XX Jornada de Virologia – Virology meeting 2021

3rd Symposium on Coronavirus Research

Organització: Secció de Virologia de la SCB

Sala Prat de la Riba/ Virtual

5 de novembre de 2021

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

Nuria Izquierdo

Juan José López-Moya

Andreas Meyerhans

Sofia Pérez del Pulgar

Josep Quer

Amb el suport de:

XX Jornada de Virologia – Virology meeting 2021/3rd Research Symposium on Coronavirus

9:00 h BENVINGUDA/WELCOME: Núria Busquets

9:05 - 9:35 h (20 + 10')

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

OPENING LECTURE: Pablo Gastaminza (CNB-CSIC): **The CNB Antiviral Screening Platform: an initiative to fight viral infections.**

09:35 - 11:00 h

Oral presentations/Flash talks

O.1. **Viral factors and host response in chronic HDV infection.** (10' + 5')

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

O.2. **Viral manipulation of the PD-1/PD-L1 axis by molecular mimicry.** (10' + 5')

Francesc Poblador. Immunology Unit, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, University of Barcelona, Barcelona, Spain.

O.3. **The temporal dynamics of spleen architecture disruption influence infection outcome.** (10' + 5')

Valentina Casella. Infection Biology Laboratory, Department of Experimental and Health Sciences (DCEXS), Universitat Pompeu Fabra, Barcelona, Spain.

O.4. **IRF7 expression regulates HIV-1 latency reversal by Janus Kinase 2 Inhibitors independent of Cytokine signalling blockade.** (10' + 5')

Ifeanyi Ezeonwumelu. AIDS Research Institute – IrsiCaixa and Health Research Institute Germans Trias i Pujol (IGTP), Hospital Germans Trias i Pujol, Universitat Autònoma de Barcelona, Badalona, Spain.

O.5. **ASFV-specific PBMC transcriptional signature from BA71ΔCD2-immune pigs reveals a role of inflammatory macrophages in protection.** (10' + 5')

Uxía Alonso. IRTA, Centre de Recerca en Sanitat Animal (CReSA, IRTA), Campus de la Universitat Autònoma de Barcelona, Bellaterra 08193, Spain.

O.6. **Single-cell RNA-sequencing of lymph node cells from ASF-immune pigs uncovers a protective role of cytotoxic CD8 T cells**. (3' + 2')

Laia Bosch-Camós. IRTA, Centre de Recerca en Sanitat Animal (CReSA, IRTA), Campus de la Universitat Autònoma de Barcelona, Bellaterra 08193, Spain.

💻 O.7. **Lung RNA-Seq unravel chicken genes potentially involved in resistance to avian influenza virus infection.** (3' + 2')

Albert Perlas. IRTA, Centre de Recerca en Sanitat Animal (CReSA, IRTA), Campus de la Universitat Autònoma de Barcelona, Bellaterra 08193, Spain.

11:00 - 11:20 h

COFFEE-BREAK

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

SESSION II: Chairs: Silvia Bofill/ Juanjo López Moya

INVITED SPEAKER: Mari Paz Sánchez-Seco (ISCIII): **Emerging arboviruses in Spain**

11:50 - 13:05 h

Oral presentations/Flash talks

O.8. **Hotspot of Crimean-Congo Hemorrhagic Fever Virus Seropositivity in Wildlife, northeastern Spain.** (3' + 2')

Laura Carrera. Wildlife Conservation Medicine Research Group (WildCoM), Departament de Medicina i Cirurgia Animals, Universitat, Autònoma de Barcelona, 08193 Bellaterra, Spain.

O.9. **Virus-induced reprogramming of translation landscape in mosquito cells.** (10' + 5')

Marc Talló-Parra. Molecular Virology group, Department of Experimental and Health Sciences, Universitat Pompeu Fabra, 08003 Barcelona, Spain.

O.10. **Elucidating the viral RNA modification landscape of Chikungunya virus.** (3' + 2')

Belinda Baquero-Perez. Molecular Virology group, Department of Experimental and Health Sciences, Universitat Pompeu Fabra, 08003 Barcelona, Spain.

O.11. **Rift Valley fever phlebovirus distribution and transmission in *Culex pipiens* and *Aedes albopictus* mosquitoes.** (3' + 2')

Karen Yautibug. IRTA, Centre de Recerca en Sanitat Animal (CReSA, IRTA), Campus de la Universitat Autònoma de Barcelona, Bellaterra 08193, Spain.

O.12. **The genomes of the Rosaceae plants contain a diverse array of endogenous pararetrovirus of different genera.** (10' + 5')

Carlos de Tomás. Center for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Barcelona, Spain.

O.13. **Production of flexuous filamentous virus-like particles (VLPs) of different sweet potato viruses.** (10' + 5')

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

O.14. **NGS Techniques Reveal a High Diversity of RNA Viral Pathogens and Papillomaviruses in Fresh Produce and Irrigation Water.** (10’ + 5’)

Marta Itarte. Laboratory of Viruses Contaminants of Water and Food. Department of Genetics, Microbiology and Statistics, Faculty of Biology, University of Barcelona. Barcelona (Spain).

13:05 - 14:00 h

DINAR / LUNCH

3rd Research Symposium on Coronavirus

14:00 - 14:50 h (5' each (20’) + 30’)

SESSION III: Chairs: Andreas Meyerhans /Ana Angulo

ROUD TABLE ON SARS-CoV-2 VACCINES.

Representatives of three academic consortia and the company Hipra that are all developing vaccines against SARS-CoV-2 in Spain, will briefly introduce their vaccine strategies, and discuss vaccine challenges and perspectives to combat viral pandemics.

INVITED SPEAKERS:

- Isabel Sola (CNB-CSIC)
- Toni Prenafeta (Hipra)
- Montse Plana (IDIBAPS)
- Jorge Carrillo (IRSI-Caixa)

14:50 - 15:55 h

SESSION IV: Chairs: Jordi Argilaguet / Andreas Meyerhans

Oral presentations/Flash talks

O15. **SARS-CoV-2 interaction with Siglec-1 mediates *trans*-infection by dendritic cells.** (10’ + 5’)

Daniel Perez-Zsolt. IrsiCaixa, Badalona, Spain.

O.16. **SARS-CoV-2 impact on maternal and neonate health, cytokine profile and IgG transplacental transfer.** (10’ + 5’)

Rocío Rubio. ISGlobal, Universitat de Barcelona, Spain

O.17. **Chronological brain lesions after SARS-CoV-2 infection in hACE2 transgenic mice.** (10’ + 5’)

Carlos López-Figueroa. IRTA, Centre de Recerca en Sanitat Animal (CReSA, IRTA), Campus de la Universitat Autònoma de Barcelona, Bellaterra 08193, Spain.

💻 O.18. **Topology characterization of the SARS-CoV-2 nsp4 protein in biological membranes.** (10’ + 5’)

Laura Gadea-Salom. Department of Biochemistry and Molecular Biology, Institute for Biotechnology and Biomedicine BioTecMed, University of Valencia, Dr. Moliner 50, 46100 Burjassot, Spain.

💻 O.19. **Repurposing drugs against SARS-CoV-2.** (3' + 2')

José Pedro Cerón. Reconocimiento y Encapsulación Molecular, Universidad Católica de Murcia, Campus los Jerónimos, 30107, Spain.

16:00 - 16:35 h (25 + 10')

SESSION V: Chairs: Nuria Izquierdo-Useros

💻 INVITED SPEAKER: Akiko Iwasaki (HHMI): Immune responses to SARS-CoV-2

16:35 - 16:45 h

BREAK

16:45 - 17:55 h

SESSION VI: Chairs: Nuria Izquierdo-Useros /Susana Guix

Oral presentations/Flash talks

O.20. **A large SARS-CoV-2 outbreak in a long-term care facility: lessons learned from a molecular epidemiological analysis.** (10' + 5')

Antoni E. Bordoy. Microbiology Department, Laboratori Clínic Metropolitana Nord, Hospital Universitari Germans Trias i Pujol, Institut d'Investigació en Ciències de la Salut Germans Trias i Pujol (IGTP), Badalona, Barcelona, Spain. CIBER in Epidemiology and Public Health (CIBERESP).

O.21. **Study of deletions in SARS-CoV-2 Spike protein of the most widespread variants in the consecutive pandemic waves in Barcelona.** (10' + 5')

Carolina Campos. Vall d'Hebron Institut de Recerca (VHIR), Malalties Hepàtiques-Hepatitis Virals - Vall d'Hebron Barcelona Hospital Campus.

