



Societat Catalana
de **BIOLOGIA**

XVIII Jornada de Virologia

Organitzada per la Secció de Virologia de la SCB

INSTITUT D'ESTUDIS CATALANS

Carrer del Carme 47
Barcelona

11 de novembre de 2019



XVIII Jornada de Virologia BCN Virology Meeting 2019

PROGRAMA

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

SOFIA PÉREZ DEL PULGAR

Amb el suport de:

CerTest
BIOTEC

VIASURE
Real Time PCR Detection Kits

Cultek
Your partner in Life Sciences



9:00 h

RECOLLIDA DOCUMENTACIÓ / REGISTRATION

9:15 h

BENVINGUDA / WELCOME: *Sofía Pérez del Pulgar*

9:30 h

OPENING LECTURE

“In vitro studies of broad-spectrum antivirals against RNA viruses”

Santseharay Ramírez. *Copenhagen Hepatitis C Program (CO-HEP), Department of Infectious Diseases, Hvidovre Hospital and Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.*

SESSIÓ I / SESSION I

MODERADOR / CHAIR: *Josep Quer*

10:00 h

O-1: Identification of host-derived circular RNAs that display pro- and anti-viral activities in flavivirus-infected cells.

Marc Talló-Parra. *Universitat Pompeu Fabra, Barcelona, Spain.*

10:15 h

O-2: Lack of association between novel astrovirus and diarrheic children and higher viral titer among asymptomatic controls

Diem-Lan Vu. *University of Barcelona, Spain; Geneva University Hospitals, Switzerland.*

10:30 h

O-3: Inhibition of the DNA damage response enzyme DNA-PK increases the susceptibility to HBV infection in cell culture.

Urtzi Garaigorta. *Centro Nacional de Biotecnología (CNB-CSIC), CIBERehd, Madrid, Spain.*

10:45 h

O-4: Biotechnological strategies to obtain Virus-Like Particles (VLPs) of Torradovirus and Fabavirus, two plant viruses from the family *Secoviridae*.

Júlia García-Mulet. *Center for Research in Agricultural Genomics CRAG, CSIC-IRTA-UAB-UB, Campus UAB Bellaterra, Barcelona, Spain.*

11:00 h

O-5: Disruption of a RNA secondary structure in HIV-1 gp41 induces viral lethality.

Ana Jordan-Paiz, IrsiCaixa, Badalona, Spain.

11:15-11:45h

PAUSA I CAFÈ / COFFE BREAK

11:45 h

KEYNOTE LECTURE

“Plant-infecting potyvirids: still surprises and unexpected findings”

Juan José López-Moya. Center for Research in Agricultural Genomics CRAG, CSIC-IRTA-UAB-UB, Cerdanyola del Vallès, Barcelona, Spain.

SESSIÓ II / SESSION II

MODERADOR / CHAIR: *Susana Guix*

12:15 h

O-6: Cytomegalovirus interferes with the ICOSL:ICOS axis to impair T cell co-stimulation.

Guillem Angulo. University of Barcelona, Barcelona, Spain.

12:30 h

O-7: A virome study on field-collected mosquitoes uncovers new insect-specific viruses circulation in Europe.

Lotty Birnberg. IRTA-CReSA, Bellaterra, Spain.

12:45 h

O-8: Virological characterisation of the hepatitis C virus epidemic in people who inject drugs by using dried blood spot samples.

Adrián Antuori. Germans Trias i Pujol University Hospital and Research Institute (IGTP), Badalona, Spain.

13:00 h

O-9: Resistance to *Cucumber mosaic virus*: a matter of relocating CmVPS41 a protein involved in intracellular trafficking-

Núria Real. Center for Research in Agricultural Genomics CRAG, CSIC-IRTA-UAB-UB, Campus UAB Bellaterra, Barcelona, Spain.

13:15 h

O-10: DNA viruses in colorectal polyps.

Marta Itarte. University of Barcelona, Barcelona, Spain.

13:30-14:15 h

DINAR / LUNCH

14:15-15:15h

SESSIÓ DE PÓSTERS/ POSTER SESSION

POSTER TOURS

- **Tour 1. Miscelania – Posters 1-5.**

Tour guide: Susana Bofill.

- **Tour 2. Viral hepatitis – Posters 6-11**

Tour guide: Juana Díez.

15:15 h

MEET THE EXPERT

“The secret of hepatitis C virus culture development: efficient systems based on adaptation of patient derived genotype 1-6 strains”

Jens Bukh. Copenhagen Hepatitis C Program (CO-HEP), Department of Infectious Diseases, Hvidovre Hospital, and Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.

SESSIÓ III/ SESSION III

MODERADOR / CHAIR: Santseharay Ramírez

16:00 h

O-11: Persistent transcriptional alterations after hepatitis C virus elimination in cell culture.

Pablo Gastaminza. Centro Nacional de Biotecnología (CNB-CSIC), CIBERehd, Madrid, Spain.

16:15 h

O-12: Super-resolution microscopy as a powerful tool for understanding the formation and inhibition of influenza virus structures in mammalian cells.

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

16:30 h

O-13: Early type I IFN response dynamics determines infection fate decision.

Jordi Argilaquet. *Universitat Pompeu Fabra, Barcelona, Spain.*

16:45 h

O-14: Supramolecular arrangement of the Zika virus NS5 protein and its role in vivo: beyond the RdRP activity.

Diego S. Ferrero. *Molecular Biology Institute of Barcelona (IBMB-CSIC), Barcelona, Spain.*

17:00 h

HOT TOPICS IN 3D CELLULAR MODELS

“Liver Organoids: Shaping the future of liver bioengineering for transplantation and beyond”

Pedro Baptista. *Health Research Institute of Aragón (IIS Aragón), Zaragoza, Spain. Health Research Institute of Fundación Jiménez Díaz (IIS FJD), Madrid, Spain. Department of Biomedical and Aerospace Engineering, Carlos III University of Madrid, Spain. Fundación ARAID. CIBERehd.*

17:30 h

SUMMARY OF THE MEETING

BEST PRESENTATION AWARDS

POSTER SESSION

P-1: Determining the extended host range of the crinivirus Sweet potato chlorotic stunt virus (SPCSV).

Ornela Chase¹, Juan José López-Moya¹

¹Laboratory of plant virology, Centre for Research in Agricultural Genomics, CRAG, CSIC-IRTA-UAB-UB, Cerdanyola del Valles, Barcelona, Spain

P-2: Position-specific OM-pBAE polymer modification of oncolytic adenovirus to improve systemic delivery.

Marc Otero, Pau Brugada-Vilà, Cristina Forneguera, Anna Cascante, Salvador Borrós, Cristina Fillat.

Teràpia Gènica i Càncer. Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS). Barcelona, 08036, Spain. Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Barcelona, Spain.

P-3: Validation of VIASURE commercial one-step real-time RT-PCR kits targeting Human Astrovirus and Sapovirus for the diagnostic of gastroenteritis.

Aurora Sabrià¹, Virginia Rodriguez Garrido², Rosa M Pintó¹, Albert Bosch¹, Susana Guix¹

¹Enteric Virus Laboratory, Department of Genetics, Microbiology and Statistics, University of Barcelona, Barcelona, Spain; Nutrition and Food Safety Research Institute (INSA-UB), University of Barcelona, Santa Coloma de Gramenet, Spain

² Microbiology Department, Hospital Universitari Vall d'Hebron, Barcelona, Spain

P-4: Non-integrating retrovirus improves Cre-recombination driven cell tracking using Brainbow2.1/Confetti mice.

Jolanda. J.D. de Roo, MSc

Leiden University Medical Center, 2333 ZA Leiden, the Netherlands

P-5: Super-resolution microscopy as a powerful tool for understanding the formation and inhibition of influenza virus structures in mammalian cells.

Maria Arista-Romero^{1#}, Annasaheb Kolpe^{2#}, Bert Schepens², Silvia Pujals¹, Xavier Saelens², Lorenzo Albertazzi^{1,3}

¹Nanoscopy for Nanomedicine Group, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), C\ Baldiri Reixac 15-21, Helix Building, 08028 Barcelona, Spain.

²VIB-UGent Center for Medical Biotechnology, Technologiepark-Zwijnaarde 71, Ghent, B-9052, Belgium.

³Department of Biomedical Engineering, Institute for Complex Molecular Systems (ICMS), Eindhoven University of Technology, 5612AZ, Eindhoven, The Netherlands.

P-6: Differential roles of lipin1 and lipin2 in the hepatitis C virus replication cycle.

Victoria Castro¹, Gema Calvo¹, Ginés Ávila¹, Marlene Dreux² and Pablo Gastaminza¹

¹Department of Cellular and Molecular Biology Centro Nacional de Biotecnología-Consejo Superior de Investigaciones Científicas, Madrid 28049 (Spain).

²CIRI, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, École Normale Supérieure de Lyon, Univ Lyon, Lyon F-69007 (France).

P-7: Analysis of Regulatory T-Cell Frequency and Phenotype in Chronic Hepatitis C patients Undergoing Interferon-Free Therapies.

Elena Perpiñán, Ivana Jordan, Mireia García-López, María-Carlota Londoño, Zoe Mariño, Sabela Lens, Concepción Bartres, Sofía Pérez-Del-Pulgar, Xavier Forns, George Koutsoudakis

Liver Unit, Hospital Clinic, IDIBAPS, CIBERehd. University of Barcelona, Spain.

P-8: Clinical and virological predictors of response after antiviral therapy interruption in HBeAg-negative chronic hepatitis B

M. García-López¹, S. Lens¹, Z. Marino¹, M. Bonacci¹, S. Rodríguez-Tajes¹, E. Perpiñán¹, F. Rodríguez-Frías², B. Testoni³, G. Koutsoudakis¹, M. Buti², F. Zoulim³, S. Pérez del Pulgar¹, X. Forns¹.

