2nd Symposium on Coronavirus Research

XIX Jornada de Virologia – Virology meeting 2020

Organització: Secció de Virologia de la SCB

Virtual/ Plataforma Zoom

29 d'octubre de 2020

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

Núria Busquets Martí

Comitè científic:

Ana Angulo

Jordi Argilaguet

Sílvia Bofill

Juana Diez

Susana Guix

Juan José López-Moya

Miguel Ángel Martínez

Andreas Meyerhans

Sofia Pérez del Pulgar

Josep Quer

Amb el suport de:

PROGRAMA

9:00 h

BENVINGUDA/WELCOME: Núria Busquets

9:10 - 9:40 h (20 + 10')

SESSION I: Chairs: Juana Diez and Josep Quer

OPENING LECTURE: **Coronavirus-host interaction and protection: vaccine development.**

Luis Enjuanes. National Center of Biotechnology (CNB-CSIC), Campus Universidad Autónoma de Madrid, Madrid, Spain.

9:40 - 10:00 h (15 + 5')

INVITED SPEAKER: **Advances in animal models for the study of SARS-CoV-2.**

Júlia Vergara-Alert. IRTA-CReSA, Campus UAB, Bellaterra, Spain.

10:00 - 11:00 h

ORAL PRESENTATIONS

O.1 - O.3 (10 + 5')

O.1. **SARS-CoV-2 induces cytokine release by human macrophages in the absence of productive viral replication**.

Daniel Perez-Zsolt. IrsiCaixa AIDS Research Institute, Badalona, Spain.

O.2. **Tissue-specific 3D genome conformation predicts novel genetic susceptibility loci for COVID-19.**

Marc A. Martí-Renom. CNAG-CRG, Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain.

O.3. **Search of SARS-CoV-2 inhibitors and their combinations within approved drugs to tackle COVID-19 pandemic.**

Jordi Rodon. IRTA-CReSA, Campus UAB, Bellaterra, Spain.

O.4 - O.6 (3 + 2') Flash talks

O.4. **Cellular models for the study of SARS-CoV-2 pseudoviral entry.**

Jordana Muñoz-Basagoiti. IrsiCaixa AIDS Research Institute, Badalona, Spain.

O.5. **Previous inoculation of SARS-CoV-2 in hamsters protects against subsequent infection by D614-homologous or G614-heterologous SARS-CoV-2 strains.**

Marco Brustolin. IRTA-CReSA, Campus UAB, Bellaterra, Spain.

O.6. **Membrane integration and topology of SARS-CoV-2 E (envelope) protein.**

Gerard Duart. Departament de Bioquímica i Biologia Molecular, Institut Universitari Biotecmed, Universitat de València, Burjassot, València.

11:00 - 11:20 h

BREAK

11:20 - 11:50 h (20 + 10')

SESSION II Chairs: Fernando Rodriguez and Ester Ballana

INVITED SPEAKER: **SARS-CoV-2 entry into cells and its inhibition.**

Stefan Pöhlmann. Infection Biology Unit, German Primate Center, Göttingen, Germany.

11:50 - 13:00 h

ORAL PRESENTATIONS

O.7 - O.10 (10 + 5')

O.7. **Low zinc levels at clinical admission associates with poor outcomes in COVID-19.**

Marc Talló-Parra. Molecular Virology Group, Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain.

O.8. **COVID Viral Beacon: Fast SARS-COV-2 Data discovery.**

Claudia Vasallo. European Genome-phenome Archive - Centre for Genomic Regulation Barcelona, Spain.

O.9. **Sentinel surveillance of SARS-CoV-2 in wastewater anticipates the occurrence of COVID-19 cases.**

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.10. **Does size matter? SARS-CoV-2 surveillance in diferent sized wastwater treatment plants**.

Eva Forés. Department of Genetics, Microbiology and Statistics, University of Barcelona, Spain. The Water Institute of the University of Barcelona, Spain.

O.11 - O.12 (3 + 2') Flash talks

O.11. **SARS-CoV-2 detection in clinical samples and urban sewage by target enrichment sequencing.**

Sandra Martínez-Puchol. Laboratory of Viruses Contaminants of Water and Food, Genetics, Microbiology & Statistics Dept., Universitat de Barcelona. Barcelona, Catalonia, Spain.

O.12. **Insertion and folding of SARS-CoV-2 M (membrane) protein.**

José M. Acosta-Cáceres. Departament de Bioquímica i Biologia Molecular, Institut Universitari Biotecmed, Universitat de València, Burjassot, València, Spain.

13:00 - 14:00 h

DINAR / LUNCH

14:00 - 14:30 h (20 + 10')

SESSION III Chairs: Ana Angulo and Jordi Argilaguet

INVITED SPEAKER: **Viral zoonoses and their reservoir hosts: the animal component of the One Health approach.**

Miguel Ángel Jiménez-Clavero. Centro de Investigación en Sanidad Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA-CISA), Valdeolmos, Spain.

14:30 - 15:30 h

ORAL PRESENTATIONS

O.13 - O. 15 (10 + 5')

O.13. **The role of visual and olfactory cues in host plant selection by the insect vector *Bemisia tabaci* in mixed infections of *Tomato chlorosis virus* (ToCV) and *Tomato yellow leaf curl virus* (TYLCV).**

Irene Ontiveros. Center for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, Barcelona, Spain. Institute for Mediterranean and Subtropical Horticulture "La Mayora" (IHSM-UMA-CSIC), Algarrobo-Costa, Málaga, Spain.

O.14. **Double targeting of two key co-signaling molecules of NK and T cells by a cytomegalovirus-encoded CD48 homolog.**

Francesc Poblador. Immunology Unit, Department of Biomedical Sciences, Medical School, University of Barcelona, Barcelona, Spain.

O.15. **Role of cross-presenting dendritic cells in anti-PD-L1 immunotherapy of chronic viral infections.**

Eva Domenjo-Vila. Infection Biology Laboratory, Department of Experimental and Health Sciences (DCEXS), Universitat Pompeu Fabra, Barcelona, Spain.

O.16 - O.18 (3 + 2') Flash talks

O.16. **Evaluation of algorithm-assisted procedures for identification of polymerase slippage motifs in the genome of members of the Potyviridae family using FIMO and gkm-SVM.**

Giannina Bambaren. Center for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Barcelona, Spain.

O.17. **Identification of a gene product with RNA silencing suppression activity in the symptomless potyvirus *Sweet potato latent virus.***

Eric Ros-Moner. Center for Research in Agricultural Genomics (CRAG-CSIC-IRTA-UAB-UB), Barcelona, Spain.

O.18. **Detection of Norovirus in saliva samples from cases and asymptomatic subjects involved in gastroenteritis outbreaks**

Eduard Anfruns-Estrada. Enteric Virus Laboratory, Department of Genetics, Microbiology and Statistics, University of Barcelona, Spain. Nutrition and Food Safety Research Institute (INSA·UB), University of Barcelona, Spain.

15:30 - 16:00 h (20 + 10')

SESSION IV Chairs: Susana Guix and Juan José López-Moya

INVITED SPEAKER: **Translational control in emerging viral infections.**

Juana Diez. Virology Unit, Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain.

