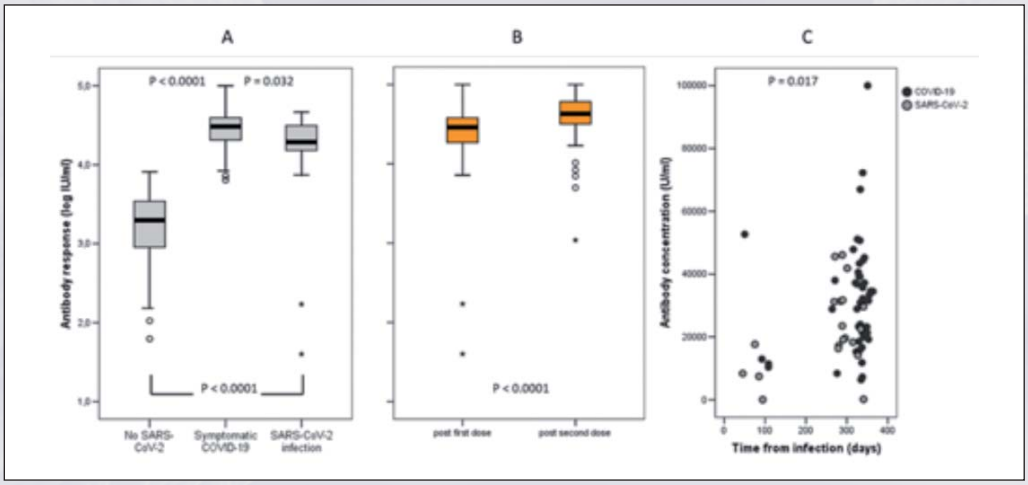
P21

Antibody response to SARS-CoV-2 vaccination is extremely vivacious in subjects with previous SARS-CoV-2 infection

A. Callegaro[1,2], D. Borleri[3], M. Arosio, C. Farina[2], G. Napolitano[1,2], D. Valenti[4,5], M. Rizzi[5], F. Maggiolo[5]

[1] Biobank - [2] Microbiology and Virology Laboratory - [3] Occupational Medicine Unit - [4] FROM Foundation - [5] Division of Infectious Diseases, ASST Papa Giovanni XXIII, Bergamo, Italy

Aim of the study: The SARS-CoV-2 pandemic calls for rapid actions, now principally oriented to a world-wide vaccination campaign. In this study we verified if, in individuals with a previous SARS-CoV-2 infection, a single dose of mRNA vaccine would be immunologically equivalent to a full vaccine schedule in naïve individuals.
Methods used: Health care workers with a previous SARS-CoV-2 infection were sampled soon before the second dose of vaccine and between 7 and 10 days after the second dose, the last sampling time was applied to SARS-CoV-2 naïve individuals, too. Antibodies against SARS-CoV-2 were measured using Elecsys® Anti-SARS-CoV-2 S immunoassay. The study was powered for non-inferiority. We used non parametric tests and Pearson correlation test to perform inferential analysis.
Results and conclusion: Out of 184 health care workers, joining the National vaccination campaign, 53 were previously diagnosed with COVID-19; 21 had been previously tested positive (nasal swab or serological test) for SARS-CoV-2 without any symptom of COVID-19 and 110 were naïve individual. After a single vaccine injection, the median titer of specific antibodies in individuals with previous COVID-19 was 30,527 U/ml (IQR 19,992-39,288) and in subjects with previous SARS-CoV-2 asymptomatic infection was 19,367.5 U/ml (IQR 14,688-31,353) (P = 0.032)(figure panel A). Both results were far above the median titer in naïve individuals after a full vaccination schedule: 1,974.5 U/ml (IQR 895-3,455) (P < 0.0001)(figure panel A). With the second dose, in previously exposed



individuals, median titers raised to 43,073 U/ml (IQR 31,605-61,903) (P < 0.0001)(figure panel B). Titers were slightly higher in subjects who acquired the infection during the first wave, observed in March/April 2020, 8-11 months before testing, compared to those with more recent infection (2-3 months) (P = 0.017)(figure panel C), but titers were not influenced by age (P = 0.083). Adverse events after vaccine injection were more frequent after the second dose of vaccine (mean 0.95, 95%CI from 0.75 to 1.14 versus mean 1.91, 95%CI from 1.63 to 2.19)(P<0.0001) and in exposed compared to naïve (mean 1.63; 95%CI from 1.28 to 1.98 versus mean 2.35; 95%CI from 1.87 to 2.82)(P=0.015). In SARS-CoV-2 naturally infected individuals a single mRNA vaccine dose seems sufficient to reach immunity. Modifying current dosing schedules would speed-up vaccination campaigns.

P22

Neutrophil to lymphocyte ratio and clinical severity in COVID-19 patients of 1st and 2nd waves

M. Gelzo[1,2], S. Cacciapuoti[3], B. Pinchera[3], A. De Rosa[4], G. Cernera[1,2], F. Scialò[1,5], M. Mormile[3], G. Fabbrocini[3], R. Parrella[4], I. Gentile[3], G. Castaldo[1,2]

[1] CEINGE-Biotecnologie avanzate, scarl, Naples, Italy - [2] Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università di Napoli Federico II, Naples, Italy - [3] Dipartimento di Medicina Clinica e Chirurgia, Università di Napoli Federico II, Naples, Italy - [4] Dipartimento di Malattie Infettive e Emergenze Infettive, Divisione di Malattie Infettive Respiratorie, Ospedale Cotugno, AORN dei Colli, Naples, Italy - [5] Dipartimento di Medicina Traslazionale, Università della Campania L. Vanvitelli, Naples, Italy

Aims: We evaluated the neutrophil/lymphocyte ratio (NLR) related to the clinical severity in COVID-19 patients from 1st wave (March-May 2020) and 2nd wave (September-December 2020).
Methods: We studied 49 patients from the 1st wave and 168 patients from the 2nd wave. The clinical severity was classified on the basis of the seven ordinal scale made by the World Health Organization (WHO)-Research and Development Blueprint expert group [1]. Blood samples were analyzed for cell count and serum interleukin (IL)-6 and myeloperoxidase (MPO) were measured by Luminex (Labospace s.r.l., Biorad, Italy).
Results and conclusions: In the patients of the 2nd wave, we found significantly lower levels of serum IL-6 and MPO, as compared to the 1st wave patients, for each WHO subgroups. In addition, among the patients of the 1st wave, the NLR was significantly higher in WHO 5-7 (severe patients) as compared to either WHO 3 (mild patients) and WHO 4 patients (moderate patients). This trend of NLR ratio was due to both neutrophils and lymphocytes counts. In fact neutrophil number was higher in WHO 5-7 subgroup as compared to WHO 3 and WHO 4 patients, on the contrary the lymphocytes number was significantly lower. These findings are in agreement with the literature highlighting that the NLR has a good predictive value on disease severity and mortality in patients with COVID-19 infection [2]. While, in 2nd wave patients, none of these parameters showed significant differences related to WHO score. Although the patients of the 2nd wave were younger than 1st wave patients, we did not found association between the age and the cytometric parameters. Therefore, the lack of association between NLR and COVID-19 severity in 2nd may depend on the different therapies performed in the two waves. In fact, the patients of the Ist wave were hospitalized and treated after the diagnosis based on symptoms and followed by molecular analysis. While, in most patients from the 2nd wave the diagnosis has been performed when they were still asymptomatic. Moreover, it could be taken into account that the result of the nasopharyngeal test was obtained more rapidly in the 2nd wave, and all the patients started to be treated several days, before hospitalization, by corticosteroids and/or azithromycin that act on circulating IL-6 and MPO levels as well as on neutrophils and lymphocytes. Overall, this study reveals the lack of NLR predictive value for COVID-19 severity and mortality in patients of the 2nd wave, probably for the used therapies. Therefore, NLR value may be an useful prognostic marker only in naïve patients, before starting therapies.

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P23 - OC 53

A real-life use of remdesivir in patients with coronavirus disease 2019: a retrospective case-control study

F. Cogliati Dezza, A. Oliva, V. Mauro, F.E. Romani, R. Aronica, F. Cancelli, C.M. Mastroianni

Department of Public Health and Infectious Diseases, Sapienza University of Rome

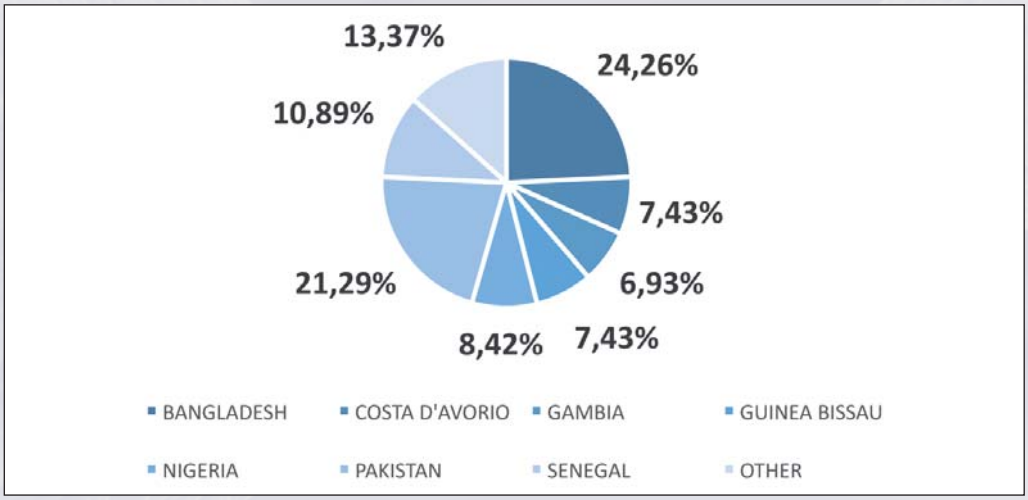
Background. Remdesivir (REM), a nucleotide analogue drug that inhibits viral RNA polymerases, has shown potent antiviral activity in vitro and efficacy in animal models of COVID-19; nevertheless, clinical trials have shown conflicting data on its effectiveness. Aims of the study were to evaluate the impact of remdesivir on Intensive Care Unit (ICU) admission and in-hospital mortality.
Methods. We conducted a retrospective, single-center, case-control (1:1) study including hospitalized patients with confirmed SARS-COV2 infection. Cases were patients treated with REM for 5 days, controls were patients not receiving REM. REM was used in patients with radiologic evidence of pneumonia and receiving oxygen support. Cases and controls were matched for age, sex, duration of symptoms (days) and severity of infection at admission (expressed by PaO2/FiO2). Univariable and multivariable analyses were performed to explore the effect of REM on ICU admission and in-hospital mortality.
Results. A total of 192 patients (96 cases and 96 controls) were included in the study, 134 males and 58 females, 13.5% were admitted to ICU with a mortality of 14.1%. Overall, median (IQR) age was 64 years (56-72), duration of symptoms was 6 days (3-8) and PaO2/FiO2 at admission was 279 (211-337). Patients receiving REM had a lower rate of ICU admission (6% vs 20%, *p*: 0.003), whereas no difference between cases and controls were observed as for mortality rate (*p*: 0.14). At multivariable analysis only high CRP (p: 0.006), severity of infection (p: 0.015), and haematological malignancies (*p*: 0.011) were independently associated with ICU admission. Presence of haematological malignancies (*p*: 0.001), lower duration of symptoms (*p*: 0.012), higher severity of infection (*p*: 0.001) and low lymphocytes count (p: 0.036) at admission were independently associated with in-hospital mortality.
Conclusions. Our real-life study showed that therapy with REM did not have an effect on either in-hospital mortality or ICU admission. Additional studies are warranted to evaluate the efficacy of REM in COVID-19 patients.

P24
Is SARS-CoV-2 spreading among undocumented migrants? A real life experience

A. Colpani[1], B. Zauli[1], A. De Vito[1], C. Fanelli[1], V. Fiore[1], G. Madeddu[1], A. Donisi[2]

[1] Unit of Infectious Disease, Department of Medical. Surgery, and Experimental Sciences, University of Sassari, Sassari - [2] Migration Health Unit, Primary Health Care Department, AUSL Piacenza, Piacenza.

Aim of the study: Since 2019, the world is experiencing the spread of a new coronavirus named SARS-CoV-2. Several measures have been put in place to curb the spreading of coronavirus disease (COVID-19). Migrants can be particularly vulnerable due to crowded living spaces, precarious hygiene conditions, informal jobs, difficult access to correct education in native language, and no access to primary care. In Piacenza, undocumented migrants were excluded from "test and trace" strategy, especially during the first wave of the pandemic.
We aimed to evaluate the virus spreading among a migrant population previously excluded by community surveillance programs.
Methods: We conducted a retrospective study, collecting data about people without SARS-CoV-2 related symptoms who attended the outpatient clinic for undocumented migrants from 1st November 2020 to 31st March 2021. Patients who performed a nasopharyngeal swab and serologic test to evaluate the presence of antibody anti-SARS-CoV-2 were enrolled. We included only patients aged 18 years old and above. We collected data about sex, age, and country of origin. People with either a positive PCR test or serology were defined as SARS-CoV-2 positive.
Results and Conclusion: We enrolled 202 patients, 11 (5.4%) women, with a median age of 26.9 (IQR 24.4-32.2) years. Twenty-nine (14.4%) patients tested positive for SARS-CoV-2 at the nasopharyngeal swab during the study period. Of these, 17 had positive or low positive results for IgG and two had positive results for both IgG and IgM testing. Besides,



27 (13.4%) participants resulted positive to serological testing but negative to PCR testing. Two of them had a previous positive PCR test.
The median age of SARS-CoV-2 positive (n=56) was significantly higher than in negative people [29.9 (IQR 25.6-35.4) vs. 26.6 (IQR 24.3-31.5)]; *p-value*=0.008].
Nationality is summarized in Figure 1. Regarding SARS-CoV-2 positive people, the most frequent nationality was Bangladeshi, with 25 (46.3%) people (*p-value*<0.001). The highest percentage of positive was found among the same nationality (51% of Bangladeshi tested positive).
Our data show an high virus spreading among the migrant population living in Piacenza. In particular, 13.4% were positive only to serology suggesting an undetected previous infection and 14.4% with a positive PCR could have been undiagnosed if not screened in our outpatient clinic. The patients found positive were put in isolation until negative to the nasopharyngeal swab and also educated through proper mediation about the most effective preventive measures. Our data underline the significantly higher prevalence of SARS-CoV-2 infection in the undocumented migrant population in respect of the general population of Piacenza province in the same period (14.4% vs. 5.9%, *p-value* <0.001). The extention of surveillance programs to the whole population, thus including undocumented people, is crucial in order to curb the spreading of the virus.

P25 - OC 50

Oral microbiome and local immune/inflammatory response in COVID-19 patients: a cross-sectional study

M. D'Accolti[1], I. Soffritti[1], C. Fabbri[2], A. Passaro[3], R. Manfredini[4], G. Zuliani[3], M. Franchi[2], C. Contini[5], E. Caselli[1]

[1] Section of Microbiology, Department of Chemical, Pharmaceutical and Agricultural Sciences, CIAS research Center and LTTA, University of Ferrara, Ferrara, Italy - [2] Section of Dentistry, Department of Biomedical and Specialty Surgical Sciences, University of Ferrara, Ferrara Italy - [3] Internal Medicine Unit, Department of Translational Medicine, University of Ferrara, Ferrara, Italy - [4] Medical Clinic Unit, Department of Medical Sciences, University of Ferrara, Ferrara, Italy - [5] Section of Infectious Diseases and Dermatology, Department of Medical Sciences, University of Ferrara, Ferrara, Italy

Background: The new human coronavirus SARS-CoV-2, responsible for the development of COVID-19 disease, has become a global pandemic emergency. Like other respiratory viruses, the primary site of the entrance is represented by the oropharynx, and the local microbiome environment may influence its capability to infect and induce the disease. The aim of the present study was therefore to characterize the oral microbiome in a cohort of COVID-19 patients with different symptom levels, to evidence the eventual association between virus-induced disease and the microbial environment of the oral cavity. Moreover, the inflammation and local immune response were also assessed in parallel.
Methods: Overall, 75 oral rinse samples were collected from 39 COVID-19 subjects and 36 controls recruited in the study. Each specimen was reassessed by digital droplet PCR to measure the load of SARS-CoV-2 at the time of withdrawal. The profile of the oral microbiome was analyzed by Whole Genome Sequencing (WGS), allowing evidencing also the non-bacterial components (mycome and virome) of the oral microbiome. In parallel, the local immune response (secretory IgA) and inflammatory cytokine release (IL-6, IL-17, TNFα, and GM-CSF) were assessed by specific ELISA assays.
Results: WGS results showed significant alpha-diversity decrease in the oral microbiome of COVID-19 patients compared with matched controls, associated with symptom severity, and oral dysbiosis was associated with the increased local concentration of inflammatory cytokines and decreased mucosal secretory IgA response. Bacterial genera associated with poor oral hygiene and periodontitis were increased in COVID-19 subjects *(Prevotella*, *Capnocytophaga*, *Porphyromonas*, *Abiotrophia*, *Aggregatibacter)*, with *Enterococcus* and *Enterobacter* spp. exclusively detectable in COVID-19 patients. In addition, also mycetes *(Candida*, *Saccharomyces)*, and viruses (EBV, HSV-1) were significantly increased, with *Aspergillus*, *Nakaseomyces*, and *Malassezia* genera exclusively detectable in COVID-19 patients.
Conclusions: The oral microbiome may be important in defining the individual susceptibility to SARS-CoV-2 infection and the subsequent development of symptomatic COVID-19. In particular, poor oral hygiene might facilitate inflammation and a worse course of COVID-19 disease. Instead, sIgA presence associated with mild symptoms may be considered as an important marker in monitoring therapy and vaccine development.

P26

Demographic and clinical characteristics of COVID-19 patients: AORN Sant'Anna e San Sebastiano of Caserta experience

M.T. Della Rocca[1,2], P. Iuliano[3,4], V. Panetta[1], A. Durante[1], F. Simeone[3], G. Di Caprio[3], R. Greco[1] and P. Maggi[3]

[1] U.O.S.D. Microbiology, AORN Sant'Anna e San Sebastiano Caserta, Via F.Palasciano, 81100 Caserta,Italy - [2] Section of Microbiology and Virology, University Hospital "Luigi Vanvitelli", Via Costantinopoli, 80138 Naples, Italy - [3] Department of Infectious Diseases, AORN Sant'Anna e San Sebastiano Caserta, Via F.Palasciano, 81100 Caserta,Italy - [4] Department of Mental and Physical Health and Preventive Medicine, University of Campania "Luigi Vanvitelli," Naples, Italy

Introduction/Aim of the study. SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), is a viral strain of the SARS-related coronavirus species, belonging to the genus *Betacoronavirus* (*Coronaviridae* family), first discovered around the end of 2019 for the first time in Wuhan, China causing a syndrome called COVID-19. On January 30, 2020, the WHO declared SARS-CoV- 2 infection as a global health emergency. There have currently been around 137 million confirmed cases globally, of which 2.97 million deaths as of 04/15/2021. The situation in Campania has about 360,000 confirmed cases. The purpose of this study was to report the demographic and clinical-laboratory characteristics of patients hospitalized from March to April 2020 at AORN of Sant 'Anna and San Sebastiano of Caserta. However, the study reported the first data of Viral RNA SARS-CoV-2 detection in other districts (urine, faces, tears) collected in the same patient's court followed in post-discharge visit. The relationships between clinical manifestations, organ involvement and the presence of virus in different districts can improve the virus detection rate and reduce the spread of the disease.
Methods. The study was conducted between March-April 2020 on patients with confirmed SARS-COV-2 infections enrolled in our hospital. From May to July 2020, in post-dismissed control patients SARS-COV-2 infection was monitored in pharyngeal, tears, faces and urine specimens. Demographic information, epidemiological and clinical characteristics (including medical history, underlying comorbidities, symptoms and signs), laboratory findings and Chest RX scan results was obtained. The RNA Real Time PCR assay was conducted in accordance to the protocol established by WHO. Statistical analysis was conducted with IBM SPSS software; categorical variables were express as the cunts and percentage.
Resulta and conclusions. A total of 70 confirmed patients were enrolled at Infection Disease, Intensive Care, Pulmonary departments in the period of March-April 2020. Fifty-one patients were male (72,9%) and 19 (27,1%) females. The average age of the patients was 63 years. Comorbidities with higher rate were hypertension (45,7%), ischemic heart disease (25,7%) and also diabetes mellitus (24,3%). The most common patient's symptoms were fever (82,9%), dispnea (82,9%) and cough (62,9%). Other symptoms included diarrhea (14,3%), sickness (5,7%) and chest pain (2,9%). Our data showed, among all the inpatients, a percentage of 11,4% of co-infections. In this study also reported the laboratory and radiologic findings on admission. As showed in several study the median value of D-Dimero, Protein C Reactive, Interleukin-6 and Lactate Dehydrogenase showed significant altera-

tions reference to the normal range. We also checked *Chest RX scan* that highlight pulmonary involvement. There were 29 patients with Bilateral Interstitial Pneumonia (41,4%); 13 with Bilateral Alveolar Pneumonia (18,5%) finally 5 (7,1%) with both of pattern. A number of 29 (41,4%) patients were identified as uncomplicated cases followed by 24,32% mild cases and 34,3% severe cases. In post demised control patients, a rate of 4,3% were tested positive to faces samples followed by tears samples (2,0%). This study reported demographical and clinical characteristics of COVID-19 patients enrolled at our hospital. In order to better understand the disease, we also evaluated the presence of SARS-COV-2 virus in different specimens in patients after demission. The first data obtained encourage future studies to better understand the presence of SARS-COV-2 in different specimens and monitoring the clinical course of COVID-19.

P27

Preliminary evidence of blunted humoral response to SARS-CoV-2 mRNA vaccine in Multiple Sclerosis patients treated with Ocrelizumab

G. Donnarumma[1], A. Gallo[2], R. Capuano[2], A. Bisecco[2], E. Grimaldi[1], M. Conte[2], A. d'Ambrosio[2], G. Tedeschi[2], M. Galdiero[1]

[1] Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy - [2] Department of Advanced Medical and Surgical Sciences, University of Campania "Luigi Vanvitelli", Naples, Italy

Objectives. Several concerns regard the immunogenicity of SARS-CoV-2 vaccines in people with multiple sclerosis (pwMS), since the majority of them is treated with immunomodulating/immunosuppressive disease modifying therapies. Here we report the first data on the humoral response to mRNA SARS-CoV-2 vaccine in a case series of 4 pwMS treated with ocrelizumab (OCR) as compared to a group of healthy subjects (HS).
Methods. We collected serum samples at 0, 14, 21 days after the first dose and 7 days after the second dose of NT162b2-mRNA-COVID-19 vaccine from 55 health-care workers and 4 relapsing pwMS on OCR, with no history of COVID-19 infection. Sera were tested using the LIAISON®SARS-CoV-2 TrimericS-IgG assay (DiaSorin-S.p.A.) for the detection of IgG antibodies to SARS-CoV-2 spike protein. The anti-spike IgG titers were expressed in Binding Antibody Units (BAU), an international standard unit.
Results. At baseline all subjects were negative for anti-spike IgG. Seven days after the second dose of vaccine all HS mounted a significant humoral response (geometric mean 2010.4 BAU/mL C.I. 95% 1512.7-2672) while the 4 pwMS showed a lower response (range <4.81-175 BAU/mL)
Discussion. Humoral response to NT162b2-mRNA-vaccine in pwMS treated with OCR was clearly blunted. Further data are urgently needed to confirm and expand these preliminary results and to develop strategies to optimize the response to SARSCoV-2 vaccines in pwMS on OCR.

P28 - OC 7

Analysis of anti-spike neutralizing antibodies titers and levels of circulating biomarkers in COVID-19 patients

F. Frasca[1], M. Scordio[1], G. Oliveto[1], A. Viscido[1], L. Sorrentino[1], L. Santinelli[2], C. Pinacchio[2], G. Ceccarelli[2], E. Gentilini[2], E. Nelson Cavallari[2], A. Gaeta[2], G. d'Ettorre[2], A. Pierangeli[1], G. Antonelli[1,3], C. Scagnolari[1]

[1] Virology Laboratory, Department of Molecular Medicine and - [2] Department of Public Health and Infectious Diseases, Sapienza University, Rome, Italy - [3] Microbiology and Virology Unit, Sapienza University Hospital "Policlinico Umberto I", Rome, Italy

Introduction: Human plasma biomarkers, such us lactate dehydrogenase (LDH), D-dimer and C-reactive protein (CRP) have been well recognized as indicators of pathophysiological changes and inflammatory status caused by SARS-CoV-2 infection. Also, individuals that become infected with SARS-CoV-2 produce neutralizing antibodies against this virus. However, the neutralizing antibody titer could be highly variable. Since a possible association between blood proteins and SARS-CoV-2 neutralizing antibodies is still poorly understood, the purpose of this preliminary study was to investigate the relationship between anti-spike-Nab titers and levels of circulating biomarkers of COVID-19 severity.
Methods: Plasma samples were collected, during the second SARS-CoV-2 wave from 67 male patients with symptomatic SARS-CoV-2 infection hospitalized at the Policlinico Umberto I Hospital in Rome. Clinical data were available for 48 out of 67 SARS-CoV-2 positive patients analyzed. Neutralization activity of heat-inactivated plasma was assessed on VeroE6 cells using a replication-competent VSV-pseudovirus espressing the SARS-CoV-2 spike protein. Two-fold serial dilutions of patients plasma were pre-incubated with 102 TCID50/100 uL and then added to cell monolayers in 96-well plates; CPE was scored at 48 hours post-infection. Statistical analysis was performed using SPSS v.20.0.
Results: Results obtained in the VSV-SARS-CoV-2 neutralization assay showed that 28.35% (n=19) of the COVID-19 patients were negative to neutralizing antibodies and 35.82% (n=24) of the patients produced antibodies with anti-spike-neutralizing activity below the dilution 1:160. By contrast, 35.82% (n=24) showed a high titer of neutralizing antibodies (i.e. greater than 1:160). No significance differences were observed among the two subgroups concerning the days elapsed from the onset of the SARS-CoV-2 infection to the collection and analysis of plasma samples. Of note, in the subgroup of SARS-CoV-2 patients with a lower titer of anti-spike-neutralizing antibodies, LDH levels were positively correlated with D-dimer (p=0.021; r=0.632) and CRP (p=0.003; r=0.714). These relationships were not found in the subgroup of COVID-19 patients with a higher neutralizing antibody response against SARS-COV-2.
Conclusions: Our results showed that SARS-CoV-2 patients with a neutralizing antibody titer below 1:160 expressed high levels of LDH, D-dimer and CRP, suggesting that the analysis of both anti-spike-neutralizing antibodies and plasma biomarkers might be helpful to better delineate the prognosis in patients with COVID-19.

P29

Absolute quantitation of IgG response to COVID-19 mRNA vaccine, a longitudinal study

A. Fucci[1], S. Giacobbe[1], E.L. D'Andrea[1], G. Vitale[2], G. Icardi[3], M. Scrima[4], M. Caraglia[4,5] & M.L. Nolli[1]

[1] Laboratory of Protein Factory, Biogem scarl, Ariano Irpino (AV) - [2] Istituto Auxologico Italiano IRCCS, Laboratory of Geriatric and Oncologic Neuroendocrinology Research, Cusano Milanino, MI, Italy and Department of Clinical Sciences and Community Health (DISCCO), University of Milan - [3] Planning and Prevention Unit, I.R.C.C.S. San Martino Hospital and Department of Health Sciences, University of Genoa, Genoa, Italy - [4] Laboratory of Molecular Oncology and Precision Medicine, Biogem scarl, Ariano Irpino (AV) - [5] Department of Precision Medicine, University of Campania "L. Vanvitelli", Naples

Aim of the study. Rapid vaccine-induced immunity is a key global strategy to control COVID-19 pandemics, also for spread of new variants. The monitoring of the amounts of serum IgG and IgM after the vaccination through a quantitative assay should be a way to check the success of the vaccination strategies.

Methods. We performed a longitudinal nested case-control analysis on 80 healthcare workers vaccinated with BNT162b2 mRNA COVID-19 vaccine (Pfizer-BioNTech, Mainz, Germany) between January and February 2021. Fifteen of them had a previous laboratory-confirmed mild or asymptomatic SARS-CoV-2 infection. All participants were tested before the first immunization dose (baseline), and then 10, 20, 30, 40 and 60 days later, for a total of 6 time points. IgG and IgM were titrated by using a novel quantitative serological assay developed in Biogem (COVID-19 QuantiGEM IgM and IgG ELISA kit, Biogem), which allows the absolute quantitation of IgG and IgM anti-SARS-CoV2. IgG were detected in the baseline sample of ten participants, who had a recent COVID-19 diagnosis (less than 6 months); five subjects with a remote infection (March 2020) where instead IgG negative.

Results and conclusions. Ten days after the first dose, antibodies were detectable in all samples who had a previous COVID-19 diagnosis, even if seronegative at baseline, with a median concentration of 336 ug/mL (CI95% 2.24 – 591.21). Three participants with no history of SARS-CoV-2 infection, had antibody levels matching those of participants with recent COVID-19 infection, suggesting an undiagnosed infection. At day 20, seroconversion was observed in 70% of cases, with concentrations ranging from 0.49 to 360 ug/mL (Median= 1.75 ug/mL). At day 30, all participants mounted a strong humoral response, with a median concentration of IgG of 206 ug/mL (N = 65, CI95% 102.2 – 424.8). These levels are higher than those measured in convalescent serum samples (N = 108, Median = 18 ug/mL, CI95%, 4.4 – 19.7). In subjects with a previous COVID-19 infection, the increase was about 10 times greater (Median= 2080 ug/mL).

Tests performed at subsequent times indicated a gradual reduction of IgG concentration, which decreased from 84.4 ug/mL at 40 days, to 18 ug/mL at 60 post-immunization.

Overall, the data obtained from the longitudinal analysis, allowed the preliminary outlining of the humoral response to the vaccine, which reaches the peak 30 days after the administration of the first dose and coincides with the start of the protection window. Subsequently the concentration of IgG gradually decreases. How this diminution relates to the

protection against the virus (or reduction thereof) is one of the key points to be clarified. The results obtained from subjects with a previous COVID-19 diagnosis, suggest that immune response is promptly boosted by vaccine administration, and that viral infection could be analogous to immune priming. The rapid and strong increase of antibodies observed in patients where virus-induced IgG were yet undetectable, support that additional immunity measures are involved in SARS-CoV2 protection. The identification of immune correlate of protection for SARS-CoV-2 vaccines in humans, as well as the evaluation of the persistence of the antibody response are pivotal for defining effective immunization programs and will need further investigation.

P30

Seroprevalence of SARS-CoV-2 assessed by four chemiluminescence immunoassays and one immunocromatography test for SARS-CoV-2

A. Furno[1], M. Cennamo[1], E. La Civita[1], A. Liotti[1], F. Russo[1], G. Carbone[1], S. Di Somma[1], F. Napolitano[1], F. Aloe[1], T. M. Sorbo[2], P. Romano[3], D. Iorio[4], V. Bruno[4], P. Filauri[4], F. Alfarano[4], E. Coluccia[4], P. Cerino, D. Terracciano, G. Portella

[1] Department of Translational Medical Sciences, University of Naples "Federico II", 80131 Naples, Italy - [2] Division of Nephrology, Department of Translational Medical Sciences, School of Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy - [3] Department of Surgical Sciences and Nephrology; University Federico II of Naples, Naples, Italy - [4] Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Via L. De Crecchio, 80138 Naples, Italy - [5] Centro di Referenza Nazionale per l'Analisi e Studio di Correlazione tra Ambiente, Animale e Uomo, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici 80055, Italy

Aim of the study: The onset of the new SARS-CoV-2 coronavirus encouraged the development of new serologic tests that could be additional and complementary to Real Time RT-PCR based assays. In such a context, the study of performances of available tests is urgently needed, as their use has just been initiated for seroprevalence assessment. The aim of this study was to compare four chemiluminescence immunoassays and one immunocromatography test for SARS-Cov-2 antibodies for the evaluation of the degree of diffusion of SARS-CoV-2 infection in Salerno Province (Campania Region, Italy).
Methods used: 3185 specimens from citizens were tested for anti-SARS-CoV-2 antibodies as part of a screening program. Four automated immunoassays (Abbott and Liaison SARS-CoV-2 CLIA IgG and Roche and Siemens SARS-CoV-2 CLIA IgM/IgG/IgA assays) and one lateral flow immunoassay (LFIA Technogenetics IgG-IgM COVID-19) were used.
Results and conclusions: Seroprevalence in the entire cohort was 2.41%, 2.10%, 1.82% and 1.85% according to the Liaison IgG, Abbott IgG, Siemens and Roche total Ig tests, respectively. When we explored the agreement among the rapid tests and the serologic assays, we reported good agreement for Abbott, Siemens, and Roche (Cohen's Kappa coefficient 0.69, 0.67 and 0.67, respectively), whereas we found moderate agreement for Liaison (Cohen's Kappa coefficient 0.58). Our study showed that Abbott and Liaison SARS-CoV-2 CLIA IgG, Roche and Siemens SARS-CoV-2 CLIA IgM/IgG/IgA assays and LFIA Technogenetics IgG-IgM COVID-19 have good agreement in seroprevalence assessment. In addition, our findings indicate that the prevalence of IgG and total Ig antibodies against SARS-CoV-2 at the time of the study was as low as around 3%, likely explaining the amplitude of the current second wave.

P31

SARS-CoV-2 mRNA detection in the cerebrospinal fluid of a COVID-19-related acute encephalitis

V. Gentili[1], F. Sganga[2], R. Rizzo[1], D. De Plano[3], M. Polastri[3], E. Fallica[4], G.P.I. Caio[5], R. De Giorgio[5], R. Cultrera[6]

[1] Microbiology, Dept. of Translational Medicine, University of Ferrara - Ferrara (Italy) - [2] Geriatric Unit, Dept. of Medicine, "S. Anna" University Hospital - Ferrara (Italy) - [3] Internal Medicine Unit, Dept. of Medicine, "S. Anna" University Hospital - Ferrara (Italy) - [4] Neurology Unit, "S. Anna" University Hospital - Ferrara (Italy) - [5] Internal Medicine, Allergology and Gastroenterology, Dept. of Translational Medicine, University of Ferrara - Ferrara (Italy) - [6] Infectious Diseases, Dept. of Translational Medicine, University of Ferrara - Ferrara (Italy)

Background: COVID-19-related neurological disease is poorly understood. Previous reports almost exclusively focused on clinical data describing post-COVID encephalitis without establishing any actual relationship between central nervous system (CNS) manifestations and viral detection.
Case description: A 70-year-old male was admitted in Emergency Department of St. Anna Hospital, Ferrara, Italy, for high fever, chills and lipothymia after an episode of diarrhea occurred two days before. On admission, the patient had respiratory rate 22 breaths/min, C-reactive protein 9.5 mg/ml and lymphopenia (900 cells/µl). Arterial blood gas analysis showed pH 7.5, PaCO2 28 mm Hg, PaO2 60 mm Hg. RT-PCR assay on nasopharyngeal swab was positive for SARS-CoV-2 infection. A chest X-Ray revealed consolidations and ground-glass opacities in the lungs. Neurological examination showed acute confusion and agitation alternating with drowsiness and lethargy (Glasgow Coma Scale = 7). T2-weighted brain MRI images showed a limbic encephalitis more evident on the left side. Electroencephalography (EEG) was compatible with diffuse non-specific encephalic pain; this pattern was confirmed 14 days later. Cerebrospinal fluid (CSF) analysis was normal and tested negative for bacteria, fungi and viruses. HIV antibodies and blood cultures were negative. qRT-PCR showed 871.3±7.13 copies/ml of SARS-CoV-2 RNA in CSF. A 4-day supernatant of VeroE6 cell culture infected with 1 ml CSF showed 1000.5±9.43 SARS-CoV-2 RNA copies/ml. A treatment with hydroxychloroquine 400 mg (the first day), 200 mg (the other 4 days), remdesivir 200 mg (the first day), then 100 mg/day, methylprednisolone 1g/day and human intravenous immunoglobulin 0.4 g/Kg/day for 5 days was established. One week after treatment discontinuation, the SARS-CoV-2 RNA detection resulted negative on CSF.
Discussion: The diagnosis of COVID-19-related encephalitis can be extremely challenging, as the definition of viral encephalitis largely depends on virus isolation from CSF. Evidence of a direct viral insult to the CNS is lacking. To our knowledge, this is one of the first accurate detection of SARS-CoV-2 dissemination in the CSF. Absence of SARS-CoV-2 RNA detection in the CSF and a significant neurological improvement were observed after treatment. Our data provide novel evidence to understand the SARS-CoV-2-related neurological manifestations, a rapidly evolving area in the field of COVID-19 wide clinical spectrum.

P32 - OC 51

The pathogenic HERV-W envelope protein is associated to the hyper-inflammation and lymphocytes exhaustion in COVID-19

C. Matteucci[1], A. Minutolo[1], V. Petrone[1], M. Fanelli[1], M. Iannetta[2,3], V. Malagnino[2,3], M. Zordan[2,3], P. Vitale[2,3], S. Bernardini[1], E. Garaci[4], P. Di Francesco[1], P. Sinibaldi Vallebona[1,5], H. Perron[6,7], L. Sarmati[2,3], M. Andreoni[2,3], E. Balestrieri[1], S. Grelli[1,8]

[1] Department of Experimental Medicine, University of Rome Tor Vergata, Rome, 00133, Italy - [2] Department of Systems Medicine, University of Rome Tor Vergata, Rome, 00133, Italy - [3] Infectious Diseases Clinic, Policlinic of Tor Vergata, Rome, 00133, Italy - [4] IRCCS San Raffaele Pisana, Rome 00163, Italy - [5] National Research Council, Rome, 00133, Italy - [6] University of Lyon, Lyon, 69007, France; Institute of Translational Pharmacology - [7] Geneuro – Innovation, Lyon-France 69008 - [8] Virology Unit, Policlinic of Tor Vergata, Rome, 00133, Italy

Aim: The identification of early biomarkers for predicting coronavirus disease 2019 (COVID-19) progression and new therapeutic intervention are needed. Recent findings demonstrated that the Human Endogenous Retrovirus-W Envelope (HERV-W ENV) is activated in response to infectious agents leading to various immune-pathological effects. The present study aimed to evaluated HERV-W involvement during severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

Methods: HERV-W ENV expression was evaluated in swab and in blood samples of COVID-19 patients and of Healthy Donors (HDs) by RT Real Time PCR and flow cytometry. Patients have been characterized for immunophenotype, biochemical and inflammatory markers, molecular and protein cytokine expression, clinical signs and disease progression. To address the contribution of SARS-CoV-2 on the activation of HERV-W ENV, *in vitro* experiments were performed stimulating Peripheral Blood Mononuclear Cells (PBMCs) from HDs with SARS-CoV-2 Spike protein and monitoring the induction of HERV-W ENV and IL6.

Results and Conclusions: HERV-W ENV was found to be highly expressed already in SARS-CoV-2 positive versus negative swabs and correlated with the expression of several cytokines (IL-1β, IL-6, IL-10, INF-α, INF-β, MCP-1) and N gene of SARS-CoV-2. In blood, HERV-W ENV mRNA and protein were found highly expressed in COVID-19 patients but not in healthy donors (HDs). Notably, the detection of HERV-W ENV protein was most significantly associated with lymphocytes, in particular in $CD3^+$ T cells. HERV-W ENV protein expression correlated with the T cell differentiation, exhaustion, and senescence markers: in particular, $CD3^+CD8^+$ T HERV-W ENV expression was associated with a decrease in naïve ($CD45RA^+CCR7^+$) and central memory (CD45RA-$CCR7^+$) cells and positively correlated with effector memory (CD45RA-$CCR7^+$) and terminal effector memory ($CD45RA^+$ $CCR7^+$) cells. Furthermore, HERV-W ENV positively correlated with $CD3^+CD4^+PD1^+$ and $CD3^+CD8^+CD57^+$ T cells. Moreover, the percentage of HERV-W ENV-positive cells in $CD4^+$ T cells significantly correlated with coagulopathy markers and with COVID-19 severity. Most importantly, ENV expression in $CD4^+$ T lymphocytes at sampling reflected the respiratory outcome during the global course of hospitalization. The in vitro experiment also confirmed a specific induction by SARS-CoV-2 Spike protein to early activate HERV-W ENV. The data demonstrated a close association between the expression of HERV-W ENV and several immunological and clinical parameters related to the severity of COVID-19 disease, supporting the role of HERV-W ENV as contributing factor in the development and progression of COVID-19 and suggesting a new potential therapeutic target.

P33
Profiling of oral microbiota and cytolines in COVID-19 patients

V. Iebba[1], N. Zanotta[2], G. Campisciano[2], V. Zerbato[3], S. Di Bella[1], C. Cason[2], R. Luzzati[1], M. Confalonieri[1,4], A.T. Palamara[5], M.Comar[1,2]

[1] Department of Medical, Surgical and Health Sciences, University of Trieste, Piazzale Europa, 1, 34127 Trieste, Italy - [2] Laboratory of Advanced Microbiology Diagnosis and Translational Research, Institute for Maternal and Child Health-IRCCS Burlo-Garofolo, Trieste, Italy - [3] Infectious Diseases Department, Udine University, 33100 Udine, Italy - [4] Pulmonology Department, University Hospital of Cattinara, 34149 Trieste, Italy - [5] IRCCS San Raffaele Pisana, 00166 Rome, Italy; Department of Public Health and Infectious Diseases, Sapienza University of Rome, Laboratory Affiliated to Institute Pasteur Italia- Cenci Bolognetti Foundation, 00185 Rome, Italy

SARS-CoV-2 presence has been recently demonstrated in the sputum or saliva, suggesting how the shedding of viral RNA outlasts the end of symptoms. Recent data from transcriptome analysis show that oral cavity mucosa harbors high levels of ACE2 and TMPRSS2, highlighting its role as a double-edged sword for SARS-CoV-2 body entrance or interpersonal transmission. Here we studied the oral microbiota structure and inflammatory profile of naive severe COVID-19 patients by 16S rRNA V2 automated targeted sequencing and magnetic bead-based multiplex immunoassays, respectively. A significant diminution in species richness was observed in COVID-19 patients, along with a marked difference in beta-diversity. Species such as *Prevotella salivae* and *Veillonella infantium* were distinctive for COVID-19 patients, while *Neisseria perflava* and *Rothia mucilaginosa* were predominant in controls. Interestingly, these two groups of oral species oppositely clustered within the bacterial network, defining two distinct Species Interacting Group (SIGs). COVID-19-related pro-inflammatory cytokines were found in both oral and serum samples, along with a specific bacterial consortium able to counteract them. We introduced a new parameter, named CytoCOV, able to predict COVID-19 susceptibility for an unknown subject at 71% of power with an AUC equal to 0.995. This pilot study evidenced a distinctive oral microbiota composition in COVID-19 subjects, with a definite structural network in relation to secreted cytokines. Our results would be usable as a theranostic approach against COVID-19, using bacterial consortia as biomarkers or to reduce local inflammation.

P34

Anti-SARS-CoV-2 vaccination strategy and immunoresponse in healthcare workers of the INT - IRCCS "Fondazione Pascale" Cancer Center (Naples, Italy)

M.A. Isgrò[1], D. Rea[1], L. Di Capua[1], G. Trillò[2], L. Russo[1], G. Botti[3], L. Miscio[3], A.A.M. Bianchi[3], F.M. Buonaguro[3], E. Cavalcanti[1]

[1] Division of Laboratory Medicine, Istituto Nazionale Tumori – IRCCS Fondazione Pascale, Napoli, Italia - [2] Specialization School in Clinical Pathology and Clinical Biochemistry, School of Medicine and Surgery, Università degli Studi di Napoli Federico II, Napoli, Italia - [3] Istituto Nazionale Tumori – IRCCS Fondazione Pascale, Napoli, Italia

Background: Coronavirus disease 2019 (COVID-19), the first pandemic of the 21st century, has been characterized by the speed of global transmission and the rapidity of the development of diagnostic reagents and vaccines. By December 2020, less than 12 months after the identification of the novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the FDA-authorized and EMA-approved mRNA anti-spike vaccine (named BNT162b2 / Pfizer) was implemented in Italy for mass vaccination starting from health workers.
Aim: In the present study, differences in the SARS-CoV-2 humoral immune response after vaccine administration were evaluated between two cohorts of healthcare professionals at the INT Tumor Center - IRCCS "Fondazione Pascale" (Naples, Italy): previously exposed and not exposed to SARS-CoV-2 subjects.
Methods: We determined specific anti-RBD (receptor-binding domain) titers against trimeric spike glycoprotein (S) of SARS-CoV-2 by Roche Elecsys Anti-SARS-CoV-2 S immunoassay in serum samples of 35 healthcare workers with a previous documented history of SARS-CoV-2 infection and 158 healthcare workers without, after 1 and 2 doses of vaccine, respectively. Moreover, geometric mean titers and relative fold changes (FC) were calculated.
Results: Both previously exposed and not exposed to SARS-CoV-2 subjects developed significant immune responses to SARS-CoV-2 after the administration of 1 and 2 doses of vaccine, respectively. Anti-S antibody responses to the first dose of vaccine were significantly higher in previously SARS-CoV-2-exposed subjects in comparison to titers of not exposed subjects after the first as well as the second dose of vaccine. Fold changes for subjects previously exposed to SARS-CoV-2 were very modest, given the high basal antibody titer, as well as the upper limit of 2500.00 BAU/mL imposed by the Roche method. Conversely, for naïve subjects, mean fold change following the first dose was low (x = 1.6), reaching 3.8 FC in 72 subjects (45.6%) following the second dose.
Conclusions: The results showed that, as early as the first dose, SARS-CoV-2-exposed individuals developed a remarkable and statistically significant immune response in comparison to those who did not contract the virus previously, suggesting the possibility of administering only one dose in previously SARS-CoV-2-exposed subjects in order to prevent a risk of vaccine-associated disease enhancement (VADE). FC for not exposed subjects, after the second dose, were 3.8 in > 45.0% of vaccinees, and ≤ 3.1 in 19.0%, the latter showing a potential susceptibility to further SARS-CoV-2 infection.

P35

COVID-19 vaccine MRNABNT162B2 elicitis human antibody response in milk of breastfeeding

E. La Civita[1], M. Cennamo[1], A. Liotti[1], A. Furno[1], L. Conte[1], C. Grillo[1], C. Gnasso[1], C. Cozzolino[1], S. Di Somma[1], F. Napolitano[1], L. Sarno[2], M. Guida[2], D. Terracciano[1], G. Portella[1]

[1] Department of Translational Medical Sciences, University of Naples "Federico II", 80131 Naples, Italy - [2] Department of Neurosciences, Reproductive Science and Dentistry, School of Medicine, University of Naples Federico II, Naples, Italy

Aim of the study: To demonstrate the release of SARS Cov-2 Spike (S) antibodies in human milk samples obtained by patients who have been vaccinated with mRNABNT162b2 vaccine.
Methods used: Milk and serum samples were collected in 10 volunteer women 20 days after the first dose and 7 seven days after the second dose of mRNABNT162b2 vaccine. Anti SARS-CoV-2 S antibody were measured by the Elecsys® Anti-SARS-CoV-2 S ECLIA assay (Roche Diagnostics AG, Rotkreuz, Switzerland), a quantitative electrochemiluminescence immunometric method.
Results and conclusions: At first sample, anti SARS Cov-2 S antibodies were detected in all serum samples (103,9±54,9 U/mL) and only in two (40%) milk samples with a low concentration (1,2±0,3 U/mL). At the second sample, collected 7 days after the second dose, anti SARS Cov-2 S antibodies were detected in all serum samples (3875,7±3504,6 UI/mL) and in all milk samples (41,5±47,5 UI/mL). No correlation was found between level of serum and milk antibodies; milk antibodies/serum antibodies ratio was on average 2% (range: 0,2 - 8,4%). We demonstrated a release of anti SARS-Cov-S antibodies in breast milk of women vaccinated with mRNABNT162b2. Vaccinating breastfeeding women could be a strategy to protect their infants by COVID-19 infection.

P36

Screening workers to prevent SARS-Cov-2 infection in the workplace: results of a year of investigation in the Marche Nord Companies

C. Orlandi[1], D. Betti[1], B. Borghi[2], A. Baroni[2], M. Papalini[2], M. Magnani[1], A. Casabianca[1]

[1] Department of Biomolecular Sciences, University of Urbino "Carlo Bo" - [2] Confindustria Pesaro Urbino

Aim of the study: During the first months of the SARS-CoV-2 pandemic, the Covid-Lab set up at the University of Urbino was authorized (on 8th May 2020) to carry out molecular SARS-CoV-2 diagnostic tests for COVID-19 by the regional reference laboratory (Virology Unit, AOU Ospedali Riuniti, Ancona, Italy). Meanwhile the Confindustria Pesaro Urbino association, as part of the procedures for implementation of the internal anti-contagion security protocols, signed an agreement with the Covid-Lab to support its companies specifically about the containment of the spread of the virus in the workplace and the health of its workers.
Serological tests, identifying exposure to the SARS-CoV-2 by detecting antibodies directed against the virus, are useful in epidemiological evaluation of viral circulation, but they do not replace the molecular method for viral RNA detection that is the only conclusively diagnostic test.
A fast and cost-effective procedure involves a double step: a positive serological rapid test (performed in the workplace, always taken on a voluntary basis) for the presence of IgM and/or IgG or both antibodies, is followed by a nasopharyngeal swab processed and analysed at the Covid-Lab on the same day as the rapid test. To ensure an optimal safety path, rapid tests are repeated periodically (every 2/4 weeks).
Methods: The detection of IgG and IgM Ab was performed using a rapid immunochromatographic test marketed by Diatheva, CE-IVD certified. The test provides the result in just 15 min with the aid of a simple lancet device used to generate a blood drop at the fingertip. Total RNA from nasopharyngeal swabs was extracted using Total RNA Purification Kit (Norgen). Three sets of primers and probes detect the RNA-dependent RNA polymerase (RdRp) specific gene and envelope gene (E) of the SARS-CoV-2, and the internal control (human RNase P) to evaluate RNA extraction and the presence of PCR inhibitors (COVID-19 PCR DIATHEVA Detection kit, Diatheva, CE-IVD certified). The one-step real-time RT-PCR multiplex reactions were carried out in a 7500 real-time PCR system (Applied Biosystems) according to the manufacturer's specifications. Results were considered valid only when the cycle threshold (Ct) value of the internal control was ≤ 40. The results were considered positive when the Ct values of RdRp target gene were ≤ 45, negative when > 45.
Results: Between May 2020 and April 2021, over 10,000 rapid serological tests had been carried out on workers of 33 companies associated with Confindustria Pesaro Urbino, and in 5% of cases IgG or IgM were found (512). Only for workers testing positive to antibodies it was necessary to perform the nasopharyngeal swabs and the virus RNA analysis. All the 512 swabs gave a valid result regarding internal control amplification (RNAse P Ct ≤ 40, mean±SD 29.81±3.88) with 102 positive results (20%) for SARS-CoV-2 with a Ct value ≤ 45 (mean±SD 23.62±7.59). Overall, only 1% of samples resulted positive for viral RNA

(102/10,000). In the months May-September 2020, were analysed 107 swabs and the positivity rate was 0%. In the months October-December 2020 the positivity rate was 19% (64/329) and in the months January-15 April 2021 50% (38/76). Based on AMCLI indications (Associazione Microbiologi Clinici Italiani, Prot. 001-2021: Indicazioni operative AMCLI su quesiti frequenti relativi alla diagnosi molecolare di infezione da SARS-CoV-2) we have evaluated the number of "very low positive" samples with RdRp Ct value ≥ 35. We observed 57 samples above (89%) and 7 (11%) below (these data are updated to December. 2020).

Conclusion: The University of Urbino set up a rapid-response diagnostic centre for Coronavirus (Covid-Lab) that guaranteed the result within 24 hours (generally <6 h) allowing the company to activate the optimal safety path to ensure the health and safety of workers in the workplace. Our observations during this first year of activity, highlight that in the workplace, the infection does not seem to spread if precautionary measures are followed and only 1% (1 worker out of 100) tested positive for the SARS-CoV-2 virus.

P37

Coinfection of tubercolosis and COVID-19 limits the ability to in vitro respond to SARS-Cov-2

L. Petrone[1], E. Petruccioli[1], V. Vanini[1], G. Cuzzi[1], G. Gualano[2], P. Vittozzi[2], E. Nicastri[3], G. Maffongelli[3], A. Grifoni[4], A. Sette[4], G. Ippolito[5], G.B. Migliori[6], F. Palmieri[2], D. Goletti[1]

[1] Translational Research Unit, National Institute for Infectious Diseases Lazzaro Spallanzani-IRCCS, Rome, Italy - [2] Respiratory Infectious Diseases Unit, National Institute for Infectious Diseases Lazzaro Spallanzani-IRCCS, Rome, Italy - [3] Clinical Division of Infectious Diseases, National Institute for Infectious Diseases Lazzaro Spallanzani-IRCCS, Rome, Italy - [4] Center for Infectious Disease and Vaccine Research, La Jolla Institute for Immunology (LJI), La Jolla, CA 92037, USA - [5] Scientific Direction, National Institute for Infectious Diseases Lazzaro Spallanzani-IRCCS, Rome, Italy - [6] Servizio di Epidemiologia Clinica delle Malattie Respiratorie, Istituti Clinici Scientifici Maugeri IRCCS, Tradate, Italy

Aim of the study. The interaction of COVID-19 and tuberculosis (TB) are still poor characterized. Here we evaluated the immune response specific for *M. tuberculosis* (Mtb) and SARS-CoV-2 using a whole-blood-based assay-platform in COVID-19 patients either with TB or latent TB infection (LTBI).
Methods. We evaluated IFN-γ level in plasma from whole-blood stimulated with Mtb antigens in the Quantiferon-Plus format or with peptides derived from SARS-CoV-2 spike protein, Wuhan-Hu-1 isolate (CD4-S).
Results. We consecutively enrolled 63 COVID-19, 10 TB-COVID-19 and 11 LTBI-COVID-19 patients.
IFN-γ response to Mtb-antigens was significantly associated to TB status and therefore it was higher in TB-COVID-19 and LTBI-COVID-19 patients compared to COVID-19 patients ($p \leq 0.0007$).
Positive responses against CD4-S were found in 35/63 COVID-19 patients, 7/11 LTBI-COVID-19 and only 2/10 TB-COVID-19 patients. Interestingly, the responders in the TB-COVID-19 group were less compared to COVID-19 and LTBI-COVID-19 groups ($p=0.037$ and 0.044, respectively). Moreover, TB-COVID-19 patients showed the lowest quantitative IFN-γ response to CD4-S compared to COVID-19-patients ($p=0.0336$) and LTBI-COVID-19 patients ($p=0.0178$).
Conclusions. Our data demonstrate that COVID-19 patients either TB or LTBI have a low ability to build an immune response to SARS-CoV-2 while retaining the ability to respond to Mtb-specific antigens.

P38 - OC 52

Study of entry checkpoints and kinetics of SARS-CoV-2 variants in veroe6 cells

P. Quaranta[1,3], B. Storti[2], C. Di Primio[3], N. Clementi[4,5], P.G. Spezia[1], V. Carnicelli[6], G. Lottini[1], E. Paolini[7], G. Freer[1], M. Lai[1], M. Costa[3], F. Beltram[2,8], A. Diaspro[9,10], M. Pistello[1,9], R. Zucchi[6], P. Bianchini[9], G. Signore[8], R. Bizzarri[2,6]

[1] Retrovirus Center, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, SS 12 Dell'Abetone e del Brennero 2 56127 Pisa, Italy - [2] NEST, Scuola Normale Superiore and Istituto Nanoscienze-CNR, Piazza San Silvestro 12, 56127 Pisa, Italy - [3] Istituto di Neuroscienze - CNR, Via Moruzzi 1, 56124 Pisa, Italy - [4] Laboratory of Medical Microbiology and Virology, University "Vita-Salute" San Raffaele, Via Olgettina, 58, 20132, Milan, Italy - [5] Laboratory of Medical Microbiology and Virology, IRCCS San Raffaele Hospital, Milan, Italy - [6] Department of Surgical, Medical and Molecular Pathology, and Critical Care Medicine, University of Pisa, Via Roma 65, 56126 Pisa, Italy - [7] Department of Mathematics, University of Pisa, Largo Bruno Pontecorvo 5, 56127 Pisa, Italy - [8] Fondazione Pisana per la Scienza, via F. Giovannini 13, 56017 San Giuliano Terme (PI), Italy - [9] Pisa University Hospital, Via Pietro Trivella, 56126, Pisa, Italy - [10] Nanoscopy, CHT, Istituto Italiano di Tecnologia, Via E. Melen 83, 16152 Genoa Italy

Aim of the study. In December 2019, a new Coronavirus named SARS-COV2 appeared in Wuhan (China), and rapidly spread worldwide causing a pandemic with devastating social consequences. Currently, SARS-CoV2 infected 136 million of people in the world and caused the death of 2.94 million of them. One year after the outbreak of the pandemic, we have several vaccines available that hold promise for a rapid end of it, nevertheless they may be ineffective against the numerous emerging variants, as consequence the development of new drugs and of a therapy against COVID-19 is needed. In this context, elucidation of structure-property relationships that modulate virus-cell host checkpoints, such as entry, replication, and egress, is crucial to assess the role of genome mutation on virus infectivity furthermore the mechanistic knowledge of virus entry in cells is relevant for developing drugs tailored to prevent infection (Seyedpour S, et al. J Cell Physiol 2021; Xia S, et al. Cell Res 2020). To this aim, we developed a multi-scale microscopy imaging toolbox to address some major issues related to SARS-CoV-2 interactions with host cells.
Methods used: Our approach harnesses both conventional and super-resolution fluorescence microscopy (Airyscan, STORM, and STED); through these techniques we investigated the entry phase of SARS-CoV-2 variants in VeroE6 cells. We also use real time PCR technique to compare the kinetics of replication of B.1 and B1.1.7 SARS CoV-2 strains. The B.1 is characterized by D614G, the dominant mutation in SARS-CoV-2 lineages that have been circulating worldwide since spring 2020; while the B.1.1.7 SARS-CoV-2 variant (Volz E, et al. Cell 2021) has become the dominant lineage in UK (Cyranoski D. Nature 2021).
Results and conclusions: Our results suggest that the variant of concern B.1.1.7, currently on the rise in several countries by a clear transmission advantage (Volz E, et al. Cell 2021), outcompetes its ancestor B.1 in terms of a much faster kinetics of entry. Both strains used the late pathway mechanism of entry and showed similar fraction of pre-cleaved S protein, accordingly, the faster entry of B.1.1.7 could be directly related to the N501Y mutation in the S protein, which is known to strengthen the binding of Spike RBD with ACE2. Remarkably, we also directly observed the significant role of clathrin as mediator of late entry endocytosis, as already suggested for other CoVs and from pseudovirus-based infection models. In conclusion, a fluorescence microscopy-based approach is a valuable tool to evaluate the entry kinetic of SARS-CoV-2 and its variants.

P39 - OC 47

SARS-CoV-2 sensing by TLR3 enhances immune response and hACE2 expression

S. Rizzo[1], D. Bortolotti[1], V. Gentili[1], G. Schiuma, F. Caccuri[2], A. Caruso[2], R. Rizzo[1]

[1] Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara (Italy) - [2] Department of Microbiology and Virology, "Spedali Civili," Brescia - Italy

Background: SARS-CoV-2 is the causative agent of COVID-19, a disease associated to a peculiar clinical case history, characterized by an inefficient immune system response and high levels of inflammatory cytokines, known as "cytokine storm". To achieve an efficient control of the infection, it is crucial that the host immune response is balanced, in order to avoid both excessive inflammation that could damage the host system as observed in COVID-19 patient lungs, and immune system low activation, that could facilitate viral spread.
Methods: In this study we used Calu-3/MRC-5 multicellular spheroids as an *in vitro* lung model. The choice to use MTCSs lung model was supported by the evidence that both epithelial and fibroblast components could participate in the inflammatory response observed during lung damage, contributing to both cytokine storm and antiviral response.
Aim: Our aim was to investigate how RNA sensors might be involved in SARS-CoV-2 infection.
Results: We observed that SARS-CoV-2 infection induced the expression of TLR3 and TLR7 RNA sensors, resulting in the up-modulation of pro-inflammatory cytokines, interferon (IFN) type 1, type 2 and lambda III. TLR3 regulates mainly the cytokine secretion via NF-kappaB and IRF3, while TLR7 controls mainly IFNs expression via NF-kappaB. The IFN Type 1 enhanced JAK/STAT pathway, that conversely induced human angiotensin-converting enzyme 2 (hACE2) expression.
Discussion: We propose that TLR3 and TLR7 RNA sensing of SARS-CoV-2 in lung environment is a key driver of inflammation and viral entry, and that the specific inhibition of innate immune pathways may beneficially mitigate inflammation-associated COVID-19. In particular, the induction of JAK/STAT pathway might have a possible implication in the deregulated immune response observed in severe COVID-19 patients supporting it as a target for a more functional therapy.

P40

Cytokine signature and prediction models in the waves of COVID-19 pandemics

S. Romano[1,2], S. Cabaro[1,2], V. D'Esposito[1,2], M. F. Di Tolla[1,2], T. Migliaccio[1,2], A. Riva[1,2], L. Atripaldi[3], U. Atripaldi[3], T. Di Matola[3], D. Terracciano[1], M. Cennamo[1], M. Sansone[4], F. Beguinot[1,2], G. Portella[1], P. Formisano[1,2]

[1] Department of Translational Science, University of Naples "Federico II", Napoli - [2] URT "Genomic of Diabetes", Institute of Experimental Endocrinology and Oncology (IEOS), National Research Council (CNR), Napoli - [3] Azienda Ospedaliera dei Colli, Napoli - [4] Department of Electrical Engineering and Information Technology, University of Naples "Federico II"

The coronavirus disease 2019 (COVID-19) is an emerging infectious disease caused by SARS-CoV-2, the novel coronavirus responsible for the onset of severe acute respiratory syndrome and associated with the current worldwide outbreak. Patients with severe COVID-19 develop a dysregulated release of cytokines, also known as "cytokine storm", associated with disease state and severity. In Europe, at least two waves of infections have been observed. In this study, we have defined cytokine patterns in individuals affected by COVID-19 in the two pandemic waves.

A panel of 27 molecules has been analyzed in serum samples from patients with moderate and severe/critical symptoms of COVID-19, compared with healthy donors. In COVID-19 patients ospedalized during the first wave, a wide variety of cytokine was increased (IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-15, IL-17, FGF-b, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, PDGF, TNF-α, and VEGF). Linear Discriminant Analysis (LDA) algorithm allowed to classify the three groups based on cytokine patterns. Classification and Regression Tree (CART) analysis further defined accurately an algorithm based on IL-6 concentration to discriminate controls and COVID-19 patients, whilst IL-8 well defined disease severity. During the second wave of pandemics, an increasing trend only for IL-1β, IL-1ra, IL-2, IL-6, IL-8, IL-10, GM-CSF, IFN-γ, and IP-10 was observed. CART analysis confirmed the role of IL-6 as better predictor of infection to discriminate controls and moderate COVID-19 patients.

In conclusion, the identification of the role of single cytokines in SARS-CoV-2 infection may allow to help envision novel therapeutic options and will pave the way to set up innovative diagnostic tools.

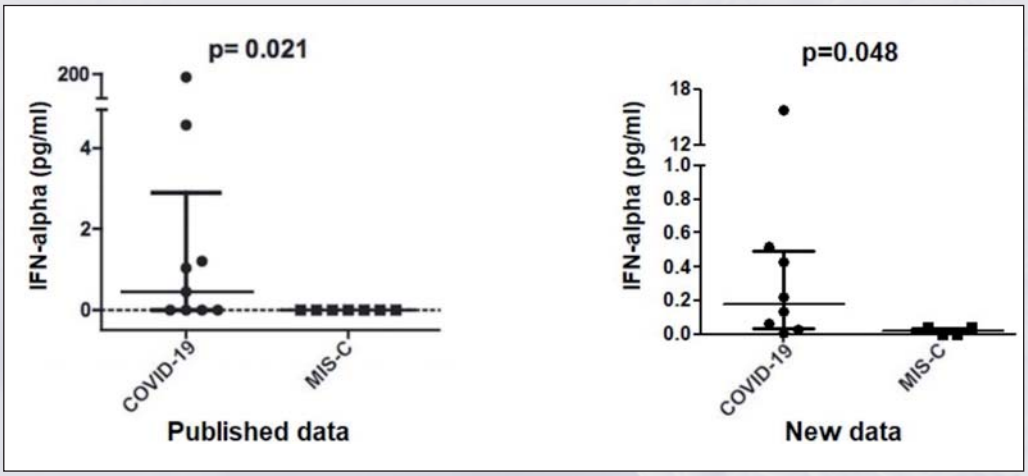
P41

Higher plasma IFN-α in children with acute COVID-19 infection as compared to multisystem inflammatory syndrome (MIS-C) patients

S. Roversi[1] , C. Sabelli[2] , S. Messali[1] , A. Caruso[1] , S. Fiorentini[1]

[1] Section of Microbiology, Department of Molecular and Translational Medicine, University of Brescia, 25123, Brescia, Italy - [2] Copan Italia Spa, Brescia, Italy

Aim of the study. COVID-19 occurs in the majority of children without symptoms or with a paucisymptomatic respiratory syndrome, but a small proportion of children develop the systemic Multi Inflammatory Syndrome (MIS-C), characterized by persistent fever and systemic hyperinflammation. In a previous study [Caldarale and Giacomelli. Plasmacytoid Dendritic Cells Depletion and Elevation of IFN-γ Dependent Chemokines CXCL9 and CXCL10 in Children With Multisystem Inflammatory Syndrome. 2021, Front.Immunol.12:654587], we presented that patients with MIS-C showed higher plasma levels of proinflammatory chemokines mainly induced by IFN-γ CXCL9 and CXCL10 than COVID-19 children. By contrast, we detected IFN-α in plasma of children with COVID-19, but not in patients with MIS-C. With this study we confirmed the previously published data, measuring the IFN-α plasma levels in COVID-19 and MIS-C patients through a new highly sensitive method.
Methods. In previous study, we investigated lymphocyte and dendritic cells subsets and chemokine/cytokine profiles in 10 children with COVID-19 and 9 with MIS-C at the time of hospital admission. New data were obtained measuring plasma IFN-α levels in COVID-19 and MIS-C patients through a new ultra-sensitive biomarker detection system, Quanterix SR-XTM based on Simoa® bead technology.
Results and conclusions. In previously published data we investigated the pathogenesis of MIS-C by evaluation of IFN-α plasmatic levels in children with MIS-C and COVID-19. Comparison between the two groups revealed a significant increase of IFN-α levels in



COVID-19 children as compared to MIS- C (p=0.021). In contrast, IFN-γ was undetectable in the plasma of both groups. Because plasmacytoid dendritic cells (pDCs) constitute the main source of IFN-α in blood, low levels of IFN-α are associated with low pDC levels in MIS-C subjects. Analysis of mDC and pDC counts revealed a profound depletion of pDCs in patients with MIS-C (p=0.01), as compared to children with COVID-19. Measurement of plasma IFN-α levels with a more sensitive method shows a reduction in plasmatic IFN-α levels in MIS-C than COVID-19 patients (p=0.048) and allowed us to confirm the data previously published: COVID-19 patients present a higher plasma IFN-α levels than MIS-C patients.

P42

High IFN-α levels in ruxolitinib-treated Aicardi-Goutieres patient during SARS-CoV-2 infection: a case report

R. Scaduto[1,2], S. Roversi[1,2], J. Galli[3], M. Cattalini[1], R.M. Ferraro[4], M. Cortesi[1], S. Giliani[4], E. Fazzi[3], A. Caruso[2], R. Badolato[1]

[1] Unit of Pediatrics, Dept of Clinical and Experimental Sciences University of Brescia and Angelo Nocivelli Institute of Molecular Medicine, University of Brescia, ASST Spedali Civili Brescia - [2] Section of Microbiology, Dept of Molecular and Translational Medicine, University of Brescia - [3] Child Neurology and Psichiatry Unit, University of Brescia and ASST Spedali Civili di Brescia, Brescia - [4] A. Nocivelli Institute for Molecular Medicine, University of Brescia - ASST Spedali Civili di Brescia, Brescia, Italy

We report a case of a five-year-old female affected by Aicardi–Goutières syndrome (AGS) with late- onset of symptoms who was under treatment with ruxolitinib, admitted at our clinic for COVID-19 infection. The clinical course was regular without any significant complication. During SARS-CoV-2 infection there was a significant increase in interferon-α (IFN-α) serum levels compared to basal levels, followed by normalization after SARS-CoV-2 test negativity.

Introduction. AGS is a rare monogenic leukodystrophy with pediatric onset. It belongs to a group of conditions called type I interferonopathies, and it is characterized by psychomotor disturbances, cerebral white matter abnormalities with basal ganglia calcification, cerebral atrophy, chronic cerebrospinal fluid (CSF) lymphocytosis, and elevated levels of IFN-α. The diagnosis of AGS is usually established in the first year of live in patients with characteristic clinical findings and typical abnormalities on cranial radiological imaging and/or by the identification of biallelic pathogenic variant.

