Interpreting in-vitro data for sofosbuvir against SARS-CoV-2: cell lineage is key

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Background

Various existing antimicrobials have been repurposed and tested to find an effective therapy for SARS-CoV-2, including sofosbuvir, which is known to have broad anti-viral effects against many viruses. Promising *in-silico* studies predicted strong binding for sofosbuvir with SARS-CoV-2 replication enzymes (RdRp). Whilst *in-vitro* studies showed mixed outcomes for sofosbuvir, small clinical trials showed potential benefits in combination with other antivirals. We summarise published *in-vitro* research on sofosbuvir efficacy when repurposed against SARS-CoV-2 and various other viruses.

Objectives and Methodology

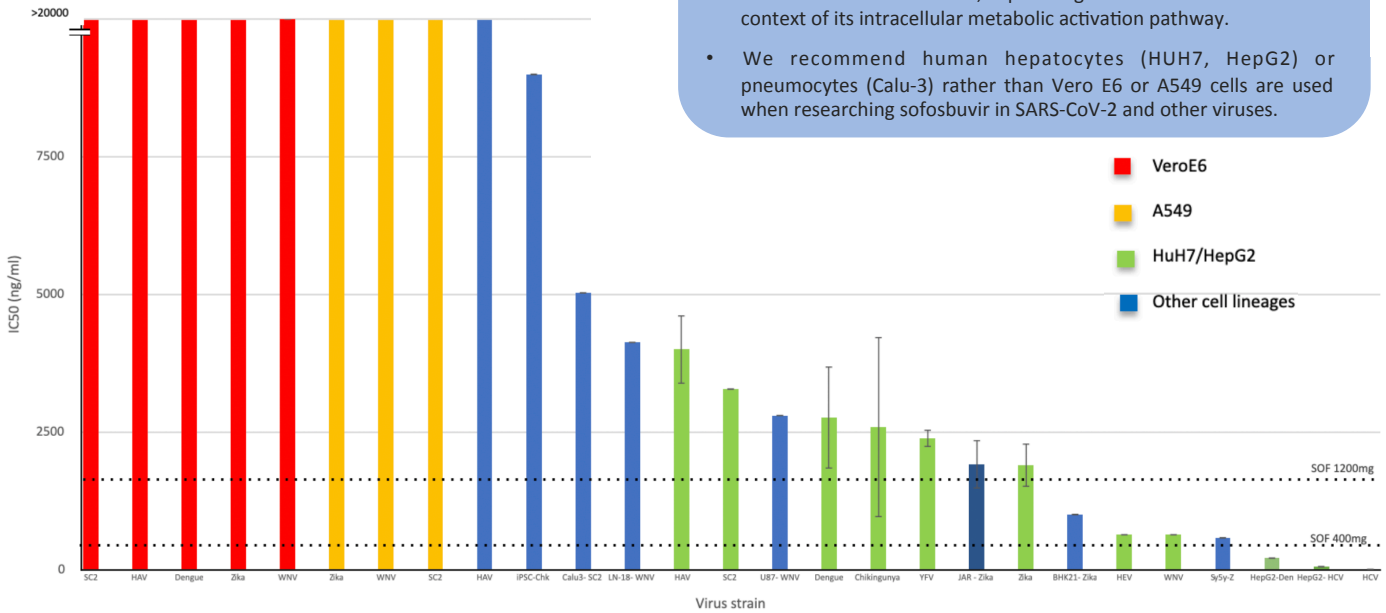
We reviewed published *in-vitro* studies of sofosbuvir against SARS-CoV-2 and eight other viruses, to identify whether differences in cell lineage, viral strains and assay readout technique impacts IC_{50} results for sofosbuvir against various viruses.

