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DI VIROLOGIA
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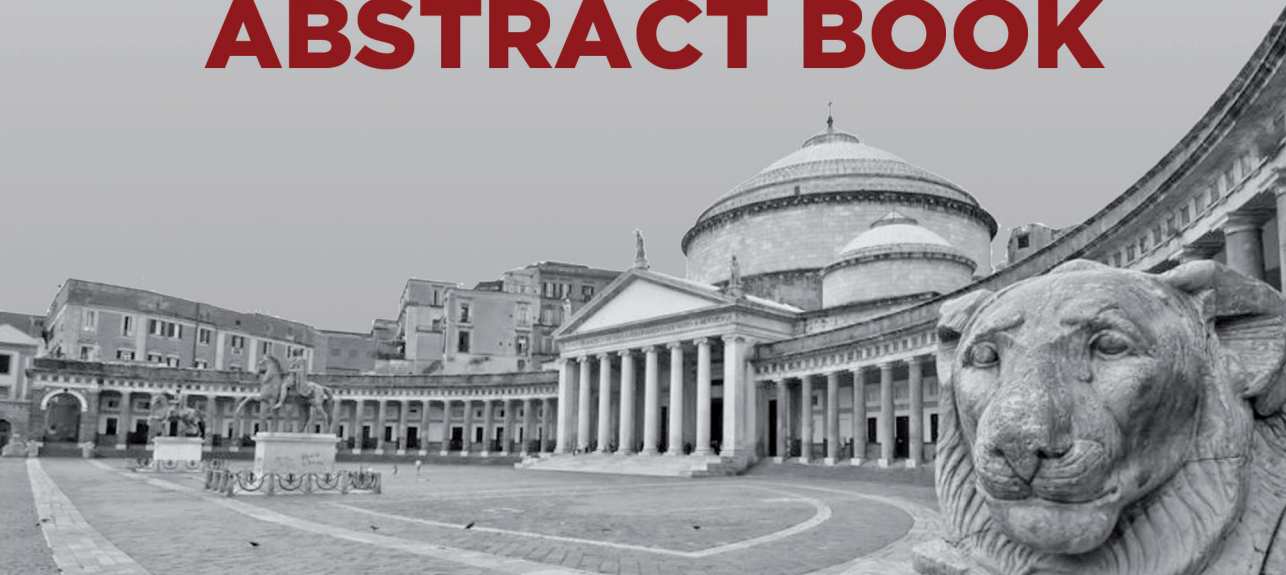
6th National Congress of the Italian Society for Virology

One Virology One Health

**Naples
July 3-5, 2022**

Hotel Royal Continental
Via Partenope, 38

ABSTRACT BOOK



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Maurizio ZAZZI, Siena, Italy
Francisco Murilo ZERBINI, Viçosa, Brazil (ICTV President)

Sunday July 3th, 2022

OPENING OF POSTER SESSION AND SPONSOR EXHIBITION

From 12.00 Registration of participants

13.15-14.00 Welcome addresses

Arnaldo Caruso, President SIV-ISV

Andrea Costa, Ministry of Health, Undersecretary of State

Greetings from local Authorities

PLENARY SESSION

Auditorium

SESSION 1 COVID-19: two years within a pandemic

Chairs: **Guido Antonelli** (Rome), **Arnaldo Caruso** (Brescia)

14.00-14.30 The pandemic through the eyes of the ISS

Paola Stefanelli (Rome)

14.30-15.00 Clinical management of COVID-19: un update

Claudio Mastroianni (Rome)

15.00-15.30 SARS-CoV-2 infection in children: Much information still missing

Carlo Federico Perno (Rome)

15.30-16.00 Long Covid: mutation of concern and transcriptional analysis

Davide Cacchiarelli (Naples)

16.00-16.30 *Coffee Break*

Sunday July 3th, 2022

PARALLEL SESSIONS	
Auditorium	Mirabilis
SESSION 2 Veterinary Virology Chairs: Vito Martella (Bari), Gabriele Vaccari (Rome)	SESSION 3 Diagnostics in human virology Chairs: Rossana Cavallo (Turin), Tiziana Lazzarotto (Bologna)
<u>16.30-16.50</u> The changing horizons of virology in cats Barbara Di Martino (Teramo)	<u>16.30-16.50</u> Antiviral drug resistance of human cytomegalovirus in immunocompromised patients Irene Cassaniti (Pavia)
<u>16.50-17.10</u> Food virology: a winding road balancing innovation and standardization Elisabetta Suffredini (Rome)	<u>16.50-17.10</u> Role of cellular immune response in viral diagnosis Cristina Costa (Turin)
<u>17.10-17.30</u> Avian Influenza - An expanding global threat Isabelle Monne (Padua)	<u>17.10-17.30</u> Strategies for testing non-SARS-CoV-2 respiratory viruses Alessandra Pierangeli (Rome)
<u>17.30-17.45</u> OC1: Detection of a recombinant torovirus in cattle Francesco Pellegrini (Bari)	<u>17.30-17.45</u> OC5: Molecular epidemiology of RSV strains causing an unusual peak of hospitalization in autumn 2021 Matteo Fracella (Rome)
<u>17.45-18.00</u> OC2: Peptides modelled on the glycoprotein Gn and Gc of Schmallenberg virus inhibit viral infectivity Carla Zannella (Naples)	<u>17.45-18.00</u> OC6: Chromosomally integrated human herpesvirus 6: laboratory and clinical features Liliana Gabrielli (Bologna)
<u>18.00-18.15</u> OC3: Circular single-stranded DNA viruses in cats Violetta Iris Vasinioti (Bari)	<u>18.00-18.15</u> OC7: Detection of measles specific IgM/IgG by in house elisa based on recombinant n protein Maedeh Kojouri (Rome)
<u>18.15-18.30</u> OC4: Detection and characterization of bopiviruses in domestic and wild ruminants, Italy Andrea Palombieri (Teramo)	<u>18.15-18.30</u> OC8: ToRCH agents retrospective 10-years seroprevalence survey in women of childbearing age in Sicily Emilia Palazzotto (Palermo)

PARALLEL SESSIONS

Auditorium	Mirabilis
18.30-19.30 <i>With the unrestricted educational grant of COPAN ITALIA</i> Advancements of sample collection and transport in virology Self-collection for HPV Screening: new approaches for the prevention of cervical cancer Clementina Elvezia Cocuzza (Milan) Detecting CMV shedding in pregnant women and newborns: are all swabs created equal? NgeeKeong Tan (London) The challenges of managing the COVID-19 pandemic pre-analytic to diagnosis Paolo Gaibani (Bologna)	18.30-19.00 <i>With the unrestricted educational grant of ELITECHGROUP</i> Chair: Carlo Federico Perno (Rome) Monitoring of cytomegalovirus infection in hematopoietic stem cell transplantation recipients undergoing letermovir prophylaxis Tiziana Lazzarotto (Bologna) 19.00-19.30 <i>With the unrestricted educational grant of DIASORIN</i> (No CME accreditation) A novel diagnostic approach to differentiate Viral from Bacterial Infections Giancarlo Giaccone (Saluggia)

Monday July 4th, 2022

OPENING OF POSTER SESSION AND SPONSOR EXHIBITION

PLENARY SESSION

Auditorium

SESSION 4 Virus-host interaction

Chairs: **Giorgio Gribaudo** (Turin), **Arianna Calistri** (Padua)

08.30-08.50 HSV entry: from virus engineering to preclinical models
Tatiana Gianni (Bologna)

08.50-09.10 Viral proteins knocking at the nuclear door: a tale of importins
Gualtiero Alvisi (Padua)

09.10-09.30 Toscana Virus and Antagonism to the innate immune response
Maria Grazia Cusi (Siena)

09.30-09.45 **OC9:** Transcriptionally active HBV integrations frequently occur in the early phases of chronic infection and mostly involve genetic regions crucial for cell proliferation
Stefano D'Anna (Rome)

09.45-10.00 **OC10:** Effect of SARS-CoV-2 on the coagulation cascade in COVID-19 associated coagulopathies
Daria Bortolotti (Ferrara)

10.00-10.15 **OC11:** IFI16 impacts metabolic reprogramming during human cytomegalovirus infection
Valentina Dell'Oste (Turin)

10.15-10.30 **OC12:** A complex signaling pathway involving NF- κ B, JAK/STAT, and gp130 is activated by CCL2 neutralization to induce the expression of HIV-1 restriction factors in primary human macrophages
Iole Farina (Rome)

PARALLEL SESSIONS	
Auditorium	Mirabilis
SESSION 5 COVID-19 Diagnosis: novel perspectives Chairs: Massimo Clementi (Milan), Fabrizio Maggi (Varese) <u>10.30-10.50</u> Upper respiratory tract and viral pathogens: what did we learn from SARS-CoV-2 infection? Nicasio Mancini (Milan) <u>10.50-11.10</u> A rapid and scalable PCR-based test to detect SARS-CoV-2 cellular immunity administration Cristina Lapucci (Monza) <u>11.10-11.30</u> Cell-intrinsic immunity to sense SARS-CoV-2 infection Roberta Rizzo (Ferrara) <u>11.30-11.45</u> OC13: Performance of home-made whole blood stimulation assays for the quantification of SARS-CoV-2 specific T-cell response: a cross-sectional study Federica Bergami (Pavia) <u>11.45-12.00</u> OC14: Interferon-gamma levels in umbilical cord blood of newborns from SARS-CoV-2 affected mothers Evelina La Civita (Naples) <u>12.00-12.15</u> OC15: Monitoring of humoral response in healthcare workers receiving Biontech/Pfizer BNT162B2 mRNA vaccination Roberta Mancuso (Milan) <u>12.15-12.30</u> OC16: Human endogenous retroviruses (HERVs) transcriptome in PBMC is modulated during SARS-CoV-2 infection and allows to discriminate COVID-19 clinical stages Nicole Grandi (Cagliari)	SESSION 6 Viral Vectors and gene therapy Chairs: Giuseppe Portella (Naples), Maria Cristina Parolin (Padua) <u>10.30-10.55</u> Development of next generation of tumour-targeted replicating oncolytic viruses using an ideal immunocompetent animal model Yaohe Wang (London) <u>10.55-11.20</u> Gene therapy of X-linked Myotubular Myopathy by systemic AAV vector Fulvio Mavilio (Modena) <u>11.20-11.45</u> Vector genome integration and gene therapy: friends or foes Pasquale Piccolo (Naples) <u>11.45-12.00</u> OC17: Synergism of the oncolytic adenovirus dl922-947 and G-quadruplex binder pyridostatin against breast cancer Fabiana Napolitano (Naples) <u>12.00-12.15</u> OC18: An improved sequencing approach to fully profile SARS-CoV-2 pandemics at regional level, identify new variants and describe infection signatures in patients Antonio Grimaldi (Pozzuoli)

Auditorium
<u>12.30-13.15</u> SIV-ISV MEMBERS' MEETING Available from 12.30 to 14.00 Lunch and Poster viewing

Monday July 4th, 2022

PLENARY SESSION

Auditorium

SESSION 7 Structural virology and biotechnology

Chairs: **Giorgio Palù** (Padua), **Massimiliano Galdiero** (Naples)

- 14.00-14.20 On the mechanism of antibody neutralization targeting the receptor binding domain of the SARS-CoV-2 spike protein
Felix Rey (Paris)
- 14.20-14.40 The potential of electron microscopy in understanding virus-host interactions: A study of HCMV morphogenesis
Clarissa Read (Ulma)
- 14.40-15.00 Structure-based drug design for antiviral drug discovery
Arianna Loregian (Padua)
- 15.00-15.15 **OC19:** Molecular and structural characterization of SARS-CoV-2-induced cellular remodeling
Mirko Cortese (Pozzuoli)
- 15.15-15.30 **OC20:** A small-molecule inhibitor of human papillomavirus E7 oncoprotein rescues cellular PTPN14 levels and possesses antitumoral activity
Chiara Bertagnin (Padua)
- 15.30-15.45 **OC21:** Structural characterization of HIV-1 matrix protein p17 variants to develop specific functional inhibitors
Alessandro Rondina (Brescia)
- 15.45-16.30 *Coffee Break*

PARALLEL SESSIONS

Auditorium	Mirabilis
SESSION 8 Viruses and Cancer Chairs: Roberto Accolla (Varese), Franco M. Buonaguro (Naples)	SESSION 9 Environmental and plant virology Chairs: Francesco Di Serio (Bari), Luisa Rubino (Bari)
16.30-16.50 Role of human papillomaviruses in carcinogenesis Massimo Tommasino (Bari)	16.30-17.00 At the interface of host-virus interactions: cleavage of plant proteins by a potyvirus protease and potential applications for novel antiviral strategies Hélène Sanfaçon (Summerland)
16.50-17.10 HTLV-1 retrovirus infection and the road to Adult T-cell Leukaemia Roberto Accolla (Varese)	17.00-17.30 A virus by any other name: The new binomial nomenclature for virus species Francisco Murilo Zerbini (Viçosa)
17.10-17.30 Convergence between viral and tumor antigens Luigi Buonaguro (Naples)	17.30-17.45 OC26: Ambivirus: a new Baltimore class of viruses? Massimo Turina (Turin)
17.30-17.45 OC22: Dissecting the basis of herpesvirus associated proliferative skin diseases in lower vertebrates. A model for DNA-virus associated proliferative and neoplastic diseases Francesco Origi (Bern, Switzerland)	17.45-18.00 OC27: Multipartite BNYVV genomic RNAs change their relative frequencies according to the infected host and organ and after transmission in the vector Yi Guo (Bologna)
17.45-18.00 OC23: First detection of a cluster of sexually transmitted HIV-1 mutants expressing a B-cell clonogenic variant of p17 Alberto Zani (Brescia)	18.00-18.15 OC28: Enteric viruses circulation in the environment and their occurrence in cases of infantile gastroenteritis Maria Grazia Amoroso (Portici)
18.00-18.15 OC24: The enrichment of positively charged amino acids in HBsAg C-terminus impairs HBsAg secretion, affects its structural stability and is correlated with HBV-induced liver cancer Lorenzo Piermatteo (Rome)	18.15-18.30 OC29: Tracking SARS-CoV-2 variants in Italy (October 2021-March 2022) using the national wastewater-based surveillance system Giusy Bonanno Ferraro (Rome)
18.15-18.30 OC25: The endogenous HBZ interactome in ATL leukemic cells reveals an unprecedented complexity of host interacting partners involved in RNA splicing Mariam Shallak (Varese)	18.30-18.45 OC30: Cutting edge application of Raman spectroscopy for the diagnosis of virus infection in two major crops, tomato and grapevine Emanuela Noris (Turin)

PLENARY SESSION

Auditorium

Chairs:	Plenary Lecture Arnaldo Caruso (Brescia), Franco M. Buonaguro (Naples)
18.45-19.15	Role of RNA viruses in human diseases - Robert C. Gallo (Baltimore)
20.30	Social Dinner

Tuesday July 5th, 2022

PLENARY SESSION

Auditorium

SESSION 10 Infection, Immunity and Vaccines

Chairs: **Fausto Baldanti** (Pavia), **Mauro Pistello** (Pisa)

08.30-08.50 Advances in Vaccine Technologies
Mauro Pistello (Pisa)

08.50-09.10 Immune Responses to Old and New Vaccines
Sergio Abrignani (Milan)

09.10-09.30 Regulatory Process Leading to Decisions on Vaccines: Focus on COVID-19
Sandra Petraglia (Rome)

09.30-09.45 **OC31:** Analysis of the immune response to measles virus in vaccinees and naturally infected subjects
Gabriele Anichini (Siena)

09.45-10.00 **OC32:** Dynamic of IgA production in saliva of healthcare workers after Biontech/Pfizer BNT162B2 mRNA vaccination
Lorenzo Agostino Citterio (Milan)

10.00-10.15 **OC33:** Mucosal immune response in BNT162B2 COVID-19 vaccine recipients
Greta Forlani (Varese)

10.15-10.30 **OC34:** The protection from CMV infection in solid organ transplants is highly dependent on CMV T-cell specific immunity and type of organ transplant
Davide Abate (Padua)

PARALLEL SESSIONS	
Auditorium	Mirabilis
SESSION 11 Viral pathogenesis and Disease Chairs: Guido Antonelli (Rome), Maurizio Zazzi (Siena)	SESSION 12 Antiviral Therapy Chairs: Arnaldo Caruso (Brescia), Gianluigi Franci (Naples)
<u>10.30-10.50</u> Update on hepatitis E virus Maria Rosaria Capobianchi (Rome)	<u>10.30-10.50</u> Tetravalent Antibody Drugs for Ultrapotent and Broad Neutralization of SARS-CoV-2 Variants Giuseppe Novelli (Rome)
<u>10.50-11.10</u> Update on HBV/HCV/HDV virology and diagnostics Francesca Ceccherini-Silberstein (Rome)	<u>10.50-11.10</u> Searching for direct acting agents targeted to SARS-CoV-2 proteins Enzo Tramontano (Cagliari)
<u>11.10-11.30</u> Update on HBV/HCV/HDV therapeutics Raffaele Bruno (Pavia)	<u>11.10-11.30</u> Estrogen Signaling in Infectious Diseases Marcello Allegretti (L'Aquila)
<u>11.30-11.45</u> OC35: Trojan horses and cassandran predictors, the role of immune cells and mediators in SARS-CoV-2 infection Josè Camilla Sammartino (Pavia)	<u>11.30-11.50</u> Fixed Dose Combinations of Drugs Synergistically Active Against SARS-CoV-2 Franco Lori (Alghero)
<u>11.45-12.00</u> OC36: The integrin $\alpha\beta3$ mediates SARS-CoV-2 entry into ACE2-negative endothelial cells Antonella Bugatti (Brescia)	<u>11.50-12.05</u> OC39: Potent antiviral activity of new generation HIV-1 maturation inhibitors on human primary cells Leonardo Duca (Rome)
<u>12.00-12.15</u> OC37: The direct cleavage of caspase-8 during HSV-1 infection is due to US11 tegument protein and triggers ATG3 degradation to support viral replication Rosamaria Pennisi (Messina)	<u>12.05-12.20</u> OC40: N-acyl ethanolamine acid amidase inhibition affects Zika virus replication Michele Lai (Pisa)
<u>12.15-12.30</u> OC38: SARS-CoV-2 infection in cystic fibrosis: the role of CFTR mutation/downregulation Valentina Gentili (Ferrara)	<u>12.20-12.30</u> OC41: Regulation of m6A methylation as a new therapeutic option against respiratory viral infection Veronica Folliero (Naples)

Tuesday July 5th, 2022

PLENARY SESSION

Auditorium

SESSION 13 Highlights in HIV pathogenesis and therapy

Chair: **Matteo Porotto** (Naples)

12.30-12.50 HIV-1 mutants expressing B cell clonogenic matrix protein p17 variants are increasing their prevalence worldwide
Francesca Caccuri (Brescia)

12.50-13.10 New frontiers in HIV therapeutics
Francesco Castelli (Brescia)

13.10-13.30 **Luria and Viruses Award 2022**

13.30 Closing remarks

Delivery and compilation CME questionnaire

Tuesday July 5th, 2022

PLENARY SESSION

14.00-17.00 **VirSudNet Symposium**

Three years of a national project on antivirals within the COVID-19 pandemic

Project Presentation

Massimiliano Galdiero (Naples, Italy)

VirSudNet: the experience of the IBB-CNR Naples

Nunzianna Doti (Naples, Italy)

VirSudNet: the experience of the University of Cagliari

Giuseppina Sanna (Cagliari, Italy)

VirSudNet: the experience of the University of Campania

Annalisa Chianese (Naples, Italy)

VirSudNet: the experience of the University of Messina

Antonio Mastino (Messina, Italy)

VirSudNet: the experience of the University of Palermo

Federica Cacioppo (Palermo, Italy)

VirSudNet: the experience of the University of Calabria

Michele Pellegrino (Cosenza, Italy)

Discussion and Perspectives:

**Massimiliano Galdiero, Antonio Mastino, Stefano Aquaro,
Giovanni Giammanco, Aldo Manzin, Nunzianna Doti**



Abstracts



THE PANDEMIC THROUGH THE EYES OF THE ISS

P. Stefanelli, A.T. Palamara

Dept. Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy

The Istituto Superiore di Sanità (ISS) is the main Italian research institute in the biomedical and public health field, as well as the technical and scientific body of the Italian National Health Service (Servizio Sanitario Nazionale, SSN). ISS cooperates with the Ministry of Health, the Italian Regions/Autonomous Provinces (A.P.) and the whole SSN, to accomplish health policies on the basis of scientific evidence, in the field of prevention and health promotion to the fight against infectious diseases, cancer, chronic and neurodegenerative diseases, from autism to rare diseases, to pathological addictions. ISS has managed national, international, and European research projects for decades, both as partner and leader. ISS also participates in the European Research Infrastructure ELIXIR (European Life-science Infrastructure for Biological Information) which deals with infrastructures for high-intensity data analysis.

In this central role, the activities to face COVID-19 comprises several aspects from surveillance, training, infection prevention and control, research activities.

In particular, ISS hosts the designated National Reference Laboratory for SARS-CoV-2 and in this task coordinates the Italian genomic laboratory network. It also hosts and manages the national genomic repository for SARS-CoV-2 sequences (Italian COVID-19 Genomic or I-Co-Gen) for the centralized analysis and collection of all SARS-CoV-2 sequences obtained through Italy. In this function, the ISS works to increase the completeness, timeliness and quality of sequences uploaded to the I-Co-Gen platform to study more in deeply SARS-CoV-2 variants. It also coordinates the Italian genomic laboratory network for the conduction of periodic prevalence surveys for the identification of circulating SARS-CoV-2 Variants of Concern (VOC) in Italy.

Moreover, the ISS hosts national SARS-CoV-2 epidemiological, and environmental virological surveillance with periodic sequencing of raw sewage samples. ISS coordinates, with the support with all the Regions/AP the integration of SARS-CoV-2 data from multiple sources for an integrated analysis approaches also through the application of statistical and mathematical models.

Despite a reduction in the number of clinical severe cases of COVID-19 observed in spring 2022, the high circulation of SARS-CoV-2 associated with the progressive waning of vaccine-induced protection and the emergence of novel lineages raises significant concern about the potential mismatch between available vaccines and circulating virus strains.

All the activities in place at ISS and, in particular those concerning the virus genomic surveillance, is in line with recommendations for EU/EEA countries issued by the ECDC and, from a broader perspective, this action supports the policy priority to respond to the COVID-19 crisis and to enhance preparedness for future health emergencies.

CLINICAL MANAGEMENT OF COVID-19: UN UPDATE

C.M. Mastroianni

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

The treatment landscape for COVID-19 is evolving rapidly. Healthcare professionals must continue to adapt and offer the best possible care based on the strongest evidence and available therapies. Two main processes are thought to drive the pathogenesis of COVID-19. Early in the clinical course, the disease is primarily driven by the replication of SARS-CoV-2. Later in the clinical course, the disease appears to be driven by a dysregulated immune/inflammatory response to SARS-CoV-2 that leads to tissue damage. Based on this understanding, it is anticipated that therapies that directly target SARS-CoV-2 would have the greatest effect early in the course of the disease, while immunosuppressive/anti-inflammatory therapies are likely to be more beneficial in the later stages of COVID-19.

The first step to timely treatment of COVID-19 is early diagnosis. We need to educate our patients not to wait out symptoms. Especially for people who are at high risk for severe outcomes, it is helpful to seek a test sooner vs later, because some therapies are more effective when offered early. For example, in the clinical trials evaluating antiviral monoclonal antibody therapies, most patients were randomized and treated within 3-5 days of symptom onset. Hence, it is imperative to move quickly when symptoms are detected.

Evidenced-based options for outpatient treatment of early-stage COVID-19 remain somewhat limited to early use of antiviral monoclonal antibodies and antivirals. When evaluating candidacy for antiviral monoclonal antibodies, remember that these agents are generally indicated for patients with ≤ 10 days of symptoms, no need for hospitalization, and high risk for severe COVID-19; in the supporting clinical trials, patients had ≥ 1 risk factor such as cardiovascular disease, obesity, diabetes, cancer, or renal failure. In addition, it is important to counsel patients about “red flags” that indicate they should be clinically assessed with disease worsening. For patients with COVID-19 who have been admitted to the hospital and require supplemental oxygen, dexamethasone is recommended. It has been shown to reduce mortality in this setting. Regarding remdesivir, which is FDA approved for patients who are hospitalized with COVID-19, international guidelines vary regarding its use, with the IDSA and the NIH recommending it in patients who are hospitalized with COVID-19 and require supplemental oxygen.

SARS-COV-2 INFECTION IN CHILDREN: MUCH INFORMATION STILL MISSING

C.F. Perno

Rome, Italy

The infection by SARS-CoV-2 in children is a very common event, whose rate is not different than that found in adults.

Recent evidences show that the rate of infection is very high, and children account for the majority of infection in the last months, mostly because of the limited vaccination so far performed at the children's age, and of the rapid and abundant circulation of the virus SARS-CoV-2 in pre-school contexts (where face mask is not required).

The molecular characteristics of the virus in children are shared with adults, with clusters and evolution rate not different with that found in older patients. However, the same virus may cause different diseases in children. IN this frame, the rate of long-term infection and damage driven by SARS-CoV-2 is greater in children, and the clinical manifestations are also different. The most typical is the MIS-C, a multiorgan disease, with potential severe outcome, that is encountered typically in children and rarely in adults.

It should also be noted that SARS-CoV-2 can be present in a child together one or more other respiratory viruses, from those of the upper respiratory tract to those of the lower one. Preliminary data suggest that the presence of other viruses sharing the same cellular target of SARS-CoV-2 in the respiratory tract might somewhat be protective against a full blown SARS-CoV-2 infection, while the infection by viruses that do not share similar cellular target can increase the damage and therefore impair the prognosis

Taken together, the pathogenesis, diagnosis, and therapeutic intervention in SARS-CoV-2 infection in children has characteristics not different than those found in adults, but the clinical manifestation differ. Major attention has to be given to the long-term effects of SARS-CoV-2 infection, and to the presence of other viruses. This latter point represents an unmet clinical need, since in the majority of cases the diagnostic pathway is stopped at the time of SARS-CoV-2 infection, without searching for other pathogens. This is an issue that deserves attention and further characterization in the near future.

LONG COVID: MUTATION OF CONCERN AND TRANSCRIPTIONAL ANALYSIS

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Genomic surveillance of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the only approach to rapidly monitor and tackle emerging variants of concern (VOC) of the COVID-19 pandemic. Such scrutiny is crucial to limit the spread of VOC that might escape the immune protection conferred by vaccination strategies. It is also becoming clear now that efficient genomic surveillance would require monitoring of the host gene expression to identify prognostic biomarkers of treatment efficacy and disease progression. We sequenced over 20,000 viral genomes since the beginning of the pandemics, producing the highest number of sequences in Italy processed by a single entity. We thus reconstructed the whole pandemic dynamics in the regional territory. In addition, we have matured and applied novel proof-of-principle approaches to prioritize possible gain-of-function mutations by leveraging patients' metadata and isolated patient-specific RNA signatures of SARS-CoV-2 from infected hospitalized patients. The aforementioned goals have all been achieved as both an optimized and cost-effective strategy that does not require automation, in an effort to allow any lab with a benchtop sequencer and a limited budget to perform integrated genomic RNA surveillance on-premises.

THE CHANGING HORIZONS OF VIROLOGY IN CATS

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Detection of viruses in animals has long been based on electron microscopy or isolation in tissue cells or in laboratory animals. Also, virological assays based on specific immuno-reagents have been largely used in human and animal virology. A revolution in the diagnostics arrived in the 1990s, with the development of novel molecular techniques based on the detection of nucleic acids, i.e. polymerase chain reaction (PCR). This technology was quickly declined in a variety of protocols and applications. In recent years, the use of broad-range consensus PCRs, able to recognize viruses at the genus or family level, has resulted in the discovery of several novel feline viruses. This is exemplified by the identification of carnivore noroviruses (NoVs), first discovered in the intestinal content of a captive lion cub that died of severe hemorrhagic enteritis [1] by using a broadly reactive primer pair targeting caliciviruses [2]. The most significant development in viral discovery has been the advent of next-generation-sequencing (NGS) technologies. Unbiased analysis of nucleic acids from feline samples (metagenomic) has enabled the identification and characterization of an unexpectedly high number of previously unknown viruses, including novel parvoviruses genetically unrelated to feline panleukopenia parvovirus (FPV), such as feline bufavirus [3] and feline chaphamaparvovirus [4]. Whether these orphan viruses have the ability to cause disease is still uncertain, and epidemiological studies and clinical investigations are necessary to gather information. The possible role of viruses in feline liver disease has long remained neglected. However, in 2018 in Australia [5], an analogue of human hepatitis B virus, designed Domestic Cat Hepadnavirus (DCH), was discovered during a transcriptomic study in a seven-year-old male neutered domestic shorthair cat diagnosed with multicentric large B cell lymphoma and concomitant infection with feline immunodeficiency virus (FIV). A correlation has been observed between DCH infection and suspected clinical signs of liver injury supporting the possible role of DCH in the development of feline liver disease, similar to HBV infection in humans [6,7]. Noteworthy, some of the novel feline viruses have also been identified in the canine virome, suggesting the possibility of inter-species circulation between the two carnivore species. Cats and dogs may harbor NoVs of the same genogroups and genotypes, GIV.2 and GVI.2 [8]. Binding of GIV.2 and GVI NoVs in dog tissues has been demonstrated to be mediated by the presence of H and A antigens of the histo-blood group antigen (HBGA) family. Accordingly, it has been hypothesized that dogs and cats share a similar pattern of HBGAs as the attachment factor for NoV infections [9]. Novel carnivore protoparvoviruses identical to each other in their capsid gene (>99.9% nt identity) have been found in stool and respiratory samples either in cats or dogs [3,10]. Likewise, novel canine hepadnaviruses with high nucleotide identity (about 98%) and similar organization to DCH, have been recently found in dog sera with altered hepatic markers [11]. The close social interactions of cats and humans in households provide a strong rationale for studying feline virome. Examples hinting to the zoonotic and reverse zoonotic potential of influenza A viruses and SARS-CoV-2 infections have been reported in several animal species, including domestic cats [12]. This raises concerns on the possible implications for public health and, at the same time, requires a One Health vision in the study and management of infectious diseases of animals.

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FOOD VIROLOGY: A WINDING ROAD BALANCING INNOVATION AND STANDARDIZATION

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Food virology is a relatively young field within both food safety and virology itself. Indeed, while the first recognition of food as a vehicle for virus transmission dates back to 1914, when a poliomyelitis outbreak was reported in association to raw milk consumption, significant progresses in the field were not possible until the '90s, when the advent of molecular techniques provided the approach for direct detection and identification of viral agents in foods.

To date, foodborne viruses are considered responsible for approximately 140 million cases per year globally, and are the leading cause of foodborne disease of known etiology in developed countries, Norovirus being the most significant contributor to reported illnesses. However, while the role of viruses in the epidemiology of foodborne diseases is no longer questioned, no consensus has yet been reached for the introduction in food legislation of specific criteria on foodborne viruses.

This enduring lack of regulation is essentially a side effect of the peculiar technical issues that have to be faced in food virology, such as: i) the low viral load in food matrices, in conjunction with the (usually) low infectious dose of foodborne viruses, which requires the development of extremely sensitive detection methods; ii) the presence in food matrices of a huge variety of inhibitors of molecular (PCR) reactions; iii) the need to optimize matrix-specific procedures to achieve an effective viral concentration from highly diverse food types; iv) the lack of technically feasible assays to discriminate virus infectivity, with the consequent failure in assessing the concrete risk associated to the detection of viral genome in a food; v) the legal implications of analysis on food and feed, which call for the use of standardized and nationally/internationally recognized methods, often in association with strict quality control procedures.

In recent years, significant methodological advancements have been made to tackle some of these problems, including the gradual formalization into ISO methods of harmonized approaches for viral analysis in food, the optimization of assays for the major foodborne viruses (NoV, HAV and more recently HEV), and the progresses in the use of viral integrity assays and cell/enteroid culture systems. All of these advancements will provide the starting point for further evolutions in the field of food virology.

AVIAN INFLUENZA - AN EXPANDING GLOBAL THREAT

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The dynamics of highly pathogenic avian influenza (HPAI) viruses of the H5 subtype have changed dramatically in recent years. These viruses constitute an increasing threat to poultry production, animal welfare, wildlife and human health as well as global food security. This worrisome trend is the consequence of the spread of the HPAI viruses of the H5 subtype descendent of the H5N1 virus A/goose/Guangdong/1/1996 (Gs/GD), which was first detected in China in 1996. Since their emergence, these viruses have dramatically expanded their geographical distribution spreading to four distinct continents and remaining well entrenched in a number of countries. They have been acquiring a broader host range with a constantly expanding list of free-living wild bird species, which can potentially be affected by the virus (Adlhoch et al. Avian influenza overview September - December 2021. EFSA J 2021, 19:e07108). The replication of these viruses in new hosts and in multiple poultry production systems increases the chance for the virus to evolve as a result of drift in individual genes and of genotypic variability through genetic reassortment. The dynamic interplay between viruses and their hosts has resulted in a wide spectrum of disease ranging from subclinical infections to mass mortality events with an unprecedented impact on the agro-ecosystem. Further, the perceived threat to human health posed by H5 has recently globally increased following the recent reports of zoonotic events in Russia, Nigeria, the UK and the USA, and the increasing numbers of human cases described in China. Based on the events occurred in the last two decades, natural extinction of Gs/GD H5 HPAIVs seems a remote possibility. Indeed, the ability of these viruses to infect wild birds without necessarily causing disease results in the generation of a vicious circle in which dangerous viruses, originally emerged in the poultry sector, spill back into the wild bird population to spread further via migratory pathways. Pre-requisites for success in control of this zoonotic disease relay on a better understanding of the biological, social, economic, political, environmental and ecological factors that are driving its spread, persistence and evolution.

ANTIVIRAL DRUG RESISTANCE OF HUMAN CYTOMEGALOVIRUS IN IMMUNOCOMPROMISED PATIENTS

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Cytomegalovirus (CMV) is still a common infection in immunocompromised subjects including solid organ (SOTRs) and hematopoietic stem cell transplant recipients (HSCTRs). Several drugs have been introduced for the treatment of prevention of CMV infection and reactivation, including ganciclovir and foscarnet or cidofovir. In particular, after prolonged exposure to antiviral agents, subpopulation of CMV with genetic mutations leading to antiviral resistance may be detected. Commonly, resistance is associated to viral phosphotransferase (UL97) or DNA polymerase (UL54). Duration of drug exposure and ongoing viral replication resulting from insufficient antiviral drug activity or impaired host defense are crucial risk factors. Additionally, CMV-seronegative recipients of seropositive solid organs (D+/R-) account for most of the cases, due to the absence of pre-existing immunity and post-transplant immunosuppression. The recent introduction of letermovir for CMV prophylaxis in HSCTRs has changed the scenario of CMV management in these patients. Even if the drug has not been routinely introduced in SOTRs management yet, this is an appealing drug because of its favorable side-effect profile, its availability in both oral and intravenous formulations, and its particular mechanisms of action, since the drug inhibits the viral terminase complex. However, reports of its use for the treatment of CMV infection remain sparse.

In this setting, the role of CMV-specific immune response should be also investigated in order to clarify a possible correlation between therapy, CMV immune response and CMV antiviral resistance. From our experience, about 70% of the UL-97 mutation leading to GCV resistance were associated with lower or undetectable CMV-specific immune response.

From a diagnostic point of view, the current gold standard for the detection of UL-97 GCV resistance is by Sanger sequencing, a method that is unable to detect viral subpopulation <20%; thus the early induction of resistance mutation might be missed by Sanger sequencing. On the other hand, next generation sequencing (NGS) techniques can detect viral subpopulations at lower frequencies. Data obtained from 343 SOTRs retrospectively collected in our laboratory using NGS techniques revealed the emergence of a great variety of minor subpopulations of proven resistance mutation.

In conclusion, the evaluation of CMV-specific immunocompetence among transplanted patients could be informative in order to define the risk of drug resistance emergence and the clinical expression of drug-resistance (uncontrolled replication of drug-resistant strains) appears to be depending on impaired CMV-specific T-cell immunity recovery. Sanger sequencing remains the most accessible and scalable solution for routine genotyping solution; on the other hand deep sequencing can be more informative and specific protocols should be implemented in different clinical contexts.

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Cellular immune response plays a relevant role in the pathogenesis and outcome of viral infections, particularly in specific clinical context such as CMV in transplantation. CMV reactivation in transplant patients may be associated to direct (systemic and organ disease) and indirect effects (opportunistic infections, rejection, graft loss, up to death). Strategies for clinic-therapeutic management of CMV include risk-adapted use of pre-emptive and antiviral prophylaxis with respect to donor/recipient serostatus. Evaluation of risk of CMV infection is based on evaluation of viral replication (virologic monitoring) as well as immunologic monitoring. Reference guidelines are available for the management of CMV and viro-immunological monitoring in transplant recipients. Some innate factors play a role in the control of CMV after transplantation, although changes in CMV management based on these factors have not been studied. Also nonpathogen specific adaptive immunity has been widely investigated in relation to outcome of CMV infection and management, including parameters such as hypogammaglobulinemia, lymphopenia, TTV load, as well as commercially-available assays measuring overall immune function as marker of immunosuppression. However, CMV-specific immune assays are likely to have a greater clinical utility than a nonspecific assay. Among the different methods for CMV-specific immune monitoring, Interferon Gamma Releasing Assays quantitatively measure T-cell specifically reactive versus CMV epitopes. The use of these assays is recommended in transplant context, particularly for pre-transplant evaluation of recipient and for therapeutic management post-transplantation. In the presence of a positive CMV-specific immune response discontinuation of antiviral prophylaxis is recommended as well as waiting strategy for spontaneous viral clearance in the occurrence of reactivation. On the other hand, a negative response indicates the need for an intensive viral monitoring and aggressive/prolonged antiviral prophylaxis or therapy. Some examples of use of IGRA assay will be discussed. In the COVID-19 era, CMV antiviral stewardship has evidenced an increasing complexity, in both virologic and immunologic monitoring. The impact of COVID-19 will be discussed.

STRATEGIES FOR TESTING NON-SARS-COV-2 RESPIRATORY VIRUSES

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Lower respiratory tract infections (RTIs) are the fourth leading cause of global Burden of Disease (BOD) calculated as disability-adjusted life-years in the world in 2019 (GBD 2019 Diseases and Injuries Collaborators, Lancet 2020). RTIs are predominantly caused by viruses; although the higher BOD is caused by human respiratory syncytial virus (RSV) in children and influenza viruses in adults, there is an underappreciated burden of RTIs caused by several other viral pathogens, including human metapneumovirus (hMPV), Adenoviruses (AdV), Coronavirus (CoV) OC43, 229E, NL-63 and HUK1, Rhinoviruses (HRV), Parainfluenza viruses PIV type 1–4, and human bocavirus (hBoV). Furthermore, classification of viral versus bacterial pneumonia etiology remains a significant clinical challenge; in fact, the symptoms of respiratory tract infections are non-specific, and empiric antibiotics are frequently started to cover possible bacterial pneumonia.

There are at least three main need for a comprehensive diagnostic of RTIs. Firstly, for the purposes of antimicrobial stewardship: rapid viral tests can therefore reduce the unnecessary prescription of antibiotics, although virus detection does not exclude bacterial co-infection. Secondly, to confirm the specific viral cause of illness and guide a specific antiviral therapy. Finally, respiratory pathogen diagnosis has a crucial role for establishing control and prevention measures at the sanitary and at the population level, in case of outbreaks caused by novel virus/variants.

In recent years, there has been a rise in the use of multiplex respiratory virus panels and of those including also bacterial pathogens (syndromic testing), using a range of technologies, which are increasingly replacing individual real-time PCR assays. This advancement reduced turn-around time and manual steps and increased the number of detected pathogens with a single sample; however, the continuous evolution of different panels in the market is not paralleled by regulatory standards and there are no specific recommendations on respiratory samples to be used. Moreover, the high costs are still a major drawback so that their use is still limited to high-risk patients.

During the COVID-19 pandemic, capacity for viral diagnostics has grown extensively at all levels of care and novel, not amplification-based, techniques have been developed quickly and received emergency use authorization. Lateral flow assays (LFAs) have become essential devices for a rapid, cost-effective testing of SARS-CoV-2 antigens. Developing LFAs that are rapid, stable, sensitive, specific, and affordable can be challenging but worthwhile to extend their use to testing diseases caused by RSV, influenza viruses and other common respiratory viruses. Rapid, point-of-care nucleic acid detection were crucial methods for controlling SARS-CoV-2, as well; they can work also on unextracted samples and use novel technologies, such as those CRISPR-based, that must be further implemented for other respiratory pathogens.

In conclusion, novel competences and technologies should be exploited for testing the other respiratory viruses in a world where SARS-CoV-2 is becoming endemic. Nevertheless, there are still concerns and challenges to be addressed; highly trained virologists should remain engaged with clinicians and continuously evaluate their diagnostic needs in order to implement effective and affordable strategies for respiratory pathogens testing at different levels.

SELF-COLLECTION FOR HPV SCREENING: NEW APPROACHES FOR THE PREVENTION OF CERVICAL CANCER

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Aim of the study: Cervical cancer represents a major public health problem worldwide, mostly occurring in women who either have no access or do not participate to cervical cancer screening.

The introduction of HPV-based primary screening has allowed for the possibility to implement HPV testing on self-collected vaginal or urine samples, improving women's acceptability to screening.

The use of HPV testing on self-collected samples has been supported by several meta-analyses demonstrating that target amplification-based HPV tests can offer a similar accuracy on self-collected as on testing cervical samples, although clinical validation of the HPV assays in combination with the self-collection devices is required¹. This study aimed to compare the clinical accuracy of HPV testing using two new PCR-based HPV assays, OncoPredict Screening (SCR) and Quantitative Typing (QT) (Hiantis), on vaginal self-samples and first-void urine samples.

Methods used: A prospective clinical study was performed based on published protocol¹ (ClinicalTrials.gov Identifier: NCT04312737). Briefly 600 women attending 4 colposcopy clinics (3 in Italy and one in Scotland) were enrolled. All participants were asked to collect 20mL of first-void urine sample using the Colli-Pee (Novosanis) device and a self-vaginal swab using FLOQSwab (Copan); clinician-collected cervical scrapings using Cervex-Brush (Rovers) were also collected prior to colposcopy. Cervical samples were immediately resuspended in 20 mL ThinPrep (Hologic); vaginal swabs travelled dry to the laboratory and then resuspended in 5 mL of ThinPrep (Hologic) or eNAT (Copan). Nucleic acid extraction was performed on all samples using Quick DNA/RNA viral MagBead (Zymo), which were subsequently tested using both OncoPredict SCR and QT assays (Hiantis) for high-risk HPV (hrHPV) screening and normalized genotype-specific viral load determination. Colposcopy and biopsy results were obtained from the 4 participating centers.

Results and Conclusions: Overall hrHPV positivity concordance between self-collected and cervical samples was good for both OncoPredict assays (Kappa values ranging from 0.70 to 0.79). Clinical accuracy of OncoPredict SCR and QT assays on vaginal self-samples and first-void urine samples was evaluated by determining the clinical sensitivity and specificity relative to cervical samples. Both assays showed good relative clinical sensitivity for CIN2+ on self-vaginal (SCR=0.96 [95%CI 0.90-1.01]; QT=0.96 [95%CI 0.91-1.01]) and urine samples (SCR=0.96 [95%CI 0.89-1.02]; QT=0.97 [95%CI 0.93-1.03]). The relative specificity estimates were <1 (except SCR assay on urine samples (SCR=1.01 [95%CI 0.95-1.08])). However, cut off optimization of the assays allowed to achieve good relative specificity for both vaginal (SCR=0.94 [95%CI 0.89-1.002]; QT=0.94 [95%CI 0.88-1.01]) and urine samples (QT=0.94 [95%CI 0.87-1.007]) with 95% CI around the relative sensitivities

still including unity (vaginal: SCR=0.95 [95%CI 0.90-1.002]; QT=0.96 [95%CI 0.91-1.008]; urine: QT=0.95 [95%CI 0.89-1.007]. In conclusion, using an optimized cut-off, both assays SCR and QT, showed similar accuracy to detect cervical precancer on self- compared to cervical samples.

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DETECTING CYTOMEGALOVIRUS SHEDDING IN PREGNANT WOMEN AND NEWBORNS: ARE ALL SWABS CREATED EQUAL?

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Human cytomegalovirus (CMV) is a leading viral cause of congenital infection. Currently, universal serological screening for CMV in pregnancy is not recommended and the investigation of maternal and fetal CMV infection is dependent on a reactive approach that has limited the early detection and management of infection. Recent evidence suggests that a proactive detection of CMV shedding in bodily fluids by PCR in seropositive women may allow early detection of active maternal CMV infection and the prediction of the risks of fetal transmission. Used in conjunction with sonography and serology, this proactive approach may also support early discussion for further investigation and potential interventions to reduce the consequence of infection. However, before this can become a reality, several knowledge gaps pertaining to the application of PCR to detect maternal CMV shedding need to be addressed. One of these is the collection and pre-analytical handling of appropriate swabs and transport media. In this talk, the evidence on the performance of commercially available swabs and transport media to detect CMV shedding is presented. Commercially available swabs do not have equal absorption efficiency, or the equivalent capability of releasing or preserving CMV DNA over time to ensure an accurate and reliable detection of CMV DNA in biological fluids. A judicious use of these swabs is required.

THE CHALLENGENS OF MANAGING THE COVID-19 PANDEMIC PRE-ANALYTIC TO DIAGNOSIS

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The SARS-CoV-2 pandemic brought multiple challenges to the virology and microbiology labs. Since 2020 and the mid-half of 2021, the general shortage of extraction and amplification reagents, together with the low number of platforms available from the manufacturers impacted dramatically the testing capacity. Also, the lack of skilled and trained personnel have pose multiple threads to the reporting capabilities. Several actions were put in place to successfully face this situation. Among them, pre-analytic step is the most labor and time-consuming phase, as well as the most unsafe from the operator stand point, the adoption of an automation platform for the sample preparation. In this context, the management of the unprecedented sample volumes for COVID-19 testing was possible only by the integration of several analytical platforms with different features and feeding them with samples prepared with a universal platform for sample preparation and management.

MONITORING OF CYTOMEGALOVIRUS INFECTION IN HEMATOPOIETIC STEM CELL TRANSPLANTION RECIPIENTS UNDERGOING LETERMОВIR PROPHYLAXIS

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Letermovir (LMV) is a novel antiviral drug used to prevent CMV infection, having a favorable side effect profile and a different viral target (ie, the viral terminase) was approved by the Food and Drug Administration in November 2017 for CMV prophylaxis in adult CMV seropositive allo-HSCT recipients.

Recently Cassaniti et al. (*Am J Transplant.*2021;21:1622-28), showed that CMV-DNAemia positivity in both whole blood and plasma detected during LMV prophylaxis in transplant recipients may not be associated with a complete replicative cycle. In fact, the study showed that the presence of active CMV reactivation during LMV prophylaxis correlated with the presence in blood of circulating viral infectious particles identified by virus isolation and the analysis of degradation of free viral DNA.

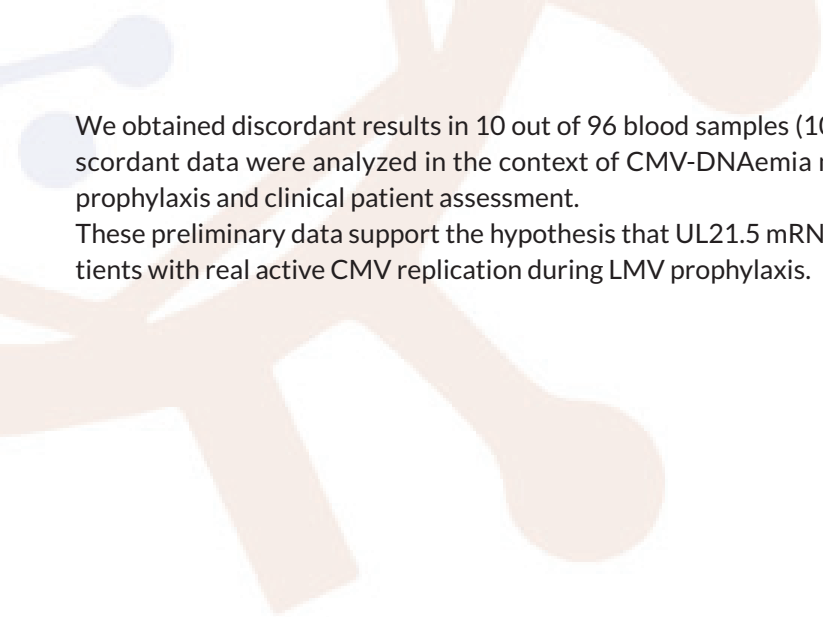
During the active viral replication, CMV virion assembly involves the incorporation of RNAs into infectious particles. These transcripts are expressed to high levels late in the replication cycle during virion assembly. The tegument-associated UL21.5 mRNA is predominantly located within the cytoplasm of HCMV-infected cells at late times during infection and it be an indicator of active rather than latent CMV infection.

The purpose of this study was to evaluate the detection of tegument-associated UL21.5 mRNA in blood samples from allo-HSCT recipients through LMV prophylaxis to appropriately identify an active CMV replication.

The study cohort comprised 127 allo-adult HSCTR undergoing LMV prophylaxis. In detail, 100 CMV-DNAemia negative samples obtained from 100 patients without active CMV infection and 96 sequential CMV-DNAemia positive samples from 27 patients with active CMV infection were retrospectively analyzed. The assay for the detection of mRNA consists of a reverse transcription and a Real-Time PCR specific for a region of the CMV UL21.5 mRNA and for a region of the genomic RNA of MS2 phage (exogenous Internal Control) (RNAemia test). The characterization of true vs abortive CMV infection during LMV prophylaxis was performed by virus isolation from peripheral blood leucocytes in shell vial cultures (viremia test) and/or quantification of encapsidated vs free-floating CMV-DNA in plasma samples after enzymatic digestion with DNase (DNase test).

The results from 100 samples obtained from patients without active CMV infection and examined with RNAemia, viremia and DNase tests showed a 100% of agreement.

Fourteen out of 96 blood samples were positive for the presence of circulating infectious HCMV particles determined by virus isolation and/or degradation of free-floating viral DNA. Ten out of this 14 blood samples (71.4%) were positive by RNAemia test. In the remaining 82 CMV-DNAemia negative, 76 (92.7%) were negative by RNAemia test, resulting in an 89.6% agreement.



We obtained discordant results in 10 out of 96 blood samples (10.4%). The individual discordant data were analyzed in the context of CMV-DNAemia monitoring during LMV prophylaxis and clinical patient assessment.

These preliminary data support the hypothesis that UL21.5 mRNA assay can identify patients with real active CMV replication during LMV prophylaxis.

A NOVEL DIAGNOSTIC APPROACH TO DIFFERENTIATE VIRAL FROM BACTERIAL INFECTIONS

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DiaSorin S.p.A. , Saluggia (VC) – Italy

It is presented the first The first CLIA, fully automated & high throughput assay for bacterial and viral infection discrimination. The Test presented is made-up of a host signature, composed by three host immune-system proteins:

TRAIL (TNF related apoptosis-inducing ligand)

- Member of the tumor necrosis factor family implicated in programmed cell death
- Important roles in the immune response to viral infections and in immune surveillance of tumors and metastases

IP-10 (interferon γ -induced protein 10 kDa)

- Chemokine implicated in cell growth inhibition, chemotaxis and innate and adaptive immune response by activation of T lymphocytes (Th1), NK cells, macrophages, dendritic and B cells
- Alterations in expression levels have been associated with inflammatory diseases including infectious diseases, immune dysfunction and tumor development

CRP (C-reactive protein)

- Acute phase protein released into the circulatory system in response to proinflammatory stimuli,
- Diverse roles in tissue injury, infection and other inflammation processes

The individual biomarkers of the host-response signature have complementary dynamics in response to bacterial and viral infections and the innovative host-based signature technology guarantees high level of accuracy performing significantly better than other clinical parameters and well established markers (diagnostic accuracy: 94% vs 68% toward PCT 1 ng/ml, 94% vs 87.6% toward CRP 80 mg/l) The signature is meant to improve the quality of patient's life by optimizing the antibiotic use.

The signature aims at improving the quality of patient's life and the Healthcare management by optimizing the antibiotic use.

HSV ENTRY: FROM VIRUS ENGINEERING TO PRECLINICAL MODELS

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Herpes simplex virus (HSV) enters cells by recognition of the cellular receptor (nectin1 or herpesviruses entry mediator), followed by fusion of the viral envelope with plasma or endocytic membranes (neutral or acidic compartments). These steps are carried out by the HSV multipartite entry/fusion apparatus made of gD, gB, gH, and gL glycoproteins. According to the current model, gD recognizes and binds the receptor, then propagates the activation to the trio of gH/gL and gB, which, in turn, executes fusion. The determinants of the routes of entry are the integrins. High levels of specific integrins enable the re-localization of nectin1 to lipid rafts and force gD to interact with its receptor at these domains, ultimately triggering the endocytic route at the lipid-rafts. In few cell types, integrins are expressed at low levels, nectin1 is not re-localized, and HSV enters the cell by fusion with plasma membrane. The most characterized integrins that interacts with HSV and its receptors are $\alpha\text{v}\beta 6$, $\alpha\text{v}\beta 8$ and $\alpha\text{v}\beta 3$. $\alpha\text{v}\beta 6$ - and $\alpha\text{v}\beta 8$ -integrins serve as co-receptors for HSV,

binding the gH/gL complex. This interaction induces the dissociation of gL, freeing gH from its inhibitor and licensing gH for fusion. Moreover, HSV-integrin interactions synchronize endocytosis at the lipid-rafts with the cascade of glycoprotein activation that culminates in execution of fusion. This strategy is exploited by HSV to avoid premature activation and exhaustion of the

glycoproteins. $\alpha\text{v}\beta 3$ -integrin, beside lipid-raft routing, represents also a sensing systems able to detect HSV and signal the incoming infection to the cell. Specifically, in cooperation with Toll-like receptor 2 (TLR2), $\alpha\text{v}\beta 3$ integrin enables the recruitment of TLR2, MAL, MYD88 at lipid rafts, assembling an antiviral signalling platform. When engaged by HSV, $\alpha\text{v}\beta 3$ -integrin triggers the innate response by boosting the MYD88-dependent TLR2 signalling and IRAK4 phosphorylation, leading to the expression of type I interferon (IFN), NF- κ B, and a specific set of cytokines. Again, gH/gL interacts with $\alpha\text{v}\beta 3$ -integrin, triggers the activation of the signalling platform and boosts the signalling cascade, interacting with $\alpha\text{v}\beta 3$ and TLR2 independently one of the other and cross- linking the two receptors. Through this strategy, HSV couples virion entry with the triggering of the signalling cascade. Seemingly detrimental to HSV infection, the antiviral pathways are readily shut- off by viral factors and the virus exploit the ability of the signal to activate the cell for virus replication.

Oncolytic virotherapy strategies based on the HSV are reaching their thirties, and a wide variety of approaches has been envisioned and tested in many different models. The vast majority of oncolytic HSV (oHSVs) in clinical trials, including the approved talimogene laherparepvec (also named OncoVEXGM-CSF), were generated by attenuation strategies, which confer cancer-selectivity and are based on deletion/mutation in virulence genes or in genes that contrast the innate and adaptive immune responses. This way, normal cells restrict virus infection, while tumor cells which are often defective in innate response allow for effective infection and viral replication. An alternative strategy pursued in our laboratory to generate oHSV consists in the detargeting of HSV from its natural receptors and the retargeting to a cancer-specific receptor of choice. The retargeted oHSVs carry no mo-

dification in genes that contrast the antiviral innate and adaptive immune responses and, like wt-HSVs, are equipped to overcome the inimical host response, likely obtaining an enhanced oncolytic effect. Once they infect the cancer cell, their replication proceeds in unattenuated fashion and is independent of defects in immune pathways typical of cancer cells. Inasmuch as the retargeted oHSVs infect specifically tumor cells and effectively spare off-tumor cells, their safety profile in mice are high, and they proved to be very effective anti-cancer agents in different clinical models.

VIRAL PROTEINS KNOCKING AT THE NUCLEAR DOOR: A TALE OF IMPORTINS

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Viruses intensively interact with the host cell nucleocytoplasmic nuclear transport apparatus at different levels. Indeed, viral proteins can be actively translocated across the nuclear pore complex or interfere with the cellular nucleocytoplasmic transport process itself. These events are crucial for viral genome nuclear targeting, expression, replication, and encapsidation, as well as for host cell function manipulation, with profound effects on cell proliferation, survival, and antiviral response. Furthermore, recent developments of broad and specific nuclear transport inhibitors suggest a potential future pharmacological implication for antiviral drug discovery. I will briefly summarize the state of the art on the topic and my contribution to the field, along with unpublished data from my group.

TOSCANA VIRUS AND ANTAGONISM TO THE INNATE IMMUNE RESPONSE

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Aim of the study: The Non Structural (NSs) protein of some members of the *Phenuiviridae* family is an important virulence factor, being a potent antagonist of type I Interferons. Our aim was to evaluate the role of Toscana Virus NSs to antagonize the innate immunity in the human host.

Methods used: Luciferase reporter gene assay, *in vitro* Ubiquitination assay, Mass spectrometry, Immunofluorescence, Immunoblot, *in vitro* Cell infection and transfection.

Results and conclusions: We revealed the inhibitory effect of NSs on RIG-I involved in the signaling cascade for type I IFN production, leading to degradation of RIG-I upon binding. In fact, NSs appears to directly bind the CARD domain of RIG-I and to mediate its degradation, thus abolishing the downstream signaling of IFN- β activation. The addition of MG-132 to the cells widely restores IFN- β promoter activation, suggesting that RIG-I degradation, mediated by NSs, is proteasome dependent and specific, since the nucleoprotein of TOSV does not show any of these effects. Moreover, we show that TOSV NSs has an E3 ubiquitin ligase activity, mapping at the carboxy-terminal domain and also involving the amino-terminal of the protein. Indeed, neither the amino- (NSs Δ N) nor the carboxy- (NSs Δ C) terminal-deleted mutants of TOSV NSs are able to cause ubiquitin-mediated proteasome degradation of RIG-I. We hypothesized a model in which NSs behaves as an atypical RING between RING (RBR) E3 ubiquitin ligases. This is the first report identifying the E3 ubiquitin ligase activity in a viral protein among negative strand RNA viruses.

UPPER RESPIRATORY TRACT AND VIRAL PATHOGENS: WHAT DID WE LEARN FROM SARS-COV-2

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SARS-CoV-2 infection and its dramatically different possible clinical outcomes has refocused our attention on the extremely important interplay occurring, at the upper respiratory tract level, between viral pathogens and the innate immune system. In my communication, I will discuss some of these interactions highlighting the thin border between an effective immune response, capable of controlling viral replication, and a dysregulated response possibly leading to the most severe clinical outcomes. I will also mention the possible concurring role of the resident microbiota in determining a basal antiviral state possibly influencing the outcome of a viral infection. All these mechanisms certainly deserve further attention, not only in SARS-CoV-2-related settings, and may lead to novel therapeutic and prophylactic strategies in the management of respiratory viral infections.

RAPID, SCALABLE ASSESSMENT OF SARS-COV-2 CELLULAR IMMUNITY BY WHOLE-BLOOD PCR

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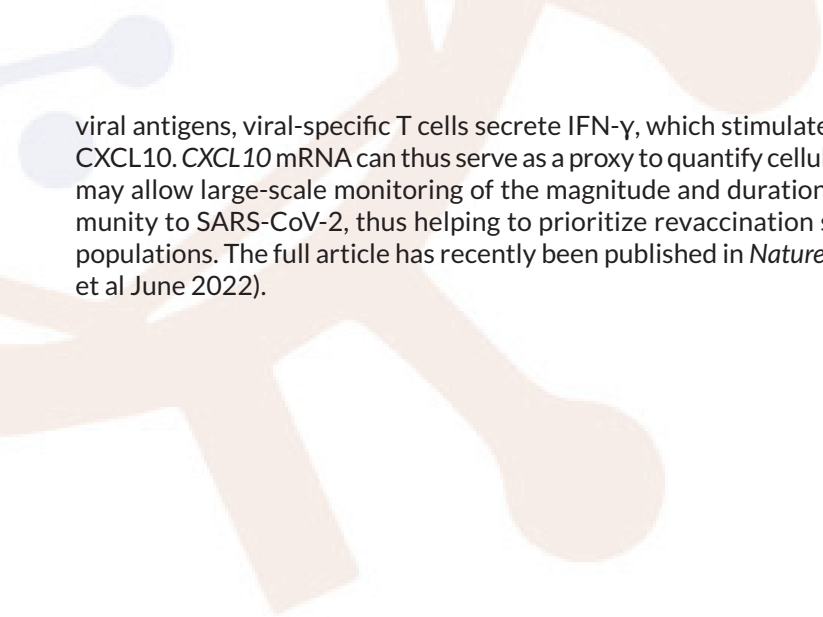
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Fast, high-throughput methods for measuring the level and duration of protective immune responses to SARS-CoV-2 are needed to anticipate the risk of breakthrough infections. Here we report the development of two qPCR assays for SARS-CoV-2-specific T cell activation. The assays are rapid, internally normalized, and probe-based: *qTACT* requires RNA extraction and *dqTACT* avoids sample preparation steps. Both assays rely on the quantification of CXCL10 mRNA, a chemokine whose expression is strongly correlated with activation of antigen-specific T cells. Upon restimulation of whole blood cells with SARS-CoV-2



viral antigens, viral-specific T cells secrete IFN- γ , which stimulate monocytes to produce CXCL10. CXCL10 mRNA can thus serve as a proxy to quantify cellular immunity. Our assays may allow large-scale monitoring of the magnitude and duration of functional T cell immunity to SARS-CoV-2, thus helping to prioritize revaccination strategies in vulnerable populations. The full article has recently been published in *Nature Biotechnology* (Schwarz et al June 2022).

CELL-INTRINSIC IMMUNITY TO SENSE SARS-COV-2 INFECTION

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Aim of the study: Acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiological agent for Coronavirus disease 2019 (COVID-19) that has led to a pandemic since March 2020. The role of SARS-CoV-2 components on innate immunity is still under research. The investigation of the possible implication of pathogen-associated molecular patterns (PAMPs)-pattern recognition receptors (PRRs) interaction and the possible different effect of the SARS-CoV-2 variants of concern (VOCs) on innate immune response are of extreme importance.

Methods: We evaluated: i) the activation of RNA sensors, transcription factors and cytokines/interferons (IFN) secretion in pulmonary organoids after SARS-CoV-2 infection (Bortolotti D, et al. *Cells*. 2020; 9(9):1975. doi: 10.3390/cells9091975; Bortolotti D, et al. *Microorganisms*. 2021; 9(9):1820. doi: 10.3390/microorganisms9091820); ii) the underlying mechanism leading to the emergence of variants, analyzing quasi-species interaction with host cells (Caccuri F, et al. *Virus Evolution*. veac042, <https://doi.org/10.1093/ve/veac042>); iii) the changes in endothelial activation biomarkers' profile and neutrophil adhesion (Bortolotti D et al. *Viruses*. 2021; 13(9):1855. doi: 10.3390/v13091855).

Results: We observed that pulmonary SARS-CoV-2 infection induces the activation of TLR3 and TLR7 RNA sensor pathways. In particular, TLR3 might act via IRF3 producing Interleukin (IL)-1 α , IL-1 β , IL-4, IL-6 and IFN- α and IFN- β during the first 24 hours post infection. Then, TLR3 activates NF κ B transduction pathway leading to pro-inflammatory cytokine secretion. Conversely, TLR7 seems to act mainly via NF κ B inducing type1 IFN, IFN- γ and IFN- λ 3, starting from the 48 hours post infection. Different quasi-species showed a different entry into target cells occurring by fusion and/or endocytosis pathways. SARS-CoV-2 activated endothelial cells controlled, via HLA-G/CD160 interaction, FGF2-induced sICAM-1 and sE-selectin expression, affecting neutrophil adhesion.

Conclusions: We showed that SARS-CoV-2 infection activates different TLRs pathways and might enter host cells via fusion and/or endocytosis pathways, modifying innate immune cell activation. These results might suggest a deep impact of cell-intrinsic immunity in controlling SARS-CoV-2 infection and the emergence of SARS-CoV-2 variants with a higher capability than their ancestors to rapidly spread around the world.

DEVELOPMENT OF NEXT GENERATION OF TUMOUR-TARGETED REPLICATING ONCOLYTIC VIRUSES USING AN IDEAL IMMUNOCOMPETENT ANIMAL MODEL

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Following our better understanding of cancer biology and cancer immunity, the last few years have seen an increased interest in immunotherapy in the treatment of malignant diseases. So far vast array of immunotherapeutic agents for the treatment of cancer have been developed. Tumour-targeted replicating Oncolytic Viruses (TOVs) have emerged as attractive therapeutic candidates for cancer treatment. TOVs have shown some promising clinical outcome, with having four TOVs approved as new drugs for cancer treatment. However, the efficacy of TOVs as monotherapy has been limited. Therefore, it is imperative to develop next generation of oncolytic viruses to improve the anti-cancer potency of these agents. Arming oncolytic virus with cytokines, such as human IL-12, is the most commonly used strategy. However, human IL-12 does not function in murine model, it is difficult to refine the new biological agents and ensure these new generation are safe and effective as the current mouse tumour models are not suitable for assessment of new generation TOVs. In this talk the speaker will address how we can develop next generation of TOVs using a novel transgenic Syrian hamster cancer models. The speaker will also discuss how the field of oncolytic virotherapy has developed, what we have learnt from pre-clinical and clinical studies thus far and what the future may hold, in particular how TOVs could potentially overcome the weaknesses of immune check point blockage therapy in order to optimise immunotherapy and boost the immune system to maximise the therapeutic potential of treatment.

GENE THERAPY FOR X-LINKED MYOTUBULAR MYOPATHY BY SYSTEMIC AAV

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Systemic administration of muscle-tropic adeno-associated viral (AAV) vectors is a clinically validated strategy to deliver genes to skeletal and heart muscle in patients affected by inherited muscle diseases. X-linked myotubular myopathy (XLMTM) is a rare, life-threatening monogenic disorder caused by mutations in the MTM1 gene. XLMTM is characterized by profound muscle weakness, respiratory failure, short life span and an extremely poor quality of life. In 2017, Audentes Therapeutics, a biotechnology company based in San Francisco (CA) initiated ASPIRO, a multicenter clinical study aimed at evaluating the safety and efficacy of a systemic, single-dose administration of AT132, an AAV8 vector expressing the human MTM1 cDNA under the control of the muscle-restricted human desmin promoter. The vector was developed and preclinically tested in mouse and dog models by Genethon in France. The trial enrolled 14 patients in two dose cohorts. AT132 has been generally well-tolerated with a manageable safety profile across cohorts for the first 11 patients, aged 0.8 to 3 years. Analysis of muscle biopsies showed robust, dose-dependent transduction of muscle fibers, MTM1 protein expression at physiological or supra-physiological levels and dramatically improved muscle histopathology. Treated patients showed clinically meaningful improvements in neuromuscular and respiratory functions and achieved motor milestones such as the ability to raise, walk with support or walk alone. There was significant and rapid reduction in ventilator use in all treated patients, with most patients reaching ventilator independence. Unfortunately, three patients aged >5 years died because of treatment-related, severe adverse events that included liver failure. The risk/benefit balance of this therapy will be discussed.

VECTOR GENOME INTEGRATION AND GENE THERAPY: FRIENDS OR FOES?

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Adeno-associated vectors (AAV) represent the most promising platform for in vivo delivery of gene therapies. AAV has reached remarkable successes in the clinics, and liver-directed gene therapy clinical trials are currently ongoing for several inborn errors of metabolism (IEM). Nevertheless, classic AAV-mediated gene replacement strategies still hold relevant limitations toward effective in vivo gene therapy. A number of IEMs manifests during childhood or are characterized by liver damage and administration of episomal AAV vectors may result in progressive loss of transgene expression due to hepatocyte proliferation associated to liver growth and/or regeneration. Moreover, low-frequency integration of AAV-ferried DNA within host genome has been associated to the development of hepatocellular carcinoma (HCC) in newborn mice, with a mechanism involving transactivation of multiple microRNAs by vector-borne promoter elements. Pre-existing liver injury appears to increase the incidence of HCC following AAV gene therapy in adult mice. Even though no evidence of HCC development arose so far from studies in non-human primates and clinical trials, long-term oncogenic potential of AAV-mediated gene therapy represents a lingering concern.

To overcome the hurdles to classic gene therapy posed by hepatocyte proliferation and potential AAV genotoxicity, we designed a liver-directed genome editing approach, based on AAV-mediated targeted integration by homology-directed recombination of a promoterless cDNA into the host albumin locus. We applied this approach to Wilson disease (WD), a life-threatening disorder of copper metabolism caused by mutations in copper transporter ATP7B and characterized by toxic copper accumulation resulting in severe and progressive liver and brain injury. We generated an AAV8 vector bearing a codon-optimized human mini-ATP7B cDNA flanked by two mouse Alb homology arms and preceded by a sequence encoding for a 2A peptide derived from porcine teschovirus-1 (AAV-Alb-mini-ATP7B). Intra-venous injection of AAV-Alb-mini-ATP7B resulted in a complete rescue of survival in *Atp7b*^{-/-} mice. At sacrifice, these mice showed extensive liver repopulation by genome edited hepatocytes, associated to an amelioration of liver injury and rescue of serum ceruloplasmin oxidase activity, compared to *Atp7b*^{-/-} mice injected with a control vector. Furthermore, we combined promoterless nuclease-free genome editing with the administration of D-penicillamine, a copper chelator currently used for the therapy of WD. *Atp7b*^{-/-} mice treated with D-penicillamine and AAV-Alb-mini-ATP7B showed a significant improvement of liver pathology and reduction of copper storage compared to *Atp7b*^{-/-} mice administered with chelation therapy alone.

In summary, promoterless nuclease-free genome editing provide a significant and sustained therapeutic benefit in WD and may represent a safer alternative to classic gene replacement strategies.

ON THE MECHANISM OF ANTIBODY NEUTRALIZATION TARGETING THE RECEPTOR BINDING DOMAIN OF THE SARS-COV-2 SPIKE PROTEIN

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Aim of the study: Since the beginning of the SARS-CoV-2 pandemic in early 2020, there have been multiple neutralizing human monoclonal antibodies (Mabs) isolated from convalescent or vaccinated individuals. Various variants of concern (VoCs) emerged during the pandemic, and only a few of these Mabs have remained active against the new variants. In this talk, I will describe the structural features of a human Mab isolated at Institut Pasteur in Paris that is capable of neutralizing all VoCs, including Omicron. I will also describe experiments on similarly potently neutralizing single-chain VH-only antibodies (VHHs) derived from immunization of alpaca, which provide complementary new insight into the mechanism of neutralization of antibodies targeting the receptor binding domain of the SARS-CoV-2 spike protein.

Methods Used: X-ray-crystallography, cryo-Electron microscopy, neutralizing assays in biosafety level 3 containment.

Conclusions: Despite its high sequence variability, the coronavirus spike has vulnerable sites that can be targeted for efficient neutralization of new variants.

THE POTENTIAL OF ELECTRON MICROSCOPY IN UNDERSTANDING VIRUS-HOST INTERACTIONS: A STUDY OF HCMV MORPHOGENESIS

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The generation and release of mature virions from human cytomegalovirus (HCMV) infected cells is a multistep process, involving profound reorganization of cellular structures and various stages of virus particle morphogenesis in different cellular compartments. These stages are categorized into *primary envelopment*, which plays a role during egress of newly formed capsids from the nucleus into the cytoplasm, and *secondary envelopment*, which provides HCMV virions with their final membrane before being released from the cell. Although the general steps of HCMV-host interaction and virion morphogenesis are known, the detailed molecular mechanisms are complex and depend on various viral and cellular factors. Thus, there are still many open questions. This emphasizes the need for imaging techniques to visualize host-cell interactions and the different stages of virion assembly. Electron microscopy (EM) is the only imaging technique that can directly visualize biological structures in virus infected cells. For this, perfect structural preservation, especially of membranes, is a prerequisite. Thus, our laboratory uses the advanced sample preparation technique of high-pressure freezing and freeze substitution (HPF-FS) instead of classical aldehyde fixation protocols.

With such high-quality samples, we performed three-dimensional (3D) EM imaging techniques to shed light upon various stages of virus infection, among others, nuclear egress and primary envelopment. We found that egress occurs on large infoldings of the inner nuclear membrane into the nucleoplasm, which exhibit an unprecedented membrane complexity. Furthermore, we applied thorough quantitative EM analyses of HCMV wild-type and mutant virus infected cells and by this identified the function of the C-terminal tetra-lysine motif of the tegument protein pUL71 for secondary envelopment.

In my presentation I will demonstrate the technical aspects of these studies and discuss the potential of EM in understanding virus-host interactions.

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Structural biology has emerged in recent years as a powerful tool for rational drug discovery. In fact, advances in high throughput strategies for structure determination have yielded a large number of three-dimensional (3D) structures for several therapeutically relevant targets. Crystal structures of protein targets alone and in complex with ligands and inhibitors have provided essential insights into the mechanisms of actions of enzymes, their conformational changes upon ligand binding, the architectures and interactions of binding pockets. In addition, structure-based drug design (SBDD) methods are becoming increasingly powerful, versatile, and more widely used for the identification of hits, generation of leads, as well as to accelerate the development of high-quality drug candidates. Several biologically active compounds discovered by structure-based design are now drugs in the market, confirming the crucial role played by structural biology in drug development. The power of SBDD has been demonstrated most clearly in the discovery of new therapeutics for HIV/AIDS, where structural knowledge of the HIV-1 protease enabled the successful design and development of several protease inhibitors that are now commercially available drugs. Another success that owes its origins to SBDD is Relenza, a drug for the treatment of influenza. Examples of applications of structure-based and ligand-based virtual screening and design to discover new antivirals will be discussed.

ROLE OF HUMAN PAPILLOMAVIRUSES IN CARCINOGENESIS

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Infectious agents represent a major group of risk factors for cancer development and contribute to about 15% of human cancers worldwide. Six viruses and one bacterium, i.e. human papillomavirus (HPV), hepatitis C virus (HCV), hepatitis B virus, Human T-lymphotropic virus type I (HTLV-1), Epstein-Barr virus (EBV), Kaposi sarcoma-associated virus (KSHV) and *Helicobacter pylori*, have been clearly associated with human carcinogenesis. The mucosal high-risk (HR) HPV types are the etiological factors of cervical cancers and sub-set of oropharyngeal cancers. In addition, ongoing studies concerning a sub-group of HPV types that infect the skin suggest their involvement, together with ultraviolet radiation (or solar exposure), in the development of squamous cell carcinoma.

Biological studies have demonstrated that the products of two early genes from the HR HPV types, E6 and E7, play a key role in cancer development. Both viral oncoproteins are able to target several cellular pathways leading to the evasion of the immune surveillance and cellular transformation. These studies also substantially contributed to our understanding of key mechanisms involved in the normal life of the cell.

In the last few years, we have performed additional studies on cutaneous and mucosal HPV types and have characterized novel oncogenic viral mechanisms involved in the evasion of the immune response and/or in cellular transformation. A few examples will be presented.

HTLV-1 RETROVIRUS INFECTION AND THE ROAD T ADULT T-CELL LEUKAEMIA

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Human T cell leukemia virus-1 (HTLV-1) is the causative agent of a severe cancer of the lymphoid lineage that develops in 3-5% of infected individuals after many years. HTLV-1 infection may also induce a serious inflammatory pathology of the nervous system designated HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Two virus-encoded proteins, the viral transactivator Tax-1 and the HTLV-1 basic leucine-zipper factor HBZ, are strongly involved in the oncogenic process. Tax-1 is involved in initial phases of the oncogenic process. Conversely, HBZ seems to be involved in maintenance of the neoplastic state as witnessed by the generation of leukemic/lymphomatous phenotype in HBZ transgenic mice and the persistent expression of HBZ in all phases of the oncogenic process. Nevertheless, the intimate molecular and cellular mechanism mediated by the two viral proteins, particularly HBZ, in oncogenesis still remain elusive. An important step toward the complete comprehension of HBZ-associated oncogenicity is the clarification of the anatomical correlates of HBZ during the various phases of HTLV-1 infection to development of HTLV-1-associated inflammatory pathology and ultimately to the establishment of leukemia. In this review, I will summarize recent studies that have established for the first time a temporal and unidirectional expression of HBZ, beginning with an exclusive cytoplasmic localization in infected asymptomatic individuals and in HAM/TSP patients and ending to a progressive cytoplasmic-to-nuclear transition in leukemic cells. These results are framed within the present knowledge of HTLV-1 infection and the future lines of research that may shed new light on the complex mechanism of HTLV-1-mediated oncogenesis.

CONVERGENCE BETWEEN VIRAL AND TUMOR ANTIGENS

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

Methods: In order to find homologies between these two classes of non-self antigens, we interrogated the BLAST looking for homology between published TAAs and microorganism-derived epitopes. Bioinformatics analyses and ex vivo immunological validations have been performed.