Clinical Case. We herein report the case of a 5-year-old female affected by AGS, diagnosed with compound heterozygous mutations in ADAR gene, because of previous Aicardi–Goutières syndrome diagnosed in her brother with the same mutations in ADAR gene. She was initially treated with intravenous immunoglobulins infusion and subsequently with ruxolitinib. In January 2021 she developed fever and cough initially treated with amoxicillin. She underwent nasopharyngeal swab with analysis of SARS- CoV-2 by reverse transcriptase polymerase chain reaction, which resulted positive. In consideration of the underlying pathology, she was admitted at our Department. At admission she was in good general clinical conditions. Blood pressure and oxygen saturation were normal. Laboratory findings showed normal white cell count, Erythrocyte Sedimentation Rate (ERS), and C-Reactive Protein (CPR). Chest- X-ray showed only a minimal bronchovascular accentuation. During the hospital stay she continued her domiciliary treatment with ruxolitinib, acyclovir and subcutaneous immunoglobulins. Her clinical course was regular, without fever, with normal respiratory dynamic, and normal saturation levels without oxygen supplementation. Pre-admission IFN-α levels were normal (one month before COVID-19 infection). During the course of the disease IFN-α was significantly increased (Interferon Score 296.01). IFN-α levels returned normal one month after recovery from COVID-19. Recently, a preliminary study has shown that the increase in blood IFN-α levels in COVID-19 patients were directly associated with improvement in disease severity and greater survival. So we hypothesized that increasing IFN-α serum levels in this patient may be an adequate response during COVID-19 infection and may have provided a benign clinical course.

P43

Effects of remdesivir on negativation time in SARS-CoV-2 disease: an open label cohort clinical trial - preliminary data

F.S. Serino[1], M. Manara[1], L. Di Donato[1], C. Guarente[1], L. Brollo[2]

[1] Department of Infectious Diseases, Hospital of Jesolo, AULSS 4 Veneto Orientale, Jesolo, (Italy) - [2] Chief of the Department of Infectious Diseases, Hospital of Jesolo, AULSS 4 Veneto Orientale, Jesolo, (Italy)

Background: Though all the factors (including the timing) that contribute to the progression of patients to severe COVID-19 are not yet fully understood, different panels suggest the potential benefit of early intervention with an antiviral (Remdesivir) in hospitalized patients with COVID-19 Severe Pneumonia. Daily experience on the use of Remdesivir in this context leads us to hypothesize that the earlier is the treatment (within the 10 days required by the guidelines) the greater will be the probability of a favorable trend in terms of mortality and need for supplemental oxygen.

Materials and methods: A total of 24 patients (16 M and 8 F) of different age groups (35% between 30-50 years; 50% between 50-70 years; 15% older than 70 years) hospitalized for COVID-19 Pneumonia and following the Remdesivir prescribing criteria according to AIFA, were enrolled. They were divided according to the timing of drug administration within 5 days (54%) or within 10 days (46%) of the first Nasopharyngeal Swab (NPS) with a Positive output. The NPSs were processed and analyzed with a PCR technique by the Lab Department of the Covid Hospital of Jesolo. Possible presence of one or more comorbidities (e.g. arterial hypertension, COPD, diabetes) were also considered as risk factors for disease progression (43% had no comorbidities; 45% had 1 comorbidities and 12% had 2 or more comorbidities).

Results: Clinical improvement and the time (in days) needed for the first negative NPS (from the first positive NPS and from the first given dose of Remdesivir) were used as parameters. We observed (Table 1) that: 76,0% (of the patients who had received the drug within 5 days were negative within 3 weeks, most of them between the 2nd and 3rd week (69,2%); 72,7% of those who had received the drug within 10 days were negative within

Table 1. Number of negative patients across time

	NUMBER OF NEGATIVE PATIENTS ACROSS TIME					
	Within 1 WEEK	Within 2 WEEKS	Within 3 WEEKS	Within 1 MONTH	MORE THAN A MONTH	TOT N PZ.
Among those who had received Remdesivir within 5 days	1	5	4	2	1	13
Among those who had received Remdesivir within 10 days	3	3	2	2	1	11
TOT Since first Remdesivir day	4	8	6	4	2	24
TOT Since First + NPS	1	6	9	3	5	24

Table 2. Number of negative patients across time, calculated since the first given Remdesivir dose and compared with the number of comorbidities

COMORBIDITIES	NUMBER OF NEGATIVE PATIENTS ACROSS TIME					TOT PZ. FOR COMORBIDITIES
	Within 1 WEEK	Within 2 WEEKS	Within 3 WEEKS	Within 1 MONTH	MORE THAN A MONTH	
0	1	3	3	2	1	10
1	2	3	3	2	1	11
2	1	1	0	0	0	2
3	0	0	0	0	0	0
4	0	1	0	0	0	1
5	0	0	0	0	0	0

3weeks, only 45,5% between the 2nd and 3rd week. Negative patients at 2-3weeks treated within 5 days vs treated within 10 days P = 0,0538. The remainder were negative for 30 days or more.

Conclusions: Although within the limits of sample size, patient heterogeneity and the methods used (lack of a comparison population), the advantage of early treatment with Remdesivir (within 5 days from the first positive NPS) could indeed help patients to have a better outcome and a shorter negativization time. It should also be noted that the presence of one or more comorbidities did not significantly affect the negativization time:data in Table 2 show comparable time to patients without comorbidities. These data represent a preliminary notice, our investigation is still ongoing. Further clinical trials could be supportive in the near future.

P44

Evaluation of a fully closed real time PCR platform for the detection of SARS-CoV-2 in nasopharyngeal swabs: a pilot study

L. Stanziola[1], M. Cennamo[1], E.A. Molinari[1], A. Liotti[1], E. La Civita[1], M. Fiorenza[1], C. De Luca[2], L. Vallefuoco[1], R. Sorrentino[1], T.M. Sorbo[3], P. Romano[4], E. Montella[2], G. Troncone[2], D. Terracciano[1], G. Portella[1]

[1] Department of Translational Medical Sciences, University of Naples "Federico II", 80131 Naples, Italy - [2] Public Health, University of Naples Federico II, Naples, Italy - [3] Division of Nephrology, Department of Translational Medical Sciences, School of Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy - [4] Department of Surgical Sciences Submission and Nephrology; University Federico II of Naples, Naples

Aim of the study: To date, reverse transcriptase PCR (RT-PCR) on nasopharyngeal swabs is the 'gold standard' approach for the diagnosis of COVID-19. The need to develop easy to use, rapid, robust and with minimal hands-on time approaches are warranted. In this setting, the Idylla SARS-CoV-2 Test may be a valuable option. The aim of our study is to evaluate the analytical and clinical performance of this assay on previously tested SARS-CoV-2 people by conventional RT-PCR based approach in different settings, including initial diagnosis and clinical follow-up.
Methods used: To evaluate the sensitivity and specificity of the Idylla SARS-CoV-2 Test, we retrieved 55 nasopharyngeal swabs, previously analysed by a fully validated assay, from symptomatic patients or from people who have been in close contact with COVID-19 positive cases. Discordant or high discrepant cases were further analysed by a third technique. In addition, a second subset of 14 nasopharyngeal swab samples with uncertain results (cycle threshold between 37 and 40), by using the fully validated assay, from patients with viral infection beyond day 21, were retrieved.
Results and conclusions: Overall, Idylla showed a sensitivity of 93.9% and a specificity of 100.0%. In addition, in the additional 14 nasopharyngeal swab samples, only five (35.7%) featured a positive result by the Idylla SARS-CoV-2 Test. We demonstrated that the Idylla SARS-CoV-2 Test may represent a valid, fast, highly sensitive and specific RT-PCR test for the identification of SARS-CoV-2 infection.

P45 - OC 54

Single-dose BNT 162B2 mRNA COVID-19 vaccine significantly boosts neutralizing antibody response in health care workers recovering from asymptomatic or mild natural SARS-CoV-2 infection

I. Vicenti[1], F. Gatti[2], R. Scaggiante[3], A. Boccuto[1], D. Zago[2], M. Basso[2], F. Dragoni[1], M. Zazzi[1], S.G. Parisi[2]

[1] Department of Medical Biotechnologies, University of Siena, Italy - [2] Department of Molecular Medicine University of Padova, Italy - [3] Belluno Hospital, Belluno, Italy

Aim of the study. To investigate the role of post-infection vaccination with BNT162b2 mRNA COVID-19 vaccine in a cohort of Italian healthcare workers (HCWs), recovered from asymptomatic or mild natural SARS-CoV-2 infection.
Methods. Two-fold serial dilutions of heat-inactivated sera were incubated with 100 TCID50 of SARS-CoV-2 virus (lineage B.1) at 37°C for 1 h in 96-well plates. Then, 10,000 pre-seeded Vero E6 cells per well (ATCC CRL-1586) were treated with serum-virus mixtures and incubated at 37°C. After 72 h, cell viability was determined through the commercial kit CellTiter-Glo® 2.0 Cell Viability Assay (Promega). The NtAb titer was defined as the reciprocal value of the sample dilution that showed a 50% protection of virus-induced cytopathic effect (ID50). Each run included an uninfected control, an infected control and a known SARS-CoV-2 neutralizing serum. Antibodies with ID_{50} titers ≥10 were defined as SARS-CoV-2 positive and neutralizing. Statistical analyses were performed using IBM SPSS Statistics, version 20.
Results. A total of 57 Italian HCWs were enrolled, including 41 SARS-CoV-2 infected individuals (median age 45 [34-51] years, male 34.1%) recovering from asymptomatic infection or mild disease and 16 uninfected controls (median age 49 years [34-59], male 31.2%). Sera from previously infected HCWs were collected on the day of first-dose vaccination (T1_inf) and 21±4 days thereafter (T2_inf), whereas sera samples from uninfected controls were collected on the day of first-dose vaccination (T1_uninf, all confirmed to be negative), 20±4 days thereafter (T2_uninf) and then further 20±4 days following the second-dose vaccination (T3_uninf). Previously infected HCW were vaccinated after a median (IQR) of 313 (285-322) days since diagnosis. NtAb titres increased significantly at T2_inf with respect to T1_inf (median values 1544 [732-2232] vs. 26 [10-88]; p < 0001) and a significant correlation between days from diagnosis and NtAb levels at T2_inf (Pearson R2 = 0.395; p = 0.011) but not at T1_inf (Pearson R2 = 0.119; p = 0.502) was found. Similarly, in the previously uninfected control group, there was a significant increase in NtAb titres at T3_uninf with respect to T2_uninf (median values 183 [111-301] vs. 5 [5-15]; p <0001). Notably, T2_inf NtAb levels (1544 [732-2232]) were significantly higher than uninfected NtAb titres, when measured both at T2_uninf (p <0.0001 for both analyses).
Conclusion. The need to vaccinate those who have recovered from natural infection is still under debate. In this work, we demonstrated that a single-dose vaccination of people with mild or asymptomatic previous infection is sufficient to significantly boost SARS-CoV-2 immunity.

P46
Monitoring anti SARS-CoV-2 antibody response after BNT162b2 vaccine

G. Anichini, C. Gandolfo, C. Terrosi, G. Gori Savellini, M.G. Cusi

Virology Unit, Department of Medical Biotechnologies, Siena University Hospital, Siena, Italy

Aim: We have designed a prospective study with the aims to explore and monitor the antibody response against SARS-CoV-2 virus induced by the COMIRNATY mRNA vaccine in a sample of 178 vaccinated volunteers.
Here, we report the interim analysis of the study, including serological results ten and thirty days, and three months (in process) after the second mRNA BNT162b2 vaccine boost among healthcare personnel at S. Maria alle Scotte University Hospital in Siena. Moreover, vaccinated sera of 60 subjects were also tested for the presence of neutralizing antibodies against the virus ten days after receiving the second dose of vaccine. Results were then compared with neutralizing titers obtained from 38 previously infected subjects ten days after receiving one dose of vaccine.
Methods: The humoral immune response of all healthcare workers was evaluated by chemiluminescent (CMIA) and, among them, 60 were also tested by live virus-based neutralization assay. Both IgG and neutralizing titers were also analyzed according to the symptoms developed after the second vaccine administration.
Results: Our results showed a significant decrease of circulating IgG levels over time between ten and thirty days after vaccination, despite neutralizing titers remained quite stable. Moreover, both IgG and neutralizing titers showed a robust correlation with the grade of clinical symptoms reported after the second dose administration. Results after three months will be processed.
Moreover, we observed a significantly lower neutralizing antibody response in naïve patients after complete vaccination compared with those previously infected by SARS-CoV-2 after receiving only a single dose1.
These findings provide evidence that after the administration of a single dose of vaccine, humoral response against SARS-CoV-2 in subjects with a past history of SARS-CoV-2 infection is greater than the one produced by naïve subjects after receiving two doses of vaccine.

Reference:

1. Anichini G, Terrosi C, Gandolfo C et al. SARS-CoV-2 Antibody Response in Persons with Past Natural Infection. N Engl J Med. 2021 Apr 14. doi: 10.1056/NEJMc2103825.

P47

Time course of neutralizing antibody in healthcare workers with mild or asymptomatic COVID-19 infection

A. Boccuto[1], F. Gatti[2], R. Scaggiante[3], E. Moldolo[3], D. Zago[2], M. Basso[2], F. Dragoni[1], N. Bartolini[1], I. Vicenti[1], M. Zazzi[1], S. G. Parisi[2]

[1] Department of Medical Biotechnologies, University of Siena, Italy - [2] Department of Molecular Medicine University of Padova, Italy - [3] Belluno Hospital, Belluno, Italy

Aim of the study. Development of neutralizing antibody (NtAb) is crucial for protection from SARS-CoV-2 reinfection. The aim of the study was to analyze the time course of NtAb titers in a cohort of 104 non-hospitalized healthcare workers (HCW) with mild or asymptomatic SARS- CoV-2 infection diagnosed after symptoms onset or for contact tracing.
Methods. Two-fold serial dilutions of heat-inactivated sera were incubated with 100 TCID50 of SARS-CoV-2 virus (lineage B.1) at 37°C for 1 h in 96-well plates. Then, 10,000 pre-seeded Vero E6 cells per well (ATCC CRL-1586) were treated with serum-virus mixtures and incubated at 37°C. After 72h, cell viability was determined through the commercial kit Cell-titer Glo 2.0 (Promega). The NtAb titer was defined as the reciprocal value of the sample dilution that showed a 50% protection of virus-induced cytopathic effect (ID50). Antibodies with ID50 titers ?5 were defined as SARS-CoV-2 positive and neutralizing. Statistical analyses were performed using MedCalc® Statistical Software version 19.7 (MedCalc Software Ltd).
Results. One hundred and four patients (34 males, 70 females) were included in the study. The median time from diagnosis to the first NtAb titration (T1) was 65 (39-86) days. Overall, the median (IQR) NtAb titre in 86 patients was 24.9 ID50 (14.0-68.1) while the remaining 18 (17.3%) patients had a negative result. Sixty-seven HCW (24 males, 43 females) had two NtAb measurements (T1 and T2). In this group the median interval between T1 and T2 was 140 (120-189) days and the median interval between diagnosis and T2 was 223 (172-262) days. No correlation between T2-T1 interval and NtAb titer at T2 (rho=-0.131, p=0.302) and between T2-diagnosis interval and NtAb titer at T2 (rho=0.0194, p=0.8771) was observed. Five (7.5%) and 13 (19.4%) HCW had no detectable NtAb titre at T1 and T2, respectively (p = 0.043). In this group, 4 individuals were negative both at T1 and at T2 (median T2-T1 interval was 52 [175-191] days) and 1 was negative only at T1 but positive at T2 (17 ID50). Nine of the 62 HCW positive at T1 had undetectable NtAb at T2 after a median interval of 184 (166-194) days: at T1, their median ID50 (9.9, [6.7-14.7]) was lower than the ID50 (28.0 [14.6-90.5]) of the 53 HCW who were positive both at T1 and at T2 (p=0.0034). Overall, the median NtAb titre decreased significantly from T1 to T2 (24 [11.7-66.0] vs. 17 [7-44.2], p=0.009) and this result was confirmed in the 53 persistently positive HCW (28 [14.6-90.5] vs. 27 [12.8-57.1], p=0.037).
Conclusion. The strengths of this study include the homogeneity of the population enrolled and the use of authentic virus neutralization with a SARS-CoV-2 isolate circulating in Italy at HCW enrolment. Following mild or asymptomatic infection, most individuals respond with neutralizing antibody to SARS-CoV-2. In our study, NtAb levels decreased over time, however serum neutralizing activity was retained after a median of 7 months from SARS-CoV-2 diagnosis in 79% of cases. An NtAb cut-off indicative of protection from infection, as well the duration over the time of NtAb titers, remain to be established.

P48 - OC 20

Analysis of adaptive immune response to SARS-CoV-2 infection and the acquired immune response after COVID-19 vaccination with different assays

F. Bossi[1], A. Vendramin[1], S. Molon[1], E. Ricci[2], V. Lucente[2], E. De Marchi, G. Spirito[2], M. Marino[3], L. Grumiro[3], M. Gasparin[1], K. Bortolozzo[1], L. Pasini[2], V. Sambri[3]

[1] Eurospital SpA, Trieste - [2] Casa di Cura Giovanni XXIII – Monastier (TV) - Unità Operativa Microbiologia del Laboratorio Unico di Pievesestina dell'Ausl Romagna

Aim of the study. The etiologic agent of COVID-19 is SARS-CoV-2, an RNA virus that consists of four kinds of structural proteins: spike glycoproteins (S1 and S2), envelope protein (E), membrane protein (M), and nucleocapsid protein (N). The diagnosis of COVID-19 is primarily made by detecting viral RNA using RT-qPCR, which is the gold standard test, or by detecting antigens of SARS-CoV-2. Serological tests, on the other hand, are useful to evaluate both adaptative immune response to SARS-CoV-2 infection and acquired immune response after vaccine administration, thereby representing a valid tool for the evaluation of the course of the disease and the immunological protection. This research study aims at investigating both adaptative and acquired immune response by means of different assays.

Method used. The detection of SARS-CoV-2 IgG antibodies was performed on serum or plasma samples collected from: i) healthy donors (n=20); ii) subjects after 1-4 months recovery from SARSCoV- 2 infection (n=10); iii) subjects before and after Pfizer mRNABNT162b2 (Comirnaty) vaccine administration at different time points (i.e., 20-21 days after the first dose and 8-10 days after second one) (n=17); and iv) subjects at 16 days after the second dose of Pfizer Comirnaty (n=100). The assays used were: i) SARS-CoV-2 IgG (Eurospital SpA) and SARS-CoV-2 RBD IgG (Eurospital SpA), ELISA tests for the detection of IgG antibodies against a nucleocapside and spike proteins or RBD region of S1 subunit of SARS-CoV-2, respectively; ii) ACCESS SARS-CoV-2 IgG II (Beckman Coulter Inc., Ref. C69057), a CLIA test for the detection of IgG antibodies against receptor binding domain (RBD) of the S1 protein. The efficacy of SARS-CoV-2 IgG neutralizing antibodies was tested on samples collected recovered COVID-19 subjects (n=25) with a Plaque Reduction Neutralization Test (PRNT), which quantifies the titre of neutralizing antibodies, and compared to SARS-CoV-2 RBD IgG (Eurospital SpA). Tests were performed following manufacturer's instructions for use.

Results and conclusions. Samples from recovered COVID-19 subjects analysed with PRNT test showed a different efficacy of neutralising antibodies in preventing the virus from creating plaques, which positively correlated with the level of RBD IgG antibodies detected with SARS-CoV-2 RBD IgG test, thereby giving rise to a stratification of the results. In agreement with these data, samples from vaccinated subjects revealed an antibody response to RBD region after the first dose, which were higher after the second one. From the analysis with SARS-CoV-2 RBD IgG, which totally agreed with ACCESS SARS-CoV-2 IgG II test, the 90% of samples collected from individuals 16 days after receiving the second dose of vaccine displayed a great amount of RBD IgG antibodies (Index > 0.8) which correlated with a high PNRT titre, according to the stratification of the results observed on recovered COVID-19 subjects. However, the remaining 10% of samples displayed a nega-

tive result (Index 0.8), but higher that the Index calculated on healthy donors (e.g., > 0.5). However, in these samples IgG antibodies against total N and S proteins were detected with SARS-CoV-2 IgG test. Overall these data point out a strong correlation between SARS- CoV-2 RBD IgG and PRNT tests results, showing a stratification of the efficacy of neutralizing antibodies. Moreover, it emerged that patients who recovered from COVID-19 produce an immune response against SARS- CoV-2 that is higher for N protein detecting antibodies, instead of RBD region.

P49

Antibody response to COVID-19 vaccination in healthcare workers of Microbiology Unit of "S. Maria della Misericordia" Hospital

S. Bozza[1,2], A. Graziani [2], A. Mencacci[1,2], B. Camilloni[1,2]

[1] Microbiology Unit of S. Maria della Misericordia Hospital, Perugia - [2] Department of Medicine and Surgery, University of Perugia

Aim: SARS CoV2 coronavirus disease (COVID-19) is an infectious disease caused by a recently discovered Coronavirus (SARS-CoV-2), a new strain that had not previously been identified in humans. The presence of IgG antibodies directed against SARS-CoV-2 is indicative of an immune response to the infection; however, it is not yet well known whether the presence of these antibodies provides lasting protective immunity and how long after infection or vaccination, they remain detectable. The transmembrane glycoprotein S of the virus forms 'trimers' that protrude from the viral surface, and is the main target of neutralizing antibodies and, therefore, fundamental in the design of vaccines *[Walls AC, et al. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell 183(6);2020]*. The recent availability of m-RNA vaccines (Pfizer BionTech & Moderna) and Adenovirus vector vaccines (AstraZeneca & Janssen J&J), has started in Italy a mass vaccination campaign. The first to be vaccinated were the healthcare workers (HCWs) of the COVID units. The aim of our study was to analyze the kinetics of the vaccine-induced antibody response in HCW working in the Microbiology Unit of S. Maria della Misericordia Hospital (Perugia).

Methods used: This study involved 40 HCWs who received the first dose of Pfizer/BioNTech (95%) or Moderna (5%) vaccine between December 27, 2020 and January 31, 2021. Sera samples were drawn before vaccination (T0), 21 days after the first vaccine dose (T1), 7 days (T2) and 30 days after the second vaccine dose (T3). The sera were analyzed by LIAISON® SARS-CoV-2 TrimericS IgG test, an indirect chemiluminescence immunoassay (CLIA). The results were expressed in BAU/mL (Binding Antibody Units/mL) calculated with respect to the first international standard WHO 20/136 *[First WHO International Standard for anti-SARS-CoV-2 Immunoglobulin (Human). NIBSC code: 20/136. Instructions for Use (Version 2.0, 17/12/2020)]*. Considered a cutoff = 33.8 BAU/mL, the presence of high LIAISON® values (ie ≥ 520 BAU/mL) was considered a sign of a good antibody response because it has been shown that this value overlaps with high neutralizing antibody concentration (ie ≥ 1:80).

Results and Conclusions: Most participants were female (77.5%) and the mean age was 45 years (range 25-68). Before the vaccination, no HCW had antibodies to SARS-CoV-2. TrimericS IgG geometric mean titers (GMTs) increased rapidly after the first vaccination, with seroconversion in all participants by day 21. Four people (20%) had an antibody value ≥ 520 BAU/mL. The effect of the second vaccine dose, evaluated at 7 and 30 days after vaccination, showed a significant increase in the GMT values ($p<0.01$) and in the number of subjects with antibodies ≥ 520 BAU/mL (97.5% at day 7 and 95.1% at day 30). Compa-

ring the results obtained at T2 and T3, 22 HCWs (55.0%) show a decrease in the antibody concentration with at least a halving of the values. In the remaining 22% the amount of TrimericS IgG is stable or higher. In conclusion, the vaccines induced anti-SARS-CoV-2 immune response in all HCWs. During the study period, characterized by a high circulation of P.1 variant in Umbria and in particular within the Perugia hospital, no cases of COVID-19 infection were recorded in the 40 vaccinated HCWs. To assess the durability of the antibody response, the analysis of a further sera sample (3 months after the second vaccine dose) is planned.

P50

Neutralization of SARS-CoV-2 variants by convalescent and post-vaccine serum

A. Cara[1], A. Rosellini[1], P. Villa[1], C. Dolfa[1], R. Fonnesu[1], F. Maggi[2], M.L. Vatteroni[1], P. Mazzetti[1], M.Pistello[1]

[1] Department of Translational Research and New Technologies in Medicine and Surgery, OU Virology, Azienda Ospedaliero-Universitaria Pisana, , Pisa, Italy - [2] Department of Medicine and Surgery, University of Insubria, Varese, Italy

Aim of the study. The pandemic caused by SARS-CoV-2 is a public health emergency of international concern.
Neutralizing antibodies (NAbs) against SARS-CoV-2 have been considered therapeutic agents for the treatment of coronavirus disease 2019 (COVID-19) so far. Nevertheless, the emergence and rapid spreading of viral variants worldwide may potentially limit NAbs therapy. This study aims to evaluate the neutralizing activity of serum collected from vaccinated (Pfizer) and convalescent donors against different variants of SARS-CoV-2.
Methods used. Serum neutralization test was performed in microtiter plates and takes 3 days to complete and was based on the cytopathic effect (CPE). Different clinical isolates of SARS-CoV 2 were used to detect the neutralization of the virus: the original and the Brasilian, English, Nigerian, and Sud-African variants.
Results and conclusions. The results indicate that NAbs titers against wild-type SARS-CoV-2 are generally higher in plasma from vaccinated subjects compared to convalescents. The neutralization power of both vaccinated and convalescent plasmas against Brasilian, English, Nigerian, and Sud-African SARS-CoV-2 variants is also discussed.

P51

Dynamics of SARS-CoV-2 neutralizing antibody response among infected healthcare workers

S. Chandel[1], G. Griffante[1], V. Caneparo[2], D. Ferrante[1], S. Bortoluzzi, D. Cappello[1], V. Bettio, C. Aleni, L. Scotti[1], C. Airoldi[1], S. Esposito, D. Cappello[1], G. Gazzola, M. De Andrea[2], C. Borgogna[1], F. Faggiano[1], M. Gariglio[1]

*UNIRSA group - [1] Department of Translational Medicine, University of Eastern Piedmont, Novara 28100, Italy - [2] CAAD Center for Translational Research on Autoimmune and Allergic Disease, University of Eastern Piedmont, Novara 28100, Italy

Rational. Understanding the nature of immunity following mild/asymptomatic infection with SARS-CoV-2 is crucial to controlling the pandemic . It is well known that neutralizing antibody is a very strong correlate of protection, and data on the extent of the humoral response generated during SARS-CoV-2 infection among patients with different disease severity, its effectiveness in terms of neutralization of the infection, and its longevity, are needed.
Materials and methods. Study design. A cohort study of subjects (n=100 ≥18 years of age) with a PCR-documented infection by SARS-CoV-2, mostly healthcare workers (HCW) of the University-Hospital in Novara (66%), proceeded with a 10-month follow-up after recovery (n=71). Among these, 36 subjects received mRNA-based vaccination during the follow up period. Laboratory analysis: Sera from this study cohort were tested for the presence of anti-SARS-CoV-2 RBD and Spike (S) IgG antibodies using Kantaro Quantitative SARS-CoV-2 IgG Antibody RUO kit (REF# DSR200, New York, USA). Serum samples of the positive patients were then tested for the presence of neutralizing antibodies (Nab) using a SARS-CoV-2 S protein-pseudotyped vesicular stomatitis virus (VSV) vector-based neutralization assay (kindly provided by Sean Whelan, USA). Statistical analysis: descriptive analysis of the immune response in the study group is being studied with longitudinal models of multilevel analysis at T1 (approximately 3 to 5 weeks after healing considering T0), and T2 (9 to 11 months after T1).
Results and conclusion. The infection clinical presentation was symptomatic in 84% of cases of which 10% were hospitalized, but none in intensive care unit (ICU). At T1, we found that 7% (n=7) were negative for anti-SARS-CoV-2 Abs by ELISA, while 93% (n=93) were found positive for both anti-SARS-CoV-2 RBD and S Abs. With regard to the neutralizing activity, a significant correlation was found between high anti-Spike Ab titers and high neutralizing titers (NT) while low Ab titers correlated with low NT. Consistent with previous reports, those patients who were hospitalized, therefore with a more severe outcome, showed higher NT titers when compared to those who developed mild disease or remained asymptomatic. On a follow-up at T2, 71 subjects were tested for the presence of anti-SARS-CoV-2 antibodies by ELISA, while we are currently performing the neutralization assays. Among the vaccinated subjects, 35 out of 36 showed increased anti-S Ab titers when compared to the T1 dosage. Among non-vaccinated subjects, those with high anti-S Ab levels (n=12, 34%) at T1 still displayed high anti-S Ab titers at T2. As expected, those with low Ab titers (n=15, 42%) showed very low anti-S Ab titers at T2. A decrease in anti-S Ab levels was seen among 8 (22%) of non-vaccinated subjects from T1 to T2, while an increase was seen in one individual from T1 to T2 (3%). Collectively, our findings demonstrate the high specificity of our assays, which may help understanding the quality or duration of the antibody response upon SARS-CoV-2 infection and determining the effectiveness of potential vaccines.

P52

The IgA immune response in COVID-19 patients and vaccines: a neglected but critical aspect of SARS-CoV-2 infection

F. De Marco[1], L. de Latouliere[1], L. Sorrentino[1,5], C. Mandoj[2], L. Conti[2], M. De Rienzo[3], M.L. Foddai[3], C. Castilletti[6], M.R. Capobianchi[6], G. Ciliberto[4]

[1] Department of Research, Technological Innovation & Advanced Diagnostics - [2] Department Clinical Pathology and Cancer Biobank - [3] Immunohematology and Transfusional Medicine Unit - [4] Scientific Director, the "Regina Elena" National Cancer Institute - IRCCS, Rome, Italy - [5] Dept of molecular medicine - Laboratory of Virology "Sapienza" Rome University - Italy - [6] Virology and Biosecurity Laboratories, National Institute for Infectious Diseases "Lazzaro Spallanzani" IRCCS, Rome, Italy

Aim of the study: In the effort to counter-act the current COVID-19 pandemic a great attention has been posed on the study of the humoral immune response to the SARS-CoV-2 infection. Despite a rather large amount of data have been rapidly generated a number of fundamental issues remain poorly known, namely: the kinetics of Ab response, their value as predictors of clinical evolution and outcome and the duration of sterilizing immunity. Within this scenario comparatively few works have been dedicated to the role of the IgA this Isotype is expected to play a crucial role in any kind of air transmitted viral infection.
Methods used: A total of 99 patients with confirmed SARS-CoV-2 infection and 275 vaccinees were enrolled. Their serial serum samples were collected at different times since clinical symptoms onset or first vaccine inoculation. Total SARS-CoV-2 Anti S + Anti N specific IgM and IgG were assayed by the SNIBE-*MAGLUMI* (CLIA) assay. Their RBD/ACE2 binding inhibiting titres were measured both by the *COVID-19 Spike-ACE2 binding assay kit* (RayBiotech Inc) and by the *SARS-CoV-2 Inhibitor Screening Kit* (Adipogen). On a selected number of cases the direct Viral Infection Inhibiting Titre was also evaluated. The specific Anti S IgA titres were evaluated by the RecombivirusTM Human Anti-SARS-CoV-2 Virus (COVID-19) Spike 1 IgA ELISA Kit (Alpha Diagnostic International). Data analyses and graphics were performed with the PRISM-6 software (GraphPad Inc).
Results: The RBD/ACE2 Binding assay kits is simple and convenient and can be used to generate quantitative results. Their use in the clinical setting might provide a swift, robust and convenient way to assess the neutralizing activity of sera without the limitations and the safety concerns linked to the labour intensive, time consuming method for direct viral infection Inhibition assay. At any time RBD/ACE2 binding inhibition titre does appear to correlated with the total Anti COVID Ab titres. Anti S1 specific serum IgA are easily detected in sera by commercial assay kits. The IgA titres are generally higher than that of IgM, appear to persist quite longer and to correlate with the neutralizing titre both in convalescent patients and in healthy vaccinees. IgA appear as a promising adjunct to the tools for COVID patients' evaluation. Both anti S IgA and anti RBD titres are sharply higher in BNT162b2 vaccine recipients than in convalescent patients.

P53
Mucosal humoral response in BNT162B2 COVID-19 vaccinated subjects

L. Azzi[1], M. Shallak[2], D. Dalla Gasperina[3], F. Maggi[4], R.S. Accolla[2], G. Forlani[2]

[1] Unit of Oral Medicine and Pathology, ASST dei Sette Laghi, Department of Medicine and Surgery, University of Insubria, Varese, Italy - [2] Laboratories of General Pathology and Immunology "Giovanna Tosi", Department of Medicine and Surgery, University of Insubria, Varese, Italy - [3] Unit of Infectious and Tropical Diseases, ASST dei Sette Laghi, Department of Medicine and Surgery, University of Insubria, Varese, Italy - [4] Laboratory of Clinical Microbiology, ASST dei Sette Laghi, Department of Medicine and Surgery, University of Insubria, Varese, Italy

Aim of the Study: Mucosal immunity, including secretory IgA (sIgA), plays an important role in early defenses against respiratory pathogens, such as SARS-CoV-2 infection. Most Recent reports have focussed on the efficacy of BNT162b2 vaccine in the induction of virus specific IgG-neutralizing/sterilizing antibodies. However, cases of reinfected health care vaccinated workers are increasingly frequent, suggesting that the specific immune response induced by the vaccination may not be sufficient to fully protect from mucosal viral infection. In order to evaluate the mucosal immune response generated after vaccination we analyzed the titer and the neutralization capacity of both IgG and IgA in sera and saliva of BNT162b2 vaccinated subjects.
Methods: The titer and the neutralizing capacity of SARS-CoV-2-specific IgG and IgA in sera and saliva of sixty healthcare worker volunteers of ASST dei Sette Laghi, who received two injection of BNT162b2 mRNA vaccina, were analyzed by using a quantitative ELISA assay specific for SARS-CoV-2 spike protein subunit S1and a viral neutralization assay, respectively. Interestingly 6% of the enrolled subjects was previously exposed to virus. Samples were collected at the day of the first dose (T0), two weeks after the first injection (T1) and two weeks after the second injection (T2). The CLIA serological assay on serum was used as a standard diagnostic reference for the evaluation of IgG neutralizing antibodies in serum.
Results and discussion: 88% of recruited subjects developed IgG neutralizing antibodies in serum after the first dose, and 100% showed serological positivity by CLIA after the second dose. The amount of total IgG in serum positively correlated with the level of neutralizing IgG antibodies detected by CLIA. In saliva we could measure a significant amount of IgG only after the second administration of the vaccine. Interestingly in the majority of the previously exposed subjects we observed high IgG levels both in serum and saliva after the first dose, reinforcing the idea that in this group a single vaccination is sufficient to protect from the infection. As far as the IgA, we were able to detect a significant amount of serum IgA in 60% and 95% of vaccinated subjects after the first and second injection, respectively. In saliva the IgA were present in 40% and 50% of subjects after the first and the second administration of the vaccine, respectively. The presence of IgA in saliva after the first vaccination was independent from previous exposure to the virus. After the second vaccination only 25% of vaccinated people presented neutralizing antibodies in saliva, being included in this group the majority of individuals previously exposed to the infection. Taken together, these results indicate that although half of vaccinated people display specific IgA antibodies in oral mucosa, these antibodies are relatively poor at neutralizing the virus highlighting the weak efficacy of the BNT162b2 mRNA vaccine to induce IgA-driven mucosal immunity.

P54

BNT162b2 efficacy: results from healthcare workers vaccination. The renaissance study

O.M. Gagliardi[1], A. Pani[2], V. Cento[2], F. Di Ruscio[3], R. Gibilisco[3], A. Zampiero[4], D. Campisi[4], A. Rimondini[1], M. Senatore[1], P.A. Schenardi[1], S. Agliardi[1], T. Conti[1], G. Gazzaniga[1], A. Schianchi[1], F. Scaglione[2-4]

[1] Postgraduate school of Clinical Pharmacology and Toxicology - [2] Department of Oncology and Hemato-Oncology, Università degli Studi di Milano, Milan, Italy - [3] Postgraduate school of Microbiology and Virology, Università degli Studi di Milano, Milan, Italy - [4] Chemical-Clinical and Microbiological Analyses, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy

Aim of the study. The BNT162b2 vaccine demonstrated 95% of efficacy at preventing severe COVID-19 versus placebo. However, the approval process required a short time by necessity, and we lack data on special populations (1). In addition, it is still unknown whether BNT162b2 reduces transmission of SARS CoV-2. To define immunogenicity of BNT162b2 and his efficacy to reducing transmission of SARS CoV-2, we evaluated antibody response in the first vaccinated individuals in Italy: the healthcare workers (HWs).
Methods. As a measure of immunogenicity of BNT162b2 we evaluated anti-Trimeric Spike glycoprotein IgG titer after 14 days from the second dose of BNT162b2 using a quantitative chemiluminescent assay (Diasorin). We excluded HWs with an incomplete vaccination course or a previous molecular, serological or antigenic test resulting positive for SARS CoV2 infection. In addition, to rule out prior exposure to SARS-CoV2 we also screened HWs for anti-Nucleocapsid (N) antibodies, through an electrochemiluminescence immunoassay (Roche). To determine the efficacy of BNT162b2 to reducing transmission of SARS CoV2 we compared the pre-vaccine and the post-vaccine trends of infected HWs until 27th March 2021.
Results and conclusions. 5,023 HWs receiving the first dose of vaccine were included in the study; 144 participants for incomplete vaccination and 871 of them for known prior exposure to SARS-CoV2 were excluded. 163 of HWs with no previous history of laboratory-confirmed COVID-19 resulted positive to anti-N (95% CI: 5.4%-7.4%). The final population consisted of 2569 HWs; of these, only 4 had a negative antibody response (0.16% prevalence; 95% CI: 0.04%-0.40%) %). Notably, these non-responders were assuming immunosuppressant drugs, especially mycophenolate; 23 participants that referred to be immunocompromised had a measurable antibody response. 3,4% of participants had titers below 1000 BAU/mL (n/N=87/2569, 3.4%), 9,8% from 1000 to 1500 BAU/mL(n/N=251/2569), 19,2% from 1500 to 2000 (n/N=494/2569), and 67,5% above 2000 (n/N=1733/2569). The female gender was associated with a significantly higher probability of having an anti-Spike titer above 800 than male gender (64.5% [1044/1618] vs. 58.3% [410/703]; p=0.005). Of note, this correlation was found also adjusting for age groups (OR [95%CI]: 1.275 [1.062-1.531]; p=0.009). Before the beginning of vaccinations in Italy the incidence of SARS CoV-2 infections within our HWs was steadily above 20%. Up to 27th March 2021, we found only one individual with a positive SARS CoV2 RT-PCR result within the vaccinated HWs, even if poorly symptomatic. Additionally, 6 vaccinated individuals with a weak-positive SARS-CoV-2 RT-PCR resulted negative to digital PCR analysis. None

of our vaccinated HWs developed a moderate or a severe form of COVID-19 (100%). Concluding, in our study in vaccinated healthcare workers BNT162b2 vaccine revealed high induction of immunogenicity and a strong efficacy to reducing transmission of COVID-19.

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SARS-CoV-2 N protein target TRIM-25-mediated RIG-I activation to suppress innate immunity

G. Gori Savellini, G. Anichini, C. Gandolfo, M.G. Cusi

Department of Medical Biotechnologies, University of Siena, Siena, Italy

Aim of the study. During the infection by the novel SARS-CoV-2 virus, a weak production of INF-β, along with an exacerbated release of pro-inflammatory cytokines, has been reported. SARS-CoV-2 encodes several proteins able to counteract the host immune system, which is believed to be one of the most important features contributing, at least in part, to the viral pathogenesis and development of a severe clinical picture (Blanco-Melo et al., 2020; Hadjadj et al., 2020; Yuen et al., 2020). Previous reports demonstrated that SARS-CoV-2 N protein efficiently suppresses INF-β production by interacting with RIG-I (Oh et al., 2021). In the present study, we better characterized the mechanism by which the SARS-CoV-2 N counteracts INF-β secretion. In particular, we focused on the involvement of the N protein on TRIM25 activity and its effects on RIG-I activation.
Methods. Plasmids. The N SARS-CoV-2 coding gene (nt 28218-29477) and the HA-tagged TRIM25 SPRY domain were cloned by standard procedure in pcDNA4HisMax-C plasmid (Life Technologies) in frame with the 6xHis tag and in pEF-Bos, respectively. Other plasmids used in the experiments were kindly provided by colleagues or purchased. Immunoprecipitation. Cell lysates from co-transfected cells were used for immunoprecipitation with anti-HA magnetic beads. For RIG-I ubiquitination detection, cell samples were lysed in 1% SDS by boiling and then diluted to 0.1% SDS final concentration with RIPA buffer. Proteins were captured with anti-FLAG M2 magnetic beads. Proteins were resolved by SDS-PAGE and probed by immunoblotting with selected antibodies. Luciferase reporter assay. Lenti-X 293T cells were transfected with indicated plasmids. Thirty-six hours post-transfection, cells were stimulated with poly(I:C) and collected after additional 12h. Luciferase activities were measured using the Dual Luciferase reporter assay reagent (Promega), according to the manufacturer's instructions. Results are given as mean values of several experiments ± standard deviations (SD).
Results and conclusions. In the present study, we demonstrated that the SARS-CoV-2 N protein significantly impaired the RIG-I signaling pathway leading to IFN-β production. By luciferase reporter assay, a marked decrease in both IFN-β- or NF-kB-promoter activation was noted when the N protein was co-expressed along with RIG-I or MDA-5. TRIM25, an E3 ubiquitin ligase involved in full RIG-I activation, was previously described to be targeted by the nucleoprotein of the SARS-CoV virus. For this reason, we next investigated the interplay between SARS-CoV-2 N protein and TRIM25. Interestingly, by reporter gene assay, we observed that TRIM25-mediated RIG-I activation was significantly reduced when the N protein was ectopically expressed. On the contrary, no activity was observed with respect to Riplet, another important E3 ligase active on RIG-I. Taken together, these data suggested a direct and specific involvement of the N protein in the TRIM25-RIG-I signaling cascade. Furthermore, as demonstrated by co-immunoprecipitation experiments, we reported a pronounced affinity of the N protein for TRIM25, in particular for its SPRY domain, harboring the RNA-binding activity necessary for its self-activation. Moreover, the

SARS-CoV-2 N protein was found associated to a multiprotein complex containing both RIG-I and TRIM25. Thus, we hypnotized that the N protein intercalates between RIG-I and TRIM25, spatially limiting RIG-I ubiquitination and activation by TRIM25. Indeed, a reduction in K63-moiety poly-ubiquitination of RIG-I was observed when the N protein was co-expressed. Here, we described new findings regarding the interplay between SARS-CoV-2 and the IFN system, filling some gaps for a better knowledge of the molecular mechanisms associated to the COVID-19 disease.

P56

Humoral response assessment in BNT162b2 vaccinated healthcare workers: an assay comparison

D. Mileto[1], M. Cutrera[1], G. Gagliardi[1], A. Rizzo[1], A. Gigantiello[1], A. Mancon[1], M. Bianchi[1], F. De Poli[1], G. Giacomel[1], I. Burgo[2], M. Longo[1], S. Grosso[1], V. Micheli[1], G. Rizzardini[3], M.R. Gismondo[1], and A. Lombardi[1]

[1] Laboratory of Clinical Microbiology, Virology and Bioemergencies, ASST Fatebenefratelli Sacco, L. Sacco University Hospital, Milan - [2] Hematology and Transfusion Medicine, ASST Fatebenefratelli Sacco, L. Sacco University Hospital, Milan - [3] Division of Infectious Diseases, ASST Fatebenefratelli Sacco, L. Sacco University Hospital, Milan

Aim of the study: On 27th December 2020 the vaccination with mRNA BNT162b2 (Comirnaty) started in Italy. We evaluated the antibody development after first and second dose according to the vaccination schedule in healthcare workers naïve at SARS-CoV-2 infection comparing four chemiluminescence immunoassays (CLIA) and plaque reduction neutralization test (PRNT) results.
Methods used: Serum samples of 37 enrolled subjects, median age 40 (range 24 - 65) years, were collected for each following time point: T0, 1 day before I vaccination dose; T1 and T2, 10 and 20 days after I dose respectively; T3, T4 and T5, 10, 20 and 30 days after II dose respectively. Each time point was analyzed using different CLIA assays: LIAISON® SARS-

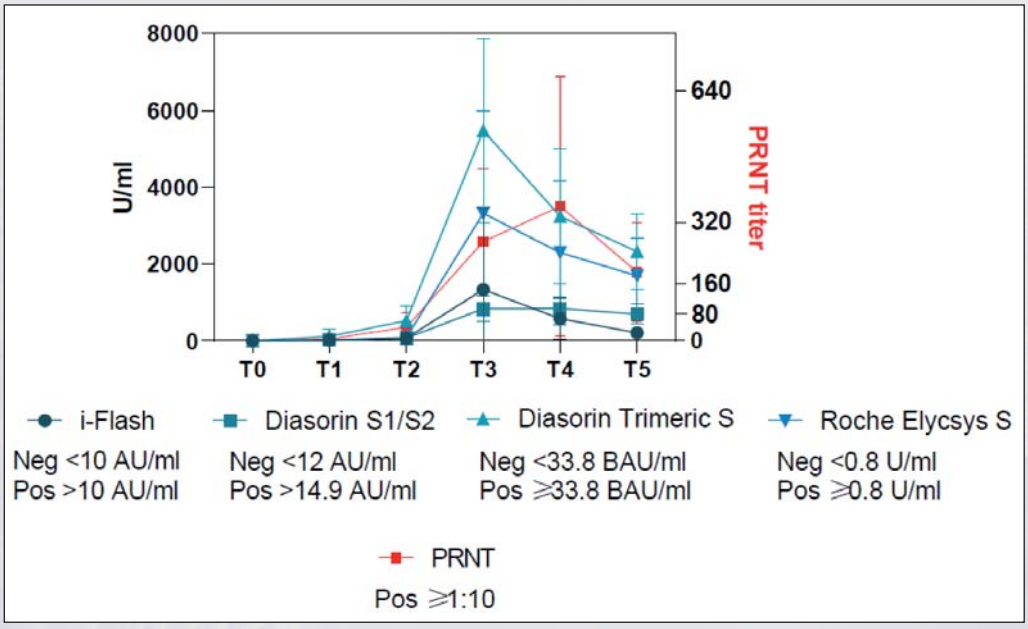

Fig. 1. Anti-SARS-CoV-2 response measured by CLIA assays and neutralizing antibodies titre anti-SARS-CoV-2 measured by PRNT at baseline (T0), 10 days after I dose (T1), 20 days after I dose (T2), 10 days after II dose (T3), 20 days after II dose (T4) and 30 days after II dose (T5).

CoV-2 S1/S2 IgG (DiaSorin Inc.) to measure antibodies against the SARS-CoV-2 native S1/S2 proteins, while iFlash-2019-nCoV NAb (Shenzhen YHLO Biotech Co), LIAISON® SARS-CoV-2 TrimericS IgG (DiaSorin Inc) and Elecsys Anti-SARS-CoV-2 S (Roche Diagnostics Rotkreuz, Switzerland) to quantify the specific RBD-binding antibodies. Furthermore, the neutralizing antibody serum titre was determined by PRNT for all samples at each time point using the European strain (D614G) and the T5 collected serum of 10 subjects were tested against the SARS-CoV-2 UK strain (501Y.V1, lineage B.1.1.7) too.

Results and conclusion: All enrolled subjects developed antibodies 10 days after II dose according to all methods: the CLIA assays showed the highest level of antibody to S1/S2 and RBD at T3 while the highest level of neutralizing antibody titre is reached at T4 according to PRNT (median 1:320; IQR 1:160-1:320) (Fig.1).

Regarding the neutralization titers against B.1.1.7, 2/10 (20%) of tested serum showed a slight decline: 1:320 for EU vs 1:160 for UK. Further evaluations on antibody titres and vaccine efficacy against SARS-CoV-2 variants will be conducted by our ongoing study.

P57

Vaccination against COVID-19 prevents disease severity and infection in a hospital in Northern Italy

C. Belgiovine[1], I. Dambrosio[2], F. Frattolillo[2], S. Sancini[2], A. Porcella[2], N. Nicodemo[2], A. Garegnani[2], R. Lacavalla[2], C. Vidello[2], M. Monari[1]

[1] Humanitas Clinical and Research Center-IRCCS, Via Manzoni 56, 20089 Rozzano Milan, Italy - [2] Mater Domini Humanitas, Via Gerenzano 2 Castellanza, Varese

Background: Recently COVID-19 vaccines based on mRNA has been approved, and the schedule for these vaccines require a first jab followed by recall immunization. The Italian immunization program identified health care workers as a priority. There is still no certain evidence about their efficacy in containing the infection, but only on their ability to avoid sever COVID-19 disease.
Aim: this study has a dual purpose: both to evaluate the efficacy of this vaccination in preventing COVID infection and disease severity in vaccinated health care workers of an Italian Hospital (Humanitas Mater Domini, Castellanza-Varese).
Methods: All samples are tested routinely for sourvelliance or in case of sympthoms. The molecular method for the detection is: SARS-COV2: Alliplex, SARS CoV-2, Seegene.
Results: In our hospital, 479 health care workers, between 1-01.21 to 8.2.21, were vaccinated, 94 of them had already contracted SARS-CoV2. During the screening of health-care workers, between 11.1.21 to 11.4.21, of the 385 who had never contracted the virus, 9 infected subjects were found.

Table1: result of infection pre and post vaccination.

Nr. of helth care workers	Past infection	1° dose vaccine	2° dose vaccine	Infection post vaccination	Re-infection post vaccination	pauci-symptomatic	asyntomatic
1	x	x	x		x		1
5		x		x		3	2
3		x	x	x			3

The mean duration of positivity was 16.5 days for these who received a single dose of the vaccine and 10 days for those who completed the vaccine schedule. No infected subjects showed any symptoms.
Discussion: The study highlights how the vaccine is able to prevent the infection of the disease (99% efficacy), and also severe symptoms of COVID-19, regardless of the number of doses made by health-care professionals. In our observation, between 9 infection post vaccination 6 are asymptomatic and only 3 paucisymptomatic (Table 1). So all health workers could return to work in 14 days despite in case of infection without vaccination where the average time of absence was estimated between 21 to 45 days with some days of hospitalization. Further studies could be made on the infectious capacity of the virus in vaccinated subjects, trying to investigate the differences between those vaccinated with a single dose or with both.
Conclusion. The vaccine shows 99% effectiveness in preventing infection and 100% in preventing COVID-19 disease in Mater Domini hospital.

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Impaired priming of SARS-CoV-2 specific naïve CD8$^+$ T cells in older subjects

E. Gallerani[1], D. Proietto[1], B. Dallan[1], M. Campagnaro[1], S. Pacifico[2], V. Albanese[2], E. Marzola[2], P. Marconi[1], A. Caputo[1], V. Appay[3], R. Gavioli[1] and F. Nicoli[1]

[1] Laboratory of Biochemistry, Immunology and Microbiology (BIM), Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, 44121 Ferrara, Italy - [2] Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, 44121 Ferrara, Italy - [3] CNRS UMR 5164, ImmunoConcEpT, Université de Bordeaux, 33000 Bordeaux, France

Advanced age is associated with severe symptoms and death upon SARS-CoV-2 infection. Virus-specific CD8$^+$ T-cell responses have shown to be protective toward critical COVID-19 manifestations, suggesting that suboptimal cellular immunity may contribute to the age-pattern of the disease. The induction of a CD8$^+$ T-cell response against an emerging pathogen like SARS-CoV-2 relies on the activation of naïve T cells. To investigate whether the primary CD8$^+$ T-cell response against this virus is defective in advanced age, we used an in vitro approach to prime SARS-CoV-2-specific naïve CD8$^+$ T cells from healthy, unexposed donors of different age groups. Compared to younger adults, older individuals display a poor SARS-CoV-2-specific T-cell priming capacity in terms of both magnitude and quality of the response. In addition, older subjects recognize a lower number of epitopes. Our results implicate that immune aging is associated with altered primary SARS-CoV-2-specific CD8$^+$ T-cell responses.

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Neutralizing antibodies responses against SARS-CoV-2 in sardinian cohort group

G. Sanna[1], A. Marongiu[1, 2], G. Franci[3], M. Galdiero[4], C. Piras[1], G. Pala[1], V. Palmas[1], R. Littera[5], M. Vacca[5], R. Jammoul[5], A. Perra[6], F. Sedda[6], M. Campagna[7], F. Pes[7], D. Firinu[7], M. Erbi[7], M. Secci[7], F. Meloni[7], L. Chessa[7], A. Manzin[1]

[1] Department of Biomedical Sciences, University of Cagliari, Cittadella Universitaria, 09042, Monserrato, Cagliari, Italy; Giuseppina Sanna; email: g.sanna@unica.it - [2] Department of Biomedical Sciences, University of Sassari, Sassari 07100, Italy - [3] Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, 84081 Baronissi, Italy - [4] Department of Experimental Medicine, University of Campania Luigi Vanvitelli, 80138 Naples, Italy - 5Department of Internal Medical Sciences, Medical Genetics, "R. Binaghi" Hospital, University of Cagliari, Italy - [6] Department of Biomedical Sciences, Unit of Oncology and Molecular Pathology, University of Cagliari, Cittadella Universitaria, 09042, Monserrato, Cagliari, Italy - [7] Department of Medical Sciences and Public Health, University of Cagliari, Cittadella Universitaria, 09042, Monserrato, Cagliari, Italy

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), the etiological agent of COVID-19, determined over 132 million cases of infections and almost 3 million deaths worldwide.

The pandemic has called for science, technology and innovation to provide solutions, and due to an incredible scientific and financial global effort several prophylactic and therapeutic apparatus, such as monoclonal antibodies, and vaccines have been developed in less than one year to fight this emergence. After SARS-CoV-2 infection, serum neutralizing antibodies are produced by B cells, and studies on virus-neutralizing antibodies kinetics are pivotal. The process of protective immunity, and the duration of this kind of protection against COVID-19, remain to be clarified. Several authors have described antibodies responses in COVID-19 patients, but the pending question is the duration of these responses. Some of them report rapid declining of antibodies, while others detected the lost of neutralizing antibodies by around 30-60 days after the beginning of illness. All this data have important implication for the duration of protective immunity from reinfection, and the success of vaccination in prevention from reinfection and disease.

We tested 100 sera from 3 groups of individuals, some of them providing multiple sequential sera (1- healthy, no previous CoV2 infected, vaccinated, 2- healthy, previous CoV2 infected, vaccinated, 3- healed, (previous CoV2 infected), not vaccinated) to assess the kinetics of antibodies neutralizing activity. We found that SARS-CoV-2 infection elicits moderate neutralizing antibody activity in most individuals; nor age and gender appear to influence antibodies responses, neither Body Mass Index (BMI) is correlated with antibodies production. BNT162b2 vaccine, when administered in two doses, results able to induce high antibodies titers endowed with potent neutralizing activity against bare SARS-CoV-2 in *in vitro* neutralizing assay. The cohort group is increasing in order to implement our database.

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Serosurvey in BNT162B2 vaccine-elicited neutralizing antibodies against authentic SARS-CoV-2 variants

A. Zani, F. Caccuri, S. Messali, C. Bonfanti, A. Caruso

Section of Microbiology, Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy

Introduction: The ongoing outbreak of coronavirus disease (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its accompanying morbidity, mortality and destabilizing socioeconomic effects have made the development of therapeutics and vaccines an urgent global health priority. Because the spike protein of SARS-CoV-2 is critical for viral entry, it has been targeted for vaccine development and therapeutic antibody interventions. Italy launched its immunization campaign at the end of December 2020 with the inoculation of the first SARS-CoV-2 spike-based vaccine approved in the Country, the BNT162b2 from Pfizer. Since the beginning of vaccine development, different SARS-CoV-2 variants characterized by multiple mutations in their spike have arisen, raising concern over vaccine efficacy.
Aim of the study: An important issue for spike-based vaccines is whether the authentic virus can escape vaccine-elicited neutralizing antibodies. Here, using human sera from recipients of the BNT162b2 mRNA vaccine, we report the impact on antibody neutralization of a panel of authentic SARS-CoV-2 variants including United Kingdom (UK-IT-BS, B.1.1.7 lineage), South African (SA- IT-BS, B.1.351 lineage), Brazilian (BR-IT-VA, P.1 lineage) and Nigerian (NI-IT-BS, B.1.525 lineage) isolates.
Material and Methods: We collected and tested a panel of human sera obtained between 10 and 20 days after the administration of the second dose of BNT162b2 from 37 volunteers with no history of natural SARS-CoV-2 infection. The presence of antibodies against the spike glycoprotein receptor binding domain (RBD) was attested by Electro-chemiluminescence immunoassay (ECLIA). All sera collected contained antibodies with values ranging from 265.7 to >5000 UI/ml. Each serum was then tested for neutralization of the wild type (WT-IT-BS, B.1 lineage), UK-IT-BS, SA-IT-BS, BR-IT- VA and NI-IT-BS authentic viruses. Neutralization was performed by cytopathic effect (CPE)-based assay, using viruses soon after having confirmed their identity by next generation whole-genome sequencing.
Results: All the serum samples efficiently neutralized WT-IT-BS and all the viral variants. In particular, as compared with neutralization of WT-IT-BS, neutralization of UK-IT-BS and NI-IT-BS was significantly higher, and neutralization of SA-IT-BS and BR-IT-VA was robust but significantly lower. Interestingly, UK-IT-BS and NI-IT-BS variants bearing a single mutation in the RBD (N501Y or E484K) were robustly neutralized by vaccine-elicited antibodies. At the same time, two close mutations in the RBD of SA-IT-BS and BR-IT-VA (E484K + N501Y) challenged antibody neutralization, possibly due to perturbation of antigen recognition.
Conclusions: Considering the capability of the BNT162b2 to elicit potent T-cell immunity against multiple variants, vaccine-mediated protection must be validated by data on clinical effectiveness collected in regions where SARS-CoV-2 variants are common.

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Robust and persistente B and T-cell responses after COVID-19 in immunocompetent and transplanted patients

F. Zavaglio[1], V. Frangipane[2], M. Morosini[2], E. Gabanti[1], P. Zelini[3], M. Gregorini[4], T. Rampino[4], A. Asti[4], E. Seminari[5], A. Di Matteo[5], C. Pellegrini[6], F. Meloni[2], D. Lilleri[1], F. Baldanti[1,7]

[1] Unit of Microbiology and Virology, IRCCS Policlinico San Matteo Foundation, Pavia, Italy - [2] Research Laboratory of Lung Diseases, Section of Cell Biology, IRCCS Policlinico San Matteo Foundation, Pavia, Italy - [3] Obstetrics and Gynecology, IRCCS Policlinico San Matteo Foundation, Pavia, Italy - [4] Unit of Nephrology, Dialysis and Transplantation, IRCCS. Policlinico San Matteo Foundation, Pavia, Italy - [5] Infectious Diseases Clinic, University of Pavia and IRCCS Policlinico San Matteo Foundation, Pavia, Italy - [6] Cardiac Surgery, Department of Intensive Medicine, IRCCS Policlinico San Matteo Foundation, Pavia, Italy - [7] Department of Clinical, Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy

Aim: Antibody and T-cell responses were evaluated in SARS-CoV-2-infected immunocompetent and transplanted patients with pneumonia or mild symptoms.
Methods: The SARS-CoV-2-specific immune response was analysed in 44 immunocompetent (IC) and 17 solid-organ transplanted (TX) patients with pneumonia (IC n=18 and TX n=12) or mild symptoms (IC n=26 and TX n=5). Anti-Spike and Nucleocapsid IgG and IgA antibodies were determined by ELISA. Antigen-specific T cell response was determined by stimulation of peripheral blood mononuclear cells with peptide pools of the Spike, Envelope, Membrane and Nucleocapsid proteins. Antigen-specific "effector" T cells were determined with a 20 hours Activation Induced Marker (AIM) assay. "Memory" T cells were determined with a 7-day lymphoproliferative response (LPR) assay.
Results: Peak antibody response was detected within the first two months after infection at similar levels in IC and TX patients. Anti-Spike IgG and IgA persisted for at least one year after infection, while anti-Nucleocapsid IgG declined earlier. Patients with pneumonia developed higher antibody levels than patients with mild symptoms. Similarly, both effector and memory T-cell responses were detected within the first two months after infection at comparable levels in IC and TX patients, and were higher in patients with pneumonia. SARS-CoV-2-specific T cells were detected mainly in $CD4^+$ and TFH than in the $CD8^+$ T-cell subset. Levels of SARS-CoV-2-specific effector T cells were significantly lower 6-12 months after infection than in the acute phase, while the LPR of memory T cells did not change significantly over time. The Spike, Membrane and Nucleocapsid proteins elicited the major $CD4^+$ and $CD8^+$ T-cell responses, whereas the T-cell response to Envelope protein was negligible. Among effector $CD4^+$ T-cells, the dominant specificity was directed towards the Nucleocapsid protein, whereas the Spike protein induced the major LPR in memory $CD4^+$ T and TFH cells.
Conclusion: After SARS-CoV-2 infection, the antibody and T cell responses develop rapidly and persist over time in both immunocompetent and transplanted patients.

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SARS-CoV-2 diagnosis: implementation of a qPCR kit on the automated platform Panther Fusion system

P. Bottino, E. Zanotto, C. Balloco, S. Varetto, S. Alizzi, F. Sidoti, C. Costa, R. Cavallo

Microbiology and Virology U., A.O.U. "Città della Salute e della Scienza di Torino"

Aim of the study. The ongoing SARS-CoV-2 pandemic has called for the use of massive qPCR tests in order to detect positive cases and to trace contacts with the aim to stop community transmission. However, many commercial kits are available in two separate stages: extraction and qPCR. For these reasons, fully automated platforms provide an important support to increase the laboratory testing capability and to reduce the turnaround time. The Panther Fusion® system (Hologic Inc.) is a high throughput, fully automated, sample-to-result in vitro diagnostic (IVD) system capable of performing various IVD tests using real-time PCR. Furthermore, the platform has an Open Access™ functionality that allows users to perform real-time PCR laboratory-developed tests (LDT) alongside IVD tests. We took advantage of Open Access functionality to automatize a commercial qPCR kit (SARS-CoV-2 ELITe MGB® Kit, Elitech) in order to increase the laboratory testing capability.
Methods. The study was performed on 51 nasopharyngeal swabs collected for diagnosis of SARS-CoV-2 infection. Samples were simultaneously tested with SARS-CoV-2 ELITe MGB® Kit adapted on Open Access System (LDT-OA) and the standard of care (SoC) consisting in extraction with DSP Midi Kit on QIAsymphony platform (Qiagen®) and amplification with the qPCR kit aforementioned on ABI 7500 Fast Dx instrument (Applied Biosystems™). Both methods were also compared with a referring test based on TMA technology (Aptima® SARS-CoV-2 Assay, Hologic Inc.). Agreement between the two methods (LDT-OA and SoC) and sensitivity and specificity towards TMA were evaluated.
Results. A total of 38 samples resulted positive for LDT-OA and SoC, 1 sample was detected by LDT-OA and 11 samples tested negative for both methods. When compared with TMA, LDT-OA was able to detected as positive 39 samples and SoC 38. Agreement between LDT-OA and SoC was 97% for positive samples and 100% for negative ones. Sensitivity and specificity of both methods in comparison with TMA are resumed in Table 1.

Table 1. Sensitivity and specificity of methods in comparison with TMA

	LDT-OA vs TMA	SoC vs TMA
Sensitivity	98%	95%
Specificity	100%	100%

The Cohen's kappa coefficient between SoC and TMA was 0.88, while for LDT-OA and TMA was slightly better (K Value= 0.94).
Difference analysis of the cycle threshold (ΔCt) between LDT-OA and SoC were respectively -0.28±0.94 for ORF8 gene and +0.31±1.06 for RdRp gene.

Conclusions. Herein, we demonstrated the successful implementation of a qPCR kit on the automated platform Panther Fusion® system with excellent performances in terms of sensitivity and specificity. This approach provides important advantages: a fully automated workflow with minimal hands-on time and a Turnaround Time of 2 hours and 30 minutes for each sample against more than 4 hours with the standard of care procedure. Furthermore, the platform is provided with a random access, thus avoiding the necessity to work with defined samples batch, allowing for fast and reliable diagnostics in clinical routine.

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Colorimetric test for fast detection of SARS-CoV-2 in nasal and throat swabs

M. Cennamo[1], B. Della Ventura[2], A. Minopoli[2], R. Campanile[2], E. La Civita[1], A. Liotti[1], A. Furno[1], L. Stanziola[1], M. Fiorenza[1], S. Di Somma[1], F. Napolitano[1], A. Russo[1], M. Pagano[1], R. Velotta[2], D. Terracciano[1], G. Portella[1]

[1] Department of Translational Medical Sciences, University of Naples "Federico II", 80131 Naples, Italy - [2] Department of Physics "E. Pancini", University of Naples "Federico II", Via Cintia 26, 80126, Naples, Italy

Aim of the study: Mass testing is fundamental to face the pandemic caused by the coronavirus SARS-CoV-2 discovered at the end of 2019. To this aim, it is necessary to establish reliable, fast and cheap tools to detect viral particles (virion) in biological material so to identify the people capable to spread the infection.
Method used: We demonstrate that a colorimetric biosensor based on gold nanoparticle interaction induced by SARS-CoV-2 lends itself as an outstanding tool for detecting virions in nasal and throat swabs. The extinction spectrum of a colloidal solution of gold nanoparticles functionalized with antibodies targeting three surface proteins of SARS-CoV-2 (spike, envelope and membrane) is redshifted in few minutes when mixed to a solution containing the virion.
Results and conclusions: The optical density of the mixed solution measured at 560 nm was compared to the threshold cycle (Ct) of a Real Time-PCR (gold standard for detecting the presence of viruses) finding that the colorimetric method is able to detect very low viral load with a detection limit approaching that of RT-PCR. Since the method is sensitive to the infecting particle (virion) rather than to its RNA, the achievements reported here open new perspective not only in the context of the current and possible future pandemics, but also in microbiology as the biosensor proves itself to be a powerful though simple tool for measuring the virion concentration.

P64

Detection of SARS-CoV-2 RNA on plastic glasses and bottles. A possible approach for non-invasive surveillance of communities?

F. Di Maria[1], T. Bonato[2], A. Pivato[3], R. Piazza[2], P. Mancini[4], G. Bonanno Ferraro[4], C. Veneri[4], M. Iaconelli[4], E. Beccaloni[4], F. Scaini[4], L. Bonadonna[4], T. Vicenza[5], G. La Rosa[4], E. Suffredini[5]

[1] LAR Laboratory – Dipartimento di Ingegneria – University of Perugia, Via G. Duranti 93, 06125 Perugia, Italy - [2] Department of Environmental Sciences, Informatics and Statistics (DAIS), Ca' Foscari University of Venice, Via Torino 155, 30172 Venezia-Mestre, Venice, I - [3] Department of Civil, Environmental and Architectural Engineering (ICEA), University of Padua, Italy - [4] Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy - [5] Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy

The diagnosis of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection relies on the detection of viral RNA by real-time reverse transcription polymerase chain reaction (rRT-PCR) performed with respiratory specimens, especially nasopharyngeal swabs. However, this procedure requires specialized and trained medical personnel, as well as centralized laboratory facilities for the analysis. For these reasons, several studies have suggested the use of other body fluids, including saliva, for the detection of SARS-CoV-2. In this study we explored an alternative approach to investigate SARS-CoV-2 presence in small communities (such as office communities, schools, canteen goers, malls, residential social and health facilities, sport centers, etc.) through indirect sampling of saliva deposited on single-use glasses and plastic (PET) bottles.
Twenty different collection points were selected based on their specific profile in generating this kind of waste, as e.g. those in which coffee and/or drink vending machines operate. The collection points were distributed on one Italian area (570 square Km, 650,000 inhabitants), and sampling took place between 1 January 2021 and 20 February 2021, a period roughly corresponding in Italy to the third COVID-19 pandemic peak. Sterile foam swabs, specifically selected for viral recovery form surfaces and moisted with sterile saline solution, were used to wipe rims and finishes of plastic glasses and bottles, respectively.
Overall, 20 swab samples were analyzed, each representing between 50 and 100 glasses and plastic bottles. Recovery of biological material from the surfaces was confirmed by analysis of human RNaseP sequence (12 samples) and inhibition of PCR was ruled out by analysis of an external amplification control. Three swabs tested positive for SARS CoV-2 by a published real-time RT-(q)PCR targeting the nsp14 region and viral load ranged from 4.8×103 to 4.0×106 genome copies/swab. Results were confirmed by conventional RT-nested-PCR amplification of a portion of the spike protein followed by sequencing. Mutations typical of some variants circulating in Italy were detected in all the positive samples (A222V and P521S).
Recent studies showed that detecting SARS-CoV-2 in wastewater can be an efficient and low-cost solution for tracking COVID-19 outbreaks (the so-called Wastewater-based epidemiology) but limited to specific communities. Similarly, information can also be obtained

by the analysis of surfaces - in this case, plastic glasses and bottles - that comes in contact with saliva.
Consequently, if applied in specific sites (e.g., airports, highway rest areas), this analytical control could also be used for monitoring and/or tracing the movements of SARS-CoV-2 and similar infective disease cross different areas and regions. This peculiarity indicates that this new approach, that we named "solid waste-based-epidemiology (SWBE)", may represent an effective support for non-invasive and dynamic health surveillance aims.