¹ Hospital Clinic, IDIBAPS, CIBERehd. University of Barcelona, Liver Unit, Barcelona, Spain;

² Hospital Vall Hebron, CIBERehd. Internal Medicine, Hepatology Section, Barcelona, Spain;

³ Cancer Research Center of Lyon (CRCL), University of Lyon, UMR, UCBL, INSERM, U1052, Lyon, France.

P-9: Conservation and amino acid changes in hepatitis B core gene in chronic hepatitis patients at different clinical stages.

Marçal Yll^{1,2}, Maria Francesca Cortese^{1,2}, Mercedes Guerrero^{1,3}, Gerard Orriols¹, Josep Gregori^{4,5,6}, Mar Riveiro Barciela^{6,7}, Rosario Casillas^{1,2}, Carolina González², Sara Sopena^{1,2}, Cristina Godoy^{1,6}, Josep Quer^{4,6}, Ariadna Rando², Rosa Lopez-Martinez², Rafael Esteban Mur^{6,7}, Maria Buti^{6,7}, David Tabernero^{1,6} and Francisco Rodríguez-Frías^{1,2,4}.

(1) Liver Unit, Vall d'Hebron Research Institute.

(2) Biochemistry and Microbiology/Liver Pathology Unit, Vall d'Hebron University Hospital.

(3) Department of Microbiology, Vall d'Hebron University Hospital.

(4) Liver Diseases, Vall d'Hebron Institute of Research, VHIR.

(5) Roche Diagnostics SL.

(6) CIBERehd, Instituto De Salud Carlos III, Madrid.

(7) Liver Unit, Department of Internal Medicine, Vall d'Hebron University Hospital.

P-10: Study of HDV effect on HBV replication and quasispecies complexity in mice model

Cristina Godoy^{1,2}, Gracián Camps³, Josep Gregori^{1,4}, David Tabernero^{1,2}, Sara Sopena^{1,4}, Maria Francesca Cortese^{1,4}, Rosario Casillas^{1,4}, Marçal Yll^{1,4}, Ariadna Rando¹, Rosa López-Martínez¹, Josep Quer^{2,4}, Rafael Esteban^{2,5}, Mar Riveiro-Barciela^{2,5}, Francisco Rodríguez-Frías^{1,2}, Gloria González-Aseguinolaza³, Maria Buti^{2,5}

¹Liver Pathology Unit, Departments of Biochemistry and Microbiology, Hospital Universitari Vall d'Hebron, Universitat Autònoma de Barcelona, Spain.

²Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Instituto de Salud Carlos III, Madrid, Spain.

³Centro de Investigación Médica Aplicada (CIMA), Universidad de Navarra, Pamplona, Spain.

⁴Liver Unit, Liver Disease Laboratory-Viral Hepatitis, Vall d'Hebron Institut Recerca-Hospital Universitari Vall d'Hebron, Universitat Autònoma de Barcelona, Spain.

⁵Department of Internal Medicine, Hospital Universitari Vall d'Hebron, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain.

P-11: Viral characterization as a tool for the treatment choice in patients with chronic hepatitis E virus (HEV) infection.

*Ruzo, SP.^a, Guerrero-Murillo, M.^a, Riveiro-Barciela, M.^b, Rando, A.^c, Gregori, J. ^d, Llorens-Revull, M. ^a, Soria, ME.^a, Rodríguez-Frías, F.^{ce}, Esteban, JI.^{aef}, Buti, M.^{bef}, Quer, J. ^{*aef}*

- a. Laboratorio de enfermedades Hepáticas-Hepatitis virales, Vall d'Hebron Institut de Recerca (VHIR)- Hospital Universitario Vall d' Hebron, Barcelona.
- b. Servicio de Medicina Interna-Hepatología. Hospital Universitario Vall d' Hebron, Barcelona
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- d. Roche Diagnostics S.L., Sant Cugat del Vallès.
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INVITED SPEAKERS' ABSTRACTS

OPENING LECTURE

In vitro studies of broad-spectrum antivirals against RNA viruses.

Santseharay Ramirez. *Copenhagen Hepatitis C Program (CO-HEP), Department of Infectious Diseases, Hvidovre Hospital and Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.*

Historically, development of antivirals has been hampered by the one virus, one drug paradigm, in which compounds are developed to be targeted against a specific virus. This approach has particularly limited the availability of treatments against emerging and re-emerging viral diseases. The use of potent antivirals active against multiple viruses, similarly to the use of broad-spectrum antibiotics in bacterial infections, represents an interesting alternative in the fight against viral disease. Thus, novel potent broad-spectrum antivirals are in preclinical and clinical investigations for the treatment of various viral diseases. However, their universal use might eventually promote the development of antiviral resistance. Thus, studies aimed at characterizing not only the potency and breadth of these antivirals but also their barrier to resistance are important to preserve their future efficacy.

Our studies focus on next-generation nucleos(t)ide analogs (nucs) with broad spectrum antiviral activity, aiming at systematically defining whether these drugs can prevent viral resistance or if viruses will rapidly evolve to develop resistance *in vitro*. For that purpose, we are using model viruses that cause emerging acute diseases, such as yellow fever (YFV) and tick-borne encephalitis (TBEV), as well as viruses leading to chronic disease, such as hepatitis C (HCV). From our panel of broad spectrum nucs, remdesivir exhibited the highest efficacy against YFV, TBEV, and HCV. For remdesivir, viral escape associated with decreased drug susceptibility was relatively fast for TBEV when compared to different genotypes of HCV. Unexpectedly, broad spectrum nucs remained highly efficient for the treatment of drug resistant HCV.

KEYNOTE LECTURE

Plant-infecting potyvirids: still surprises and unexpected findings.

Juan José López-Moya. Centre for Research in Agricultural Genomics, CRAG, CSIC-IRTA-UAB-UB, Cerdanyola del Vallès, Barcelona, Spain.

The family *Potyviridae* includes more than 200 plant-infecting virus species currently distributed into the largest genus *Potyvirus* (up to 175 members) and 9 more genera: *Bevemovirus*, *Brambyvirus*, *Bymovirus*, *Ipomovirus*, *Macluravirus*, *Poacevirus*, *Roymovirus*, *Rymovirus*, and *Tritimovirus* (ranging from 1 to 8 viruses in each genus). Common features shared by all members of the family, collectively known as potyvirids, include: i) their positive sense single-stranded (ss)RNA genomes, monopartite in all genera except for bipartite bymoviruses; ii) their elongated and flexuous rod-shaped viral particles; iii) the presence of pinwheel-shaped cytoplasmic inclusions in infected cells; and iv) their gene expression strategy, with a large polyprotein further processed by viral-encoded proteases, yielding 10 or more mature products.

The extraordinary variability of potyvirids make them attractive candidates for biotechnological applications in plant systems, such as the production of foreign proteins using virus-based expression vectors. For that, considerable efforts have been dedicated to understand their molecular biology. However, and despite more than a century of work with potyvirids, new discoveries were still awaiting. A paradigmatic example followed the finding of a conserved extra ORF, embedded out-of-frame in the P3 cistron, and encoding for PIPO (meaning 'Pretty Interesting Potyviral ORF'). Our group has contributed recently to reveal the expression mechanism of P3N-PIPO through RNA polymerase slippage occurring in a conserved $G_{1-2}A_6$ motif to generate genome variants with $G_{1-2}A_7$. Interestingly, editing of genomic templates with insertion of +1A was only described before in the genus *Ebolavirus*. Currently, we are pursuing the characterisation of similar mechanisms in other genome regions found in different potyvirids, where they could contribute to express additional truncated/overlapping gene products, such as P1N-PISPO. To summarize, potyvirids constitute a successful group of viruses with rather unique aspects in terms of molecular biology. Of course, this story is far from being complete: which will be the novel and unexpected features that we are yet to discover?

MEET THE EXPERT

The secret of hepatitis C virus culture development: efficient systems based on adaptation of patient derived genotype 1-6 strains.

Jens Bukh. *Copenhagen Hepatitis C Program (CO-HEP), Department of Infectious Diseases, Hvidovre Hospital, and Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.*

Hepatitis C virus (HCV), classified within the Flaviviridae family, is a small enveloped virus that causes liver cirrhosis and hepatocellular carcinoma. The health and economic burden of hepatitis C is enormous, with an estimated 500.000 deaths annually. Thus, availability of experimental systems is essential to study antivirals and vaccines. However, an efficient infectious system to study HCV in vitro, using human hepatoma derived cells, has only been available since 2005, and was initially limited to a single unique replication competent isolate, named JFH1. At the Copenhagen Hepatitis C Program (CO-HEP), we have adapted patient derived HCV strains of genotypes 1-6 to efficiently spread in Huh7.5 cells resulting in robust HCVcc recombinants with fast spread kinetics and relatively high infectivity titers.

Initially we generated full-length consensus clones of genotype 1a, 1b, 2a, 2b, 2c, 3a, 4a, 5a, and 6a patient strains. These genomes were consistently non-viable in vitro (negative for HCV antigen after transfection of RNA into Huh7.5 cells). However, by various methods of viral adaptation, using chimeric JFH1 genomes or mutations selected by various approaches, we have defined sets of adaptive mutations that allow culture of full-length and/or JFH1-based (genotype specific 5'UTR-NS2, 5'UTR-NS5A, Core-NS2, Core-NS5A, Core-NS5B, NS3/4A and NS5A) recombinants. Through cell culture adaptation of HCV, we have discovered substitutions that primarily rescue viral replication and/or assembly and release.