Results: Several homologies between TAAs and microorganism-derived epitopes have been found. Moreover, structural similarities between paired peptides as well as comparable patterns of contact with HLA and TCR α and β chains have been observed. Cross-reactive T cells have been identified by ex-vivo immunological evaluations. Therefore, the two classes of non-self antigens (tumor and microorganism antigens) may converge, eliciting cross-reacting CD8⁺ T cell responses which possibly drive the fate of cancer development and progression.

Conclusions: An established anti-microorganism T cell memory may turn out to be an anti-cancer T cell memory, able to control the growth of a cancer developed during the lifetime if the expressed TAA is similar to the microorganism-derived epitope. This may ultimately represents a relevant selective advantage for cancer patients and may lead to a novel preventive/therapeutic anti-cancer vaccine strategy with antigens not affected by the immunological tolerance.

AT THE INTERFACE OF HOST-VIRUS INTERACTIONS: CLEAVAGE OF PLANT PROTEINS BY A POTYVIRUS PROTEASE AND POTENTIAL APPLICATIONS FOR NOVEL ANTIVIRAL STRATEGIES

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Potyvirus constitute a large and economically important group of plant positive-strand RNA viruses. The potyvirus NIa protease (NIa-Pro) is a chymotrypsin-like cysteine protease closely related to the 3C protease of human and animal picornaviruses. Like the 3C protease, NIa cleaves the viral polyprotein at multiple sites. The picornavirus 3C protease also cleaves hundreds of host proteins to facilitate its infection. However, whether or not the NIa protease also targets plant proteins was not known. To address this question, we used a bioinformatic approach to identify putative NIa cleavage sites in the plant proteome. We focused on the NIa proteases from plum pox virus (PPV) and turnip mosaic virus (TuMV), for which consensus cleavage site sequences are similar and well-characterized. Regular expression search using the consensus cleavage site sequence [EQN]xVxH[QE]₁[SGTA] for the PPV NIa protease allowed the identification of 90-94 putative cleavage events in the proteomes of *Arabidopsis thaliana*, *Nicotiana benthamiana* and *Prunus persica*. Using *in vitro* processing assays, we confirmed cleavage of 11 host proteins by purified PPV and TuMV NIa proteases. To examine cleavage in infected cells, we transiently expressed epitope-tagged versions of candidate plant proteins and simultaneously launched virus infection from a TuMV infectious clone. Using this method, we demonstrate *in vivo* cleavage of AtEML2 (At5g06780), an Emsy-like protein belonging to a family of histone readers known to be involved in pathogen resistance and AtUFD1 (At4g15420), a ubiquitin fusion degradation-like protein probably involved in the endoplasmic reticulum (ER)-associated protein degradation (ERAD) pathway. AtUFD1 also includes a TRAF-like domain, a domain family which has been associated with the regulation of plant immune responses. Cleavage of AtEML2 and AtUFD1 was also observed in plant cells that co-express the PPV or TuMV NIa-Pro. Mutation of the predicted cleavage sites prevented the processing of these host proteins. Using a differential centrifugation fractionation method, we investigated the subcellular localization of the uncleaved proteins and of the cleaved fragments. Uncleaved AtEML2 protein was found predominantly in nuclear-enriched fractions while the N- and C-terminal cleaved fragments accumulated in cytoplasmic-enriched fractions. Full-length AtUFD1 and the cleaved AtUFD1 fragments were detected predominantly in cytoplasmic-enriched fractions. These results suggest that NIa-Pro is able to cleave plant proteins at diverse subcellular localization in infected cells, consistent with the known association of mature or precursor NIa-Pro with the nucleus, cytoplasm and ER membranes. We are now examining the biological impact of these cleavage events. It is anticipated that mutation of the NIa-Pro cleavage sites to prevent cleavage of the identified plant proteins could result in increased resistance to potyvirus infection.

A VIRUS BY ANY OTHER NAME: THE NEW BINOMIAL NOMENCLATURE FOR VIRUS SPECIES

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President of the International Committee on Taxonomy of Viruses (ICTV)

Virus taxon names are highly standardized. In virus taxonomy, the names of taxa above the rank of species begin with a capitalized first letter, are italicized in their entirety, and end in suffixes such as *-virus* for genera, *-viridae* for families, *-virales* for orders, etc. In all cellular organismal taxonomies, species names have always been highly standardized, following a binomial format spearheaded by Carl Linnaeus in 1753. This format consists of two ("binomial") italicized and Latinized words, separated by a space, with the first (capitalized) word being the name of the genus to which the species belongs ("genus name") and the second (lower-case) word denoting the species ("species epithet"). However, in virus taxonomy, species names were, until recently, not standardized, following a plethora of formats. This inconsistency in species name formats and styles made it difficult for virologists, let alone non-virologists (such as educators, policy makers, data analysts, students, or journal copy editors) to identify a given name as a virus species name or to differentiate it from the virus name. The International Committee on Taxonomy of Viruses (ICTV) started discussing the adoption of a standard format for virus species during its 48th annual meeting, in 2016. The idea of adopting the same standard of all other taxonomies slowly gained traction (although not without controversy). A taxonomy proposal to this effect was submitted in 2018. The decision was deferred to 2019 and then to 2020, with a "consultation" paper published (Sidell et al., Arch Virol 165:519, 2020) in which the virology community was invited to comment on the three binomial formats being considered: (i) genus name + Latin or Latinized epithet, (ii) genus name + alphanumeric epithet, and (iii) genus name + freeform epithet. A public forum was established at the ICTV website, which became the most viewed and commented forum in the history of the ICTV. Feedback was also received from several national virology societies. A consensus emerged to adopt the third option (freeform species epithet). Thus, since the ratification vote in March 2021, a standard two-part binomial nomenclature with a freeform species epithet is now the norm for naming virus species. All species created since then must have binomial names, and the names of existing species must be changed to comply with the new format no later than July 2023. It is important to highlight that the binomial format applies to species names only, not to virus names. Thus, virus names do not change (in fact, the ICTV has no mandate over virus names). A paper reiterating the differences between virus names and species names, and to provide guidelines for using and writing them correctly, has recently been published (Zerbin et al., Arch Virol 167:1231, 2022).

ADVANCES IN VACCINE TECHNOLOGIES

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Aim of the study: Vaccination is a key component of public health policy with demonstrated cost-effective benefits in protecting both human and animal populations. Recently, the WHO issued the “Immunization agenda 2030 (IA2030)” aiming to reduce mortality and morbidity from vaccine-preventable diseases for everyone and throughout the life course. WHO strategic priorities include a research and development plan aimed to improve products and services for immunization programmes, develop new vaccines and technologies, and scale up innovations. This presentation aims to summarize the recent advances in vaccine technologies and delivery.

Methods: Examination of the different vaccine technologies and manufacturing processes available starting from the types of vaccines available, e.g., inactivated, live attenuated, virus-like particles, synthetic peptides, toxoids, polysaccharides, vector-based, nucleic acids, etc.

Results and conclusions: This presentation will show the recent developments in biology, medical/biomedical engineering, immunology, and vaccinology that have led to the emergence of innovative vaccines, novel strategies of immunization, and selection of vaccine targets. The

recent advances in vaccine technologies and platforms will be also discussed to describe their mechanisms of action, advantages, and possible drawbacks. The different vaccine options will be also discussed according to IA2030's goals to strengthen immunization policies, adjust health interventions to different target age groups, rapidly respond to vaccine preventable and emerging disease outbreaks, and ensure immunization services to all people across the world.

IMMUNE RESPONSES TO OLD AND NEW VACCINES

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Istituto Nazionale Genetica Molecolare "Romeo ed Enrica Invernizzi" Milano

Vaccines are the most effective therapeutic strategy that has ever been put in place, saving more than 25 million lives worldwide in the decade 2011-2020. Vaccines aim at stimulating the adaptive arm of the immune system, whose main features are specificity and memory, and elicit the production of neutralizing antibodies, CD4+ and CD8+ T cells.

Classical vaccination strategies rely on chemically inactivated or live attenuated microorganisms, and purified or recombinant proteins, all cases in which antigens are produced in the lab, purified, formulated and then injected.

Genetic vaccines have been the most disruptive approach in the history of vaccine development, in that genetic information is injected into individuals who then produce vaccine antigens by themselves. This strategy has taken the stage over the last two years, when genetic vaccines have been brought to human use and employed to fight COVID-19 pandemics.

Genetic vaccination strategies against SARS-CoV-2 were adenoviral particles and mRNA vaccines. In particular, the mRNA-based vaccine technology is highly precise, transient, non-infectious and non-mutational. Most importantly, COVID-19 pandemics has shortened vaccine development from 7-10 years to 7-10 months, due to massive investments and parallel task development.

SARS-CoV-2 vaccines demonstrated efficacy both in preventing infection (in 60-90% of cases, depending on the virus variant considered) and in protecting from severe disease (e.g., ICU requirement and death, in 90% of the vaccinees).

REGULATORY PROCESS LEADING TO DECISIONS ON VACCINES: FOCUS ON COVID-19

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In this presentation a general overview of the regulatory pathway leading to final approval of vaccines is presented, with a specific focus on the procedures applied for COVID-19 vaccines and highlights on the streamlined processes put in place to face the health crisis during the COVID-19 pandemic. Also reference to the still ongoing discussions at international level on the necessary evidence to support emergency use authorisations or full authorisations of vaccines in a health emergency situation and subsequent lessons learnt. A brief comparison of the regulatory approaches in different geographical areas will also be presented.

UPDATE ON HBV/HCV/HDV VIROLOGY AND DIAGNOSTICS

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Hepatitis B virus, HBV, Hepatitis C virus, HCV, and Hepatitis D virus, HDV contribute substantially to the global burden of hepatic diseases. According to the latest WHO Global Hepatitis Report, Hepatitis B is a major public health problem, with 257 million chronic hepatitis B, followed by an estimated 70 million of individuals living with HCV, and around 10 to 15 millions of individuals with HBV/HDV coinfection. In this presentation will be discussed the different characteristics of these 3 viruses, providing an update on viral pathogenesis and their diagnostics.

UPDATE ON HBV/HCV/HDV THERAPEUTICS

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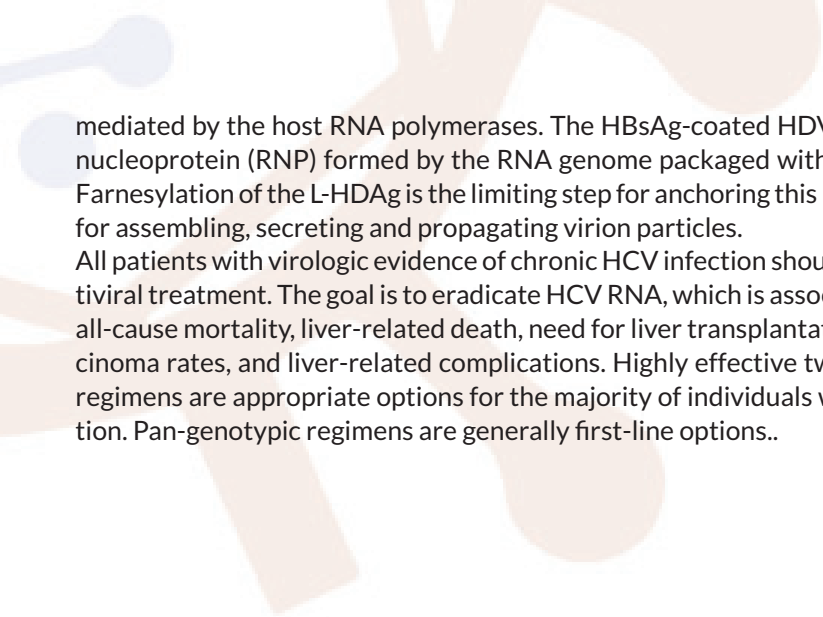
Rapid advances in the management of patients with hepatitis B/D virus (HBV/HDV) or hepatitis C virus (HCV) have occurred during the past several years with the availability of new treatment options and continued clinical research, making it possible for medical practitioners to improve outcomes for infected patients. With contemporary HCV treatment regimens, HCV elimination is now possible, but implementation of effective diagnostic and treatment strategies toward this goal requires awareness, knowledge, and the clinical acumen of medical care providers. On the contrary, current recommended treatment of chronic hepatitis B (CHB) rarely achieve the so-called “HBV functional cure”, which was defined by clinical guidelines as seroclearance of hepatitis B surface antigen (HBsAg) with or without anti-HBs.

Currently, the recommended treatment of choice for CHB regardless of the severity of the liver disease is the long-term administration of a nucleos(t)ide analog (NA) with a high barrier to resistance, such as entecavir and tenofovir; alternatively, for patients with mild to moderate CHB, a 48 week-therapy with peginterferon alfa (PegIFN α) can be considered. According to the latest update, more than 50 compounds are currently being tested for CHB. The most promising is bulevirtide which acts upon the sodium taurocholate cotransporting polypeptide (NTCP), a receptor of both HBV and HDV. Antiviral treatment can reduce the risk of long-term complications from chronic HBV (eg, liver failure and hepatocellular carcinoma) as well as the transmission of HBV to others. For some patients, immediate antiviral therapy is indicated, whereas for others, treatment may be deferred with careful monitoring. The main endpoint of all current treatment strategies is suppression of HBV DNA, loss of HBeAg (in patients who were initially HBeAg-positive), and loss of HBsAg. A sustained viral response, particularly in those who clear both HBeAg and HBsAg, is almost invariably accompanied by normalization of serum ALT, a decrease in necroinflammatory activity, and over time, a decrease in fibrosis as well. Long-term therapy with NA is effective in achieving viral suppression; however, this is not indicative of HBV eradication; with this in mind, finite NA therapy should be considered in a specific subset of patients. After reviewing the current literature NAs should be discontinued in not-cirrhotic patients with undetectable levels of HBVDNA (on three separate occasions, 6 months apart) after 12–18 months from HBeAb seroconversion (consolidation therapy), after HBsAg loss.

Conversely, NAs discontinuation should be avoided in patients who are not motivated to adhere to close monitoring or in patients with HIV or HDV coinfection.

The ideal goal for HDV treatment is the clearance of HBsAg, but a reasonably achievable goal is a sustained HDV virological response (negative HDV RNA 6 months after stopping treatment). New drug development must take into account the interaction of HBV and HDV.

The HDV genome is a circular single-stranded RNA which encodes for a single hepatitis delta antigen (HDAg) that exists in two forms (S-HDAg and L-HDAg), and its replication is



mediated by the host RNA polymerases. The HBsAg-coated HDV virions contain a ribonucleoprotein (RNP) formed by the RNA genome packaged with small and large HDAG. Farnesylation of the L-HDAG is the limiting step for anchoring this RNP to HBsAg, and thus for assembling, secreting and propagating virion particles.

All patients with virologic evidence of chronic HCV infection should be considered for antiviral treatment. The goal is to eradicate HCV RNA, which is associated with decreases in all-cause mortality, liver-related death, need for liver transplantation, hepatocellular carcinoma rates, and liver-related complications. Highly effective two- to three-month oral regimens are appropriate options for the majority of individuals with chronic HCV infection. Pan-genotypic regimens are generally first-line options..

TETRAVALENT ANTIBODY DRUGS FOR ULTRAPOTENT AND BROAD NEUTRALIZATION OF SARS-COV-2 VARIANTS

G. Novelli, F. Caccuri, P. Spitalieri, A. Zani, F. Sangiuolo, G. Citro, M. Murdocca, S. Miersch, A. Caruso, S. Sidhu

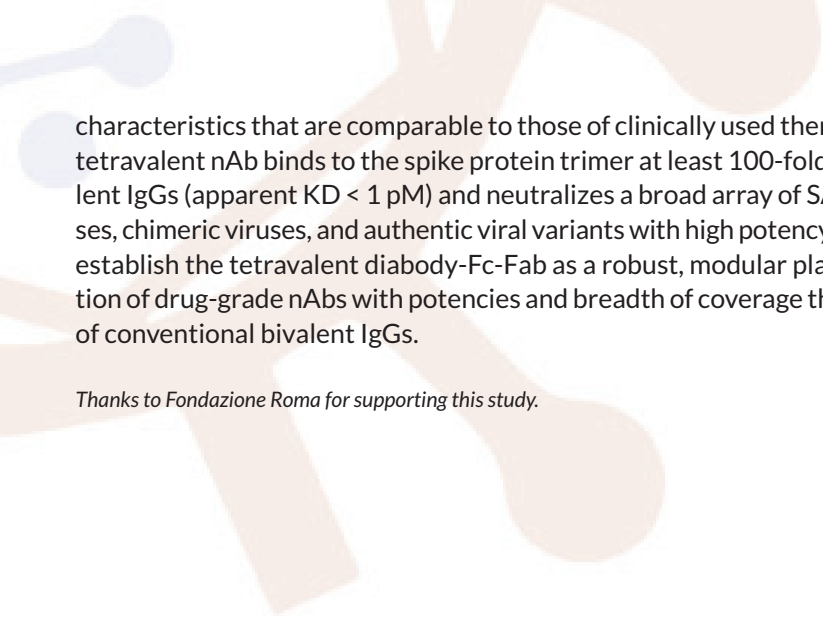
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SARS-CoV-2, the cause of the ongoing COVID-19 pandemic, relies on its spike protein (S-protein) to mediate interaction with and entry into host cells. The S-protein contains a receptor-binding domain (RBD) that is responsible for host cell recognition through interactions with the human cell-surface protein angiotensin-converting enzyme 2 (ACE2). Most of the natural neutralizing antibodies (nAbs) that arise in response to SARS-CoV-2 infection target the RBD and block ACE2 binding, and thus block infection. Cloning and recombinant production of these natural nAbs has led to the development of several therapeutic nAbs that have proven successful for inhibiting virus replication in patients and have been authorized for therapy (e.g., Casirivimab from Regeneron and Bamlanivimab from Eli-Lilly).

Even with the increasing availability of effective vaccines, the world continues to endure hundreds of thousands of infections daily due to active spread of the virus through contact. Moreover, the original SARS-CoV-2 virus continues to evolve and a growing number of variants of concern (VOCs) have proven to be not only more infective, but also, at least partially resistant to current vaccines. In addition, immune compromised individuals, the elderly, and other vulnerable groups may not be able to mount an effective immune response even after vaccination, or the immune response may wane over time. Importantly, passive immunization with recombinant nAbs can complement vaccination strategies and alleviate many of these issues. Thus, increasing the availability and utilization of more potent and broadly effective nAbs should improve patient outcomes, reduce the burden on the health care system, and contribute to increased health equity.

Unfortunately, despite the promising clinical success of bivalent nAbs for treatment of COVID-19, they suffer from two significant problems. First, even nAbs that are highly potent must be administered at extremely high doses. Second, approved IgG drugs that were derived from nAbs that arose in response to the original strain of the virus have proven to be ineffective against many VOCs that have arisen since, and even more concerning, there is evidence that nAb therapy may promote evolution of VOCs. Thus, there is an urgent need for new nAb formats and technologies that can build on the success of first-generation bivalent nAbs, while addressing the limitations that have prevented this class of drugs from having a strong beneficial impact on the COVID-19 pandemic.

We have developed a modular platform that allows us to rapidly assemble tetravalent nAbs with drug-grade quality and scalable manufacturability. We first selected antibodies targeting the RBD of SARS-CoV-2 from a synthetic phage-displayed library and used them as building blocks to generate bi-paratopic tetravalent nAbs with antigen-binding sites from two distinct nAbs. The tetravalent nAb purifies in high yield and exhibits biophysical



characteristics that are comparable to those of clinically used therapeutic antibodies. The tetravalent nAb binds to the spike protein trimer at least 100-fold more tightly than bivalent IgGs (apparent $K_D < 1$ pM) and neutralizes a broad array of SARS-CoV-2 pseudoviruses, chimeric viruses, and authentic viral variants with high potency. Together, these results establish the tetravalent diabody-Fc-Fab as a robust, modular platform for rapid production of drug-grade nAbs with potencies and breadth of coverage that greatly exceed those of conventional bivalent IgGs.

Thanks to Fondazione Roma for supporting this study.

SEARCHING FOR DIRECT ACTING AGENTS TARGETED TO SARS-COV-2 PROTEINS

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Aim of the study: Since early 2020, when the COVID-19 pandemic emerged as a global threat, efforts to identify COVID-19 treatments and preventives had ramped-up at an unprecedented speed. In addition to vaccines, the development of therapies to control SARS-CoV-2 infection had been a major goal to treat COVID-19 patients and reduce the pandemic life burden. In particular, the search for direct acting antiviral agents involved many research teams and was accomplished by two major biological approaches. A first approach was aimed to perform drug repurposing campaigns using approved or shown to be safe in man drugs. Indeed, compound repurposing allows fast identification of drug candidates that, originally developed for other purposes but having suitable safety profiles, might be effective on SARS-CoV-2 proteins. Drug repurposing antiviral campaigns have involved both cell-based viral replication and viral proteins biochemical assays. A second approach was aimed to design target-based inhibitors based on knowledges of similar targets and/or structural information. Scope of this study was to accomplish both approaches in the attempt to identify direct acting SARS-CoV-2 inhibitors.

Methods used: Cell-based SARS-CoV-2 replication assays have been established in different cells lines and used to perform drug repurposing phenotypic screening campaigns. Recombinant SARS-CoV-2 nsp3 (PL-Protease), nsp5 (3CL-Protease), nsp12 (RNA-dependent RNA polymerase) and nsp13 (Helicase) proteins, all validated drug targets, have been purified, biochemical assays have been established for each of the enzyme functions and used for compounds screening.

Results and conclusions: Large-scale repurposing collections were tested on SARS-CoV-2 replication leading to > 100 compounds showing anti-cytopathic EC₅₀ values < 20 μ M. Some of them were suitable for further study as potential therapies against COVID-19. Recombinant SARS-CoV-2 3C-like protease (3CL-Pro) was screened against the same collections leading to the identification of > 50 drugs with IC₅₀ values < 1 μ M. Selected compounds were profiled for their selectivity toward chymotrypsin and the Middle East respiratory syndrome virus (MERS) 3CL-Pro, and structural and biochemical characterization of their binding mode was also performed. In addition, a series of newly synthesized tripeptides, rationally designed as covalent reversible SARS-CoV-2 3CL-Pro inhibitors, were assayed, leading to compounds able to inhibit enzyme activity and viral replication in the low/sub nanomolar range.

Recombinant SARS-CoV-2 Helicase was screened both *in silico* and *in vitro* against a natural compound collection identifying molecules able to inhibit both enzyme-associated functions in the micromolar range. Computational studies suggested that they can bind both nucleotide and 5' -RNA binding sites, laying the bases for further drug design.

Recombinant SARS-CoV-2 PL-Pro, having multiple roles in the virus replication cycle related to both its polypeptide cleavage function and its capacity to antagonize host cellular ubiquitination and ISGylation processes, was screened using different substrate peptides and a number of compounds were found active in the nano/micromolar range.

Overall, both drug repurposing and target-based approached were used to identify diverse SARS-CoV-2 inhibitors to be explored in clinical setting and to be used for further drug developments.

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The new coronavirus SARS-CoV-2 is the causative agent of COVID-19 pandemic which so far has caused more than 6.3 million deaths (<https://covid19.who.int/>) despite new vaccines and antiviral medications. There is a high unmet need for novel targets and new effective treatments to reduce the mortality rate of COVID-19 and also for treating the early mild to moderate disease. In this scenario, the emerging of SARS-CoV-2 clinically relevant variants raised concerns about vaccine efficacy, reinfections, increased transmissibility and disease severity, boosting the search for broad-spectrum drugs or vaccines to prevent, treat and control COVID-19 also in the long term.

A gender disparity exists in COVID-19, as it is evident that male patients have a greater tendency to symptomatic COVID-19 and progression to more severe disease. The role of estrogen and estrogen receptors (ERs) signaling pathways is now quite clear in the outcome of COVID-19, probably due to their ability to regulate the viral entry protein expression and activity, thus regulating innate and adaptive immune responses as well as controlling proinflammatory cytokines, and macrophage and monocyte migration. The immune-mediated effects, both ER-dependent and ER-independent, of the class of ER modulators (SERMs) to their efficacy against a wide spectrum of pathogen infections is known (Sfogliarini C, et al., *Front Pharmacol.* 2022, 30;13:879020. doi: 10.3389/fphar.2022.879020).

In the context of the H2020 project “EXSCALATE4CoV” (E4C), an extensive virtual screening campaign on SARS-CoV-2 target proteins based on the EXSCALATE platform for drug repurposing, allowed to identify several active molecules, among them the well-known SERM raloxifene used to prevent postmenopausal osteoporosis, previously characterized for its antiviral activity (Johansen LM et al., *Sci Transl Med* 2015, 5(190): 190ra179; Montoya MC et al., *mBio* 2018, 9(6): e02272-02218.). The multiple links between ER modulation and host response to viral infections suggest beneficial effects in controlling the viral replication and may explain the observed activity.

Further, an in-depth in vitro characterization of raloxifene has shed new light on its pleiotropic mechanism of action that may account for the observed inhibition of viral replication independent from viral variant specificity (Iaconis D et al, *Cell Death Dis* 2022, 13(5):498. doi: 10.1038/s41419-022-04961-z.). Based on these findings, a phase 2/3 clinical trial (NCT05172050) in early mild-to-moderate COVID-19 outpatients confirmed evidence of efficacy of raloxifene in the virologic endpoint and in shortening the time of viral shedding (Nicastri E. et al. *EClinicalMedicine* 2022;48:101450 doi: 10.1016/j.eclinm.2022.101450.). Here we present the results of recent studies on previously unknown interactions between viral proteins and ERs, that will advance knowledge of SARS-CoV-2 biology, COVID-19 pathology, and mechanisms of sex differences in the pathology of infectious diseases, thus paving the way for potential new developments of clinical and therapeutic relevance.

FIXED DOSE COMBINATIONS OF DRUGS SYNERGISTICALLY ACTIVE AGAINST SARS-COV-2

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Aim of the study: Despite new antivirals are being approved against SARS-CoV-2 they suffer of significant constraints and are not indicated for hospitalized patients, who are left with few antiviral options. Repurposed drugs have previously shown controversial clinical results due to suboptimal drug exposure and incomplete virus suppression, however, they have mostly been used as add-on monotherapies. Similar to other viruses (e.g. HIV and HCV) identifying synergistic combinations among such drugs could overcome monotherapy-related limitations.

Methods: In a cell culture model for SARS-CoV-2 infection stringent criteria were adopted to assess drug combinations: 1) identify robust, synergist antiviral activity with no increase in cytotoxicity, 2) identify the lowest drug concentration inhibiting the virus by 100% (LIC100) and 3) extrapolate whether the LIC100 could be reached in the lung at clinically indicated drug doses.

Results and conclusions: Among several combinations tested, remdesivir with either azithromycin or ivermectin synergistically increased the antiviral activity with no increase in cytotoxicity, improving the therapeutic index and lowering the LIC100 of every one of the drugs to levels that are expected to be achievable and maintained in the lung for a therapeutically relevant period of time.

These results could have immediate implications for the treatment of hospitalized patients with COVID-19: the proposed “drug cocktails” should have antiviral activity against present and future SARS-CoV-2 variants without significant overlapping toxicity, while minimizing the onset of drug resistance.

HIV-1 MUTANTS EXPRESSING B CELL CLONOGENIC MATRIX PROTEIN p17 VARIANTS ARE INCREASING THEIR PREVALENCE WORLDWIDE

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8 Centre for Cancer Immunotherapy, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia; ilnstitute of Human Virology, University of Maryland, Baltimore, MD, USA

Aim of the study: Despite the induction of virologic suppression and immune recovery obtained by the combined antiretroviral therapy (cART), lymphoma incidence still remains high in human immunodeficiency virus-infected patients (HIV+). These findings suggest that HIV may contribute to B-cell lymphomagenesis by promoting a pro-lymphomagenic microenvironment. The HIV-1 matrix protein p17 (p17) accumulates and persists in lymphoid tissues even during cART. We have recently demonstrated that amino acid insertions at the p17 C-terminal region cause protein destabilization leading to conformational changes. P17 variants (vp17s) carrying these amino acid insertions, differently from wild-type p17, strongly impact on clonogenic B-cell growth properties that may contribute to B-cell lymphomagenesis as suggested by the significantly higher frequency detection of vp17s in plasma of HIV-1-infected patients with than without Non-Hodgkin Lymphoma (NHL). Aim of this study was to expand our previous observations by assessing the prevalence of vp17s in large retrospective cohorts of patients with lymphoma, including HIV-NHL, patients with Hodgkin's lymphoma (HIV-HL) and without lymphoma.

Methods: Plasma samples from two distinct cohorts of HIV-1-infected patients were investigated. The HIV lymphoma cohort was obtained from HIV-1-infected patients with lymphoma treated at the Centro di Riferimento Oncologico of Aviano, Italy. The control cohort included plasma samples obtained from HIV-1-infected patients undergoing routine monitoring of viremia at the Brescia Civic Hospital, Italy. RNA was extracted from plasma samples using the QIAamp DSP Virus kit and then reverse transcribed and PCR amplified using the Superscript III One-Step RT-PCR system with PlatinumTaq DNA polymerase. Then, PCR products were subjected to a nested-PCR reaction. Nested-PCR products were Sanger sequenced. All the sequences were aligned with HIV-1 subtype B p17 pNL4-3 reference genome. Phylogenetic analysis was performed using IQ-TREE. The Maximum likelihood (ML) phylogeny was used as a starting tree for Bayesian time-scaled phylogenetic analysis using BEAST. Recombinant viral proteins were purified by HPLC. B cell colony formation assay and Anchorage-Independent Growth Assay were used to evaluate the B cell clonogenic activity of vp17 on Raji cells.

Results and conclusions: Our results confirm and expand previous observations dealing with the prevalence of vp17s characterized by amino acid insertion, in the protein's C-terminal region and endowed of B cell clonogenic activity, in large retrospective cohorts of patients with and without lymphoma. In particular, we confirm the significantly higher pre-

valence of vp17s in lymphoma patients than in HIV-1-infected individuals without lymphoma. We also show that prevalence of HIV-1 mutants expressing B cell clonogenic vp17s is increasing worldwide over time. Moreover, we describe the first cluster of HIV-1 mutant expressing a B cell clonogenic vp17 and highlight that amino acid insertions can be fixed in HIV-1 and that mutant viruses displaying B cell clonogenic vp17s are actively spreading. This knowledge advocates for an extensive genomic surveillance program to monitor the evolution of such mutant virions worldwide.

NEW FRONTIERS IN HIV THERAPEUTICS

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Since the introduction of combined antiretroviral therapy (cART), AIDS-related mortality and morbidity has dramatically decreased.

From a therapeutic point of view, cART allows not only to prevent the individual progression of the disease but also, when stable and complete virological suppression is achieved, to prevent secondary sexual transmission of the infection.

New classes of drugs have been progressively made available with high efficacy and tolerability, as well as now administration modes that make chronic antiretroviral therapy more friendly for long-term use

These innovations are important as not only new molecules are being studied but also a rational change in the modality of administration of the therapy is being studied.

In fact, the first long-acting antiretrovirals are on the way, which involve the intramuscular administration of the therapy (bi-monthly injections) and make it possible to definitively shift from daily oral therapy. Along with the new long-acting drugs cabotegravir and rilpivirine, further long-acting therapeutic strategies with different drugs and classes are being studied. These strategies will be available for both treatment-naïve patients and experienced or multi-failed patients. Finally, the horizon of a possible eradication of the infection for the moment does not find a clinical applicability, but on the other hand the knowledge on preventive strategies is increasing. In fact, the use of long-acting drugs in PrEP seems to allow excellent control of transmission among HIV-negative subjects.

Unfortunately, however, as many as 12-13% of HIV infected individuals are not aware of their infection, and many more in low-income countries where HIV infection may affect sometimes even more than 10% of the adult population. Wider access to testing, prevention campaign and access to drugs is key to achieve the 95-95-95 targets set by the United Nations by 2030.



Poster & Oral Communications



TRENDS OF HIV-1 DRUG RESISTANCE AND APOBEC RELATED STOP CODONS IN PBMC COMPARTMENT OVER THE LAST DECADE

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Aim of the study: The evaluation of HIV-1 drug-resistance in PBMCs has been increased in clinical practice in the last years, especially in subjects with poor information about previous resistance. Genotypic resistance testing (GRT) in PBMCs is also useful to explore APOBEC editing in HIV-DNA. We aimed at evaluating the temporal trend of HIV-1 drug-resistance and APOBEC editing detected in PBMC GRTs in a context of suppressed viremia.

Material and methods: We included virologically suppressed individuals for whom protease (PR)/reverse-transcriptase (RT) and integrase (IN, when available) Sanger GRTs in PBMCs were available over the period 2010-2021. Temporal trends of major resistance mutations (MRM) to PI, NRTI, NNRTI and INI and APOBEC-related mutations (APO-M) were evaluated through Stanford HIVdb algorithm version 9.0. Among APO-M were considered also those substitutions related to stop codons (APO-stop) and to drug-resistance (APO-DRM).

Results: Overall, 1126 individuals with a PBMC GRT were included (724 for PR/RT/IN; 402 for PR/RT). At GRT, patient's characteristics [median (IQR)] were: age: 50 (43-56) years; time of virological suppression: 44 (4-98) months; number of regimens experienced: 4 (2-7); nadir CD4 count: 180 (60-307) cells/mm³; zenith viremia: 5.3 (4.7-5.7) log₁₀ copies/mL. Around half of individuals (45.2%) were previously exposed to INIs (raltegravir: 24.2%; dolutegravir: 22.4%; elvitegravir: 6.7%; bictegravir: 2.4%) and were highly treatment experienced (46.5%).

Concerning drug-resistance, 35.2% of individuals harboured at least one MRM (23.4% to NRTI, 18.8% to NNRTI, 7.7% to PI and 1.4% to INI). APO-M were observed in 11.4% and 15.4% of individuals in PR/RT and IN, respectively, while APO-stop were observed in 6.2% and 5.2% in PR/RT and IN, respectively. APO-DRMs related to PI, NRTI, NNRTI and INI were observed in 3.7%, 9.7%, 3.7% and 4.4% of individuals, respectively. From 2010 to 2021 no significant changes were found in the proportion of individuals harbouring MRM to any drug-class (37.5% to 30.8%), to PI (5.0% to 6.7%), NRTI (17.5% to 17.9%), NNRTI (25.0% to 16.5%) and to INI (0.0% to 1.4%). No significant changes in the prevalence of specific MRMs were found over-time. Concerning APOBEC editing in PR/RT, no significant changes in trends of APO-M (7.5% to 7.6%), APO-Stop (7.5% to 4.0%), APO-DRM related to PI (5.0% to 2.2%), NRTI (7.5% to 8.9%) and NNRTI (7.5% to 1.8%) were observed from 2010 to 2021. No significant changes in the prevalence of specific APO-DRMs were found

over-time. Concerning APOBEC editing in integrase, a slight increase of APO-M (11.1% to 16.1%), APO-Stop (3.7% to 5.7%) and APO-DRMs (3.7% to 6.8%) was observed, however without statistical significance. Among specific INI APO-DRMs, only G163R significantly increased from 2012 to 2021 (from 0% to 4.7%, $P=0.008$), while G140R significantly decreased (3.7% to 0%, $P=0.016$). Individuals who were previously exposed to INI showed a similar prevalence of INI APO-DRMs compared to others (4.4% vs. 4.5%).

Conclusions: In virologically suppressed individuals, resistance in PBMC and the extent of APOBEC editing were generally stable in the last decade. Interestingly, a slight increase of APOBEC editing in integrase was observed, particular with a G163R significative increase. The low and stable prevalence of APO-Stop underlines that Sanger HIV-DNA GRT provides reliable information to manage treatment switch in individuals under virological control.

EVALUATION OF THE QUANTIFERON SARS-COV-2 INTERFERON- γ (INF- γ) RELEASE ASSAY IN TWO COHORT OF BNT162B VACCINATED FRAGILE PATIENTS

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Aim: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccination is the standard of care for the prevention of COVID-19 disease, with a positive impact in countries in which vaccination has been promoted. Since the emergence of variants of concern (VOCs) European Medicines Agency (EMA) recommended an extra dose of the COVID-19 vaccines Comirnaty (BioNTech/Pfizer) and Spikevax (Moderna) for patients with severely weakened immune system and booster doses for subjects with normal immune system to ensure a lasting response. Although Vaccination triggers both humoral and cellular immune response, COVID-19 vaccination efficacy is evaluated by measuring antibodies only, whereas adaptative cellular immunity is unexplored. Our aim is to test this new kit QuantIFERON SARS-CoV-2 to evaluate the immune response after three doses of BNT162b vaccine in healthy donors compared to two cohort of fragile patients: Common Variable Immunodeficiency (CVID) patients and Kidney Transplant Recipients (KTR) patients.

Methods: Blood samples were collected from eight health care workers in our department, fourteen CVID patients and eight KTR patients. All the individuals recruited were naïve to SARS-COV2 and immunized by three doses of BNT162b vaccine. We examined humoral responses to vaccinations using the LIAISON DIASORIN "SARS-COV-2 S1/S2 IgG". Next blood from all participants was subjected to the novel Interferon γ (INF- γ) Release Assay (IGRA) from Qiagen, measuring INF- γ release induced by two proprietary SARS-CoV-2 peptide pools (Ag1 and Ag2) encompassing the spike protein and designed to stimulate CD4+ and CD8+ T cells and induce the releases of INF- γ .

Results and Conclusions: We confirm that in healthy subjects BNT162b third dose had successfully mounted humoral immune response with a S1/S2 IgG mean of 17100 BAU/ml. Conversely, the CVID group and KTR group shown a statistically significant reduction of IgG levels with a mean of 978 BAU/ml and 1029 respectively. Notably seven patients (five CVID and two KTR) presented IgG levels below the cut-off (33,8 BAU/ml). Next, we evaluated the INF- γ response to SARS-CoV-2 Ag1 and Ag2 observing seven non-reactive subjects (three CVID and four KTR). Surprisingly three of non-reactive patients showed a good humoral response, whereas three patients with a negative humoral immune response showed reactivity to IGRA assay. Assessing cellular immunity for SARS-COV-2 in addition to humoral immunity is important taking into account that cellular immunity plays a pivotal role against the virus and likely its variants. Some patients with weakened immune response have no correlation between humoral and cellular immunity, suggesting that the evaluation of T cell responses could be a more sensitive clinical marker of immunization. In this scenario the evaluation of cellular immunity might be informative for clinicians to identify patients more susceptible to a severe COVID-19 disease.

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Aim: The prevalence in the Campania region of HDV infection in patients with chronic hepatitis B is estimated to be around 6.4% in native subjects. However the actual prevalence is underestimated as the test is not performed in all subjects with chronic hepatitis B but it is usually performed upon a clinical order. To assess the true prevalence of HBV HDV, total anti-HDV antibodies were tested in all HBsAg positive subjects in the period from 02/21 to 03/22.

Methods: 309 patients of A.O.U. Federico II routinely tested for HBV markers with chronic hepatitis B were enrolled and a reflex test for the detection of total anti-HDV antibodies was carried out. Sera were evaluated with ADVIA Centaur HBsAgII Qualitative, Liaison Murex HBsAg Quantitative and Liaison Murex Anti-HDV Qualitative.

Results and conclusion: We found 276 HDV negative subjects and 33 HDV positive. 25 positive were already diagnosed as HBV-HDV infected, whereas in 8 patients HDV infection was detected for the first time. Our data confirm that a percentage of HBV-HDV infections are unnoticed. Due the forthcoming approval of specific anti-HDV drugs, our approach could be useful to identify patients that could benefit of anti HDV treatments.

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PO 04
COMPARISON OF SARS-COV-2 RNA DETECTION IN SALIVA OR NASOPHARYNGEAL SWAB SPECIMENS

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Laboratory of Clinical Pathology of Urbino Hospital (ASUR Marche AV1) a one-step real-time reverse RT PCR multiplex assay (RT PCR) efficiencyFor saliva samples, undiluted RNA and 1:10 dilutions were tested.

Results. All NPS and saliva samples (100%) gave a valid result for (Ct ≤ 35 according to the supplier's indications). For 24 NPS samples already diagnosed positive for the presence of viral RNA, all (100%) the paired saliva tested SARS-CoV-2 RNA positive and the median [IQR] Ct values of N gene were 21.08 [19.75-25.79] and 25.95 [19.75-25.79], of ORF1b gene were 26.06 [23.28-29.99] and 30.37 [25.78-32.82], for undiluted and 1:10 dilution samples, respectively. The is reported in the Table 1.

ID	NP SWAB (Ct)	SALIVA		SARS CoV-2 RNA	
		Undiluted	1:10 dilution	NP SWAB	SALIVA
#01	POS <40	TND	36.55	Positive	Positive
#02	POS <40	24.00	28.09	Positive	Positive
#03	34.66	TND	TND	Positive	Negative
#04	TND	37.74	TND	Negative	Positive
#05	POS <40	31.35	32.99	Positive	Positive
#06	29.2	31.02	30.79	Positive	Positive
#07	35.59	37.50	TND	Positive	Positive
#08	33.85	TND	TND	Positive	Negative
#9	34.64	TND	TND	Positive	Negative
#10	34.34	21.61	25.29	Positive	Positive
#11	31.2	24.51	28.13	Positive	Positive
Mean	33.35	29.68	30.31		
SD	2.29	6.52	4.03		

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Aim of the study. A change in the clinical epidemiology of the common seasonal respiratory pathogens affecting paediatric population has been observed during COVID-19 pandemic also in Italy (Cason et al., 2022). To note, no case of bronchiolitis caused from respiratory syncytial virus (RSV) has been evaluated in children from Trieste geographical area, compared to pre-pandemic season (Amaddeo et al., 2021). Knowing RSV clinical impact on younger children management, it was crucial to understand the virus behaviour after the spread of COVID-19 pandemic. Although restriction measures have impacted notably on RSV transmission, it has been supposed that the low rate of spread could be due to viral interference, a process that may have happened between SARS-CoV-2 and RSV (Zheng et al., 2017). The aim of this study is to monitor the epidemiology of RSV and its relationship with a wide range of respiratory pathogens, including SARS-CoV-2, in the period from May 2021 to April 2022, after the removal of pandemic social distancing measures.

Methods used. A total of 1172 nasopharyngeal swabs from symptomatic paediatric patients, were analysed at the Institute for Maternal and Child Health IRCCS "Burlo Garofolo", Trieste. Swabs were tested by a molecular multiplex platform for the simultaneous presence of RSV, SARS-CoV-2, and other respiratory pathogens including influenza A and B, adenovirus, other coronaviruses (229E, HKU-1, NL63, OC43), parainfluenza virus 1-4, enterovirus, bocavirus, metapneumovirus, rhinovirus, *Bordetella pertussis*, *Bordetella parapertussis* and *Mycoplasma pneumoniae*.

Results and conclusions. RSV was detected in 113/1172 children (9,6%), of which 94% under 5 years of age. After the observed drop during the 2020-21 winter season, RSV circulated from May 2021 reaching a peak in November of the same year and causing a large number of bronchiolitis.

Coinfections were found in 49/113 samples (43%), frequently associated with rhinovirus (18/49) followed by bocavirus (7/49). A globally decreased of the prevalence of other pathogens was observed. In conclusion, these data highlighted the risk of future changes of RSV epidemiology, and the potential for alarming epidemics when restrictive measures will be totally abolished.

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SEROPREVALENCE OF TORCH INFECTIONS IN WOMEN OF CHILDBEARING AGE OVER THE 2012-2022 DECADE IN SICILY

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Background: Infections in pregnancy can cause congenital abnormalities with serious fetal consequences. The acronym ToRCH (*Toxoplasma gondii*, other (including *Treponema pallidum*), rubella virus (RV), cytomegalovirus (CMV), herpes simplex virus) refers to pathogens directly associated with the development of congenital diseases. Primary infected pregnant women are mostly asymptomatic but severe disease due to transplacental, perinatal, or postnatal transmission can affect the unborn or newborn child.

Aim of the Study: The aim of this study was to retrospectively evaluate the seroprevalence for ToRCH infections over 10 years spanning period and define the risk population among women of childbearing age in Sicily.

Methods: Serology results were retrieved from laboratory database for a total of 2,317 sera collected from women of childbearing age (16-45 years) at the first hospital access in gynecologic ward or ambulatorial visit, over 10 consecutive years (from 01/01/2012 to 01/01/2022) at the AOUP "P. Giaccone" of Palermo, Sicily. Chemiluminescent immunoassay was performed to detect ToRCH IgM and/or IgG antibodies against the following agents: *T. gondii*, RV, CMV, B19V, and *T. pallidum*. IgM positivity in the absence of IgG antibodies was considered indicative of acute infection.

Results and conclusions: Among the 2,317 samples analyzed, 62.4% (1,445) had been tested for *T. gondii*, 55.2% (1,280) for CMV, 45.9% (1,065) for RV, 39.5% (915) for *T. pallidum*, and 1.6% (37) for B19V. The IgG seroprevalence was 21 % for *T. gondii*, 72.1% for CMV, 81.2% for RV, 1.9% for *T. pallidum*, and 81.2% for B19V. Acute infections were found in 1.9% of sera tested for *T. gondii*, 0.1% for CMV, 0.1% for rubella, 2.5% for *T. pallidum*, and 3.1% for B19V. No noteworthy differences were found in IgG and IgM seroprevalence among different age groups. According to epidemiological surveys similar seroprevalence levels were found among European childbearing age women, but few data are available in literature on the epidemiology of the ToRCH complex pathogens in Italy. In this study high seroprevalence levels were found for CMV, RV and B19V starting from 16-26 age group showing a low risk for primary infection during pregnancy. However, the relatively low seropositivity for *T. gondii* and *T. pallidum* suggests to maintain screening campaigns and to promote proper behaviours for the prevention of congenital infections.

VALIDATION AND COMPARISON OF FOUR MOLECULAR KITS FOR IN VITRO DIAGNOSIS OF SARS-COV-2 INFECTION

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Aim of the study: This study aimed to compare the performance of four molecular diagnostic assays to detect the SARS-CoV-2 RNA genome using WHO International Standard and nasopharyngeal swabs.

Methods used: A ten-fold serial dilutions of WHO International Standard for nucleic acid amplification techniques (NIBSC code: 20/146), ranging from 1000000 to 0,1 UI/ml, were analyzed with Aptima SARS-CoV-2 (Panther System, Hologic), Cobas SARS-CoV-2 (Cobas 6800, Roche), SARS-CoV-2 AMP Kit (Alinity-m, Abbott), and Allplex SARS-CoV-2 assays (Nimbus, Seegene). Assays were tested for analytical sensitivity and precision. Nasopharyngeal swabs, collected from anonymized specimens referring for analysis at the Virology unit, were used to evaluate specificity and compare overall performance and concordance between different platforms.

Results and conclusions: Allplex SARS-CoV-2 assay showed reduced sensitivity (95% LoD 100000 UI/ml of WHO International Standard) as opposed to Cobas SARS-CoV-2 Assay and Abbott SARS-CoV-2 AMP Kit. The last two were the most sensitive and reached 95% LoD with 10 UI/ml. Aptima SARS-CoV-2, which uses Transcription Mediated Amplification, showed 95% LoD with approximately 100 UI/ml. Sample to sample comparison of all platforms were carried out with 100 randomly selected routine swab samples. Both negative and positive (Ct < 35) samples showed complete concordance between Cobas 6800, Alinity-m, and Panther. The Allplex SARS-CoV-2.

Assay confirmed a lower sensitivity probably due to the extraction method (STARMag Viral DNA/RNA 200/c Kit). Allplex assay sensitivity increased using the TANBead Nucleic Acid Extraction Kit. Comparison among diagnostic platform did not show complete agreement with samples containing low input virus, e.g. Ct > 35.

In conclusion, validation of the four assays using the WHO International Standard allowed to compare the performance of each platform in our settings. Comparison spanned several months and enrolled different technicians to take into consideration lot-to-lot variability among diagnostic kits and inter-operator variability, thus providing a robust and consistent results of overall performance. Good to excellent agreement between SARS-CoV-2 RNA assays from Roche, Abbott and Hologic were achieved while small differences were obtained with low viral load samples. The latter results were expected due to the different LoDs of the systems examined and different genes targeted from each assay.

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Aim. Chromosomally integrated human herpesvirus 6 (ciHHV-6) is a condition in which the complete HHV-6 genome is integrated into the host germline genome and is vertically transmitted in a Mendelian manner. The frequency of ciHHV-6 varies from 0.2 to 2.9%, depending on the population investigated. HHV-6 levels in whole blood that exceed 6 Log₁₀ copies/ml are strongly suggestive of ciHHV-6. However, when the white blood cell count is significantly decreased, as observed in transplant recipients, HHV-6 load results in blood may be ambiguous.

The aims of this study were to identify: i) the appropriate laboratory diagnostic approaches for the diagnosis of ciHHV-6 and ii) clinical features in individuals carrying this integration.

Methods. HHV-6 PCR testing of hair follicles was used to confirm ciHHV-6 status when the viral load in whole blood was more than 5 Log₁₀ copies/ml. Extraction of nucleic acids and real-time PCR amplification from whole blood and hair follicles were performed using HHV-6 ELITE MGB® Kit (ELITechGroup, Italy) on ELITeInGenius® instrument. Clinical data from patients was collected to evaluate already known or new diseases.

Results. From January 2012 to March 2022, HHV-6 DNAemia was investigated in 1944 patients and a viral load higher than 5.0 Log₁₀ copies/ml was observed in 54 patients. In particular, 46 had a viral load higher than 6 Log₁₀ copies/ml (range: 6.0-7.2) and HHV-6 DNA in hair follicles was positive. Among the remaining 8 patients (range: 5.0-5.9), 3 were transplant recipients with leukopenia and positive HHV-6 DNA in hair follicles, and 5 were newborns with active HHV-6 infection and negative HHV-6 DNA in hair follicles. Therefore the ciHHV-6 frequency in our population was 2.5% (49/1944). We collected clinical information in the 49 ciHHV-6 patients. In 24 patients (24/49; 49%) a haematological, autoimmune or neurological pathology was identified. The remaining patients were either healthy children or adults not affected by the afore mentioned diseases.

Conclusions. Our results confirm that HHV-6 DNAemia values more than 6.0 Log₁₀ copies/ml are correlated with ciHHV-6. In case of viral loads between 5.0 and 5.9 Log₁₀ copies/ml, particular attention should be paid to leukopenic patients, in whom these loads can be associated with ciHHV-6, and to pediatric patients, in whom instead they can be associated with active neonatal infections. Therefore in this cases it is necessary to test hair follicle to confirm or exclude ciHHV-6.

In this small study population, a high percentage of patients with ciHHV-6 (49%) are affected by neurological, haematological or autoimmune diseases. In literature it is reported that ciHHV-6 might be related to many disorders, but large-scale studies are needed.

**DETECTION OF SARS-CoV-2 IN SALIVARY SAMPLES
BY IMMUNOFLUORESCENCE ASSAY**

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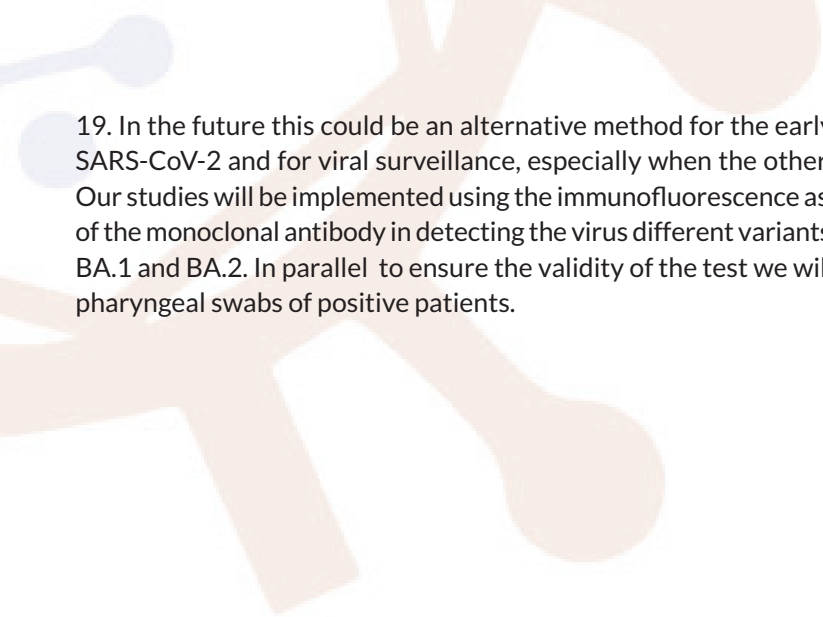
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Aim of the study: The COVID-19 pandemic caused by Severe Acute Respiratory Coronavirus 2 (SARS-CoV-2) remains a global emergency due to the rapid spread of its variants. Despite all efforts and the mass vaccination programs, achieving global vaccine coverage remains a major hurdle. SARS-CoV-2 continued to evolve under immune selective pressure, and while transmission levels remain high, there is an increased likelihood of vaccine escape variants evolving. There is a need of strengthen the control of the COVID-19 epidemics through the developing of more reliable and easy-to-use diagnostic tests essential for more rapid detection of patients and for optimized prevention and treatment in both industrialized countries and low-resource settings. In a previous work we described production of four monoclonal antibodies (mAb) recognize receptor-binding domain RBD (rRBD) of SARS-CoV-2 spike glycoprotein from the Wuhan-Hu 1 reference with neutralizing activity (Mariotti S. et al., 2021). We chose one of these mAb, R590, to develop a rapid test based on immunofluorescence assay using, saliva samples.

Methods: Viral isolate BetaCov/Italy/CDG1/2020|EPI ISL 412973|2020-02-20 obtained from a COVID-19 patient, was propagated by inoculation of Vero E6 cells. Stocks of SARS-CoV-2 virus were harvested at 72 h post infection, and supernatants were collected, clarified, and aliquoted (Magurano F. et al, 2021). This isolate was used to performed serial dilutions starting from 10^4 TCID₅₀/ml to 10^1 TCID₅₀/ml in a pool of salivary samples. These samples were spotted in duplicate in 15-well slides and were firstly incubated with R590 and with the fluorescein conjugated anti-rabbit secondary antibody. The fluorescence imaging was acquired on Zeiss LSM 900 microscope. In parallel a Real Time PCR targeting the N gene of SARS-CoV-2 was performed to evaluate the viral load of the different salivary dilutions.

Results: The results obtained from fluorescence observation showed that mAb R590 recognize SARS-CoV-2 on each dilution sample and immunofluorescence intensity was strongly correlated with virus load. Our rapid immunofluorescence test was able to detect virus even in the most diluted sample (10^1 TCID₅₀/ml) that correspond to Ct 33 value on a RT-PCR assay that a rapid antigen-test cannot detect.

Conclusions: In brief, our preliminary results was very promising to develop a new diagnostic assay to detect SARS-CoV-2. Compared with the current methods it will represent a promising immunofluorescence assay for rapid and less expensive diagnosis of COVID-



19. In the future this could be an alternative method for the early and rapid detection of SARS-CoV-2 and for viral surveillance, especially when the other tests are not available. Our studies will be implemented using the immunofluorescence assay to test the potential of the monoclonal antibody in detecting the virus different variants such as Delta, Omicron BA.1 and BA.2. In parallel to ensure the validity of the test we will use samples and nasopharyngeal swabs of positive patients.

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Aim of the study: The World Health Organization (WHO) has set a goal of eradicating measles globally through mass vaccination. High-quality surveillance is required for efficient monitoring of eradication aims (Coughlin MM et al. 2021). The identification of measles-specific IgM/IgG antibodies is widely utilized for laboratory confirmation within the WHO worldwide measles and rubella laboratory network (Cohen BJ et al. 2006; Hiebert J et al. 2021). A specific IgM/IgG in-house ELISA was developed using a recombinant measles nucleocapsid (N) protein expressed in *Escherichia coli*.

Methods: Viral RNA was extracted from Measles virus genotype B3 strain MVs/Padova.ITA/24.17/1[B3] isolated from infected Vero E6 cells. The N gene was amplified from viral RNA to produce N recombinant protein. Human serum was diluted and commercial secondary monoclonal anti-human IgM-HRP goat anti-human IgG-HRP antibodies was used. Seventy-six human serum specimens, collected during the surveillance activity for measles disease, were first screened using a commercial IgM/IgG ELISA (SERION) kit. These sera were then processed using our new in-house assay for the presence of anti-measles IgG and IgM and the results were compared with those obtained with the commercial assay.

Results: The N protein was easily purified from *E. coli* cell lysates by a one-step protocol at high level of homogeneity. It was stable at -80°C TN buffer, and used as coating antigen in ELISA. For the development of the assay several quantities of coating antigen were tested (0.5, 0.25, 0.125 µg/well). Sensitivity and specificity of our in-house assay were evaluated and resulted comparable to those obtained with the commercial assay.

Conclusions: The in-house IgM/IgG ELISA presented here is a specific and sensible tool in diagnosis of measles (Arista S et al. 1995; Pedersen IR et al. 1982), in seroprevalence and epidemiological investigations on protective immunity in different populations.

PO 11

TWO YEARS OF ONGOING OBSERVATIONS OF RESPIRATORY VIRUSES' INFECTION RATE IN PANDEMIC SARS-COV-2 PERIOD USING BIOFILMARRAY RESPIRATORY PANEL 2.1PLUS IN SYMPTOMATIC PATIENTS

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Aim: In the last two years the SARS-CoV-2 pandemic has determined radical changes in human behaviors and lifestyles. These changes have also reflected the epidemiological patterns of common respiratory viruses. This study investigated the rate of infection of common respiratory viruses in the SARS-CoV-2 epidemic situation from October 2019 to October 2021 in the "Tor Vergata" Hospital of Rome (Italy), by using BioFilmArray RP2.1plus as a diagnostic test. The results were compared with incidence from previous years, taking into account social changes as mask use or domestic isolation, as well as also by vaccination campaigns that take place during pandemics and that could influence the results.

Methods: Nasopharyngeal swabs of 2554 patients, with clinically suspected of Acute Respiratory Infections (ARIs) from October 2019 to November 2021, were collected to detect one or more of 23 common respiratory pathogens, especially viruses, via BioFilmArray RP2.1plus, including SARS-CoV-2. Demographical characteristics were acquired from electronic medical records.

Results: 2300 patients (254 patients were excluded for data missing) were enrolled in this observational study. Among them, men were 1560 and women 760, with a median age of 64,5 years. Considering the respiratory virus research request from different departments of hospital, the most of patients went to the Emergency Medicine Department (41,2%, of patients), whereas patients in the Infectious Diseases Department were 29,5% of patients. The most frequently detected pathogens included SARS-CoV-2 (31,06%, 707/2300, from March 2020 to November 2021), InfA-B (1,86%, 43/2300), HCoV (2,17% 50/2300) and HSRV (1,65%, 38/2300). Interestingly, coinfection rates dramatically decreased in the SARS-CoV-2 pandemic period.

Conclusion: The results could be important for many aspects: diagnostic approach, therapeutical choices, and health policies strategies. We concluded that in the pandemic period, particularly in the seasonal peaks of ARI in 2020-2021, SARS-CoV-2 prevailed as a mono virus for several reasons, among these, the ability of SARS-CoV-2 to bind with greater greed to membrane receptors of cells of the respiratory system. Furthermore, the timely reporting of cases, updates on clinical status and genetic predisposition of patients, the real-time analysis of data, and the appropriate dissemination of information are essential for outbreak-managing decisions.

PO 12

EVALUATION OF JCPyV VIRURIA, VIREMIA, SEROSTATUS AND MICRORNA EXPRESSION AS BIOMARKERS TO MANAGE MULTIPLE SCLEROSIS PATIENTS UNDERGOING IMMUNOSUPPRESSIVE TREATMENT

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Markers of JC polyomavirus (JCPyV) activity can be used to evaluate the risk of progressive multifocal leukoencephalopathy (PML) in multiple sclerosis (MS) patients undergoing immunosuppressive treatment. In this study, we evaluated the diagnostic value of JCPyV viruria, viremia, serostatus and microRNA expression in treated MS patients to estimate the feasibility of using these different biomarkers for JCPyV infection and for PML. The presence of JCPyV DNA and microRNA (miR-J1-5p), the anti-JCV index and the sequence of the non-coding control region (NCCR) in urine and plasma were determined in 42 MS subjects before treatment (T0), 6 months (T6) and 12 months (T12) after natalizumab, ocrelizumab, fingolimod or dimethyl-fumarate administration and in 25 healthy controls (HC). The number of MS patients with viruria increased from 43% at T0 to 100% at T12, whereas it remained similar for the HC group (35–40%). Viremia first occurred 6 months after treatment in MS patients and increased after 12 months, whereas it was absent in HC. The viral load in urine and plasma from the MS cohort increased over time, mostly pronounced in natalizumab-treated patients, whereas it persisted in HC. The archetypal NCCR was detected in all positive urine, whereas mutations were observed in plasma-derived NCCRs resulting in a more neurotropic variant. The prevalence and miR-J1-5p copy number in MS urine and plasma dropped after treatment, whereas they remained similar in HC specimens. Viruria and miR-J1-5p expression did not correlate with anti-JCV index. In conclusion, results emphasize that the molecular monitoring of JCPyV may allow early identification of patients at risk for PML among MS population and underline that the evaluation of the immunological status alone is not sufficient to limit the risk of PML. JCPyV miRNAs could be strongly proposed as biomarkers since they are stable, robustly detected, and their levels in body fluids may reflect viral perturbations in tissue sites.

PO 13

EARLY DETECTION OF SARS-COV-2 VARIANT OF CONCERN BY SNPs MUTATION AS DRIVER OF APPROPRIATE MONOCLONAL ANTIBODY THERAPY IN PEDIATRIC PATIENTS

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Aim: According to Center for disease control and prevention (CDC) a SARS-CoV-2 variant concern (VOC) critically affect patient outcome and clinical management. The impact of different VOCs specially relates to interference with diagnostic test targets decreased susceptibility to one or more class of therapies. Anti-SARS-CoV-2 monoclonal antibodies (mAbs) that target the spike protein have been shown to have clinical benefit in treating SARS-CoV-2 infection. Some anti-SARS-CoV-2 mAbs have been found to be effective as post-exposure prophylaxis (PEP) after a potential exposure to SARS-CoV-2 and during SARS-CoV-2 outbreaks. At the end of 2021 a new SARSCoV2 VOC formal named as Omicron variant has made its first appearance. In a flash survey performed in the first week of January 2022, the omicron variant was predominant with an estimated prevalence of 81% compared with the delta variant (19%) which a total replacement of Gamma and Alpha clades.

Characterization by NGS_WGS technique is the gold standard for lineage, clade and sub-clade assignment of SARS-CoV-2. Unfortunately, this technique is particularly labor-intensive (7-10 days) and expensive (>120 euro/test). Since the efficacy of mAbs increases if administration is made within 72 hours of the onset of infection and no later than 10 days after molecular detection of the virus, the time factor plays a key role. The main objective of this research was to optimize and apply operative a Multiplex RT PCR Assay able to target the different Variants by SNPs technique, in order to obtain reliable results, in a clinically useful time on NPS specimens resulted positive at SARS CoV2 RNA RT-PCR.

Method: 156 NPS specimens referred to 154 children admitted at Bambino Gesù Children Hospital were analysed by using Allplex SARS-CoV-2 Variants (Seegene) able to detect:

- Delta variant by identification of L452R-

- Omicron variant and sub-lineages by a series of combinations as follow. The deletion 69_70 found in the main lineage B.1.1.529 and in the sub-lineages BA.1, BA.1.1, BA.3 and NOT in the lineage BA.2; the N501Y mutation occurring in the main lineage B.1.1.529 and in the sub-lineages BA.1, BA.1.1, BA.2 and NOT in the lineage BA.3; the E484A mutation present in the main lineage B.1.1.529 and sub-lineages BA.1, BA.1.1, BA.2 and NOT in lineage BA.3.

Results and Conclusions: 14/156 samples (9%) were identified as VOC Delta and 127/156 (81%) were identified as VOC Omicron (respectively 93%sub-lineage BA.1 and 7% of BA.2). For 13 specimens (8 %) the assay failed due to low viral load (> 31 CT). Finally for two samples the match has not been interpretable.

In order to evaluate the accuracy of this method in VOCs identification, we compared PCR-based lineage assignment with WGS-based one used as reference for 55 specimens. Per-

formance analysis showed a lineage match of 0.95% and a Clade match of 0.98%. The Mismatch was 0.05 for lineages and 0.02 for clades.

Among samples for which we performed PCR-based lineage detection we identified 16 patients with oncological comorbidities. Among them, 12 received an appropriate mAB treatment, 3 received an empirical treatment, a therapy was corrected after VOC identification and 6 additional candidates to mAB therapy could actually benefit from VOC lineage assignment.

PCR-based approach revealed indeed to be a potential great innovation for VOC detection in clinical setting since rely on a fast procedure with a very limited TAT and a simple result interpretation further than a great gain in economic terms. The great limitation of this technique only consist in its dependency by more informative approaches (such as NGS) for a correct primer design of specific pathogen lineages.

EVALUATION OF A SARS-COV-2 QUANTITATIVE ANTIGEN TEST IN THE FOLLOW-UP OF INFECTED PATIENTS

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Introduction - Aim of the study: On October 2020, a European Commission Recommendation on COVID-19 testing strategies included the use of rapid antigen (Ag) test as a fast and convenient alternative to PCR for SARS-CoV-2 detection. FDA approved several commercial antigenic test, with different features and performances, including immunochromatographic test (ICT), immunofluorescent and chemiluminescent assays. In this study was evaluated the clinical reliability of a quantitative commercial chemiluminescent assay (Lumipulse® G SARS-CoV-2 Ag) during the follow up of 12 infected patients.

Materials and Methods: A total of 38 samples obtained from the upper airways (nasopharyngeal swabs - NPS) of 12 patients was analyzed during the second wave of SARS-CoV-2 at the Virology laboratory of Bambino Gesù Pediatric Hospital.

Samples were tested with both antigenic and molecular assay. In particular, Ag detection was performed by CLEIA technology (Lumipulse® G SARS-CoV-2 Ag), using the automated Lumipulse G1200 System (Fujirebio INC), and RNA detection was carried on by real time RT-PCR using the automated Starlet-Tanbead-CFX96 platform, with Allplex™ SARS-CoV-2 Assay (Seegene). Referring to an external standard curve, the corresponding RNA viral load was calculated for each average CT and was expressed in log 10 copies/ml.

Results: For seven patients with a NPS characterized by both SARS-CoV-2 antigen concentration <1 pg/ml and a corresponding viral load higher than 2,5 log 10, samples were all collected at least 14 days after first SARS-CoV-2 detection. For five patients with a NPS characterized by SARS-CoV-2 antigen concentration >50 pg/ml, the corresponding viral load was higher than 5 log 10 and samples were always collected at the beginning of the infection.

Conclusion: As recently shown in a previous work, the analysis of Ag concentration in relation to the timing of infection highlighted a clear distribution of samples: those with Ag ≥50 pg/ml were collected in the first ten days from the first SARS-CoV-2 positivity and samples with Ag <1 pg/ml, after 14 days. *P value* < 0.001.

Here we report the application of the quantitative SARS-CoV-2 antigen detection as a tool to stage the infection in patients with positive molecular test.

This assay allows distinguishing potentially contagious subjects from those with low chances of being contagious.

COMPARISON OF TWO SARS-COV-2 RAPID ANTIGENIC TESTS DURING A POPULATION SCREENING

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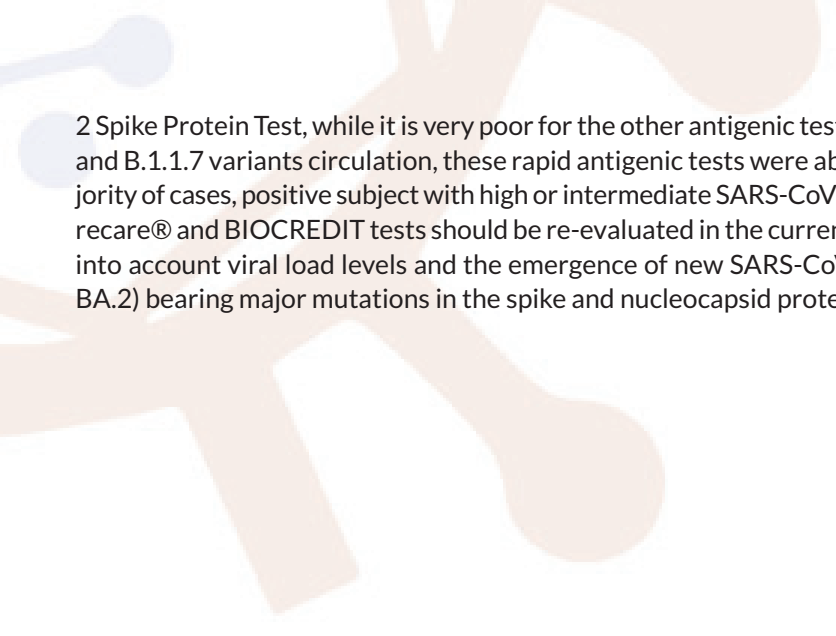
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Aim of the study: SARS-CoV-2 detection on nasopharyngeal swabs by molecular (RT-PCR) assay is the gold standard to diagnose infection. Currently, the World Health Organization (WHO) suggested the use of rapid antigen tests for population screening. Antigenic tests are able to identify acute active infection, then to lead fast isolation of positive individuals. Herein, we evaluated the performance of two rapid antigenic tests during a population screening in the Calabria Region, Southern Italy.

Methods: In January 2021, a total of 159 subjects were enrolled in one day. There was no age restriction. The screened people provided demographic characteristics, symptoms, reason for testing and informed consent. Nasopharyngeal swabs were collected in triplicate. Samples were tested using BIOCREDIT COVID-19 Ag (RapiGEN INC, Anyang, Korea), Allplex™ SARS-CoV-2 Assay Kit (Seegene, Republic of Korea) and Fluorecare® SARS-CoV-2 Spike Protein Test (Microprofit Biotech, Shenzhen, China). Quantitative fluorescence was performed by Fluorecare MF-T1000 (Microprofit Biotech, Shenzhen, China). The cycle threshold (Ct) value was related to SARS-CoV-2 RNA viral load as following: Ct < 25 = high, 25 < Ct < 30 = intermediate, Ct > 30 = low. Statistics was carried out by ROC analysis.

Results and Conclusions: Among 159 enrolled people, 69 (43.4%) were males and 90 (56.6%) females. Median age was 44 (range 8 - 89) years old. Two of the enrolled subjects claimed to have symptoms, while ten of enrolled subjects referred contacts with positive subjects. None of them resulted positive to the three tests. Seven out of 159 (4.4%) subjects were RT-PCR positive by Allplex™ SARS-CoV-2 Assay Kit, the mean Ct values were 23, 25, 33, 35, 36, 37 and 39, respectively. Only two subjects who showed mean Ct values < 30 were positive for the two antigenic tests or one test (Fluorecare® test), respectively. BIOCREDIT COVID-19 Ag identified two positive subjects, but only one was confirmed by RT-PCR (mean Ct value 25). Fluorecare® SARS-CoV-2 Spike Protein Test, detected nine positive subjects, but only three were confirmed by RT-PCR (mean Ct values were 23; 25; 39, respectively). BIOCREDIT COVID-19 Ag (targeting nucleocapsid protein) showed a sensitivity of 14% and a specificity of 99% with AUC of 0.568, while Fluorecare® SARS-CoV-2 Spike Protein Test showed a sensitivity of 42% and a specificity of 95% with AUC of 0.690. Our studies report that both antigenic test shows low sensitivity in contrast to the high sensitivity declared by manufacturer (90% and 92%, respectively). However, the area under the curve (AUC = 0.690) in our study is acceptable for Fluorecare® SARS-CoV-



2 Spike Protein Test, while it is very poor for the other antigenic test evaluated. During B.1 and B.1.1.7 variants circulation, these rapid antigenic tests were able to detect, in the majority of cases, positive subject with high or intermediate SARS-CoV-2 RNA viral load. Fluorecare® and BIOCREDIT tests should be re-evaluated in the current pandemic era, taking into account viral load levels and the emergence of new SARS-CoV-2 variants (BA.1 and BA.2) bearing major mutations in the spike and nucleocapsid proteins.

PO 16

IMMUNE PROFILING OF SARS-COV-2 EPITOPES IN ASYMPTOMATIC AND SYMPTOMATIC PAEDIATRIC AND ADULT PATIENTS

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Aim: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has unpredictable clinical manifestations of coronavirus disease (COVID-19) ranging from asymptomatic infection to mild illness of upper respiratory tract and severe respiratory distress or even death, mostly depending on the age and health conditions. We aimed to evaluate the possible correlation between Covid-19 severity and immune response against specific epitopes of SARS-CoV-2 proteome as well as of other human coronaviruses.

Methods: We included in the study 49 patients with SARS-CoV-2 confirmed infection by RT-PCR, comprising 19 paediatric and 30 adult patients with variable course of disease from mild to critical symptoms. Thirty subjects without previous infection but vaccinated against SARS-CoV-2 were also included in the analysis. Serum samples were probed against a peptide microarray made of 5828 overlapping 15-mer synthetic peptides, spanning the full SARS-CoV-2 proteome and selected peptides of the spike (S), nucleoprotein (N) and membrane (M) proteins of Middle East respiratory syndrome-related coronavirus (MERS) and common-cold coronaviruses 229E, OC43, NL63 and HKU1 (isolates 1,2 and 5).

Results and conclusions: The antibody reactivity against domains of the entire SARS-CoV-2 proteome was detected in the sera of all SARS-CoV-2-positive patients. The signal intensities varied significantly between and within patient groups and were significantly higher among severe symptoms patients. By setting the threshold above 25'000 units, all sera samples (100%, 16 out of 16) of hospitalized patients, 36% (5 out 14) of mild symptoms, 8% (1 out of 12) of 8-years old and none of 8-12 children reacted against specific peptides of N protein. Conversely, the percentage of sera samples reacting against SARS-CoV-2 M peptides was very high among children below 8-years (67%, 8 out of 12), high among hospitalized patients (31%, 5 out 16) and low in the remaining groups ($\leq 7\%$). Importantly, the antibody levels against non-structural antigens NS7B, NS8 and ORF10 were high only among adults with mild (63%) and severe symptoms (7%). IgG against S and N proteins of other coronaviruses (MERS, 229E, OC43, NL63 and HKU1) were detected in all groups without correlation with SARS-CoV-2 antibody levels.

Overall, our results show that antibodies against linear epitopes of SARS-CoV-2 S and N proteins, particularly N aa 157-175, N aa 221-235 and S aa 785-799, are age dependent and correlate with clinical severity. These epitopes are of particular interest for the development of diagnostic tests to predict Covid-19 clinical outcome.

PO 17

SARS-CoV-2 AND INFLUENZA CO-INFECTION DURING COVID-19 PANDEMIC: DATA FROM SEASONS 2020/2021 AND 2021/2022

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Up to now, little is known about coinfection and its epidemiology. In this descriptive study we retrospectively analyzed the proportion of influenza and SARS-CoV-2 virus co-infection among patients admitted to our IRCCS Sacro Cuore Don Calabria Hospital, during the flu-seasons 2020/2021 and 2021/2022. Real-time RT-PCR tests were performed to confirm COVID-19 and Influenza A/B (INF-A/B). In the PCR test, nasopharyngeal swab samples were studied in the CFX96 Real time PCR device and Real-Time PCR SARS-CoV-2/Flu/RSV Panel Kit (Anatolia Geneworks, Turkey or Qiagen, NeuMoDx, USA) was used. In the device, 200 RFU values in each channel were taken as threshold values. RNaseP was studied as an internal control and sigmoidal curves with a Ct value of ≤ 38 in the FAM channel (Orf1ab/N) and in Cy5 (INF-A/B) were considered positive. INF-A/B genotyping was performed by Regional lab service. We tested 18112 case-patients in 2020/2021 season and 18008 in the current 2021/2022 season (from week 42 until week 13). Patients age distribution was similar between the two seasons: the highest proportion of population was for patients aged 19-59 years (56.3% and 49.1% respectively for 2020/2021 and 2021/2022) and 60-99 (34.8% and 34.1% respectively for 2020/2021 and 2021/2022) with a medium age of 49.1 years. Female accounted for 52.8% and 53.8% whereas male for 47.1% and 46.2% respectively for 2020/2021 and 2021/2022.

In the course of the season 2020/2021, 35.6% patients were infected with SARS-CoV-2 and the total seasonal prevalence of non-SARS-CoV-2 respiratory viruses for all specimens tested was exceptionally low. None of sample tested resulted positive to INF-A/B regardless patient age, sex or week number season. However, in contrast with previous season, 0.43% influenza cases were detected during the 2021/2022 season (from week 42/2021 to 13/2022) in our area, despite the number of SARS-CoV-2 infected cases were similar (32.6%). Samples were then genotyped as Influenza subtype A; among these, one subtype was H1N1 whereas others were H3N2. InFluNet data recorded until the 10th week 2021/2022 season showed low levels of circulation of influenza viruses in Italy (1). Interestingly during the 2021/2022 season, we observed two cases of co-infection, the first case was detected during week 2 in a 37 years old woman patient with a Ct for SARS-CoV-2 of 32.4 and 36.4 for INF-A/B whereas the second case was detected during week 11 in a 36 years old man patient with a Ct for SARS-CoV-2 of 18.4 and 21.6 for INF-A/B. The genotype resulted to be A/H3N2.