16:00 - 16:50 h

ORAL PRESENTATIONS

O.19 - O.21 (10 + 5')

O.19. **Viruses in motion: a close look at virus maturation through cryo-electron microscopy**

Roger Castells-Graells. Department of Biological Chemistry, John Innes Centre, Norwich, UK.

O.20. **Nanostructural characterization of influenza virus-like particles with super resolution microscopy.**

Maria Arista-Romero. Nanoscopy for Nanomedicine Group, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology, Barcelona, Spain.

O.21. **Serum and intrahepatic HBV markers and HBV-specific CD8 T cell responses after nucleos(t)ide analog therapy discontinuation in HBeAg-negative chronic hepatitis B patients.**

Mireia García-López. Liver Unit, Hospital Clínic, University of Barcelona, IDIBAPS, CIBERehd, Barcelona, Spain.

O.22 (3 + 2') Flash talk

O.22. **HDAg abundance in hepatitis delta patients before liver transplantation is associated with HDV infection of the graft.**

Ester García-Pras. Liver Unit, Hospital Clinic, University of Barcelona, IDIBAPS, CIBERehd, Barcelona, Spain.

16:50 - 17:00h

BREAK

17:00 - 17:30h

SESSION V: Chairs: Sílvia Bofill and Andreas Meyerhans

CLOSING LECTURE **(20 + 10')**

Enabling Ecosystem Scale Viral Metagenomics.

James M. Pipas. Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, USA.

ORAL AWARDS

INVITED SPEAKERS' ABSTRACTS

OPENING LECTURE

Coronavirus-host interaction and protection: vaccine development.

Luis Enjuanes

National Center of Biotechnology (CNB-CSIC), Campus Universidad Autónoma de Madrid, Madrid, Spain.

Viruses are part of the ecosystem and exist where life exists. Mathematical models with some experimental support indicate that there are still more than 300000 new viruses to be discovered. The barriers between animal and human infections are very small and viruses constantly cross these barriers. In fact, almost two-thirds of all human communicable diseases are zoonotic diseases. There are several viral transmission routes, some of which are markedly affected by climate change. In the presentation we will review the emergence of deadly coronaviruses for humans, including the new virus from Wuhan (China).

Protection against viral epidemics has many aspects, but prevention through vaccination is one of the most effective, probably followed by antiviral treatment. Our laboratory has investigated in last years the development of these strategies, what facilitated us the description of different experimental approaches developed through reverse genetics and virus-host interaction studies. Deletion of non-essential genes in human coronaviruses resulted in attenuated viruses that protect against infection as they were highly promising vaccine candidates. From these attenuated viruses we have selected SARS-CoV-2 derived RNA replicons which promoted viral RNA replication and transcription but that were unable to produce propagation competent infectious progeny, which mean that they are safe and effective vaccine candidates. Besides, virus-host interaction studies allowed us to identify antivirals that protect against these viruses, facilitating therapeutic treatments to respond to pathogenic human coronavirus emergency.

KEYNOTE LECTURES

Advances in animal models for the study of SARS-CoV-2.

Júlia Vergara-Alert

IRTA-CReSA, Campus UAB, Bellaterra, Spain.

The coronavirus disease 2019 (COVID-19) is an emerging respiratory infection caused by the introduction of a novel coronavirus (named severe acute respiratory syndrome coronavirus 2 or SARS-CoV-2) into humans late in 2019. SARS-CoV-2 was first detected in Hubei province, in China and has spread rapidly worldwide. As of 11 October 2020, SARS-CoV-2 has infected over 37 million people and has caused more than 1 million deaths. As humans do not have pre-existing immunity to SARS-CoV-2, there is an urgent need to develop therapeutic agents and vaccines to mitigate the current pandemic and to prevent the re-emergence of COVID-19. In February 2020, the World Health Organization (WHO) assembled an international panel to develop animal models for COVID-19 to accelerate the testing of vaccines and therapeutic agents. This presentation will summarize the findings to date and provides relevant information for preclinical testing of vaccine candidates and therapeutic agents for COVID-19.

SARS-CoV-2 entry into cells and its inhibition.

Stefan Pöhlmann

Infection Biology Unit, German Primate Center, Göttingen, Germany

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of coronavirus disease 2019 (COVID-19). The pandemic spread of SARS-CoV-2 poses a severe burden on economies and health systems and therapeutics are urgently needed. Entry into target cells is the first step in SARS-CoV-2 infection and the viral and cellular factors involved in this process constitute potential targets for intervention. My laboratory demonstrated that SARS-CoV-2 employs the cellular protein angiotensin-

converting enzyme 2 (ACE2) as receptor for host cell entry. Moreover, we found that entry depends on the cleavage-activation of the viral S protein by the cellular proteases furin and TMPRSS2 and can be blocked by the clinically-proven serine protease inhibitor camostat. Our recent research focused on the question whether proteases other than TMPRSS2 can activate SARS-CoV-2 and whether these proteases are inhibited by camostat. Moreover, we addressed whether camostat metabolization compromises antiviral activity and, jointly with partners at Göttingen University Hospital, we investigated whether camostat is suitable for treatment of COVID-19. The results of these studies will be presented.

Viral zoonoses and their reservoir hosts: the animal component of the One Health approach.

Miguel Ángel Jiménez-Clavero

Centro de Investigación en Sanidad Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA-CISA), Valdeolmos, Spain.

It is widely recognized that a high proportion (over 70%) of emerging infectious diseases (EIDs) have an animal origin, that is, are zoonotic. The concept “One Health” stands for a multidisciplinary approach in which human and veterinary medicine as well as environmental disciplines are concerned, so that this concept is considered as the most appropriate to tackle zoonotic diseases and by extension EIDs. Amongst pathogens giving rise to EIDs, viruses are the most frequently involved, much more often than bacteria or parasites. Viruses are endowed with high plasticity. Their rapidly changing nature is a consequence of higher mutation rates as compared with other microorganisms, giving them their outstanding ability to adapt to new environments, i.e. hosts (and vectors wherever applicable). In fact, adaptation to a new host or vector, i.e. crossing the species barrier, is considered the “original sin” or landmark leading to EIDs. Therefore, zoonotic viruses must be kept under close surveillance since they are often involved in episodes of emerging or re-emerging infectious diseases throughout the world. Abundant examples can be found recently, even close our life settings. One of the most outstanding ones is the recent spread of West Nile fever/encephalitis in vast

regions of the world. While only two decades ago this disease was restricted to Sub-Saharan Africa, with very sporadic incursions to the Mediterranean area, now it is widespread in Europe, parts of Asia, Oceania and the Americas and constitutes a serious threat for both animal and human health. Although we are far from understanding why this pathogen has spread so vastly, the One Health perspective, and, particularly, its animal component, may contribute to identify the reasons behind this phenomenon. The study of virus-host interactions points out essential features that might be crucial to explain the expanding behaviour of certain West Nile virus strains.

Translational control in emerging viral infections.

Juana Diez

Virology Unit, Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain.

How viruses, such as the emerging mosquito-borne Chikungunya virus (CHIKV), express their genomes at high levels despite an enrichment in suboptimal codons remains a puzzling question. By integrating subcellular fractionation and transcriptome-wide analyses of translation in CHIKV-infected human cells, we demonstrate an unanticipated virus-induced reprogramming of the host translation machinery to favor translation of viral RNA over cellular genes featuring optimal codon usage.