Results

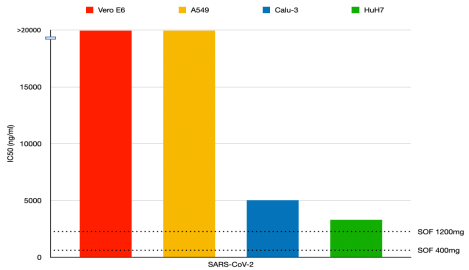
Data was available for published IC_{50} results for sofosbuvir across twelve different cell lines, for nine viruses; dengue, chikungunya, zika, yellow fever virus (YFV), west nile virus (WNV), SARS-CoV-2, and hepatitis A, C and E. We found cell lineage is a crucial factor when interpreting data from *in-vitro* trials of sofosbuvir, whilst other *in-vitro* lab methodology factors are also important, such as MOI, assay readout technique and viral isolate polymorphisms. Sofosbuvir seems inactive against all viruses in Vero E6 and A549 cells but is effective in other human cells, especially hepatocyte lineages such as HUH7 and HepG2 cells. This is also true for SARS-CoV-2. These findings are most likely because the commonly used Vero E6 and A549 cells lacks the key enzymes that are involved in the metabolism of sofosbuvir into its active form.

Conclusion

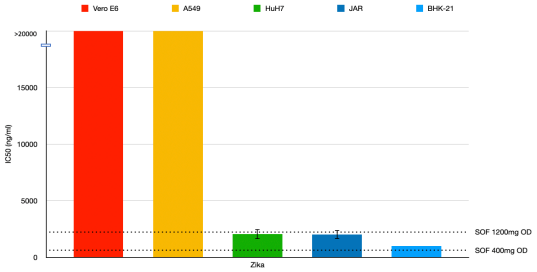
- The IC50 for a medication against a specific virus varies significantly depending on lab methodology, especially cell lineage.
- In order to effectively screen repurposed therapies as possible trial candidates for SARS-CoV-2, a pro-drug must be understood in the context of its intracellular metabolic activation pathway.
- We recommend human hepatocytes (HUH7, HepG2) or pneumocytes (Calu-3) rather than Vero E6 or A549 cells are used when researching sofosbuvir in SARS-CoV-2 and other viruses.



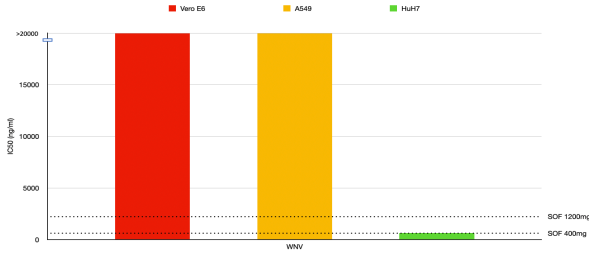
IC50 with sofosbuvir in different cell types for SARS-CoV-2



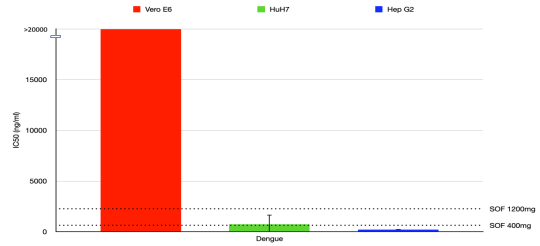
IC50 with sofosbuvir in different cell types for Zika Virus



IC50 with sofosbuvir in different cell types for Western Nile Virus (WNV)



IC50 with sofosbuvir in different cell types for Dengue virus



Sofosbuvir

As shown on the right, Sofosbuvir, is a Pro-Tide molecule requiring intracellular activation. Sofosbuvir is metabolized to its active triphosphate in a multistep pathway, starting with the removal of monophosphate protections by the enzymes CES1/CatA and HINT1. Next, sofsobvuir-monophosphate is converted to the active compound GS-461203. CES1/CatA and HINT1 are strongly expressed in human hepatocytes; hence sofosbuvirs' tropism for hepatic viruses, namely hepatitis C virus (HCV) for which it's a licensed treatment. The CES1 enzyme is also expressed in alveolar epithelial cells, a cell tissue of interest in potential treatments for SARS-CoV-26 due to its damaging impact in the respiratory system.