In this study we have determined that in 2020/21 winter seasonal the Influenza virus is circulating again but also it can be seen as a co-infection with SARS-CoV-2 and other seasonal respiratory virus. Our findings suggest that co-infection with SARS-CoV-2 and influenza virus was not very common in our context and little is known about the biology and the clinical features of coinfection that might be addressed in future studies.

1. InFluNet (Istituto superiore di Sanità, <https://w3.iss.it/site/RMI/influnet/pagine/rapportoInflunet.aspx>)

PO 18

EVALUATION OF CELLULAR RESPONSE AND PRODUCTION OF INTERFERON- γ TO CMV IN TRANSPLANT PATIENTS

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Aim of the study: Cytomegalovirus (CMV) is a herpetic virus that infects about 50-85% of the adult population. This virus can lead to complications, particularly after transplants. The enumeration of CD8 + cytotoxic T lymphocytes specific for CMV in immunosuppressed patients and the production of Interferon- γ (IFN- γ) may be predictive of the risk of developing CMV disease. QuantiFERON-CMV (QF-CMV) is a test that measures cell-mediated immune responses to peptide antigens that mimic the CMV proteins. The detection and subsequent quantification of IFN- γ represent the basis of this test. The aim of our study is to evaluate the cellular response to CMV, and therefore the production of IFN- γ in critically ill patients who have undergone a solid organ or bone marrow transplant.

Methods used: In the period between July 2018 and March 2022, 1227 tests for QuantiFERON-CMV were performed at Microbiology and Virology Unit, University Hospital City of Health and Science of Turin. In particular, 923 tests belong to patients with kidney transplantation, 228 to patients with lung transplant and 76 to patients with bone marrow transplant. Whole blood was first collected in each of the QF-CMV blood collection tubes (QuantiFERON-CMV kit, Qiagen) which include a null control tube, a CMV antigen tube, and a Mitogen tube. The Mitogen tube is used in the QF-CMV assay as a positive control. The tubes were incubated at 37 ° C for a period of 16 to 24 hours, the tubes were centrifuged, the plasma was removed and the amount of IFN- γ (IU/mL) was measured with the QF-CMV ELISA.

Results and conclusions: Of the 923 tests performed for kidney transplant patients, 54% were Reactive, thus indicating a good T response to CMV; 44% is Not Reactive and 2% undetermined (absence of Interferon production also in the mitogen tube). The 228 tests for lung transplant patients showed that 50% are Reactive, 40% Not Reactive and the remaining 10% undetermined. In the 76 bone marrow transplant patients, 58% were Reactive, 24% Not Reactive and 8% undetermined. The trend of the results is similar in the three categories of patients, indicating that a good part of them is able to respond and therefore defend themselves from CMV infection. Subsequently, it will be useful to evaluate the change in T response in each patient and carry out monitoring from the early pre-transplant stages until the patient is fully recovered.

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Aim of the study. High-Risk human papillomaviruses (HR-HPVs) are responsible for virtually all cases of cervical carcinoma worldwide and several other types of cancers, including ano-genital and head-and-neck cancers. Unfortunately, despite substantial advancement in anti-HPV vaccination, there is still an urgent need of treatments for the multitude of already infected patients, since no specific anti-HPV drug is available yet.

The carcinogenic potential of HR-HPVs is related to two major viral oncoproteins, E6 and E7. It was recently demonstrated that E7 interacts with and leads to the proteasomal degradation of the cellular phosphatase PTPN14, which is an inhibitor of YAP, a well-established oncogenic determinant in solid tumors.

The goal of this study was to discover and characterize small-molecule compounds able to inhibit the protein-protein interaction (PPI) between E7 and PTPN14 and hence rescue PTPN14 levels in HPV-positive cells, paving the way for the development of new therapeutic approaches to treat HPV-associated cancers.

Methods. By using the co-crystal structure of HPV18 E7 with the C-terminal domain of PTPN14, we performed an *in silico* screening of small-molecule libraries to search for compounds directed against the C-terminal domain of E7. Hit compounds were then tested in an ELISA assay to assess their capability to inhibit the E7-PTPN14 interaction and by MTT cell viability assay in different HPV-positive and HPV-negative cancer cell lines, as well as non-tumoral cells as a control. PTPN14 rescue in HPV-positive cells treated with test compounds was assessed by means of Western Blot and immunofluorescence experiments. Antitumoral activity was investigated through clonogenic assays, transwell migration and invasion assays, wound healing assays, and 3D spheroid formation assays.

Results and conclusions. Starting from the 46 compounds emerged from the *in silico* screening, we identified three compounds (compound 20, 23, and 28) that specifically block the E7-PTPN14 interaction *in vitro* and inhibit the growth of HPV-positive cells without affecting non-tumoral and HPV-negative cancer cell lines. Among them, compound 20 was able to rescue PTPN14 protein levels in HPV-positive cells in a dose-dependent manner. Moreover, compound 20 was also able to inhibit the proliferation, migration, invasion and stem potential of HPV-positive cells, thus showing a promising antitumoral activity. Finally, treatment with compound 20 was able to rescue PTPN14 levels in a panel of cancer cell lines transformed with four different HR-HPV genotypes (HPV16, 18, 45, and 68), suggesting a broad-spectrum activity of the compound.

With this work, we provide the proof-of-principle that the E7-PTPN14 interaction can be targeted by means of small molecules, making this protein-protein interaction a new attractive drug target.

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In humans, 90% of head and neck cancers (HNC) overexpress epidermal growth factor receptor (EGFR) [1]. Therefore, the chimeric monoclonal anti-EGFR antibody Cetuximab is employed in the treatment of these tumours in combination with radiation therapy (RT) and chemotherapy (CT) and it has been considered the leading candidate in the de-escalation strategies aimed at establishing targeted and less invasive treatments, particularly in the sub-group of HNC associated with human papillomavirus (HPV) infection (20-25%) [1-2]. Feline oral squamous cell carcinoma (FOSCC) is a malignant tumour characterized by high invasiveness and poor prognosis, indeed surgery, CT and RT are mostly unsuccessful [3]. FOSCC shares several biological properties with human counterpart, such as expression of EGFR and, in a subset of tumours, association with PV infection, mainly by *Felis catus* PV type 2 (FcaPV-2) [4-6]. The aim of this study was to assess the effects of Cetuximab on proliferative and invasive abilities of FcaPV-2 FOSCC cell lines expressing EGFR associated to *Felis catus* papillomavirus infection [6-8]. To this purpose, cell lines were treated with different concentrations of Cetuximab and, after different incubation times, subjected to cell proliferation assay in 96-well plates by using a commercially available CCK-8 kit. Adsorbance at 460 nm was measured with a plate reader and changes in treated cells expressed as percentage vs control wells. Furthermore, cells monolayers seeded in 6-well plates were subjected to the scratch test; reduction of the width of wounds was measured at different time points and expressed as percentage with respect to time 0 in treated vs control cells. Then, cell pellets were collected by tripsinization and subjected to western blotting (WB) for markers of invasiveness and epithelial-mesenchymal transition (EMT). Results from CCK-8 assay showed that Cetuximab inhibited cell proliferation in all the analyzed cell lines at variable concentrations and different incubation times. Moreover, data from the scratch test revealed that the drug slowed migration of cells at different degrees and WB results indicated that these biological responses were associated with variable changes in markers of invasiveness and EMT. Taken together, these preliminary results point out that Cetuximab exhibits potential anti-cancer activities in pre-clinical models of FOSCC, targeting cell proliferation and invasive abilities of tumour cells. In conclusion, this work paves the way for future translational studies aimed at evaluating Cetuximab as a potential candidate for treatment of FOSCC.

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PO 21

DUAL CYTOPLASMIC AND NUCLEAR LOCALIZATION OF HTLV-1-ENCODED HBZ PROTEIN IS A UNIQUE FEATURE OF T ADULT CELL LEUKEMIA

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Aim of the study: 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 of HTLV-1 asymptomatic carriers and patients with HAM/TSP chronic neurologic disease, HBZ is exclusively localized in the cytoplasm 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.

Methods: 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. Quantitative real time PCR was used to measure the relative abundance of spliced and unspliced HBZ mRNA isoforms in ATL patients.

Results: 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. Tax-1 localized also in both compartments. ATL characterized by a prevalent cytoplasmic HBZ, similarly to HAM/TSP patients and AC carriers, mainly expressed the spliced HBZ isoform. Conversely, in ATL patients with a predominant HBZ nuclear localization, we observed a more abundant or similar unspliced vs spliced HBZ.

Conclusion: 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 by the capacity of HBZ to translocate to the nucleus, which suggests a role of cytoplasmic-to-nuclear translocation in HTLV-1-mediated oncogenesis.

SYNERGISM OF THE ONCOLYTIC ADENOVIRUS dl922-947 AND G-QUADRUPLEX BINDER PYRIDOSTATIN AGAINST BREAST CANCER

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Aim of the study: The oncolytic dl922-947 adenovirus demonstrated potent anticancer effects in breast cancer models. This adenoviral mutant bears a 24 bp deletion in the E1A-Conserved Region 2 (CR-2), therefore the mutant virus can replicate only in cells with a non-functional retinoblastoma pathway, a defect observed in almost all the human malignancies. We proved the anticancer efficacy of dl922-947 as a single agent or in combination with other agents in several experimental models. We here investigated in breast cancer cells a novel combinatory approach using dl922-947 with the G-quadruplex binder, pyridostatin (PDS) known to stabilize G4 motifs with consequent induction of cancer cell death.

Methods used: We used breast cancer MCF-7 cell line to evaluate the anticancer effects of dl922-94, PDS and/or their combination. We assessed cytotoxicity by sulforhodamine B assay. Cells were treated with dl922-94 and PDS or their combination at concentrations ranging from 0,2 to 4µM for 6 days. To address synergism, isobolograms were generated by computational methods using CompuSyn software. G4 motif formation was determined treating the cells as above described followed by intracellular staining with anti-G4 antibody and propidium iodide. THP-1 monocytic cells were incubated for 24h with the conditioned media (CM) of MCF-7 (treated/untreated) cells to assess phagocytosis by flow cytometry.

Results and conclusions: dl922-947 and PDS efficiently inhibited MCF-7 cell proliferation and their combination induced a synergic cytotoxic effect. Surprisingly, we observed that dl922-947 induced G-motif formation. Additionally, its combination with PDS potentiated this effect according to the synergism observed. In order to assess a potential effect on tumor cell microenvironment, we evaluated the effect of dl922-94, PDS and/or their combination in THP-1 cells exposed to the CM of MCF-7 cells. We observed a reduced phagocytic activity of monocytes exposed to CM of MCF-7 alone or in CM with dl922-94 or PDS compared to normal control medium. Surprisingly, the combination in the tumor CM of dl922-94 and PDS increased the phagocytic activity of THP-1 cells in agreement with the synergic effect observed, result suggesting the induction of an antitumor inflammatory microenvironment. Finally, our data provide evidence that the novel combination dl922-94 and PDS might represent a novel therapeutic approach against breast cancer.

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Amphibians are undergoing an unprecedented global decline with major loss of biodiversity. Infectious agents have been recently recognized as critical contributors to this threat to conservation. Among them, chytrid fungi and ranavirus have been shown to be primary pathogens leading to the local extinction and extirpation of several species of amphibians. However, it is unlikely that the several thousands of amphibian species present on the globe, might be threatened by three infectious agents only. Accordingly, we have started to close monitor emerging infectious diseases in free-ranging amphibians. This work led to the recent discovered two novel herpesviruses, named Ranid herpesvirus 3 and Bufonid herpesvirus 1, which are associated with a strikingly similar pathology phenotype consistent with a proliferative skin disease in common frog (*Rana temporaria*) and common toad (*Bufo bufo*), respectively. The disease is seasonal and affected animals show extensive skin lesions. The lesions are characterized by an inconspicuous to absent cellular immune response and with a remarkable compartmentalization of the viral replication, which appears to be confined to the upper layers of the thickened epidermis, sparing the germinal layer, suggesting the possibility of a recovery (recurrent disease). Ultrastructural pathology revealed that the viral particles are shuttled within the cells with a different transport system, which provides a higher output with Ranid herpesvirus 3, resulting in a larger number of virions accumulating extracellularly. Transcriptomic data revealed that the host genes predominantly affected are those associated with cell signaling and cell remodeling and that a number of predicted viral immunomodulatory genes are actively transcribed. Of the host genes affected, seven homologous to human genes known to be relevant in oncogenesis are strongly downregulated both in infected frogs and in toads, suggesting that they might have a role in the pathogenesis of the disease, which is characterized by a proliferative nature, similarly to cancer. Interestingly, the first frog herpesvirus to be discovered (Ranid herpesvirus 1) was the first herpesvirus shown to be the etiologic agent of a neoplastic disease (real adenocarcinoma). In conclusion, the proliferative skin disease associated with the infection of either Ranid herpesvirus 3 or Bufonid herpesvirus 1 appears to be a concerted process, where a significant epidermal cell remodeling occurs together with extensively altered cell signaling and cell remodeling gene expression. Furthermore, there is a strikingly compartmentalized viral replication associated with the expression of putative immunomodulatory viral genes. The changes in the skin are suggestive of a regeneration potential, making of these diseases potential models for DNA virus-associated proliferative diseases including neoplastic diseases, besides a great opportunity for a better understanding of amphibian disease ecology and its impact on conservation.

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DETECTION OF HUMAN NEUROTROPIC JCPyV DNA SEQUENCE IN PEDIATRIC PLEOMORPHIC XANTHOASTROCYTOMA

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Due to its peculiar histopathological findings, the Pleomorphic xanthoastrocytoma (PXA), a rare cerebral tumor of young adults with a slow growth and a good prognosis, resembles to the lytic phase of progressive multifocal leukoencephalopathy (PML), a fatal neurodegenerative disease in an immunosuppressed state caused by JC virus (JCPyV). For these reasons, the presence of JCPyV DNA was searched in a 11-year-old child with PXA, WHO grade 3, by means quantitative polymerase chain reaction (qPCR). Nested polymerase chain reactions (nPCRs) were performed for amplification of the N and C-terminal region of large T antigen (LTA_g), which is known to be involved in DNA replication, transcription and in cellular transformation, Non-Coding Control Region (NCCR) which contains the promoter/enhancer elements for expression of the early and late genes and the origin of viral DNA replication, and Viral Protein 1 (VP1), essential for early events of the viral life cycle, such as attachment to a cellular receptor, adsorption, and penetration. qPCR results showed an amount of viral DNA of 6×10^4 confirming the presence of specific JCPyV DNA in the tumor. nPCR for the amplification of the LTA_g region gave a positive result for the N-terminal region whereas, the C-terminal region was not detected. These results could be explained assuming that, while the N-terminal region is highly conserved among the primate polyomaviruses such as SV40 and BK including JC, the absence of C-terminal domain could lead to the inactivation of p53 and deregulation of cell cycle homeostasis, contributing to the development of PXA. Although in most cases, either the Mad-1 or the Mad-4 NCCRs have been identified in association with human brain neoplasms, interestingly, in this preliminary study, the archetype NCCR structure was observed. No amplification of VP1 sequence was obtained, ruling out productive infection by JCPyV. Although the expression of viral proteins T-antigen reinforces the notion of JCPyV as a key player in malignant transformation at an early stage of tumorigenesis, further studies are warranted to better understand JCPyV association with oncogenesis of pediatric brain tumors.

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Aim of the study: This study is aimed at analysing the structural and conformational dynamics of the HIV-1 matrix protein p17 (refp17) and of its variants (vp17s). The HIV-1 matrix protein is a structural protein characterized by five α -helices, four are compacted to form a packed globular domain, while the N-terminal region of the fifth helix is destabilized due to its high flexibility. On the contrary of refp17, vp17s, characterized by amino acids (aa) insertions at their C-terminal region, are endowed by B cell growth promoting activity. Functional analyses have shown that the epitope responsible for this activity is located in the N-terminal region of the viral protein. Specifically three residues Arg15, Lys18 and Arg20 are mainly involved in vp17s biological activity. These evidences represent essential knowledge to provide predictions on the effects of disease-causing mutations and positively help drug-design of novel anticancer agents, focusing the attention mainly on inhibiting the protein activity rather than counteracting the mere interaction with one of the possible receptors.

Methods used: Starting from p17 protein structures (pdb ID: 1TAM, 2HMX and 2H3Q), refp17 and vp17s were modelled by homology modelling approaches, based on single or multi templates. The models obtained were evaluated based on stereochemical parameters and energy profiles. To elucidate the mechanism that regulates epitope accessibility in the vp17s and to understand how a distal mutation in the C-terminal region of vp17s may trigger the lymphogenic activity of the epitope in the N-terminal portion, an exhaustive conformational sampling was performed by an integrated study of 200 nanoseconds (ns) classic molecular dynamic (cMD), followed by 500 ns accelerated dynamic (aMD). All systems have been solvated with tip3p water box model recreating the correct aa protonation state in the cellular environment and following an in-house protocol of minimization, heating and equilibration. All analyses were carried out with Amber18's cpptraj program.

Results and conclusions: MD simulations of refp17 and NHL-a101 (aa insertion at position 117-118) showed that vp17s increase the hydrophobic profile and alpha helix propensity in the disordered region compared to refp17. Aa insertion thus gives vp17 a lower fluctuation level in the areas corresponding to the functional epitope and helix 5. To detect correlative motions in vp17, PCA analysis followed by dynamics cross correlation analysis revealed a prominent motion of helix 5 toward helix 3, simultaneously with a loss of secondary structure in the first 3 residues of Helix 1 (residues 10 to 12), not evident in refp17. Data suggest a pronounced spatial movement and a subsequent coupling of helix 3 by the C-terminal region, affecting also the functional epitope. Hence, to perform drug repositioning studies, preliminary evidence suggests that potential druggable pockets are close to 10 to 20 residues, key residues involved in the regulation of the functional epitope. Further support for this first task will be given by the development of artificial intelligence (AI) models able to improve 3D conformations of vp17s and to perform a first binding pockets analysis.

THE ENDOGENOUS HBZ INTERACTOME IN ATL LEUKEMIC CELLS REVEALS AN UNPRECEDENTED COMPLEXITY OF HOST INTERACTING PARTNERS INVOLVED IN RNA SPLICING

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Aim of the study: Adult T-cell leukemia/lymphoma (ATL) is a T-cell lymphoproliferative neoplasm caused by the human T-cell leukemia virus type 1 (HTLV-1). Two viral proteins, Tax-1 and HBZ play important roles in HTLV-1 infectivity by altering key pathways of cell homeostasis. Tax and HBZ were recently suggested to reprogram the host cell transcriptome by perturbing the splicing landscape in T-cells, targeting cancer genes that are also altered in ATL patients. To investigate in detail the involvement of HBZ in deregulation of cell homeostasis we analyzed the endogenous HBZ interactome in leukemic cells.

Methods: The investigation of the HBZ interactome was carried out by immunoprecipitation with the 4D4-F3 anti-HBZ mAb in ATL-2 leukemic cells, followed by tandem mass spectrometry analyses. RNA seq analysis was performed to decipher the differential gene expression and the alternative splicing modifications upon HTLV-1 infection. The DEAD-Box RNA helicases DDX5-p68 and DDX17-p72 and their interaction with HBZ were confirmed by immunofluorescence followed by confocal microscopy analysis and by co-immunoprecipitation assay.

Results: The HBZ interactome of ATL-2 cells identified three main nodules corresponding to protein families mainly involved in mRNA splicing, nonsense-mediated RNA decay (NMD) and JAK-STAT signaling pathway. Here we analyzed RNA splicing. RNAseq analysis showed that HBZ specifically altered the transcription of many genes including crucial oncogenes by affecting different splicing events. Consistently, the two RNA helicases, members of the RNA splicing family, DDX5 and its paralog DDX17, which has been recently shown to be involved in alternative splicing of cellular genes after NF- κ B activation by HTLV-1 Tax-1, interacted and partially co-localized with HBZ.

Conclusion: For the first time, a complete picture of the endogenous HBZ interactome was elucidated. The wide interaction of HBZ with molecules involved in RNA splicing and the subsequent transcriptome alteration strongly suggests an unprecedented complex role of the viral oncogene in the establishment of the leukemic state.

FIRST DETECTION OF A CLUSTER OF SEXUALLY TRANSMITTED HIV-1 MUTANTS EXPRESSING A B-CELL CLONOGENIC VARIANT OF P17

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Aim of the study: Human immunodeficiency virus type 1 (HIV-1) infection is associated with an increased risk of developing lymphoid malignancies. Despite the control of HIV-1 infection has improved thanks to combined antiretroviral therapy (cART), lymphomas are still elevated in HIV-1-seropositive (HIV⁺) patients, with non-Hodgkin's lymphoma (NHL) as the most frequent. A large amount of data has been accumulated on the crucial role of circulating viral proteins in high incidence of lymphoma among HIV⁺ patients. We have recently demonstrated that amino acid insertions at the COOH-terminal region of the HIV-1 matrix protein p17 (p17), a Gag-encoded 132 amino acids-long protein with a structural role within HIV-1 virions, cause protein destabilization leading to conformational changes that strongly impact on clonogenic B-cell growth properties that may contribute to B-cell lymphomagenesis. In this study we describe the first cluster of HIV-1 mutant expressing a B cell clonogenic vp17 and highlight that amino acid insertions can be fixed in HIV-1 and that mutant viruses displaying B cell clonogenic vp17s are actively spreading.

Methods: Plasma samples were obtained from HIV⁺ patients undergoing routine monitoring of viremia at the Brescia Civic Hospital. All the sequences, obtained from Sanger sequence analysis, were aligned with HIV-1 subtype B p17 pNL4-3 reference genome. The Maximum likelihood (ML) phylogeny was used as a starting tree for Bayesian time-scaled phylogenetic analysis using BEAST. B cell colony formation assay and Anchorage-Independent Growth Assay were used to evaluate the B cell clonogenic activity of vp17s on Raji cells.

Results and conclusions: In 2019 we found a cluster of three patients, 2 females (patient C6 and C7) and 1 male (patient C2) infected by a mutant HIV-1 expressing a vp17 characterized by a Glu-Lys (EK) insertion at position 114-115 (insEK¹¹⁴⁻¹¹⁵ vp17). Phylogenetic inference showed that the three insEK¹¹⁴⁻¹¹⁵ vp17s form an independent cluster that assigns to patient C7 the role of ancestor at the beginning of the branch. Recombinant insEK¹¹⁴⁻¹¹⁵ vp17 was obtained from the dominant sequence detected in patient C7 and then investigated for its ability to enhance clonogenic activity of the Raji lymphoma B-cell line. InsEK¹¹⁴⁻¹¹⁵ vp17 was able to significantly increase cell proliferation of Raji cells in both single cell cloning assay and anchorage-independent growth assay. To better define the role of specific amino acid insertions in conferring a clonogenic property to insEK vp17, we engineered the inactive refp17 by insertion of EK at position 114-115 (insEK¹¹⁴⁻¹¹⁵ refp17). InsEK¹¹⁴⁻¹¹⁵ refp17, similarly to its natural counterpart InsEK¹¹⁴⁻¹¹⁵ vp17, signifi-

cantly increased the area of Raji cell colonies and their proliferation in the single cell cloning assay. Altogether, these data indicate that amino acid insertions can be fixed in HIV-1 and that mutant viruses displaying B-cell clonogenic vp17s are actively spreading. This knowledge calls for a wide genomic surveillance program to monitor the evolution of such mutant virions worldwide. Prompt identification of clonogenic vp17s would make possible to realize therapeutic or preventive strategies aimed at avoiding their long-term cancer-promoting activity.

TITLE: AN IMPROVED SEQUENCING APPROACH TO FULLY PROFILE SARS-COV-2 PANDEMICS AT REGIONAL LEVEL, IDENTIFY NEW VARIANTS AND DESCRIBE INFECTION SIGNATURES IN PATIENTS

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Aim: Genomic surveillance of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the only approach to rapidly monitor and tackle emerging variants of concern (VOC) of the COVID-19 pandemic. Such scrutiny is crucial to limit the spread of VOC that might escape the immune protection conferred by vaccination strategies. The resources required to realize an efficient genomic surveillance program, however, impers its implementation, especially at regional levels. We aimed at developing a framework for large-scale, rapid and cost-effective analysis of SARS-CoV-2 to use for the genomic surveillance in Campania, the most densely inhabited region in Italy.

Methods: We have matured and applied novel proof-of-principle approaches to prioritize possible gain-of-function mutations by also leveraging patients' metadata. By optimizing the libraries construction and sequencing strategies, we were able to significantly enhance the throughput of our approach while reducing the costs and retaining high-quality results

Results: We sequenced over 25,000 viral genomes 1 year and a half, producing the highest number of sequences in Italy. We thus reconstructed the whole pandemic dynamics in the regional territory. In addition, we have matured and applied novel proof-of-principle approaches to prioritize possible gain-of-function mutations by leveraging patients' metadata and isolated patient-specific signatures of SARS-CoV-2 infection. As further prove of our approach potential, we we identified three new viral variants specifically originated in Campania.

Conclusions: The aforementioned goals have all been achieved in a cost-effective manner that does not require automation, in an effort to allow any lab with a benchtop sequencer and a limited budget to perform genomic surveillance on premises.

AN INDUCIBLE CRISPR/Cas9 SYSTEM FOR THE GENE EDITING OF HIV-1 LATENTLY INFECTED CELLS**G. Magro¹, V. Poletti^{2,3}, A. Garzino-Demo^{1,4}, A. Crisanti¹, A. Calistri¹, C. Parolin¹**

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Background: One of the main barriers to achieve a definitive eradication of HIV-1 infection is the persistence of the proviral DNA in a subset of latently infected host cells. If the antiretroviral therapy is interrupted, cell reactivation can lead to viral rebound. The CRISPR/Cas9 gene editing system has been already adopted with success to directly target and disrupt the provirus in latently infected cells both *in vitro* (Ebina et al., 2013; Kaminski et al., 2016) and *in vivo* (Dash et al., 2019; Mancuso et al., 2020). Although this strategy showed promising results towards possible clinical applications, there are still issues to address, including possible off-target effects due to Cas9 constitutive expression.

Aim of the study: In this study we developed and validated a strategy aimed at limiting Cas9 expression in latently infected cells upon their activation that is known to favour HIV-1 replication. This approach should reduce the likelihood of off-target effects and, at the same time, lead to an effective disruption of the viral reservoirs.

Methods: All-in-one lentiviral vectors expressing an array of gRNAs targeting the host co-receptor CCR5 and HIV-1-specific genes (*gag*, *tat/rev*), along with the SpCas9 nuclease under a cell-activation inducible promoter, were designed. Specifically, cytokine-induced promoters were selected and validated by luciferase assay. Next, stably transduced lymphoblastoid Jurkat T cells were generated and the vector copy number (VCN) was determined in single cell clones by digital droplet PCR. Cas9 nuclease expression was also evaluated under un-stimulated or stimulated conditions along with the ability of the generated vectors to affect the expression of the gRNA targets. Finally, the antiviral efficacy of the developed approach was investigated in latently infected cell models. To this end, stably transduced cells were cloned, characterized and adopted to test the effect of Cas9 expression on viral replication upon cell stimulation compared to the appropriate controls.

Results and conclusions: Among the assayed promoters, an NFAT-regulated one showed the best performance in terms of fold of expression change (14 folds) upon activation. This finding was confirmed in uninfected cell clones stably transduced with the all-in-one lentiviral vector encompassing the NFAT-Cas9 cassette. Furthermore, the Cas9 activity against its targets was demonstrated by fluorescent-based assay in one of the developed clone (VCN=2) only upon induction of nuclease expression. Finally, we were able to show in monocyte derived latently infected cells that the developed strategy is effective against viral replication upon cell activation. Work is currently underway to further validate the feasibility and efficacy of adopting a cell-activation-inducible Cas9-based approach for the potential functional disruption of the HIV-1 latent reservoir.

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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. Cancer cells are more susceptible than healthy cells to viral replication, thus OVs were derived by attenuation of different human viruses, including herpes simplex virus type 1 (HSV-1). Oncolytic HSV-1 (oHSV1) talimogene laherparepvec was approved for the treatment of unresectable melanoma in the USA and the EU. Currently, 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. Regardless of the value of intratumoral delivery, a strategy to achieve systemic delivery and targeting of metastases involves using carrier cells which can be infected *ex vivo*, shielding the OV from the immune system. Here we propose an approach which to our knowledge was never attempted before, i.e the use of monocytes as carrier cells for oHSV1. Human monocytes can be infected by HSV-1 but are relatively resistant to infection. At the same time, in many solid tumors, circulating monocytes are the source of tumor-associated macrophages (TAMs), and are actively recruited by cancer cells. Human monocytic THP-1 cells were susceptible to infection with an EGFP-expressing oHSV1 with a backbone similar to talimogene, and could transfer infection to human breast cancer MDA-MB-231 cells both in coculture and transwell migration experiments. Primary human monocytes were also susceptible when infected with a higher viral load and could transfer infection to both MDA-MB-231 and human squamous head-and-neck UM-SC-11B cancer cells. We confirmed the migration of infected monocytic cells towards human tumor cells in a more relevant setting we injected THP-1 cells in chicken embryos with UM-SC-11B cells growing on the chorioallantoic membrane (CAM).

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A CASE OF XE SARS-COV-2 RECOMBINANT VARIANT IDENTIFIED AT THE LABORATORY OF MICROBIOLOGY AND VIROLOGY OF THE COTUGNO HOSPITAL

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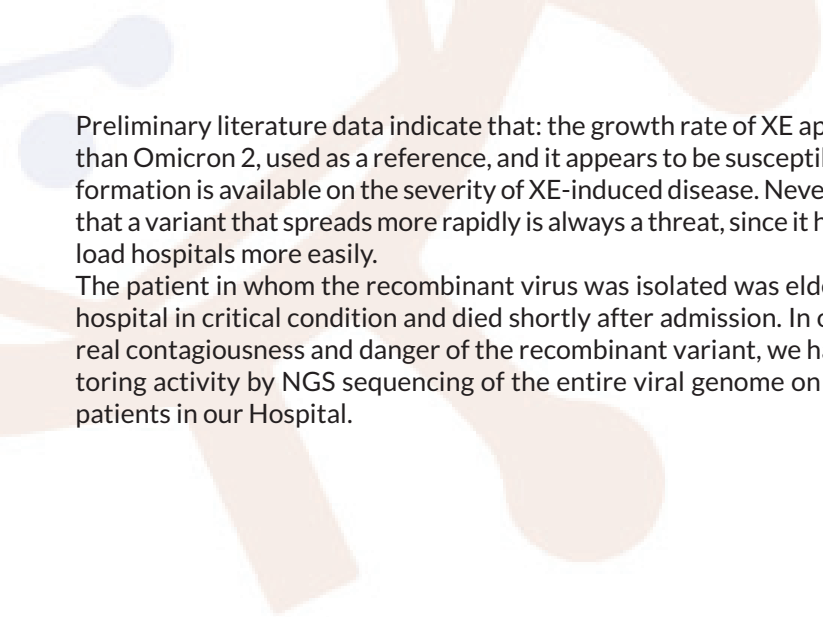
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Aim of the study: On November 26, the WHO designated the B.1.1.529 variant as 'Variant of Concern' (Voc), with the name of Omicron variant, characterized by the presence in the Spike protein of several and numerous mutations with a potential impact on the behavior of the virus, both in terms of its ability to spread and the severity of the disease. Isolated for the first time, in the first half of November in South Africa, since then it has rapidly spread throughout the world, co-circulating to the point of completely replacing the B.1.617.2 Delta variant. Each individual virus is typically an almost exact copy of the previous one. However, viruses can undergo a process called recombination. Recombinant viruses can emerge when two or more variants infect the same cell in an individual, allowing the variants to interact during replication. This mechanism involves the growth of new combinations of viruses. Over the last few months, compared to the first two years of the Sars-CoV-2 pandemic, we are witnessing a bigger recombination. This is probably due to the fact that compared to the beginning of the pandemic, recently more viruses have circulated at the same time (Delta, BA.1, BA.2) and the lineages have therefore had the opportunity to co-infect. The Laboratory of Microbiology and Virology of the P.O. Cotugno, carries out a constant and continuous monitoring activity of the SARS-CoV-2 variants circulating in the Campania region by means of NGS sequencing. During one of the last sequencing sessions we isolated a recombinant variant (Omicron1 / Omicron2) XE.

Methods: In the Laboratory of Microbiology and Virology of the Cotugno hospital, the samples that resulted positive for SARS-CoV-2, are selected for genotyping by NGS sequencing based on the requests of the departments, the viral load, the severity of the disease and the vaccination status. The Laboratory is equipped with two different systems for NGS Sequencing system: Illumina MiSeq Dx and the IonTorrent Genexus.

During a routine screening of new hospitalized patients, we sequenced 24 samples from patients hospitalized from April 4th to April 16th with Illumina MiSeq Dx. One sample, out of these 24, was found to be an Omicron 1 and Omicron 2 hybrid named XE. In order to confirm the recombinant genotype, the sample was subjected to new extraction and sequencing with Genexus Ion torrent and also in this case it was found to be a recombinant variant XE.

Results and discussion: XE recombinant results to be a combination of BA.1 (Omicron 1) and BA.2 (Omicron 2) "The recombinant XE sample isolated in our laboratory shows the following mutations in the coding region for the "S-protein": "T19I; LPPA24S_del; G142D; V213G; G339D; S371F; S373P; S375F; T376A; D405N; R408S; I410; K417N; N440K; S477N; T478K; E484A; Q493R; D614G; H655Y; N679K; P681H; N764K; D796Y; Q954H; N969K; D1146; "In addition to the mutations listed above and related to the S-protein, XE has three mutations not seen in other variants and located in two non-structural proteins (other than Spike): Nsp3 and Nsp12.



Preliminary literature data indicate that: the growth rate of XE appears to be 9.8% higher than Omicron 2, used as a reference, and it appears to be susceptible to the vaccine. No information is available on the severity of XE-induced disease. Nevertheless, it must be said, that a variant that spreads more rapidly is always a threat, since it has the potential to overload hospitals more easily.

The patient in whom the recombinant virus was isolated was elderly, arrived at Cotugno hospital in critical condition and died shortly after admission. In order to understand the real contagiousness and danger of the recombinant variant, we have increased the monitoring activity by NGS sequencing of the entire viral genome on every new hospitalized patients in our Hospital.

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Oncolytic viruses (OVs) are emerging therapeutics that selectively replicate in cancer cells, thus can combine cancer cell-killing activity with immunotherapy and gene therapy when the viral genome is modified to express therapeutic genes. We modified a strain 17+ $\Delta\gamma34.5$ HSV-1, containing a firefly luciferase (FLuc)-expressing cassette in the UL55-UL56 intergenic region, by BAC mutagenesis to obtain a deletion of the Us12 gene (Δ Us12) analogous to the one present in talimogene laherparepvec (T-VEC), the only clinically approved oncolytic herpes simplex virus type 1 (HSV-1), approved in the US in 2015 and in the EU in 2016 for the treatment of unresectable melanoma. T-VEC has deletions in the neurovirulence $\gamma34.5$ gene and Us12 gene ($\Delta\gamma34.5/\Delta$ Us12) and is further armed with human granulocyte-monocyte colony stimulating factor (hGM-CSF) gene. Our research group developed several oncolytic HSV-1 (oHSV1s) with a $\Delta\gamma34.5/\Delta$ Us12 backbone, armed with an array of immunotherapeutic genes other than GM-CSF. However, a specific question which has not been addressed yet, to the best of our knowledge, is that of the best strategy which should be employed to express multiple therapeutic genes using oncolytic viruses. While inserting different genes in a single vector is indeed feasible with large viruses such as HSV-1 and minimizes the number of viruses that must be produced, it is technically more demanding. At the same time, the use of multiple vectors potentially allows a flexible and rapid combination of therapeutic genes. Here we report that coinfection of human breast cancer cells with two different oncolytic HSV-1 vectors with a $\Delta\gamma34.5/\Delta$ Us12 backbone, each expressing an individual foreign gene (enhanced green fluorescent protein, firefly luciferase, or human interleukin 12) leads to high-level expression of both transgenes at the same compared to infection with a single virus. So, our data suggesting that vector combination genes is a potentially feasible strategy to express multiple therapeutic genes, which deserves further investigation in more advanced *in vitro* and *in vivo* models.

SARS-COV-2 INFECTION OF HUMAN OVARIAN CELLS: AN IN VITRO MODEL FOR THE DETECTION OF THE VIRUS ENTRY INTO THE HOST CELLS

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Aim: The impact of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic on female reproductive health has not been yet comprehensively investigated. To enter host cells, SARS-CoV-2 uses Spike S1 subunit to bind the receptor angiotensin-converting enzyme2 (ACE2), S2 subunit is cleaved by the host transmembrane serine protease 2 (TMPRSS2) or by cathepsin L (CSTL) to produce fusion-catalyzing viral forms. CD147 (BSG) has been proposed as an additional host receptor for SARS-CoV-2. This in vitro study aimed to investigate the potential of SARS-CoV-2 to infect the follicular micro-environment, in particular granulosa (GCs) and cumulus cells (CCs).

Methods: GCs and CCs were collected from 25 patients from March 2020 to October 2021 at the Center of Couple Sterility, Siena University Hospital. GCs were recovered from the follicular fluid and after oocyte denuding CCs were isolated. The in vitro experiments aimed to assess the permissibility of GCs and CCs to SARS-CoV-2 infection were performed in 3 patients: both GCs and CCs were infected with SARS-CoV-2 viral stock at a MOI of 0.5 and the supernatants were collected at different time point (0, 24, and 48) to be titrated in VERO E6 cell line by Reed and Muench method, evaluating the cytopathic effect (CPE) by Cell Titer (Promega). Host factors and SARS-CoV-2 expression/localization was assessed by real time quantitative PCR (qRT-PCR), Western blot (WB) and Immunofluorescence. SARS-CoV2 infection and effect on GCs and CCs were evaluated by Transmission and Immuno-electron microscopy (TEM).

Results: qRT-PCR analysis and WB showed that ACE2, TMPRSS2, BSG and CTSL transcripts were expressed in both GCs and CCs cells. ACE2 transcript was significantly increased in the CCs (0.43 vs 0.15; $p < 0.05$) respect to GCs. Coreceptor BSG and CTSL were the most expressed in GCs with respect to TMPRSS2 (0.7 vs 0.3 and 0.8 vs 0.4 respectively; $p < 0.05$). Exposure of CCs and CGs to SARS-CoV-2 in vitro did not result in any visible CPE however viral RNA titers in collected supernatants at T₂₄ (Figure 1A) significantly increased (347 ± 140 fold) with respect to T₀ in all patients (6.6×10^5 copies/ml at T₂₄ vs 1.6×10^3 copies/ml at T₀; $p = 0.004$). Globally, no significant differences were observed in viral RNA amount between infected CCs and CGs. In the figure 1B are indicated the titers obtained, expressed as TCID₅₀/ml: all cells were permissive for the infection and produce infectious virus, without significant differences with respect to the cell type. By TEM, infected human GCs showed spherical cell-associated virus-like particles, with a diameter ranging from 50-150 nm. Complete virions were also observed inside the cytoplasm as single or small groups of particles. Immunoelectron microscopy confirmed these particles as SARS-CoV2.

Conclusions: GCs and CCs are susceptible to SARS-CoV-2 infection. These in vitro findings raise concerns since CCs establish intimate connections with the oocyte. It is necessary to unveil the role of SARS-CoV-2 infection on reproductive health and ensure safety in reproductive medicine.

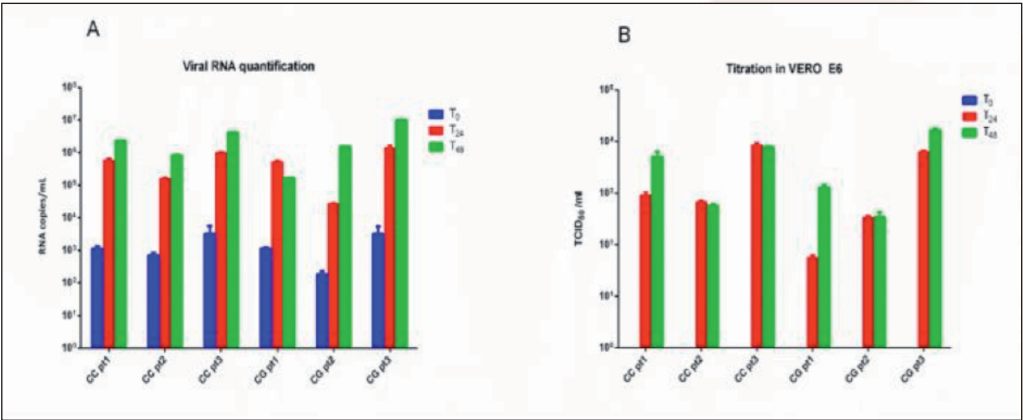


Figure 1: SARS-CoV-2 replication in CCs and CGs cells is productive. (A) Viral RNA starts to be produced at 24h and increases with respect to baseline (T₀). (B) CCs and CGs supernatants collected at 24h (T₂₄) and 48h (T₄₈) are infective and quantifiable in VERO E6 cell lines.

HERPESVIRUS INFECTIONS IN KIR2DL2 POSITIVE MULTIPLE SCLEROSIS PATIENTS: MECHANISMS TRIGGERING AUTOIMMUNITY

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Background: In multiple sclerosis (MS), there is a possible relationship with viral infection, evidenced by clinical evidence of an implication of infectious events with disease onset and/or relapse.

Aim: The aim of this research is to study how human herpesvirus (HHVs) infections might dysregulate the innate immune system and impact autoimmune responses in MS.

Methods: We analyzed 100 MS Relapsing Remitting patients, in remission phase, 100 healthy controls and 100 subjects with other inflammatory neurological diseases (OIND) (Neuro-lupus) for their immune response to HHVs infection. We evaluated NK cell response, levels of HHVs DNA, IgG and pro- and anti-inflammatory cytokines.

Results: The results demonstrated that the presence of KIR2DL2 expression on NK cells increased the susceptibility of MS patients to HHVs infection. We showed an increased susceptibility mainly to EBV and HHV-6 infections in MS patients carrying KIR2DL2 receptor and HLA-C1 ligand. The highest HHV-6 viral load was observed in MS patients, with an increased percentage of subjects positive for IgG against HHV-6 in KIR2DL2 positive MS and OIND subjects compared to controls. MS and OIND patients showed the highest levels of IL-8, IL-12p70, IL-10 and TNF-alpha in comparison with control subjects. Interestingly, MS and OIND patients showed similar levels of IL-8, while MS patients presented higher IL-12p70, TNF-alpha and IL-10 levels in comparison with OIND patients.

Conclusions: We can hypothesize that HHVs reactivation, by inducing immune activation via also molecular mimicry, may have the ability to induce autoimmunity and cause tissue damage and consequent MS lesion development.

COMPETITION AMONG TWO SARS-CoV-2 QUASI-SPECIES USING DIFFERENT MECHANISMS TO ENTER INTO HUMAN LUNG CELLS

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It is well-known that in a host the RNA viral population includes a collection of replicating viruses characterized by related sequences, named as quasi-species, instead of a sole dominating sequence. Within quasi-species there are a master mutant and minor ones continuously in competition to gain a fitness advantage and to become the dominating mutant. We were able to isolate 2 different master mutants from nasopharyngeal swabs collected from an immunocompromised patient affected by B-cell lymphoma and SARS-CoV-2 persistent infection at day 0 (MB61⁰) and at day 222 (MB61²²²). MB61⁰ represents the wild-type SARS-CoV-2 strain, while MB61²²² is characterized by two critical mutations (Q493K, N501T) in the Spike Receptor Binding Domain. The aim of this study was to understand the mechanisms leading to the dominance of MB61²²² on MB61⁰.

At first, we evaluated their growth kinetics through ddPCR on two different epithelial cell lines, Calu-3 and Caco-2. Interestingly, MB61²²² showed a significant faster replication as compared to MB61⁰ in both these cell lines.

Subsequently, to reproduce quasi-species competition *in vitro* in Calu-3 and Caco-2 cells, we performed coinfection experiments with a mixture of each virus whose relative abundance was detected through Next Generation sequencing. Of note, MB61²²² was able to replace MB61⁰ in both these cell lines. In particular, in Caco-2 cells as soon as 12 h post infection (p.i.), MB61²²² represents 70% of the virus released.

SARS-CoV-2 may gain access into host cells following endocytic or fusion pathway, thus we hypothesized that MB61²²² and MB61⁰ may use different cell entry mechanisms. To test this hypothesis, we inhibited endocytosis in Calu-3 cells through Chlorpromazine and Cathepsin L inhibitor III and we observed that MB61⁰ entry was significantly reduced while no inhibitory effects were observed when cells were infected with MB61²²². Moreover, the inhibition of the fusion pathway, obtained by blocking the activity of TMPRSS2 with Camostat, led to the significant reduction of both MB61⁰ and MB61²²² replication. This finding suggests that MB61⁰ mainly exploits the endocytosis mechanism, while MB61²²² the fusion one.

Different host cell entry modalities lead to the activation of distinct RNA sensing in infected cells. Indeed, gene expression analysis on RNA through quantitative real-time qPCR showed that MB61⁰ infection triggers the endosomal RNA sensors TLR3 and TLR7, while MB61²²² not only enhances the expression of the endosomal sensors but also of the cytosolic one, RIG-I. This data proposes a differential down-stream transduction caused by these SARS-CoV-2 isolates. In fact, different RNA sensors activate distinct transcriptional factors: TLR3 and TLR7 recruit NF- κ B, whereas RIG-I activates IRF3 and also NF- κ B. Coherently, in ELISA experiments MB61⁰-infected cells showed an increased NF- κ B transcription only, while in MB61²²²-infected cells both NF- κ B and IRF3 transcription were enhanced.

Consistently with the differential effects exerted by the two SARS-CoV-2 master mutants on RNA sensors and, therefore, on the down-stream transduction, quantitative ELISA assays showed that MB61²²² infection determines increased levels of proinflammatory cytokines (IL-1 β , IL-6, TNF- α and IFNs (IFN- α , IFN- β , IFN- γ), whereas MB61⁰ infection enhances IFN- γ transcription and secretion only.

In summary, our data suggest that MB61²²² enters into host cells not only via endocytosis but also through the fusion pathway, recruiting both endosomal (TLR3, TLR7) and cytosolic (RIG-I) RNA sensors. Therefore, either IFR3 or NF- κ B transcription factors are phosphorylated and trigger secretion of proinflammatory cytokines (IL-1 β , IL-6, TNF- α) and IFNs (IFN- α , IFN- β , IFN- γ). Differently, MB61⁰ exploits endocytosis to enter target cells, engaging TLR3 and TLR7 only, which induce NF- κ B pathway with IFN- γ production.

Altogether, our analysis points to the crucial role played by SARS-CoV-2 quasi-species in intra-host evolution aimed at achieving the best fitness to adapt to the human host. Moreover, our results propose that during SARS-CoV-2 infection host innate immunity exerts a selective pressure on quasi-species, leading to the establishment of the SARS-CoV-2 master mutant able to escape host innate responses.

DIFFERENTIAL TLR9 AND TYPE I IFNS EXPRESSION IN UPPER AIRWAYS OF MERKEL CELL POLYOMAVIRUS POSITIVE CYSTIC FIBROSIS PATIENTS

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Introduction: Merkel cell polyomavirus (MCPyV) has been detected in respiratory specimens including those from Cystic Fibrosis (CF) patients, raising questions about its immunological and clinical relevance in the respiratory tract. MCPyV might promote an inappropriate antiviral response contributing to a chronic inflammatory response and resulting in detrimental effects in CF. Therefore, our aim was to study MCPyV prevalence in a large cohort of CF patients and to assess the expression of Toll like receptor 9, one of the most important pattern recognition receptors involved in response to dsDNA viruses, and type I interferon genes (IFN α , IFN β and IFN ϵ).

Methods: Respiratory samples (n = 1138) were randomly collected from CF patients (n = 539) attending the Lazio Regional Reference Center for CF, Policlinico Umberto I Hospital, for routine visits during July 2018 - October 2019. MCPyV-DNA detection was performed by Real Time-PCR and positive samples were characterized by sequencing of the NCCR genomic region. The transcript levels of TLR9 and IFN α , IFN β and IFN ϵ were examined by RT/Real Time-PCR assays.

Results: MCPyV-DNA was detected in 268 out of 1138 respiratory specimens (23.55%) without any difference in MCPyV prevalence according to age, gender or bacteriological status of CF individuals. Thirteen out of 137 CF patients remained positive for MCPyV-DNA over the time (a median follow-up period of 8.8 months). Detection of MCPyV-DNA was not associated with the occurrence of exacerbation events. Both MCPyV positive adolescents (11-24 years) and adults (>25 years) had lower mRNA levels of TLR9, IFN β , IFN ϵ and IFN α than the negative patients of the same age group, while MCPyV positive children produced increased levels of TLR9 and IFN-I genes (p<0.05 for TLR9, IFN β , IFN ϵ) with respect to the negative ones. There were statistically significant differences in levels of TLR9 (p<0.01), but not in those of IFNs, between MCPyV-DNA positive and negative patients suffering from *S. aureus*, *P. aeruginosa* or both bacterial infections.

Conclusion: MCPyV is frequently detected in the respiratory samples of CF patients and may influence type I IFN levels in an age dependent manner. The combination of MCPyV, *S.aureus* and/or *P.aeruginosa* can affect transcript levels of TLR9 suggesting that virus-bacteria coinfection might contribute to alter airway antiviral innate immunity in CF patients.

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Aim of the study: In this study, we showed that SARS-CoV-2 gains access into primary human lung microvascular endothelial cells (HL-mECs) lacking angiotensin-converting enzyme 2 (ACE2) expression through the interaction between the conserved Arg-Gly-Asp (RGD, aa 403–405) motif endowed in the receptor binding domain (RBD) of its Spike protein and integrins.

Methods used: To demonstrate the involvement of integrins in SARS-CoV-2 Delta variant (alias of B.1.617.2) entry into ACE2-negative HL-mECs, cells were pretreated with integrin-ligand inhibitors, such as the RGD peptide or neutralizing mAbs, for 30 min at 37° before SARS-CoV-2 infection. Twenty-four h post infection, the presence of SARS-CoV-2 mRNA at intracellular level was evaluated by qRT-PCR. The Surface Plasmon Resonance (SPR) assay was used to evaluate the direct binding of $\alpha_v\beta_3$ integrin to the entire RBD of the Spike protein, while mechanisms leading SARS-CoV-2 entry into HL-mECs were scrutinized by immunofluorescence.

Results and conclusions: Using the RGD peptide as inhibitor of integrin-ligand interactions, we demonstrated the involvement of integrins in SARS-CoV-2 entry into ACE2-negative HL-mECs. $\alpha_v\beta_3$ integrin is highly expressed on the surface of HL-mECs. To gain deeper insight into the integrin mainly involved in Spike/cell interaction, we performed SARS-CoV-2 infection upon inhibition of $\alpha_v\beta_3$ integrin. Either pretreatment of the virus with $\alpha_v\beta_3$ integrin or of the HL-mECs with mAb against $\alpha_v\beta_3$ were able to strongly inhibit SARS-CoV-2 entry into HL-mECs. SPR analysis, used to evaluate the binding of $\alpha_v\beta_3$ to the entire RBD domain of the Spike protein immobilized on a sensor chip, showed that $\alpha_v\beta_3$ /RBD interaction is dose-dependent with a high kinetic affinity value, equal to 6.3 nM. We also demonstrated that, pretreating cells with different inhibitors of the endocytic pathways the virus, after binding to $\alpha_v\beta_3$, is internalized into ACE2-negative cells following a clathrin-mediated endocytic pathway. Our data highlight the RGD motif in the Spike protein of SARS-CoV-2 as a functional constraint aimed to maintain the interaction of the viral envelope with integrins and could represent a new and important therapeutic target for counteracting the presence of the virus at the systemic level and helping to limit the severity of the disease.

HUMAN PAPILLOMAVIRUS TYPE 16 E6 INDUCES THE ACTIVATION OF PELP1 PROTO-ONCOGENE CAUSING OVER-PRODUCTION OF E6*I ISOFORM IN CERVICAL CANCER

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Background: Persistent infection with oncogenic human papillomaviruses (HPVs), particularly HPV16, and constitutive expression of E6 and E7 viral genes represent the necessary cause of cervical intraepithelial neoplasia (CIN) and invasive cervical carcinoma (ICC). The host cell splicing factors regulate the differential production of HPV E6, E6*I and E7 oncoproteins during cervical neoplasia progression. The proto-oncogene PELP1 (Proline, glutamate and leucine rich protein 1) was recently shown to be involved in the alternative splicing process through the interaction with PRMT6 (Protein arginine methyltransferase 6) and SRSF2 (Serine and arginine rich splicing factor 2). In this study we investigated the role of HPV16 E6 on the upregulation of PELP1 and the production of E6*I in CIN, ICC and derived cell lines.

Materials and Methods: We analysed the expression of HPV16 E6, E6*I, PELP1, PRMT6 and SRSF2 in 14 ICC and 25 CIN samples. Squamous cell carcinoma cell lines SiHa, HeLa, HT-3, NTERA-2, PCA-5 and PCA-23 were transduced with retroviruses carrying the HPV16 E6/E7 genes and the levels of E6, E6*I, PELP1, PRMT6 and SRSF2 were evaluated by RT-PCR and digital droplet PCR (ddPCR). The production of HPV16 E6 and E7 proteins has been quantified by western blot analysis.

Results and conclusions: Overall, the HPV16 E6*I/E6 ratio was higher in ICC than CIN samples. The expression of PELP1 was statistically significant higher in ICC than CIN samples ($p=0.005$). The PRMT6 and SRSF2 expression was higher in CIN than in ICC ($p=0.0007$ and $p=0.01$, respectively). The stratification of samples in high and low risk HPVs showed that PELP1 mRNA level was higher in high-risk HPV-positive ICC samples compared to high-risk ($p=0.01$) and low-risk ($p=0.03$) HPV-positive CIN. Conversely, PRMT6 and SRSF2 were overexpressed either in high or in low risk HPV CIN compared to ICC. The transduction of LXSNE6 in cell lines induced between 2 and 8 folds upregulation of PELP1. Notably, PELP1 expression was found to correlate with E6*I expression ($R=0.88$, $p<0.05$) in LXSNE6-transduced cells.

In conclusion, our results indicate that the HPV E6 induces the expression of PELP1, which in turn causes the increase of E6*I spliced isoform and ICC development.

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Like other positive-sense single-strand RNA viruses, SARS-CoV-2, the etiologic agent of the COVID-19, replicates its genome within specialized membrane compartments called viral replication organelles (ROs). To date, very little is known about the molecular constituents or mechanisms that regulate SARS-CoV-2-induced cytopathogenicity and host-cell alterations. Here we combined advanced light and electron microscopy techniques, to obtain an integrative view of the SARS-CoV-2 ROs architecture and biogenesis. Moreover, we characterize the impact of virus replication on the cellular organelle morphology and function. Using these integrative approaches, we have identified key macromolecular complexes and cellular processes that contribute to SARS-CoV-2 cytopathogenicity.

PROPER SELECTION OF *IN VITRO* CELL MODEL AFFECTS THE CHARACTERIZATION OF THE NEUTRALIZING ANTIBODY RESPONSE AGAINST SARS-COV-2

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Significant efforts have been made to characterize the protection induced by the different COVID-19 vaccine formulations. Both clinical and epidemiological evidence demonstrated how the neutralizing activity of vaccine-induced serum antibodies is not the sole correlate of protection against SARS-CoV-2 viral variants. Nevertheless, serum neutralizing antibody titer of vaccinees still represents one of the surrogate markers of protection under the light of possible escape of SARS-CoV-2 variants from neutralizing antibodies elicited by both natural infection and vaccination. In this study, we investigate reliable *in vitro* cellular models for studying SARS-CoV-2 entry dynamics, by focusing on the evaluation of the neutralizing activity of the BNT162b2 mRNA vaccine-induced antibodies when tested in different cells.

The entry mechanisms of SARS-CoV-2 in the three cell lines (Vero E6, Vero E6/TMPRSS2, and Calu-3) were evaluated with both pseudoviral particles and whole-virus. The neutralizing capability of sera collected from vaccinated subjects a month after the second dose was characterized through microneutralization assays.

Results showed that the virus can enter both Vero E6 and Vero E6/TMPRSS2 cells mainly by using one out of two possible entry pathways. In contrast, Calu-3 cells allowed the evaluation of both viral entry mechanisms. The choice of an appropriate cellular model can have a decisive influence on the determination of the neutralizing activity of serum antibodies against the different SARS-CoV-2 variants. Our results suggest how the use of a sole system allowing only one of the two viral entry pathways may not fully reflect the neutralizing activity of vaccine-induced antibodies moving further and further away from possible correlates of protection from SARS-CoV-2 infection.

TRANSCRIPTIONALLY ACTIVE HBV INTEGRATIONS FREQUENTLY OCCUR IN THE EARLY PHASES OF CHRONIC INFECTION AND MOSTLY INVOLVE GENETIC REGIONS CRUCIAL FOR CELL PROLIFERATION

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Aim of the study: HBV integration into the human genome can mediate oncogenic activity. Limited data are available on HBV integration in the early phases of infection. Here, we characterize chimeric HBV-human RNAs in order to evaluate HBV-DNA integrations with transcriptional activity in eAg positive phases of HBV infection.

Methods: RNA-seq of liver tissues from 42 eAg+ chronically infected patients (27 with eAg+ hepatitis [eAg+CH] and 15 with eAg+ infection [eAg+CI]) was performed by NGS [Illumina, median(IQR) reads per sample: 22(18-27) millions]. An ad-hoc bioinformatic pipeline was applied to recognize chimeric HBV-human transcripts (present in >2 reads). Role of genes involved in HBV integration was assessed by GeneCards and Protein Atlas.

B: Patients with eAg+CI were significantly younger than eAg+CH [Median(IQR): 27(22-29) vs 29(25-35) years; $P < 0.001$]. Median(IQR) serum HBV-DNA and HBsAg were 8.0(5.7-8.6) logIU/ml and 4.4(4.1-4.8) logIU/ml respectively, with no significant difference according to HBV infection status. Overall, >1 chimeric HBV-human RNA was revealed in almost all patients (98%) for a total of 1048 unique HBV-human transcripts, reflecting the abundance of transcriptionally active HBV integrations. The number of chimeric transcripts in each patient did not differ in eAg+CH and eAg+CI groups [median(IQR): 13(11-22) and 14(10-39); $P = 0.46$], highlighting an early occurrence of HBV integration in young patients with limited fibrosis.

The landscape of transcriptionally active HBV integrations was similar in both CI and CH patients, involving all chromosomes in both groups. Notably, HBV integration was revealed in mitochondrial transcriptome (at levels of genes with a role in electron transport chain) only in CH group but never in CI group (48% vs 0; $P < 0.001$).

69.6% of integrations originated from HBx/Core while 20.7% of chimeric transcripts involved HBsAg coding region, resulting in production of truncated/aberrant HBsAg forms. By analyzing the number of chimeric transcripts according to HBV genotypes, we found that gen-C, -D and -E have a trend to harbor a higher number of transcriptionally active HBV integrations respect to gen-B and -A ($P = 0.07$).

Notably, by gene ontology, 18% (189/1048) of chimeric transcripts involved human exons and splicing signals, crucial for gene expression. Among them, 35.4% corresponds to genes playing a pivotal role in modulating cell survival/proliferation, including genes (BIRC6, SYF2, EVI5, BTG1) known to be dysregulated in HCC.

Transcriptionally active HBV integrations occur frequently in the eAg-positive phases of HBV infection, even in young patients with limited liver fibrosis. These events lead to the production of chimeric HBV-human aberrant transcripts, that mostly involve genes with a role in crucial intracellular pathways, conferring a proliferative advantage to the hepatocytes. This further supports early treatment initiation in eAg positive chronic infection.

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DISSECTING THE NRF2 PATHWAY AND ITS IMPACT ON THE REDOX CHANGES AND THE INFLAMMATORY RESPONSE UPON SARS-COV-2 AND INFLUENZA VIRUS INFECTIONS

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The modulation of the intracellular redox state is a key event during respiratory virus infections because of its regulation of virus-life cycle and host responses. We recently demonstrated that influenza virus infection decreases the protein expression and impairs the nuclear localization of Nrf2, a transcription factor that regulates the expression of antioxidant genes, such as those involved in glutathione (GSH) restoration and in the inflammatory response (De Angelis M et al. 2022). Other reports have shown a suppression of the Nrf2 signalling also by SARS-CoV-2, leading to a dysregulation in GSH level. However, nowadays the respiratory virus proteins involved in redox modulation as well as the mechanisms through which they regulate the antioxidant pathway remain to be clarified.

Our study was aimed at: i) dissecting the antioxidant response and inflammatory pathways during SARS-CoV-2 infection; ii) identifying potential influenza virus and SARS-CoV-2 proteins responsible of the regulation of Nrf2 and inflammatory signalling.

Methods: African green monkey kidney cell line (Vero E6), human epithelial lung cells (A549) or human embryonic kidney 293 cells (HEK-293T) were infected with SARS-CoV-2, influenza A/Puerto Rico/8/34 H1N1 virus (PR/8) or transfected with different PR/8 or SARS-CoV-2 plasmids encoding for selected respiratory virus proteins. The whole cellular extract or the nuclear/cytoplasmic fractions were then analysed for the expression of Nrf2 and G6PD by western blot. The activation of Antioxidant Response Element (ARE) promoter (pARE) was analysed by reporter gene assay in HEK-293T cells expressing the viral proteins. Reactive Oxygen Species (ROS) were measured by the DCFDA assay in A549 cells transfected with plasmids encoding for the viral proteins. The total glutathione level and its reduced form (GSH) were analysed by using a colorimetric assay kit. **Results and Conclusions:** Nrf2 and G6PD protein expression levels were decreased after SARS-CoV-2 infection indicating a modulation of the antioxidant response similar to influenza virus. A549 cells were transfected with three main SARS-CoV-2 accessory proteins individually: ORF3, wild-type (wt-) ORF6, ORF6 M58R mutant or with ORF8. In parallel the cells were also transfected with the nucleocapsid (N) structural protein. We reported a reduction of Nrf2 protein expression, along with its related genes SOD1/2 and G6PD, only when the wt-ORF6 but not the M58R mutant protein was ectopically expressed. At the same time, the total level of glutathione, as well as the reducing form of GSH, was decreased in the same experimental condition. Accordingly, the activation of the ARE promoter, reflecting the Nrf2 activity, was down-regulated by the wt-ORF6 protein compared to the respective mutant. On the other hand, the production of ROS was proportionally modulated at 48 h after viral protein over-expression, as demonstrated by the DCFDA assay. On the contrary, transfection with N protein increased pARE activation at both 24 and 48 h, suggesting a different virus-induced regulation of the antioxidant response.



In influenza virus infection model, we observed an inhibition of the pARE activation after the nonstructural NS1 protein transfection at 24 h while the pARE activation was increased at 48 h in particular after the transfection with NS1, nucleoprotein (NP), matrix protein 1 and 2 (M1, M2) and hemagglutinin (HA) proteins suggesting a rescue of the antioxidant response.

Overall, these preliminary data have allowed to identify some respiratory virus proteins involved in the modulation of the host antioxidant response and to understand the relationship between the antioxidant pathways and inflammatory response during viral infection. Further experiments will be necessary to better understand the timing through which the viral proteins are able to modulate these pathways and how the proteins interfere with antioxidant response functions.

IFI16 IMPACTS METABOLIC REPROGRAMMING DURING HUMAN CYTOMEGALOVIRUS INFECTION

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Cellular lipid metabolism plays a pivotal role in human cytomegalovirus (HCMV) infection as increased lipogenesis in HCMV-infected cells favors the envelopment of newly synthesized viral particles. As all cells are equipped with restriction factors (RFs) able to exert a protective effect against invading pathogens, we asked whether a similar defense mechanism would also be in place to preserve the metabolic compartment from HCMV infection. Here we show that the IFN- γ -inducible protein 16 (IFI16), an RF able to block HCMV DNA synthesis, can also counteract HCMV-mediated metabolic reprogramming in infected primary human foreskin fibroblasts (HFFs), thereby limiting virion infectivity. Specifically, we find that IFI16 downregulates the transcriptional activation of the glucose transporter 4 (GLUT4) through cooperation with the carbohydrate-response element-binding protein (ChREBP), thereby reducing HCMV-induced transcription of lipogenic enzymes. The resulting decrease in glucose uptake and consumption leads to diminished lipid synthesis, which ultimately curbs the *de novo* formation of enveloped viral particles in infected HFFs. Consistently, untargeted lipidomic analysis shows enhanced cholesteryl ester levels in IFI16 KO vs. wild-type (WT) HFFs. Overall, our data unveil a new role of IFI16 in the regulation of glucose and lipid metabolism upon HCMV replication and uncover new potential targets for the development of novel antiviral therapies.

A COMPLEX SIGNALING PATHWAY INVOLVING NF- κ B, JAK/STAT, AND GP130 IS ACTIVATED BY CCL2 NEUTRALIZATION TO INDUCE THE EXPRESSION OF HIV-1 RESTRICTION FACTORS IN PRIMARY HUMAN MACROPHAGES

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Aim of the study: CC chemokine ligand 2 (CCL2) is a main inflammatory chemoattractant directing the mobilization and homing of monocytes/macrophages and effector T lymphocytes to sites of inflammation. CCL2 and its receptor CC chemokine receptor 2 (CCR2) are involved in different diseases characterized by chronic inflammation, among which AIDS (Fantuzzi et al, Cell Mol Life Sci 2019, 76(24):4869-4886). We previously found that exposure of monocyte derived-macrophages (MDMs) to CCL2 neutralizing antibody (Ab) strongly inhibited HIV-1 replication at post-entry steps of the viral life cycle (Sabbatucci et al, Retrovirology 2015, 12:4). This effect was associated with up-regulation of several mRNA coding for factors involved in innate antiviral responses, among which APOBEC3A, RSAD2 and ISG15. These transcripts were enriched for RELA, NF κ B1 and STAT targets, thus suggesting the activation of the canonical NF- κ B and JAK/STAT pathways upon CCL2 neutralization (Covino et al, Front Immunol 2020, 11:2129). This study aimed at identifying the signal transduction pathways involved in the induction of host restriction factors by CCL2 blocking in MDMs.

Material and methods: CD14⁺ monocytes were isolated from the peripheral blood of healthy donors by immunomagnetic selection and cultivated in vitro for 6 days to obtain MDMs. The signaling pathways underlying CCL2 blocking-mediated induction of APOBEC3A, RSAD2 and ISG15 were investigated in MDMs exposed to anti-CCL2 Ab by using a combination of pharmacologic inhibition, qPCR, western blot and confocal laser scanner microscopy.

Results and Conclusions: CCL2 neutralization mediated APOBEC3A, RSAD2 and ISG15 transcripts accumulation was inhibited by actinomycin D, thus indicating that it requires active transcription. Moreover, exposure of MDMs to anti-CCL2 Ab induced a time dependent phosphorylation of I κ B, STAT1, and STAT3, as well as p65 nuclear translocation, thus experimentally confirming activation of the canonical NF- κ B and JAK-STAT signaling pathways. Interestingly, the inhibitor of I kappa B kinase BMS-345541 strongly inhibited APOBEC3A and RSAD2, but not ISG15, transcript accumulation elicited by anti-CCL2 Ab treatment, while Jak inhibitor I reduced induction of all three genes. Furthermore, CCL2 neutralization mediated APOBEC3A and RSAD2 transcripts accumulation was inhibited by cycloheximide, thus suggesting the involvement of newly synthesized proteins. Finally, the exposure of MDMs to anti-CCL2 Ab resulted in induction of IL-6 family cytokines and interfering with gp130 signaling inhibited APOBEC3A and RSAD2 up-regulation triggered by CCL2 neutralization.

These results provide novel insights into the signal transduction pathways regulating the expression of HIV-1 restriction factors in primary human macrophages and unravel new mechanisms by which CCL2 may deregulate macrophage antiviral responses and contribute to AIDS pathogenesis. Therapeutic targeting of CCL2 may thus represent an opportunity to strength host innate immunity and restrict HIV-1 replication.

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Aim of the study: In this study, we scrutinized the ability of the SARS-CoV-2 Omicron variant, alias of B.1.1.529 lineage, to infect human primary pulmonary microvascular endothelial cells (HL-mECs). We studied the mechanism of entry and the capability to promote angiogenesis of the SARS-CoV-2 Omicron variant using the HL-mECs as a cellular model. Furthermore, we highlighted the possibility to inhibit SARS-CoV-2-entry into HL-mECs to counteract its biological effects.

Methods used: HL-mECs were infected with SARS-CoV-2 Omicron variant, at high multiplicity of infection. Viral RNA was quantified by RT-PCR at different time points. The angiogenic capability of SARS-CoV-2-infected HL-mECs was explored by seeding cells on 48-well plates containing polymerized plugs of growth factor-reduced Basal Membrane Extract and by spheroidal system aimed at mimicking the correct 3D assembly of the lung capillary blood vessel wall. Immunofluorescence experiments and quantifications of viral RNA by RT-PCR were performed to study and clarify the mechanism of SARS-CoV-2 Omicron variant entry into endothelial cells.

Results and conclusions: Recently, it has been demonstrated that SARS-CoV-2-infected HL-mECs play a role in sustaining vascular dysfunction during the early stages of infection. Here we demonstrated that the SARS-CoV-2 Omicron variant, is able to remodel the cellular phenotype and to promote angiogenesis in the absence of productive infection. In fact, we observed that supernatant collected at 1, 24, 48, and 120 h post infection did not show any increase in viral RNA over time. On the contrary, viral RNA was found at the intracellular levels. We hypothesized that the internalization of SARS-CoV-2 Omicron variant into ACE2-negative cells could be ascribed to an endocytic mechanism. To prove this hypothesis, we carried out SARS-CoV-2 infection on HL-mECs after their pretreatment with different inhibitors of the endocytic pathways. Data obtained indicate that the mechanism leading to SARS-CoV-2 entry into ACE2-negative cells follows a clathrin-mediated endocytic pathway. HSPGs may act as coreceptors for SARS-CoV-2 infection. Our data showed that pretreatment of SARS-CoV-2 or HL-mECs with heparin or heparinase, respectively, significantly inhibits integrin-mediated SARS-CoV-2 Omicron variant entry into ACE2-negative cells. Our findings highlight the importance of targeting Spike-HSPGs interactions in order to counteract SARS-CoV-2 infection of ECs.

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Aim of the study: In the recent decades, several emerging and re-emerging viral respiratory pathogens, including severe acute respiratory syndrome coronaviruses 2 (SARS-CoV-2) and measles virus (MeV), caused high morbidity and mortality globally, defying the public health systems. Therefore, the development of approaches capable of limiting the spread of these diseases is urgently required. Epigenetic mechanisms have aroused considerable interest. Recent evidence has reported the involvement of N6-methyladenosine (m6A) in the replication of several viruses, although its role needs to be fully elucidated. The m6A pathway could represent a potential target to develop novel antiviral drugs.

Methods used: In the present study, the catalytic activity of the fat mass and obesity associated protein (FTO) was inhibited using the selective inhibitor Rhein. The cytotoxicity of Rhein (1.5 - 200 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 (ATCC CCL-81) after 24 hours of treatment. The antiviral activity against MeV, Human Coronavirus OC43 (HCoV-OC43), Human Coronavirus 229E (HCoV-229E) and SARS-CoV-2 was evaluated via the co-treatment assays. Then, Vero cells were infected with SARS-CoV-2 and MeV (MOI 0.01) for 12 hours and treated with rhein at 50 and 200 µg / mL. RNA was extracted by TRIzol reagent and quantified using the NanoDrop spectrophotometer. Finally, the m6A levels were obtained via the m6A RNA methylation assay kit.

Results and conclusions: Cytotoxicity data showed a reduction in cell viability of approximately 40% at the highest concentration tested. Rhein exhibited a strong antiviral activity against HCoV-OC43, HCoV-229E, SARS-CoV-2, and MeV. In detail, the drug reduced the replication of HCoV-229E and MeV by 90% at the concentration of 200 µg/mL. Higher antiviral effects were found against beta coronaviruses: total inhibition of the replication cycle of HCoV-OC43 and SARS-CoV-2 was obtained after exposure with 100 µg/mL of Rhein. Once the antiviral activity of the FTO inhibitor was demonstrated, the modulation of m6A modification was investigated. Methylation levels increased in a dose-dependent manner when cells were treated with Rhein at 200 µg/mL inducing an approximately two-fold increase in the RNA m6A rate in infected cell lines. The antiviral effect of Rhein could be due to the regulation of m6A pathway, demonstrating a novel therapeutic target for the treatment of viral respiratory infections.

ALTERATIONS IN IFN-OMEGA EXPRESSION AND ANTI-IFN-OMEGA NEUTRALIZING ANTIBODIES IN SEVERE COVID-19 PATIENTS

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Introduction: Type I interferons (IFNs) are cytokines that play a key role in antiviral responses. Among these, IFN ω (omega) activity to fend off viruses has been studied. However, the role of IFN ω in COVID-19 disease is poorly characterized. Thus, our aim was to assess the expression of IFN ω in the airway and peripheral blood of COVID-19 patients, also evaluating the presence of anti IFN ω neutralizing antibodies (NAB).

Methods: Respiratory specimens from COVID-19 patients (n=50) and healthy donors (HD, n=43) were collected at Policlinico Umberto I Hospital in Rome. Furthermore, blood samples from COVID-19 subjects (n=360), SARS-CoV-2/HIV-1 co-infected patients (n=8) and HD (n=19) were included. Evaluation of the mRNA level of IFN α (alpha), IFN β (beta), IFN ω and IFN stimulated genes (ISGs) was carried out by RT/Real Time PCR. Investigation of respiratory and circulating anti-IFN-I NAB and their ability to neutralize IFN ω was performed using antiviral bioassay.

Results: Results showed that IFN ω was highly expressed in respiratory cells of COVID-19 patients compared to HD, $p < 0.0001$. Levels of IFN ω were similar in COVID-19 patients stratified according to their clinical phenotypes: non oxygen support (n=14), non-invasive ventilation (n=18) and invasive mechanical ventilation (n=4), $p = 0.3071$. By contrast, blood transcript of IFN ω was reduced in COVID-19 patients with respect to HD, $p < 0.0001$. Anti-IFN-I autoantibodies screening from sera of COVID-19 patients (n=360) revealed that 3.6% (13/360) had anti-IFN α NAB. Of note, while only 42.9% (3/7) of sera with titers of anti-IFN α NAB < 10.000 TRU/ml were able to neutralize IFN ω , all serum samples (100%, 6/6) with titers of anti-IFN α NAB ≥ 10.000 TRU/ml had anti-IFN ω NAB. Anti-IFN ω NAB persisted in all patients. Remarkably, anti-IFN ω NAB were associated with male sex, admission to the intensive care unit and fatal outcome, $p < 0.01$. Furthermore, anti-IFN ω NAB patients exhibited raised levels of C-reactive protein, lactate dehydrogenases, D-Dimer, and higher counts of hematological parameters compared to COVID-19 patients without anti-IFN-I Abs, $p < 0.05$. With respect to IFN response, anti-IFN ω NAB patients had a trend toward lower expression of IFN α , IFN β and ISG56, as well as a significant decrease of IFN ω and ISG15 compared to HD, $p < 0.01$. ISGs were abolished during hospitalization in patients with persistent anti-IFN ω NAB. NAB to IFN-I were also detected in 3/17 (17.6%) of respiratory samples but no one neutralized IFN ω . Remarkably, analysis of anti-IFN-I NAB in a group of COVID-19 patients living with HIV infection (n=8) revealed that 87.5% (7/8) exhibited anti-IFN-I NAB and the 71.4%, (5/7) had anti-IFN ω NAB.

Conclusion: A dysregulation in the respiratory and systemic production of IFN ω characterized COVID-19 patients. Blood periphery, especially of male patients, is depicted by the presence of anti-IFN ω NAB associated with laboratory parameters, predictive for COVID-19 outcome, and a defective in IFN response.

AKT ACTIVATION IS INVOLVED IN FOXG1 DOWNREGULATION DURING ZIKA VIRUS INFECTION

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Aim of the study: Zika virus (ZIKV) infection during pregnancy causes microcephaly and other defects in brain development, as shown by the 2016 outbreak of "congenital Zika syndrome" in Brazil. Congenital ZIKV syndrome recapitulates birth defects found in "FOXG1-syndrome", triggered by mutations in the transcription factor Forkhead box g1 (FOXG1) coding gene. Indeed, the presence of a functional FOXG1 in the nucleus keeps neural cells in a state that allows them to replicate and the forebrain to reach its full size. In this work, we investigate the involvement of FOXG1 in ZIKV microcephaly induction.

Methods: We used FOXG1 naturally expressing human neural stem/progenitor cells and A549 cells transfected with FOXG1-GFP encoding plasmids. Western blot analysis and confocal microscopy were used to assess viral infection and FOXG1 and other protein expression. Infection was carried out with ZIKV Brazilian strain.

Results: We show that ZKV infection impacts on FOXG1 fate by altering FOXG1 nuclear localization in FOXG1-transfected A549 cells and in human neural stem/progenitor cells, naturally expressing FOXG1. Nuclear export of FOXG1 was not observed when other virus species were used to infect cells, nor when polyI:C was delivered to mimic the activation of intrinsic immunity caused by infection. In addition, another transcription factor, SOX2, was unaffected by ZKV infection, showing the effect was ZKV- and FOXG1-specific.