O.22. **Monitoring natural SARS-CoV-2 infection in lions (*Panthera leo*) at Barcelona Zoo: viral dynamics and host responses.** (10' + 5')

Jordi Rodon. IRTA, Centre de Recerca en Sanitat Animal (CReSA, IRTA), Campus de la Universitat Autònoma de Barcelona, Bellaterra 08193, Spain.

O.23. **Dynamics of SARS-CoV-2 Alpha (B.1.1.7) variant spread: the wastewater surveillance approach.** (10' + 5')

Albert Carcereny. Enteric Virus Laboratory, Section of Microbiology, Virology and Biotechnology, Department of Genetics, Microbiology and Statistics, School of Biology, University of Barcelona. Enteric Virus Laboratory, Institute of Nutrition and Food Safety (INSA), University of Barcelona.

O.24. **Cetylpyridinium chloride mouthwashes to reduce the shedding of viable SARS-CoV-2** (3' + 2')

J. Muñoz-Basagoiti. IrsiCaixa AIDS Research Institute, 08916, Badalona, Spain.

O.25. **Reduced detection of Delta and other VOCs by antigen-detecting diagnostic tests** (3’ + 2’)

Dàlia Raïch-Regué. IrsiCaixa AIDS Research Institute, 08916, Badalona, Spain.

18:00 h Summary of the *XX Jornada de Virologia* – Virology meeting 2021/3rd Research Symposium on Coronavirus and **best presentation award**.

(Presentacions en remot/on-line presentations)

INVITED SPEAKERS' ABSTRACTS

OPENING LECTURE

The CNB Antiviral Screening Platform: an initiative to fight viral infections.

Pablo Gastaminza

National Center of Biotechnology (CNB-CSIC), Madrid, Spain

The current pandemic is having devastating medical and economic consequences worldwide despite the rapid generation of highly effective vaccines. One of the important lessons derived from this pandemic is the lack of preparedness to fight new pathogens that are expected to emerge in the coming decades. This puts an enormous pressure on society in order to get ready to fight future pandemics with proper chemical, biological and biotechnological tools, including effective new antivirals. In response to this need the CNB antiviral screening platform was created in March 2020. Our mission is to stablish a long-term infrastructure that allows the identification, optimization and characterization of antivirals against human pathogenic viruses, with special interest in those with the potential to produce a pandemic. To do so, we promoted a nationwide collaborative network giving us access to thousands of compounds that we screened in miniaturized cell culture infection systems. On one hand, I will explain the pipeline we have established to identify new antiviral molecules and describe in more detail few examples of molecules with antiviral activity against SARS-CoV-2 infection. On the other hand, I will briefly discuss our resources and capabilities to apply this antiviral search efforts to other virus infections.

KEYNOTE LECTURES

Emerging arboviruses in Spain

Mari Paz Sánchez-Seco

Instituto de Salud Carlos III (ISCIII), , Madrid, Spain.

CCHFV is an emerging virus in Spain and other regions. It has been circulating in ticks in Spain at least since 2010 where *Hyalomma lusitanicum* ticks obtained from reed deer were collected and the genome of the virus could be amplified. However, the first case of CCHFV was prospectively detected in 2016, when a man was infected by a tick while walking in the field and during his stay at hospital a nurse taking care of him got infected. After that, cases have been detected before (2013 retrospectively) and after: 2018 (2 cases), 2020 (3 cases) and 2021 (2 cases). Apart from these 10 human cases, studies in animals had shown very high levels of seropositivity in a big area of the country and studies in ticks had shown that the virus is wide distributed in central and southwestern Spain. Moreover, a high genetic variability has been detected in our country. Genotypes III, IV and V have been detected in humans, being one of them a reassortant with segments M and L from genotype III and S from genotype V. In ticks, all these genotypes and genotype II had been described. Results obtained until now, shows a wide distribution of the virus in Spain and a high variability that indicates probable multiple introductions of the virus.

Immune responses to SARS-CoV-2

Akiko Iwasaki

Howard Hughes Medical Institute (HHMI), Yale University School of Medicine, USA

The clinical presentation of COVID-19 involves a broad range of symptoms and disease trajectories. Understanding the nature of the immune response that leads to recovery over severe disease is key to developing effective treatments for COVID-19. In this talk, I will discuss immune responses in COVID-19 patients with moderate and severe disease. I will compare viral load, immune phenotype and cytokines that are predictive of mortality, and

discuss signatures of cytokines and growth factors that associate with recovery vs. disease exacerbation. I will discuss impact of mutations of variants on vaccine-induced immunity, and the key adaptive immune players in clearance of primary infection and prevention of infection induced by vaccines. Finally, I will touch on long COVID disease pathogenesis and ongoing studies.

ORAL PRESENTATIONS/FLASH TALKS ABSTRACTS

O.1. **Viral factors and host response in chronic HDV infection.**

Thais Leonel (1), **Ester García-Pras** (1), Sergio Rodríguez-Tajes (1), Mireia García-López (1), Sabela Lens (1), Zoe Mariño (1), Yabetse G. Tessema (1), Françoise Berby (2), Barbara Testoni (2), Francisco Rodríguez-Frías (3), Fabien Zoulim (2), Xavier Forns (1), Sofía Pérez-del-Pulgar (1).

(1) Liver Unit, Hospital Clínic, University of Barcelona, IDIBAPS, CIBERehd, Barcelona, Spain.
(2) Cancer Research Center of Lyon (CRCL), University of Lyon, UMR_S1052, UCBL, INSERM, U1052, Lyon, France.
(3) Liver Pathology Unit, Department of Biochemistry and Microbiology, Hospital Universitari Vall d'Hebron, Universitat Autònoma de Barcelona, CIBERehd, Barcelona, Spain.

Background and aims: Chronic hepatitis delta is the most severe form of viral hepatitis and a risk factor for cirrhosis and hepatocellular carcinoma. Little is known about the pathobiology of HBV/HDV infection, due in part to limited experimental models to study HBV and HDV infection and the lack of studies using liver tissue samples from HBV/HDV infected patients. The aim of this study was to explore viral and host factors contributing to chronic hepatitis delta pathogenesis.
Methods: Thirty-eight anti-HDV positive patients were included in the study. Intrahepatic and serological HBV and HDV replication markers were determined in prospectively collected liver biopsies and serum samples. To investigate the host response towards HDV, Nanostring gene expression analysis was performed on 22 FFPE liver samples.
Results: The median age of the patients was 51 years; 65% were male, 71% had liver cirrhosis and 50% were on nucleos(t)ide analog (NA) treatment. All patients had detectable levels of HBsAg (1.8-4.4 Log IU/ml) and 94% were HBeAg negative. Serum HDV-RNA assessment revealed ongoing HDV infection in 26 (68%) patients (HDV+), whereas the remaining 12 (32%) had undetectable HDV-RNA (HDV-). The percentage of patients receiving NA therapy was similar between HDV+ and HDV- patients (46% and 58%, respectively). HDV viral load significantly correlated with intrahepatic levels

of HDV-RNA (r=0.78, p<0.0001) and with HBsAg (r=0.62, p<0.001). HBcrAg levels were significantly higher in HDV+ patients (p=0.001) and correlated positively with intrahepatic levels of HDV-RNA (r=0.76, p=0.0001). No differences in HBV-DNA and HBV-RNA between groups were observed. Only 3 (12%) HDV+ patients had circulating 3.5kb HBV-RNA (none of the HDV- patients). In contrast, 3.5 kb HBV-RNA in the liver was detected in 72% of HDV+ versus 43% of HDV- patients (p= 0.038). cccDNA levels were significantly lower in HDV+ patients (p=0.001), detected in 22% of HDV+ versus 86% of HDV-patients. Host response gene expression analysis revealed increased innate and adaptative responses in HDV+ patients. Genes involved in leukocyte recruitment, cytotoxicity, cell proliferation and cell death were significantly upregulated in HDV+ patients.

Conclusions: Our results suggest a dissociation between cccDNA levels and the intrahepatic levels of HBV-DNA and HBV-RNA, and HBcrAg. This could be due to the transcription of HBV-DNA integrated into the host genome, or to the presence of very low levels (less than 1 copy per 10.000 cells) of transcriptionally active cccDNA. HDV appears to elicit a proinflammatory and cytotoxic response, mediated by innate and adaptative cells. The overexpression of genes involved in cell proliferation and apoptosis may account for the increased risk of hepatocellular carcinoma in HDV+ patients.

O.2. **Viral manipulation of the PD-1/PD-L1 axis by molecular mimicry.**

Francesc Poblador[1], Pablo Martínez-Vicente[1], Judith Leitner[2], Peter Steinberger[2], Pablo Engel[1,3], and Ana Angulo[1,3].