Robust and efficient infectious culture systems for HCV like the ones described here are highly relevant for studies of the viral life cycle, and for studies of resistance of HCV drugs.

HOT TOPICS IN 3D CELLULAR MODELS

Liver Organoids: Shaping the future of liver bioengineering for transplantation and beyond.

Pedro Baptista. *Laboratory of Organ Bioengineering and Regenerative Medicine, Health Research Institute of Aragón (IIS Aragón), Zaragoza, Spain. Health Research Institute of Fundación Jiménez Díaz (IIS FJD), Madrid, Spain. Department of Biomedical and Aerospace Engineering, Carlos III University of Madrid, Spain. Fundacion ARAID.CIBERehd.*

Liver transplantation is presently the only proven therapy able to extend survival for end-stage organ disease. It is also the only treatment available for severe acute liver failure and to some forms of inborn errors of metabolism. Nevertheless, the waiting list for organ transplantation is extensive and many patients will not survive long enough to receive an organ due to the dramatic shortage of donors or lack of eligibility. This distressing donor shortage is common to most solid organs like the lung, heart and particularly the kidneys. In light of the worrying situation of organ transplantation, our laboratory has developed novel methods to generate an entire liver scaffold from donor livers, using tissue decellularization. This same method is also able to decellularize other solid organs generating specific acellular kidney, lung, intestine, pancreas or heart scaffolds. These scaffolds can then be recellularized with vascular and specific tissue cells. Furthermore, we have been isolating and expanding fetal and adult liver stem cells in organoid cultures. These hepatic organoids, although immature, are able to recreate several features of adult liver tissue once properly differentiated in vitro in hepatic extracellular matrix (ECM). Finally, with our recent development of a novel method of large-scale cell expansion of liver progenitor organoids, we are now able to produce the necessary billions of cells to recellularize human-sized decellularized liver scaffolds for transplantation and generate large numbers of hepatic organoids suitable for drug discovery and basic research. Despite the multiple challenges, a path to the clinic will be described, truly shaping the future of liver bioengineering for transplantation and beyond.

ORAL ABSTRACTS

O-1

Identification of Host-derived Circular RNAs that Display Pro- and Anti-Viral Activities in Flavivirus-Infected Cells.

Tzu-Chun Chen^{1,5}, **Marc Talló-Parra**^{2,5}, Sebastian Kadener⁴, Rene Böttcher², Pakpoom Boonchuen³, Kunlaya Somboonwiwat³, Juana Diez^{2†} and Peter Sarnow^{1†}

¹ Department of Microbiology & Immunology, Stanford University SOM, Stanford, CA 94305, USA.

² Molecular Virology Group, Department of Experimental and Health Sciences, Universitat Pompeu Fabra, 08003, Barcelona, Spain.

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⁵ These authors contributed equally to this work.

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It is known that viruses subvert macromolecular pathways in the infected host to aid in viral gene amplification or to counteract innate immune responses. Recently, roles for host-encoded noncoding RNA, such as microRNAs, have been found to encode pro- and anti-viral functions. One class of noncoding RNAs are circular RNAs that are generated by a nuclear back-splicing mechanism of pre-mRNAs. This study examines the circular RNA landscape in uninfected and hepatitis C virus-infected liver cells. Results showed that the abundances of distinct classes of circular RNAs were up-regulated or down-regulated in infected cells. The identified circular RNAs displayed both pro- and anti-viral effects. One particular up-regulated circular RNA, cPSD3, displayed a minor inhibitory effect on HCV mRNA translation, but a very pronounced pro-viral effect on viral RNA replication. Surprisingly, cPSD3 inhibited the cellular nonsense-mediated decay (NMD) pathway in liver cells. Thus, enhanced abundance of cPSD3 in virus-infected cells aids in viral replication and likely contributes to the known viral core-mediated inhibition of NMD in infected cells. Findings from the global analyses of the circular RNA landscape argue that both pro- and anti-viral functions are executed by circular RNAs that modulate viral gene expression and host pathways. Because of their long half-lives, circular RNAs likely play hitherto unknown, important roles in viral pathogenesis.

Lack of association between novel astrovirus and diarrheic children and higher viral titer among asymptomatic controls.

Diem-Lan Vu^{1,2}, Aurora Sabrià¹, Núria Aregall¹, Kristina Michl¹, Jaume Sabrià³, Virginia Rodriguez Garrido⁴, Lidia Goterris⁴, Albert Bosch¹, Rosa M Pinto¹, Susana Guix¹

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² Department of Infectious Diseases, Geneva University Hospitals, Switzerland

³ Primary Health Care Center El Serral, Generalitat de Catalunya, Sant Vicenç dels Horts, Spain

⁴ Microbiology Department, Hospital Universitari Vall d'Hebron, Barcelona, Spain

Novel human astroviruses (HAstV) were discovered 10 years ago and have been associated with fatal cases of central nervous system infections. Their role in gastroenteritis is controversial, as they have been identified in symptomatic and asymptomatic subjects. The aim of the study was to investigate novel HAstV in a gastroenteritis case-control study including a pediatric population in Spain over a one-year period. We included stool samples from patients with gastroenteritis and negative results for viruses screened by routine diagnostics, and stool samples of control subjects who sought for a routine medical consultation. All samples were screened by real-time RT-PCR assays for novel HAstV. An additional screening for rotavirus, norovirus GI, GII, sapovirus, classic HAstV and adenovirus was also performed for the control group.

Overall, 23/363 stool samples from case patients (6.3%) and 8/199 stool samples from control patients (4%) were positive for ≥ 1 novel HAstV. MLB1 was predominant (64.5% of positives). Seasonality was observed for the case group ($p=0.015$), but not the control group ($p=0.95$). No difference was observed in the prevalence of novel HAstV between the case and control groups (OR 1.78, 95% CI 0.68-5.45; $p=0.30$). Nevertheless, MLB genome copy number/ml of fecal suspension was significantly lower in the control group than in the case group ($p=0.008$). Our study identified a lack of association between novel HAstV and gastroenteritis in children, which could indicate a potential role of reservoir for children, especially given the higher viral load observed in the asymptomatic group.

Inhibition of the DNA damage response enzyme DNA-PK increases the susceptibility to HBV infection in cell culture.

Pilar Gomollón Zueco¹ and Urtzi Garaigorta^{1,2}

¹Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain. ²CIBER de Enfermedades Hepáticas y Digestivas, Madrid, Spain.

Hepatitis B virus (HBV) represents an important human pathogen causing both acute and chronic hepatitis. It is estimated that over 240 million people are chronically infected and a million people die every year due to complications of HBV, including liver cirrhosis and hepatocellular carcinoma. Despite of our understanding of many aspects of the HBV life cycle, details of the identity and function of cellular and viral factors that regulate the establishment of infection are poorly understood. The recent identification of the HBV receptor (NTCP) and the establishment of a new cell culture HBV infection system suitable for genetic and chemical manipulation (i.e. HepG2-NTCP), allows the identification and characterization of cellular pathways regulating the infection. It is believed that the establishment of the infection is a highly regulated process where cellular DNA damage response (DDR) pathways may participate. To test this hypothesis we chemically manipulated DDR pathways and analyzed the effect on the establishment of the infection. We identified NU7441, a DNA-dependent protein kinase (DNA-PKc) inhibitor, as a compound that when used before the HBV inoculation increased the susceptibility of HepG2-NTCP cells to the infection. Pre-treatment with NU7441 led to increased levels of intracellular HBV cccDNA, and mRNA and extracellular antigen production, suggesting that DNA-PK restricts the infection. Moreover, shRNA-mediated silencing of XRCC5 and XRCC6, the regulatory subunits of the catalytic subunit of DNA-PK enzyme, showed similar effects. Collectively, these results suggest that activation of DNA-PK leads to changes in the cellular homeostasis that negatively affect the establishment of HBV infection.

Biotechnological strategies to obtain Virus-Like Particles (VLPs) of Torradovirus and Fabavirus, two plant viruses from the family *Secoviridae*.

Garcia-Mulet, Júlia¹; Ferriol, Immaculada¹; Ruiz, Tarik¹ and López-Moya, Juan José¹

¹Center for Research in Agricultural Genomics (CIRAG-CSIC-IRTA-UAB-UB), Campus UAB, Bellaterra, Barcelona 08193, Spain

The family *Secoviridae* comprises several genera of viruses, all of them with non-enveloped icosahedral particles. Within the family, viruses of the genera *Fabavirus* and *Torradovirus* have two ssRNA⁺ molecules which are translated as polyproteins, and processed into mature products by a viral-encoded protease present in the larger segment RNA1. The RNA2 encodes proteins needed for virus movement and encapsidation of virions. We have worked with tomato apex necrosis virus (ToANV), an isolate of *Tomato marchitez virus* (ToMarV, genus *Torradovirus*), and with an isolate of *Broad bean wilt virus 1* (BBWV-1, genus *Fabavirus*). Although both viruses have virions with a pseudo 3 triangulation number, the torradovirus has three coat proteins (CP) while the fabavirus has only two CPs. We wanted to explore for these viruses if their CPs have the ability to self-assemble and produce virus-like particles (VLPs) such as empty capsid structures that would be used to study their structural properties, and also serve as potential molecular carriers. Here we present some methods to obtain VLPs from the mentioned viruses, starting with the true identification of cleavage sites in the polyprotein leading to generate mature CPs by action of specific viral proteases, confirming suspected or previously described sites. The strategy designed includes plasmid constructs containing multiple virus proteins, either complete or partial, and molecularly tagged to test the hypothetical cleavage sites, previously predicted bioinformatically with assistance of the published available bibliography regarding other viruses within the family *Secoviridae*. The constructs are being prepared in different binary plasmids adequate for transient expression of the proteins in leaves of *Nicotiana benthamiana* plants, using agroinfiltration procedures. Next, we are planning to analyze through N-terminal sequencing the protein products generated, in order to confirm the predicted cleavage sites in the polyprotein. Moreover, the constructs will serve to evidence if a putative protease cofactor encoded in the virus genome is strictly necessary to successfully process the viral polyproteins. In a final step, the complete CPs will be expressed and purified to test their capacity to self-assemble into VLPs.

