XXI Jornada de Virologia – Virology meeting 2022

Organització: Secció de Virologia de la SCB

Sala Prat de la Riba (Carre del Carme 47, Barcelona) / Virtual

21 de novembre de 2022

Coordinadora de la Secció i responsable de la coordinació de la Jornada:

Núria Busquets Martí

Comitè científic:

Ana Angulo

Sílvia Bofill

Susana Guix

Nuria Izquierdo-Useros

Juan José López-Moya

Andreas Meyerhans

Sofia Pérez del Pulgar

Josep Quer

Amb el suport de:

XXI Jornada de Virologia – Virology meeting 2022

8:50 h BENVINGUDA/WELCOME: Núria Busquets

SESSION I: Chairs: Sofia Pérez del Pulgar/Josep Quer

9:00 - 9:30 h

CONFERÈNCIA D'OBERTURA: Maite Muniesa (Universitat de Barcelona): **Novetats en l'ús de bacteriòfags com antimicrobians.**

09:30 - 10:30 h

Oral presentations

O.1. **Adaptation of viral vectors to different plant species for production in molecular farming systems.** (10' + 5')
Adrià Bugeda. CRAG, CSIC-IRTA-UAB-UB, Campus UAB, Bellaterra, 08193 Barcelona, Spain.

O.2. **Novel therapies for rapid responses to pandemic viral threats.** (10' + 5')
Florencia-Evelin Alonso. Molecular Virology Group, Department of Medicine and Life Sciences, Universitat Pompeu Fabra, 08003, Barcelona, Spain.

O.3. **Human chimeric monoclonal antibodies targeting ACE2 to neutralize SARS-CoV-2 variants and other coronaviruses for therapeutic use**. (10' + 5')
Pablo Hernández-Luis. Immunology Unit, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, University of Barcelona, Barcelona, Spain.

O.4. ***In vitro* screening platform to quickly assess the antiviral and immunomodulatory activity of potential anti-SARS-CoV-2 compounds.** (10' + 5')
Daniel Perez-Zsolt. IrsiCaixa AIDS Research Institute, Badalona, Spain.

SESSION II: Ana Angulo/ Juanjo López Moya

10:30 - 11:00 h

KEYNOTE LECTURE: Marion Koopmans (Erasmus MC department of Viroscience, Rotterdam, The Netherlands): **From response research to pre-emergence research: is it possible to prevent emerging Infectious diseases (EID) outbreaks?** (💻)

11:00 - 11:30 h

COFFEE-BREAK

11:30 - 13:00 h

Oral presentations

O.5. **Whole-genome sequencing for the epidemiological surveillance of Monkeypox outbreak in Barcelona.** (10' + 5')
S. Martínez-Puchol. Microbiology Department, Laboratori Clínic Metropolitana Nord. Hospital Universitari Germans Trias i Pujol, Germans Trias i Pujol Research Institute (IGTP), Badalona, Spain.

O.6. **The Catalan Surveillance Network of SARS-CoV-2 in Sewage**. (10' + 5')
Laura Guerrero-Latorre. Catalan Institute for Water Research (ICRA), Emili Grahit 101, E-17003, Girona, Catalonia, Spain.

O.7. **Evaluation of the ONT MinION Mk1C platform vs Illumina MiSeq for the genomic epidemiology study of SARS-CoV-2.** (10' + 5')
Panisello Yagüe D. Microbiology Department, Laboratori Clínic Metropolitana Nord. Germans Trias i Pujol University Hospital, Germans Trias i Pujol Research Institute (IGTP), Badalona, Spain.

O.8. **Validation of a passive sampler as an affordable and easy-to-use tool for wastewater based epidemiology**. (10' + 5')
Cristina Mejías-Molina. Laboratory of Viruses Contaminants of Water and Food, Departament de Genètica, Microbiologia i Estadística, Facultat de Biologia, Universitat de Barcelona (UB), Barcelona, Spain.

O.9. **The frequency of defective genomes in Omicron differs from that of the Alpha, Beta and Delta variants**. (10' + 5')
Carolina Campos. Liver Diseases-Viral Hepatitis, Liver Unit, Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Hospital Universitari.

O.10. **Evolution and Reassortments Analysis of Swine Influenza Virus H1N1 and H3N2 in Vaccinated Pigs after Simultaneous Infection by contact**. (10' + 5')
Álvaro López-Valiñas. Unitat mixta d'Investigació IRTA-UAB en Sanitat Animal. Centre de Recerca en Sanitat Animal (CReSA). Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, Barcelona, Spain.

13:00 - 14:00 h

DINAR / LUNCH

SESSION III: Chairs: Sílvia Bofill / Andreas Meyerhans

14:00 - 14:30 h

INVITED SPEAKER: Cristina Risco (Centro Nacional de Biotecnología, Madrid): **Imaging viral infections: identifying new targets for antiviral treatments.**

14:30 - 16:30 h

Oral presentations

O.11. **ß-Cyclodextrins as affordable antivirals against coronavirus infection.** (10' + 5')
Marçal Gallemí. IrsiCaixa, Hospital Germans Trias i Pujol, Can Ruti Campus, 08916, Badalona, Spain.

O.12. **PBMC immunophenotyping and plasma inflammatory profile of children with Long COVID.** (10' + 5')
Jon Izquierdo-Pujol. IrsiCaixa AIDS Research Institute, Badalona, Spain.

O.13. **Development and characterization of a new human ACE2 knock-in mouse model for SARS-CoV-2 infection and pathogenesis.** (10' + 5')
Anna Pons-Grífols. IrsiCaixa AIDS Research Institute, Can Ruti Campus, UAB, Badalona, Spain.

O.14. **Towards a functional cure for chronic virus infection by shifting the virus – host equilibrium state.** (10' + 5')
Paula Cebollada Rica. Infection Biology Laboratory, Department of Medicine and Life Sciences (MELIS), Universitat Pompeu Fabra, Barcelona, Spain.

O.15. **The combination of Gapmers and/or siRNA as a potential hepatitis B virus gene therapy strategy against hepatitis B virus: preliminary in vitro results.** (10' + 5')
Selene Garcia-Garcia. Liver Pathology Unit, Departments of Biochemistry and Microbiology, Vall d'Hebron University Hospital, 08035 Barcelona, Spain.

O.16. **RAB GTPases: key players in HAV egress from hepatocytes.** (10' + 5')
Gemma Chavarria-Miró. Enteric Virus laboratory, Department of Genetics, Microbiology and Statistics, Section of Microbiology, Virology and Biotechnology, School of Biology, and Institute of Nutrition and Food Safety, University of Barcelona, Barcelona, Spain.