[1]Immunology Unit, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, University of Barcelona, Barcelona, Spain. [2]Division of Immune Receptor and T-Cell Activation, Institute of Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria. [3]Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain

Viruses employ molecular mimicry as a way to subvert host immunity. In particular, herpesviruses, encode multiple genes implicated in immune responses hijacked from their host via horizontal gene transfer. Programmed cell death receptor-1 (PD-1) and its ligand PD-L1 are membrane receptors belonging to the immunoglobulin superfamily. PD-1 is expressed by activated T cells, and PD-L1 is constitutively present on both hematopoietic

and non-hematopoietic cells. Importantly, the PD-1/PD-L1 axis plays a pivotal role attenuating T-cell responses and regulating immune cell tolerance. Here we report the discovery of a γ-herpesvirus PD-L1 homolog (vPD-L1) with immunosuppressive properties. Phylogenetic analyses indicate that the viral capture of the PD-L1 gene leading to vPD-L1 occurred around 50 million years ago. vPD-L1 exhibits a close homology with its cellular counterpart, including a 94% amino acid identity in the N-terminal immunoglobulin domain involved in PD-1 interaction. Accordingly, we show that this viral homolog encodes a cell-surface glycosylated protein that directly interacts with host PD-1. Moreover, employing a fluorescence-based cellular assay, we demonstrate that vPDL-1 inhibits T-cell signaling. Thus, this viral molecule represents the first functional PDL-1 homolog found in a pathogen.

O.3. **The temporal dynamics of spleen architecture disruption influence infection outcome.**

Valentina Casella[1]*, Eva Domenjo-Vila[1], Mireia Pedragosa[1], Anna Esteve-Codina[2], Enric Vidal[4], Monica Perez[4], Jordi Argilaguet[1,4]* and Andreas Meyerhans[1,5]*.

[1]Infection Biology Laboratory, Department of Experimental and Health Sciences (DCEXS), Universitat Pompeu Fabra, Barcelona, Spain.

[2]CNAG-CRG, Center for Genomic Regulation (CRG), Barcelona Institute of Science and Technology, Barcelona, Spain.

[3]Universitat Pompeu Fabra, Barcelona, Spain

[4] IRTA, Centre de Recerca en Sanitat Animal (CReSA-IRTA-UAB), Campus de la Universitat Autònoma de Barcelona, Bellaterra, Spain.

[5]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.

*Corresponding authors

Spleen architecture is known to be critical for the coordination of a proper immune response against viral infections in mouse. In particular, an intact splenic marginal zone (MZ) is instrumental for the production of type I Interferon (IFN-I) after the infection and the subsequent establishment of an efficient adaptive antiviral response. The splenic MZ is

a complex anatomic compartment that separates the white pulp from the red pulp and comprises two macrophage populations: marginal zone macrophages (MZMs) and metallophilic marginal zone macrophages (MMMs). Using Lymphocytic Choriomeningitis Virus (LCMV) infection of mice as a model, we previously showed that MMMs are lost 5 days post-infection (p.i.) with chronic infection. However, MMMs are still present in acute infection, where they produce a second wave of IFN-I. Here, we characterize the mechanism behind the early disappearance of MMMs and its implications for the infection outcome. Immunofluorescence analysis showed differential disruption dynamics of the splenic architecture during acute and chronic LCMV infection. In particular, we could link the disappearance of MMMs in chronic infection starting from day 3 p.i. to the differential virus distribution. Moreover, the loss of MMMs was partially avoided by anti-CD8α antibody treatment at days -1 and 2 p.i. indicating that rapid killing of infected macrophages was mediated by antiviral CD8+ cells. To assess the consequences of this event, we depleted MMMs in LCMV acute-infected CD169-DTR transgenic mice, by administration of diphtheria toxin (DT) at day 3 p.i.. DT treatment dampened the induction of pro-inflammatory macrophages at day 6 p.i and LCMV-specific IFNg-producing CD8+ T cells at days 9-15 p.i, with a consequent increase in virus titres. Importantly, blockade of IFNAR demonstrated that this poly-functional role of MMMs was mediated by the second wave of IFN-I production. All these results demonstrate that the differential dynamics of spleen architecture disruption in acute versus chronic LCMV infection, determine differential IFN-I kinetics which accounts for divergent infection outcomes.

O.4. **IRF7 expression regulates HIV-1 latency reversal by Janus Kinase 2 Inhibitors independent of Cytokine signalling blockade.**

Ifeanyi Ezeonwumelu[1], Edurne Garcia-Vidal[1], Roger Badia[1], Lucia Gutierrez-Chamorro[1], Eudald Felip[1], Marta Massanella[1], Bruna Oriol-Tordera[1], Marta Ruiz-Riol[1], Eva Riveira-Muñoz[1] and Ester Ballana[1]*

[1] AIDS Research Institute – IrsiCaixa and Health Research Institute Germans Trias i Pujol (IGTP), Hospital Germans Trias i Pujol, Universitat Autònoma de Barcelona, Badalona, Spain

Background: The mechanisms governing HIV persistence remain to be fully elucidated. Understanding these mechanisms is key for developing successful strategies that aim to target and eliminate the viral reservoir, and ultimately cure HIV infection. Increasing interest has focused on the development of strategies that target the immune response to HIV latent infection, including modulation of the JAK/STAT pathway, which plays a crucial role in mediating innate immune responses. Here, we identified a subclass of Janus kinase inhibitors (JAKinibs) as novel latency reactivation agents, exerting their function through modulation of IRF7 expression.

Methods: Non-clonal GFP-expressing cellular models of HIV-1 latency were used to evaluate the HIV-1 reactivation capacity of a panel of JAKinibs. The *ex vivo* effect of JAKinibs on immune activation and CD4+ T-cell subsets in uninfected donors and ART-suppressed HIV+ subjects was determined by flow cytometry. Latency-reversal was measured by determining HIV-1 RNA in the supernatant using nested PCR. Transcriptomic profiles were analyzed by RNA-Seq, and qPCR and Western blotting were used to validate gene and protein expression.

Results: Latency reactivation capacity of JAKinibs with different JAK1/2 selectivities was evaluated in cellular models of HIV-1 latency. A subclass of selective JAK2inibs, including fedratinib, consistently reversed HIV-1 latency in all tested *in vitro* models (2-fold increase, $p<0.001$) and also in CD4+ T cells of HIV infected subjects *ex vivo*. No significant changes in CD4+ T cells´ immune activation were observed upon treatment with fedratinib, although with a higher proportion of effector memory and central memory CD4+T populations. Whole transcriptomic data indicated a significant differential expression of IRF7 upon fedratinib treatment (6-fold increase, $p<0.001$), despite the blockade of the JAK/STAT signalling pathway and downregulation of proinflammatory cytokines and chemokines. Interestingly, latency reversal by JAK2inibs positively correlated with IRF7 expression (Pearson $r=0.86$, $p=0.006$) and knockdown of IRF7 by siRNA limited HIV reactivation by fedratinib, thus, pointing towards a new signalling pathway governing HIV-1 latency reactivation.

Conclusion: Selective JAK2 inhibitors showed strong HIV-1 latency reversal potential across all models of HIV latency. Our findings indicate that IRF7 could be an important modulator of HIV-1 latency and might play a crucial role in HIV-1 eradication strategies.

O.5. **ASFV-specific PBMC transcriptional signature from BA71ΔCD2-immune pigs reveals a role of inflammatory macrophages in protection.**

Uxía Alonso [1,2◊], Laia Bosch-Camós [1,2◊], Beatriz Martin [3], Marta Muñoz [1,2], Anna Barceló [1,2], María J. Navas [1,2], Chia-Yu Chang [1,2], Enric Vidal [1,2], Sonia Pina-Pedrero [1,2], Francesc Accensi[4], Anna Esteve-Codina [3], Jordi Argilaguet [1,2]*, Fernando Rodríguez [1,2]*.

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2. OIE Collaborating Centre for the Research and Control of Emerging and Re-Emerging Swine Diseases in Europe (IRTA-CReSA), Bellaterra 08193, Barcelona, Spain
3. CNAG-CRG, Center for Genomic Regulation (CRG), Barcelona Institute of Science and Technology & Universitat Pompeu Fabra, Barcelona 08003, Spain
4. UAB, Centre de Recerca en Sanitat Animal (CReSA, IRTA-UAB), Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

◊ *Both authors contributed equally to this work.

