#688 ONCOLYTIC VIRAL THERAPY WITH RETARGETED GLYCOPROTEIN AND GENETIC ON SWITCH FOR PRECISE TARGETING OF LIVER CANCER USING A SYNTHETIC VIROLOGY PLATFORM.

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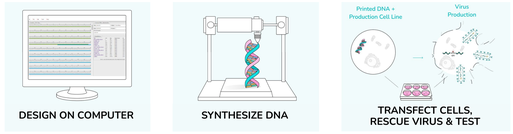
INTRODUCTION

- Hepatoblastoma (HB) and hepatocellular carcinoma (HCC) are common pediatric liver tumors with low survival rates.
- Currently, there is no effective therapy for HB.
- Oncolytic virus therapy (OVT) is a promising modality that leverages the propensity of natural or engineered viruses to selectively replicate in and kill cancer cells.
- (Pre)clinical studies have shown that OVT is generally safe, but therapeutic efficacy has fallen short of expectations.
- We have developed a synthetic biology platform to engineer oncolytic virus therapies with high specificity and efficacy.
- Vesicular stomatitis virus (VSV) is an ideal candidate for virus engineering. It is highly lytic, it is retargetable, has low seroprevalence, a small genome that's well understood and has a relatively large carrying capacity for therapeutic transgenes.

GOALS

- Engineer liver cancer-specific glycoprotein and aptazyme components.
- Determine selectivity and efficacy of engineered VSV *in vitro*.
- Evaluate safety and biodistribution of engineered VSV *in vivo*.

METHODS



SYNTHETIC VIROLOGY PLATFORM

Genome design of oncolytic viruses was completed using our proprietary software. DNA fragments were synthesized and then assembled to construct the complete viral genome, which was verified by nanopore sequencing. Viruses were rescued and propagated in 293T, isolated and titered in Vero, and confirmed by nanopore sequencing.

COMPONENT TESTING - GLYCOPROTEIN

Glycoproteins were designed that target glypican-3 (GPC3), a cell surface proteoglycan currently used as a diagnostic biomarker to distinguish cancer from normal liver tissue. DNA fragments were synthesized, assembled in a pCAG expression plasmid, and then verified. 293T were seeded in a 6-well, incubated overnight, and then transfected with expression plasmid for 24 hours. Cells were infected with VSV-ΔG-RFP (MOI=5 IU), harvested after 24 hours, and then titered on 293T. Target HepG2 and healthy primary human hepatocytes (PHH) were infected with serial dilutions of VSV pseudotyped with natural and retargeted glycoprotein.

COMPONENT TESTING - REPLICATION SWITCH

Aptazymes, composed of a ribozyme and proprietary cancer-specific aptamer, were designed and constructed in a mammalian GFP reporter plasmid. In the absence of target cancer protein, ribozyme cleavage would occur, leading to loss of p(A) tail and GFP stability. In the presence, cleavage would be blocked and GFP expression observed. Cells with high (HepG2), medium (293T), and low (NCF) target expression were transfected with plasmids, and imaged for GFP expression after 24 hours.

ONCOLYTIC VIRUS - *IN VITRO*

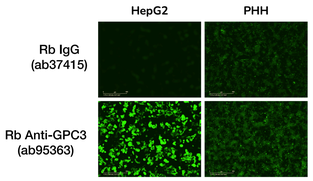
We evaluated HG001 efficacy in Hep3B and patient HB17 cells, selectivity in off target (GPC3 negative) SNU-449 & HepT1 cancer cells, and safety in healthy NIH3T3 (mouse) and WI-38 (human) cells. IncuCyte live cell imaging & analysis software was used to measure cell area per well (μm²/well) and determine cell health and proliferation. Student's t-test was used to assess statistical significance.

ONCOLYTIC VIRUS - *IN VIVO*

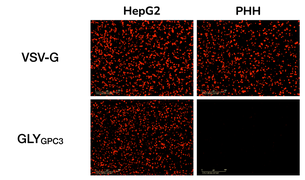
Female NSG mice (5-6 weeks old) were administered one intraperitoneal (IP) injection of 50μL PBS, HG001 (10^7 PFU), or HG001 (10^8 PFU) (n=5/group). Mice were monitored and weighed daily, and euthanized at day 7 post-treatment. Organs (brain, kidney, liver, lung) were harvested at necropsy. RT-qPCR was conducted to determine biodistribution.