O.17. **Pharmacological inhibition of IKK to tackle latency and hyperinflammation in chronic HIV-1 infection.** (10' + 5')
Roger Badia. IrsiCaixa AIDS Research Institute Hospital Universitari Germans Trias i Pujol, Can Ruti Campus, 08916 Badalona, Spain.

O.18. **Niemann Pick Protein C1, a cholesterol transporter involved in Cucumber mosaic virus infection in melon**. (10' + 5')
Irene Villar. Centre for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, C/ Vall Moronta, Edifici CRAG, Bellaterra (Cerdanyola del Vallés), 08193 Barcelona, Spain.

SESSION IV: Chairs: Núria Izquierdo/ Susana Guix

16:30 - 17:00 h

CLOSING LECTURE: César Muñoz Fontella (Bernhard-Nocht-Institut für TropenmedizinI): **Ebola virus disease immunology.**

17:00

AWARDS

End of the XXI Jornada de Virologia – Virology meeting 2022

💻 (Presentacions en remot/on-line presentations)

INVITED SPEAKERS' ABSTRACTS

OPENING LECTURE

Maite Muniesa.

Universitat de Barcelona.

Novetats en l'ús de bacteriòfags com antimicrobians.

La resistència als antibiòtics és un dels grans problemes de salut pública a nivell global. L'ús de virus bacterians, bacteriòfags (o fags) apareix com a una valuosa alternativa terapèutica als antibiòtics. La recerca per l'aplicació de fags com a antimicrobians està experimentant actualment un renaixement i alguns països ja estan adoptant noves regulacions *ad hoc* per afavorir la implantació a curt termini de la fagoteràpia en la pràctica clínica. Els aspectes favorables i desfavorables de l'ús dels fags es discuteixen aquí juntament amb els requisits pel seu aïllament. L' eficàcia del tractament amb fags per a reduir bacteris depèn de la generació de resistents i per això hi ha consens en la convinença d'usar cocktails de fags. Parlarem dels avantatges i desavantatges de la seva especificitat, evolubilitat, capacitat d'autodosificació, i la seva seguretat, inclosa la capacitat dels fags de mobilitzar gens i la possible resposta immunològica que poden estimular. Finalment, es revisa possibles mètodes d'administració dels fags i els productes disponibles.

Novelties in the use of bacteriophages as antimicrobials.

Antibiotic resistance is one of the major public health problems globally. The use of bacterial viruses, bacteriophages (or phages) appears as a valuable therapeutic alternative to antibiotics. Research into the application of phages as antimicrobials is currently experiencing a renaissance and some countries are already adopting new *ad hoc* regulations to favour the short-term implementation of phage therapy in clinical practice. The favorable and unfavorable aspects of the use of phages are discussed here along with the requirements for their isolation. The effectiveness of phage treatment to

reduce bacteria depends on the generation of resistances and therefore there is consensus on the agreement to use phage cocktails. We will talk about the advantages and disadvantages of their specificity, evolution, self-dosage, and their safety, including the ability of phages to mobilize genes and the possible immune response they can stimulate. Finally, possible methods of administration of phages and available products are reviewed.

KEYNOTE LECTURES

Marion Koopmans.

Erasmus MC department of Viroscience, Rotterdam, The Netherlands.

From response research to pre-emergence research: is it possible to prevent emerging Infectious diseases (EID) outbreaks?

Our current approach to emerging infectious disease outbreaks is focused mostly on response to notifications of human disease. With a high proportion of outbreaks resulting from spillover of viruses from animals to humans, refocusing early warning surveillance to ecosystems is a logical choice. However, a key question is how to operationalize this. In the VEO project, we are exploring the use of publicly available data on drivers for disease emergence to assess trajectories of disease emergence. The overall aim is to improve surveillance of outbreak RISK to help focus where enhanced surveillance could be targeted. I will present some of the ongoing case studies.

Cristina Risco.

Cell Structure Laboratory, National Center for Biotechnology, CNB-CSIC, Madrid, Spain.

Imaging viral infections: identifying new targets for antiviral treatments.

As a result of recent advances in light and electron microscopy, we are starting to be aware of the variety of structures that viruses assemble inside cells. Viruses remodel cellular organelles to build their replication factories. These intracellular compartments harbor viral replication complexes and the sites of assembly and maturation of new virus

particles, that later move to organelles specialized in virus egress. Live cell microscopy, correlative light and electron microscopy (CLEM), and three-dimensional imaging methods are unveiling how viruses manipulate cell organization to assemble their factories. In particular, methods for molecular mapping in situ in two and three dimensions, are showing how viruses build functional macromolecular complexes inside infected cells. The combination of all these imaging approaches is uncovering the viral lifecycle events with a detail never before seen and helping to identify new targets for antiviral therapies.

CLOSING LECTURE:

César Muñoz Fontella

Bernhad-Nocht-Institut für Tropenmedizinl.

Ebola virus disease immunology.

Ebola virus disease (EVD) is caused by infection with Ebola virus (species Zaire ebolavirus) or other members of the Ebolavirus family. The high mortality of EVD is associated with poorly effective host immune responses and high levels of inflammation. In this lecture we will see that defects in the cross-talk between dendritic cells and T cells are at the center of this immune dysregulation, are a biomaker of severe disease and result in poor virus clearance and virus dissemination. Research data gathered during field studies and in chimeric mice indicate that the DC-T cell cross-talk is at the center of EVD pathophysiology and suggest new targets for post-exposure therapies.

ORAL PRESENTATIONS ABSTRACTS

O.1. **Adaptation of viral vectors to different plant species for production in molecular farming systems.**

Adrià Bugeda[1], Laia Castillo[1], Arcadio García[2], José Antonio Daros[2], Juan José López-Moya[1], María Coca[1].

[1]CRAG, CSIC-IRTA-UAB-UB, Campus UAB, Bellaterra, 08193 Barcelona, Spain.

[2]IBMCP, CSIC, Valencia, Spain.

Many natural products and organic compounds with desirable characteristics for treatments or industrial processes are rare or difficult to produce efficiently through conventional systems. To address this demand, we are testing and improving plant-based transient expression production systems, usually referred to as molecular farming. Currently, we are using two different systems based on viral vectors in *Nicotiana benthamiana and* developing a new system to be used mainly in lettuce (*Lactuca sativa*), a robust and rapid growing crop plant that is also considered a GRASS organism (Generally Regarded as Somewhat Safe), making it a desirable host.