To shed light on the mechanism activated by ZKV infection, the aminoacid residue Thr271, located in FOXG1 was mutated abolishing the effect and showing that it took part in this event. Because this residue is phosphorylated by AKT to regulate shuttling of FOXG1 from the nucleus to the cytoplasm, we quantified the amount of phosphorylated AKT in infected cells and found that early in infection it increases considerably, returning to normal soon after.

After nuclear export, the amount of FOXG1 in the cell rapidly decreased, hinting at the fact that it was destroyed after it exited the nucleus. By progressive deletion of a GFP-tagged FOXG1 sequence, we identified the C-terminus and the residues 428-481 as critical domains for FOXG1 possible degradation. Collectively, our data suggest that ZIKV infection might induce microcephaly also by nuclear export possibly mediated by AKT and downregulation of FOXG1 in neural cells.

SARS-COV-2 INFECTION IN CYSTIC FIBROSIS: THE ROLE OF CFTR MUTATION/DOWNREGULATION

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Aim of the study: The baseline lung inflammatory status of cystic fibrosis patients seems to modulate the individual susceptibility to SARS-CoV-2 infection (1). Particularly interesting in this regard is the observation of a chronic presence of neutrophils in CF lung that may indeed play an essential role in the control of infections and in tissue repair (2). The aim of this study was to clarify whether CFTR mutation/downregulation in airway epithelial cells might influence SARS-CoV-2 infection.

Methods: We used different cell models: CF bronchial epithelial cells (CFBE41o-) with or without stable expression of F508del-CFTR or wild-type CFTR, human bronchial epithelial cell line expressing wild-type CFTR (16HBE14o-) and Calu-3 cell line, wild type or downregulated for the expression of CFTR through microRNA post-transcriptional control. To assess the SARS-CoV-2 replication in the different cell models, cells were infected at 0.1 m.o.i. and analyzed at 24, 48 and 72 hours post infection. RNA was extracted from both supernatants and cells and quantified by q-PCR. Virus was also titrated by plaque count assay.

Results and conclusions: SARS-CoV-2 replication was significantly reduced in CF cell models and Calu-3 downregulated for CFTR expression in comparison with wild type cells ($p < 0.001$; Fisher exact test). The unpaired expression and function of CFTR, as well as affecting SARS-CoV-2 infectivity, also decreased the expression of ACE2 (angiotensin-converting enzyme 2), the SARS-CoV-2 main receptor. CFTR seems to influence both ACE2 expression and cellular localization. The reduction of ACE2 expression and membrane localization in CF epithelial cells, due to CFTR mutations/downregulation, might mitigate the severity of SARS-CoV-2 infection, as observed in clinical follow-up. CFTR targeted-modulation, e.g. via miRNA therapy, might represent a potential strategy to control the severity of SARS-CoV-2 infection.

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Extracellular vesicles (EVs) are a heterogeneous group of cell-derived membranous structures present in many biological fluids and involved in multiple physiological and pathological processes. During microbial infections, the cellular response to pathogens influences the production of different factors like cytokines, chemokines and EVs that represent an important cell-cell communication strategy. Between these factors IFNs are fundamental effectors of antimicrobial innate immunity and important regulators of the adaptive immune response. Aim of the present work was to analyze whether type I (i.e., IFN α 2b and IFN β) and type II (IFN γ) can influence the composition of the released EVs. As cellular models we used THP-1 differentiated cells, a monocytic cell line that assumes the phenotype of macrophages when treated with PMA (phorbol-12-myristate-13-acetate). The supernatant of the IFNs-treated cells was harvested after 20 hours of treatment. Two categories of EVs were isolated through several ultracentrifugation session: small-EVs which included exosomes (Exo), and medium/large-EVs which included microvesicles (MV). EVs content were examined by western blot focusing on two proteins involved in the inflammation: i) ISG15, an ubiquitin-like modifier that is induced in response to type I and type III IFNs, viral and bacterial infections, and ii) TNF- α converting enzyme (TACE/ADAM17), a metalloprotease involved in the maturation of TNF α from the TNF- α precursor in order to release soluble TNF- α (sTNF- α) and whose presence is associated with pro-inflammatory activity. We observed that in differentiated THP-1, TACE-active form is detected in small-EVs and medium/large-EVs, but is predominantly associated with the medium/large-EVs. Treatment with IFN β significantly decreases the amount of the TACE active-form in medium/large-EVs, while IFN- α and IFN- γ did not. ISG15 was barely detected only in the small-EVs without any modulation of its presence induced by IFNs treatment. Then we performed treatments of PMA-differentiated THP-1 with the collected EVs and the preliminary results indicate that the IFNs-EVs induce the activation of P-STAT1 pathway in target cells. Altogether, our results show that EVs are affected by the IFNs treatments, that can alter their “message” inducing different outcome in target cells.

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Aim of the study: During infection by the SARS-CoV-2 virus, a weak and delayed production of IFN- β 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, although contradictory, demonstrated that IFN- β production was differentially modulated upon infection by different virus lineages (Shalamova et al., 2022; Bojkova et al., 2022). Our study aimed to improve the knowledge on virus-lineage correlation to IFN- β production. In detail, we focused on the effect of the Omicron (B.1.1.529) variant, in comparison to the Wuhan-1 lineage, in terms of time and strength induction of IFN- β upon viral infection of cultured Calu-3 cells.

Methods: Cells and viruses. Wuhan-1 and Omicron 1 (B.1.1.529) SARS-CoV-2 lineages were isolated from clinical specimen on Vero E6 cells (ATCC CRL-1586). Calu-3 and HEK-293T cells were both from ATCC (HTB-55 and CRL1573, respectively). Plasmids. Reporter plasmids were provided by colleagues or purchased. The ACE-2 expressing plasmid was prepared by standard cloning procedure. Luciferase reporter assay. HEK-293T cells were transfected with ACE-2 expressing plasmid along with the IFN- β promoter reporter plasmid. Twenty-four hours later, cultures were infected with a MOI=0.1 of indicated SARS-CoV-2 lineages. Samples were collected at 24h and 48h post-infection (p.i.) and luciferase activities were measured by using the Dual Luciferase reporter assay (Promega). IFN- β detection. IFN- β was quantified in cell supernatant of infected Calu-3 by using the Veri-Kine-HS Human IFN Beta TCM ELISA Kit following manufacturer's instruction. Cellular pellets were conserved and used to assess cytokine expression by commercial TaqMan assay after total RNA extraction.

Results and conclusions: In the present study, we investigated how the emergent Omicron (BA.1.1.529) SARS-CoV-2 variant modulated the type I interferon response. As a first step, Calu-3 cell model was used for quantitative detection of secreted IFN- β . We identified that the Omicron variant led to a reduced induction of the IFN- β cytokine release by in vitro infected Calu-3 cells compared to the Wuhan-1 lineage, at 48h post-infection (p.i.). Conversely, none of the selected viral lineages determined a precocious release of IFN- β , such as at 24h p.i. Of note, levels of infectious viral load and viral RNAs produced at 48h p.i. were comparable for all samples tested, excluding the possibility that Omicron generated lower amount of dsRNAs to induce IFN- β . To further address the innate immunity modulation by the Omicron variant, IFN- β expression was additionally assessed at transcriptional level by RT-qPCR. Results confirmed that the Wuhan-1 and Omicron SARS-CoV-2 strains differentially modulated the IFN- β gene expression. Indeed, a ~2-fold reduction in specific mRNA was detected in Omicron infected cells compared to the Wuhan-1 infected samples. These results were in contrast with the IFN- β promoter (pIFN- β) activation experiments. Indeed, preliminary results of a reporter gene assay showed a similar acti-



vation rate of the pIFN- β when the Wuhan-1 or Omicron infections were established. In conclusion, the Omicron variant exerted an increased antagonistic activity on innate immunity leading to a reduction in IFN- β production at transcriptional level which, in turns, reflects a decreased cytokine release. Notably, SARS-CoV-2 proteins known to inhibit the host cell interferon response are mutated in the Omicron variant. Further experiments will be addressed to test whether the antagonistic behavior of the Omicron variant occurs at post-transcriptional steps, such as on mRNA stability, or at upstream levels, such as on pattern recognition receptors (PRRs), e.g. RIG-I/MDA-5, activation. The reported data are important to elucidate the immune system escape by SARS-CoV-2 in the light of virus evolution.

HUMAN ENDOGENOUS RETROVIRUSES (HERVs) TRANSCRIPTOME IN PBMC IS MODULATED DURING SARS-CoV-2 INFECTION AND ALLOWS TO DISCRIMINATE COVID-19 CLINICAL STAGES

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COVID-19 pandemic is affecting the global population since December 2019, when the secondly identified human severe acute respiratory syndrome coronavirus (SARS-CoV-2) gave rise to an epidemic in Wuhan city (China). Since then, the virus has rapidly spread to the rest of the World, leading to about 480 million confirmed cases of COVID-19 and more than 6 million deaths (WHO).

SARS-CoV-2 infection is known to trigger an important inflammatory response, which has a major role in COVID-19 pathogenesis. In infectious and inflammatory contexts, the modulation of Human Endogenous Retroviruses (HERV) has been broadly reported, being able to further sustain innate immune responses due to the expression of immunogenic viral transcripts – including dsRNA – and, eventually, immunogenic proteins. This might also account, at least in part, for the different clinical manifestations seen in COVID-19 patients, ranging from asymptomatic infections to severe systemic manifestations.

To gain insights on the still poorly characterized interplay between SARS-CoV-2 infection and HERV transcriptome, we performed a high-throughput analysis of about 3300 specific HERV loci, assessing their differential expression in publicly available RNA-seq profiles from the PBMC of healthy controls (HC, n=10) and individuals exposed to COVID-19 being either convalescent after the first infection (C, n=6) or re-testing positive after convalescence (RTP, n=10) (GEO dataset GSE166253).

Results showed that the exposure to COVID-19 manifestations determine a specific modulation of HERV expression, allowing to distinguish the different clinical stages based on HERV transcriptional signature. The differential expression analysis between HC and COVID-19 patients allowed to identify a total of 282 differentially expressed HERV loci (deHERV) in the individuals exposed to SARS-CoV-2 infection, independently from the clinical form. In addition, 278 and 60 deHERV loci that were specifically modulated in C and RTP patients as individually compared to HC, respectively, as well as 164 deHERV loci between C and RTP patients. The identified HERV loci belonged to 36 different HERV groups, including members of all three retroviral classes.

The present study provides an exhaustive and detailed picture of HERV transcriptome in PBMC and its variation in the presence of COVID-19, revealing specific modulation patterns according to the infection stage that can be relevant to the disease clinical manifestation and outcome.

**PREVALENCE OF HEPATITIS C VIREMIA IN HEART TRANSPLANT RECIPIENTS:
A SINGLE-CENTER EXPERIENCE**

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Aim of the study: The existence of occult hepatitis C virus (HCV) infection, irrespective of the presence of anti-HCV antibodies and/or elevated ALT levels, has been shown in several studies. Diagnosis of such occult, subclinical HCV infection, using high-sensitivity nested-reverse transcriptase polymerase chain reaction (hs-n-rtPCR) has been described [1]. HCV persistence in an occult form could be related to immune suppression condition and anti-neoplastic chemotherapy and may have prognostic relevance in the immunocompromised host [2, 3]. Accordingly, the aimed to estimated the prevalence of HCV-RNA positivity, even in trace, in a cohort of heart transplant recipients (HTRs) irrespective of the presence of anti-HCV-Ab or elevated ALT.

Methods used: We studied a retrospective cohort of 134 HTRs, for 55 of whom we also studied samples from the organ donors, and a prospective cohort of 45 HTRs. As controls, we studied a cohort of seronegative blood donors (n = 126), and a group of patients with known HCV hepatitis. Sera were tested for HCV-RNA with a non-commercially available (i.e., a "homemade"), high-sensitivity-nested-reverse transcriptase polymerase chain reaction (hs-n-rtPCR), HCV antigen, HCV antibodies, and markers of liver damage. Where HCV-RNA was positive, genotyping was performed and random samples were sequenced to confirm specificity of the test.

Results and Conclusions: HCV-RNA was positive in 5.5% of blood donors, 100% of hepatitis C patients, 21% of HTRs before transplant and 24% of HTRs after transplant in the retrospective group, 22% of HTRs before transplant and 27% of HTRs after transplant in the prospective group. There was no evidence of a consistent transmission from donors to HTRs. An absolute concordance was found between negative HCV-RNA on hs-n-rtPCR and absence of HCV antigen. In contrast, antigen test was positive in HCV-RNA-positive samples after transplant for 53% compared to before transplant for 33% (P = 0.19) and 58% compared 40% (P=0.66) for retrospective group and prospective group, respectively. In the prospective group, HCV serology was positive in only one patient, also HCV.RNA-positive (2.2%) before transplant compared to 5 patients (42%) positive for two tests after transplant (P=0.16). HTRs with positive HCV-RNA with hs-n-rtPCR showed a significantly higher level of each of the three markers of liver damage (ALT-AST-GGT). In HTRs, there is a very high prevalence of positive, low level HCV viremia. This might be a consequence of health-care exposures and post-transplant immune suppression. The clinical consequences of this virologic phenomenon need to be further investigated.

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PNPLA3 AND HSD17B13 POLYMORPHISMS INFLUENCED LIVER FIBROSIS DEVELOPMENT IN A SMALL COHORT OF ITALIAN PATIENTS WITH VIRAL HEPATITIS

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Aim of the study: Liver steatosis is an histological element that can be present in viral hepatitis, especially by HCV [1]. The pathogenesis of liver damage can be influenced by the genetic background of patients; PNPLA3/HSD17B13 gene polymorphisms have been associated in Northern Europe and US with evolution to- or protection of- liver fibrosis, respectively [2-4]. In this study we evaluated the effects of PNPLA3 and HSD17B13 polymorphisms on the development of liver cirrhosis by single or dual HBV and HCV infections in patients in Italy.

Methods used: 280 patients with chronic hepatitis by HBV, HCV or HBV-HCV, naive for antiviral therapy, were genotyped for PNPLA3 I148M and HSD17B13 rs72613567:TA variants. All individuals were genotyped for HSD17B13 and PNPLA3 gene polymorphism with the single nucleotide polymorphism (SNP) PCR-method and using pre-designed primers respectively, in the Real Time PCR system. Serum markers and liver function tests were performed with routine methods in use at our hospital.

Results and Conclusions: 112 patients had liver cirrhosis and 168 were without.

The PNPLA3 mutants were prevalent in our population, while HSD17B13 gene was mostly wild type. The PNPLA3 mutant correlates with elevated ALT levels ($p=0.005$) and the HSD17B13 mutant with elevated HCV RNA serum levels ($p=0.04$). PNPLA3 mutants were significantly associated with absence of cirrhosis in the global population ($p=0.01$) and in the HCV-infected group ($p<0.001$), while HSD17B13 was associated with the absence of cirrhosis only in HCV-infected patients ($p<0.05$) (Fig.1). At univariate analysis age was a factor favoring cirrhosis while PNPLA3 mutants were protective of liver cirrhosis ($p<0.01$ and 0.017 , respectively); at multivariate analysis only age was confirmed as an independent factor promoting liver cirrhosis (OR 7.79; $p<0.001$). The presence of any mutant allele of both PNPLA3 and HSD17B13 was protective in the development of cirrhosis ($p=0.02$) only at univariate analysis.

In conclusion, in our Italian population PNPLA3 and HSD17B13 show a protective role in the development of fibrosis in chronic viral hepatitis, particularly by HCV, but larger studies on this specific population are needed.

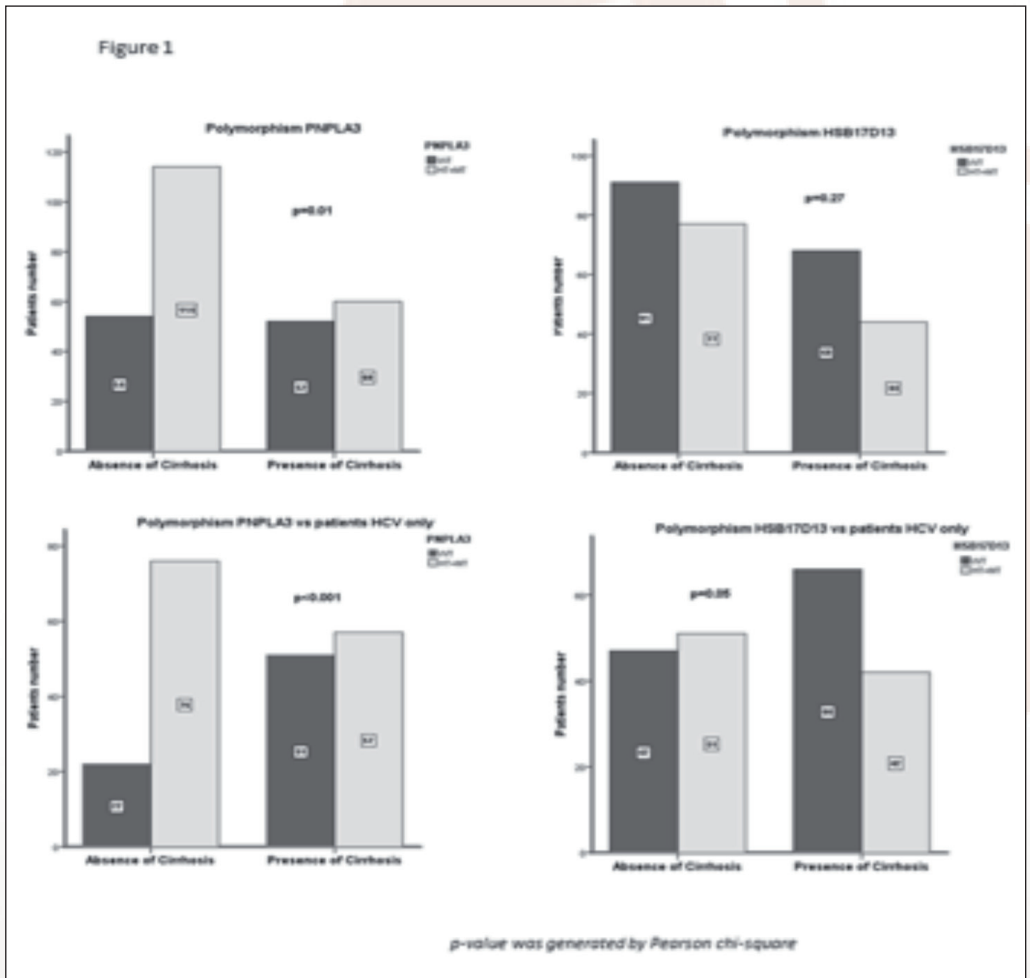


Figura 1

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PRE-PANDEMIC HUMAN SERA FROM INDIVIDUALS WITH NATURAL MEASLES INFECTION SHOW REACTIVITY AGAINST SPIKE AND N PROTEINS SARS-CoV-2

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Aim of the study: Human sera of individuals natural infected with measles, collected in the acute phase of infections during a pre-pandemic period, showed a certain grade of neutralizing activity to SARS-CoV-2 (Gold, J. E. et .al 2020; López-Martin et. al 2021.). It was investigated if these measles sera reacted to recombinant SARS-CoV-2 proteins.

Methods: Human sera, previously tested for measles RNA, measles-specific IgM and IgG antibodies with the ELISA immunoassays (SERION), were used in this study. Plaque reduction naturalization assay was performed to quantify the titer of neutralizing antibody for measles and SARS-CoV-2. *E. coli* expressed SARS-CoV2 N and S proteins were loaded on 4-15% gradient gel and subjected to SDS-PAGE. After electrophoresis the gels were blotted onto PVDF membranes, cut into stripes and incubated with human sera in corning tube with human sera diluted 1:500 in 3ml of TBST 3% NFDM. Antigen-antibodies complexes were detected by an anti-Human IgG-HRP. Immunoblotting of human sera was also performed with protein lysate of SARS-CoV-2, HCoV-OC43 and HCoV-229E

Results: Most of the measles sera showed reactivity to stripes containing either the recombinant N-CoV2 expressed in *E. coli* or N from SARS-CoV-2 virus lysate. The intensity of reactivity varies between samples. The positive sera also reacted with the N stripes of HCoV-OC43 and HCoV-229E virus lysates. The N-reactivity observed in immunoblot was always lower than that observed with COVID-19 sera or with an anti-HIS tag. Some of the sera also demonstrate reactivity to other proteins including S1 and S2 of the virus lysate. Surprisingly, also negative sera for measles virus, used as control showed some positive reactivity to N-SARS-CoV-2, OC43 and 229 E virus lysates.

Conclusions: This study showed that the reactivity observed in measles sera collected in pre-pandemic period toward SARS-CoV-2 proteins is present also in control sera, suggesting the presence of a cross-reactive immune response possibly due to previous infections with other coronaviruses (Guo, L. et .al. 2022; Tamminen K et .al. 2021).

INTERFERON-GAMMA LEVELS IN UMBILICAL CORD BLOOD OF NEWBORNS FROM SARS-COV2 AFFECTED MOTHERS

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Aim: Pregnant women are at high-risk for severe COVID-19 and mortality. SARS-CoV-2 infection during pregnancy induces a peculiar inflammatory response at the maternal-fetal interface largely managed by maternal T cells and fetal stromal cells. Little is known about the effect of SARS-CoV-2 infection on the inflammatory response of the fetus. To address this issue, we evaluated the levels of anti-SARS-CoV-2 antibodies, IL-6 and IFN-gamma in umbilical cord blood (UCB).

Methods: In this prospective study, 93 UCB were collected: 54 from newborns with mothers infected with SARS-CoV-2 and 38 newborns from healthy mothers. SARS-CoV-2 Total anti-SPIKE antibodies in UCB were measured using the ADVIA Centaur SARS-CoV-2 Total assay, Interferon-gamma (IFN γ) performing ELISA assay (Human IFN gamma Uncoated ELISA for quantitative detection of human IFN gamma INVITROGEN) and IL-6 using IMMULITE 2000 IL-6 Assay.

Results and Conclusion: IFN γ concentration was significant lower in UCB from infected mothers compared with controls (5,32 pg/ml vs 0,79 pg/ml; $p < 0,05$). Conversely, IL6 and anti-SARS-CoV-2 antibodies showed no significant difference in UCB samples of the two groups (15,86 pg/ml vs 12,52 pg/m; $p > 0,05$). Our results showed that maternal SARS-CoV-2 infection affected the ability of the fetus to produce IFN γ . Further studies are needed to assess the molecular mechanisms involved and short- and long-term consequences on the health status of the offspring.

N-ACYLETHANOLAMINE ACID AMIDASE INHIBITION AFFECTS ZIKA VIRUS REPLICATION

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Background: ZIKA virus (ZIKV) is an emerging arboviral causative agent representing a public health concern, as no approved antiviral therapy is available to date. ZIKV replication relies on lipid host metabolism to build its replication sites. Thus, counteracting host lipid pathways crucial for viral replication offers a potential target for new therapy.

Aim of the study: our aim was to investigate the antiviral activity of a class of inhibitors of N- Acylethanolamine acid amidase (NAAA) with anti-inflammatory effect but not yet tested as an antiviral compound. NAAA catalysed the hydrolysis of Palmitoylethanolamide (PEA), an endogenous fatty acid amide well known for its analgesic, anti-inflammatory, and neuroprotective properties. Its biological activity is exerted by peroxisome-proliferation transcription factor (PPAR α). PPAR α regulates the degradation of lipid droplets, used by ZIKV as replication sites, and autophagy activation, also known to affect viral replication.

Methods: We used genetical and pharmacological approaches to inhibit NAAA by using CRISPR/Cas9 system or NAAA inhibitor drugs respectively, in A549 cells. The expression of NAAA in ZIKV infected cells was assessed by Real-Time PCR and High-Content confocal imaging. ZIKV yield was determined by Plaque reduction assay and Real-Time PCR from the supernatants. Autophagy activation was assessed by Western Blot on LC3 I-II and P62 proteins and confocal imaging analysis. We also analysed whether NAAA ablation affects lipid droplet content by using confocal imaging analysis.

Results and conclusions: We first investigated whether ZIKV infection affects NAAA expression: we observed a 3-fold increase in NAAA expression after 48 h post infection in A549 and Huh-7 cells. We also observed co-localization of ZIKV NS1 protein and NAAA into cytoplasmatic vesicles. Next, we assess whether NAAA has a role during ZIKV replication in NAAA ablated cells, where we observed a 5-fold reduction of ZIKV yield compared to control cells. In agreement with this result, we observed a significant 20-folds decrease in ZIKV Envelope protein and a 5-times lower quantity of ZIKV genome in supernatants. Surprisingly, we found that ablation of NAAA counteracts the ZIKV-induced autophagy blockade, leading to high levels of autophagy-related proteins in infected cells. Moreover, we found that NAAA ablation decreases lipid droplets by the activation of β -oxidation. Our finding suggests that NAAA inhibition and consequent PEA build up has a potential antiviral activity, affecting lipid vesicles that sustain ZIKV replication by inducing lipid droplet dismantling and keeping autophagy active.

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Background: SARS-CoV-2 is the etiologic cause of COVID-19. The severe clinical manifestations of COVID-19 were mainly observed in subjects who display several comorbidities including preexisting lung disease (Zavascki and Falci 2020). Since cystic fibrosis leads to the inability to clear bacteria and other pathogens from the lungs and in turn causes inflammation and chronic infection, people with cystic fibrosis should show an increased risk of developing severe manifestations in case of SARS-CoV-2 infection. However, several studies indicate a reduction in the spread of SARS-CoV-2, and in case of infection, milder manifestations (Mathew et al. 2021; Colombo et al. 2021, 2020; Fainardi et al. 2020), suggesting complex regulation of SARS-CoV-2 replication.

Aim: We analyzed SARS-CoV-2 replication in wild-type and CFTR-modified human bronchial epithelial cell lines and primary cells to investigate the mechanism behind the lower virus spread and the less severe manifestation in case of SARS-CoV-2 infection in people with cystic fibrosis. The clinical importance of characterizing the effects of SARS-CoV-2 infection in people with cystic fibrosis, and understanding the possible underlying protective effects, could shed light on novel targets and new therapeutic approaches.

Methods. Both immortalized and primary human bronchial epithelial cells expressing wt- or F508del- CFTR were infected with SARS-CoV-2. Real-time RT-PCR was used to evaluate viral load in both supernatant and cell extracts from 24 to 72 h post-infection. In primary cells (wt/wt-CFTR MucilAir™ and F508del/F508del-CFTR MucilAir™) variations in transepithelial electrical resistance (Δ TEER) were measured using a volt/ohm-meter and STX 2 electrodes.

Results: SARS-CoV-2 replication was reduced in CFTR-modified bronchial cells compared with wild-type cells. The sharp increase in wt/wt-CFTR MucilAir™ viral replication correlated with a reduction in epithelium integrity mostly at 72 hpi, while in F508del/F508del-CFTR MucilAir™ the TEER values remain consistent with the passing of hours after infection.

Conclusions: With this study, we provide new insights into the role of CFTR in SARS-CoV-2 infection, demonstrating that CFTR expression/function is involved in the regulation of SARS-CoV-2 replication. Thus, CFTR may represent a potential target to decrease viral pathogenicity, opening new paths in the comprehension and management of SARS-CoV-2 infection.

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ZIKA VIRUS INDUCES FOXG1 NUCLEAR DISPLACEMENT AND DOWN-REGULATION THAT CAN BE PREVENTED WITH GROWTH FACTORS

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Aim of the study: Zika virus (ZIKV) is a positive-sense single-stranded RNA (ssRNA+) virus belonging to *Flaviviridae* family. ZIKV infection can induce several congenital brain malformations in the fetus, including microcephaly.

Several key features of brain development impairment caused by ZIKV can be found also in congenital alterations or in down-regulation of the transcription factor Forkhead box G1 (FOXG1). In vertebrates, *FoxG1* expression orchestrates forebrain development. Although FOXG1 is predominantly nuclear, its subcellular localization is controlled post-translationally and alternates between nucleus and cytoplasm, following different stimuli, including growth factors (GFs), such as Fibroblast Growth Factors-2 (FGF-2).

The aim of the study is to assess whether a common mechanism that might exist between microcephaly caused by congenital ZIKV infection and FOXG1 defects. We investigated FOXG1 protein localization in telencephalic neural progenitor cells derived from human induced pluripotent stem cells (hiPS-NPCs) and human neocortical NES cells, both expressing endogenously FOXG1, as well as in A549 cells, transiently expressing FOXG1.

Methods: hiPS-NPCs and NES cells were infected with 1 MOI of ZIKV and monitored up to 3 days post infection (DPI). A549 were transiently transfected with FOXG1 fused to GFP (FOXG1-GFP). 1-day post transfection, A549 were infected with 1 MOI of ZIKV and 1 DPI they were fixed. As controls, hiPS-NPCs and A549 were also infected with 1 MOI of two different ssRNA+ Arboviruses: Usutu (USUV), belonging to the same genus as ZIKV, and the Asian strain of Chikungunya (CHIKV), belonging to *Togaviridae*. To assess the role of GFs, we exposed hiPS-NPCs and A549 to EGF and FGF-2 and infected them with ZIKV in the presence of both GFs, alone and in combination.

Results: In uninfected hiPS-NPCs, FOXG1 was mostly localized within the nucleus, while in ZIKV-infected cells, FOXG1 shifted towards the cytosol in a time-dependent manner. Similarly, in A549 transiently expressing FOXG1 and after ZIKV infection, FOXG1 was displaced from the nuclear compartment to the cytoplasm.

Next, to evaluate whether FOXG1 relocation was a consequence of ZIKV, and not a non-specific effect following any viral infection, both hiPS-NPCs and A549 were infected with USUV and CHIKV. Interestingly, we did not detect significant changes in FOXG1 localization after either infection. These data show that ZIKV infection, but not other viruses, specifically perturbs FOXG1 nuclear pattern.

To further explore FOXG1 nuclear pattern disruption, we investigated the role of GFs and intrinsic immunity in ZIKV-induced FOXG1 subcellular localization. We performed kinetic studies to elucidate their impact on FOXG1 shuttling. Our data suggest that GFs play a role in the effect of ZIKV in our model.

THE US12 PROTEIN IS THE SECOND VIROPORIN ENCODED BY THE US12 GENE FAMILY OF HUMAN CYTOMEGALOVIRUS: WHEN TWO IS BETTER THAN ONE

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Background and aim: The US12 gene family of Human Cytomegalovirus (HCMV) includes a set of 10 contiguous tandemly arranged genes (US12 to US21) each predicted to encode a membrane-associated 7TMDs protein. Some US12 members show low level of homology to the cellular Transmembrane Bax-Inhibitor 1 Motif-containing (TMBIM) proteins that modulates cellular Ca^{2+} homeostasis and adaptive responses to stress conditions. However, despite the confirmed evolutionary importance of the US12 genes to HCMV biology, only a few functions have been associated with the gene family. Previously, we observed that inactivation of individual US12 genes in a clinical strain of HCMV does not affect viral replication in fibroblasts, while the disruption of US16, US18, US20, and US21 members prevented viral growth in endothelial and epithelial cells, thus suggesting a role of these US12 genes in regulating HCMV cell tropism (Bronzini et al., J. Virol. 2012; Cavaletto et al., J.Virol. 2015; Luganini et al., J. Virol. 2017). Moreover, we characterized the US21 protein as an ER-resident viroporin that dysregulates intracellular Ca^{2+} homeostasis, inhibits apoptosis (Luganini et al., PNAS 2018), and stimulates adhesion and motility of HCMV-infected cells (Luganini et al., submitted 2022). Here, we report on the functional characterization of another US12 family member, the US12 protein as the second viroporin of the gene family.

Methodology: The BAC-Recombineering technology was employed to generate recombinant viruses on the HCMV TR background: TRUS12HA (HA tag at the C-terminus of the ORF), US12NV5-CHA (V5 tag at N-terminus and HA tag at C terminus), TRΔUS12 (deletion of the entire US12 ORF), and TRUS12stop (stop codon at the 5th codon of the ORF). The Trans-Membrane Domain (TMD) topology of pUS12 was predicted using five different algorithms, and a selective epitope accessibility immunofluorescence assay was then used to verify the *in silico* predictions. The Tetracycline-Regulated Expression (TREx) 293 cell system was used to achieve tetracycline-inducible expression of pUS12HA. Intracellular Ca^{2+} content was determined by ratiometric cytosolic Ca^{2+} ($[\text{Ca}^{2+}]_i$) measurement with the Fura-2 AM probe.

Results and conclusions: Using recombinant TR-HCMV encoding different tagged versions of the US12 ORF, we observed that pUS12 is a 7TMD protein (with the N-terminal in the cytosolic side and the C-terminal in the luminal side of the membrane) and its expressed as early as 24h p.i. pUS12 co-localizes with Golgi-derived membranes and accumulates late in infection at the periphery of the cytoplasmic Virion Assembly Complex (cVAC). Recombinant TR-HCMV in which the US12 ORF was removed (TRΔUS12) or inactivated (TRUS12stop) exhibited major growth defects in endothelial and epithelial cells, whereas in fibroblasts their replication was not significantly different from that of parental

TRwt. Calcium imaging experiments then showed that the tetracycline-induced expression of pUS12 in TREx-293 cells significantly decreased the amount of releasable Ca^{2+} from intracellular stores, thus indicating that pUS12 acts as a Ca^{2+} -conducting viroporin. In addition to pUS21, pUS12 therefore represents the second protein of the US12 family able to hijack cell's Ca^{2+} homeostasis. Studies are ongoing to determine the cytobiological consequences of the pUS12-mediated alteration of intracellular Ca^{2+} content.

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Aim: Infection by herpes simplex viruses (HSV) of fully permissive cells is characterized by effective counteraction of host cell innate response, including NF- κ B activation and apoptosis, and completion of the replication cycle in infected cells, which ultimately undergo lytic death. Conversely, cell types that can be infected by HSV, but fail to optimally support their replication, including human immune cells, undergo apoptosis as an exclusive cytopathic effect. Molecular mechanisms involved in the different outcome have not been fully elucidated. MicroRNAs (miRNAs) play important roles in the modulation of key cellular processes, including innate response regulatory pathways. The aim of this study was to collect information on possible cellular miRNAs involved in the regulation of the restriction/apoptotic processes in cells infected by HSV-1 using dual bioinformatic and experimental approaches.

Methods: Two bioinformatics tools based on different criteria of selection for most predictable miRNAs involved in HSV-1/cell interaction were utilized. One software analysis (BIOINFO1) utilized as selection criteria: i) data from a gene expression array, already available in the lab, reporting miRNAs levels in U937 cells with functional NF- κ B (U937-pcDNA) and in U937 cells where NF- κ B activation was stably inhibited by a DN form of I κ B α and undergoing high levels of apoptosis following HSV-1 infection (U937-I κ B α -DN), infected or not with HSV-1, ii) known, innate-response-related targets of the differentially expressed miRNAs. The other software analysis (BIOINFO2) utilized as selection criterion the predicted interaction of human miRNAs with HSV-1 transcription products, based on data available on databases. The methodological strategy for validation of the *in silico* generated data consisted of the detection by qRT-PCR of the actual expressions levels of selected miRNAs with high score of predictability in cells mock-infected or infected with HSV-1, and/or under different, restriction/apoptosis related experimental conditions.

Results and conclusions: In a first phase, the two different bioinformatics procedures to select the putative miRNAs with high probability to perform the required functions, gave two independent lists of predictable miRNAs, ordered by arbitrary scores. Based on the scores obtained by BIOINFO1 and BIOINFO2, miR-99a-5p and miR-762, respectively, were then selected for validation by qRT-PCR. The main results of qRT-PCR assays can be summarized as it follows. The miR-99a-5p was highly expressed (about 16 fold-change) in HSV-1-infected U937-I κ B α -DN, showing high levels of apoptosis, with respect to mock-infected cells, while quite equally in mock-infected and HSV-1-infected U937-pcDNA, showing very low levels of apoptosis following infection, thus indicating its NF- κ B-dependent involvement in modulation of restriction/apoptosis in monocytic cells infected by HSV-1. Expression of miR-762 following infection with respect to mock-infected cells in U937-pcDNA, U937-I κ B α -DN, and HEP-2 cells, showing progressively increasing levels of permissiveness to HSV-1, was inversely correlated with cell permissiveness, thus indicating a possible involvement of this miRNA in modulation of cellular restriction to HSV-1 infec-

tion. In conclusion, results of our study account for the reliability of the strategy adopted to select miRNAs involved in the regulation of the restriction/cell death processes in cells infected by HSV-1, also indicating two, previously unconsidered, cellular miRNAs showing characteristics highly suggestive for their possible role in the phenomena of interest.

THE TRANSACTIVATION OF ENDOGENOUS RETROVIRUSES CORRELATES WITH HIV RESERVOIR, LYMPHOCYTES ACTIVATION AND LOW CD4 COUNT IN VIROLOGICALLY SUPPRESSED PATIENTS

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Aim of the study: In the context of the long-term therapy in virologically suppressed HIV-1 infected patients, the identification of new biomarkers associated with viro-immunological discordance and/or the risk of disease progression is needed. Human endogenous retroviruses (HERVs) are relics of ancestral exogenous retroviral infections and comprise nearly 8% of the human genome. HERVs have been co-opted in physiological roles, but could be reactivated by exogenous viruses such as Herpesviruses, SARS-CoV-2, HTLV-1 and HIV-1. The aim of the study was to investigate HERVs expression in association with viro-immunological parameters for the identification of novel markers for the clinical monitoring of virologically suppressed HIV-1 infected patients towards a personalized approach.

Methods: 40 HIV-1+ viral suppressed patients (HIV RNA <20 cp/mL; CD4 > 400 cell/mm³) and 10 Healthy Donors (HD) were enrolled. Blood HIV-DNA levels and residual plasma viremia were quantified by droplet digital-PCR system (Biorad). The expression of the env gene of two elements of the HERV-K HML-2 group (HERV-K113 and HERV-K111), of pHERV-W, HERV-W Syncytin-1 (SYN-1) and HERV-H have been analysed by RT-Real time PCR. The immunophenotyping have been evaluated by flow cytometry. The non-parametric Mann-Whitney test and the Spearman Correlation analysis were used for statistical analysis.

Results: HIV-1 infected patients median age (IQR) was 37 (32-45) years, 39 (97.5%) were males. At HIV-1 diagnosis, median (IQR) viremia was 4.7 (4.3-5) Log₁₀cps/mL and CD4+ were and 469 (299-627) cells/μL. 27 (67.5%) patients had HIV-1 B subtype. At the enrolment, median (IQR) CD4+ was 720 (583-1041) cells/μL and residual viremia was 3 (2-6) cps/mL. All patients were on 2 NRTI-regimen with a 3rd drug (20 INSTI, 15 NNRTI, 5 PI). The relative expression of pHERV-W, HERV-K113 and HERV-K111 was significantly higher in patients compared to healthy donors ($p \leq 0.001$). Interestingly, the expression of HERVs directly correlated with HIV-DNA (HERV-K113: $\text{Rho} = 0.534$, $p < 0.010$; HERV-K111: $\text{Rho} = 0.706$, $p < 0.010$; SYN-1: $\text{Rho} = 0.623$, $p < 0.010$). Moreover, an inverse correlation was found between HERV-K111 and CD4 NADIR ($\text{Rho} = -0.366$, $p < 0.050$), and between HERV-K113 with absolute CD4 count at collection ($\text{Rho} = -0.421$, $p < 0.050$). HIV-DNA values correlated directly with the percentage of peripheral CD8 lymphocytes expressing the activation marker CD38 (CD8+CD38+) ($\text{Rho} = 0.400$; $p = 0.026$). A significant direct correlation between the CD8+CD38+ and the expression of HERV-K113 ($\text{Rho} = 0.526$, $p < 0.010$), HERV-K111 ($\text{Rho} = 0.513$, $p < 0.010$) and SYN-1 ($\text{Rho} = 0.403$, $p < 0.050$) was found. Activated B lymphocytes (CD19+CD38+) were also found to be po-

sitively correlated with the expression of pHERV-W ($\text{Rho}=0.580$, $p<0.010$), HERV-K113 ($\text{Rho}=0.418$, $p<0.050$) and HERV-K111 ($\text{Rho}=0.382$, $p<0.010$).

Conclusion: The analysis reveals a complex dynamics between HERVs, HIV reservoir and lymphocytes activation. In particular, HERV-K111 correlated directly with HIV-DNA and inversely with CD4 NADIR. HERV-K111 is known to be reactivated during HIV infection and its centromeric deletion has been identified in long term nonprogressors and elite controllers. HERV-K113 correlated positively with HIV-DNA and inversely with CD4 count, suggesting its role in disease pathogenesis. In addition, the correlation of HERVs with the activation of CD8 T lymphocytes and B lymphocytes provides new information to the potential role of HERVs in modulating the immune response during HIV infection, potentially leading to the identification of new prognostic markers useful in monitoring virologically suppressed patients.

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Abstract: Zika virus (ZIKV) is a member of the *Flaviviridae* family mainly transmitted among human populations by mosquito vectors. In pregnant women, ZIKV is able to replicate and cross the placenta barrier causing adverse consequences to the fetus ranging from miscarriages to fetal malformations called congenital ZIKV syndrome (CZS). It has been demonstrated that the placental susceptibility to ZIKV infection decreases during pregnancy; nevertheless, ZIKV can impair fetal development throughout the entire pregnancy, supporting the hypothesis that, depending on the gestational phase, ZIKV could use different unknown pathogenic mechanisms to undermine the placenta barrier. Recent works (Martín-Acebes et al., 2016) underlined the important role of both host cell membrane and viral envelope lipids in Flavivirus pathogenesis, pointing out how the success of host cell infection strictly depends on the type of viral and cell lipids and their reciprocal interactions. ZIKV envelope lipids derive directly from the infected host cell membranes, and host cells, as placental cells, mainly derive their lipids from dietary fat. From this perspective, we decided to investigate whether dietary lipids could affect ZIKV pathogenesis at the level of placenta in pregnant women by performing a translational in vivo study in immuno-competent mice.

To test our hypothesis, we fed mice with different diets that reproduce the lipid content of the main human dietary patterns, namely: a) diets with a high content of either saturated or unsaturated fatty acids to mimic a Western and a Mediterranean diet, respectively; b) diets with a low content of saturated or unsaturated fatty acids to represent an African or an Asian diet, respectively. Six-week-old female C57BL/6J mice were divided into four groups and each group was fed with one of the four experimental feeds. After four weeks of feeding, females were mated and successively checked for vaginal plugs to define the gestation time. For each diet group, ten female mice were sacrificed at 15 days of gestation to perform biochemical and lipidomic analyses on serum, liver and placental tissues; ten females were intraperitoneally infected with French Polynesia ZIKV strain at gestational day 12 and subsequently sacrificed at 6 days post-infection to collect lung, spleen, placentas and fetal brains for virological analyses.

Biochemical parameters of pregnant mice resulted comparable among the four diet-groups demonstrating that, despite the different lipid content, the four feed formulations represented diverse but healthy diets suitable for sustaining a normal pregnancy. On the contrary, the lipidomic analysis revealed that the lipid composition of placentas significantly differed among the four diet-groups. This result demonstrated for the first time how the lipid composition of placental tissues can be severely modified by the quality and quantity of dietary lipids. Virological analyses identified the placenta as the main tissue target of ZIKV and most importantly revealed a lower susceptibility to infection in animals fed with a low content of saturated fatty acids, recording a significantly lower number of infected placentas in mice fed with Mediterranean and African diets compared to mice fed with a

Western and Asian diet.

To date, due to the lack of ZIKV vaccines and effective antiviral drugs, prevention of infection mainly relies on chemical and biological vector control. For the first time, our study has unveiled maternal diet as a fundamental determinant of ZIKV pathogenesis in a pre-clinical animal model of placental infection. These results not only present new perspectives on the neglected interplay between dietary lipids and Flavivirus pathogenesis, but open the way to a new line of research in the field of prophylaxis for the control of ZIKV.

**DYNAMIC OF QUASISPECIES IN LONG TERM SARS-COV-2
IMMUNOCOMPROMISED INFECTED HOST**

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Aim of the study: In October 2020, the United Kingdom faced a rapid rise in positive COVID-19 cases sustained by a new SARS-CoV-2 lineage, namely B.1.1.7, promptly defined as a variant of concern (VOC). Soon after, in South Africa, Brazil and India, new circulating lineages defined VOCs were also described. All VOCs are characterized by a typical mutational pattern in the spike receptor binding domain (RBD), leading to human angiotensin-converting enzyme 2 (ACE2) increased affinity and enhanced infectivity. It has been suggested that the emergence of VOCs worldwide has likely occurred in immunocompromised patients who allowed uncontrolled persistent infection. The RNA virus population in a host is not represented by a single dominating sequence, but rather consists of an ensemble of replicating viruses termed quasispecies. This allows RNA viruses to have a greater possibility to find the best adapting quasispecies to the specific host. Here we describe the case of an immunocompromised patient affected by B-cell lymphoma where the persistent replication of SARS-CoV-2 led to the accumulation of critical amino acid changes within the spike RBD.

Methods: 12 nasopharyngeal swabs obtained from an immunocompromised patient were collected for diagnostic purposes, processed using the InGenius automatic system and evaluated with the GeneFinder™ COVID-19 PLUS RealAmp Kit. Then, total RNA was extracted using the QIAamp DSP Virus kit. The Paragon Genomics' CleanPlex multiplex PCR Research and Surveillance Panel was used to obtain the full-length SARS-CoV-2 genome. The created libraries were sequenced on an Illumina MiSeq platform. Raw data were checked for quality and analyzed with the software SOPHiA GENETICS' SARS-CoV-2 Panel. Lineage assessment was conducted using the Phylogenetic Assignment of Named Global Outbreak LINEages tool. For phylogenetic analysis, the 12 viral genomes were aligned together with 2419 GISAID sequences representative of the lineage B.1.1 globally circulating. Sequences were aligned using MAFFT (FF-NS-2 algorithm) employing default parameters. Phylogenetic analysis was performed using the maximum likelihood method implemented in IQ-TREE2 employing the GTR+I model of nucleotide substitution.

Results and conclusions: Using deep sequencing data, we were able to follow the dynamics of the intra-host evolution during a SARS-CoV-2 prolonged infection in an immunocompromised patient. Genetic screening of all samples revealed 610 intra-host single nucleotide variants (iSNVs), mainly scattered over ORF1ab and S genes. The comparison of iSNVs down to low frequencies has revealed an elevated accumulation of intra-host variants emerged out of a population that existed previously. Many of the iSNVs with a small re-

presentation in the early samples appeared with almost the same frequencies also in later samples, suggesting that the intra-host competition was maintained. In addition, the presence of iSNVs with similar growth rates but appearances in different time points confirmed the co-existence of viral minor mutants and reflected the dynamic interplay with the master mutant. In particular, after 222 days the original master mutant was replaced by a minor quasispecies expressing two critical mutations in the S gene: Q493K and N501T. The time stamped tree revealed that all of 12 strains belonged to an ancestral B.1.1 lineage. The sequences isolated at day 0, 7, and 20 scattered on different branches of the phylogenetic tree, while sequences from day 26 to 222 established an independent cluster. This finding further suggests an intra-host virus evolution, in which genomic diversity is relatively heterogeneous during the early stage of infection, but becoming more homogeneous over time as a consequence of virus adaptation. In conclusion, the preexistence of potential fitness mutants calls for the importance of studying the mutation spectra of different hosts, and minor mutants in particular, to predict the emergence of new variants with a higher capability than their ancestors to rapidly spread around the world and with neutralizing antibody or antiviral drug escape ability.

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Aim of the study: The human coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is associated with elevated morbidity and mortality, and currently with long-term complications. Considering the recent evidence that the Human Endogenous Retroviruses (HERVs) were activated in response to infectious agents leading to immune-pathological effects, the present study aimed to evaluate HERVs in the immune-pathogenesis of COVID-19, for the identification of new biomarkers for the diagnosis, prognosis, and follow-up of COVID-19 patients and their prioritization for targeted therapy.

Methods: Blood and plasma samples were collected at Tor Vergata University Hospital of Rome from Acute COVID-19 (A-COV) patients and Healthy Donors (HD). Blood from Post COVID-19 (P-COV) was also collected (range: 7-48 weeks post-infection). The expression of the envelope (ENV) of HERV-K and HERV-W, and of cytokines have been analyzed by RT-Real time PCR. HERVs gene and protein expression in blood was analyzed by flow cytometry and correlated with clinical signs, immunophenotyping, inflammatory markers, and disease progression.

Results: HERV-W and HERV-K ENV proteins have been found expressed in blood samples from A-COV but not in HDs. Despite the immunological recovery, even after several weeks' post-infection the expression of HERVs remained elevated in P-COV. HERV-K and HERV-W ENV gene and protein expression positively correlated with IL-6, IL-10, and TNF-alpha in A-COV. While in P-COV the expression of HERVs positively correlated with high expression of IL-17 and IFN-g.

Among leukocytes, in A-COV lymphocytes displayed the highest percentage of HERV-W ENV positive cells, which correlated with T cell differentiation, exhaustion, and senescence markers. HERV-W ENV positive CD4+ T cells significantly correlated with coagulopathy and biochemical parameters associated with COVID-19 severity. Instead, HERV-K ENV resulted in highly expressed in granulocytes.

In P-COV patients, HERVs protein expression remained high, especially in granulocytes. To date, long-term health problems, including neurological symptoms such as headache, fatigue, dizziness, memory loss, confusion, and difficulty focusing, are associated with post-

COVID-19 infection. Notably, HERVs expression was found higher in patients with specific neurological symptoms such as paraesthesia and tremors, suggesting their involvement in neurological alterations related to COVID-19.

Conclusion: These data suggest HERVs as contributing factors in the development, progression, and long-term complications of COVID-19 describing disease evolution and opening avenues for novel therapeutic strategies.

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Introduction and aim: Among the multiple factors that may contribute to the pathogenesis of Alzheimer's disease (AD), numerous experimental and epidemiological evidence suggests that recurrent herpes simplex virus-1 (HSV-1) infection reaching the brain is one of the risk factors for disease onset and/or progression. However, the molecular mechanisms linking HSV-1 infection to neuronal dysfunctions have yet to be fully elucidated. Genetic, proteomic, and immunologic evidence suggests that dysregulation of the complement cascade, a key component of the innate immune system that is rapidly recruited to allow the clearance of pathogens and promote tissue repair, is involved in the pathogenesis of AD (Park et al, 2020). More specifically, hyperactivation of the classical complement pathway plays a role in the elimination of CNS synapses leading to synaptic dysfunction and consequent neurodegeneration.

This study aims to investigate whether complement activation occurs after HSV-1 infection in cultured brain cells and to evaluate HSV-1 induced complement-dependent synaptic deficits.

Methods: Primary neuronal cultures were isolated from the brain of rat embryos (E18). Neurons were mock- and HSV-1-infected at different multiplicity of infection and the efficacy of HSV-1 infection was evaluated by standard plaque assays (SPA). Cells were lysate and analyzed by Western blot and RT-PCR to study complement components expression at protein and mRNA levels. Neutralization assay was performed with the aid of a specific antibody directed against the complement component c3.

Results and Conclusion: We found that HSV-1 infection in cultured rat brain cells induces a dose-dependent increase in the expression of components of the classical complement pathway, such as c1q, c3 and c4, and a decrease in the synaptic marker PSD-95 levels. In addition, results from a neutralization assay showed that in HSV-1 infected neurons the inhibition of the c3 component rescued the HSV-1 induced decrease of PSD-95, suggesting that the complement protein c3 may be involved in the process of synaptic reduction that follows HSV-1 infection. Overall, these data indicate that complement plays a role in the immune response to HSV-1 infection in rat brain and suggest that c3 may be involved in triggering the synaptic damage that occurs after infection.

THE DIRECT CLEAVAGE OF CASPASE-8 DURING HSV-1 INFECTION IS DUE TO US11 TEGUMENT PROTEIN AND TRIGGERS ATG3 DEGRADATION TO SUPPORT VIRAL REPLICATION**R. Pennisi¹, M.P. Tamburello¹, D. Lombardo², M.T. Sciortino¹**

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The viruses can subvert the host intracellular pathways to support better replication. Thus, the aim of this work was to investigate the role of viral tegument protein Us11 in the modulation of innate immunity by caspase-8. Caspase-8 is an initiator caspase which normally triggers the apoptotic event following its activation mediated by proteolytic cleavages (Kallenberger et al., 2014). The Us11 protein counteracts the immune response by interacting with the protein kinase-R (PKR) and interfering with the FADD/caspase-8 death-signalling pathway and autophagy response (Balachandran et al., 2000; Cassady and Gross, 2002; Tsapras and Nezis, 2017). Based on this, we firstly investigated the role of US11 on canonical caspase-8 functions related to apoptosis response. A combinatory approach of HSV-1 mutant (R3630- Δ Us11/ Δ Us12) infection and Us11- and Us12-encoding plasmid transfection was employed to investigate the ability of Us11 to interact with caspase-8. Moreover, GST-Us11 and GST-caspase-8 recombinant proteins were produced by using the Baculovirus Expression Vector System (BEVS) technology proteins and used to verify the interaction of these proteins in a cell-free system. The human monocytic leukemia cells (THP-1), embryonic kidney cells (293T), and wild type and caspase-8 deficient (CASP8^{-/-}) epithelial cells derived from a larynx carcinoma (HEp-2) were employed as models with different permissivity to viral infection in order to study the role of caspase-8 during HSV-1 replication. Our findings report for the first time the direct cleavage of caspase-8 mediated by US11 protein. In particular, we found that HSV-1 accumulates caspase-8-p18 active fragment in US11-dependent manner in both monocytes and epithelial cells without triggering apoptosis. To individuate the biological role of Us11-dependent p18 accumulation during HSV-1 replication, we analysed the expression of Atg3 protein as a representative substrate of activated caspase-8 protein. We found that HSV-1 specifically cleaves Atg3 protein as shown by the reduction in the full-length protein compared to the basal levels and by detecting cleavage fragment. Otherwise, the addition of the caspase-8 inhibitor z-IETD-fmk as well as the transfection with a pool of chemically synthesized caspase-8 siRNAs resulted in the block of Atg3 cleavage suggesting a direct connection between the activation of caspase-8 mediated by HSV-1 and Atg3 degradation. Based on this, we investigated the role of caspase-8 during viral replication by comparing the cytopathic effect, viral title, viral DNA accumulation and the HSV-1 proteins cascade expression in wild type HEp-2 (CASP8^{+/+}) and CASP8^{-/-}. Our finding report that the accumulation of viral proteins and DNA, as well as virus yield, were affected in CASP8^{-/-} suggesting that caspase-8 might have a pro-viral role during HSV-1 replication. Therefore, we speculate that the “non-canonical” activation of caspase-8 by HSV-1 can be a new viral immune escape mechanism to, through the fragmentation of ATG3, block autophagy and support better replication.

THE ENRICHMENT OF POSITIVELY CHARGED AMINO ACIDS IN HBsAg C-TERMINUS IMPAIRS HBsAg SECRETION, AFFECTS ITS STRUCTURAL STABILITY AND IS CORRELATED WITH HBV-INDUCED LIVER CANCER

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Aim: Patients with chronic hepatitis B have a 100-fold increased risk of developing hepatocellular carcinoma (HCC). An impairment in HBsAg secretion is a mechanism mediating HBV related oncogenesis.

HBsAg C-terminus is a hydrophobic transmembrane domain, crucial for HBsAg secretion and the gain of charged amino acids (aa) in this domain can alter its folding in the ER membrane, thus hampering HBsAg secretion. In this light, the aim of this study is to investigate the role of HBsAg C-terminus (aa189-226) mutations, associated with a gain of charged aa, on HBV-induced HCC onset.

Material: We analyze 807 HBV chronically infected patients collected from routine clinical practice with an available HBsAg sequence: 28 with HCC (78.6% D; 21.4% A), and 779 patients without HCC (79.8% D; 20.2% A). Multivariable logistic regression model is used to assess the association of identified mutations with HCC. The impact of identified mutations on HBsAg-secretion is analyzed *in vitro* by transfecting HepG2 cells with plasmids encoding wt- and mutated-HBsAg. Extracellular and intracellular HBsAg is quantified by an immunoassay (LiaisonXL, Diasorin) and used to define HBsAg secretion factor (ratio between extracellular and intracellular HBsAg). I-Tasser is used to assess HBsAg structures and its structural stability ($\Delta\Delta G[\text{wt-mutated}] < 0$ indicating decreased stability in presence of mutation based on Quan, 2016).

Results: The acquisition of >1 positively charged amino acid at positions 204, 207, and 210 of HBsAg C-terminus strongly correlates with HCC (71.4% with HCC vs 30.2% without HCC, $P < 0.001$). Multivariable analysis confirms this association stratifying for patients' demographics, HBV genotype, serum HBV-DNA and anti-HBV drugs use ($\text{OR}[95\% \text{CI}]: 6.3[2.6-15.3]$, $P < 0.001$). The acquisition of positively charged amino acids results from S204R, S207R and S210R mutations, found in 14.3%, 28.6% and 28.6% of HCC-patients, respectively. By *in vitro* experiments, all these mutations determine a significant decrease in extracellular HBsAg amount compared to wt (42% for S204R, 39% for S207R and 32% S210R, $P < 0.0001$ for all comparisons). Moreover, S204R and S210R also cause a 58% and 28% reduction in HBsAg secretion factor compared to wt ($P < 0.0001$ and $P = 0.009$), further reinforcing their detrimental role in HBsAg release. *In silico*, S204R, S207R and S210R decrease structural stability of HBsAg compared to wt ($\Delta\Delta G[\text{S204R-wt}] = -0.27$; $\Delta\Delta G[\text{S207R-wt}] = -0.11$; $\Delta\Delta G[\text{S210R-wt}] = -0.14$) and determine a shortening of membrane-spanning alpha-helix motif (predicted alpha-helix length: aa209-224 for

S204R, S207R and S210R vs 205-225 for wt), suggesting an impaired HBsAg C-terminus stability.

Conclusions: Gain of positively charged amino acids at specific HBsAg C-terminus positions tightly correlates with HBV-induced HCC, hampers HBsAg release in vitro and alters the proper folding of this domain. This could favour an intracellular HBsAg retention, posing the bases for HBV-driven hepatocarcinogenesis.

The detection of these mutations may help identifying patients at higher HCC-risk, deserving more intense liver monitoring.

HSV-1 INFECTION AND DYRK1A KINASE: A POTENTIAL TARGET FOR VIRUS-RELATED NEURODEGENERATIVE EVENTS

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Background and aims: The dual-specificity tyrosine phosphorylation activated kinase 1A (Dyrk1A) is known to phosphorylate the microtubule-associated protein tau at several sites correlated with different neurodegenerative disorders, including Alzheimer's disease (AD), the most common type of dementia in the elderly affecting millions of people worldwide (Wegiel et al, FEBS J, 2011). Among the environmental risk factors for AD onset and progression, a growing body of evidence supports the involvement of herpes simplex virus type-1 (HSV-1) infection/reactivation reaching the brain. In particular, HSV-1 replication in neuronal cells and mouse brains induces tau hyperphosphorylation, aggregation, and cleavage reminiscent of AD-like pathology (De Chiara et al, Plos Pathogens, 2019). Here we checked whether Dyrk1A is involved in HSV-1-induced tau phosphorylation and verified the efficacy of pharmacological inhibitors of Dyrk1A in inhibiting this event, as well as HSV-1 replication in neuronal cells.

Methods: Pharmacological inhibitors of Dyrk1A and inactive compounds (designed and synthesized by Perha pharmaceuticals) were used to study Dyrk1A involvement in HSV-1-induced tau phosphorylation in human neuroblastoma SH-SY5Y cells. MTT assay was used to check their cytotoxicity on SH-SY5Y cells. Mock- and infected-cells were treated with different concentrations of compounds both during the absorption phase of HSV-1 infection and after the infection for 24 hours. Levels of tau phosphorylation were evaluated by Western blotting (WB) with antibodies directed against specific phosphorylated sites of the protein. The efficacy of HSV-1 infection was evaluated by standard plaque assays (SPA) and in-Cell western (ICW) assay.

Results and conclusions: We found that: 1) HSV-1 induced tau phosphorylation in T217 (pT217) and T205 (pT205), both sites known to be directly targeted by Dyrk1A, and S214 (pS214); 2) among the 6 inhibitors tested, 3 resulted effective in inhibiting HSV-1-induced pT217 in a dose-dependent manner, whereas the inactive compounds were ineffective; two of these inhibitors also affected viral replication. Overall, these results indicate that HSV-1 exploits Dyrk1A to induce tau phosphorylation in neuronal cells and suggest Dyrk1A pharmacological inhibitors as possible drug candidates for counteracting HSV-1-induced neurodegeneration.

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Aim of the study: It is becoming evident that Covid-19 disease is much more than a simple respiratory disease. Other organs can be affected with unexpected and silent deterioration including the CNS. Recent data have reported viral proteins and RNA in brain biopsies. SARS-CoV-2 in the brain and cerebrospinal fluid of patients, providing an explanation for neurological symptoms. Specifically, recovered patients report long-term cognitive symptoms, likely due to hidden damages in the brain. Nevertheless, the effects of the virus on CNS are not fully understood and few data are available. As for other neurotropic viruses, it is believed that SARS-CoV-2 entry and replication into cells are successfully accomplished through a fine interplay between viral proteins and cytoskeletal elements. Understanding how SARS-CoV-2 manipulate the cytoskeleton and disrupt the cellular homeostasis will be crucial to unveil short- and long-term damages in neurons. Thus, it is a high priority to understand the molecular bases of the neurotropism and the cellular biology of SARS-CoV-2 in healthy neurons as well as in cells already primed for neurodegeneration diseases. To this aim we investigated SARS-CoV-2 trafficking and related damages in healthy and in pathological neuronal models of Alzheimer disease. Moreover, we investigated the contribution of viral proteins in the activation of kinases involved in neurodegeneration.

Methods used: SH-SY5Y neuroblastoma cells were infected with SARS-CoV-2 B.1(Wuhan), B.1.1.7(alpha), B.1.617.2 (delta) and BA.1 (Omicron) variants; infected cells were examined at different time points from infection for the presence of viral proteins and biomarkers of neurodegeneration by confocal microscopy and western blot.

Results and conclusions: Neuroblastoma cells are efficiently infected by all SARS-CoV-2 variants; both Spike and Nucleocapsid proteins of SARS-CoV-2 are expressed in infected cells and N and Matrix induced the activation of specific cellular kinases presumably involved in the pathological phosphorylation of neurodegeneration biomarkers. These findings indicate that SARS-CoV-2 play a direct role in neurodegeneration and may therefore responsible for neurological symptoms observed in substantial number of COVID patients. Further studies are warranted to confirm this observation.

CIRCULATING MICRORNA SIGNATURES ASSOCIATED WITH DISEASE SEVERITY AND OUTCOME IN COVID-19 PATIENTS

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Background: SARS-CoV-2 induces a spectrum of clinical conditions ranging from asymptomatic infection to life threatening severe disease. Host miRNAs, key regulators of innate and adaptive immune cell development, have been involved in the cytokine storm driven by SARS-CoV-2 infection and proposed a candidate diagnostic and prognostic biomarkers for COVID-19 patients.

Aim of the study: To identify signatures of circulating serum miRNAs associated with SARS-CoV-2 infection, disease severity and mortality in COVID-19 patients and to search for possible sources of the differentially expressed serum miRNAs through an *in vitro* study.

Material and Methods: Circulating miRNAs and isomiRs were analysed by small RNA sequencing in the serum of patients with mild (n = 26), moderate (n = 29) and severe (n = 34) COVID-19 collected at the time of hospital admission and in serum samples collected from healthy control subjects (HC) before 2019 (n = 45). DE analysis was performed using GLM and TMM normalized counts (CPM) as input data, considering significant Benjamini-Hochberg adjusted p-values ≤ 0.05 . Gene targets for candidate miRNAs were identified using MIENTURNET and miRWalk 3.0. Significant target genes were selected using GSEA and implemented with STRING v11.5, through GO functional analysis and annotation databases, setting FDR < 0.05. ROC and survival curve analyses were performed to identify candidate miRNA biomarkers. Then, differentially expressed miRNAs were investigated in human cell types (Calu-3, Caco-2, HUVEC, and PBMC), characterized by different permissiveness to SARS-CoV-2 infection and responsiveness to type I IFN.

Results: DE analysis identified 23 upregulated and 27 downregulated miRNAs in each group of COVID-19 patients (mild, moderate and severe) vs HC. ROC curve analysis showed that high levels of miR-320 family members and miR-483-5p and low levels of miR-30d-5p, miR-25-3p, miR-93-5p, miR-16-5p showed >90% sensitivity and >90% specificity to discriminate between COVID-19 patients from HC. Additionally, high levels of miR-21-5p, miR-22-3p and miR-451a, miR-92a-3p, miR-101-3p, miR-224-5p, and miR-194-5p could distinguish severe COVID-19 from mild/moderate disease, while low level of miR-155-5p was identified as a candidate prognostic biomarker of ICU admission, long-term sequelae and death in COVID-19 patients. Network analysis showed that the modulated miRNAs were involved in different cellular pathways including cell response to oxidative stress, autophagy, mitophagy, apoptosis, cell senescence, and angiogenesis. The *in vitro* study showed cell-specific expression of some miRNAs. Changes of miRNAs induced by SARS-CoV-2 infection were more prominent in the highly permissive Calu-3 cells and were associated with antiviral and pro-inflammatory responses triggered by

sensors of RNA viruses, while treatment with IFN α led to a down-regulation of most of the selected DE miRNAs.

Conclusion: We discovered signatures of circulating miRNAs associated with COVID-19 severity and mortality. The identified DE miRNAs provided clues on COVID-19 pathogenesis, highlighting signatures of impaired IFN and antiviral responses, inflammation, organ damage and cardiovascular failure as associated with severe disease and death. *In vitro* experiments showed that some of the DE miRNAs were modulated directly by SARS-CoV-2 infection or indirectly by type I IFN stimulation.

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Aim: The evaluation of the effect of SARS-CoV-2 infection during pregnancy has raised interest. Even if virus vertical transmission is still controversial, several research have focused on the identification of possible distinctive markers associated to a different susceptibility to SARS-CoV-2 infection, particularly on placenta. The aim of this study was to further investigate the placental SARS-CoV-2 infection and the potential effect on protein expression as ACE2, CD147, HLA-G and CD56, as a marker of NK cells, in placental tissues from symptomatic and asymptomatic COVID19 women, positive for SARS-CoV-2 infection at the hospital admission.

Methods: Placental tissues from 18 women with asymptomatic and 9 women symptomatic respiratory SARS-CoV-2 infection and a control cohort made up of 11 women with physiological pregnancy were analyzed for SARS-CoV-2 NP, Human Leukocyte Antigen-G (HLA-G), CD147, ACE2 and CD56 expression by immunohistochemistry.

Results: The 78% of the placenta samples from the COVID-19 symptomatic group were positive for SARS-CoV-2 NP expression in comparison with the 39% of COVID-19 asymptomatic group ($p < 0.0001$). SARS-CoV-2 NP positivity showed a different localization between syncytiotrophoblast (ST) and extra-villous trophoblast (EVT). SARS-CoV-2 NP positivity was preferentially found in the ST of symptomatic group samples (100% vs 57%, $p < 0.01$), while the infection was at EVT level in COVID-19 asymptomatic women (86% vs 71%, $p < 0.01$). Both ACE2 and CD147 receptors were expressed in all the placenta samples. CD147 diffuse and strong expression was found in ST of all the three groups, with a strongest staining in symptomatic women, independently to SARS-CoV-2 NP positivity. ACE2 expression is mainly present in EVT of all the placenta samples, but with a different cellular distribution. SARS-CoV-2 negative samples presented a diffused expression of ACE2, while SARS-CoV-2 positive samples, independently from the symptomatic or asymptomatic condition of the mother, showed a membrane-associated staining. HLA-G expression was evident in ST of all the placental samples, with a significant increase in ST of COVID-19 symptomatic SARS-CoV-2 positive samples. HLA-G expression was also evident in EVT, with an induced expression in COVID-19 symptomatic SARS-CoV-2 positive and negative samples and in COVID-19 asymptomatic SARS-CoV-2 positive samples, but without reaching a significant difference in comparison with control samples (asymptomatic $p = 0.31$; symptomatic $p = 0.21$). We observed the presence of CD56+ cells in the uterine tissues. COVID-19 women, independently to symptoms and SARS-CoV-2 positivity of placental tissues, showed a significant decrease in CD56+ cells compared to controls ($p < 0.001$).

Conclusions: COVID19 symptomatic women showed and increased SARS-CoV-2 NP and CD147 positivity in the ST, with a decrease in CD56+ resident cells. These results support previous observations on the role of CD147 during SARS-CoV-2 infection and the significant effect on placental immune environment.

TROJAN HORSES AND CASSANDRAN PREDICTORS, THE ROLE OF IMMUNE CELLS AND MEDIATORS IN SARS-COV-2 INFECTION

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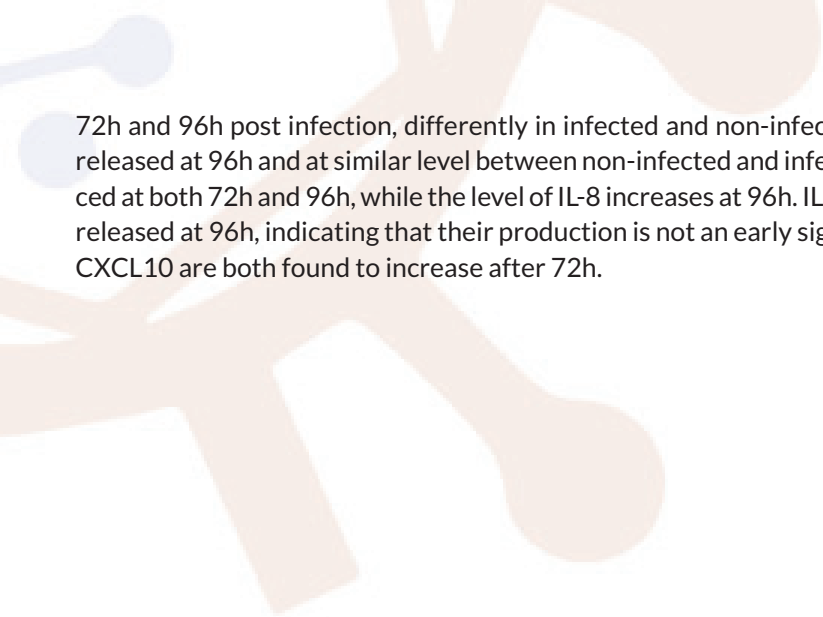
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Aim: The severe acute respiratory syndrome virus 2 (SARS-COV-2) continues to be an issue for the public health despite the advances in understanding its biology and pathogenesis. During infections, the principal actors of the immune innate system are the antigens presenting cells, such as monocytes (MN), macrophages (MDM), dendritic cells (DC) and the released stimuli of cytokines and chemokines. Our aim is to identify the role of immune cells in SARS-COV-2 dissemination, and the indirect effect of local/systemic inflammatory and immune responses. Our hypothesis is that SARS-COV-2 can be preserved in immune cells, promoting its dissemination to different organs and that the release of cytokines/chemokines can define infection outcomes and serve as predictive markers for COVID-19.

Methods: Several SARS-CoV-2 variants were isolated from infected patients. VERO E6 cells were used to prepare viral stock and virus isolation or infection by coculture of MN, MDM and DC differentiated from peripheral blood mononuclear cells of healthy subjects. ACE2 receptor block with monoclonal antibodies was done to evaluate the viral mechanism of entry. Evaluation of immune cells infection at various time points was performed by CLSM, EM, RT-qPCR (intra/extracellular) and viral titration. To study viral transmission and cytokines/chemokines release, VERO E6, 16HBE cells or pulmonary tissue were infected with SARS-COV-2 and cocultured with uninfected immune cells. Then, MN, MDM and DC ability to migrate in response to chemoattractants was exploited. Migrated MN, MDM and DC were used to verify their function as "Trojan horses" by coculture with permissive cells, while cytokines/chemokines levels were analysed in the supernatants of each condition using ELISA/ELLA kits.

Results and conclusions: Infected VERO E6 with active viral replication transmit the virus to MN, MDM and DC, where the virus persists but does not replicate. Nonetheless, infected immune cells can transmit the infection to permissive cells. Therefore, MN, MDM and DC can host the viable virus but are not permissive to viral replication. These in vitro observations confirm that the immune cells could be key players in viral dissemination and persistence. In EM, there was a discrepancy between the amount of viral proteins (high) and mature viral particles (low), which may be a consequence of an initial but abortive replication. From the ACE2 receptor block assays, we determined that more than one mechanism is involved in the internalization of SARS-COV-2 in immune cells, but this aspect requires further investigation. As for the cytokines/chemokines release in experiments using pulmonary biopsies from healthy subjects, we found that CCL2 is released both at



72h and 96h post infection, differently in infected and non-infected cells, while CCL5 is released at 96h and at similar level between non-infected and infected cells. IL-6 is produced at both 72h and 96h, while the level of IL-8 increases at 96h. IL1-b and TNF-a are more released at 96h, indicating that their production is not an early sign of infection. IFNg and CXCL10 are both found to increase after 72h.

INTERACTION OF U24 FROM HUMAN HERPES VIRUS 6B WITH NEDD4 WW AND FYN-SH3 DOMAINS: THE IMPORTANCE OF PHOSPHORYLATION AND POTENTIAL LINK TO MS

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Background: Recent work has shown that U24, a putative tail-anchored membrane protein unique to *Roseoloviruses* (*i.e.*, Human Herpesvirus (HHV) type-6A, -6B and -7), may play a role in multiple sclerosis (MS). Two hypotheses on how these viruses may be implicated in MS have been proposed: molecular mimicry and endocytic recycling. In particular, studies indicated how charges and phosphorylation influence binding affinities between U24 from HHV-6A (U24-6A) and Fyn-SH3 and Nedd4 WW domains for the two respective models.

Aim: Herein, the interaction of a peptide consisting of the first fifteen residues of U24-6B and its phosphorylated analogue (pU24-6B) with hNedd4L-WW3*, as well as Fyn-SH3, were quantified.

Methods: We examined the interaction of U24 from HHV-6B and its phosphorylated form with hNedd4L-WW3* and Fyn-SH3. We established the role of KIRs in MS evaluating natural killer (NK) cell activation in MS patients upon exposure to U24-6A and -6B and their respective phosphorylated versions.

Results: Results obtained from isothermal titration calorimetry (ITC) experiments showed that U24-6B and pU24-6B bind to human Nedd4L-WW3* with affinities similar to U24-6A and pU24-6A, respectively. ¹H-¹⁵N HSQC nuclear magnetic resonance (NMR) titrations showed that the binding interaction between U24-6B and Fyn-SH3 is stronger than the interaction between U24-6A and Fyn and that phosphorylation weakens the interaction. Finally, a comparison of natural killer (NK) cell activation in MS patients upon exposure to U24-6A, U24-6B, pU24-6A and pU24-6B showed that HHV-6A and phosphorylation are important.

Conclusions: These results support the highly prevalent HHV-6A and 6B variants to MS and the involvement of U24.

HUMAN PAPILLOMAVIRUS E6*I EXPRESSION AND TERT PROMOTER MUTATION IN DISTINCT SUBTYPES OF HEAD AND NECK CANCERS

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Background and aim: Head and neck cancers (HNC) include a heterogeneous group of tumors originating from lip and oral cavity (OC), salivary glands (SGC), oropharynx (OPC), nasopharynx (NPC) and hypopharynx (IPC). The main risk factors are heavy smoke for OC and high-risk human papillomavirus (HPV) infection for OPC. Somatic mutations in cancer driver genes as well as constitutive expression of HPV E6, E7 and E6* isoforms are known to be necessary for neoplastic transformation in cervical neoplasia. The aim of this study was to evaluate the role of HPV16 E6*I expression and of TERT promoter (TERTp) mutation in the pathogenesis of different HNC histotypes.

Material and methods: Genomic DNA was extracted from 38 HNC fresh frozen biopsies and paired peri-tumor tissues as well as from 60 archived formalin fixed paraffin embedded (FFPE) HNC tissues. In addition, ten HN hyperplastic lesions were also analyzed. Detection of HPV DNA and TERTp mutations was carried out by end-point PCR and Sanger sequencing analysis. Total RNA from fresh frozen samples was retro-transcribed and used to quantify TERT and HPV16 E6*I expression by droplet digital PCR (ddPCR).

Results and conclusions: HPV DNA was identified in 21 out of 98 (21.4%) HNCs, in 10 out of 22 (45.5%) paired peri-tumor tissues and in 3 out of 10 (30%) hyperplastic samples. The most common genotype was HPV16 (90.5% of all positive samples) followed by HPV33 (9.5%), and genotype concordance was always observed between HNC and peri-tumor tissues. HPV was more frequent among OPC (43.5%, 10/23) than OC (10.5%, 6/57) samples. The single cases of SGC and NPC were both positive for HPV16. Interestingly, the HPV16 E6*I expression was higher in the tumor biopsies than peri-tumor tissues. Mutations in the TERTp region were highly frequent in OC (10/16, 62.5%) while undetected in OPC and associated with a 2-fold increase in telomerase expression in HNC biopsies compared to paired TERT wild type peri-tumor and hyperplastic samples. In conclusion, we confirm that HPV is frequently detected in OPC. The HPV16 isoform E6*I is highly expressed in OPC biopsies compared to peri-tumor and precancerous lesions. Moreover, TERTp mutations are very common in OC but not in other HNC histotypes. These findings suggest an important role for HPV E6*I and for TERTp mutations in the development of OPC and OC, respectively. Further studies are needed to determine the earliness of these potential biomarkers and their diagnostic potential in the monitoring of head and neck evolving precancerous lesions.