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Post vaccinale evaluation of the increase in IgG anti SARS-CoV-2 in a sample of health care workers of the ASL Napoli 3 Sud

M. Ferrigno, C. Maiello, A. Rainone, M. A. Sorrentino, L. Riccardi, N. Occhiati, L. Di Maio, L. Lovriso, E. Policano, L. Folliero, V. Rodriguez, G. Pagliara, A. Cimino, M. Aloisio, P. Petrosino, F. Maiello, M. D'Orazio, C. Furfaro

Azienda Sanitaria Locale Napoli 3 Sud Presidio Ospedaliero "Cav. R. Apicella" Pollena Trocchia (Na) - Laboratorio Di Patologia Clinica

Introduction. The introduction in laboratory diagnostics of quantitative tests for the detection of anti-SARS CoV 2 IgG and IgM antibodies has significantly changed the use of the qualitative ones previously adopted. If before the advent of vaccines the dosage of these antibodies was aimed at identifying recent and / or previous infections, currently, the test, and particularly the detection of IgG antibodies, and their quantification, is useful above all in the evaluation of the post vaccine immune response.
The aim of our study was to evaluate the production / increase of anti SARS CoV 2 IgG secondary to vaccine administration.
Materials and methods. A representative sample of Health care workers of our ASL subjected to anti SARS CoV 2 vaccination was examined and, after the prescribed period, the quantitative test for the detection of IgG and IgM was performed. Prior to the advent of vaccination campaign, in order to contain the infection, the same test was performed periodically for healthcare workers, first qualitative and subsequently quantitative test. It should be noted that, prior to vaccine administration, the protocol provided the execution of a rhino-pharyngeal swab for the diagnosis of SARS CoV 2 infection, when IgM was detected.
The tests were carried out on Abbott Architect ci 8200 instrumentation using the Abbott SARS-CoV-2 IgG , SARS-CoV-2 IgG II Quant and SARS-CoV-2 IgM kits
Results

Anti SARS CoV 2 IgG absent pre vaccination	Anti SARS CoV 2 IgG present post vaccination
568	568
Anti SARS CoV 2 IgG present (low titre) pre vaccination (infection or contact)	IgG anti SARS CoV 2 present (high titre) post vaccination
91	91
Anti SARS CoV 2 IgG present post vaccination without pre vaccination IgG dosage	2.525

Conclusions. The examination of the results highlights the importance of the vaccination campaign carried out early and wisely in our ASL as early as December 2020.
The antibody titer will be checked periodically over time.

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SARS-CoV-2 serological diagnosis: comparison of Liaison® vs Abbott® chemiluminescent assay and Roche® electrochemiluminescent assay

R. Gibilisco[1], F. Di Ruscio[1], A. Zampiero[2], A. Nava[2], D. Fanti[2], C. Vismara[2], A. Pani[3], V. Cento[3], F. Scaglione[2,3], D. Campisi[2]

[1] Residency in Microbiology and Virology, Università degli Studi di Milano, Milan, Italy - [2] Clinical Chemistry and Microbiology, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy - [3] Department of Oncology and Hemato-Oncology, Università degli Studi di Milano, Milan, Italy

Aim of the study: Highly sensitive and specific SARS-CoV-2 serological assays are needed to guide clinical decision-making, the vaccination efficacy, epidemiological studies. Our aim was to compare the analytical performance of 2 different chemiluminescent and 1 electro-chemiluminescence serological assays.
Methods used: Chemiluminescent assay Abbott (S1-RBD protein-based) and electro-chemiluminescent assay Roche (S protein-based) were compared with chemiluminescent assay Diasorin (TrimericS spike protein-based).
LIAISON® SARS-Cov-2 TrimericS IgG uses immunoassay technology in chemiluminescence (CLIA), a quantitative assay for detection of SARS-CoV-2 anti-Trimerics spike glycoprotein antibodies. Alinity i SARS-CoV-2 IgG II Quant is a qualitative and quantitative microparticles-capture chemiluminescent immunological assay (CMIA) used for determination of antibodies directed against the receptor-binding domain (RBD) of the S1 subunit.
Elecsys Anti-SARS-CoV-2 (including IgM) on platform Cobas is a qualitative and semi-quantitative electro-chemiluminescent immunoassay intended for antibodies detection against SARS-CoV-2 spike (S) protein receptor binding domain (RBD).
Serological tests were performed on anonymised residual serum samples withdrawn from 153 patients: 23/153 were COVID-19 patients whith a PCR-positive , 130/153 were COVID-19 patients with a PCR-negative or without PCR. Cohen's kappa coefficient was used to measure the agreement between Diasorin vs Abbott, between Diasorin vs Roche and between Abbott vs Roche.
Results: Using Alinity i SARS-CoV-2 IgG II Quant VS LIAISON® SARS-Cov-2 TrimericS IgG among the 153 samples, sensitivity was 100% (95%CI:97%-100%), specificity was 100% (95%CI: 92%-100%). Liaison declareed analytical Sensitivity (days post PCR) was 98.7% (≥15 days), declared Specificity was 99.5% (95% CI: 99.0% - 99.7%). Alinity i's SARS-CoV-2 IgG II Quant declared sensitivity was 99.37% (95%CI:96.50%-99.97%), declared specificity was 99.60% (95%CI:99.15%-99.76%). Using Elecsys Anti-SARS-CoV-2 VS LIAISON® SARS-Cov-2 TrimericS IgG among the 153 samples, sensitivity was 100% (95%CI:97%-100%), specificity was 100% (95%CI: 85%-99%). Elecsys® Anti-SARS-CoV-2 declared sensitivity was 98.8 % (95% CI: 98.1 – 99.3 %) and declared specificity was 100 % (95 % CI: 99.7 – 100 %). Using Elecsys Anti-SARS-CoV-2 vs Alinity i SARS-CoV-2 IgG II Quant among the 153 samples, sensitivity was 100% (95%CI:97%-100%), specificity was 100% (95%CI: 85%-99%).
Results and conclusions: The agreement between Diasorin and Abbott chemiluminescent assay is "Excellent" (κ-values 0.80-1.00), between Diasorin and Roche is "Excellent" (κ-values 0.81-1.00), between Abbott and Roche assays is "Excellent" (κ-values 0.81-1.00). Theese compared qualitative and quantitative essays show high sensitivity and specificity.

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Parvovirus B19: role in the differential diagnosis of measles and rubella

S. Gioacchini, M. Baggieri, A. Marchi, P. Bucci, M. Kojouri, E. D'Ugo, F. Magurano

Dipartimento di Malattie Infettive, Istituto Superiore di Sanità, Rome, Italy

Aim of the study. In the context of measles and rubella elimination strategy (plan), it is crucial that all countries confirm every suspected measles and rubella case in the community for an effective surveillance of these diseases (World Health Organization (WHO). Strategic plan for measles and congenital rubella infection in the European Region of WHO 2003).
Considering that near to the eradication and measles and rubella cases are not expected, the differential diagnosis assumes a key role. Since the etiology of double negative cases is still unknown, we focused on Human Parvovirus B19 (B19V), responsible for exanthemic disease and rash illness that can be confused with measles and rubella infection (Nicolay N. et al., 2009; Toshev A. et al., 2014).
In Italy, samples from measles and rubella suspected cases are tested in the National Reference Laboratory for measles and rubella (NRL) at the National Institute of Health and in laboratories belonging to the network MoRoNet (Magurano F. et al., 2015). The aim of our retrospective study is to evaluate the role of B19V as causative agent of rash illness in measles and rubella negative cases.
Methods. From April 2008 to December 2019, urine, oral fluid and dried blood samples were collected from patients with rash/fever were tested and sent to the NRL/MoRoNet labs to confirm measles or rubella infection. Samples from a total of 157 double negative cases/patients were further investigated for B19V by molecular methods. Overall, 223 samples from these 157 patients, were tested by Real-Time PCR. Those samples with a positive result were further amplified by PCR for genotyping. Then, genetically characterization was performed.
Results. A total of 14 samples (7 blood, 3 urine, 4 oral fluid samples) resulted B19V positives and 7 sequences were obtained. Phylogenetic analysis showed that all the sequences belonged to genotype 1, the most prevalent B19V genotype in the world. For the first time B19V has been identified in sample of oral fluid, even if no data had been available in literature about the use of this sample.
Conclusions. As several countries already do, the NRL has introduced in Italy the possibility to investigate B19V on double negative cases as routinary diagnosis in support of measles and rubella surveillance. Our results showed that the B19V differential diagnosis, in cases where measles and rubella can be involved, is an accurate tool not only for case management but also for public health control activities (Goncalves G. et al., 2005; Toshev A. et al., 2014; Cubel R. et al., 2017). Furthermore, oral fluid can be used as an alternative sample for molecular diagnosis of B19V even if more studies are needed to establish how this sample is valid.

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Detection of uncommon G3P[4] group a rotavirus strains in symptomatic children in Italy, during 2019

G. Ianiro[1], A. Chiereghin[2], L. Gabrielli[3], T. Lazzarotto[3], M. Monini[1]

[1] Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy - [2] Section of Microbiology, Department of Specialized, Experimental, and Diagnostic Medicine, University of Bologna, Italy and Department of Public Health, Local Health Authority of Bologna, Italy - [3] Microbiology Unit, Department of Specialized, Experimental, and Diagnostic Medicine, IRCCS St. Orsola Polyclinic, University of Bologna, Bologna, Italy

Background: Group A rotaviruses (RVAs) are a major cause of dehydrating acute gastroenteritis (AGE) in children <5 years of age worldwide, with high mortality rate mainly in developing countries. The RVA genome is composed of 11 double-stranded RNA segments, encoding 6 structural viral (VP) and 5 or 6 nonstructural (NSP) proteins. The nucleotide sequence differences of genes encoding the VP7 and VP4 proteins define the G- and P-genotypes, with a binary classification system. Since 2008, a more complete genotyping system has been developed aiming to classify all the genomic segments and to highlight possible reassortment events in the circulating strains.

Methods: During the 2019 RotaNet-Italy surveillance, 4 stool specimens from children hospitalized with AGE symptoms at the IRCCS St. Orsola Polyclinic of Bologna and tested positive for RVA with a commercial chemiluminescent immunoassay, were collected and sent to the Istituto Superiore di Sanità of Rome for nucleotide sequencing and genotyping.

Results: After RT nested PCR and agarose gel genotyping, the 4 RVA strain revealed the uncommon G3P[4] genotype, and were then subjected to nucleotide sequencing of the 11 genomic segments in order to define the complete viral genomic backbone. Nucleotide sequencing showed for all strains a complete genomic constellation 2 (DS-1-like) in combination with the G3P[4] genotype. Phylogenetic analyses posed the evidence that the occurrence of multiple reassortment events originated the detected strains.

Conclusions: This study reports the complete genotyping and phylogenetic analysis of an uncommon rotavirus A strain presenting a genotype never detected before in Italy, and able to cause acute dehydrating gastroenteritis in four patients. The genome characterization highlighted possible reassortment events between G3P[8] and G2P[4] strains previously circulating in Italy. Also, the genomic backbone could be generated by additional intra genotype reassortment events. The monitoring of the circulation of RVA genotypes is essential to predict the possible spreading of novel epidemic strains in the pediatric population.

P69

Enhanced liver fibrosis (ELF) score and fibroscan: a comparison between two noninvasive methods in patient with chronic liver disease HCV-related

E.A. Molinari[1], L. Stanziola[1], L. Vallefuoco[1], R. Sorrentino[1], S. Di Somma[1], F. Napolitano[1], A. Furno[1], A. Russo, M. Pagano, V. Cossiga[2], G. Pontillo[2], M. Guarino[2], A. Fiorentino[2], D. Terracciano[1], F. Morisco[2], G. Portella[1]

[1] Department of Translational Medical Sciences, University of Naples "Federico II", 80131 Naples, Italy - [2] Department of Clinical Medicine and Surgery, Gastroenterology Unit, University of Napoli "Federico II", Napoli, Italy

Aim of the study: Liver fibrosis is an important predictor of clinical outcome, as overall survival and also liver-related morbidities and mortality in patient with chronic HCV infection. Liver biopsy is still considered the gold standard to evaluate liver fibrosis. Noninvasive methods are also available, such as Fibroscan. Recently ELF score has been developed. ELF score is an algorithm based on tissue inhibitor of metalloproteinases 1 (TIMP-1), amino-terminal propeptide of type III procollagen (PIIINP) and hyaluronic acid (HA) serum levels. The aim of this study is to compare ELF score with Fibroscan in patients with chronic liver disease HCV-related treated with direct acting antiviral agents (DAAs).
Methods used: We enrolled 119 patients with chronic liver disease HCV-related (66 male, 53 females; mean age of 64.7 ± 9.6 years). All patients were treated with DAAs and most of them (94.1%) achieved a sustained viral response (SVR). Serum samples were obtained before DAA treatment and 24 weeks after the end of treatment (SVR24). ELF score was determined using an ADVIA Centaur XPT (Siemens Healthcare USA) as recommended by manufacturer.
Results and conclusions: At baseline, Pearson coefficient between ELF score and Fibroscan showed a moderate and direct significant correlation ($r=0.35$. $p<0.001$), the weighted kappa coefficient indicated a fair agreement (WK= 0.28, 95% C.I.: 0.16 to 0.4) between ELF score and Fibroscan values with a tendency of the former to overestimate the severity of fibrosis compared to the latter. In particular 20 patients (16.8%), graded as moderate by Fibroscan (9.5-12 kPa), were classified as severe fibrosis by ELF. Same trend was observed when comparing the two parameters at SVR24; the correlation coefficient was statistically significant ($r=0.31$, $p=0.002$) and weighted kappa revealed an even lower agreement between ELF and Fibroscan (WK= 0.13, 95% C.I.: -0.08 to 0.35). Of note 39 (37.5%) patients classified as severe fibrosis by ELF were either mild (n=25) or moderate (n=14) according to Fibroscan (7-12 kPa). Our findings suggest that ELF score shows a good correlation with Fibroscan and could be used in patients with HCV related chronic liver disease candidate to DAA treatment and in subsequent follow-up. This finding is of particular interest in relation to the simplified approach to HCV therapy, with the objective of total eradication of the population.

P70

Two years surveillance of Norovirus and Rotavirus acute gastroenteritis in healthcare associated infections in Italy

M. Monini[1], I. Di Bartolo[1], G. Ianiro[1], A. Di Pierro[2], G. Giammanco[3], S. De Grazia[3], F. Bonura[3], G.M. Rossolini[4,5], S. Pollini[4,5], V. Rabensteiner[6], E. Pagani[2] and the NosoVirNet group

[1] Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy - [2] Laboratory of Microbiology and Virology, Azienda Sanitaria dell'Alto Adige, Bolzano, Italy - [3] Dipartimento di Scienze per la Promozione della Salute e Materno Infantile "G. D'Alessandro", Università di Palermo, Palermo, Italy - [4] Department of experimental and Clinical Medicine, University of Florence, Florence, Italy - [5] Microbiology and Virology Unit, Florence Careggi University Hospital, Florence, Italy - [6)] Provincia Autonoma di Bolzano

Nosocomial or healthcare associated infections (HCAIs) are a major concern for public health and can be acquired during hospitalization or in long-term care facilities or less frequently during ambulatory care. Infections appearing 48 hours after hospitalization or within 24 hours after having received health care are considered HCAI. Hospital-acquired acute gastroenteritis (AGE) is frequently cause by Clostridium but several studies have demonstrated that rotavirus A (RVA) and norovirus (NoV) are also common causes of nosocomial AGE.

Surveillance of gastroenteritis in healthcare settings is lacking and the occurrence of RVA and NoV is probably underestimated. To overcome this gap, the "Surveillance of norovirus and rotavirus acute gastroenteritis in healthcare associated infections" was conceived in the framework of the CCM program 2018 with the financial support of the Italian Ministry of Health. From march 2018 to march 2020, a surveillance program was established to collect data on NoV and RVA HCAIs. The national network comprises 11 hospitals/local health authority/department of the Italian National Sanitary System (SSN) and located in eight regions distributed along the North, Centre and South of Italy. Fecal Samples were collected and viruses typed by molecular methods.

A total of 541 fecal samples were collected and analyzed for the presence of enteric viruses. Over two years of surveillance, four outbreaks occurred in four different long term care facilities (LTCF) for elderly caused by NoV infections, as confirmed by nucleotide sequencing of NoV strains involved. Overall, 137/541 samples (25.3%) resulted positives for RVA, and 117/541 (21.6%) for NoV GII.

Among samples resulted negative for RVA and NoV, 18/541 were positives for AdV (3.3%), 9/541 for Sav (1.7%) and 2/541 for AstV (0.4%).

Concerning NoV, 54 sequences were obtained on the capsid region (ORF2) and 14 on the polymerase region (ORF1) and 10 recombinant genotypes were identified. Among the 137 samples resulted positive for RVA, the predominant genotype detected was G12P[8] (45/137 cases, 32.8%), followed by G1P[8] (26/137, 19.%).

During the surveillance a lack of awareness of viral infections in HCAI was observed. Besides this, the occurrence of these viral infections has been observed rarely in some hospitals. Occurrence of GE outbreaks in LTCF could be even more frequent and deserves

attention because infections caused by NoV can spread fast and can be fatal in elderly. Preliminary data obtained during this surveillance provide insight into the burden of healthcare-associated AGE in order to be helpful for future public health action.

NosoVirNetGroup:
Farina Claudio, Martinelli Monica; Calderaro Adriana; Aprea Giuseppe; Binda Sandro, Pellegrinelli Laura, Campanini Giulia, Baldanti Fausto, Turriziani Ombretta, Sciandra Ilaria, Hallgass Maria Elena, Giannotta Alberto, Coltella Luana, Concato Carlo, Esposito Susanna, Camilloni Barbara, Tamara Brunelli, Castiglia Paolo, Chiereghin Angela, Lazzarotto Tiziana, Gabrielli Liliana, Contarini Marianatalia, Defrancesco Maria, Zoppelletto Maira, Valentini Diletta.

P71
A case of CMV pancreatitis in an immunocompetent host

A. Morea[1], A. Forniti[2], C. Caroselli[2], S. Verdenelli[2], F. Amadori[3], L.R. Suardi[2]

[1] Infectious Diseases Unit, Azienda Usl Toscana nord ovest. Livorno, Italy - [2] Infectious Diseases Unit, Azienda Ospedaliera Universitaria Pisana. University of Pisa, Italy - [3] Infectious Diseases Unit, Azienda Usl Toscana nord ovest Massa, Italy

An immunocompetent 43-year-old man with no medical history was admitted to the internal medicine ward of the University Hospital in Pisa for fever of unknown origin (FUO). A chest and abdomen computed tomography (CT) scan was performed, reporting diffuse bilateral lymphoadenomegaly and splenomegaly. Among the numerous tests he also performed a Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) serology and viraemia assays. CMV serology documented a positive immunoglobulin M (IgM) and a weak positive immunoglobulin G (IgG). EBV serology was also positive for IgM and IgG, however only CMV viraemia was positive with 7350 copies/ml viral load. HIV status proved negative; lymphocytes subpopulations and Ig classes levels tested normal. Peripheric blood smear showed lymphocytosis with a prevalence of granulated lymphocytes with no atypical findings. During the hospital stay there was a progressive rise in amylases and lipases meanwhile, the patient reported a sense of epigastric tenderness and dyspepsia. An esophagogastroscopy was then performed revealing the presence of antral gastric ulcers, which were biopsied reporting nonspecific inflammation findings. Proton pump inhibitor therapy was started however due to lack of clinical improvement a second abdominal CT was performed and documented an inflammatory process affecting the pancreas. CMV serology was repeated during hospitalization in several moments, documenting a progressive reduction in IgM and a progressive increase in IgG; also IgG avidity initially tested low then increased thus confirming the hypothesis of a primary CMV infection.
Clinicians initially opted for an observational monitoring with intravenous hydration; however, amylases and lipases did not spontaneously decrease and since pancreatic pseudocysts or any obstructive cause were excluded by magnetic resonance (MR), antiviral treatment with ganciclovir 5 mg/kg twice daily was initiated. In the following days there was finally a reduction in pancreatic enzymes and clinical improvement, so the patient was discharged after 14 days of intravenous therapy, with continuation of antiviral therapy with oral valganciclovir.
Like other cases described in scientific literature[1], CMV pancreatitis was also accompanied by gastric ulcers in this one. These clinical scenarios are more frequent in immunocompromised patients but can also occur in healthy subjects especially in CMV primary infections hospitalized for FUO[2] and often requiring antiviral treatment[3].

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P72
Assessment the feasibility of pool testing for SARS-CoV-2 infection screening

I. Paganini[1], C. Sani[1], E. Burroni[1], S. Bisanzi[1], G. Pompeo[1], F. Cellai[1], J. Viti[1], A. Antonelli[2,3], C. Chilleri[2,3], M. Coppi[2,3], M. Baccini[4], F. Mealli[4], G. M. Rossolini[2,3], F. Carozzi[5]

[1] Regional Laboratory of Cancer Prevention; ISPRO, Florence, Italy - [2] Microbiology and Virology Unit, Careggi University Hospital, Florence - [3] Dep. of Experimental and Clinical Medicine, University of Florence - [4] Dep. of Statistics, Computer Science, Applications, University of Florence - [5] University of Florence, contract professor for Master degree in molecular and applied biology

Background and Aim of the study. SARS-CoV-2 pandemic represented a huge challenge for national health systems worldwide.
Molecular testing to identify infected patients and avoid new infections is a crucial tool in containing the pandemic, so the high demand of tests may result in the lack of reagent supplies. Recently, rapid antigen detection (RAD) tests for qualitative determination of SARS-CoV-2 antigen, easy interpreted within 30 minutes, were introduced. However, RAD in most cases presents a lower sensitivity compared to Real Time PCR (RT-PCR) and may produce more false negative results, causing negative consequences for pandemic spreading. Pooling nasopharyngeal swabs (NPS) seems to be a promising strategy, saving time and resources but it could reduce the sensitivity of the RT-PCR and exacerbate sample management in terms of automation and tracing. In this study, we evaluated the feasibility of pool testing for SARS-CoV-2 infection diagnosis, even in low viral load samples.
Methods. NPS were firstly tested singularly using an automated system for RNA extraction followed by RT-PCR assay targeting four viral genes (E, RdRP/S and N). 349 negative and 60 positive samples were selected for pool preparation, anonymized after results registration and stored at 4°C. Only positive samples showing a Ct value of at least 28 for each gene were chosen for pool preparation; Ct values of positive samples selected were on average about 35 for the four genes analysed. Pools were prepared with an automated instrument (Opentrons OT-2, Biomedical Service), mixing 4, 6 or 20 NPS, including one, two or none positive samples and processed under the same conditions of the singular test. The instrument software allows to trace samples included in each pool. Sensitivity was estimated as the proportion of pools resulting positive, among those including at least one specimen classified as positive by singular test.
Results and Conclusions. A total of 80 pools (35 pools of 4 samples, 37 pools of 6 samples and 8 pools of 20 samples) were processed. The ΔCt between the single test and the pools were on average, for 4-samples pools 1.51, 1.27 and 2.32 higher for E, RdRP/S and N gene, respectively, and 1.3, 1.52 and 2.58 for 6-samples pools, approximately consistent with the dilution factor; ΔCt for pools of 20 samples did not shown a correlation with the dilution factor, but they are numerically too low to obtain reliable data. The overall sensitivity was 86% (90% CI =[78-91]), with all the 8 pools with more than one positive samples with low viral load resulting positive. The overall sensitivity in pools with only one positive sample was 84% (90% CI =[76-90]). The sensitivity of 4-sample pools with 1 positive was 82%

(90% CI =[67-90]), and in 6-sample pools with 1 positive was 87% (90% CI =[74-94]). Considering only pools including positive samples showed Ct value <34, the sensitivity reached 98% (90% CI =[90-99]) (100% and 92% for pools of 6 and 4 samples, respectively).
Our study demonstrated an overall good sensitivity of pool testing with low viral load NPS, increasing sample traceability thank to automated preparation. Pool testing needs time, specific instrument and trained personnel, but could be fully automated with dedicated instruments and the sensitivity still remains higher, allowing good results in terms of false negative rate due to low viral load. Finally, a reduction in prevalence and in symptomatic cases is expected after vaccination campaign, making the theoretically ideal contest for the use of pool testing in large screening.

P73 - OC 9

Exploratory analysis to identify the best antigen and the best immune biomarkers to study SARS-CoV-2 infection

E. Petruccioli[1], S. Najafi Fard[1], A. Navarra[2], L. Petrone[1], V. Vanini[1,3], G. Cuzzi[1], G. Gualano[4], L. Pierelli[5], A. Bertoletti[6], E. Nicastri[4], F. Palmieri[4], G. Ippolito[7], D. Goletti[1]

[1] Translational Research Unit, National Institute for Infectious Diseases Lazzaro Spallanzani - IRCCS - Rome, (Italy) - [2] Clinical Epidemiology Unit, National Institute for Infectious Disease Lazzaro Spallanzani - IRCCS – Rome, (Italy) - [3] UOS Professioni Sanitarie Tecniche National Institute for Infectious Diseases Lazzaro Spallanzani – IRCCS - Rome, (Italy) - [4] Clinical Division of Infectious Diseases, National Institute for Infectious Diseases Lazzaro Spallanzani - IRCCS – Rome, (Italy) - [5] UOC Transfusion Medicine and Stem Cell Unit, San Camillo Forlanini Hospital - Rome, (Italy) - [6] Programme in Emerging Infectious Diseases, Duke-National University of Singapore Medical School - Singapore (Singapore) - [7] Scientific Direction, National Institute for Infectious Disease "Lazzaro Spallanzani"- IRCCS – Rome, (Italy)

Background: recent studies proposed the whole-blood based IFN-γ-release assay to study the antigen-specific SARS-CoV-2 response. Since the early prediction of disease progression could help to assess the optimal treatment strategies, an integrated knowledge of T-cell and antibody response lays the foundation to develop biomarkers monitoring the COVID-19. Whole-blood-platform tests based on the immune response detection to SARS-CoV2 peptides is a new approach to discriminate COVID-19-patients from uninfected-individuals and to evaluate the immunogenicity of vaccine candidates, monitoring the immune response in vaccine trial and supporting the serological diagnostics results. Here, we aimed to identify in the whole-blood-platform the best immunogenic viral antigen and the best immune biomarker to identify COVID-19-patients.
Methods: whole-blood was overnight-stimulated with SARS-CoV-2 peptide pools of nucleoprotein- (NP) Membrane-, ORF 3a- and Spike-protein. We evaluated: IL-1β, IL-1Ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL- 15, IL-17A, eotaxin, FGF, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF, RANTES, TNF-α, VEGF. By a sparse partial least squares discriminant analysis we identified the most important soluble factors discriminating COVID-19- from NO-COVID-19-individuals.
Results: we identified a COVID-19 signature based on six immune factors: IFN-γ induced by Spike, IP-10 induced by Spike, IL-2 by Spike; RANTES by NP; IP-10 by NP and IL-2 by ORF3a. We demonstrated that the test based on IP-10 induced by Spike had the highest AUC (0.85, $p < 0.0001$) and that the clinical characteristics of the COVID-19-patients did not affect IP-10 production. Finally, we validated the use of IP-10 as biomarker for SARS-CoV2 infection in 2 additional COVID-19-patients cohorts.
Conclusions: evaluating patients with acute COVID-19 and recovered patients, we set-up a whole-blood assay identifying the best antigen to induce a T-cell response and the best biomarkers for SARS-CoV-2 infection and we focused on IP-10, already described as a potential biomarker for other infectious disease such as tuberculosis and HCV. An additional application of this test is the evaluation of immune response in SARS-CoV-2 vaccine trials: the IP-10 detection may define the immunogenicity of a Spike-based vaccine, whereas the immune response to the virus may be evaluated detecting other soluble factors induced by other viral-antigens.

P74

Development and evaluation of two serological ELISA assays based on SARS-CoV-2 N protein

G. Pezzoni[1], S. Grazioli[1], R. Savoldi[3] , A. Bregoli[1], L. Capucci[1], D. Lelli[1], E. Foglia Alessandro[1], M.B. Boniotti[1], B. Colitti[2], S. Rosati[2], E. Brocchi[1]

[1] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy - [2] Università degli Studi di Torino, Dipartimento di Scienze Veterinarie, Grugliasco, Torino, Italy - [3] Istituto Clinico Città di Brescia, Brescia, Italy

Aim of the study: Coronavirus Disease 2019 (COVID-19), which is caused by the novel SARS-CoV-2, continues to spread worldwide more than one year after its discovery. So far, a lot of studies about its diffusion in the human population have been conducted, and many serological assays have been developed for use in the human field. However, there is a need for diagnostic tools to study SARS-CoV-2 distribution in animals. The most immunogenic proteins of SARS-CoV-2 are the Nucleocapsid protein (Np) and the Spike protein, both are viral structural proteins and the latter is most externally exposed since located in the viral envelope.
This study describes the development and evaluation of two serological ELISA assays based on SARS-CoV-2 Np and designed with two different principles, namely a species and isotype independent double antigen (DA) ELISA and a human IgG specific assay (IgG ELISA).
Methods: SARS-CoV-2 Np was expressed in E. coli, purified by Immobilized Metal Affinity Chromatography and conjugated with horseradish peroxidase (HRPO). For the DA assay, unconjugated Np was coated onto the ELISA microplates and then incubated with test sera. After washing, the Np-HRPO was added and, after incubation followed by washing, a colorimetric reaction was developed. For the second assay, immunoglobulins purified from an anti-human-IgG polyclonal serum were coated onto the ELISA microplates, then test sera and Np-HRPO were simultaneously incubated; after washing the colorimetric reaction was developed. For both assays the results were expressed as a percentage of reactivity versus the positive control included in each plate.
Four groups of human sera were collected and tested: 379 sera from blood donors, sampled in the period 2015-2016 and thus considered SARS-CoV-2 negative; 24 sera from individuals sampled one to three months after a positive result to the SARS-CoV-2 molecular assay; 70 sera collected in May 2020 from hospitalized patients, positive for SARS-CoV-2 according to the molecular test; 85 sera collected in June 2020 from healthcare workers previously tested with a commercial serological test able to detect human IgG against SARS-CoV-2 Spike protein (Liaison-DiaSorin).
Results and conclusions: Cut-off values were set for the two assays, mainly based on the results obtained by testing the 379 SARS-CoV-2 negative sera, of which more than 99% resulted below the established thresholds. Both tests consistently detected as positive all the 24 sera collected from one to three months after SARS-CoV-2 positive result by molecular test. A total of 56 and 46 out of 70 sera from hospitalized patients were identified as positive by DA assay and IgG ELISA respectively. Thanks to its peculiar design the DA ELISA detects all immunoglobulin isotypes, thus it resulted able to discover an earlier hu-

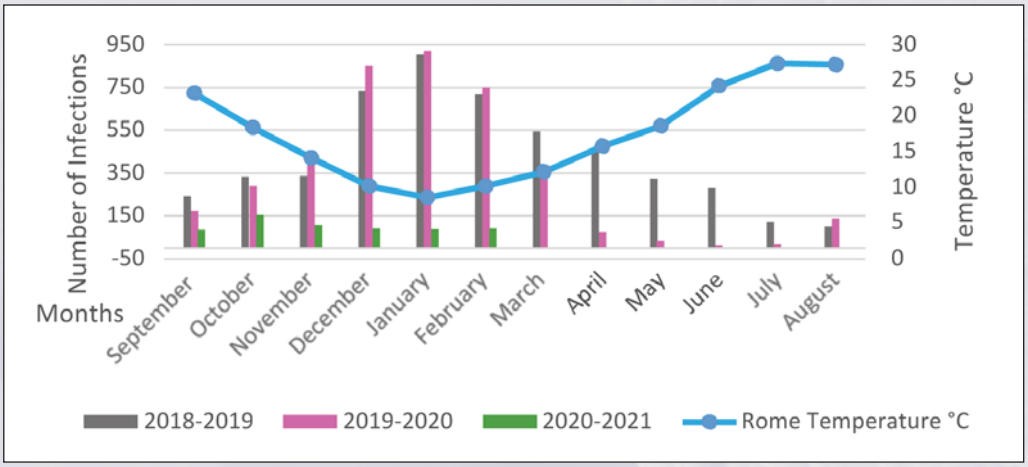
moral response than the IgG ELISA, succeeding to detect the IgM, while sampling was plausibly too early even for the detection of IgM in patients who scored negative.When the 85 sera from healthcare workers were tested, DA and IgG ELISA showed a concordance of 92% and 89% respectively with the commercial ELISA assay, both tests missed four positive sera, of which three scored as borderline with the commercial test. Overall, the results obtained highlight that both assays can correctly differentiate SARS-CoV-2 human positive and negative sera. Furthermore, ELISAs based on Np can also be useful to differentiate infected from vaccinated individuals when vaccines based on the Spike protein are used. The DA ELISA, which is species independent, is a promising tool for the evaluation of the diffusion of SARS-CoV-2 in different animal species. Studies heading towards this objective are currently ongoing.

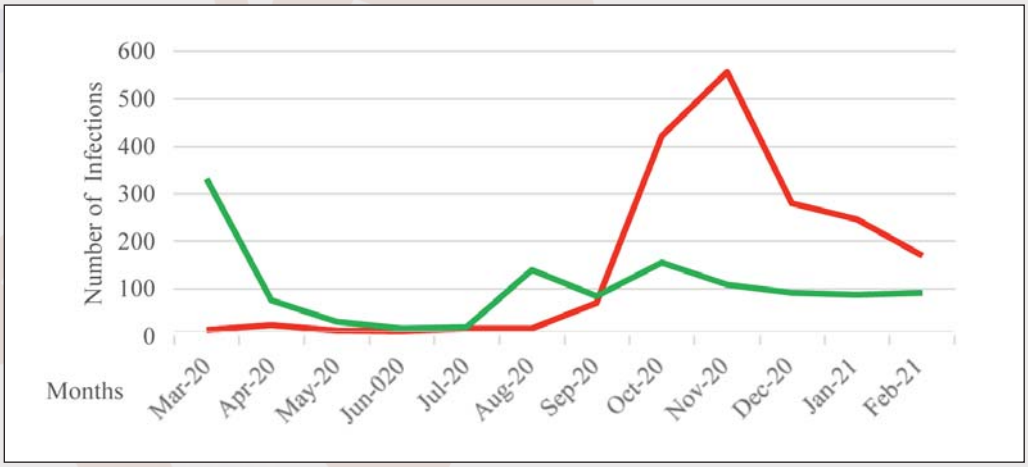
P75
Respiratory infections pre and post SARS-CORONAVIRUS 2 era

L. Piccioni, S. Ranno, L. Coltella, G. Linardos, G. Pizzichemi, S. Chiavelli, C. Concato, C.F. Perno

Microbiology and Diagnostic Immunology Unit, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

Aim of the study. Respiratory infections remain the most common cause of infant and child morbidity and mortality worldwide. Among respiratory virus, Respiratory Syncytial virus is the main cause of hospitalization in children followed by Adenovirus, Rhinovirus and Influenza A virus (Gaunt ER et al. "Disease burden of the most commonly detected respiratory viruses in hospitalized patients calculated using the disability adjusted life year (DALY) model." J Clin Virol. 2011;52(3):215-221.)
The aim of our study is to analyze the trend of viral respiratory infections in the Bambino Gesù Children's Hospital (Rome, Italy) before and after COVID-19 pandemic in Italy.
Methods. We retrospectively analyzed results obtained from nasopharyngeal swabs belonging to patients under 18 years of age, admitted to our hospital in the last three years. Samples were processed for respiratory virus detection by multiplex real-time PCR.
Results and conclusions. A total of 9100 patients ca. was screened for suspected virus respiratory infections in the last three years (2018-2021) at the Bambino Gesù Children's Hospital. Data collected corroborated that the main cause of respiratory infections in children are due to Rhinovirus and Respiratory Syncytial virus. The first graph shows the number of respiratory infections compared to the external medium temperature of the city of Rome: as the temperature increases, the percentage of infections decreases.
The graph underlines a decrease from March 2020 compared to the previous season. On 11 March 2020, World Health Organization declared COVID-19 as a pandemic and Italian government imposed several public health measures to prevent the transmission.





From the onset of the COVID-19 pandemic in our hospital around 35,700 paediatric patients were screened for SARS CoV2. The second graph shows the trend of SARS-CoV-2 infections in comparison to the other respiratory virus infections during the last year. In conclusion, data highlight that safety procedures undertaken for pandemic containment had as results both the reduction of SARS-CoV-2 transmission and of the number of respiratory infections compared to previous years.

P76

KI and WU polyomavirus in respiratory samples of SARS-CoV-2 infected patients

C. Prezioso[1,2], U. Moens[3], G. Oliveto[4,5,6], F. Frasca[4,6], A. Viscido[4,5], G. Brazzini[1], F. Piacentini[1], M. Scordio[4,6], G. Guerrizio[4,5], D.M. Rodio[4,5], A. Pierangeli[4,6], O. Turriziani[4,5], G. d'Ettorre[1], G. Antonelli[4,5,6], C. Scagnolari[4,6], V. Pietropaolo[1,5]

[1] Department of Public Health and Infectious Diseases, "Sapienza" University of Rome, Italy - [2] IRCSS San Raffaele Pisana, Microbiology of Chronic Neuro-degenerative Pathologies, Rome, Italy - [3] Department of Medical Biology, Faculty of Health Sciences, University of Tromsø-The Arctic University of Norway, 9037 Tromsø, Norway - [4] Laboratory of Microbiology and Virology, Department of Molecular Medicine, "Sapienza" University of Rome, Italy - [5] Microbiology and Virology Unit, "Sapienza" University Hospital "Policlinico Umberto I", Rome, Italy - [6] Istituto Pasteur Italia, Rome, Italy

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) has been declared a global pandemic. Our goal was to determine whether coinfections with respiratory polyomaviruses, such as Karolinska Institutet polyomavirus (KIPyV) and Washington University polyomavirus (WUPyV) occur in a significant subset of SARS-CoV-2 infected patients.
Oropharyngeal swabs from 150 individuals, 112 symptomatic COVID-19 patients and 38 healthcare workers not infected by SARS-CoV-2, were collected during the period from March 2020 through May 2020 and tested for KIPyV and WUPyV DNA presence. Of the 112 SARS-CoV-2 positive patients, 27 (24.1%) were co-infected with KIPyV, 5 (4.5%) were positive for WUPyV, and 3 (2.7%) were infected simultaneously by KIPyV and WUPyV. Neither KIPyV nor WUPyV DNA was detected in the oropharyngeal samples of the 38 healthcare workers.
A correlation was found in patients concurrently infected with SARS-CoV-2 and KIPyV, but not WUPyV. These results suggest that KIPyV and WUPyV may behave as opportunistic respiratory pathogens. Additional studies are needed to establish whether KIPyV and WUPyV, simultaneously detected in SARS-CoV-2 patients, could potentially drive viral interference or influence disease outcome.

P77

Detection of cytomegalovirus with molecular biology technique in transplanted and/or immunodepressed subjects in a representative sample of the Campania region

A. Rainone*, C. Maiello, M. Ferrigno*, M.A. Sorrentino*, R. Angrisani**, L. Di Maio*, N. Occhiati*, L. Lovriso*, E. Policano*, F. Maiello**, L. Folliero*, M. Aloisio *, P. Petrosino*, M. Ametrano**, M. D'Orazio*, C. Furfaro***

Azienda Sanitaria Locale Napoli 3 Sud, Presidio Ospedaliero "Cav. R. Apicella", Pollena Trocchia (NA) - * Laboratorio di Patologia Clinica, Direttore: Dott. M. D'Orazio, Azienda Sanitaria Locale Salerno - Presidio Ospedaliero di Agropoli (SA) - ** Laboratorio di Patologia Clinica, Direttore Dott. M. Ametrano

Introduction. Cytomegalovirus (CMV) is a globally widespread virus belonging to the Herpesvirus family.
Man is the only reservoir for Cytomegalovirus infection, whose transmission occurs from person to person through blood and biological fluids. Currently, the causes of mortality, as a complication of liver transplantation, see opportunistic infections secondary to immunosuppression at the top. Among these, Cytomegalovirus (CMV) is the most frequent infection in solid organ transplant patients. CMV infection is currently considered a complex phenomenon, concerning humoral and cellular immunity mechanisms, and innate and adaptive responses.
Methods. Our study contemplated the quantitative assay of CMVDNA in whole blood of approximately three hundred and fifty (350) patients, of which 90% were liver transplants and 10% were on immunosuppressive therapy. Cobas X 480 - Roche instrument was used for nucleic acid extraction, while Cobas Z 480 - Roche instrument was used for its amplification, with dedicated diagnostics Cobas CMV - Roche and Cobas Control Kit CMV - Roche.
Results. The results obtained showed the absence of viral replication in about 84% of patients, viral replication not quantizable, but not absent, in about 3% and viral replication in about 13%.
Conclusions. CMV is a potential threat to the hepatotransplant and / or immunosuppressed patient, but the effective preventive strategies that can be implemented today have reduced the possible complications related to the infection.
A further study is underway for the evaluation of Cytomegalovirus infection in pregnant women who show the presence of anti-CMV IgM on serological examination.

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Comparison of three commercial diagnostic tests for antibody anti SARS-CoV-2 spike protein detection

S. Ranno[1], G. Linardos[1], L. Piccioni[1], L. Coltella[1], M. Agosta[1], E. Galeno[1], S. Gobbi[1], M. D'Agostini[2], F. Antonelli[4], R. Veralli[4], S. Angeletti[3], O. Porzio[2], C.F. Perno[1], E. Riva[3]

[1] Microbiology and Diagnostic Immunology Unit, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy - [2] Clinical Laboratory, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy - [3] Unit of Clinical Laboratory Science, University Campus Bio-Medico of Rome, Italy - [4] Unit of Virology, University Campus Bio-Medico of Rome, Italy

Aim of the study. A lot of serological diagnostic tests have been promptly developed in response to the SARS-CoV2 pandemic with the aim to evaluate both natural or post vaccine protection. This study purpose was to compare three different commercial assays: Elecsys-anti SARS-CoV-2 S (Roche Diagnostics) Architect SARS-CoV-2 IgG anti-S II (Abbott); Liaison SARS-CoV2 TrimericS IgG (DiaSorin).

Methods. A total of 100 serum samples have been selected on the basis of patients clinical history: 17 negative subjects; 40 positive subjects with SARS-CoV2 RNA molecular detection in naso-pharingeal swab and 43 vaccinated subjects. For all samples a qualitative detection of antibodies direct against the nucleocapsid (N) protein of SARS CoV 2 with Elecsys anti SARS-CoV-2 (Roche Diagnostic) and a quantitative detection direct against the Spike (S) protein with the three compared assays was performed. In table 1 are summarized the test characteristics.

Table 1: Assays characteristics. Electro-chemiluminescence immunoassay (ECLIA); Chemiluminescence immunoassay (CLIA); Chemiluminescence Microparticle Immunoassay (CMIA); Receptor Binding Protein (RBD); Binding Arbitrary Units (BAU)

	anti-N (Roche)	Anti-S (Roche)	TrimericS (DiaSorin)	Anti-S (Abbott)
Technology	ECLIA	ECLIA	CLIA	CMIA
Protein Target	Nucleocapsid	RBD	Trimeric S	RBD
Antibodies	IgA, IgM, IgG	IgA, IgM, IgG	IgG	IgG
Instrument	Cobas e801	Cobas e801	Liaison XL	Architect
Cut-off	≥1 Index	≥ 0.80 BAU/mL	≥ 33.8 BAU/mL	≥7.1 BAU/mL

Results and conclusions. Overall, positivity to anti-S antibody was revealed in 77/100 samples by Anti-S (Roche), 67/100 samples by TrimericS (DiaSorin) and 75/100 samples by Anti-S (Abbott).

The results for anti-N antibody confirm clinical infection diagnosis: 40/100 positive samples.

On the basis of clinical data and of antibody isotypes detected by the test we have discriminated true positives and true negatives results, and we have calculated sensitivity, specificity, positive predictive value and negative predictive value of each: 100%,100%, 100%, 100% (anti-S Roche); 85%,88%, 96%, 67% (TrimericS DiaSorin); 99%, 96%, 99%, 96 % (anti-S Abbott).
Most of the discordant results are very close to the cut off declared by manufacturers and in some cases the quality of sample (i.e. high concentration of hemoglobin) may have influenced antibody detection.
In conclusion, in our experience all the assays have proved suitable for the detection of the specific anti-S antibody response.

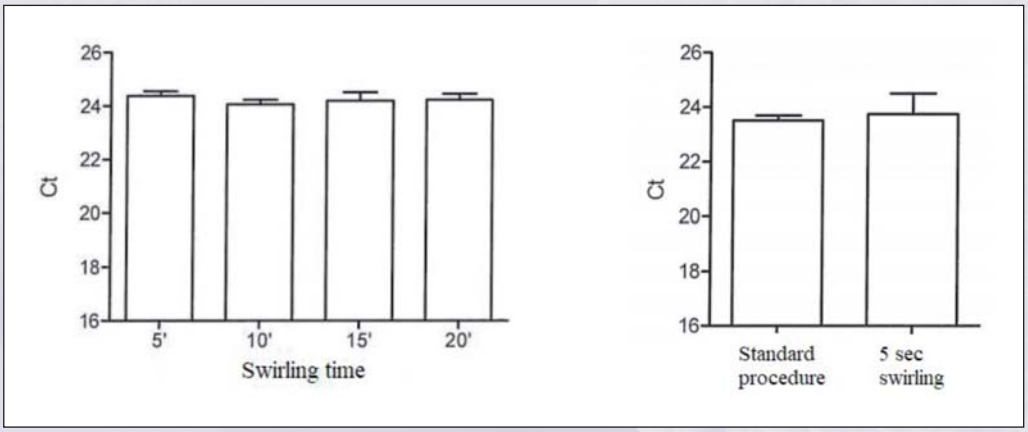
P79

Evaluation of analytical results obtained with different elution procedures from simulated nasal matrix collected with Copan Nasopharyngeal Floqswabs

S. Roversi[1], C. Sabelli[2], S. Messali[1], A. Caruso[1], S. Fiorentini[1]

[1] Section of Microbiology, Department of Molecular and Translational Medicine, University of Brescia, 25123, Brescia, Italy - [2] Copan Italia Spa, Brescia, Italy

Rationale and Aim of the study. Molecular testing for SARS-CoV-2, the etiologic agent of COVID-19, preferentially utilize nasopharyngeal sampling with flocked swabs. This sampling method is presumed to have the highest diagnostic yield, as evidenced by its use as the reference method by Food and Drug Administration. Many analytical systems require the direct allocation of primary collection tubes and, therefore, is often necessary to throw away the swab before sample processing. This manipulation may impede fastening of high throughput procedures and can be an important contamination source. It would be therefore important to eliminate the swab soon after collection, avoiding to leave it within the collection tube.
FLOQSwabs® developed by Copan are designed for a rapid spontaneous release of the collected material into the collection tube filled with universal transport medium (UTM®, Copan Italia). Aim of this study was to identify the shortest swirling time that allow the complete release of the virus in the transport medium.
Methods. A simulated nasal matrix produced by Copan was spiked with a known amount of SARS-CoV-2. Swab was immersed in the SARS-CoV-2-positive matrix then swirled in UTM® for 5', 10', 15' or 20'. A total of 10 replicates for each time points were tested. Comparison with the standard method was obtained evaluating 94 replicates.
For each sample, 200 µl of UTM® were extracted using the DSP Virus/Pathogen Kit (QIAGEN) and the Qiasymphony instrument (QIAGEN). One-step RT-PCR has been performed on CFX Real time system (Biorad) using the Allplex SARS-CoV-2 Assay (Seegene) as specific reagent.



Results and conclusions. The results obtained using different swirling times were comparable and have shown no differences in virus release. Standard deviations among Ct values were within acceptable limits (≤15%) so we can conclude that optimal release was already obtained in 5' (Figure, left panel). Moreover, no sensitivity differences were observed when 5'-long swirling was compared with data obtained with the standard collection procedure (Figure, right panel).
Results make feasible sampling protocols where FLOQSwabs® are eliminated at the time of nasopharyngeal sample collection.

P80

Analysis and validation of a rapid one-step RT-PCR assay for specific detection of SARS-CoV-2 on nasopharyngeal swabs

S. Fontana[1], S. Roversi[2], S. Messali[2], R. Negrini[3], G. Flamminio[1], A. Caruso[2], S. Fiorentini[2,3]

[1] ASST Spedali Civili Brescia, Presidio Ospedaliero di Montichiari, Laboratorio di Analisi - [2] University of Brescia, Department of Molecular and Translational Medicine, Section of Microbiology - [3] ASST Spedali Civili Brescia, Presidio Ospedaliero di Gardone Val Trompia, Laboratorio di Analisi

Rationale and aim of the study. COVID-19 global pandemic is an unprecedented health emergency, calling an urgent need of reliable diagnostic tools. Admission from emergency department to the COVID-free zone of the hospital is, in fact, dependent from SARS-CoV-2 testing results. In this context, antigenic tests are inadequate because their lack of sensitivity, therefore RT-PCR assays remain essential to avoid high incidence of false negative results. On the other side, a short turn around time (TAT) allows to rapidly differentiate admissions, thus optimizing emergency workflow. In this study we evaluated the sensitivity and specificity of the rapid point-of-care RT-PCR assay VitaPCR™ (Credo Diagnostics Biomedical, Singapore) whose sample's analysis time is around 20 min. Performances were compared both to another extraction-free system and a standard diagnostic test.
Methods. From August 2020 to April 2021, 1190 patients presenting to the 2 spoke Emergency Departments of the Spedali Civili Hospital were included in the study. Each patient benefited from two nasopharyngeal swab samplings. One for the two swab was evaluated on VitaPCR™ SARS-CoV-2. Results were systematically compared to those obtained using RT-PCR Simplexa™ COVID-19 Direct (Diasorin) and to a standard diagnostic system that use MGISP-960 system. For the latter platform RNA extraction was performed with SARS-CoV-2 MGIEasy Nucleic Acid Extraction Kit (EuroClone) and amplification was made with SARS-CoV-2 ELITe MGB® KIT (ELITechGroup). VitaPCR™ has single position with a 30-min TAT; Diasorin has 8 positions with a 110-min TAT and MGISP-960 plus Elite MGB® amplification is a high throughput method with a 4 hours TAT.
Results and conclusions. We have analyzed 1190 patients, 46% of them (N=553) were processed at Montichiari site (Site A) and 54% (N=637) were evaluated at Gardone Val Trompia hospital (site B). Site A have run VitaPCR™ in comparison with the MGISP-960 plus Elite MGB® amplification standard assay whereas results from site B were compared with data obtained from RT-PCR Simplexa™ COVID-19 Direct (Diasorin).
Among the 1190 analyzed samples, 258 were positive and 898 negative using both assays, while 34 were discordant: 27 resulted as negative and 7 as positive samples. Interestingly, more than 80% of discordant samples showed high Ct values (greater than 33), attesting for the presence of low viral load. Therefore, the lack of presumptive positive samples falls into a category of patients with a presumable low infectivity.
The great value of a very short delay in supporting clinicians taking decision for emergency patients, together with acceptable performances, make this method eligible for small emergency department needs.

P81 - OC 8

Vaginal self-collected vs cervical clinicians collected samples for cervical cancer screening

C. Sani[1], G.F antacci[1], E. Burroni[1], S. Bisanzi[1], G. Pompeo[1], F. Cellai[1], I. Paganini[1], A. Iossa[2], C. Di Pierro[2], C.B. Visioli[3], C. Nicolai[4], A. Lombardi[4], F. Carozzi[5]

[1] Regional Laboratory of Cancer Prevention; ISPRO, Florence, Italy - [2] Screening and secondary prevention, ISPRO, Florence, Italy - [3] Clinical Epidemiology, ISPRO, Florence, Italy - [4] Tuscany North-West Local Health Unit - [5] Università degli Studi di Firenze - Professore a contratto.- Corso di Laurea Magistrale Biologia Molecolare ed Applicata

Aim of the study. Substantial reduction in worldwide cervical cancer mortality is going to be realized through two crucial strategies: an effective HPV vaccination campaign and the implementation of cervical cancer screening programs. A key feature of a self-collected HPV testing strategy is the move of the primary screening activities from the clinic to the community with the efforts to increase the affordability and availability of HPV DNA tests. The aim of this study, conducted in ISPRO Florence, was to evaluate whether self-sampling can increase attendance of non responder-women, to guarantee a comparable clinical sensitivity with the HPV test performed on clinician samples; to evaluate the performance of molecular triage in a screening setting; and to investigate the acceptability of self-collection respect of clinician-collection of the samples. The study is founded by Regional Institute of Cancer (public health institution).

Methods. Women aged 34–64 years, residents in Florence, Massa Carrara and Viareggio who have not responded to the previous screening invitation, were eligible for the self sampling project.

A total of 8800 women were randomly assigned to one of the following arms:

- control arm (4400 women) with a standard invitation to perform HPV test (clinical usual care);
- two intervention arms (2200 women for each arm): a group received at home the “dry” self-sampler device FLOQSwab® (Copan); another group received at home the “wet” self-sampler device FLOQSwab® (Copan) and 1 ml of preservation solutions mSWAB ® (Copan).

We used an intent-to-treat analysis to compare invitation compliance between the control and intervention arms. Women in the intervention arms received a satisfaction questionnaire. In the control arm, only HPV positive women with positive triage cytology were sent to colposcopy, according to the standard protocol. In the intervention arms, HPV positive women were sent directly to colposcopy and a new cervical sample were taken to perform Pap test Triage, genotyping, methylation and to repeat HPV test.

Results and conclusions. Compliance to the invitation within 90 days, according to intention-to-treat analysis, was 18.1%, 13.5% and 13.9% for control, “Dry” and “Wet” arms, respectively. The relative compliance was: 0.75; [95% Confidence Interval (95%CI): 0.66-0.85] and 0.77 (95%CI: 0.68-0.87) for the dry and wet devices, respectively, therefore, 25% and 23% lower than standard recall, respectively. The analytical sensitivity and reproducibility of self collected vs conventional samples were comparable. The HPV positivity rate was: 10.7%, 11.7% and 8.6% for “Dry”, “Wet” and control arms, respectively.

Concordance (K Cohen) between self collected and clinicians samples collected during colposcopy was 0.705. The colposcopy referral rate was 8.7% for self collected samples, 6.3% for clinicians samples (HPV HR positive) and 2.4% for the standard protocol. No significant differences were found in genotyping and methylation data between self and clinicians samples, probably due to the low number of CIN 2+ lesions in study population. A total of 560 satisfaction questionnaires were completed: 90.9% found that the self-collection system was very easy/easy to use; 97.6% found easy to do and understood the instructions; and 92.6% have no pain, discomfort or bleeding related to the self collection. Finally, 81.1% of women preferred self-collection method rather than cervical clinician collection.
Our study did not confirm greater adherence to self sampling than the standard invitation. The reasons are still to be investigated. Although, the results confirmed the potential of HPV self–collection testing demonstrating its acceptability and a good agreement between self and clinician-sampled HPV test. This strategy assumes particular relevance in the Covid-19 era, guaranteeing adherence to screening but limiting patients' access to points of care.

P82

In-house validation of a real-time RT-qPCR and a droplet digital RT-PCR assay gor SARS-CoV-2 detection in nasopharyngeal swabs

B. Pierri[1,2], Y.T.R. Proroga[3], F. Capuano[3], P. Cerino[1], A. Mancusi[3], S. Girardi[3], L. Vassallo[1], M. Tafuro[1], G. La Rosa[4], F. Beikpour[5,6], S. Di Pasquale[6], T. Vicenza[6], L. Cozzi[6], E. Suffredini[6]

[1] Centro di Referenza Nazionale per l'Analisi e Studio di Correlazione tra Ambiente, Animale e Uomo, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici (NA), Italy - [2] Department of Medicine, Surgery and Dentistry 'Scuola Medica Salernitana', University of Salerno, Baronissi (SA), Italy - [3] Department of Food Microbiology, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici (NA), Italy - [4] Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy - [5] Department of Veterinary Medicine, University of Bari 'Aldo Moro', Bari, Italy - [6] Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) emerged in December 2019 in China, and subsequently spread worldwide, till the recognition of COVID-19 as a pandemic by WHO. In a previous study a real-time RT-qPCR targeting the nsp14 of SARS-CoV-2 genome region (3′-to-5′ exoribonuclease, ExoN) was developed for the detection and quantitation of this virus in wastewaters [1]. Aim of the present study was the assessment of the performance of this RT-qPCR assay and of a droplet digital PCR (ddPCR) based on the same primers/probe combination for the diagnostic of SARS-CoV-2 in nasopharyngeal swabs.

The clinical specimens were taken with the COPAN UTM-RT system within the GEN-COVID study protocol. A total of 258 nasopharyngeal swabs collected between April and July 2020 in Campania Region were selected for the study, covering the variability of the Ct values obtained with the reference diagnostic RT-PCR assay (Real Quality RQ-2019-nCoV kit, AbAnalitica). RNA was extracted using a magnetic bead-based protocol (GeneQuality X120 Pathogen kit) according to manufacturer's instructions. RNA was analyzed with the aforementioned reference assay (targeting the RdRp gene of SARS-CoV-2 and gene E of Sarbecovirus), with the developed real-time RT-qPCR and with the corresponding ddPCR, the latter performed on a Bio-Rad QX200 system using the One-Step RT-ddPCR Supermix (BioRad). Conflicting results were further investigated by analyzing the samples with two additional protocols (the CDC protocol for N1 and N2 [2], and a RT-nested-PCR for the spike region [3] followed by sequencing). The performance characteristics, i.e. sensitivity (Se), specificity (Sp) and accuracy (Ac), were calculated for the methods.

Agreement of results between the reference diagnostic assay and the two assays based on nsp14 was achieved on 226 samples (156 positive and 70 negative); further 8 samples were positive only using the reference assay and the nsp14 real-time RT-qPCR. Considering the identifications obtained with the additional protocols on the 24 conflicting results, the calculated performance characteristics (and their 95%C.I.) were: Se 100.0% (97.4-100.0), Sp 98.7% (92.1-100.0), and Ac 99.6% (97.5-100.0) for the real-time RT-qPCR nsp14

assay, Se 92.4% (87.4-95.6), Sp 100.0% (94.2-100.0), and Ac 94.7% (91.1-97.0) for the ddRT-PCR assay.
The results of the study support the use of the nsp14 real-time RT-qPCR and ddPCR assays for the detection of SARS-CoV-2 in nasopharyngeal swabs.

[1] La Rosa et al., 2021 https://doi.org/10.1016/j.scitotenv.2020.141711
[2] CDC https://www.fda.gov/media/134922/download
[3] Shirato et al., 2020 https://doi.org/10.7883/yoken.JJID.2020.061

P83

Prevalence analysis of respiratory viruses during SARS-CoV-2 pandemic in a hospital in north-east Italy

D. Treggiari, C. Piubelli, E. Pomari, F. Formenti, R. Silva, and F. Perandin

Department of Infectious, Tropical Disease and Microbiology, IRCCS Sacro Cuore Don Calabria Hospital, Negrar di Valpolicella, Verona, Italy

Aim of the study: SARS-CoV-2 virus spread in Italy and the rest of the northern hemisphere during the influenza epidemic season 2019/2020 and persisted even during the season 2020/2021. Considering only symptoms, SARS-CoV-2 infection cannot be distinguished from other respiratory viruses, therefore the differential diagnostic analysis was mandatory (i.e. molecular diagnostic tests). Here, we aimed to describe retrospectively the prevalence of influenza (INF-A/B) and respiratory syncytial (RSV-A/B) viruses observed in our hospital during the COVID-19 pandemic 2020/2021 season and to compare our data with national and global virus surveillance data.
Methods used: This study is an observational retrospective analysis of INF-A/B and RSV-A/B molecular diagnostic test collected from hospitalized patients and outpatients referring to IRCCS Sacro Cuore Don Calabria Hospital (Negrar di Valpolicella, Verona, Italy) for nasopharyngeal swab sampling between November 2019 and March 2021. The specimens were screened for respiratory viruses using GeneXpert Xpress Flu/RSV multiplex (Cepheid) during season 2019/2020, whereas Bosphore SARS-CoV-2/Flu/RSV kit (Anatolia geneworks) during season 2020/2021. Descriptive statistics was generated to define the study population and $p<0.05$ was defined as the level of significance.
Results and conclusions: Out of 6618 respiratory specimens, 182 (2.7%) came from season 2019/2020, whereas 6436 (97.2%) from season 2020/2021. According to age, we observed the highest proportion of population for 2019/2020 season in patients aged 0–10 years (27.2% female, 33.6% male, $p<0.001$) and in aged 60-80 years (41.5% female, 36.9% male, $p=0.10$). For 2020/2021 season, the highest proportion of population was in patients aged 30-80 years (84.0% female, 84.2% male, $p<0.01$). The most common ethnicity was Italian, represented by 94% patients. In the course of the season 2020/2021, none of the samples tested resulted positive to both INF-A/B and RSV-A/B regardless patient age, sex or season week number. For comparison, we retrieved non-SARS-CoV-2 respiratory viruses data collected in our site during 2019/2020 season, from week 46/2019 to week 14/2020. Overall, 19 out of 182 nasopharyngeal swabs (10.4%) were found to be positive for at least one INF-A or B virus, whereas 27 out of 182 swabs (14.8%) were found to be positive for RSV-A/B. The percentage of positive samples for INF-A/B was highest in week 8/2020 (3.3%) and lowest in week 10/2020 (0.5%). The highest prevalence of INF-A/B infection was observed in the age 0-4 years (4.9%) and the lowest rates of infection were in patients aged 18-65 (1.0%) years. It should be noted that in week 10/2020 Italy started nationwide lockdown measure and school closure (9th March 2020). Therefore a sharp decrease of positive cases was observed after the lockdown (week 10) in which the circu-

lation of INF-A/B detected was significantly different compared to previous weeks ($p<0.05$). Analyzing prevalence of RSV-A/B for the same periods, we did not observe a more relevant prevalence of RSV in overall population compare to INF-A/B. However, between the two viruses, RSV-A/B was the most prevalent virus detected in the 0-4 years cohort population ($p<0.05$). In addition, we observed a decrease in RSV-A/B positive cases after week 10 due to lockdown implementation.

Our findings, clearly demonstrated the extremely low prevalence of INF-A/B and RSV-A/B among hospitalized patients and outpatients referring to our hospital. This was consistent with national and global data which also reported the lowest ever INF-A/B prevalence recorded by the surveillance network (InfluNet and FluNet). Mitigation measures and travel restriction may have played an important role in temporarily stopping circulation of respiratory viruses.

P84

Assessment of HIV-1 DNA quantification methods in the italian HIV DNA network

I. Vicenti[1], F. Dragoni[1], A. Giannini[1], F. Saladini[1], O. Turriziani[2], D. Di Carlo[2], S. Belmonti[3], S. Di Giambenedetto[3,13], F. Lombardi[3], F. Ceccherini-Silberstein[4], R. Scutari[4], C. Alteri[5], M. Antonello[5], A. Lai[6], A. Bergna[6], S. Racca[7], A. Ardemagni[7], A. Casabianca[8], C. Orlandi[8], I. Bon[9], A. Bertoldi[9], P. Bagnarelli[10], L. Di Sante[10], I. Abbate[11], G. Rozera[11], M.R, Capobianchi[11], S. Paolucci[12], F. Giardina[12], M. Zazzi[1]

[1] Department of Medical Biotechnologies, University of Siena - [2] Department of Molecular Medicine, Sapienza University of Rome - [3] Department of Safety and Bioethics, Infectious Disease Section, Catholic University of Sacred Heart, Rome; [4] Department of Experimental Medicine, University of Rome Tor Vergata, Rome - [5] Department of Oncology and Hemato-oncology, University of Milan, Milan - [6] Department of Biomedical and Clinical Sciences L. Sacco, University of Milan, Milan - [7] Laboratory of Microbiology and Virology, IRCCS San Raffaele Scientific Institute, Milan - [8] Department of Biomolecular Sciences, University of Urbino "Carlo Bo", Urbino, PU - [9] Microbiology Section, Department of Experimental, Diagnostic and Specialty Medicine, School of Medicine, University of Bologna - [10] Virology Unit, Department of Biomedical Sciences and Public Health, Polytechnic University of Marche, Ancona - [11] Virology and Biosafety Laboratories Unit, National Institute for Infectious Diseases IRCCS L. Spallanzani, Rome - [12] Molecular Virology Unit, Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, Pavia - [13] Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome Italia, UOC Infectious Disease

Aim of the study. Total cell-associated HIV-1 DNA is considered a surrogate marker of the HIV-1 reservoir, however, certified systems for its quantification are not available. The "Italian HIV DNA network" was launched to create a collaborative network to validate HIV-1 DNA quantification methods in use at University and Hospital labs, including homebrew systems and commercial kits not yet marked for in vitro diagnostic use.

Materials and Methods. As participants to the first external quality assurance program, 12 labs received from the Coordinator Lab a comprehensive panel of HIV-1 DNA standards, reconstructed blood samples (RBS) and DNA extracts from different HIV-1 subtypes. Detailed instructions were provided to each Lab, concerning the stocks manipulation and the number of dilutions and replicates to test. Results obtained by each lab were analyzed at Coordinator Lab. Reference values for the RBS and subtypes were defined as the median value of the measurements provided by all the participating labs. Data are reported as mean copies per test or copies per million cells ± SD or median (IQR) values, as indicated. Statistical analysis was performed with SPSS version 20.0.

Results and conclusions. Of the 12 labs, 4 performed homebrew real-time (rt) PCR, 2 different commercial rtPCR kit, 3 digital droplet (dd) PCR, and 3 both homebrew rtPCR and ddPCR. Based on Probit analysis, performed on 4 replicates for each 10-fold dilution series (5 to 50000 nominal standard copies), the global 95% hit rate was 3.5 ± 1.3. The R2 value for linear regression was 0.993 ± 0.020. The ratio between nominal and measured values was 1.5 ± 1.4, 1.6 ± 1.2, 1.7 ± 1.2 and 2.0 ± 1.4 for the 2560, 320, 40 and 5-copy standards and 1.4 ± 1.0, 1.4 ± 1.0, 1.5 ± 1.4 and 2.2 ± 2.6 for the 16949, 1801, 605 and 133-copy/million RBS, respectively. The coefficient of variation (CV) was 14.7 ± 6.5, 15.7 ± 7.4, 24.2 ±

9.7, 48.3 ± 18.7 for the 2560, 320, 40 and 5-copy standards and 31.6 ± 22.9 and 38.8 ± 22.7, 61.8 ± 38.3 and 65.7 ± 28.6 for the 16949, 1801, 605 and 133-copy/million RBS, respectively. A significantly higher CV was scored for rtPCR vs. ddPCR with the 40-copy (25.9 ± 9.8 vs. 17.3 ± 10.1, P=0.026) and 5-copy standard (55.5 ± 33.2 vs. 37.4 ± 28.0, P=0.088) but not with RBSs. The HIV-negative clinical sample was scored as positive by 3 labs (16/54 replicates), 2 using ddPCR and one using a commercial rtPCR, with a mean value of 1149 ± 1243 HIV-1 DNA copies per million cells. Importantly, HIV-1 subtypes were frequently missed (CRF01_AE by three lab) or underestimated by >1 log (subtypes A, C, D, F by one lab; CRF01_AE by one lab; CRF02_AG by one lab). The overall performance was excellent with HIV-1 DNA standards and good with the RBS, however the detection and quantification of different HIV-1 subtypes needs to be improved.

P85
Association between HSV-1 and Parkinson's disease

S. Agostini[1], R. Mancuso[1], L.A. Citterio[1], A.S. Costa[1], M. Meloni[2], M. Clerici[1]

[1] Laboratory of Molecular Medicine and Biotech, IRCCS Fondazione Don Gnocchi – ONLUS - [2] Centro Diagnostico e Riabilitativo per la Malattia di Parkinson e i Disordini del Movimento, IRCCS Fondazione Don Gnocchi – ONLUS - [3] Department of Pathophysiology and Transplantation, University of Milan

Parkinson's disease (PD) is a progressive nervous system disorder that affects movement. The most common symptoms of the disease are resting tremors, unstable posture, bradykinesia, rigidity, and non-motor symptoms, as dysphagia. PD is characterized by the loss of neurons in the substantia nigra, in association with the accumulation of misfolded and aggregated forms of α-Synuclein protein. The etiology of PD is still unknown, but epidemiological studies suggest a strong association between genetics and environment in the onset and development of the disease. Regarding environment factors, HSV-1 is one of the more suspected to have a role in the disease. Paired Immunoglobulin-like type 2 receptor alpha (PILRA) is a cell surface inhibitory receptor expressed on innate immune cells down-regulating inflammation. The PILRA rs1859788 A/G single nucleotide polymorphism (SNP) causes the 78 Glyicin (G) substitution, coded by G allele, with Arginine (R), coded by the A alleles. This SNP is an AD risk locus. PILRA plays a key role for HSV-1 infection, as the virus binds this cellular protein to infect cells, and, in presence of R78 the viral capacity to infect macrophage is lower in comparison with G78, suggesting that R78/R78 people are less susceptible to infection and, even in case of infection, are able to block HSV-1 recurrence and reactivation. Recently we found that the rs1859788 polymorphism of *PILRA* gene is associated with the HSV-1 specific IgG antibodies in Alzheimer's disease (AD), suggesting that HSV-1 infection and the host genetic pattern is fundamental on the role of HSV-1 in AD.
The aim of the present work is to verify whether HSV-1 and HSV1-related host genes are associated with PD. The HSV-1, CMV and HHV-6 antibody titers were measured in serum of 51 PD patients and 73 HC by ELISA. For the same subjects the genotype distribution of *PILRA* rs1859788 polymorphism was analyzed. The HSV-1 seropositivity was higher in PD (98 %) than HC (92 %), whereas for the other two herpesviruses it was the opposite (PD: 88 %; HC: 98 % for CMV and PD: 56 % and HC: 67 % for HHV-6), but without statistical difference for any virus. Regarding the antibody titers, the HSV-1 levels were significantly higher in PD compared to HC (p=0.05). No significantly difference was observed regarding the antibody titers of CMV and HHV-6 between the two groups. Regarding the *PILRA* genotyping, the rs1859788 polymorphism was not differentially distributed between PD and HC, but a trend of significativity (p=0.06) was found regarding the minor allele A (AA+AG), resulted more frequent in PD (67.4 %) compared to HC (50 %). Moreover, splitting the study population in relation with gender, if no differences were observed among female, the frequency was significant different in male PD compared to male HC (p=0.05), with, again, the minor allele A (AA+AG) statically more frequent in male PD (64.7 %) compared to male HC (37.1 %) (p=0.036). No relation was found between HSV-1 IgG and *PILRA* genotype. In conclusion, results herein confirm the possible involvement of HSV-1 in the Parkinson's disease, and indicate an intriguing interaction between *PILRA* gene and the pathology, in relation either with HSV-1 infection or with the inflammation typical of the disorder.