The first system is a deconstructed viral vector based on the tobamovirus Tobaco mosaic virus (TMV) that retains the ability to self-replicate and to spread from cell-to-cell, in which the viral coat protein (CP) has been substituted by the protein of interest. This protein is preceded by a potent subgenomic promoter that will generate subgenomic RNAs, leading to the accumulation of large amounts of proteins (Shi et al, 2019, Plant Biotechnol J 6, 1069-80). The system also allows tagging the products with one or more subcellular compartmentalization signals to target them to different organella, as confirmed by transmission electron microscopy (TEM).

The second vector is the pEFF system (Mardanova et al, 2017, Front Plant Sci 8, 247). It consists in a quimera built on fragments of different viruses, with the replication machinery of the potexvirus Potato virus X (PVX), the enhancer region of the alfamovirus Alfalfa mosaic virus (AMV) and other viral elements, like the silencing suppressor of the closterovirus Grapevine leafroll-associated virus two (GLRaV2). This vector is restricted to the agroinfiltrated tissues because it cannot spread through the plant, still producing good yields of protein in *N. benthamiana* plants. As a further advantage, pEFF can be used also to produce heterologous proteins in lettuce, although the conditions for

optimal use in this host require optimization. Finally, we are currently generating a new vector expected to be better adapted to lettuce based on the potexvirus Lettuce virus X (LeVX), aiming to overcome the limitations found in *N. benthamiana*.

(Funded by RTI2018-101115-B-C22 and PID2019-105692RB-I00).

O.2. **Novel therapies for rapid responses to pandemic viral threats.**

Marc Talló-Parra[1], Florencia-Evelin Alonso[1], Gemma Vilaró-Pérez[1], Nereida Jiménez de Oya[2], Estela Escribano-Romero[2], Ana-Belén Blázquez[2], Miguel A Martín-Acebes[2], Alfonso Gutiérrez-Adán[3], Juan Carlos Saiz[2], Ivan Dotu[4], Juana Díez Antón[1].

[1] Molecular Virology Group, Department of Medicine and Life Sciences, Universitat Pompeu Fabra, 08003, Barcelona, Spain.

[2] Department of Biotechnology, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Consejo Superior de Investigaciones Científicas (CSIC), 28040 Madrid, Spain.

[3] Department of Animal Reproduction, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Consejo Superior de Investigaciones Científicas (CSIC), 28040 Madrid, Spain INIA-CSIC 4 Moirai Biodesign SL. 08028, Barcelona, Spain

Humanity faces an almost unlimited pool of viral threats. To handle and contain upcoming and unpredictable outbreaks before they spread worldwide it is fundamental to rapidly generate specific antivirals or to count with broad-spectrum antivirals that can be stockpiled. Neither of these options are functional today. We have addressed these two needs by generating a novel circular RNA (circRNA)- based antiviral platform. Artificial circRNAs were designed to hybridize and interfere with highlyconserved RNA structures within RNA viral genomes that are essential for their translation and/or replication. Using an in-house developed software, as a proof of principle we designed circRNAs that highly inhibited HCV, DENV, WNV or SARS CoV-2 in cell culture. CircRNA-based with broadspectrum activities were also generated by including sequences targeting different viruses. Importantly, as predicted by the lack of free 5´or 3´ ends accessible to exonucleases for degradation, circRNAs were highly stable in cell culture and in mice models without the need of including any RNA modification. Moreover, circRNA-based therapies are predicted to hamper the emergence of resistant mutants, a major problem of traditional antivirals, because they target highly conserved structures and multiple structures can be targeted with the same circRNA molecule. In

sum, the high stability and versatility of circRNAbased antivirals together with the in-house developed methods for their designed and low-cost production point at circRNAs as a novel RNA-based strategy for ultrarapid generation of antivirals against novel viral threats.

O.3. **Human chimeric monoclonal antibodies targeting ACE2 to neutralize SARS-CoV-2 variants and other coronaviruses for therapeutic use**.

Pablo Hernández-Luis[1], Mar Tolós[1], Pablo Engel[1], and Ana Angulo[1].

[1] Immunology Unit, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, University of Barcelona, Barcelona, Spain.

SARS-CoV-2 has produced unprecedented global health problems with devastating economic consequences and the situation is far from being yet under control. Moreover, the emergence of new viral variants has put the world on an even higher alert. Thus, there is an urgent need to find effective therapeutics to fight infections caused by present and future SARS-CoV-2 variants. SARS-CoV-2 enters host cells through binding of its spike protein (S protein) to the cell surface receptor human angiotensin-converting enzyme 2 (ACE2). In this study, we have developed neutralizing chimeric monoclonal antibodies (mAbs) that recognize and block the human ACE2 receptor. BALB/c mice were immunized with a stably transfected murine cell line (300.19) that expresses at high levels human ACE2 on its cell surface. 649 murine hybridoma supernatants were initially screened by flow cytometry for their capacity to interact with the receptor, using the ACE2-transfected cells, and 26 showed specific ACE2 binding. Employing an in vitro pseudo-neutralization flow cytometry-based assay with RBD (receptor binding domain)-Fc fusion proteins, we obtained two mAbs, B1 and B2, able to block the RBD-ACE2 interaction. Both mAbs recognized different ACE2 genetic variants. Moreover, they presented neutralizing activity against multiple RBD variants. Cross-competition assays showed that B1 and B2 targeted overlapping receptor epitopes. The variable regions of the two blocking antibodies and one non-blocking mAb were sequenced and subcloned in an expression vector containing the constant regions of human IgG4 and light chain k, to generate human chimeric antibodies. We are producing these chimeric mAbs to analyze their neutralizing potential in a transgenic mouse expressing human ACE2 with

S-protein pseudotyped lentiviruses. We predict that a therapy based on this type of mAbs could be especially useful for infected elderly persons or immunocompromised patients, which present poor immune responses to vaccination.

O.4. ***In vitro* screening platform to quickly assess the antiviral and immunomodulatory activity of potential anti-SARS-CoV-2 compounds.**

Daniel Perez-Zsolt[1,*], Dàlia Raïch-Regué[1,*], Jordana Muñoz-Basagoiti[1], Marçal Gallemí[1], Bonaventura Clotet[1,2,3,4,5], Nuria Izquierdo-Useros[1,3,5,&].

[*]Equal contribution

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[3]Germans Trias i Pujol Research Institute (IGTP), Badalona, Spain.

[4]Fundació Lluita contra la SIDA, Infectious Diseases Department, Hospital Germans Trias i Pujol, Badalona, Spain.

[5]Consorcio Centro de Investigación Biomédica en Red de Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, Madrid, Spain.