THE NRF2 ANTIOXIDANT RESPONSE IS DOWN-MODULATED IN UNDER 10-YEARS OLD CHILDREN POSITIVE TO SARS-COV2: NEGATIVE CORRELATION WITH THE CLASS I AND III INTERFERON (INF) GENE EXPRESSION

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Abstract: Several host factors, including the redox state environment and the activation of related pathways,

play a key role in regulating the respiratory virus replication and the pathogenesis of infection. In fact, we have recently found a down-regulation of NRF2 expression during influenza virus infection, that leads to inhibition of Glucose-6-Dehydrogenase (G6PD) gene expression and to depletion of the intracellular glutathione (GSH) levels (De Angelis M. et al 2022). Some authors (Olagnier D. et al 2020) have also demonstrated that SARS-CoV-2 mediated down-regulation of NRF2 caused suppression of inducible genes in lung biopsy samples obtained from patients with severe disease. In addition to antioxidant responses, NRF2 is involved in the regulation of other cellular processes, including inflammation and interferon (IFN) response.

In the present study, we evaluated the expression of NRF2 and some related redox genes by qPCR in an under 20 years old population of 64 individuals, who came to the hospital to perform a control nasopharyngeal swab.

We found a significant down-regulation of NRF2 and its related genes, G6PD and Apurinic/aprimidinic Endodeoxyribonuclease 1 (APE-1), in SARS-COV-2 positive group (31 children). Indeed, the mean expressions of NRF2, G6PD and APE1 decreased by 95.7, 67.7 and 67.9% respectively compared to negative group (33 children). Surprisingly, the expression levels of some class I and III INF genes was not increased with respect to the negative control group, but the mean expression of ISG56 was increased by 32%. We then stratified the negative and positive children by age, under-10 (31 children: 17 negative, 14 positive) and over-10 (33 children: 16 negative, 17 positive) years. In positive under-10 group we found a significant increase of the IFN $\alpha 2$, β and $\lambda 2$ genes (with mean expressions equal to, respectively, 2.56, 1.38 and 2.41) compared to negative group (with mean expressions equal to 1.98, 0.97, and 1.56); while these increases were not found in the over-10 group. Furthermore, the under-10 population undergoes a more drastic downregulation of NRF2 (the median decreased by 93.7%) and related redox genes (G6PD median decreased by 64.9% and APE-1 median decreased by 78.5%) following infection than the over-10 (where the NRF2 and G6PD medians decreased respectively by 90% and 32.1% and the APE-1 median was increased by 16.4%). Interestingly, by comparing the negative groups, we found that NRF2 gene expression was higher in under-10 children with respect to over-10 group, thus suggesting a strong drop of the antioxidant response during infection in under-10 compared to over-10 years old. Since it has been shown that SARS-COV-2 infection can activate the inflammasome in order to induce hyperinflammation, we also examined the expression of the NOD-like receptor family pyrin domain containing 3 (NLRP3),

but we did not detect any increase in both groups. Further studies are in progress to evaluate whether the iperactivation of the interferon response mediated by down- regulation of antioxidant response is important in the severity of disease.

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LATENT INFECTION OF RESTING AND ACTIVATED CD4+ T CELLS BY HIV-1 PSEUDOTYPED WITH ENV MOLECULES CARRYING A HETEROLOGOUS GP41 C-TERMINAL TAIL

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Background: A small pool of naive and memory CD4+ T-cells harboring latent HIV and persisting indefinitely despite anti-retroviral therapy represents the major barrier toward a cure for HIV-1 infection. It is, therefore, important to investigate the pathways involved in the establishment of HIV-1 latency in these cells. Recent studies indicated that HIV-1 is capable of establishing latent and productive infection directly in either activated or resting CD4+ T-cells (Chavez L et al. PlosPathog 2015). Other studies indicate that latent infection in resting CD4+ T cells is favored by chemokine-signaling, and that X4-tropic HIV-1 Env can signal through the cognate CXCR4 chemokine receptors (Cameron PA et al. PNAS 2010; Balabanian K et al. J Immunol 2004). It is known that the Env gp41 C-terminal tail is a key determinant of Env conformation and antigenicity, suggesting that it might affect Env-CCX4 interaction and signalling. Based on these data, we aimed to determine whether the gp41 C-terminal tail affects the efficiency of HIV-1 latency establishment in CD4+ T cells. To this purpose, we infected activated or resting CD4+ T cells with tier-1 and tier-2 HIV-1 isolates encompassing homologous or heterologous gp41 C-terminal tail sequences. Our preliminary data suggest that the gp41 C-terminal tail might contribute to determine HIV latency in activated CD4+ T cells.

Material and methods: Resting or activated CD4+ T cells were infected with a non-replicating HIV-1 dual-fluorescent reporter vector (HIV-GKO) capable of identifying latently and productively infected cells (Battivelli et al. eLife 2018). In particular, HIV-GKO expresses the GFP protein under the control of the virus LTR and the MKO2 protein under the control of the EF1-alpha constitutive transcriptional promoter. Hence, GFP expression marks productive replication, whereas expression of MKO2, coupled to absence of GFP expression, identifies latently infected cells. The HIV-GKO backbone was co-transfected in HEK 293T cells with vectors encoding R5-tropic tier-1/2 Env carrying homologous or heterologous (HXB2-derived) gp41 C-terminal tail sequences. CD4+ T cells were isolated from PBMCs of normal donors by negative depletion and left unstimulated or activated through CD3/CD28 engagement. Latent and productive infection was visualized and quantified by flow cytometry.

Results: No difference in the efficiency of latency establishment was observed in resting CD4+ T cells using two tier1 R5-tropic pseudoviruses carrying their homologous gp-41 C-terminal tail or the HXB2 heterologous tail. However, the tier-2 R5-tropic pseudovirus showed an inversion in the ratio of latent versus productive infection in activated CD4+ T cells, with the chimeric-gp41 pseudovirus yielding a higher proportion of latent infection as compared to the native gp41 pseudovirus.

Conclusion: If further substantiated, these preliminary data might point to crucial effects of the gp41 C-terminal tail in determining the fate of HIV-1 productive versus latent infection in primary CD4+ T cells.

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Aim of the study: In the last decades the interest in bioactive compounds derived from natural sources including bacteria, fungi, plants, algae, and spongi, has increased significantly. It is well-known that aquatic or terrestrial organisms are able to produce, in special conditions, secondary metabolites with a wide range of biological properties, such as anticancer, antioxidant, anti-inflammatory and antimicrobial activities [1]. In addition, the current pandemic has brought out the importance of searching new antivirals as quickly as possible. In this scenario, the present study was focused on the extremophilic microalga *Galdieria sulphuraria* as possible producer of bioactive compounds with antiviral activity.

Methods: The algal culture was subjected to an organic extraction with acetone. Then the extract was dried and dissolved in dimethyl sulfoxide (DMSO) at the concentration of 10 mg/mL. Cytotoxicity of the raw extract was evaluated by the 2,5-diphenyl-2H-tetrazolium bromide (MTT) assay on Vero cells (ATCC CCL-81). The same cell line was used to propagate all the viruses. The antiviral activity was assessed through plaque assay against *Herpesviridae* (HSV-1 strain SC16; HSV-2 strain 333) and *Coronaviridae* (HCoV-229E strain VR740; SARS-CoV-2 strain VR-PV10734) members as enveloped viruses, and poliovirus (PV-1 strain CHAT) as non-enveloped virus. The monolayer was treated with different extract concentrations, ranging from 200 µg/mL to 1 µg/mL, and infected with viruses at 0.01 multiplicity of infection (MOI). To understand in which stage of the infection the extract could act, the assay was performed in four conditions: co-treatment, virus pre-treatment, cell pre-treatment and post-treatment. The inhibitory effect of the algal extract was confirmed by the quantitative RT-PCR in which we analysed the relative expression of viral genes. Finally a preliminary chemical profiling of the extract was performed by HPLC Mass spectrometry.

Results and Conclusion: The algal extract displayed a strong antiviral activity at the non-toxic concentrations against all the tested enveloped viruses, in particular against the members of *Coronaviridae* family, i.e., HCoV-229E and SARS-CoV-2 with an IC₅₀ of 0.8 and 6.3 µg/mL, respectively. On the contrary, it did not show any activity against the non-enveloped poliovirus (PV-1). Then, our results demonstrated that *Galdieria sulphuraria* extract exhibited a very strong antiviral activity directly on the viral particles by interacting with the envelope during the early stages of infection and preventing the virus entry. Molecular test confirmed the data obtained *in vitro*. The HPLC Mass spectrometry analysis revealed that the extract was enriched in primary fatty acid amides (PFAA) such as oleamide, palmitamide and pheophorbide. These compounds have been previously re-

ported to show relevant antiproliferative and antiviral properties [2]. These interesting results encourage the purification of the mixture in order to evaluate its potential application in the pharmaceutical fields as an antiviral agent.

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Aim: Aim of the study was to evaluate the differences in the cellular immune response against measles virus, among subjects vaccinated with two doses of MMR vaccine, naturally infected subjects, unvaccinated subjects who never had the infection and subjects who were seronegative to measles after two-dose MMR vaccination-

Methods: First of all, levels of functional neutralizing antibodies, which is considered a good correlate of protection from infection during measles outbreaks, was investigated among either vaccinees or naturally infected subjects. In order to investigate cellular immunity, peripheral blood mononuclear cells (PBMC) drawn from the four categories of subjects and seeded in quadruplicate at a density of 1×10^6 cells in 500 μ l in 24-well plates. After 6 hours, UV-inactivated measles virus (Edmonston B strain) was added in one well from each subject at a concentration of 1 MOI, while the other well was kept as the unstimulated control. T-cell populations were analyzed 48 hours after antigen stimulation. After that, cells were harvested and analyzed by flow cytometry. The transcriptome-level characterization (mRNA-Seq data) of responses to measles virus stimulation in antibody responders (either vaccinated or naturally infected) and non-responders to the vaccine is in progress.

Results and conclusions: Data showed a significant increase in the percentage of CD3+ T cells among naturally infected subjects in comparison with the correspondent unstimulated samples ($p=0.003$). This increase was higher than that observed in the vaccinated cohort ($p=0.01$), highlighting a stronger T cell induction in naturally infected subjects than in vaccinees upon antigen exposure. Subsequently, the similar analyses were evaluated in CD4+ and CD8+ T cell subsets. While the CD4+ and CD8+ cell response was almost unvaried among vaccinated subjects, it was heterogeneous in naturally infected subjects. Finally, a deeper analysis of CD4+ subsets (Th1, Th2, Th17) was performed. An unvaried Th17 response was observed in both groups, however differences in Th1/Th2 responses were observed. Indeed, while a preferential induction of the Th1 response and a correspondent decrease of the Th2 response was recorded in vaccinees, an opposite profile, with an overall induction of Th2 response, was observed among naturally infected subjects. These data could explain why the neutralizing response observed in naturally infected subjects was higher than in vaccinated ones (Anichini et al., 2020). We also tried to investigate the role of CD4+ and CD8+ naïve, memory and effector subsets; no differences were observed after antigen stimulation in both groups.

Finally, transcriptomic analysis to find the immune pathways that could be involved in the 'non-response' to the vaccine among the four non-responder subjects is still ongoing. At the moment, the complex transcriptome data are very preliminary and under analysis (data not shown).

This study might be very helpful to develop a panel of biomarkers to easily monitor the immune response to measles vaccine, besides the antibody response, with the aim to identify and protect the subjects, who eventually become seronegative along the time, with a vaccine booster.

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PROSPECTIVE EVALUATION OF HUMORAL AND T-CELL RESPONSE ELICITED BY THIRD mRNA VACCINATION IN KIDNEY TRASPLANT RECIPIENTS

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Aims of work: In this study we prospectively investigated the role of third mRNA vaccine against SARS-CoV2 in a cohort of kidney transplant recipients (KTRs), analysing both humoral and cell-mediated response.

Methods: mRNA-vaccinated KTRs were prospectively enrolled at time of first vaccination dose and immune response elicited by vaccination was analysed according to the following time-points: baseline (before vaccination; T0); three weeks after second dose (T1); six months after second dose and before the administration of the third dose (T2), 21 days after third dose (T3) 3 months after the third dose (T4) and after 6 months (T5). Chemiluminescent assay (Liason SARS-CoV-2 trimeric, Diasorin) was used for Spike IgG quantification. Results higher than 33,8 BAU/ml were given as positive. SARS-CoV-2 neutralizing antibody (NT Abs) titer was measured and results were given as the maximum dilution with the reduction of 90% of CPE (Cytopathic Effect). Results higher or equal to 1:10 serum titer were considered positive. Peripheral blood mononuclear cells (PBMCs) were isolated from heparin-treated blood by standard density gradient centrifugation. Then, PBMC were stimulated in duplicate with peptide pools representative of the whole spike protein (S). Response ≥ 10 INF- γ producing cells/ 10^6 PBMC were considered positive.

Results and conclusions: The rate of patients with detectable Spike-specific T-cell response increased over time reaching at T3 75% of responsiveness after the third dose of vaccine. Otherwise, the rate of so-called "humoral responders" was 50% at same time point. Thus, the evaluation of T-cell mediated response elicited by vaccination represent a valuable tool for the characterization of the overall immune response in KTRs. Looking at immunosuppressive regimen, the administration of mycophenolate was associated to increased rate of humoral non responders (84.2% vs 41%; $p=0.009$). Prospective enrolment at 6 months after third dose is still ongoing, in order to evaluate the long-term persistence of humoral and cell-mediated response.

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NOVEL ANTIVIRAL ACTIVITY OF PADS INHIBITORS AGAINST HUMAN BETA-CORONAVIRUSES SARS-COV-2 AND HCoV-OC43

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Emerging zoonotic RNA viruses have repeatedly attracted the attention of researchers over the past few decades. Novel coronaviruses (CoVs) in particular need special attention, due to the high mortality rates, the lack of effective therapies, the potential to spillover from a large reservoir of animal hosts, and the high rate of transmissibility that allows them to cause epidemics. To date, seven human CoVs (HCoVs) have been identified: among them, HCoV-OC43 and SARS-CoV-2, the causative agent of the ongoing epidemic of atypical pneumonia (COVID-19), belong to beta genus. A very recent study described the putative roles of a family of cellular enzymes called peptidylarginine deiminases (PADs) in COVID-19 disease. PADs are a family of calcium dependent enzymes that catalyze the post-translational modification citrullination, a process in which the guanidinium group of a peptidyl-arginine is hydrolyzed to form peptidyl-citrulline, a non-genetically coded amino acid. PADs dysregulation leads to an aberrant citrullination which is a characteristic biomarker of several inflammatory conditions. Moreover, a correlation has recently emerged between PADs dysregulation and other viral infections, including human rhinovirus and cytomegalovirus. Based on these evidences, the aim of this work was to evaluate whether PAD inhibitors were a reliable new class of host-targeted antivirals against coronaviruses. By using the HCoV-OC43 and SARS-CoV-2 strains as models of infection in human lung fibroblasts (MRC-5) and monkey kidney cells (Vero-E6), we tested the antiviral activity of well characterized PAD inhibitors. We used real time quantitative PCR to quantify copies of the viral genomes, Western blot analysis to evaluate the expression of viral proteins, and plaque assay to evaluate the production of new virions. Furthermore, we assessed the pattern of citrullination upon infection by using a citrulline-specific rhodamine phenylglyoxal (RhPG)-based probe. HCoV-OC43 and SARS-CoV-2 infections were significantly associated to PAD-mediated citrullination in vitro and to an increase of PAD expression, both at mRNA and protein levels. Moreover, the pharmacological inhibition of PAD enzymes led to a significant reduction of viral replication, suggesting that PAD4 isoform in particular might play a major role in OC43 replication. Our results suggested that 1) citrullination is a process that can be induced by RNA viruses, such as HCoV-OC43 and SARS-CoV-2, as a mechanism to foster their replication, and 2) that increase of PADs activity is central for beta-coronavirus replication. Taken together, we provide evidence that PADs inhibitors deserve consideration against human beta-coronaviruses infection.

HUMORAL AND CELL-MEDIATED RESPONSE ELICITED BY TRIPLE DOSE VACCINATION IN HEALTHCARE WORKERS: A PROSPECTIVE LONGITUDINAL STUDY

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Aim: Severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) still represents a crucial health problem despite the introduction and the massive use of vaccines. So far, vaccination is the only effective chance to control the coronavirus disease 2019 (COVID-19). We reported the long-term kinetics of humoral and cell-mediated response after vaccination and evaluated the immunogenicity after third dose of mRNA vaccine in healthcare workers.

Methods: 86 whole blood samples from Healthcare Worker were collected starting before first dose of vaccine. The follow up times were defined as: i) T0 (baseline, the day of first dose administration), T1 (day of second dose administration), T2 (21 days after second dose), T3 (six months after complete vaccination schedule) and T4 (21 days after third dose). Chemiluminescent assay was used for Spike IgG quantification (positive ≥ 33.8 BAU/ml). NT Abs titre was determined with neutralization test (positive $\geq 1:10$). Peripheral blood mononuclear cells (PBMC) were used for quantification of IFN- γ response ELISpot assay (positive ≥ 10 netspots/million) and for enumeration of memory B cell secreting IgG antibodies specific for the SARS-CoV-2 Receptor Binding Domain (RBD) and B cell secreting total IgG (positive ratio ≥ 2.85 specific RBD IgG / total IgG).

Results and conclusions: At twenty-one days after second dose, in all naïve subjects, positive values of IgG were detected and about 54.4% showed levels upper limits of quantifiable range of assay. Even if a decrease of humoral response was observed at six months, all the subjects maintained positive values. Finally, after third dose we observed an increase in median response, and the percentage of subjects that showed levels upper limits was 93%. As regards NT Abs titre, we showed that at 21 days after second dose positive results were detected in all naïve subjects and the 81.8% showed values upper than 1:320. Six months after second dose only 7.7% of subjects showed values upper than 1:320. After third dose in 92.2% values upper than 1:320 were detected. In SARS-CoV-2 naïve subjects, the median at T2 was eighth-fold upper than T3. Moreover, the median at T4 was sixteen-fold upper than T3. Looking at T cell response, about 23% of unexposed subjects showed a positive response at baseline, suggesting a cross reactive response with other common coronaviruses.

At T2 all but one subjects were positive, but at six months after second dose the percentage of negative subjects decreased to 9.4%. At T4, all subjects were positive. Next, RBD-specific B cell were investigated also after second dose. We showed an increase at T2, as well as, at T3. Furthermore, the third dose increased the median of RBD-specific B cell of 2.7-fold between after second and third dose.

In conclusion in our study we showed that triple dose of vaccine induced a humoral and T cell mediated response against SARS-CoV-2 higher than that observed after second dose. Analyses of long-term of persistence immune response after vaccination are needed.

POTENT INFLUENZA VIRUS PA-PB1 INTERACTION INHIBITORS EXHIBIT BROAD-SPECTRUM ACTIVITY AND SYNERGISM WITH ANTI-INFLUENZA DRUGS

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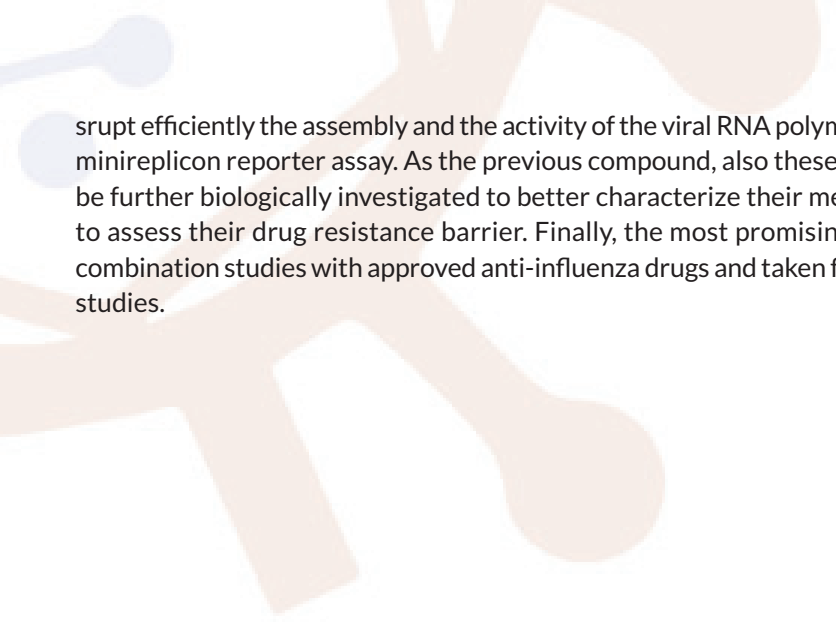
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Study aim: Influenza viruses still pose a large threat to public health by causing yearly seasonal epidemics and occasionally worldwide pandemics. The existing therapeutic and prophylactic approaches present poor efficacy, mainly due to the high viral genomic variability. Therefore, there is an urgent need for new anti-influenza drugs endowed with novel mechanisms of action. A new intriguing and promising target is the interaction between the three subunits of the viral RNA polymerase since they are very conserved among influenza A and B viruses. In the last years, our research group has been focusing on targeting the interaction between the polymerase PA and PB1 subunits. Previously, an *in silico* screening of a library of small molecules and subsequent hit-to-lead optimization studies led to the identification of a chemical structure capable to accommodate very efficiently in the PA cavity, the cycloheptathiophene scaffold. Starting from this chemical scaffold, many derivatives have been then designed, synthesized and *in vitro* characterized to evaluate their therapeutic potential and to establish Structure-Activity Relationships (SARs). These data allowed us to develop more potent PA-PB1 inhibitors.

Methods: The abilities of the compounds to inhibit the PA-PB1 interaction and the viral polymerase assembly were assessed by an ELISA-based interaction assay and by a cell-based minireplicon reporter assay, respectively. The anti-Flu activity was evaluated by plaque reduction assays (PRAs). MTT assays were performed to test the toxicity of the compounds. The combination studies results were assessed by employing Chou-Talay method (Chou T.C. 2006. Pharmacol. Rev. 58: 621-681) according to mass-action law base dynamic theory computed in the CalcuSyn software.

Results and conclusions: From the *in vitro* characterization of a series of derivatives obtained from the cycloheptathiophene scaffold, some promising hits emerged which exhibited both anti-Flu activity in PRA and in cell-based minireplicon reporter assay and inhibitory activity in the ELISA PA-PB1 interaction assay in the low micromolar/nanomolar range. In particular, one compound showed broad-spectrum anti-influenza activity and a high drug resistance barrier. Given the encouraging results obtained for this compound, it was further characterized by performing combination experiments with three approved drugs against influenza, i.e. Oseltamivir, Favipiravir, and Baloxavir. In these studies, this compound exhibited a synergistic effect with all the three drugs. Furthermore, this small molecule demonstrated virucidal activity *in vivo* in chicken embryonated egg model against the avian subtypes H5N1 and H5N8. Furthermore, other three cycloheptathiophene scaffold derivatives resulted very promising hits since they exhibited antiviral activity in PRA against several influenza A and B strains in the nanomolar range, and showed ability to di-



srupt efficiently the assembly and the activity of the viral RNA polymerase in the cell-based minireplicon reporter assay. As the previous compound, also these three hits are going to be further biologically investigated to better characterize their mechanism of action and to assess their drug resistance barrier. Finally, the most promising hits will be tested in combination studies with approved anti-influenza drugs and taken forward into preclinical studies.

T-CELL ASSAY AFTER COVID-19 VACCINATION COULD BE A USEFUL TOOL? A PILOT STUDY ON INTERFERON-GAMMA RELEASE ASSAY IN HEALTHCARE WORKERS

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Aim of the study: According to recent research, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) specific memory T cells are believed to be crucial for long-term immune protection against COVID-19 (Sette & Crotty, 2021, Murugesan et al., 2021). Our aim was to demonstrate that most vaccinated individuals have a robust T cell reactivity, evaluated through Interferon-gamma Release Assay (IGRA) test (Martínez-Gallo et al. 2021), which could be a useful diagnostic tool for vaccination schedule monitoring.

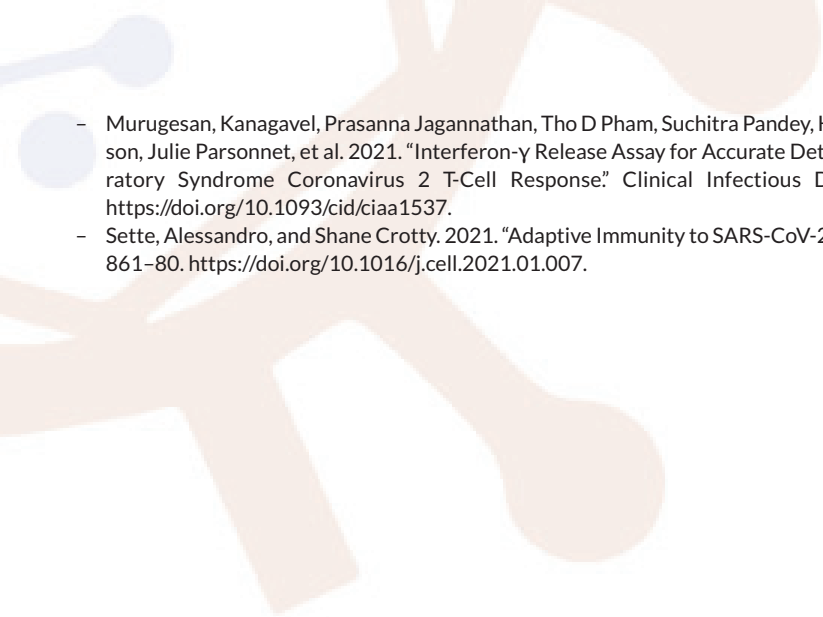
Methods: In a prospective cohort of 98 vaccinated healthcare workers for SARS-CoV-2 were selected 23 people in low-antibodies (Group 1, N=8), high-antibodies (Group 2, N=9), and negative control groups (Group 3, N=6). SARS-CoV-2-specific humoral and cellular responses were analyzed at 8 months after two doses of Pfizer BioNTech, evaluating anti-RBD (Receptor Binding Domain) and ACE-2 (Angiotensin Converting Enzyme-2) antibodies in sera through a Chemiluminescence Immunoassay (CLIA) and T cells through IGRA test in heparinized plasma. Moreover, lymphocyte subtyping was executed by a flow cytometer. Statistical analysis was performed.

Results: Antibody data confirmed that the median concentration value of RBD and ACE-2 competitive antibody levels of Group 1 (28.90 BAU/mL, and 56.55 IU/ml, respectively) were significantly lower than Group 2 (645.29 BAU/mL, and 836.82 IU/ml, respectively) with a $p < 0.001$. However, T cells, measured by IGRA test showed no significant difference (p : no significance, n.s.) between Group 1 and Group 2 (median values: 633.43 mLU/mL, and 1791.65 mLU/mL, respectively). Group 3 is the negative control group, health workers not previously infected and without vaccination, who showed RBD median levels of 0.562 BAU/mL, ACE-2 competitive 2.16 IU/mL, and IGRA test 0.5 mLU/mL, significantly different vs Group 1 and Group 2 ($p < 0.01$). As so, it was not observed significances in all lymphocyte subtyping.

Conclusion: The vaccination built robust circulating and memory T cell response, notwithstanding antibody levels. This work increases the studies on SARS-CoV-2 immunity and suggests the need for new strategies on booster doses administration.

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Aim of the study: This work has been carried out under Tuscavir, an interdisciplinary consortium aimed at performing research and providing qualified services for the development of novel broad spectrum antiviral therapies. Tuscavir is founded by Tuscany Region and includes the University of Siena (UNISI), Azienda Ospedaliera Universitaria Senese (AUOP), Azienda Ospedaliera Universitaria Pisana (AOUP), and the University of Florence (UNIFI) as team members. As part of the project, UNISI has developed different antiviral compounds capable of inhibit SARS-CoV-2 infection *in vitro*. These compounds named TUS (1-14) are small molecules that act against different targets of the viral mechanism of replication. Some of these TUS (1, 2, 3, 4, 5, 6, and 7) are entry inhibitors, and others (TUS 11-14) are Kinase inhibitors. Here we investigate the antiviral activity of single or combined compound against SARS-CoV-2.

Methods: Measurement of the cytotoxicity of the molecules was performed by WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt], a cell-based assay used for screening a library of compounds to determine if the investigational molecules have effects on cell proliferation or show direct cytotoxic effects, eventually leading cell to death. For every compound, the IC 50 was calculated. The antiviral activity against SARS-CoV-2 of selected molecules was evaluated by limiting dilution assay. The viral titer was calculated by applying the Reed and Muench formula. In this case, the ability of the molecules to inhibit the entry and/or replication of viral particles was revealed by measuring the cytopathic effect (CPE) on the cellular platform. Moreover, differences in the viral genome production were determined by Real time PCR measurements. VERO E6 cells were adopted for both antiviral activity and cytotoxicity assays.

Results and conclusion: The molecules investigated are generally well-tolerated by VERO E6 cells up to a concentration of 50 μ M. This concentration was the maximum used to assess antiviral activity. Inhibition of the levels of infection of SARS-CoV-2 on VERO E6 in the presence of drugs was evaluated by using different protocols of administration, according to the supposed mechanism of actions. The reduction of CPE and of the viral genome in the supernatants of infected cells demonstrated the ability of some of the compounds to reduce infection. TUS 3 and 7 (entry inhibitors) have proven effective in preventing SARS-CoV-2 infection *in vitro*.

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Aim of the study: The worldwide outbreak of coronavirus disease 2019 (COVID-19) highlighted the urgent need to discover innovative broad-spectrum antiviral therapies that could be implemented for the future emergence of viral infections. Current antivirals are therapeutic molecules designed to act on a specific viral target and interfere with a precise stage of the replication cycle. Antimicrobial peptides (AMPs), also known as host defense peptides (HDPs), represent an emerging class of therapeutic agents in several fields; they are used as antibacterial, antiviral, antifungal, antiparasitic, antioxidant and anticancer agents [1,2]. One of the natural sources of AMPs is represented by amphibian skin secretions. Their antibacterial and antifungal activities have been widely reported, but their exploitation as potential antiviral agents have not been thoroughly investigated. In this study, the antiviral activity of peptides Hylin-a1, RV-23, HS-1 and its analogs, derived from the secretion of the *Rana* genus, has been evaluated against different human viruses.

Methods used: Peptides have been synthesized using the solid-phase Fmoc chemistry method, followed by purification by reversed-phase HPLC. In addition, HS-1 analogs have been synthesized performing ala-scanning mutagenesis, in which each residue was systematically replaced by alanine. Cytotoxic activity was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on VERO cells. The antiviral activity was evaluated against different members of the *Herpesviridae* (Herpes simplex virus type 1, HSV-1), *Paramyxoviridae* (Measles Virus, MeV; Human parainfluenza virus type 2, HPIV-2), *Coronaviridae* (Severe acute respiratory syndrome coronavirus 2, SARS-CoV-2; Human coronavirus 229E, HCoV-229E) and *Picornaviridae* (Poliovirus, PV-1) families, using plaque assays, molecular test, and Transmission electron microscopy (TEM) analysis.

Results and conclusions: Preincubation of peptides with viruses has determined a significant antiviral activity, demonstrating that they could disrupt the viral envelope, as confirmed by TEM. Screened peptides act on the extracellular phases of the viral lifecycle, probably by blocking the viral attachment and entry phases. Furthermore, HS-1 analogs exhibited reduced toxicity and improved antiviral properties compared to the native peptide. Our results show possible novel applications of amphibian skin peptides in the field of antivirals. Further studies will focus on their specific mechanism of action to clarify the viral target on which the peptides act.

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Severe acute respiratory syndrome coronavirus 2 (Sars-CoV-2) is the causative agent of coronavirus disease 2019 (COVID-19), a severe and even fatal disease, in particular for the elderly and frail population, that affect the global community.

mRNA vaccine BNT162b2 (Comirnaty), by BioNTech/Pfizer, is one of the vaccines developed by the scientific community in order to face the ongoing COVID-19 pandemic and its administration has started in Italy at December 27th, 2021. The aim of this project is to monitor the salivary levels of IgA specific for Sars-CoV-2 in healthcare workers (HW) with (HW+) or without (HW-) history of documented Sars-CoV-2 infection during BioNTech/Pfizer vaccination. For the study, saliva samples of 47 HW- and 12 HW+, collected before the second dose (baseline), 1, 3, 6 and 9 months after the second dose of vaccination, and 10 days after the booster dose, were utilized to evaluate IgA levels (AU/mL). IgA were detected in the majority of HW already at the baseline (87% in HW- and 92% in HW+), as well after one month from the second dose (97% vs 100%); the totality of HW- and HW+ showed salivary IgA after 3 and 6 months. To note, after 9 months from the second dose, our results showed that IgA remained detectable in all HW+, whereas they became undetectable in 43% of HW- ($p=0.01$). Importantly, ten days after the booster dose, IgA became again visible in the majority of subjects of both groups without any statistical difference (97% in HW- and 100% in HW+).

For quantitative data, our results showed that the peak of salivary IgA levels were reached 1 month after the second dose in HW- as well in HW+; after this time-point their levels constantly decreased during time following a similar trend in both groups. To note, IgA levels were constantly and significantly higher in HW+ compared to HW- in any time-point ($p<0.05$).

Ten days after the booster dose, we noticed that IgA amounts were augmented in HW- as well in HW+: in HW- this increase was statistically significant ($p=0.001$), whereas in HW+ this increase did not reach the statistical difference. Nevertheless, in this time-point IgA levels remain statistically higher in HW+ compared to HW- ($p<0.05$).

In conclusion, our results confirm that the administration of two doses of BNT162b2 vaccination promotes the production of salivary IgA; IgA are constantly higher in HW+ compared to HW-; their levels decreased in time up to 9 months, but, importantly, the vaccine booster dose is able to increase their titers.

CHARACTERIZATION OF THE ANTI-RHINOVIRUS ACTIVITY OF 25-HYDROXYCHOLESTEROL AND 27-HYDROXYCHOLESTEROL, AND VALIDATION ON NASAL AND BRONCHIAL HISTOCULTURES

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Aim of the study: Human rhinovirus (HRV) is a quasispecies, a highly antigenically diverse virus population endowed with a high rate of mutations.

The aim of this study is to provide an empirical proof of principle of the actual greater genetic barrier of 25-hydroxycholesterol (25OHC) and 27-hydroxycholesterol (27OHC), two physiologic oxysterols and host-targeting antivirals, using HRV as a quasispecies model. Moreover, we selected 27OHC for further studies aiming at exploring further its putative potential of preclinical development.

Methods: The ability of 25OHC or 27OHC to generate resistant strains of HRV was explored by exploiting clonal or serial passages approaches, and compared with the one of pleconaril and rupintrivir. Moreover, both the efficacy and biocompatibility of 27OHC were further validated in a challenging and predictive model, i.e. 3D in vitro fully reconstituted human nasal and bronchial epithelia from cystic fibrosis patients.

Results and conclusions: In this study we demonstrate with two different approaches that 25OHC and 27OHC do not select HRV oxysterol-resistant variants. Moreover, we demonstrate the ability of 27OHC to inhibit HRV yield in both nasal and bronchial epithelia, preventing virus-induced cilia damage.

The complex of these characteristics suggests that 27OHC antiviral potential should be considered further, and provide a rationale for further studies aiming at exploring its potential of preclinical development.

SARS-CoV-2 ANTIBODY RESPONSE IN SOLID ORGAN TRANSPLANT PATIENTS AFTER mRNA BNT162b2: DATA FROM AN OBSERVATIONAL PROSPECTIVE STUDY

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Background: The impact of COVID-19 in Solid Organ Transplant (SOT) patients is still an open issue. A significant increased risk of complications, Intensive Care Unit (ICU) admission and mortality has been reported, however the real burden on the mortality rate is debated. Currently, SARS-CoV-2 vaccination has become the standard of care to prevent COVID-19 hospitalization and mortality. In the SOT recipients, vaccine is strongly recommended as high-risk patients due to their immunocompromised status and underlying diseases. Unfortunately, the data described a lower seroprevalence after the second vaccine and booster dose compare to general population. The aim of the study was to assess over-time the antibody response in the Kidney and Lung Transplant Recipients (KTR and LTR, respectively) after the mRNA booster dose and the factors associated with humoral response.

Material and methods: We conducted an observational, monocentric, prospective study including KTR and LTR that received the third mRNA BNT162b2 vaccine dose. We investigated clinical status and vaccine-induced humoral response of the study population before booster dose (T0), after 45 days (T1) and after 180 days (T2). At T0, T1 and T2 we performed a clinical questionnaire and collected blood samples to assess serum anti-Spike SARS-CoV-2 antibodies. LIAISON SARS-CoV-2 S1/S2 IgG chemiluminescent assay against a recombinant Spike (S) protein (S1/S2) was used. Results below 33.8 BAU/mL were considered negative. Patients with or without seroconversion were defined responder or no responder, respectively. Preliminary univariate and multivariable analyses were performed in order to explore the factors associated with seroconversion after the booster dose.

Results: A total of 38 patients were included in the study, 29 (76%) KTR and 9 (24%) LTR, 14 (37%) females and 24 (63%) males. Overall, median age was 56 years (IQR 49-61), months from the transplant were 76 (55-169), days from 2nd to booster dose were 179 (172-204). No serious adverse effect was reported. Concerning maintenance immunosuppression regimen, 25 (66%) included an antimetabolite and 31 (82%) steroids. Generally, 20 (52%) were responder at T0, 28 (74%) at T1, 8 had a confirmed SARS-CoV-2 infection after T1. The T2 assessment is still on going. At T1 evaluation, no responder patients were more likely to be LTR ($p: 0.02$), no responder at T0 ($p: 0.02$), aged > 60 years ($p: 0.01$) and to receive the transplant within 48 months from T0 ($p: 0.03$). Whereas, no differences were observed regarding sex, comorbidities and immunosuppression regimen. At the multivariable analysis only time (months) from transplant ($p: 0.041$) and be responder at T0

(*p*:0.036) were protective factors independently associated with the responder patients at T1 (Table1).

Conclusions: SOT recipient’s seroconversion after booster dose is associated with time from transplant and be already responder. These patients still remain a vulnerable population and strict adherence to non-pharmacological interventions are mandatory. Further studies are needed in order to assess vaccine-induced humoral response, considering, above all, the ongoing fourth vaccine dose campaign.

Table 1. Multivariable analysis with factors independently associated with the responder patients at T1

	OR	95%CI	P value
Responder at T0	0.073	0.006-0.838	0.036
Time from transplant (months)	0.977	0.955-0.999	0.041
Constant	3.584	-	0.191

PO 90

DEEP-LOOK INTO DOLUTREGRAVIR THERAPY FAILURE MECHANISM: COULD MUTATIONS IN THE 3'-PPT SUPPORT HIV-1 REPLICATION WITHOUT INTEGRATION?

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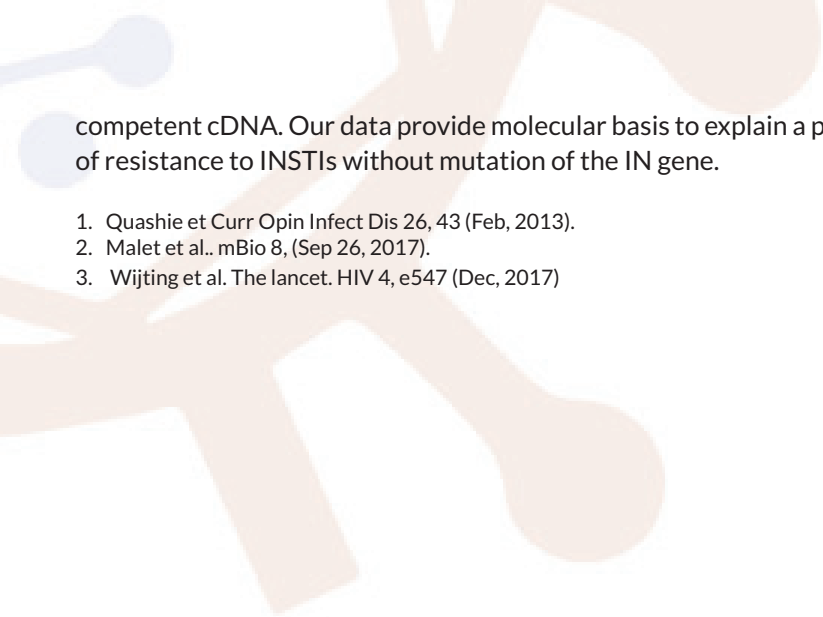
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Integration of the reverse-transcribed genome is a critical step of the retroviral life cycle. HIV-1 integrase strand transfer inhibitors (INSTIs) are in first-line treatment and rescue therapy. Treatment with INSTIs such as dolutegravir (DTG) can lead to the selection of mutations in the IN gene¹. Recently an HIV-1 INSTIs highly resistant strain bearing mutations not on the target IN gene² but only in the 3'-PPT (poly-purine tract) was in vitro selected. Such mutations were retrieved also in a patient failing to respond to DTG³, representing a new resistance pathway for INSTIs. We, therefore, investigated the mechanism of this INSTIs resistance involving the 3'-PPT region. The PPT is highly conserved in most retroviruses and is used as the site of (+) strand initiation during viral reverse transcription.

Molecular and cellular virology methodologies were applied to determine whether the mutated 3'PPT (3'-mPPT) could be still functional as primer for (+) strand synthesis, assessing its i) recognition by HIV-1 Reverse Transcriptase (RT), ii) elongation potential and iii) strand-displacement synthesis. We also investigated if 3'-mPPT virus could still integrate its genome into the host-cell genome.

Results showed that 3'-mPPT is recognized by RT but undergoes additional internal cuts with respect wt PPT and it is not elongated. Differently strand displacement synthesis is not significantly affected. On cell-based assays we found that 3'-mPPT virus could replicate, generating a virus progeny, although with lower efficiency. A strong accumulation of unintegrated 1-circular LTR viral DNA was observed while no accumulation of 2-circular LTR, clear sign of direct IN inhibition, was observed. It was not possible to detect the presence of integrated viral DNA or the presence of viral insertion in the host cell genome by the sequencing of integration sites.

Overall, data suggest that 3'-mPPT cannot serve as a primer for the start of (+) strand synthesis at that spot, preventing the correct accomplishment of canonical reverse transcription. Most probably, the (+) strand synthesis could start to form the central PPT, and the length of the so formed DNA fragment impedes the occurrence of the (+) strand transfer needed for the formation of the complete LTRs at both ends required for an integration-



competent cDNA. Our data provide molecular basis to explain a possible new mechanism of resistance to INSTIs without mutation of the IN gene.

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ANTIMICROBIAL PEPTIDES ACTIVITY ON MURINE NOROVIRUS AND HEPATITIS A VIRUS INFECTIVITY

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Antimicrobial peptides (AMPs) are important components of the innate immune system with antibacterial, antifungal and antiviral activities. Therefore, AMPs represent an exciting option taken progressively into consideration as new antimicrobial substances to replace the conventional drugs, food additives and sanitizers.

In this study different AMPs have been tested on Hepatitis A Virus (HAV) and murine norovirus (MNV-1), a surrogate for human norovirus to evaluate their antiviral potential. In particular, peptides HCAT, MTP1, RiLK1, RiLK3, RiLK30, AVP1, AVP2 and AVP3 were tested.

Solutions 40 μ M and 80 μ M for each peptide were prepared to treat HAV and MNV-1 at a final concentration of 4.6×10^4 TCID₅₀/ml and 3.2×10^4 TCID₅₀/ml respectively. Incubation of virus with the peptide was carried out for 1 hour at two temperature conditions (room temperature and 4°C). Then the residual viral infectivity was evaluated on Frp3 and RAW 264.7, permissive cell lines for HAV and MNV-1 respectively. Untreated HAV and MNV-1 suspensions and 40 μ M and 80 μ M AMPs solutions, incubated at the same conditions, were used as positive and negative controls.

The results showed a reduction of the infectivity of MNV-1 between 0.9 and 1.5 log. The higher reduction was obtained by the treatment with RiLK1 at room temperature. Instead, HCAT, MTP1, RiLK3, AVP1 and AVP3 didn't exert any significant effect on viral infection (<0.5 log reduction). The infectivity of HAV showed a reduction between 0.7 and 2.0 log. Also in this case the most significant reduction of infectivity was obtained by the treatment with 80 μ M RiLK1 at room temperature. The others peptides and temperature conditions of treatment displayed a lower reduction. No effect was obtained with RiLK3 and AVP3. This study highlights that AMPs could provide a partial inhibition of MNV-1 and HAV infectivity. Further studies, to evaluate the effect of the AMPs, when they are used in synergy, could be useful for their potential future use to control contamination by foodborne viruses.

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Aim of the study: Despite the continuous efforts into studying HIV-host relationship and the strong evolution of the vaccinology, up-today there is not an effective anti-HIV vaccine. One of studied vaccinal strategies is based on the identification of epitopes recognized by broadly neutralizing antibodies to include in a vaccinal preparations¹. Since Nisonoff et al. first proposed the use of anti-idiotypic antibodies as immunogen, anti-idiotypic vaccine candidates have been developed². We previously reported the isolation of a murine anti-idiotypic Fab fragment antibody, P1, able to react specifically with b12, a broadly anti-HIV neutralizing antibody³, and to induce a neutralizing immune response in rabbits. Based on these data, we investigated the biological features of different antibody formats, including single chain (scFv) and minibody (Mb) as immunogens to improve the immunogenicity of P1.

Methods: P1 scFv was designed by linking the variable region of the light chain (V_L) and of the heavy chain (V_H) of P1 through an 18 amino acid, while Mb was generated by adding the hinge and the CH3 genes to scFv encoding genes. We designed two different orientations for both antibody formats: V_H - V_L (5'-3') and V_L - V_H (5'-3'). The different molecules were produced, tested for their binding to b12, and used as immunogen for rabbits' immunization. The collected rabbits' sera were analyzed for the binding to gp120/HIV. Statistical analysis was conducted to highlight significant differences among the obtained data.

Results and conclusions: scFv and Mb displayed different binding results to b12, but we also reported differences between the two orientations (V_H - V_L and V_L - V_H) within the same antibody format. As expected, due to the homodimerization, the best signal was obtained using Mb. For both formats, the higher signals were obtained using the V_H - V_L orientation, indicating that the structure in this orientation is better recognized by b12, and this result was also confirmed by analyzing the sera obtained from the immunized rabbits. Our results showed that Mb V_H - V_L P1 immunization elicits a good humoral response against gp120/HIV, and compared to the classic anti-idiotypic, the Mb presents further advantages: it is bivalent as the whole antibody, but it is smaller and monocistronic, both features are helpful for the development of an mRNA-based vaccine.

In conclusion, we demonstrated for the first time that the minibody format can be used as an immunogen to induce an immune response against a virus, and it can be considered a starting point for the constitution of a polyclonal anti-idiotypic minibody vaccine.

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POTENT ANTIVIRAL ACTIVITY OF NEW GENERATION HIV-1 MATURATION INHIBITORS ON HUMAN PRIMARY CELLS

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Aim of the study: Drug resistance emergence can seriously affect the effectiveness of anti-HIV therapy, highlighting the need of new drug classes. Among them, maturation inhibitors (MIs), targeting gag-pol cleavage sites, represent a new promising approach. Here, we evaluated the antiviral activity of new generation MIs against HIV-1 wild type (wt) and mutated viral strains in human primary cells.

Methods: Monocyte-derived macrophages (MDM) and PBMC-derived lymphocytes were infected with wt CCR5-tropic 81A and CXCR4-tropic NL4-3 laboratory viral strains, respectively. Lymphocytes were also infected with four gag-mutated NL4-3 strains (A364V, V370A, V370Δ and V362I+V370A) known to confer resistance to the first generation MI Bevirimat (BVM).

Four compounds (provided by ViiV Healthcare), defined as MI-1, MI-2, MI-3 and MI-4 were tested at different concentrations (1000 nM, 100 nM, 10 nM, 1 nM). BVM was used as a control. Antiviral activity was assessed by quantifying p24 protein in supernatants by ELISA at 7 (for lymphocytes) or 14 (for MDM) days after infection. EC₅₀ was assessed for wt (EC_{50-wt}) and mutated viruses (EC_{50-mut}). Fold change (FC) resistance values were defined as ratio of EC_{50-mut}/EC_{50-wt}.

Transmission electron microscopy (TEM) was used to visualize mature/immature viral particles in presence/absence of tested MIs.

Results and conclusions: In lymphocytes, all tested MIs showed a good antiviral activity against wt, with MI-1 and MI-2 having an EC₅₀ significantly lower ($p < 0.002$) than BVM (EC₅₀ 20.38±0.59 nM for BVM vs 2.03±0.55 nM for MI-1, 3.31±0.78 nM for MI-2, 37.57±26.78 nM for MI-3, 36.51±31.40 nM for MI-4). As expected, BVM showed no activity against NL4-3 gag-mutants with high level resistance observed for A364V and V362I+V370A (FC resistance: >500 for both).

Conversely, the antiviral activity of MIs was not significantly affected by V370Δ (FC resistance: <2). Similarly, the antiviral activity of MI-3 and MI-4 was not reduced at all by A364V, V370A and V362I+V370A (FC resistance: ~1). A low level resistance was observed for MI-1 and MI-2 showing 12.2, 3.3 and 3.4 and 4.4, 8.3 and 1.8 FC resistance for A364V, V370A, V362I+V370A, respectively.

Furthermore, all MIs showed a good antiviral activity also in 81A-infected MDM with EC₅₀ lower than BVM (23.89±14.26 nM for BVM vs 4.61±0.40 nM for MI-1, 9.26±9.41 nM for MI-2, 19.85±3.46 nM for MI-3, 3.57±1.96 nM for MI-4).

Finally, by TEM, treatment with different MIs determined an intracellular accumulation of immature viral particles more marked than that observed for untreated 81A-infected MDM, compatible with the inhibited processing of gag polyprotein (Fig. 1).

These results highlight a potent antiviral activity of new MIs against both wt and tested

gag-mutated viruses, higher than BVM. The capability of these compounds to suppress HIV replication in both lymphocytes and MDM supports the role of MIs as promising new class of antiretroviral drugs.

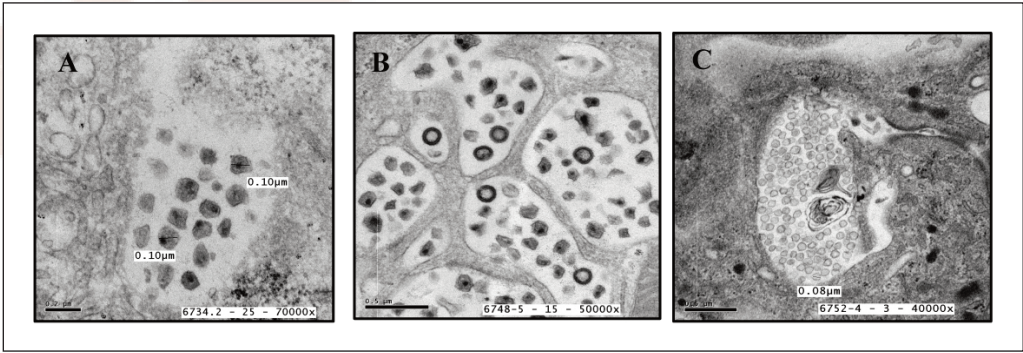


Figure 1. TEM images of human primary macrophages infected with HIV-1 (81A strain) after 14 days post-infection. (A) untreated, (B) treated with MI-1 1 nM and (C) with MI-3 10 nM. Insight into intracellular vesicles containing mature viral particles (A) or immature viral particles (B,C).

STRUCTURAL AND BIOCHEMICAL ANALYSIS OF THE DUAL INHIBITION OF MG-132 AGAINST SARS-COV-2 MAIN PROTEASE (Mpro/3CLPro) AND HUMAN CATHEPSIN-L

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After almost two years from its first evidence, the COVID-19 pandemic continues to afflict people worldwide, highlighting the need for multiple antiviral strategies. SARS-CoV-2 main protease (Mpro/3CLpro) is a recognized promising target for the development of effective drugs. Because single target inhibition might not be sufficient to block SARS-CoV-2 infection and replication, multi enzymatic-based therapies may provide a better strategy.

Here we present a structural and biochemical characterization of the binding mode of MG-132 to both the main protease of SARS-CoV-2, and to the human Cathepsin-L, suggesting thus an interesting scaffold for the development of double inhibitors.

X-ray diffraction data show that MG-132 well fits into the Mpro active site, forming a covalent bond with Cys145 independently from reducing agents and crystallization conditions.

Docking of MG-132 into Cathepsin-L well-matches with a covalent binding to the catalytic cysteine.

Accordingly, MG-132 inhibits Cathepsin-L with nanomolar potency and reversibly inhibits Mpro with micromolar potency, but with a prolonged residency time. We compared the apo and MG-132- inhibited structures of Mpro solved in different space groups and we identified a new apo structure that features several similarities with the inhibited ones, offering interesting perspectives for future drug design and in silico efforts.

EFFICACY OF LICENSED MONOCLONAL ANTIBODIES AND ANTIVIRAL AGENTS AGAINST THE SARS-COV-2 OMICRON SUB-LINEAGES BA.1 AND BA.2

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Background: Newly emerging SARS-CoV-2 variants have the potential to escape monoclonal antibodies (mAbs) and antiviral drugs. We assessed the ex vivo inhibition of omicron and delta sub-lineages by sera obtained from patients treated with licensed mAb preparations including bamlanivimab/etesevimab (LYC), casirivimab/imdevimab (REG) and sotrovimab (SOT). In addition, we assessed the in vitro susceptibility of the same variants to remdesivir (RMD), nirmatrelvir (NRM) and EIDD-1931, the active form of molnupiravir (EIDD).

Materials and Methods: Of 30 patients treated with mAbs (14 males, 59±18 years) one was asymptomatic while the others had mild symptoms. Patients were treated with LYC (n=10), REG (n=10), or SOT (n=10), 3.5±1.7 days from diagnosis. To test mAb activity, paired sera were obtained before (baseline) and 1 hour post mAb infusion and used in a live virus neutralization assay in VERO E6 cells with automated cell viability readout. To assess antiviral drug activity, viral isolates were used at MOI 0.005 to infect VERO E6 cells treated with 0.5 µM of P-GP inhibitor (CP-100356) and with decreasing concentrations of RMD, EIDD and NRM. Challenge viruses included the B.1 wild type (WT), delta, delta sub-lineage AY.4.2, omicron BA.1 and BA.2. Neutralizing antibody titers (ID₅₀) and drug activity (IC₅₀) were defined as the reciprocal value of the serum dilution and as the drug concentration, respectively, showing 50% protection of virus-induced cytopathic effect.

Results: All pre-infusion sera were negative for SARS-CoV-2 NtAb activity. In post-infusion sera REG, LYC and SOT showed activity against the WT (19,814 [17,459-23,471]; 6,792 [4,736-8,328] and 456 [259-592] ID₅₀), the delta (58,858 [41,585-79,971]; 12,145 [10,840-18,667] and 1,023 [798-1,134] ID₅₀) and the AY.4.2 (58,602 [42,941-82,960]; 11,067 [10,757-12,614] and 1,333 [708-1,714] ID₅₀).

Notably, SOT was the only active treatment against the BA.1 (216 [118-233] ID₅₀) while the BA.2 was neutralized by REG (185 [120-211] ID₅₀) and SOT (9 [5-20] ID₅₀) but not by LYC (Figure 1). No significant intervariant IC₅₀ differences were observed for EIDD (1.5 ±0.1/0.9±0.1/0.7±0.3/0.8±0.3/0.8±0.1 µM for WT/delta/AY.4.2/BA.1/BA.2 respectively); NRM (0.04±0.02/0.06±0.01/0.07±0.03/0.02±0.01/0.04±0.01 µM) or RMD (0.1±0.04/0.1±0.1/0.1±0.03/0.1±0.1/0.1±0.01).

Conclusions: Although designed based on the ancestral virus, licensed mAbs retain activity against the main delta variant and its sublineage AY.4.2, which has been circulating in Italy. The BA.1 and BA.2 variants fully escape the LYC cocktail, while REG retains partial activity only against BA.2 with a fold-change decrease (FCD) of 131±45 with respect to WT. Inte-

restingly, SOT retains significant activity against BA.1 (2.8 ± 1 FCD) and BA.2 (23 ± 9 FCD on the 5 paired sera with measurable activity). Since omicron has rapidly replaced past variants, the mAbs arsenal should be updated accordingly. By contrast, currently approved antiviral drugs are not affected by SARS-CoV-2 variability at this time.

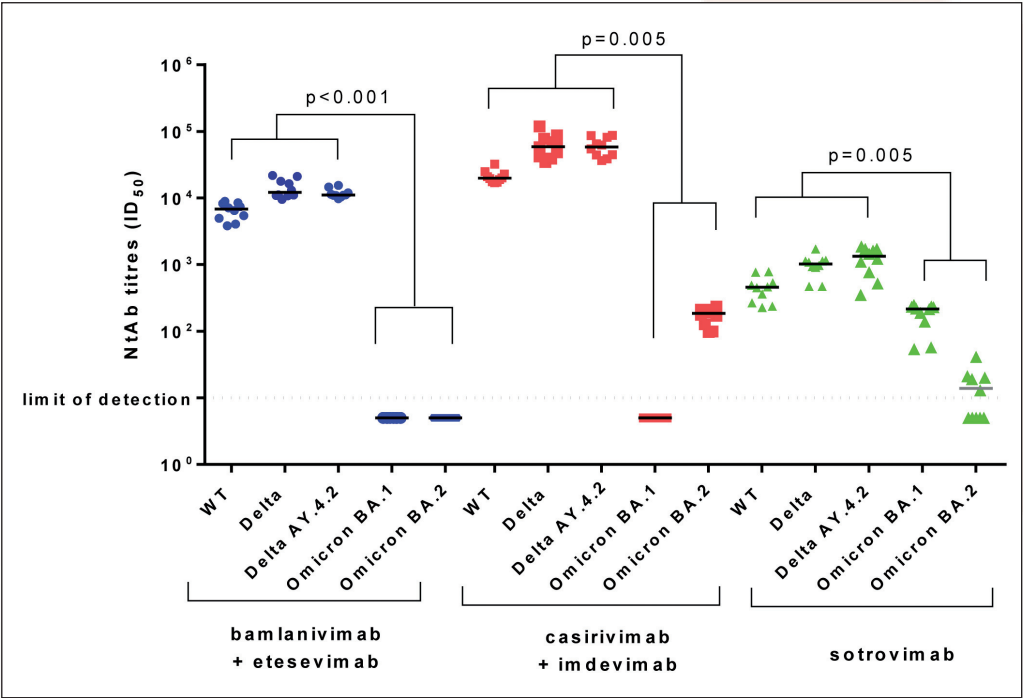


Figure 1: *Ex vivo* anti-SARS-CoV-2 wild type, delta, delta AY.4.2, BA.1 and BA.2 neutralizing antibody (NtAb) titers measured in sera from 30 patients following infusion of the bamlanivimab/etesevimab (in blue) or casirivimab/imdevimab (in red) or sotrovimab (in green) monoclonal antibody cocktails. Paired data were analyzed by the non-parametric Wilcoxon Signed Rank Sum test. NtAb: neutralizing antibody; ID₅₀: the reciprocal value of the sera dilution showing the 50% protection of virus-induced cytopathic effect.

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Introduction: Severe cases of COVID-19 are mainly associated with strong inflammation and lung injury. However, the best therapeutic responses against SARS-CoV-2 might be obtained by combining anti-inflammatory and antiviral properties (Rahman et al, 2021). Therefore, we explored the antiviral and anti-inflammatory properties of a class of compounds that inhibit N-Acylethanolamine acid amidase (NAAA). This enzyme catalyzes the hydrolysis of palmitoylethanolamide (PEA), which in turn activates the peroxisome proliferator receptor- α (PPAR- α) (Musella et al, 2017). Thus, the inhibition of NAAA enzyme induces the PEA accumulation in cells. PEA then interacts with the transcription factor PPAR- α and induces the transcription of genes involved in autophagy, lipid metabolism and inflammation.

Aim of the study: Considering the importance of monocytes in SARS-CoV-2 pathogenesis, our aim was the evaluation of the antiviral and anti-inflammatory effect of NAAA inhibitor on THP-1 monocyte and peripheral blood mononuclear cells (PBMCs) extracted from healthy donors.

Methods: We analyzed the effect of SARS-CoV-2 infection on THP-1 cells and on PBMCs by measuring expression of pro-inflammatory genes. SARS-CoV-2-infected cells were treated with NAAA inhibitor to assess the antiviral and anti-inflammatory activity of the compound during SARS-CoV-2 infection. We performed qRT-PCR on cell lysate to quantify SARS-CoV-2 genomes and the activation of pro-inflammatory pathways. We also confirmed the inhibition of SARS-CoV-2 replication through the count of SARS-CoV-2 Spike-positive cells using high content confocal microscopy.

Results and conclusions: We show a 70% reduction in SARS-CoV-2 genome release and a strong decrement in pro-inflammatory mediator transcription when cells were treated with the NAAA inhibitor ARN077. In particular, the inhibition of NAAA decreases SARS-CoV-2 TNF- α transcription by 3-fold, compared to mock-treated infected cells. We also observed a 2-fold down-regulation in the expression of CXCL10, a key gene related to cytokine storm, after NAAA inhibition. Additionally, IL-6, IL-10, TGF- β mRNA turned out to be down-regulated after drug administration, confirming its anti-inflammatory activity also during viral infections.

Therefore, the efficacy of NAAA inhibition as an antiviral strategy is based on the combination between its anti-inflammatory activity and antiviral effect. Since COVID-19 severe consequences are due to excessive inflammation, the use of a drug that blocks both inflammation and viral replication might be effective in preventing lung injury and severe cases of COVID-19. Additional studies might help to evaluate the role of NAAA inhibitors

in the transition between different macrophage phenotypes (M1 and M2). Future experiments will evaluate if NAAA inhibitors might also decrease the expression of CD163, a marker for pro-fibrotic macrophages, which are associated with the aberrant macrophagic activation, one of the major causes of COVID-19 lethality.

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Aim of the study: In 2020, a global pandemic was declared following the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the pathogen belongs to the *Coronaviridae* family responsible for COVID-19 [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 augment preventive measures and reduce viral spread [3]. In the present study, we analyzed the potential reduction of ocular infection and transmission caused by the spread of SARS-CoV-2, testing the antiviral activity 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 μ L) in the presence of the virus (10^3 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. Furthermore, the expression levels of early and late genes were evaluated through molecular assays (Real-time PCR) to quantified the antiviral potential of eye drops.

Results and conclusions: 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 the spike protein (S). The significant inhibitory effect of the SARS-CoV-2 infection recorded by the treatment of cells with eye drops, it is possible to conclude that these ophthalmic solutions could represent a preventive resource for viral ocular infections.

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PALMITOYLETHANOLAMIDE (PEA) INHIBITS SARS-COV-2 ENTRY BY INTERACTING WITH S PROTEIN AND ACE-2 RECEPTOR

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Introduction: The rearrangement of lipid membranes is a crucial step in the replication of several positive-strand RNA viruses such as Flaviviridae and Coronaviridae. Due to the close connection between lipids, inflammation and viral infection, lipid metabolism has become an area of intense research. The goal is to better understand how viruses seize lipids and design antiviral drugs targeting lipid pathways.

Aim of the study: Our work investigates the antiviral potency of Palmitoylethanolamide (PEA), an endogenous lipid mediator with analgesic and anti-inflammatory activity, mediated by the activation of peroxisome proliferator receptor alpha (PPAR- α). PEA has a consolidated efficacy in preventing or treating bacterial and viral infections.

For those characteristics, PEA has been considered a valid candidate to inhibit the pathogenesis of SARS-CoV-2 infections.

Materials and Methods: Infection of Huh-7 cells was performed with 0.1 MOI of clinical strains of SARS-CoV-2 VR PV10734, B.1.617.2, and B.1.1.529 obtained from the UO of Virology, AOUP, Pisa. Micronized PEA was used at various concentrations to pretreat cells prior to the infection or in a solution with the viral strains. Viral infections were assessed using qRT-PCR performed on cell-substrate extracts and immunocytochemistry performed on infected cells, using high content confocal microscopy.

Results and conclusions: A preliminary molecular docking *in silico* analysis shows a potential interaction between PEA and both ACE-2 and Spike protein. This binding might inhibit SARS-CoV-2 entry in the host cell.

We confirmed this prediction by *in vitro* assays that revealed how PEA causes a reduction of viral infection by nearly 70% when administered both to cells or incubated with SARS-CoV-2 prior to infection. This reduction is observed either in the WT strain of SARS-CoV-2 that in Delta and Omicron variants. Two different mechanisms could explain the activity of PEA. The first is the inhibition of viral entry, as suggested by the molecular docking studies and corroborated by the reduction by 40% of the transduction efficiency of a lentiviral vector pseudotyped with SARS-CoV-2 S protein when administered with PEA. Cell-based ELISA also confirmed the interaction between PEA and the RBD domain of Spike protein. The other proposed mechanism is ascribable to the activation of lipolysis mediated by PPAR- α . The activation of this pathway led to the disruption of fatty acid droplets required by SARS-CoV-2 for its replication. Indeed, the administration of PEA to Huh-7 and Calu-3 cells prior to infection led to a reduction of lipid droplets by 40%.

In conclusion, the present work demonstrates a novel mechanism of action for PEA as a direct and indirect antiviral agent against SARS-CoV-2. This evidence reinforces the idea that this compound might significantly impact the COVID-19 course.

Further preclinical and clinical tests will be needed to fully consider this lipid as a promising adjuvant therapy in the current COVID-19 pandemic or against emerging RNA viruses that share the same route of replication as Coronaviruses.

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Aim of the study: Although the BNT162b2 COVID-19 vaccine is known to induce IgG neutralizing antibodies in serum protecting against COVID-19, it has not been studied in detail whether it could generate specific immunity at mucosal sites, which represent the primary route of entry of SARS-CoV-2. In order to evaluate the mucosal immune response generated after vaccination we analyzed the titer of both IgG and IgA and their neutralization capacity against SARS-CoV-2 wild-type, Delta, Delta Plus and Omicron variants in sera and saliva of BNT162b2 vaccinated subjects after the first cycle of vaccination and after the booster.

Methods: Samples of serum and saliva of 60 BNT162b2-vaccinated healthcare workers were collected at baseline, two weeks after the first dose, two weeks after the second dose, six months after the second dose and 14 days after the booster. Anti-S1-protein IgG and IgA total antibodies titers and the presence of neutralizing antibodies against the Receptor Binding Domain (RBD) of wild-type and Delta variants in both serum and saliva were measured by quantitative and by competitive ELISA, respectively. The Neutralizing antibodies activity was also investigated against wild-type, Delta, Delta-Plus and Omicron RBD variants after the booster both in serum and saliva.

Results and Conclusion: Complete vaccination cycle generates a high serum IgG antibody titre as a single dose in previously infected seropositive individuals. Serum IgA concentration reaches a plateau after a single dose in seropositive individuals and two vaccine doses in seronegative subjects. After the second dose IgA level was higher in seronegative than in seropositive subjects. In saliva, IgG level is almost two orders of magnitude lower than in serum, reaching the highest values after the second dose. IgA concentration remains low and increases significantly only in seropositive individuals after the second dose. Neutralizing antibody titre were much higher in serum than in saliva. After six months of the second doses both IgG and IgA levels and their neutralizing activity decreased, but significantly increased after the booster both in serum and in saliva. At least 60% of enrolled subjects present neutralizing antibodies against wild-type, Delta, Delta plus but not against SARS-CoV-2 Omicron variants in saliva after the booster.

The first cycle of mRNA BNT162b2 vaccination elicits a strong systemic immune response by drastically boosting neutralizing antibodies development in serum, but not in saliva, indicating that at least oral mucosal immunity is poorly activated by this vaccination protocol, thus failing in limiting virus acquisition upon its entry through this route. However, individuals boosted with mRNA vaccines exhibited potent neutralization against wild-type, Delta, Delta Plus and SARS-CoV-2 variants both in serum and saliva. Neutralization of Omicron was only 4-6-fold lower than wild type in serum, suggesting enhanced cross-reactivity of neutralizing antibody responses. However, the absence neutralizing antibodies against SARS-CoV-2 Omicron variant in saliva might contribute to virus spreading and infection.

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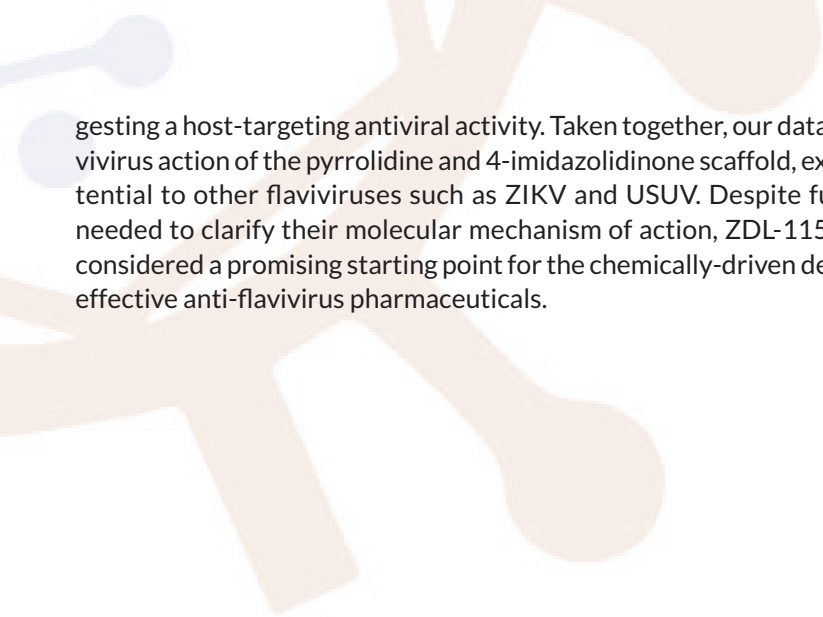
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Aim of the study: Zika virus (ZIKV) and Usutu virus (USUV) are two emerging flaviviruses for which no antivirals or vaccines are currently available. They may cause conditions ranging from mild febrile diseases to severe outcomes. Indeed, ZIKV is associated with microcephaly in newborns and the recently reported USUV neurotropism represents a growing concern for human health. In this context, the search for new effective antivirals against ZIKV and USUV results to be of great scientific interest. In previous studies, the fused bicyclic derivative of pyrrolidine and 4-imidazolidinone was discovered as an active scaffold against Dengue virus (DENV) and Japanese encephalitis virus (JEV), with its vitamin D receptor (VDR) modulatory activity associated to the antiviral properties. Nevertheless, the first set of compounds had some unfavorable chemical characteristics, including low hydro-solubility, limiting their application. With the aim of identifying new molecular entities targeting flaviviruses, we designed and synthesized an improved library of fused bicyclic derivatives of pyrrolidine and 4-imidazolidinone mounted on 2-deoxyribose. The anti-ZIKV and anti-USUV activity of these compounds was analyzed *in vitro* and the preliminary mechanism of action of two hit compounds was investigated.

Methods used: A library of 150 molecules based on the abovementioned scaffold was synthesized and chemically characterized. All molecules were initially screened against two ZIKV strains (MR766 and HPF2013) and against USUV (Strain: 3345 Isolate: Arb276) by means of focus reduction assays. After the identification of active and not toxic compounds, an additional library of 25 analogs was synthesized and screened. The antiviral activity of the identified hit compounds was further evaluated on three different cell lines (Vero, Huh7, A549) and by means of plaque assays and virus yield reduction assays. A preliminary investigation of the mechanism of action was performed with specific antiviral assays. *In silico* docking analyses were carried out on VDR and viral enzymes to identify the putative molecular target of the selected hit compounds.

Results and Conclusions: We identified two molecules, named ZDL-115 and ZDL-116, endowed with anti-ZIKV and anti-USUV activity (EC_{50} ranging 2.13 μ M from to 9.73 μ M). They showed favorable selectivity indexes ($CC_{50}/EC_{50} > 100$) on three different cell lines and were able to significantly reduce ZIKV and USUV progeny production at low micromolar concentrations. Additionally, we observed a reduction in the ZIKV plaque size, suggesting an inhibition of the viral spread. We demonstrated that ZDL-115 and ZDL-116 exert their antiviral activity when added on cells 24 hours prior to the infection or immediately after viral entry into the host cell. The combination of the two active treatments (pre- and post-infection) did not improve the antiviral efficacy. *In silico* docking analyses identified VDR as the best binder of both molecules, according to their predictive K_d s, sug-



gesting a host-targeting antiviral activity. Taken together, our data confirmed the anti-flavivirus action of the pyrrolidine and 4-imidazolidinone scaffold, expanding its antiviral potential to other flaviviruses such as ZIKV and USUV. Despite further experiments are needed to clarify their molecular mechanism of action, ZDL-115 and ZDL-116 could be considered a promising starting point for the chemically-driven development of novel and effective anti-flavivirus pharmaceuticals.

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Background: Ibalizumab (IBA) is the first humanized IgG4 monoclonal antibody targeting CD4 receptor recently approved for the treatment of multi-drug resistant HIV-1 infection in adult individuals. Given the importance of the selection of companion drugs in salvage therapy, in this study we aimed to evaluate the *in vitro* combinatorial activity of IBA together with licensed or investigational antiretrovirals.

Methods: The combinatorial effect of IBA with either tenofovir alafenamide (TAF, NRTI), lamivudine (3TC, NRTI), etravirine (ETR, NNRTI), darunavir (DRV, PI), dolutegravir (DTG, INSTI), temsavir (TMV, AI) or lenacapavir (LEN, CI) was evaluated in a checkerboard assay. MOLT4-CCR5+ cells were infected with the wild-type NL4-3 strain and exposed to a 6x6 drug concentration matrix for 8 days, then the supernatants were used to infect reporter TZM-bl cells. The matrix including the combination of IBA plus IBA was used as control of additive activity. Luminescence values were normalized to calculate the percentage of inhibition of viral replication and elaborated with the SynergyFinder2.0 software. Synergy scores were determined as the mean of at least two replicates and were calculated with ZIP, Bliss, Loewe and HSA models. Values <-10, from -10 to 10 and >10 were likely associated with antagonism, additive effect, and synergy between drugs, respectively.

Results: Globally, all the drugs tested in combination with IBA were predicted to have additive or synergistic activity independently by the synergy model (Table 1). The combinations IBA+ETR, IBA+LEN and IBA+DTG were associated with stronger synergistic effects as determined by all models, while the values calculated with IBA+DRV, IBA+TAF, IBA+TMV and IBA+3TC were mostly predictive of additive activity. The effect of control combination IBA+IBA were correctly identified as additive with all models, resulting in an average synergy score close to zero.

Conclusions: These preliminary data suggest that IBA positively interacts with other antivirals against the replication of the wild-type HIV-1 NL4-3 strain, irrespective of the drug class.

Table 1. Synergy scores (mean $\pm$ standard deviation) of drug combinations

Model	Drug combinations							
	IBA+DRV	IBA+DTG	IBA+LEN	IBA+TMV	IBA+ETR	IBA+3TC	IBA+TAF	IBA+IBA
ZIP	10.5 $\pm$ 1.2	15.6 $\pm$ 0.6	18.4 $\pm$ 3.1	12.6 $\pm$ 2.4	21.3 $\pm$ 6.3	9.6 $\pm$ 3.4	13.0 $\pm$ 0.8	-3.1 $\pm$ 6.4
Bliss	7.8 $\pm$ 1.8	13.3 $\pm$ 2.1	17.1 $\pm$ 1.8	6.1 $\pm$ 11.2	19.3 $\pm$ 4.7	9.6 $\pm$ 3.3	12.8 $\pm$ 1.2	-3.2 $\pm$ 7.1
Loewe	6.7 $\pm$ 2.1	11.9 $\pm$ 1.4	15.0 $\pm$ 2.2	9.7 $\pm$ 5.8	12.3 $\pm$ 2.3	7.7 $\pm$ 1.1	9.6 $\pm$ 2.0	-0.2 $\pm$ 2.7
HSA	6.0 $\pm$ 4.1	10.8 $\pm$ 2.7	13.7 $\pm$ 0.7	3.5 $\pm$ 12.3	12.5 $\pm$ 0.8	5.2 $\pm$ 5.5	8.9 $\pm$ 0.4	6.6 $\pm$ 1.6
Mean synergy score ^a	7.7	12.9	16.1	8.0	16.3	8.0	11.1	0.03

THE ANTIVIRAL POTENTIAL OF GRAPES AND SAFFRON PLANT EXTRACTS AGAINST SARS-CoV-2: THE TUSCAVIA PROJECT

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Introduction: Sustainable development is the driving force behind a global action program aimed at limiting the exploitation of natural resources. In this scenario, circular economy and a smarter waste management play a key role. In this perspective, industrial waste from plant processing may be exploited for a wide range of purposes, because they are enriched in secondary metabolites with multiple properties. The TUSCAVIA project was conceived with this purpose.