P86
Overexpression of endogenous retroviruses in children with celiac disease

P.A. Tovo[1], A. Opramolla[2], A. Pizzol[2], G. Calosso[2], V. Daprà[3], I. Galliano[3], C. Calvi[3], M. Pinon[2], F. Cisarò[2], C. Rigazio[2], P.L. Calvo[2], M. Bergallo[1,3*]

[1] Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy - [2] Pediatric Gastroenterology Unit, Regina Margherita Children's Hospital, Azienda Ospedaliera-Universitaria Città della Salute e della Scienza, Turin, Italy - [3] Pediatric Laboratory, Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy

Background: Human endogenous retroviruses (HERVs) represent 8% of our genome. Although no longer infectious they can regulate transcription of adjacent cellular genes, produce retroviral RNAs and encode viral proteins that can modulate both innate and adaptive immune responses. Based on this, HERVs have been studied and proposed as contributing factors in various autoimmune disorders. Celiac disease (CD) is considered an autoimmune disease, but HERV expression has not been studied in celiac patients.
Aim: To assess the transcription levels of pol genes of HERV-H, -K, and -W and of their TRIM28 repressor in WBCs from celiac children and age-matched control subjects.
Methods: A PCR real time Taqman amplification assay was used to evaluate HERV and TRIM28 transcripts with normalization of the results to glyceraldehyde-3-phosphate dehydrogenase.
Results: The RNA levels of pol genes of the three HERV families were significantly higher in WBCs from 38 celiac patients than from 51 control subjects. TRIM28 transcription was comparable between the two study populations.
Conclusions: Present results show, for the first time, that pol genes of HERV-H, -K, and -W are overexpressed in patients with CD. Given their proinflammatory and autoimmune properties this suggests that HERVs may contribute to the development of CD in susceptible individuals.

Key words: Celiac disease, human endogenous retroviruses, autoimmune diseases, children.

P87 - OC 18

Molecular features of the measles fusion complex: infection and spread in the central nervous system

F.T. Bovier[1,2,3], B. Corneo[4], G. Franci[5], M. Galdiero[6], N.A.P. Lieberman[7], A.L. Greninger[7], M. Porotto[1,2,3]

[1] Department of Pediatrics, Columbia University Vagelos College of Physicians and Surgeons, New York, NY, USA - [2] Center for Host–Pathogen Interaction, Columbia University Vagelos College of Physicians and Surgeons,New York, NY, USA - [3] Department of Experimental Medicine,University of Campania “Luigi Vanvitelli,” Caserta, Italy - [4] Stem Cell Core Facility, Columbia University Vagelos College of Physicians and Surgeons, New York, USA - [5] Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana",Salerno, Italy - [6] Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy - [7] Department of Laboratory Medicine, University of Washington Medical Center, Seattle, WA, USA

Aim of the study. Measles virus (MeV) infection causes an acute systemic disease, associated in some instances with central nervous system (CNS) infection leading to lethal neurological disease. A clinical isolate of MeV bearing a single amino acid change -L454W- in the envelope viral fusion protein (F) was identified in the brain of two patients who died of MeV CNS infection. We hypothesized that this mutation conferred an advantage over wild-type virus in the CNS, contributing to brain dissemination in these patients.
Methods. We tested our hypothesis in human induced pluripotent stem cell-(hiPSC)-derived brain organoids with the combination of transcriptome analysis.
Results and conclusions. In human brain organoids derived from three separate hiPSC, the virus bearing the F L454W spread more efficiently than wild type in this model. The increased spread is associated with an enhanced innate immunity response. Next-generation sequencing reveals a positive selection of F L454W in these models of CNS infection. Our data suggest that this single mutation in MeV F renders the virus more neuropathogenic, allowing it to propagate in the face of the innate immune response.

P88 -OC 39

Binding to PI(4,5)P2 is indispensable for secretion of B cell clonogenic HIV-1 matrix protein P17 variants

A. Bugatti[a], F. Caccuri[a], F. Filippini[a], C. Ravelli[b], A. Caruso[a]

[a] Section of Microbiology, Department of Molecular and Translational Medicine, University of Brescia medical School, Brescia, Italy - [b] Section of Experimental Oncology and Immunology, Department of Molecular and Translational Medicine, University of Brescia medical School, Brescia, Italy

Introduction: HIV-1 matrix protein p17 is released from infected cells and can be detected in blood at nanomolar concentrations in the blood of patients even under successful combined antiretroviral therapy (cART). Extracellularly, p17 deregulates the biological activity of different immune cells. Secretion of p17 is dependent on the interaction of Gag precursor polyprotein (Pr^{55}Gag) with phosphatidylinositol-(4,5)-bisphosphate (PI(4,5)P2) and its subsequent cleavage from Pr^{55}Gag by cellular aspartyl proteases. Recently, we demonstrated that p17 variants (vp17s) derived from non-Hodgkin lymphoma (NHL) tissues of HIV-1+ patients possess B cell growth-promoting activity by activating the Akt signaling pathway.

Aim of the study: Due to the partial unfolding state of vp17s we asked whether interaction of Pr^{55}Gag carrying vp17s with PI(4,5)P2 occurs, leading to Pr^{55}Gag cleavage by cellular or viral proteases and secretion as biologically active proteins.

Methods used: Jurkat T-cells, were transiently nucleofected with pNL4-3 plasmids expressing two vp17s, namely NHL-a101 and NHL-a102, characterized by different amino acid insertions at the C-terminal region. Vp17s secretion was then quantified by cellular ELISA assay. To determine if vp17s are secreted in their biologically active form, we performed a single cell cloning assay on Raji B-cells and a wound healing assay on endothelial cells. Cellular localization of vp17s was performed by an indirect immunofluorescence assay and confocal microscopy analysis, while a SPR assay was used to evaluate the binding of vp17s to heparan sulfate proteoglycans (HSPGs).

Results: In this study, we show that both vp17s are secreted from cells either in the absence or in the presence of active HIV-1 protease. Secretion of biologically active vp17s also occurred in HIV-1-infected Jurkat T-cells during the active phase of viral replication. Binding to PI(4,5)P2 was indispensable for the subsequent cleavage of p17 and vp17s from the precursor Pr^{55}Gag polyprotein and their secretion. We also demonstrated that HSPGs expressed at the cell surface were involved in tethering of different viral matrix proteins.

Conclusions: This finding stimulates at investigating whether p17 tethered on the surface of HIV-1 reservoirs may represent a likely target for immune-mediated killing.

P89
Role of Immediate Early Proteins ICP0 and ICP27 in Apoptotic pathway

M. Buratto[1], M. Sicurella[1], A. Stanchina[1], A. Caproni[1], F. Salvatori[1], M. Pappadà[1], R. Fontana[1,2], R. Manservigi[2], P. Marconi[1]

[1] Department of Chemical, Pharmaceutical and Agricultural Sciences (DOCPAS), University of Ferrara - [2] Department of Life Sciences and Biotechnology, University of Ferrara, Italy

Herpes simplex virus (HSV) type 1 and type 2 are human neurotropic, host-adapted pathogens whose lifestyle is based on a long-term dual interaction with the infected host that can establish both lytic and latent infections. These viruses establish latent infections in sensory ganglia; such infections can undergo reactivations that can be either asymptomatic or symptomatic. In this case, cold sores, keratitis, blepharitis, meningitis, encephalitis, genital infections or systemic and severe conditions in immune compromised patients can be observed. Dissemination is very common in human communities owing to latent infection, periodic reactivation, and asymptomatic virus shedding. HSV has evolved in the manner to modulate apoptosis in different cell types with anti- apoptotic genes to promote the generation of new viral progenies or with pro-apoptotic genes to promote cell death to favor viral release and shedding.
The aim of our research was to evaluate the modulation of the expression of genes and respective proteins involved in apoptotic pathway after HSV1 infection. Nevertheless we studied the possible role of HSV1 immediate early genes ICP0 and ICP27.
We analyzed gene and protein expression in two cell strains: HRPE (Human Retinal Pigment Epithelial cells) and SHSY5Y (bone marrow cell line, derived from neuroblastoma, used as model for neuronal function and differentiation). All cells were infected with wild type HSV1 (strain F), HSV1 ICP0- and ICP27- (two mutants, deleted for ICP0 or ICP27 genes, respectively) and no infected cells were used as controls. We retrotranscribed total mRNA at 4 and 8 hours post infection (including non-infected cells) and performed relative qPCR using TaqMan™ Array Human Apoptosis Panel Assay, a 384 wells plate with 93 apoptosis related genes and 3 housekeeping genes spotted. The+ data were confirmed by looking at the protein expression by Western blot.
Results indicate that in HRPE infected cells, BIRC1, BIRC2, BIRC7 and BIRC8 genes were over expressed 8h after infection, while BIRC2, CFLAR, NFKB1 and NFKB2 genes were upregulated in SHSY5Y cells 4 hours after infection. Interestingly all upregulated HRPE genes belong to intrinsic apoptotic pathway, and are involved in preventing the Apoptosome to activate caspase 9, or blocking the activity of caspase 9 itself. The results on gene expression were also confirmed by the increase of the respective proteins. Moreover, many upregulated genes in SHSY5Y cells belongs to extrinsic apoptotic pathway, and are involved in promoting cells survival or possibly preventing caspase 8 activity. Therefore, in the SHSY5Y cell line infected with the mutants lacking ICP0 or ICP27 we observed the cut and, consequently, the activation of pro caspase 8, while this was not seen in non-infected or infected with the wt virus, confirming the role of these very early proteins in blocking the extrinsic apoptotic pathway during the early stages of infection.

P90 - OC 24

Dissecting lyssavirus-host interaction in the Syrian Hamster model

M. Castellan*, M. Zorzan*, R. Mura#, L. Brandao#, A. Pastori*, S. Leopardi*, S. Crovella§, P. De Benedictis*

* Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy - # Federal University of Pernambuco (UFPE), Brazil - § Department of Biological and Environmental Science – Qatar University, Doha, Qatar

Aim of the study. Understanding the molecular links between a virus and the host immune system represents a key point for the prevention and treatment of infectious diseases. Rabies virus (RABV) is the prototype of the Lyssavirus genus, which accounts for more than 17 identified species, mostly adapted to specific bat ecological niches. Lyssaviruses are generally transmitted through saliva, following a biting event, and are expected to trigger a 100% fatal neurological disease that can be spread from reservoir species (like bats) to other mammals, including humans[1]. Of note, what is known about lyssaviruses has been deduced from RABV that, differently from the other mostly unknown members of the genus, is widely widespread among mammals. The Syrian hamster *(Mesocricetus auratus)* has been employed as an animal model to study human and animal diseases caused by emergent pathogens. Although not extensively characterized, it has peculiar features related to the immune system, which makes it more similar to humans rather than to the well-established mouse model[2]. Currently, host response against lyssaviruses has only partially been unveiled, exploiting the mouse as animal model and the intracerebral way as preferred route of viral challenge[3]. The available data regarding lyssaviruses behaviour in the infected host are associated to RABV, assuming all lyssaviruses perform the same. Therefore, the main purposes of this study were: (i) to further characterize the Syrian hamster as an animal model to investigate the host-pathogen interaction and (ii) to investigate and compare the immune response elicited by different lyssavirus species (i.e. RABV vs divergent lyssaviruses).
Methods. Eight-week old Syrian hamster females were infected by intramuscular route with Duvenhage lyssavirus (DUVV – a bat associated lyssavirus circulating in Africa and responsible for rare human infections) and a dog-associated RABV from Western Africa, often spilling over to humans and other accidental hosts; clinical score was registered in the three weeks after infection. At the onset of symptoms, animals were sacrificed and brain immediately collected. Half of the brain was formalin fixed for immunofluorescence examination, while the remaining half was stored for RNA-seq analysis. The obtained RNA-seq reads were aligned on the partially annotated Syrian hamster genome publicly avaialble[4] to identify differentially expressed genes between infected and control animals. Real-time PCR and immunofluorescence were performed to validate RNA-seq results.
Results and conclusions. The transcriptomic analysis revealed activation of the classical pathways related to lyssaviruses infection[3] both in RABV and DUVV-infected animals, underlying a comparable behaviour. We observed the activation of the type-I IFN response and a clear upregulation of inflammosome components and activators of the pyroptosis,

with the key effector Gasdermin-D being up regulated in infected animals. Moreover, the obtained RNA-seq data were also employed to improve the Syrian hamster genome annotation. To validate the transcriptomic results in such a poorly characterized animal model, we also developed *ad hoc* protocols to identify immune markers in the Syrian hamster model. In conclusion, we describe a mammalian system of lyssavirus infection, closely simulating the natural disease progression and we set up experimental procedures that could be used as standard protocols for future studies on other emergent viral pathogens.

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P91

A novel nanobret protease sensor to sense HIV-1 PR activity in living cells

M. Centazzo, H. Ghassabian, G. Alvisi

Department of Molecular Medicine, University of Padova, Padova Italy

HIV-1 is one of the most relevant viral pathogens of our times, and after more than 40 years since its discovery it is still impossible to clear the infection from affected individuals. Despite the essential role of viral protease in virion maturation and viral polyprotein processing, a few aspects of its activity remain overlooked. To this end we developed a new NanoBRET sensor, which allows to monitor HIV-1 protease activity in real time in living cells. In addition, such novel is endowed with a threefold increase in signal to noise ratio a as compared to a traditional BRET based sensor employing Renilla luciferase and YFP. Expression of wild type but not catalytically inactive protease resulted in a significant decrease of the BRET ratio, and allowed validation of the inhibitory activity of several well-known protease inhibitors. The possible applications of this system are numerous, and include (i) validation of different putative HIV-1 cleavage sites in cells to better characterize host protein targeted by the protease; (ii) real time-live cells kinetic analysis of incoming protease activity in HIV-1 virion upon infection; (iii) screening and identification of new antiviral protease inhibitors. Additionally, the design of this molecular probe makes it possible to rapidly exchange the protease-cleavable linker, thus allowing a great flexibility and adaptability to other viral proteases. As a proof of principle, we developed different probes sensitive to both the SARS-CoV-2 proteases, putting this kind of design on the forefront of current research in virology.

P92

Chronic HCV infection is associated with overexpression of human endogenous retroviruses that persists after drug-induced viral clearance

V. Daprà[3], P.A. Tovo[1*], S. Garazzino[2], C. Alliaudi[1,3], C. Calvi[1,3], P. Montanari[1,3], I. Galliano[1,3], M. Bergallo[1,3*]

[1] Department of Pediatric Sciences and Public Health, University of Turin, Piazza Polonia 94, Turin, Italy - [2] Department of Pediatrics, Infectious Diseases Unit, Regina Margherita Children's Hospital, Piazza Polonia 94, Turin, Italy - [3] Pediatric Laboratory, Department of Pediatric Sciences and Public Health, University of Turin, Turin, Italy

Aim of the study: Chronic HCV infection is associated with several hepatic and extra-hepatic complications, including cancers and autoimmune disorders, whose frequency is reduced but not abolished after drug-induced viral clearance. The causes of these complications and of their persistence are ill-defined. Human endogenous retroviruses (HERVs) are remnants of ancestral infections and constitute 8% of the human genome. Most HERV elements are inactive, but some are transcribed. HERV overexpressions are associated with many cancers and autoimmune diseases with putative pathogenetic role. Several viral infections induce HERV activation, but there are no studies in HCV infected subjects.
Methods: We assessed, through a PCR real time amplification assay, the transcription levels of pol genes of HERV-H, -K, and -W and of their repressor TRIM28 in WBCs of vertically infected children both before and after direct acting antivirals (DAA) therapy.
Results: The results documented significantly higher expressions of HERV-H-pol and HERV-K-pol, not of HERV-W-pol, in HCV infected subjects as compared to age-matched controls. HERV RNA levels remained unchanged after DAA-driven viral clearance. No significant variations in transcription levels of TRIM28 were observed in infected subjects.
Conclusions: Our findings demonstrate HERV-H-pol and HERV-K-pol overexpressions in subjects with chronic HCV infection, without variations after positive response to DAA, suggesting that this might justify their predisposition to cancers and autoimmune disorders that persists after DAA-induced resolution of viremia.

Keywords: Hepatitis C virus infection, human endogenous retroviruses, viral clearance, cancers, autoimmune diseases.

P93 - OC 19

Nrf2 and G6PD as key players in modulating cell antioxidant response and influenza virus replication

M. De Angelis[a], D. Amatore[b], P. Checconi[c] , G. De Chiara[d], A.T. Palamara[a], L. Nencioni[a]

[a] Department of Public Health and Infectious Diseases, Sapienza University of Rome, Laboratory affiliated to Istituto Pasteur Italia-Fondazione Cenci Bolognetti, P.le Aldo Moro 5, 00185, Rome, Italy - [b] Scientific Department, Army Medical Center, Rome, Italy - [c] Department of Human Sciences and Promotion of the Quality of Life, IRCCS San Raffaele Pisana, San Raffaele Roma Open University, Via di Val Cannuta 247, 00166, Rome, Italy - [d] Institute of Translational Pharmacology, National Research Council, Rome, Italy

Respiratory viruses, like influenza virus, use different strategies to manipulate host cell machinery to their advantage, including the modulation of intracellular redox state, mainly characterized by an increase of Reactive Oxygen Species and a decrease of intracellular glutathione (GSH) content. One of the key players for intracellular redox homeostasis is Nuclear factor erythroid 2–related factor 2 (Nrf2), known to be activated under a stressful condition to transcribe antioxidant genes. Glucose-6- phosphate dehydrogenase (G6PD) is an enzyme involved in glucose metabolism and GSH regeneration, whose transcription is under Nrf2 control. G6PD is also regulated at the activity level by SIRT2, a NADPH-dependent deacetylase that acts at the lysine regulatory site.

Different viruses, including human coronavirus HCoV-229E, decrease G6PD expression to favour their replication. Our preliminary results also showed that the expression and the activity of G6PD were decreased during influenza virus infection, but the mechanisms underlying this down- modulation, its dependence by Nrf2 activity and whether these pathways contribute to virus- induced oxidative stress and viral replication remain to be clarified.

Our study was then aimed at evaluating the contribution of G6PD in influenza virus mediated modulation of the antioxidant response, as well as the potential crosstalk with Nrf2 and SIRT2. The role of these pathways in influenza virus replication has been also investigated.

Methods: Human epithelial lung cells (A549) and Madine Darby canine kidney cells (MDCK) were infected with influenza A/Puerto Rico/8/34 H1N1 virus. The expression of G6PD, Nrf2 and SIRT2 proteins and mRNA levels were evaluated by western blot and real-time PCR. Viral titer was measured by using TCID50 assay in G6PD-silenced A549 cells. GSH level and G6PD activity were evaluated by using colorimetric assays.

Results and Conclusions: G6PD expression and activity were significantly decreased following influenza virus infection and their down-regulation correlated with the reduction of Nrf2 and SIRT2 expression, respectively. The decreased expression of SIRT2 in infected cells caused the increased acetylation of G6PD, likely contributing to the loss of its enzymatic activity. This hypothesis was confirmed by the use of a selective inhibitor of SIRT2 deacetylase that caused a further decrease in the G6PD activity in comparison with untreated cells. Furthermore, the use of a SIRT2 activator rescued G6PD activity, GSH con-

tent and Nrf2 expression and induced, at the same time, a decrease in the influenza virus replication. The treatment with specific antioxidant molecules was also able to impair the replication of influenza virus and restore the intracellular redox balance by increasing the expression of antioxidant factors and the production of GSH. Finally, it was investigated whether SARS-CoV-2 infection determines a modulation of the antioxidant response similar to that by influenza virus. It was found a reduction in the expression of the same antioxidant factors thus suggesting similar antioxidant mechanisms involved in regulating SARS-CoV-2 replication.

Overall these data identify a novel mechanism used by influenza virus to tip the intracellular redox balance towards increased oxidative conditions that favour different steps of its replicative cycle. Furthermore, the use of molecules able to restore the activity of specific redox-related pathways may open the way to novel cell-targeted strategies against respiratory virus infections.

P94 - OC 22

The first high detailed Human nuclear proteome and the HPV16 genome interaction

G. Franci[1], A. Chianese[2], F. Dal Piaz[1], A. Nebbioso[3], G. Donadio[1], G. Greco[2], M.V. Morone[2], G. Sanna[4], S. Amitrano[5], L. Altucci[3], M. Galdiero[2]

[1] Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, 84081 Baronissi, Italy - [2] Department of Experimental Medicine, University of Campania Luigi Vanvitelli, 80138 Naples, Italy - Department of Precision Medicine, University of Campania Luigi Vanvitelli, 80138 Naples, Italy - [4] Department of Biomedical Sciences, University of Cagliari, Cittadella Universitaria Monserrato (CA), 09042 Monserrato, Italy - [5]Fondazione "Bartolo Longo III millennio" Via Lepanto, 153, 80045 Pompei (NA), Italy

Aim of studies: Human Papilloma Virus (HPV) is a member of the *Papillomaviridae* family that infects actively proliferating cells such as epithelial cells, and its replication cycle is associated with the differentiation of the host cell [1]. It can cause asymptomatic infections or clinical manifestations, ranging from mild (such as warts or benign skin lesions) to severe (such as anal, oropharyngeal and cervical cancer). To date, over 200 different HPV genotypes have been identified, and it has been shown that HPV infections are responsible for 5% of human cancers; in particular, HPV genotypes 16 and 18 are responsible for 70% of cervical cancers in the world. Currently there are 3 vaccines against HPV approved by the Food and Drug Administration (FDA), but effective treatments are not available. The aim of this project is to identify all human proteins interacting with the HPV16 genome in order to stratify patients and search new potential therapies through a new approach. In order to obtain that we setup an innovative method for long DNA regions modified with biotinylated oligos. We call this innovative and high performant method: Long Regions of DNA Pull-down (LDP).
Methods: Firstly, hybrid oligos were designed using Primer3 software for all HPV16 sequence genome, in which part of them were specific to each sequence, and the other part corresponded to unique sequences not present in the human genome and HPV. pHPV-16 plasmid in *E. coli* bacterium (ATCC® 45113™) was amplified, extracted and used as template for the first step of amplification through PCR pull-down. The 500 bp amplifiers were used as templates in the second PCR step, to obtain fragments with 5' biotinylated ends. SiHa cell lines (Elabscience EP-CL-0210), containing ~3 integrated HPV16 copies per cell, were propagated and nuclear extraction was performed. Subsequently, DNA pull-down was carried out: biotinylated dsDNA fragment interacted with streptavidin Sepharose beads and the nuclear cell extract was added to this complex. In this way the specific interacting proteins can be isolated and at the end the eluate proteins will be processed by mass spectrometry.
Results and Conclusion: About 4350 proteins have been identified by mass spectrometry analysis, and among them 310 were unique. Gene ontology (GO) was carried out by analysing the biological processes, the molecular functions and the cellular components in which the identified interactors participate. A major part of them is involved in DNA replication, transcription and translation. Some interactors are already known and take part in the HPV associated diseases, but the rest are unknown. For example, the Chromodomain-helicase-DNA-binding protein 4 (CHD4), component of the histone deacetylase

NuRD complex, participates in the chromatin remodelling by deacetylating histones [2]. Also, Histone deacetylase 1 and 2 (HDAC 1 and HDAC2) have been identified and as reported in literature, it interacted with HPV E7 protein. However, since the integration mechanism of the virus in the host cell is unknown, the important feature could be provided by the two proteins identified with our method with specific functions in the genome integration. Further studies will be conducted to find new potential and efficacy therapies to fight HPV16 infection, progression and pathogenic.

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P95 - OC 23

Parallel G-quadruplexes recruit the HSV-1 transcription factor ICP4 to promote viral transcription in infected human cells

I. Frasson, M. Nadai, S.N. Richter

Department of Molecular Medicine, University of Padova, Padova Italy

Background: Human *Alphaherpesviruses* are characterized by a lytic cycle in epithelial cells and by a latent state in the nervous system (Everett, 2014). Human herpes simplex (HSV) is the a prominent member of the family and its infection in humans has historically been associated with mild oral sores and to much more severe syndromes (Saleh et al., 2021). Indeed, HSV is the leading cause of infectious blindness and encephalitis in humans (Duarte et al., 2019; Toma et al., 2008). Genital herpes and neonatal encephalopathy linked to HSV infection do cause medical concern (James and Kimberlin, 2015; Sauerbrei, 2016). Antiviral treatments, facing worrisome resistance traits, cure the symptoms but not the infection (Taylor and Gerriets, 2021). Thus, there is an imperative need to identify innovative antiviral targets. Elucidation of the viral/cellular proteins that control viral gene expression in infected cells may provide new essential targets.
Non B-forms of DNA, called non-canonical DNA secondary structures, have gained much attention as unique regulators of many biological processes; they include G-quadruplexes (G4s), i-motifs, cruciform, triplexes and others (Bochman et al., 2012). G4s have become one of the most exciting nucleic acid secondary structure, formed in guanine-rich DNA and RNA sequences. G4s can readily form under physiologically relevant conditions and have been proved to finely regulate important biological processes, such as transcription, replication and genome maintenance (Spiegel et al., 2020). Their pivotal role in the regulation of viral cycles of important human pathogens such as HIV-1, HCV, Zika and Ebola has been described. G4s do also represent feasible antiviral targets (Ruggiero and Richter, 2018). We have demonstrated that G4s are abundantly embedded in the HSV-1 genome (Artusi et al., 2016, 2015), with high enrichment at promoter regions. Indeed, G4s are present and possibly regulate transcription in all immediate early genes in infected cells (Frasson et al., 2019).
Aim of the study: Here we aimed at identifying and characterizing the viral and cellular proteins that, by specifically recognizing the G4 structures embedded in the viral genome, regulate HSV-1 viral cycle.
Methods: Human U-2 OS cells were infected with HSV-1 virus, and nuclear extracts at 8 and 16 h.p.i were obtained. Nuclear extracts were used in pull down assays coupled to LC-MS analysis using established viral and cellular G4s as baits. Results were confirmed by cross-linking assays. CD spectroscopy and FRET analysis revealed protein effect on DNA secondary structures, while PLA assays and immune-FISH fluorescent microscopy confirmed protein-G4 binding in infected cells.
Results and conclusions: By cross-linking/pull-down assay we identified ICP4, the major HSV-1 transcription factor, as the protein that most efficiently interacts with viral G4s du-

ring infection. ICP4 was proved to specifically bind and unfold a particular G4 structure, known as parallel G4. ICP4 unfolding pattern was recorded not only on the viral G4s but also on the cellular ones, like those regulating the expression of c-myc and VEGF oncogenes. ICP4 activity on the G4 structures present in HSV-1 immediate early gene promoters boosted transcription in vitro and in infected cells. We showed that this mechanism was also exploited by ICP4 to promote its own transcription. Proximity ligation assay allowed visualization of G4-protein interaction at the single selected G4 in cells. Moreover, G4 ligands inhibited ICP4 binding to G4s and reverted its activity on its own promoter.

In conclusion, our results indicate the existence of a well-defined G4-viral protein network that regulates the productive HSV-1 cycle. They also point to G4s as elements that recruit transcription factors to activate transcription in cells.

P96 - OC 16

Identification of the nuclear proteome from all human viruses by a comprehensive analysis of classical nuclear localizations

H. Ghassabian, G. Cassioli, M. Allegro, M. Centazzo, N. Ambkari, G. Palù, E. Lavezzo, G. Alvisi

Department of Molecular Medicine, University of Padova, Padova Italy

We defined the nuclear proteome of all human viruses, discriminating between viral proteins translocated in an IMPα/β dependent or independent process by combining a bioinformatics analysis with extensive functional characterization of viral cNLSs. Our study represents an unprecedented opportunity.to compare how viruses differently interact with the host cell nuclear transport machinery, with important implications for the development of broad-range host targeted antivirals. In depth functional validation of putative cNLSs identified led to the discovery of more than 20 novel viral cNLS. Among them, two extremely well conserved cNLS in orthologues of Vaccinia Virus proteins A19 and N2. Both proteins localized in the cell nucleus via energy and IMPα/β-dependent process, and their nuclear import could be abolished by site specific mutagenesis of the cNLSs. A19 and N2 mutant derivatives impaired in nuclear transport were tested for their ability to interfere with IRF3 activation upon poly (I:C) stimulation, suggesting a role for nuclear transport during Poxviridae members life cycle. Furthermore, we also report the first characterization of the nuclear import process of Human Polyomaviruses (HPyVs) Large T antigens (LT) as well as of the cNLS involved. Although LT from all 14 HPyVs bear a functional cNLS, the latter are extremely heterogenous, both in terms of activity and structural organization. Importantly, cNLS activity mirrored the levels of nuclear accumulation of full length proteins, with lowest activity associated to HPyV7. Surprisingly, while most HPyVs bear one or more monopartite cNLS, four of them bear a bipartite cNLS. Clearly, such structural differences suggest an important role in conferring binding abilities to specific IMPα isoforms with potential implication for viral tropism determination.

P97

HIV-1 NefSF2 protein increases vesicles production in THP-1 human monocytic cells differentiated with PMA

F. Giannessi[1], A. Aiello[1], Z.A Percario[1], K. Fecchi[2], M. Carollo[3], M. Sargiacomo[2] and E. Affabris[1]

[1] Dept.of Science, University Roma Tre, Rome, Italy - [2] Global Health Center, Istituto Superiore di Sanità, Rome, Italy - [3] Core Facilities Cytometry Unit, Istituto Superiore di Sanità, Rome, Italy

Extracellular vesicles (EVs) are lipid bilayer-enclosed entities containing proteins and nucleic acids that mediate intercellular communication both in physiological and pathological conditions (Schorey et al., 2016). EVs resemble enveloped viruses in both structural and functional aspects. In full analogy with viral biogenesis, some of these vesicles are generated inside cells and once released into the extracellular milieu, are called "exosomes" (size 30-100nm), whereas others bud from the plasma membrane and are generally referred to as "microvesicles" (size >100nm) (Wurdinger et al., 2012). The protein Nef of Human Immunodeficiency Virus (HIV) is a virulence factor that acts as an adaptor molecule inside the infected cells. It regulates viral production, induces immunoevasion and importantly can be transferred to uninfected cells through exosomes (Percario et al., 2015). In this study we examined if the recombinant myristoylated HIV-1 Nef_{SF2} was able to induce the production of vesicles in THP-1 cells differentiated with PMA. To this aim, we used commercially available BODIPY®-C16 fatty acid to label the cells, that once incorporated, produces fluorescent exosomes and microvesicles that we examined and quantified as reported by Sargiacomo and colleagues (Coscia et al., 2016). The supernatant of the treated and untreated cells was harvested after 20 hours of treatment. Two categories of EVs were isolated through several ultracentrifugation session: small-EVs (Exo) which included exosomes, and medium/large-EVs (MV) which included microvesicles (Théry et al., 2018). We observed that treatment of cells with myrNef_{SF2} increases about twice the production of small-EVs but not that of medium/large-EVs. In addition, Nef is incorporated into the small-EVs fraction. The analysis of the vesicular production induced by cell treatment with Nef mutants suggests that some of its conserved domains are involved in small-EVs production. Also, we have verified that small-EVs produced by cells can be transferred and influence cell activation when used to treat cells of same cell line. Further studies are in progress to analyze the pro-inflammatory effects induced by the small-EVs containing myr-Nef_{SF2}.

P98

Human cytomegalovirus-induced host protein citrullination is crucial for viral replication

G. Griffante[1], F. Gugliesi[1], S. Pasquero[1], V. Dell'Oste[1], M. Biolatti[1], A. J. Salinger[2,3], S. Mondal[2], P.R. Thompson[2], E. Weerapana[3], R. J. Lebbink[4], J. A. Soppe[4], T. Stamminger[5], V. Girault[6], A. Pichlmair[6], G. Oroszlán[7], D. M. Coen[7], M. De Andrea[1,8] and S. Landolfo[1]*

[1] Department of Public Health and Pediatric Sciences, University of Turin, Turin 10126, Italy - [2] Department of Biochemistry and Molecular Pharmacology, UMass Medical School, Worcester, Massachusetts 01605, United States - Department of Chemistry, Boston College, Chestnut Hill, Massachusetts 02467, United States - [4] Department of Medical Microbiology, University Medical Center Utrecht, Utrecht 3584 CX, The Netherlands - [5] Institute of Virology, Ulm University Medical Center, Ulm 89081, Germany - [6] Institute of Virology, Technical University of Munich, Munich 81675, Germany - [7] Department of Biological Chemistry & Molecular Pharmacology, Blavatnik Institute, Harvard Medical School, Boston, Massachusetts 02115, United States - [8] CAAD Center for Translational Research on Autoimmune and Allergic Disease, University of Eastern Piedmont, Novara 28100, Italy

Citrullination is the conversion of arginine-to-citrulline by protein arginine deiminases (PADs), whose dysregulation is implicated in the pathogenesis of various types of cancers and autoimmune diseases. Consistent with the ability of human cytomegalovirus (HCMV) to induce post-translational modifications of cellular proteins to gain a survival advantage, we show that HCMV infection of primary human fibroblasts triggers PAD-mediated citrullination of several host proteins, and that this activity promotes viral fitness. Citrullinome analysis reveals significant changes in deimination levels of both cellular and viral proteins, with interferon (IFN)-inducible protein IFIT1 being among the most heavily deiminated one. As genetic depletion of IFIT1 strongly enhances HCMV growth, and in vitro IFIT1 citrullination impairs its ability to bind to 5'-ppp-RNA, we propose that viral-induced IFIT1 citrullination is a novel mechanism of HCMV evasion from host antiviral resistance. Overall, our findings point to a crucial role of citrullination in subverting cellular responses to viral infection.

P99

The envelope protein of Usutu virus attenuates West Nile virus virulence in immunocompetent mice

L. Jurisic[1], G. Zaccaria[2], D. Malatesta[3], G. Di Teodoro[4], A. Conte[5], A. Lorusso[6]

[1] Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise (IZSAM), Teramo, Italy; Faculty of Veterinary Medicine, University of Teramo, Teramo-Italy - [2.3.4.5] Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise (IZSAM), Teramo, Italy - [6] Istituto Zooprofilattico [1/ 3] Submission Sperimentale dell'Abruzzo e Molise (IZSAM), Teramo, Italy

West Nile virus (WNV) and Usutu virus (USUV) are the two most widespread mosquito-borne flaviviruses in Europe with significant public health impact causing severe neuroinvasive disease in humans. Although USUV is an emerging pathogen, little is known about its pathogenesis, biologic features, virulence factors and host spectrum. Moreover, despite the existence of several reverse genetics studies on flaviviruses, only one study involved USUV.

Here we first compared the kinetics of infections of three wild type (wt) WNV and one USUV isolates at different doses in adult immunocompetent mice. While all WNV isolates showed the same severe neurovirulence properties, USUV did not cause clinical signs in mice. Then, we engineered WNV and USUV genome by reverse genetics. While recombinant wild type WNV (r-wt WNV) was successfully rescued, despite several attempts, r-wt USUV was not rescued. In turn, a recombinant virus carrying the 5'-UTR of WNV within the USUV genome backbone (r-USUV5'UTR WNV) was rescued and when administered to mice, it did not cause signs or disease as wt USUV, suggesting that 5'UTR of a marked neurotropic parental WNV was not per se a virulence factor. Interestingly, a chimeric virus carrying the E protein of USUV in the WNV genome backbone
(r-WNVE USUV) showed an attenuated profile in mice compared to wt WNV but significantly more virulent than wt USUV. Therefore, we demonstrated that the E protein of USUV was likely responsible for the observed attenuated profile in mice after administering with r-WNVE USUV. Moreover, except when is tested against serum samples originating from a live WNV infection, r-WNVE USUV showed the same antigenic profile of wt USUV, confirming that this protein is the major immunodominant protein of USUV.

P100 - OC 25

The US21 viroporin of human cytomegalovirus regulates cell adhesion and migration

A. Luganini[1], V. Serra[1], S. M. Bhat[1], G. Scarpellino[2], L. Munaron[2], A. Fiorio Pla[2], G. Gribaudo[1]

[1] Laboratory of Microbiology and Virology - [2] Laboratory of Molecular Angiogenesis, Department of Life Sciences and Systems Biology, University of Turin, Turin, Italy

Background. During coevolution with its host, the Human Cytomegalovirus (HCMV) has invested a large part of its protein coding potential to ensure the dysregulation of the majority of cellular homeostatic circuits. Among the viral genes involved, the US12 gene family includes a set of 10 contiguous tandemly arranged genes (US12 to US21) and constitutes about 5% of HCMV's genetic content. The identification of putative seven-transmembrane hydrophobic domains in each of the US12 ORFs predicts a common structural framework that associates these proteins with cellular membranes. However, despite the confirmed evolutionary importance of the US12 proteins to HCMV biology, only a few functions in the regulation of virus cell tropism, virion composition, and immunoevasion have been associated with the family to date. Previously, we characterized the function of the US21 protein as a HCMV homolog of cellular TMBIM proteins and an ER-resident virus-encoded calcium-permeable channel able to dysregulate intracellular Ca^{2+} homeostasis and that inhibit apoptosis (Luganini et al., PNAS, 115, E12370, 2018).
Aim of the study. Given the role of Ca^{2+} in controlling cell adhesion and motility, in this study we investigated whether the pUS21-mediated Ca^{2+} release from intracellular stores might influence these important cytobiological responses.
Resuts and conclusions. Using engineered human cell lines to express pUS21 in an inducible manner, that allows to investigate cell responses without non-specific toxic effects due to a prolonged pUS21 expression, we observed a significant increase in the migration rate of cells expressing pUS21wt, while its mutation in the critical D201 residue that define the pUS21's Ca^{2+} channel leaking function, affected the ability to stimulate cell motility, thus suggesting an involvement of its ability to reduce the Ca^{2+} content in intracellular stores. To test this hypothesis, we performed migration assays in the presence of an inhibitor of calpain 2, a Ca^{2+}-activated cysteine protease, that regulates the disassembly of cellular focal adhesions and promotes cell motility. The addition of the calpain 2 inhibitor abrogated the pUS21-mediated increase of migration in cells expressing this HCMV protein. As a further confirmation of a calpain 2 involvement, its biochemical activity was measured in cell expressing either pUS21wt or its mutated forms in the two critical amino acid residues that define the TMBIM architecture of pUS21, D178 and D201. An increase of calpain 2 activity was observed in extracts from cells expressing pUS21wt or pUS21-D178N, while the expression of pUS21-D201N was unable to stimulate calpain 2 activity, thus confirming an involvement of pUS21 Ca2+ channel function in the control of calpain 2 activation. Moreover, the functional relationship between pUS21 and calpain 2 was fur-

ther suggested by the observation that talin 1, a known calpain proteolytic substrate, interact with pUS21 as observed by mass spectrometry and co-immunoprecipitation. Together, these findings highlight a novel role of the pUS21 viroporin of HCMV as a regulator of focal adhesion dynamics, cell adhesion, and migration as a consequence of the dysregulation that pUS21 exerts on the localized Ca^{2+} dependent activation of calpain 2 and the subsequent talin activity at newly forming protrusions.

P101

Nicotine upregulates ACE2 expression and increases competence for SARS-CoV-2 in human pneumocytes

L. Macera[1,2], A. Rosellini[2], P. Russo[3], C. Tomino[4], P.G. Spezia[1], M. Pistello[1,2], F. Maggi[5]

[1] Department of Translational Research, Retrovirus Center and Virology Section, University of Pisa, Pisa, Italy - [2] Virology Division, Pisa University Hospital, Pisa, Italy - [3] Clinical and Molecular Epidemiology, IRCSS San Raffaele Pisana, Rome, Italy - [4] Scientific Direction, IRCSS San Raffaele Pisana, Rome, Italy - [5] Department of Medicine and Surgery, University of Insubria, Varese, Italy

Aim of the study. The coronavirus disease 2019 (COVID-19) pandemic has a variable degree of severity according to underlying comorbidities and life-style. Several studies have reported an association between cigarette smoking and increased severity of COVID-19 (Gulsen et al., "The effect of smoking on COVID-19 symptom severity: Systematic review and meta-analysis", 2020, in press; Dorjee et al., "Epidemiological risk factors associated with death and severe disease in patients suffering from COVID-19: a comprehensive systematic review and meta analysis", 2020, preprint). The exact mechanism of action is nowadays largely unclear. We investigated whether angiotensin-converting enzyme 2 (ACE2) is overexpressed in pneumocytes after exposure to nicotine (Wang et al., "Structural and functional basis of SARS-CoV-2 entry by using human ACE2", 2020), and if this leads in turn to increased SARS-CoV-2 replication and cytopathic effect.

Methods. Nicotine was assayed using in vitro experiments for its ability to stimulate ACE2-expression in human pulmonary adenocarcinoma A549 epithelial cells. We exposed low ACE2-expressing A549 cells to nicotine and assessed ACE2 expression at different time points. Furtherly, we used the nicotine-exposed cells in plaque reduction neutralisation test (PRNT).

Results and Conclusions. Nicotine exposure induces rapid and long-lasting increases in gene and protein expression of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) receptor ACE2, which in turn translates into increased competence for SARS-CoV-2 replication and cytopathic effect.

These findings show that nicotine makes worse SARS-CoV-2 pulmonary infection and have implications for public health policies.

P102

Role of the HSV-1 US11 protein in the pathway of apoptotic response in monocytic cells

M. Musarra-Pizzo[1] , R. Pennisi[1,2] , M.T. Sciortino[1]

[1] Department of Chemical Biological Pharmaceutical and Environmental Sciences, University of Messina, Messina, 98166, Italy - [2] Shenzhen International Institute for Biomedical Research, 1301 Guanguang Rd. 3F Building 1-B, Silver Star Hi-tech Park Longhua District, Shenzhen, Guangdong, 518116, China

In response to viral infection, host cells undergoing apoptosis to ensure elimination of infected cells and limit the release of progeny virus. Among other, wild-type herpes simplex virus type 1 (HSV-1) modulate the apoptosis pathways to subvert cellular antiviral defences and promote viral propagation (Yu et al., 2017). The viral protein Us11, a true late gene product, acts as an inhibitor of PKR thus preventing the shutdown of protein translation and therefore interfering with PKR mediated host cell responses, including the FADD/caspase 8 death-signalling pathway (Khoo et al., 2002; Cassady and Gross, 2002; Balachandran et al., 2000). Therefore, we used an HSV-1 strain F Us11/Us12-null virus (R3630) in order to learn about the impact of Us11 deletion on apoptosis response in monocytes cells, which show appreciable apoptosis response following infection (Mastino et al., 1997; Ito et al., 1997; Bosnjak et al., 2005; Iannello et al., 2011; Kather et al., 2010). To this purpose, we infected a human monocytic leukaemia cell line (THP-1) in vitro with the wild type HSV-1 and the R3630 viruses and we analyse the apoptotic marker by western blot. We found that the cleaved forms of caspase 3, caspase-8 and PARP were detected in both HSV-1 wild type and R3630 infected cells at later time point post infection. However, a different cleavage pattern of caspase-8 was observed in absence of Us11/Us12 proteins. Transfection experiments confirmed that the overexpression of Us11, but not Us12, was responsible for the specific cleavage pattern of caspase-8, observed during the infection process. Consistently, we found that the Us11-mediated cleavage of caspase-8 was abrogated when the accumulation of γ2 proteins was inhibited by phosphonoacetic acid (PAA) treatment. However, the cleavage mediated by the Us11 protein was not prevented by the treatment with the caspase-8 inhibitor z-IETD-fmk, which is known to block the canonical caspase-8 cleavage. This evidence indicates that an alternative cleavage of caspase 8 was observed during infection and requires viral DNA synthesis and accumulation of γ2 Us11 protein. Hence, our results demonstrated that the Us11 viral protein specifically modulates the cleavage of caspase-8 during the infection of monocytic cells. However, further investigations are required to better investigate this alternative cleavage and its implications for viral replication, cell death, and immune evasion.

P103 - OC 26

The innate immunological response mediated by PKR is counteracted by herpes virus tegument proteins

R. Pennisi[1,2], M. Musarra-Pizzo[2], G. Zhou[1], M. T. Sciortino[2]

[1] Shenzhen International Institute for Biomedical Research, 1301 Guanguang Rd. 3F Building 1-B, Silver Star Hi-tech Park Longhua District, Shenzhen, Guangdong, 518116, China - [2] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale SS. Annunziata, 98168 Messina, Italy

PKR is the best-characterized component of the human innate immunity which establishes and maintains an antiviral state stopping the proteins synthesis process. The present study stemmed from the observation that HSV-1 encodes for VHS, Us3 and UL13 tegument proteins with additional enzymatic activity useful in the immune escape mechanism. VHS is packaged to the virus as a tegument protein and released into the cytoplasm of infected cells, where it works as an endoribonuclease provoking the degradation of mRNAs (Taddeo et al. 2006; Sciortino M. T. et al. 2013). Otherwise, Us3 and UL13 are tegument proteins with kinase activity involved in the viral transport and maturation and in the regulation of host and viral molecular pathways (Gershburg, S. et al. 2015). A combined approach of viral plasmids transfection and mutant viruses infection was employed to investigate the role of VHS, Us3 and UL13 in the regulation of PKR at the transcriptional and translational level. Besides, the cycloheximide (CHX) and actinomycin D (Act.D) treatment were used as inhibitors of protein synthesis to indirectly simulate in vitro accumulation/ depletion of mRNAs potentially targeted by VHS and activators of PKR. Lastly, Phosphonoacetic acid (PAA) inhibitor was used to block the viral DNA polymerase and abrogate the γ2 gene accumulation during viral infection. The results report that: i) VHS RNase activity affects PKR activation. Indeed, VHS mutant virus (R2621), defective in the RNAse activity, accumulates phospho- PKR in several cell types compared to total abrogation by HSV-1 wt. Similarly, the transient transfection with the VHS mutant plasmid, encoding the catalytically inactive form of VHS protein, accumulates phospho-PKR compared to the wild-type VHS; *ii*) the overload of viral mRNAs and the abrogation of γ2 genes induce phospho-PKR accumulation suggesting that, during HSV-1 replication both mechanisms are involved in the phospho-PKR modulation; *iii*) Us3 and UL13 control PKR activation. Indeed, the infection with Us3 mutant virus (R7041) and UL13 mutant virus (R7356) provokes the accumulation of phospho-PKR similarly to R2621 infection. Otherwise, the Us3 and UL13 overexpression, by plasmid transfection, blocks PKR activation. *iv*) the accumulation of VHS and Us3 transcripts and proteins correlates with PKR depletion at the transcriptional and translational level. In this contest, it was studied the reciprocal regulation between VHS and Us3 protein and it was find that Us3 kinase activity is responsible for the VHS phosphorylation. A shifted high molecular weight VHS band was detected in western blot analysis following Us3 transfection. Moreover, multiple transfections experiments using VHS/Us3 and VHS mutant/Us3 plasmids showed that VHS wild-type but not VHS mutant protein limits the accumulation of Us3 protein. This finding suggests that the RNase activity of VHS affects

US3. Therefore, this study proposes a new immunological escape mechanism used by HSV-1. Since that the activation of PKR needs double-stranded RNA binding, it is possible to assume that HSV-1 uses the RNase activity of VHS to steal RNAs useful for PKR activation together with the enrollment of Us3 and UL13 to limit PKR (Pennisi R. et al 2020). Further studies are in progress to clarify the reciprocal interplay between Us3 and VHS. Collectively, our findings remark the PKR role as a biological sensor in the virus-host interaction and the HSV-1 capability to deceive innate immune response by viral protein-protein modulation.

P104 - OC 21

The expression of the truncated ACE2 isoforms only is related to the interferon response in airway epithelial cells from young adults

G. Oliveto[1], A. Viscido[1], F. Frasca[1], M. Scordio[1], L. Sorrentino[1], A.M. Zicari[2], A. Salvatori[2], F. Salvatori[2], L. Matera[2], A. Febbo[2], R. Nenna[2], F. Midulla[2], C. Scagnolari[1], G. Antonelli[1], A. Pierangeli[1]

[1] Virology Laboratory, Department of Molecular Medicine, and [2] Department of Pediatrics and Infantile Neuropsychiatry "Sapienza" University

Introduction: SARS-CoV-2 entry into respiratory tract cells starts with the binding to its main receptor, the angiotensin-converting enzyme 2 (ACE2). ACE2 has been proposed to be an interferon (IFN)-stimulated gene (ISG) in epithelial tissues; however, more recent studies demonstrated the existence of a truncated ACE2 isoform (dACE2) of unknown functions, not able to serve as SARS-CoV-2 entry receptor. Moreover, the authors observed that IFNs and viruses induced dACE2, but not full-length ACE2, in several, but not all, cell types. Our previous study also supported this acquisition in the A549 and Calu3 cell lines but data in SARS-CoV-2 infected-patients are not yet available. Hence, we measured the expression of both ACE2 isoforms in respiratory mucosal cells from SARS-CoV-2 infected, and not-infected individuals in relation to the activation of type I and III IFN-stimulated genes (ISG).
Methods: from young adults attending Policlinico Umberto I University Hospital for SARS-CoV-2 molecular/serological testing, residual nasopharyngeal swabs and blood samples were collected. Anti-SARS-CoV-2 IgG antibodies were measured in all individuals resulted negative to SARS-CoV-2 molecular tests, by a semi-quantitative ELISA. Patient data (i.e. smoke, atopic asthma) were collected and anonymized. Quantitative expression of genes coding for the full-length ACE2 isoform (f-lACE2) and dACE2 using specific primers and probes [Scagnolari et al. Cytokine. 2021] and the ISG15 and ISG56 were measured in nasopharyngeal swabs from enrolled individuals.
Results: 63 young adults (18-30 years) were enrolled: 36/63 (57.1%) resulted never-infected (negative to SARS-CoV-2 molecular and serological tests), 14/63 (22.2%) were negative to SARS-CoV-2 when swabs were collected but positive to anti-SARS-CoV-2 antibodies (past-infection) and 13/63 (20.6%) were positive to SARS-CoV-2 RNA (present-infection, all had no or little respiratory symptoms). In 19 samples (of which 17 were negative to SARS-CoV-2), neither ACE2 isoforms were detectable and in three, only flACE2 expression was measured at low levels; in the remaining samples both isoforms were detectable so that their expression levels and the ratio between flACE2 and dACE2 was calculated. Overall, participants ever SARS-CoV-2 infected had a significantly lower ratio between flACE2 and dACE2 expression ($p=0.041$) with respect to those never infected. Moreover, individuals presently positive to SARS-CoV-2 and those with a past infection, had a significantly higher (Kruskal-Wallis test, $p=0.015$) dACE2 expression with respect to the never-infected group. Interestingly, both ISGs were significantly more expressed in

the group with a dACE2 level higher than flACE2 (i.e. ratio flACE2/dACE2 < 1), indicating a positive relation of dACE2 levels with the ISGs.
Conclusions: we evidence in the upper respiratory mucosal cells, an ISG-type induction of dACE2, but not of the flACE2, in young adults during or after a mild SARS-CoV-2 infection. The induction by IFNs of the truncated ACE2 isoform only would not enhance the risk of further progression of SARS-CoV-2 in the respiratory tract.

P105
Nrf2 expression differs in bronchiolitis caused by RSV-A, RSV-B or HRV

A. Viscido[1]*, W. Toscanelli[2]*, G. Oliveto[1], R. Nenna[3], M. De Angelis[2], F. Midulla[3], G. Antonelli[1], A.T. Palamara[2], A. Pierangeli[1], C. Scagnolari[1]* and L. Nencioni[2]*

[1] Virology Laboratory, Department of Molecular Medicine, [2] Department of Public Health and Infectious Diseases, and 3Department of Pediatrics and Infantile Neuropsychiatry, "Sapienza" University of Rome - *equally contributed to the work

Introduction. Respiratory Syncytial Virus (RSV) and Rhinovirus (HRV) are the first and second most common cause of bronchiolitis, an illness characterized by acute inflammation and respiratory distress in infants. The first line of defense against respiratory pathogens is the immune response occurring in the naso-pharyngeal mucosa. The antiviral innate immune response is largely dependent on the proper activation of type I and III Interferons (IFN) pathways. In turn, the immune response is modulated by oxidative stress and inflammatory processes in which a crucial role is played by the Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2) activation, able to reduce inflammation and to interfere with the IFN response. As far as these interactions are concerned, most previous data were obtained from in vitro primary cell cultures and in the BALs of severe cases of RSV and HRV infections. Accordingly, the aim of this study was to correlate the activation of several genes of the type I and III IFN pathways with the expression of Nrf2 and some of its antioxidant related-genes, including Glucose-6-phosphate dehydrogenase (G6PD), in naso-pharyngeal cells from bronchiolitis cases.
Methods. From prospectively enrolled, previously healthy, term infants hospitalized for bronchiolitis in 2016-17 and 2017-18 epidemic seasons, cases positive to RSV and HRV were selected. Quantitative expression of genes coding for IFNs beta and lambda 1-3, for the ISGs MXA, ISG15 and ISG56 (markers of type I and III IFN activation), and of Nrf2 and G6PD were measured in nasopharyngeal washings from full-term infants hospitalized with bronchiolitis caused by RSV-A (N=33), RSV-B (N=28) and HRV (N=21).
Results. Significantly higher levels of the ISGs, but not of the IFN-coding genes, were found in RSV than in HRV patients, whereas Nrf2 levels, but not of G6PD, were decreased in the RSV with respect to the HRV group. Interestingly, when analyzing separately RSV-A and -B cases, the latter group showed values intermediate between RSV-A and HRV (Jonckheere-Terpstra Test, $p<0.05$ for the comparison of any of the ISGs and of Nrf2 values among the three ordered groups).
Conclusions. The elevated level of the IFN-stimulated genes observed in infants infected with RSV-A, may be associated to a more severe bronchiolitis course, confirming our previous findings. On the other hand, we showed for the first time in the upper respiratory tract, that Nrf2 is lowered in RSV-more severe cases, whereas Nrf2 activation may be effective in controlling bronchiolitis severity during HRV infections.

P106

Human Endogenous Retroviruses (HERVs) and Mammalian Apparent LTRs Retrotransposons (MaLRs) are dynamically modulated in different stages of immunity

M.P. Pisano, N. Grandi and E. Tramontano

Laboratory of Molecular Virology, Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy

Human Endogenous retroviruses (HERVs) and Mammalian Apparent LTRs Retrotransposons (MaLRs) are remnants of ancient retroviral infections that represent a large fraction of our genome. The HERV and MaLR transcriptional activity is regulated in developmental stages, adult tissues, and pathological conditions. In this work, we used a bioinformatic approach based on RNA-sequencing (RNA-seq) to study the expression and modulation of HERVs and MaLR in a scenario of activation of the immune response. We analyzed transcriptome data from subjects before and after the administration of an inactivated vaccine to investigate the HERV and MaLR expression and differential expression in response to the administration of the vaccine. Specifically, we described the HERV transcriptome in PBMCs and identified HERV and MaLR loci differentially expressed after the 2nd, 3rd and 4th inactivated vaccine administrations. We found that the HERV and MaLR expression increased in response to the vaccine and that the activation of several individual HERV and MaLR loci is indicative of each vaccine administration and correlated to different genes and immune-related pathways.

P107 - OC 46

Role of extracellular vesicles in propagating HSV-1 induced brain neurodegenerative damage

V. Protto[1], M. Miteva[1], V. Ardovini[1], M.E. Marcocci[1], A.T. Palamara[1,2], G. De Chiara[3]

[1] Department of Public Health and Infectious Diseases, Sapienza University of Rome, Laboratory affiliated to Istituto Pasteur Italia-Fondazione Cenci Bolognetti, Rome, Italy - [2] San Raffaele Pisana, IRCCS, Telematic University, Rome, Italy - [3] Institute of Translational Pharmacology, CNR, Rome, Italy

Introduction and Aim: Extracellular vesicles (EVs) are involved in cell-to-cell communication, spreading of intracellular proteins and miRNA, including neurotoxic proteins such as amyloid beta (Abeta) and Tau, key players in Alzheimer's disease (AD). A growing body of evidence supports the role of Herpes Simplex Virus-1 (HSV-1) infection in AD pathogenesis and we recently showed the accumulation of Tau-related brain damages in an *in vivo* model of multiple HSV-1 reactivations in mice. Recent studies suggest that viruses could exploit EVs to pass from cell to cell along with harmful proteins, such as the phosphorylated and aggregated forms of Tau. These can be transmitted among neurons and enter inside cells acting as seeds for the aggregation of the endogenous protein, thus propagating Tau-dependent damages in the brain. Hence, the aim of our study was to investigate whether HSV-1 infection in the brain could promote virus diffusion and Tau spreading among neuronal cells via EVs.

Methods used: EVs were purified from supernatants of human neuroblastoma and primary cultures of rat neurons following 24 hours (h) of HSV-1- or Mock-infection and analysed in western blot (WB). Alternatively, purified EVs were treated with neutralizing anti-HSV-1 antibodies and/or UV to block virus infection and incubated on uninfected neurons for 24h-48h. Cell lysates were then analysed in WB for phosphorylated Tau (pTau) content and compared to untreated cells.

Brains were isolated from HSV-1- and Mock-infected BALB/c mice undergone cycles of hyperthermia to induce virus reactivations within their life. Brain exosomes were isolated from the dissociated tissues through several steps of differential centrifugations and ultracentrifugation of a sucrose gradient. EVs were then analysed in WB to characterize their protein content.

Results and Conclusions: Results showed that EVs isolated from HSV-1-infected cells (HSV-EVs) contained both viral proteins and increased levels of pTau. To evaluate whether EVs contain infective virions, control cells were layered with HSV-EVs and treated with anti HSV-1 antibodies to neutralize free virus. The analysis of viral titre in the supernatant 24h p.i. showed the occurrence HSV-1 productive infection, indicating that EVs are exploited for viral spreading.

Moreover, in cells layered with HSV-EVs that were previously treated with UV to inactivate intra- or extra-vesicles viral particles, we found higher levels of pTau, with respect to cells incubated with EVs isolated from control cells. These results indicate that EVs derived

from infected cells could carry neurotoxic proteins and promote Tau phosphorylation and aggregation in uninfected neurons.
Finally, we characterized for their content EVs isolated from the brains of mice that were HSV-1- or Mock-infected. Preliminary results show that the EVs enriched fractions contains several forms of pTau whose levels are modulated in HSV-1-infected mice compared with matched controls. Overall, these data indicate that HSV-1 can promote pTau propagation, as well as viral spreading among neurons via EVs, and support the hypothesis that repeated HSV-1 reactivations may concur to neurodegeneration.

P108
HPV infection: the role of vaginal microbiota

B. Santella[1], E. Serretiello[1], V. Folliero[1], M.T. Schettino[2], P. De Franciscis[2], N. Colacurci[2], A. Schiattarella[2], M. Galdiero[1], G. Franci[3]

[1] Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy - [2] Department of Woman, Child and General and Specialized Surgery, University of Campania "Luigi Vanvitelli", 80138 Naples, Italy - [3] Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, 84081 Baronissi, Italy

Aim of study: Human papillomaviruses (HPV) are naked dsDNA viruses belonging to the *Papillomaviridae* family. The World Health Organization (WHO) estimates that the prevalence of HPV infection is between 9 and 13 % of the world population and only in the United States (USA), more than 6.2 million are positive every year. There are more than 100 types of HPV, among them two serotypes (16 and 18) are related to 70% of cervical cancers and pre-cancerous cervical lesions. The vaginal microbiota could play a considerable role in HPV infection and the genesis of cervical tumors caused by HPV. Moreover, bacteria are strongly associated with vaginal inflammation and oncogenic mutations in human cells. We aim to investigate if HPV infection could influence the bacterial microbiota composition in the uterine cervix.
Methods used: A total of 31 women were enrolled in the study. The vaginal swabs had been collected as mucosal scrapings with a brush. The HPV-DNA was extracted with QIAamp DNA Microbiome. The V3-V4-V6 region of the 16S rDNA gene was amplified by Polymerase Chain Reaction (PCR) followed by sequencing with MiSeq Illumina.
Results and conclusions: The main phylum identified in the vaginal microbiota were *Firmicutes*, *Bacteroidetes*, *Proteobacteria* and *Actinobacteria*. The *Firmicutes* occupied most of the commensal vaginal flora, with a lower percentage in HPV positive women. The phylum of *Actinobacteria*, *Proteobacteria* and *Bacteroides* was more represented in HPV positive patients. *Lactobacilli* represented the dominant genus, with a high percentage of *L. iners*, *L. jensenii* and *L. crispatus* as species. *Gardnerella vaginalis*, *Enterococcus* spp., *Staphylococcus* spp., *Proteus* spp. and *Atopobium* were the most represented in HPV positive patients. An altered vaginal microbiota might play a functional role in HPV cervical infection, progression and clearance. The relationship between infection and microbiota could spur the development of new probiotic-based therapies to fight infection. However, further studies are needed to clarify the role of the vaginal microbiota in HPV infection.

P109
Kinetic of IL-35 and IgA antibodies in COVID-19 patients

A. Quirino[1], N. Marascio[1], C. Peronace[1], G.S. Barreca[1], L. Gallo[1], A. Giancotti[1], A.G. Lamberti[1], M. Mazzitelli[2], E.M. Trecarichi[2], C. Torti[2], E. Garofalo[3], A. Bruni[3], F. Longhini[3], F. Divenuto[1], G. Pavia[1], G.G.M. Scarlata[1], G. Matera[1], M.C. Liberto[1] and Microbiology COVID-19 Group*

*** E. Colosimo, L. Martino, C. Zangari, E. La Ratta, C. Di Cello, N. Raso, R. Sinopoli, V. Tancrè, E. Gambardella, V. Esposito, C. Maida, F. Mongiardo**

[1] Department of Health Sciences, Unit of Microbiology, "Magna Graecia" University, Viale Europa, 88100 Catanzaro, Italy - [2] Department of Medical and Surgical Sciences, Unit of Infectious and Tropical Diseases, "Magna Graecia" University, Viale Europa, 88100 Catanzaro, Italy - [3] Department of Medical and Surgical Sciences, Intensive Care Unit, "Magna Graecia" University, Viale Europa, 88100 Catanzaro, Italy

Introduction and aim of the study: SARS-CoV-2-specific antibodies (Abs) used in diagnostic routine are IgM and IgG, while IgA is less considered in the diagnosis of COVID-19 (Huang Z. et al., 2020, doi: 10.1111/cei.13500). Both specific IgM and IgA antibodies are produced in the early response with their peaks observed 7 days from illness onset; while specific IgG antibodies develop from 10 to 18 days after and are presumably long-lasting protective antibodies (Nuccetelli M. et al., 2020, doi: 10.1080/08820139.2020.1823407). There are few data regarding the long-term kinetics of IgA antibodies during symptomatic COVID-19, as well as during convalescence. Interleukin (IL)-35, is a Breg cytokine that plays an important regulatory role in inflammatory diseases (Zhang J. et al., 2019, doi: 10.1111/imm.13044). The increase of IL-35 could determine the reduction of antibodies in the late stages of infection. There are no published data regarding the inhibition of antibodies release associated with IL-35 during COVID-19 disease. The aims of our study were to evaluate the kinetic of anti-SARS-CoV-2 IgA antibodies and IL-35 at different time points (T_0 0-4 days; T_1 7-10 days; T_2 15-20 days; T_3 25-40 days; T_4 45-60 days), in COVID-19 patients. Also a group of healthy control subjects was enrolled.
Materials and Methods: We enrolled a total of 58 patients admitted to “Mater Domini” University Hospital of Catanzaro from March to December 2020. We selected sera from 37 COVID-19 patients confirmed by rRT-PCR. Also a group of 21 healthy control subjects with a negative SARS-COV-2 rRT-PCR was included. The kinetic of IgA-Abs and IL-35 was tested using Anti-SARS-CoV-2 IgA ELISA Kit (Euroimmun®) and Human Interleukin 35 (IL-35) ELISA Kit (Cusabio®), respectively.
Results and conclusions: The Anti-SARS-CoV-2 IgA levels of COVID-19 patients were significantly higher than the control subjects. IgA levels significantly increased starting from day 4 and peaked between 7-20 days; afterward such IgA significantly decreased without achieving undetectable levels after 60 days. The IgA durability, as well as the early onset may be very interesting besides the diagnostic purposes, also for pathogenic implications, because IgA may defend the mucosal barriers against viruses (Paces J. et al., 2020, doi: 10.33549/physiolres.934492). IL-35 levels were significantly higher than the healthy control subjects. The IL-35 levels started to increase during the first 4 days, peaked within 15-20 days from symptoms onset and remained at substantially high levels after 45-60 days, suggesting that IL-35 could be used as a new COVID-19 early marker. Also such cytokine might play a role in reducing durability of SARS-CoV-2 specific antibodies.

P110
Late onset intra-uterine growth restriction: role of HHV-6 infection

G. Schiuma[1], D. Bortolotti[1], V. Gentili[1], S. Rizzo[1], E. Santi[2], C. Taliento[2], A. Vitagliano[2], S. Beltrami[1], G. Lanza[3], R. Gafà[3], P. Greco[2], R. Rizzo[1]

[1] Department of Chemical and Pharmaceutical Sciences, University of Ferrara (Italy) - [2] Department of Medical Sciences, Obstetric and Gynecological Clinic, University of Ferrara (Italy) - [3] Department of Translational and for Romagna Medicine, University of Ferrara (Italy)

Background: Late onset intra-uterine growth restriction (IUGR) refers to an impaired growth and development of the fetus and/or its, characterized by placental morphological abnormalities that affect fetus' supply nutrients supply.
Human Leukocyte Antigen-G (HLA-G) is physiologically expressed during pregnancy, but in normal placenta decreased during the last weeks of gestation induce childbirth. Several viruses involved in congenital infection, such as herpesviruses, exploit HLA-G expression as an immune-escape mechanism. To date, despite different congenital herpetic infections have been associated with late IUGR, no direct implication of HHV-6 infection, has been reported.
Aim and Methods: We evaluated HLA-G expression and HHV-6 infection in 11 placentas from late onset IUGR newborns and 11 placentas from uncomplicated pregnancies by histopathological and immunohistochemistry analysis.
Results: We found higher levels of HLA-G expression and HHV-6 presence in IUGR placentas compared with physiological placentas and a co-localization of HLA-G expression and HHV-presence in IUGR samples, associated with maternal and newborn clinical characteristic.
Conclusions: We report HLA-G expression alteration in IUGR placentas in presence of HHV-6, suggesting the direct involvement of HHV-6 in HLA-G deregulation that affects vessels remodeling and prevents the correct pregnancy carry-over observed in IUGR. Although these findings demonstrate HHV-6 association with IUGR, further studies are required.

P111

HSV-1 as a risk factor in the pathogenesis of Alzheimer's disease: expression of nuclear tau in different cell lines and in mouse model neurons

M. Sicurella[1], D. Cecchi[1], M. Buratto[1], A. Caproni[1], F. Salvatori[1], M. Pappadà[1], R. Fontana[1,2], M.C. Tomasini[3], R. Manservigi[2], P. Marconi[1]

[1] Department of Chemical, Pharmaceutical and Agricultural Sciences (DOCPAS), University of Ferrara - [2] Department of Life Sciences and Biotechnology, University of Ferrara, Italy - [3] Department of Biomedical and Specialized Surgical Sciences, Physiology division, University of Ferrara, Italy

Initial observations of Herpes simplex virus (HSV1) type 1 DNA in Alzheimer's disease (AD) brains, suggested a possible involvement of the virus in the pathogenesis of AD. The main pathological hallmarks of the AD are: chronic inflammation, calcium dyshomeostasis and the presence of the aberrant highly insoluble protein aggregates neurofibrillary tangles (NFT) and senile plaques (SP), respectively composed by the amyloid-β peptide (Aβ) and the hyperphosphorylated protein tau (P-tau). Previous studies have suggested a link between HSV1 and abnormally phosphorylated tau in the development of AD. This work is focused on the possible involvement of HSV1 in perturb protein tau physiology, paying particular attention to nuclear tau since several viral functions such as transcription, DNA replication, assembly of new capsids and packaging of DNA occur in the host cell nucleus. Therefore, a possible interference of HSV-1 with nuclear tau stems from different observations such as: i) the virus induces oxidative stress, a type of injury to which neurons respond by recruiting tau in the nucleus; ii) HSV-1 induces chromatin relaxation, by promoting histone acetylation, and tau was found to bind DNA in a similar manner to histones.