Work funded by project AGL2016-75529-R, "Severo-Ochoa" SEV-2015-0533.

Disruption of a RNA secondary structure in HIV-1 gp41 induces viral lethality.

Ana Jordan-Paiz, Maria Nevot, Kevin Lamkiewicz, Marie Lataretu, Sandra Franco, Manja Marz, Miguel Angel Martinez

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Synonymous genome recoding has been widely used to study different aspects of virus biology. Previous studies have demonstrated HIV-1 attenuation by reduction in protein expression after synonymous recoding. We aim here to explore the impact of synonymous codon usage on HIV-1 Env expression and virus replication capacity.

The codons AGG, GAG, CCT, ACT, CTC and GGG of HIV-1 *env* gene were synonymously changed to CGT, GAA, CCG, ACG, TTA and GGA, respectively. Different recoded *envs* were generated. Viral replication and viability was measured after transfection in MT-4 cells by quantifying HIV-1 p24 antigen production. Replication capacity assays were performed in MT-4 cells and PBMCs. WT, recoded *env* genes and HIV-1 *rev* were cloned in an expression vector (pcDNA3.1). Env expression plasmids were cotransfected with Rev expression plasmid in 293T cells. Immunoblot analyses and qPCR were performed to quantify protein expression and Env mRNA production. RNA secondary structures were obtained using Vienna RNA package.

A recoded *env* variant containing 39 mutations was lethal for the virus. WB analysis of Env expression revealed that protein expression of the recoded variant was highly reduced. To further study the mutations responsible for this phenotype, new mutants were designed by reverting substitutions to WT or reducing the number of newly generated CpG dinucleotides. Most of the new virus variants were viable, although they showed different replication capacities. Interestingly, one variant that only reverted two nucleotides that belong to the same codon showed indistinguishable replication capacity when compared to WT. Moreover, after transfection, other virus variants generated compensatory mutations next to this codon or reverted this codon to WT. Computational analyses revealed a severe disruption in a RNA secondary structure of variants containing this mutated codon. Importantly, the disrupted RNA structure was restored when this codon was reverted to WT or new mutations were introduced in the proximity.

We show here that codon usage of the HIV-1 *env* strongly impacts the replication capacity of the virus. Moreover, synonymous recoding of HIV-1 *env* gene has identified, in the gp41 coding region, an evolutionary conserved local RNA secondary structure that may be essential for virus viability. Disruption of this RNA secondary structure leads to severe reduction in mRNA translation and virus replication capacity.

Cytomegalovirus interferes with the ICOSL:ICOS axis to impair T cell co-stimulation.

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As part of their immune evasion strategies to remain latent in their hosts, cytomegaloviruses (CMVs) have evolved mechanisms to alter the function of antigen presenting cells (APCs). APCs play a main role triggering different arms of the adaptive immunity against pathogens. Indeed, induction of optimal T-cell responses depends on the interaction of T-cell costimulatory receptors with their ligands expressed on APCs. One important costimulatory receptor-ligand pair is ICOS-ICOSL. Here, we report that ICOSL is targeted during murine CMV (MCMV) infection of APCs. The use of a set of MCMV deletion mutants led to the identification of a unique viral gene responsible for the suppression of this molecule at the cell surface. We show that this viral protein interacts with ICOSL, preventing its trafficking to the cell membrane and redirecting it to lysosomal degradation. *In vitro* functional experiments revealed that the viral product is capable to dampen the ability of infected APCs to stimulate CD8⁺ T cell responses during antigen presentation. Furthermore, *in vivo* assays injecting an anti-ICOSL antibody to infected mice led to a significant decrease in both T helper follicular cells and germinal center B cells, impairing the generation of MCMV-specific antibodies. Notably, a reduction of neutralizing antibodies was also observed. These results demonstrate that ICOSL-dependent costimulation is critical for the antiviral immune response to MCMV infection. Finally, our findings that additional herpesviruses have also developed strategies to efficiently target ICOSL suggest that the blockade of the ICOSL:ICOS axis is a broader immune evasion tactic crucial in the maintenance of life-long viral persistence.

A virome study on field-collected mosquitoes uncovers new insect-specific viruses circulation in Europe.

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To assess new alternatives for arthropod-borne viral diseases surveillance, as well as, the detection of new viruses, honey-soaked FTA cards were used as sugar bait in mosquito traps during entomological surveys at the Llobregat River Delta (Catalonia, Spain) and subjected to Next Generation Sequencing (NGS) metagenomics analysis. The virome present in FTA cards was characterized and virus-specific primers were designed from sequences corresponding to mosquito-associated viruses. Circulation of Alphamesonivirus, Bunyaviridae environmental virus, Dezidougou virus and Wuhan-like Mosquito virus was confirmed in culicid mosquitoes linked to the FTA cards subjected to metagenomics. Phylogenetic analysis revealed that the viruses herein detected are related with viruses previously reported in other continents. To our knowledge, our findings constitute the first distribution record of these viruses in European mosquito species. In conclusion, NGS applied on honey soaked FTA cards showed to be a valid approach for the detection of circulating viruses in mosquitoes and set up a new tool for arbovirus surveillance and control programs.

Virological characterisation of the hepatitis C virus epidemic in people who inject drugs by using dried blood spot samples.

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Background and Aims: Incident hepatitis C virus (HCV) cases identified in Europe occur mostly among people who inject drugs (PWID). The identification and characterization of recent infections is necessary for understanding mechanisms of current HCV transmission in this population. Dried blood spot (DBS) samples have demonstrated their usefulness in facilitating the HCV diagnosis and could help to characterize the local epidemic among PWID. Thus, we aimed to (i) to validate the use of DBS samples for the assessment of HCV genetic variability both inter- and intra-host by next-generation sequencing (NGS), (ii) to explore key HCV epidemiological parameters, including circulating subtypes, and acute infections and transmission clusters.

Method: HepCdetect II is a cross-sectional study of 410 active PWID in four harm reduction settings in Barcelona. DBS were collected from all participants, and plasma was additionally collected in 300 cases. DBS were qualitatively tested for HCV RNA [Saludes V, J

Viral Hep 2018], and viral loads were quantified from plasma (Abbott). For positive samples, a 389-bp region of the HCV NS5B gene was amplified. Sequence libraries were indexed and subjected to Illumina paired-end sequencing (2x250 cycles, MiSeq). Inter and intra-host HCV genetic variability estimates and cluster analysis based on patristic distances were performed from NGS data [Montoya V, Infect Genet Evol 2016]. NGS was performed from plasma when available or from DBS when not; for 16 participants both plasma and DBS were sequenced.

Results: Excluding samples with viral loads <10,000 UI/mL, 93.4% of 183 plasmas were successfully characterized by NGS. Additionally, 83.1% of 59 DBS samples were also sequenced. The major circulating subtypes were 1a (37.1%) and 3a (32.7%), followed by 1b (16.3%) and 4d (6.9%). Five mixed infections were identified. The genetic variability estimates (Shannon entropy and single nucleotide variants) were not significantly different between plasma-DBS pairs. Based on Shannon entropy measures observed in acute and chronic infection controls, 29/215 (13.5%) viremic participants excluding mixed infections were identified as acute, and 103/220 (46.8%) were identified as part of a transmission cluster.

Conclusion: DBS represent a valuable tool for facilitating HCV diagnosis, and also for characterising and monitoring HCV epidemics, since they can be successfully used to identify acute HCV infections for incidence estimation, and transmission clusters. HCV preventive strategies directly targeted to PWID populations currently experiencing ongoing HCV transmission could inform public health interventions.

Resistance to Cucumber mosaic virus: a matter of relocating CmVPS41 a protein involved in intracellular trafficking.

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Cucumber mosaic virus (CMV) is one of the plant viruses with the broadest host range. The Spanish melon (*Cucumis melo* L.) cultivar Piel de Sapo (PS) is susceptible to all CMV strains, producing a systemic infection. However, the Korean cultivar Songwhan Charmi (SC) encodes one gene, *cmv1*, which confers resistance only to CMV strains from subgroup II (SG II), but not to subgroup I (SG I) strains. In the lines containing *cmv1*, the SG I strain FNY is able to reach the phloem and develop a systemic infection, whereas SG II strain LS can replicate, and move cell to cell but it is restricted in the bundle sheath cells (BS) and does not reach the phloem (Guiu-Aragonés et al, 2016). The viral virulence factor that communicates with *cmv1* is the Movement Protein (MP) (Guiu-Aragonés et al, 2015). *cmv1* encodes a Vacuolar Protein Sorting 41 (CmVPS41), a protein involved in intracellular trafficking to the vacuole, which carries a single L to R amino acid change causing the resistance in the SC genotype (Giner et al, 2017). We have examined the relationship between CmVPS41 and the viral MP by studying their cellular localization. CmVPS41 from PS (susceptible) and SC (resistant) genotypes show significant differences in their localization pattern, with structures such as nuclear speckles, membrane dots and transvacuolar bridges that are numerous in CmVPS41PS, but in CmVPS41SC there are much fewer. These CmVPS41 characteristic structures co-localize with the late endosome. Moreover, CmVPS41 from the exotic resistant melon accessions Freeman's Cucumber and Pat-81, harbouring the causal L to R mutation for resistance, show a pattern similar to that of CmVPS41SC. Finally, the presence of MP from CMV-FNY produces changes in CmVPS41SC localization, which becomes similar to the susceptible CmVPS41PS localization pattern. Those changes are not produced in the presence of MP from CMV-LS. Therefore, this suggests that those structures are involved in susceptibility to CMV.