African swine fever (ASF) pandemics is currently causing enormous economic losses to swine industry worldwide. Lack of vaccines hampers its control, and the insufficient knowledge regarding the immunological mechanisms underlying protection hinders rational vaccine design. Macrophages are the main target cells of ASF virus (ASFV), which results in disruption of antiviral immune responses and make them central players in ASF pathogenesis. Virus-specific CD8+ T cells play a key role in ASF protection, but the underlying mechanisms remain elusive. Live attenuated viruses (LAVs) induce a broad protective immunity in pigs, thus becoming useful tools to analyze ASFV-specific immune responses. We previously generated a LAV vaccine prototype consisting of a deletion mutant lacking CD2v, namely BA71ΔCD2, which confers protection against homologous and heterologous challenge infection. To better characterize ASFV-specific cellular responses, here we performed RNA-seq analysis to obtain the transcriptomic signature of PBMC from BA71ΔCD2 immunized animals after specific *in vitro* stimulation with ASFV. Compared to non-immunized pigs, cells from immunized animals showed an upregulation of genes corresponding to a robust Th1 response. Interestingly, concomitant with this adaptive immune response, we also distinguished an innate immune response characterized by the expression of macrophage-related inflammatory genes. Further

analyses by flow cytometry demonstrated that IFNG signaling by polyfunctional CD4+CD8+ memory T cells is necessary to induce the production of TNF by myeloid cells. We hypothesize that early activation of macrophages mediated by vaccine-induced ASFV-specific T cells contributes to the protection afforded against virulent ASFV.

O.6. **Single-cell RNA-sequencing of lymph node cells from ASF-immune pigs uncovers a protective role of cytotoxic CD8 T cells**.

Laia Bosch-Camós [1,2], Uxía Alonso [1,2], María J. Navas [1,2], Marta Muñoz [1,2], Chia-Yu Chang [1,2], Francesc Accensi [3], Anna Esteve-Codina [4], Fernando Rodríguez [1,2]*, Jordi Argilaguet [1,2]*

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4. CNAG-CRG, Center for Genomic Regulation (CRG), Barcelona Institute of Science and Technology & Universitat Pompeu Fabra, Barcelona 08003, Spain

* Corresponding authors.

African swine fever (ASF) is currently the number one threat for the swine industry. No vaccines nor treatments are commercially available against ASF virus (ASFV), being live attenuated viruses (LAV) the most advanced vaccine prototypes. Although biosafety concerns still hamper their implementation in the field, the experimental use of LAV are an essential tool to gain knowledge on the processes involved in protective immunity against ASF. Indeed, previous studies using attenuated strains have shown a major role of CD8 T cells in ASF immunity, as their depletion *in vivo* results in abrogation of protection. Taking advantage of a LAV previously generated in our lab which confers homologous and heterologous protection, namely BA71ΔCD2, we intend to elucidate the ASFV-specific cellular responses underlying ASF immunity. Previous RNA-seq and flow cytometry data from our group revealed a recall Th1 response in PBMC from BA71ΔCD2-immunized animals, concomitant with a myeloid-mediated inflammatory response. Here we aimed to validate and expand these results by performing single-cell RNA-sequencing (scRNA-seq) in submandibular lymph node cells. Samples from either a non-immunized or a BA71ΔCD2-

immunized animal were *in vitro* stimulated with ASFV and their cell transcriptomic signatures were compared. This approach uncovered cell clusters that were only present or overrepresented in the immunized animal. scRNA-seq data validated the induction of a rapid inflammatory response characterized by the upregulation of interferon-stimulated genes and the Th1 chemokine CXCL10 in several cell subsets. Importantly, the analysis also revealed a potent cytotoxic response mediated by CD8 T cells, which was further validated by flow cytometry analysis of perforin in T cells.

Overall, scRNA-seq allowed deciphering the complex protective immune response against ASFV infection, demonstrating a major role of an early Th1-mediated inflammatory response concomitant with a rapid expansion of cytotoxic CD8 T cells. These results represent a step forward in the understanding of ASF immunology and provide important clues that might help in the rational development of future vaccines.

O.7. **Lung RNA-Seq unravel chicken genes potentially involved in resistance to avian influenza virus infection.**

Albert Perlas[1,2*], Jordi Argilaguet[1], Kateri Bertran[1], Raúl Sánchez-González[1,2], Miquel Nofrarías[1], Rosa M. Valle[1], Antonio Ramis[1,2], Martí Cortey[2 †], Natàlia Majó[1,2 †].

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Highly pathogenic avian influenza viruses (HPAIV) cause severe systemic disease and high mortality rates in chickens, with a huge economic impact in the poultry sector. However, some chickens are resistant to the disease. This study aimed at evaluating the mechanisms behind HPAIV disease resistance. Chickens were challenged with either H7N1 HPAIV or clade 2.3.4.4b H5N8 HPAIV, euthanized at 3 days post-inoculation (dpi), and classified as resistant or susceptible depending on the following criteria: chickens that presented i) clinical signs, and ii) histopathological lesions, and iii) presence of HPAIV antigen in tissues were classified as susceptible, while chickens lacking all these criteria were classified as resistant. The host gene expression between resistant and susceptible chickens was

compared using lung and spleen samples. Few transcriptomic changes were identified in tissues from resistant chickens, while numerous transcriptomic changes were identified in susceptible chickens. Interestingly, six differentially expressed genes were downregulated in resistant birds and upregulated in susceptible birds. Some of these genes belong to the NF-kappa B and/or mitogen-activated protein kinase signalling pathways. Among these six genes, a serine protease-encoding gene was of particular interest, being the most significantly downregulated gene in resistant chickens. Expression levels of this protease were further validated by RT-qPCR in lung tissues from experimentally infected chickens. Altogether, our results suggest that an early inactivation of important host genes could prevent an exaggerated immune response and/or viral replication, conferring resistance to HPAIV in chickens.

O.8. **Hotspot of Crimean-Congo Hemorrhagic Fever Virus Seropositivity in Wildlife, northeastern Spain.**

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Crimean-Congo Haemorrhagic Fever (CCHF) is a tick-borne disease caused by an arbovirus from the genus *Nairovirus*, belonging to the family *Bunyaviridae*. It was first recognised in 1944 in a human outbreak in the Crimean Peninsula although phylogenetic analysis indicates that the virus was already circulating in many locations for more than 2,500 years ago without causing noticeable disease outbreaks. The Crimean-Congo haemorrhagic fever virus (CCHFV) has been reported in several countries of Asia, Africa, the Middle East and south-eastern Europe, being its range similar to that of its main vector and reservoir, *Hyalomma* species ticks, which are expanding their habitat range. People become infected

by tick bites and, less frequently, though blood and infected animal fluids. It also can be transmitted by direct contact from human-to-human.

Infection induces a haemorrhagic systemic disease manifested in petechiae and haematomas over the gastrointestinal, genitourinary and respiratory systems with mortality rates ranging between 5 and 80%.

The natural cycle of CCHFV involves ticks from different stages that include several domestic and wild animal species. As ticks become long-life infected, they are considered both vectors and reservoirs of CCHFV. Ticks in larvae and nymph stages feed on small mammals such as hedgehogs, lagomorphs, rodents or ground-feeding birds, whereas adults feed on large wild and domestic ungulates. Infections in animals are generally subclinical but induce enough viremia to allow CCHFV transmission to uninfected ticks. Moreover, infected animals produce antibodies which enable mapping regions of CCHFV endemicity through serological studies. CCHFV has recently emerged in south-western Europe. In Spain, CCHFV was first detected in 2010 in *Hyalomma lusitanicum* ticks from a red deer (*Cervus elaphus*) and since 2016 at least eight CCHF cases in humans have been reported in the country. Viral strains identified in Spain showed genetic variability, which suggest repeated introductions of strains from different origins – i.e., northern Africa and eastern Europe. Serological studies in humans and animals show evidence of virus circulation in south-western Spain, which coincides with geographical areas that encompass suitable ecological and environmental conditions for the presence of *Hyalomma* ticks. In eastern Spain, which comprises the north-western Mediterranean Basin, the presence of either these tick species or CCHFV is unknown. To address these gaps of knowledge, we conducted a serosurvey for Crimean-Congo hemorrhagic fever virus antibodies in various wildlife species in Catalonia, northeastern Spain. We detected high seroprevalence in southern Catalonia, close to the Ebro Delta wetland, a key stopover for birds migrating from Africa. Our findings could indicate that competent virus vectors live in the region.