CLOSING LECTURE

Enabling Ecosystem Scale Viral Metagenomics.

James M. Pipas

Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, USA.

The Earth's biome is changing rapidly. The pressures of expanding population and development coupled with climate change are likely to lead to the emergence of novel pathogens that threaten human health, agriculture, and the overall stability of critical ecosystems. Our laboratory is interested in uncovering the basic science behind viral emergence. The current focus is on developing tools to detect viruses and microorganisms in the environment and associating them with specific hosts, and in understanding the barriers to cross-species transmission. This presentation will outline our efforts on viral detection. We seek to leverage advances in computer power, robotics, and artificial intelligence to enable the detection and surveillance of viruses at the ecosystem scale. This involves the development of novel cloud-based computational methods for detecting viral sequences in nucleic acid sequencing data sets, and robotic methods for collecting large numbers of samples for analysis.

ORAL PRESENTATIONS ABSTRACTS

SESSION I

O.1. **SARS-CoV-2 induces cytokine release by human macrophages in the absence of productive viral replication**.

Daniel Perez-Zsolt[1,*], Jordana Muñoz-Basagoiti[1,*], Jordi Rodon[2,*], Alfonso Valencia[3,4], Víctor Guallar[3,4], Jorge Carrillo[1], Julià Blanco[1,5,6], Joaquim Segalés[7,8], Bonaventura Clotet[1,5,6], Júlia Vergara-Alert[2,+], Nuria Izquierdo-Useros[1,5,+]

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[8]Departament de Sanitat i Anatomia Animals, Facultat de Veterinària, UAB, Bellaterra (Cerdanyola del Vallès), Spain.

*Equal contribution; [+] Dual senior authorship.

Background: Acute respiratory distress syndrome (ARDS) is a common complication of the coronavirus infection disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). ARDS is associated with a disproportionate pro-inflammatory response to SARS-CoV-2. Myeloid antigen-presenting cells such as macrophages and dendritic cells (DCs) are key to induce antiviral immunity, but also secrete pro-inflammatory cytokines upon viral sensing. Here, we have assessed SARS-CoV-2 replication in myeloid cells and their capacity to secrete cytokines upon viral exposure.

Methods: Primary monocyte-derived macrophages and DCs were pulsed with SARS-CoV-2 at a MOI = 2, and cell-associated SARS-CoV-2 and viral accumulation in the supernatant were measured by a nucleocapsid ELISA over time. Pro-inflammatory

cytokines such as IL-6, TNF-□ and MIP-1□ were collected kinetically and assessed with Luminex technology.

Results: Cell-associated viral nucleocapsid was mostly detected in macrophages as compared to DCs, and levels deceased over time. However, no viral accumulation was detected in the supernatant, suggesting that these cells are able to efficiently trap SARS-CoV-2 particles and degrade them in the absence of viral replication. Pro-inflammatory cytokines were mostly detected in supernatants from SARS-CoV-2-exposed macrophages.

Conclusions: Among human myeloid antigen-presenting cells, macrophages are able to recognize SARS-CoV-2 particles. Although this interaction does not lead to productive viral replication, it triggers the release of pro-inflammatory cytokines that might contribute to the development of ARDS in COVID-19 patients.

O.2. **Tissue-specific 3D genome conformation predicts novel genetic susceptibility loci for COVID-19.**

Juan A. Rodriguez[1], Grégoire Stik[2,3], Ralph Stadhouders[4,5], Oscar Lao[1], Thomas Graf2[,3] and **Marc A. Marti-Renom**[1,2,3,6]

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Since late 2019, SARS-CoV-2 has infected millions world-wide with extreme variable disease outcome between individuals. Age, disease co-morbidities such as obesity or diabetes, and gender have a strong effect in the course of the disease. However, these aspects do not explain all the observed variability. In the quest to identify genetic variants, standard GWAS approaches have suggested a few loci associated to the disease

susceptibility[1]. We have now developed a novel tissue-aware GWAS (TA-WAS) approach that leverages cell-specific three-dimensional (3D) genome conformation[2] to increase the power of detecting disease susceptibility loci. The rationale behind TA-GWAS is that groups of genetic variants, with non-detectable disease penetrance in the context of the linear genome, converge into tissues specific 3D hubs that may play a role in the susceptibility and/or severity of a disease. Here, we applied TA-GWAS to COVID19 disease by combining a recently released GWAS analysis (B2 dataset from the http://covid19hg.org initiative[3] with ~3,200 hospitalized patients compared to the general population) with chromosome conformation capture datasets from six cell types involved in the SARS-CoV-2 infection (that is, lung epithelial cells, venous endothelial cells, B-lymphocytes, CD4+/CD8+ T-lymphocytes and macrophages) from our labs and other available sources[4-6]. Our TA-GWAS analysis identified dozens of novel cell type-specific loci that predict a patient's predisposition to develop severe COVID19. These loci constitute possible novel therapeutic targets.

References

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6. Consortium, E. P. An integrated encyclopedia of DNA elements in the human genome. *Nature* **489**, 57-74, doi:10.1038/nature11247 (2012).

O.3. **Search of SARS-CoV-2 inhibitors and their combinations within approved drugs to tackle COVID-19 pandemic.**

Jordi Rodon[1], Jordana Muñoz-Basagoiti[2], Daniel Perez-Zsolt[2,*], Marc Noguera-Julian[2], Roger Paredes[2,5], Lourdes Mateu[5], Carles Quiñones[5], Itziar Erkizia[2], Alfonso Valencia[3,4], Víctor Guallar[3,4], Jorge Carrillo[2], Julià Blanco[2,5,6], Joaquim Segalés[7,8], Bonaventura Clotet[2,5,6], Júlia Vergara-Alert[1], Nuria Izquierdo-Useros[2,5]

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Different therapies are used for clinical treatment of SARS-CoV-2 infection, but little is known about their efficacy yet. Here we have screened existing drugs that have been shown safe to human use and are applied in a variety of diseases, to compare how they counteract SARS-CoV-2-induced cytopathic effect *in vitro.* Among the 72 drugs tested herein, only eight compounds including remdesivir and hydroxychloroquine had an IC_{50} below 25 µM or 10^2 IU/mL. Plitidepsin was the only clinically approved drug displaying nanomolar efficacy. Since the most effective antiviral treatments usually combine therapies that tackle the virus at different steps of the infection cycle, we also tested several drug combinations. Although no particular synergy was found, inhibiting combinations did not reduce antiviral activity and could reduce the potential emergence of resistant viruses. Antivirals prioritized here will require further evaluation in adequate animal models of COVID-19, to provide a solid evidence-driven rationale and inform forthcoming clinical trials.

O.4. **Cellular models for the study of SARS-CoV-2 pseudoviral entry.**

Jordana Muñoz-Basagoiti[1,*], Daniel Perez-Zsolt[1,*], Jordi Rodon[2,*], Marc Noguera-Julian[1,6], Roger Paredes, Lourdes Mateu, Carles Quiñones, Itziar Erkizia[1], Alfonso Valencia[3,4], Víctor Guallar[3,4], Jorge Carrillo[1], Julià Blanco[1,5,6], Joaquim Segalés[7,8], Bonaventura Clotet[1,5,6], Júlia Vergara-Alert[2,+], Nuria Izquierdo-Useros[1,5,+]

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[*]Equal contribution, [+] Dual senior authorship.