In order to endorse the hypothesis of a possible correlation between HSV1 and nuclear tau, Western blot and Immunofluorescence analysis were conducted using Tau-1 antibody that recognizes Tau when serine residues (195-202) are dephosphorylated. Despite several groups showed that HSV1 leads to an increase in the hyperphosphorylated form of Tau, we demonstrated that HSV-1 infection induces the accumulation of Tau-1 positive isoforms in the nucleus of mitotic SH-SY5Y and HRPE cells and in the axons of differentiated neuronal cells. In particular, in SH-SY5Y and HRPE cell lines, HSV-1 rapidly affected nucleolar organization promoting the distribution of Tau-1 in the whole nucleus, suggesting that HSV-1 infection interfere with the localization of Tau and its association with cellular DNA. Subsequently, the temporal profile of Tau expression in primary cultures of cerebral cortex neurons from the triple-transgenic mouse model of AD (3xTg-AD) was determined following HSV-1 infection. Primary HSV-1 infected neurons showed a marked increase of Tau-1 positive isoforms that appeared clustered in dots and dispersed along the axons. These results indicate that Tau levels, regardless of its cell-dependent localization, increase and accumulate in the presence of viral infections, suggesting that HSV-1 may contribute at the outbreak of the pathological phenotype in neuronal cells.

P112

Modulation of mirnome by HCMV and HHV-6 infection in human dermal fibroblasts: possible significance in systemic sclerosis

I. Soffritti[1], M. D'Accolti[1], G. Ravegnini[2], C. Arcangeletti[3] and E. Caselli[1]

[1] Department of Chemical, Pharmaceutical and Agricultural Sciences and LTTA, University of Ferrara, 44121 Ferrara, Italy - [2] Department of Pharmacy and Biotechnology, University of Bologna, 40126 Bologna, Italy - [3] Department of Medicine and Surgery, University of Parma, 43126 Parma, Italy

Background: Human cytomegalovirus (HCMV) and Human herpesvirus 6 (HHV-6) have been reportedly suggested as triggers of the onset and/or progression of many autoimmune diseases. Among them, we recently reported a possible involvement of both viruses in systemic sclerosis (SSc), a severe autoimmune disorder characterized by vasculopathy and multi-organ fibrosis. The etiology and pathogenesis of SSc are still largely unknown but virological and immunological observations support a role for these beta-herpesviruses. We recently observed a direct impact of HCMV and HHV-6 infection on the expression of cell factors associated with fibrosis at the cell microenvironment level. Since in SSc patients miRNA expression has been found deregulated at the tissue or blood level, here we aimed to investigate the impact of HCMV and HHV-6 infection on the miRNome of in vitro infected primary human dermal fibroblasts, which represent one of the main SSc target cells.

Methods: Human primary dermal fibroblasts were infected in vitro with cell-free inocula of HCMV and HHV-6, and at different times post infection (0, 4, 7, 10, and 14 d.p.i.) were collected to extract RNA. The analysis was performed by Taqman arrays detecting and quantifying 754 miRNAs.

Results: The miRNome analysis showed that both viruses significantly modulated miRNA expression in infected cells, with effects evident at both early and late times p.i.. PCA analysis showed a significantly different clusterization of miRNA at all time tested. Up to 106 miRNAs were up-regulated and 170 down-modulated by HCMV infection; HHV-6 infection up-regulated the expression of up to 117 miRNA and down-modulated 112 miRNAs. Several altered miRNAs belong to those already recognized for their key function in fibrosis; several other miRNAs appear potentially involved in the process leading to cell function impairment and apoptosis.

Conclusion: HCMV and HHV-6 infection profoundly remodulate cell miRNome in human dermal fibroblasts, and the correlation between these in vitro results with in vivo observations is strongly suggestive of a role of HCMV and HHV-6 in the multistep pathogenesis of fibrosis in SSc.

Keywords: miRNome; HCMV; HHV-6; systemic sclerosis; fibrosis

P113 - OC 40

Potential of latency reversing agents in NK cell-mediated eradication of the HIV reservoir

D.A. Covino, M.G. Desimio, M. Doria

Primary Immunodeficiency Unit, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

Aim of the study: The antiretroviral therapy (ART) effectively reduces HIV viral loads to undetectable levels, but viral persistence in latently infected CD4+ T cells makes actual drug regimens unable to cure infection. 'Shock and kill' is an actively pursued strategy towards HIV eradication that implies administration of latency reversing agents (LRAs) associated with the elimination of cells harbouring reactivated HIV by host immune responses or viral-induced apoptosys. However, concluded LRA-based trials have failed to reduce the HIV reservoir size of ART patients and clearly demonstrated the need for interventions improving both latency reversal and immune-mediated killing. Here we searched for 2-LRA combinations that could enhance NK-cell mediated clearance of latent HIV reservoir via the potentiation of the NKG2D/NKG2D ligands (NKG2DL) axis. The rational behind this approach stands on the reported ability of various LRAs to induce both expression of latent HIV and NKG2DLs, thus potentially enhancing the virus-mediated NKG2DL induction and increasing the susceptibility of T cells that exit viral latency to recognition and killing by NK cells via their activating NKG2D receptor.
Methods: Using experimental T cell models of latency, we screened 2-LRA combinations for their capacity to reactivate HIV and up-regulate NKG2DLs while preserving cell viability. LRA combinations consisted in one leading candidate histone deacetylase inhibitor (HDACi) (Vorinostat, VOR; Romidepsin, ROM; Panobinostat, PAN) associated with a PKC agonist (Prostratin, PRO), proteasome inhibitor (Bortezomib, BOR) or TLR-7 agonist (GS-9620). Selected combinations were then tested for their impact on NK cell viability, phenotype, and function. The effects of LRA combinations were evaluated in NK-cell mediated killing of autologous CD4+ T cells that exit HIV latency using an experimental system in which both effector and target cells were equally exposed to the drugs.
Results and conclusion: In the J1.1 latently infected T-cell line, HIV reactivation and expression of NKG2Ls were induced at higher levels by combinations of VOR or ROM with PRO and of any HDACi with BOR as compared with single drug treatments; on the other hand, GS-9620 impaired the capacity of each HDACi to induce HIV and NKG2DL expression. In primary cells, BOR was highly toxic for T and NK cells at a clinically relevant dose, whereas PRO, which by itself increased the viability of CD4+ T and NK cells, attenuated the toxic effects of VOR or ROM in CD4+ T cells as well as the toxicity of ROM and PAN in NK cells. In addition, while VOR and PAN had deleterious effects on the NK-cell phenotype, the NKG2D receptor was up-modulated by ROM and, to a higher level, by the ROM/PRO combination. By using a primary CD4+ T cell-based model of latency, we demonstrated that HDACis did not reactivate HIV in terms of Gag p24 protein expression and partly antagonized the latency reversal activity of PRO when used in combination; nevertheless,

ROM/PRO resulted in a significantly higher HIV reactivation and further NKG2DL up-modulation as compared to ROM alone. Finally, we showed that ROM did not stimulate NK-cell mediated suppression of T cells that exit viral latency, whereas PRO and, even better, ROM/PRO increased suppression; importantly, by pre-incubating NK cells with anti-NKG2D blocking antibody, we were able to demonstrate that ROM/PRO augmented the function of NKG2D in NK cell-mediated suppression of p24+ target cells as compared with single drug treatments.

Overall, this study disclosed limitations of current LRA candidates and emphasizes the importance of considering the effects on NK cells of potential interventions to eliminate the latent HIV reservoir. Moreover, our results provided evidence that the pathways mediating NK cell recognition and killing of T cells harboring reactivated HIV may be specifically modulated by LRAs.

P114
Rhein and oncolytic herpes simplex virus in HCC treatment

A. Ambrosino[1], D. Stelitano[1], F. Dell'Annunziata[1], F. Caruso[1], B.M. Nastri[1], A. Chianese[1], B. Santella[1], G. Franci[2], M. Galdiero[1]

[1] Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", 80138, Napoli, Italy - [2] Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, 84081 Baronissi, Italy

Aim of the study. Hepatocellular carcinoma (HCC) is a global health burden representing the fourth cause of death among tumors worldwide [1]. Actually the only approved drugs for liver cancer treatments are sorafenib, regorafenib and lenvatinib [2]. These drugs often result in an unsuccessful outcome in advanced liver cancers, so new approaches occur. The combination of oncolytic viruses and epigenetic drugs has been proposed as a valid solution for different types of cancers. Rhein (4,5-dihydroxyanthraquinone-2-carboxylic acid), a natural compound obtained from rhubarb, has been suggested as a valid instrument for HCC therapy [3]. We investigated the potential of the combination between an oncolytic Herpes simplex virus type 1 (oHSV-1) and rhein in HCC treatment. oHSV-1 is an engineered virus modified to infect and to replicate only in cancer cells.
Methods used. Huh-7 and HepG2 cell lines were treated with different concentrations of rhein and oHSV-1 in a post-treatment assay for 24, 48 and 72 hours. Cell viability was evaluated through MTT assay. Apoptosis and the cell cycle arrest was evaluated after the combinatorial treatment. In particular, apoptosis was analyzed through the evaluation of the nuclear fragmentation, and chromatin condensation by DAPI staining, the expression of caspase 3, 9 and 8 by Western blotting analysis. Cell cycle arrest and cell death were evaluated by FACS and Annexin V analysis.
Results and conclusions. Rhein and oHSV-1 combinatorial therapy triggered apoptosis of cancer cells. We observed an increased nuclear fragmentation and chromatin condensation compared to the non-combinatorial treatment. This stronger effect is remarkable already after 24 hours. Together these data confirm the role of rhein as a pro-apoptotic compound in HCC cell lines. Moreover, this effect is enhanced when rhein is combined with oHSV-1 in post-treatment. These results suggest that our combinatorial approach may represent a valid solution to face the problem of resistance to treatments in advanced liver cancer and so give hopes to the future research.

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P115

Increased susceptibility to skin carcinogenesis and UVB-induced damage in immunodeficient human paillomavirus (HPV)-8 transgenic mice

C. Borgogna[1], L. Martuscelli[1], C. Olivero[2], G. Patel[2], B. Akguel[3], R. Boldorini[4], M. De Andrea[5,6], and M. Gariglio[1]

[1] Virology Unit, Department of Translational Medicine, Novara Medical School, Italy - [2] European Cancer Stem Cell Research Institute, School of Biosciences, Cardiff University, UK - [3] Institute of Virology, University of Cologne, Medical Faculty and University Hospital Cologne, Germany - [4] Pathology Unit, Department of Health Sciences, Novara Medical School, Italy - [5] Virology Unit, Department of Public Health and Pediatric Sciences, Turin Medical School, Italy - [6] CAAD-Center for Translational Research on Autoimmune and Allergic Disease, Novara Medical School, Italy

A large body of experimental and epidemiological evidence indicates that infection by skin-tropic papillomavirus belonging to the β-genus (β-HPV genotypes whose prototype is HPV8) contribute to increased skin cancer risk, especially in the immunocompromised setting, e.g. organ transplant recipients (OTR). Despite these findings, a causal role of these viruses has been difficult to verify because of their ubiquitous prevalence in the general population, their absence in some cancers, and the lack of experimental animal models. To provide mechanistic insights into β-HPV-induced skin carcinogenesis in the immunosuppressed setting, a mouse model that recapitulates the events occurring in organ transplant recipient (OTR) was generated by crossing the β-HPV8 transgenic mice (K14-HPV8, that express the entire early region of the HPV8 genome in a skin specific manner) with Rag2 deficient mice ($Rag2^{-/-}$) that lack functional B and T lymphocytes. Our findings provide proof of concepts that immunosuppression accelerates HPV8-induced skin carcinogenesis. Indeed, skin cancer development in the $Rag2^{-/-}$: K14-HPV8 has been more aggressive in terms of incidence profile, lesion extension, and progression to overt cancer when compared to the immunocompetent counterpart, $Rag2^{+/+}$:K14-HPV8. As β-HPV-induced skin cancer occurs mainly in sun-exposed areas of the body with the contribution of UVB-induced DNA damage, we used our mouse model to demonstrate a causal link between HPV8 infection, immunosuppression, UVB exposure, and skin cancerogenesis. We show that exposure to a single low dose of UVB is sufficient to induce skin hyperplasia and inflammation in a remarkable short period of time in $Rag2^{-/-}$:K14-HPV8 while nothing relevant is happening in $Rag2^{+/+}$:K14-HPV8. UVB-induced DNA damage was still evident when the mice were sacrificed at 30 days post UVB-exposure as documented by immunohistochemical staining with the DNA-damage marker γH2AX. A statistically significant accumulation of γH2AX-positive cells was observed when UVB-irradiated $Rag2^{-/-}$:K14-HPV8 were compared to $Rag2^{+/+}$:K14-HPV8. Histologically, the skin of UVB-irradiated $Rag2^{-/-}$:K14-HPV8 was thicker, hyperplastic, and showed increased accumulation of mast cells in the dermis when compared to $Rag2^{+/+}$:K14-HPV8. Next, Bio-Plex immunoarrays have been used to compare the relative levels of 23 cytokines in skin extracts from $Rag2^{-/-}$:K14-HPV8 vs $Rag2^{+/+}$:K14-HPV8 mice that revealed an abundance of MCP-1, CXCL-1, IL-6, G-CSF, GM-CSF in the immunosuppressed mice when compared to the normocompetent counterpart.

P116

Involvement of microRNAs and extracellular vesicles in human papillomavirus-induced carcinogenesis

M.V. Chiantore[1], R.M. Mongiovì[1], M. Iuliano[2], G. Mangino[2], L. Capriotti[2], M. Tommasino[3], P. Di Bonito[1], G. Romeo[1,2]

[1] Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy - Department of Medico-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Italy - [3] Infections and Cancer Biology Group, International Agency for Research on Cancer, Lyon, France

Background: Human Papillomaviruses (HPVs) of beta genus infect cutaneous keratinocytes. Emerging biological evidence indicates that some β-HPV genotypes share similar biological, transforming properties with certain anogenital high-risk (HR)-HPVs of alpha genus. In fact, the β3-species HPV49 and 76 have been shown to induce immortalization of primary keratinocytes with similar efficiency to HPV16.
Aim of the study: This study aims to investigate whether different HPV types (alpha and beta) can perturb cellular processes through the deregulation of microRNA (miRNA) expression profile. Moreover, since the extracellular vesicles (EVs) may contribute to the reprogramming of the cancer microenvironment, it has been tested if HPV expressing cells could support cellular transformation by EV delivery of HPV oncogenes as well as significant miRNAs.
Methods: TaqMan Array Human MicroRNA Card assay was used to detect the miRNA expression profiles on keratinocytes transduced with E6/E7 from HPV16, -49 and -76 (K16, K49 and K76 cells) and in the EVs isolated by differential centrifugation (i.e. 500 x g per 10', 2000 x g per 10', 100000 x g per 60') from the supernatant of two of these cell types (EV_K16, EV_K49). The expression of selected oncogenic miRNAs and the E6/E7 viral oncogenes were validated by Real Time RT-PCR, while Western Blot analyses were performed to study the microRNA target expression.
Results: The expression of E6 and E7 from the different HPV types studied affects the expression of specific miRNAs compared to control keratinocytes. In particular, 35 and 28 miRNAs were shared among K16 and K49 or K76 cells, respectively, while 19 microRNAs were found to be deregulated in all the three cell types. The analysis of the expression of miRNAs in the EVs was first performed in EV_K16 compared to EVs silenced for E6/E7 (silenced EV_K16). Twenty-six miRNAs appear to be upregulated in EV_K16 while 6 are downregulated. Moreover, 18 miRNAs are exclusively expressed in EV_K16 whereas 16 in silenced EV_K16. A selection of deregulated miRNAs (miR-17, -21, -429, -518b, -590-5p), validated by RT-qPCR, showed an upregulated expression in EV_K16. Subsequently, miRNA expression was analyzed in EV_K49 compared to EVs silenced for E6/E7 (silenced EV_K49). MiR-17, -19a, -21, -31, -34a, -99a and -590-5p were upregulated in EV_K49 compared to silenced EV_K49. These specific HPV-related miRNAs show a similar series of targets. Interestingly, Cyclin D1 is a target of all of the miRNAs analyzed in EV_K49, whereas p21 and CDK4/CDK6 are targets of three of these miRNAs.
The expression of E6 and E7 mRNAs into EV_K16 and EV_K49 has been also investigated. HPV16 E6 and E7 mRNA is detected in EV_K16 whereas more experiments are needed to confirm the oncogenes expression in EV_K49.

Conclusions: The results showed that the expression of E6 and E7 from different HPVs in keratinocytes affects the expression of specific miRNAs. This highlights the plasticity of the virus/host interaction of different genotypes during a persistent HPV infection. Moreover, the observation of the presence of specific miRNAs as well as of viral oncogenes in EVs released by HPV-positive cells suggests that the EV delivery may potentiate the virus oncogenic effects in the tumor cell microenvironment.

P117

Virotherapy as a novel tool for reshaping tumor microenvironment in aggressive breast cancer

S. Di Somma, F. Napolitano, G. Castellano, I. Cimmino, M. Cennamo, M. Fiorenza, A.M. Malfitano, P. Formisano, G. Portella

Dipartimento di Scienze Mediche Traslazionali, Università degli Studi di Napoli Federico II

Aim of the study. Oncolytic virotherapy based on the use of oncolytic viruses (OVs) has received significant attention since the approval of an engineered Herpes Simplex Virus Talimogene Laherparepvec (T-VEC) in 2015 by FDA and EMA. Our group has extensively evaluated the anticancer activity of the oncolytic adenovirus *dl*922-947 in different cancer models. Our hypothesis is that the oncolytic adenovirus *dl*922-947 might be an efficient direct anticancer agent in aggressive breast cancer. Moreover, *dl*922-947 represents also a tool to dissect the complex interactions within BC tumor microenvironment (TME), the interaction of OVs with TME is quite unexplored and needs to be elucidated to unveil the efficacy of virotherapy, since TME as well cancer cells influence therapeutic response and patient prognosis.
Methods used. We first assessed the cytotoxic effect of *dl*922-947 on two BC cell lines MDA-MB231 (ER-/triple negative, aggressive) and MCF7 (ER+, less aggressive) 72 hours post infection trough SRB assays. We have evaluated apoptosis/necrosis by Annexin V/Propidium Iodide staining followed by flow cytometry analysis. Immunogenic cell death (ICD) has been evaluated testing the release of immunomodulatory molecules such as adenosine triphosphate (ATP) by a luminescence assay, high mobility group box 1 (HMGB1) intracellular content and/or surface exposure of calreticulin (CALR) by flow cytometry. Finally, we have assessed *dl*922-947 modulation of CCL5 and IL-6 secretion, cytokines highly released in aggressive BC, in MDA-MB231 and MCF7 infected cells.
Results and conclusions. *dl*922-947 infection exhibits cytotoxic effects in aggressive BC cell lines (MDA-MB231, IC 50 3 pfu/cell), inducing apoptosis and regulating main hallmarks of ICD. *dl*922-947 affects the release of ATP and HMGB1 expression which could enhance the recruitment and activation of dendritic cells, and CALR exposure which could promote phagocytosis. Breast TME is partly composed of macrophages that markedly infiltrate the tumor and present a pro-tumorigenic (M2) phenotype usually associated with poor prognosis. OVs infection and modulation of multiple factors secreted by tumor cells may shift M2 activated macrophage toward M1 macrophages, contributing to stimulation of antitumor immunity. We will assess whether *dl*922-947 affects BC-TME cells crosstalk, focusing on the BC cells-macrophages interaction. Our results suggest that dl922-947 may affect immune cells recruitment by regulation of CCL5 and IL-6 secretion. We will evaluate other cytokines and chemokines highly expressed in triple negative breast cancer (TNBC) and playing important role in homing and migration of immune cells such as GM-CSF, M-CSF, IL-6, TGFβ, TNF-α, IL-8. Moreover, we are setting up differentiation protocols of THP-1 cells, an acute monocytic leukemia cell line, largely used as a human model of

monocytes/macrophages, that will help us to define the effects of *dl*922-947 on the crosstalk between BC cells and macrophages. The study of OVs effects in BC microenvironment could unveil its therapeutic efficacy providing a complete evaluation of BC-TME crosstalk. Moreover, it could reveal unknown mechanisms responsible for acquisition of drug resistance, and help to predict therapy response.

P118

Dual Cytoplasmic and nuclear localization of HTLV-1-encoded HBZ protein is a unique feature of adult cell leukemia

G. Forlani[1], M. Shallak[1], A. Tedeschi[1], I. Cavallari[2], A. Marais[3], O. Hermine[3], R.S. Accolla[1]

[1] Laboratories of General Pathology and Immunology "Giovanna Tosi", Department of Medicine and Surgery, University of Insubria, Varese, Italy - [2] Istituto Oncologico Veneto IOV - IRCCS, Padua, Italy - [3] Department of Hematology, Necker-Enfants Malades, University Hospital, Assistance Publique Hopitaux de Paris, Paris Descartes University, Paris, France

Adult T-cell leukemia-lymphoma (ATL), is a highly malignant T-cell neoplasm caused by human T-cell leukemia virus type 1 (HTLV-1), characterized by a poor prognosis. Two viral proteins, Tax- 1 and HBZ play important roles in the pathogenesis of ATL. While Tax-1 can be found in both cytoplasm and nucleus of HTLV-1 infected patients, HBZ is exclusively localized in the cytoplasm of HTLV-1 asymptomatic carriers and patients with chronic neurologic disease HAM/TSP, and only in the nucleus of ATL cell lines, suggesting that the nuclear localization of HBZ can be a hallmark of neoplastic transformation. To clarify this crucial point, here we investigated in detail the pattern of HBZ expression in ATL patients. We made use of our monoclonal antibody 4D4-F3, that at present is a uniquely reported reagent, among the few described, able to detect endogenous HBZ by immunofluorescence and confocal microscopy in cells from asymptomatic carriers, HAM/TSP and ATL patients. We found that HBZ localizes both in the cytoplasm and in the nucleus of cells of ATL patients irrespective of their clinical status, with a strong preference for the cytoplasmic localization. Also Tax-1 localized in both compartments. As HBZ is exclusively localized in the cytoplasm in asymptomatic carriers and in non-neoplastic pathologies, this finding shows that neoplastic transformation consequent to HTLV-1 infection is accompanied and associated with the capacity of HBZ to translocate to the nucleus, which suggests a role of cytoplasmic-to-nuclear translocation in HTLV-1-mediated oncogenesis

P119 - OC 27

Immunotherapeutic effectiveness of retargeted HSV oncolytic virus against the prostate membrane antigen

T. Gianni[1], A. Vannini[1], D. Bressanin[1], F. Parenti [2], A. Zaghini [2] and G. Campadelli-Fiume[1]

[1] Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, 40126 Bologna, Italy - [2] Department of Veterinary Medical Sciences, University of Bologna, 40064 Bologna, Italy

The FDA and EMA approval of the oncolytic herpes simplex virus (oHSV) OncovexGM-CSF drove renewed interest in oncolytic virotherapy. By definition, oncolytic viruses (OVs) infect and kill tumor cells and spare healthy tissues. Most frequently, the cancer-selective OVs generated by deletion of virulence genes grow to high yields in tumor cells, which are defective in mounting an antiviral innate response, and replicate very poorly in the cells which sustain the natural infection. Inasmuch as OVs elicit an antitumor immune response, they serve as immunotherapeutic agents, and can be combined with additional immunomodulatory molecules, including immune checkpoints inhibitors. Our laboratory has pursued the generation of cancer-specific rather than cancer-selective oHSVs by retargeting HSV tropism to cancer-specific receptors selected from the family of Tumor Associated Antigens. This strategy fully preserves virulence in the targeted cancer cells, prevents off-tumor and off-target infections, and results in high immunotherapeutic efficacy and improved safety profile.

Prostate cancers account for about 20% of all cancers diagnosed in men over the age of 50. PSMA (prostate specific membrane antigen) is expressed at high frequency and at high level in prostate cancer cells, and at low level in non-cancerous prostate cells. These properties make PSMA an interesting candidate receptor for retargeted oHSVs. The oHSV R-405 is retargeted to PSMA, through the insertion in gD of a single-chain variable-fragment antibody to PSMA, and is detargeted from the HSV natural receptors HVEM and nectin-1. R-405 is armed with murine IL-12 to increase its immunotherapeutic profile. In vitro, R-405 specifically infected PSMA-positive cells and failed to infect PSMA-negative cells that express the HSV natural receptors. When administered intratumorally, R-405 fully inhibited the growth of LLC-1-PSMA tumours implanted in immunocompetent mice, and conferred long-term protection against PSMA-positive as well as PSMA-negative untreated distant tumors.

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Human papillomavirus (HPV) testing of vaginal self-collected samples: evaluation of different resuspension media

C. Giubbi[1], M. Martinelli[1], I. Triva[1], R. Musumeci[1], F. Perdoni[1], G.E. Calcagno[1], S. Canali[1], R. Fruscio[1,2], F. Landoni[1,2], S. Castriciano[3], C. Cocuzza[1]

[1] University of Milano-Bicocca, Monza, Italy - [2] San Gerardo Hospital, ASST Monza, Monza, Italy - [3] Copan Italia, Brescia, Italy

Aim of the study. Human Papillomavirus (HPV) testing in cervical cancer screening offers the possibility to introduce self-sampling in screening programs to improve coverage rates. ThinPrep (Hologic) medium has been widely used to suspend cervical and vaginal self-samples for HPV testing. However, this medium is toxic and flammable, requiring special handling and additional costs for the transport. This study aims to evaluate MSwab™ and eNat™ (Copan) as alternative resuspension media for HPV detection in self-collected vaginal samples.

Methods used. Two self-collected vaginal swabs (FLOQSwab®, Copan) and a physician administered cervical sample (L-shaped Endocervical/Esocervical FLOQSwab®, Copan) were collected from 20 women referred for colposcopy at San Gerardo Hospital (Monza, Italy) because of an abnormal cervical cytology. The self-collected vaginal samples were transported dry to the laboratory; from 10 women swabs were resuspended in 5 ml of ThinPrep and 5 ml of MSwab™ respectively and from the remaining 10 women in 5 ml of ThinPrep and 5 ml of eNat™.

All samples were extracted using Microlab Nimbus starting from 200 ul of sample and HPV detection performed with AnyplexII HPV28 (Segeene). Vaginal samples were tested on the day of the collection, after one (T1) and two (T2) weeks stored at room temperature to evaluate the stability of different media. Concordance in HPV detection between sample types was determined using Cohen's Kappa value (k).

Results and conclusions. A good concordance in the results of HPV test was observed between cervical and vaginal self- samples resuspended in MSwab™ and eNat™; with an excellent agreement (k=1.000) between vaginal self-samples suspended in ThinPrep as compared to both MSwab™ and eNat™ even after one- and two-weeks storage at room temperature. These preliminary data suggest that eNat™ and MSwab™ may be a valid alternative to ThinPrep for the resuspension of vaginal samples for HPV testing. Further studies on a larger number of samples are necessary to confirm these findings and to assess the stability of viral nucleic acids in these media.

P121 - OC 29

The cellular deacetylase SIRT1 contributes to P53 curbing by HPV16 and 18 and its targeting inhibits cancer cell proliferation

I. Lo Cigno[1], F. Calati[1], C. Girone[1], G. Loi[2], M. Krengli[3], M. Niebler[4], F. Rösl[4], A. Venuti[5], M. Gariglio[1]

[1] University of Piemonte Orientale, Medical School, Department of Translational Medicine, Molecular Virology Unit, Novara, Italy - [2] Medical Physics, University Hospital Maggiore della Carità, Novara, Italy - [3] Division of Radiotherapy, University Hospital Maggiore della Carità, Novara, Italy; Department of Translational Medicine, University of Piemonte Orientale, Novara, Italy - [4] Division of Viral Transformation Mechanisms, German Cancer Research Center (DKFZ), Heidelberg, Germany - [5] HPV Unit, UOSD Tumor Immunology and Immunotherapy, IRCCS Regina Elena National Cancer Institute, Rome, Italy

Human papillomavirus (HPV)-associated cancers of the genital and head&neck (HN) region represent ~5% of all cancers worldwide and are set to remain a major health concern for the foreseeable future, thereby requiring novel effective therapeutic solutions. Currently, the treatment for these tumors involves radiotherapy, chemotherapy, or surgery, all with devastating effects on the targeted anatomical sites. Thus, alternative antiviral therapies with fewer side effects are urgently needed to improve patient outcomes. Sirtuins are an evolutionarily conserved family of NAD+- dependent deacetylases and ADP-ribosyltransferases playing important roles in a broad range of biological activities. SIRT1, the main NAD+-dependent deacetylase in mammalian cells, functions through deacetylation of its substrates, which include histone substrates and non-histone targets, such as p53. It is well known also that SIRT1 is a crucial regulator of HPV replication in cervical keratinocytes, it is involved in E1-E2-mediated viral DNA replication, and it is strongly upregulated in HPV+ cells in an E7-dependent fashion as well as in cervical intraepithelial neoplasia (CIN), with an increasing trend from low-to high-grade CIN1 to 3. By contrast, much less is known about SIRT1 expression in head and neck cancer (HNC). Here, we have characterized the mechanistic details and function of a novel signaling pathway involving the cellular deacetylase SIRT1 in HPV-induced carcinogenesis and assessed whether targeting of this pathway is an effective anticancer strategy against HPV-associated cancer. Two cervical carcinoma derived cell lines, HeLa and CaSki— harboring integrated HPV18 and HPV16 genome, respectively—and NOKE6E7 cells, a newly established model of HPV-induced HN carcinogenesis have been used. SIRT1 inhibition by either the pharmacological inhibitor EX527 or gene silencing restored functional p53 in HPV+ cells. Much to our surprise, we also found that SIRT1 inhibition downregulates E6 and E7 expression levels suggesting that SIRT1 may be targeted to not only rescue p53 activity but also revert the malignant phenotype. Consistently, SIRT1 inhibition by EX527 treatment alone promoted cell death of HPV+ cell lines and increased their sensitivity to sublethal doses of doxorubicin (DX). Likewise, SIRT1 inhibition also increased sensitivity to cisplatin or radiotherapy-induced apoptosis.

In addition, the impact of SIRT1 inhibition on HPV16-driven tumorigenesis has been also assessed using an in vivo tumorigenicity assay based on dorsal subcutaneous (s.c.) injection

of syngeneic C3 cells, which harbor an integrated HPV16 genome, in C57BL/6J mice. Upon s.c. injection, mice were treated or not with EX527, and tumor growth was measured overtime by means of a Vernier caliper. Remarkably, EX527 treatment led to a significant reduction of tumor growth when compared to vehicle-treated animals while rescue of p53 expression was also observed by immunohistochemistry. Altogether, our findings uncover an essential role of SIRT1 in HPV-driven carcinogenesis and provide evidence that inhibition of such process can sensitize transformed cells to genotoxic agents or ionizing radiation.

P122 - OC 28

dl922-947 adenovirus and G-quadruplex binder combination against breast cancer

A.M. Malfitano[1], F. Napolitano[1], S. Di Somma[1], J. Amato[2], B. Pagano[2], A. Randazzo[2], G. Portella[1]

[1] Department of Translational Medical Sciences, University of Naples Federico II, 80131 Naples, Italy - [2] Department of Pharmacy, University of Naples Federico II, 80131, Naples, Italy

Aim of the study: G-quadruplex (G4) are secondary structures characterized by the formation of G-tetrads, planar cyclic arrays of four guanine bases. G4 motifs are observed at the telomeres and in the promoter regions of oncogenes. Stabilization of G4 motifs with specific ligands induces DNA damage along with induction of cancer cell death. Small molecules targeting G4 are the focus of clinical investigation as anticancer agents. We hypothesized that DNA destabilization induced by G4 ligands, might favor or potentiate the anticancer activity of other agents that targeting DNA may better elicit their antineoplastic activity or cooperate with G4 ligands to counteract cancer cell growth and diffusion. To this aim, we used oncolytic viruses (OVs), in particular, the adenovirus dl922-947 able to replicate and exert a direct lytic activity in cancer cells. We approached combinatory studies using Braco-19 and Pyridostatin as G4 ligands in combination with dl922-947 to address potential enhanced anti-tumor effects in breast cancer.
Methods used: We used breast cancer MDA-MB-231 and MCF-7 cell lines to evaluate the anticancer effects of G4 ligands, dl922-947 and/or their combination. We assessed cytotoxicity induced by sulforhodamine B assay. Cell cycle progression and G4 structure formation were evaluated by flow cytometry. Viral entry using a non-replicating reporter adenovirus transducing GFP (AdGFP) was also assessed by flow cytometry after 24 and 48 hours post infection. dl922-947 DNA amplification was quantified by Real-Time PCR. SA-β-gal staining was used to assess cell senescence evaluated by light microscope.
Results and conclusions: G4 ligands and dl922-947 efficiently inhibited breast cell proliferation. G4 binders induced G4 motifs in breast cancer cells, in particular, in MCF-7 cells, G4 motif distribution was detected in the S and G2/M phases for both molecules, additionally, Braco-19 induced G4 motifs also in the subG0/G1 phase. The combination G4 binders-dl922-947 increased viral entry in both the cell lines, such effect is particularly interesting in MCF-7 cells that we found not susceptible to adenovirus entrance. Additionally, dl922-947 efficiently replicated in these cells, as revealed by the increase of viral copies in the intracellular fraction with respect to the extracellular release. Enhanced cytotoxicity was observed using the combination dl922-947-pyridostatin with respect to the single agents in MDA-MB231 cells, other combinations: dl922-947/pyridostatin, dl922-947/braco-19 (except in MCF-7 cells) increased cytotoxicity with respect to the virus used as single agent. These effects were associated with variation of the cell cycle progression with increase in the subG0/G1 phase using G4 binders and dl922-947 in combination. Indeed, all the agents used singularly or in combination enhanced cell senescence. Noteworthy, we observed that dl922-947 induced G4 structure formation and the combination with pyridostatin potentiated this effect. In conclusion, our results suggest that the combination G4 binders /virotherpy might represent a novel therapeutic approach against breast cancer.

P123
U94 inhibits tumorigenesis of prostate cancer cells lines

F. Giordano[1], F. Caccuri[2], M.L. Panno[3], S. Marsico[4]

[1] Dipartimento di Farmacia e Scienze della Salute e della Nutrizione, Università della Calabria, Arcavacata di Rende (CS), [2] Dipartimento di Medicina Molecolare e Traslazionale, Università di Brescia, Brescia - [3] Dipartimento di Farmacia e Scienze della Salute e della Nutrizione, Università della Calabria, Arcavacata di Rende (CS), [4] Dipartimento di Farmacia e Scienze della Salute e della Nutrizione, Università della Calabria, Arcavacata di Rende (CS)

Advanced-stage prostate cancer progression and insensitivity to androgen ablation is a major problem in clinical therapy because the prognosis remains poor and no effective specific targeted therapy is readily available. Recently, we demonstrated the ability of U94, the latency gene of human herpes virus 6 (HHV-6), to exert its anticancer activity on three different triple- negative breast cancer cell lines by inhibiting DNA damage repair genes, cell cycle and eventually leading to cell death following activation of the intrinsic apoptotic. Interestingly, we found that U94 acted synergistically with DNA-damaging drugs. The role of U94 in favouring a less aggressive phenotype in cancer cells was confirmed on cervical cancer cells (HeLa), whereas the antitumor capability of U94 was further demonstrated in glioma cell lines U251 and U87. Furthermore, U94 suppressed transformation mediated by the oncogene H-ras in NIH 3T3 and tumorigenesis of prostate cancer PC3 cell line. In the current study, we demonstrate that U94 gene expression obtained by an HSV-1-based amplicon in prostate cancer cell lines (LnCap and PC3), is able to exert anticancer activity by inhibiting cell proliferation (by MTT assay), cell cycle (by flow cytometry) and leading to cell death following activation of the apoptotic pathway (by western blotting analysis). The induction of apoptosis by viral protein signifies a key target for cancer therapy. Viruses represent a cancer cause and, why not, a solution.

P124

Intra-tumor delivery of HPV therapeutic vaccines in an oral orthotopic preclinical model

F. Paolini [1,2], S. Falcucci [1,2], R. Covello[3], S. Massa[4], G. Coppola[5], C. Bonomo[3], V. Balzano[2], R. Franconi[6], A. Venuti [1,2]

[1] HPV-Unit - [2] UOSD Tumor Immunology and Immunotherapy - [3] Department of Pathology, IRCCS Regina Elena National Cancer Institute, Rome Italy - [4] Division of Biotechnology and Agroindustry - Department of Radiological, Oncological and Pathological Sciences, Sapienza University of Rome, Rome, Italy - [6] Division of Health Protection Technology, Department for Sustainability, ENEA, Rome, Italy

Aim of the study. High-risk Human Papillomaviruses (HPVs) are considered the causal agent of ano-genital cancers, and of a subset of head-neck cancers. Persistent/latent oral HPV infection, especially HPV16 infection, is associated with development of oropharyngeal squamous cell carcinoma (OPSCC) [Wu R et al, Oral Oncol. 2018]. In the last decades, a significant increase in the incidence of HPV-associated OPSCC has been observed in several western countries. Noteworthy, the presence of HPV in OPSCC is related with a better response to therapy and a better prognosis. Nevertheless, it should be noted this type of cancer doesn't have a true effective therapy which is burdened with a slew of comorbidities and high recurrence rates. New approaches such as immunotherapy appear to be valid alternative treatments. Prophylactic/therapeutic vaccines as well as genetic (DNA-based) vaccination are long lasting ideas that can be applied also for preventing and treating HPV-associated OPSCC. Our previous studies showed that a chimeric DNA vaccine formulation, the signal sequence of the Polygalacturonase-inhibiting protein (PGIPss) from Phaseolus vulgaris, fusion with L2 and E7 genes of HPV16 (Italian/European patent) was able to enhance humoral responses to HPV antigens [Massa S et al, Hum Vaccin Immunother. 2017; Franconi R et al, Cancers (Basel). 2020].
Goal of this study was to explore the possibility to enhance the activity of this chimeric DNA vaccine formulation by introducing an intra-tumor delivery of following HPV DNA vaccines: PGIPss-L2-E7 and E7-SAP (Italian and International Patent) where E7 gene of HPV16 was fused to mutated Saporin (SAP) from Saponaria officinalis. [Massa et al, Hum. Vaccines. 2011]. This last formulation was supposed to induce apoptotic effects that in turn may stimulate immune responses.
Methods. Vaccination was performed in a well-established pre-clinical animal model of HPV-associated oral cancer, AT-84 E7-Luc cells [Venuti et al, Cancer Immunol Immunother. 2015]. The following therapeutic vaccination schedule was applied by inoculating AT-84 Luc E7 cells in oral pavement of C3H mice (150x103 cell/inoculum). When tumors reached a palpable volume (about 1 mm in the longest diameter), mice were vaccinated via intramuscular plus electroporation (EP) and intra-dermal routes using 50 µg and 10 µg of PGIPss-L2-E7 DNA vaccine, respectively. After 7 days, mice were boosted with the same dose/scheduling. In addition, an intra-tumor injection of PGIPss-L2-E7 (50µg) or E7-SAP (50µg) DNA vaccines plus EP was made. Three days after last vaccination, mice were euthanized and tumor, serum and splenocytes collected for histological and immunological analyses, respectively.
Results. Systemic administration of PGIPss-L2-E7 DNA vaccine was able to decrease

tumor growth. Additional intra-tumor delivery of DNA vaccines improved tumor growth control only with PGIPss-L2-E7 vaccine whereas E7-SAP did not modify the response against tumor.
Histology showed presence of apoptosis in tumors injected with PGIPss-L2-E7 and surprisingly no apoptosis in E7-SAP treated tumors.
Humoral and cell-mediated immune responses upon these treatments were detected. Strong IgG response against L2 capsid protein (cross-reacting antigen for protection against different HPV) confirmed the possible double activity (preventive and therapeutic) of the chimeric formulation.
Conclusions. Additional intra-tumor treatment showed that PGIPss-L2-E7 vaccine was able to improve immune responses whereas E7-SAP did not. This effect can be ascribed to the presence of apoptosis within the tumor and/or to the introduction of a new antigen (L2) inside tumor, rendering it more immunogenic. This last scenario would open new perspectives of therapy by introducing neo-antigens inside tumors.

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Association between polymorphism in the enhancer gene and hepatitis C virus-induced hepatocellular cancer risk

E. Serretiello[1], G. Greco[1], L. Rinaldi[2], F. Pinto[1], M. Manfedini[1], F. Caruso[1], G. Franci[3], M. Galdiero[1]

[1] Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy - [2] Department of Advanced Medical and Surgical Sciences, University of Campania "L. Vanvitelli", Naples, Italy - [3] Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, 84081 Baronissi, Italy

Aim of study. Hepatocellular carcinoma (HCC) is an important complication of cirrhosis related to the hepatitis C virus (HCV) and is reported with an average incidence of 1-4% per year [1]. Furthermore, HCV proteins exert a direct carcinogenic effect by deregulating host cell cycle checkpoints and increasing immune-mediated oxidative stress, which in turn leads to a higher frequency of DNA mutations in liver cells [2]. As reported in the literature, HCV infection induces epigenetic changes that can increase the risk of HCC. These changes persist as an epigenetic signature even after treatment with direct acting antivirals (DAAs) [3]. Although there are surveillance programs for HCC, these are not effective because many cancers are diagnosed in the late stages and the patient is already in a terminal state. The purpose of our work is to analyze patients who develop HCC, even if eradicated by the virus after effective antiviral therapies, and to evaluate the relationship between polymorphism in the enhancer gene and risk of developing HCC.
Methods used. The pipelline consists of four main phases. First of all, the GWAS SNPs related to HCV- induced HCC were downloaded from the GRASP2 database, then we associated the LD SNPs related to HCC by querying the SNiPRA3 database. At this point, we have identified the enhancer regions in three HCC cell lines (Hep G2, SNU387, Li-7) thanks to the HACER4 database. After that, we mapped the SNPs on the enhancer thus obtaining the SNP enhancers characteristic of HCV-associated HCC and, finally, we evaluated our results by querying the rVarBase5 database.
Results and conclusions. The 64 SNPs related to HCV-associated HCC were used to query the SNiPA database to obtain 612 SNP-LDs, for a total of 676 SNPs associated with HCV-induced HCC. Subsequently, having mapped the SNPs to the enhancer regions, we obtained 32 SNP enhancers associated with HCV-induced HCC. Then, by interrogating the rVarBase5 database, we analyzed these 32 SNP enhancers and we discovered that only 4 of these had evidence of SNP enhancers and 3 of these were involved in distal transcriptional regulation. In conclusion we can say that the SNPs associated with HCV-induced HCC are different, and SNP chr17: 29141558 was significantly associated with the risk of developing HCV-induced HCC. This demonstrates that the TRIL gene plays an important role in the risk of HCV-related HCC. These data will be confirmed by the analysis on blood samples from patients who developed HCC after HCV infection. This study is currently underway in our research laboratory.

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P126 - OC 30

The unprecedented wide interaction of HTLV-1-encoded HBZ protein with the RNA splicing and stability machineries in leukemic cells

M. Shallak[1], I. Iacobucci[2], M. Monti[2], T. Alberio[3], M. Fasano[3], F. Mortreux[4], R.S. Accolla[1], G. Forlani[1]

[1] Laboratories of General Pathology and Immunology "Giovanna Tosi", Department of Medicine and Surgery, University of Insubria, 21100 Varese, Italy - [2] Department of Chemical Sciences, University Federico II of Naples, Strada Comunale Cinthia, 26, 80126 Naples, Italy; CEINGE Advanced Biotechnologies, Via G. Salvatore 486, 80145 Naples, Italy - [3] Laboratory of Biochemistry and Functional Proteomics, Department of Science and High Technology, University of Insubria, Busto Arsizio, VA, Italy - [4] Laboratory of Biology and Modelling of the Cell, Univ Lyon, ENS de Lyon, Univ Claude Bernard, CNRS UMR 5239, INSERM U1210, 46 Allée d'Italie Site Jacques Monod, 69007, Lyon, France

Aim of the study: Adult T-cell leukemia/lymphoma (ATL) is a rare T-cell lymphoproliferative neoplasm caused by the human T-cell leukemia virus type 1 (HTLV-1) and characterized by poor prognosis. The detailed mechanism behind ATL occurrence remains unresolved. Two viral regulatory proteins, Tax-1 and HTLV-1 bZIP factor (HBZ), encoded by the sense and antisense viral transcripts, respectively, play important roles in HTLV-1 infectivity by altering key pathways of cell homeostasis. However, an organic picture of the host factors interacting with the viral proteins is still lacking. Toward this goal, here we investigated the HBZ interactome on the patient derived leukemic cell line ATL-2s.

Methods: Enrichment of endogenous HBZ from the nuclear fractions of ATL-2s cells was carried out by immunoprecipitation with the 4D4-F3 anti HBZ mAb, followed by mass spectrometry. The DEAD-Box RNA helicases DDX5-p68 and DDX17-p72 and their interaction with HBZ were studied by immunofluorescence followed by confocal microscopy analysis and by coimmunoprecipitation assay.

Results and Conclusion: The interactome was mostly distributed into three nodules of interactions corresponding to protein families mainly involved in mRNA splicing, nonsense-mediated nRNA decay (NMD) and JAK-STAT signaling pathway. We focused our attention on DDX5 and DD17, two RNA helicases recently shown to be involved in alternative splicing induced by their recruitment on cellular genes after the NF-kB activation by HTLV-1 Tax-1.

By immunofluorescence and confocal microscopy, we found a partial co-localization between HBZ and DDX5 or DDX17. Furthermore, by co-immunoprecipitation assay we could demonstrate a direct interaction of DDX5 and DDX17 with HBZ in ATL-2s and in HBZ-transduced Jurkat T cells (Jurkat-HBZ).

The wide interaction of HBZ with molecules involved in RNA splicing and stability and particularly DDX5 and DDX17, often altered in cancer, strongly suggests a more complex and diffuse role of the endogenous viral oncogene in the establishment of the oncogenic phenotype.

P127

Role of HPV infection and tert promoter mutation in vulvar squamous cell carcinoma

N. Starita[1], A. Cerasuolo[1], V. Gigantino[2], G. Aquino[2], L. Dassi[1], S. Greggi [3], C. Scaffa[3], N.S. Losito[2], F.M. Buonaguro[1], M.L. Tornesello[1]

[1] Molecular Biology and Viral Oncology Unit, Istituto Nazionale Tumori IRCCS Fond Pascale, Napoli, Italy - [2] Pathology Unit, Istituto Nazionale Tumori IRCCS Fond Pascale, Napoli, Italy - [3] Gynecology Oncology Unit, Istituto Nazionale Tumori IRCCS Fond Pascale, Napoli, Italy - [4] Cancer Immunoregulation Unit, Istituto Nazionale Tumori IRCCS Fond Pascale, Napoli, Italy

Aim: Human papillomavirus (HPV)-related tumors show distinct clinical features and different mutational signatures in coding and non-coding regions of the human genome compared to HPV-negative neoplasia. High-risk HPVs, especially genotype 16, are the main risk factor for lower genital tract tumors, including a significant fraction of vulvar squamous cell carcinoma (VSCC). In the present study we analyzed the distribution of HPV genotypes, the HPV16 E6 and E7 viral genes expression as well as mutations in TERT promoter (TERTp) and TP53 genes of VSCC, in order to identify significant molecular events associated or not with HPV-driven carcinogenesis.
Methods: A total of 52 VSCC cases, including fresh frozen (n=17) and archived formalin fixed paraffin embedded (FFPE) (n=35) samples, were analyzed for HPV DNA as well as somatic mutations in TERTp and TP53 gene by PCR and nucleotide sequencing analysis. TERTp mutations allele frequencies were quantified by droplet digital PCR (ddPCR). The expression of TERT, HPV16 E6 and E7 genes was analyzed by Real-time PCR.
Results: HPV DNA was detected in 36.5% of VSCC with HPV16 representing the 73.7% of all infections. Recurrent mutations at nucleotide positions -124 G>A and -146 G>A upstream TERT ATG start site were identified in 42.3% of VSCC by standard PCR/Sanger sequencing and in 53.8% of VSCC by ddPCR with mutant allele frequencies ranging from 5% to 70%. Nucleotide changes in TP53 exon 7 were found in 19.2% of VSCC. The TERTp and TP53 exon 7 mutation frequencies were higher in HPV negative (60.6% and 24.2%, respectively) than HPV positive cases (42.1% and 10.5%, respectively). The TERT mRNA levels were 2-fold higher in TERTp mutated versus not-mutated VSCC, irrespective of HPV16 E6 and E7 expression.
Conclusions: TERTp mutations are highly frequent in VSCC. The mutation frequency is to some extent lower in HPV-related VSCC than virus negative tumours. TERTp mutations are associated with increased telomerase expression irrespective of HPV16 E6 and E7 levels.

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Antibody response to peptide microarray of HCV proteome identifies specific epitopes with diagnostic potential for disease progression in HCV infected subjects

A.L. Tornesello[1], U. Reimer[2], P. Holenya[2], T. Knaute[2], F. Izzo[3], L. Buonaguro[1], M.L. Tornesello[1] and F.M. Buonaguro[1]

[1] Molecular Biology and Viral Oncology Unit, Istituto Nazionale Tumori – IRCCS Fondazione Pascale, Napoli, Italy - [2] JPT Peptide Technologies GmbH - Volmerstrasse 5 - 12489 Berlin, Germany - [3] Hepatobiliary Surgery, Istituto Nazionale Tumori – IRCCS Fondazione Pascale, Napoli, Italy

Aims: Hepatitis C virus (HCV) infection is a progressive disease that can cause chronic active hepatitis, cirrhosis, hepatocellular carcinoma (HCC), and lymphoproliferative disorders. Currently, non invasive diagnosis of liver disease is mainly based on biochemical assays testing for increased levels of transaminases and alpha-fetoprotein, which are indicative of liver damage and inflammation but not specific for liver neoplasia or lymphoproliferative disorders. The main objective of this study was to identify new biomarkers specific for HCV-related neoplasia among chronically HCV infected subjects by HCV-based peptide array analysis.
Method: A retrospective cohort of 71 HCV-positive subjects, at different disease stages were included in this study. Epitope mapping of IgG antibodies, which have been produced against HCV proteins in the serum of patients diagnosed with HCC (n=48), mixed cryoglobulinemia (MC, n=10) and with HCV chronic infection (n=13), was performed by probing a microarray composed of 5952 overlapping peptides which cover the whole proteome of eight viral genotypes and viral subtypes (1a,1b, 2, 3, 4, 5, 6, 7; coverage 80-94%).
Results: Sera of HCC and MC patients showed a significant stronger immune response against C, E2, NS3, NS4A, NS4B, NS5A and p7 proteins compared to HCV positive subjects. The antibody responses against E2 (aa 155-169 and aa 218-232), NS3 (aa 44-58 and 348-362), NS4A (aa 25-39), NS4B (aa 17-31), NS5A (aa 19-33, aa 29-43, aa 149-163 and aa 196-210) and p7 (aa29-43) peptides was statistically significant higher in HCC or MC patients compared with chronically infected HCV infection subjects.
Conclusion: Our results suggest that immune response against specific HCV protein domains may represent useful biomarkers of disease progression among HCV-positive patients and suggest that peptide microarrays are good tools for the screening of immunotherapy targets in preclinical HCV research.

P129

Oncolytic adenovirus in combination therapeutic approaches with epigenetic modulators

C. Zannella[1], A.M. Malfitano[2], A. Ambrosino[1], B. Santella[1], S. Di Somma[2], F. Napolitano[2], A. Chianese[1], G. Franci[3], M. Galdiero[1], G. Portella[2]

[1] Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", 80138 Naples, Italy - [2] Department of Translational Medical Sciences, University of Naples Federico II, 80131 Naples, Italy - [3] Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, 84081 Baronissi, Italy

Aim of the study: According to the World Cancer Report, cancer rates are increasing by 50% with 15 million new cases in 2020. Cancer research is taking great steps and is constantly looking for new strategies for the treatment of lesions effective therapies, such as anaplastic thyroid carcinoma (ATC). Among these, oncolytic virotherapy represents a therapeutic option with a wide range of approaches and applications [1]. Oncolytic viruses (OVs) are natural or engineered viruses characterized by the unique characteristics of preferentially infecting, replicating and lysing cancer cells, as well as activating the immune response. Considerable interest is arousing the combinatorial use of oncolytic viruses and epigenetic modulators to enhance the immune response and allow viral entry, replication and spread between proximal cells.
Methods used: Two different ATC cell lines (8505-c and BHT-101) were treated with the oncolytic adenovirus dl922-947 alone and in combination with different epigenetic drugs, i.e. Suberoylanilide hydroxamic acid (SAHA) and toxoflavin (PKF118-310), and the cytotoxicity was analysed via sulforhodamine B assay. Furthermore, protein extracts were collected and Western Blot analysis was performed.
Results and conclusions: The combination strategy gave promising results, showing a considerable increase of cytotoxicity. The best data were obtained with the combination constituted by PKF and dl922-947 especially on 8505-c cell line at both lower and higher plaque-forming units (PFU). The level of H3K27ac were strongly reduced when cells were infected and treated with PKF compared to the virus alone, and, on the contrary, the levels of H3K9me2 and H3K9me3 showed a high increase in the combinatorial treatment respect to cells infected with dl922-947. All together, these results demonstrated that oncolytic adenovirus in combination with epigenetic modulators improves viral performance in ATC cells, and it is influenced by a fine epigenetic regulation.

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P130

Deep next-generation sequencing (NGS) data analysis of SARS-CoV-2: detetion of defective viral genomes

G. Campisi, S. Messali, C. Arnaldo

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

Introduction: RNA viruses, during viral replication, generate truncated forms of viral genomes, also called defective viral genomes (DVGs). DVGs possess the minimum nucleotide sequence that interferes with the innate immune host response and are highly relevant to viral pathogenesis.
Aim of the study: In this study, we evaluated if SARS-CoV-2 is able to generate defective viral genomes, particularly copy-back (DVGs), that are the primary initiators of the antiviral immune response during host infection.
Materials and Methods: Total RNA was extracted from nasopharyngeal swabs of two positive SARS-CoV-2 patients, one collected from a symptomatic patient at the early epidemic phase (March 2020) and the other collected from an asyntomatic patient at the late epidemic wave (May 2020). Randomly amplified cDNA was generated using Sequence-independent Single-Primer Amplifcation (SISPA). PCR- product were purified and quantified using the Qubit DNA HS Assay Kit (Termo Fisher Scientifc), then genomic libraries were prepared using Nextera DNA Flex kit (Illumina, San Diego, CA). Sequencing was performed using an Illumina MiniSeq® platform (Illumina) generating 2x150 bp paired-end reads. DI-Tector, an open-source python script, was used to analyze row reads of genome sequences for the identification of DVGs using default parameters.
Results: DI-Tector output gave a multitude of SARS-CoV-2 DVG structures over the whole genomes, mainly in the non-structural region. All the results were evaluated according to the mean reads coverage, viral mapping region, and the percentage of DVGs types. We selected several significant DVGs that computationally have been detected. Our analysis was focused particularly on helicase domain (NSP13) 3'-copy-back 17141-17195 DVGs, which was the most representative for both samples. The presence of several defective viral genomes in this region were confirmed by digital droplets PCR. It is worth noting that significant difference in the amount of 3'-copy-back 17141-17195 DVGs types in the two tested samples was observed.
Conclusion: Our data show that SARS-CoV-2 is able to generate several defective viral genomes. This evidence may led to speculate increased amounts of 3'-copy-back 17141-17195 DVGs, could be related to suppression of clinical symptoms. Therefore 3'-copy-back 17141-17195 DVGs may have immunomodulatory effects and sustain milder forms of covid-19.

P131 - OC 14

Genomic characterization of SARS-CoV-2 variants in Campania, Italy

A. Grimaldi[1], P. Annunziata[1], F. Panariello[1], T. Giuliano[1], B. Pierri[2], P. Cerino[2], A. Ballabio[1] and D. Cacchiarelli[2]

[1] Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Naples, Italy - [2] Istituto Zooprofilattico Sperimentale del mezzogiorno (IZSM), Portici, Naples, Italy

SARS-CoV-2, the etiological agent of COVID-19 pandemic, is a positive-sense, single-stranded RNA virus belonging to the genus *Betacoronavirus* [1]. SARS-CoV-2 spreading around the globe in last two years promoted the natural selection of viral variants harbouring beneficial mutations [2]. The substitution of aspratate-614 with a glycine in the Spike protein (Spike D614G) is emblematic from this point of view. While not identified until February 2020, viruses carrying this mutation represent today 99% of all SARS-CoV-2 samples [3]. Indeed, the D614G mutation guarantees a competitive advantage over the wild-type genotype, both in terms of replication efficiency and of infectivity [3]. Similarly, the B.1.1.7 viral lineage, characterized by a set of 17 mutations, first appeared in November 2020 in England and in March 2021 it was responsible for the 97% of English COVID-19 cases [4,5]. These data demonstrate that the SARS-CoV-2 genome is under active natural selection and they remark the importance of performing genomic surveillance both at the international and national level. While being one of the first countries hit by the pandemic, till December 2020 Italy missed an efficient program to profile viral genetic diversity, especially in the south of the country. To fill this gap, we developed a genomic framework to characterize SARS-CoV-2 variants in Campania, the most populous region in the south of Italy. Taking advantage of a network of hospitals and laboratories providing clinical specimens, we were able to sequence and publish on GISAID [5] more than 6000 samples, allowing Italy to become the 7th country depositing the highest number of sequences in Europe. Our data revealed the dynamics of COVID-19 pandemic in Campania, showing that the three main peaks of infections correspond to the arise of three specific lineages. Furthermore, we were able to identify, as soon as it appeared, a new viral lineage (designated as B.1.177.88) specifically located in Campania and carrying the Spike E484K substitution. Such mutation is known to decrease viral sensibility to both monoclonal antibodies and human serum upon BNT162b2 vaccine treatment[6]. Our approach, in conclusion, highlights the power of genomic surveillance for the detection and characterization of new viral variants, an extremely important activity to face SARS-CoV-2 rapid evolution, especially as new selective stimuli, such as vaccines and antibodies therapies, are being introduced in the environment.

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P132 - OC 49

Isolation and characterization of monoclonal antibodies specific for SARS-CoV-2 and its major variants useful for developing innovative diagnostic assays and immunotherapy

S. Mariotti, A. Capocefalo, M.V. Chiantore, A. Iacobino, R. Teloni, A. Marchi, M. Baggieri, P. Bucci, M.F. Pirillo, A. Gallinaro, A. Di Virgilio, M. Sgarbanti, S. Sandini, C. Acchioni, F. Grasso, F. Tosini, M. Borghi, M.L. De Angelis, D. Negri, F. Magurano, A. Cara, P. Di Bonito and R. Nisini

Istituto Superiore di Sanità, Roma

Aim of the study. The emergence of the new pathogen SARS-CoV-2 determined a rapid need of monoclonal antibodies (moAb) for viral detection in biological liquids as a rapid tool to identify infected individuals to be treated or quarantined. Isolation of carriers is a pivotal public health measure for outbreak control until herd immunity is achieved through mass vaccination programs. Numerous assays are now commercially available, however the rapidly spreading of SARS-CoV-2 variants worldwide causes a continuing need for new reagents for diagnosis and immunotherapy. Since the first months of the new emergency, a working group at ISS has developed a strategy to produce SARS-CoV-2 specific moAb, choosing SARS-CoV-2-Spike (S) and its Receptor Binding Domain (RBD) proteins as targets and using novel immunization procedures.
Methods. Balb/c mice were primed with the recombinant SARS-CoV-2 (Wuhan isolate) furin site-mutated ectodomain (ECDm) of S glycoprotein with a trimerization domain produced in a mammalian cell system. Mice were boosted twice with in-house produced recombinant RBD or with the same ECDm. The splenocytes from animals showing high titers of specific IgG were fused with mouse myeloma cells, following the standard procedure and specific hybridomas were cloned by limiting dilutions. Different recombinant S domains corresponding to RBD, S1 and S2 were generated in mammalian cells and used together with the furin site-mutated ECDm to select clones producing S-specific moAb by ELISA. Specific purified moAb were characterized by 1) western blot using full S wild-type ectodomain and several S domains produced in eukaryotic and prokaryotic systems to evaluate the ability of moAb to detect linear, conformational, glycosylated or not-glycosylated proteins and to provisional map the recognized epitopes; 2) western blot using SARS-CoV-2 in the supernatant of infected VERO cells to assess the capacity of moAb to recognize the virus and 3) flow cytometry analysis using HEK-293T cells expressing the full-length S of Whuan isolate or UK-B.1.1.7 and SA-B.1.351 variants (plasmids for the expression of S variants were kindly provided by Paul McKay, Imperial College, London) to show the capacity of moAb to bind the viral proteins of SARS-COV-2 and its variants in their native conformation. Finally, the viral neutralization capacity of moAb was investigated by both S-pseudotyped lentiviral vectors (Dispinsieri S. et al., Nat Commun 2021. In press) or the SARS-CoV-2 Italian isolate BetaCov/Italy/CDG1/2020jEPI ISL 412973j2020-02-20 (Magurano et al. 2021).
Results. The immunization procedure was highly efficient and a huge panel of different SARS-CoV-2-specific moAb were isolated, purified and characterized. We obtained moAb

recognizing different epitopes along the sequence of S protein including diverse glycosylated or non-glycosylated sites. The majority of isolated moAb were able to bind SARS-CoV-2 Whuan isolates as well as the UK-B.1.1.7 and SA-B.1.351 SARS-CoV-2 variants. The most efficient moAb of different isotype were selected to develop sandwich assay for antigenic tests. Three moAb specific for the RBD protein were shown to bind UK-B.1.1.7 and SA-B.1.351 SARS-CoV-2 variants as efficiently as the Whuan isolate. RBD specific moAb inhibited the SARS-CoV-2 Italian isolate infection of VERO cells at high titers. Experiments are in progress to test the neutralization activity of the three moAb in the neutralization test using lentiviral vectors pseudotyped with S from UK-B.1.1.7 and SA-B.1.351 variants. **Conclusions**. The isolated moAb represent important additional tools for the diagnosis of SARS-CoV-2 infection. Cooperation with innovative biotech companies is ongoing to develop immunoassay to detect SARS-CoV-2 antigens and its more common variants. Studies are ongoing to evaluate the role of the RBD-specific neutralizing moAb as possible new tools for the COVID-19 immunotherapy

P133 - OC 15

Plant-produced VP2-based particles provide protection against very virulent Infectious Bursal Disease Virus

C. Marusic[1], A. Bortolami[2], M. Donini[1], C. Lico[1], C. Terregino[2], S. Baschieri[1]

[1] Laboratory of Biotechnology, ENEA Casaccia Research Center, Rome, Italy - [2] Specialized Virology and Experimental Research Department Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy

Aim of the work. Infectious Bursal Disease Virus (IBDV), the etiological agent of Gumboro disease, causes mortality and immunosuppression in chickens and major losses to poultry industry worldwide. The IBDV major capsid protein VP2 is considered the best candidate for the production of novel subunit vaccines. This structural protein contains the major conformational epitopes responsible for the induction of IBDV neutralizing antibodies in chickens and was demonstrated to be able to form supramolecular structures in yeast and insect cells. The aim of this study was to express an engineered version of the VP2 protein (His-pVP2) in order to verify its ability to self-assemble into virus-like particles in plants and to protect animals from a very virulent IBDV strain.
Methods. The *His-pVP2* synthetic gene was cloned in a pBI-based plant expression vector and the resulting construct was used to transform LBA4404 *A. tumefaciens*.
Transient production of the recombinant pVP2 was obtained by vacuum agro-infiltrating the plants at the 6-7 leaf stage. The plant tissues were collected at different time points and crude plant extracts were analysed by ELISA and Western blot. The assembly of His-pVP2 into supramolecular structures in plant tissues was demonstrated by transmission electron microscopy (TEM). VP2-based particles were used to immunise SPF chicks of 8 days of age. After three intramuscular injections the animals were challenged via the oculonasal route with a suspension of a Moroccan vvIBDV strain. Anti-IBDV antibody titers were determined using a commercial kit.
Results and conclusions. An engineered version of the pVP2 derived from a vvIBDV field isolate, fused to a polyhistidine-tag (His-pVP2) was transiently expressed in *N. benthamiana*. The expressed His-pVP2 was demonstrated to assemble in plant cells not only into VLP (T=13) but also into a range of different supramolecular structures such as SVP with a diameter ranging from ~15 nm to 20 nm (T=1 shell), particles with a larger diameter (~35-50 nm), possibly corresponding to T=7, and tubular structures of variable lengths (in the range of 100-400 nm) and ~22 nm in diameter.
The recombinant VP2-based particles when used for the intramuscular immunization of specific-pathogen-free chicks resulted able to induce the production of anti-IBDV specific antibodies at titers comparable to those registered in the control group immunized with an inactivated commercial vaccine. Moreover, all the immunized birds survived to the challenge with a Moroccan very virulent IBDV strain with no major histomorphological alterations of the Bursa of Fabricius, similarly to what obtained with the commercial inactivated vaccine.

In conclusion in this work we demonstrate the formation of VP2-based supramolecular structures in plant cells that when administered to chikens are able to activate immune responses conferring 100% of protection.

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P134

SARS-CoV-2 genome reconstruction: a method for obtaining accurate data from low viral load clinical samples and a simple targeted methd for variants identification

C. Piubelli[1], L. Marcolungo[2], C. Beltrami[2], C. Degli Esposti[2], G. Lopatriello[2], A. Mori[1], E. Pomari[1], M. Deiana[1], S. Scarso[1], Z. Bisoffi[1,3], V. Grosso[2], E. Cosentino[2], S. Maestri[2], D. Lavezzari[2], B. Iadarola[2], M. Paterno[2], E. Segala[2], B. Giovannone[2], M. Gallinaro[2], M. Rossato[2,4] and M. Delledonne[2,4]

[1] Department of Infectious, Tropical Diseases and Microbiology, IRCCS Sacro Cuore Don Calabria Hospital, Negrar di Valpolicella, 37024 Verona, Italy - [2] Department of Biotechnology, University of Verona, Strada le Grazie 15, 37134 Verona, Italy - [3] Department of Diagnostics and Public Health, University of Verona, 37134 Verona, Italy - [4] Genartis srl, via IV Novembre 24, 37126 Verona, Italy

Aim of the study: This work focused on two goals:
1- to develop an amplicon-based viral genome sequencing workflow (Accurate SARS-CoV-2 genome Reconstruction, ACoRE) for the complete and accurate reconstruction of SARS-CoV-2 sequences from clinical samples, including suboptimal ones that would usually be excluded even if unique and irreplaceable.
2- to develop a cost/time-effective sequencing-based workflow (STrain-Amplicon-Seq, STArS) applicable in the clinical routine for both SARS-CoV-2 diagnostics and genotyping, starting from the well-established ARTIC protocol coupled to nanopore sequencing.
Methods used: for ACoRE protocol, total RNA was extracted from nasopharyngeal swabs collected from 172 COVID-19 patients diagnosed at the Department of Infectious, Tropical Diseases and Microbiology of the IRCCS Sacro Cuore Don Calabria Hospital. RNA samples were subjected to reverse transcription producing 2 or 3 cDNAs per samples and subjected to amplification using the ARTIC nCoV-2019 V3 panel. The number of amplification cycles was adapted according to the Ct obtained by SARS-CoV-2-specific real-time PCR (CDC protocol). Full length or fragmented amplicon sequencing was obtained using KAPA Hyper prep kit and the MiSeq platform (Illumina) or Illumina DNA Prep kit and Novaseq 6000 device (Illumina) respectively. A pipeline for data filtering, reference genome alignment and variant calling was developed. For STArS protocol A set of 10 amplicons was selected from the ARTIC tiling panel, to cover all the main biologically relevant genetic variant located on the Spike gene, a minimal set of variants to uniquely identify the current circulating strains, genomic sites usually amplified by RT-qPCR method to identify SARS-CoV-2 presence. PCR-amplified clinical samples (both positive and negative for SARS-CoV-2 presence) were pooled with a serially diluted exogenous amplicon at known concentration and sequenced on a MinION device. The study was approved by the competent Ethical Committee for Clinical Research of Verona and Rovigo Provinces (Prot N° 39528/2020).
Results and conclusions: The ACoRE protocol was optimized to improve flexibility and the combination of technical replicates was established as the central strategy to achieve accurate analysis of low-titer/suboptimal samples. We demonstrated the utility of the approach by achieving complete genome reconstruction and the identification of false-positive variants in >170 clinical samples, with Ct values in the range 15–40, thus

avoiding the generation of inaccurate and/or incomplete sequences. Most importantly, ACoRE was crucial to identify the correct viral strain responsible of a relapse case, which would be otherwise mis-classified as a re-infection due to missing or incorrect variant identification by a standard workflow. STArS method allowed to effectively genotype strain-specific variants. Thanks to the reduced turnaround time and costs, the proposed approach will simplify the clinical application of sequencing for viral genotyping, thus helping in contrasting the global pandemic.

P135
Validation of monocytes as carrier cells for intravascular delivery of oncolytic HSV-1

A. Reale[1], A. Vitiello[1], M. Cadamuro[1], L. Krutzke[2], S. Kochanek[2], G. Palù[1], A. Calistri[1]

[1] Department of Molecular Medicine, University of Padova, Via A.Gabelli, 63 35121 Padova (Italy) - [2] Department of Gene Therapy, University of Ulm, Helmholtzstrasse 8/1, 89081 Ulm (Germany)

Oncolytic viruses (OVs) are therapeutics which can combine cancer cell-killing activity, immunotherapy and gene therapy, if the viral genome is modified to encode therapeutic genes. Oncolytic Herpes Simplex Virus type 1 (oHSV1) talimogene laherparepvec was approved for the treatment of unresectable melanoma in the USA and the EU, while OV research is focusing on increasing effectiveness both in melanoma and other solid tumors. The most employed delivery method is intratumoral administration, both in preclinical and clinical studies. In fact, following intravenous injection, the host's immune system can clear the attenuated viruses before they can reach the target. A strategy to achieve systemic delivery involves using carrier cells which can be infected ex vivo, shielding the OV from the immune system. Human monocytes can be infected by HSV-1 but are relatively resistant to infection. At the same time, in many solid tumors, circulating monocytes are the source of tumor-associated macrophages (TAMs), and are actively recruited by cancer cells.