O.9. **Virus-induced reprogramming of translation landscape in mosquito cells.**

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Viruses are obligatory intracellular parasites that intimately adapt to the host they infect. How mosquito-borne viruses such as chikungunya virus (CHIKV) expand so efficiently in humans and mosquitoes, two organisms one million years apart in evolution, and why they kill the human cells but chronically infect the mosquito ones remain unsolved fundamental questions. Previously, we showed that CHIKV alters the human tRNA epitranscriptome to adapt the host translational machinery to the viral RNA genome enriched in sub-optimal codons in such a way that they become optimal for the viral gene expression. Here, we address whether this mechanism is conserved in mosquito-infected cells. By using RNA-seq and ribosome profiling we obtained a high-resolution time course analyses of the transcriptome and translatome in CHIKV-infected mosquito cells. Interestingly, CHIKV-infection did not induce changes in codon optimality but favoured translation of host mRNAs mainly expressing aminoacyl tRNA synthetases and proteins related to odorant response. The aminoacyl tRNA synthetases are enzymes that attach the appropriate amino acid onto its corresponding tRNA. Thus, their overexpression would favour viral RNA translation without major alterations in the host expression programme, reaching the virus-host equilibrium required to establish chronic infection. The overexpression of odorant-related proteins strongly suggests that virus-induced changes in the host translatome might ultimately affect the mosquito behaviour to favour viral expansion.

O.10. **Elucidating the viral RNA modification landscape of Chikungunya virus.**

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There are over 100 different chemical RNA modifications, collectively known as the epitranscriptome. Recently, transcripts encoded by positive-sense single-stranded (+ss) RNA viruses that replicate in the cytoplasm have been reported to be decorated by N^6-methyladenosine (m^6A), which is the most commonly found internal RNA modification in cellular mRNAs. However, the cellular machinery that adds m^6A modification to the viral RNA is located in the nucleus, therefore a clear understanding of how this RNA epitranscriptomic mark is added in these viruses is still missing.

In this work, we endeavoured to elucidate the currently unexplored epitranscriptome of the exclusively cytoplasmic-replicating chikungunya virus (CHIKV). CHIKV recently re-emerged in 2005 as an epidemic in Reunion Island and India and no current vaccine or antiviral treatment is available. We firstly isolated the genomic CHIKV RNA (11,8 kB) and the subgenomic CHIKV RNA (4,1 kB) from virus-infected cells and subjected these RNAs to mass spectrometry analysis. The m^6A modification stood out as a potential modification present in CHIKV RNA. In addition, we mapped m^6A modification using the latest cutting-edge technologies, which involved antibody-dependent methods (m^6A-immunoprecipitation followed by deep-sequencing) and antibody-independent methods (nanopore sequencing). To our surprise, while we could detect many thousands of m^6A sites in cellular RNAs, we did not observe m^6A modification in the viral RNA. In line with this, knockdown experiments of the cellular m^6A-related machinery did not affect CHIKV replication.

Our results highlight that m^6A modification might not be as widely spread as previously thought in single-stranded cytoplasmic-replicating RNA viruses and that analysis of RNA

modifications through mass spectrometry must be carefully evaluated and validated with different approaches, as very small amounts of contaminating cellular RNAs in the samples can give rise to misleading results. We are currently evaluating the presence of m^6A in other RNA viruses.

O.11. **Rift Valley fever phlebovirus distribution and transmission in *Culex pipiens* and *Aedes albopictus* mosquitoes.**

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The Rift Valley fever phlebovirus (RVFV) (*Phenuiviridae* family) is an emerging zoonotic arbovirus that mostly affects ruminant livestock and has serious economic consequences in Africa. It also affects humans, occasionally causing severe disease that can lead to haemorrhagic fevers. Mosquitoes belonging to the genera *Culex* and *Aedes* are the most common vectors associated to outbreaks and epidemics of this virus. In several European countries *Culex pipiens* and *Aedes albopictus* are well-established mosquito species that might transmit the virus if introduced. The goal of our study was to better understand RVFV infection within these mosquito species and detect its potential implications on vector capacity as well as the mechanisms of virus transmission.

Mosquitoes were inoculated intrathoracically with RVFV, and the viral antigen distribution in different anatomical structures of the mosquitoes and viral load in saliva were examined using immunohistochemistry and viral titration, respectively. When *A. albopictus* and *C. pipiens* were compared, the inoculated mosquitoes all showed disseminated infection, with specific variations in the digestive and reproductive systems. Both species' nervous systems were highly infected with RVFV, which could have an impact on vector capacity. Moreover, viable virus was found in mosquito saliva. The finding of higher transmission rate of *C. pipiens* suggests that *C. pipiens* may play an important role in the horizontal transmission of virus among mammal hosts. Furthermore, the identification of a RVFV-positive

chorionated egg in *C. pipiens* reveals for the first time the passage of RVFV from infected adult females to their eggs (transovarial transmission) within this mosquito species, highlighting its potential as a possible vector transmitting the virus vertically or as an exogenous source of infection via ingestion of infected mosquito larvae.

O.12. **The genomes of the Rosaceae plants contain a diverse array of endogenous pararetrovirus of different genera.**

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Plant pararetroviruses (family *Caulimoviridae*) is a class of non-enveloped dsDNA viruses that replicate by transcription in the nucleus followed by reverse transcription in the cytoplasm. *Caulimoviridae* double-stranded non-convalently closed circular genome between 7 and 10 Kbp can be encapsidated by viral coat proteins into isometric virions or bacilliform-shaped virions. *Caulimoviridae* includes about 10 genus differing in the nucleocapsid and in the number of ORFs and in their encoded proteins. *Caulimoviridae* reproduction cycle does not involve integration in the genome, but a number of sequences with high identity to pararetroviruses have been detected in different plant genomes and they were termed Endogenous ParaRetroViruses (EPRVs). Some of them have been classified inside one of the currently existing genus, but the majority are included in genus from which none currently active viruses are known. Many of these EPRV sequences were possibly integrated into plant genomes several years ago and represent fossil records of ancient pararetroviruses. They can be used to study the evolution of the *Caulimoviridae.* These EPRVs can also act as a reservoir of virus in some plants and they can contribute to the virus resistance.

Rosaceae is a family of flowering plants, which includes a number of species with relatively small genomes and many of them with an economic importance as food crops or as ornamentals. In this study, we identified and classified the EPRVs present in 11 genomes of

9 *Rosaceae* species including *Prunus persica*, *Prunus dulcis*, *Prunus mira, Prunus armeniaca*, *Prunus salicina*, *Prunus avium, Malus x domestica*, *Pyrus communis* and *Fragaria vesca*. A collection of RT domain sequences from typical pararetroviruses was used to search for related sequences across the *Rosaceae* genomes using tBLASTn. After manual curation to remove the closely related *gypsy* LTR retrotransposons, we obtained a total of 919 EPRTV-RT sequences. We then classified them using a phylogenetic reconstruction based in their sequence similarity to the RT sequences of known *Caulimoviruses* and EPRV-RTs obtained in other species. Most of them correspond to the genus *Florendovirus* exclusively composed by EPRVs, but sequences corresponding to other genera were also found, in special in the species outside the genus *Prunus*. The presence of genera-specific EPRVs suggest they have been integrated relatively recently. This study has been complemented with an analysis of the distribution of EPRVs throughout each genome, to analyze whether there are specific regions that are enriched with EPRVs. We did not find any special chromosomal distribution but we found they are usually integrated into other types of repetitive sequences (transposons or simple repeats), and they are frequently integrated in tandem arrays including complete and partial sequences in both orientations. This analysis would provide important insights into the coevolution of *Caulimoviruses*, their EPRVs forms and their plant hosts.

O.13. **Production of flexuous filamentous virus-like particles (VLPs) of different sweet potato viruses.**

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The *Potyviridae* constitutes one of the largest families of plant RNA viruses, including many socioeconomically relevant viruses, being able to infect a wide range of plant species with worldwide distribution. Due to its high agricultural relevance, the family has been extensively studied for understanding the molecular mechanisms governing virus infection and dispersal processes. Some members of the family can infect sweet potato (*Ipomoea batatas*), an important staple crop for food security, leading to devastating consequences

due to high yield losses and crop quality degradation, especially in coinfections with unrelated viruses. Nonetheless, sweet potato infecting viruses are comparatively less studied than other viruses within the family and there are still many aspects awaiting to be solved on their infectious cycle.

All members of the family share a characteristic virion structure as flexuous elongated particles which are composed by hundreds of copies of a single protein (coat protein, CP) surrounding the RNA genome in helical arrangement.

Several studies about plant virus-like particles (VLPs) have been published recently because they present a great potential for nanobiotechnological uses, although the production of flexuous particles still remains a major challenge. Therefore, our purpose was the production of VLPs of three sweet potato infecting viruses of the family *Potyviridae*, two belonging to the genus *Potyvirus*, namely *Sweet potato feathery mottle virus* (SPFMV) and *Sweet potato virus 2* (SPV2), and one member of the genus *Ipomovirus, Sweet potato mild mottle virus* (SPMMV), through transient expression of their respective CPs in *Nicotiana benthamiana* plants. Initially, the CP coding region of each virus was cloned into a potato virus X (PVX)-based vector and delivered to *N. benthamiana* plants using *Agrobacterium tumefaciens*. CP expression was confirmed by Western blot analysis using specific antibodies at 3, 5 and 7 days post-agroinfiltration, and crude extracts of agroinfiltrated tissues were imaged under electron microscopy (EM), showing that the overexpressed CPs were assembled into VLPs resembling flexuous filaments. Our results demonstrated the successful production of flexuous VLPs for the three tested viruses, providing evidence that their CPs were able to form flexuous filamentous particles without the presence of other potyviral factors. The produced VLPs could serve for further studies aiming to solve the atomic structure of virions, and to better understand their contribution to key processes of the biology these viruses, such as infection of sweet potato plants and dissemination by natural insect vectors.