Background: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is causing a respiratory disease and has spread all over the world. Only remdesivir has proven efficacy in randomized controlled clinical trials, highlighting a urgent need to identify novel and effective therapeutic approaches to treat the coronavirus infectious disease 2019 (COVID-19). SARS-CoV-2 entry requires viral binding and spike protein activation via interaction with the cellular receptor ACE2 and the cellular protease TMPRSS2. However, when cellular proteases are not expressed in the plasma membrane, alternative mechanisms can favor viral internalization via endocytosis. It is essential to identify effective compounds tackling the different mechanisms of viral entry to help improve the clinical outcome.

Methods: An HIV-1 virus-like particle encoding the luciferase reporter gene and pseudotyped with the SARS-CoV-2 Spike protein was used to detect viral fusion on HEK-293T cells transfected with the cellular receptor ACE2 and with or without the serine protease TMPRSS2. The antiviral effect of clinically approved compounds that

potentially abrogate viral fusion with host cell membranes were tested *in vitro* by measuring the luminescence upon pseudoviral entry.

Results: By using this pseudoviral system, viral fusion on cells expressing TMPRSS2 could be blocked by
the protease inhibitor camostat, mimicking what has been described in pulmonary cells. Nevertheless, in cells lacking TMPRSS2 expression, chloroquine derivatives and cathepsin inhibitors were identified as the most promising candidates to impair viral fusion and block pseudoviral entry into renal cells expressing ACE2.

Conclusions: Our results point out that alternative routes govern SARS-CoV-2 viral entry, and these pathways vary depending on the cellular target. Thus, effective treatments may need to block both plasma membrane fusion and endosomal routes to fully achieve viral suppression.

O.5. **Previous inoculation of SARS-CoV-2 in hamsters protects against subsequent infection by D614-homologous or G614-heterologous SARS-CoV-2 strains.**

Marco Brustolin[1,*], Jordi Rodon[1,*], María Luisa Rodríguez de la Concepción[2], Carlos Ávila-Nieto[2], Guillermo Cantero[1], Marc Noguera-Julián[2,4] , Núria Roca[1], Nuria Izquierdo-Useros[2,3,+] Julià Blanco[2,3,4], Bonaventura Clotet[2,3,4], Jorge Carrillo[2], Júlia Vergara-Alert[1,+], Joaquim Segalés[5,6,+].

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The first evidence of SARS-CoV-2 re-infection in human was described on August 25th, 2020, in South Korea and subsequent cases were also found in different countries worldwide. Experimental studies on re-infection have been performed in non-human primate, transgenic mice expressing human angiotensin-converting enzyme 2 (hACE2) and cat. In all these models, animals challenged with SARS-CoV-2 develop protective immunity against re-challenge with the same viral strain. Despite these promising results, no data are currently available about the induction of protective immunity against a different viral strain. To address this question, we first challenged Golden Syrian hamsters using a viral strain isolated from a human patient in Spain (Cat/01), and 21-day post infection (dpi), animals were exposed to the same Cat/01 strain or to a different strain isolated from a human patient in the US (WA/01). Importantly, Cat/01 is characterized by the presence of a single point mutation in the position 614 of the Spike protein gene (D614G) that has been recently correlated with a higher transmission capacity. Animals were sacrificed at 2, 4, 7 dpi and 2 and 4 days post-reinoculation (dpre). Viral RNA levels, isolation of infectious virus and immunohistochemistry were performed in different target organs. In addition, we investigated the development of specific humoral response in these animals. Our results demonstrated that animals exposed for a first time to SARS-CoV-2 developed a protective but no sterilizing immune response against a second infection event. Loss of weight and interstitial pneumonia were recorded during the first week post-inoculation. Development of specific humoral response began at 7 dpi and was able to neutralize both viral strains. Upon re-challenge, viral RNA level and infectious viral loads decreased in all tested organs, especially those of the lower respiratory tract (trachea and lungs) where viral isolation was not possible. Isolation of infectious virus in re-infected animals was possible for both viral strains, but limited to nasal turbinate and/or oropharyngeal swabs collected at 2 dpre. These results suggest that, in the hamster model used, animals were protected against development of lesions in the respiratory tract and lack of infectious virus in lung upon reinfection. In contrast, infectious SARS-CoV-2 was still present, to low levels, at the upper respiratory tract. In conclusion, the infection/re-infection hamster model indicates that a primo-infection generates a protective but not sterilizing immune response against re-infection with homologous and heterologous viral strains.

O.6. **Membrane integration and topology of SARS-CoV-2 E (envelope) protein.**

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Coronavirus E protein is a small membrane protein found in the virus envelope. Different coronavirus E proteins share striking biochemical and functional similarities, but sequence conservation is limited. In this communication, we studied the E protein topology from the new SARS-CoV-2 virus both in microsomal membranes and in mammalian cells. Experimental data reveal that E protein is a single-spanning membrane protein with the N-terminus being translocated across the membrane, while the C-terminus is exposed to the cytoplasmic side (Ntlum/Ctcyt). We also challenged E protein topology by replacing charged amino acids close to the transmembrane segment, considered the fore most topological determinants according to the positive inside rule. The defined membrane protein topology of SARS-CoV-2 E protein may provide a useful framework to understand its interaction with other viral and host components and contribute to establish the basis to tackle the pathogenesis of SARS-CoV-2.

SESSION II

O.7. **Low zinc levels at clinical admission associates with poor outcomes in COVID-19.**

Marc Talló-Parra[*1], Marina Vogel-González[*2], Víctor Herrera-Fernández[2], Gemma Vilaró-Pérez[1], Miguel Chillón[3,4,5], Xavier Nogués[6], Silvia Gómez-Zorrilla[7], Inmaculada López-Montesinos[7], Judit Villar[7], Maria Luisa Sorli-Redó[7], Juan Pablo Horcajada[7], Natalia García-Giralt[6], Julio Pascual[8], Rubén Vicente[2¶], Robert Güerri-Fernández[6¶], Juana Díez[1¶]

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Background: Biomarkers to predict COVID-19 outcome early at infection are urgently needed to improve prognosis and treatment. Zinc balances immune responses and also has a proven direct antiviral action against some viruses. Importantly, zinc deficiency is a common condition in elderly and individuals with chronic diseases, two groups with more severe COVID-19 outcomes. We hypothesize that serum zinc content (SZC) influences COVID-19 disease progression and thus might represent a useful biomarker.

Methods: We run a retrospective observational study with 249 COVID-19 patients admitted in Hospital del Mar. We have studied COVID-19 severity and disease progression attending to SZC at admission. In parallel we have studied SARS-CoV2 replication in the Vero E6 cell line modifying zinc concentrations.

Findings: Our study demonstrates a correlation between serum zinc levels and COVID-19 outcome. Serum zinc levels lower than <50 µg/dl at admission correlated with worse clinical presentation, longer time to reach stability and higher mortality. Our *in vitro* results indicate that low zinc levels favor viral expansion in SARS-CoV2 infected cells.

Interpretation: SZC is a novel biomarker to predict COVID-19 outcome. We encourage performing randomized clinical trials to study zinc supplementation as potential prophylaxis and treatment with people at risk of zinc deficiency.