Antivirals such as remdesivir are clinically effective when administered early upon SARS-CoV-2 infection. On the other hand, immunomodulators like dexamethasone and baricitinib reduce mortality of COVID-19 patients ameliorating the hyperinflammation developed at later stages of the disease. Different compounds have been proposed as potential antivirals and/or immunomodulators against SARS-CoV-2, including the antimicrobial clofazimine, the antidepressants fluoxetine and fluvoxamine, and the corticosteroid budesonide. To assess the antiviral and immunomodulatory activity of these compounds *in vitro*, we set up a screening platform based on Vero E6 cells and primary human macrophages, key inflammatory cells in the lungs.

To test antiviral activity, Vero E6 cells were pulsed with the D614G and Omicron SARS-CoV-2 variants in the presence of increasing concentrations of the different compounds. Drug-associated and viral-induced cytopathic effect was measured with a luminometric assay 3 days post-infection. For immunomodulation studies, monocyte-derived human

macrophages (MDM) were exposed to the D614G SARS-CoV-2 variant in the presence of the candidates. 24 hours later, cytokines released to the supernatant were measured by Luminex, while drug-associated cytotoxicity was assessed by luminometry.

Clofazimine was the only compound that exerted certain antiviral activity, partially blocking infection by D614G and Omicron SARS-CoV-2 variants at the non-toxic concentration of 2 □M. Regarding immunomodulation, exposure of MDMs to SARS-CoV-2 induced the secretion of pro-inflammatory cytokines MCP-3, IL-1RA and IL-8, which was completely blocked by dexamethasone and baricitinib. Clofazimine, fluoxetine and fluvoxamine failed to inhibit the secretion of these cytokines. In contrast, budesonide fully inhibited cytokine release by SARS-CoV-2-exposed MDMs.

To sum up, we demonstrated that clofazimine and budesonide have antiviral and immunomodulatory activities, respectively. The ability to discriminate between clinically relevant immunomodulators (dexamethasone and baricitinib) from those that failed to be protective (fluoxetine and fluvoxamine) validates the screening platform employed here as a promising tool to quickly assess the immunomodulatory activity of new candidates.

O.5. **Whole-genome sequencing for the epidemiological surveillance of Monkeypox outbreak in Barcelona.**

S. Martínez-Puchol[1,2], A. C. Pelegrin[1], A. E. Bordoy[1], A. Not[1], D. Panisello Yagüe[1], L. Soler[1], S. González-Gómez[1], G. Clarà[1], M. Vall[5,6], A. Alemany[5,6,7], M. Ubals[5,6,7], A. Mendoza[5,8], C. Suñer[5,6], Á. Rivero[6,8], P. Coll[6,8], J. M. Cabrera[6,8], M. Carrasco[1], C. Casañ[1], Á. Hernández[1], P. J. Cardona[1,3], V. Saludes[1,4], E. Martró[1,4].

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[5]Skin Neglected Diseases and Sexually Transmitted Infections Section, Hospital Universitari Germans Trias i Pujol, Badalona, Spain.

[6]Fight Infectious Diseases Foundation, Badalona, Spain.

[7]Facultat de Medicina, Hospital Clinic, Universitat de Barcelona, Barcelona, Spain.

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Background

Since May 2022, human monkeypox virus (hMPXV) has broadly spread resulting in the first multi-country outbreak without known epidemiological links to endemic regions. When no hMPXV-targeted Whole Genome Sequencing (WGS) assays were available, a virome-sequencing approach allowed us to obtain the first Spanish draft genome sequence (ON622718.1). However, this approach is time consuming, expensive and provides low yield for the sequences of interest, compared with amplicon-based protocols. In this study, we aimed to perform a genomic epidemiology study from a subset of hMPXV positive samples from the Northern Metropolitan area of Barcelona using tiling amplicon sequencing.

Methods

Ninety-three samples from single pustular lesions representing 14 weeks (June-September 2022) were included in the study, following the criteria of Ct< 26 for hMPXV real-time PCR. WGS was performed using a set of 88 primers (NextGenPCR) for the generation of tiled amplicons covering the whole hMPXV genome. Sequencing libraries were prepared using the Rapid Barcoding Kit 96 kit (Oxford Nanopore Technologies, ONT) and sequenced for 72h in four independent runs using MinION Mk1C (ONT) with R9.4.1 flow cells (ONT). Raw sequencing data was analysed using a custom Nextflow pipeline. Briefly, reads were length and quality trimmed and aligned against a hMPXV reference genome (MT903343.1) using minimap2 (v2.24-r1122). Draft consensus sequences were built using TrueConsense (v0.5). Finally, Nextclade (v2.5.0) was used to assess consensus quality and assign hMPXV lineages.

Results & Discussion

Sequenced samples corresponded to 92 male and 1 woman aged 17 to 60 years old, representing 37.20% of positive patients diagnosed at our hospital. hMPXV genomic sequences were successfully obtained for 96.80% (90/93) samples, with a mean genome coverage of 97.10% (range 71.86–99.96%) and a median sequencing depth of 623x (IQR, 334.5–978.75). hMPXV1 lineages identified were B.1 (86.70%), B.1.3 (6.70%), B.1.7 (3.30%) B.1.8 (2.20%) and B.1.1 (1.10%). Lineages B.1.1 and B.1.7 had not previously been detected in Spain but have been described through Europe. The tiling amplicon

sequencing approach enabled us to obtain highquality hMPXV genomic sequences, which are essential for tracking the spread and evolution of viral lineages.

O.6. **The Catalan Surveillance Network of SARS-CoV-2 in Sewage**.

Laura Guerrero-Latorre[1,2], Neus Collado[1,2], Nerea Abasolo[3], Gabriel Anzaldi[4], Sílvia Bofill-Mas[5], Albert Bosch[6,7], Lluís Bosch[1], Sílvia Busquets[1], Antoni Caimari[8], Núria Canela[3], Albert Carcereny[6,7], Carme Chacón[9], Pilar Ciruela[9], Irene Corbella[9], Xavier Domingo[4], Xavier Escoté[10], Yaimara Espiñeira[4], Eva Forés[5], Isabel Gandullo-Sarró[11], David Garcia-Pedemonte[6,7], Rosina Girones[5], Susana Guix[6,7], Ayalkibet Hundesa[5], Marta Itarte[5], Roger Mariné-Casadó[10], Anna Martínez[9], Sandra Martínez-Puchol[5], Anna Mas-Capdevila[10], Cristina Mejías-Molina[5], Marc Moliner i Rafa[11], Antoni Munné[11], Rosa Maria Pintó[6,7], Josep Pueyo[1,2], Jordi Robusté-Cartró[11], Marta Rusiñol[12], Robert Sanfeliu[4], Joan Teichenné[10], Helena Torrell[3], Lluís Corominas[1,2], Carles M. Borrego[1,13].