Here we propose an approach which to our knowledge was never attempted before, i.e. the use of monocytes as carrier cells for oHSV1. We aim to demonstrate that human monocytic cells are susceptible to oncolytic HSV1 infection, can migrate in vitro towards cancer cells and transfer the virus. We also aim to assess the migration of uninfected and infected monocytic cells towards human tumor cells in a biologically more relevant setting, such as in chicken embryos with human head and neck cancer cells (UMSC11B cells) growing on the chorioallantoic membrane (CAM). Experiments in ovo were performed in collaboration with Dr. Lea Krutzke at the Department of Gene Therapy of the University of Ulm (Germany).

Human monocytic THP-1 cells were susceptible to infection in vitro with an EGFP-expressing oHSV1 with a backbone similar to talimogene, and could transfer infection to human breast cancer MDA-MB-231 cells both in coculture and 5µm-pore-transwell migration experiments. After infection, THP-1 cells were washed three times with phosphate buffer saline (PBS) solution, and supernatant from the final washing was titrated to ensure that no infectious extracellular virions were present. Primary human monocytes were also susceptible when infected with a higher viral load. Both MDA-MB-231 and UMSC11B cells supported productive oHSV1 infection in vitro. All cells were infected for 1 hour at 37°C in serum-free medium. The multiplicity of infection (MOI) varied according to different cell types, ranging from 0.01 plaque forming units (PFU)/cell for UMSC11B cells to 1-3 PFU/cell for monocytic cell lines, to 10 PFU/cell for primary monocytes.

Following intravascular injection in chicken embryos of up to 5x105 uninfected THP-1 cells resuspended in PBS solution, no significant toxicity could be observed over several days, while human CD14+ human cells were detected by immunohistochemistry and im-

munofluorescence in sections of UMSC11B tumors on the CAM. A similar experiment is being performed with EGFP-oHSV1-infected THP-1 cells to evaluate biodistribution and transmission of infection to UMSC11B cells in ovo. Our results show that monocytes are promising carrier cells for oHSV1, deserving further investigation in an immunocompetent animal model. As a preliminary step in this direction, we infected a murine monocytic cell line (J774A.1 Balb/c cells) which was also susceptible to oHSV-1 in vitro.

P136 - OC 17

High-throughput platforms to assess neutralizing antibodies and antiviral molecules against SARS-CoV-2

D. Stelitano[1,2,3], C. Mathieu[4], F.T. Bovier[1,2,3], G. Franci[5], G. Portella[6], M. Cennamo[6], B. Horvat[4], M. Galdiero[1], M. Porotto[1,2,3]

[1] Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", 80138Napoli, Italy - [2] Department of Pediatrics, Columbia University Irving Medical Center, NY, NY, USA - [3] Center for Host–Pathogen Interaction, Columbia University Irving Medical Center, NY, NY, USA - [4] CIRI, International Center for Infectiology Research, INSERM U1111, University of Lyon, University Claude Bernard Lyon 1, CNRS, UMR5308, Ecole Normale Supérieure de Lyon, France - [5] Department of Medicine, Surgery, Dentistry, University of Salerno "Scuola Medica Salernitana", 84081, Salerno, Italy - [6] Department of Translational Medical Sciences, University of Naples Federico II, 80131 Naples, Italy

Aim of the study. The current SARS-CoV-2 virus pandemic has caused more than 132,000,000 confirmed cases and 2,800,000 deaths worldwide. Flexible and rapid platforms for evaluating the neutralizing activity of the human serum to monitor population immunity after infection or vaccination is an absolute priority. Moreover, the identification of antiviral agents effective against SARS-CoV-2 is urgently required.
Methods. We developed a cell-based platform to screen neutralizing antibodies (nAb) and antiviral intervention under low containment (BSL2). A key element of this platform is cDNA encoding the spike protein (S) to mimic multiple rounds of infection. SARS-CoV-2 spike protein is the main target of nAb. Our high-throughput assay miming various cycles of infection allows the identification of neutralizing antibodies and antiviral molecules targeting the SARS-CoV-2 S protein. Additionally, we developed a cell-based ELISA to assess the potential neutralization activity of sera in BSL1 conditions.
Results and conclusions. We tested sera from patients with disease from moderate to severe for the presence of nAb. We evaluated the SARS-CoV-2 S binding of the different sera through our cell-based ELISA. We assessed the capability of these antibodies to recognize the spike proteins of other beta-coronaviruses (e.g., SARS-CoV-1 and MERS), showing that our assay allows the detection of cross-reactivity against their spike proteins. The results obtained through our platforms statistically correlated with the results wiht live SARS-CoV-2 virus, confirming the validity of our assays. This platform is seamlessly adaptable to the variants of concern that are constantly emerging during the COVID-19 pandemic, providing a real-time tool to evaluate neutralization activity of convalescent and immunized sera.

P137

Optimization of a protocol for the production of pseudotyped vectors with SARS-CoV-2 spike glycoprotein

G. Lottini[a,b], M. Sidoti[a], E. Plicanti[a], M. Lai[a], P. Quaranta[a], M. Pistello[a], G. Freer[a]

[a] Retrovirus Centre, Department of Translational Research, University of Pisa, Pisa, Italy - [b] Department of Medical Biotechnologies, University of Siena, Siena, Italy

COVID-19 is a severe respiratory disease caused by SARS-CoV2, a positive-sense single-stranded RNA beta-coronavirus. Because of its high pathogenicity and transmissibility, the virus is classified as a biosafety level 3 (BSL3) agent and cannot be manipulated in BSL2 settings. To overcome this problem, but also to study viral entry or to set up drug screening and neutralization tests, it is possible to produce lentiviral vectors pseudotyped with SARS-CoV2 spike (S) glycoprotein. Pseudotyped lentiviral vectors are vector particles that have a replication-defective core of a lentivirus and the envelope protein of another virus on their surface.
In this work, we produced vector particles with an HIV structural backbone, bearing the SARS-CoV2 S glycoprotein and encoding only for a reporter gene.
The aim of this work is the optimization of a protocol to produce pseudotyped lentiviral vectors with SARS-CoV2 S glycoprotein. We started from a codon-optimised Spike glycoprotein without the 19 aminoacids coding for the ER retention signal.
We started from testing different transfection protocols, with different plasmid amounts and reporter genes and different plasmid combination, to find the most performing one. Initially, we tried to use a reporter plasmid coding for the GFP and evaluated, the transfection efficiency of different protocols by FACS. Then we performed transduction assays on HuH7 cell line, with pseudotyped lentiviral vectors produced in Hek 293T cell line, evaluating transduction efficiency of different vectors produced.
Once we found the best protocol, we started to improve the transduction method. We used polybrene (10 µg/ml), fresh vector and performed spinoculation for 1h, 1000 x g, at 35°C. We also produced Hek 293T stably expressing ACE-2 receptor. We found that transfection efficiency was not directly proportional to transduction efficiency. Therefore, we chose the protocol that showed the best transduction efficiency on HuH7 cells. Then we found that the polybrene and the spinoculation improved the process of transduction by 50 and 20 %, respectively.
Pseudotyped vectors with SARS-CoV 2 S did not work as well as VSV-G pseudotyped vectors, produced as controls. Moreover, when we transduced HuH7 cells, we obtained big syncytia that FACS was unable to acquire, so we produced a cell line stably expressing ACE-2 successfully transduced with vectors.
Moreover, we also tried to produce vectors coding for the luciferase reporter gene, and we found that this assay was more sensitive and could detect vectors also in small quantities.
In conclusion, we found that to quantify pseudotyped lentiviral vectors with SARS-CoV2 S glycoprotein, the transduction protocol is important; it must include spinoculation and the use of fresh vectors. Transduction of the right cell line expressing high levels of ACE-2 protein is also fundamental. Moreover, the choice of the right gene reporter is important to have a good titration of particles and to use them in serological and entry inhibition assays.

P138

Quinolinonyl Non-Diketo Acid Derivatives as Inhibitors of the HIV-1 Ribonuclease H Function of Reverse Transcriptase

A. Messore[1], A. Corona[2], V.N. Madia[1], F. Saccoliti[3], V. Tudino[1], A. De Leo[1], D. Ialongo[1], L. Scipione[1], D. De Vita[1], G. Amendola[4], E. Novellino[5], S. Cosconati[4], M. Metifiot[6], M.L. Andreola[6], F. Esposito[2], N. Grandi[2], E. Tramontano[2], R. Costi[1], R. Di Santo[1]

[1] University of Rome La Sapienza, Chimica e Tecnologie del Farmaco, Roma - [2] Universita degli Studi di Cagliari, Life and Environmental Sciences, Cagliari - [3] Italian Institute of Technology, D3 PharmaChemistry - [4] University of Campania Luigi Vanvitelli, Dipartimento di Scienze e Tecnologie Ambientali Biologiche e Farmaceutiche - [5] University of Naples Federico II, Pharmaceutical and Toxicological Chemistry, Napoli - [6] Laboratoire MFP, UMR-5234, Bordeaux

Novel anti-HIV agents are still needed to overcome resistance issues, in particular inhibitors acting against novel viral targets. The ribonuclease H (RNase H) function of the reverse transcriptase (RT) represents a validated and promising target and no inhibitor reached the clinical pipeline yet. Here we present rationally designed non-diketo-acid selective RNase H inhibitors (RHI) based on quinolinone scaffold starting from former dual integrase (IN)/RNase H quinolinonyl diketo
acids. Several derivatives were synthesized and tested against RNase H and 1viral replication and found active at micromolar concentration. Docking studies within RNase H catalytic site, coupled with site-directed mutagenesis, and Mg2+ titration experiments, demonstrated that our compounds coordinate the Mg cofactor and
interact with amino acids of the RNase H domain that are highly conserved among naïve and treatment-experienced patients. In general, the new inhibitors influenced also the polymerase activity of RT but were selective against RNase H. Hence providing important insight for the development of the scaffold.

P139

Cysteamine and its oxidised form cystamine reduces SARS-CoV-2 production in culture and cytokines release from immune cells

T. Alonzi*, A. Aiello*, S. Najafi Fard*, L. Petrone*, M.R. Capobianchi§, M. Piacentini*#, D. Goletti*

* Translational Research Unit, National Institute for Infectious Diseases "L. Spallanzani" IRCCS, Rome - § Laboratory of Virology, National Institute for Infectious Diseases "L. Spallanzani", IRCCS, Rome - # Laboratory of Electron Microscopy, National Institute for Infectious Diseases "L. Spallanzani", IRCCS, Rome. Department of Biology, University of Rome "Tor Vergata", Rome

Background. The aminothiol cysteamine, derived from coenzyme A degradation in mammalian cells, and its disulfide product of oxidation, cystamine have been demonstrated to have anti-infective properties targeting viruses, bacteria, and even the malarial parasite. To determine if cysteamine and cystamine exert an antiviral action against SARS-CoV-2, the causative agent of COVID-19, we tested their efficacy in a cell-based assay, while their immunomodulatory properties were tested on both whole-blood and peripheral blood mononuclear cell (PBMC).
Methods. Cysteamine and cystamine antiviral effect was evaluated using the VERO-E6 cells-based cytopathic effect (CPE) inhibition assay. The immunomodulatory effects of these compounds were assessed using the whole-blood followed by multiplex cytokine assay or PBMC by flow cytometry.
Results. We found that in SARS-CoV-2-infected Vero-E6 cells cysteamine and cystamine decrease both the virus-induced cytopathic effects, with EC50 values of 180±53 and 80± 39μM respectively, and the in vitro viral production.
We also found that cysteamine and cystamine significantly decreased the magnitude of cytokine production induced by a broad stimulus such as Staphylococcal Enterotoxin B antigen (SEB). Moreover, we found a reduced TNFα and IL-2 production by SEB-stimulated CD4+ cells.
Conclusions. Overall our findings suggest that cysteamine, an already human applied drug, and cystamine exert an anti-viral effect against SARS-CoV-2 and have immunomodulatory effect in vitro, thus providing a rationale to test these compounds as novel therapy for COVID-19.

P140 - OC 13

In silico and *in vitro* combined approaches identified promising candidates as SARS-CoV-2 and HCoV-OC43 inhibitors

I. Arduino[1], M. Donalisio[1], M. Milani[2], E. Mastrangelo[2], F. Boni[2], A. Marcello[3], R. M. Bonotto[3], E. Schneider[4] , D. Lembo[1]

[1] Department of Clinical and Biological Sciences, Laboratory of Molecular Virology and Antiviral Research, University of Turin, Orbassano, Turin, Italy - [2] CNR-IBF, Istituto di Biofisica, Milano, Italy; Dipartimento di Bioscienze, Università di Milano, Milan, Italy - [3] International Centre for Genetic Engineering and Biotechnology, Laboratory of Molecular Virology, Padriciano, Trieste, Italy - [4] High Throughput Screening Facility of the International Centre for Genetic Engineering and Biotechnology, Padriciano, Trieste, Italy

Aim of the study: Coronaviruses are a large family of viruses that already challenged humanity in at least two other previous outbreaks and are likely to be a constant threat for the future. The current pandemic of the novel coronavirus SARS-CoV-2 urged the need for broad-spectrum antiviral drugs as the first line of treatment. In particular, drug repositioning represents an effective drug discovery strategy from existing drugs that could shorten the time and reduce the cost compared to *de novo* drug discovery. In this context, the aim of this study was to identify effective compounds with anti- betacoronavirus activity to tackle the current and also future pandemics.

Methods used: In this work we developed a pipeline based on *in silico* docking of known drugs on SARS-CoV-1/2 RNA-dependent RNA polymerase (RdRp) combined with *in vitro* antiviral assays on both SARS-CoV-2 and the common cold human coronavirus HCoV-OC43. We analyzed *in silico* the public database of approved/investigational drugs (DrugBank library), targeting a wide region around the active site of SARS-CoV-1/2 RdRp. A reasonable number of compounds (together with suramin, known to inhibit several RNA viruses) was selected for further investigation, taking into account commercial availability and solubility properties of compounds. The selected compounds were subjected to an additional *in silico* analysis targeting the active site of CoV main protease. Next, we assessed *in vitro* the antiviral activity of these compounds against two pathogenic betacoronavirus strains, SARS-CoV-2 and HCoV-OC43, by means of antiviral assays and viral yield reduction assays.

Results and conclusions: We identified certain drugs with activity against both betacoronavirus strains at a similar inhibitory concentration, while other drugs exhibited inhibitory activity only against a specific strain. In particular, the antipsychotic drug lurasidone and the antiviral drug elbasvir showed promising activity in the low micromolar range against both SARS-CoV-2 and HCoV-OC43 with good selectivity indexes. Thus, our approach allowed the identification of lead candidate drugs for further *in vitro* and clinical investigation to contain the present outbreak. Furthermore, it could contribute to the identification of broad-spectrum anti-CoV inhibitors/therapies that would allow for a rapid and effective reaction to future epidemics. Moreover, treatment of CoV infections with drugs that could inhibit different viral targets, as predicted for lurasidone and elbasvir, would be an effective way to lower chances of the emergence of drug resistant viral strains.

P141 - OC 44

Antiviral and antioxidant activities of *Artemisia annua* against SARS-CoV-2

M. Baggieri[1], A. Marchi[1], P. Bucci[1], M. Kojouri[1], S. Gioacchini[1], D. Nuzzo[2], P. Picone[2], A. Pinto[3], S. Dallavalle[3], F. Ubaldi, F. Magurano[1]

[1] Dipartimento di Malattie Infettive, Istituto Superiore di Sanità, Rome, Italy - [2] Consiglio Nazionale delle Ricerche, Palermo, Italy - [3] Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente, University di Milano, Italy

Aim of the study. The COVID-19 pandemic caused by severe acute respiratory coronavirus 2 (SARS-CoV-2) has taken a heavy toll on public health and global economy. Unfortunately, there are currently no specific and effective antiviral drugs available to treat the large number of infected patients.
Artemisinin isolated from the plant *Artemisia annua*, sweet wormwood, is a sesquiterpene lactone with an endoperoxide bridge and has been widely used as antimalarial drug. It showed multiple pharmacological activities, including anticancer, antiviral, and immune modulation.
The antiviral broad-spectrum potential of artemisinins has been demonstrated by several studies on the inhibition of several DNA and RNA viruses, including SARS-CoV (Shi You Li et al., 2005; Efferth T. et al., 2008; Flobinus A. et al., 2014). Our study focused on testing in vitro the antioxidant and antiviral activities of crude extracts of *Artemisia annua* against SARS-CoV-2.
Methods. Crude plant extract was obtained from harvested leaves soaked in 96% ethanol and incubated in the dark at room temperature. After filtration, alcohol was removed from the mother tincture by liquid nitrogen, to preserve thermo labile components, obtaining a product of 227mg.
The essential oil was resuspended with 30% DMSO to obtain a concentration of 100mg/ml and serial dilutions (from 10 mg/ml) were prepared with sterile PBS. The dilutions were tested to assess the cytotoxic potential on Vero E6 cells by cytotoxicity test assay (XTT Cell Viability Assays, Roche).
The antiviral potential was assessed by plaque reduction neutralization test (PRNT80) on Vero E6 cells. The presence of SARS-CoV-2–infected cells was indicated by the formation of viral plaques. Moreover, the capacity of the *Artemisia* extract to reduce ROS generation induced by TBH was analyzed by DCFH-DA assay.
Results. According to results from XTT, plant extract showed no cytotoxic effect. The infectivity in PFU of SARS-CoV-2 challenge virus in the PRNT80 was reduced as direct result of the plant extract capable of neutralizing infectious virus. The dilutions used to treat the virus, from 1mg/ml to 1ul/ml, were effective more than 80%. To analyze whether the *Artemisia* extracts were able to protect the A549 cell, we treated the cells with the oxidative agent tert-Butyl hydroperoxide (TBH) 500 µM in combination with increasing concentrations (25, 50, 100 and 200 µg/ml) of the *Artemisia* extract after 24 hours.
Conclusions. Our study demonstrates the antiviral activity of *Artemisia annua* extract in vitro confirming previous results on Sars-CoV-2 infections in vitro (Kerry G. et al., 2020; Ruiyuan Cao et al., 2020). Moreover, the plant extract plays a significant role as antioxidant agent.

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P142
Inhibitory effect of ophthalmic solutions against SARS-CoV-2

A. Chianese[1], F. Petrillo[1], M. De Bernardo[2], C. Zannella[1], MV. Morone[1], M. Galdiero[1], G. Boccia[2], N. Rosa[2], G. Franci[2]

[1] Department of Experimental Medicine, section of Virology and Microbiology,University of Campania "Luigi Vanvitelli", Naples, Italy - [2] Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, 84081 Baronissi, Italy

Aim of the study. SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) belongs to the *Coronaviride* family and represents the etiological agent of COVID-19. In March 2020, a state of a global pandemic was declared, due to the marked spread of the virus and the significant increase in cases [1]. Transmission of the virus can occur by direct or indirect contact, through air droplets, and affect oral and ocular areas [2]. To cope with this situation, it is important to increase preventive measures and reduce viral spread [3]. In this work we will focus on trying to reduce the ocular infection and trasmission, testing the antiviral activity against SARS-CoV-2 of some already marketed eye drops (Iodim, Ozodrop, DROpsept, and Septavis).
Methods used. SARS-CoV-2 (strain VR PV10734) was propagated on Vero cells, epithelial kidney cells of *Cercopithecus aethiops* (ATCC CCL-81). Co-treatment and virus pre-treatment assays were performed. Different volumes of compounds (12.5, 25, 50, 100 µL) in the presence of the virus (103 PFU/cell) for different time points (15 sec, 30 sec, 1 min, 5 min, 10 min, 15 min, 30 min, 1 h, and 2 h) were evaluated. Also, molecular tests were performed to evaluate the antiviral potential of eye drops through Real-time PCR.
Results and conlusions. The obtained data indicate that only 3 of 4 ophthalmic solutions (Iodim, Ozodrop, and DROpsept) exhibited a high inhibitory activity against SARS-CoV-2 at different volumes and times tested. Also, molecular tests confirmed data obtained *in vitro*. Indeed, eye drops inhibited the expression of the nucleocapsid protein (N) and reduced the expression of spike protein (S). Following the significant inhibitory effect of ophthalmic solution against the virus, it is possible to reach the conclusion that these solutions could represent a preventive resource for ocular infections.

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P143

The cholesterol metabolite 27-hydroxycholesterol inhibits SARS-CoV-2 and is markedly decreased in COVID-19 patients

A. Civra[1], A. Marcello[2], R. Milan Bonotto[2], M. Costantino[1], L. Nascimento Alves[2], S. Rajasekharan[2], C. Giacobone[3], C. Caccia[4], R. Cavalli[5], M. Adami[6], P. Brambilla[3], V. Leoni[3], G. Poli[7], D. Lembo[1]

[1] Department of Clinical and Biological Sciences; Laboratory of Molecular Virology and Antiviral Research; University of Turin; San Luigi Hospital; Orbassano, Turin; Italy - [2] Laboratory of Molecular Virology; International Centre for Genetic Engineering and Biotechnology (ICGEB); Trieste; Italy - [3] Laboratory of Clinical Chemistry; Hospitals of Desio and Monza, ASST-Monza and Department of Medicine and Surgery, University of Milano-Bicocca; Monza; Italy - [4] Unit of Medical Genetics and Neurogenetics; Fondazione IRCCS, Istituto Neurologico Carlo Besta; Milan; Italy - [5] Department of Drug Science and Technology; University of Turin; Turin; Italy - [6] Department of Pharmacological and Biomolecular Sciences; University of Milan; Milan; Italy - [7] Department of Clinical and Biological Sciences; Unit of General Pathology and Physiopathology; University of Turin; San Luigi Hospital; Orbassano, Turin; Italy

Aim of the study. There is an urgent need to identify antivirals against the human coronavirus (HCoV) SARS-CoV-2 in the current COVID-19 pandemic and to contain future similar emergencies early on. Specific side-chain cholesterol oxidation products of the oxysterols family have been shown to inhibit a large variety of both enveloped and non-enveloped human viral pathogens. In this study we focused on investigating the anti-CoV potential of the oxysterol 27-hydroxycholesterol (27OHC), a cholesterol oxidation product of enzymatic origin, constitutively present in several human biologic fluids, and capable of inhibiting or even preventing the infection of both enveloped and non-enveloped viral pathogens.

Materials and Methods. We tested the in vitro antiviral activity of 27OHC against a clinical isolate of SARS-CoV-2 and a second member of beta-CoV subfamily and common cold etiological agent, OC43, by plaque reduction assay. Importantly, we integrated these in vitro data with relevant clinical evidences, by assessing the oxysterols hematic profile in 67 consecutive COVID-19 adult patients. Only SARS-CoV-2 cases confirmed through real-time reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assays of nasal and pharyngeal swabs were included in the analysis.

Results and conclusions. The results of antiviral assays demonstrate the in vitro inhibitory activity of the redox active oxysterol 27OHC against SARS-CoV-2 and against HCoV-OC43 without significant cytotoxicity. Interestingly, physiological serum levels of 27OHC in SARS-CoV-2 positive subjects were significantly decreased compared to the matched control group, reaching a marked 50% reduction in severe COVID-19 cases. Moreover, no correlation was observed between 24-hydroxycholesterol and 25-hydroxycholesterol serum levels and the severity of the disease. Opposite to that of 27OHC was the behavior of two recognized markers of redox imbalance, i.e. 7-ketocholesterol and 7β-hydroxycholesterol, whose serum levels were significantly increased especially in severe COVID-19 patients. These results show that low blood levels of 27OHC are associated with the more severe forms of COVID-19, and suggest that the exogenous administration of 27OHC may represent in the near future an antiviral strategy in the worsening of diseases caused by present and emerging CoVs.

P144

Lactoferrin as potential supplementary nutraceutical agent in COVID-19 patients: in vitro and in vivo preliminary evidences

M.P. Conte[1], E. Campione[2], L. Rosa[1], C. Lanna[2], C. Del Vecchio[3], F. Iacovelli[4], M. Falconi[4], L. Bianchi[2], P. Valenti[1]

[1] Department of Public Health and Infectious Diseases, University of Rome "La Sapienza", Rome, Italy - [2] Dermatology Unit, Tor Vergata University Hospital, Department of Systems Medicine, Rome, Italy - [3] Department of Molecular Medicine, University of Padova, Padua, Italy - [4] Department of Biology, Structural Bioinformatics Group, University of Rome "Tor Vergata", Rome, Italy

Aim of the study: Lactoferrin (Lf), a multifunctional cationic glycoprotein, secreted by exocrine glands and neutrophils, possesses pleiotropic activities. The Lf is able to chelate two ferric ions per molecule influencing both bacterial and viral replication and reactive oxygen species formation. The binding of Lf to the anionic surface compounds of host cells and/or of bacteria or viruses, thanks to its cationic feature, is associated to the host protection against bacterial and viral adhesion and entry. Moreover, Lf is able to access into host cells and to translocate into the nucleus restoring iron and inflammatory homeostasis disorders. Therefore, based on these evidences, we investigated the role of Lf in i*n vitro* counteracting SARS-CoV-2, in in silico putative binding with viral structure(s) and in COVID-19 patients as unique or supplementary treatment.

Methods used: The *in vitro* experiments were carried out in different cell line models (Vero E6 and Caco-2 cells) infected with SARS-CoV-2 strain.

Lf, used at different concentration (100 or 500μg/ml), was pre-incubated with cell monolayers or virus prior of the infection or added at the moment of infection or in post-infection phase in order to verify its protective, neutralizing and therapeutic activity against SARS-CoV-2, respectively. Furthermore, the SARS-CoV-2 Spike trimer structure was used to perform a protein-protein molecular docking analysis to verify a putative direct interaction between the two glycoproteins: Spike and Lf.

Regarding the clinical trial, a total of 92 mild-to-moderate (67/92) and asymptomatic (25/92) COVID-19 patients were recruited and divided in 3 groups according to the administered regimen. Thirty-two patients, 14 hospitalized and 18 in home-based isolation received oral and intranasal liposomal bovine Lf (bLf) as unique treatment; 32 hospitalized patients were treated with standard of care treatment and 28, in home-based isolation, did not take any medication. Furthermore, 32 healthy subjects, COVID-19 negative, were added as a control group for ancillary analysis.

Results and conclusions: The Lf antiviral activity varies according to concentration (100 or 500μg/ml), multiplicity of infection (0.1/0.01) and cell type (Vero E6/Caco-2 cells). Our experimental results indicate that Lf, in a dose-dependent manner, exerts its antiviral activity either by direct binding to the SARS-CoV-2 particles or host cells obscuring viral receptors.

Moreover, the results obtained through the molecular docking strongly support the hypothesis of a direct recognition between the Lf and the Spike glycoproteins.

Regarding the clinical trial, bLf-treated COVID-19 patients obtained an earlier and significant ($p < 0.0001$) median rRT-PCR SARS-COV-2 RNA negative conversion compared to

the standard of care- treated and non-treated COVID-19 patients (14.25 vs 27.13 vs 32.61 days, respectively).
In addition, bLf-treated COVID-19 patients showed significant fast clinical symptoms recovery than standard of care-treated and non-treated COVID-19 patients. Of note, in bLf-treated patients, a significant decrease of serum ferritin and IL-6 levels, parameters characterizing inflammatory processes, were observed. Serum D-dimers levels was also found significantly decreased following bLf supplement. No adverse events were reported. Overall, these *in vitro* and *in vivo* observations led us to speculate a potential and safe unique or supplementary treatment of bLf in the management of mild-to-moderate and asymptomatic COVID-19 patients.

P145

Antiviral effect of a cationic antimicrobial peptide against murine norovirus and hepatitis A virus

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

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

Antimicrobial peptides (AMPs) are amphiphilic agents with a broad-spectrum antimicrobial activity, accomplished by mechanically damaging the integrity of bacterial membranes [1]. The AMPs have been extensively used as substitutes of antibiotics for bacterial infections, but recently their use has been extended to antiviral therapy [2]. In order to investigate the antiviral potential of these peptides, this study evaluated the effect of a cationic antimicrobial peptide belonging to the IDR (innate defense regulator) family, named pAVP (potential-AntiViral-Peptide) against Hepatitis A Virus (HAV) and murine norovirus (MNV-1), the latter used as a surrogate for human norovirus.

Preliminary results were obtained on pAVP cytotoxicity on cell lines permissive for HAV and MNV-1, namely Frp3 and RAW 264.7. pAVP solutions with concentrations ranging from 10 µM to 80 µM were assayed and no cytotoxic effect was observed.

The pAVP solutions 40 µM and 80 µM were selected for antiviral tests, treating viral suspensions of HAV and MNV-1 at two different titrations (4.6×10^6 $TCID_{50}$/ml and its 100-fold dilution for HAV, and 3.2×10^4 $TCID_{50}$/ml and its 100-fold dilution for MNV-1). Briefly, the pAVP-virus solutions were incubated with viral stocks for 1 hour at room temperature and residual viral infectivity was evaluated by titration based on cytopathic effect. Untreated HAV and MNV-1 suspensions and 40 µM and 80 µM pAVP solutions, incubated at same conditions, were used as positive and negative controls, respectively.

The results showed for HAV and MNV-1 a decrease of viral infectivity between 0.66 and 1.66 log. In detail, in presence of pAVP 40 µM, the two HAV suspensions decreased by 0.66 and 1.33 log (higher concentration and 100-fold dilution, respectively) and MNV-1 viral stocks decreased by 1.00 and 0.84 log. As regard to the antiviral activity of pAVP 80 µM, the two HAV suspensions decreased by 1.66 and 1.33 log, while for MNV-1 a reduction of 1.00 log was obtained regardless of the viral concentration.

This study highlights that the peptide pAVP exerts a partial inhibitory effect on MNV-1 and HAV infectivity. Further studies are necessary to optimise the peptide effect with a look at its potential future use, to control contamination by foodborne viruses.

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P146

Antiviral activity of hydrolates from *Citrus limon*, *Thymus vulgaris* and *Thymus serpyllum* against norovirus surrogates

L. Cozzi[1], T. Vicenza[1], R. Battistini[2], C. Masotti[2], E. Suffredini[1], S. Di Pasquale[1], C. Ercolini[2], L. Serracca[2]

[1] Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, National Reference Laboratory for Foodborne Viruses, Rome, Italy - [2] Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, La Spezia, Italy

Hydrolates are natural compounds obtained as co-products of essential oils extraction, from the steam-distillation or hydro-distillation of fresh medicinal plants [1]. Due to their antimicrobial, antiviral and antifungal features, they are suggested to be suitable for food safety applications [2]. In this study we assessed the potential antiviral effect of *Citrus limon*, *Thymus vulgaris* and *Thymus serpyllum* hydrolates on murine norovirus (MNV-1), a surrogate for human noroviruses that can be cultivated in vitro on RAW 264.7 cell line.

Times of exposure and concentrations of hydrolates were evaluated. Preliminary assays demonstrated that *Citrus limon*, *Thymus vulgaris* and *Thymus serpyllum* hydrolate solutions at 1% and 2% concentrations do not have a cytotoxic effect on RAW 264.7. Therefore, to evaluate the effect of these hydrolates on viral infectivity, a MNV-1 suspension (2.14×10^5 $TCID_{50}$/ml) was incubated with 1% and 2% v/v of each hydrolate in serum-free culture medium. Treated virus aliquots were stored at -80°C immediately (t=0) and after 24 h of incubation at 20±2°C (t=24). An untreated MNV-1 suspension, and 1% and 2% hydrolate solutions kept at the same conditions were used as positive and negative controls, respectively. The decrease of viral infectivity was calculated by comparison of the $TCID_{50}$/ml of the untreated virus with that of the viral suspension treated with the three different hydrolates.

The results showed that *Thymus vulgaris* and *Thymus serpyllum* hydrolates caused an immediate (t=0) decrease of the MNV-1 infectivity of almost 2 log both at 1% and 2% concentrations. In detail, both hydrolates achieved a 1.83 log reduction at 1% and 1.67 reduction at 2%. Prolongation of the incubation time (t=24) provided further reduction, but displayed limited efficacy considering the natural loss of infectivity of untreated virus. As regard to *Citrus limon*, this hydrolate showed an immediate (t=0) reduction of viral infectivity of 1 log and 1.33 log at 1% and 2% concentration, respectively, followed by a further reduction of approximately 1 log after 24h of incubation. These results showed that *Thymus vulgaris* and *Thymus serpyllum* exert their antiviral effect almost immediately and a longer exposure time does not provide a significant increase of their antiviral activity, regardless of the concentration of use.

In conclusion, the results obtained are relevant for the use of the hydrolates in the field of food technology and food safety, as they support their efficacy at low concentrations and with the use of short contact times.

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P147 Peptidodendrimer designed on the viral spike protein inhibits SARS-CoV-2 infectivity

A. De Filippis[1], C. Zannella[1], V. Folliero[1] D. Stelitano[1], V. Fiore[1], G. Franci[2], M. Galdiero[1]

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

Aim of study. The Coronavirus disease 2019 (COVID-19) appeared in Wuhan City (China) on December 2019 and it spreads worldwide with great speed. Everyday the number of deaths grows drastically, reaching 3 millions deaths globally and over 137 millions reported cases since the start of the outbreak. The etiological agent of COVID-19 was identified in a novel coronavirus called Severe acute respiratory syndrome-associated coronavirus 2 (SARS-CoV-2). It belongs to the large family of Coronaviridae, consisting of enveloped RNA viruses with a broad host range and the property of causing zoonotic diseases, such as SARS and Middle East respiratory syndrome (MERS).
The infections lead to mild to severe respiratory illness and even death. It was widely reported that the surface spike (S) protein is essential for the coronavirus binding and entry of host cells. It is a class I fusion protein, characterized by two subunits: S1 binds to the human receptor angiotensinconverting enzyme 2 (hACE2), meanwhile the S2 subunit takes part in the viral and target cell membrane fusion. The S2 subunit contains several main elements, including a hydrophobic fusion peptide, a pair of heptad repeat (HR) helices and a pre-transmembrane domain. The S protein undergoes different conformational changes: the fusion peptide inserts into the target cell membrane and the two HRs rearrange to form a six-helices bundle (6-HB) leading to the fusion between the viral envelope and cellular membrane.
Methods used. Starting from the entire aminoacidic sequence of the viral S protein (NCBI YP_009724390.1), a peptide designed on the C-terminal HR (HGINASVVNIQKEIDRLNEVAKNLNESL-OH) was synthetized by solid-phase methodology and purified by reversed-phase high performance liquid chromatography. Then a solution of peptide was added to dendrimer, the mixture was concentrated and purified by size-exclusion chromatography. The anti-SARS-CoV-2 activity and the cytotoxicity of the peptidodendrimer were evaluated through viral plaque reduction and MTT assays, respectively.
Results and Conclusions: The peptidodendrimer was examinated for its ability to inhibit SARS-CoV-2 plaque formation on Vero cells, at peptide concentrations of ~30 μM. To validate that the observed antiviral activity was not a consequence of cellular cytotoxicity, a MTT assay was performed. Vero cells treated with ~30 μM of peptidodendrimer showed no difference in absorbance as compared to untreated cells. Peptides derived from the HR helices of the class I viral fusion proteins have been demonstrated to possess a strong antiviral activity, also against SARS and MERS viruses. The addition of dendrimer allows the peptide to be targeted close to the spike C-terminal HR preventing the formation of the 6-HB and, as a result, the fusion virus-cell membranes. These findings allow for the identification of possible therapeutic peptides for the treatment of SARS-CoV-2 infections.

P148 - OC 45

Antiviral activity against HSV-1 and SARS-CoV-2 of leaf extract derived from *Vitis vinifera*

F. Dell'Annunziata[1], F. Sarno[2], A. Nebbioso[2], L. Altucci[2], C. Buonocore[3], D. de Pascale[4], G, Vitale[4], R. Giugliano[1], A. Chianese[1], G. Franci[1], M. Galdiero[1]

[1] Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy - [2] Department of Precision Medicine, University of Campania "Luigi Vanvitelli, Naples, Italy - [3] Institute of Biochemistry and Cell Biology (IBBC), National Research Council (CNR) Naples, Italy - [4] Department of Marine Biotechnology Stazione Zoologica "Anton Dohrn", Naples, Italy

Aim of the study. *Vitis vinifera* represent a species widely studied to produce molecules with powerful biological activity. In addition to wine, also winery bioproducts, such as grape pomace, skins, and seeds are rich in molecules with high anti- bacterial, viral and fungal activity. However, to date the knowledge of vine leaves biological properties is not completely clarified. In this scenario, the present study evaluated phenolic composition and antiviral activity of *Vitis vinifera* leaf extract against two human viruses, the Herpes simplex virus type 1 (HSV-1) and the pandemic and currently widespread Severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2).
Methods used. Green healthy leaves were harvested in April 2019 in Portici (Naples, Italy) from a clone of *V. vinifera*. Growth conditions are normal open field conditions of the Campania (Italy) region. Polyphenols were extracted using a solution of 1.5 ml of 75% (v/v) methanol / 0.05% (v/v) trifluoroacetic acid (TFA). The extract was assessed by LC-MS analysis. The cytotoxicity of extracts (65-500 µg/mL) was evaluated through the metabolic activity of viable cells via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H tetrazolium bromide (MTT) assay on HCT-116, A549, H9c2, MCF7, HepG2 and Hacat after 48 hours of treatment. The antiviral activity against HSV-1 and SARS-CoV-2 via plaque assay and RT Real-time PCR. Relative target Ct (the threshold cycle) values of UL54, UL52 and UL27 (for HSV-1) and S protein (for SARS-CoV-2) was normalized to GAPDH as housekeeping gene.
Results and conclusions. About 40 phenolic compounds were identified in the phenolic vine extract by HPLC-MS / MS analysis. Most of them were derivatives of quercetin, others included derivatives of luteolin, kaempferol, apigenin, isoramnetin, myricetin, chrysoeriol, biocanin, isookanin, and scutellarin. The leaf extract inhibited replication of both HSV-1 and SARS-CoV-2 in the early stages of infection by directly blocking proteins on the viral surface, at a very low concentration of 10 µg/mL. These evidences highlight the powerful antiviral activity of natural extracts and lay the foundations for potential antiviral drug design and vaccine development derived from natural compounds.

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Identification of inhibitors of SARS-CoV-2 3CLpro enzymatic activity using a small molecule in vitro repurpusing screen

F. Esposito[1], M. Kuzikov[2], E. Costanzi[3], J. Reinshagen[2], L. Vangeel[4], M. Wolf[2], B. Ellinger[2], C. Claussen[2], G. Geisslinger[2,11], A. Corona[1], D. Iaconis[5], C. Talarico[5], C. Manelfi[5], R. Cannalire[6], G. Rossetti[7], J. Gossen[7], S. Albani[7], F. Musiani[8] , K. Herzog[9], Y. Ye[10], B. Giabbai[3], N. Demitri[3], D. Jochmans[4], S. D. Jonghe[4], J. Rymenants[4], V. Summa[6], A. R. Beccari[5], P. Leyssen[4], P. Storici[3], J. Neyts[4], P. Gribbon[2], A. Zaliani[2] and E. Tramontano[1]

[1] Dipartimento di Scienze della vita e dell'ambiente, Cittadella Universitaria di Monserrato, SS-554, Monserrato, Cagliari, Italy -[2] Fraunhofer Institute for Translational Medicine and Pharmacology (ITMP) and Fraunhofer Cluster of Excellence for Immune mediated diseases (CIMD), Schnackenburgallee 114, 22525 Hamburg, and Theodor Stern Kai 7, 60590 Frankfurt, Germany - [3] ElettraSincrotrone Trieste S.C.p.A., SS 14 - km 163, 5 in AREA Science Park 34149 Basovizza, Trieste, Italy - [4] KU Leuven, Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, Herestraat 49 - box 1043, 3000- Leuven, Belgium - [5] Dompé Farmaceutici SpA, via Campo di Pile, 67100 L'Aquila, Italy - [6] Department of Pharmacy, University of Naples Federico II, Via D. Montesano, 49, 80131 Naples, Italy - [7] Institute of Neuroscience and Medicine (INM-9)/Institute for Advanced Simulation (IAS-5) and Jülich Supercomputing Centre (JSC) Forschungszentrum Jülich D-52425 Jülich, Germany - [8] Laboratory of Bioinorganic Chemistry, Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy - [9] EU-OPENSCREEN ERIC, Robert-Rössle-Straße 10, 13125 Berlin, Germany - [10] University of Chinese Academy of Sciences, Beijing, 100049, China - [11] Institute of Clinical Pharmacology, Goethe-University, Theodor Stern Kai 7, 60590 Frankfurt, Germany

Compound repurposing is an important strategy for the identification of effective treatment options against SARS-CoV-2 infection and COVID-19 disease. In this regard, SARS-CoV-2 main protease (3CL-Pro), also termed M-Pro, is an attractive drug target as it plays a central role in viral replication by processing the viral polyproteins pp1a and pp1ab at multiple distinct cleavage sites.

We here report the results of a repurposing program involving 8.7 K compounds containing marketed drugs, clinical and preclinical candidates, and small molecules regarded as safe in humans.

We confirmed previously reported inhibitors of 3CL-Pro and have identified 62 additional compounds with IC50 values below 1 µM and profiled their selectivity toward chymotrypsin and 3CL-Pro from the Middle East respiratory syndrome virus. A subset of eight inhibitors showed anticytopathic effect in a Vero-E6 cell line, and the compounds thioguanosine and MG-132 were analyzed for their predicted binding characteristics to SARS-CoV-2 3CL-Pro. The X-ray crystal structure of the complex of myricetin and SARS-Cov-2 3CL-Pro was solved at a resolution of 1.77 Å, showing that myricetin is covalently bound to the catalytic Cys145 and therefore inhibiting its enzymatic activity.

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Evaluation of phenolic profile and antimicrobial activity of grapevine waste from typical cultivars of Campania

V. Folliero[1], A. Morana[2], G. Squillaci[2], F. La Cara[2], F. De Caro[3], V. Carbone[4], P. Minasi[4], F. Dell'Annunziata[1], G. Franci[3], M. Galdiero[1]

[1] Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy - [2] Research Institute on Terrestrial Ecosystems (IRET), National Research Council of Italy, (CNR) - [3] Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, 84081 Baronissi, Italy - [4] Proteomic and Biomolecular Mass Spectrometry Center, Institute of Food Sciences (ISA), National Research Council of Italy (CNR)

Aim of the study. The present study aimed to determine the phenolic composition and antiviral activity of grape cane extracts from three typical cultivars of Campania.
Methods used. Aqueous extracts at different pH (1÷13) were obtained from Aglianico, Fiano, and Greco grape canes. The phenolic contents were measured by the Folin-Ciocalteu method. The grape cane extracts at different pH were analyzed by Reverse-Phase High-Performance Liquid Chromatography Ultraviolet (RP-HPLC-UV) and HPLC-Electrospray Ionization Multistage Ion Trap Mass Spectrometry (HPLC-ESI-ITMSn). The cytotoxicity of extracts (1-1000 µg/mL) was evaluated through the metabolic activity of viable cells via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay on Vero cells after 24 hours of treatment. The antiviral activity against herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) via co-treatment, virus pre-treatment, cell pre-treatment and post-treatment assays were evaluated.
Results and conclusions. The findings showed that pH 13 produced extracts with higher polyphenolic contents, recording a yield greater than twice as much as the respective extracts prepared at pH 1. Greco grape cane extracts contained the highest amount of phenolic compounds at each pH, ranging from 42.7 ± 0.4 to 104.3 ± 3.0 mg of Gallic Acid Equivalent / g of dry extract for pH 1 and 13, respectively. Seventy-five compounds were detected in the extracts via HPLC-MS of which six were first described in grape canes. Cytotoxicity data showed that none of the extracts at pH 7.00 were (was?) toxic to cell monolayers after 24 hours at maximum concentration. Conversely, a significant reduction in viability with extracts at pH 1 was observed, less marked with extracts at pH 13.00. All extracts showed a strong virucidal activity against HSV-1 and HSV-2. Greco grape cane extracts at pH 7 exhibited high antiviral activity with 100% inhibition of virus plaques at 10 µg/mL. At pH 13, Greco extract was the most active by showing an IC_{50} at 0.9 µg/mL. The extracts showed slightly different antiviral activity against HSV-2. All neutral and alkaline extracts exhibited a strong antiviral effect against HSV-1 and HSV-2. These data suggested suggest a potential application of grape cane extracts as new natural antiviral preparations and emphasize the importance of investigating the biological effects of agricultural waste.

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Drug repurposing: palmitoylethanolamide in the treatment of SARS-CoV-2

R. Fonnesu, V. La Rocca, E. Iacono, G. Freer, M. Pistello, M. Lai

Centro Retrovirus. Dipartimento di Ricerca Traslazionale e delle Nuove Tecnologie in Medicina e Chirurgia, Università di Pisa, Pisa

Introduction: Drug repurposing is a strategy for identifying new uses for approved or investigational drugs that are outside the scope of the original medical indication. This strategy allows saving money and time, shortening preclinical tests and clinical trials [1].
The global SARS CoV-2 pandemic offered a new goal: finding effective drugs to fight or prevent this infection since no specific drugs have been identified yet [2].
Molecular docking high throughput analysis has identified Palmitoylethanolamide (PEA) as a potential ligand for Spike protein of SARS CoV-2. This bind could inhibit the attack of the virus to ACE2 and its entrance into cells.
PEA is an endogenous amide, largely present in various living organisms, his production occurs constitutively but is diminished following various pro-inflammatory stimuli.
Micronized PEA preparations are commonly used as analgesic, anti-inflammatory, and neuroprotective mediator [3].
This study aims to test PEA efficacy to inhibit SARS CoV-2 entry into cells and characterize a possible antiviral role of this molecule.
Materials and Methods: A clinical strain of SARS CoV-2 was incubated at different times with different concentrations of PEA, and used for the infection of susceptible HuH-7 cells. Viral infections were assessed using qRT-PCR performed on supernatants and immunocytochemistry performed on infected cells, using high content confocal microscopy.
Results: Preliminary data show 2-fold reduction in viral infection rate when SARS CoV-2 virions are exposed to PEA prior infection. Interestingly, infectivity reduction increases with the time of incubation of SARS CoV-2 virions with PEA.
Discussion: Early clinical trials with PEA suggested that the compound reduced the incidence of acute respiratory infections [3].
Our data are still ongoing, but promising results united with the historical use of PEA, make this molecule a good candidate to fend off SARS CoV-2 infection.

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Antiviral activity of human milk and derived extracellular vesicles against known and emerging viruses

R. Francese[1], M. Donalisio[1], A. Civra[1], P. Tonetto[2], S. Sottemano[2], A. Coscia[2], S. Cirrincione[3], C. Lamberti[3], L. Cavallarin[3], G.E. Moro[4], E. Bertino[2], D. Lembo[1]

[1] Department of Clinical and Biological Sciences, Laboratory of Molecular Virology and Antiviral Research, University of Turin, Orbassano (TO), Italy - [2] Department of Public Health and Pediatrics, Neonatal Intensive Care Unit, University of Turin, Turin, Italy - [3] National Research Council - Istituto di Scienze delle Produzioni Alimentari, Grugliasco (TO), Italy - [4] Italian Association of Human Milk Banks (AIBLUD), Milan, Italy

Aim of the study: Human milk (HM) is a complex biofluid that has evolved over millennia to nourish infants, support their growth and to protect them from numerous diseases. It contains macro- and micro-nutrients and a myriad of biologically active components, including antimicrobial factors, which have a profound role in infant survival and health. In particular, HM antiviral factors are numerous and multi-functional and science is just beginning to clarify their function. The general aim of this study is to evaluate the specific antiviral activity of HM against viral pathogens common in newborns and children (i.e. cytomegalovirus –CMV-, respiratory syncytial virus –RSV-, and rotavirus –HRoV-) and against emerging flaviviruses (i.e. zika virus –ZIKV- and usutu virus –USUV-). Furthermore, the study is addressed to investigate the role and contribution of specific HM components, i.e. the extracellular vesicles (EVs), to the HM overall protective activity.
Methods used: Different samples of colostra were collected from mothers admitted to Sant'Anna Hospital of Turin for delivery. Colostra were centrifuged at high speed to obtain the aqueous fractions and subsequently EVs were purified and characterized by means of Nanosight, TEM and Western blot analyses. Both colostra and EVs were tested *in vitro* against HCMV, RSV, HRoV, ZIKV and USUV and the effective concentrations inhibiting the 50% of infection (EC_{50s}) were calculated and compared between them. The anti-ZIKV and anti-USUV activity was also evaluated at different stages of HM maturation, by testing transitional and mature milk samples *in vitro*. Furthermore, we explored the mechanism of action of EVs against the three pediatric clinically relevant viruses, CMV, HRoV and RSV. Specific antiviral assays were performed to identify the step of viral replication inhibited by EVs and shaving experiments of these vesicles and proteomic analysis of the shaved peptides were carried out to investigate the role of EV surface proteins in impairing viral infection.
Results and conclusions: We demonstrated that human colostrum was endowed with a significant antiviral activity against both pediatric clinically relevant viruses and emerging flaviviruses. HM protection against ZIKV and USUV was maintained through lactation, with no significant difference in the activity of colostrum, transitional and mature milk. We showed that EVs substantially contributed to the overall protective activity of HM against all the viruses on study. Furthermore, our results revealed that EVs were able to impair the early steps of HCMV, RSV and HRoV cell infection, with EVs surface proteins playing an important role in mediating this activity. This study discloses the intrinsic antiviral activity of HM against known and emerging viruses and contributes to unravelling novel mechanism underlying the functional role of HM as protective and therapeutic agent in infant.

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Therapeutic efficacy of hyperimmune immunoglobulins isolated from the plasma of convalescent COVID-19 subjects

C. Fusco[1], C. Zannella[2], G. Franci[3], M. Galdiero[3], A. Leonardi[1], I. Gargiulo[4], R. Parrella[4], G. Matarese[1,5]

[1] Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli "Federico II", Naples, Italy - [2] Dipartimento di Medicina Sperimentale, Università degli Studi della Campania "Luigi Vanvitelli", Naples, Italy - [3] Dipartimento di Medicina Chirurgia ed Odontoiatria "Scuola Medica Salernitana", Baronissi, Italy - [4] Dipartimento delle Malattie Infettive e Urgenze Infettivologiche, Azienda Ospedaliera Specialistica dei Colli – Ospedale Cotugno, Naples, Italy - [5] Istituto di Endocrinologia e Oncologia Sperimentale, Consiglio Nazionale Delle Ricerche (IEOS-CNR), Naples, Italy

The COVID-19 pandemic has now become a major global health threat. Originating in Wuhan, China, in December 2019 it has spread with great speed and aggression, causing more than 3 million deaths to date according to the World Health Organization (WHO), as well as numerous problems in the national and regional health system. Although many therapies are available today for hospitalized patients with SARS-CoV-2 infection in medium-critical conditions, currently there is no therapy that has proven to be certainly effective in the treatment of SARS-CoV-2 infection. The aim of our study was to evaluate the therapeutic efficacy of the administration of highly purified hyperimmune immunoglobulins from convalescent donors in COVID-19 patients, with a moderate to severe pulmonary involvement. Hyperimmune immunoglobulins are a biomedical preparation consisting of an enriched anti SARS-CoV-2 gamma-globulin fraction isolated from the plasma of subjects recently recovered with acclaimed infection. Therapy with hyperimmune immunoglobulins is characterized by the induction of a "passive immunization" thanks to the infusion of highly purified IgGs. Therefore, we firstly generated a database of potential donors recovered from both severe and mild/asymptomatic forms of COVID-19 infection, enrolled and screened by the Azienda Ospedaliera dei Colli, with a specific antibody title for SARS-CoV-2, measured by chemiluminescence method. Next, we analyzed the ability of the anti SARS-CoV-2 antibodies, present in the collected sera, to bind the virus and prevent its ability to infect cells at different dilutions (1:10; 1:40; 1: 160; 1: 640), through an in vitro neutralization test. Preliminary results show that in a cohort of 100 convalescent subjects, 30% of the subjects with a low antibody title (< 60 AU / ml) have a high capacity to neutralize the SARS-CoV-2 virus *in vitro*. On the contrary, 10% of subjects with a high antibody title (> 60 AU / ml) showed a low neutralizing capacity. Our preliminary data suggest that a low antibody title not always corresponds to a low neutralization efficacy of the virus, as well as a high antibody title not always corresponds to a strong ability to neutralize the virus, a parameter that should be considered as a fundamental factor during hyperimmune immunoglobulin isolation.

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Comparison of immunocytometric pattern in COVID-19 patients of 1st and 2nd waves

G. Scalia[1], M. Raia[1], M. Gelzo[1,2], S. Cacciapuoti[3], A. De Rosa[4], B. Pinchera[3], R. Scotto[3], G. Fabbrocini[3], I. Gentile[3], R. Parrella[4], G. Castaldo[1,2]

[1] CEINGE-Biotecnologie avanzate, scarl, Naples, Italy - [2] Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università di Napoli Federico II, Naples, Italy - [3] Dipartimento di Medicina Clinica e Chirurgia, Università di Napoli Federico II, Naples, Italy. 4Dipartimento di malattie infettive e emergenze infettive, Divisione di malattie infettive respiratorie, Ospedale Cotugno, AORN dei Colli, Naples, Italy

Aims: This study describes the immunocytometric pattern of COVID-19 patients from the 2nd wave (September 2020-January 2021; n = 170) in comparison with those of the 1st wave (March-May 2020; n = 35) [1].
Methods: We studied COVID-19 patients at hospital admission. The clinical severity of enrolled patients was classified on the basis of the seven ordinal scale made by the World Health Organization (WHO)-Research and Development Blueprint expert group [2]. We grouped the patients as follows: WHO 3, not requiring supplemental oxygen; WHO 4, requiring supplemental oxygen; and WHO 5-7, requiring non-invasive or invasive mechanical ventilation or death. We analyzed a large panel of lymphocyte subpopulations by cytofluorimetry. In addition, serum interleukin (IL)-6 was measured by ELISA assay.
Results and conclusions: In comparison with 1st wave, the patients of 2nd wave showed: i) significantly lower levels of IL-6 for each WHO stage; ii) a decreasing trend of total lymphocytes, T cells, T helper and T suppressor lymphocytes in WHO 3 subgroup, while these parameters were higher for WHO 4 and WHO 5-7 subgroups; iii) a higher number of B lymphocytes, naïve lymphocytes, and total activated lymphocytes in WHO 4 and WHO 5-7 subgroups. Interestingly, we also observed that these parameters showed an increasing trend related to the clinical severity (WHO score) in the 2nd wave, as opposed to the 1st wave which showed a decreasing trend; iv) a decreasing trend of T activated, TH1 and TH1 activated lymphocytes. Our findings reveal that the 2nd wave patients with severe disease had a better immunocytometric pattern compared to the 1st wave. Instead, 2nd wave patients with a mild disease (WHO 3) resulted more immunosuppressed than 1st wave. These differences on immunocytometric parameters may be due to some factors. In particular, the differences between therapeutic approaches for the patients of the two waves could be taken into account. In fact, the most of 2nd wave patients were treated by steroids, azithromycin [3,4] and/or hydroxychloroquine before hospitalization admission, while the patients of the 1st wave were hospitalized before starting therapies. Further studies are necessary to better define the therapies in COVID-19 patients. Immunocytometry may contribute to predict the disease severity and to select a personalized treatment.

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New antiviral glycolipidis from a marine *Rhodococcus* sp.

R. Giugliano[1], F. Palma Esposito[2], C. Buonocore[3], D. de Pascale[2], BM. Nastri[1], V. Folliero[1], G. Franci[4], M. Galdiero[1]

[1] Department of Experimental Medicine, section of Virology and Microbiology, University of Campania "Luigi Vanvitelli", Naples, Italy - [2] Department of Marine Biotechnology, Stazione Zoologica Anton Dohrn, Naples Italy - [3] Institute of Biochemistry and Cell Biology (IBBC), National Research Council (CNR), Naples, Italy - [4] Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Baronissi, Italy

Aim of study. To counter the spread of enveloped viruses, such as SARS-CoV-2, it is worth interrupting the chain of infection using molecules with detergent-like action, such as biosurfactants. Among them, glycolipids, that are structurally constituted by one or more fatty acid chains linked to one or more sugars, are the most studied and well-characterized. Actinobacteria belonging to the *Rhodococcus* genus are among main producers of trehalose lipids, a class of biosurfactant in which the sugar moiety is represented by the trehalose disaccharide, and other glycolipids.
This study aimed to evaluate the antiviral activity of a trehalose and trihexose lipids mixture produced by a novel marine *Rhodococcus* sp. "IR2" towards enveloped viruses belonging to the *Coronaviridae* and *Herpesviridae* families. In particular these glycolipids were tested against Human Coronavirus 229E (HCoV-229E), belonging to the *Alphacoronavirus* genus, the Human Coronavirus OC43 (HCoV-OC43), belonging to the genus *Betacoronavirus*, and Herpes simplex virus type 1 (HSV-1).
Methods used. Strain IR2 was isolated from Mediterranean marine sediments and it was identified by 16S and whole genome analysis. This bacterium was cultivated in 22 different growth media, following the One Strain MAny Compounds (OSMAC) approach. The cell free supernatants were collected and extracted by ethyl acetate, then the organic solvent was evaporated, and the obtained extracts were investigated through Liquid Chromatography coupled to High Resolution Mass Spectrometry (LC-HRMS). The extract containing the glycolipids was fractionated by Solid Phase Extraction (SPE) and the obtained fractions investigated for the antiviral activity towards HCoV-229E and HSV-1 by plaque assay, and towards HCoV-OC43 by cell viability assay.
Results and conclusions. IR2 strain was identified as a novel species of the *Rhodococcus* genus. Through the OSMAC approach, the culture medium containing glycerol showed to be the only one that led to the production of biosurfactants among 22 tested conditions. In particular, these molecules were identified as trehalose and trihexose lipids by LC-HRMS and most of them were never reported previously. The extract containing this new mixture was fractionated by SPE at 50%, 90%, and 100% of methanol in water, and each fraction was subjected to antiviral assay. The fraction 90% MeOH showed best results, in fact, it was able to inactivate the enveloped viruses, showing a complete viral inhibition at 7.5 and 30 µg/ml for HSV-1 and HCoV-229E, respectively. Finally, when cells were infected with HCoV-OC43, only 20% of cytopathic effect was observed in presence of 60 µg/ml of glycolipids. In conclusion, the isolated *Rhodococcus* strain is a promising bacterium because it produces a new biosurfactant mixture with antiviral activity. Considering that the antiviral action of biosurfactants is not specific, but directed against the envelope and its proteins, these molecules can target a broad spectrum of viruses. In particular, biosurfactants could play a key role in the fight against SARS-CoV-2 pandemic.

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Combating actions of green 2D-materials against herpes simplex virus type 1

A. Mali[1], P. More[1], F. Foglia[1], C.A. Lanza[1], R. Giugliano[1], A. Ambrosino[1], M. Singh[2], C. Altucci[2], B. Santella[1], A. De Filippis[1], M. Galdiero[1]

[1] Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", 80138 Naples, Italy - [2] Laboratory of Bio-Nano-Photonics, Department of Physics "Ettore Pancini", University of Naples "Federico II", Naples, 80126, Italy

Aim of the study. Bi-dimensional (2D) nanomaterials constitute an interesting topic due to the their biological activity, such as their bactericide and antiviral actions. In detail, we studied the interaction between 2D nanoflakes, i.e. MoS_2 and graphene oxide (GO), and Herpes simplex virus type-1 (HSV-1). HSV-1 is the causative agent of the common cold sores but is also associated with lesions affecting the genital areas. Very rarely HSV-1 can spread to the ocular area, causing herpetic keratitis, and also to the central nervous system and thus can determine neurological sequelae, including cognitive dysfunction and learning difficulties.

Methods used. Firstly MoS2 and GO nanoflakes cytotoxicity was investigated via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT); on the other side, their antiviral abilities was evaluated through plaque assays (co-treatment, cell pre-treatment, virus pre-treatment and post-treatment assays) and Transmission Electron Microscopy (TEM).

Results and conclusions. The experimental results are really novel and somewhat surprising, highlighting a stronger antiviral action of GO as compared to MoS2. In particular, GO showed a strong antiviral activity in the virus pre-treatment, that only detects the direct NSs-virus interaction, and co-treatment experiments; on the other side, MoS_2 only induced some antiviral action in virus the pre-treatment experiment. No antiviral effect was noted in either cell pre- and post-treatment case for both nanomaterials. We interpreted this as caused by the presence of specific glycoproteins on the cell membrane that have high affinity with the oxygen functionalized groups on the GO NSs surfaces. All together these data indicate the importance of green 2D-materials as innovative antiviral compounds.

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The cranberry extract Oximacro® prevents Hazara virus infection by inhibiting the attachment to target cells

M. Mirandola[1], M.V. Salvati[1], C. Rodigari[1], A. Mirazimi[2,3], M.E. Maffei[4], G. Gribaudo[4] and C. Salata[1]

[1] Department of Molecular Medicine, University of Padova, 35121 Padova, Italy - [2] Department of Laboratory Medicine, Karolinska Institutet, 17177 Stockholm, Sweden - [3] National Veterinary Institute, 75189 Uppsala, Sweden - [4] Department of Life Sciences and Systems Biology, University of Turin, 10123 Turin, Italy

Hazara virus (HAZV) belongs to the *Nairoviridae* family and is included in the same serogroup of the Crimean-Congo haemorrhagic fever virus (CCHFV). CCHFV is the most widespread tick-borne arbovirus responsible for a serious haemorrhagic disease for which specific and effective treatment or preventive system are missing. Bioactive compounds derived from several natural products may provide a natural source of broad-spectrum antiviral agents and, in addition, provided by a good tolerability and minimal side effects. Previous in vitro studies showed that Oximacro®, a cranberry (*V. macrocarpon* Ait.) extract with a high polyphenol content, inhibits the replication of herpes simplex and influenza viruses by hampering their attachment to target cells. Given the broad-spectrum antimicrobial activity of polyphenols and the urgency to develop therapies for the treatment of CCHF, we investigated the antiviral activity of Oximacro® against the Hazara virus (HAZV), a surrogate nairovirus model of CCHFV that can be handled in Level 2 Biosecurity Laboratories (BSL-2). The results of time-of-addition experiments indicated that Oximacro® exerts an antiviral activity against HAZV by targeting early stages of the viral replication cycle, such as the initial adsorption to target cells. Although the details of the molecular mechanism of action remain to be clarified, Oximacro® exerts a virucidal effect through a direct interaction with HAZV particles that leads to the subsequent impairment of virus attachment to cell-surface receptors. As a whole, the evidence obtained suggest Oximacro® as a valuable candidate to be considered for the development of therapeutic strategies for CCHF infections.

Keywords: Hazara virus; Crimean-Congo haemorrhagic fever virus; Vaccinum macrocarpon ex-tract; Oximacro®; A-type proanthocyanidins; virucidal and antiviral activities.

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Evaluation of antiviral effect of silver nanoparticles from sour cherry leave extract

P. More[1], S. Shinde[1], A. Mali[1], A. Ambrosino[1], A. Santangelo[1], A. Zaino[1], C.A. Lanza[1], F. Foglia[1], S.A. Maiella[1], G. Franci[2], M. Galdiero[1]

[1] Department of Experimental Medicine, section of Virology and Microbiology, University of Campania "Luigi Vanvitelli", Naples, Italy - [2] Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana" University of Salerno, Baronissi (SA), Italy

Aim of study. Last decade a variety of viruses, such as influenza, hepatitis, herpes simplex virus (HSV), and human immunodeficiency virus (HIV) are becoming life threatening and there is no cure for this viral infection, we can reduced the effect, but complete cure in not available. For the reason alternative treatment for those viral infection is necessary. Silver nanoparticles are promising alternative for antiviral systems as they bring a significant improvement in antiviral activity through effective adsorption of them on the viral surfaces. In this study we are focusing on formation of green synthesis silver nanoparticles with the help of sour cherry leaves and will evaluate antiviral activity against HSV-1 and Canine distemper virus (CDV).

Methods used. Finely powdered cherry leaves were boiled in distilled water at 80 °C for about 20 min. The supernatant was filtered using Whatman filter paper No.1 to remove the particulate matter. To the extracted solution of sour cherry leaves, $AgNO_3$ was added and a brown colour change indicated the formation of silver nanoparticles. Characterization of silver nanoparticles were carried out by different methods like UV-VIS Spectroscopic Analysis, Dynamic light scattering (DLS), Zeta potential, Fourier-transform infrared spectroscopy (FTIR), and transmission electron microscope (TEM).

Results and conclusions. Absorption spectra of aqueous precursors and synthesized AgNPs were recorded with UV-VIS spectrometer. The chemical compositions of plant extract, and the synthesized silver nanoparticles were studied using FTIR. Sizes of nanoparticles were measured with the help of DLS and Zeta potential and it gives idea about potential stability of colloidal system. To detect size and shape of nanoparticles, TEM was used. To check antiviral activity of AgNps against HSV-1 and CDV pre- and co-treatment assays were performed and cell viability was determined by MTT assay. AgNPs were active in inhibiting viral infection both in pre- and co-treatment assays against two different viruses, i.e. HSV-1 and CDV. We did not observe a decrease in cell viability in MTT assay. We can conclude that the green synthesis of silver nanoparticles from sour cherry leaves was capable of controlling viruses infectivity most likely by blocking interaction of the virus with the cell, which might be dependent on the size and zeta potential of the AgNPs.

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SmCo nanoparticles produced for microbiological applications

M.V. Morone[1], G. Corvino[1], E. Serretiello[1], S. Shinde[1], R. Giugliano[1], F. Foglia[1], G. Greco[1], M. Galdiero[1], A. Morone[2]

[1] Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", 80138 Naples, Italy - [2] National Research Council - Institute of Structure of Matter U.O. di Tito Scalo, Industrial Zone of Tito Scalo, Italy

Aim of the study. Samarium-cobalt (SmCo) is a permanent magnetic material having peculiar characteristics. Magnetic SmCo nanoparticles (SmCo Nps) can be used in different application fields such as sensors and biosensors for environmental and biological measurements and medical application, but little is known about their antimicrobial properties (1).
Methods used. SmCo Nps were produced using liquid phase Pulsed laser ablation technique (PLD) (2) and they were resuspended in Phosphate Buffer Saline (PBS). They were analyzed for their potential microbiological applications to cause the death of bacteria and viruses (3, 4). First of all, we evaluated SmCo Nps toxicity through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on Cellosaurus cell line Vero 76 after 24 hours.
Results and conclusions. Nanoparticles resulted non toxic at any concentration tested. Our preliminary data are very encouraging: SmCo Nps in fact were active on Gram-positive bacteria to the highest concentrations, but they did not show any activity on Gram-negative bacteria, most likely due to the differences that they have in the structural composition. Furthermore, we also evaluated nanoparticles' activity against Herpes Simples Virus Types 1 (HSV-1). SmCo Nps antiviral activity was assessed in co-exposure with the virus, inhibiting the viral replication during the early phases of infection at the highest concentrations. At the final, these preliminary data appear very interesting, emphasizing the innovative properties and applications of nanoparticles to act as antibacterial and antiviral agents. Our future aim is to extend the analysis towards microorganisms resistant to the common therapies, as well as verify SmCo Nps's activity against the pandemic virus responsible of Coronavirus Disease 2019 (COVID-19).

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P160

A new therapeutic approach in HSV-1 infection: synergic effect of YY1 and HDACs

B.M. Nastri, C. Zannella, M.V. Morone, G. Greco, C. Del Vecchio, C. Russo, C. De Biasio, M. Galdiero

Department of Experimental Medicine, section Virology and Microbiology, University of Campania "Luigi Vanvitelli", Naples, Italy

Aim of the study. Yin Yang 1 (YY1) is a ubiquitously distributed mediator implicated in several processes such as cell growth, differentiation, tumor development and apoptosis. The origin of its name derives from its capability to activate or inhibit gene expression depending on the cofactors that it enrolls. In fact, YY1 is able to recognizes a consensus sequence in promoter gene region characterized by not less than one core binding elements as CCAT and ACAT. In addition, it is involved in the regulation of some epigenetic modifications as histone acetylation, deacetylation and methylation thanks to the interaction with some factors as p300, HDACs, Ezh2, Ezh1 and PRMT1. YY1 represents a regulation factor in HSV-1 gene expression through the transactivation of different viral β and γ genes. HSV-1 is a dsDNA virus belonging to Herpesviridae family. This virus behaves as a common human pathogen that can establish productive lytic infection in epithelial cells and latent infection in the nervous system.
Methods. Vero cells (ATCC CCL-81) were pre-treated with YY1 inhibitor, called NPI-0052 (Sigma SML1916), combined with several HDAC and Ezh2inhibitors (as valproic acid, trichostatin A, entinostat, vorinostat and GSK126). Then, HSV-1, carrying an EGFP gene fused to the HSV-1 tegument protein VP22, was used to infect the cell monolayer. Via plaque assay and fluorescence microscopy it has been possible to monitor the course of infection and via Annexin V assay it was evaluated the putative apoptosis effect. A Real-Time PCR, after a Chromatin immunoprecipitation (ChIP), was carried out using β and γ primers.
Results and conclusions. The obtained data highlight how HSV-1 infection is regulated synergistically by YY1 inhibitor together with HDAC and Ezh2 inhibitors. Definitely, there is a reduction in HSV-1 infection after the treatment of NPI-0052 in combination with several epigenetic drugs and no apoptosis was detected after the treatment. This encourage the idea that the reduction in HSV-1 infection was due only to the antiviral activity of the different combinations. ChIP analysis helps to sustain the results highlighting that NPI-0052, together with the epigenetic drugs, prevent the recognition and the modulation of HSV-1 genome.
During the past two decades antivirals have been used for the treatment of herpesvirus infections, especially acyclovir, penciclovir, and their prodrugs. However, recently, antiviral resistance has rapidly emerged making necessary the research of new antiviral drugs. The synergistic action between YY1 inhibitor NPI-0052 and several epigenetic drugs potentially represent a new and efficient therapeutic approach in the prevention and healing from HSV infection.