O.8. **COVID Viral Beacon: Fast SARS-COV-2 Data discovery.**

Claudia Vasallo, Dietmar Fernández, Mauricio Moldes, Babita Singh, Sabela de la Torre, Marta Ferri, Umuthan Uyan, Frederic Haziza and Jordi Rambla

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CRG Covid Viral Beacon is a tool to find SARS-CoV-2 variability at genomic, amino acid and motif level. It offers the possibility to (i) search in detail about the genomic variants, (ii) filter queries and find unique cases, (iii) filter data based on strain/country level variants, and (iv) explore associated metadata among other information. It has been developed as a branch of the GA4GH Beacon standard, as a special use case for testing and demonstration of new features in Beacon v2. We have analysed SARS-Cov-2 genomic variants for more than 150,000 samples covering more than 180 countries for an in-depth variant discovery. The Viral Beacon utilises data from different resources (European Nucleotide Archive, NCBI/SRA and GISAID), generated by different platforms (Oxford Nanopore, Illumina). Most available databases, such as GISAID, only provide consensus sequences which mask individual variations that exhibit a low proportion in the viral population of each individual. The Viral Beacon sheds some light on this matter, and it can help challenge hypothesis and unveil insights hidden in the vast amounts of

COVID-19 data. Utilising Viral Beacon features, we will present some use cases where researchers can quickly analyse both raw and consensus data to discover individual level changes, for instance, real-time tracing of variant evolution, interesting cases of low-frequency intra-host variants resulting in crucial changes at protein level etc. Moreover, this demonstration of Viral Beacon will also open the channel of communication with experts in the field to utilise Beacon specifications and API to design pathogen-specific Beacon platforms for other infectious diseases.

O.9. **Sentinel surveillance of SARS-CoV-2 in wastewater anticipates the occurrence of COVID-19 cases.**

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Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is responsible for the global pandemic coronavirus 2019 disease (named as COVID-19), which was declared a public health emergency worldwide by the WHO on 30 January 2020. The outbreak emerged on the 31st of December 2019 in Wuhan, China, but rapidly spread to other countries, causing a considerable number of cases and deaths globally. Unfortunately, Spain is one of the most affected countries worldwide. While COVID-19 is a respiratory disease, coronavirus can also be shed in feces and consequently reach sewage. Sentinel surveillance of SARS-CoV-2 in wastewater, also referred as wastewater-based epidemiology (WBE), has been suggested as a tool to support existing COVID-19 surveillance systems and therefore enabling rapid adoption of mitigation measures. Two large wastewater treatment plants were weekly analyzed during the first peak of the epidemics to track the occurrence of SARS-CoV-2 infections in Barcelona. Samples were

processed and analyzed by RT-qPCR using five different set of primers targeting the RNA-dependent RNA polymerase (RdRp) gene (IP2 and IP4 fragments), the envelope protein (E) gene (E fragment), and the nucleoprotein (N) gene (N1 and N2 fragments). Besides, analysis of archival samples from January 15 to March 4, 2020 were also performed. Unexpectedly, SARS-CoV-2 was present in Barcelona sewage in January 15, 41 days before the first case imported case of COVID-19 was reported. Similarly, the second wave of COVID-19 in the metropolitan area of Barcelona, could be anticipated 30 days before. Additionally, the effectiveness of the confinement was very well delineated by the generated WBE data, confirming its usefulness in pandemic situations.

O.10. **Does size matter? SARS-CoV-2 surveillance in diferent sized wastwater treatment plants**.

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Wastewater has become a potential surveillance tool for COVID-19 incidence regrowth in the frame of wastewater based-epidemiology. There is an urgent need on data on the recovery performance of viral concentration methods, developed and validated for nonenveloped viruses testing in wastewater samples. The aim of this study was to characterize the performance of the concentration methods used to quantify the occurrence of SARS-CoV-2 in wastewater samples as well as to evaluate the effect of WWTP size in early warning predictions.

Seventy-seven 24h-composite wastewater samples (70-80ml) were collected during July and August 2020, when the infection was present all over the studied areas, in 10 different sized wastewater treatment plants (WWTP) and concentrated using the Centricon® Plus-70 with a 30 kDa cutoff (CeUF) as well as by applying a rapid automatic

ultrafiltration device that uses 150 kDa ultrafiltration tips (Concentrating Pipette, CP-Select™) with. A selection of samples were concentrated in parallel by both methods in order to compare the performance of both methods and to characterize and validate the Concentrating Pipette procedure for SARS-CoV-2 surveillance in wastewater by amplifying N1 and N2 genes. Human adenoviruses (HAdV) and JC polyomavirus (JCPyV), indicators of the presence of human fecal contamination, were also quantified in these analyses.

No statistically significant differences were observed between both concentration methods (*p*-value 0.783). Mean recovery values using two surrogate viruses for spiking the samples (MS2 bacteriophage and Murine Hepatitis Virus, MHV) were 31.91% and 29.02% (max, 79.99 and 69.11; min, 7.82 and 6.35) for the Centricon Plus-70 device and 24.87% and 6.63% (max, 86.69 and 28.50; min 3.90 and 1.24) for the Concentrating Pipette respectively.

Concentrations of JCPyV were constant during the time-period analyzed and showed expected levels in wastewater, whereas concentrations of HAdV with some serotypes being transmitted via the respiratory pathway and some others by oral-fecal route, decreased in comparison to commonly detected concentrations probably as a result of the first COVID-19 lockdown during March-May of the same year and due to the measures adopted since then to avoid SARS-CoV-2 transmission. Mean values for SARS-CoV-2 in the largest WWTP, treating 1 million Hab.Eq., was 5.52E+04 GC/L whereas WWTP serving smaller municipalities (100.000 Hab.Eq.) presented mean values of 6.07E+03 GC/L. SARS-CoV-2 concentrations, within WWTPs which treated wastewater below 15.000 Hab.Eq., were under the limit of quantification of the technique (LOQ for N1, 9.66 gc/rx; LOQ for N2, 9.44 gc/rx). These results suggest that wastewater surveillance for SARS-CoV-2 might not be useful as early warning tool in WWTPs treating wastewater volumes lower than 50.000 Hab.Eq despite presenting similar incidence of COVID-19 per 100.000 inhabitants. The relation among incidences, WWTP size and SARS-CoV-2 concentration in wastewater is being analyzed with larger data bases.

O.11. **SARS-CoV-2 detection in clinical samples and urban sewage by target enrichment sequencing.**

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In the wake of SARS-CoV-2 pandemics, Next Generation Sequencing (NGS) applications has raised for the genetic characterization of clinical isolates from this virus. Apart from patient-focused investigations, wastewater-based epidemiology has proven to be a useful tool to complement clinical studies and to serve as an early warning tool for outbreak prevention. The use of NGS in wastewater could be the key for an in-depth study of the excreted virome, not only focusing on SARS-CoV-2 presence and typing, but also to detect other members of the same family.

With this aim, a 24-hours composite wastewater sample from March 2019, containing mean values of 3,92E3CG /ml of N1 and 2,71E3 CG/ml of N2 SARS-CoV-2 genes, was sequenced by applying specific viral NGS as well as target enrichment NGS that favours, through probe-capture, the sequencing of vertebrate viral families.