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[11]Catalan Water Agency (ACA). Provença, 260, E-08008, Barcelona, Catalonia, Spain.

[12.] Institute of Environmental Assessment & Water Research (IDAEA), CSIC, Barcelona, Catalonia, Spain.

[13] Group of Molecular Microbial Ecology, Institute of Aquatic Ecology, University of Girona, E-17003, Girona, Catalonia, Spain.

Wastewater-based epidemiology has shown to be an efficient tool to track the circulation of SARS-CoV-2 in communities assisted by wastewater treatment plants (WWTPs). The Catalan Surveillance Network of SARS-CoV-2 in Sewage monitors, weekly or biweekly, 56 WWTPs evenly distributed across the territory and serving 6M inhabitants (80% of the Catalan population). Since July 2020, samples from 45 WWTPs are weekly collected, analysed, results of SARS-CoV-2 concentrations reported to Health authorities, and finally published within less than 72 hours in an online dashboard (https://sarsaigua.icra.cat). After 20 months of monitoring (July 20 - March 22), the standardized viral load (gene copies/day) in all the WWTPs monitored fairly matched the cumulative number of COVID-19 cases during the pandemic (Spearman Rho = 0.69). In November 2021, the network implemented the periodical (biweekly or monthly) analysis of SARS-CoV-2 genomic variants by sequencing the Spike gene using subARTIC primers and the Oxford nanopore platform. By doing so we could monitor the early introduction of Omicron in the territory and the progressive spread of its evolved lineages throughout the country. The Catalan Surveillance Network of SARS-CoV-2 in Sewage is particularly relevant under the current scenario where clinical reporting has been discontinued and emerging SARS-CoV-2 variants can drive to new pandemic waves.

O.7. **Evaluation of the ONT MinION Mk1C platform vs Illumina MiSeq for the genomic epidemiology study of SARS-CoV-2.**

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Background

Whole-genome sequencing (WGS) has become a key element of SARS-CoV-2 genomic epidemiology. In Spain, this has been mostly driven by Illumina-based sequencing, while the process could be simplified using Oxford Nanopore Tecnologies' MinION Mk1C, an economic, fast, and user-friendly sequencer. We aimed to evaluate the applicability of the Mk1C device for SARS-CoV-2 genomic surveillance and rapid outbreak characterization, by comparing its technical and practical performance with Illumina's MiSeq platform, including turn-around time (TAT) and costs.

Methods

SARS-CoV-2-positive nasopharyngeal swabs (Ct<30) were processed with both sequencing technologies, including: i) cases selected from weekly routine surveillance (RS: 94 samples plus positive and negative controls in a single run), and ii) 44 samples from nine different nosocomial outbreaks (NO). For Illumina, reverse transcriptase and multiplex tiling PCR (ARTIC) was used, followed by library preparation (Illumina DNA prep kit) and MiSeq sequencing (v2, 2x150 cycles, 27 h). For Nanopore, the Midnight RT-PCR Expansion protocol was used, followed by library preparation (Rapid Barcoding Kit 96) and Mk1C sequencing with an R9.4 flow cell run overnight for RS, and reduced for the NO runs to approximately 2-3 h, when 20,000 reads per sample were obtained (12-26 samples plus controls per run). Data were analyzed using the nf-core/viralrecon pipeline (v2.4.1).

Results

Good quality sequences were obtained by both methods in 131/138 (94.9%) clinical samples. Their mean genome coverage was 99% for both techniques, while mean depth of coverage was 1.84-fold lower for MinION (914 vs 498). Nevertheless, Pango lineage assignments were totally concordant (including several Delta and Omicron lineages). No differences in SNPs were observed between NO paired MiSeq and MinION sequences, but three different aminoacid substitutions were missed by the Nanopore sequencing

method in four RS samples. Nanopore sequencing had a 1.3-fold shorter lab TAT for RS and 4.1-fold for NO, and a 3-fold lower cost.

Conclusions

These results support the use of MinION Mk1C for a rapid response to nosocomial outbreaks as well for its implementation into SARS-CoV-2 genomic surveillance, as a reliable alternative to Illumina MiSeq.

O.8. **Validation of a passive sampler as an affordable and easy-to-use tool for wastewater based epidemiology**.

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Wastewater-based epidemiology (WBE) has raised as an important tool for the prevention and early warning of infectious disease outbreaks within a community. Most of the published WBE studies use active sampling (by means of autosamplers) to collect composite samples, from the inlet of municipal wastewater treatment plants (WWTP). Although autosamplers collect flow dependent samples over 24-h, their use in smaller settings can be limited by their cost, the need of a power supply and, in some cases, limited access to the sampling area. Passive samplers have been purposed as affordable and easy-to-use devices for virus surveillance in smaller scale scenarios.

In this study, we validated a passive sampling method, for the detection and quantification of a diversity of viruses, in a large urban WWTP and in an aged care facility. A total of 15 passive samplers, containing 2 electronegative membranes, were deployed, and retrieved 24h later. Using a traditional autosampler, 24-h composite

samples were collected in parallel in both settings. Electronegative membranes were used for direct nucleic acid (NA) extraction using a Qiagen RNeasy Power Microbiome Kit. Twenty-four-hour composite samples were concentrated using the automatic CP-SelectTM device with ultrafiltration tips and NA were extracted with QiAamp Viral RNA Kit. The detection and quantification of several viruses (SARS-CoV-2 (N1 and N2 assays), JC Polyomavirus, Human adenovirus, Rotavirus, Norovirus genogroup I and II and Enterovirus) was assessed using specific qPCR and massive sequencing using a Targetted Enrichment Approach. Both methods were also compared as for their performance in subsequent massive viral sequencing. The results indicated no differences in the detection of JCPyV and HAdV in the WWTP by applying any of the sampling-methods. Although active sampling demonstrated to have greater sensitivity over the WWTP samples, the proposed passive sampling protocol performed equally at the building level. In the event of new epidemic outbreaks, the use of passive samplers can be used for targeted actions or to track back specific facilities with vulnerable population (e.g., schools, universities or aged care facilities).

O.9. **The frequency of defective genomes in Omicron differs from that of the Alpha, Beta and Delta variants**.