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DNA viruses in colorectal polyps.

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Several DNA viruses have been reported to occur in colorectal cancer (CRC) and their role as a cofactor in this malignancy remain unclear. Human papillomaviruses (HPV) and adenoviruses (HAdV) are prevalent in environmental samples and, as a consequence, in food and drinking water sources. This study aims to investigate the presence of these viruses in colorectal polyps that might be precursors of CRC.

The detection and characterization of these viruses was assessed by specific quantitative PCR (qPCR), nested PCR (nPCR) and subsequent sequencing. Colorectal polyp formalin-fixed paraffin-embedded (FFPE) samples were obtained from routine colonoscopies from CIMA hospital in Barcelona and 148 samples were analyzed. In addition, 13 raw sewage samples were collected from a wastewater treatment plant in the North of Barcelona over one year. Viral particles from sewage samples were concentrated and DNA was extracted. Total DNA was also extracted from 20 μ m sections of polyp samples. A qPCR amplifying the human house-keeping gene β -globin was run in all polyp samples as an endogenous control. All polyp samples were analyzed by HAdV qPCR. All sewage samples and 32 polyp samples were analyzed by α -HPV, β -HPV and HAdV nPCR. Amplicons obtained by nPCR were purified and sequenced.

Regarding sewage samples, 30,77% samples were positive for α -HPV (4/13), 84,62% samples were positive for β -HPV (11/13) and 69,23% samples were positive for HAdV (9/13). Quantitative PCR showed all polyp samples containing β -globin and 7,43% being positive for HAdV (11/148) in a range from $6,59 \times 10^1$ to $5,80 \times 10^2$ GC/20 μ m section. Regarding nPCR assays, 9,37% polyp samples were positive for α -HPV (3/32) and 28,12% were positive for β -HPV (9/32). Nucleotide sequences obtained from HAdV and HPV nPCR amplicons from both sewage and polyp samples showed different serotypes being present including carcinogenic ones.

HPV and HAdV serotypes detected in colorectal polyps have been identified also in urban wastewater and environmental fecal contamination could be a source of viruses potentially associated to the development of polyps and CRC. These preliminary results suggest a new role of the infections of intestinal cells by these viruses, some of them with oncogenic properties, or, alternatively, viral DNA uptake by these cells. Further studies should be conducted to elucidate if these viruses play a role in the transformation of normal colonic tissue to colorectal polyps or CRC.

Persistent transcriptional alterations after hepatitis C virus elimination in cell culture.

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Chronic hepatitis C virus (HCV) infection causes liver inflammation and fibrosis, which can lead to development of cirrhosis and hepatocellular carcinoma (HCC). Recent approval of direct acting antiviral (DAA) drug combinations has revolutionized antiviral therapy against HCV. These drugs enable virus eradication in virtually all treated patients, regardless of the genotype and liver disease status. Based on clinical parameters, it has been proposed that elimination of infected cells by reactivated immune responses may be dispensable for virus eradication, in contrast to previously used interferon-based therapies. It is thus formally possible that patients that are declared cured, indeed carry formerly infected cells that display irreversible alterations due to prolonged chronic HCV infection.

Although transcriptional profiles of biopsies from cured patients have been previously studied, it is difficult to determine the precise mechanisms by which permanent alterations are established in the context of a complex tissue often in patients with an underlying liver disease. Thus, we used cell culture models of persistent HCV infection to determine if HCV infection causes permanent transcriptional alterations in host cells after virus eradication. In these models, HCV infection causes profound alterations of host cell transcriptome, that aim at regaining homeostasis in the context of intracellular membrane rearrangements, interference with homeostatic processes and persistent stress conditions permitting cell survival even though the virus has completely colonized the host cell. In this context, we asked the question of whether the original homeostasis and original transcriptomic profile is regained in the formerly infected cells after DAA treatment-mediated virus eradication. Our results indicate that a minor subset of transcriptional alterations persists even after virus eradication, suggesting that DAA-mediated eradication does not ensure normalization of formerly infected cell homeostasis. Combined analysis of the transcriptional profiles in proliferating and growth arrested cells suggest that several mechanisms underlie the establishment of permanent alterations.

Super-resolution microscopy as a powerful tool for understanding the formation and inhibition of influenza virus structures in mammalian cells.

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Super-resolution microscopy is a powerful tool that has the ability to study fluorescence samples beyond the diffraction limit, achieving resolutions around 20 nm. The study of viruses can greatly benefit from super-resolution due to their small average size, between 50 and 200 nm. Here we show that, with the correct labeling and sample preparation, we are able to identify two relevant viral structures: filament formation of influenza using super-resolution microscopy (STORM) and virus-like particles formed from influenza using DNA-PAINT.

Influenza A virus is highly pleomorphic, and virions can have either spherical or filamentous morphology. Influenza A virus strain A/Udorn/72 (H3N2) produces copious amounts of long and thin filaments on the surface of infected cells, led mainly by the matrix protein M1 and the membrane protein M2. However, due to the small size of these filaments (200 nm of width), they are hard to characterize in detail using immunofluorescence microscopy.

Here, we show with super-resolution microscopy that the ion channel M2 is localized in basilar part of filaments on the membrane of infected cells. Also, filament formation was inhibited by the treatment of cells with M2e-specific IgG2a and IgG1 antibodies but was not inhibited with the isotype control antibodies.

Additionally, the M2e-specific IgG1 and IgG2a antibodies can reduce influenza A/Udorn/72 (H3N2) virus plaque growth and infectivity *in vitro*.

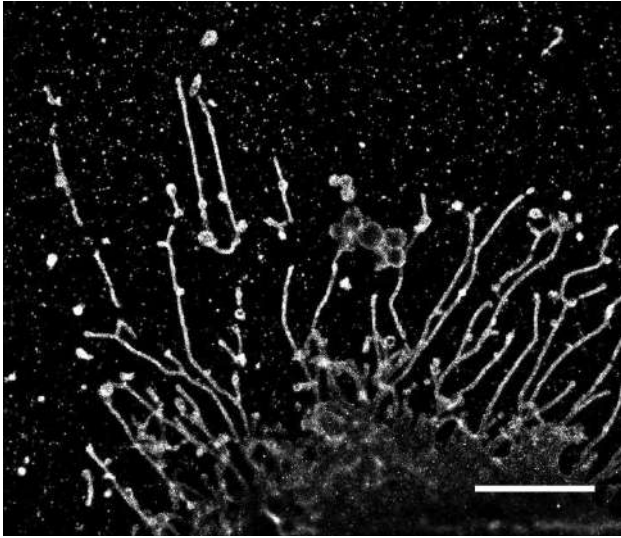


Figure 1: Filaments of influenza A Udorn strain produced on MDCK cells using STORM. Scale bar 5 μm .

Our results¹ demonstrate that M2e-specific IgGs reduces the level influenza A virus replication *in vitro* and suggest that the inhibition of virus replication lead by M2e-specific antibodies is due to the fragmentation of filamentous virions and the loss of filament formation from the surfaces of infected cells.

Moreover, we study virus-like particles produced from influenza proteins transfected on mammalian cells. These structures are exactly as virus particles but they lack viral genetic material, for this reason they are the perfect model to study influenza particles without risks. Influenza expresses 3 different proteins on the surface of the particle and the distribution and homogeneity between particles is not well understood. To study this distribution, we are analyzing with DNA-PAINT the differential expression and distribution of these 3 proteins on the surface of the particles.

Overall we show how super-resolution is suitable to study nanoscale viral structures and can provide new insights into anti-viral therapies.

1-“Super-resolution microscopy reveals significant impact of M2e-specific monoclonal antibodies on influenza A virus filament formation at the host cell surface.” Maria Arista-Romero*, Annasaheb Kolpe*, Bert Schepens, Silvia Pujals, Xavier Saelens, and Lorenzo Albertazzi. Scientific Reports, 9(1), 4450, 2019. <http://doi.org/10.1038/s41598-019-41023-5>

Early type I IFN response dynamics determines infection fate decision.

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Acute viral infections are usually resolved within a few weeks. By contrast, persistent infections, such as those caused by HIV and HCV, are not resolved and develop when immune responses are not sufficient to eliminate the invading virus. Type I interferons (IFN-I) play a critical role in shaping the antiviral immune response early after an infection. However, the dynamics by which different immune cell subsets regulate the IFN-I response during the early stages of acute and chronic infections is not completely understood. Here we used the Lymphocytic Choriomeningitis Virus (LCMV)-infection mouse model system to characterize the dynamics of the IFN-I response in acute and chronic infections. Time-resolved spleen-transcriptomes of mice were determined by RNA-seq to analyze the expression kinetics of IFN-I genes. Interestingly, during an acute infection, IFN-I showed two peaks of expression at days 2 and 5 post-infection (p.i.). In contrast, in chronically infected mice, only a single peak of IFN-I genes appeared at day 1. Further analysis revealed that IFN-I genes in acute infection were co-expressed with genes related to inflammatory macrophages, suggesting an important role of these cells determining infection fate. Indeed, we identified metallophilic macrophages as an important source of *Ifnb* only during acute infection and demonstrated that the subsequent IFN-I receptor (IFNAR) signaling is necessary to induce pro-inflammatory macrophages. In contrast, during chronic infection, early depletion of marginal zone macrophages results in a lack of IFN-I production and the inflammatory response is not induced. Importantly, blockage of the second peak of IFN-I response by IFNAR blockage during an acute infection also resulted in exhaustion of virus-specific CD8 T cells and appearance of lymphoid tissue fibrosis. Further studies are ongoing to decipher the regulatory mechanisms underlying the characterized events, thus revealing universal concepts related to infection fate decisions that are also relevant for persistent human infections such as HIV or HCV.