RESULTS

COMPONENT TESTING - GLYCOPROTEIN

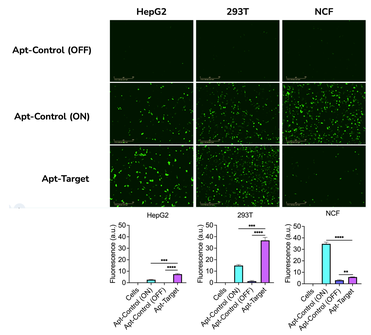


Immunofluorescence staining with Anti-GPC3 and Isotype Control antibodies shows high GPC3 expression in HepG2 cells, but not in healthy hepatocytes.



Infection with pseudotyped VSV is indicated by RFP expression. VSV-ΔG-RFP pseudotyped with wildtype VSV-G glycoprotein infects target HepG2 and healthy PHH cells. In contrast, our GPC3-retargeted glycoprotein shows selective infection in HepG2 only.

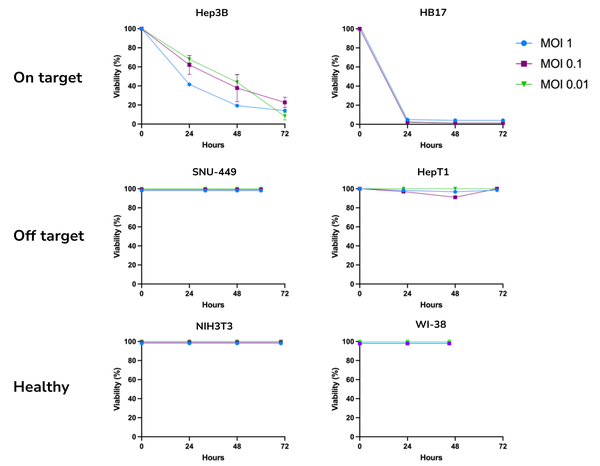
COMPONENT TESTING - REPLICATION SWITCH



Plasmid-based transfection shows the replication switch functions as designed. GFP expression increases with expression of target cancer protein. Both are high in HepG2, medium in 293T, and low in NCF.

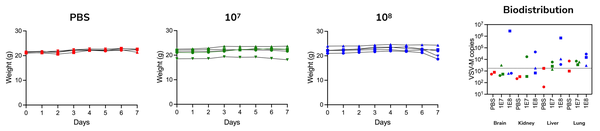
RESULTS

ONCOLYTIC VIRUS - *IN VITRO*



- Infection and lysis observed in target Hep3B and patient HB17 cells, even with low input of MOI=0.01 (PFU).
- No signs of cytopathic effects in off target (GPC3 negative) SNU-449 and HepT1 cancer cells.
- Healthy mouse cells (NIH3T3) show no signs of toxicity at MOI=1 (PFU), supporting further studies *in vivo*.
- Healthy human lung cells (WI-38) are highly susceptible to virus infection, but there are no signs of toxicity at MOI=1 (PFU).

ONCOLYTIC VIRUS - *IN VIVO*



At the 10^7 PFU dose, no toxicity was observed. There was no significant decrease in weight, and organs appeared normal at necropsy. VSV-M mRNA was detected by RT-qPCR in the kidney of one mouse. However, at the 10^8 PFU dosage, two mice experienced a decrease in weight on Day 7, and VSV-M mRNA was similarly detected in brain and liver tissue. These indicate toxicity at 10^8 PFU dosing. Taken together, 10^7 PFU doses were tolerated and will be used in upcoming *in vivo* efficacy studies.

CONCLUSIONS & FUTURE DIRECTIONS

- We have shown that a rationally designed oncolytic virus can be made efficiently with high specificity and efficacy.
- Initial testing is showing high promise for future *in vivo* testing.
- With further iterations we hope to develop a strong therapeutic for liver cancer.
- We are actively looking for collaborations with oncology centers on other types of cancer we could target using our platform.