Aim of the study: The TUSCAVIA project is a sustainable project that aims at exploiting the antiviral, antibacterial and antifungal properties of vegetable matrices produced in Tuscany. Our aim is to evaluate the antiviral potential of plant extracts. To choose the most promising compounds, we relied on chemical-analytical analyses carried out by the University of Pisa, which revealed the presence of liposoluble active ingredients, such as N-Acylethanolamines (NAEs) or water-soluble ones, such as phenols, flavonoids, phenolic acids and tannins.

Material and Methods: VERO E6 cells were pre-treated with increasing concentrations of vegetable extracts for 48h. Cytotoxicity assays and a CFSE cell proliferation assay were performed. VERO E6 cells were infected with 0.1 MOI of the SARS-CoV-2 clinical strain VR PV10734 obtained from the Unit of Virology, AOUP, Pisa. The antiviral activity of plant matrices was tested by counting the number of infected cells in the presence and absence of treatment using High Content confocal microscopy. In addition, SARS CoV-2 genomes were quantified by Real-Time PCR.

Results: We first analyzed cell viability after the treatment with artichoke, grape extracts, soy, alfalfa, cooked in the field or stewed, and saffron and we observed that no extract showed cytotoxic effect on VERO-E6 cells. We then assessed the antiviral activity of these compounds and we observed that grapes significantly reduced SARS-CoV-2 viral genomes and frequency of cell infection by 4-fold. We also found that saffron caused a 2-fold reduction of SARS CoV-2 infection.

Discussion: Grape extract antiviral activity against SARS-CoV-2 is confirmed in the Literature. Phenolylphenols, such as flavan-3-ols, dimeric proanthocyanidins and tannic acid, are proposed as ingredients acting as SARS-CoV-2 main protease inhibitors. Starting from this data, we will try to investigate the mechanism behind the antiviral action of grape extracts.

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The SARS-CoV-2 is the causative agent of COVID-19 pandemic that can cause acute respiratory disease (ARD). It has been demonstrated that SARS-CoV-2 is able to escape immune responses, an event generally associated with a poor clinical outcome. Immune escape is also associated with the failure of the development of T cell memory compartments. Currently it has been found that SARS-CoV-2 variants do not significantly disrupt the total SARS-CoV-2 T cells reactivity. Nevertheless, the decrease of humoral immunity response observed highlights the importance of active monitoring of T cell reactivity in the context of SARS-CoV-2 evolution. Fatal ARD is highly decreased among vaccinated people but the pandemic persistence, due to the rise of newest SARS-CoV-2 variants, leads to wonder whether SARS-CoV-2 could impact on the effective distribution of memory T cells subsets.

The aim of this study is to characterize the T memory subsets testing the immune response against class I and II restricted immunodominant epitopes shared by ancestral and variants SARS-CoV-2 strains. Naïve T cells as well as T memory subsets were analyzed by multiparametric flow cytometry on 9 fully vaccinated healthy donors (HDV) and 14 COVID-19 recovered patients (CD). Blood samples were withdrawn after booster dose for HDV or negativization for CD. Memory T subsets were identified by using specific surface markers (i.e., CD45RA, CCR7 and CD62L). In particular, we enumerated naïve (Tn: CD45⁺CCR7⁺CD62L⁺), central memory (Tcm: CD45⁺CCR7⁺CD62L⁺), effector memory (Tem: CD45⁺CCR7⁺CD62L⁺) and recently activated effector memory T cells (Temra: CD45⁺CCR7⁺CD62L⁺). Specific cell recognition of SARS-CoV-2 S epitopes (S-epitope) was performed by using ProT2 MHC-II tetramers (for CD4⁺ cells) and ProT5 MHC-I pentamers (for CD8⁺ cells) (both from ProImmune).

The results obtained by comparing the two cohorts of subjects indicate no statistically significant difference in the percentage of SARS-CoV-2 antigen restricted T clones in both CD4⁺ and CD8⁺ subsets even if there is a small increase in the CD cohort compared to HDV. Looking at CD4⁺ T memory subsets no difference was recorded between the two cohorts of analyzed patients whereas, in the case of CD8⁺ T cells a significant decrease of the Tn subset (HDV: 49,76±16,49% vs. CD 19,72±14,76%, p<0,0015) is associated with a parallel significant increase of the Tem subset (HDV: 24,87±13,72% vs. CD 46,19±17,51%, p<0,0074) in the CD cohort compared to HDV.

Collectively, these results suggest that, even if both the vaccination and the natural infection are equally able to induce the activation of T cell clones restricted for immunodominant peptides, recovered subjects display, at least in the case of CD8⁺ T cells, an improved expansion of the effector memory T cell subset compared to vaccinated people. This feature probably reflect the broader T cell repertoire stimulated by the virus during the natural infection compared to the spike-restricted one activated during the vaccination schedule. A larger study is in progress to further substantiate the evidence of the modulation of T cell compartment in the immune response to SARS-CoV-2.

**N-ACYLETHANOLAMINE ACID AMIDE HYDROLASE INHIBITION DECREASES
RNA+ VIRAL REPLICATION BY DISMANTLING LIPID DROPLETS
AND ACTIVATING SELECTIVE AUTOPHAGY**

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Aim of the study: Lipids play a crucial role in the entry and egress of viruses, no matter whether they are naked or enveloped. Recent evidence shows that lipid involvement in viral infection goes much further, providing energy and protected niches in which viruses replicate. We hypothesized that derangement of lipid metabolism during viral infections might be counteracted by anti-inflammatory compounds which also destroy niches of viral replication.

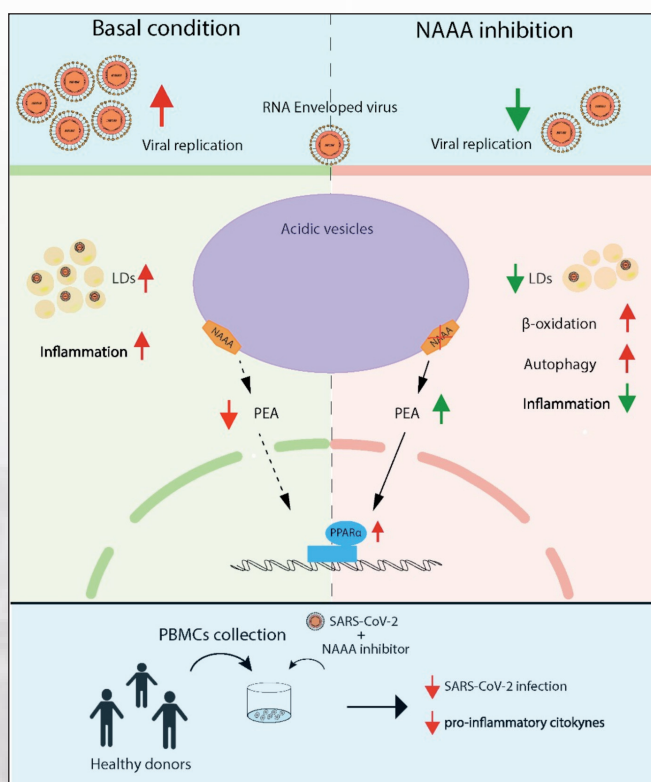
To this aim, we investigated N-Acylethanolamine acid amidase (NAAA) inhibition as a novel antiviral strategy. NAAA catalyzes the hydrolysis of palmitoylethanolamide (PEA), a potent anti-inflammatory ethanolamide. Once NAAA is inhibited, PEA accumulates and activates peroxisome proliferator-activated receptor- α (PPAR- α) which steers β -oxidation, blocking inflammation and disrupting lipid droplets, niche for viral replication.

Methods: The role of NAAA in viral replication was addressed with positive-sense RNA viruses known to use lipidic vesicles during their replication: Zika Virus (ZIKV), SARS-CoV-2 and Cocksackie B type 5 (COXB5) virus. Infections were measured by qRT-PCR and/or Western blots performed on both supernatants and cell lysates. Data were confirmed by high-content confocal imaging screenings using Operetta CLS (Perkin Elmer). This system was also used to dissect autophagy and to evaluate molecular interactions among NAAA and viral proteins.

Results and Conclusion: We show that once NAAA is blocked both ZIKV and SARS CoV-2 replication decrease by 10 folds, which parallels a sudden decrease in virion release. These effects occurs concomitantly with stimulation of autophagy during infection, despite both ZIKV and SARS-CoV-2 hijack this pathway. Moreover, NAAA inhibitors act synergistically with Ribavirin, dropping its IC50 by 90 times. Remarkably, parallel antiviral and anti-inflammatory effects of NAAA antagonism were confirmed ex-vivo, within SARS-CoV-2 infected human PBMC cells, in which the amount of infected cells and chemokines decrease in the very same cells involved in the cytokine storm, one of the major cause of COVID-19 lethality.

Importance: The pathogenesis of severe COVID-19 consists of an initial infection phase elicited by SARS-CoV-2 replication and a strong inflammation derangement characterized by aberrant macrophage activation and uncontrolled release of chemokine and cytokines, which lead to tissue damage, acute respiratory failure or even death. While several anti-inflammatory agents used in clinical practice are beneficial to switch-off inflammation (e.g corticosteroids), they might favor viral replication.

We highlight the repurposing of NAAA inhibitors, extremely potent anti-inflammatory and anti-nociceptive compounds, as antiviral agents. We show that they are capable to counteract the replication of ZIKV and SARS-CoV-2, but not Coxsackievirus, RNA+ viruses. These results were confirmed on NAAA KO cells and above all, on natural host cells such as primary human neural cells (ZIKV) and monocytes (SARS-CoV-2): the latter, once infected with SARS-CoV-2 and treated with NAAA inhibitors showed a decrease of intracellular viral genomes and inflammatory cytokine release, the immune signature of the cytokine storm. Moreover, we show that NAAA inhibitors act synergistically with ribavirin, one of the most used antiviral drugs to treat RNA+ viral infections, decreasing its IC₅₀ by at least 90 times. In addition with the potential therapeutical application of our findings, we highlight a novel cellular player in the replication of the aforementioned viruses: for the first time, we describe that NAAA is involved in fueling viral replication by increasing LDs. We also shed light into the mechanism, showing that NAAA inhibition prevents the degradation of PEA which in turn, activates PPAR- α . Once activated, PPAR- α maintains active autophagy and leads LDs dismantling through peroxisomal and mitochondrial β -oxidation.



Graphical Abstract

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Aim of the study: To develop promising polymer conjugates and polymeric nanoparticle showing effective *in-vitro* inhibition of SARS-CoV-2.

Methods used: Chemical conjugation strategy was developed using the maleimide-thiol conjugation chemistry. Initially, maleimide conjugation to Sodium hyaluronate was performed with N-hydroxy succinimide (NHS) and 1-(3-Dimethylaminopropyl)-3-ethyl carbodiimide (EDC). The reaction mixtures were dialyzed with a membrane (MWCO-7500Da) to remove impurities and lyophilize them for further use. The maleimide modification of Sodium hyaluronate and subsequent peptide conjugation (either with peptide VFIC or peptide-THLC) was confirmed by proton NMR and nanoparticle was prepared by ionic interaction between the Modified polymer-peptide conjugate and a counter-chitosan in a mild aqueous condition. The resulting nanoparticles were fully characterized by dynamic light scattering and Nanoparticle tracking analysis. For the plaque assay experiments, nanoparticles were incubated with SARS-CoV-2 for 2 hours, then the mixture was added on Vero-76 cells and incubated for 1 hour. Later these cells were washed and overlaid with carboxymethyl cellulose (CMC). About 48 hours of post infection, the cells were stained with crystal-violet 0.5%, and plaques were counted.

Results and conclusions: High degree of maleimide conjugation was obtained with Sodium hyaluronate; then the polymer-peptide conjugates were obtained using thiol-maleimide click chemistry with a very high efficiency of conjugation (more than 95%). The polymeric nanoparticles with peptide-modified polymer and chitosan was prepared. All the formulations with high concentration exhibited a size of 360 ± 5 nm; PDI: 0.094; Zeta-potential: 21 ± 2 mV whereas formulation with low concentration exhibited a size less than of 200 ± 5 nm; PDI: 0.115; Zeta-potential: 21 ± 2 . Both polymer-peptide conjugates and polymeric nanoparticles were able to enhance the antiviral activity at lower concentrations compared to the free peptides as observed in the *in-vitro* virus pre-treatment assay. Interestingly, the nanoparticles showed the highest antiviral activity, while the control nanoparticles without peptides did not show strong antiviral effects. This indicates the need for both the antiviral peptide and a suitable formulation to enhance the antiviral activity. Finally, in the present study we proposed new materials with anti-SARS-CoV-2 activity. If they are effective *in-vivo*, they could be promising candidates for prophylactic and/or therapeutic nasal wash/spray for human application.

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Severe acute respiratory syndrome coronavirus 2 (Sars-CoV-2) virus infection was declared a Public Health Emergency by World Health Organization (WHO) in January 2020: it can cause a very contagious and severe disease, in particular for elderly and frail subjects, the Coronavirus disease 2019 (COVID-19). The first vaccine to receive approval by European Medicines Agency (EMA) was the BioNTech/Pfizer BNT162b2 mRNA vaccine and in Italy its administration has started at December 27th, 2021. The aim of the present work is to monitor during BNT162b2 vaccination the humoral response of healthcare workers (HW) with (HW+) or without (HW-) history of documented Sars-CoV-2 infection. Fifty-nine HW (47 HW- and 12 HW+) were included in this study, and serum was collected before the vaccination, 10 and 20 days after the first dose, 1, 3, 6 and 9 months after the second dose of vaccination, and 10 days after the booster dose. In each time point, IgM and IgG titers, as well IgG neutralizing activity, were evaluated. Twenty days after the first dose as well 1 month after the second dose, the IgM titers were detected in the majority of HW, without any differences between HW- and HW+. Twenty days after the first dose, IgG titers were significantly higher in HW+ (293.75; 205.62-343.19 UA/ml) than HW- (80.95; 42.01-205.62 UA/ml; $p < 0.05$). In HW-, the peak of IgG titers was reached 1 month after the second dose of vaccine (278.80; 183.76-426.04 UA/ml), whereas in HW+ already 20 days after the first dose. The IgG titers gradually significantly decreased during the time for both groups; ($p < 0.05$) after 6 and, in particular, after 9 months the second dose, IgG titers are undetectable for about half of HW-, whereas they remain detectable for 11/12 of the HW+ after 6 months, and for 9/12 after 9 months. However, ten days after the booster dose, the IgG titers are detectable in all the HW, without any differences between HW- and HW+ regarding the titers.

Twenty days after the first dose, the IgG neutralizing activity (Nab) was statistically higher in HW+ compared to HW- ($p < 0.05$), but this difference disappeared 1 month after the second dose, when the Nab of HW- reached those of HC+. The Nab significantly decreased ($p < 0.05$) in HC- 6 months after the second dose, whereas in HC+ only 9 months after the second dose. For both groups the Nab statistically increased ($p < 0.05$) 10 days after the booster dose, without any statistical differences between the two groups.

Our results confirm that the two doses of BNT162b2 vaccination induce a strong humoral response in subjects with or without history of documented Sars-CoV-2 infection, but in this latter group a more intense and prolonged response was observed up to six months after the second dose; however, the booster dose is able to quickly stimulate the humoral response, leading to IgG or Nab levels comparable in both groups.

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Introduction and aim: Bombinins H (H for hydrophobic and hemolytic properties) are 20-residues amphibian antimicrobial peptides (AMP) originated from *Bombina variegata* skin secretion. These peptides are rich in glycine (25%) and can assume different conformations in addition to the typical amphipathic α -helical structure in a membrane-mimicking environment, as shown by their circular dichroism spectra. Their most striking feature is the presence of a D-amino acid in the second N-terminal position in some of them, representing the first example of natural AMPs with a single D-amino acid in their sequence; moreover, the isomers coexist in the same secretion (Coccia et al, 2011). In our study we focussed on Bombinin H2 (IIIGPVLGLVGSALGGLLKKI-NH₂) and its diastereomer H4 (I-(D-allo- I)GPVLGLVGSALGGLLKKI-NH₂), known to exhibit high membrane-perturbing activity against Gram-negative bacteria (Mangoni et al, 2000). The aim of this study was to investigate the antiviral activity of Bombinin H2 and H4 against enveloped viruses, such as herpes simplex virus type 1 (HSV-1) and Influenza A virus (IAV).

Methods: Vero and A549 cells were infected with HSV-1 and IAV, respectively. Infected cells were subjected to time-of-addition assay and virucidal test with Bombinin H2 and H4 at doses 10 and 20 μ g/mL after testing by MTT assay. The efficacy of infection was evaluated by standard plaque assays (SPA) and In-Cell Western assays in cellular supernatants. Cellular monolayers were lysed and analysed by Western blot.

Results and Conclusion: We found that bombinin H2 and H4 exert virucidal effect on both enveloped viruses, but bombinin H2 was more effective than H4. No significant decrease in HSV-1 and IAV infection was observed either when the peptides were used at different times of infection, nor when used to pre-treat the cells. Synergy experiments between Bombinin H2/H4 and Temporin G, an amphipathic AMP that in our previous studies inhibited the attach/entry phases of HSV-1 and IAV into the host cells, were performed and the results showed that peptides with different targets inhibited more viral infections. Overall, these data indicate that Bombinin H2 and H4 could be promising AMPs for the treatment of enveloped virus infections.

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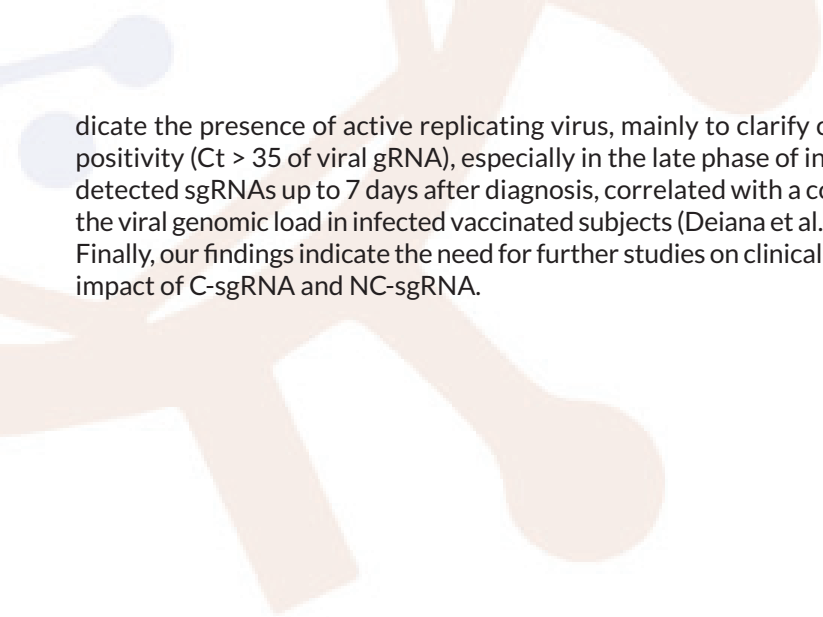
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Aim: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for COVID-19 disease and the on-going pandemic. Coronaviruses are characterized by “discontinuous transcription” of an array of distinct subgenomic mRNAs (sgRNAs) during their life cycle. SgRNAs are only transcribed in infected cells and not packaged into virions. As viral mRNA, sgRNAs are translated into structural (nucleocapsid, spike surface, matrix, envelope proteins) and accessory proteins. Therefore, sgRNAs have been proposed as marker of viral replication. However, contradictory results have been reported in literature about diagnostic on clinical samples. Some authors concluded that detection of sgRNAs correlate with active virus replication (Wölfel et al., Nature. 2020), whereas others asserted that sgRNAs are poor marker of active infection due to their highly stable nature (Alexandersen et al., Nat Commun. 2020). In order to contribute for clarifying these questions, this preliminary study aimed at evaluating the expression of canonical (C-sgRNA) and non-canonical sgRNAs (NC-sgRNA) in infected human Caco-2 cell line, treated and untreated with the antiviral drug Remdesivir (RdRp inhibitor).

Methods: The study was carried out by infecting Caco-2 cells with 0.1 MOI of SARS-CoV-2 lineage B.1. Then, Caco-2, untreated and treated with 10 μ M Remdesivir, were analyzed in parallel to investigate the presence of genomic (gRNA) and sgRNAs at 0 and 24 hour post-infection (hpi). Infectious SARS-CoV-2 load was measured in cell pellets and in cell culture supernatant by TCID₅₀ assay. SgRNA analysis was performed by NGS technique based on amplicon approach (ARTIC nCoV-2019 V3 panel) performed by MiSeq platform, and bioinformatic analysis by “periscope” tool (Parker et al., Genome Res. 2021). To verify NGS results, a Real Time PCR (RT-PCR), using the REALQUALITY SARS-CoV-2 SubG (AB ANALITICA) were performed. The kit detects two targets of the SARS-CoV-2 gRNA (ORF1ab and N genes) and three targets of SARS-CoV-2 sgRNAs (E+M and N).

Results and conclusions: In untreated cells, NGS reported a total of 7 types of C-sgRNA (N, M, S, E, orf6, orf3a, orf7a). Globally, all C-sgRNA showed an increasing trend. In treated cells, the NGS results showed that virus replication was drastically reduced and blocked at 24 hpi by Remdesivir. C-sgRNA synthesis was detected until 24 hpi, showing a decreasing trend in S, M, orf3a and orf7a, whereas sgRNA N instead showed a low unvaried expression. Both in untreated and treated cells, the NGS results were in accordance with the viral titration by TCID₅₀ assay, viral gRNA and sgRNA RT-PCR detection. Interestingly, in untreated Caco-2, NGS detected also NC-sgRNA, which resulted to be characterized by wide heterogeneity both in terms of types, onset time-points (0 and 24 hpi), and trend. Some NC-sgRNAs showed an increasing trend, other NC-sgRNAs decreasing and some were detected only at 0 hpi or at 24 hpi. Contrariwise, few NC-sgRNAs were detected in treated cells. In conclusion, the results supported the application of sgRNA analysis to in-



indicate the presence of active replicating virus, mainly to clarify clinical cases of dubious positivity ($Ct > 35$ of viral gRNA), especially in the late phase of infection. Indeed, we also detected sgRNAs up to 7 days after diagnosis, correlated with a concomitant reduction in the viral genomic load in infected vaccinated subjects (Deiana et al., Microorganisms 2021). Finally, our findings indicate the need for further studies on clinical samples to assess clinical impact of C-sgRNA and NC-sgRNA.

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Aim of study: Despite increasing prevention by vaccination, viral infections still represent a serious threat to humanity. Since 2019, a new viral infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread around the world. On March 11, 2020, the World Health Organization (WHO) declared a global pandemic status. Therefore, it is mandatory to search for new molecules as potential antiviral drugs to counter this pandemic emergence. Recently, antimicrobial peptides (AMPs) have attracted the interest of many researchers. They are natural molecules derived by the innate immune system of almost all living organisms, including invertebrates and vertebrates. In the present study, we evaluated the antiviral activity of AR-23, an AMP isolated by the Japanese frog of Ranid (*Rana tagoi*). Its antiviral potential has been investigated against a broad spectrum of viruses, both with DNA and RNA genome, and with or without envelope.

Method used: Cell viability was analyzed on Vero CCL-81 cells using a colorimetric assay, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Different concentrations of the peptide ranged from 0.39 to 100 μ M were tested. Subsequently, to understand the mechanism of action of AR-23, different antiviral assays were performed (co-treatment, virus pre-treatment, cell pre-treatment, and post-treatment). The antiviral activity was evaluated against enveloped DNA (Herpes simplex virus type 1) and RNA viruses (measles virus, human parainfluenza virus type 2 and 3, human coronavirus 229E, and SARS-CoV-2), and also against the naked virus (poliovirus type 1).

To confirm the data, a molecular analysis was conducted by Real-time PCR. The expression of genes involved in viral infection has been evaluated. In detail the genes UL54, UL27 and UL52 for HSV-1, and the spike (S) and nucleocapsid (N) genes for HCoV-229E and SARS-CoV-2, were quantified. Finally, the morphology of viral particles were analyzed after treatment with AR-23 by Transmission Electron Microscope (TEM).

Results and conclusions: Our results suggest that AR-23 showed a broad-spectrum activity against all enveloped viruses tested. In contrast, the peptide did not inhibit naked virus infection. Data confirmed a strong antiviral effect in virus pre-treatment assay, suggesting that AR-23 could act directly on the viral surface.

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Aim of the study: The monoclonal antibody (mAb) Sotrovimab is used under the emergency authorization for the treatment of patients at risk for severe COVID-19 progression. Sotrovimab can neutralize all sarbecoviruses, including SARS CoV-2, by binding to a highly conserved epitope within the receptor-binding domain. However, its use, as well as of other SARS CoV-2-specific mAbs, warrants caution being frequent in the development of mutations that confer viral resistance.

Methods: 46 patients were treated with Sotrovimab at the ASST Settelaghi (Varese, Italy) during the B.1.1.529 (Omicron) variant outbreak between January and March 2022. From 12 (26%) of these patients, two nasopharyngeal swabs (NPS) were obtained just before the mAb infusion, and on day 10 post-therapy. The presence of SARS CoV-2 RNA was evaluated by the real-time Alinity mSARS-CoV-2 Assay (Abbott). Positive NPS with cycle thresholds (Ct) below 25 were assayed by next-generation sequencing (NGS) of the whole SARS CoV-2 genome on the Miseq platform (Illumina). Genomic analysis was performed by the software platform BaseSpace Sequence Hub (Illumina).

Results and conclusions: Of the 12 outpatients, 9 (75%) had persistently positive PCR at day 10, while 3 patients resulted in SARS COV-2 RNA negative. Six of 9 positive samples harbored virus levels at day 10 with Ct < 25, and thus they were furtherly analyzed by whole-genome NGS. Although all the sequenced samples resulted infected by the same Omicron BA.1 VOC, consecutive NPS from 2 subjects revealed Sotrovimab treatment-emergent E340D at genomic analysis. Case 1 was a 51-years old HIV-positive female with cerebral toxoplasmosis, and Case 2 was a 43-years old male kidney transplant recipient. The analysis of Omicron sequences deposited in GISAID on March 28, 2022, revealed that E340D mutation was present in only 732 out of 2,390,087 sequences [1]. Notably, mutations at position S:E340K/A/V have been associated with a significant reduction in neutralization by Sotrovimab.

Our data show that SARS CoV-2 RNA persists for at least 10 days in a significant number of Sotrovimab-treated patients and that the appearance of drug-induced mutations is a relatively frequent event. Overall, the findings underscore the importance of mAbs stewardship, particularly because Sotrovimab is one of the few mAbs with retained activity against the B.1.1.529 variant. Genomic surveillance of patients receiving mAbs for the SARS-CoV-2 treatment is essential to minimize the risk of both treatment failure and transmission of potentially resistant SARS-CoV-2 variants in the health care settings and community [2].

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IN VITRO ANTIVIRAL ACTIVITY OF A CRANBERRY *VACCINIUM MACROCARPON* EXTRACT AGAINST EBOLA VIRUS AND RABIES VIRUS

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Emerging and reemerging viral infections represent a major concern for human and veterinary public health and there is an urgent need for the development of broad-spectrum antivirals.

We have recently observed that a cranberry (*Vaccinium macrocarpon*) extract, which contains high levels of A2-type proanthocyanidins (PAC-A2), inhibits influenza A and B viruses [1], herpes simplex virus type 1 and 2 [2], and Crimean-Congo Hemorrhagic Fever Virus [3] *in vitro* replication by interfering with the adhesion/internalization stage of the viral life cycle. In this work, we have evaluated the antiviral activity of the cranberry extract against two highly pathogenic viruses, such as Ebola Virus (EBOV) and Rabies Virus (RABV).

To evaluate the antiviral activity of the cranberry extract, a recombinant Vesicular Stomatitis Virus (pVSV) vector expressing the luciferase reporter was adopted. The pVSV was pseudotyped either with the EBOV or RABV glycoproteins. Time-of-addition, viral attachment, and entry assays were performed on Vero CCL-81 in the presence of different concentrations of the cranberry extract. Finally, experiments with infectious EBOV and RABV were performed to validate the results obtained with pseudovirus.

The cranberry extract showed an inhibitory activity against both the pVSV-EBOV and pVSV-RABV infection. Treating target cells or the pseudovirus with the compound before or during the infection phase determined a significant reduction of viral infectivity. In contrast, only a modest inhibition was detected when cells were treated with the extract after virus internalization. The antiviral activity of the cranberry extract was confirmed against the live EBOV, while experiments are ongoing for RABV.

In Conclusions:

- The cranberry extract inhibits EBOV and RABV infection acting at the early stages of their replicative cycles.
- This broad-spectrum antiviral activity suggests this cranberry extract (or its components) as a promising antiviral candidate against emerging and re-emerging viral infections.

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IN SEARCH OF NOVEL ANTIVIRAL STRATEGIES: NEWLY IDENTIFIED MOLECULES INDUCE TYPE-I INTERFERON EXPRESSION THROUGH STING DEPENDENT PATHWAY**A. Paulis¹, P.O. Vidalain², V. Lotteau², S. Distinto¹, M.G. Delogu¹, E. Tramontano¹**¹ Department of Life and Environmental Sciences, University of Cagliari, 09042 Monserrato, Italy² CIRI, Centre International de Recherche en Infectiologie, Univ Lyon, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, Lyon, F-69007, France

The STING is a transmembrane protein localized in the endoplasmic reticulum (ER) involved in the innate immune response by stimulating type I Interferons (IFNs) transcription. When cytosolic DNA is detected, cGAS (cyclic GMP-AMP synthase) produces 2'3' cGAMP (2'3' cyclic GMP-AMP), such cyclic dinucleotide binds hSTING triggering IFN Regulatory Factor 3 (IRF3) phosphorylation and dimerization, this complex is translocated into the nucleus where stimulates type I IFN (IFN-I) transcription. Moreover, hSTING recognizes bacterial cyclic dinucleotide 3'3' cGAMP, demonstrating its relevance during both viral and bacterial infections. Recent studies identified STING agonists which showed antiviral activity, for this reason, a selection of putative STING-ligands was tested to evaluate their ability to induce IFNs and to inhibit viral replication.

Starting from a small in-house library through a virtual screening approach we identified eleven molecules potentially interacting with STING. A gene reporter assay in cells knock-out for STING (HEK293T) has been used to investigate compounds' dependent IFN- β transcription in presence of reconstituted STING and a luciferase reporter gene driven by the human IFN- β promoter. In this system, we determined the ability of the tested compounds to induce IFN- β transcription. Of note, compounds were not able to induce IFN- β transcription in presence of mutated and inactive STING suggesting a specific protein-ligand interaction. To exclude that STING activation could be due to indirect genotoxic effect inducing the cGAS-STING pathway, we investigated the DNA damage response, showing that p53 levels were unchanged by compound treatment. In addition, since it is known that pyrimidine biosynthesis is strongly active during viral infections, producing dihydroorotate dehydrogenase, the key enzyme in pyrimidine biosynthesis and a target of antivirals, we evaluated the ability of the compounds to block pyrimidine biosynthesis showing no effects. Studies are ongoing to verify the effect of selected compounds on viral replication.

DEVELOPMENT OF SARS-COV-2 IGM AFTER 1ST VACCINE DOSE PREDICTS LONGER IMMUNITY

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Background: In our previous work, we demonstrated that individuals developing SARS-CoV-2 specific IgM following vaccination show higher levels of SARS-CoV-2 neutralizing IgG.

Aim: To study whether development of SARS-CoV-2 IgM following vaccination predicts longer immunity.

Methods: We analysed SARS-CoV-2 specific humoral response in 1873 health care worker (HCW) recipients of the BNT162b2, longitudinally: before administration (week 0, W0), at the second dose (W3), three weeks (W6) and 6 months (W27) after the second dose. The cohort included 1584 immunologically naïve subjects to SARS-CoV-2 (IN) and 289 individuals with a history of previous infection (PI). We measured IgG antibodies specific for the SARS-CoV-2 spike protein (S), specifically against the receptor binding domain RBD (IgG-S; Quant assay, Abbott, Ireland) and anti-S IgM (IgM-S; SARS-CoV-2 IgM-S assay, Abbott). For IgM-S, the patients were classified negative (<1 BAU/ml) or positive (≥1 BAU/ml) as indicated by the manufacturer. Two-level linear regression models were used to assess differences of IgG-S titers according to time of examination (W0-W27) and IgM-S group, separately for IN and PI subjects.

Results: In IN, we identified three patterns of responses: (a) IgG-S positive/IgM-S negative (36%); (b) IgM-S positive after the first dose together with IgG-S (38%); (c) IgM-S positive after the first dose and after IgG-S (26%). In PI, 41% were IgM-S negative at T0 (a); the remaining IgM-S positive developed IgM-S at T1(19%, b) or at T2 (2%, c); a proportion of patients had detectable IgM-S already at T0, probably as reflection of the recent infection (38%, d). In IN (Fig. 1A), expression of both IgM-S/IgG-S responses were associated with higher IgG-S titers at short (W6, $p<0.0001$) and long (W27, $p<0.001$) follow-up. In PI, although pre-existing immunity may differentially modulate antibody response, the generation of vaccine-induced IgM-S after the first vaccine dose was associated with a trend for higher IgG-S titres in subjects that developed Ig-M at follow-up (Fig. 1B).

Conclusion: SARS-CoV-2 vaccination induces (1) absence of IgM-S, (2) appearance of IgM-S after 1st vaccination dose and together with IgG-S, or (3) IgM-S following IgG-S appearance. The coordinated expression of IgG-S and IgM-S was associated with a more efficient response in both antibody titers and virus-neutralizing activity up to 6 months following vaccination schedule completion, representing a potential correlate of protection.

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Aim: Letermovir is a new anti-CMV drug, recently approved by FDA and EMA for treatment of adult allogenic HSCT patients (Marty, 2017). Differently from traditional antivirals, Letermovir acts by inhibiting the terminase-complex, thus blocking the packaging of the viral genomes and maturation of virions (Ligat, 2018). This implies that the CMV DNA accumulates during the treatment and its direct detection by QNAT assay becomes inadequate marker of the infection state. Pretreatment of plasma samples with DNase have been recently adopted to remove the free circulating DNA and to allow the detection only of CMV DNA in infective viral particles (Weinberger, 2020). However, an *in vitro* diagnostic method for direct detection and measurement of CMV infective virions would be desirable in association to Letermovir prophylaxis.

Here we report the preliminary result of CMV RNA ELITE MGB Kit, a new assay that allows direct detection and quantification of CMV RNA in infective virions directly in plasma samples of patients under Letermovir treatment.

Method: In total 31 plasma samples of patients belong to a set of 14 transplanted children were analyzed. All Children were under Letermovir treatment due to a previous CMV infection. Surveillance post-transplant was carried out for CMV, by monitoring CMV Viral Load DNA as recommended by guidelines at OPBG Virology Unit. Samples were tested CMV DNA according to routine procedures. The leftover was tested for CMV RNA. RNA extraction was carried out by ELITE InGenius SP 1000, using an input volume of 0.6 mL. CMV RNA was detected by quantitative real time PCR using CMV RNA ELITE MGB Kit. The entire process was carried out by ELITE InGenius system, using a fully automated procedure.

Results and Conclusions: Before Letermovir treatment, all plasma samples were positive for CMV DNA. After treatment, CMV RNA was found in 7/31 samples while 24/31 were still positive with RT-PCR for CMV DNA.

The average quantity of CMV DNA was 2.82 log copies/mL before treatment and 2.66 Log copies/mL in the Letermovir group while the average quantity of CMV RNA was 1.41 Log copies/mL.

Looking at CMV DNA VL detection before and after treatment we do not found a significant difference, otherwise, between the CMV RNA and CMV DNA groups ($P < 0.001$). As expected, no inter-rater agreement or correlation was observed between the CMV RNA and CMV DNA detection.

These preliminary data suggest that detection of CMV RNA in plasma samples by quantitative real time RT PCR can be used as a direct marker of active CMV infection in association to the Letermovir prophylaxis. Due to the difference with the current practice for CMV viral load follow-up, new criteria are needed for a correct clinical interpretation of the results. The study is ongoing.

**IDENTIFICATION OF NEW SARS-COV-2 MAIN PROTEASE INHIBITORS
BY AN INTEGRATED *IN-SILICO* AND *IN-VITRO* STRATEGY**

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By February 2020 SARS-CoV-2 has spread from China (Wuhan) to countless countries around the world with devastating effects for public health and global economy. In December 2020, following an unprecedented effort of the pharmaceutical industries, new vaccines able to fight SARS-CoV-2 were developed and approved for emergency use. However, the short immunological protection, vaccine hesitancy, and the continuous occurrence of virus variants limited their efficacy to control the virus circulation. At the present time, approved drugs are limited, and the development of new therapeutics is the highest valuable tool to fight this pandemic outburst.

In this view, the main protease of SARS-CoV-2, M^{pro}, is an appealing target for the development of inhibitors, due to its essential role in the viral life cycle and high conservation among different coronaviruses. Recently, it has been approved the first oral antiviral against COVID-19 (Paxlovid) based on a peptidomimetic M^{pro} inhibitor. However, the active compound suffers of a high metabolic instability and must be co-administered with Ritonavir that increases side effects.

In this prospect, the aim of the present work is the identification of novel inhibitors of SARS-CoV-2 M^{pro} to develop new and more effective lead compounds. To this end, a structure-based virtual screening was performed on a library of commercially available compounds, followed by FRET-based screening, biophysical and crystallographic analyses on the isolated recombinant target. Ten hits - including covalent and not-covalent M^{pro} inhibitors with different chemical scaffolds - were selected for the evaluation of antiviral activity by a phenotypic cell-based assay. Six out of the ten hits protected Vero E6 cells from SARS-CoV-2-induced cytopathic effects confirming their antiviral activity. At the moment, further assays are ongoing on different cell lines to validate and characterize the antiviral activity of the hits. These good preliminary results might pave the way to discover novel inhibitors that could lead to the development of clinically relevant inhibitors.

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DEVELOPMENT OF PROTECTIVE IMMUNITY AFTER BNT162B2 THIRD DOSE IN SUBJECTS WITH IMMUNO-MEDIATED INFLAMMATORY DISEASE (IMIDs) AND TREATED WITH ANTI-CD20 DRUGS

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Abstract: Due to the high rate of morbidity and mortality related to Sars-CoV-2 infection, the main focus of the entire scientific community over the past two years has been the COVID-19 pandemic.

Up to now, vaccinations represent the only dependable means to reduce the spread and relieve the impact of COVID-19 in the approaching period.

Although the overall immune effects of the BNT162b2 vaccine have been reported, the humoral immune profile requires further investigation in selected subgroups, such as patients affected by immune-mediated inflammatory diseases (IMID). In these patients, the risk of infection may be even higher due to both the altered regulation of the immune system itself and the immunosuppressive effects of medications.

To prospectively investigate the responses in a cohort of patients treated with anti-CD20, we have measured humoral and cellular immunity using quantitative IgG anti-SARS-CoV-2 Spike antibody (anti-S-IgG) and neutralization assay and specific interferon-gamma (IFN-γ) release assay (IGRA) before and after the third dose of BNT162b2. The response was then compared with healthy controls (HC) and patients treated with drugs different from anti-CD20.

This study shows that a third BNT162b2 dose is highly immunogenic in IMID patients, naïve to anti-CD20, as 100% of the subjects have seroconverted. Among responders, IMID patients have shown a significant difference in levels of anti-S-IgG and neutralization titers compared to HC, whereas no significant difference was observed when comparing anti-CD20 and HC. Furthermore, 13% of anti-CD20 and about 7% of IMID were simultaneously negative for both neutralizing antibodies and detectable IFN-gamma response, which highlights the potential role of a fourth vaccine dose for these at-risk groups of patients.

This data draws attention to the immunogenicity and importance of COVID-19 vaccination in patients with IMID as a measure to contrast pandemics, taking specific groups such as those treated with B-cell depleting agents into consideration.

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Aim of the study: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a significant challenge to human health. Recently, the research has been animated by the pressing need for new therapeutic and prophylactic treatments [1]. Peptide inhibitors are a valid alternative approach for treating emerging viral infections mainly due to their low toxicity and high efficiency. The present study was focused on two small peptides, each consisting of only three amino acid residues, deriving from two recurrent nucleotide signatures recently identified in many pathogenic microorganisms [2], including in different members of the *Coronaviridae* family. We hypothesized that the corresponding amino acid sequences could play a relevant role during the infection.

Methods used: Several viral genomes were analyzed in order to search the two recurrent nucleotide sequences identified only in the genome of different human coronaviruses. The amino acids produced were synthesized and, the stability and cytotoxicity of peptides were evaluated. In addition, antiviral activity and molecular docking were performed.

Results and conclusions: Surprisingly, the peptides inhibited the infection of both the human coronavirus OC43 and SARS-CoV-2. Their small size does not allow their structuring, but we observed that both could interact with the SARS-CoV-2 spike through in silico models. Our results demonstrated that tripeptides could occupy pockets inside S1 and S2 domains, potentially blocking all downstream events and the entire SARS-CoV-2 replication cycle. The two tripeptides were involved in the inhibition of beta coronaviruses entry mechanism, probably by interfering with the viral spike protein. In addition, the tripeptides had a very low toxicity profile and a long half-life in serum, suggesting their potential use as innovative and safe options in the prevention and antiviral therapy against SARS-CoV-2 infection.

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Aim of the study: SARS-CoV-2 is a positive-sense, single stranded-RNA virus. It is the aetiological agent of COVID-19 and it caused a pandemic. Due to its high pathogenicity and transmissibility SARS-CoV-2 must be manipulated in biosafety-level-3 (BSL-3) facilities. Lentiviral vectors pseudotyped with SARS-CoV-2 S protein (PLV-S) can be a useful model to study the entry process of highly pathogenic viruses in a safer way, because PLV-S do not replicate and can be handled in BSL-2 [1]. The aim of this study was the evaluation of the antiviral activity of a panel of compounds synthesized by the University of Sassari, using PLV-S and SARS-CoV-2. These compounds were designed as entry inhibitors for various RNA viruses.

Methods: Cytotoxicity of the panel of compounds was evaluated on 293T/ACE2 cells, Huh7 and A549 cells using Alamar Blue cell viability assay. To produce PLV-S, HEK-293T cells were transfected using PEI and a second-generation packaging system. As a reporter gene, firefly luciferase was used. To perform drug screening using PLV-S, 293T/ACE2 cells were transduced with PLV-S in the presence of various concentrations of compounds. Time of administration was also assessed. 72h post transduction cells were lysed and firefly luciferase activity was determined. To evaluate inhibition of SARS-CoV-2 infection performed by these compounds, Huh7 cells were infected with 0.1 multiplicity of infection in the presence of various concentration of drugs. Supernatants of infected cells were analyzed using qRT-PCR, while infected cells were analyzed using Western Blot. These compounds were tested also against other two viruses, Cocksackie serotype B5 (CoxB5) and VSV, on A549 cells.

Results and conclusions: Some of these compounds shown a strong inhibition of the transduction efficiency of PLV-S, especially when cells were treated with the compounds prior to transduction with PLV-S. The most promising compounds were tested also against SARS-CoV-2, VSV and CoxB5. Real-time PCR on supernatants of infected cells and Western Blot on cell lysates confirmed inhibition of SARS-CoV-2 infection, but not of VSV or Cocksackie virus. Some selected derivatives may be considered as lead compounds for future improvements aimed at COVID-19 treatment.

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EARLY IMMUNE SIGNATURE AS CORRELATE OF PRETECTIVE ANTIBODY RESPONSE TO THE BNT162b2 mRNA VACCINE

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Aim of the study: Cytokine and chemokine and key drivers of inflammation and innate immunity play a crucial role in the development and maintenance of adaptive immunity induced by infection and vaccination. Identification of cytokine and chemokine signatures early after vaccination that are predictive of vaccine-induced protection and sustained immunity would be fundamental for vaccine optimization. Given these premises, aim of this study was to identify early cytokine and chemokine markers of successful vaccination with COVID-19 mRNA vaccines.

Methods: To assess the early immune events occurring post mRNA vaccine administration, we conducted an observational study in 50 healthcare workers who were longitudinally followed up before and after the first, second and third boosting doses of BNT162b2 mRNA vaccine. A panel of 48 cytokine and chemokines was measured by a multiplex immunoassay in serum samples collected at baseline and at 24 h after each vaccine dose. Expression of interferon (IFN)-inducible genes was analyzed by qRT-PCR in PBMCs collected at baseline and 24 h after each vaccine dose. SARS-CoV-2 RBD binding IgG antibodies and neutralizing antibodies (NTAb) were titrated in serum at baseline, 21 days after the first dose, 2 weeks and 6 months after the second dose, on the day of the third dose and 1 month after the third dose.

Results: We identified an early type I and II IFN-inducible gene transcription in PBMC and innate cytokine/chemokine profile in sera, that included the cytokines IL-15, IL-6, TNF- α and IFN- γ and the chemokines IP-10, MCP-1, MIP-1 β and MIG, strongly induced 24 h after the second vaccine dose, that correlated with the magnitude of binding and NTA response to vaccination in healthy individuals. Analogous results were also observed after the third boosting dose. Individuals with prior SARS-CoV-2 immunity showed a strong cytokine signature after the first vaccine dose. Baseline levels of some cytokines (type I IFN, IL-9, IL-13) were lower in males than in females, but vaccination led to a significantly higher increase of pro-inflammatory cytokines (IL-1 β , IL-2, IL-5, IL-6, IL-8, IL-18) in males than in females. Higher induction of pro-inflammatory cytokines correlated with the occurrence of systemic adverse events to vaccination.

Conclusions: This study suggests that the early regulation of specific immune factors affects the magnitude and the quality of vaccine-induced adaptive responses and that it might be predictive of vaccine-induced humoral protection.

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Background: The origin of the COVID-19 pandemic has been due to a new SARS-CoV-related Betacoronavirus. From the first cases of patients who developed symptoms on 1 December 2019, rapidly spreading inter-human transmission around the world was declared a pandemic by the WHO in March 2020. Today it is increasingly evident that, although vaccines are the main solution to counter the pandemic, their long-term efficacy and their worldwide availability is limited. Furthermore, existing antivirals and treatment options against COVID-19 have shown only limited efficacy to date. It is known that most RNA viruses can often develop large deletions in their viral genome during viral replication. These defective interfering genomes have been described and appear to be common even in coronaviruses where they have been used to locate the functional packaging elements of their genomes that are critical to the process of mature virion formation.

Aim of the study: The main objective is the development of new antiviral strategies based on the use of nucleic acids (Nucleic acid based) drawn on the packaging genomic regions of SARS-CoV-2.

Materials and Methods: The cells used were Vero E6 cells passed in the laboratory using the DMEM culture medium supplemented with 10% fetal serum. The viruses used were SARS-CoV-2 clinical isolates grown on Vero E6 cells and titrated by the plaque method. The sequences of phosphorothioate oligonucleotides (S-ON) were designed by selecting the SARS-CoV-2 genomic RNA stem-loop packaging structures within the 3' end of the ORF1ab. The synthesis of S-ONs of variable length from 6 to 19 nt were performed according to traditional methods and using the modifications to make them stable. The selected S-ON compounds were tested for the inhibitory capacity of SARS-CoV-2 variants. Compounds were tested at different concentrations (0.1-100 μ M) on 24-well plates containing Vero E6 cells using different multiplicities of infection (MOI). To facilitate the entry of S-ON into cells, Lipofectamine is used as a carrier lipid. The inhibitory activity was analysed by seeding the viral supernatants treated with S-ONs collected after 72 hours on 6-well plates containing Vero E6 cells and analysing plaque reduction (PRA) at 3 days compared to the viral control grown in absence of S-ONs. The cytotoxicity of candidate S-ON compounds were evaluated by the MTT reduction assay on Vero E6. All experimental procedures were conducted under biosafety level 3 containment.

Results: The S-ONs used in this study were derived from the SARS-CoV-2 RNA stem-loop structures within the 3' end of the ORF1ab previously identified as packaging sequences and common to SARS-CoV and other CoVs. The 20 S-ONs were synthesized by using the genomic and complementary sequence and were partially overlapped. Among the S-ONs tested in the PRA assay, several compounds exhibited a fifty percent inhibitory concentration antiviral activity ranged from 0.01 to 34 μ M. The S-ON with a scrambled sequence used in the same condition was not active. Moreover, selected S-ON compounds were tested using different MOI and also against different SARS-CoV-2 variants reported comparable antiviral activity. Importantly, under the experimental conditions used, neither the

S-ONs associated with liposomes at any concentration nor liposomes alone exerted more than 12% reduction in cell viability, thus excluding that inhibition of was merely a consequence of compound cytotoxicity. Experiments to evaluate the intracellular localization of the S-ONs and a potential target of the inhibitory activity are ongoing.

Conclusions: Collectively the data indicate that S-ONs derived from the SARS-CoV-2 packaging region in the 3' end of the ORF1ab have the ability to potently and specifically suppress SARS-CoV-2 growth and are promising candidates for further investigation to development nucleic-acid viral interfering antiviral therapy.

GLICOPRO, NOVEL STANDARDIZED AND STERILE SNAIL MUCUS EXTRACT FOR MULTI-MODULATIVE OCULAR FORMULATIONS: NEW PERSPECTIVE IN MANAGEMENT OF EYE DISEASES

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Aim: This study aimed to evaluate the mucoadhesive, regenerative and anti-microbial properties of a novel lubricating multimolecular ophthalmic solution (GlicoPro®) extracted from snail mucus and its potential anti-inflammatory and analgesic role in the management of eye diseases, in comparison with sodium hyaluronate and trehalose.

Methods: GlicoPro, sodium hyaluronate and trehalose (Thealoz duo) bio-adhesive efficacy was assessed using a lectin-based assay, and its regenerative properties were studied in a human corneal epithelial cell line. *In vitro* DED was induced in human corneal tissues; the histology and mRNA expression of selected genes of inflammatory and corneal damage biomarkers were analyzed in DED tissues treated with GlicoPro. Anti-viral properties were assessed on *in vitro* experimental models of Herpes simplex 1 virus (HSV-1) infections (0.01 PFU/cell) on Vero cell line (ATCC-CCL81).

Results: A higher percentage of bio-adhesivity was observed in corneal cells treated with GlicoPro than with sodium hyaluronate and trehalose-based compounds. In the scratch test GlicoPro improved *in vitro* corneal wound healing. Histo-morphological analysis revealed restoration of cellular organization of the corneal epithelium, microvilli, and mucin network in DED corneal tissues treated with GlicoPro. A significant reduction in inflammatory and ocular damage biomarkers was observed. High-performance liquid chromatography-mass spectrometry analysis identified an endogenous opioid, opiorphin, in the peptide fraction of GlicoPro. After 2 hours from HSV-1 infection of Vero cells, the treatment with GlicoPro was able to reduce 3 log the HSV-1 titre, similar to the effect obtained with acyclovir 10^{-5} M. Interestingly, the anti-viral effect is dose-dependent, and it is already evident 24 hours after HSV-1 infection.

Conclusions: GlicoPro induced regeneration and bio-adhesivity in corneal cells; moreover, considering its anti-inflammatory, analgesic anti-viral properties, this novel ophthalmic lubricating solution may be an innovative approach for the management of eye diseases.

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Even though acyclovir and related nucleoside analogues are regarded first-line pharmacological therapies for herpetic infections, frequent drug treatments tend to result in drug-resistant Herpes Simplex (HSV) strains [Piret J. et al. 2011]. Therefore, the discovery and development of antiviral drugs represent the primary challenge to combat viral infections and prevent resistance. In this perspective, natural products, such as crude extracts and bioactive molecules derived from plants, have been recognized as effective in fight against various viral infections [Musarra Pizzo M. et al. 2021]. Naturally derived molecules have been recognized as highly promising sources of novel antiherpetic compounds due to lower toxicity and mechanisms of action alternative to nucleoside analogues [Schnitzler P. et al. 2010, Ben-Amor I. et al., 2021, Bisignano C. et al. 2017].

Thus, in this study, the effectiveness of polyphenols-rich extracts of natural raw (NRRE) and roasted unsalted (RURE) pistachios was evaluated on HSV- infected Vero cells. Pistachio polyphenolic extracts were obtained using two distinct extraction procedures, with or without *n*-hexane.

The RP-HPLC-DAD analysis were carried out to identify the phenolic components in both extracts. Cytotoxicity studies were performed to measure cell viability following the treatment time course. Besides, plaque reduction assay, Real Time PCR and western blot analysis were employed to detect viral DNA and viral proteins and test the anti-HSV activity of NRRE and RURE.

The RP-HPLC-DAD results reported a higher concentration of polyphenolic compounds in NRRE than RURE extracts. Besides, cell viability was determined by tracking the metabolic activity of cells after treatment with NRRE and RURE at different doses (0.2, 0.4 and 0.8 mg/mL), and the NRRE and RURE extracts prepared without the use of *n*-hexane have a better profile of cellular compatibility. Furthermore, non-toxic doses of NRRE and/or RURE (0.6, 0.4 and 0.3 mg/mL) were used in a plaque reduction assay to monitor the anti-viral effect. Both extracts were used for 1-hour pre-treatment on cells and virus, then blended to enable viral adsorption. Excess unabsorbed virus was removed after 1 hour and the monolayer was covered with Dulbecco's Modified Eagle's Medium containing 0.8% methylcellulose with both extracts, separately. It was observed that, at 0.6 mg/mL, NRRE and RURE had a considerable inhibitory effect in Vero cells, and that treatment with *n*-hexane-extracted compounds resulted in a significant reduction of plaque numbers and size, as demonstrated by morphological change and micro-plaques detection. Accordingly, NRRE and RURE *n*-hexane were employed to treat cells and virus suspensions and thus to detect viral DNA and genes. At 0.4 and 0.6 mg/mL, NRRE dramatically reduced viral DNA and genes, while viral protein expression was only reduced at higher concentrations. Conversely, at 0.6 mg/mL, RURE had little effect on viral DNA, however did lower viral transcripts and protein. These results highlight the anti-herpetic potential of pistachio polyphenols and remark their role in the formulation of innovative topical or oral pharmaceutical preparations to treat HSV-1 infections, alone or in addition to conventional antiviral medicines.

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Hydrolates and Essential Oils (EOs) are natural compounds obtained from the steam distillation or hydro-distillation of fresh medical plants, widely used for their antimicrobial activity. In recent years, the food industry is evaluating new ways to use effective natural antimicrobials to improve food safety and is exploring the antibacterial, antiviral and antifungal properties of hydrolates and EOs. This study aimed to compare the antiviral effect of EOs and hydrolates obtained from *Citrus limon* and *Thymus serpyllum* on Murine Norovirus (MNV-1), a surrogate to human noroviruses.

Concentrations, from 0.01 to 1% v/v of EOs (emulsified in 33% sunflower oil, 0.1% tween 80 and physiological saline solution) and hydrolates did not exert cytotoxic effect on cell line RAW 264.7 (MNV-1 permissive cells). The highest concentration (1%) was used to treat an MNV-1 suspension (2.7×10^5 TCID₅₀/ml) to evaluate the effect on viral infectivity. In detail, treated virus aliquots were analysed immediately (t=0) and after 24 hours (t=24) of incubation at $20 \pm 2^\circ\text{C}$ with each of the EO or hydrolate. Untreated viral suspensions, hydrolates solutions and EOs emulsions were used as positive and negative controls, respectively.

The EO and hydrolate derived from *T. serpyllum* reduced MNV-1 infectivity by about 1.8 log directly after treatment (t=0), but did not provide a further significant decrease at t=24. Instead, the EO and hydrolate of *C. limon* exerted an immediate (t=0) reduction of the viral infectivity of about 1.3 log and 1 log, respectively, followed by a further reduction of infectivity of 1 log after 24h for the treatment with the hydrolate. A natural loss of infectivity (approximately 1 log) of untreated virus after 24 h was also observed.

In conclusion, the results obtained with *C. limon* and *T. serpyllum* showed relatively similar antiviral activity, both for EOs and hydrolates. This opens encouraging perspectives on using hydrolates, which are easier to use, especially in food technology, as they support their efficacy at low concentrations and short contact times.

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**POTENTIAL ACTIVITY OF FUNGAL SECONDARY METABOLITE
6-PENTYL- α -PYRONE AGAINST CANINE CORONAVIRUS**

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Aim of the study: Genotype II of canine coronavirus (CCoV-II), an alphacoronavirus, can provoke moderate to severe enteric disease in dogs. The recent pandemic of SARS-CoV-II has turned the spotlight on coronaviruses, that are able to mutate into new and more dangerous strains. Indeed, it has been detected a novel canine-feline recombinant alphacoronavirus isolated from a human patient with pneumonia. Remarkably, if confirmed as a pathogen, it might be the eighth unique coronavirus established as agent of disease in human. To date, drugs such as indomethacin has been shown to have potent antiviral properties against CCoV. Thus, no toxic antiviral compounds are considered necessary to fight CoVs infections. Fungi produce many secondary metabolites (SMs), several of which have been developed for application like antibiotics, fungicides, plant growth regulators and hormones. Recent screening performed on benzo- γ -pyrone 3-O-methylfunicone, a SM produced by *Talaromyces pinophilus*, demonstrated that it reduces infectivity of hepatitis C virus, as well as of bovine herpesvirus 1. 6-pentyl- α -pyrone (6PP), a SM produced by fungi belonging to *Trichoderma* genus, plays an important role in defense against plant pathogens. In addition, 6PP it has been revealed anti-biofilm-producing bacteria activity. Up to now, no studies about the potential antiviral activity of 6PP are known. Consequently, herein to evaluate the antiviral efficacy of 6PP, we performed *in vitro* tests against CCoV-II.

Methods used: In this study, during infection with CCoV-II (reference strain S/378) in a canine fibrosarcoma (A-72) cell line, *in vitro* bio-screen, immunofluorescence staining, cytomorphological and virus yield analyses were performed.

Results and conclusion: Briefly, monolayers of A72 cells were either infected or not infected with CCoV, in the presence or absence of 6PP at different concentrations (0.001, 0.01, 0.05, 0.1, 0.5 and 1 μ g/mL) to obtain four groups: untreated uninfected cells; untreated infected cells; 6PP-treated uninfected cells; 6PP-treated infected cells. 6PP at the concentration of 0.1 μ g/mL did not significantly modify A72 cell viability and cell proliferation as compared to untreated uninfected cells. Following infection for 48 h, the non-toxic concentration of 0.1 μ g/mL of 6PP markedly increased cell viability as well as cell proliferation. These results were accompanied by reduced cell death features, as highlighted by Giemsa staining. Moreover, 6PP noticeably diminished the cytopathic effect and significantly decreased the expression of the nucleocapsid protein NP, a structural protein of CoVs, responsible for binding the viral RNA genome and commonly more stable than CoV spike protein, which may have a higher mutation rate.

Taken together, our preliminary results suggest that, at nontoxic concentration, 6PP shows potential antiviral activity during CCoV infection. Of note, in the screening of potential antivirals, our *in vitro* animal model of CoVs avoids the manipulation of extremely hazardous CoVs (SARS-CoVs, MERS-CoV).

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The zoonotic transmission of HEV-3 has been confirmed by sequencing identical or strictly related viral strains in humans, wild boar, and derived food. The HEV sequences classified within the HEV-3 genotype are highly variable, and the observed differences allow for the further classification of the HEV-3 genotype into subtypes. The biological role of subtypes is still unknown but it could determine host specificity or different outcomes of the disease.

Aim of the study: The aim of this study was to evaluate the genetic variability and the evolution of HEV-3 strains isolated from a wild boar population from Central Italy (Lazio and Umbria, two neighbouring regions). The study was conducted on eight HEV-3 strains subjected to whole genome sequencing and analyzed by phylogenetic methods.

Methods: Total RNA from eight wild boar livers, HEV-3 positive, was extracted by QIAmp Viral Mini kit (Qiagen, Milan, Italy) and used to prepare the libraries for Next Generation Sequencing by metagenomic approach. The libraries were sequenced by Ion System S5 (Thermo Fisher Scientific, Rome, Italy) on a 520 Ion chip and the complete genome built using Galaxy instance (Aries). Sequence comparison of complete genomes and short ORF2 genomic fragments were performed using BlastN. The Maximum Likelihood tree was built using IQtree2, with 1000 bootstraps and the best model chosen by IQtree2ModelTest.

Results and conclusions: The complete genomes obtained from wild boar were classified within the HEV-3a, HEV-3c, HEV-3f subtypes and two new recently proposed subtypes (HEV3*, HEV-3n). In Italy, HEV-3f is the subtype most frequently found in wild boar, included in a cluster with other swine and human strains. HEV-3a is rare in Italy in both wild boar and humans, it is unknown if it is not adapted to humans. Furthermore, detection of HEV-3* and HEV-3n only in Italy and in wild boar, suggest that the two subtypes (3*, 3n) could have originated only recently in Italy and could not have spread to other countries yet or for a low circulation of this subtypes, not yet reported, abroad. Sequence analyses confirmed a main role of wild species in maintenance of viral diversity.

In conclusion, extensive sequencing of HEV in humans and animal reservoirs is needed to fully understand diversity and evolution of these viruses.

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Aim: EU is the second worldwide producer of pig meat after China and the biggest exporter. In 2020, 23 million tons of pig meat were produced and 5.5% comes from Italy. Due to the importance of this production for economy and public health, being pork widely consumed, pork food chain is strictly monitored to guarantee food safety. Zoonotic viruses can be foodborne and pigs are frequent reservoirs. Hepatitis E virus (HEV) is recently recognized as an emerging viral zoonotic foodborne pathogen and represents the cause of foodborne outbreaks related to pork consumption.

Methods: In the framework of the European Joint Programme "Biosecurity practices for pig farming across Europe (BIOPIGEE)", the presence of HEV in pig farms over 16 EU countries was evaluated. In Italy, 47 pig farms were investigated, including both indoor and outdoor. In particular, 22 finishing, 18 breeding, 7 farrow-to-finish farms were included in the study, and 958 pooled fecal samples were collected. HEV viral genome was detected using RT-real time PCR.

Results and Discussion: HEV was detected in 16/47 (34.0%) farms with the highest prevalence in finishing farms (9 farms positive out of 22, mean individual prevalence 53.9%). In conclusion, according to our results, HEV is widespread and difficult to prevent, requiring constant monitoring. One approach to prevent the occurrence of HEV-contaminated food at point of sale is reducing the occurrence of the infection at farm. The implementation of biosecurity measures at farms remains an important strategy to reduce the risk impact of diseases linked to food products of animal origin.

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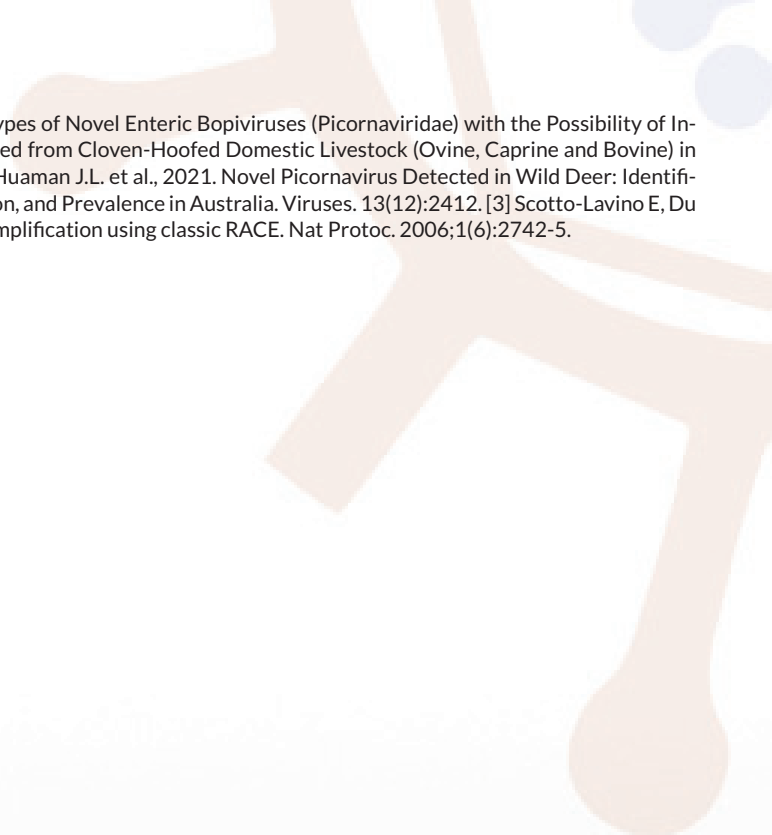
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Highly divergent picornaviruses (PVs) classified in the genus *Bopivirus* have been recently discovered by next-generation sequencing on faecal samples from sheep and goats in Hungary [1] and from fallow and red deer in Australia [2]. On full genome sequence analysis, these novel PVs displayed the highest relatedness (57.0-79.0% nucleotide [nt] identity) to the bovine PV TCH6/2013/USA (NC_026249), prototype strain of the species *Bopivirus A*. Following strictly the ICTV classification criteria for PVs species demarcation, the ovine/goat and deer bopiviruses have been tentatively classified into two novel species designated respectively *Bopivirus B* and *Bopivirus C* [1,2]. In this study, we investigated the epidemiology of these novel viruses in domestic and wild ruminants from Northwestern Italian Alps by molecular screening of 508 faecal samples collected from 128 sheep, 167 goats, 61 red deer (*Cervus elaphus*), 77 roe deer (*European roe deer*), 43 chamois (*Rupicapra rupicapra*) and 32 Alpine ibex (*Capra ibex*). Total RNA was extracted by TRIzol LS procedure and analysed by conventional RT-PCR using consensus primers HBG/F and HBG/R [1]. *Bopivirus* RNA was detected in a total of 19 animals, including 14 sheep (10.9%), 2 red deer (3.3%), 1 roe deer (1.3%), 1 chamois (2.3 %) and 1 Alpine ibex (3.3 %), but not in goats (0/167, 0%). On BLAST analysis, all sequences detected in ovine, chamois, roe deer and Alpine ibex faecal specimens shared 94.1-100% nt identity to each other and displayed the closest identity (94.7-98.5%) to sheep and goats bopiviruses (*Bopivirus B*) previously detected in Hungary [1], whilst the strains found in red deer exhibited the highest identity to deer bopiviruses (*Bopivirus C*) identified in Australia [2]. The full-length genome of strains ITA/Sheep/1473/2020 and ITA/Alpine ibex/12/2020 detected in a sheep and in an Alpine ibex, respectively, were determined by combining a modified 3' - RACE protocol [3] with Oxford Nanopore Technologies sequencing platform. Sequence trimming and assembly of reads was performed by using Genome Detective Virus Tool (<https://www.genomedetective.com/app/typingtool/virus>). Both sequences generated included a 5' and 3' untranslated regions (UTRs) and a unique ORF of 6618 nt in length encoding a polyprotein of 2202 amino acids. On phylogenetic analysis, both the strains segregated into the proposed species *Bopivirus B* along with ovine and caprine strains detected in Hungary [1]. The finding of this study provides firm evidence that these novel PVs are common component of the faecal virome of domestic and wild ruminants. Furthermore, the identification of bopivirus in roe deer, chamois and Alpine ibex expands the host range of these novel viruses and hints to a possible virus circulation between domestic ruminants and wildlife animals. Large structured epidemiological surveys and comprehensive characterization of bopiviruses strains identified from other geographic areas might help clarify their global distribution, genetic heterogeneity and the possible clinical impact on ruminants.

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Aim of the study: Coronaviruses (CoV) comprise a large number of enveloped, positive-sense single-stranded RNA viruses causing respiratory, enteric, hepatic and neurological diseases of varying severity in domestic and wild animals, as well as in humans. CoVs are constantly evolving, and may acquire the ability to cross host species barriers, posing a serious threat to humans, as shown by the currently ongoing devastating COVID-19 pandemic. With the exception of the recent advances in the antiviral treatment of SARS-CoV-2 infection, no effective antiviral drug or vaccines are presently available for most CoV. Cyclopentenone metabolites of arachidonic acid (AA) have been known for several years to possess a broad-spectrum antiviral activity; in particular, an effective antiviral action of the cyclopentenone prostaglandin A1 (PGA1) has been demonstrated against respiratory viruses, including influenza and parainfluenza viruses; however, there is still no information on the effect of cyclopentenone prostaglandins on coronavirus infection. Herein we investigated the effect of PGA1 and other AA metabolites on coronavirus replication, in two selected *in vitro* models of canine (CCoV) and feline (FCoV) coronavirus infection.

Methods: Canine adenocarcinoma (A72) and feline kidney (CRFK) cells were infected, respectively, with CCoV (strain S-378) and FCoV (strain 25/92), kindly provided by Prof. Canio Buonavoglia, University of Bari, under single-step and multi-step growth conditions. PGA₁, PGE₂, PGF₂ α , 15-deoxy- Δ 12,14-Prostaglandin J₂ (15d-PGJ₂) and AA (Cayman) were dissolved in ethanol, unless differently specified. Virus yield was determined by TCID₅₀ infectivity assay, and cell viability was determined by MTT assay. Viral and cellular proteins were characterized by Western-blot analysis, and CCoV and FCoV genomic RNA levels were determined by qRT-PCR. For RNA transfection experiments, cell monolayers were transfected with CCoV or FCoV genomic RNA, and treated with the selected compounds after transfection.

Results and Conclusions: PGA1 was found to possess a remarkable antiviral activity against both canine and feline coronaviruses at submicromolar concentrations (CCoV: IC₅₀ = 0.56 μ g/ml; FCoV: IC₅₀ = 0.69 μ g/ml), and to protect the host cell against the virus-induced damage. PGA1 treatment does not affect virus adsorption or entry into the host cell, but acts at a postentry level, as confirmed by viral genomic RNA transfection experiments. Analysis of viral proteins shows that PGA1 treatment reduces the expression of CCoV and FCoV structural proteins; in addition, CCoV and FCoV RNA analysis shows that PGA1 causes a significant reduction in viral RNA levels in both models, starting at early stages of viral infection. As expected, the antiviral activity of PGA₁ was associated with the activation of the heat shock response and the induction of heat shock proteins in infected cells. Similar results were obtained with the cyclopentenone prostanoid 15d-

PGJ₂, whereas the non-cyclopentenone prostaglandins PGE₂ and PGF_{2α} had no effect. Surprisingly arachidonic acid was found to significantly inhibit both CCoV and FCoV replication. In conclusion, we demonstrate for the first time an anti-coronavirus activity of cyclopentenone prostanoids; these results may represent the basis for investigating new therapeutic strategies against emerging CoV of zoonotic origin.

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The family Tobaniviridae, subfamily Torovirinae comprehends enveloped viruses. Toroviruses (ToVs) possess a linear, non-segmented, positive-sense single-stranded RNA genome (25-30kb), containing two large overlapping ORF1a and ORF1ab encoding two non-structural proteins and four small ORFs coding for structural proteins, namely the spike (S), membrane (M), haemagglutinin-esterase (HE), and nucleocapsid (N) [1]. Currently, human torovirus (HToV), equine torovirus (EToV), bovine torovirus (BToV) and porcine torovirus (PToV) have been officially recognized at the species level. ToVs have been associated with gastroenteric signs, ranging from subclinical to severe enteritis.

In this study we report the identification of a recombinant BToV from a 20-day old calf with depression and bloody diarrhea during a metagenomic investigation. A total of 38 fecal samples (21 from animals with enteric signs and 17 from clinically healthy animals) were examined using Illumina technology (NextSeq 500 platform). The data were analyzed with the online platform: Genome Detective Viral Tool, version 1.136 (<https://www.genomedetective.com>) as well as with the software package Geneious Prime v.2022.1.1. In sample #572/19-5, a total of 1405062 reads were classified as viral of which 103676 reads were mapped to ToV, obtaining a contig of 28007 nucleotide (nt) in length encompassing the full-length genome with a depth of coverage of 474X. The consensus sequence was analyzed with FASTA (<https://www.ebi.ac.uk/Tools/sss/fasta/>) and BLAST Tool Basic Logic Alignment Search Tool (BLAST, <https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to find homologous hits. The sequence showed a 97.59% nt identity to the strain BToV SC-1 Sichuan/2018 (GenBank accession MN073058) [2] and 96.34-96.56% nt identities to BToV Ishikawa/2010 (accession LC088094) and BToV Kagoshima/2014 (accession LC088095). Moreover, the BToV sequence of sample #572/19-5 showed a lower nt identity (81.6%) to the prototype BToV Breda/1979 (accession AY427798) [3]. BToV Ishikawa/2010 and Kagoshima/2014 have been characterized as recombinant strains between BToV Breda/1979 and PToV, with two recombinant breakpoints, located at the 3' end of the ORF1b coding region and at the 3' end of HE coding-region. As a result, the nearly 76% of the genome is similar to PToV genome whilst the S gene is of bovine origin [4]. Accordingly, BToV strain #572/19-5 is a porcine-bovine ToV recombinant, similar to viruses described in Japan and China [3,4]. This finding points out a remarkably diversity between BToVs. Improve diagnostics to detect and discriminate the two different BToV types could be helpful to better assess the epidemiology of these enteric viruses and to understand if there are relevant phenotypical differences. This information could be helpful to understand better the possible zoonotic origin of HToVs.

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LOW PREVALENCE OF HEPATITIS E VIRUS IN FOOD PRODUCTS FROM A HYPERENDEMIC REGION (ABRUZZO, ITALY)

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Hepatitis E virus (HEV) is a zoonotic virus whose transmission dynamics vary worldwide, genotypes G1 and G2 being restricted to humans and mostly to low-income countries (waterborne transmission), and G3 and G4 being zoonotic, and often associated to foodborne transmission in developed countries. Aim of this work was the investigation of HEV occurrence in widely consumed foods in a known hotspot for HEV circulation, the region of Abruzzo (Italy).

A total of 129 food samples (including 58 ready-to-eat meat products, 45 fruits/vegetables and 26 bivalve shellfish) were collected at primary production sites or at retail between January and December 2021 in the four provinces of the region of Abruzzo. Meat products included sausages, salami and other cured meat products without liver cuts inside, fruits and vegetables included products usually consumed without cooking or other inactivating treatments (strawberries, lettuce, baby spinaches, tomatoes, etc.), and bivalve shellfish samples comprised mussels and clams. Virus concentration was carried on using different matrix-specific protocols based on Szabo et al. (2015) for meat products, and on ISO 15216 for the other food matrices. Screening for HEV presence was done by a real-time RT-PCR targeting the ORF3, and included specific controls for viral extraction efficiency and for PCR inhibitors.

None of the analysed food products tested positive for HEV RNA (prevalence: 0%, 95%CI: 0 – 3.5%), hence displaying a low risk of exposure to the virus through these food categories. However, as genotype 3 HEV is widely present in swine and in the wild boar populations of Central Italy and pig and wild boar liver may be used as an ingredient in traditional and homemade cured meat products, further studies are required to specifically assess HEV occurrence and levels in liver-containing foods in this hyperendemic area.

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Astroviruses are single-stranded, positive sense RNA viruses mainly associated with gastroenteritis in humans and animals. A variety of unusual astrovirus strains, different from classical human astroviruses, that are commonly associated with acute gastro-enteritis (AGE), have been identified from the stools of patients with and without AGE and from the central nervous tissues of patients with neurological disease. Astrovirus-associated neurological disease has also been identified in several animal species. The *Astroviridae* family show high genetic plasticity and diversity, posing a challenge for classification. Also, several examples of inter-species transmission have been gathered during epidemiological studies in different hosts. Bivalve shellfish filter their surrounding waters to absorb nutrients and are therefore a good indicator to monitor viral presence in marine environments. The present study aims to investigate the presence of astroviruses in the aquatic environment through shellfish testing.

The aim of this study was to investigate the presence of non-classical human astroviruses (Mamastrovirus species 1) in shellfish. A total of 48 shellfish samples (*Crassostrea gigas*) were collected between November 2016 and October 2018 in four locations in the northern and southern Adriatic Sea and in the northern and southern Tyrrhenian Sea, respectively. Samples were prepared by digestive tissue dissection and homogenization, and nucleic acid extraction. A multi-target strategy was used, based on different primer sets specific for MLB 1 and 2 human astroviruses and consensus primers (pan-astrovirus) optimized to recognize most members of the Family *Astroviridae* targeting the RdRp region of the genome.

Eleven samples (23%) and 7 samples (14.5%) yielded weak signals by the MLB-specific and pan-astrovirus primers, respectively. While the uttermost part of the amplicons did not generate good-quality sequences, likely due to mixed viral populations or to nonspecific signals, three samples yielded astrovirus sequences. Two sequences had 78.8% and 77.1% nucleotide identity to Beihai astro-like virus (accession KX884564.1) and the other had 74.6% nucleotide identity to marine animal astrovirus (accession KM587711.1).

These findings, whilst highlighting the diversity of astroviruses, suggest the possibility that in raw or poorly cooked food destined to human consumption, there may be astroviruses of unknown nature that go undetected with current diagnostic protocols.

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STUDY ON THE CIRCULATION OF CORONAVIRUSES IN HEDGEHOGS (*ERINACEUS EUROPAEUS*) IN THE MUNICIPALITY OF ROME: PRELIMINARY RESULTS

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Aim of the study: To clarify the circulation and evolution of Coronaviruses in Italy, 41 feces from European hedgehogs (*Erinaceus europaeus*) were collected from a rehabilitation center in central Italy (Rome) and tested for the presence of EriCoV species. The feces from three animals have been collected for 2 months to establish the minimum duration of infection from EriCoV.