Supramolecular arrangement of the Zika virus NS5 protein and its role in vivo: beyond the RdRP activity.

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Zika virus (ZIKV) has explosively emerged over the past years, causing a series of epidemics across the western world. Neonatal microcephaly associated with ZIKV infection has already caused a public health emergency of international concern. As with other members in the *Flaviviridae* family, ZIKV relies on its non-structural protein 5 (NS5) for RNA capping (by methyltransferase N- terminal domain) and genome replication (by RNA-dependent RNA polymerase, (RdRP) domain), being an attractive crystallisable and antiviral target.

The crystal structures of the ZIKV NS5 protein in two different space groups presented in this work evidenced conserved protein self-interactions to form dimers and also higher order fibrillar oligomers. Complementary biophysical analyses by small-angle X-ray scattering (SAXS), analytical ultracentrifugation (AU), mass spectrometry, negative staining TEM, and atomic force microscopy (AFM) have been employed to further characterize the NS5 assemblies and verify their existence in solution. Additionally, point and deletion mutations in NS5 allowed us to confirm the interfaces between NS5 monomers within a dimeric structure, as well as the dimer–dimer interactions to form higher-order assemblies that have less RdRP activity than its monomeric mutant counterpart.

In order to extend our findings, we analyse the effects of ZIKV NS5 and mutants *in vivo* showing that this protein is able to affect cell division of neural progenitor cells (NPC) in model embryos. We identified NS5 host partners that prompted us to trace a molecular mechanism in which NS5 contributes to the microcephaly phenotype observed in virus infection. We also identified that the nonomeric mutant of NS5 is unable to produce the ciliopathy.

The quaternary arrangement of ZIKV NS5 provides a model to explain the coordination between the different protein activities, and paves the way for exploring new structure-based inhibitors that would interfere with the intermolecular NS5–NS5 interactions.

POSTER ABSTRACTS

P-1

Determining the extended host range of the crinivirus *Sweet potato chlorotic stunt virus* (SPCSV).

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Plant viruses cause diseases of great economic importance. Sweet potato (*Ipomea batatas*, family *Convolvulaceae*), the third most important root crop worldwide, is significantly affected by many viral pathogens due to its vegetative propagation. The most devastating and well characterised disease of sweet potato is a synergistic mixed infection of the potyvirus *Sweet potato feathery mottle virus* (SPFMV) and the crinivirus *Sweet potato chlorotic stunt virus* (SPCSV). While the potyvirus has been extensively studied, the information available for the crinivirus is still scarce. SPCSV is a phloem limited virus transmitted uniquely by the whitefly *Bemisia tabaci* in a semi-persistent manner, two features that act as limiting factors for experimental studies requiring virus management.

In our current project we are interested in the characterisation of the biological properties of SPCSV and its dynamics in mixed infections. To determine the natural host range of the virus, an experimental inoculation system was tested in different plants using virouliferous *B. tabaci* vectors MEAM 1 or MED (formerly biotypes 'B' and 'Q', respectively), followed by a sensitive RT-PCR diagnostic protocol. So far, transmissibility rates of SPCSV were assayed in 9 species belonging to 5 different botanical families, and our preliminary results revealed that the virus was efficiently transmitted to several new hosts not described before, expanding the range of susceptible plant species beyond the convolvulaceae family. In many cases infections were symptomless, at least in our conditions, and the virus presence required confirmation through molecular detection, which could explain why all these potential hosts have remained unnoticed until now.

Our results expand the host range of SPCSV to new plant species, although further experiments and surveys will be required to determine the incidence in natural conditions. Nevertheless, the possibility of these and other new hosts acting as reservoirs of SPCSV must be considered among the epidemiological risks for the dissemination of the virus and the diseases in which it participates.

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P-2

Position-specific OM-pBAE polymer modification of oncolytic adenovirus to improve systemic delivery.

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Adenoviruses are common pathogens widely used in gene therapy as delivery vectors or in the treatment against cancer as oncolytic viruses. However, pre-existing humoral immunity represents a major limitation for systemic delivery. The presence of anti-adenovirus neutralizing antibodies (Nabs), especially for the most commonly used adenovirus (Ad5) is very high in the population. Several strategies have been explored to help the virus escape from Nabs, however many of them have led to a reduction in the viral infective capacity. The site-specific genetic modification of the hypervariable region 1 (HVR1) of the hexon adenoviral protein offers the possibility to covalently link polymers and shield the virus. We have explored the capacity of oligopeptide end-modified pBAEs (OM-pBAEs) to overcome the limitations associated to adenoviral systemic delivery. We have engineered an adenoviral genome containing the EGFP gene with the specific modification in the HVR1 region. A battery of adenoviruses with OM-pBAEs and polietilenglicol (PEG) linked at different ratios and by different reactive groups were generated. The coated viruses form discrete nanoparticles, maintain their infective capacity and effectively replicate. Preliminary results show that OM-pBAEs/PEG partially protect adenovirus from neutralizing antibodies and the degree of PEGylation is a key factor.

Validation of VIASURE commercial one-step real-time RT-PCR kits targeting Human Astrovirus and Sapovirus for the diagnostic of gastroenteritis

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Human Astroviruses (HAstV) and Sapoviruses (SaV) are two main causes of acute viral gastroenteritis, especially in children. The aim of the study was to validate VIASURE Real Time PCR Detection Kits to detect HAstV and SaV in stool samples. VIASURE Real Time PCR Detection Kit contains in each well all the components necessary for the reaction in an stabilized format that can be stored at room temperature, as well as an internal control to monitor PCR inhibition. Performance of VIASURE kits was compared to available published “in-house” RTqPCR assays and other available commercial kits. A total of 216 stool samples from sporadic cases of gastroenteritis in children ≤ 5 years old collected between January and April 2016 were included. All samples were negative for Rotavirus, Adenovirus and HAstV by immunochromatography; 96,7% of them had been tested and confirmed negative for common bacterial pathogens; and 31% of them had been tested and confirmed negative for parasites. HAstV testing was performed following a reference published protocol (Sano *et al.*, 2009), a reference commercial kit (RIDA®GENE Viral Stool Panel II, R-Biopharm), and the VIASURE Astrovirus Real Time PCR Detection Kit (Certest Biotec). SaV testing was performed following a reference published protocol (Sano *et al.*, 2011), a reference commercial kit (RIDA®GENE Sapovirus, R-Biopharm), and the VIASURE Sapovirus Real Time PCR Detection Kit (Certest Biotec). Discrepancy analysis was performed for any conflicting results repeating all used RT-PCR assays performed in triplicate. For HAstVs, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the VIASURE Astrovirus Real Time PCR Detection Kit were calculated to be 88%, 100%, 100% and 99%, respectively as compared to the published protocol; and 100%, 100%, 100% and 100%, respectively as compared to the other commercial kit. For SaVs, sensitivity, specificity, PPV and NPV of the VIASURE Sapovirus Real Time PCR Detection Kit were calculated to be 100%, 98%, 81% and 100%, respectively as compared to the published protocol; and 100%, 99%, 91% and 100%, respectively as compared to the other commercial kit. Because of the high sensitivity and specificity of the assays with a rapid and simple procedure, the VIASURE RT-PCR assays will be useful as routine assays for the clinical diagnosis of HAstV and SaV infection.

Non-integrating retrovirus improves Cre-recombination driven cell tracking using Brainbow2.1/Confetti mice

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Cell tracking models have gained much interest to understand clonal lineage relationships in stem cell differentiation studies. Even though increasingly complex genetic barcoding methods are being developed, little consideration is devoted to the required PCR purification steps on top of phenotypic analysis. Hence fluorescent markers offer a simpler and low-cost alternative to demonstrate clonal relationships via spatio-temporal confocal imaging and flow cytometry.

The Brainbow2.1/Confetti mice are well known for their elegant stochastic expression of multiple fluorescent proteins (XFPs). The *Brainbow2.1* DNA cassette offers four color possibilities upon Cre-recombination per allele insertion into the genomic DNA. Prior studies with this *in vivo* mouse model have been unsuccessful to fully benefit the full marking potential, most likely due to inadequate Cre enzymatic activity. Conditionally expressed

Cre models (ERT2-Cre) or promoter-driven Cre expression have shown to be suboptimal with relatively low XFP marking.

In the present study we show the potential of a homozygous *R26R-Confetti* mouse model in combination with a gammaretroviral iRV-Cre-GFP vector to successfully express 10 XFPs by recombination. This approach allows for efficient recombination of all potential XFPs and sufficient clonal tracking resolution for early B- and T-cell development and other lineage relationships of blood cells.

The use of a gammaretroviral vector to drive *Brainbow2.1* cassette XFP expression offers the desired near-equal ratio for the four primary colors (Green, Yellow, Red and Blue). In fact, our data reports a low fraction of double XFP expressing cells following Cre expression, indicating a decreased XFP expression probability according to recombination event complexity.

Our approach shows the possibility of enhancing an existing cell tracing model with a more efficient gammaretroviral Cre recombination method. Previously reported XFP limitations were not observed, demonstrating its application for complex recombination strategies.

Super-resolution microscopy as a powerful tool for understanding the formation and inhibition of influenza virus structures in mammalian cells.

