#946 NOVEL ONCOLYTIC VIRUS TARGETING GPC3 AND CTNNB1 DEMONSTRATES COMPLETE RESPONSE IN HEPATOCELLULAR CARCINOMA (HCC) XENOGRAFT WITH A SINGLE, SYSTEMIC ADMINISTRATION

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INTRODUCTION

- Hepatoblastoma (HB) and pediatric hepatocellular carcinoma (HCC) are aggressive childhood liver cancers with limited treatment options and poor survival outcomes.[1, 2]
- Oncolytic virus therapies (OVTs) have shown promise in cancer treatment, though efficacy and selectivity challenges remain.[3]
- Humane Genomics has developed a synthetic virology platform enabling the advanced engineering of vesicular stomatitis virus (VSV)-based therapies.[4] The resulting therapy shows robust therapeutic efficacy and selectivity using innovative two-factor authentication (2FA) strategy combining engineered glycoproteins for cancer-specific infection and protein-responsive aptazymes for selective replication.
- HGI627 is our lead candidate for liver cancer featuring a synthetic glycoprotein retargeted to glypican-3 (GPC3) and a beta-catenin (CTNNB1)-dependent aptazyme as a genetic ON switch.

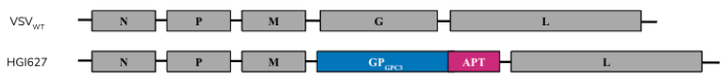
GOALS

- Validate the target specificity of HGI627 in vitro using cancer versus normal cell lines.
- Assess therapeutic efficacy of HGI627 in vivo in a Hep3B hepatocellular carcinoma model.
- Evaluate safety profile and potential maximum tolerated dose (MTD) of HGI627 in vivo.

METHODS

VIRUSES

- HGI627 is a vesicular stomatitis Indiana virus (VSIV) engineered using our platform for targeted treatment of liver cancer. Key modifications include deletion of the native VSV-G glycoprotein, replacement with detargeted, engineered glycoprotein, incorporation of a synthetic anti-GPC3 nanobody for tumor-specific retargeting, and integration of a cancer protein–responsive aptazyme that acts as a genetic ON switch to restrict replication to tumor cells.



IN VITRO

- HGI627 was constructed and rescued using a synthetic virology platform [Moles et al., Viruses 2024], and viral titers were measured by $TCID_{50}$ assay on Hep3B cells and calculated using Spearman-Karber.
- Endpoint dilution assays were performed and immunocytochemistry (ICC) staining was carried out using anti-VSV (REA005; Imanis Life Sciences) to evaluate selectivity.
- Cytotoxicity and selectivity were evaluated using real-time imaging (IncuCyte system) on liver cancer (e.g. Hep3B, HepG2) and off-target/normal cells.

IN VIVO

- Efficacy of HGI627 was assessed in NSG mice bearing subcutaneous Hep3B hepatocellular carcinoma xenografts. A single intraperitoneal (IP) dose of 10^8 $TCID_{50}$ was administered when average tumor volume reached 80-100mm^3.
- Histology was performed by HistoWiz (histowiz.com) using a Standard Operation Procedure and fully automated workflow. Staining with anti-mitochondria (ab92824) was performed to differentiate human Hep3B xenograft from mouse cells.
- Acute toxicity and maximum tolerated dose (MTD) were evaluated in NSG mice (n=4/group) following single and multiple IP doses of 10^9 $TCID_{50}$ to assess safety and tolerability.

RESULTS

IN VITRO

- HGI627 demonstrated robust lytic activity in vitro MOI 1 and 0.1 in GPC3+/CTNNB1+ cells with minimal to no cytotoxicity in off-target and normal cells (FIG. 1).

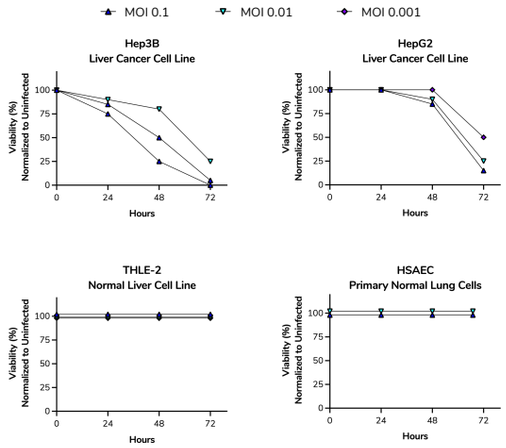
IN VIVO

- In vivo, a single intraperitoneal (i.p.) injection of 1×10^8 $TCID_{50}$ HGI627 (n=9) achieved a 100% complete response and significantly extended survival to day 49 (end of study) compared to median survival of 15 days in vehicle group ($p<0.005$) (FIG. 2-4).
- In the safety study, even multiple (n=3) high-dose (1×10^9 $TCID_{50}$) administrations of HGI627 were well tolerated, and no signs of clinical toxicity were observed up to 28 days; Importantly, this highest dosing group received an equivalent of 300-fold more than the proposed final first-in-human dose (FIG. 5).