Methods: Feces from 41 hedgehogs (*Erinaceus europaeus*) hosted in a “Wildlife Rescue Center (LIPU)” in Rome were collected each two days until the released of the animal or the dead, from Rome Province. Total RNA was extracted by QIAmp Viral Mini kit (Qiagen, Milan, Italy) from 150µl of a 10% fecal suspension. The Reverse Transcription-PCR (RT-PCR) amplification was performed using the QIAGEN One-Step RT-PCR Kit (Qiagen) to amplify a short genomic region between the 3´ end of the spike gene and the 5´ of ORF3a gene for the detection of EriCoV and the CD200 ortholog insertion (1). Amplicons were sequenced with sanger sequencing. The Maximum Likelihood phylogenetic tree has been built using MEGAX.

Results and discussion: Twenty-eight animals (68%, 28/41) were positive for EriCoVs, as confirmed by PCR and sequencing analysis confirm a high prevalence of EriCoV positivity. Strains detected showed a strict phylogenetic relationship and were correlated to EriCoV strains reported in Germany, Great Britain and Northern Italy (1). None of the sequences reported the CD200 ortholog insertion observed in animals from Northern Italy (2). Three animals were monitored for 6-8 weeks and showed a persisted shedding of EriCoV in feces up to 18 days, however, the positivity of some animals was recurrent.

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Rep-encoding single-stranded (CRESS) DNA viruses are the smallest known viral pathogens that can infect eukaryotes and are widely distributed in different ecosystems. The rolling circle replication mechanism of these viruses is based on the replication initiation protein Rep. CRESS DNA viruses include the *Circoviridae* family that is further classified into two genera, *Circovirus* and *Cyclovirus*. Circoviruses (CVs) are nonenveloped spherical viruses with a small single-strand circular DNA genome of approximately 2 kb length. CVs have been found in several animal species, and they are largely known for causing serious health conditions in pigs and birds. CVs have been found also in human stools, blood, and cerebrospinal fluid (CSF). In 2012 a novel CV was described in dogs (CaCV) related to respiratory and gastrointestinal disorders and in some case to systemic disease involving vasculitis and hemorrhage (1). In cats, a feline cyclovirus has been reported in pooled fecal samples in a metagenomics study (2). Lately, a novel unclassified feline CRESS DNA virus has been retrieved from fecal samples of cats with diarrhea (3).

In this study, a total of 530 samples from cats (361 sera, 131 stools, and 38 respiratory swabs), collected at the Department of Veterinary Medicine, University of Bari, Italy, during the 2011- 2021 period, were screened for CVs using a nested PCR protocol with broadly reactive primers able to amplify a portion of (~400bp) the Rep sequence (4). Overall, CV DNA was detected in 47 (8.9%) of 530 samples. In detail, CV DNA was identified in 9/361 (2.5%) sera, 32/131 (24.4%) stool samples, and 6/38 (15.8%) respiratory swabs. The positive samples were sequenced, obtaining 29 (61.7%) sequences of acceptable quality. The Rep sequences were characterized by interrogation of NCBI and EBI sequence databases using BLASTN and FASTA Nucleotide online software tools. In addition, a nucleotide alignment, using selected CV sequences retrieved from the databases, was used to analyze the data set with Geneious Prime vs 2021.1. On sequence and phylogenetic analyses based on the partial rep-gene, the sequences formed separate clusters, along with other identified CV strains. A large group of sequences (n=10) were tightly clustered together and showed similarity with viruses detected in bats, whilst one sequence was similar to another bat CV. Sequences similar to CV detected in bird (n=2), in fish (n=1), in pig (PCV3) (n=2), in dog (CaCV) (n=3) and in mollusks (Avon-like) (n=2) were obtained. Interestingly, sequences resembling to human-associated cycloviruses NG12 (n=2), TN9 (n=2), TN12 (n=2) and PK2111 (n=2) were also identified.

The majority of the CVs sequences (n=24) were recovered from feces and likely reflected a dietary origin or environmental contamination due to the wide range of animals preyed by cats. A large cluster with high genetic homogeneity (>98.3% nt identity) from the bat-like sequences was identified, and this could indicate a common nutrition source or a feline-adapted virus circulating in this mammal. Moreover, the detection of CVs sequences in serum, including CaCV (n=3), human cyclovirus TN12 (n=1), and Avon-like CRESS DNA

virus (n=1) might suggest virus replication in the animal host. Overall, these findings indicate a wide genetic diversity of CRESS DNA viruses in cats and warrant further investigation.

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TICK-BORNE ENCEPHALITIS ASSOCIATED TO THE CONSUMPTION OF CHEESE FROM RAW GOAT MILK: A CASE REPORT

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Tick-borne encephalitis virus (TBEV) is a single-stranded RNA virus of the Flavivirus genus. TBEV transmission is mainly through tick bites (particularly of the *Ixodes* species), but foodborne transmission through consumption of raw milk and fresh or cottage cheese from unpasteurized milk has also been increasingly reported in Europe. This study reported the investigation of a small TBE outbreak associated to goat cheese consumption.

On 26th December 2021 a man, age 49, was admitted to the hospital in Tione di Trento (Italy) with fever, nausea, headache, and myalgia. Based on patient declarations, symptoms had started four days before (22.12.2021). TBE infection was serologically diagnosed (IgM and IgG positive assay) on 27.12.2021. The patient did not remember having been bitten by ticks during open air activities, but during the investigation of the case a sibling of the man was also reported to have the same symptoms. According to patient declarations, both men run a small goat farm as a hobby and produced cheese for personal/family consumption. The goats never showed symptoms of disease. Four batches of soft cheese (*caciotta*) prepared by the siblings between 12th November and 3rd December 2021 using unpasteurized goat milk were collected from the patient's household by the Veterinary department of Local Health Unit of Tione di Trento. Analyses were carried out by the NRL for Foodborne Viruses at ISS following the viral concentration procedure described in Hennechart-Collette et al. (2017) and based on cheese homogenization, dilution with TGBE buffer pH 9.5, proteinase K digestion, and centrifugation to separate liquid phase from fats. Nucleic acids were extracted using the semi-automatic MiniMag platform (bioMérieux) and were further purified with an inhibitor removal kit (Zymo Research). TBEV RNA was then detected by a real-time RT-qPCR based on Schwaiger & Cassinotti (2003) with quadruplicate reactions. Appropriate recovery, inhibition and negative controls were included in the assay for quality assurance. While viral recovery from the samples was on average very low (<1%) due to the high fat content of the cheese, one of the batches (production on 12.11.2021) tested positive in all replicates (estimated TBEV concentration: 1.7×10^2 genome copies/g), hence confirming the presence of the virus in one of the products that had been presumably consumed by the siblings.

To our knowledge, this is the first reporting of virus detection in a dairy food suspected for TBEV transmission in Italy. Further studies (including estimation of infectious viral particles) are needed to improve our understanding of the risk of foodborne TBE.

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PEPTIDES MODELLED ON THE GLYCOPROTEIN Gn AND Gc OF SCHMALLENBERG VIRUS INHIBIT THE VIRAL INFECTION

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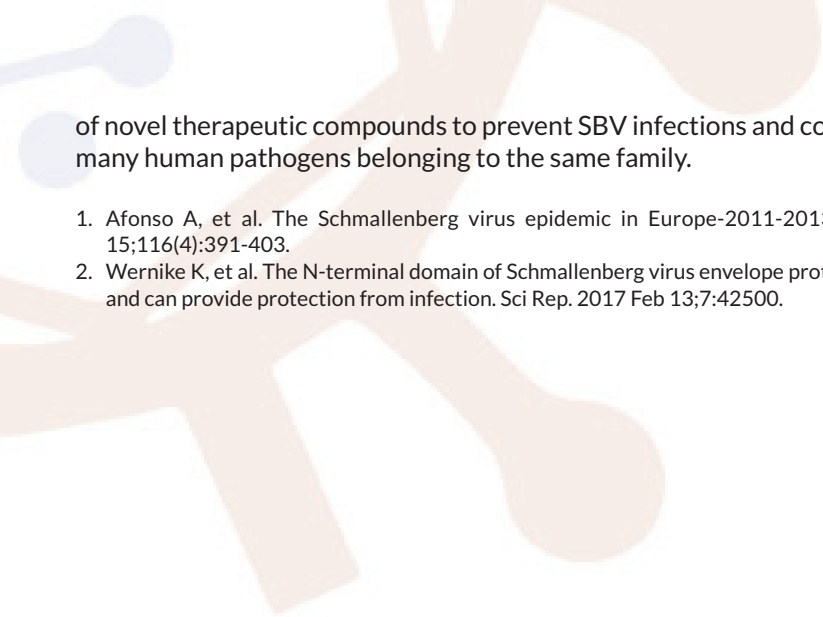
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Aim of the study: In recent years arthropod-borne viruses are emerging as serious threat to human and animal health worldwide. Among the Orthobunyaviruses, the Schmallenberg virus (SBV) represents a novel and major member, appeared in central Europe during the summer of 2011 and rapidly spread all over the continent [1]. The virus affects the ruminants causing abortion and malformation in newborns, as well as febrile episodes, decreased milk production and diarrhea in adults. The viral particle is enclosed in a membrane formed by the glycoproteins N (Gn) and C (Gc) able to mediate the viral entry by means of their fusogenic potential. The fusion peptide is located on Gc, a class II fusion protein, but cell fusion requires the expression also of Gn, a chaperone essential for the correct trafficking of Gc to the Golgi complex before viral budding [2]. Therefore, both Gn and Gc glycoproteins may represent a target for antiviral development. In the present study, we investigated the inhibitory activity mediated by synthetic overlapping peptides designed on the amino acid sequences of the proteins. SBV can be used as a model virus for studying peribunyaviruses, a complex and highly divergent family of RNA viruses.

Methods used: We have synthesized peptides by standard 9-fluorenylmethoxycarbonyl polyamine solid-phase synthesis. The crude peptides were purified by preparative reverse-phase high-pressure liquid chromatography. The identity of purified peptides was confirmed by Maldi spectrometry. Peptides cytotoxicity were evaluated on hamster kidney cells (BHK-21) at different concentrations starting by 100 μ M via the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Then cells were simultaneously treated with each peptide at 100 μ M and infected by SBV (gently provided by University Federico II of Naples). After 2 hours of incubation, cells were washed to remove all the unattached virus, and then were overlaid with a viscous medium. To better understand how peptides could interfere with the SBV lifecycle, different time of addition and temperature shift assays were performed at scalar concentrations. Furthermore, the expression levels of the viral M gene were analyzed.

Results and conclusions: We have performed a brute analysis of both glycoproteins in order to explore the inhibitory activity of such peptides against SBV infection. Five out of the Gc peptides at a concentration of 100 μ M reached the 50% of inhibition arbitrary cut-off. None of the Gn peptides had a consistent inhibiting effect and no peptide toxicity was observed by the MTT assay at the concentrations used in our experimental conditions. Our data indicate the possible direct involvement of the described domains in the process of virus penetration; therefore, these results are of relevance to the potential development



of novel therapeutic compounds to prevent SBV infections and could serve as a model for many human pathogens belonging to the same family.

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Introduction: Infective gastroenteritis (GA) represent a world public health relevant problem. Enteric viruses are the pathogens mainly involved in the episodes of GE, causing about 70% of the cases. In Italy most of the GA are not notified because of a mild symptomatology which does not require medical support. Only in cases of hospitalization (prevalently children) the cause of the disease is further investigated. However the viruses usually searched are the pathogens more commonly associated to GE (rotavirus, norovirus, and adenovirus) and very often (in 1/3 of the cases) the patient is discharged without knowing the infective agent. The diagnostic gap lead us to hypothesize that there are other viruses involved, as demonstrated by recent surveillance studies which show interesting prevalences of emerging viruses. In the present study we investigated the presence of 10 different viruses in the faeces of children hospitalized with GE in the Campania Region (Southern Italy). The same viruses were also investigated in samples of marine water and shellfish from the coastal area surrounding the Region with the aim to trace a picture of their circulation in the environment.

Materials and methods: We analyzed 70 faecal samples taken from children (5 months-10 years old) hospitalized with gastroenteric symptoms. We furthermore investigated 155 sea water samples taken from discharge points situated along the coast of the Region and 34 mussels samples (made of around 30 animals each) farmed in the Gulf of Naples. Waters were preliminary concentrated from 10 liters to 50 ml by filtration; faeces (100 mg) were first suspended in 900 µl phosphate saline buffer; hepatopancreas of 10-15 mussels (per sample) were pooled together and underwent a preliminary virus extraction step. Nucleic acids extraction was carried by KingFisher Flex automatic extraction system using the MagMax viral/pathogen II nucleic acid isolation kit. Each extract underwent Real time PCR analyses for the detection of the following viruses: adenovirus (AdV), norovirus GI and GII (NGI, NGII) astrovirus (AsV), sapovirus (SaV), aichivirus (AiV), rotavirus (RV), parechovirus (ParV), salivirus (SalV) and enterovirus (EV). For each viral target a protocol with specific primers and probes was carried out. NoV and AdV positive samples were further characterized by sequencing.

Results: Results showed that 58.6% of the feces (41/70) were positive to at least one virus. The virus most frequently identified was AdV, present in 24/41 samples (58.5%), followed by NGII (22/41, 53.7%), RV (11/41, 26.8%) and ParV (8/41, 19.5%); SaV, AiV and SalV were found only in 1-2 samples, while the other viruses (NGI, AsV and EV) were never identified. Looking at seawaters, 45/155 (29%) were positive to at least one virus with RV the preva-

lent one (22/45, 48.9%), followed by AiV (14/45, 31.1%), NGI and SaV (both 7/45, 15.5%), ParV (5/45, 11.1%) and NGII (3/45, 6.6%). EV, SaV and AdV were found only in few samples (1-2) while AsV was always negative. Mussels were 38.23% (13/34) positive to at least one virus. Results revealed the predominant presence of the two NoVs: GI 84.6% (11/13 samples), and GII 23.1% (3/13). SaV and RV were the only other two viruses identified (in 1-2 samples). Characterization of NGII discovered the occurrence of various genotypes: GII.33, GII.2, GII.1 (GII.P33), GII.17, with GII.2 the most frequent one. As to AdV we identified the serotypes 41 and B3. Of the NGI positive samples only one mussel gave a good sequence and the strain was genotyped as GI.3.

Discussions: Environmental samples showed an intense circulation of the enteric viruses, with the presence of all the investigated ones (but AsV). More than 50% of the feces exhibited at least one virus, some of them (17) showing the co-presence of more than one pathogen, with NGII and AdV almost always found together. One sample, belonging to a 8 months years old baby, was even contaminated by 4 viruses (NGII, SaV, AdV and ParV). The pathogens more prevalent in the feces where AdV and NGII, (both revealed in more than 50% of the samples). RV showed a definitely lower prevalence (around 1/4 of the samples) even if it was the main circulating virus in the environment. Considering that RV is the most common cause of GE in children < 5 years in the world, with a full range of severity of clinical presentations often requiring hospitalization, we could conclude that its lower involvement in GE (also with respect to high viral circulation) could be attributed to implementation of the vaccination plan in our Region. Surprisingly we identified ParV, an emerging virus, in 1/5 of the samples: further investigations would clarify its involvement (if any) in the GE episodes.

THE NUCLEOCAPSID PROTEIN OF COGUVIRUSES ACTS AS A WEAK RNA SILENCING SUPPRESSORS. Li¹, F. Ferilli^{2*}, M. Minutolo³, D. Alioto³, B. Navarro², E. Di Serio²

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Citrus concave gum-associated virus (CCGaV) and citrus virus A (CiVA) are two negative stranded RNA viruses (genus *Coguvirus*, family *Phenuviridae*) infecting citrus and also reported in apple and pear trees, respectively. CCGaV has been associated with concave gum disease in sweet orange, whereas CiVA has been associated with symptoms of impie-tratura on fruits of sweet orange and grapefruit. These viruses have a bipartite genome, with the negative-stranded RNA1 encoding the RNA-dependent RNA polymerase, and the ambisense RNA2 coding for the movement protein and the nucleocapsid protein (NP) in the genomic and anti-genomic strands, respectively. RNA silencing is a major plant defence mechanism against viruses. To achieve infection, most plant viruses counteract such a mechanism by expressing RNA silencing suppressor (RSS) proteins. Whether coguviruses encode RSSs is still unknown. In this study, the role of the NP of CCGaV and CiVA as RSS has been explored using a silencing suppression assay based on *Agrobacterium tumefaciens* infiltration. For this purpose, leaves of *Nicotiana benthamiana* plants, wild type (wt) or expressing constitutively the green fluorescent protein (GFP) (transgenic line 16c), were co-infiltrated with i) *A. tumefaciens* transformed with a binary vector containing the GFP reporter gene, and ii) *A. tumefaciens* transformed with a binary construct harboring the CCGaV NP or CiVA NP gene. HC-Pro of tobacco etch virus, a strong RSS, and the empty binary vector were used as positive and negative controls, respectively. In both plant systems (wt and 16c), the GFP fluorescence in the areas co-agroinfiltrated with CCGaV-NP and CiVA-NP was more intense than in those agroinfiltrated with the empty vector, where the fluorescence almost disappeared at 4-6 days post-agroinfiltration, suggesting that CCGaV and CiVA NPs may act as RSSs. However, such an activity was weaker than that observed in the positive controls co-agroinfiltrated with HC-Pro. Northern-blot hybridization of total RNA preparations from agroinfiltrated patches revealed that the steady-state levels of GFP mRNA correlated positively with the observed fluorescence intensity of GFP. Moreover, Northern-blot hybridization showed lower accumulation of the GFP-derived small RNAs of 21-, 22-, 24-nt, the hallmark molecules of RNA silencing, in the CCGaV-NP and CiVA-NP agroinfiltrated areas compared with negative controls harboring the empty vector, and similar or slightly higher accumulation than in the HC-Pro positive control. Based on these data we concluded that NP proteins of CCGaV and CiVA act as weak suppressors of RNA silencing, thus providing a first evidence of multifunctionality of a coguvirus-encoded protein.

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Aim of the study: Recent advances on NGS approaches allowed a broad exploration of viromes from different fungal host, unveiling a great diversity of mycoviruses with interesting evolutionary aspects. Moreover, the majority of virome studies are focused on mycoviruses infecting true fungi, with less mycoviruses found and characterized in oomycetes, particularly in the obligatory biotrophs (Sutela et al. 2019). In this context, the study of mycoviruses infecting biotrophic hosts is complicated by the difficult manipulation of the fungal isolates in lab condition, but a recent work focused on the characterization of the virome associated to grapevine downy mildew (*Plasmopara viticola*) lesions showed an outstanding amount of new viral taxa (Chiapello et al. 2020). Aim of our work was to characterize the virome associated to the lettuce downy mildew *Bremia lactucae*, which is an important biotrophic pathogen for lettuce production and that was studied in details for the molecular aspects of the plant-pathogen interactions (Parra et al. 2016).

Methods: *B. lactucae* isolates were maintained on susceptible lettuce seedlings and zoospores were collected to extract total RNA. Virome characterization was performed through illumina sequencing of pooled RNA samples and obtained metatranscriptome was analyzed through similarity searches and ORFan ad hoc analysis to detect new viruses or ORFan sequences of viral origin. Viruses identified were detected in the single isolates of origin through qRT-PCR.

Results and conclusions: Homology approaches allowed the identification of 14 new RNA viruses. These viruses are sufficiently distant from the closest hit in the database to be established as new viral species with the only exception of a partitivirus which is almost 90% identical to *Plasmopara viticola* lesion associated partitivirus 7. Among the 14 viruses, we could identify 2 new negative sense ssRNA viruses related to the yueviruses; one example of a bipartite toti-like dsRNA virus which accumulates more negative strand RNA and could be a link to the origin of negative strand RNA from dsRNA; a new splipalmivirus (narna-like viruses with the active site of the RNA-dependent-RNA-polymerases divided on two proteins); a positive sense ssRNA virus called *Bremia lactucae* lesion associated ssRNA virus 1 which only shares homology with a positive sense RNA virus isolated from diatom colonies and some recent flavi-like viruses detected in metatranscriptomic studies. Interestingly, a virus that we called *Bremia lactucae* associated ssRNA virus 2 shows an ORF encoding for a putative protein that doesn't show any hit against the NCBI database, but could find a hit against permutotetraviruses RdRP when investigating the folded structure prediction. Investigation of the ORFan contig from the metatranscriptome allowed the identification of a virus whose RdRP shows distant homology to a mycovirus previously characterized in *Phytophthora infestans* and two viral contigs encoding for putative proteins that can not be associated to a viral genome. The results obtained shown a great diversity of viruses previously unreported for oomycetes and set up the basis to study tripartite interactions between plants, pathogen and viruses infecting the pathogen using *Bremia lactucae* as a model.

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**NOROVIRUS GENOGROUPS I AND II AND RELATED GENOTYPES
IN URBAN WASTEWATERS IN ROME, ITALY, DETECTED BY NEXT GENERATION
AMPLICON SEQUENCING**

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Human Norovirus (HNoV) belongs to the *Caliciviridae* family and is the most common etiological agent responsible of acute gastroenteritis, causing an estimated 685 million cases worldwide annually. About 200 million cases are reported among children under 5 years old, leading to an estimated 50,000 child deaths every year, mostly occurring in developing countries. Currently, HNoVs are divided into 10 genogroups and 49 genotypes, and recently, two tentative genogroups and three new genotypes have been proposed. Genogroups GI, GII, and GIV are known to infect humans. HNoV is transmitted primarily through the fecal-oral route, either by direct person-to-person contact or fecally contaminated food or water. Among GII NoV, despite the broad genetic diversity, most outbreaks are caused by GII.4, which is the predominant genotype responsible for NoV infections worldwide. New GII.4 variants have emerged every 2–4 years since 2002; in addition, non-GII.4 epidemic variants emerged recently, such as the GII.17 Kawasaki 2014 in years 2014/2015. This study aimed to explore the genetic diversity of GI and GII NoV in urban wastewater in Rome during 2021. Overall, 60 24h-composite wastewater samples were collected from the five main wastewater treatment plants of the city. Viral concentration was performed by polyethylene glycol (PEG) precipitation and viral nucleic acids were extracted using the NucliSENS MiniMag semi-automated extraction system (bioMerieux). Nested PCR targeting the region C of the capsid gene, coupled with a next generation (NGS) sequencing-based approach was used for molecular characterization. NGS was carried out on MiSeq II sequencer (Illumina). Subsequent bioinformatic analysis was carried out with a similarity base approach, using a joined reads method with high base calling reliability.

NGS revealed in wastewater samples the presence of six different HNoV GI genotypes (GI.1, GI.2, GI.3, GI.4, GI.5 and GI.6), with GI.1, GI.2 and GI.3 prevalent, and 12 HNoV GII genotypes (GII.2, GII.3, GII.4, GII.6, GII.7, GII.9, GII.10, GII.12, GII.13, GII.16, GII.17, GII.21). This study showed a wide variety of major and minor norovirus GI and GII genotypes detected from raw sewage in Rome during 2021. Complementary data obtained from both clinical and environmental samples can be an effective strategy for understanding the genotypic diversity and the evolutionary dynamics of HNoV at the population level.

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Hepatitis E virus (HEV) infection can cause both acute and chronic hepatitis in humans and represents an emerging public health concern worldwide. In industrialized countries, sporadic autochthonous cases, unrelated to travel in endemic areas are rising. Pigs and wild boars are considered the main reservoirs. An ongoing project entitled "Improving understanding of autochthonous Hepatitis E transmission routes: a focus on foodborne and waterborne pathways" focuses on the Abruzzo Region in Central Italy.

Here we show the results obtained in the first phase of the project by analyzing untreated sewage samples collected throughout the Region ($n^{\circ}= 83$, period December 2019 - July 2020), coastal marine water samples ($n^{\circ}= 10$, five from bathing areas and five from non-bathing areas) and raw slurry samples collected from swine farming activities (solid and liquid fractions, $n^{\circ}= 36$, period 27-30 July 2021). Urban sewage and swine slurries were concentrated using a standard PEG-centrifugation-based protocol, while coastal marine waters were filtered using electropositive membranes. After filtration, a second concentration step was performed via acid flocculation. Nucleic acids extraction was performed using magnetic silica beads with the MiniMag platform (bioMerieux). Broadly reactive primers targeting ORF1 region were used to detect HEV by RT-nested-PCR followed by amplicon sequencing. Typing in the ORF2 region with specific primers is ongoing to further characterize the isolates.

HEV RNA was detected in 10 urban sewage samples (12%) from five Wastewater Treatment Plants; all samples were characterized as genotype G3. In addition, sequences showed high identity with human sequences already detected in autochthonous cases (no travel history) and swine sequences in Italy. Marine water samples were all negative for the presence of HEV. As for the pig slurries, five samples from four different farms tested positive for HEV G3 (14%).

In conclusion, HEV RNA genotype 3 was found in 12% of urban wastewaters collected in the Region of Abruzzo, reflecting a not negligible viral circulation in the population. Samples of pig slurry were also found positive for HEV. Furthermore, similarity was found between HEV sequences of human and swine origins.

An integrated Food-Animal-Environmental surveillance is fundamental for gathering data on HEV epidemiology and studying transmission routes for autochthonous Hepatitis E.

THE RAPID SPREAD OF SARS-COV-2 OMICRON VARIANT IN ITALY REFLECTED EARLY THROUGH WASTEWATER SURVEILLANCE

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The SARS-CoV-2 Omicron variant emerged in South Africa in November 2021 and has later been identified worldwide, raising serious concerns. It was classified as Variant of Concern by the World Health Organization on 26 November 2021.

In this study, a real-time RT-PCR assay was designed for the rapid screening of the Omicron variant, targeting key mutations of the spike gene. After assessing the specificity of the assay using characterized variants from clinical samples, the real-time PCR was used to test 737 sewage samples collected from 19 Italian Regions/Autonomous Provinces between 11 November and 25 December 2021, with the aim of tracking the spread of the Omicron variant in the country. For comparison purposes, positive samples were also tested with a real-time RT-PCR developed by the European Commission, Joint Research Centre (JRC). A nested RT-PCR followed by Sanger sequencing was also performed to characterize positive samples.

Overall, 115 samples tested positive for the Omicron variant. The first occurrence of Omicron was found in a sample collected in Veneto, North Italy, on 7 December 2021. Later on, the variant spread extremely fast in three weeks, with a prevalence of positive wastewater samples rising from 1.0% (1/104 samples) in the week 5-11 December, to 17.5% (25/143 samples) in the week 12-18 December, to 65.9% (89/135 samples) in the week 19-25 December. This is in line with the increase of the Omicron variant prevalence observed in nasopharyngeal swabs in Italy. Similarly, the number of Regions/Autonomous Provinces where the variant was detected increased from one in the first week, to 11 in the second, and to 17 in the third.

The JRC real-time RT-PCR protocol confirmed the presence of the Omicron variant in 79.1% (91/115) of the positive samples, and by Sanger sequencing in 66% (64/97) of PCR amplicons.

In conclusion, we designed a RT-qPCR assay capable of distinguishing the Omicron variant from other SARS-CoV-2 variants which can be successfully used for rapid screening of clinical samples, as well as for wastewater-based epidemiology studies. The description of the introduction and diffusion of the Omicron variant in the Italian population and territory confirms the effectiveness of wastewater monitoring as a powerful surveillance tool.

**TRACKING SARS-COV-2 VARIANTS IN ITALY (OCTOBER 2021-MARCH 2022)
USING THE NATIONAL WASTEWATER-BASED SURVEILLANCE SYSTEM**

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The current COVID-19 pandemic caused by SARS-CoV-2, characterized by a high rate of morbidity and elevated mortality, has emerged as one of the most important human threats in the last centuries. A number of SARS-CoV-2 variants have been identified thus far and are driving new infections globally. Wastewater-based epidemiology (WBE) can be successfully used to study the trend of the disease and describe the circulation of SARS-CoV-2 variants, with the potential to predict the rising of new variants. Following the EU Commission Recommendation 2021/472 on a common approach to establish a systematic surveillance of SARS-CoV-2 and its variants in wastewaters in the EU, the network of the environmental surveillance in Italy established “flash surveys”, i.e. periodic (monthly) sampling campaigns to be held in all regions in Italy over the course of a brief period (the first week of the month), aimed at tracking presence and spread of SARS-CoV-2 variants.

Here we describe the results of the first six months (October 2021 - March 2022) of official wastewater surveillance. Wastewater samples were processed by the laboratories of the national network located in each Region/Autonomous Province. The purified RNAs were shipped in dry ice to the National Institute of Health, where they were subjected to a RT-nested-PCR assay targeting a 1583 bp fragment of the spike region. PCR amplicons were individually subjected to Sanger sequencing, and to NGS (pooled by Region), using long-read Amplicon Sequencing, based on Oxford Nanopore Technology.

Overall, 889 samples collected from 162 WTPs located in 18 regions and 2 autonomous provinces were tested for SARS-CoV-2 variants between October 2021 and April 2022. Predominant viral variants found in wastewaters changed over the six-month monitoring, according with changes in the epidemiological condition: only the Delta variant found in October 2021 (including sublineage AY.4.2) and only the Omicron variant in March 2022 (sublineage BA.2 prevalence), with a switch between the two variants between December 2021 and January 2022, mirroring the variants profiles reported in clinical data.

These results confirm that WBE can contribute tracking the evolution of SARS-CoV-2 genome at population level, providing key information for effectively controlling the pandemic and contributing to vaccine development.

TRANSCRIPTOMIC PROFILES OF SOUR ORANGE SEEDLINGS INOCULATED WITH TWO CITRUS TRISTEZA VIRUS ISOLATES OF THE SAME GENOTYPE AND DIFFERENT PHENOTYPE

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Citrus tristeza virus (CTV) is a phloematic Closterovirus, responsible for tremendous economic losses to the citrus industry. Seven major CTV genotypes, including several isolates, have been described. In Sicily, severe VT isolates are prevalent along with a percentage of mild T30 and few mixed. Some variants of VT genotype, totally asymptomatic have been also reported. In the last two decades, many transcriptomic studies have been undertaken on different hosts and CTV genotype combinations, but never on sour orange (Gandia et al., 2007; Fu et al., 2016; Cheng et al., 2016). A preliminary investigation on the expression level of selected genes by quantitative real-time PCR was carried out in different citrus hosts, including sour orange (Licciardello et al., 2010). Here, we compared the transcriptome profiles of sour orange seedlings infected with two VT CTV isolates (SG29 and M39), differing for only 13 nucleotides. SG29 causes severe seedling yellows on sour orange and grapefruit seedlings, as well as tristeza decline on sweet oranges grafted on sour orange (SO) while M39 is totally asymptomatic. Each isolate was bark inoculated into four one year-old sour orange seedlings and four additional were left as mock-non inoculated controls. Several months later, bark samples were singly collected from young twigs of still asymptomatic seedlings, but ELISA positive to CTV, and RNA extracted and sequenced. In parallel, the transcriptome of sour orange was for the first time obtained by sequencing the RNAs of bark, leaves, whole plant, flowers, rind, and juice, by combining a short-reads (Illumina) and a long-reads (Oxford Nanopore) technology. Compared with the non-inoculated seedlings, transcriptome profiles obtained through RNA-seq revealed 4718 differentially expressed transcripts (DETs) after infection with SG29 and 33 after M39. The comparison between SG29 and Mac39 inoculated seedlings highlighted a total of 4197 DETs belonging to a wide range of pathways involved in signalling and stress response, cell wall modification, transcription factors, transport, hormone response and secondary metabolism. Overall fold change was between -7.97 and +10.4 Log₂ (P < 0.01). Many disease resistance (DS) genes were differentially expressed in response to SG29 infection. Nineteen CC-NBS-LRR receptors and proteins containing NB-ARC domain were upregulated (2.71-1.01 Log₂) along with 101 leucine rich repeat (LRR) kinases (5.0-1.0 Log₂). Twenty-seven transcripts belonging to peroxidase superfamily (ROS) protein were also over-expressed as well as 25 ankyrin (ANK) repeat-containing proteins and 14 pentatricopeptide repeat (PPR) proteins. On the contrary, transcripts containing the Eukaryotic RNA Recognition Motif (RRM) profile, Armadillo repeat (ARM) and Transducin/WD40 repeat-like superfamily proteins were downregulated. Real-time PCR undertaken on a subset on 10 transcripts, selected among the most representative gene families (DS, ROS, ARM, RRM,

PPR, WD40, ANK, AMR), showed a comparable trend. Overall, results are not consistent with most of the investigations carried out on host and virus genotype combinations different from this first transcriptomic study on sour orange. As expected, the inoculation of sour orange with two isolates of the same genotype but differing in the phenotype, induced a relevant number of DETs despite non symptom was present at the time of sample collection. This might help to better understand host-pathogen interactions occurring on sour orange and to explore new potential control strategies of CTV.

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Citrus variegation virus (CVV) (genus *Ilarvirus*, family *Bromoviridae*) has been reported from several citrus-growing areas worldwide, including the Mediterranean Basin and is associated with variegation disease in citrus. CVV induces mild symptoms on orange and mandarin but can determine fruit malformation and yield reduction in citron and lemon. Two main types of symptoms have been associated with CVV in the field: chlorotic mottle (variegation) and crinkle on leaves. Only one complete genome sequence of CVV (an isolate from USA) is available (Scott and Ge, 1995, J Gen Virol 76:957–63; Li et al., 2008, Arch Virol 153:385–8). CVV was reported in southern Italy (Campania region) in 2005 (Damiano et al., 2005, J. Plant Pathol. 87:292). Based on phylogenetic analysis using the sequence of the viral capsid protein (CP), it was proposed that the Italian CVV isolates were divergent from other isolates of different geographic origin, which were associated with variegation or crinkle leaf symptoms (Barone et al, 2009, Phytopathol. Mediterranea 48:469-73). Here we report the full-length genome sequence of an Italian isolate of CVV (CVV-IMP4) identified in the frame of a study aimed to characterize the virome associated with a lemon tree (*Citrus limon* [L.] in Campania region (southern Italy). The complete genome of the CVV-IMP4 isolate was determined by high-throughput sequencing using *de novo* assembling and mapping of reads to a reference. The three genomic RNAs of CVV-IMP4 isolate showed 95.45, 96.29 and 97.01% nucleotide (nt) sequence identity with the RNA1, RNA2 and RNA3 of the CVV reference sequence, respectively. RNA1 (3442 nt), which codes for the methyltransferase and helicase, showed an insertion of twelve nt (at position 805-817), generating an insertion of four amino acids in the methyltransferase domain, and a deletion of three nt in 3' untranslated region. Phylogenetic analysis of the CP confirmed that the isolate CVV-IMP4 clustered with the other Italian isolates from Campania region characterized previously. In fact, the CVV-IMP4 CP contains three out the four amino acid parsimonious sites identified as characteristics of this phylogenetic group. Moreover, the two amino acid changes associated with crinkle symptoms are absent in the CVV-IMP4 isolate. To the best of our knowledge, this is the second full-length complete genomic sequence of CVV.

THE C4 PROTEIN OF TOMATO YELLOW LEAF CURL SARDINIA VIRUS PRIMES DROUGHT TOLERANCE IN TOMATO

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Plant viruses can interfere with the ability of their hosts to overcome abiotic and biotic stresses, underpinning the existence of common molecular networks that regulate the responses towards these stimuli. A geminivirus causing the tomato yellow leaf curl disease was recently shown to increase plant tolerance to heat and drought stress, possibly through the intervention of the virus-encoded C4 protein. Here, we elucidated physiological and molecular events underlying the drought tolerance induced in tomato by the C4 protein of tomato yellow leaf curl Sardinia virus (TYLCSV), using transgenic plants overexpressing C4. Combining morphometric and physiological parameters and hormone contents with transcriptional analysis of genes involved in water stress response and hormone metabolism, we assessed that TYLCSV C4 indeed supports the increased drought tolerance of tomato plants. We report that specific anatomical and hydraulic traits, rather than biochemical signals, are the keynote of the C4-associated stress resilience. Overall, these data allow to conclude that TYLCSV C4 exerts a priming role on plants towards abiotic stresses, opening to new perspectives for managing the effects of climate change in horticultural crops.

CUTTING EDGE APPLICATION OF RAMAN SPECTROSCOPY FOR THE DIAGNOSIS OF VIRUS INFECTION IN TWO MAJOR CROPS, TOMATO AND GRAPEVINE

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Global population forecasts dictate to rapidly adopt multifaceted approaches to fulfill increasing food requirements, ameliorate dietary value and food security, preserving the cultural heritage linked to traditional products. All this must be accomplished using sustainable and economically feasible agricultural processes. Plants face a multitude of biotic stresses, induced by different kinds of pathogens including viruses, which are responsible for major crop losses. Plant react to viral infections deploying a plethora of metabolic changes. Early detection of viral infections contributes to limit their spread and economic burden. Beyond the time-consuming, destructive, and expensive diagnostic procedures based on immunological and molecular techniques, Raman spectroscopy (RS) is emerging as an alternative tool for a non-destructive and rapid diagnosis, as it generates a chemical fingerprinting of a sample, at low operating costs. We assessed the effectiveness of RS combined to chemometric analysis to monitor the presence and progress of infection in two major crops. We tested the RS diagnostic potential on tomato plants infected by two different viruses, tomato yellow leaf curl Sardinia virus (TYLCSV) and tomato spotted wilt virus (TSWV), and on grapevine plants harboring grapevine fan leaf virus (GFLV) and grapevine rupestris stem pitting-associated virus (GRSPaV). Overall, RS allowed to discriminate healthy from virus-infected leaf specimens with accuracy greater than 70 and 85% for TYLCSV and TSWV, respectively, in tomato and with precision up to 100 and 80% in vine plants affected by GFLV and GRSPaV, respectively, at stages when symptoms were not visible. Metabolic changes occurring in leaves related to chlorophylls, carotenoids, and polyphenolic compounds were the major biomarkers identified by this technique. The potential applications of this cutting edge technique for a real-time dynamic monitoring of virus infections in crops will be discussed.

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Background and aims: Positive-strand RNA [(+)RNA] viruses are agents of important diseases in humans, animals and plants, including COVID-19. Regardless of the host, the replication of all (+) RNA viruses occurs in association with the host endomembrane system. Based on this common replication mechanism, we have used the yeast model to express the replication-associated proteins of one plant and one human virus to decipher virus-membrane interactions, with the final aim of identifying host factors co-opted for viral replication, to develop new antiviral strategies.

Methods: We expressed the (+)RNA carnation Italian ringspot virus (CIRV) replication-associated protein p36 under the control of the inducible GAL 1 promoter in *Saccharomyces cerevisiae*. Similarly, we cloned and expressed in yeast the SARS-CoV-2 non-structural proteins nsp3, nsp4 and nsp6, having a role in the formation of the double-membrane vesicles, which likely represent the virus replication site. Protein expression was analyzed by Western blot and immunofluorescence analyses.

Results and conclusions: It was shown that p36 expression in *S. cerevisiae* strain YPH499 (i) increased necrotic cell death and concomitantly decreased regulated cell death in response to acetic acid; (ii) decreased respiratory yeast cell growth; (iii) altered the mitochondrial network; (iv) decreased oxygen consumption due to respiratory chain complex impairment.

SARS-CoV-2 non-structural proteins nsp3, nsp4 and nsp6 expression was obtained in *S. cerevisiae* strain W303-1B and viability was measured as a function of time. Cells expressing nsp3 and nsp4 showed significantly reduced viability compared to control cells at all time points, whereas the viability of yeast cells expressing nsp6 was reduced only up to 24 h. Cell growth rate was determined and shown to be reduced. The expression of SARS-CoV-2 nsp3, nsp4 and nsp6 was verified at all time points by Western blotting and the intracellular localization of the expressed proteins was determined by immunofluorescence analysis.

We show that the yeast model system can be successfully employed to study both plant and human virus replication-associated proteins. *S. cerevisiae* was confirmed as an invaluable model host to study the molecular pathogenesis of (+)RNA viruses and the evasion of the host immune system. Elucidating virus-host cell interaction complexity is crucial to the identification of novel druggable targets for the development of broad-spectrum antivirals.

**EFFECT OF ULTRASONIC PREMIXING ON HEPATITIS A VIRUS INFECTIVITY
A PRELIMINARY STUDY**

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The Hepatitis A virus (HAV) is the leading cause of acute viral hepatitis worldwide. It is transmitted through fecal-oral route or person-to-person contact and the transmission typically occurs by the exposure to contaminated food or water. Furthermore, HAV transmission is supported by high virus stability in the environment and with respect to extreme physicochemical conditions. In the last few years there has been an increasing number of outbreaks worldwide caused also by food imports from HAV-endemic areas.

New strategies for the control and prevention of HAV outbreaks are necessary. In this preliminary study, the effects of an innovative sonication method on HAV infectivity were evaluated, using an ultrasonic premixer (Thinky Nano Premixer PR-1, Poly Dispensing Systems, France). The instrument is designed to rotate the container containing the virus at an angle of 45° and at different speeds (80 to 600 rpm) while ultrasounds (40 kHz) irradiate the viral suspension from the bottom and side of the ultrasonic bath.

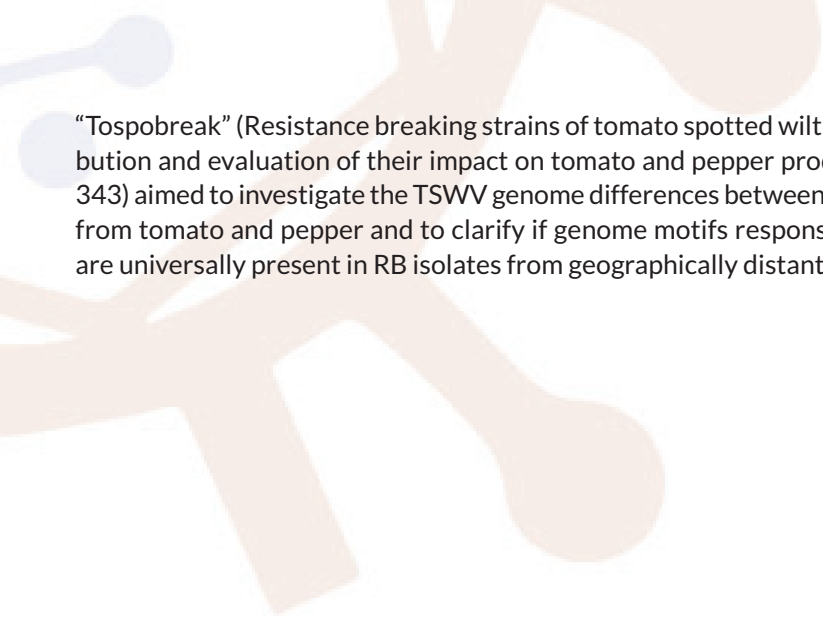
To assess the inactivating effect of the sonication, a HAV suspension (3.2×10^4 TCID₅₀/ml final concentration in PBS) was treated for 2 and 5 minutes in combination with two rotation speeds (80 and 600 rpm), under temperature-controlled conditions (the temperature was kept below 25 °C for all the experiments). The evaluation of the inactivation effect was estimated by comparing the titration (TCID₅₀/ml) on Frp3 cell line of the treated and untreated viral suspensions. Treated and untreated PBS solutions were used as a negative control.

In this preliminary study, none of the four tested conditions (2 and 5 minutes, 80 and 600 rpm) exert a significant reduction of HAV infectivity, all differences in viral titre being below 0.5 log. Further experiments, however, are necessary to assess the effect of such treatment on foodborne viruses with capsid structures significantly different from HAV, such as noroviruses, which may display a higher sensitivity to the disruptive effects of ultrasound waves.

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UPDATE ABOUT THE PRESENCE OF TOMATO SPOTTED WILT ORTHOTOSPOVIRUS (TSWV) RESISTANCE BREAKING (RB) ISOLATES IN TOMATO AND PEPPER CROPS IN CENTRAL-SOUTH ITALY**A. Tiberini¹, A. Manglli¹, M. Allegrezza², L. Sigillo³, F. Cillo⁴, A. Gentili¹**¹ Council for Agricultural Research and Economics - Research centre of plant protection and certification² Università degli studi di Milano La Statale³ Council for Agricultural Research and Economics - Research centre for Vegetable and Ornamental Crops⁴ National Council for Research - Institute for Sustainable Plant Protection

Tomato spotted wilt virus (TSWV), belonging to *Orthotospovirus* genus, (family *Tospoviridae*, order *Bunyavirales*) is present in all countries with temperate, tropical and subtropical climate conditions. It could affect a wide range of host plants and represents the most economically important virus for tomato and pepper, especially in the Mediterranean basin. Despite the availability of integrated management approaches for containing TSWV disease, including the use of resistant varieties, emergence of TSWV resistance-breaking strains (RB) alarmed producers and stakeholders. RB strains could induce significant damages up to 100% and nowadays are reported from Spain, Italy, Hungary, Turkey, USA, Brazil, Argentina, Australia, Serbia and South Africa in peppers and/or tomatoes. Since data on the presence and distribution of RB TSWV strains is not available in most of the tomato and pepper growing countries inside the EPPO region, in this study an intensive surveys activity was conducted in central-south Italy, including some of the most important cultivation areas. Surveys were performed in 16 different tomato fields (Campania, Molise and Apulia) and in 13 pepper fields (Lazio, Campania, Molise and Apulia), mainly focusing on resistant varieties. A total number of 70 tomato and 57 pepper samples from symptomatic and asymptomatic plants were collected. Severe symptomology occurred mainly in no resistant tomato varieties or in those carrying only the Sw5 resistance gene, affecting in some cases up to 80% of the production. In all surveyed pepper fields, despite the use of varieties characterized by IR resistance profile (intermediate resistance) a wide range of symptoms like stunting, yellowing of leaves and damage of fruit were observed, affecting up to 30% of plants. After the first identification of TSWV, confirming the presence of the virus in 40 and 18 samples of tomato and pepper, respectively, further analysis were performed to assess the presence of RB strains. Sequence and phylogenetic analysis were performed including NSm, N and NSs genes. In tomato, all the characterized and sequenced isolates from the different production areals showed a mutation at position 118 (C118Y) and none of them at position 120 (T120N) on NSm region. These mutations are known to have a role in the establishment of resistance breaking phenotype in TSWV isolates. In view of above it could be stated that the TSWV RB-strain seems to be prevalent in all the production areas surveyed. In pepper, there is not a clear determinant factor that could be identified in establishing the RB-phenotype in TSWV, although recent studies hypothesize a role of NSs region or whole reassortment of S segment. Since TSWV was retrieved in all pepper cultivars with IR profile, it could be assumed that those isolates could have an RB phenotype. All these activities were performed in the frame of Euphresco project



“Tospobreak” (Resistance breaking strains of tomato spotted wilt orthotospovirus: distribution and evaluation of their impact on tomato and pepper production – code 2020-A-343) aimed to investigate the TSWV genome differences between RB and non-RB isolates from tomato and pepper and to clarify if genome motifs responsible for RB phenotypes are universally present in RB isolates from geographically distant areas.

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Aim of the study: Ambivirus are a group of recently characterized mycovirus with unique features. We initially characterized them from ORFan sequences of orchid mycorrhizal fungi of the species *Ceratobasidium* and *Tulasnella* [4]. Later, we also found them in *Cryphonectria parasitica* [1], the model species for mycovirus-host interaction. Since our discovery, ambiviruses have shown to be widespread in fungi [3]. Ambivirus have the unique characteristic of a single stranded ambisense genome whose length is remarkably conserved among different ambiviruses (circa 4.5-5 kb). Their genomes encodes two main non-overlapping ORFs in opposite orientation (one from the putative plus strand and one from the minus strand). Aim of this study is understanding the replication and expression strategy of this possible new Baltimore's class of viruses [2].

Methods: Here we used different in silico approaches and molecular biology approaches to characterize the distinct viral species accumulating during ambivirus infection of the host fungus *Tulasnella* spp. and *C. parasitica*.

Results and conclusions: Although the two genomic-encoded proteins are ORFans when compared to the current databases, alignment of homologues among different ambiviruses show that one protein (protein encoded by ORF-A) has conserved domains that could be the hallmark of the A, B and C palm subdomains of an RdRP. Nevertheless, structural prediction with Alpha-fold does not result in a reliably conserved RdRP. ORF B is also conserved in some motifs, but we could not provide evidence of any functionally conserved characterized domain. Surprisingly in all but one cases the most abundant RNA species that accumulates during fungal infection is an RNA dimer. 5' RACE on the plus strand and minus strand RNA accumulating during infection shows the presence of a stretch of circa 250 UTR that is complementary, implying a so far unreported replication/transcription mechanism for RNA viruses where an incomplete dimer sequence is the template for two long subgenomic RNAs, encompassing almost the complete monomer sequence. Currently we are testing the function of such conserved region.

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Aim of the study: The Wastewater Based Epidemiology (WBE) is a “water fingerprinting” technique that provides an objective assessment of public and environmental health status in real time. During the SARS-CoV-2 pandemic, it has been recognized as an effective tool to monitor viral circulation, to early detect epidemic peaks and the introduction of VoC (variants of concern) (Bivins et al., 2020). To this aim the European Commission recommended a WBE surveillance. In Italy, a national project of the Center of Diseases Control (CCM) started in March 2021 and has been followed by the national surveillance program from October 2021 onwards. The environmental and clinical surveillance together can provide timely data to get a clearer picture of the pandemic situation. In this work, we conducted an environmental monitoring of SARS-CoV-2 in Tuscany, in the period from February to December 2021, to investigate the relationships between clinical cases of COVID-19 and virus’ environmental circulation, the critical aspects of WBE too.

Methods used: Four wastewater treatment plants (WWTPs) that serve areas with more than 50.000 inhabitants, in north-west Tuscany were selected. One included a large university hospital (WTTP1), one was a small city with a large surrounding area (WTTP2), one was a very touristic city, where population strongly increases during summer (WTTP4), and another one was a city without the above-mentioned characteristics (WTTP3). Composite samples, collected weekly, were transferred on ice to the laboratory, kept refrigerated at 4 °C and concentrated within 24 h. The ministerial protocol by Italian National Health Institute (ISS) was adopted but it was changed during the study, with a more performing method of concentration, inducing a variation in reliability of results. After a thermal inactivation at 56 °C for 30 min, the first protocol was based on precipitation of polyethylene glycol (PEG) and dextran and recovery phase through biphasic separation. The second one, implemented starting from the beginning of June 2021, used the same principle of precipitation through PEG and NaCl but the virus was recovered by centrifugation.

The viral genome was quantified by RT-qPCR (Genomic Copies for Liter - CG / L) basing on gene target ORF1 ab, nsp14. Clinical data on the number of new cases of COVID-19 in the territories linked to the four WWTPs were obtained from the public databases of the competent Local Health Authorities.

Results and conclusions: Overall, positivity was detected in 80 of the 172 samples tested (46,5%). The concentration of the viral genome resulted from a minimum of 1.03×10^3 GC/L to a maximum of 4.55×10^4 GC/L (geometric mean $3.27 \times 10^2 \pm 1.13 \times 10^3$ GC/L). The correlation between new weekly clinical cases and GC resulted statistically significant ($r=0,3046$; $p < 0,0001$). The correlations between the two data sets, showed a significant correspondence for the WWTPs 1 and 3, not for the 2 and 4 ones. WWTP 2 covers an area

that not corresponding exactly with the municipality published clinical data and the population served by the WWTP 4 highly increased during summer months, but clinical data referred to resident population. These data confirmed that wastewater monitoring is a strong indicator of SARS-CoV-2 spreading in the population and its potential use as early warning system.

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MULTIPARTITE BNYVV GENOMIC RNAS CHANGE THEIR RELATIVE FREQUENCIES ACCORDING TO THE INFECTED HOST AND ORGAN AND AFTER TRANSMISSION IN THE VECTOR

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Multipartite viruses possess genomes split in more than two genomic segments each one packaged into independent particles. One of the benefits of being a multipartite virus is the gene expression modulation via changes of the segment copy number. The soil-borne *Beet necrotic yellow vein virus* (BNYVV) may be considered a model of multipartitism since, with its 4 to 5 genomic RNAs, it has the highest number of genomic segments among RNA viruses infecting plants. In this work, we investigate the ratio of the four genomic segments of BNYVV type B in different host types analyzing tissues from infected roots and leaves by a validated protocol of dual step reverse transcriptase droplet digital (RT-dd)-PCR. BNYVV genome formula was also calculated within the vector *Polymyxa betae* after zoospore purification from infected *Beta vulgaris* roots evaluating the plant rate of contamination.

Results showed that some viral gene segments accumulate at low frequency, whereas others dominate. BNYVV segment copy numbers change according to the type of host and organ infected, moreover the virus seems to reach a dedicated set-point genome formula also within its vector. These data together with the biology of this virus raise questions about the genome integrity preservation of BNYVV during the host infection and transmission by the vector.

THE PROTECTION FROM CMV INFECTION IN SOLID ORGAN TRANSPLANTS IS HIGHLY DEPENDENT ON CMV T-CELL SPECIFIC IMMUNITY AND TYPE OF ORGAN TRANSPLANT

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Introduction: The CMV specific immune recovery is known to control viral infection and disease. The CMV specific cell-mediated immunity (CMI) and CMV specific humoral immune response (IgG titer and IgG avidity) was explored as candidate biomarker predictive of CMV infection.

Methods: 653 observations from 297 SOT including 103 kidney (K), 60 liver (L), 47 heart (H), 87 Lung (Lu) transplants. The follow-up was 0-400 days after transplant. The following parameters were examined: 1) CMV serostatus before transplant 2) CMV IgG titer 3) CMV IgG avidity 4) CMV-ELISPOT 5) primary (D+/R-) or non- primary (D±/R+) CMV infection 6) CMV viremia. Data were statistically analyzed using ANOVA and linear regression analysis and spline model.

Results: The main findings of the study are: 1) CMV IgG titers and avidity are not predictive biomarker for CMV infection. The CMV IgG titer and IgG avidity levels are comparable either in infected and non-infected patients. 2) CMV viral load is statistically different between infected D±/R+ and D+/R-. The viral load in infected D+/R- is 1 Log higher compared to D±/R+ (10^5 vs 10^4 respectively) 4) The CMV immune reconstitution is more rapid and efficient in D±/R+ compared to D+/R- 5) D

±/R+ and D+/R- Kidney and Lung transplants display a statistically significant lower CMI compared to heart and liver transplants; 6) Kidney and Lung transplants display a similar pattern of immune recovery 7) The CMV cell mediated immunity biomarker is predictive of infection in all organs with exception of lung transplants.

Conclusions: The study shows a marginal role of humoral immunity in controlling CMV infection. The pattern of CMI immune recovery is highly dependent upon pre- transplant CMV serostatus, type of organ transplant and immunosuppression therapy. High levels of CMV specific cell-mediated immunity correlate with a reduced CMV infection risk in heart, kidney and liver transplants. A different scenario emerged in lung transplants: high levels of circulating CMV specific T-cells can be found in lung transplants but cannot prevent CMV infection and clear the virus. In this view the lung may be considered a "sanctuary site" for CMV replication.

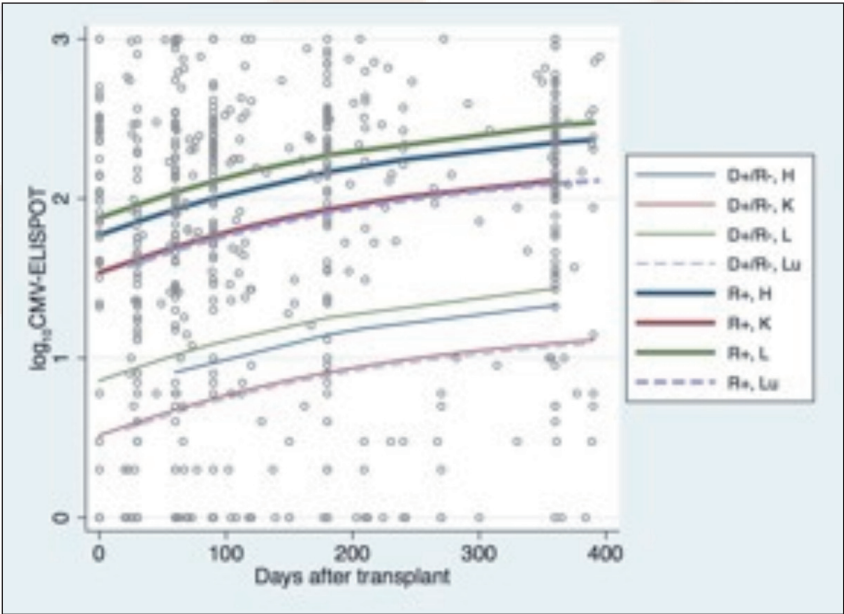


Figure 1. CMV specific T-cell responses in heart (H), kidney (K), liver (L) and lung (LU) seronegative recipients (R) or seropositive recipients (R+) SOTs in 0-400 days after transplant. Spline model.

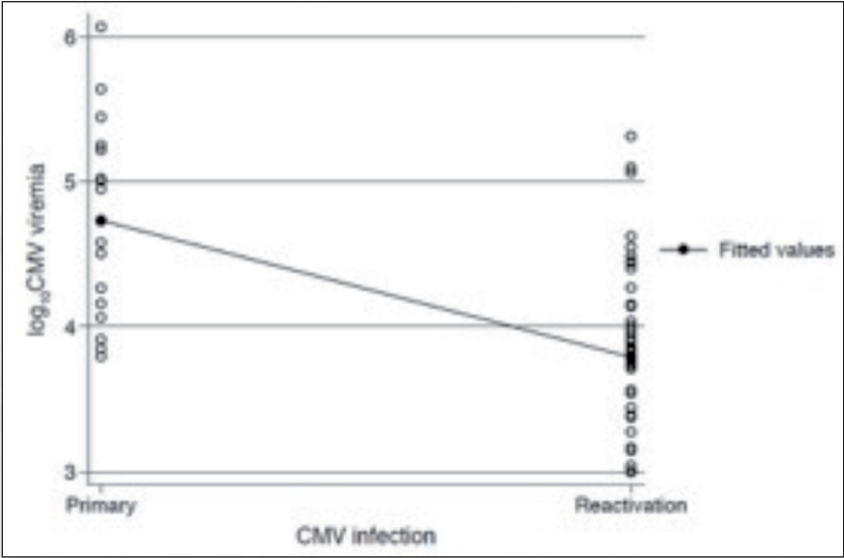


Figure 2. CMV viremia level during primary CMV infection (D+/R-) and R+ SOTs.

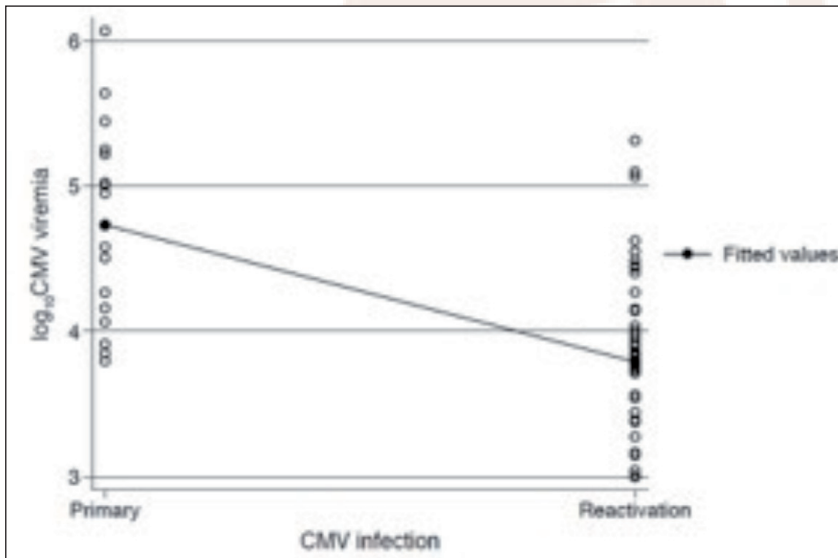


Figure 3. CMV humoral responses in SOTs. CMV IgG titers and CMV IgG avidity in heart, kidney, liver transplants at day 0,30,60,90, 180 and >180 after transplant. The graph included only patients who did not receive CMV Immunoglobulins, for this reason all lungs transplants were excluded. D+/R- SOTs were also excluded since by definition CMV IgG were undetectable.

COMPLEX EXPRESSION PROFILE OF HERVs AND INFLAMMATORY MEDIATORS IN NASAL MUCOSA AS EARLY BIOMARKER OF COVID-19 SEVERITY

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Aim of the study: Human endogenous retroviruses (HERVs), genetic elements, derived from ancestral exogenous retroviruses infecting the germline, currently make up ~8% of the human genome. During co-evolution with humans, the majority of HERVs were silenced by negative pressure; conversely their activation by external stimuli (eg viruses, drugs, cytokines) has been associated with complex diseases. Recently, we demonstrated the presence of a higher expression of pHERV-W in leukocytes from COVID-19 patients compared to healthy donors, which correlated with immune-dysfunction and reflected patients' respiratory outcome during hospitalization. Aim of this work was to study the expression of HERVs and cytokines in nasopharyngeal swab samples and the association with respiratory outcome in COVID-19 patients. Finally, to clarify whether HERVs can be activated by SARS-CoV-2, hypopharyngeal carcinoma cells were in vitro stimulated with the SARS-CoV-2 Spike protein.

Methods: Residuals of nasopharyngeal/oropharyngeal swabs samples [20 SARS-CoV-2 negative and 43 SARS-CoV-2 positive of which 29 from individuals who did not require medical treatment (not-HOSPs) and 14 from individuals who were immediately hospitalized at the time of the swab result (HOSPs)] were collected at Policlinico Tor Vergata, (Rome), in the period from the end of March until the begin of May 2020 and the expression of HERVs (HERV-H, HERV-K, pHERV-W, pHERV-W), pro-inflammatory mediators, Angiotensin-converting enzyme 2 receptor (ACE2) and Nucleocapsid (N) of SARS-CoV-2 have been analyzed by RT-Real time PCR. Multivariate general linear models were used to assess the association of gene expression with clinical parameters of COVID-19 patients.

Results: The expression of HERV-K, pHERV-W, HERV-H as well as IL-1 β , IL-6, TNF α , INF γ , TLR3, TLR7 and ACE2 were higher in SARS-CoV-2 positive swabs (independently from the hospitalization of the patients) compared to negative. Among positive samples, HERV-K and HERV-H expression was highly expressed in HOSPs with respect to other groups. Higher levels of IL-1 β , IL-6, IL-17, TNF α , MCP1, TLR7 and N gene of SARS-CoV-2 have been found in the HOSPs group, while IL-10, INF α , INF β and TLR4 expression was significantly higher in not-HOSPs group. In the group of the hospitalized the expression of pHERV-W ENV and also IL-1 β , IL-6, INF α and INF β was higher in individuals who needed oxygen support. Moreover, the Analysis of the Principal Component (PCA) on data of the hospitalized, yielded 5 factors explained 89% of the variance, with the first component that included N

gene SARS-CoV-2, IL-10, pHERV-W, IL-6 and TNF α (38% of variance), the second with TLR3, TLR7, IL17RA, HERV-K, MCP1 (27%) and the third that comprised TNF α , IL-1 β , INF β (11%). Spearman's correlation analysis by using data from PCA showed a significant correlation between the first three components with markers of coagulation and inflammation (fibrinogen, C-reactive protein and brain natriuretic peptide) and with the T cell-mediated immune response (CD3, CD4 absolute count), which are the main factors involved in the severity of COVID-19. Finally, we demonstrated that spike protein induced the expression of HERVs and inflammatory mediators in hypopharyngeal carcinoma cells *in vitro*.

Conclusions: The results support the role of HERVs in COVID-19 etiopathogenesis and suggest the expression profile of HERVs and mediators of inflammation and immune response in nasal mucosa as early complex biomarker associated to COVID-19 severity and able to predict disease outcome.

PERFORMANCE OF HOME-MADE WHOLE BLOOD STIMULATION ASSAYS FOR THE QUANTIFICATION OF SARS-COV-2 SPECIFIC T-CELL RESPONSE: A CROSS-SECTIONAL STUDY

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Aim: In the context of SARS-CoV-2 vaccination, there is an urgent need of standardization of methods for the assessment of adaptive T-cell response elicited by vaccination, especially in immunocompromised subjects. The aim of our study was to set-up a simple “home-made” method for the quantification of Spike-specific T-cell response in vaccinated healthy subjects and immunocompromised patients using whole blood sample

Methods: Samples from 95 healthcare workers (HCWs) and 55 immunocompromised subjects (IC) were analyzed. Whole blood and serum samples were collected six months after BNT162b2 vaccination in both groups. Peripheral blood mononuclear cells (PBMC) were isolated from heparin-treated blood by standard density gradient centrifugation and used for ELISpot assay against Spike [S] peptide pools (1). Home-made whole blood interferon-gamma release assay (HM-WB IGRA; pg/mL) against the same antigen was performed. As gold standard we used the ELISpot assay (SFU/million PBMC) developed in our institute.

Results and conclusions: Overall, 116/150 subjects were positive for ELISpot assay; of them, 93 (80.2%) were positive also for HM-WB IGRA. On the other hand, 34/150 subjects were negative for ELISpot assay and, of them, 28 (82.4%) were also negative for HM-WB IGRA.

The overall 150 subjects were stratified according to IFN γ production level obtained by HM-WB IGRA: 34.7% showed a negative IFN γ production level (IFN γ <10 pg/mL), 31.3% tested positive for IFN γ at medium level (IFN γ level ranging from 10-100 pg/mL) and 34.0% showed high IFN γ (higher than 100 pg/mL). The median level of SFU/million PBMC in group IFN<10pg/ml was significantly lower than medium levels for each group of IFN γ response was compared in both HCWs (p=0.0178) and ICs (p=0.001).

In this study we setted a simple and easy-to-perform assay for the evaluation of S-specific cell-mediated response, by stimulating whole blood and quantifying IFN γ release. The method showed a good correlation with our previously developed in-house ELISpot assay, in both HCWs (r=0.74, p<0.001) and IC (r=0.45, P=0.002) subjects. Overall, 90% and 83% of HCWs tested positive for ELISpot assay and HM-WB IGRA, respectively, suggesting the lower sensitivity of the latter one. Similarly, a higher percentage of responder subjects was reported for IC subjects with ELISpot assay (56%) than respect to HM-WB IGRA (33%).

1. Cassaniti et al. Clin Microbiol Infect. 2021 Jul;27(7):1029-1034.

EFFECT OF SARS-CoV-2 ON THE COAGULATION CASCADE IN COVID-19 ASSOCIATED COAGULOPATHIES

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Background: Coagulation decompensation is one of the complications most frequently encountered in COVID-19 patients and particularly the onset of thrombi and ischemia are often associated with a poor prognosis. Although the evidence for an association between SARS-CoV2 infection and coagulopathies is known (Levi et al, 2020), to date the mechanism underlying the alteration of the coagulation cascade in some COVID-19 patients remains misunderstood. Recently, protein S (PROS1), an anticoagulant plasma protein involved in the correct homeostasis of the coagulation cascade, has been described as a potential risk factor for complications related to COVID19 (Lemke et al, 2020) and represents a potential target for PLpro SARS-CoV-2 enzyme proteolysis (Ruzika, 2020).

Aim: This study aims to identify peculiar expression patterns in COVID19-associated coagulopathies, to identify possible pharmacological targets, focusing on PROS1 protein alteration.

Methods: Thrombotic, arteriosclerotic plaques, venous \ arterial, perivascular fat samples and blood samples were collected from COVID-positive and COVID-negative subject, and from COVID-positive subjects with no coagulopathies. SARS-CoV-2 presence will be evaluated by Real time PCR and by IHC and gene expression, and ELISA analysis will be performed to identify specific expression profiles associated with coagulation imbalances, with particular attention to protein S (PROS1).

Results and Conclusions: We reported substantial differences in the activation of the coagulative cascade, and particularly a significant decrease of PROS1, in the COVID-19 cohort experiencing coagulative disorders, in association with SARS-CoV-2 positivity by IHC and real time PCR. These data suggested that, possibly, SARS-CoV-2 associated thrombotic/ischemic events might involve PROS-1 cleavage by viral PLpro, leading to the loss of its anticoagulant function. Basing on this evidence, the use of PLpro inhibitors might be suggest as a therapeutical tool for COVID-19 coagulopathies.

**DIFFUSION OF SARS-COV-2 OMICRON VARIANT IN CAMPANIA REGION
BETWEEN NOVEMBER 2021 AND DECEMBER 2021**

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Introduction: SARS-CoV-2, like other RNA viruses, is prone to genetic evolution while adapting to their new human hosts with the development of mutations over time, resulting in the emergence of multiple variants that may have different characteristics. By sequencing viral RNA from SARS-CoV-2 positive samples we reconstructed the trend of Delta variant and Omicron variant in Campania region between November 2021 and January 2022.

Materials and Methods: RNA was extracted from 246 nasopharyngeal positive swabs obtained in Campania region between 01/11/2021 and 04/01/2022. 72 samples were collected in November 2021; 95 samples were collected in December 2021; 79 samples were collected in January 2022. Sequencing was performed using the MGI DNB-Seq G400. The SARS-CoV-2 complete genomes were built using the pipeline MGI SARS-CoV-2 analysis pipeline for multiplex-PCR MPS (Massive Parallel Sequencing) data.

Results and Conclusions: Out of 32 samples collected between 01/11/2021 and 10/11/2021, all the samples were Delta variant. Out of 26 samples collected between 11/11/2021 and 20/11/2021, 23 (88.5%) were Delta variant and 3 (11.5%) were other non-Omicron variants. Out of 14 samples collected between 21/11/2021 and 30/11/2021, 11 (78.6%) were Delta variant, 1 (7.1%) was Omicron variant and 2 (14.3%) were other non-Omicron variants. Out of 4 samples collected between 01/12/2021 and 10/12/2021, 3 (75%) were Delta variant and 1 (25%) was other non-Omicron variants. Out of 14 samples collected between 11/12/2021 and 20/12/2021, 10 (71.4%) were Delta variant, 2 (14.3%) were Omicron variant and 2 (14.3%) were other non-Omicron variants. Out of 77 samples collected between 21/12/2021 and 31/12/2021, 17 (22.1%) were Delta variant, 59 (76.6%) were Omicron variant and 1 (1.3%) was other non-Omicron variants. Out of 79 samples collected between 01/01/2022 and 04/01/2022, 7 (8.9%) were Delta variant, 71 (89.9%) were Omicron variant and 1 (1.3%) was other non-Omicron variants.

Our data show that between 01/11/2021 and 10/12/2021 there was a gradual decline in the cases of Delta variant in favor of Omicron variant. Starting from the 11/12/2021, the cases of Omicron variant quickly escalated until almost completely replacing the Delta variant from January 2022.

CIRCULATING COLLAGEN METABOLITES AND THE ENHANCED LIVER FIBROSIS (ELF) SCORE AS DISEASE SEVERITY MARKERS IN SARS-COV-2 INFECTION

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Aim: Serum markers for severity of SARS-CoV-2 infection remain limited. The Enhanced Liver Fibrosis (ELF) score is calculated combining the values of a collagen metabolites set including procollagen type III amino terminal propeptide (PIIINP), tissue inhibitor of metalloproteinases 1 (TIMP-1), and hyaluronic acid (HA). This study aimed to examine the association of the ELF score and its single analytes as surrogate outcome measures of severity of COVID-19.

Methods: Ninety COVID-19 patients with the absence of chronic liver diseases were enrolled. Serum PIIINP, TIMP-1, HA, and the ELF score were measured and correlated with inflammatory indices and clinical variables. Patients were stratified for disease severity according to WHO criteria in two groups, based on the requirement of oxygen support.

Results and Conclusion: Serum TIMP-1, but not PIIINP, HA and ELF score were significantly higher in patients with WHO score > 5 compared to patients with WHO score < 5 [PIIINP: 7.2 (5.4–9.5) vs. 7.1 (4.5–9.9), $p = 0.782$; TIMP-1: 297.7 (20.5–460) vs. 236.7 (28.5–452.8), $p = 0.029$; HA: 117.1 (55.4–193.7) vs. 75.1 (36.9–141.8), $p = 0.258$; ELF: 10.2(9.1–10.5) vs. 9.6 (8.7–10.4), $p = 0.266$]. Even higher levels of PIIINP, TIMP-1, and ELF score were found in patients with SARS-CoV-2 infection than controls. TIMP-1 showed good correlation with PCR ($r = .312$, $p = 0.003$) and with LDH ($r = 0.263$, $p = 0.009$). PCR and serum LDH were significantly higher in COVID patients with WHO score > 5 compared to the matched group of patients with WHO score < 5 [15.8 (9–44.5) vs. 9.3 (3.4–33.8), $p = 0.039$ and 373 (282–465) vs. 289 (218–383), $p = 0.013$, respectively].

In patients with COVID-19, circulating TIMP-1, but not ELF score, was associated with disease severity and with systemic inflammatory index as PCR. In the future, circulating collagen metabolites may potentially be used to select the patients for therapeutic approaches targeting matrix metalloproteases pathway.

ENHANCED LIVER FIBROSIS SCORE AS NONINVASIVE BIOMARKER IN HCV PATIENTS AFTER DAAS

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Aim: In more than 90% of chronic viral hepatitis C (HCV) patients treated with direct-acting antiviral agents (DAAs) sustained viral response (SVR) was observed. Unfortunately, there are subgroups of subjects who display enduring liver fibrosis and are at high risk to develop hepatocellular carcinoma (HCC). Thus, liver fibrosis evaluation during the follow-up of these patients plays a pivotal role. The gold standard to evaluate hepatic fibrosis is liver biopsy an invasive procedure, imaging techniques and serum biomarkers were proposed as more safe procedures. In this study we evaluated the concordance of Transient Elastography (TE) with ELF score (Enhanced Liver Fibrosis) in a cohort of patients with HCV before and after direct-acting-antiviral (DAAs) treatment. ELF score has been validated in other chronic liver diseases; evidence is not available in HCV patients treated with DAAs.

Methods: We prospectively recruited all consecutive HCV patients candidate to DAAs therapy at University of Naples "Federico II" between April 2015 and July 2016. Liver fibrosis was evaluated with both TE and ELF test at baseline, 24 weeks after DAAs treatment (SVR24) and 48 weeks after DAAs treatment (SVR48). Enrolled patients were submitted to TE by Fibroscan® and to a fasting blood sample in the same days. Patients were divided into two groups, according to TE: the F1-F2 group with mild/moderate fibrosis and the F3-F4 group with advanced fibrosis. Samples were assayed in an automated analyzer that performs magnetic separation enzyme immunoassay tests (ADVIA Centaur; Siemens Healthcare Diagnostics, Tarrytown, NY). Results were entered into the manufacturer's published algorithm to derive an ELF score [$ELF = 2.278 + 0.851 \ln(HA) + 0.751 \ln(PIIINP) + 0.394 \ln(TIMP-1)$].

Results and Conclusion: One-hundred-nineteen patients were treated with DAAs and 94.1% of them reached SVR. 55.5% of patients were males with a mean age of 64.7 ± 9.6 years. TE results revealed that 12 patients (10%) had F1-2 mild/moderate fibrosis, 107 (90%) had F3-4 advanced fibrosis. At baseline, SVR24 and SVR48, the concordance between ELF test and TE was poor: 0.11 ($p = 0.086$), 0.15 ($p = 0.124$) and 0.00 ($p = 0.002$) respectively. However, at SVR24 and SVR 48 both methods showed a significant amelioration of liver fibrosis compared to baseline ($p < 0.001$). In addition, both ELF index and TE were significantly associated with portal hypertension at baseline, but not with varices and ascites. Our findings suggested that ELF test can predict independently of TE changes in liver fibrosis. In case of TE unavailability, ELF score could represent an appropriate substitute and for HCV therapy "simplification" ELF score could help to reduce TE exams. Notably, in the context of COVID-19 pandemic, ELF testing should be encouraged to reduce unnecessary access to the hospital and prolonged physical contact.

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Aim of the study: Pregnancy induces changes in immune function that may cause an increased risk of infections and severe sequelae. Understanding the behavior of the SARS-CoV-2 infection during pregnancy is crucial for the maternal-infant dyad's health.

Methods: During the second year of the pandemic (2021), at the Institute for Maternal and Child Health – IRCCS "Burlo Garofolo", Trieste, Italy, women diagnosed with SARS-CoV-2 infection during pregnancy were characterized for the presence of anti-SARS-CoV-2 IgG in sera and funicular blood and for the levels of 27 soluble immune markers at the time of delivery. In addition, the bacterial composition of vaginal and rectal swabs was profiled together with the placental gene expression of ACE2 and TMPRSS2 receptors. To assess vertical transmission, the diagnosis of SARS-CoV-2 infection in the newborns was performed at the time of birth.

Results and conclusions: Based on the time of diagnosis, we recognized the COVID-19 group showing SARS-CoV-2 infection in the last trimester of pregnancy (n=60) and the past-COVID-19 group showing the infection in the first or second trimesters of pregnancy (n=40).