A full virome of the analysed sample was obtained showing no significant differences with sewage viromes previously described, with the exception of members of the *Coronaviridae* family detected after applying target enrichment NGS: One contig (235bp) of a Betacoronavirus 1, typed as HCoV-OC43, and 8 contigs (2020bp) identified as SARS-CoV-2, with 913bp mapped against the nucleoprotein. These SARS-CoV-2 sequences were compared with sequences obtained in the same period from clinical specimens from the same geographical area and with the same target enrichment technique. In this case, the 90.3-91.1% of SARS-CoV-2 genome was obtained. The results

showed that NGS enrichment-based protocols might serve to describe the occurrence of members of the *Coronaviridae* family in clinical samples as well as in the excreted virome present in wastewater samples.

However, for a successful detection of SARS-CoV-2 and other coronaviruses in complex matrices such as sewage, further efforts should be directed towards designing adequate capture panels that would favour coronavirus diversity studies in a single sequencing assay.

O.12. **Insertion and folding of SARS-CoV-2 M (membrane) protein.**

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The glycosylated membrane (M) protein of SARS-CoV2 is the main structural component of the viral envelope and plays a central role in virus morphogenesis and assembly via its interactions with other viral proteins. In this communication, we studied the M protein membrane topology applying different approaches: first, experimental data derived from a topological dual-reporter assay in *E. coli* showed that M protein has three membrane-spanning domains along its sequence.

This results in the N-terminus being translocated across the membrane whereas the C-terminus faces the citoplasmatic side, displaying an Ntlum-Ctcyt topology. We have also confirmed some of these results in eukaryotic microsomal membranes, and in mammalian cells, where we confirmed the cytoplasmic location of the C-terminus. In addition, molecular dynamics simulations provide critical insights into the folding of the transmembrane segments within DOPC (dioleoylphosphatidylcholine) bilayers. In the absence of a high resolution structural model for SARS-CoV-2 M protein, having the present topological and folding model is essential to search for both viral and cellular proteins that interact with this principal component of the virus envelope.

SESSION III

O.13. **The role of visual and olfactory cues in host plant selection by the insect vector *Bemisia tabaci* in mixed infections of *Tomato chlorosis virus* (ToCV) and *Tomato yellow leaf curl virus* (TYLCV).**

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Viruses are widespread in both natural and agricultural plant communities and can significantly alter diverse traits of their host plants that mediate key interactions with other organisms, inducing direct or indirect effects which generally lead to phenotypic changes. Among these alterations, the host selection process by insect vectors can be affected by changes on both visual cues, such as plant color and olfactory cues, such as plant volatiles, with attraction of vectors to the host plant being a beneficial strategy for the transmitted viruses. Furthermore, the frequent occurrence of mixed viral infections with more than one virus can theoretically modify the virus-host-vector relationships, and consequently the epidemiology of viral diseases. To explore the importance of visual and olfactory alterations associated with mixed infections, we focused in the host selection process within the pathosystem of *Tomato chlorosis virus* (ToCV, a crinivirus) and *Tomato yellow leaf curl virus* (TYLCV, a begomovirus), two unrelated viruses transmitted by the same whitefly *Bemisia tabaci*.

We first compared viral loads and symptoms induced on susceptible tomato plants both by single and mixed infections, finding a dynamic response with initial antagonism and milder symptoms at first stages, that later evolved towards synergism with exacerbation of TYLCV symptoms. Then, a series of choice assays were designed and performed under controlled conditions comparing mock, single and mix-infected plants to determine the influence in whitefly preference responses of visual and olfactory cues, using respectively common arenas and an olfactometer device. The different outcomes suggested that the presence of TYLCV in the mixed infections was a key element in the preference behavior of *B. tabaci*. In addition, our results were compatible with neutral

effects for the olfactory cues, while visual stimuli associated with a severe expression of TYLCV symptoms seemed to be more important in preference responses by enhancing the attractiveness to the whiteflies. The ecological relevance of these findings will be discussed.

O.14. **Double targeting of two key co-signaling molecules of NK and T cells by a cytomegalovirus-encoded CD48 homolog.**

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Cytomegaloviruses (CMVs) have developed highly efficient strategies to evade immunity and stablish persistence. Some of these strategies are based on genes originally stolen from their host, molded and functionally optimized through evolution in order to interfere with specific immune processes. CD48 is a cell surface protein of the immunoglobulin (Ig) superfamily. Via its N-terminal Ig domain, CD48 recognizes the cell surface receptor 2B4, triggering signal transduction events that regulate T lymphocyte and NK-cell cytotoxicity. Our group recently identified A43, a CMV-encoded CD48 homolog, which is capable to bind human 2B4 with exceptional higher affinity and slower dissociation kinetics than human CD48. A43 acts as a functional viral decoy receptor, masking the 2B4 receptor and impairing NK-cell cytotoxicity and IFN-? production through interfering with the immune synapse. Here, we present that this viral protein also interacts, via its N-terminal Ig domain, with human CD2, a T and NK-cell co-stimulatory molecule whose primary ligand is CD58. We have performed a series of structural-functional analysis of these molecular interactions using site-directed mutagenesis and modeling of the N-terminal Ig domain of A43 to understand the basis for this receptor unique binding. The nature of A43, being a soluble molecule with the potential to inhibit both 2B4- and CD2-mediated NK- and T cell-mediated responses, provides an excellent basis to develop new immunosuppressive biotherapeutics for pathologies such as autoimmune diseases.

O.15. **Role of cross-presenting dendritic cells in anti-PD-L1 immunotherapy of chronic viral infections.**

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Continuous antigen stimulation in chronic infections and cancer leads to persistent immune activation which results in the loss of effector CD8+ T cell functionality known as exhaustion. Current efforts to develop therapies largely rely on reversing the exhausted CD8+ T cell phenotype by blocking checkpoint inhibitory receptors such as PD-1 and its ligand PD-L1. However, the contribution of cross-presenting XCR1+ dendritic cells (DC) to the maintenance of CD8+ T cell function during exhaustion and their role in immunotherapeutic interventions remains unclear. Here we addressed this issue using the Lymphocytic Choriomeningitis Virus (LCMV) mouse model system. We found that in LCMV-chronically infected mice XCR1+ DC were less susceptible to infection and highly activated, suggesting that they actively participate in the

maintenance of an antiviral T cell response. Indeed, Flt3L-mediated *in vivo* expansion of XCR1+ DC resulted in an increase in virus-specific CD8+ T cell activity and improved viral control. Furthermore, therapeutic vaccination of infected mice targeting the LCMV nucleoprotein to XCR1+ DC had a similar effect. Upon PD-L1 checkpoint blockade, XCR1+ DC were not required for the proliferative burst of CXCR5+ PD1+ CD8+ T cells, but proved indispensable to sustain the functionality of exhausted CXCR5- PD1+ CD8+ T cell subsets. Together, this demonstrates that XCR1+ DC are crucial for the success of checkpoint inhibitor-based therapies, and thus increasing their functionality holds great promises for immunotherapeutic treatments of chronic infections and cancer.