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Background: Omicron has promptly emerged showing higher transmissibility and probably higher resistance to current COVID-19 vaccines than other variants dominating the global SARS-CoV-2 pandemics. In a study in March 2020 involving patients with COVID-19 infection of varying severity, we found that a portion of genomes in the SARS-CoV-2 viral population accumulated deletions at the S1/S2 cleavage site (PRRAR/S) of the spike gene, generating a frameshift and appearance of a premature stop codon. The main aim of this study was to determine the frequency of defective deletions in prevalent variants from the first to the sixth pandemic waves and discuss whether the observed changes might support epidemiological proposals.

Methods: The complete SARS-CoV-2 spike gene was deeply studied by next generation sequencing using the MiSeq platform by the methodology of overlapped amplicons. More than 148 million reads have been obtained from respiratory swab specimens of 119 COVID-19 patients mildly infected with the most relevant variants circulating in Barcelona city area during the seven pandemic waves: B.1.5, B.1.1, B.1.177, Alpha, Beta, Delta and Omicron BA.1, BA.1.1, BA.2, and BA.5 variants.

Results: The frequency of defective genomes found in Omicron BA.1 subvariant was similar to that seen in variants dominating the first and second waves, but differed from the frequencies seen in the Alpha, Beta and Delta variants. Surprisingly, the frequency of defective deletions found in Omicron BA.1.1, BA.2 and BA.5 subvariants was identical to that found in Alpha, Beta and Delta variants.

Conclusions: Our results concur with findings from previous studies indicating that the S1/S2 cleavage site is an important region for the biology of the virus, affecting the capability of SARS-CoV-2 to readily infect humans. Here we discuss how defective deletions naturally occurring before S1/S2 cleavage site might have putative effects during adaptation of the virus to human infection.

O.10. **Evolution and Reassortments Analysis of Swine Influenza Virus H1N1 and H3N2 in Vaccinated Pigs after Simultaneous Infection by contact**.

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Influenza viruses (IVs) can infect wide variety of bird and mammal species. Its genome is characterized by having 8 RNA single stranded segments. The low proofreading activity of its polymerases and the genomic reassortment between different IVs subtypes allow virus to be continuously evolving, constituting a constant threat to human and animal health. In 2009, a pandemic of an Influenza A virus highlighted the importance of the swine host in IVs adaptation between humans and birds. The swine population and the incidence of swine influenza (SI) is constantly growing. In previous studies, despite vaccination, SI virus (SIV) growth and evolution was proven in vaccinated and challenged animals. However, how vaccination can drive the evolutionary dynamics of SIV after coinfection with two subtypes is poorly studied. In the present study, vaccinated and nonvaccinated pigs were challenged by direct contact with H1N1 and H3N2 independent SIVs seeders. Nasal swab samples were daily recovered and BALF was also collected at necropsy day from each pig for SIV detection and whole genome sequencing. In total, 39 SIV whole genome sequences were obtained by NGS from samples collected from both experimental groups. Subsequently, genomic and evolutionary analyses were carried out to detect both, genomic reassortments and single nucleotide variants (SNV). Regarding the segments found per sample, we observed that the simultaneous presence of segments from both subtypes was much lower in vaccinated animals, indicating that vaccine reduced the possibility of genomic reassortment events. In relation to SIV intra-host diversity, a total of 230 and 77 SNV were detected in H1N1 and H3N2 subtypes, respectively. Different proportion of synonymous and nonsynonymous substitutions was found, indicating that vaccine may

be influencing the mechanism of natural, neutral, and purifying selection that play the main role in SIV evolution. SNV were detected along the whole SIV genome with important non-synonymous substitutions on polymerases, surface glycoproteins and non-structural protein, which may have an impact on virus replication, immune system escaping and virulence of virus, respectively. The present study further emphasized the vast evolutionary capacity of SIV both under natural infection and vaccination pressure scenarios.

O.11 .**ß-Cyclodextrins as affordable antivirals against coronavirus infection**

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The impact of global pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, the causative agent of the COVID-19) made evident that the arsenal of virus-fighting drugs is limited. In an interdisciplinary approach involving both *in silico* and *in vitro* settings, we aimed to identify a cost-effective antiviral with broad spectrum activity and high safety and tolerability profiles. We began elaborating a list of 116 drugs previously used or in clinical trials to treat human pathologies with potential to treat coronavirus infections. We next employed molecular modelling tools to rank the most promising inhibitors and tested their efficacy as antivirals against α and ß coronavirus, e.g., the HCoV-229E and SARS-CoV-2. Four drugs, OSW-1, U18666A, hydroxypropyl-ß-cyclodextrin (HßCD) and phytol, showed antiviral activity against both HCoV-229E (in MRC5 cells) and SARS-CoV-2 (in Vero E6 cells). The mechanism of action of these four compounds was studied by transmission electron microscopy (TEM) and by testing their capacity to inhibit the entry of SARS-CoV-2 pseudoviruses in hACE2-expressing HEK-293T cells. The entry was inhibited by HßCD and U18666A, but only HßCD was able to inhibit SARS-CoV-2 replication in pulmonary Calu-3 cells. With these results and given that cyclodextrins (CD) are widely used in drug encapsulation and are considered safe to be used in humans, we further tested 6 native and modified CDs, which confirmed ß-CD as the most potent inhibitor of SARS-CoV-2 replication in Calu-3 cells. To further elucidate the mechanism of action, we performed a lipidomic analysis, revealing that methyl-β-CD reduced the free and ester cholesterol in biological membranes. Taken together, our results highlight the potential of different CDs to inhibit SARS-CoV-2 replication either by interfering with viral fusion via cholesterol depletion or by potentially inhibiting M^{pro} catalytic activity. Our data suggest that ß-CDs are very promising candidates to be used in therapeutic and prophylactic treatments for SARS-CoV-2 and possibly other respiratory viruses.

O.12. **PBMC immunophenotyping and plasma inflammatory profile of children with Long COVID.**

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Background

Long COVID can be developed by individuals after an infection with SARS-CoV-2 as described by the WHO. Although this condition is more commonly described in adults, it can occur in children and adolescents with a wide range of estimated prevalence of 1-25%. Little is known about the role of the immune system in long COVID. However, one of the main hypotheses about the underlying mechanism in long COVID is that there is an immune and inflammatory dysregulation that persists after the acute infection. The objective of this study is to compare immune cells populations, and inflammatory biomarkers in paediatric populations with and without long COVID.