O.14. **NGS Techniques Reveal a High Diversity of RNA Viral Pathogens and Papillomaviruses in Fresh Produce and Irrigation Water.**

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Fresh fruits and vegetables are susceptible to microbial contamination at every stage of the food production chain, and as a potential source of pathogens, irrigation water quality is a critical factor. Next-generation sequencing (NGS) techniques have been flourishing and expanding to a wide variety of fields. However, their application in food safety remains insufficiently explored, and their sensitivity requires improvement. In this study, lettuce, strawberry, and parsley samples grown under organic agricultural practices as well as water irrigation sources were analyzed by quantitative polymerase chain reaction (qPCR) assays for the presence and concentration of Human Adenovirus (HAdV), which was used as a fecal viral indicator, and other relevant viral pathogens, such as Norovirus (NoV) and Hepatitis E Virus (HEV). Results obtained by qPCR showed low but frequent contamination of HAdV and/or NoV in 46.9% of fresh produce (6/12 lettuce samples, 4/12 strawberries samples, and 5/8 parsley samples) and 50% of irrigation water samples, with NoV GII being the principal agent detected. Furthermore, two different NGS approaches, target enrichment sequencing (TES) for the analysis of viruses that infect vertebrates and amplicon deep sequencing (ADS) for NoV and Human Papillomavirus (HPV), were applied to the studied samples to explore the potential application of NGS techniques for viral detection, characterization, and discovery, especially in fresh fruits and vegetables. TES enabled the detection of human and other vertebrate viruses in all irrigation water samples, specifically viruses belonging to *Astroviridae*, *Caliciviridae*, *Circoviridae*, *Parvoviridae* and *Picornaviridae*, and the obtention of sequences assigned to the *Caliciviridae* member NoV GII.17 from a parsley sample. Additionally, ADS provided a better characterization of the NoV diversity present in the studied samples and demonstrated to be a suitable tool for HPV detection and in-depth characterization of the diversity of these specific viral pathogens. A wide diversity of cutaneous HPV was detected in fresh produce and a wider diversity of HPV was identified in more polluted water samples, such as river

and treated wastewater samples. The high-risk type HPV-8 was found in treated wastewater. In this study, irrigation water may be the most probable source of viral pathogens in food samples, since all contaminating viruses found in fresh fruit and vegetable samples were also detected in irrigation water sources.

O15. **SARS-CoV-2 interaction with Siglec-1 mediates *trans*-infection by dendritic cells.**

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Background: myeloid antigen-presenting cells (APCs) such as monocyte-derived macrophages (MDM) and dendritic cells (MDDCs) can contribute to SARS-CoV-2 pathogenesis via alternative pathways. APCs do not need to be productively infected to capture and transfer viruses to by-stander cells, which become infected via *trans*-infection. Indeed, this is a pathway co-opted by viruses such as HIV-1, which are captured by APCs through the sialic acid-binding Ig-like lectin 1 (Siglec-1). This lectin is an interferon-induced receptor that recognizes sialylated gangliosides exposed on the viral membrane. Here, we assessed the role of Siglec-1 in SARS-CoV-2 uptake and *trans*-infection by myeloid APCs.

Methods: we used functional blocking assays with specific monoclonal antibodies, as well as super-resolution and confocal microscopy to assess the contribution of Siglec-1 in viral uptake and *trans*-infection. These experiments were done with replication competent

SARS-CoV-2 variants and pseudovirions using primary myeloid APCs and transfected Raji-B cell lines expressing Siglec-1 or other lectins (DC-SIGN, Siglec-5 and Siglec-7). Statistical differences were assessed with paired and one-sample *t* tests.

Results: the amount of cell-associated SARS-CoV-2 found on APCs was higher in interferon-alpha-treated MDM or MDDCs as compared to mock-treated cells (p=0.0312), suggesting a role for Siglec-1 in viral uptake as this receptor is induced upon cellular activation. Super-resolution microscopy showed the presence of sialylated gangliosides such as GM1 recognized by Siglec-1 on the SARS-CoV-2 membrane. The role of Siglec-1 on viral uptake was confirmed using an anti-Siglec-1 antibody, which blocked viral capture in both MDMs and MDDCs ($p \leq 0.0016$), and in Raji cells expressing Siglec-1 but not the other lectins. Siglec-1 captured different SARS-CoV-2 variants. Viruses captured by MDDCs accumulated in sac-like virus-containing compartments previously described for other viruses such as HIV-1 and Ebola virus. Finally, both Raji-Siglec-1 cells and MDDCs efficiently transmitted captured viruses and pseudoviruses to target 293T cells overexpressing ACE2 and TMPRSS2 via Siglec-1, while MDMs failed to do so.

Conclusions: targeting Siglec-1 could offer cross-protection against SARS-CoV-2 and other enveloped viruses that exploit APCs for viral dissemination and bring new broad-spectrum antivirals for future outbreaks.

O.16. **SARS-CoV-2 impact on maternal and neonate health, cytokine profile and IgG transplacental transfer.**

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Introduction: Pregnant women are at increased risk of severe and adverse outcomes of COVID-19, and have higher risk of adverse pregnancy outcomes like intrauterine/fetal distress and preterm birth. However, little is known about the immune response in pregnant women infected by SARS-CoV-2 and the impact of the infection in maternal and neonatal outcomes. In this study, we investigated the inflammatory and humoral responses to SARS-CoV-2 in maternal and cord blood samples collected from pregnant women.

Methods: Thirty-six pregnant women, who had tested positive or negative for SARS-CoV-2 during the third trimester of pregnancy, were recruited at delivery at Hospital Sant Joan de Déu, Barcelona, Spain. Maternal and neonatal nasal swabs for SARS-CoV-2 detection via rRT-PCR, and maternal and cord blood samples for serology were collected at the time of delivery. Plasma IgM, IgG and IgA levels to 5 SARS-CoV-2 antigens (spike [S], S2, RBD, nucleocapsid [N,] full length and C-terminus), IgG to N full length from 4 human coronaviruses (OC43, HKU1, 229E, and NL63), and the concentrations of 30 cytokine, chemokine and growth factors, were measured by Luminex.

Results: Twenty-three pregnant women were exposed to SARS-CoV-2 (positive by rRT-PCR during the third trimester and/or serology at delivery) and 13 were non-exposed. Forty-three % (10/23) of the exposed pregnant women showed pregnancy complications vs 15% (2/13) of the non-exposed; and 22% (5/23) of the newborns from exposed pregnant women had complications vs none of the 13 born from non-exposed mothers. None of the newborns from the study tested positive by rRT-PCR at delivery. Among the exposed mothers, those with symptoms had higher

antibody levels compared to asymptomatic ones, and plasma IgG levels showed very high correlations with levels in cord blood. However, rRT-PCR positive mothers at delivery showed lower trans-placental transfer of SARS-CoV-2-specific IgGs than rRT-PCR negative exposed mothers. SARS-CoV-2 exposed mothers had higher plasma concentrations of several cytokines than unexposed mothers: EGF, IL-13, IL-2R, IL-17, IP-10, MIG and MIP-1β; and even higher levels were found in the symptomatic ones: EGF, G-CSF, HGF, FGF, IL-1β, IL-2, IL-2R, IL-6, IL-15, TNF, MIP-1α, MIP-1β, IP-10, MCP-1 and MIG. IFNα was increased in cord blood of exposed mothers, and EGF, FGF, IL-17 and IL-15 in cord blood of the symptomatic ones, whereas RANTES was decreased in cord blood of symptomatic mothers. **Conclusion:** SARS-CoV-2 infection during the third trimester of pregnancy has a negative impact on the health of the mother and the neonate, and causes a significant reduction of the trans-placental transfer of SARS-CoV-2-specific IgGs, probably related to the inflammatory process going on in the mother and in the placenta. Children born to mothers with SARS-CoV-2 during pregnancy should be longitudinally observed to assess long-term outcomes. Results support vaccination of pregnant women.

