MOLECULAR AND CELLULAR VIROLOGY OF PATHOGENIC PLUS-STRAND RNA VIRUSES

Gastaminza Laboratory Overview

Dr. Gastaminza's group focuses on the study of the molecular mechanisms that underlie viral pathogenesis mediated by virus-cell interaction in cell culture models. The main objective of the laboratory is to understand the cellular and molecular mechanisms underlying efficient viral infection, with the ultimate goal of identifying novel diagnostic and therapeutic approaches to fight viral infections and their pathogenesis. The lab uses multidisciplinary approaches studying functional as well as structural aspects of the virus-infected cells. The lab has been focused on the hepatitis C virus infection model, a field in which he has contributed for the last 17 years, with the establishment of the first cell culture system for HCV. This system was based on the generation of infectious molecular clones, a biotechnological tool that enables generation of recombinant viruses, using reverse genetics, with multiple applications.

Although focused mainly on the hepatitis C virus, the approaches and methodologies implemented in his laboratory are already being applied to human pathogenic viruses such as the influenza virus and emerging Arboviruses, mainly Flaviviruses and recently SARS-CoV-2 in the context of international collaboration programs (MOPGA 2018 or iMOVE2023). Since March 2020 he is co-director of the CNB-CSIC Antiviral Discovery and Characterization Platform (CNB-SC), an initiative launched by the CNB in response to the SARS-CoV-2 pandemic. For this reason, Dr. Gastaminza's group has developed various cell culture systems to identify new compounds with antiviral activity against this important pathogen. This initiative has involved the approach of numerous academic and industry collaborations. The CNB-SC is integrated in the PTI Salud Global WP9, coordinated by Dr. Garaigorta, co-director of the CNB-SC. PTI Partners provided the chemical candidates as well as the in vivo models. The activities of the CNB Platform have produced a substantial number of publications, patents and contracts with Industry. Currently CNB-SC is integrated in an international consortium coordinated by the European Research Infrastructure on Highly pathogenic agents (ERINHA), together with the Max Planck and Karolinska Institutes, INSERM, Glaxo-Smithkline, Erasmus University, KU Leuven among others to continue the identification and characterization of antivirals. Dr. Gastaminza has participated as inventor in more than 20 patent applications.

Gastaminza´s group has been collaborating with different groups within CNB (Roberto Solano, Juan G. Arriaza, JM Casasnovas, Luis Angel Fernandez) within CSIC (of note the activities of the CNB-SC with IQM-CSIC) as well as with other national and International Institutions. Of interest is its association within CIBERehd with Dr. Forns group from Hospital Clinic, with whom he has published and obtained joint grants. Dr. Gastaminza is part of the CIBERehd steering committee. Interaction with CIBERehd has been instrumental for the modeling of clinically relevant aspects of HCV infection in cell culture. For instance, we have recently submitted a manuscript describing the existence of permanent transcriptional alterations in persistently infected cells after infection elimination. Some of these transcriptional traits are compatible with epithelial-mesenchymal transition (EMT) caused by persistent viral replication that cannot be reverted by clinically relevant therapies, proposing a direct contribution of viral replication in tumoral transformation. Remarkably, some of these alterations were found in biopsies of patients that were declared virologically cured after antiviral therapy.

As an example of the multidisciplinary approach of the lab, Dr. Gastaminza has been actively collaborating with ALBA synchrotron Mistral Beamline for the generation of 3D maps of infected cells at nanometric resolution in quasi-native conditions using soft X-ray tomography, including a recent report on SARS-CoV-2. Currently, Dr. Gastaminza y co-supervising a Ph.D. Student at ALBA in the context of a funded MSCA Training Network (CLEXM, HORIZON-MSCA-2022-DN-01-01) where cells infected with West Nile Virus, a mosquito-borne pathogen of increasing relevance in Southern Europe will be imaged using

correlative fluorescence-synchrotron radiation X-ray microscopy. This long-standing collaboration prompted the recruitment of the Gastaminza lab to an international consortium funded by EU for the development of a laboratory size cryo-SXT microscope. Within this project, the Gastaminza lab, in collaboration with Sirius SXT has documented early ultrastructural events following antiviral treatment of cells persistently replicating HCV using multimodal microscopy, including correlative fluorescence, soft X-ray microscopy. Thus, the Gastaminza lab has implemented reverse genetics systems for the study of virus-host interactions in the context of a broad range of RNA viruses. Dr. Gastaminza also teaches virology and drug discovery classes in three Masters: Virology from U. Complutense Madrid, Biotechnology from U. Autónoma de Madrid and Gestión y Desarrollo de Tecnologías Biomédicas from U. Carlos III.

Relevant publications:

-Gema Calvo, Víctor Venturini, Carlos Garcia-Crespo, Victoria Castro, Emma Díaz and **Pablo Gastaminza.**
Sigmar1 Silencing Interferes with The Formation HCV-Induced Double-Membrane Vesicles *(Manuscript in preparation).*

-Victoria Castro*, Gema Calvo*, Ana Pérez-Berná*, Kevin Mamprin, Sergey Kapishnikov, David Rogers, Stephen O'Connor, Paul Sheridan, Kenneth Fahy, Eva Pereiro and **Pablo Gastaminza**
Rapid HCV replication machinery removal after antiviral treatment with DAA monitored by multimodal imaging *(Submitted August 2024).*

-Victoria Castro, Gema Calvo, Xavier Forns, Sofía Pérez-del-Pulgar and **Pablo Gastaminza**. Epithelial-Mesenchymal Transition Transcriptional Traits After Persistent Hepatitis C Virus Infection Elimination by Direct-Acting Antivirals In Cell Culture. *J Med Virol. 2024 Jul;96(7):e29787. doi: 10.1002/jmv.29787.PMID: 38988177* **(Gastaminza 5/5)**

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Índice Impacto: 5.4 (Quartile 1)