P161 - OC 48

Molecular dynamics simulations to investigate the antiviral effect of heparin in SARS-CoV-2 spike infection

G. Paiardi[1,3], S. Richter[1], M. Rusnati[3], R.C. Wade[1,2]

[1] Molecular and Cellular Modeling Group, Heidelberg Institute for Theoretical Studies (HITS), Heidelberg, Germany - [2] Zentrum für Molekulare Biologie (ZMBH), DKFZ-ZMBH Alliance and Interdisciplinary Center for Scientific Computing (IWR), Heidelberg University, Germany - [3] Experimental Oncology and Immunology, Department of Molecular and Translational Medicine, Brescia, Italy

Heparin is a linear anionic polysaccharide chain administered intravenously to COVID-19 patients [1] and via aerosol for the treatment of other lung diseases [2]. Experimental data indicate that heparin acts as an antiviral agent against SARS-CoV-2 by binding the viral spike glycoprotein, which is involved in both attachment and fusion to the human host cells, through the interaction with the host cell angiotensin-converting enzyme 2 (ACE2) receptor and heparan sulfate proteoglycans, co-receptors indispensable for SARS-CoV-2 infection that may influence the host susceptibility [2,3].
The aim of this study is to investigate the antiviral effect of heparin using molecular dynamics (MD) simulations as a "computational microscope" that allows a real-time visualization at an atomistic level of the interaction mechanism.
For this purpose, we modelled the prefusion configuration of the homotrimeric spike glycoprotein
in both active (open) and inactive (closed) states in the presence of zero, one or three linear polyanionic chains of heparin, performing several replica microsecond simulations of each system. Our models reveal long basic groove patches on the spike head that can accommodate linear anionic polysaccharide chains. Some N-glycans of the spike are also involved in these interactions. We identify direct and allosteric mechanisms by which heparin and HSPGs can affect the spike- host cell interaction. Our results provide a basis for understanding the role of HSPGs in SARS- CoV-2 pathogenesis and for the rational optimization of heparin derivatives for new antiviral therapies.

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P162

In-vitro evaluation of the immunomodulatory effects of baricitinib: implication for COVID-19 therapy

L. Petrone[a], E. Petruccioli[a], T. Alonzi[a], V. Vanini[a], G. Cuzzi[a], S. Najafi Fard[a], C. Castilletti[b], F. Palmieri[c], G. Gualano[c], P. Vittozzi[c], E. Nicastri[d], L. Lepore[d], A. Grifoni[e], A. Antinori[f], A. Vergori[f], G. Ippolito[g], F. Cantini[h], and D. Goletti[a]

[a] Translational Research Unit, National Institute for Infectious Diseases Lazzaro Spallanzani-IRCCS, Rome, Italy - [b] Laboratory of Virology, National Institute for Infectious Diseases Lazzaro Spallanzani-IRCCS, Rome, Italy - [c] Respiratory Infectious Diseases Unit, National Institute for Infectious Diseases Lazzaro Spallanzani-IRCCS, Rome, Italy - [d] Clinical Division of Infectious Diseases, National Institute for Infectious Diseases Lazzaro Spallanzani-IRCCS, Rome, Italy - [e] Center for Infectious Disease and Vaccine Research, La Jolla Institute for Immunology (LJI), La Jolla, CA 92037, USA - [f] HIV/AIDS Department, National Institute for Infectious Diseases Lazzaro Spallanzani-IRCCS, Rome, Italy - [g] Scientific Direction, National Institute for Infectious Disease "Lazzaro Spallanzani"-IRCCS, Rome, Italy - [h] Rheumatology department, Azienda USL Toscana Centro, Hospital of Prato, Italy

Aim of the study: Baricitinib seems a promising therapy for COVID-19. To fully-investigate its effects, we *in-vitro* evaluated the impact of baricitinib on the SARS-CoV-2-specific-response using the whole-blood platform.
Methods: We evaluated baricitinib effect on the IFN-γ-release and on a panel of soluble factors by multiplex-technology after stimulating whole-blood from 39 COVID-19 patients with SARS-CoV-2 antigens. Staphylococcal Enterotoxin B (SEB) antigen was used as a positive control.
Results: *In-vitro* exogenous addition of baricitinib significantly decreased IFN-γ response to spike- (median: 0.21, IQR: 0.01-1; spike+baricitinib 1000 nM median: 0.05, IQR: 0-0.18; $p<0.0001$) and to the remainder-antigens (median: 0.08 IQR: 0-0.55; remainder-antigens+baricitinib 1000 nM median: 0.03, IQR: 0-0.14; $p=0.0013$). Moreover, baricitinib significantly decreased SEB-induced response (median: 12.52, IQR: 9.7-15.2; SEB+baricitinib 1000 nM median: 8, IQR: 1.44-12.16; $p<0.0001$). Baricitinib did modulate other soluble factors besides IFN-γ, significantly decreasing the spike-specific-response mediated by IL-17, IL-1β, IL-6, TNF-α, IL-4, IL-13, IL-1ra, IL-10, GM-CSF, FGF, IP-10, MCP-1, MIP-1β ($p\leq0.0156$). The baricitinib-decreased SARS-CoV-2-specific-response was observed mainly in mild/moderate COVID-19 and in those with lymphocyte count $\geq 1x10^3$/μl.
Conclusions: Exogenous addition of baricitinib decreases the *in-vitro* SARS-CoV-2-specific response in COVID-19 patients using a whole-blood platform. These results are the first to show the effects of this therapy on the immune-specific viral response.

P163

Antiviral activity of silver nanoparticles from Campania Region red grape cultivars (aglianico, fiano and greco)

S. Shinde[1], A. De Filippis[1], P. More[1], A. Schettino[1], R. Manente[1], G. Giordano[1], M. Galdiero[1], G. Franci[2]

[1] Department of Experimental Medicine, Section of Virology and Microbiology, University of Campania "Luigi Vanvitelli", Naples, Italy - [2] Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Baronissi (SA), Italy

Aim of study. Herpes simplex virus 1 (HSV-1) is a neurotropic virus that after primary infection of epithelial cells becomes latent in neurons of the peripheral nervous system and can be periodically reactivated resulting in recurrent clinical or subclinical episodes throughout life. Canine distemper virus (CDV) is considered as a highly contagious and an acutely febrile disease in dogs. In the prevention of epidemic and pandemic emerging and neglected viral infections, alternative antiviral agents are need. In the last 20 years, nanotechonology have emerged as potential alternative antimicrobial agents and for drug delivery, both *in vitro* that *in vivo* models.

The interaction between silver nanoparticles and viruses is attracting great interest due to the potential antiviral activity of these particles. In this work, we demonstrate that stable, non-cytotoxic silver nanoparticles synthesized from bark extracts of Aglianico, Fiano, Greco (vine verities of Campania region). We show that production of silver nanoparticles from different vine verities is feasible and capable of reducing viral infectivity of Herpes Simplex Virus (HSV) and Canine Distemper Virus (CDV), probably by blocking interaction of the virus with the cell.

Methods used. The bark extracts of Aglianico, Fiano, Greco were challenged with 1mM silver nitrate solution and colour changes from colourless to brown is due to the formation of silver nanoparticles and the excitation of surface plasmons. Different techniques has been used to characterize silver nanoparticle i.e. UV–VIS Spectroscopic Analysis, dynamic light scattering (DLS), Zeta potential Analysis, Fourier-transform infrared spectroscopy (FTIR) and transmission electron microscope (TEM). To evaluate cytotoxicity of nanoparticles MTT assay was performed based on the reduction of the yellowish MTT to the insoluble and dark blue formazan by viable and metabolically active cells. Further, to test whether AgNPs can affect HSV and CDV infectivity *in vitro*, we used a co-treatment assay in which the different AgNPs of interest and viruses were concomitantly added to the cell culture in order to have both of them present during and after viral adsorption. For pretreatment, virus was pretreated with the different AgNPs for 1 hour before infection. The extent of HSV and CDV replication was assessed by plaque titration after addition of carboxymethyl cellulose.

Results and conclusions. From the present study it was observed that AgNPs were capable of controlling viral infectivity, which might be dependent on characteristics of the AgNPs. It was observed that AgNPs produced by Aglianico, Fiano, Greco have a size of 57nm, 61 nm and 58 nm, respectively. The FTIR analysis showed presence of protein capping agent when AgNPs scanned 400-4000 cm^{-1}. Nanoparticles have considerable antiviral activity against HSV and CDV and were less cytotoxic to Vero cells. The synthesized silver nanoparticles may have important advantage over conventional antibiotics to which the viruses got resistance.

P164

Effectiveness of polyphenols in improving virucidal properties of surgical masks

M. Verani[1], I. Federigi[1], E. Bramanti[2], B. Campanella[2], F. Cicogna[2], S. Coiai[2], E. Passaglia[2], B. Casini[3], B. Tuvo[3], A. Carducci[1]

[1] Laboratory of Hygiene and Environmental Virology, Department of Biology, University of Pisa, Via S. Zeno 35/39, 56127, Pisa, Italy - [2] Institute of Chemistry of Organometallic Compounds, National Research Council, via Giuseppe Moruzzi 1, Pisa, 56124, Italy - [3] Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Via San Zeno 35/39, 56127 Pisa, Italy

Aims of the study. The use of face masks has been suggested as important mitigation strategies against respiratory virus spread, like influenza viruses and recently SARS CoV2. However, the real effectiveness of these devices is still in discussion, underlining the necessity to improve filtering ability with the use of alternative materials for their production. Regarding this point, the antiviral properties of polyphenolic compounds suggest that their application on the external surface of disposable masks could increase viral inactivating properties, reduce cross-contamination of operator's hands and extend their lifetime. The aim of the study was the evaluation of virucidal activity of surgical masks containing polyphenol molecules extracted from olive leaves and dry clove buds towards two respiratory transmitted viruses: Human Adenovirus 5 (HAdV5) and Human Coronavirus 229E (HCoV229E).

Methods. Tests were performed according to UNI EN ISO 18184:2019, briefly, external surface of sterilized surgical masks coupons were soaked with polyphenol extracts and, after drying, known concentration of viruses was distributed on these. The coupons were then eluted and viral titers were estimated by coltural method. Each combination of extracts and viruses were tested in triplicate and negative controls were added.

Results and conclusion. The obtained data confirmed a virucidal effect of the soaked masks comparing to control due to polyphenols. In particular, the tests on HAdV5 revealed a similar abatement percentage for olive leaves and dry clove buds, of 91.5% and 95.6%, corresponding to 1.07 and 1.36 Log reduction, respectively. While HCoV229E resulted more susceptible to olive leaves extracts action, with an abatement of 89.1% and 0.96 log reduction, than dry clove buds (32.2% - 0.16). Comparing the two viruses, our experimental data underlined a more resistance of HCoV229E than HAdV5. The work confirmed the possible role of natural compounds as antiviral molecules to use for improving the barrier effect of masks, further experiments can better clarify the sensibility of different type of respiratory viruses to polyphenol activity.

P165 - OC 41
Antiviral therapeutics from amphibian skin peptides

C. Zannella[1], G. Franci[2], P. Grieco[3], A. Mali[1], MT. Della Rocca[1], M. Galdiero[1]

[1] Department of Experimental Medicine, section of Virology and Microbiology, University of Campania "Luigi Vanvitelli", Naples, Italy - [2] Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, 84081 Baronissi, Italy - [3] Department of Pharmacy, University of Naples "Federico II", Italy

Aim of the study. In the era of the new COVID-19 pandemic that has locked the world for its serious economic, social and deadly implications, it is mandatory the search for new molecules and therapies able to fight infectious viral diseases. Antimicrobial peptides (AMPs) constitute an emerging class of therapeutic agents in a number of different fields: they are used as antibacterial, antiviral, antifungal, antiparasitic, antioxidant and antitumor peptides [1,2]. They are small peptides, of no more than 100 amino acids, with a large presence of positively charged and hydrophobic residues. In particular, amphibian skin secretions represent a rich arsenal of peptides capable of defending against different microorganisms. Starting from megainin, discovered in 1987, hundreds of AMPs have been found by amphibians [3,4]. Temporins, isolated from *Rana temporaria*, belong to a family of short (8–17 amino acids), hydrophobic, C-terminally α-amidated peptides with well known antibacterial and antifungal properties. They adopt an α-helical conformation in hydrophobic environments and have the ability to perturb the integrity of target cell membranes.
Methods used. Peptides have been synthesized using solid-phase Fmoc chemistry method, followed by purification by reversed phase HPLC. The cytotoxic activity was determined via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The antiviral activity was evaluated against different viruses, including the Herpes Simplex Virus type 1 (HSV-1) and Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) through both plaque assays, molecular test and Transmission electron microscopy (TEM) analysis.
Results and conlusions. When peptides were preincubated with viruses, significant antiviral activity was observed demonstrating that they were able to disrupt the viral envelope, as observed also by TEM. Temporins influenced the extracellular phases of viral lifecycle, probably by blocking the viral attachment and entry steps. Our results show novel possible applications of amphibian skin peptides in the field of antivirals. Further studies will be focused on their specific mechanism of action in order to understand the viral target on which the peptides act.

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P166 - OC 43

In vitro investigation of the mechanism of action of two broadly neutralizing human monoclonal antibodies against rabies virus

M. Zorzan*, M. Castellan*, B. Zecchin*, A. Minola#, F. Benigni#, D. Corti#§, P. De Benedictis*

* Istituto Zooprofilattico Sperimentale delle Venezie, Padua, Italy - # Humabs BioMed SA, a subsidiary of Vir Biotechnology, Bellinzona, Switzerland - § Vir Biotechnology, San Francisco, CA, USA

Aim of the study. Rabies virus (RABV), family Rhabdoviridae, genus Lyssavirus, is the etiological agent of rabies and is found ubiquitously worldwide in different animal reservoirs. Despite being almost 100% fatal following the onset of symptoms in non-vaccinated individuals, the disease can be prevented by mass vaccination of animal reservoirs and proper pre- or post-exposure prophylaxis in humans. However, currently available antibodies do not provide full protection against all non-RABV lyssavirus strains. In the present study, the mechanism of action of two potent and broadly neutralizing human monoclonal antibodies (mAbs, RVC58 and RVC20 [1]), was i*n vitro* investigated.
Methods. Human neuroblastoma SH-SY5Y cells and Challenge Virus Standard (CVS-11) rabies virus strain were used as *in vitro* model of rabies infection. AlamarBlue® HS cell viability assays were performed to evaluate cell viability/metabolism. Fiji-ImageJ software was used for analysis of immunofluorescence images acquired by confocal microscope. Commercial kits (Promega) were used for evaluation of effector functions activated by mAbs.
Results and conclusions. Interactions between infected cells and rabies virus were investigated from different points of view. First of all, cell cytotoxicity assays indicated that on day 4 post-infection and following 72 hour mAbs treatment, both untreated uninfected and mAbs treated infected SH-SY5Y cells had a significantly increased cell proliferation compared to untreated infected cells, suggesting that mAbs treatment allows cells to maintain a higher level of metabolic activity. Secondly, experiments based on pre-incubation of rabies virus with mAbs prior to cell infection indicated that RVC58 and RVC20 can neutralize extracellular virus and/or inhibit a very early phase of viral lifecycle preceding the formation of inclusion bodies (i.e. virus endocytosis, fusion of the viral envelope with endosomal membranes). Thirdly, mAbs treatment of previously infected cells significantly inhibited virus spreading in cell culture, suggesting a potential inhibitory effect of RVC58 and RVC20 also on late phases of viral lifecycle (i.e. virus assembly, budding). In this regard, immunofluorescence analysis indicated that both mAbs co-localized with a plasma membrane marker in CVS-11 infected cells, thus binding viral Glycoprotein (G) spanning the cell membrane, with no evidence of cell internalization. In 3D view rendering analysis of confocal images viral Ribonucleoprotein typically appeared as a compact complex below or embedded in the cell membrane of infected cells, in line with the expected "pull" effect of G from the outside of the plasma membrane based on current model of rhabdovirus assembly and budding [2]. Conversely, some Ribonucleoprotein complexes in RVC20 treated infected cells apparently lost their compact features to assume a basket-like conformation, clearly in contrast with the aforementioned model. As RVC20 is demonstrated to prevent fusion of viral envelope with endosomal membranes by locking G prefusion state [3], a similar

mechanism can be hypothesized for RVC20 inhibition of viral budding based on the known involvement of G membrane ectodomain, containing RVC20 target site, in both processes of membrane fusion and nipple evagination during viral budding. Besides virus neutralization, activation of effector functions by RVC58 and RVC20 through their Fc portion was also investigated to gain more insight into viral clearance mechanisms. Both mAbs, but not their Fab forms (lacking Fc portion), specifically induced complement-dependent cytotoxicity in a dose-dependent manner in a cell-based assay. In addition, RVC20 efficiently activated *in vitro* antibody-dependent cell cytotoxicity (ADCC) as well as antibody-dependent cell phagocytosis (ADCP), whereas RVC58 induced only mild ADCC and no ADCP. In conclusion, this study indicates that RVC58 and RVC20 can act through multiple mechanisms of action, including inhibition of several phases of RABV lifecycle and activation of different effector functions, further strengthening their potential value for an efficient rabies post-exposure prophylaxis.

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P167 - OC 35

Identification of different cress DNA viruses in lizards and geckos

P. Capozza[1], G. Lanave[1], G. Diakoudi[1], F. Pellegrini[1], R. Cardone[1], V. I. Vasinioti[1], N. Decaro[1], A. Alberti[2], J. A. Mendoza-Roldan[1], K. Bányai[3], D. Otranto[1], C. Buonavoglia[1], V. Martella[1]

[1] Department of Veterinary Medicine, University of Bari. - [2] Department of Veterinary Medicine, University of Sassari - [3] Institute for Veterinary Medical Research, Centre for Agricultural Research, Budapest, Hungary

Circovirus (family Circoviridae, CVs) are non-enveloped, spherical viruses. The viruses have small, covalently closed, circular, single-stranded DNA (ssDNA) genomes and represent the smallest known autonomously replicating, capsid-encoding animal pathogens (ICTV-https://talk.ictvonline.org/ictv-reports/ictv_online_report/ssdna-viruses/w/circoviridae/659/genus-circovirus). CVs cause fatal diseases in birds and pigs, and they have been identified in the stools, blood and cerebrospinal fluid (CSF) of humans, although the pathogenic role, if any, is unclear (1, 2).

Squamata reptiles, in particular geckos (i.e., *Tarentola* spp., *Hemidactylus* spp.) and lacertid lizards (i.e., *Podarcis* spp.), have become synanthropic in urban and peri-urban areas of the Mediterranean basin (3).

In order to better understand the ecology and potential zoonotic role of CVs and CV-like viruses, in this study we extended the research of CVs in biological samples of squamates. Faecal samples from lizards and geckos, collected in different Italian regions (Apulia, Basilicata, Calabria and Sicily), were screened using a degenerate PCR protocol and direct sequencing. Genome sequences were generated after enrichment of circular DNA with rolling cycle amplification (RCA) and inverse PCR.

We identified CRESS DNA viruses in about 31.7% (33/104) samples, including avian-like (N=3), dog (n=4), bat-like (n=1), rodent-like (n=4) and insect-like (n=2) viruses, all which likely reflected dietary or environmental contamination.

Rep sequences of at least two types of human-like cycloviruses (CyCV) were identified consistently, regardless of geographic location, namely TN9-like (n=10) and TN12-like (n=6).

A third human-like CyCV, TN25-like, was detected in a unique sample. A potential recombinant CyCV (TN9xTN25) strain was also recognized.

The complete genome of TN9-, TN12- and TN25-like CyCVs, of a rodent-like CV, of an insect-like CyCV and of a bat-like CyCV were generated.

CRESS DNA viruses were not detected in sera of volunteers (n=100) living in an island where sampling in squamates revealed human-like CyCV sequences.

Whether the CRESS DNA viruses identified in squamates are only contaminant viruses of the intestinal content or they can replicate, to some extent. in these animals remains unclear. Also, since these animals are synanthropic, and since CyCVs have been identified repeatedly in humans with and without clinical signs, squamates could play a role in sustaining CyCV circulation and increasing CyCV pressure in the environment.

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P168 - OC 34

Unrevealed genetic diversity of GII norovirus in the swine population of North East Italy

L. Cavicchio[1], L. Tassoni[1], A. Laconi[2 3], G. Cunial[4], L. Gagliazzo[4], A. Milani[2], M. Campalto[1], G. Di Martino[4], M. Forzan[5], I. Monne[2], M. S. Beato[6]

[1] Diagnostic Virology Laboratory, Department of Animal Health, Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Viale dell'Università 10, 35020, Legnaro, Padua, Italy - [2] EU, OIE/FAO and National Reference Laboratory for Avian Influenza and Newcastle Disease, Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Viale dell'Università 10, 35020, Legnaro, Padua, Italy - [3] Epidemiology Department, Istituto Zooprofilattico Sperimentale Delle Venezie (IZSVe), Viale dell'Università 10, 35020, Legnaro, Padua, Italy - [4] Department of Veterinary Virology, University of Pisa, Viale delle Piagge 2, 56124, Pisa, Italy - [5] Department of Comparative Biomedicine and Food Science, University of Padua, Legnaro, Padua, Italy

Noroviruses (NoVs) are one of the major causative agents of non-bacterial gastroenteritis in humans worldwide. NoVs belong to the *Caliciviridae* family, which includes small non-enveloped viruses with single stranded positive RNA genome. NoVs are classified into ten genogroups (G) based on the variation of the major capsid protein (VP1), and are further divided into 49 genotypes. Due to the recombination events that occur between ORF1 and ORF2, a dual nomenclature based on VP1 and RNA-dependent-RNA-polymerase (RdRp) was recently proposed. Based on the RdRp sequence, NoVs are classified into eight P (polymerase)-groups and further divided into 60 P-types. In swine, the main genogroup and P-group identified are GII and GII.P; which can infect humans. To date, only one case of GIIP.11 have been identified in swine in Italy while the circulation of other P-types is currently unknown. Human NoV infections are caused by GI, GII, and GIV genotypes, with a higher frequency of the GII.4. Animal enteric caliciviruses genetically related to human NoVs have been detected in pigs and cows. Since the first report of GII NoVs in pigs in the USA, other countries in Europe and Latin America have reported the presence of this genogroup in diseased and healthy pigs. The detection of NoVs in swine fecal samples, retail and imported raw meat samples, has raised public health concerns about the zoonotic potential of porcine NoVs and the role of swine in the epidemiology of this infection. However, the contribution of swine NoVs to human infections remains unclear and to be elucidated. In the present study we investigated the presence and distribution of NoVs in healthy pigs of the Veneto region. Between January 2018-2019, 225 swine faecal samples were collected from 74 swine through on-farm monitoring. Faeces homogenate supernatant was used for viral RNA extraction. The eluted RNA was used for a one-step RT-PCR with p290-p110 primer pair, targeting a 300 bp fragment of the RdRp gene, conserved in the Caliciviridae family. A primer walking approach was used to amplify and Sanger sequence a larger fragment of the RdRp and VP1 gene of the NoV positive samples. The evolutionary relationships of the identified NoV were reconstructed by phylogenetic analysis using nt sequences. Presence of unique aa mutations of the RdRp sequences was investigated and the nt sequence entropy was calculated to evaluate the genetic variability between the GII.11 P NoVs identified.
NoV circulation was particularly high in older pigs. The phylogenetic analysis showed the co-circulation of NoVs belonging to two different P-types: GII.P11 and GII.P18, here de-

scribed for the first time in Italy, presenting an extensive genetic diversity, never described before worldwide. Distinct NoV genetic subgroups and unique amino acid mutations were identified for each P-type for the first time. This study demonstrated the co-circulation of diverse swine NoVs subgroups in Italy, raising questions on the origin of such diversity and suggesting that continuous monitoring of swine NoVs is needed to track the emergence of potentially zoonotic viruses by recombination events.

P169
Unexpected genetic diversity of two novel swine MRVS in Italy

L. Cavicchio[1], L. Tassoni[1], G. Zamperin[2], M. Campalto[1], M. Carrino[1], F. Tonon[3], S. Leopardi[2], P. De Benedictis[2], M. S. Beato[1]

[1] Diagnostic Virology Laboratory, Department of Animal Health, Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Viale dell'Università 10, Legnaro, 35020 Padua, Italy - [2] OIE Collaborating Centre for Diseases at the Animal/Human Interface, Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Viale dell'Università 10, Legnaro, 35020 Padua, Italy - [3] Suivet, Via Ernesto Che Guevara 55, 42123 Reggio Emilia, Italy

Mammalian Orthoreovirus (MRV) are segmented dsRNA viruses in the family Reoviridae. MRVs are divided into at least three serotypes: MRV1, MRV2 and MRV3. Reassortant strains are frequently detected because of their segmented genome. They are capable of infecting all types of mammals; in particular, they have been detected in pigs, bats and humans, being associated with both asymptomatic and symptomatic infections. However, in recent years, MRVs have often been described as being the only pathogen in human hosts causing severe enteritis, acute respiratory infections and encephalitis. Recently, reports on human MRVs have increased worldwide, describing human MRVs as reassortant strains involving strains detected in the animal reservoirs. In Europe, swine MRV (swMRV) was first isolated in Austria in 1998 and subsequently reported more than fifteen years later in Italy. The role of MRV in diarrheal manifestation in pigs remains uncertain and seems to be in relation to the age of infected animals; however, evidence suggests that MRV may also contribute to the severity of gastrointestinal manifestation. In the present study, we characterized two novel reassortant swine MRV identified in one same Italian farm over two years.

In February 2016, six fecal samples were collected from fattening pigs during an acute episode of gastroenteritis reported by a field veterinarian in a farm located in the Treviso province. The IZSVe researchers visited the same farm two years later, where eight faeces samples were collected from healthy pigs. Faeces homogenate were processed for viral RNA extraction. The eluted RNA was used for MRV detection by a one-step RT-PCR targeting a L1 conserved region. For MRV characterization, one-step RT-PCRs targeting the S1 gene of serotype 2 (S1.2) and 3 (S1.3) were applied. Virus isolation was attempted for all samples collected in 2016 and 2018, using African green monkey kidney (Vero). Negative staining Electron Microscopy (EM), RT-PCR targeting the L1 of the MRVs and typing RT-PCRs targeting the S1.2 and S1.3 genes were carried out on cell cultures presenting cytopatic effect (CPE). Next generation sequencing (NGS) was carried out on two viral isolates, one from 2016 and one from 2018 and, since genome termini of some swine MRV segments was poorly sequenced in NGS, they were Sanger sequenced with a primer walking approach. The evolutionary relationships between the two identified swine MRVs were reconstructed by phylogenetic analysis using nt sequences of the ten gene segments. The homology modelling of the S1 protein of the identified swine MRVs was carried out to identify the position of aa mutations on a three-dimensional structure of the S1 protein. The two viruses shared the same genetic backbone but showed evidence of reassortment in the S1, S4, M2 segments and were therefore classified into two serotypes: MRV3 detected in 2016 and MRV2 detected in 2018. A genetic relation of both MRVs to pig, bat

and human MRVs and other unknown sources was identified. A considerable genetic diversity was observed in the Italian MRV3 and MRV2 compared to other available swine MRVs. The S1 protein presented unique amino acid signatures in both swine MRVs, with unexpected frequencies for MRV2. The remaining genes formed distinct and novel genetic groups that revealed a geographically related evolution of swine MRVs in Italy. This is the first report of the complete molecular characterization of novel reassortant swine MRVs in Italy and Europe, which suggests a greater genetic diversity of swine MRVs never identified before. Further monitoring in swine, bats and humans are necessary to identify the zoonotic potential of MRVs.

P170

Tick-borne-encephalitis virus: seroprevalence in blood donors and in sentinel animals in the autonomous province of Bolzano

P. Ceschi[1], S. Spertini[2], C. Holzner[2], S. Gamper[2], E. Bernini[2], R. Vaccaro[2], G. Venturi[+], L. Busani[3], E. Suffredini[4], T. Vicenza[4], G. Capelli[5], M. Toson[5], M. Bregoli[6], C. Piffer[1], G. Morosetti[1]

[1] Veterinary Services, Local Health Unit of South Tyrol, Bolzano, Italy - [2] Hygiene and Public Health Services, Local Health Unit of South Tyrol, Bolzano, Italy - [3] Istituto Superiore di Sanità, Department of Infectious Diseases, Rome, Italy - [4] Istituto Superiore di Sanità. Department of Food Safety, Nutrition and Veterinary public Health, Rome, Italy - [5] Italian health authority and research organization for animal health and food safety, Padua, Italy - [6] Italian health authority and research organization for animal health and food safety, Udine, Italy

Aim of the study. Tick-borne-encephalitis virus (TBEV) is a positive-sense single stranded RNA virus belonging to the Flavivirus genus, whose main transmission routes are tick bites (Ixodes species) and the consumption of nonpasteurized milk. TBE cases were first confirmed in the Autonomous Province of Bolzano in 2000 and an increasing number of cases has been reported during the last decade. TBE infection may also occur in domestic animals and livestock. In these cases, the disease is often asymptomatic and some species can be considered as indicators for risk of infection in humans. As comprehensive data on TBE circulation among both the human and animal populations in the Province of Bolzano were lacking, the aim of this study was to evaluate TBE seroprevalence in blood donors and in sentinel animals as dogs and horses. Furthermore, circulation of TBEV in dairy producing animals (goats) was investigated.
Methods. A total of 600 samples from blood donors, and respectively 475 samples from dogs and 178 samples from horses (representative of local animals populations) were collected in 2018 and tested for anti-TBEV antibodies. Moreover, 123 raw bulk goat milk samples, collected between spring and autumn 2018 from 23 farms, were also tested for anti-TBEV antibodies, as well as for presence of viral RNA. Detection of anti-TBEV antibodies was undertaken with commercially available kits; RNA detection was performed by screening with a previosly published real-time RT-qPCR, followed by confimation with RT-nested-PCR. Appropriate quality controls were included in the analysis for data validation.
Results. Overall, 1,1% of blood donors, 3,3% of dogs and 3,4% of horses tested positive for anti-TBEV. Antibodies were not detected instead in bulk goat milk samples and viral RNA presence could not be confirmed in samples testing positive by the screening assay.
Conclusions. This study indicates circulation of TBEV in the Autonomous Province of Bolzano, both in the human population and in sentinel animals as dogs and horses. Further studies are required to assess relative relevance of foodborne, occupational and leisure exposure in TBE transmission in the Autonomous Province of Bolzano .

P171
Evaluation of infectivity of hepatitis E virus in fermented dried salami

D. De Medici[1], M.N. Losio[2], S. Di Pasquale[1], E. Cosciani Cunico[2], L. Cozzi[1], P. Monastero[2], T. Vicenza[1], A. Di Sandro[3], R. Lena[3], E. Dalzini[2], E. Suffredini[1], E. Pavoni[4]

[1] Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, National Reference Laboratory for Foodborne Viruses, Rome, Italy - [2] National Reference Centre for Emerging Risks in Food Safety (CRESA), Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy - [3] Italian Ministry of Health, Directorate General for Food hygiene and safety and Nutrition, Rome, Italy - [4] Department of Food Safety, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy

Hepatitis E virus (HEV) is an increasingly recognized zoonotic pathogen, transmitted to humans through contact with infected animals or through consumption of contaminated food. The aim of this study was the evaluation of the retention of HEV infectivity in experimentally contaminated fermented salami.
A HEV viral stock (strain 47832c, titer ≈103 TCID50/mL and 4.1×107 genome copies/mL) was used to contaminate salami batter (minced pork meat, spices, salt, additives, etc.) prepared according to an industrial recipe. The HEV-spiked mixture was then used to prepare twelve salami; salami batter without HEV contamination was used to prepare an equivalent number of negative controls. During the ripening the salami were maintained in a climatic chamber for 38 days. Two HEV-spiked and two negative control samples were taken at different time (0, 5, 10, 20, 25 and 38 days) and samples were analyzed as follows: a) by a standardized RT-(q)PCR to quantify viral RNA; b) by a 'viability PCR assay' (EMA RT-(q)PCR) to estimate number of viral particles retaining capsid and structural integrity; c) by an integrated cell culture-PCR method (replication on cell monolayers confirmed by real-time RT-PCR) to assess infectious virus. Sample extractions were performed using: i) a Trizol-Chloroform treatment for the detection by real-time RT(q)PCR; ii) a proteinase K – chloroform – PEG digestion/concentration protocol, followed by ethidium bromide monoazide treatment (300 µM) and photoactivation for the detection by EMA RT-(q)PCR; iii) homogenization with cell culture medium, centrifugation and filtration for infectivity assay on A549/D3 cell line.
Results showed a limited decrease of the associated RT-(q)PCR signal for HEV during the curing process, from 4.8 log genome copies (g.c.)/g to values between 4.1 and 4.3 log g.c./g after 20 days of seasoning. The number of structurally intact virions (EMA RT-(q)PCR), on the other hand, decreased from 3.2 log particles/g at the beginning of the process to 1.7 log after 10 days of seasoning, with a slight increase in the subsequent days. Finally, HEV displayed a total loss of infectivity between the 20th and the 25th day of curing, with no growth detectable on cell lines (qualitative assay).
In conclusion, this study showed a decrease in both infectivity and integrity of HEV viral particles during the curing process. These data will be useful for modelling of HEV infectivity, as well as for risk assessment purposes.

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P172
Identification of a canine homolog of human hepatitis B virus

G. Diakoudi[1], P. Capozza[1], G. Lanave[1], F. Pellegrini[1], B. Di Martino[2], G. Elia[1], M. Camero[1], M. Tempesta, V.R. Barrs[3], K. Banyai[4], C. Catella[1], S. Lucente[1], C. Buonavoglia[1], V. Martella[1]

[1] Department of Veterinary Medicine, University of Bari, Valenzano (Bari), Italy - [2] Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy - [3] City University of Hong Kong, Kowloon Tong, Hong Kong - [4] Institute for Veterinary Medical Research, Centre for Agricultural Research, Budapest, Hungary

Hepadnaviruses (family *Hepadnaviridae*) are DNA viruses with a partially double-stranded circular genome that infect a variety of animal species. The prototype species, hepatitis B virus (HBV), is a major public health problem causing chronic liver infections in humans and increased risk of hepatocellular carcinoma (1). In 2018, a novel hepadnavirus, similar to HBV, was identified in a domestic cat (domestic cat hepadnavirus, DCH) in Australia (2). Since canine and feline viromes partially overlap, we hypothesized that DCH-like viruses might be also harbored in dogs.

We screened 619 sera from dogs collected between 2018 and 2019, using quantitative PCR (qPCR) designed on the basis of the sequence of DCH. The complete sequence of a canine hepadnavirus strain (domestic dog hepadnavirus, DDH) was generated, after enriching the DNA with rolling circle amplification. Sequence analysis was performed using the Geneious v. 10.2.4 software packages.

We detected DDH DNA in 40/619 (6.5 %) of the canine samples, although in most samples DDH DNA was at low titer (< 103 copies). In this subset, 12/40 (30.0%) of the animals were also positive for leishmania, 15/40 (37.5%) had hepatic impairment based on clinical signs and/or biochemical analysis. The complete genome of 3.2 kb in length of DDH was generated. Upon sequence analysis, the virus displayed 98% nucleotide identity to a DDH strain ITA/2018/165-83 (3), and 96.9% nucleotide identity to the Australian feline reference strain Sydney2016 (2), respectively.

The findings of this study confirm the presence of hepadnaviruses in dogs. The data reported a possible correlation between the presence of the virus and pathological conditions that might cause immunosuppression, a pattern similar to what has been already reported for cats and humans (2, 3). Further investigation to better understand the impact of DDH on canine health, as well as its correlation to immunosuppressive infectious diseases, is needed.

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P173
Pilot investigation of astroviruses in squamates

G. Diakoudi[1], P. Capozza[1], F. Pellegrini[1], V. I. Vasinioti[1], G. Lanave[1], C. Catella[1], M. Camero[1], A. Parisi[2], L. Capozzi[2], J. A. Mendoza-Roldan[1], D. Otranto[1], C. Buonavoglia[1], V. Martella[1]

[1] Department of Veterinary Medicine, University of Bari, Valenzano (Bari), Italy - [2] Istituto Zooprofilattico Sperimentale di Puglia e Basilicata, Sezione di Putignano, Bari, Italy

Astroviruses (family *Astroviridae*) are small, non-enveloped, positive sense, single-stranded RNA viruses that are associated with acute gastroenteritis in children worldwide. Since their first description in children with diarrhea, astroviruses have been identified in several animal species causing a wide variety of diseases like hepatitis in birds and encephalitis in human and animal hosts (1, 2). In this study, we investigated the circulation of astroviruses in synanthropic squamates, mainly geckos and lacertid lizards, found in urban and peri-urban areas in Italy.

We collected a total of 100 fecal samples from lizards and geckos found in different Italian regions (Apulia, Basilicata, Calabria and Sicily). Using a degenerate RT-PCR protocol and direct sequencing we identified astrovirus RNA in 11% (11/100) of the samples. The virus was also detected in the brain of a gecko (RI111). Using a 3'RACE RT-PCR protocol, we generated information for the complete capsid (ORF2) and partial RdRp genomic (ORF1b) regions for 6 of the 11 positive samples. Sequence and phylogenetic analysis was conducted using the Geneious v. 10.2.4 software packages.

On sequence analysis, the astrovirus strains identified in this study were up to 48.2% identical at the nucleotide level to other astroviruses identified in squamates (1). The Italian strains shared 41.8-97.7% nucleotide and 29.3-99.1% amino acid identity to each other in the ORF2 gene. Upon phylogenetic analysis of the ORF2 genomic region, the Italian strains formed three distinct clusters highlighting the variability of the circulating astroviruses. Interestingly, similar astrovirus strains were identified in different geographic locations, a pattern suggestive of species-specificity or dietary exposure, rather than a mere environmental exposure.

Astroviruses have long been considered to infect in a species-specific manner. However, lately they have been shown to emerge through cross-species transmission and recombination events (3), highlighting the potential threat of animal-to-animal or animal-to-human circulation. Since squamates are synanthropic and often part of the diet of feral and domestic cats, they might play a role as carriers, maintaining the circulation of some pathogens in the ecosystem.

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P174

Dioxin modifies the expression of nucleocapsid protein during canine coronavirus infection

C. Cerracchio[1], F.P. Nocera[1], M. Ambrosio[1], S. Lambiase[2], L. De Martino[1] and F. Fiorito[1]

[1] University of Naples Federico II, Department of Veterinary Medicine and Animal Production, Naples - [2] Istituto Zooprofilattico del Mezzogiorno, Portici, Naples

Aim of the study: Current evidence suggests that environmental contaminants may increase the health risk assessment of infectious diseases.
2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), known as dioxin, is a widespread environmental contaminant that can suppress humoral and cell-mediated immunity, making humans and animals more susceptible to infectious agents. In vivo and in vitro findings indicate that dioxin might interfere with the replication of both human and animal viruses, such as influenza A viruses, coxsackie virus B3, cytomegalovirus, herpes simplex II and bovine herpesvirus 1 (Fiorito et al., 2017). Moreover, it has been reported a remarkable correlation between the level of environmental pollutants with the rate of mortality in COVID-19 pandemic, due to severe acute respiratory syndrome-coronavirus-2 in some European countries, including Italy (Bornstein et al., 2020).
Canine coronavirus (CCoV) may cause self-limiting enteric infections, accompanied by high morbidity and low mortality in dogs. Recently, it has been described that emerging pantropic strains of CCoV could cause hypervirulent and multi-systemic fatal disease, in contrast to classical enteric coronavirus infections (Buonavoglia et al., 2006; Timurkan et al., 2021).
The nucleocapsid protein (NP) is a structural protein of CoVs responsible for various functions, including binding the viral RNA genome, packing viral genome RNA into ribonucleoproteins, and compressing it into a compact virion core. In addition, NP is generally more stable than CoV spike protein, which has a higher mutation rate (Tseng et al., 2021).
Methods used: To elucidate the effects of dioxin in enteric CCoV infection, herein, following infection for 48 h with the reference CCoV strain S/378 in in a canine fibrosarcoma cell line (A-72) cell line, in the presence of very low concentrations of TCDD (0.01, 1 and 100 pg/mL), *in vitro* bio-screen, immunofluorescence (IF) staining, cytomorphological and virus yield analyses, were performed.
Results and conclusions: During CCoV infection, cell viability was analyzed by using Trypan Blue exclusion test while cells were attached to wells. Our results confirmed that during infection CCoV decreased total cells number (Ruggieri et al., 2007; De Martino et al., 2010). In the presence of TCDD, a considerable reduction of cell viability was found during infection, whereas dioxin enhanced cell viability in uninfected cells. For virus production assay, viral cytopathic effect was assessed. We observed morphological changes, due to syncytia development, which appeared as clusters in infected cells (Ruggieri et al., 2007; De Martino et al., 2010). These features were markedly increased by TCDD, that induced the formation of super-clusters. IF assay indicated that NP was localized in the nucleus as

well as in the cytoplasm of infected cells. These cells showed nuclei weakly counterstained with DAPI. Dioxin noticeably induced NP expression in all compartments of infected cells, whose nuclei appeared strongly labeled with DAPI.

Taken together, our preliminary investigations suggest that dioxin exposure may interfere with regulation of CCoV infection. Indeed, TCDD intensified the expression of NP, detected in more viable super-clusters cells.

Future investigations are necessary to clarify the mechanism by which an environmental contaminant, as TCDD, is involved in CCoV infection.

P175 - OC 36

Isolation of HEV-3 strains from swine fecal samples on human A549 cell line

G. Ianiro[1], M. Monini[1], L. De Sabato[1], M.G. Ammendolia[1], F. Ostanello[2], I. Di Bartolo[1]

[1] Istituto Superiore di Sanità, Rome, Italy - [2] University of Bologna, Ozzano dell'Emilia, Bologna, Italy

Background: Hepatitis E virus is an emerging virus, recently recognized as zoonotic. The disease is considered emerging and, in humans, the infection can be asymptomatic or causes a disease generally self-limiting that can become chronic in immunocompromised. The zoonotic genotypes, named HEV-3 and HEV-4, infect pigs and wild boar that are the main reservoirs. A few data are available on virus replication and mechanism of infection, due to lack of an efficient system of cultivation. This study aimed to cultivate different strains of HEV-3 detected in Italian pigs and to develop a reproducible protocol of isolation of wild-type HEV strains on cell cultures.

Methods: fecal suspensions from HEV-positive pig samples were obtained in Tris-HCl 0,1 M (10% w/v) and used for the cell inoculum. Cell lines A549, ST100 and PK15 were growth in MEM (10% FBS + 2.5 g/mL amphotericin B) at 34.5 °C and 5% CO2 for 3 days before being infected.

Afterwards, cell monolayers were inoculated at MOI 0.1 (starting tire of inocula ≥10^5 genome copies GC/ml).The supernatant was refreshed with MEM every 3-4 days. The infected cell monolayers were split regularly, 1:2 at the same growth conditions. The HEV GCs of supernatant were quantified by quantitative real-time RT-PCR and viral particles were visualised by transmission electron microscopy (TEM).

Results: three wild-type HEV-3 strains derived from fecal samples collected from Italian pigs were isolated on A549 cell monolayers. No growth was observed with HEV-3 strain from positive wild boar liver. Neither ST100 nor PK15 cell lines were permissive for the growth of the same isolated strains. At 60 days post infection, the isolates, namely 2BN5_IT20, 3AC47_IT21 19M2_IT21, reached titres comprises between 10^5-10^6 HEV RNA GC/ml. Infected cells were successfully split as confirmed by virus production in the supernatant for at least 14days post-split. Electron microscopic observations of supernatants showed assembled structured viral particles with size ranging from 28 to 33 nm.

Conclusions: the protocol developed for human HEV virus propagation was also successfully used for swine HEV-3 cultivation from fecal samples. The protocol is troublesome but ensures reproducible cultivation of wild type viruses. Further studies will be performed to ameliorate the protocol and to produce the virus for future study focusing on the replication cycle, immune responses induced by the virus and its resistance to temperature and other treatments.

P176 - OC 32

Avian reovirus P17 suppresses angiogenesis by promoting DPP4 secretion

E. Manocha[1], A. Bugatti[1], M. Belleri[2], A. Zani[1], F. Caccuri[1], M. Presta[2], A. Caruso[1]*

[1] Section of Microbiology, Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy - [2] Section of Experimental Oncology and Immunology, Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy

Aim: Avian reovirus p17 (ARV p17) is a non-structural protein displaying no sequence similarity with other known proteins. It was shown to activate autophagy, interfere with gene transcription and induce a significant tumor cell growth inhibition *in vitro* and *in vivo*. In this study we show the potent antiangiogenic properties of ARV p17 in human endothelial cells.
Methods: The anti-migratory and anti-angiogenic activity of the viral protein was investigated by wound healing, 2-D and 3-D Matrigel, and spheroid assay. Aortic ring assay and CAM assay were performed to demonstrate the same *ex vivo* and *in vivo*, respectively.
Results: The viral protein was found to significantly inhibit human endothelial cell (EC) migration, capillary-like structure and new vessel formation. ARV p17 was not only able to suppress the EC physiological angiogenesis but also rendered ECs insensitive to two different potent proangiogenic inducers, such as VEGF-A and FGF-2. ARV p17 was found to exert its antiangiogenic activity by upregulating transcription and release of the well-known tumor suppressor molecule dipeptidyl peptidase 4 (DPP4).
Conclusion: The ability of ARV p17 to impact on angiogenesis is completely new and highlights the "two compartments" activity of the viral protein that is expected to hamper the tumor parenchymal/stromal cross-talk. The complex antitumor activities of ARV p17 open the way to a new promising field of research aimed to develop new therapeutic approaches for treating tumor and cancer metastasis.

Keywords: ARV p17; angiogenesis; DPP4; VEGF-A; FGF-2; antiangiogenic activity

P177 - OC 33

SARS-CoV-2 virus dynamic in a mink farm in Italy: lessons learned

A. Moreno[1], D. Lelli[1], T. Trogu[1], A. Lavazza[1], I. Barbieri[1], M.B. Boniotti[1], C. Salogni[1], S. Giovannini[1], G. Alborali[1], S. Bellini[1], M. Boldini[1], M. Farioli[2], L. Ruocco[3], O. Bessi[3], A. Maroni Ponti[3], G. Belli[4], A. Margutti[4], M. Giorgi[4]

[1] IZSLER - [2] Regione Lombardia - [3] Direzione Generale Sanità animale e Farmaci veterinari, Ministero della Salute - [4] ATS Valpadana

Since the first report in December 2019, SARS-CoV2 (Sars2) has affected more than 128 million people, causing over 2.8 million deaths worldwide. Animals (dogs, cats, felids) could also be naturally infected with Sars2 from humans, but there is no evidence that they can infect humans. Farmed minks were also found infected following exposure from affected humans in Canada, Denmark, France, Greece, Italy, Lithuania, Netherlands, Spain, Sweden and USA, but they can also act as reservoir of Sars2, transmitting the virus between them and posing a risk for virus spill-over to humans (1). This study describes the Sars2 virus dynamic in the only virus-positive mink farm diagnosed in Italy. According with the note 11120 of Ministry of Health (14/05/20), Sars2 surveillance was performed in a farm of 28000 minks in Cremona province for 4.5 months starting from the end of July 2020 when a farm worker tested positive for Sars2 PCR. The first clinical examination revealed no clinical signs and no increase of mortality. In late August, a slight increase in mortality and diarrhea but no respiratory signs were observed in some animals. A total of 1879 samples, 593 oropharyngeal (OR) and 535 rectal (R) swabs from dead animals and 251 OR and 500 R swabs from live animals, were collected. All animals were then sacrificed in December 2020 following the approval of the OM 21/11/20, which introduced Sars2 infection as one of the notifiable diseases for which stamping out is compulsory. At that time, 74 OR and R swabs and sera were collected from sacrificed animals. The sera were collected for research purposes only, since official protocols at that time did not include serological tests. Anatomopathological and histological examinations and bacteriological cultures were performed on dead animals. Real-time RT-PCR for Sars2 targeting the E gene was performed on all samples. Partial sequencing of S gene (1400bp) of both the mink and human positive samples detected respectively in November and in August was performed using Sanger protocol. Antibody detection against N (Ab-N) and S (Ab-S) Sars2 proteins was performed using a double-antigen ELISA and indirect ELISA for Ab-N, and a surrogate neutralization assay (SNA) and the virus neutralization assay (VNT) for Ab-S. Necroscopic investigation showed catarrhal enteritis, hepatic steatosis, and splenomegaly in some animals. Histology evidenced pulmonary edema and plasma cells infiltrate in the spleen. Samples, analyzed following the slight increase of mortality, revealed a septicemic *S. equi* as well as *S. enteritidis var. enteritidis* from feces. Initial PCR testing for Sars2 of 20 animals that died in early August showed an OR swab positive near the cutoff value. One week later, both OR and R swabs from another dead animal resulted low positive. All other samples were negative until the end of October, when one R swab was low positive. Phylogenetic analysis of the mink and human sequences showed that they belonged to two different clades. The genomic features of the S protein were: i) absence of the Y453F mutation (mt) in both sequences; ii) presence of the N501T mt only in the human sequence; iii) presence of the S477N

mt in the mink sample. Serological analysis detected Ab-S in 100% of animals by SNA and VNT but no Ab-N.
The high Sars2 seroprevalence confirmed virus circulation in the mink farm without specific clinical sings or increase of mortality even with a very low number of PCR-positive samples. Thus, the course of infection may be asymptomatic and mortality due to other causes may make the Sars2 diagnosis difficult. From the data here gathered, it is possible to assume that a syndromic surveillance associated with genome detection may not be sufficient to make diagnosis in asymptomatic animals. The serology may be useful for better investigating virus circulation in the farm. However, Ab-N are likely produced for a short time at very low titers or below detection levels, especially in asymptomatic animals, and therefore serologic testing should be aimed to detect Ab-S. The use of SNA serological tests that can be performed in BSL2 is a viable option over VNT tests using live viruses that must be performed in BSL3. A correlation between human and mink sequences was not demonstrated and therefore taking all facts together it could be hypothesized that Sars2 could had been introduced unnoticed into the farm earlier than the start of the surveillance period.

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Dynamic evolution of H1 swine viruses: what can we expect in the near future?

A. Moreno[1], G. Alborali[1] , L. Baioni[2], S. Faccini[1] , A. Luppi[2], A. Prosperi[2], C. Rosignoli[1], C. Salogni[1], S. Canziani[1], S. Salvato[1], T. Trogu[1], C. Chiapponi[2]

[1] IZSLER - [2] OIE Reference Center for Swine Influenza, IZSLER, Parma

Swine influenza is a highly contagious viral infection of pigs caused by influenza A viruses (SwIAVs) belonging to three different subtypes H1N1, H1N2, and H3N2. Its evolution is different and more complex than that of human AVIs due to the geographical separation of pig populations, multiple introductions of viruses and continuous circulation in pig farms. Three lineages (Lin) and multiple genotypes (with internal genes of different origin) of SwIAVs, have emerged and some become established in swine. Of these, SwIAV H1 have been found to be the most variable viruses in Europe. A new classification system, based on genetic characteristics, subdivides H1 swIAVs in 3 Lin 1A, 1B and 1C and more than 28 clades (1). Lin1A includes classical swine and 2009pdm-H1N1 viruses derived from the human 1918pdmH1N1 virus. Human seasonal Lin1B contains swIAVs with an H1 derived from human seasonal IAVs. Eurasian avian Lin1C contains swIAVs that originated from avian IAVs, which emerged in Europe in 1979. In this study, we analyzed the H1 SwIAVs isolated in Italy in 2019-2020 with particular reference to the new H1 clades. IAV surveillance was based on investigation of pigs showing respiratory disease by genome detection using M gene RT-PCR, virus isolation, multiplex PCR typing and whole genome sequencing. Molecular analysis of the HA protein was made comparing H1 SwIAV sequences using the H3numberig (A/Aichi/2/68 H3N2).

A total of 3946 samples were tested for IAV genome with 568 positives (14.4%). Of these, 341 SwIAVs were typed by PCR. Most of them were H1 swIAVs (82,4% in 2019 and 92,9% in 2020) while only a few were typed as H3N2. Regarding H1A viruses, a new 2009pdmH1 HA sub-clade (1A.3.3.2) has recently been detected in our country characterized by very little or no cross-reactions with human seasonal vaccines including A/Cal/04/09. Thirty-eight aa mutations, some located in the receptor binding site (RBS) and in the antigenic sites (Ca1, Cb and Sa), were observed compared to A/Cal/04/09 (2). Within H1B viruses, the Italian clade 1B.1.2.2, first detected in 2003, has been successfully adapted to the Italian pigs and continues to be the predominant human-like H1 clade. It is characterized by two aa deletions (del) in the 130 loop of RBS. A very interesting situation were observed in recent avian-like H1C viruses with the detection also in Italy of the two new European clades, provisionally named 1C.2.4 and 1C.2.5, which showed lower cross-reactivity to other contemporary swine 1C strains (2). Three aa del patterns, all including one del at pos 133, were identified in 1C.2.4 strains, characterized by this del alone or with others at 125 or 158.

The current situation is characterized by the circulation of a large number of genotypes but with a predominance of H1 viruses of Lin 1B and 1C. H1A pdm viruses continue to circulate in pig farms but with a lower incidence than other H1 viruses, however a new genetic and antigenic clade has been detected and it appears that it may stabilize in the swine population in the future. In the Lin1B, a great diversity of genotypes is circulating in Italian pigs but the 1B.2.2 clade, which appeared in 2003, seems to be the one with a greater sta-

bility and selective advantage over all others. The Lin1C, which seemed to be more stable in the previous years, is now showing a great variability with the report of two new clades with interesting antigenic and genetic characteristics. Many of the deletions were in the 130 loop, which together with the 220 loop and 190 helix form the structural elements that define the RBS edges. Since pigs and humans share the same pattern of IAV receptors in their respiratory tract, inter-species transmission of IAV between pigs and humans can occur in both directions. Therefore, effective prevention and control of swIAV may not only be a benefit for pig health but also for human health. Vaccination is the main tool for controlling IAV in pigs and therefore surveillance of the antigenic variants circulating is essential to better understand the efficacy of vaccination.

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Bovine *Delta* papillomavirus E5 oncoprotein interacts with TRIM25 and hampers antiviral innate immune response

F. De Falco[1], A. Cutarelli[2], P. Cerino[2], V. Uleri[1], S. Roperto[1]*

[1] Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli Federico II, Napoli, Italia - [2] Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Napoli, Italia

The recognition of microbial nucleic acids is a major mechanism by which the immune system detects pathogens. Pattern recognition receptors (PRRs) are responsible for sensing the presence of pathogens, including viruses, since they recognize conserved features of microbes known as pathogen-associated molecular patterns (PAMPs). Retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) are one of the four different classes of PRRs. The RLR family is composed of retinoic acid-inducible gene I (RIG-I), melanoma differentiation-associated gene 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2). RLRs localize to the cytosol, and their expression is greatly increased after virus infection. The activation of RIG-I and MDA5 is regulated by multiple ubiquitin E3 ligases of the tripartite motif (TRIM) proteins such as TRIM containing 25 (TRIM25) and Riplet. Downstream signaling pathway is mediated by mitochondrial antiviral signaling adaptor (MAVS) leading to phosphorylation of IKK complex and TBK1 which are responsible for the activation of transcription factors such as NF-kB and IRFs ultimately resulting in producing interferon type I and III. RLRs play a major role in triggering and modulating antiviral immunity by detecting exogenous viral RNAs. RLRs recognize also DNA ligands from DNA viruses as well as those derived from bacteria. Bovine papillomaviruses (BPVs), a heterogeneous group of species-specific viruses distributed worldwide, comprise 29 types assigned to five genera. Bovine Delta papillomavirus (δPVs) show their transforming activity through the E5 protein, a highly conserved oncoprotein, believed to be the major δPV oncoprotein. In this study, we showed, for the first time, in a spontaneous model of bovine papillomavirus disease that the interaction between E5 oncoprotein and E3 ubiquitin ligase TRIM25 can downregulate multiple downstream effectors of the host antiviral response pathway mediated by the RLRs.

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Preliminary results from an environmental investigation for orthohepevirus C (HEV-C) in Italy

V. Sarchese[1], P. Fruci[1], F. Di Profio[1], A. Palombieri[1], G. Lanave[2], G. La Rosa[3], E. Suffredini[4], F. Marsilio[1], V. Martella[2], B. Di Martino[1]

[1] Faculty of Veterinary Medicine, Università degli Studi di Teramo, Italy - [2] Department of Veterinary Medicine, Università Aldo Moro di Bari, Valenzano, Italy - [3] Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy - [4] Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy

Background. HEV is a quasi-enveloped, icosahedral, single-stranded positive-sense RNA virus classified in the family *Hepeviridae*, genus *Orthohepevirus*, which comprises 4 species, *Orthohepevirus A* to *D*. HEV-A is a recognised agent of human acute hepatitis worldwide and in developed countries the infection has mostly a zoonotic origin. In addition to HEV-A, recent studies have documented zoonotic infection of humans with *Orthohepevirus* species *C* (HEV-C) presumably derived from rats (HEV-C1). HEV-C caused persistent hepatitis in a liver transplant recipient in Hong Kong and induced severe acute hepatitis in an immunocompetent individual from Canada. Furthermore, based on comprehensive clinical and epidemiological analyses, additional seven human infections by HEV-C have been identified in Hong Kong between January 2018 and October 2019. In order to collect information on the circulation of HEV-C in Italy, a pilot study was initiated by assessing untreated sewage samples collected from wastewater treatment plants (WWTPs) located in a geographical area considered at high risk for HEV infection (Abruzzo Region).
Material and methods. Fifty-six influent sewage specimens were collected during 2016-2017 in four WWTPs distributed over the province of Teramo (Abruzzo Region), by swabbing different surface points of the separation grids used for primary wastewater treatment. Total RNA was extracted by TRIzol LS procedure and analysed by heminested RT-PCR using broadly reactive primers targeting a region of 338-bp of the viral RNA-dependent RNA polymerase (RdRp) complex, highly conserved among members of the family *Hepeviridae*. Furthermore, all the specimens were screened with specific primer sets for HEV-C, designed to amplify a fragment of 314-bp of the ORF2 gene.
Results. Overall, HEV-C RNA was detected in 7.1% (4/56) sewage samples. Three specimens tested positive with broad-range PCR primers for *Hepeviridae*, whilst one sample was detected using specific primers for HEV-C ORF2. By BLAST and FASTA analyses the amplicons displayed the highest nucleotide identity (85.3-92.7%) to HEV-C strains previously detected in rats in Spain, Greece, Austria and China. Upon phylogenetic analyses based either on the RdRp or ORF2, all strains segregated into the species HEV-C genotype C1, along with other HEV strains of rodents.
Conclusions. The results obtained in this study provide firm evidence for the circulation of HEV-C in Italy. There is concern in global and national health bodies that HEV is an under-recognised pathogen with an overall notification rate increasingly over the last few years. Meanwhile, evidence indicates that HEV-C of rodent origin is an additional possible cause of viral hepatitis in humans. Accordingly, we cannot exclude that the high prevalence found in this study may be also subsequent to human viral excretion. It will be crucial to improve epidemiological surveillance with the inclusion of HEV-C in the diagnostic algorithms of human hepatitis.

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Occurrence and trend of human viruses in wild bivalves growing in Venice channels during the COVID-19 pandemic

M. Sigovini[1], D. Tagliapietra[1], I. Guarneri[1], G. La Rosa[2], F. Beikpour[3,4], T. Vicenza[3], S. Di Pasquale[3], M. Losardo[5], L. Cozzi[3], E. Suffredini[3]

[1] National Research Council of Italy - Marine Sciences Institute (CNR - ISMAR), Venice, Italy - [2] Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy - [3] Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy - [4] Department of Veterinary Medicine, University of Bari 'Aldo Moro', Bari, Italy - [5] President office, Istituto Superiore di Sanità, Rome, Italy

Bivalve shellfish are filter-feeding animals that, as a part of their nutrition process, may accumulate biological and chemical contaminants present in their growing waters. Therefore, they are often used as sentinel organisms for the indirect monitoring of human pathogens. The historical city of Venice and smaller inhabited islands are not provided with a centralized sewage system, with a large number of sewage discharges flowing without treatment directly into the canals. Aim of the present study was to assess occurrence and trends of human viruses in bivalve shellfish of the Venice lagoon during the first COVID-19 lockdown and of the subsequent progressive reopening of the activities.

Wild bivalve shellfish (oysters, *Crassostrea gigas*) were collected monthly between April and December 2020 in 8 sites over the network of urban canals and channels of Venice lagoon. For detection of human viruses, digestive tissue (DT) was prepared according to ISO 15216 and nucleic acids were extracted using the MiniMag system (bioMerieux). The remaining body tissues were also extracted by a modified PEG-glycine precipitation protocol [1]. Samples were analyzed by real-time RT-qPCR for Norovirus (NoV GI and GII), hepatitis A and E viruses (HAV, HEV) Aichi virus (AiV), Astrovirus (AV), and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) with protocols reported elsewhere [2, 3, 4]. SARS-CoV-2 analysis was conducted on both nucleic acids extracted from DT and from the other residual body tissues. Appropriate controls for viral recovery and PCR inhibition assessment were included in the analysis. Furthermore, samples were tested for Pepper Mild Mottle Virus (PMMV), a plant virus transient of the human intestine, candidate as a viral indicator of fecal contamination for waters [5].

Fecal contamination of the waters surrounding the sampling points was confirmed by PMMV. NoV were the most frequently detected viruses (GI 81.9% and GII 84.7% of samples), followed by AiV (27.8%) and AV (15.3%). Fluctuations of NoV concentrations were detected in August and September, depending on the sampling point. HAV and HEV were detected twice and once, respectively, and the occurrence of HAV was associated to two samples taken in the same point a month apart, possibly as a consequence of a small HAV cluster in the surrounding area. SARS-CoV-2 was never detected in the tested samples, despite the evidence of its diffusion in the area during the observed period (first and second wave of the COVID-19 pandemic). This may be due to a lower bioaccumulation of this respiratory virus in bivalve shellfish or to a lower endurance in the aquatic environment compared to enteric viruses.

These results show a significant diffusion of several enteric viruses in the human population living on the urban areas of Venice lagoon and highlight the relevance of environmental surveillance to understand viral circulation dynamics.

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Chronic wasting disease: present situation and surveillance of the Italian National Reference Center for Animal Encephalopathies

L. Tripodi*, G. Ru*, F. Lazzara*, L. Florio*, D. Meloni*, M. Mazza*, E. Bozzetta*, M.G. Perrotta**, M. Caramelli*, C. Casalone*, B. Iulini***

* Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Italy - **Ministero della Salute, Italy - *** S.C. Sanità Animale Dip. Prevenzione, ASL 3 Genovese, Italy

Chronic Wasting disease (CWD) is a prion disease that affects cervids and belonged to the Transmissible Spongiform Encephalopathies (TSEs). TSE are neurodegenerative diseases that affect humans and animals. In cervids, CWD is particularly contagious and eradication in endemic areas is very difficult, therefore it's becoming a serious problem for their conservation or their breeding. While the number of cases of bovine spongiform encephalopathy (BSE) has been steadily declining, CWD, a relatively new arrival in Europe in cervids (EFSA, 2019), has confronted public agencies and the general public with the management of an emerging public health risk. Given the presence of a large population of cervids in Italy and the growing concern about the spread of the infection in Europe, the Italian Ministry of Health has established a surveillance plan to evaluate the risk of CWD at national level. Sampling is carried out by the local health authority and entails sampling of the brainstem (obex) and medial retropharyngeal lymph nodes of wild and farmed cervids found dead or in emaciated condition. The samples are sent to the local Istituto Zooprofilattico (public health institute) and then to the National Reference Center for Animal Encephalopathies (CEA). From 2017 to 2019 a total of 1005 samples (736 roe deer, 216 deer, 37 fallow deer, and 16 reindeer) were analyzed at CEA with a rapid screening test and all resulted negative for CWD. A total of 113 brains belonging to the 1005 samples were subjected to histopathological examination to investigate the main patterns of neuropathological lesions and possible correlated pathogens. Brain lesions were detected in 20 animals, 10 were unsuitable for examination due to autolysis or tissue freezing, while 83 were without lesions. Neuropathological examination revealed in most cases encephalitis or nonsuppurative meningoencephalitis (84%). The results deriving from the application of the surveillance plan in Italy demonstrated the absence of cases of CWD, however our work has allowed us to investigate the possible causes of death of the animals examined. Our study underscored the advantage of brain sampling during necropsy of wild animals, as it can yield scienti?c information relevant for animal health. Furthermore, through our analysis we demonstrated that the examination of the brain is essential to detect neuropathological lesions in animals whose clinical status and symptoms are unknown.

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P183

Comparison of the egeneup and the minimag nuclisens semi-automatic systems for viral nucleic acids extraction from food matrices

T. Vicenza, L. Cozzi, E. Suffredini, S. Di Pasquale

Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, National Reference Laboratory for Foodborne Viruses, Rome, Italy

Foodborne transmission of enteric viruses like Hepatitis A virus (HAV) and Norovirus (NoV) is an important public health issue. The method ISO 15216 allows harmonized detection of HAV and NoV in food matrices like bivalve shellfish, soft fruits, leafy vegetables and bottled water. An interlaboratory validation of the method was undertaken in 2017 with the use of the platform available at the moment (the MiniMag system, bioMerieux), but the use of alternative nucleic extraction systems is allowed, following demonstration of equivalence. The aim of this study was the assessment of the performance of an alternative semi-automatic nucleic acids extraction system, the eGeneUP platform (also supplied by bioMerieux), and its comparison with the MiniMag system.
Four different matrices (mussels, strawberry, lettuce and still water) were treated according to ISO 15216 to prepare concentrates suitable for nucleic acid extraction. The concentrate of each matrix was experimentally contaminated with 5 different inoculum levels of HAV (strain HM175), NoVGI (GI.3) and NoVGII (GII.4 2006b). For each inoculum level, RNA extraction was performed in triplicate with the two semi-automatic bioMerieux platforms. All samples and all viral targets were then analysed by real-time RT-(q)PCR assay as described in ISO 15216-1:2017 annexes.
Both extraction systems allowed detection of the viral targets up to the last inoculum level. Overall, the eGeneUP extraction provided positive results for HAV, NoVGI and NoVGII in 57/60 (95.0%), 54/60 (90.0%) and 59/60 (98.3%) of the replicates, respectively. The MiniMag platform, instead, achieved detection of HAV in 54/60 (90.0%), NoVGI in 49/60 (81.7%) and NoVGII in 58/60 (96.7%) of the analysis. Therefore, considering the three viral targets together, eGeneUP extraction outperformed MiniMag extraction (170 vs. 161 positive detection; 98.3% compared to 89.4%). With regard to the different kinds of matrices included in the study, inhibition was acceptable for all the tested samples, but significant differences were associated to the inhibition registered on leafy vegetables (lettuce) samples, in which high values were recorded for both MiniMag and eGeneUP. Furthermore, higher LOD50 values were also calculated for leafy vegetables extracted with MiniMag.
This study show that extraction systems may perform differently depending on food matrices and that, therefore, systematic analysis of performance should be undertaken when replacing platforms. The obtained results support the use of the eGeneUP system in the application of ISO 15216 under accreditation.

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Hepatitis E virus: retention of infectivity in presence of additives used for the production of cured meats

D. De Medici[1], T. Vicenza[1], A. Di Sandro[2], R. Lena[2], L. Cozzi[1], S. Di Pasquale[1], E. Suffredini[1]

[1] Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, National Reference Laboratory for Foodborne Viruses, Rome, Italy - [2] Italian Ministry of Health, Directorate General for Food hygiene and safety and Nutrition, Rome, Italy

Foodborne viruses are recognised as a priority for consumer safety by the Competent Authorities and the food industry. Hepatitis E virus (HEV) is a zoonotic disease transmitted through pork meat and cured pork products consumption. Additives are commonly used during the preparation of food to prevent spoilage and extend shelf-life, while ensuring control of pathogenic microorganisms. The aim of the study was to evaluate the persistence of viral infectivity, capsid integrity and viral RNA in presence of additives commonly adopted in the production of cured meat products (salami, sausages, etc).
HEV (strain 47832c, titer ≈10^3 $TCID_{50}$/mL and 4.1×10^7 genome copies/mL) was added to water suspensions of the different additives as follows: i) NaCl (1.0% to 3.0% with 0.5% increments); ii) $NaNO_2$ (100, 150, and 250 mg/ L); iii) $NaNO_3$ (80, 150 and 250 mg/L); iv) aqueous solutions pH 4.5 to 6.5 with 0.5 pH unit increments to simulate the pH changes that occur during the seasoning process of cured meats. All samples were incubated at 14°C±1°C for 0, 10, 35 and 60 days (and additionally for 3 and 45 days for pH solutions). For each condition and incubation time the following parameters were evaluated: a) viral infectivity using an integrated cell culture-PCR method (viral infection on A549/D3 cell monolayers confirmed by real-time RT-PCR; b) capsid and structural integrity of viral particles using a 'viability PCR' assay (ethidium bromide monoazide treatment 300 µM followed by photoactivation and real-time RT-(q)PCR; c) concentration of viral RNA by a standardized RT-(q)PCR.
Viral infectivity of HEV displayed a significant reduction (≥1 log) from the 10th day of incubation regardless of the type or concentration of additive considered (NaCl, $NaNO_2$, and $NaNO_3$), as well as for all pH values. In detail, viral infectivity remained unaltered until the 3rd day at pH values between 4.5 and 6.5, then losing infectivity in the subsequent days. As regards to NaCl, $NaNO_2$, and $NaNO_3$, HEV displayed loss of infectivity in presence of the highest salt concentrations already at day 0, and a further decrease to undetectable levels was recorded with longer incubation times. Significantly, a reduction of HEV infectivity was observer also for the untreated virus (positive control), though it occurred from the 35th day. The analyzes carried out to determine the persistence of viral RNA and the integrity of the viral particles, showed a progressive decrease in the PCR starting from the 35th day of observation, with the 'viability PCR' method consistently providing lower values compared to conventional real-time RT-(q)PCR.
Overall, the combination of the results suggests that, while a progressive, non-specific deterioration of HEV occurs over time, additives and pH exert a reduction of infectivity, pro-

bably through an action on viral receptors rather than on RNA or capsid integrity. Furthermore, while it is not possible to provide information on the functionality of the viral receptors through molecular methods, the values obtained with the 'viability PCR' assay consistently show lower values than those obtained by real-time RT-(q)PCR, and is therefore preferable for the purposes of an assessment of the risk associated with the detection of HEV.