O.17. **Chronological brain lesions after SARS-CoV-2 infection in hACE2 transgenic mice.**

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes respiratory disease, but it can also affect other organs including the central nervous system. Several animal models have been developed to address different key questions related to the disease caused by this agent: the coronavirus infectious disease 2019 (COVID-19). Since wild type mice are minimally susceptible to certain SARS-CoV-2 lineages (Beta variant), hACE2-transgenic mice, previously developed to study SARS-CoV, have been used. These mice succumb to SARS-CoV-2 and develop a fatal neurological disease. Here, we aimed to chronologically characterize the SARS-CoV-2 neuroinvasion and neuropathology. Necropsies were performed at different time points and the brain and olfactory mucosa were processed for histopathological analysis. SARS CoV-2 virological assays, including immunohistochemistry, were performed along with a panel of antibodies to assess neuroinflammation. At 6-7 days post inoculation (dpi), brain lesions were characterized by non-suppurative meningoencephalitis and diffuse astro- and microgliosis. Vasculitis and thrombosis were also seen associated with occasional microhemorrhages and spongiosis. Moreover, neuronal vacuolar degeneration of virus-infected neurons was observed. At 2 dpi SARS CoV-2 immunolabeling was only found in the olfactory mucosa, but at 4 dpi intraneuronal virus immunolabeling had already reached most of the brain. Maximal distribution of the virus was observed throughout the brain at 6-7 dpi except for the cerebellum which was mostly spared. Our results suggest an early entry of the virus through the olfactory mucosa and a rapid inter-neuronal spread of the virus leading to acute encephalitis and neuronal damage in this mouse model.

O.18. **Topology characterization of the SARS-CoV-2 nsp4 protein in biological membranes.**

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Coronavirus proteome includes structural and non-structural proteins (nsps). Among the nsps, only three of them have been described as integral membrane proteins, and are

thought to be involved in one of the key steps of the viral cycle, the formation of viral replication factories. lauraDeciphering the molecular details of these proteins is essential to fully understand the biology of theses viruses. In this study, we have focused on the topological characterization of the nsp4 protein of the SARS-CoV-2. Up to now only *in silico* approaches have been used to predict nsp4 topology. Here, we have used two different assays to determine the topology of nsp4 in bacterial and eukaryotic cells, respectively. Both assays rely on the information given by a reporter located in the C-terminal end of several truncates of the protein. Thus, we can accurately locate the position of the C-terminal end either in the cytoplasmic or the extra-cytoplasmic side of the membrane. Additionally, our assays provide insights regarding the folding of the protein in the membrane. Our results proved that nsp4 protein contains 4 transmembrane domains, leaving both N- and C-terminal ends oriented toward the cytoplasm. Furthermore, our assays indicate that each of the four transmembrane domains is inserted into the lipid bilayer in a sequential manner.

O.19. **Repurposing drugs against SARS-CoV-2.**

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The accumulated experience in our groups during last years has led to a home library of compounds that includes molecules active against a wide panel of viruses, bioactive molecules from plant extracts as well previously repurposed drugs in several frameworks. We decided to use such background to assess their application against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the treatment of its disease (COVID-19).

As illustrated in Figure 1, there is an increasing number of available structures of SARS-CoV-2 proteins essential for the viral life cycle. In this talk, we will discuss how such structural data might help to discover novel drugs able to halt virus progression by implementing computational models, including virtual screening and molecular dynamic levels of theory. Of course, molecular models are not enough; laboratory confirmation remains a must if one looks for real medical applications. Our experiments upon cell infection with HCoV-229E will be also touched.

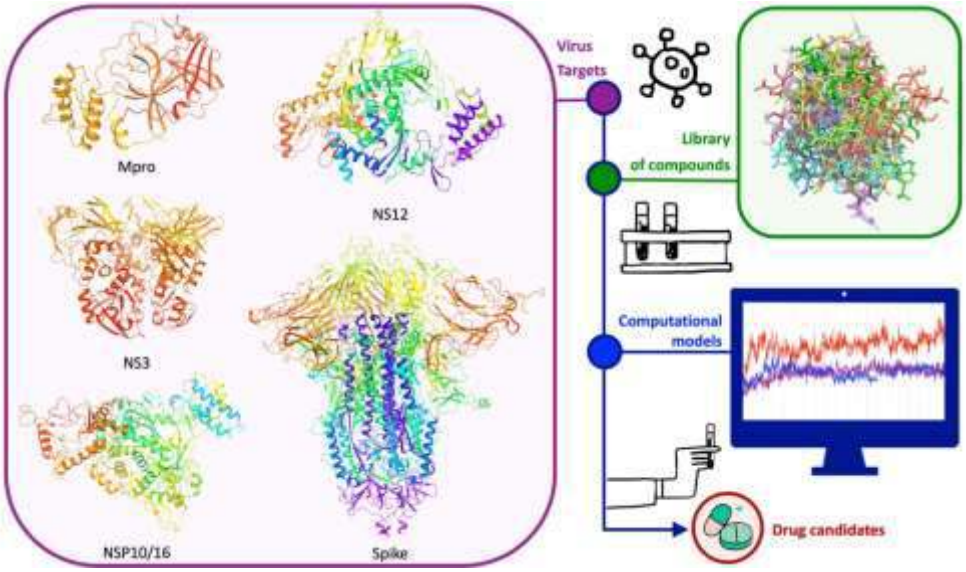

Figure 1: Schematic representation of the proposed workflow, including molecular modelling and experimental assessments.

O.20. **A large SARS-CoV-2 outbreak in a long-term care facility: lessons learned from a molecular epidemiological analysis.**

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Background: Since 2019 SARS-CoV-2 has become a globally spread pathogen and has caused more than 4.5 million deaths. Older people living in long-term care facilities (LTC) have been, by large, the population most severely affected by COVID-19. Preventing deadly outbreaks in these premises is a cornerstone strategy to avoid an excess of COVID-19 related mortality and morbidity and to keep the health system safe from collapse. A detailed study of such outbreaks may help to better shape the measures of control and prevention.

Methods: We carried out an epidemiological study of a large outbreak occurring in a LTC with 96 residents and 70 health workers, using methods of classical and molecular epidemiology analysis. The SARS-CoV-2 genome was reverse transcribed and amplified from 53 residents and three health workers, and then subjected to next generation sequencing. Phylogenetic analysis of the resulting consensus sequences was carried out including other sequences circulating in our geographical area and globally. Pairwise single nucleotide polymorphisms were calculated among outbreak sequences.

Results: The outbreak occurred in an interval of 31 days, with an attack rate of 97% among residents and a case fatality rate of 16% (N=15). The attack rate among workers was 47% (N=32). The longitudinal analysis revealed an induction phase followed by a super-spreader event and a remission stage that lasted for two additional weeks; cases among health workers paralleled the pattern observed among residents. Epidemiological analysis revealed a lack of early detection of transmission inside the institution, fostered by some failures in the management of patients' isolation. Sequencing of samples from residents

and health workers revealed three different introductions of the virus. Two residents were infected by lineage B.1.177.22, while the rest and the three health workers were classified as B.1.177. Among the latter, one was a unique case while 51 sequences clustered in two closely related groups with a monophyletic origin and high genetic similarity.

Conclusions: Unnoticed chains of transmission should be considered early during an outbreak in an LTC. Inadequate use and interpretation of diagnostic tests may misguide the management of an outbreak. Molecular epidemiological analysis could be the key to understand the dynamics of unusual and extensive outbreaks in LTC.

O.21. **Study of deletions in SARS-CoV-2 Spike protein of the most widespread variants in the consecutive pandemic waves in Barcelona.**

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Genomic studies using NGS technology have become an essential tool to monitor the circulation of the SARS-CoV-2 lineages across the population. In a previous study with patients of several clinical outcomes by March 2020, we described that a minority portion of genomes accumulates deletions in the S1/S2 cleavage site (PRRAR/S), generating a frameshift with the appearance of a premature stop codon, which might result in truncated Spike protein composed by the S1 including the receptor binding domain (RBD). As the pandemics has evolved over time, new viral variants have emerged, some of them becoming variants of concern such as the Alfa (B.1.1.7-like), Beta (B.1.351-like), Gamma (P.1-like) and Delta (B.1.617.2-like) variants. In Barcelona, the prevalence of SARS-CoV-2

variants has been different throughout the consecutive pandemic waves. The main objective of our work was to study whether these highest prevalent variants show differences in the pattern of the production of defective deletions by deep-sequencing of the complete spike gene. Our results show that emerging variants modulate the frequency of defective genomes and we discuss that this might be a consequence of the viral adaptation during human-to-human transmission.