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Super-resolution microscopy is a powerful tool that has the ability to study fluorescence samples beyond the diffraction limit, achieving resolutions around 20 nm. The study of viruses can greatly benefit from super-resolution due to their small average size, between 50 and 200 nm. Here we show that, with the correct labeling and sample preparation, we are able to identify two relevant viral structures: filament formation of influenza using super-resolution microscopy (STORM) and virus-like particles formed from influenza using DNA-PAINT.

Influenza A virus is highly pleomorphic, and virions can have either spherical or filamentous morphology. Influenza A virus strain A/Udorn/72 (H3N2) produces copious amounts of long and thin filaments on the surface of infected cells, led mainly by the matrix protein M1 and the membrane protein M2. However, due to the small size of these filaments (200 nm of width), they are hard to characterize in detail using immunofluorescence microscopy.

Here, we show with super-resolution microscopy that the ion channel M2 is localized in basilar part of filaments on the membrane of infected cells. Also, filament formation was inhibited by the treatment of cells with M2e-specific IgG2a and IgG1 antibodies but was not inhibited with the isotype control antibodies.

Additionally, the M2e-specific IgG1 and IgG2a antibodies can reduce influenza A/Udorn/72 (H3N2) virus plaque growth and infectivity *in vitro*.

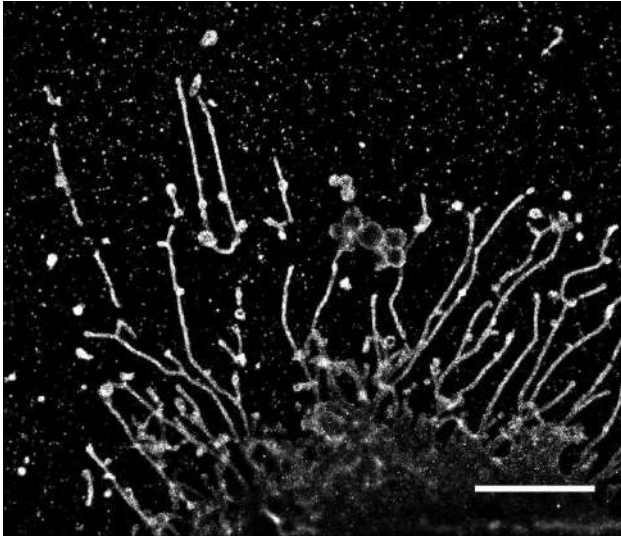


Figure 1: Filaments of influenza A Udorn strain produced on MDCK cells using STORM. Scale bar 5 μm .

Our results¹ demonstrate that M2e-specific IgGs reduces the level influenza A virus replication *in vitro* and suggest that the inhibition of virus replication lead by M2e-specific antibodies is due to the fragmentation of filamentous virions and the loss of filament formation from the surfaces of infected cells.

Moreover, we study virus-like particles produced from influenza proteins transfected on mammalian cells. These structures are exactly as virus particles but they lack viral genetic material, for this reason they are the perfect model to study influenza particles without risks. Influenza expresses 3 different proteins on the surface of the particle and the distribution and homogeneity between particles is not well understood. To study this distribution, we are analyzing with DNA-PAINT the differential expression and distribution of these 3 proteins on the surface of the particles.

Overall we show how super-resolution is suitable to study nanoscale viral structures and can provide new insights into anti-viral therapies.

1- "Super-resolution microscopy reveals significant impact of M2e-specific monoclonal antibodies on influenza A virus filament formation at the host cell surface." Maria Arista-Romero*, Annasaheb Kolpe*, Bert Schepens, Silvia Pujals, Xavier Saelens, and Lorenzo Albertazzi. Scientific Reports, 9(1), 4450, 2019. <http://doi.org/10.1038/s41598-019-41023-5>

Differential roles of lipin1 and lipin2 in the hepatitis C virus replication cycle.

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Although their origin, nature and structure are not identical, a common feature of positive-strand RNA viruses is their ability to subvert host lipids and intracellular membranes to generate replication and assembly complexes. Recently, lipin1, a cellular enzyme that converts phosphatidate into diacylglycerol, has been involved in the formation of the membranous web that hosts hepatitis C virus (HCV) replicase. Lipin1 cooperates with lipin2 in the liver to maintain glycerolipid homeostasis. We extended our previous study of the lipin family on HCV infection, by determining the impact of the lipin2 silencing on viral replication. Contrasting with the specific impact of lipin1 silencing on HCV replication, our data suggest a broader function of lipin2 not only for HCV infection, but also for replication of other RNA viruses. Moreover, lipin2-, but not lipin1-deficient cells display alterations in mitochondrial and Golgi morphology, suggesting that lipin2 contributes to the maintenance of the overall organelle architecture. Coinciding with Golgi fragmentation, our data reveal that lipin2 silencing mainly interferes with HCV virion secretion at late stages of the infection, without significantly affecting viral replication or assembly. Overall, this study reveals distinctive functions of lipin1 and lipin2 in cells of hepatic origin, a context in which they are often considered functionally redundant.

Analysis of Regulatory T-Cell Frequency and Phenotype in Chronic Hepatitis C patients Undergoing Interferon-Free Therapies.

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Background and hypothesis: Chronic hepatitis C infection has been associated with elevated frequency of CD4⁺ regulatory T cells (Tregs). High Tregs count contributes to antiviral immunity inhibition and promotes liver fibrosis. Interferon (IFN)-free therapies rapidly eliminate hepatitis C virus (HCV) regardless the degree of fibrosis/cirrhosis state. Since the liver plays a major role in maintaining immune system homeostasis, we hypothesize that the degree of liver disease may have an impact on Tregs frequency and phenotype even after successful IFN-free therapies.

Objective: To characterize the potential changes of Tregs in HCV-infected patients stratified by the fibrosis/cirrhosis state during and after IFN-free therapies.

Methods and design: Frequency and phenotype of peripheral CD4⁺CD25⁺Foxp3⁺ Tregs were analyzed by flow cytometry in HCV-infected patients with (n=21) and without (n=31) cirrhosis, at baseline, week 4 during therapy (W4) and, weeks 12 and 48 after the end-of-therapy (FU12 and FU48, respectively). Samples obtained from 12 healthy individuals and 19 cirrhotic patients from other aetiologies served as controls.

Results: At baseline, frequencies of CD4⁺CD25⁺Foxp3⁺ Tregs in HCV patients with cirrhosis were elevated in comparison to healthy controls (mean 3.49% vs. 1.03%, $P=.0023$). Furthermore, the frequency of Tregs in cirrhotic patients from other aetiologies did not differ from that of HCV patients with cirrhosis. Strikingly, the frequency of Tregs in HCV patients without cirrhosis did not significantly differ from that of healthy controls (0.83% vs. 1.03%, $P= ns$). Longitudinal analysis revealed that Tregs frequency did not normalize in HCV patients with cirrhosis even at FU48. Phenotypical analysis of the naïve marker CD45RA, memory marker CD45RO, inhibitory receptor CTLA-4 and activating molecule HLA-DR showed that only CD45RO-expressing Tregs were elevated in HCV patients (with and without cirrhosis) at all time-points compared to healthy controls.

Conclusions: Tregs frequency persisted elevated for several months after HCV eradication in cirrhotic patients, which may play a role in maintaining altered immunosurveillance and inhibiting fibrosis/cirrhosis regression. Furthermore, since the elevated expression of the CD45RO also persisted in HCV patients without cirrhosis, these data suggest that HCV may have a sustained imprint in Tregs phenotype post IFN-free therapies.

Clinical and virological predictors of response after antiviral therapy interruption in HBeAg-negative chronic hepatitis B

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Introduction: The possibility of stopping nucleos(t)ide analog (NA) therapy in virologically suppressed HBeAg-negative (HBeAg-) patients has been considered in the recent European clinical guidelines. However, the variables predicting a successful discontinuation of NA in these patients have not been defined yet. Consequently, our aim is to investigate the epidemiological, virological and clinical factors associated to a successful NA therapy discontinuation in HBeAg- patients.

Material & methods: Prospective study including non-cirrhotic patients with HBeAg- chronic hepatitis B with viral suppression for more than 3 years under NA therapy. Patients underwent a liver biopsy at inclusion to exclude the presence of advanced fibrosis and to analyze total intrahepatic HBV-DNA (iHBV-DNA) and cccDNA by an optimized qPCR. Standard liver tests with viral serum markers (HBV-DNA, pgRNA, HBsAg and HBcrAg) were quantified at 1-month intervals after NA discontinuation.

Results: Twenty-eight patients have been included. Most were male (79%) and infected by genotype D (79%) with a median age of 56 years-old. The median duration of NA therapy was 8 (IQR 6-13) years, 20 (71%) received tenofovir. At baseline median HBsAg levels were 1439 IU/mL (IQR 558–3379) and correlated significantly with iHBV-DNA levels ($\rho = 0.7$, $p < 0.001$), but not with cccDNA, pgRNA or HBcrAg levels. Moreover, there was an association between HBcrAg and pgRNA levels in serum at baseline. After a median follow-up of 105 weeks, 23 patients (82%) remained off-therapy and 5 of them (18%) presented HBsAg loss. ALT [>3 UNL (40U/L)] and DNA peaks ($>20,000$ IU/ml) were frequently observed during follow-up in 40% and 50% of patients, respectively. Patients with HBsAg loss had significantly lower baseline HBsAg (63 vs 2122 IU/ml, $p < 0.01$) and iHBV-DNA levels (0.04 vs 0.98 copies/cell, $p < 0.01$). Of note, no adverse events related to therapy discontinuation were observed.

Conclusion: Antiviral therapy discontinuation is feasible in a high proportion of HBeAg- Caucasian patients under NA therapy. Interestingly, low HBsAg and iHBV-DNA levels are associated with a high likelihood of HBsAg loss, whereas cccDNA, pgRNA or HBcrAg levels not.