Methods

We analyzed 55 blood samples from the pediaCOVID cohort (Hospital Germans Trias i Pujol), which includes more than 130 children diagnosed with long COVID and 23 controls. We measured different immune cell populations using spectral cytometry with a panel of 37 cellular markers, and 42 inflammatory markers using Luminex or ELISA. EdgeR was used for statistical analysis of the spectral data; p-values of inflammatory markers were calculated using the likelihood ratio test and they were corrected for multiple comparisons.

Results

The study cohort had a median age of 14.3 (IQR, 12.5-15.2) and 69.1% female. Patients had at least 3 symptoms associated with long COVID (median [IQR]; 10 [7-16]). The most common symptom was asthenia/fatigue (98.2%). Compared to the control cohort, children with long COVID had increased numbers of CD4+CD8+ T cells, IgA+CD21+CD27+ memory B cells, and IgA+CD21–CD27– memory B cells, while CD4+ TEMRA cells (CD45RA+ , CCR7–), intermediate monocytes (CD14+ , CD16+) and classical monocytes (CD14+ , CD16–) were decreased (all $p < 0.05$; q=n.s.). None of the 42 inflammatory

biomarkers showed significant differences between children with and without long COVID.

Conclusion

The results of this study suggest that specific populations of peripheral blood immune cells might be involved in the mechanisms underlying prolonged COVID in children and adolescents. The increase in both IgA+CD21–CD27– and IgA+CD21+CD27+ memory B cells could be associated with the persistence of viral antigen in the gut and/or gut dysbiosis. Moreover, the decrease in CD4+ TEMRA cells could be related to autoantibodies against G-protein coupled receptors (GPCRs), since this cell population can express GPR56, and autoantibodies against GPCRs were previously reported to be elevated in adults with long COVID.

O.13. **Development and characterization of a new human ACE2 knock-in mouse model for SARS-CoV-2 infection and pathogenesis.**

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Since the start of the COVID-19 pandemic, animal models have been instrumental in studying different aspects of the disease and testing vaccines and therapeutics. Being not naturally susceptible to ancestral SARS-CoV-2, mice have to be humanized for the ACE2 receptor (hACE2) through different approaches. Currently, one of the most used models is the K18-hACE2 transgenic mice (B6.Cg-Tg(K18-ACE2)2Prlmn/J Strain #:034860) in which infection curses with progressive weight loss starting from days 2-4 post-inoculation (dpi), followed by severe clinical signs by 6 dpi, primarily due to brain and lung lesions. These mice possess 8 full copies of the hACE2 transgene, which might explain the severity of the disease observed in this model. In this study (Ethical Committee Aproval Code DMAH 10960), we generated a new knock-in (KI) mouse model inserting the original K18-hACE2 transgene into the collagen Col1A1 locus using a recombinase mediated cassette exchange (RMCE) FLP-FRT system in KH2 cells via blastocyst injection. This locus was chosen as it is known to provide good and widespread expression of inserted sequences. Additionally, RMCE allowed the insertion of a single transgene copy which should better mimic physiological expression. Once the Col1A1-K18-hACE2 colony was established, mice were infected with a B.1 SARS-CoV-2 (D614G) isolate and followed until 14 dpi. KI mice showed a decrease in weight similar to the K18-hACE2 transgenic model, however, after 6 days, a progressive weight recovery was observed in the majority of animals. Besides weight loss, clinical symptoms were mostly limited to light dyspnea and animal remained fully responsive during the entire experiment follow-up. Importantly, despite this apparent mild pathogenicity, lung lesions increased over time and were still elevated after 14 days. High viral RNA loads could be observed in lung, oro-pharyngeal swab and nasal turbinate upon intranasal infection, but no viral replication was detected in the brain of these animals in contrast with K18-hACE2 mice. The longer disease follow-up, possible in this milder infection model, will allow a better characterization of the recovery phase. Overall, and with further ongoing characterization, this newly developed Col1A1-K18-hACE2 KI animal model could be a valuable tool to study COVID-19 therapies and long-term COVID-19 sequelae.

O.14. **Towards a functional cure for chronic virus infection by shifting the virus – host equilibrium state.**

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Chronic viral infections like those caused by Human Immunodeficiency Viruses (HIV) and Hepatitis B (HBV) and C (HCV) remain a wide-world challenge. During chronic virus infections, the state of the host is characterised by a partial shut-off of the immune system known as T cell exhaustion. Although it is a host protective mechanism to avoid immunopathology caused by an overactivation of the system, it also limits viral load reduction. We aim to find general immunological ways for shifting the dynamic virus-host immune system balance into one in which the virus is sufficiently controlled without causing pathology. Using our LCMV-chronic infection model, we have tested different immunotherapeutic drug combinations for its impact on virus control. We show that anti-PDL1-mediated reinvigoration of exhausted T cells and viral load reduction can be improved by the addition of the TLR7/8 and TLR3 agonists R848 and Poly I:C, respectively. Based on the system biology concept of multi-stability and the described prediction of multiplicative cooperativity between virus-specific cytotoxic T cells and neutralising antibodies we hypothesize that in order to reach a chronic infection functional cure, multiple immune system components need to be engaged. For this reason, further experiments focusing on the role of exogenous virus-specific neutralising antibodies are ongoing.

O.15. **The combination of Gapmers and/or siRNA as a potential hepatitis B virus gene therapy strategy against hepatitis B virus: preliminary in vitro results.**

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Background and Aims: Hepatitis B virus (HBV) infection cannot be eradicated due to the persistence of its covalently closed circular DNA, which supports HBV proteins expression, contributing to hepatocellular carcinoma development. Gene silencing could be a valuable strategy and HBX gene, thanks to its co-terminal localization, an optimal target. Here we present a gene therapy strategy based on antisense locked nucleic acid (LNA) Gapmer (GP) and siRNA targeting HBX hyper-conserved regions.

Method: HepG2-NTCP cells were treated with DMSO 2.5% at least 14 days and later infected with HBV at 500 multiplicities of genome equivalents by centrifugation at 37ºC for 1h and incubating overnight. Infected cells were treated with GP (GP1 and/or GP4 at 50nM, Qiagen) and/or siRNA (50nM) using TransIT-X2 at 48h or 5d post infection (pi). A scrambled control gapmer was used in each experiment. Cells and supernatants were collected after 72h of treatment. Pregenomic RNA (pgRNA) was extracted from cells through RNAeasy Plus mini kit (Qiagen) and quantified by RT-Qpcr using TaqMan probe (Roche, Lightcycler®480). HBsAg and HBeAg were quantified in supernatants by chemilumiscent enzyme assay (Roche, COBAS®8000).