O.22. **Monitoring natural SARS-CoV-2 infection in lions (*Panthera leo*) at Barcelona Zoo: viral dynamics and host responses.**

Hugo Fernández-Bellon[1,*], **Jordi Rodon**[2,*], Leira Fernández-Bastit[2], Vanessa Almagro[1], Pilar Padilla-Solé[1], Cristina Lorca-Oró[2], Rosa Valle[2], Núria Roca[2], Santina Grazioli[3], Tiziana Trogu[3], Albert Bensaid[2], Jorge Carrillo[4,5], Nuria Izquierdo-Useros[4,5], Julià Blanco[4,5,6], Mariona Parera[4], Marc Noguera-Julián[4,6] Bonaventura Clotet[4,5,6], Ana Moreno[3], Joaquim Segalés[7,8,*], Júlia Vergara-Alert[2,*].

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To date, no evidence supports that animals play a role in the epidemiology of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of the coronavirus infectious disease 2019 (COVID-19). However, several animal species are

naturally susceptible to SARS-CoV-2 infection. Besides pets (cats, dogs, Syrian hamsters, and ferrets) and farm animals (minks), different zoo animal species have tested positive for SARS-CoV-2 (large felids and non-human primates). After summer 2020, a second wave of SARS-CoV-2 infection occurred in Barcelona (Spain), reaching a peak of positive cases in November. During that period, four lions (*Panthera leo*) at Barcelona Zoo and three caretakers developed respiratory signs and tested positive for SARS-CoV-2 antigen. Lion infection was monitored for several weeks, and nasal, fecal, saliva, and blood samples were taken at different time-points. SARS-CoV-2 RNA was detected in nasal samples from all studied lions, and viral RNA was detected up to 2-weeks after the initial viral positive test in 3 out of 4 animals. SARS-CoV-2 genome was also detected in feces from animals at different times. Virus isolation was successful only from respiratory samples of two lions at an early time-point. The four animals developed neutralizing antibodies after infection that were detectable four months after initial diagnosis. The partial SARS-CoV-2 genome sequence from one animal caretaker was identical to the sequences obtained from lions. Chronology of the events, viral dynamics and the genomic data support human-to-lion transmission as the origin of infection.

O.23. **Dynamics of SARS-CoV-2 Alpha (B.1.1.7) variant spread: the wastewater surveillance approach.**

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Wastewater based epidemiology (WBE) offers an overview of the SARS-CoV-2 variants circulating among the population thereby serving as a proper surveillance method.

The variant of concern (VOC) Alpha was first identified in September 2020 in the United Kingdom, and rapidly became dominant across Europe. Our objective was to elucidate the Alpha VOC outcompetition rate and identify mutations in the spike glycoprotein (S) gene, indicative of the circulation of the Alpha VOC and/or other variants in the population through wastewater analysis.

In the period covered by this study (November 2020-April 2021), forteen wastewater treatment plants (WWTPs) were weekly sampled. The total number of SARS-CoV-2 genome copies per L (GC/L) was determined with a Real-Time qPCR, targeting the N gene. Surveillance of the Alpha VOC circulation was ascertained using a duplex RT-qPCR, targeting and discriminating the S gene. Our results showed that in a period of 6 weeks the Alpha VOC was present in all the studied WWTPs, and became dominant in 11 weeks on average. The outcompetition rates of the Alpha VOC were estimated, and their relationship with different parameters statistically analyzed. The rapid spread of the Alpha VOC was influenced by its initial input and by the previous circulation of SARS-COV-2 in the population. This latter point could be explained by its higher transmissibility, particularly advantadgeous when a certain degree of herd immunity exists. Moreover, the presence of signature mutations of SARS-COV-2 variants were established by deep-sequencing of the complete S gene. The circulation of the Alpha VOC in the area under study was confirmed, and additionally two combinations of mutations in the S glycoprotein (T73A and D253N, and S477N and A522S) that could affect antibody binding were identified.

O.24. **Cetylpyridinium chloride mouthwashes to reduce the shedding of viable SARS-CoV-2.**

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Background: SARS-CoV-2 is spread via airborne transmission. Mouthwashes containing virucidal compounds can help reduce viral spread. Here we show that cetylpyridinium chloride (CPC), a quaternary ammonium present in many oral mouthwashes, reduces SARS-CoV-2 infectivity by disrupting viral membranes both *in vitro* and *in vivo*.

Methods: We tested the capacity of CPC-containing mouthwashes to inhibit SARS-CoV-2 entry into target cells by using a luciferase-based assay with a reporter lentivirus pseudotyped with the SARS-CoV-2 spike protein. The replication-competent SARS-CoV-2 B.1.1.7 and D614G variants were also assayed. Viral envelope disruption by CPC's virucidal effect was measured by dynamic light-scattering analyses (DSL). We confirmed these results modifying an ELISA that detects the SARS-CoV-2 nucleocapsid (NC), which was used in the absence of its own lysis buffer. Effect of CPC in the saliva of individuals with Covid-19 was assessed in a double-blind, placebo-controlled, randomized clinical trial. SARS-CoV-2 positive patients were randomized to gargle either water or 0.07% CPC mouthwash. The study outcomes were the SARS-CoV-2 log10 viral RNA load by RT-PCR and the NC protein levels by ELISA, both in saliva at 1h and 3h post-intervention.

Results: CPC-containing mouthwashes inhibited SARS-CoV-2 viral fusion *in vitro* in a dose-dependent manner and decreased more than a 1000 times the viral TCID50 in target cells,

regardless of the variant tested. The ELISA and the DSL analyses pointed to the effective disruption of the integrity of viral membranes after treatment with CPC. The clinical study performed with 105 patients showed no significant differences in viral RNA load at 1h and 3h post-treatment in saliva between placebo and CPC-treated groups. However, the levels of SARS-CoV-2 NC protein of lysed viruses were significantly higher in the CPC group at 1h and 3h post-intervention.

Conclusions: CPC decreased more than a 1000 times the infectivity of SARS-CoV-2 *in vitro* and was effective against different SARS-CoV-2 variants. In Covid-19 patients, a 0.07% CPC mouthwash correlated with a statistically significant increase of NC protein levels in saliva, indicating enhanced disruption of viral particles. CPC-containing mouth rinses could represent a cost-effective measure to reduce SARS-CoV-2 infectivity in saliva, aiding to reduce viral transmission from infected individuals regardless of the variants they are infected with.

O.25. **Reduced detection of Delta and other VOCs by antigen-detecting diagnostic tests.**

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Background: SARS-CoV-2 antigen-detecting rapid diagnostic tests (Ag-RDTs) are emerging globally as an easy-to-use diagnostic tool for the identification of contagious individuals to halt transmission and novel infections. Yet, to be effective, Ag-RDTs must be able to detect distinct variants of concern (VOC) constantly arising. Given that novel VOC genetically diverge, Ag-RDTs may vary in their performance. Here we aimed to dissect if such variation could impact SARS-CoV-2 nucleocapsid detection.

Methods: Five SARS-CoV-2 VOC (D614G, Alpha, Beta, Gamma and Delta) where isolated from clinical nasopharyngeal swabs and grown in Vero E6 cells. Viral supernatants were recovered and used for performing: 1) Genomic sequencing using standard ARTIC v3 based protocol on Illumina; 2) Quantification of SARS-CoV-2 nucleoprotein with an ELISA; 3) Reverse transcription and PCR amplification of the N2 using extracted RNA. The following commercial Ag-RDTs were compared: Nesapor (Mareskit®), Roche, Siemens Healthineers (Clinitest®) and Abbott. Viral stocks were serially diluted 1:10 in PBS, and mixed with the corresponding test lysis buffer at 1:19 and 1:1 ratios, respectively. Each detection was performed in duplicates, adding 4 drops of the indicated mixtures to each Ag-RDTs and incubated for 15 minutes before readout. The highest dilution detected as positive by each Ag-RDTs was recorded as its lower detection capacity for each viral variant.

Results: Genomic sequencing revealed no changes in the nucleocapsid for the D614G variant compared to Wuhan sequence, 2 non-synonymous changes for the Beta, and up to 4 non-synonymous changes in the Alpha, Gamma, and Delta VOC. We next tested whether these non-synonymous variations could impact the detection capacity of SARS-CoV-2 nucleocapsid by the antibodies of mentioned Ag-RDTs. All VOC were used at a similar nucleocapsid concentration measured both by qPCR and ELISA. Relative to the original D614G variant, at a 1:1 ratio we found a 10-fold loss of detection for the Delta variant in two commercial tests, for the Alpha variant in the four commercial tests analyzed herein, and for the Gamma variant in one test. This 10-fold reduction was above the threshold required to isolate replication-competent virus from nasopharyngeal swabs.

Conclusions: The reduction of the SARS-CoV-2 detection by some Ag-RDTs could hamper the identification of contagious individuals. As new VOC arise, successful contact tracing will require continuous Ag-RDTs performance surveillance.