Conservation and amino acid changes in hepatitis B core gene in chronic hepatitis patients at different clinical stages.

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Background: The Hepatitis B Virus (HBV) HBc protein (encoded by the *HBC* gene) is a structural protein that covers functional activity, controlling viral expression and interfering with the cellular activity. The aim of this study is to compare *HBC* and HBc sequence conservation in a group of patients at different clinical stages.

Methods: The study included 39 chronic hepatitis B patients with viremia >3.8 log IU/mL, grouped according to their clinical stages: chronic hepatitis (CHB, n=17), hepatocarcinoma (HCC, n=17), and liver cirrhosis (LC, n= 5). A genome region between nucleotide (nt) 1863 and 2483 (including *HBC* gene) was analyzed by Next Generation Sequencing (NGS). Conservation was obtained by calculating the information content of each nt or aminoacidic (aa) position in the multiple alignment of all the unique sequences (haplotypes) collected. The presence of aa changes was studied by aligning the haplotypes with their genotype-specific consensus.

Results: In HCC group the HBC was usually conserved at both nt and aa level. CHB showed a limited nt sequence conservation (mainly between nt 1960-1980 and 2070-2090) accompanied by a high aa conservation. In these 2 groups the nt changes usually involved the 3rd nt of the aa codon. Contrarily, in LC group a high nt conservation was observed, followed by a low aa conservation. In these patients the nt substitutions usually involved the 1st or the 2nd nt of the aa codon.

Some nt hyperconserved regions were shared by all 3 groups (nt 1900-1932; 2252-2281; 2369-2398) and also one aa region (the first 29 aa of HBc). Two group-specific aa hyperconserved regions had been detected: between aa 165-167 in CHB, and aa 170-175 in HCC. The mutation P79Q was significantly detected in HCC group (median of frequency 15.8, pvalue <0.05 related to CHB group).

Conclusion: HBc sequence in HCC patients was usually conserved. In LC, although a high nt sequence conservation was observed, the presence of nt substitutions involving the first two nt in the codons generated a low conserved HBc protein sequence, whose role in cirrhosis should be investigated in depth. Further studies are required to evaluate the role of the hyperconserved regions detected (both shared and group-specific) and the effect of the aa change detected in HCC group in the protein function, viral replication and cell proliferation.

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Study of HDV effect on HBV replication and quasispecies complexity in mice model

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Introduction: It is well known that hepatitis delta virus (HDV) infection is associated with suppression of hepatitis B virus (HBV) replication. However, HDV needs the HBV surface proteins (HBsAg) for virion packaging. Thus, beyond interacting with HBsAg, other mechanisms may be involved in the HBV inhibition by HDV replication while allowing expression of HBV proteins. In this sense, in a previous study (Godoy *et al.* 2019) we showed that in chronic hepatitis delta (CHD) patients, with lower HBV replication levels, the HBV quasispecies in the 5' end of *HBX* exhibited a trend towards higher complexity than in patients with higher viral replication levels [chronic hepatitis B (CHB)]. This increase of quasispecies complexity could be due to the activation of the host innate immune response under the effect of HDV stimulation. The aim of this study was to explore the potential influence of innate immune system over HBV quasispecies complexity in a mouse model of HBV/HDV co-infection.

Material and Methods: The HBV quasispecies complexity and the expression of mutagenic host immune enzymes was analyzed in C57BL/6 (WT) and IFN alpha receptor KO (IFN α R KO) mice: 8 WT and 8 IFN α R KO mice were mono-infected with 1.3x HBV genome adeno-associated vector (rAAV-HBV 1.3x), and 9 WT and 10 IFN α R KO mice co-infected with rAAV-HBV 1.3x + rAAV-HDV 1.2x. Half of each group were sacrificed at 7 days post-infection (dpi) and the other half were sacrificed at 14 dpi.

Total RNA from liver samples was isolated using TRIzol Reagent (Invitrogen). Total RNA was pre-treated with DNase I using the TURBO DNasefree kit (Applied Biosystems) and retro-transcribed into complementary DNA (cDNA) using M-MLV reverse-transcriptase (Invitrogen). mRNA relative levels of ADAR1 and APOBEC3 were measured by qPCR using SYBR green (CFX Real-Time Detection

System, BioRad) and normalized to GAPDH mRNA expression using the $2^{-\Delta\Delta Ct}$ method. Viral nucleic acids were extracted from serum samples using High Pure Viral Nucleic Acid kit (Roche). HDV and HBV genomes in serum were quantified by quantitative reverse transcription polymerase chain reaction (RT-qPCR) and qPCR in a CFX96 Real-Time Detection System (BioRad), respectively, at 7 and 14 dpi, with known amounts of HDV- or HBcAg sequence-containing plasmid as a standard curve for quantification.

Analysis of the HBV quasispecies complexity at 14 dpi was carried out by Next-Generation sequencing (MiSeq platform, Illumina) in the 5' end of the *HBX* gene (nt 1255-1611), as previously reported (Godoy *et al*, 2019). In the present study we used the number of haplotypes (nHpl) and the mutation frequency (Mf) to describe complexity of HBV quasispecies.

Results and Conclusions: HBV-DNA levels were lower in HBV/HDV co-infected wt mice in relation to HBV mono-infected wt mice (median 7dpi HBV/HDV 4.37 vs. HBV mono-infection 6.20 log IU/mL, $p=0.0286$; median 14 dpi 4.12 vs. 6.65 log IU/mL, $p=0.0159$). In contrast, HBV-DNA levels were not significantly different between IFN α R KO HBV mono-infected and HBV/HDV co-infected mice at 7 dpi (median HBV/HDV 6.75 vs. HBV mono-infection 7.15 log IU/mL, $p=n.s.$); while they were lower in HBV/HDV co-infected mice at 14 dpi (median 5.71 vs. 7.05 log IU/mL, $p=0.0159$). This would suggest that the innate immune system may play a role in HBV inhibition.

The analysis of HBV quasispecies complexity at 14 dpi showed a trend towards a greater HBV quasispecies complexity in terms of nHpl and Mf in HBV/HDV co-infected wt mice (median nHpl HBV/HDV 17.00 vs. HBV mono-infection 14.50, $p=n.s.$; median Mf HBV/HDV $4.95E-04$ vs. $5.25E-04$, $p=n.s.$), a similar behaviour to that previously observed in patients. Surprisingly, HBV/HDV co-infected IFN α R KO mice showed lower HBV quasispecies complexity than HBV mono-infected mice (median nHpl HBV/HDV 10.50 vs HBV mono-infected 14.00, $p=n.s.$; median Mf HBV/HDV $5.25E-04$ vs HBV mono-infected $3.16E-04$, $p=n.s.$). The possible decrease of HBV quasispecies complexity in HBV/HDV co-infected IFN α R KO mice remains unexplained, and should be assessed in further studies including additional factors.

The overexpression of APOBEC3 and ADAR-1 in co-infected wt mice in relation to IFN α R KO mice (median APOBEC3 7 dpi wt $1.70E+03$ vs. IFN α R KO $7.27E+02$, $p=n.s.$; 14 dpi $2.66E+03$ vs $1.34E+03$ $p=0.0159$; median ADAR1 7 dpi wt $2.76E+03$ vs. IFN α R KO $8.13E+02$, $p=0.0159$; 14 dpi $6.94E+02$ vs. $2.57E+03$ $p=0.0159$), suggest that HDV would activate the innate immune system and consequently also the mutagenic enzymes of the host, not only for editing its own genome, but also to cause a greater quasispecies variability to HBV.

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Viral characterization as a tool for the treatment choice in patients with chronic hepatitis E virus (HEV) infection.

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Introduction: Viral hepatitis has become a public health problem that needs an urgent response. In case of hepatitis E virus (HEV), infections are a growing concern with more than 3 million cases of acute infection per year. Chronic HEV infection can occur in immunocompromised patients whom only available treatment is ribavirin (RBV). The end of RBV treatment have been subscribed to the negative detection of HEV-RNA in plasma, but relapse of viral infection usually occurs.

Recent studies suggest the use of feces as a predictor of treatment duration. The objective of this study is to compare the viral population of plasma and feces samples of two patients with chronic infection of our hospital and study which is the best biological source to monitor patient's viral elimination during antiviral treatment.

Material and methods: Plasma and feces samples were collected from two patients with chronic infection in their first (Px.1) and third (Px.2) RBV treatment respectively. HEV-RNA was extracted and the ORF1 region was amplified and sequenced by Next Generation Sequencing (NGS) using Miseq from Illumina (Illumina, Inc. San Diego, CA). Viral loads were determined using Cobas 6800 (Roche, California CA), and Fold-Change were calculated. Diversity and complexity indices and phylogenetic analysis were performed in order to compare quasispecies isolated in plasma and feces.

Results: After his third treatment with RBV, Px.2 accumulated a greater number of mutations and haplotypes in both plasma and feces than Px.1, who had only received one RBV treatment. Plasma and feces are more genetically related intra-patient (plasma-feces) than inter-patient. This situation is confirmed with the phylogenetic analysis where the haplotypes are distributed in two very differentiated clusters corresponding to both patients regardless of the sample's etiology. In both patients, diversity and complexity indices (Shannon's entropy, the average mutation frequency per entity, the FAD and the nucleotide diversity index) are higher in feces samples, which also have a higher viral load with a Fold-change between 7.1 and 8.56 intra-patient.

Conclusions: The values of the indices of diversity and complexity and the greater viral load in feces with respect to plasma, suggest that viral population in feces is a reflection of the population in plasma, and testing viral load in feces could be the best source of study for the evaluation of the dose and duration of treatment with the antiviral RBV in patients with chronic HEV infection.