RESULTS

IN VITRO

FIGURE 1. SELECTIVE LYSIS OF GPC3+/CTNNB1+ LIVER CANCER CELLS



IN VIVO

FIGURE 2. EFFICACY STUDY OF HGI627 IN SUBCUTANEOUS HEP3B CDX MODEL

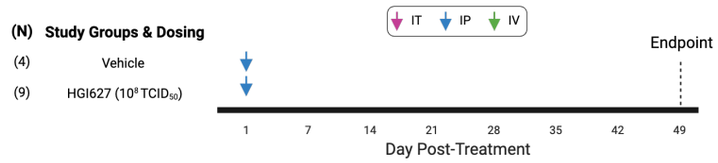
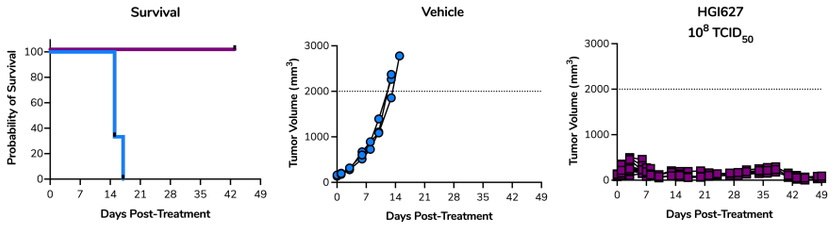


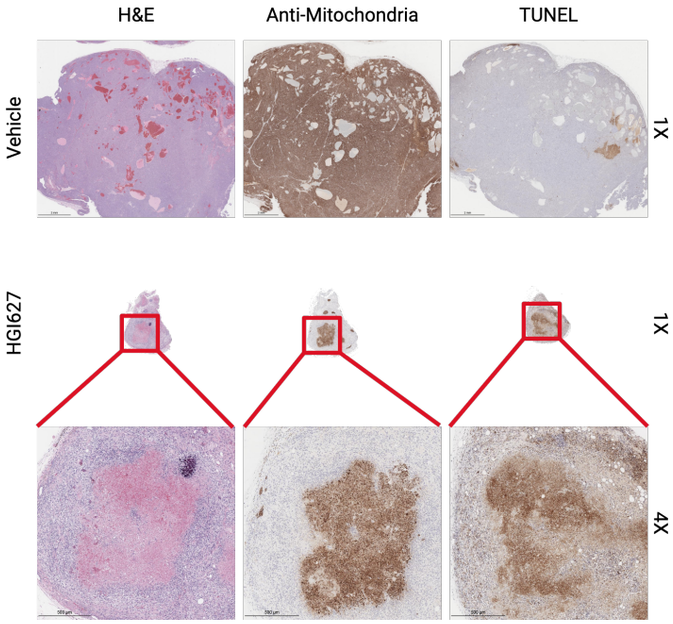
FIGURE 3. SURVIVAL AND TUMOR BURDEN ANALYSIS OF HGI627



- HGI627 treatment achieved 100% survival through Day 49, compared to a median survival of 15 days in the vehicle group.
- Vehicle-treated tumors progressed rapidly to endpoint (≥2000 mm³), while HGI627-treated tumors peaked in volume at Days 3–6 post-treatment and then exhibited a durable, complete response through Day 49.

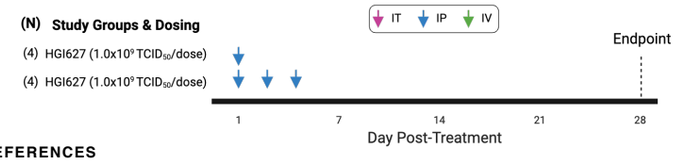
RESULTS

FIGURE 4. HISTOPATHOLOGY ANALYSIS OF HEP3B EFFICACY STUDY



- HGI627-treated tumors exhibited a 99.9% reduction in tumor burden relative to vehicle-treated controls, as measured by anti-human mitochondria IHC staining.
- TUNEL staining and analysis showed that the remaining 0.1% human-positive cells were apoptotic, indicating complete tumor eradication at the cellular level.

FIGURE 5. SAFETY STUDY OF HGI627 IN NON TUMOR BEARING NSG MICE



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CONCLUSIONS & FUTURE DIRECTIONS

- These results highlight HGI627's potency, selectivity, and safety, overcoming previous OVT challenges.
- HGI627 exemplifies a promising approach for liver cancer treatment using a dual-targeting mechanism for enhanced precision.
- We are focused on advancing HGI627 through rigorous preclinical development and in vivo validation, and are actively seeking clinical collaborators and trial site partners to support its translation into first-in-human studies. We also welcome discussions with academic and industry partners interested in expanding this platform to new indications and combination strategies.