Results: Early after infection (at 48hpi), GP1 treatment allowed an inhibition of more than 70% for both pgRNA, HBeAg and HBsAg (respectively 75.5±9.9; 74.7±6.4 72.4±7.7%). Differently, GP4 showed an inhibition between 7.4 and 13 percentage

points lower than GP1 (62±17; 66.1±11.2 and 64.7±12% for respectively pgRNA, HBeAg and HBsAg). When GP were introduced at late timepoint (5dpi), the inhibition of pgRNA decreased for both GP1 and GP4 (respectively 53.5±29% and 58.5±17%). The efficiency was lower if considering viral proteins expression (less than 48% for both GP). Of note, the combination of both GP or GP with a siRNA increased the inhibition efficiency of pgRNA (69.1±16.5%; 66.7±12.2%; 63.8±21% for respectively GP1+GP4; GP1+siRNA; GP4+siRNA), just partially improving viral proteins inhibition.

Conclusion: Gapmers seem to be valuable molecules to inhibit HBV expression *in vitro*. Their limited efficiency after the establishment of a productive infection (5dpi) could be improve by combining GP with each other or with siRNA targeting the same hyper-conserved region. Further experiments are required to confirm these results and other delivery systems should be tested to improve treatment efficiency. Funding: Instituto de Salud Carlos III (grant PI18/01436), co-financed by the European Regional Development Fund (ERDF).

O.16. **RAB GTPases: key players in HAV egress from hepatocytes.**

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Hepatitis A virus (HAV), a classic non-enveloped virus, has recently been found to be released from infected cells mostly through exosomes, forming quasi-enveloped particles (eHAV) by hijacking an ALIX-related exosome-like pathway. Exosome packaging and secretion are still poorly understood and deserves further study. RAB GTPases proteins are believed to be essential for syndecan–ALIX–mediated exosome release, however their role in this process remains unclear. In this work, we have examined the expression patters of multiple RAB genes that encode for proteins that may be implicated in exosome release in the hepatocyte-derived Huh7-AI cell line. Gene expression patterns were not significantly different between non-polarized and polarized hepatocytes, with high expression of RAB11, followed by RAB35 and RAB7. To

determine how viral replication affects gene expression, two HAV strains with different replication capacities were used: the HM175 strain (L0) and the fast-replication HM175-HP (HP). Independently of the polarization status, expression of RAB7A and RAB35 increased after infection, notably with HP. Confocal microscopy was performed to identify any possible co-localization of HAV capsids with RAB proteins. HP capsids clearly co-localized with RAB35, and to a lesser extend with RAB11A and RAB7A. Contrary, L0 capsids preferentially co-localized with RAB7A and to a lesser extent with RAB11A. These findings suggest that RAB35 and RAB7 are preferred for eHAV egress in HP and L0-infected cells, respectively. In polarized cells, RAB7A, RAB11A and RAB35 co-localized with markers for the basolateral and apical membrane, but interestingly, RAB35 was preferentially located at the basolateral membrane. We postulate that RAB35 would be involved in the fast traffic pathway occurring at the basolateral membrane, which would therefore help to explain the more efficient HP strain release via this membrane.

O.17. **Pharmacological inhibition of IKK to tackle latency and hyperinflammation in chronic HIV-1 infection.**

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HIV latent infection may be associated with disrupted viral RNA sensing, interferon (IFN) signaling, and/or IFN stimulating genes (ISG) activation. Here, we evaluated the use of IKK kinase inhibitors (IKKis) targeting distinct IκB kinase (IKK) complex subunits and related kinases (TBK1) as a novel pathway to reverse HIV-1 latency. We evaluated HIV reactivation in latently infected lymphoid Jurkat (J-HIG) and myeloid HL-60 (HL-HIG) cells by flow cytometry. Anti-HIV activity on in vitro acute infection was monitored in Jurkat and HL-60 cells infected with VSV-Gpseudotyped NL4-3-EGFP. HIV-1 reactivation in ex vivo CD4+T cells from HIV+ individuals was quantified by ultrasensitive real time semi nested RT-qPCR. Cell surface activation markers were measured by flow cytometry in PBMCs from healthy volunteers. Immune innate activation and signalling pathways were assessed by western blot and RT-qPCR. Regardless of combination or not with anti-HIV drugs, IKKis triggered up to 1.8-fold increase in HIV reactivation in J-HIG and HL-HIG cells. IKKis targeting TBK-1 (MRT67307) and IKKβ (TCPA-1) significantly induced viral reactivation in HIV+ CD4+T cells ex vivo. IKKis did not upregulate cell activation markers and innate immune signaling was partially blocked, resulting in downregulation (mRNA) of proinflammatory cytokines. Our results support a dual role of IKKis as immune modulators tackling both the HIV latent reservoir in lymphoid and myeloid cellular models and hyperinflammatory responses in chronic HIV-1 infection.

Keywords: HIV-1, latency reversing agents, IKK, TANK binding kinase-1.

O.18. **Niemann Pick Protein C1, a cholesterol transporter involved in Cucumber mosaic virus infection in melon**.

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Plant viruses, as obligate parasites, need host factors to complete their life cycle and achieve a successful infection. However, plants have a complex defence system that enables them to defend against those viruses. In melon, the main QTL for systemic resistance corresponds to cmv1 that encodes the Vacuolar Protein Sorting 41 (VPS41), which acts as a gatekeeper for resistance to CMV by either restricting the virus in the bundle sheath cells or allowing phloem entry 1. This resistance is regulated by the VPS41 and the Movement Protein (MP) of the virus. To unravel the mechanism beyond VPS41-MP, a Y2H screening was performed in a CMV-infected melon library. Results revealed 22 clones corresponding to one specific domain of a Niemann-Pick protein C1 (NPC1). NPC1 is a transmembrane cholesterol transporter related to VPS41 and to local changes in membrane composition. Moreover, it acts as receptor of Ebolavirus and other Flaviviruses in animals. The interactor domain contains four exons and retains two introns, and, some of them, have a polyA- tail at the end of the sequence. To investigate if the introns are necessary for the interaction, we have done several modifications in the clone removing either one or two introns or one exon. The resulting constructs have been tested for interaction with the MP by Y2H and by BiFC. The result will be fundamental to envisage the parts of the interaction domain that are fundamental for the virus and can subsequently be edited in the plant to impair the viral infection.

References:

[1]Cèlia Guiu-Aragonés et al (2016) Molecular Plant Pathology, 17: 973-984. Acknowledgments. I.V. is the recipient of a Margarita Salas contract from Ministerio de Universidades and funded by “Union Europea- Next Generation EU”.