O.16. **Evaluation of algorithm-assisted procedures for identification of polymerase slippage motifs in the genome of members of the Potyviridae family using FIMO and gkm-SVM.**

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Designing control strategies against plant viruses requires to understand the molecular basis underlying their replication and gene expression. Here we focus on the family *Potyviridae*, which includes many devastating plant viruses. The genome of potyvirids contains a large ORF and they follow a polyprotein expression strategy. However, expression of smaller out-of-frame ORFs can result after an additional mechanism of polymerase slippage occurring at conserved motifs in the P3 region of all potyvirids (PIPO), and in the P1 region of several potyviruses infecting sweet potato plants (PISPO).

We have designed and tested a pipeline using the tools FIMO (Find Individual Motif Occurrences) and gkm-SVM (gapped k-mers Support Vector Machine) to identify regions in the genome of potyvirids putatively prone to polymerase slippage. First, the MEME-suite for motif discovery was used to create sequence patterns based on already known polymerase slippage sites within P1 and P3. Next, FIMO was applied to search for occurrences in three sets of genomic viral sequences corresponding to: i) viruses infecting sweet potatoes; ii) viruses of the genus *Potyvirus*; and iii) viruses of the family

Potyviridae. Finally, the gkm-SVM classification algorithm was trained on the positive sequences previously obtained. When comparing the q-values of the crude occurrences of FIMO versus the classification scores for each sequence generated with gkm-SVM, it was confirmed that gkm-SVM was a required algorithm in the search for polymerase slippage motifs, providing results considerably more accurate than FIMO alone. Despite its lower computational costs, this confirmed that FIMO required a refinement algorithm trained to search for the desired patterns. As expected, our analysis showed that motifs in P3 presented higher scores than the motifs in P1. Overall, the combination of the two tools allowed us to perform accurate explorations for polymerase slippage motifs, representing the plasticity and evolutionary adaptability of these viruses.

O.17. **Identification of a gene product with RNA silencing suppression activity in the symptomless potyvirus *Sweet potato latent virus.***

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Sweet potato latent virus (SPLV) is a member of the aphid-transmitted genus *Potyvirus*, family *Potyviridae,* and as indicated by its name, causes symptomless infections in sweet potatoes. The genomic RNA of SPLV is a positive-sense single-stranded molecule of about 10 kb, encoding a large ORF translatable as a polyprotein proteolytically processed into at least 10 mature gene products. It also contains an additional embedded ORF named PIPO that yields a partially transframe gene product P3N-PIPO after polymerase slippage in a conserved motif found in the P3 region of all members of the family. Unlike other closely related potyviruses infecting the same host, such as *Sweet potato featherly mottle virus* (SPFMV) or *Sweet potato virus 2* (SPV2), SPLV lacks a second transframe gene product P1N-PISPO, again produced by a polymerase slippage event in the P1 protease encoding region. While in most potyviruses the RNA silencing suppression (RSS) function is provided by the HCPro protein located as the second gene product in the polyprotein, previous and ongoing work in our laboratory is showing that for sweet potato potyviruses like SPFMV and SPV2 the RSS activity is shifted to P1 and/or P1N-PISPO. To determine which proteins are involved in the RSS

activity of SPLV, we focused on P1, HCPro and the combination of the two P1+HCPro, both in cis and trans, and tested whether they exhibited activity in co-agroinfiltration experiments, transiently expressing the aforementioned constructs together with a reporter GFP in *Nicotiana benthamiana* leaves. Our results showed that HCPro conferred a strong RSS activity, while P1 did not act as a suppressor under our experimental conditions. Understanding the peculiarities of the RSS function in SPLV can provide more insights about the complex pathogenicity of potyviruses infecting sweet potatoes.

O.18. **Detection of Norovirus in saliva samples from cases and asymptomatic subjects involved in gastroenteritis outbreaks**

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Norovirus infections are a leading cause of acute gastroenteritis outbreaks worldwide and across all age groups, with 2 main genogroups (GI and GII) infecting humans. Infections are mainly transmitted through the fecal-oral route, although transmission through vomit is also well documented. The aim of our study was to investigate the occurrence of norovirus in saliva samples from individuals involved in outbreaks of gastroenteritis, in correlation with the virus strain, the occurrence of symptoms and virus shedding in stool, and the secretor status of the individual. Epidemiological and

clinical information was gathered from norovirus outbreaks occurring in closed and semi-closed institutions in Catalonia, Spain, during 2017-2018, and stool and saliva samples were collected from affected and exposed resident individuals and workers. Presence of norovirus in feces and saliva samples was assessed by real time RTqPCR. Saliva samples were also used to determine individual secretor status by FUT2 genotyping. A total of 385 saliva samples were collected from 355 individuals from 25 outbreaks. 83,7% of individuals also provided a stool sample. For GII infections, norovirus was detected in 18,37% of saliva samples from symptomatic individuals and 3,2% of asymptomatic individuals. Positivity in saliva occurred in both secretors and nonsecretors. None of the individuals infected by norovirus GI was positive for the virus in saliva. Individuals which were positive in saliva showed higher levels of virus shedding in stool than individuals which were saliva negative (meant Cq values 22,45±5,02 versus 24,89±4,91, p=0.019). The detection of norovirus in saliva raises the possibility of oral-to-oral norovirus transmission during the symptomatic phase, and although to a lesser extent, even in cases of asymptomatic infections.

SESSION IV

O.19. **Viruses in motion: a close look at virus maturation through cryo-electron microscopy**

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Dynamic virus-like particles have great potential as protein-based nanomaterials that allow the development of applications which require Mobile as opposed to static structures such as, for example, nanomachines with controlled motion. We are studying the maturation of such a VLP by analysing the changes in the structure of the particles by techniques including near-atomic resolution cryo-electron microscopy (cryo-EM) and SAXS. Nudaurelia capensis omega virus (NωV) is an insect virus with a pH-controlled maturation mechanism that involves a precise autocatalytic cleavage within the capsid protein. This cleavage converts the full-length protein (α) to the β and γ peptides and is accompanied by a dramatic decrease in the diameter of the particles from 48 to 40 nm and the ability of the capsids to lyse liposomes. The ability to control the movement of the subunits by changing pH has potential uses in nanobiotechnology. We have used plant based transient expression of the full-length NωV coat protein (α) to produce procapsids and have shown that reduction in pH results in cleavage of the α protein and compaction to mature particles. Furthermore, we have shown that these mature particles are able to break lipid bilayers of liposomes, confirming the biological relevance of the plant-produced material. In order to generate a model of virus maturation we are performing near-atomic resolution cryo-EM analysis of the process of capsid maturation

of NωV VLPs which occurs as a result of pH change. With these cryo-EM models we can resolve the large conformational changes in the structure of the particles that accompany maturation; this will allow both a deeper understanding of the process of virus maturation and suggest how to use the process for potential applications in synthetic biology and viral nanotechnology.

O.20. **Nanostructural characterization of influenza virus-like particles with super resolution microscopy.**

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As we unfortunately know, respiratory viruses present risks against human beings, with influenza being one of the most common. The effects of influenza virus in our society are remarkable, being the cause of at least 5 pandemics between the XX and the XXI century, causing more than 60 million deaths[1] and creating the need of seasonal yearly vaccination.