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Identification of a bacteriophage in the phytoplasma insect vector *Euscelidius variegatus*

S. Abbà[1], M. Rossi[1], S. Ottati[1,2], G. Martino[1], M. Turina[1], L. Galetto[1], C. Marzachì[1], M. Vallino[1]

[1] Istituto per la Protezione Sostenibile delle Piante, CNR, Torino, Italia - [2] DISAFA, Università degli Studi di Torino, Grugliasco, Torino, Italia

Plant pathogen transmission by insect vectors represent a global economic issue due to their negative impact on crop production. Insect vectors, like all other organisms, are associated with a dynamic and interactive microbial community, called microbiome, which includes not only living members (microbiota), but also elements considered to be non-living organisms (viruses, plasmids, prions, viroids, and free DNA) and the whole spectrum of molecules produced by those microbes. Insects are colonized by a diverse microbial community, such as bacteria, protozoa, fungi and viruses, which can influence their physiology, ecology and evolution as well as their competence as vectors. Insect–virus associations, in particular, are not limited to insects that act as vectors of viral pathogens. Viruses, in fact, may also be pathogenic to their insect host, cause a covert infection or infect members of the insect microbiome, as in the case of bacteriophages, i.e. viruses that infect bacteria. The environmental impact of bacteriophages in various ecosystems has been attracting considerable interest for the past ten years, but only recently their role in the dynamics of microbial communities has been investigated. However, only a few phages have been described so far in insect microbiomes (e.g. the phage APSE-1 of the pea aphid endosymbiont *Hamiltonella defensa*, or phage WO of Wolbachia). In this work, metatranscriptomics was integrated with classical microbiological and microscopy techniques to identify a DNA tailed-phage and its bacterial host within the microbiome of the phytoplasma insect vector *Euscelidius variegatus* Kirschbaum (Hemiptera, Cicadellidae). Phytoplasmas are plant pathogenic bacteria transmitted by insects, which can cause severe losses in agriculture. *E. variegatus* is used as a laboratory vector of the phytoplasma causing Flavescence dorée, a quarantine pest that threatens viticulture in Europe, because of the difficulties in rearing its monovoltine natural vector *Scaphoideus titanus*. During the electron microscope (TEM) observation of a partial viral purification obtained from E. variegatus, we serendipitously observed some bacteriophage-like particles with head-and-tail morphology typical of the viral family of *Siphoviridae*. In order to identify the observed viral particles, we re- analyzed two RNA-seq libraries that were originally constructed without any prior phage enrichment step to explore *E. variegatus* transcriptome. Stringent selection of only phage hallmark genes resulted in the identification of three transcripts coding for three putative major capsid proteins. PCR amplifications allowed us to reliably associate one of these sequences to the observed phage, which was thereafter named *Euscelidius variegatus* phage 1 (EVP-1). EVP-1 host was isolated in axenic culture and identified as the bacterial endosymbiont of *E. variegatus* (BEV), which was recently renamed as

Candidatus Symbiopectobacterium sp. This bacterial isolate proved to harbour multiple prophages that become active on axenic media; in fact, in addition to siphovirus-like particles, TEM observations of the colony also revealed the presence of phages with podovirus- and myovirus-like morphology, suggesting that different environments, namely the insect microbiome and axenic cultures, can trigger different mechanisms that finely regulate the within-host interactions among phages. Unraveling the microbiome of insect vectors and understanding the complex relationships within its components may help to unveil possible microbe influences on pathogen transmission, and it is a crucial step toward innovative sustainable strategies for disease management in agriculture.

P186 Identification of possible new viruses in bumble bees by next-generation sequencing

R.Abou Kubaa[1], A.K. Alabdullah[2], A. Giampetruzzi[1], R. Addante[3], M. Saponari[1]

[1] CNR, Italian National Research Council, Institute for Sustainable Plant Protection, Bari-Italy - [2] Crop Genetics Department, John Innes Centre, Colney, Norwich NR4 7UH, UK - [3] Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy

The past decade has witnessed an increasing interest in studying bumble bee (*Bombus* spp.) populations due to their role in agricultural food production and natural ecosystems. Some studies showed that some diseases have been transmitted from commercially reared bumble bees to wild populations and led to noticeable population reduction in some species. Other studies demonstrated that RNA viruses can be transmitted to bumble bees by sharing diverse foraging sites with honey bees (*Apis mellifera* L.). A recent study showed that bumble bee infection in the wild does not occur by spillover from honey bees. High throughput sequencing (HTS) technologies can provide comprehensive insights into the bee virome, leading to a better understanding of the bee-pathogen interactions, including the viral diseases and their impacts. In the present study, we used HTS technologies to characterize the RNA virome from a pool of five forage bumble bees collected from garden spaces. Bumble bees were homogenized in TRIzol reagent (Thermo Fisher), followed by isopropanol precipitation. The total RNAs were purified by RNeasy Plant Mini Kit (QIAGEN) and treatment with DNAse (Promega). PolyA enrichment of total RNAs, Illumina TruSeq RNA library construction, followed by 2 × 100 NovaSeq sequencing were outsourced to Macrogen Inc. (Seoul, Korea). The obtained reads (36,919,326 reads with 44.531% GC contents) were checked for their quality by FastQC and assembled using metaSPAdes version 3.9.0 with the "only-assembler" parameter and multiple kmers (-k, 71, 81, and 91). A total of 55831 contigs were assembled and blasted against the NCBI viral sequences using Blast+ package (v. 2.9.0; Blastx with E-value < 1e-10). Bioinformatic analyses showed that 4.8% (2663 contigs) were of non-host origin, out of which 31 contigs showed significant similarity to viral sequences. The results demonstrated the occurrence of a number of viruses belonging to different genera, such as *Partitiviridae*, *Secoviridae*, *Riboviridae*, *Hepeviridae*, *Dicistroviridae* and other belonging to unclassified *Riboviria* realm. Most of the viruses found are common in invertebrates; others are common on vertebrates and/or plants. Furthermore, further investigations by RT-PCR confirmed the presence of some viruses infecting honey bees, like *Black queen cell virus (Triatovirus)* and also, the presence of Mayfield virus 1-like (unclassified *Riboviria*). The use of HTS technologies allowed discovering viruses that may pose threats to the bumble bees worldwide.

Keywords: Bumble bees, Virus, HTS, NGS, RT-PCR

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Plant viruses go global: the european virus archive

G.P. Accotto[1], M. Ciuffo[1], R. Pierro[1], L. Rubino[2], A.M. Vaira[1], C. Prat[3], J.L. Romette[3]

[1] CNR, Institute for Sustainable Plant Protection, 10135 Torino, Italy - [2] CNR, Institute for Sustainable Plant Protection, 70126 Bari, Italy - [3] Unité des Virus Emergents (UVE), Aix-Marseille University-IRD 190-INSERM 1207-IRBA IHU Infection, 13005 Marseille, France

The current virus pandemic has brought to the general attention the need of sharing resources for the development of antiviral strategies. In this framework, key players are collections of biological resources and derived products, which can provide different laboratories and Institutions with virological material of utmost importance.
The European virus Archive - Global (EVA-GLOBAL; https://www.european-virus-archive.com/) is a non profit organisation including a global network with expertise in virology, consisting of 38 partner laboratories both based at EU member state and non-EU institutions (Romette et al., Antiviral Res 2018;158:127–134; Coutard et al., Biopreserv Biobank 2020;18:561-569). This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 871029. The aim of the project is to collect, amplify, characterize, standardize, authenticate, and distribute viruses and derived products.
The main strengths of the network rely on the synergic collaboration between laboratories, the quality of the offer in terms of viruses and derived products, the availability of diagnostic material and protocols. New tools, techniques and concepts are continuously developed in the frame of all possible virological branches. An on-line catalogue, including products and services, is available to the scientific community.
Starting in 2020, plant viruses have been included in EVAg catalogue, and synergic actions are being taken amongst the most important European plant virology institutions to establish a wide collection of viruses and isolates, as well as derived products and associated services. Quality standards are being envisaged and adapted to plant viruses, in order to maintain the high profile of the EVA-GLOBAL products. This will support the advancement of knowledge in plant virology. Moreover, the project will represent an essential contribution to the preservation of virus biodiversity.

P188 - OC 12

Transcriptomics to reveal the genetic basis of CMD2 resistance in cassava

A.V. Carluccio[1,2*], R.O. Adeoti[1], I. Rabbi[1], M. Gedil[1], A. Gisel[1,3] and L. Stavolone[1,2*]

[1] Bioscience Centre, International Institute of Tropical Agriculture, Ibadan, Nigeria - [2] Institute for Sustainable Plant Protection, CNR, Bari, Italy - [3] Institute for Biomedical Technologies, CNR, Bari, Italy

Cassava mosaic disease (CMD) is the most severe and widespread cassava *(Manihot esculenta)* viral disease and the most important factor limiting cassava production in Sub-Saharan Africa, causing huge economic losses. After cereals, cassava is the most important source of calories worldwide, and more than 800 million people use it as a source of food and income generation in Africa, Asia, and Latin America

Leaf symptoms of CMD in cassava vary from mild mosaic to more severe symptoms of leaf curl, leaf distortion, yellowing, and plant stunting depending on the infected cassava cultivar, the virus genotype(s) (mixed infections are common), and the climatic conditions.

CMD is caused by several circular ssDNA cassava mosaic geminivirus species belonging to the genus *Begomovirus* in the family *Geminiviridae* and is transmitted by the polyphagous cryptic whitefly *Bemisia tabaci*.

Cultivated cassava species are protected from CMD by a polygenic resistance introgressed from the wild species *Manihot glaziovii*, a dominant resistance conferred by a single QTL on chromosome 12, named CMD2. However, the gene(s) involved in regulation of CMD resistance have not yet been uncovered.

To investigate in detail the gene(s) involved in CMD2 resistance, genome wide RNA sequencing analysis was performed focusing on chromosome 12, and in particular on the CMD2 locus. This locus contains 406 annotated transcripts, that were exposed to a differential expression analysis between two susceptible (TMEB117 and TMS4 (2)1425) and two resistant (TMS961089A and TMS011412) lines. Our comparative analyses revealed the existence of a unique gene within the CMD2 locus differentially regulated between susceptible and resistant lines.

To confirm that this gene is indeed involved in CMD2 mediated resistance to CMD, quantitative real time PCR was performed on samples selected within progeny lines of a mapping population obtained from one susceptible (TMEB117) and one resistant (TMS961089A) line. In addition, the expression of the gene of interest was also investigated in several different genotypes with opposite CMD resistance property. Taken together these results confirmed the involvement of the gene in CMD2-mediated resistance in cassava and support the hypothesis that its overexpression is triggered upon virus infection to favor virus spread and onset of the infection. The identification of a susceptibility gene for CMD, opens new possibilities not only for a marker-assisted selection but also for a biotechnological and gene editing approach both needed for cassava improvement.

P189

A complex virome associated to tospovirus-transmitting thrips species offers new approaches to contain the damage they cause

M. Chiapello[1], L. Bosco[2], M. Ciuffo[1], S. Ottati[1, 2], L. Tavella[2] and M. Turina[1*]

[1] Institute for Sustainable Plant Protection, CNR, Strada delle Cacce 73, 10135 Torino, Italy - [2] Dipartimento di Scienze Agrarie, Forestali e Alimentari, University of Torino, Largo P. Braccini 2, 10095 Grugliasco, Torino, Italy

Aim of the study. *Franklinella occidentalis* (western flower thrips, or WFT) and *Thrips tabaci* (onion thrips, or OT) are insect species that greatly impact horticultural crops through their efficient transmission of tomato spotted wilt virus and iris yellow spot virus. The holobiont is the complex of organisms constituting a specific ecological unit. Insect holobionts include the insect species and the microorganisms associated to them. Particularly interesting are some mutualistic interactions between the insects and the microorganisms they host. Interfering with such obligatory mutualistic interactions can result in new approaches to contain their populations or the damage they cause transmitting viruses of medical, veterinary, or agricultural importance. Persistent insect viruses are a neglected component of the insect holobiont. Purpose of this work is to characterize the virome associated to the two model systems for tospovirus-transmitting thrips species.
Methods used. We collected adults of these thrips species from different host plants in 12 sites in various regions in Italy. Total RNAseq from high-throughput sequencing (HTS) was used to assemble the virome following a bioinformatic pipeline we developed and then we assigned putative viral contigs to each thrips sample by qRT-PCR. A thorough analysis of ORFan contigs was carried out to investigate their possible viral origin. Small RNA analysis was also performed by NGS on a specific population of WFT from Piedmont (NW Italy).
Results and conclusions. Excluding plant and fungal viruses, we were able to identify 64 viral segments, corresponding to 42 viruses: 15 were assigned to WFT, 17 to OT and 9 viruses could not be assigned to any species based on our stringent criteria for host association. All but one of these viruses are putative representative of new species and some viruses are the type member of new higher-ranking taxa. These additions to the viral phylogeny suggest new evolutionary trajectories specific for thrips. Most of the Baltimore's classes of RNA viruses were present (positive- and minus- strand and dsRNA viruses), but only one DNA virus was identified in our collection. Endogen virus elements were also identified and the small RNA population they originate was characterized. Repeated sampling in a subset of locations in 2019 and 2020 and further virus characterization in a subset of four populations maintained in laboratory through rearing on a controlleddiet allowed us to provide evidence of a locally persistent thrips core virome that characterizes each population. A web-based application was also developed to explore the results of the research.

P190
Looking for novel species demarcatin criteria for viroid classification

M. Chiumenti[1], B. Navarro[1], T. Candresse[2], R. Flores[3], and F. Di Serio[1]

[1] Istituto per la Protezione Sostenibile delle Piante, Consiglio Nazionale delle Ricerche, Via Amendola 122/D, Bari 70126, Italy - [2] Univ. Bordeaux, INRAE, UMR BFP, Villenave d'Ornon Cedex, CS20032 33882, France - [3] Instituto de Biologıa Molecular y Celular de Plantas, Consejo Superior de Investigaciones Cientıficas– Universidad Politecnica de Valencia, Valencia 46022, Spain

Viroids are small, circular, non-coding RNAs that infect plants. Based on the rules established by the International Committee on Taxonomy of Viruses (ICTV), more than thirty viroid species have been classified in two families and eight genera. However, at least 13 viroids are still waiting for a conclusive classification. To classify a viroid in a new species, the species demarcation criteria currently adopted by ICTV ask for less than 90 per cent sequence identity on the overall genome and evidence of biological divergence compared to the closest related viroids. When a viroid infects latently its natural or experimental hosts, and/or infects woody plants and/or has a narrow host range, biological studies are difficult, delaying its conclusive classification. Several new viroids have been discovered in the last few years by high-throughput sequencing technologies, which are expected to identify many other viroids in the next future. Therefore, to avoid the proliferation of unclassified viroids, the need of new reliable species demarcation criteria mainly based on molecular signatures of viroids, is urgent. To this aim, we have recently tested for viroids a sequence-based approach built on pairwise-sequence identity matrices that were used to identify a threshold identity score (TIS) for each viroid genus. We showed that the current classification would not be modified if TISs are used as the main species demarcation criterion. Moreover, the application of pairwise identity matrices and TISs allows the immediate classification of the unclassified viroids, allocating them to a known or to a new species. Therefore, by this criterion the necessity of additional biological data is limited to few ambiguous situations. The pairwise identity score approach has the indisputable advantages that the proposed TISs for species demarcation are not arbitrarily established, the request for biological evidence is not mandatory, and the current viroid classification scheme is not modified. The complete study supporting this proposal has been recently published (Chiumenti et al., 2021, Virus Evolution 7: veab001. Doi: 10.1093/ve/veab001). We regret very much that Prof. Ricardo Flores, who actively contributed to this study, passed away and could not read this abstract. We dedicate this work to Ricardo who has been an eminent scientist and a generous person. He deeply investigated viroids strongly contributing to the current knowledge on these infectious agents. He will be greatly missed

P191

TSWV-targeted VIGS in plants and thrips as possible virus control strategy: preliminary results

S. Daghino[1], S. Ottati[1,2], G. Forestello[1,2], M. Ciuffo[1], L. Tavella[2], M. Turina[1]

[1] Institute for Sustainable Plant Protection, CNR, Strada delle Cacce 73, 10135 Torino, Italy - [2] Dipartimento di Scienze Agrarie, Forestali e Alimentari, University of Torino, Largo P. Braccini 2, 10095 Grugliasco, Torino, Italy

Aims of the study. Tomato spotted wilt virus (TSWV) is one of the most important plant viruses, infecting more than 1000 plant species over 80 families and posing major threats to global food security. TSWV is transmitted by thrips in a persistent-propagative manner. TSWV control strategies are based on integrated disease management approaches (e.g. use of insecticides, scheduling the planting to avoid the thrips peaks) and on the use of plant varieties carrying resistance genes. All these strategies have drawbacks, such as the environmental damages caused by chemicals and the raising of pesticide-resistant vector species or resistance-breaking viral strains. A promising alternative strategy of insect pest control, currently under exploration by biotechnology industries, is the exploitation of RNA interference (RNAi)-mediated natural defenses, with the aim of specifically suppressing the expression of genes essential for the growth, development, or reproduction of the target specie, without adversely affecting non-target species (Christiaens et al., 2020, Front. Plant Sci. 11: 451). The RNAi molecular machinery naturally targets invading pathogens, including viruses, that, on the other hand, can silence the insect defenses, in a constant arm race. RNAi can be achieved by virus-induced gene silencing (VIGS), based on viruses engineered to produce the target dsRNA during virus replication. VIGS is an established silencing-strategy in plants, and suitable plasmids are available (Dommes et al., 2019, J. Exp. Bot. 70 (1): 757–770). The aim of this work was to test whether TSWV can be silenced by VIGS in model plant species as well as in thrips after feeding on replicative dsRNAs produced in plant cells.

Methods used. We engineered VIGS plasmids based on tobacco rattle virus vector to accumulate TSWV-derived short dsRNA (test) and Tomato Brown Rugose Fruit Virus (ToBRFV)-derived short dsRNA (negative control). The plasmids were expressed *in planta (D. stramonium* and *N. benthamiana)* by agroinfiltration together with a plasmid expressing TRV RNA1; plants were later challenged either mechanically with TSWV to directly observe the infection, or used for the oviposition of thrips, later fed with TSWV and tested for their transmission rate.

Results and conclusions. We could observe a clear and specific silencing of the TSVW infection in *N. benthamiana* by the specific TRV-TSWV VIGS-vector, both locally and systemically, resulting in effective control of TSWV. In *D. stramonium* we could observe a delay of the infection in the test plants with respect to the control, but not in complete prevention of TSWV infection. Preliminary data suggest that preliminary feeding of the thrips with test *D. stramonium* leaves agroinfiltrated with TRV-TSWV VIGS vector reduced their ability to transmit TSWV to healthy plants to the 40%. Further experimental approaches are needed to support our phenotypical evaluation, e.g. the molecular quantification of both the VIGS-vector and TSWV in plants.

Our results suggest the possibility of using the RNAi technology mediated by plant-virus vectors, with the aim to reduce the spread of viruses from infected to healthy plants.

P192 - OC 11

A tripartite narna-like mycovirus reveals the existence of a split RNA-dependent RNA polymerase palm domain hosted by two distinct proteins

M. Forgia[1], M. Daghino[1], S. Perotto[2], S. Turina[1]

[1] IPSP-CNR, Strada delle Cacce 73 10135, Torino, TO - [2] Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino, Viale Mattioli 25, 10125, Torino, TO

Aim of the study. Narna-like viruses are commonly found infecting fungi. Fungal narnaviruses are characterized by a monopartite genome encoding for a single ORF hosting the typical Palm- domain present in RNA-dependent-RNA-polymerases. Recently, a group of mycoviruses have been reported showing a monopartite genome with the predicted RdRp carrying only A and B motifs from the Palm-domain and lacking the typical C motif with conserved GDD residues and involved in the catalytic site of the RdRP. Our studies on the metatranscriptome obtained from a collection of ericoid mycorrhizal fungi revealed one narna-like virus segment lacking the palm-domain C motif (EricV1) together with a viral contig encoding for an ORF with little homology to fungal narna-like RdRp (EricV2). By looking at the conserved ends and after in vivo characterization, we observed that the two contigs were part of the same viral genome (called Oidiodendron maius splipalmivirus 1=OmSV1), and analyses on the predicted protein from EricV2 showed the presence of the C motif that was missing in the EricV1 segment. A recent study confirmed our evidence showing other examples of fungal narna-like split palm-domain viruses (here called splipalmiviruses). Aim of this study was to complete the molecular characterization of OmSV1 and to attempt at assembling a full length cDNA infectious clone.
Methods. A metatranscriptomic approach was used to study the virome associated to a collection of different mycorrhizal fungi. After *in silico* characterization of the viruses infecting the fungal collection, we performed qRT-PCR analysis, RACE and northern blot analysis to obtain the full-length sequence of the viral genome. Viral genome was then cloned with the aim to obtain an infectious clone. At the same time, attempt to cure the virus was made using single conidia isolation, axenic culture on antiviral molecules and hyphal tipping.
Resulta and conclusions. Our study reveals the existence of a completely new organization of the viral replication module for RNA viruses. The positive sense RNA virus related to narnaviruses that we called OmSV1 hosts the RdRp palm-domain on two different proteins carrying half of the conserved motifs on each of them. Our study also reveals a third RNA fragment associated to the virus that encodes for a protein of unknown function. Current work is focused on attempts to cure the virus or produce an infectious clone in order to generate isogenic isolates that would be useful to study the phenotype associated to the virus which infects the mycorrhizal fungus *Oidiodendron maius*. Our study reports the first evidence of a division between two different proteins of the subdomains present in the RdRp Palm-domain, raising questions about the mechanics of viral replication and of the evolution of this enzyme likely at the origin of life as we know it.

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Genetic diversity of norovirus genogroups I and II in urban sewage through a next generation sequencing-based approach: four years monitoring

G. Bonanno Ferraro[1], P. Mancini[1], C. Veneri[1], M. Iaconelli[1], E. Suffredini[2], D. Brandtner[3], G. La Rosa[1]

[1] Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy - [2] Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy - [3] Independent researcher, Rome Italy

Human Norovirus (NoVs) belong to the *Caliciviridae* viral family. It is responsible of foodborne and waterborne gastroenteritis worldwide in humans of all ages. Noroviruses are genetically highly heterogeneous including 10 genogroups (GI-GX) and 48 capsid genotypes.
Viruses from the GI, GII, and GIV genogroups are known to infect humans. Norovirus can be transmitted by the fecal-oral pathway through contaminated environments, food, water, and person-to-person contact. Among GII NoVs, despite the broad genetic diversity, GII.4 has been identified as the predominant genotype responsible for 65-80% of NoV infections worldwide. New GII.4 variants have emerged every 2–4 years since 2002; in addition, non-GII.4 epidemic variants emerged recently have drawn extensive attention, especially the GII.17 Kawasaki 2014 in 2014/2015.
The aim of this study was to provide data on genetic diversity of GI and GII NoVs along a four year period, testing urban sewage by nested PCR coupled with a next generation (NGS) sequencing-based approach.
Between 2017 and 2020 a total of 200 sewage samples were collected from five wastewater treatment plants in Rome: 35 in 2017, 60 in 2018, 45 in 2019 and 60 in 2020.
Sewage concentration was performed by the two phase polyethylene glycol (PEG), dextran separation method, according to the WHO guidelines for environmental poliovirus surveillance. Viral nucleic acids were extracted using the NucliSENS MiniMag semi-automated extraction system (BioMerieux). All samples were analysed by RT-nested PCRs targeting the region C of the capsid gene, followed by NGS for a deeper analysis of the genotypes circulating in the study period. The amplicons from positive samples were pooled per year and further analyzed by NGS carried out on MiSeq II sequencer (Illumina). Subsequent bioinformatic analysis was carried on with a similarity base approach, using a joined reads method with high base calling reliability. Similarity threshold for genotypes and variant assignation was guided by a cross similarity study applied to reference sequences.
NGS revealed 6 different GI capsid genotypes (GI.1, GI.2, GI.3, GI.4, GI.5, and GI.7) and 9 GII genotypes (GII.2, GII.3, GII.4, GII.5, GII.6, GII.7, GII.12, GII.13, and GII.17) circulating during the study period. With regard to genogroup I, the most abundant and steady genotypes found in wastewaters were GI.1, GI.2 and GI.4. Instead, GI.3 and GI.5 were found to be less abundant and sporadic. GI.7 presence was minor and discontinued. For genogroup II, the first two years of the study period was characterized by a prevalence of GII.2 and

GII.4, which decreased with the appearance of GII.5, GII.6, GII.7 and GII.12 in 2019, the year showing the widest viral diversity. Across the years, GII.3, GII.13 and GII.17 were always present, and the GII.4 Sydney was the prevalent variant.
Our results show a significant circulation of a wide variety of NoV genotypes in raw sewage and the prevalence of the GII.4 Sydney variant along time. Wastewater surveillance, coupled with NGS, is a powerful tool to study the genetic diversity and to identify new genotypes or variants responsible of human infections.

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SARS-CoV-2 variants of concern (UK and Brazilian variant) detected in urban wastewaters in central Italy

G. La Rosa[1], P. Mancini[1], G. Bonanno Ferraro[1], C. Veneri[1], M. Iaconelli[1], L. Lucentini[1], L. Bonadonna[1], S. Brusaferro[2], D. Brandtner[3], A. Fasanella[4], L. Pace[4], A. Parisi[4], D. Galante[4], E. Suffredini[5]

[1] Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy - [2] Office of the President, Istituto Superiore di Sanità, Rome, Italy - [3] Independent Researcher, Rome, Italy - [4] Experimental Zooprophylactic Institute of the Apulia and Basilicata Regions, Foggia, Italy - [5] Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy

New SARS-CoV-2 mutations are constantly emerging, raising concerns of increased transmissibility, virulence or escape from host immune response. Several new variants emerged in the fall of 2020, most notably:

i. In the United Kingdom (UK), a new variant of SARS-CoV-2 (known as 20I/501Y.V1, VOC 202012/01, or B.1.1.7) emerged with a large number of mutations.
ii. In South Africa, another variant of SARS-CoV-2 (known as 20H/501Y.V2 or B.1.351) emerged independently of B.1.1.7
iii. In Brazil, a variant of SARS-CoV-2 (known as P.1 or 20J/501Y.V3) emerged that was first was identified in four travellers from Brazil, who were tested during routine screening at an airport in Tokyo, Japan.

In this study, a long nested RT-PCR assay (~1500 bps) was developed to detect multiple key spike protein mutations distinctive of the three Variants of Concern (VoCs) 20I/501Y.V1, 20H/501Y.V2, and 20J/501Y.V3, as well as of the 20E.EU1 variant (Spain), the CAL.20C recently identified in California, and the mink-associated variant. To extend variant detection to challenging matrices such as sewage, where long fragments are difficult to amplify, two short nested RT-PCR assays (~300 bps) were also designed, targeting internal portions of the region spanned by the long nested assay.

After validation on clinical samples (nasopharyngeal swabs), the three assays were tested on 34 urban wastewater samples: 20 urban sewage samples collected from WTPs in Rome in 2020, positive for SARS-CoV-2, and 14 sewage samples collected in January and February 2021 in areas in Central Italy, where circulation of VoCs had been reported.

None of these samples collected in Rome displayed mutations indicative of VoCs. On the other hand, 6 of the samples collected in outbreak locations (43%) showed mutations indicative of the UK and Brazilian variant or other variants (Spanish). To our knowledge, this is the first evidence of sequences harbouring key mutations of 20I/501Y.V1 and 20J/501Y.V3 in urban wastewaters, highlighting the potential contribution of wastewater surveillance to explore SARS-CoV-2 diversity.

Combination of the developed protocol with next generation sequencing (NGS) may provide additional depth to the analytical approach, overcoming the limitations of Sanger sequencing, which underestimates less common sequences that may be present in complex matrices like wastewater.

In conclusion, we demonstrated the potential of this approach, based on nested amplification followed by conventional Sanger sequencing, to allow the rapid identification of specific VoCs in the catchment of wastewater treatment plants, to quickly identify areas where the intensification of clinical surveillance and/or targeted preventive intervention are required. The approach proposed here can be applied to better understand SARS-CoV-2 variant diversity, geographic distribution and impact worldwide.

P195

Emerging viruses in raw and treated wastewaters, surface waters, and drinking waters

P. Mancini[1], M. Iaconelli[1], C. Veneri[1], G. Bonanno Ferraro[1], L. Meucci[2], F. Bersani[2], G. La Rosa[1]

[1] Istituto Superiore di Sanità, Department of Environment and Health, Rome, Italy - [2] Centro Ricerche SMAT, Turin, Italy

Enteric viruses are among the commonest waterborne pathogens, causing both sporadic and outbreak-related illness. The occurrence of enteric viruses (norovirus, adenovirus, enterovirus, hepatitis A and E Viruses) was investigated in the water supply network serving the city of Turin and surrounding municipalities (Piedmont). Emerging or potentially emerging viruses (saffold virus, salivirus, aichivirus, bocavirus, sapovirus) and the non-pathogenic pepper mild mottle virus, recently proposed as a potential indicator of human fecal pollution, were also studied.

A total of 69 water samples were collected and analysed in 2019-2020: 19 raw and 19 treated sewage samples, and 31 surface, ground water, and drinking water samples. 24-hour composite wastewater samples were collected from two water treatment plants at the inlet, before treatments. The other waters samples were collected in selected points: 8 samples from the Po river, 4 samples collected at the outlet of a lagoon where part of the river water flows in, to let sediments separate, 7 samples of groundwater collected from two wells, and 12 drinking water samples. Sewage samples were concentrated by the two-phase polyethylene glycol separation method according to the WHO Guidelines for Environmental Poliovirus Surveillance. The other water samples were filtrated on site using electropositive charged cartridges and eluted within 24h as described in USEPA Method 1615. After genome extraction, samples were tested for eleven different viruses by specific nested RT-PCR protocols followed by amplicon sequencing.

All untreated wastewaters were contaminated with at least one viral family. Multiple virus families and genera were frequently detected. The most prevalent viruses found were salivirus and bocavirus (18/19 each, 95%), followed by norovirus GI (17/19, 89%), aichivirus and sapovirus (15/19 each, 79%); adenovirus (12/19, 63%), enterovirus (10/19, 53%), norovirus GII (6/19, 32%), Hepatitis E virus (3/19, 16 %), hepatitis A virus and saffold virus (1/19 each, 5%). Pepper mild mottle virus was detected in 100% of untreated wastewater samples. Treated wastewater samples were found negative for all the viral pathogens as well as for pepper mild mottle virus.

As expected, surface water samples (Po river and riverside artificial lagoon) showed higher percentages of viral contamination in comparison to groundwaters. The frequency of positives was as follows: adenovirus and norovirus GI+GII (9/12 each, 75%); enterovirus (7/12, 58%); bocavirus Aichivirus, sapovirus, salivirus and hepatitis E virus were only sporadically observed (1/12 each, 8%). Fifty percent (6/12) of the samples tested positive for the non-pathogenic pepper mild mottle virus. Most river and lagoon water samples sho-

wed multiple contamination (up to 7 positives in the same sample). Only three ground water samples revealed single contamination for norovirus (2/7, 28%) and enterovirus (1/7, 14%). All the tested drinking water samples were found uncontaminated. The results of this short term study indicates that the advanced water supply network and wastewater treatment facilities operating in the area efficiently remove all viral pathogens after treatment, offering thus an excellent barrier for human health safety and environmental protection of water ecosystems.

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One-year monitoring of SARS-CoV-2 in urban sewage in Rome, Italy

G. La Rosa[2], G. Bonanno Ferraro[1], P. Mancini[1], C. Veneri[1], M. Iaconelli[1], L. Lucentini[1], L. Bonadonna[1], E. Suffredini[2]

[1] Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy - [2] Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) wastewater-based epidemiology (WBE) has been applied as a complementary tool for studying trends of COVID-19 cases and for early warning of the occurrence of infected populations. The European Commission asks the Member States to put in place wastewater surveillance systems and ensure that relevant data on the spread of the virus and its variants is promptly provided to the competent health authorities (Recommendation on monitoring COVID-19 and its variants in wastewaters in the EU, March 2021). In Italy, a national project is ongoing to study SARS-CoV-2 occurrence and concentration in urban wastewaters throughout the country. The present study shows results of one year monitoring of SARS-COV-2 in urban wastewaters collected in Rome, Italy.

A total of 301 sewage samples (250 mL, 24 hours composite raw sample) were collected between 1st April 2020 and 31 March 2021 in four major wastewater treatment plants (WTPs) of Rome. Samples were stored at −20 °C, and dispatched frozen to the lab for analysis. Before sample concentration, a viral inactivation procedure (56 °C for 30 min) was undertaken as a safety measure. Sample concentration was performed using the two-phase (PEG-dextran) separation method recommended by the WHO Guidelines for environmental surveillance of poliovirus circulation (WHO, 2003), with slight modifications. Nucleic acids were extracted with the MiniMag platform (bioMerieux) and SARS-CoV-2 detection and quantitation was performed using a real-time RT-qPCR targeting the ORF1ab (nsp14 region) of SARS-CoV-2 genome. Trend analysis was assessed using a 14-day rolling average for both the single WTP and for the whole city (i.e., considering the overall results of the four WTPs).

A total of 218 samples (72.4%) provided positive results for SARS-CoV-2, concentration ranging from below the quantification limit of 5.9×103 genome copies (g.c.)/L of sewage to 1.6×10^6 g.c./L. Positive results were equally distributed among the four WTPs (no statistically significant difference was detected: chi-quare test = 0.1395, *p*-value = 0.987). As regard to trends of SARS-CoV-2 concentrations, a first peak of concentration in sewage could be detected in correspondence of the first wave of COVID-19 pandemic (spring 2020), and was followed by a steady decrease of viral concentration between July and August 2020. Since September, an inversion of the trend was observed and an increase of SARS-CoV-2 concentration in sewage was recorded up to December 2020 (second wave of the pandemic). After that, a slight decrease of the signal was observed but viral concentrations remained substantially unaltered between the second half of January and March 2021, possibly in relation to high viral circulation in this period, following the emergence

of SARS-CoV-2 variants (UK and Brazilian) in Italy and in the city area.
WBE is an effective approach for monitoring SARS-CoV-2 temporal and geographical trends and a helpful complementary surveillance tool to guide public health response. Studies are ongoing for normalization of data by considering different variables (e.g. dilution effects due to climatic conditions, WTP flow rate etc), to improve correlation/matching between epidemiological data and SARS-COV-2 data in wastewater, to use the pilot study in implementing a national WBE system. This type of approach may also be applicable for surveillance of other viruses and pathogens in wastewaters.

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Fast, simple and cost-effective concentration method for SARS-CoV-2 detection in wastewaters

G. La Rosa[1], G. Bonanno Ferraro[1], P. Mancini[1], C. Veneri[1], M. Iaconelli[1], E. Nicosia[2], S. Rosatto[3], G. Allaria[3], E. Iervasi[4], I. Tomesani[3], L. Dondero[3], E. Grasselli[3], E. Poznanski[6], A. Stenico[6], A.M. Prast[6], M. Rossi[6], E. Suffredini[7]

[1] Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy - [2] ARPAL-Regione Liguria, Italy - [3] DISTAV-University of Genoa, Italy - [5] DIMES-University of Genoa, Italy - [6] Biological laboratory, Agency for Environment and Climate Protection of the Autonomous Province of Bolzano, Italy - [7] Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy

The coronavirus disease 2019 (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was declared a pandemic by the WHO on March 11, 2020. The wastewater-based epidemiology (WBE) constitutes a useful tool to forecast pandemic trend. For this reason, the EU published (17th March 2021) a recommendation to all Member States asking to adopt precise strategies aimed to monitor SARS-CoV-2 and its variants in wastewaters. The WBE constitutes a challenge, considering that RNA is extracted from a matrix with a particular composition because of the many sources of RNases and the great variability due to geography, population and treatment processes. The most critical step of this procedure consists in concentration process, allowing to reduce the wastewater volume for the following RNA extraction step. To this aim, many methods have been considered and published, nevertheless, given the high variability of the starting material, the validity of each method should be tested in the specific wastewater's context. Within the national project coordinated by Istituto Superiore di Sanità "Sorveglianza di SARS-CoV-2 in reflui urbani" (SARI) two different protocols for concentrating wastewaters were compared in three different territorial laboratories.

Seventy-five wastewater samples, collected in three different Italian regions between October 2020 and March 2021, were concentrated using: i) a two-phase (PEG-dextran) biphasic separation based on the WHO Guidelines for environmental surveillance of poliovirus circulation [1], performed on 250 mL; ii) a PEG precipitation based on high speed centrifugation, described by Wu and colleagues [2], with slight modifications, performed on 40 mL of sewage. Nucleic acids were extracted with the MiniMag NucliSens (bioMerieux) and analyzed by a real-time RT-PCR targeting the ORF1ab nsp14 region of SARS-CoV-2 genome [3].

Agreement of results between the two concentration methods was obtained in 43 samples (9 negative and 34 positive results). In the remaining tests, SARS-CoV-2 was detected either only by the biphasic separation method (3 samples) or with the modified Wu method (29 samples). The difference between the two methods was statistically significant (McNemar's test p value <0.0001). Among the 34 samples providing positive results, 25 displayed higher *Ct* values in samples prepared with the biphasic concentration and the average *Ct* difference between the biphasic and the Wu protocol was 2.14 cycles. Moreover, compared to the two-phase separation, the PEG precipitation by centrifugation is faster, simpler

to perform, and less expensive. The results of this study support the use of the modified PEG precipitation protocol with centrifugation for the concentration of wastewater samples for SARS-CoV-2 detection.

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P198 Monitoring enteric viruses, somatic coliphages, protozoa and *campylobacter*, in surface waters (Tiber river) for drinking water production in Rome

C. Veneri[1], P. Mancini[1], G. Bonanno Ferraro[1], M. Iaconelli[1], R. Briancesco[1], S. Della Libera[1], R. Paradiso[1], A.M. Coccia[1], L. Bonadonna[1], C. Ottaviano[2], M. Arizzi[2], M. Segatori[2], C. Ceci[3], A. Zitelli[3], G. La Rosa[1]

[1] Istituto Superiore di Sanità, Department of Environment and Health, Rome, Italy - [2] ACEA Elabori SpA, Engineering laboratories research innovation SpA - [3] ACEA-ATO-2 Spa

Rome's drinking water supply comes mostly from springs and wells. The water system, fed by the new Grottarossa plant for the treatment of water from the Tiber, has been developed to supply the drinking water network of the central areas of Rome only in case of emergency, by serving 350,000 inhabitants. The advanced treatment provided by the plant consists of different steps: uptake, flocculation and disinfection in mixing thank, sedimentation, filtration with sand filter and then activated carbon filters and finally post disinfection.
Laboratories of the water utilities routinely carry out analyses for the traditional bacterial indicators in water supplies, but other infectious agents such as viruses, parasitic and free living protozoa and particular pathogenic bacteria are not included in the drinking-water routine monitoring. The Water Safety Plan (WSP) is an approach to prevent and reduce risks associated with the drinking water service, assessing dangerous events along the entire water supply chain including from collection to distribution. As part of the WSP implemented in 2019 focused on the water system supplied by the new Grottarossa plant, a study was performed to investigate the occurrence of enteric viruses: norovirus (NoV), adenovirus (HAdV), enterovirus (EV) in the Tiber River, and viral removal after treatment. Contemporaneously, the following parameters were investigated by culture-based methods: somatic coliphages (UNI EN ISO10705-2:2001 and BS ISO 10705-3:2003), *Campylobacter* (ISO 179995:2019) and free-living amoebas (culture and microscopic examination) while the parasitic protozoa *Giardia* and *Cryptosporidium* were detected by an immunofluorescence microscopic method.
Thirteen river waters samples at the Tiber River, and 13 drinking water effluents from the new Grottarossa drinking water plant, near the storage facility, were collected during 2018-2020. Samples were filtered on site by Nanoceram electropositive cartridge and concentrated according to USEPA Method 1615. After extraction of nucleic acid using magnetic silica beads, viruses were detected by nested-PCR and by real-time PCR.
Both Genogroup I (GI) and genogroup II (GII) NoV were detected in river waters: 3 samples were positive for GI (capsid type GI.1), and 3 for GII (types GII.1, GII.4, and GII.8). HAdV were detected in 8 river water samples (61%), characterized as type 41 and type 12. Enteroviruses, *Giardia* and *Cryptosporidium* were detected in river waters respectively 69%, 54% and 39%. Enteric viruses, *Giardia* and *Cryptosporidium* were not detected in treated drinking water samples. Similarly, somatic coliphages were detected only in river water samples (100%) in concentration ranging from 40 to 1500 PFU/100 mL. Free-living amoebas were detected in all river waters and only in one treated water sample; a chlorine dioxide disinfection system failure was reported in conjunction with these findings. Both raw

and treated waters tested negative for the presence of *Campylobacter*.
Rivers are important for drinking water supply worldwide. However, they are often impacted by pathogen discharges via wastewater treatment plants, combined sewer overflows and surrounding urbanized and countryside areas. This study shows that although viruses and other pathogens may be present in even high concentrations in rivers, their presence can indeed be reduced, or even eliminated, with more advanced treatment processes at municipal drinking water treatment plants. Data obtained will also be useful for the elaboration of a WSP of the investigated water system to intercept critical issues and prevent them in order to safeguard the health of users.

P199

Minimal mutations of *citrus tristeza* virus P23 cause substantial changes in the phenotypic expression in specific citrus hosts

G. Licciardello[1], R. Ferraro[2], A.F. Catara[3]

[1] Council Agricultural Research and Economics (CREA) - Research Centre for Olive, Citrus and Tree fruit, 87036 Rende (CS), Italy - [2] Agrobiotech Soc. Coop. z.i. Blocco Palma I, Stradale Lancia 57, 95121 Catania, Italy - [3] Formerly, Department of Phytosanitary Science and Technologies, University of Catania, 95123 Catania, Italy

Citrus tristeza virus (CTV; genus Closterovirus, family *Closteroviridae*) is a flexuous filamentous virus transmitted in a semi-persistent manner by a few aphid species. Its +ssRNA genome (approximately 19.5 kb in length), includes twelve open reading frames (ORFs) flanked by 5'- and 3'- untranslated regions (UTRs). CTV infects citrus, causing decline of trees grafted on sour orange (SO) rootstock, seedling yellow (SY) of lemon and grapefruit, and stem pitting (SP) in grapefruit and sweet orange. Unexpectedly, SO trees on their own roots overcome the SY when naturally infected with CTV isolates. These isolates then induce decline on trees grafted on sour orange, and field growing sweet-on-sour aged trees showing signs of decline eventually recover after intensive pruning. To understand this phenomenon, we have studied the genetic structure of CTV SY and non-SY isolates in Sicily by sequencing some field isolates by high throughput sequencing (HTS) associated with molecular assays and extensive bioindexing. Our results show that some non-SY variants of the virus are naturally spread in citrus orchards. Others, closely related to the severe variant of the virus, protect the trees from a subsequent infection, as result of interactions between the genetic traits of the protecting and challenging viruses (Folimonova et al., 2020). Sequence comparisons revealed that the non-SY isolates differ from the prevalent SY isolate SG29 (severe) in terms of only eight non-silent point mutations. These were distributed on five ORFs (Orf1a, p33, p25, p18 and p23), and within one intergenic region between p33 and p6. Of these, four amino acid changes shared the same position. These included a single change from isoleucine to threonine at position 1589 of the Orf1A, two changes from alanine to valine and from glycine to alanine at positions 242 and 299, respectively, of the p33 and a single change from asparagine to serine at position 54 of the p23, reported as one of the three polymorphic regions that differentiate between mild and severe CTV isolates (Sambade et al., 2003; Ferraro et al., 2017). With regard to the replacement of two amino acids in p33 associated in the observed transition from SY of SG29 with the recovery stage of the mutated isolates, p33 has a major role in CTV-T36 infection of sour orange plants (Folimonova, 2012). It will thus be interesting to see whether the expected minimal changes play a role either in escaping cross protection from the parent genome or allowing the recovered SY isolates to effectively replicate in sour orange tissues. In conclusion, these results highlight that the substitution of Ser54 with Asn54 in the 50-54 region of the asymptomatic Sicilian is typical of mild recovered SY isolates and causes substantial changes in the phenotypic expression in specific hosts (Sambade et al, 2003; Ruiz-Ruiz et al, 2019). Overall, the recovery of some citrus genotypes, infected with CTV-SY, results from a genetic adaptation of the virus to overcome host resistance. In addition, the SY phenotype is determined by specific changes in p23, either directly or associated with specific host factors. Further studies are needed to clarify the role of p33, which affects the viral pathogenicity of CTV-T36 (Sun and Folimonova 2019).

P200
Isolation of new phages and biocontrol of plant bacterial diseases

G. Martino, M. Vallino, M. Turina, M. Ciuffo

Istituto per la Protezione Sostenibile delle Piante - Consiglio Nazionale delle Ricerche, Strada delle Cacce 73 10135, Torino, TO

Aim of the study. Phytopathogenic bacteria outbreaks are generally complicated by lack of effective bactericides and resistance development. The genus *Pseudomonas* includes the agents of the bacterial kiwifruit vine disease *(Pseudomonas syringae* pv. *actinidia*, *Psa)* and of the halo blight of bean *(Pseudomonas syringae* pv. *phaseolicola*, *Pph). Psa* can infect both *Actinidia deliciosa* and *A. chinensis* and is broadly spread in Italy and New Zealand, the two major production areas. *Pph* is prevalent worldwide. The use of antibiotics like streptomycin as pesticides is forbidden in Europe, where the only approach allowed routinely against bacterial diseases is copper treatment in high quantities. A possible alternative control method is the use of bacteriophages, which are viruses that infect specific bacterial targets causing their lysis. Phages have the advantages to be very specific and to leave no residues on plants. Phage cocktails allow a broader host-range and possible synergy between actions of different phages. Purpose of this work is to develop phage-based new biocontrol strategies to fight effectively *Psa* and *Pph* on kiwifruit and bean.
Method used. Bacteriophages were isolated from soil near infected bean and kiwifruit plants. Phages were preliminarily isolated and identified through double agar assays. The particles were observed through electron microscopy and the genetic material was sequenced through Illumina Miniseq. The obtained genomes were assembled and annotated through PATRIC platform, Artemis and a manual curation through Blastp. Phage detection in plants after treatment were performed with titration with double agar assay and RT-PCR amplification with specific primers.
Results and conclusions. We have already isolated bacterial strains from beans and kiwifruit mainly from Piedmont (Italy). At the same time, we have collected 13 lytic phage isolates active against Pph and 7 lytic phage isolates against Psa. A morphological observation of the plaques and of the capsid structures coupled with a molecular characterization revealed that we have isolated one novel species of Pph phage, phiB1_1(*Autographiviridae*) and four species of Psa phages, two of which are novel to literature, phiK7A1 (*Myoviridae*) and phiK7B1 (*Siphoviridae*). The annotation of these three phages did not reveal the presence of genes associated to lysogeny, bacterial virulence or toxins, so they are eligible for possible biocontrol strategies.
Isolated phages were tested for stability to abiotic stresses, as temperature, pH and UV-C exposition. For treatment of the halo blight of bean we are testing the application of the phage phiB1_1 via irrigation. Regarding the kiwifruit bacterial canker, in addition to irrigation we are testing a trunk infusion delivery approach to reach the bacteria in the xylem. For both patho-systems a delivery system through non-pathogenic carrier bacteria is also under evaluation. We are performing tests with phage phiK7B1 and not pathogenic strains of *Pseudomonas syringae* pv. syringae, to assess the difference in persistence of the phage on the leaves surface, with or without carrier bacteria.
This work will be useful for an estimation of a future commercialization of phage-based products/techniques against *Psa* and *Pph*, and more generally, plant bacterial diseases.

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P201
Viroid-like RNAs containing hammerhead ribozymes associated with fig trees

B. Navarro[1], A. Olmedo-Velarde[2], J.S. Hu[2], M.J. Melzer[2] and F. Di Serio[1]

[1] Istituto per la Protezione Sostenibile delle Piante, Consiglio Nazionale delle Ricerche, Bari, Italy - [2] Plant and Environmental Protection Sciences, University of Hawai'i at Mānoa, Honolulu, HI, United States

Viroids and viroid-like satellite RNAs are infectious non-coding RNAs reported from plants. On the one hand from a biological point of view, they differ from each other: while infectivity of viroid-like RNAs depend on a co-infecting helper virus, viroids are autonomous in this respect. On the other hand, some viroids and viroid-like satellite RNAs share many structural features, including the presence in both RNA polarity strands of hammerhead ribozymes involved in replication. Analysis of high-throughput sequencing (HTS) data revealed the possible presence of small circular RNAs, with typical features of viroids and viroid-like RNAs, in fig trees grown in the Hawaiian Islands. Cloning and sequencing of the corresponding amplified cDNAs confirmed the existence of at least one of these RNAs and showed that it is circular, has a size of 357-360 nucleotides, contains a hammerhead ribozyme (HRz) in each polarity strand, and has very low sequence identity with previously reported sequences in databases. The tentative name fig hammerhead viroid-like RNA (FHVd-LR) has been proposed for this RNA associated with fig trees. Circular forms were detected by Northern-blot hybridization assays that also showed that one of the two strands, designed as (+) polarity, accumulated at higher level in vivo. The RNA self-cleaving activity during transcription, mediated by the hammerhead ribozymes contained in both polarity strands of FHVd-LR, was confirmed *in vitro*. Sequence variability of FHVd-LR was also studied, showing that this viroid-like RNA has the typical features of quasi-species. Although FHVd-LR has been detected by RT-PCR in RNA preparations from several fig trees grown in Hawaii, bioassays aimed to confirm its autonomous infectivity failed so far. Therefore, the possibility that FHVd-LR would be a viroid-like satellite RNA cannot be dismissed. At the same time whether fig or an organism associated with fig is the actual host of FHVd-LR needs further investigation.

P202

SARS-CoV-2 and Coronavirus 229E detection in atmospheric particulate matter

A. Pivato[1], G. Formenton[2], F. Di Maria[3], T. Baldovin[4], I. Amoruso[4], T. Bonato[5], P. Mancini[6], G. Bonanno Ferraro[6], C. Veneri[6], M. Iaconelli[6], L. Bonadonna[6], T. Vicenza[7], E. Suffredini[7], G. La Rosa[6]

[1] Department of Civil, Environmental and Architectural Engineering (ICEA), University of Padua, Italy - [2] Environmental Agency of Veneto Region (ARPAV), Mestre (VE), Italy - [3] LAR5 Laboratory – Dipartimento di Ingegneria – University of Perugia, Perugia, Italy - [4] Department of Cardiac, Thoracic, Vascular Sciences and Public Health, Hygiene and Public Health Unit, University of Padua, Italy - [5] Società Estense Servizi Ambientali (S.E.S.A. S.p.A.), Este (PD), Italy - [6] Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy - [7] Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a respiratory virus transmitted through droplets emission. Sneezing, coughing, speaking, etc. contributes to the emission of the virus from infected subjects, and disseminates the viral particles with the developed bioaerosol. The analysis of airborne particles has been recently suggested as another useful tool for detecting the presence of SARS-CoV-2 in given areas (Anand et al., 2021). Based on this evidence, the present study aims to investigate SARS-CoV-2 and Coronavirus 229E occurrence in atmospheric PM. A total of 38 air filter samples were included in the study. Thirty-two were taken in compliance with the standard EN 12341:2014 whereas 6 were taken with a high volumetric rate sampler *(i.e.* 500 l/min) (Techora/Echo PM Hi Vol) using Teflon filters with 3 µm porosity hydrophobic PTFE, 142 mm.

Sampling was performed in the province of Venice (Italy) between 21.02.2020 and 08.03.2020 (*n*=16, 7 on 47 mm quartz fiber filters for PM_{10} and 9 on 47 mm quartz fiber filters for $PM_{2.5}$) and between 27.10.2020 and 25.11.2020 (*n*=22, 6 on Teflon filters and 16 on quartz fiber filters for $PM_{2.5}$). To assess viral recovery, filters were spiked with 100 µl of a process control virus (Mengovirus) and left to dry. Filters were then transferred to an extraction tube containing 2 ml of lysis buffer (30 min incubation on an orbital shaker followed by a brief centrifugation) and RNA was extracted with the NucliSENS miniMAG semi-automated extraction system (bioMerieux), followed by purification with the OneStep PCR Inhibitor Removal Kit (Zymo Research). Samples were then tested with previously developed real-time RT-PCR for the alfa-Coronavirus 229E and for SARS-CoV-2 (ORF1ab, nsp14 region). A total of 14 samples were positive for Coronavirus 229E, 11 of which collected between October and November 2020 (11 positives on 22 samples, 50%) and 3 collected in February and March 2020 (3 positives on 16 samples, 19%). Seven samples were positive for SARS-CoV-2 genome, concentration ranging from 4.9×10^2 to 2.7×10^3 genome copies/filter with an estimated concentration in air of about 10 genome copies/m^3. SARS-CoV-2 detection was prevalently associated to high volume rate samplers using Teflon filters collected in November 2020 (4 positive samples).

These findings suggested that the analysis of outdoor particulate matter, if properly performed, can represent another possible tool for the surveillance of communities. Finally, it is also worth to mention that the SARS-CoV-2 concentration in outdoor atmo-

sphere, when detected, is very low indicating low potential probability of contributing to the spreading of the infection; the survival of SARS-CoV-2 in outdoor conditions and on PM is limited.

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P203

The International Committee on Taxonomy of viruses (ICTV) adopts a binomial nomenclature for virus species

L. Rubino[1], S. Sabanadzovic[2], F. M. Zerbini[3]

[1] Istituto per la Protezione Sostenibile delle Piante, Consiglio Nazionale delle Ricerche, Bari, Italy - [2] Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, Mississippi State University, Mississippi State, Mississippi, USA - [3] Departamento de Fitopatologia, Instituto de Biotecnologia Aplicada à Agropecuária, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil

The International Committee on Taxonomy of Viruses (ICTV), formerly the International Committee on Nomenclature of Viruses, founded in 1966, is responsible for developing and maintaining an internationally agreed system of hierarchical classification of viruses and naming of taxa. The advancements in virus taxonomy are made available to the scientific community through a website (https://talk.ictvonline.org) and published reports/papers.

Over the years the ICTV has adapted its taxonomic framework to reflect current knowledge on the evolution of global virosphere. In particular, the ICTV now allows the classification of viruses known only from genomic data. Taxonomic ranks have been expanded beyond orders, paving the way to comprehensive studies on evolutionary connections of viruses. A "megataxonomy" approach was established, unifying all currently classified RNA viruses in the realm *Riboviria*, whereas ssDNA and dsDNA viruses were afterwards classified into the realms *Monodnaviria*, *Varidanaviria* and *Duplodnaviria*.

The expansion of taxonomic ranks brings virus taxonomy closer to other biological taxonomies. Species naming in all these taxonomies follow a Latinized binomial format (i.e., binomial nomenclature) first introduced by Carl Linnaeus in 1753, consisting of two italicized words indicating the genus ("genus name") and the species ("specific name/species epithet"), respectively. Typical examples of binomial species names are *Arabidopsis thaliana*, *Saccharomyces cerevisiae*, *Homo sapiens*, and *Escherichia coli*. In contrast, viral species names did not follow a uniform format, except for a requirement to be italicized and to have the first letter of the first word capitalized. The ICTV recognized the need for a standardized nomenclature and, after years of internal discussions, in 2020 launched a call for public discussion and feedback on adopting a uniform binomial momenclature.

Following open debates, a binomial nomenclature for virus species has been ratified by the ICTV in the 2021 vote. Thus, a virus species name will consist of two (and only two) italicized words, the first one being the genus name and the second one consisting of a "free-form" species epithet. In this framework, Linnaean-style, Latinized virus species are permitted, but not mandated. The species names can be composed using the standard Latin-script English alphabet containing 26 letters and/or Arabic numbers.

Importantly, this change in nomenclature will apply to virus species only. Common virus names (for example human immunodeficiency virus 1, tobacco mosaic virus, grapevine virus A, etc) will not be affected and will remain unchanged.

P204

The interplay between carnation italian ringspot virus p36 replicase protein and mitochondria alters the mitochondrial function in yeast cells

L. Rubino[1], G. Petrosillo[2], D. Marzulli[2], A. Antonacci[1], A. De Stradis[1], S. Giannattasio[2]

[1] CNR, Istituto per la Protezione Sostenibile delle Piante, UOS Bari - [2] CNR, Istituto di Biomembrane, Bioenergetica e Biotecnologie Molecolari, Bari

Positive-strand RNA [(+)RNA] viruses, which are agents of important diseases in humans, animals and plants, depend on the host endomembrane system for their replication. (+)RNA virus invariably replicate in association with specific host cell membranes, which are extensively rearranged to form partially enclosed vesicular enclaves, constituting the virus replication site. Carnation Italian ringspot virus (CIRV) genomic (+)RNA replication occurs on the mitochondrial outer membrane which is induced to proliferate and invaginates to produce numerous vesicles between the inner and outer membrane. CIRV p36 protein is required for targeting and anchoring the virus replication complex to the mitochondrial outer membrane in plant and when ectopically expressed in *Saccharomyces cerevisiae*. In yeast cells, CIRV p36 is able to increase necrotic cell death and concomitantly decrease regulated cell death in response to acetic acid.
p36-mitochondria interaction was analyzed in *S. cerevisiae* cells expressing CIRV p36 under the control of the inducible *GAL1* promoter. p36-expressing cell growth on a non-fermentable carbon source was negatively affected, compared to that of control cells, indicating that CIRV p36 ectopic expression decreased respiratory yeast cell growth. Accordingly, the viral protein effect was more striking at temperatures favoring p36 expression. Confocal and electron microscopy analyses demonstrated that p36 expression in yeast cells altered the mitochondrial network. Measuring several parameters of mitochondrial function showed that heterologous expression of p36 decreased oxygen consumption in yeast cells due to respiratory chain complex impairment. Basal respiration rate and CCCP-stimulated maximal respiration rate were dramatically reduced in p36-expressing yeast cells as compared with control cells. Similar results were obtained in ADP-stimulated state 3 respiration measured in freshly isolated mitochondria, using succinate as a substrate. A significant reduction of the activity of complexes II + III and IV was observed in yeast.
Immunoblot analysis of either whole cell lysates or cell membrane-enriched fractions from p36-expressing or control cells showed that mitochondrial proteins are not affected by p36 overexpression, since the level of marker proteins of mitochondrial matrix, inner and outer membrane was not changed by p36 expression. These data suggest that p36 alters mitochondrial function, without affecting mitochondrial biogenesis in yeast.

P205

Survey for citrus tristeza virus in the region of Apulia, Southern Italy

L. Manco[1], P. Pollastro[1], M.R. Silletti[1], D. Angione[1], D. Valenzano[1], A. Percoco[2], V.N. Fornarelli[2], V. Cavalieri[1], F. Palmisano[1], M. Saponari[3], G. Loconsole[3]

[1] Centro di Ricerca, Sperimentazione e Formazione in Agricoltura "Basile Caramia" - [2] Regione Puglia, Area Politiche per lo Sviluppo Rurale, Osservatorio Fitosanitario Regionale - [3] Institute for Sustainable Plant Protection, National Research Concil, Italy

Citrus Tristeza Virus (CTV) is one the most important viruses infecting citrus. Although the negative impact of CTV-induced diseases is mitigated by the use virus-tolerant/resistant rootstocks, the spread of severe strains of the virus is of major concern for all Mediterranean citrus-growing areas. As recently, published by the European Food Safety Agency, although the virus is widespread in several EU/Mediterranean Countries where citrus is present, strains associated with severe field syndrome or strains overcoming the resistance of the tolerant/resistant rootstock (the so called resistant-breaking strains) are rare or never reported in the Mediterranean basin. As such, these strains have been categorized as "non- EU" strains, for which the conditions foreseen in the EU Regulation 2031/2016 apply (i.e. mandatory monitoring programs and eradication measures). In Apulia region, citrus are one of the major crops, represented by a wide panel of species and several local cultivars. In 2020, in compliance with the new EU Regulation an extensive monitoring program has been implemented, targeting the virus and its quarantine insect vector (the aphid Toxoptera citricidus). Surveys in the field included visual inspections and sampling using the scheme designed by Gottwald and Hughes (2000). Sampling was performed in spring and autumn by collecting 4 twigs from each tree. First screening tests included serological tests using broad-spectrum antisera, namely ELISA "Enzyme-Linked Immunosorbent Assay" and DTBIA "Direct Tissue ImmunoBlot Assay". A total of 400 hectares were inspected and more than 7000 samples collected and tested in ELISA or DTBIA, resulting in approx.. 650 positive samples. Among this panel of CTV-positive samples, samples representing different species/orchards/age of the trees, were re- tested by real time PCR to assess the presence of non-EU strains. Briefly, a strain specific real time PCR assay was used to detect isolates of the virus genetically related to strains harbouring VT, T3, T68 (commonly associated to biologically severe strains) and RB genotypes (associated to resistant-breaking strains) (Yokomi et al., 2010). None of the real time PCR tests yielded positive reactions, indicating that the infections detected in the monitored citrus- growing area were not associated to strains categorized as non-EU strains, and more specifically to strains potentially inducing severe syndromes in biological tests (i.e. stem pitting, seedling yellow, etc). Nonetheless, the occurrence of the virus in this area where sour orange, in the highly susceptible combination with sweet orange, is still the predominant rootstock, raise concern for the old orchards, where phenomena of tree decline can be observed. No specimens of T. citricidus were identified on any of the surveyed orchards where aphid's infestations were recorded. Gottwald T.R., Hughes G., 2000. A new survey method for citrus tristeza virus disease assessment. Proc. of the 14th Conf. of the Int. Org. of Citrus Virologists IOCV: 77–87. Yokomi R. K., Saponari M., and P. J. Sieburth P. J. (2010). Rapid Differentiation and Identification of Potential Severe Strains of Citrus tristeza virus by Real- Time Reverse Transcription-Polymerase Chain Reaction Assays. Phytopathology, 100 (4) : .319-327.

P206

Effect of onion yellow dwarf virus infection on the composition of volatile organic fraction in "Rossa di Tropea" onion

A. Taglienti[1], F. Araniti[2], A. Piscopo[3], A. Tiberini[1]

[1] Research Centre for Plant Protection and Certification, Council for Agricultural Research and Economics, Via C.G. Bertero 22, 00156 Rome, Italy - [2] Department of Agricultural and Environmental Sciences (DISAA), University of Milan, Via Celoria, 2, 20133 Milan, Italy - [3] Department of Agricultural Science, University 'Mediterranea' of Reggio Calabria, loc. Feo di Vito, 89122 Reggio Calabria, Italy

'Rossa di Tropea' onion is a particular onion cultivar cropped in Calabria region (Southern Italy), characterized by pink/red coloured bulbs, worldwide known for their organoleptic features as mild to sweet flavour, representing one of the Italian most important vegetable crops and granted with Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI) trademarks. OYDV (genus *Potyvirus*, family *Potyviridae*, ssRNA (+)) has been reported to be the most widely spread virus in onion and *Allium* spp., also found in 'Rossa di Tropea' and causing detrimental effects on crop yield and bulb quality. This virus represents the most limiting biotic stressor in onion, inducing severe symptoms as yellowing, dwarfing and stem twirling. Several studies address the effect of virus interference on host cellular metabolism and biosynthetic pathways; in particular, volatile organic compounds (VOCs) contribute to overall sensory characteristics of food products, often determining their acceptability. Hence, the impact of virus infection on the presence of such compounds may compromise bulb's sensory quality. This work aims at investigating the effect of OYDV infection on the organoleptic properties in onion, mainly focusing on the determination of VOCs profile and its evolution during postharvest storage, and on sensory analysis.

Plant material (i.e., onion bulbs) was obtained from an experimental trial involving 180 plants organized in 6 randomized blocks, 3 healthy and 3 OYDV-infected, respectively. Samples were collected at two analytical time points, mirroring the 'Rossa di Tropea' onion bulb production steps: at harvesting time (t0), i.e., at the complete development of plants and bulbs, and after storage (t2), i.e., 3 months after harvesting, when the bulbs reach the marketable stage. Each time point was represented by collecting three biological replicates.

The VOCs produced by healthy and OYDV-infected bulbs were chemically characterized using a HS/SPME GC-MS method; relative quantitation of these compounds was also accomplished by evaluating the relative percentage for each peak area over the total ion chromatogram (TIC) area. Statistical analysis, both univariate and multivariate, was applied for highlighting significant differences among groups, observing clustering of samples based on phytosanitary status and sampling time, and identifying the most responsible compounds for such classification. Sensory analysis by means of a panel test was also performed on samples at t_2 in order to compare the perceived attributes of healthy and infected bulbs and to correlate them to the VOCs profile.

Seven out of 11 quantified VOCs were observed to undergo regulation in infected bulbs with respect to healthy control at t_0 or t_2; some were significantly underrepresented, while others were upregulated upon infection. The multivariate models based on Partial

Least Squares Discriminant Analysis (PLS-DA) and Hierarchical Clustering Analysis (HCA) showed a degree of separation between groups, according to phytosanitary status and sampling time; particularly remarkable differences between healthy and infected samples were observed at t2. The sensory analysis indicated some descriptors to be significantly different between healthy and infected samples; interestingly, among the general attributes of acceptability, texture rating was remarkably higher in healthy onions than infected ones. This can be correlated with some effect of infection to the vegetable tissues, since viral infection is know to induce structural alteration starting from the cell wall.

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Biological and molecylar characterization of cryphonectria ambivirus 1, type member of a recently characterized orfan RNA virus clade with unprecedented genome organization

M. Funmilayo Akinyuwa[1], M. Forgia[3], D. Bulgari[2], E. Gobbi[2], M. Turina[3]

[1] University of Padua, Via 8 Febbraio, 2 - 35122 Padova (PD) - [2] Agri-food and Environmental Microbiology Platform (PiMiAA), Department of Molecular and Translational Medicine, University of Brescia, Brescia 25123, Italy - [3] IPSP-CNR, Strada delle Cacce 73 10135, Torino, TO

Aim of the study. Ambivirus are a group of viruses that we have recently characterized infecting a number of fungal species, among which *Cryphonectria parasitica*, a model system for mycovirus-host interactions and the causal agent of chestnut blight. Ambivirus have an unprecedented genome organization: a circa 5 kb fragment encoding for two ORFs in ambisense orientation (ORF-A and ORF-B). ORF-A is more conserved among different ambivirus species and has some (but not all) of the conserved domains generally associated to an RNA dependent RNA polymerase (RdRP). Both plus and minus strand accumulate abundantly during replication, and in some species, a dimer of the genome is often the most abundant RNA species that accumulates in vivo. The virus does not seem to be encapsidated. Cryphonectria ambivirus 1 (CpAV1) is among the first ambivirus we have isolated. Aim of this study is the evaluation of the molecular and biological effects of ambivirus infection on *C. parasitica* life cycle and on its ability to cause disease on chestnut.
Methods. In order to evaluate the biological effect of the virus on his host, a first step was obtaining isogenic virus-infected and virus free strains: for this purpose, monoconidial isolates were obtained and screened for virus presence by qRT-PCR. We then proceeded to analyse possible biological effects on a number of different growth conditions and abiotic stress: high temperature (35°C), low temperature (15°), high salt (3%), conidia production, stromatal pustules on wood, virulence on apples and chestnut cuttings. The saprophytic qualities of ambivirus-infected, and ambivirus-free *C. parasitica* on digestate substrate are also under evaluation. We also wanted to determine the full-length sequence performing 5' and 3' RACE on the infected isolate. RNAseq for DGE analysis are also being performed.
Results and conclusions. So far, we can not reveal any specific phenotype strictly associated to the presence-absence of the virus, with the exception of some effect on the lesion size caused on apples. Surprisingly RACE revealed that 5' and 3' UTR have a circa 250nt long terminal repeat in the same orientation: this feature is unprecedented among RNA viruses. The ongoing molecular and biological characterization will rely on sRNA and DGE characterization and attempts at assembling an infectious cDNA clone.

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