In the COVID-19 and in the past-COVID-19 groups, 40% and 47% of sera and matched funicular blood tested positive for IgG, respectively. When compared to a control group of twenty healthy pregnancies (from a pre-COVID-19 era), COVID-19 and past-COVID-19 groups showed a systemic inflammatory status characterized by the increase of IL-6, IL-1 β and TNF- α . In the past-COVID-19 group, a milder inflammatory scenario mitigated by the anti-inflammatory cytokine IL-10 was observed. A dysbiotic vaginal microbiome was distinctive of the COVID-19 group with a high prevalence of *Bacteroides* and *Enterococcus faecalis*, identified also in rectal swabs, and of *Aerococcus*, an urinary tract pathogen. In the vaginal and rectal swabs from the past-COVID-19 group, the beneficial *Phascolarctobacterium* was detected. When compared to available placental tissues from a pre-COVID-19 era, COVID-19/past-COVID-19 pregnant women showed a decrease in the expression levels of ACE2 and TMPRSS2. Among newborns, one positive nasopharyngeal swab was identified in the COVID-19 group.

Taken together, the rare SARS-CoV-2 vertical transmission is likely due to the maternal antibodies transplacental passage in combination with a prolonged inflammatory anti-viral response and a reduced viral receptor expression. The vaginal dysbiosis observed at the time of delivery could have adverse effects on newborns' health, though, this point needs further studies.

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Introduction: The ongoing COVID-19 pandemic has highlighted the need for effective decontamination sanitation procedures, but these should consider the even larger threat of spread of antimicrobial resistance (AMR), already killing hundreds of thousands of people around the world and often complicating the care of COVID-19 patients. Indeed, the sanitation procedures based on high concentrations of chemical disinfectants show limitations, as they have a temporary effect, high environmental impact, and might further spur AMR.

Previous results obtained by our group showed that an eco-sustainable sanitation system involving remodulation of hospital microbiome by selected probiotic *Bacillus* (probiotic cleaning hygiene system, PCHS) could stably reduce resistant pathogens (-80%) and related infections (-52%).

The aim of this study was to assess the antiviral properties of such system.

Materials and Methods: The antiviral activity of PCHS was assayed *in vitro* against enveloped viruses, including human coronavirus 229E, vaccinia virus, herpesvirus type 1, flu viruses (human and animal strains), and SARS-CoV-2. PCHS activity was analyzed following the standard procedures required to define the antiviral properties of a product, both in solution and on hard non-porous surfaces (UNI EN 14476, UNI EN 16777:2019). Different concentrations and times of contact were tested, evaluating the effectiveness both in removing and preventing virus contamination.

Results and conclusions: PCHS significantly decreased the virus contamination in solution and on surfaces, with times of application comparable to those required to certify the antiviral action certification of products by standard procedures. In detail, PCHS induced a >4Log decrease of virus load within 1-2 hours, depending on the virus type and resistance. Full action was observed against SARS-CoV-2 within 1 hour. Notably, the effect was maintained during time up to 24 hours, preventing subsequent virus contamination, contrary to control disinfectants (ethanol and sodium hypochlorite).

Based on our results, PCHS could respond to the urgent need of systems able to stably decontaminating from SARS-Cov-2 without worsening AMR concern, preventing the effects of an eventual future pandemics of secondary bacterial AMR infections.

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The current pandemic is obviously an international public health problem and it remains a challenging task to fight. Despite SARS-CoV-2 rapid transmission, the scale up country readiness, speedy response teams and the capacity of all laboratories are reducing the spread of the virus as well as its mortality rate. In our patient's cohort all the clinical and laboratory information was reported in detail from January 2020 to December 2021 at AORN of Sant’Anna and San Sebastiano of Caserta. The SARS-CoV-2 gene targeting RT Real Time PCR assay was conducted in accordance to the protocol established by WHO. Consent was obtained from all involved patients. Patients' negative test results for COVID-19 in the pharyngeal swab were excluded. Demographic information and clinical characteristics (including medical history, underlying comorbidities, symptoms and signs), laboratory findings and Chest RX scan results were obtained. Stata analysis was performed. A total of 267 confirmed patients were enrolled at Infection Disease Unit, Intensive Care, Pulmonary departments. Sixty-nine patients were male and the average age 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%), dyspnea (82,9%) and cough (62,9%). Laboratory findings on admission showed median value of D-Dimer, Protein C Reactive, Interleukin-6 and Lactate Dehydrogenase significant alterations compared to normal values. As regard pulmonary involvement, 41,4% patients had bilateral interstitial pneumonia than 18,5% bilateral alveolar pneumonia. Syndromic testing was tested for multiple respiratory pathogens in hospitalized patients with SARS-CoV-2 infection allows the rapid detection of viral and bacterial pathogens co-infection. Three patients were positive for SARS-CoV2 and Human Rhinovirus / Enterovirus and one patient for SARS-CoV2 and Parainfluenza Virus 2. As several study we isolated in COVID-19 patients' atypical bacteria like *Legionella pneumophila* and *Streptococcus pneumoniae*. Pneumococcal and legionella urinary antigen test resulted positive for four and one patient respectively. Bacterial co-infection pathogens depending on the site of infection. The most common pathogens in blood cultures were gram-positive bacteria: *E. faecalis* and *S. aureus*. Gram negative isolates was only *A. baumannii*. Instead, respiratory tract infections in COVID-19 patients, were caused in high prevalence by gram-negative bacteria, particularly *P. aeruginosa* (41%). Significant co-infection rates and pathogens were founded in females compared with males. As regards the age, mainly co-infected pathogen isolated was *P. aeruginosa* in 48- 58 years old age group than *A. baumannii* in 59-69 years old age group. We also reported antibiotics susceptibility percentages. Aminoglycosides, β lactams / β lactamase inhibitor showed the highest resistance rates in gram negative co-infections. Gram-positive bacteria showed resistance percentage to quinolones antibiotics class. The aim of this study was highlighting the importance of using all the laboratory and clinical data for the evaluation and management of Sars-CoV-2 symptoms. Furthermore, timely and simultaneous identification of viral and bacterial co-infections, with antimicrobial susceptibility profile, can contribute to patient management and antibiotic stewardship during the current pandemic.

**ASSESSMENT OF MATERNAL AND NEONATAL SARS-COV-2 ANTIBODIES
IN NORTHERN ITALY IN PRE-VACCINATION ERA**

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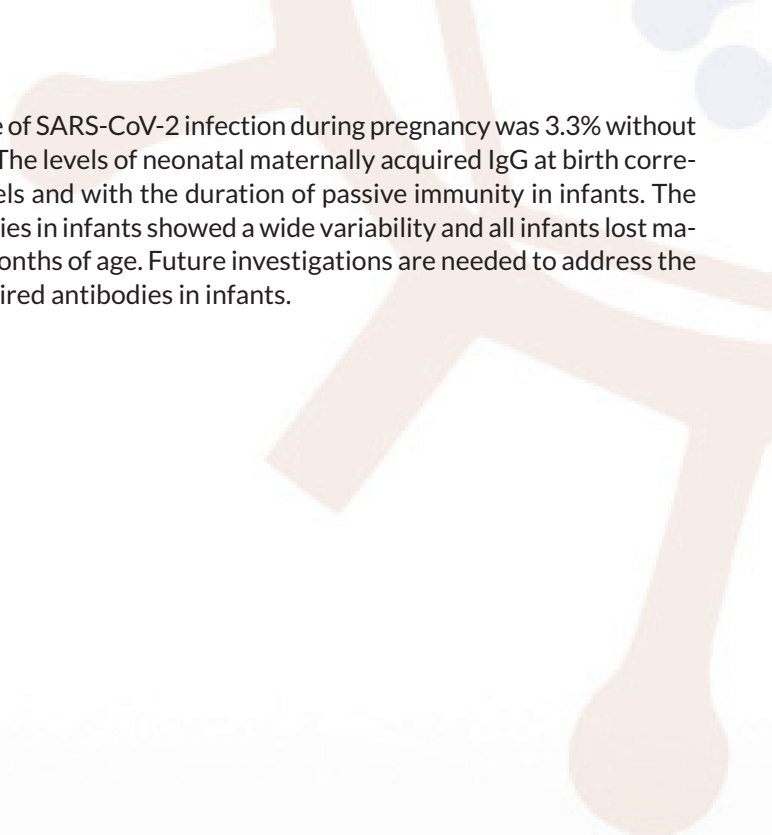
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Aim: Few studies reported that maternal antibodies produced in response to SARS-CoV-2 infection during pregnancy cross the placenta, likely conferring some degree of passive protection to the neonates. The aims of this study were: i) to explore the prevalence of SARS-CoV-2 infection during pregnancy in the pre-vaccination era, ii) to assess the efficiency of placental transfer of maternal antibodies to the neonate and their persistence during the first months of life.

Methods: Pregnant women admitted at delivery to 3 hospitals in Bologna, between July 1, 2020, and March 31, 2021, were enrolled in the study. Women were screened for SARS-CoV-2 by nasopharyngeal swab RT-PCR and by serology. All newborns were tested for SARS-CoV-2 by nasopharyngeal RT-PCR and, in case of maternal positive results, also by serological tests. The Elecsys Anti-SARS-CoV-2 ECLIA assay (Roche Diagnostics, Switzerland) was used for qualitative detection of all classes of immunoglobulins. In case of positive results, blood samples were tested for specific SARS-CoV-2 IgM (Shenzhen YHLO Biotech, China) and for quantitative detection of SARS-CoV-2 S1/S2 IgG (DiaSorin, Italy).

Results: SARS-CoV-2 antibodies were detected in 134 of 4045 mothers (3.3%): 34/134 (25%) had both specific IgM and IgG antibodies; 100/134 (75%) were IgG positive and IgM negative. Only 53/134 (40%) women were aware of their SARS-CoV-2 infection acquired during pregnancy. Samples from 67 neonates born to 67 mothers with positive serological tests were available. All neonates had negative nasopharyngeal RT-PCR during the first 24 hours of life. All were SARS-CoV-2 specific IgM negative. Seven of 67 newborns (10%) were also IgG negative; the other 60/67 (90%) were IgG positive. The median IgG level in maternal blood at birth was significantly higher than IgG level in neonatal blood (35 AU/mL versus 28 AU/mL, $P=0.04$). There was a significant positive correlation between maternal and neonatal SARS-CoV-2 IgG levels ($P<0.02$). The transplacental transfer ratio (infant IgG concentration divided by maternal IgG concentration) was 0.92. This value was higher when maternal infection occurred in the second trimester compared to third trimester maternal infection (mean 1.27 versus 0.70, $P=0.0015$). SARS-CoV-2 IgG levels in neonatal blood progressively decreased during the first months of life and 91% of the babies had lost maternal antibodies at 4 months of life. Only 3 newborns (9%) showed seroreversion at 6-7 months. The persistence of maternal antibodies was positively correlated to the SARS-CoV-2 IgG levels at first sampling.



Conclusions: The prevalence of SARS-CoV-2 infection during pregnancy was 3.3% without cases of neonatal infection. The levels of neonatal maternally acquired IgG at birth correlated with maternal IgG levels and with the duration of passive immunity in infants. The persistence of these antibodies in infants showed a wide variability and all infants lost maternal antibodies within 7 months of age. Future investigations are needed to address the protection of passively acquired antibodies in infants.

CHANGE IN THE PREVALENCE OF HEPATITIS C VIRUS (HCV) GENOTYPES IN THE AREA LARIANA DURING THE COVID-19 PANDEMIC

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Introduction and Aim: Italy is the European country with the highest prevalence of HCV infection and genotype 1, subtype 1b, is the most widespread (51% of HCV positive subjects). Although the introduction of therapy based on direct acting antiviral drugs (DAAs) has led to a reduction of infection related clinical complications (such as hepatocellular carcinoma HCC, liver failure and the need for liver transplantation), the Sars-CoV-2 pandemic caused, unfortunately, a reversal of the trend due to a drastic reduction in medical visits for patients with consequent non monitoring of clinical and pharmacological status of patients.

Our study is aimed at capture the variations both in the numbers and prevalence of genotypes of HCV infection in the Como's area during the period of the Covid-19 pandemic.

Materials and Methods: We included in the study all patients in the ASST Lariana's hospitals who tested positive for the quantitative test for HCV-RNA and we took into consideration the period from 01/01/2019 to 31/03/2022, for a total of 3977 tests performed for HCV-RNA quantification.

The quantification of virus' RNA was performed with Roche™ automated systems which provide for extraction of the genetic material on the Cobas X 480 platform and amplification in Real Time PCR on the Cobas Z 480 platform. HCV-RNA genotyping was performed using an hybridization test on nitrocellulose strips after retro-transcription in cDNA with automated detection on a scanner (Profilblot T48, Nuclear Laser Medicine).

Results and Discussion: The first fact that our results show is that, during the observed period, there was a gradual reduction in the requests for quantitative HCV-RNA (879 in 2021, 1139 in 2020, 1965 in 2019). A second interesting datum shown is the increase of the incidence of positivity on the total of requests, with percentages respectively of: 21% in 2021, 15% in 2020 and 18% in 2019. As regards genotypes, we found a change in their distribution: in the period 2019-2020 genotype 1b prevails (21%); in 2021, on the other hand, the genotype 3a (12%) prevails, with most of new discovered infection, and 1a (10%). Furthermore, in particular for the 4c/d genotype, we observed the finding (2% in 2021) or the increase in viral load in patients previously vireosuppressed or with low viral load, respectively.

Based on the observed variations, both in terms of absolute numbers and percentages, and by the crossing of laboratory data with clinical-anamnestic data, we can therefore state that in the three years period examined, in the Como's area there was a change in the distribution of HCV genotypes and a progressive recovery of HCV infections caused by genotypes considered more virulent due to their lower response to drugs but also more aggressive due to their more rapid evolution towards cirrhosis and hepatocellular carcinoma (HCC).

Conclusions: Therefore we hope for a gradual resumption of visits and monitoring and compliance on therapy of these patients so that we can once again aim for the historical lows of the spread of this infection and its complications.

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Aim of the study: The aim of this study was to investigate the genetic heterogeneity of SARS-CoV-2 circulating in Umbria region from January 2021 to December 2021 with the main objective to identify the possible introduction of new "*variants of concern*" (Voc).

Methods used: A total of 2949 SARS-CoV-2 whole genomes were newly characterized from an equal number of patients affected by COVID-19, attending different clinical centers in Umbria region. SARS-CoV-2 RNA was extracted from nasopharyngeal samples using the Kit QIAasymphony DSP Virus/Pathogen Midi kit on the QIAasymphony automated platform or manually with QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany). The library preparation was carried out with a Hamilton Microlab STAR Liquid Handling System (Hamilton Robotics, Reno, NV, USA) using COVIDSeq Test (Illumina). Deep sequencing was performed on the NextSeq 500 platform (Illumina Inc., San Diego, CA, USA) using the NextSeq 500/550 Mid Output Reagent Cartridge v2, 300 cycles, and standard 150 bp paired-end reads. After quality control and trimming of the reads by FastQC and Trimmomatic, mapping to Wuhan-Hu-1 reference genome (Acc no NC_045512) was performed by BWA tool and the consensus sequence was obtained using iVar (v1.3.1) (intra-host variant analysis of replicates; github.com/andersen-lab/ivar). All analysis steps were automatically performed at the end of the sequencing run on the GENPAT platform at IZS-Teramo. The identification of the occurring SARS-CoV-2 lineage was confirmed by using the PANGO tool via web (<https://pangolin.cog-uk.io/>).

Results and conclusions: Our results showed that from January 2021 to July, the Alpha variant (B.1.1.7) appeared with a peak of cases in May (72%). On February, the gamma variant (P.1) emerged independently from B.1.1.7 until August. In June, a new variant named Delta (B.1.617.2) appears with a peak between September and November, causing 100% of analyzed cases. Delta was still present in December with a 79% when appeared the Omicron variant (B.1.1.529). The sequences obtained in this study were deposited in GISAID (<https://www.gisaid.org/>) and I-CO-GEN database (<https://irida.iss.it/irida21-aries/projects>). The sharing of the sequencing data obtained from the different regions and the application of molecular epidemiology has made it possible to detect the emergence of new variants also in the Umbria region in the course of 2021. This information has been and still is extremely vital to detect the distribution in time and space of different variants. This data represent the critical point for the integrated surveillance and for the development of public health interventions and viral spread control policies.

TRACKING SARS-COV-2 VARIANTS OF CONCERN IN NASOPHARYNGEAL SWABS FROM MILITARY AND CIVILIAN PERSONNEL OF AN AIR FORCE AIRPORT IN CENTRAL ITALY: ONE YEAR MONITORING

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Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is responsible for the coronavirus disease COVID-19, a public health emergency worldwide. On March 11th 2020, the World Health Organization declared COVID-19 a pandemic. The emergence of variants that posed an increased risk to global public health driven the characterisation of specific Variants of Interest (VOIs) and Variants of Concern (VOCs) during late 2020, to prioritise monitoring and research.

This study aimed at tracking the occurrence and genetic variability of SARS-CoV-2 in nasopharyngeal swabs from asymptomatic and paucisymptomatic military and civilian personnel of the “Mario De Bernardi” Air Force airport in Central Italy, over more than one year. It was carried out in the frame of a Collaboration Agreement between Experimental Flight Center - Italian Air Force Logistic Command - and Istituto Superiore di Sanità (ISS), to control transmission of SARS-CoV-2 infection in the workplace.

The airport personnel was tested locally by a rapid antigen test, then samples were sent to ISS for confirmation by Real Time PCR (RealStar® SARS-CoV-2 RT-PCR Kit 1.0, Altona Diagnostics). The screening resulted in a total of 91 positive nasopharyngeal swabs collected between December 2020 and March 2022.

Total RNA was extracted by the QIAamp® MinElute® Virus Spin Kit (QIAGEN) and subjected to a RT-nested-PCR assay previously designed, followed by Sanger sequencing for molecular characterization. The assay, targeting a ~1600 bp fragment of the spike protein, is able to detect multiple nucleotide changes resulting in key spike protein mutations distinctive of the major known circulating SARS-CoV-2 variants.

Of the 91 samples positive to SARS-CoV-2, 68 were successfully amplified and characterized as VOCs or other variants. Alpha, Gamma and Delta variants were detected during 2021. The Alpha variant was detected in February and March in 11.8% of the 68 positive samples; the Gamma variant in March in 3% of samples, and the Delta variant in December 2021, in 4.4% swabs. Only the Omicron variant was detected since January 2022, in 35/68 (51.5%) samples with high degree of variability. Within the Omicron variant, key mutations of sublineages BA.1, BA.1.1, and BA.2 were detected. The results showed progressive replacement of SARS-CoV-2 variants in the study period, mirroring the variant replacement in the general population in 2020/2022. This approach can be used to better understand SARS-CoV-2 variant diversity and spatial-temporal circulation of SARS-CoV-2 VOCs and VOIs.

RAPID SARS-COV-2 INTRA-HOST AND WITHIN-HOUSEHOLD EMERGENCE OF NOVEL HAPLOTYPES

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In February 2020, the municipality of Vo', a small town near Padua (Italy) was quarantined due to the first coronavirus disease 19 (COVID-19)-related death detected in Italy. To investigate the viral prevalence and clinical features, the entire population was swab tested in two sequential surveys. Here we report the analysis of 87 viral genomes, which revealed that the unique ancestor haplotype introduced in Vo' belongs to lineage B, carrying the mutations G11083T and G26144T. The viral sequences allowed us to investigate the viral evolution while being transmitted within and across households and the effectiveness of the non-pharmaceutical interventions implemented in Vo'. We report, for the first time, evidence that novel viral haplotypes can naturally arise intra-host within an interval as short as two weeks, in approximately 30% of the infected individuals, regardless of symptom severity or immune system deficiencies. Moreover, both phylogenetic and minimum spanning network analyses converge on the hypothesis that the viral sequences evolved from a unique common ancestor haplotype that was carried by an index case. The lock-down extinguished both the viral spread and the emergence of new variants.

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West Nile virus (WNV) is a mosquito-borne virus causing disease in humans and other animals. Originally limited to Africa, in recent years this pathogen has rapidly emerged and spread globally also in temperate areas, such as North America and Europe. Only two strains have been significantly detected in Europe so far, named WNV lineage 1 (WNV L1) and lineage 2 (WNV L2). Since 2004, several studies have highlighted a progressive increase of WNV L2 in Europe, associated with a reduction of WNV L1 circulation. This trend has also been observed in Italy, one of the European countries which has registered the highest number of human and animal cases.

To better characterize the WNV L2 epidemiological trend in Italy we analyzed 2107 samples collected during a 10-year period under the National surveillance plan, implemented by the Ministry of Health since 2001 to monitor WNV and USUV circulation in the whole country. Among these samples the National Reference Centre for Foreign Animal Diseases (CESME) confirmed 2093 real time PCR WNV L2 and 14 WNV L1 positive samples.

Our ecological and epidemiological analyses confirm a progressive increase of WNV L2 circulation on the Italian territory during the last years, with a peak observed for the 2018 epidemic season, which was probably influenced by abiotic factors such as particularly warm spring temperatures and higher rainfalls.

Furthermore, we downloaded all available WNV sequences from NCBI and we extracted 46 WNV L2 full genome sequences obtained at CESME between 2011 and 2022. We used this dataset to reconstruct WNV-L2 Italian phylogeny and evolution using molecular clocks. Molecular data clearly show a sharp separation between different WNV lineages, with the L1 and L2 clades representing most strains in our dataset. Among WNV L2 we detected two main WNV L2 groups, previously identified as i) the Central-Southern-Eastern European clade, which is further divided into the Central-Southern and the Central-Eastern European clusters, and ii) the Russian-Romanian clade. The newly obtained WNV L2 Italian sequences clustered with old Italian samples in the Central Eastern-European clade, showing that the circulation of WNV in Italy is endemic, with no significant introductions from other countries in recent years. Resident birds and vector competent mosquitoes (mainly belonging to the *Culex* genus) seem to play a major role in the WNV endemic circulation, while migratory birds seem responsible for sporadic introductions and do not significantly affect the Italian scenario.

Our results represent an overview of the epidemiological and genetic situation of WNV on the Italian territory, which can be used as a reference scene to identify new strategies to limit the impact of the pathogen into new regions and to identify new critical questions to be addressed in future studies.

Title: SARS-COV-2 VARIANTS AND THE INFERENCE OF CORRELATES OF PROTECTION BASED ON NEUTRALIZING ANTIBODIES AND ANTI-S-RBD IGG LEVELS

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The appearance of omicron variants BA.1 and BA.2 has aroused serious concerns due to the unprecedented high diffusivity of these strains and their ability to break through population immunity. In a state of endemicity, the emergence of novel variants will pose recurrent challenges to the decision process guiding the prescription of seasonal boosts. Ideally, serosurveys aimed at assessing variant-specific immune-protection levels would help tailor the immunization schedule, as well as the identification of eligible candidates for additional vaccinations among fragile categories and subjects with high occupational risks.

Relying on a live virus plaque reduction neutralization test (PRNT) and a commercial chemiluminescence immunoassay (CLIA) targeting antibodies against the receptor binding domain (RBD) of the parental Spike protein, we tested the efficacy of homologous and heterologous booster vaccinations at inducing antibodies against SARS-CoV-2 parental, delta, beta and omicron BA.1 and BA.2 variants, in groups with a different history of SARS-CoV-2 infection. Booster vaccination was performed with the BNT162b2 vaccine, while individuals who underwent heterologous booster vaccination were primed with the ChAdOx1 nCov-19 vaccine. To translate neutralization data into variant-specific estimates of protection, we relied on published predictive mathematical models that interpolate mean neutralization titers of convalescent individuals as calibrator for the inference of protective cut-off values in vaccinated individuals (Khoury et al, 2021). Moreover, we performed ROC analyses of PRNT titers and CLIA binding antibody units to identify tentative cut-offs for protection for the CLIA assay.

In our study, we observed that homologous and heterologous booster vaccination can induce neutralizing antibodies that correlate with protection against infection with parental, delta, beta and the omicron variants, in both naïve adults and convalescent adult populations. Moreover, we verified that a high-throughput anti-RBD CLIA assay retained semi-quantitative characteristics against omicron, beta and delta variants. Our correlation analyses confirmed that despite severe drops in neutralization and antibody binding reactivity, quantification of anti-RBD antibodies against omicron correlated with neutralizing titers ($r=0.7530$; $p<0.0001$), similarly to the other variants. When ROC curves were generated, the area under the curve (AUC) was comparably high for the parental (0.98), beta (1.00), delta (0.96) and omicron (0.96) variants ($p<0.0001$).

Identification through serological commercial assays of thresholds of protection against new emerging variants (as well as for the already existent Omicron BA.1 and BA.2) is a cru-

cial step towards large-scale serosurveys to finely assess infection risk both at population and individual level, and to prioritize future vaccinations for those individuals with higher risk of infection.

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Since the start of the COVID-19 pandemic, public health measures revealed that the control of SARS-CoV-2 modified the epidemiology of other respiratory viruses including Respiratory syncytial virus (RSV). Consequently, in Italy, as well as in other countries, a strong reduction in all viral respiratory infections was observed in the last season (2020–2021), compared with the previous seasons. Nevertheless, after a relative absence during the past season, a large resurgence of RSV detections occurred in recent months in Italy. RSV is the most prevalent cause of viral respiratory infections in children up to the age of 2 years and causes a wide range of clinical manifestations. It circulates seasonally with variable epidemiology, depending on geographic area and climate. In temperate regions of the Northern Italy, the virus diffusion generally occurs in late fall and early spring, with a peak incidence in January/February. This retrospective study involved pediatric patients (0–10 years old) admitted with respiratory symptoms at AORN Sant'Anna and San Sebastiano of Caserta during the last three winter seasons (September to March 2021–2022). Samples collected included nasopharyngeal swabs were processed. The identification of respiratory viruses was accomplished by with BioFire Respiratory Panel 2.1 plus (BioFire, Salt Lake City, Utah). This test included respiratory pathogens: Adenovirus, Coronaviruses 229E, HKU1, NL63, OC43, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), Severe Acute Respiratory Coronavirus 2 (SARS-CoV-2), Human Metapneumovirus, Human Rhinovirus/Enterovirus, Influenza A, Influenza B, Respiratory syncytial virus (RSV), parainfluenza virus 1,2,3,4. In the Northern Hemisphere, the RSV season starts in November and ends in March, with peaks in January and February, while in the Southern Hemisphere it lasts from June to September. During the season 2020–2021, the total number of pediatric viral infection cases was 46. RSV infection was 53,5% than, Adenovirus and Human Rhinovirus/Enterovirus were 16,3% and 34,9% respectively. Similarly, the total parainfluenza virus 3 infections were 20,9% while we did not detect any case of influenza virus. Compared to the last

seasons, 2019–2020 and 2020–2021 we registered a decrease of 75% and 57,3% in the total number of respiratory infections. Notably, RSV increased trend infection from October to December 2021 that represent the typical RSV season but no RSV cases were recorded from January to March 2022. Although an important decrease in Rhinovirus cases emerged. Mixed RSV-adenoviral infections (3), RSV-parainfluenza virus 3 (3) infection and Rhinovirus infection (3) were founded. Our study showed sudden and earlier start of the RSV epidemic season, compared to the previous years and almost no cases detected in February and March months. This mono-centric study highlights an important epidemiological change due to current strategies adopted against COVID-19 and focuses on the importance to continue the active surveillance against respiratory viruses in pediatric patients.

MOLECULAR EPIDEMIOLOGY OF RSV STRAINS CAUSING THE UNUSUAL PEAK OF HOSPITALIZATION IN AUTUMN 2021

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Respiratory syncytial virus (RSV) is the main cause of children hospitalization for severe bronchiolitis. Since March 2020, a remarkably low circulation of respiratory viruses other than SARS-CoV-2 has been reported worldwide, due to pandemic restrictions. After a nearly complete absence of RSV cases during 20/21 winter season, a resurgence of cases was expected in autumn 2021 after school re-opening and removal of the most restrictive measures.

The aim of this study was to monitor respiratory viruses' resurgence in the 21/22 epidemic season, the incidence of RSV severe cases and the circulation of RSV-subtypes and variants.

Methods: previously healthy children consecutively hospitalized for bronchiolitis and other acute respiratory infections were enrolled from September 1, 2021 to March 15, 2022 at the Sapienza University Hospital of Rome. RSV and other 13 respiratory viruses were tested in nasopharyngeal (NP) washing from infants up to one year of age or in NP swabs from older children by home-made PCR-based molecular methods. After sequencing the second half of the G gene of about 70 samples of RSV-A and -B, a phylogenetic analysis was performed.

Results: We consecutively enrolled 218 children admitted for respiratory tract infection; the median age was 0.6 years (IQ: 0.18-2.2) and 131/218 (60.1%) were males. The most frequent symptoms were cough, rhinitis, respiratory distress and fever; the mild forms were predominant (54%), and only 2.3% were severe. The RSV positive cases were 114/218 (54.3%), followed by Rhinoviruses (7.8%) and Metapneumoviruses (6.9%), and a few other virus infections. The total hospital admissions increased from the second half of October 2021 to the first half of December 2021 with a peak at the beginning of November 2021. The RSV incidence peak strictly followed the peak of total admissions; their number exceeded the median number of hospitalizations observed in pre-pandemic seasons. Unlike 2018/2019, RSV-A was more prevalent (70% of the total cases) than RSV-B. The phylogenetic analysis of the G gene identified several variants of the genotype ON1 (RSV-A) and of the genotype BA10 (RSV-B); some of them were not found in the preceding seasons.

Conclusions: The 21/22 seasonal peak of RSV hospitalizations occurred much earlier and was more intense than in pre-pandemic years, probably caused by the decline in population immunity due to the disappearance of respiratory viruses during the winter 20/21. Interestingly, this unusual peak had an abrupt drop in coincidence with SARS-CoV-2 surge during December 2021. Molecular surveillance of respiratory infections may help to effectively plan RSV passive and, in the future, active prophylaxis and to build resilience against future pandemic threats.

WHEN AN OPPORTUNISTIC INFECTION GIVES THE OPPORTUNITY TO MAKE A DIAGNOSIS: A CASE REPORT

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We report a case of a fifty-four years old male arrived in emergency room with fever and subsequent appearance of left hemisindrome who hid many others disease.

Clinical Scenario: At first, the clinical status directed the attention toward a vascular event. So, waiting for patient's collaboration for the anamnesis, the emergency colleagues proceeded to blood chemistry tests, chest x-ray and brain CT scan. Incidentally, the research of Sars-CoV-2 was negative, always.

The full blood count showed anemia and a normal count of leukocytes with neutrophilia and lymphopenia and the blood profile showed aspecific alterations.

Even the x-ray showed aspecific finds: "thickening of the interstitium in mid-upper lung fields bilaterally, in the absence of frank parenchymal thickenings with an ongoing focus".

Brain CT scan, instead, showed: "in the right temporo-parietal site, inhomogeneous parenchymal hypodensity is recognized with associated smoothing of the periencephalic sulci in the first hypothesis with ischemic significance which cannot be excluded with certainty the hypothesis of expansive alteration".

Clinical Case: The findings not matched because in the differential diagnosis there weren't univocal elements that could lead to one disease. For these reasons, own laboratory equipe according to ward equipe started to do some diagnostic insights to understand if the clinical scenario was due to ischemic or infectious disease.

The patient was transferred in neurology department where news blood serological tests were requested. In particular, it was requested HIV serology which unexpectedly turned out to be positive, even to confirmatory test. The suspect, therefore, went to a complicated neurotoxoplasmosis but the serological tests were negative.

We also investigated lymphocytic subpopulations and we found CD4+: 26/mmc.

At this point, in according to infectious disease and neurological equipe, we analysed liquor sample for other microorganisms. The liquor analysis revealed positivity for Koch Bacillus (BK) and, consequently, this positivity was confirmed with QuantiFERON-TB Gold Plus (Diasorin) in peripheral blood.

However, a serotin low-grade fever persisted. In others insights, in fact, we found IgG and IgM positivity for Citomegalovirus, as it was a reactivation.

To complete the diagnosis, it was requested a chest TC that showed the typical alterations of miliary tuberculosis.

It was started pharmacological therapy and, gradually, there was an improvement in clinical scenario.

When the patient became collaborative for the anamnesis, he reported a silent history for risk factors for the infectious disease and he didn't know his positivity for HIV.

Conclusion: Nowadays we never would like to comment about advanced features of AIDS but, unfortunately, there are still some of this cases.

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COMMON RESPIRATORY VIRUSES AND COVID-19, DID SOMETHING CHANGE?

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Aim of the study: Influenza and Respiratory Syncytial viruses dominate winter months in temperate regions. There is evidence that factors such as temperature, humidity and human behaviour interfere with the seasonality of respiratory viruses [Moriyama M et al. Seasonality of Respiratory Viral Infections. Annu Rev Virol. 2020]. During the pandemic, their trend changed. In Italy in 2020-21 the incidence of Flu and other respiratory infections [<https://www.epicentro.iss.it/influenza/flunews>] remained below 3,16/1000 compared to 2019-2020 (12,6/1000). In 2021-22, the relaxation of the public health measures probably contributed to a new increase of respiratory infections SARS-CoV-2 unrelated. We report how the spread of Influenza A/B and RSV has changed before and after the COVID-19 pandemic in patients attending ASST-FBF-Sacco Hospital in Milan.
Methods: A total of 1855 nasopharyngeal swabs collected from 1st January 2018 to 31st March 2022 in hospitalized and emergency-room patients were tested by Real-Time PCR Xpert®Xpress Flu/RSV.

Results and conclusion: Table 1 shows the results of Flu A/B/RSV test in 2018-2022. Figure 1 shows samples that tested positive for each respiratory virus compared to the total in the four quarters/year. The results showed that the percentage of positivity for the respiratory viruses varied widely in 2018-2022. In details, there was a clear difference in the percentage of Flu A positivity in the pre-pandemic (2018, 15%; 2019, 24%) vs pandemic (2020, 10%; 2021, 0%) years. The disappearance of Flu A in 2021 coincided with the finding of RSV in 13% of samples. Vice versa, in the first quarter of 2022 there is an inversion in the trend with the detection of Flu A in 22% of cases. Our data confirmed that the emergence of SARS-CoV-2 and public health measures may have interfered with the spread of Flu A and RSV in Milan.

Table 1.

	2018	2019	2020	2021	2022 (Q1)
Sample size	386	655	226	348	240
Negative	307 (80%)	446 (68%)	189 (84%)	302 (87%)	186 (77%)
Positive	79 (20%)	209 (32%)	37 (16%)	46 (13%)	54 (23%)
Flu A	59 (15%)	160 (24%)	22 (10%)	0	52 (22%)
Flu B	8 (2%)	2 (0.3%)	7 (3%)	1 (0.3%)	0
RSV	12 (3%)	47 (7%)	8 (4%)	45 (13%)	2 (1%)

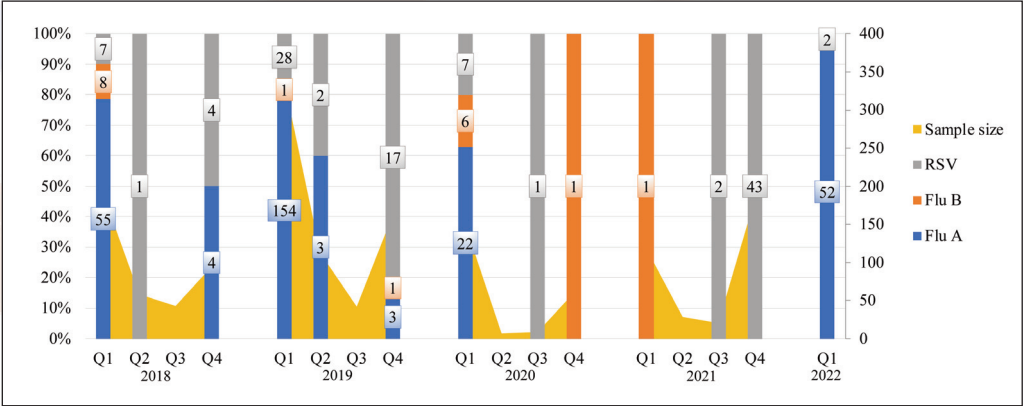


Figure 1.

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Aim of the study: In the first phase of the SARS-CoV-2 pandemic, children and adolescents represented a tiny portion of cases. However, more transmissible variants and school reopening have contributed to an increase in the diffusion of SARS-CoV-2 infection in this population. In Italy, patients aged 0-19 years old were 1.2% of all positive cases in March 2020, 12.2% in January 2021 and 28% in January 2022 [SARS-CoV-2 dashboard, Istituto Superiore di Sanità]. Here we retrospectively investigated the epidemiological and clinical characteristics of non-hospitalized patients under 19 years old tested for SARS-CoV-2 RNA at ASST-Fatebenefratelli-Sacco Hospital in Milan, Italy.

Methods: A total of 1838 nasopharyngeal swabs collected from December 2021 to January 2022 were analysed by Real-Time PCR using the PerkinElmer® SARS-CoV-2 Assay, which targets two SARS-CoV-2 genomic regions: N and ORF1ab.

Epidemiological and clinical data were collected using a self-administered questionnaire designed by Lombardy Region.

Results and conclusion: The rate of patients who tested positive for SARS-CoV-2 RNA was 35.6% (654/1838), with a median time length of negativization of 10 days (min 2; max 27). Patients were divided into three age groups: 0-5 (A), 6-12 (B), 13-19 (C) years old, showing a positivity rate of 32% (260/810), 40% (310/778) and 34% (84/250), respectively. The median Ct was 24 (min: 15; max: 37) in group A and 23 (min: 15; max: 37) in B and C groups. Asymptomatic patients were 67% (173/260) in group A, 59% (184/310) in group B and 50% (42/84) in group C, respectively, while those reporting two or more symptoms were 10% (26/260, group A), 15% (47/310, group B) and 16% (13/84, group C).

Our study showed a high rate of SARS-CoV-2 among children and adolescents during the Omicron-dominated pandemic wave that hit Italy from December 2021 to January 2022 due to this extremely high transmissible variant, as already described [Hospitalizations of Children and Adolescents with Laboratory-Confirmed COVID-19- COVID-NET, 14 States, July 2021–January 2022. CDC]. Most of the tested positive patients were asymptomatic or presented mild symptoms like nasal congestion, cough and headache; only a small fraction of patients showed more than two symptoms. Our data show that the highest prevalence of asymptomatic patients was detected in ages 0-5 in accordance with other studies that reported a less severe outcome in the Omicron infection compared to the Delta ones [Wang, Lindsey et al. "COVID-19 infection severity in children under 5 years old before and after Omicron emergence in the US;13 Jan. 2022].

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Aim of the study: During the global spread of the COVID-19 a number of genetic variants of the SARS-CoV-2 have emerged. Several variants have increased transmissibility or exhibit an increased ability to escape from host immunity, and consequently cause an increased risk to global public health (1). The aim of present study was to monitor the molecular epidemiology of variants of concern (VOCs) of SARS-CoV-2 in the province of Matera, Italy, between June and September 2021

Methods: A total of 137 nasopharyngeal/oropharyngeal swab samples positive for SARS-CoV-2 were investigated by molecular biology (2) using MagMAX Viral RNA Isolation Kit followed by qPCR. The qPCR was performed by GenFinder COVID-19 Plus RealAmpKit and cDNA synthesis with SuperScript Vilo cDNA synthesis kit. Lineages were assigned from alignment file using Ion Chef System Ion Torrent™ and sequencing by Ion GeneStudio S5 S5-0465 Semiconductor Sequencer.

Results and conclusions: By analyzing samples, were derived some relevant observations concerning the lineages of SARS-CoV-2 in infected subjects. For instance, the SARS-CoV-2 lineages belong to percentage (%) different variants, as B. 1.1.7 alpha (English) in the 35,7 %, and to P.1 Gamma (Brazilian) for 1,5%, B.1.617.2 (Delta) for 57,6%, AY7.2 (Delta-Like) 2,2% and B.1.177 Spanish (3%) of the investigated sample (137 subjects), between June and September 2021 in the province of Matera (Italy). Novel SARS-CoV-2 variants will emerge through the time. Specific mutation profiles deserve further investigations to clarify potential effects on vaccination efficacy (3). The epidemiological profile of the variants we provided here is valuable for comparative study on the infectivity of other future emerging variants, also evaluating their epidemic risks.

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EPIDEMIOLOGY OF VIRAL RESPIRATORY INFECTIONS DURING TWO YEARS OF THE SARS-COV-2 PANDEMIC IN A TEACHING HOSPITAL, SOUTHERN ITALY, USING MULTIPLEX PCR PANELS

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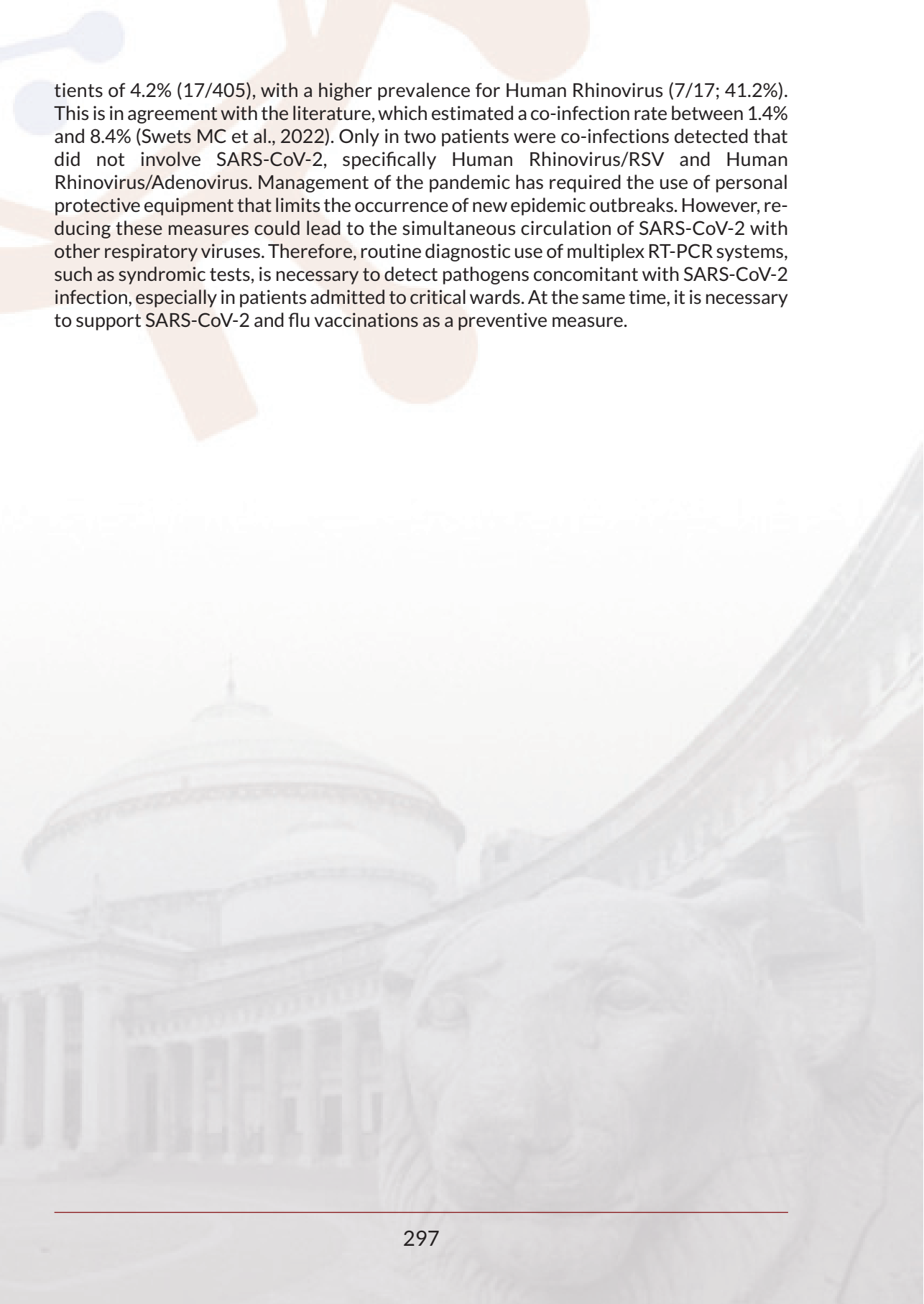
Aim of the study: Infections caused by respiratory viruses can often be difficult to differentiate clinically from SARS-CoV-2 infection. Multiplex PCR panels are able to rapidly identify single or co-infections in the respiratory tract to facilitate proper patient management. SARS-CoV-2 infection alone may often result in mild or absent clinical symptoms, as is the case of most COVID-19 patients, while coinfection of SARS-CoV-2 with other respiratory viruses can aggravate the clinical course of the disease (Aghbash PS et al., 2022). Herein, we describe the distribution of respiratory viruses and the occurrence of co-infections during the pandemic in the Teaching Hospital of Catanzaro, Southern Italy.

Methods: Between January 2020 and February 2022 we collected 2126 nasopharyngeal swabs from patients attending our Hospital: 1.278 swabs were processed using the FilmArray® RP2plus v1.0/RP2.1plus v1.0 panel (bioMérieux Italia), and 848 swabs were analyzed using the QIAstat-Dx Respiratory SARS-CoV-2 Panel (QIAGEN S.r.l., Italy).

Results and Conclusions: Overall, 405/2126 (19%) patients were positive for at least one respiratory virus, 234 (57%) were male and 171 (43%) were female. Mean age was 63 years (range 3 - 92 years). Most of the patients affected by respiratory viral infections were in the 61-70 age group (97/405; 24%). The prevalence of viral infections during the study period is shown in Table 1. We observed a co-infection rate in SARS-CoV-2 positive pa-

Table 1. Prevalence of viral infections over the years (2020-2022).

VIRAL RESPIRATORY INFECTIONS	2020	2021	2022
	%	%	%
Adenovirus	0,9	0,4	0
Human Metapneumovirus	0,9	0	1,4
Human Rhinovirus/Enterovirus	48,1	25,3	1,4
Influenza A (H1N1)	3,7	0,4	0
Influenza B	0,9	0	0
RSV	3,7	3,1	2,8
Bocavirus	0	0,4	0
SARS-CoV-2	33,3	56,9	83,3
Coronavirus other then SARS-CoV-2	1,8	10,2	4,2
Co-infections with SARS-CoV-2	6,5	2,2	6,9
Co-infections without SARS-CoV-2	0	0,9	0



tients of 4.2% (17/405), with a higher prevalence for Human Rhinovirus (7/17; 41.2%). This is in agreement with the literature, which estimated a co-infection rate between 1.4% and 8.4% (Swets MC et al., 2022). Only in two patients were co-infections detected that did not involve SARS-CoV-2, specifically Human Rhinovirus/RSV and Human Rhinovirus/Adenovirus. Management of the pandemic has required the use of personal protective equipment that limits the occurrence of new epidemic outbreaks. However, reducing these measures could lead to the simultaneous circulation of SARS-CoV-2 with other respiratory viruses. Therefore, routine diagnostic use of multiplex RT-PCR systems, such as syndromic tests, is necessary to detect pathogens concomitant with SARS-CoV-2 infection, especially in patients admitted to critical wards. At the same time, it is necessary to support SARS-CoV-2 and flu vaccinations as a preventive measure.

MODULATION OF IFN PATHWAYS AND ANTIBODY RESPONSE IN HIV INFECTED PATIENTS RECEIVING COVID-19 VACCINES

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Background: COVID-19 vaccine has been reported to elicit humoral and T cell immune response in HIV-1 patients. Remarkably, some studies suggest that Interferon (IFN) signature might correlate with immunological and clinical efficacy of COVID-19 vaccines, as reported for Influenza vaccines. Understanding the early innate response to vaccine exposure and the humoral activation is needed to characterize mechanisms related to immunization in HIV-1 individuals. Therefore, the aim of this study was to evaluate the humoral response and IFN signature in HIV-1 positive individuals receiving COVID-19 vaccine.

Material and methods: Longitudinal effects of mRNA-based SARS-CoV-2 vaccine on humoral response in HIV-1 patients (n=82) were investigated measuring antibodies levels using LIAISON SARS-CoV-2 Trimerics IgG assay in serum samples collected before vaccination (T0), at the time of the second dose (T1), and 1 or 6 months following the second dose (T2). Expression level of mRNA encoding for distinct type I IFNs (i.e. IFN-Alpha2, IFN-Beta, IFN-Epsilon and IFN-Omega), and IFN-stimulated genes (ISGs), ISG15 and ISG56, were quantified by RT/Real time PCR assays in peripheral blood mononuclear cells (PBMCs) collected at T0, T1 and T2.

Results: Eighty-two HIV-1 patients (57 males (70%), 25 females (30%), mean age 56 years) on long-term ART were enrolled in this study. Results showed that humoral response following immunization increased significantly from T0 to T1 ($p<0.001$), and from T1 to T2 ($p<0.001$). Stratifying patients according to CD4 T cell count, no differences in antibody production were observed up to 1 month after the second dose. However, individuals with CD4 T cell count <400 cells/mm³ maintained a lower anti spike-antibody response 5 months after full vaccination compared to those with high (>400 cells/mm³) CD4 T cell count ($p<0.05$). Gene expression analysis performed in 55 HIV-1 infected patients before and after COVID-19 vaccination, indicated an overall increased of IFN response up to 1 month after the second dose. Also, transcript levels of IFN-Alpha2, IFN-Beta, IFN-Epsilon and IFN-Omega, and of ISG15 and ISG56 were positively correlated with antibody production. By contrast, IFNs and ISGs mRNA levels decreased ($p<0.05$) at 5 months after the second dose, concomitantly with the reduction of antibody levels.

Conclusion: Our results confirmed that mRNA-based SARS-CoV-2 vaccine successfully promote antibody production in ART-treated HIV-1 patients and that the levels of CD4 T cells can impact on the rate of humoral immune response activation. Moreover, we demonstrated that transcript levels of IFN related genes changed in patients receiving COVID-19 vaccine according to the amount of anti-spike antibodies.

CHARACTERIZATION OF SARS-COV-2 EPIDEMIC AND TRANSMISSION DYNAMICS IN CHILDREN OVER THE FOUR COVID-19 WAVES

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Aim of the study: No definite data were published so far regarding SARS-CoV-2 epidemic in children and potential correlation with clinical presentation. Here, we aim to fill these gaps thanks to the characterization of viral diversity, transmission dynamic and clinical presentation of 1291 SARS-CoV-2 positive children over the four COVID-19 waves in Italy.

Methods: This study included 1291 SARS-CoV-2 positive nasopharyngeal-swabs, obtained from patients aged ≤ 12 years referred for the diagnosis at Bambino Gesù Children Hospital IRCCS from March 2020, to February, 2022. Whole SARS-CoV-2 sequences, obtained by Multiplex PCR system (Illumina MiSeq) were analysed by Maximum Likelihood and Bayesian coalescent methods, to define the phylogenetic structure of the paediatric epidemic against time, and to define potential transmission clusters. To describe the relatedness of the paediatric sequences against SARS-CoV-2 diversity, 722 SARS-CoV-2 sequences belonged to adolescent and adult population (> 12 years) living in the same area of paediatric population were also included.

Results: Among paediatric individuals, 722 (55.9%) patients were male, with a median age of 2 (Interquartile-range, IQR: 1-6) years. Mild infections were the most prevalent (82.8%), followed by moderate/severe (10.9%), and asymptomatic infections (6.3%). 184 (14.3%) patients were hospitalized and 108 (16.1%) had comorbidities.

At least five clades circulated widely in the paediatric population during the four COVID-19 waves. Most of SARS-CoV-2 infections (36.6%) belonged to delta clade (B.1.617.2 and AY sublineages), followed by omicron (26.6%), EU (19.6%), alpha (9.8%) and gamma (2.7%) clades. At SARS-CoV-2 diagnosis, delta and gamma clades were characterized by higher SARS-CoV-2 RNA load respect to omicron, alpha and EU clades (viral load: 8.3 [7.3-8.7] vs 8.0 [6.1-8.6] vs 7.8 [7.1-8.3] vs 7.7 [6.2-8.5] vs 7.2 [6.1-8.4] copies/mL, respectively, $P < 0.0001$). No significant association was found between clades and COVID-19 presentation, even if a lower number of moderate/severe cases were found during alpha epidemic (4.0% vs 13.0%). 12.6% of pediatric SARS-CoV-2 sequences were involved in local clusters, 6 of them large (≥ 10 sequences) and involving mainly alpha and delta clades, and 5 small (5-7 sequences) clusters, involving only the omicron clade. No cluster was significantly associated with moderate/severe manifestations, and no cluster carried mutations able to increase pathogenicity, except for one delta chain, characterized by the Spike-Q677H mutation known to enhance viral infectivity. Adult population was present exclusively in the 6 large chains.

Multivariate logistic regression analysis showed that age < 5 , gamma and delta clades were positively associated with transmission clusters (adjusted odds ratio, AOR [95% CI]: 1.51 [1.31-1.84] $P = 0.008$; 6.51 [2.71-15.660] $P < 0.0001$; 2.72 [1.45-5.11], $P = 0.002$). Differen-

tly, comorbidities and alpha clade resulted positively and negatively associated with a moderate/severe COVID-19 presentation (AOR: 4.59 [2.69-7.82] $P < 0.001$; 0.33 [0.12-0.92] $P = 0.034$).

Conclusions: This study provides an increased knowledge of SARS-CoV-2 dynamic in children over the four COVID-19 waves, showing definite correlations among community transmission, children's age, and specific variants of concern (also characterized by enhanced infectivity). These results also emphasise that the molecular surveillance in this partially vaccinated population will be essential to closely monitor SARS-CoV-2 evolution and to define potential correlations between SARS-CoV-2 variability and disease manifestations.

**UPDATE ON THE PREVALENCE AND DISTRIBUTION OF HPV GENOTYPES
IN THE METROPOLITAN AREA OF NAPLES**

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Aim of the study: the double-stranded DNA HPVs (Human Papilloma viruses) are able to infect cutaneous epithelia in humans, provoking benign lesions (warts and laryngeal papilloma), and malignant lesions (cancers of cutaneous epithelial, anogenital, vagina, vulva, penis, and oropharynx). HPV infection represents one of the most common sexually transmitted infections (STI) and the major risk factor for cervical cancer. The present study proposes an update of Martora F. et al.(1) published data on HPV age/genotype distribution of women living in the metropolitan area of Naples (2011-2017), to track differences about the incidence of HPV infections and the related distribution of genotypes by age groups. Methods used: From January 2018 to December 2019, 502 cervical scrape specimens from women with abnormal cytological indication, were collected and analyzed for HPV DNA identification. Linear Array HPV genotyping test was used to identify the presence of 37 anogenital HPV-DNA genotypes.

Results and conclusions: The HPV infection rate was 24.1%. Among the 121 HPV-positive patients, 38% were infected with a single type (ST) of HPV, 62% with multiple infections (MT). Out of them, 76.0% with ≥ 1 HR-HPV genotype. The most common HR-HPV (high risk human papillomavirus) genotype identified was HPV-16 (14.6%), followed by HPV-31 (13.8%), -18 (9.2%), and -51 (8.5%). HPV-42 (16.4%) resulted the most prevalent LR-HPV (low risk human papillomavirus) genotypes, followed by HPV-53 (10.2%), HPV-62, -73, -CP6108 (9.4%). HPV-11 resulted absent. HPV-33, -35 and -68 displayed the highest rate for ST-HR (single type) (33.3%), while HPV-39 was present only in MT (multiple type) infections. HPV-56, -31 and -16 resulted for $>84\%$ in MT infection. The LR genotypes HPV-26, -40, -55, -67 were notice only in MT, followed by HPV-42 (90.5%). HPV-70 resulted for 50.0% present in MT and in ST infections. HPV-82 (100% in ST infections), was followed by HPV-81 and -84 (33.3%). The highest HPV infections incidence fell into 23-29 years age group (42.5%), followed by the younger (<23 years, 41.2%) and the lowest into age group with ≥ 30 years (19.3%). Several variations were observed from the reference paper. In particular, the decrease of HPV incidence percentage (24.1% versus 44.5%), the increase of HPV-18 rate (from 6.9% to 9.2%), an increase of HPV-42 genotype rate (from 9.4% to 16.4%), a decrease of HPV-53 (from 12.41% to 10.2%), and the absence of -11 were recorded. About the ST and MT distribution, HPV-68 changed from 100% presence in MT infection to 66,7% and 33,3% in ST. HPV-39 went from 30% to 100% in MT. HPV-18 presence in MT showed a minimal decrease (75% compared to the previous 87.2%) while -16 an increase (84.2% versus 70%). The both HR-HPV and LR-HPV distribution shows a higher prevalence in the 23-29 age group (HR-HPV 52.9%; LR-HPV 51.7%) compared to the reference study. Some fluctuations observed could be due to the administration of vacci-

nes although a real estimate of the vaccinated is complex to draw up. To appreciate variations over time is useful to evaluate the infection frequency rate changes and its trend in one's own metropolitan areas, also to evaluate the vaccination plan improvement.

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In late November 2021 a new SARS-CoV-2 variant with increased transmissibility emerged. The new variant B.1.1.529 was first discovered in a patient in Southern Africa (PMID:35042229) and then it spread worldwide becoming today the most represented one. Consequently, the World Health Organization (WHO) classified this variant as Variant of Concern (VoC) and named it Omicron. Our Institute is actively involved in the SARS-CoV-2 genomic surveillance, therefore we have been able to monitor the spread of the variant throughout the Apulia Region, in South East of Italy, which by late December 2021 appeared to be the predominant one. Currently, more than 590 Omicron SARS-CoV-2 genomes have been sequenced in Apulia and made available on the GISAID platform. Of these, 386 genomes have been sequenced by our laboratory following an established procedure (doi:10.3390/v13050731) and analyzed through the I-Co-Gen platform (<https://irida.iss.it/irida21-aries>). We conducted a genetic diversity analysis focusing on identified amino acid mutations, mainly in structural proteins. Particularly, the mutations in Spike protein are able to enhance infection and immune escape (PMID:35337047), that can explain the wide spread of the Omicron VoC.

We identified in our dataset two of the three genetically distinct Omicron lineages: BA.1 (274/386 – 71%) and BA.2 (112/386- 29%), with several related sub-lineages. We performed a mutation analysis with respect to the SARS-CoV-2 reference genome (Entrez AC:NC_045512.2). As expected, the protein that accumulated the highest number of mutations was the Spike (S) protein. For all the strains sequenced we calculated the total number of non-synonymous mutations detected in genes encoded structural proteins. We identified a total of 73 unique amino acid changes in the Spike protein; 22 in the Nucleocapsid protein; 9 in the Membrane protein; 2 in the Envelope protein. Seven S-protein mutations, S:D614G, S:H655Y, S:N679K, S:P681H, S:N764K, S:D796Y, S:Q954H and S:N969K, were detected in all sequenced strains, and were typically described in the Omicron variant. In the other genes, the most frequently observed mutations were E:T9I, M:19E, M:A63T, N:P13L, N:R203K, N:G204R, S:A67V, S:S373P, S:S375F, S:K417N, S:N440K, S:S477N, S:T487K, S:E484A, S:Q493R, S:Q498R, S:N501Y and S:Y505H. We also identified the majority of mutations peculiar of each Omicron sublineage. Finally, we also detected previously unobserved mutations, e.g. N:S413R identified in strains belonging to lineage BA.2. This result suggests the existence of ongoing evolution occurring within the Omicron variant.

Some mutations detected with high frequency were previously identified in other VoC (S:A67V in Alpha and Beta, S:P681H in Alpha, S:N679K in Gamma). Together with the other mutations described, these might be related to transmissibility and immune escape of the virus. Considering the lower clinical implications of the Omicron variant, we could hypothesize that such mutations are not correlated with disease severity. The continuing surveillance efforts, together with more transcriptomic and/or proteomic analyses of SARS-CoV-2 variants should be conducted to better explore the effect of mutations and understand the evolution of the virus.

THE EFFECTS OF VITAMIN A ORAL SUPPLEMENTATION IN VIRAL INFECTIONS: A SYSTEMATIC REVIEW OF RANDOMIZED CLINICAL TRIALS

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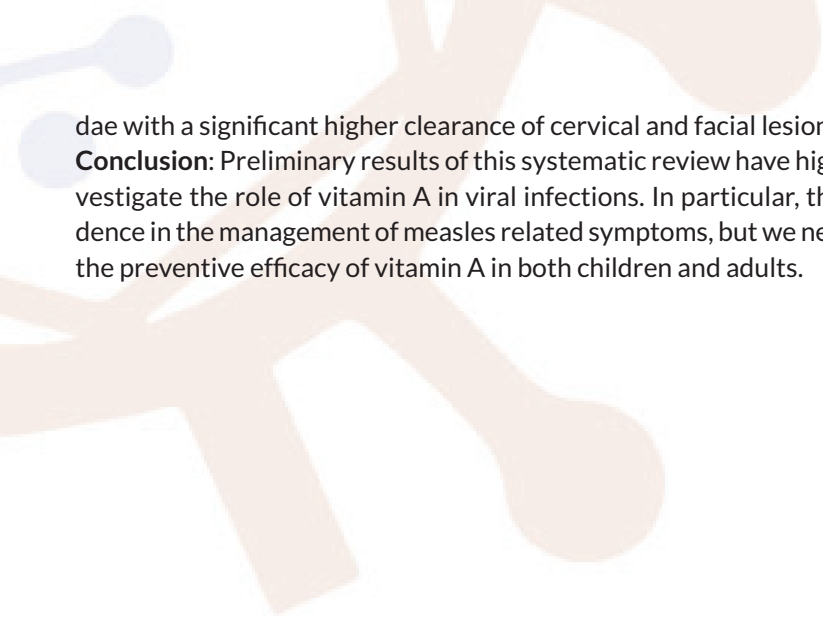
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Aim of the study: The aim of our systematic review was to identify the effects of orally administered vitamin A against viral infections in adults and children to provide a synthesis of the results and support clinicians in the evaluation of supplemental treatments for viral diseases.

Methods: This study was conducted according to the Cochrane Handbook for systematic reviews and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement. To reach adequate coverage of the clinical research conducted on the topic, two reviewers independently searched PubMed, Scopus and Web of Science from database inception to 21st May 2021 using the following terms: virus OR disease OR infection OR viral AND retinoidal OR retinol OR vitamin A OR tretinoin OR retinoic acid. No minimum vitamin A dosage was specified. For each eligible study retrieved from the literature search, two reviewers independently extracted the following information: first author, year of publication/submission, country, virus family, characteristics of the target population, study design, type and duration of the intervention, form of vitamin A administered, area of evaluation (prevention or management of viral infections), main findings and side effects. Articles were grouped according to the virus family, and a narrative synthesis of the results was performed.

Results: After the removal of duplicates, 7447 records resulted from the initial search. Screening by title and abstract selected 100 articles eligible for full-text analysis, from which 64 records were excluded with reasons. Six records were added to the previous from the reference lists of relevant articles for a total of 42 records included in the systematic review.

Evidence was grouped based on the viral family. We found data on infections sustained by Retroviridae (human immunodeficiency virus, HIV, n=19), Caliciviridae (Norovirus, n=2), Pneumoviridae (respiratory syncytial virus, RSV, n=4), Papillomaviridae (n=3), Paramyxoviridae (n=13), Flaviviridae (n=1). Studies were published between 1995 and 2017 and carried out mostly in Africa (n=27), followed by United States (n=5), India (n=4), Mexico (n=2), Australia (n=1), Chile (n=1), Japan, (n=1) and Greece (n=1). The clinical trials predominantly enrolled a population aged >18 years, while 13 articles considered children. Studies conducted on HIV positive individuals showed heterogeneous results. By contrast, more consistent results about the effects of orally administered vitamin A were observed in measles's studies with a significant improvement of some clinical outcomes (duration of pneumonia and diarrhea, significant lower measles croup incidence) and in Papillomaviri-



dae with a significant higher clearance of cervical and facial lesions.

Conclusion: Preliminary results of this systematic review have highlighted the need to investigate the role of vitamin A in viral infections. In particular, there are convincing evidence in the management of measles related symptoms, but we need to better investigate the preventive efficacy of vitamin A in both children and adults.

INTRODUCTION OF PROBIOTIC-BASED SANITATION IN THE EMERGENCY WARD OF A CHILDREN'S HOSPITAL DURING THE COVID-19 PANDEMIC

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Aim of the study: The massive use of disinfectants to prevent COVID-19 transmission might worsen the antimicrobial resistance (AMR) threat, especially in the hospital environment, possibly leading to future AMR pandemics. However, the control of microbial and viral contamination is crucial in hospitals, since hospital microbiomes can cause healthcare-associated infections (HAIs), which are particularly frequent and severe in pediatric wards due to children having high susceptibility.

We have previously reported that a probiotic-based sanitation (probiotic cleaning hygiene system PCHS) could stably decrease bacterial pathogens and their AMR in the hospital environment, reducing associated HAIs in adult hospitals. Since we recently showed that PCHS can also inactivate enveloped viruses in vitro, the aim of this study was to test the effect of PCHS in the emergency room (ER) of a children's hospital during the COVID-19 pandemic.

Methods: Conventional chemical disinfection was replaced by PCHS for 2 months during routine ER sanitation; the level of environmental bioburden was characterized before and at 2, 4, and 9 weeks after the introduction of PCHS. The presence of SARS-CoV-2 was monitored by PCR, and microbial contamination was monitored simultaneously by conventional culture-based CFU count and molecular assays, including 16S rRNA NGS for bacteriome characterization and microarrays for the assessment of the resistome of the contaminating population.

Results and Conclusions: PCHS usage was associated with stable absence of SARS-CoV-2 in the treated environments (similarly to what obtained with conventional virucides). Meanwhile, a stable 80% decrease in bacterial/fungal pathogens was observed compared to the levels detected with chemical disinfection ($P < 0.01$), accompanied by an up to 2 log decrease in resistance genes ($P_c < 0.01$). The effects were reversed when reintroducing chemical disinfection, which counteracted the action of the PCHS. Since the control of microbial contamination is a major issue during the pandemic emergency, collected data suggest that PCHS may be successfully used to control virus spread without simultaneous worsening of the AMR concern.

LONGITUDINAL STUDY OF TYPE I IFN RESPONSE IN PBMC FROM CHILDREN AND ADOLESCENTS AFTER SARS-COV-2 INFECTION

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Introduction: Upon SARS-CoV-2 infection, children generally develop asymptomatic/mild COVID-19, mounting a rapid and effective innate immune response during which interferon (IFN) is important in inhibiting viral replication, contributing to both innate and cell-intrinsic immunity (Ziegler, C. G. K. et al. Cell 2021; Yoshida, M., et al. Nature 2022). Nonetheless, the IFN response in peripheral blood mononuclear cells (PBMCs) from children infected by SARS-CoV-2 has not been completely characterized yet. Hence, we measured type I IFNs (IFN-I) and several IFN Stimulated Genes (ISGs) expression in PBMCs from children and adolescent with mild or asymptomatic SARS-CoV-2 infection and compared with serum and salivary antibodies levels.

Methods: Children and adolescent attending Umberto I hospital of Rome to perform a molecular test for SARS-CoV-2 diagnosis after a contact with SARS-CoV-2 positive subjects, were enrolled in this study. Blood samples were collected at T1 (a first follow-up visit after 20-30 days from SARS-CoV-2 diagnosis) and at T2 (20-30 days after T1); RNA was purified from PBMC and gene expression of IFN alpha (IFNA), IFN beta (IFNB), IFN omega (IFNO), IFN epsilon (IFNE), IFNAR1, IFNAR2, ISG15, ISG56, IFI27 and MXA was measured by quantitative Real Time PCR. Statistical analysis was performed using SPSS software.

Results: 110 subjects (mean age 11,65 years, range 1-19 years) positive to SARS-CoV-2 were enrolled; we measured IFN-I and ISGs expression in PBMCs at T1 and T2. Expression of IFN alpha (IFNA), IFN beta (IFNB), IFNE and IFNO in PBMC was higher at T1 with respect to T2 ($p=0.008$ for IFNA and $p<0.001$ for other IFNs); differently, IFNAR2 subunit expression level was higher at T2 ($p<0.001$). The IFN-related gene expression did not differ by sex and age of the infected patients nor with IgG antibody levels measured in serum at T1 and T2. Analysing gene expression on the basis of the presence of symptoms during SARS-CoV-2 infection, we observed higher expression of IFI27 and IFNAR1 at T1, in subjects that reported fever ($p=0.027$ and $p=0.011$ respectively) and higher expression of IFI27 in subjects that reported anosmia ($p=0.027$). Interestingly, in the 8/108 (7.4%) children that developed long-COVID, a significantly higher expression of IFNE and IFNO was detected ($p=0.014$ and $p=0.011$, respectively).

Discussion: Overall, our findings suggest that IFN-I genes expression in children and adolescents may be still active and relevant in PBMC at one month after SARS-CoV-2 symptomatic and asymptomatic infections but apparently not associated with serum IgG levels. It should be explored which is the possible role of IFNAR2 upregulation in children two months after SARS-CoV-2 infection; in adults, an altered expression of IFNAR2 has been found in severe COVID-19 patients, but this seems not to be the case in this group of children. Children experiencing long COVID were characterized by higher IFNE and IFNO, the less well-characterized type-1 IFNs; this novel finding has to be investigated further in larger groups of children.

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Aim of the study: Influenza (also known as Flu) is a contagious respiratory illness caused by influenza viruses. There are two main types of human flu viruses: types A and B. The Flu A and B viruses that routinely spread in people are responsible for seasonal Flu epidemics each year, in particular Flu viruses are most common during the fall and winter. Respiratory Syncytial Virus (RSV) is the most common cause of inflammation of the small airways in the lungs (bronchiolitis) and pneumonia in babies and is more common in winter and early spring months. The transmission of these viruses occurs inhaling droplets from an infected person's sneeze or cough or contact with fluid from an infected person's nose or mouth. During the SARS CoV 2 pandemic period, the use of masks has limited the transmission of various viruses, so the purpose of our work is to quantify the number of tests performed for Flu A, B, RSV and evaluate the percentages of positive and negatives.

Methods used: In the period between January 2019 and December 2021, 4531 tests for Flu A, B and RSV were performed at the Microbiology and Virology Unit, University Hospital City of Health and Science of Turin using the Xpert® Xpress Flu / RSV Cepheid kit (RT-PCR). The samples belonged to both hospitalized patients and emergency room access.

Results and conclusions: In 2019, 1437 tests were performed for Flu A, B, RSV. Flu A was positive in 13% of cases, Flu B in only 0.2% of cases and RSV was positive in 13% of cases; the greatest incidence of positives occurred in the winter months. In 2020, 2106 tests were performed for Flu A, B, RSV with the following results: Flu A was positive in 6% of cases, Flu B in 3% of cases and RSV in 8% of cases. The positive trend was concentrated in the first months of the year with the total absence of cases in the autumn-winter season. In 2021 only 988 tests were carried out of which 1% was positive for Flu A, 0.4% positive for Flu B and 24% positive for RSV. In the first half of the year, all tests were negative. These data indicate that the use of masks and the restrictions imposed for the SARS CoV 2 pandemic have significantly affected the epidemiology of other respiratory viruses, reducing their transmission. Only RSV increases at the end of 2021, probably due to the decrease of restrictions and the greater contact between children. Subsequent studies will allow analyzing the epidemiology of numerous other respiratory viruses.

FATAL HUMAN RABIES IMPORTED TO ITALY FROM THE ISLAND OF ZANZIBAR HIGHLIGHTS THE NEED FOR ACCURATE RABIES PREVENTION IN TRAVELLERS

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Aim of the study: In October 2019, an Italian man was admitted to a public hospital in Bisceglie, Italy with acute respiratory distress. Due to deterioration of his clinical conditions, he was transferred to a public hospital in Bari, Italy, where he died after 42 days of intensive care treatment. Rabies was suspected based on symptoms and anamnesis. Indeed, a stray dog displaying aggressive behaviour had bitten the patient in the island of Zanzibar (Tanzania) while he was on holiday one month before his admission to hospital. The patient immediately underwent wound washing with an antiseptic solution and rabies vaccination started in Zanzibar, but he did not receive rabies immunoglobulin (RIG). Rabies diagnosis was confirmed ante mortem by the FAO and National Reference Centre for Rabies, IZSve (Padova, Italy).

Methods: Ante mortem rabies diagnosis was achieved by real-time and conventional RT-PCR followed by sequencing analysis performed on total RNA extracted from salivary swab, salivary sputum and nuchal skin biopsy. A direct fluorescent antibody (DFA) test on nuchal skin biopsy enabled to detect viral antigens. Whole genome sequencing of isolated virus was performed using a metagenomic approach implemented in the Illumina platform. Blood serum (collected on days 3, 14, 22 and 34 after hospitalisation, a.h.) and cerebrospinal fluid (CSF) (on day 14, 22 and 34 a.h.) were tested to titrate rabies-neutralizing antibodies (RVNA) through Fluorescent Antibody Virus Neutralization (FAVN), and to differentiate IgG/IgM rabies-binding antibodies (RVBA) through indirect fluorescent antibody (IFA) test.

Results: Rabies infection was first demonstrated by PCR on salivary sputum collected 3 days a.h. and on salivary swab and nuchal skin biopsy collected 14 days a.h. DFA test confirmed the presence of rabies virus nucleoprotein in the nuchal skin biopsy. According to literature, rabies virus excretion in saliva was intermittent, as indicated by tests performed on subsequent samples collected during hospitalization. Rabies virus was isolated from salivary sputum by mouse intracerebral inoculation. Whole genome sequencing and phylogenetic analysis were performed for complete characterization of virus isolate by comparison with reference rabies strains. RVNA were detectable since day 14 a.h. in blood and CSF, with progressively increasing titres up to 25.35 IU/ml in serum on day 34 a.h.. CSF displayed much lower RVNA levels compared to serum (up to 4.83 IU/ml), indicating either production of antibodies within the central nervous system or their infiltration from the periphery across blood-brain barrier with altered permeability. IgG RVBA were detectable in serum since day 14 a.h. confirming a late immunological response to viral invasion. Results for IgM RVBA were inconclusive for blood serum samples, while they were detected in all CSF samples analysed, confirming the involvement

of central nervous system into antiviral immunological response.

Conclusions: This fatal case of human rabies highlights the fundamental importance of proper pre-exposure prophylaxis (PrEP) when travelling in rabies-endemic countries. Recently updated PrEP protocols include the administration of only two vaccine doses (0-7 days); however this abbreviated vaccination scheme should not be applied to immunocompromised people, for whom serological post-vaccination testing is also recommended. Considering the short timeframe to achieve post-vaccination protection, we do expect that rabies PrEP will be considered more often when addressing travel associated sanitary risks, instead of relying on post-exposure prophylaxis (PEP). In both healthy and immunocompromised people, PEP includes the prompt administration of multiple doses of rabies vaccines and RIG in case of severe exposures. However, PEP might be less effective in immunocompromised patients, with consequently increased risk of death. In conclusion, proper PrEP, possibly coupled with RVNA titration before travel, might save lives to people who are accidentally exposed to rabid animals during their stay in rabies-endemic countries, as it could have been for the case herein described. Unfortunately, PEP is charged to the patient in many endemic countries and RIG availability is far away from the actual need especially in endemic areas, undermining health equality in resource-limited regions. Despite worldwide efforts to achieve the global goal of zero human dog-mediated rabies deaths by 2030 through an improved control of rabies in dog population, the main reservoir of the disease, simple prevention protocols like proper PrEP are also able to save lives and should not be neglected in both endemic and non-endemic areas.

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We know little about viruses compared to the other lifeforms but, recently, metagenomics has helped us to gain a better understanding of the virosphere. This research aims to discover the virome diversity and composition in *Fusarium poae* and *Fusarium proliferatum* collections, to characterize the mycovirus that may have an effect on the host pathogenicity, and to provide potential materials for the biological control of *Fusarium* spp. pathogens.

Next-Generation Sequencing (NGS) analysis of 30 *F. poae* isolates revealed an extreme diversity of mycoviruses. Bioinformatic analysis shows that contigs associated with viral genome belong to the families: *Hypoviridae*, *Mitoviridae*, *Partitiviridae*, *Polymycoviridae*, proposed *Alternaviridae*, proposed *Fusagraviridae*, proposed *Fusariviridae*, proposed *Yadokariviridae*, and *Totiviridae*. In *F. poae* collections, 15 viruses were obtained and eight of them are new viruses.

Moreover, all the *F. poae* isolates analyzed are multi-infected. *Fusarium poae* partitivirus 1 appears in all the 30 strains, followed by *Fusarium poae* fusagravirus 1 (22), *Fusarium poae* mitovirus 2 (18), *Fusarium poae* partitivirus 3 (16), and *Fusarium poae* mitovirus 2 and 3 (11).

Using the same approach, the virome of *F. proliferatum* collections resulted in lower diversity and abundance. Four new viruses belong to the family *Mitoviridae* and *Mymonaviridae*. Interestingly, most *F. proliferatum* isolates are not multi-infected.

By multiple liner regression and the principal component analysis of the virome composition and the growth rate of 30 *F. poae*, *Fusarium poae* mitovirus 3 is significantly correlated with the growth rate among *F. poae* collection, its presence could increase the growth rate. The curing experiment and pathogenicity test in Petri indicated that *Fusarium poae* hypovirus 1 might be associated with the host hypovirulence phenotype, while *Fusarium poae* fusagravirus 1 and *Fusarium poae* partitivirus 3 may have some beneficial effect on host pathogenicity.

From the results obtained so far, it is interesting to further explore the differences between the two fungal collections and the virus-virus interaction in a single fungus.

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