

# Elimination of HCV replication machinery early after antiviral treatment with DAA monitored by multimodal microscopy

**Dr. Victoria Castro**<sup>1</sup>, Gema Calvo<sup>1</sup>, Ana Pérez-Berna<sup>2</sup>, David Rogers<sup>3</sup>, Stephen O'Connor<sup>3</sup>, Sergey Kapishnikov<sup>3</sup>, Paul Sheridan<sup>3</sup>, Eva Pereiro<sup>2</sup>, Kenneth Fahy<sup>3</sup>, Dr. Pablo Gastaminza<sup>1</sup>  
<sup>1</sup>Department of Molecular and Cellular Biology; Centro Nacional de Biotecnología-Consejo Superior de Investigaciones Científicas, Madrid, Spain, <sup>2</sup>ALBA Synchrotron Light Source, Cerdanyola del Valles, Spain, <sup>3</sup>SiriusXT, Dublin, Ireland

## Background incl. aims

Hepatitis C virus (HCV) infection in cell culture constitutes an excellent model of persistent infection whereby the virus takes control of the infected cell without killing it. This strong interference with host cell homeostasis is manifested by a profound remodeling of the host endomembrane system as well as with a strong induction of virtually all stress response pathways in the cells. The availability of specific direct-acting antiviral (DAA) drugs against HCV provides a unique opportunity to revert this process and to define the ultrastructural events that follow viral replication blockade short after antiviral treatment.

## Methods

Using confocal immunofluorescence and transmission electron microscopy (TEM) as well as the correlation of cryo-fluorescence microscopy and cryo-soft X-ray tomography (cryo-FM-SXT), we monitored the HCV replication machinery removal after antiviral treatment with DAA of a surrogate cell culture model of viral replication.

## Results

To assess the impact of antiviral treatment of HCV-replicating cells, we treated cells bearing an HCV subgenomic replicon with a DAA combination of sofosbuvir, a polymerase inhibitor targeting NS5B, and daclatasvir, an NS5A-targeting antiviral. Analysis of DAA-treated HCV replicons indicate that most viral antigens and RNA are eliminated within the first 48 hours of treatment, concomitant with the reversion to baseline expression of HCV-induced stress markers, such as ATF3. A general survey of control cells and HCV replicons using correlative cryo-FM-SXT indicates that HCV-induced membranous alterations are no longer visible after 24 hours of treatment and that a substantial fraction of NS5A, a viral component of the replicase is located in pleomorphic, high-absorption contrast organelles in DAA-treated cells. Three-dimensional reconstruction of these cells suggest that these organelles are spatially organized in layers proximal to the cell nuclei in areas with reduced mitochondrial content. TEM and cryo-FM-SXT studies confirmed the rapid elimination of the viral machinery, and the concurrent appearance of large endo-lysosomes and multivesicular bodies, suggesting a major role for this recycling machinery in the elimination of HCV-induced membranous compartments. These and results by others suggest that HCV replication compartment is constantly recycled by the endo-lysosomal system and that this equilibrium is unbalanced by DAA treatment, resulting in a transient activation of the endo-lysosomal system to achieve rapid viral machinery removal.

## Conclusions

Overall, these TEM and correlative cryo-FM-SXT studies suggest that HCV replication machinery removal after DAA treatment entails transient proliferation of endo-lysosomes and MVB, but not that of double-membrane autophagosomes. Moreover, live fluorescence confocal microscopy indicates that NS5A remnants co-localize with an acidic compartment labeled with lysotracker green. Given that a fraction of NS5A is found in endo-lysosomes also before antiviral treatment it is reasonable to propose that HCV replicase compartment size is balanced by a constant flux through lysosomal/MVB compartments.

**Keywords:**

HCV, DAA, cryo-FM-SXT, TEM, recycling-machinery