The lack of a universal vaccine boosted the industry to develop novel methods to produce them. Currently, the industry is starting to focus attention on vaccines based on recombinant proteins that can be produced in a safe environment with the DNA of the virus but lacking risks. One type of recombinant vaccine we are focusing on is virus-like particles (VLP), structures that mimic the viral particles without the genetic material, selfassembling and releasing spontaneously from recombinant viral proteins expressed on cells[2]. VLPs contain repetitive, high density and natural motives from the virus showing also promising results as nanovectors since resemble the infectivity machinery

of the virus[3]; they are so promising that there are 13 preclinical candidates for COVID-19 vaccine[4].

Despite the interest of VLPs, there is a lack of understanding of how the influenza VLP structure distribute the three main viral envelope proteins along the surface, due to the small size of influenza (100 nm). To overcome this limitation, single molecule localization microscopy (SMLM), a super-resolution technique, has burst in the last years since allows the analysis of the spatial arrangements of molecules in the nanoscale by the localization of fluorophores[5].

Here, we study with SMLM the spatial distribution of each of the three proteins of influenza VLP in the nanoscale. By using DNA-PAINT[6], a SMLM technique that allows us the quantification of target structures, we characterized the differential expression of the three transfected proteins of the VLP on the membrane of mammalian cells. We could identify a heterogeneous expression of the three proteins expressed within the transfected cell population, not being constant for each protein. We also imaged the population of the VLPs produced, measuring semiquantitatively the amount of each protein intra-and interparticle and characterizing the distribution of them inside the VLP. Further, we could detect a huge heterogeneity of the amount and distribution of proteins; not only they are not evenly distributed but the amount of proteins per VLP is very variable. To conclude, the heterogeneity detected by SMLM in the protein expression of VLPs could be crucial to understand the hits and miss of VLPs for clinical purposes.

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O.21. **Serum and intrahepatic HBV markers and HBV-specific CD8 T cell responses after nucleos(t)ide analog therapy discontinuation in HBeAg-negative chronic hepatitis B patients.**

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Background and Aims: Previous studies have reported that up to 20% of patients with chronic hepatitis B (CHB) may achieve functional cure (HBsAg loss) after nucleos(t)ide analog (NA) treatment withdrawal. CD8 T cells play an important role in the immune control of HBV infection. The objective of this study was to analyze HBV-specific CD8 responses in parallel with peripheral and intrahepatic virological markers after NUC discontinuation in patients with HBeAg-negative (HBeAg-) CHB.

Methods: Twenty-seven HBeAg- CHB patients with complete viral suppression (>3 years) and without cirrhosis were prospectively studied. A liver biopsy was taken at the time of treatment withdrawal (baseline). PBMC and serum samples were collected at baseline and various time-points during follow-up. Intrahepatic HBV-DNA (iHBV-DNA), covalently closed circular DNA (cccDNA) and serum HBV-DNA, HBsAg, core-related antigen (HBcrAg) and pregenomic RNA (pgRNA) levels were determined. HBV-specific T cell responses (IFNy, TNF and CD107a) were analyzed by multiparametric flow cytometry

after *in vitro* expansion in the presence of overlapping peptides (OLP) spanning core, envelope and polymerase.

Results: After a median follow-up of 34 months (IQR 26-37), 22 (81%) patients remain off-therapy, with 8 (30% of the total cohort) losing HBsAg; whilst 5 (19%) required NA reintroduction due to relapse. Although all patients were iHBV-DNA and cccDNA positive at baseline, only 41% and 48% had detectable serum pgRNA and HBcrAg, respectively. Baseline HBsAg levels correlated significantly with iHBV-DNA ($r = 0.7$, $p < 0.0001$) and both markers were lower in patients who lost HBsAg ($p < 0.001$). Baseline intrahepatic (iHBV-DNA, cccDNA) or serum (HBsAg, HBcrAg or pgRNA) viral markers did not show any association with peripheral CD8 T cell responses. Importantly, degranulating CD8 T cells (CD107a+) or those co-producing IFNy and TNF in response to stimulation with core OLP were significantly higher ($p = 0.05$ and $p = 0.039$, respectively) at baseline in patients remaining off-therapy compared to those requiring NA reintroduction. Interestingly, the enhanced frequency of CD8 T cells co-producing IFNy and TNF persisted up to 1 year of follow-up ($p = 0.009$). Notably, CD8 T cell responses to polymerase or envelope failed to associate with outcome as robustly as those against core.

Conclusions: NA discontinuation is feasible in a high proportion of HBeAg- patients, particularly in those with low HBsAg levels. Higher frequencies of CD8 T cells with cytotoxic and non-cytolytic anti-HBV (core) reactivity are detectable at baseline in those patients who maintain viral control after therapy withdrawal. These data support HBsAg levels and HBV-specific CD8 T cell frequencies as correlates of HBV control off-therapy, requiring validation in larger studies.

O.22. **HDAg abundance in hepatitis delta patients before liver transplantation is associated with HDV infection of the graft.**

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Background & aim: Hepatitis delta is a chronic inflammatory liver disease caused by the hepatitis delta virus (HDV). HDV is a small defective virus that needs hepatitis B virus (HBV) to propagate. As it lacks its own envelope proteins, HDV requires the HBV surface antigen (HBsAg) to ensure its assembly and infectivity. Hepatitis delta is the most severe form of viral hepatitis, being liver transplantation (LT) the only therapeutic option for patients with decompensated cirrhosis or hepatocellular carcinoma infected by HDV. LT is a valuable model to study some aspects of the HDV replication cycle. The aim of this study was to characterize the presence of viral antigens in liver biopsies from HBV/HDV co-infected patients undergoing LT.

Materials & methods: Eighteen VHB/VHD co-infected LT recipients were included in the study. HBsAg, hepatitis B core antigen (HBcAg) and hepatitis delta antigen (HDAg) were detected in paraffin-embedded liver biopsies by immunofluorescence and confocal microscopy. Liver biopsies obtained during the reperfusion of the graft were used as negative controls.

Results: HDAg was detected in 76% of liver biopsies obtained before LT (explants). The median percentage of HDAg+ hepatocytes was 6% (range 5-20%) in 4 patients and < 0.1% in the rest of patients. The presence of HDAg was predominantly nuclear. Clusters of HDAg+ hepatocytes were found in liver biopsies with a higher abundance of HDAg (> 5%). Although HBsAg was detected in 12 of 17 liver biopsies before LT, co-expression of HBsAg and HDAg in the same hepatocyte was a rare event (< 0.1%). Furthermore, HBcAg was not observed in any of the liver explants. All reperfusion biopsies were negative for viral antigens. Neither HBsAg nor HBcAg were detected in liver biopsies obtained after LT (n= 15). In contrast, HDAg was present in post-LT liver biopsies: 3 days (P1, 4%), 1 month (P2, ≤0.1%), 5 months (P3, 2%) and at several time points between 2 days and 3 months (P4, range 0.1-1%). Interestingly, patients with detectable HDV in post-LT biopsies showed a high proportion of HDV-infected hepatocytes in the corresponding explant sample.

Conclusion: Graft infection by HDV is a frequent event in patients with a high expression of HDAg in the explant. Low HBsAg expression levels in liver explants suggest that minimal amounts of HBsAg are needed for HDV virion assembly and further graft

infection. Further studies with more sensitive techniques are needed to solve this question.