Development of Allogeneic, Potency-edited, Anti-GPC3 CAR T Cells for the Treatment of Hepatocellular Carcinoma

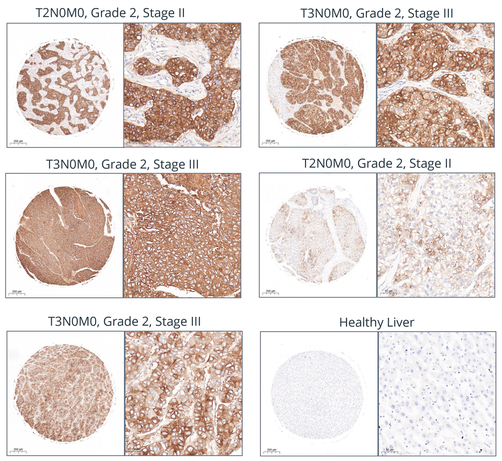
Daniel Henderson[1], Minh Thu Pham[1], Dan Li[2], Mitchell Ho[2], Jason Sagert[1]

[1]CRISPR Therapeutics, Boston, MA [2] National Cancer Institute, Bethesda, MD

Introduction

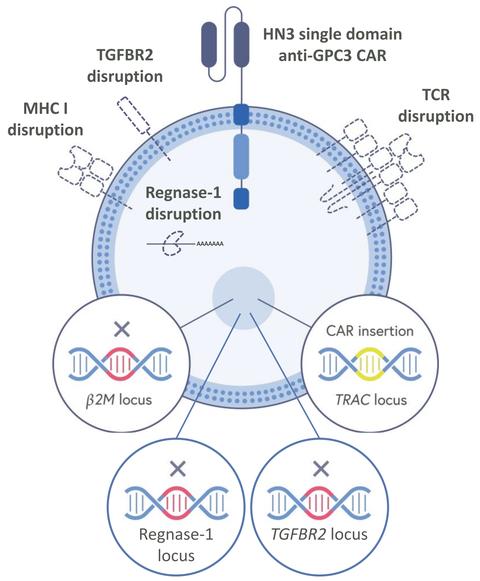
- Glypican-3 (GPC3) is an oncofetal heparan sulfate proteoglycan and is associated with cell proliferation, differentiation, and mobility through Wnt, Yap, and other signaling pathways.
- GPC3 expression is highly restricted in adults, and aberrant overexpression of GPC3 is associated with several cancers, most prominently hepatocellular carcinoma (HCC).
- The HCC tumor microenvironment is strongly immunosuppressive. Allogeneic CRISPR/Cas9 gene-edited CAR T cells incorporating potency edits to enhance anti-tumor activity in suppressive tumor microenvironments can address this challenge.

Figure 1: GPC3 Expression in HCC and Healthy Tissues



- GPC3 is overexpressed in ~75% of all hepatocellular carcinoma cases (Moek et al. 2018) and is not detected in healthy liver tissue (bottom right).
- A formalin fixed paraffin embedded hepatocellular carcinoma tissue microarray (TissueArray.Com, LV1501) was stained using anti-GPC3 mouse monoclonal antibody (Abcam, ab216606) on the Leica BOND™ RX autostainer.

Figure 2: CRISPR/Cas9 Gene-edited Allogeneic CAR T Chassis



Donor-derived allogeneic anti-GPC3 CAR T cells incorporate the following edits:

- **TCR KO:** Minimizes risk of GvHD
- **β2M KO:** Eliminates MHC class I expression to mitigate host T cell-mediated clearance of CAR T cells
- **CAR KI:** Precise insertion of CAR transgene into the TRAC locus
- **Regnase-1:** Removes intrinsic “brake” on T cell function
- **TGFBR2 KO:** Removes key extrinsic “brake” on T cell anti-tumor activity

Figure 3: CRISPR-edited Anti-GPC3 CAR T Cells Show Robust and Specific Activity *In Vitro*

- Anti-GPC3 CAR T activity and specificity was assessed via a co-culturing assay with a GPC3(+) HepG2 cell line and a GPC3(-) A498 cell line at the indicated ratios.
- GPC3-targeted CAR T cells, with and without potency edits, showed robust activity against the GPC3(+) cell line. In contrast, CAR T cells show no activity towards the GPC3(-) cell line as evidenced by the lack of cytotoxicity and cytokine secretion

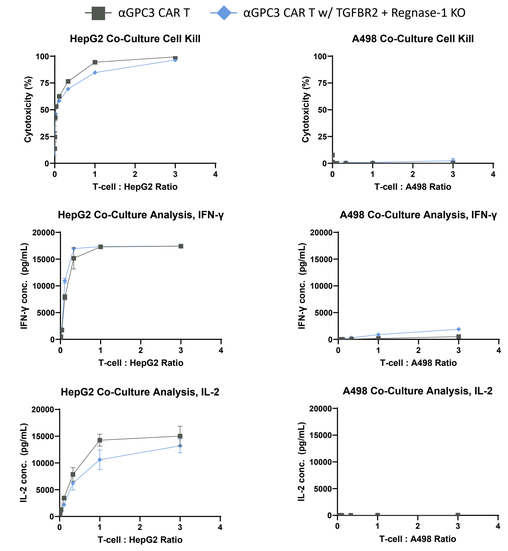
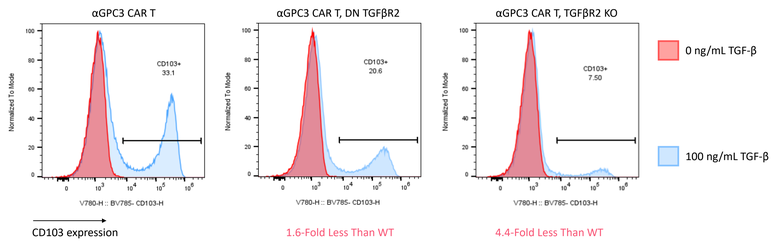


Figure 4: TGFBR2 Knock-out Exhibits Better Attenuation of TGF-β Induced Signaling Than the Dominant Negative Knock-in



- GPC3-directed CAR T cells were generated either through our allogeneic method with the endogenous TGFBR2 KO or via lentiviral transduction with a vector containing the GPC3 CAR T2A dominant negative (DN) TGFBR2 construct.
- CD103 is upregulated as a result of TGFBR2 signaling. CAR T cells with the DN receptor showed a decrease in CD103 expression vs. those without the receptor in the presence of TGF-β. CAR T cells with TGFBR2 KO via CRISPR exhibit even lower CD103 expression.

Figure 5: Endogenous TGFBR2 KO Diminishes TGF-β-mediated CAR T Cell Inhibition

- Anti-GPC3 CAR T cells with the DN receptor or TGFBR2 KO were evaluated for their ability to kill HepG2 cells in a co-culture assay in the presence of TGF-β.
- Knocking out the endogenous TGFBR2 gene wholly rescues TGF-β-mediated inhibition, whereas the DN TGFBR2 construct does not

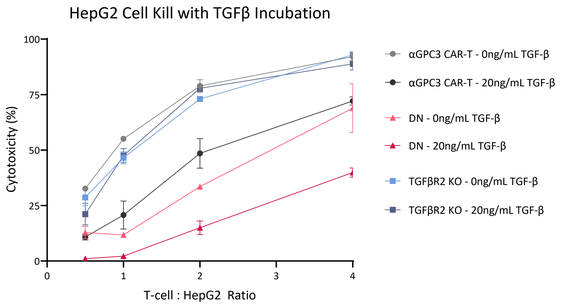
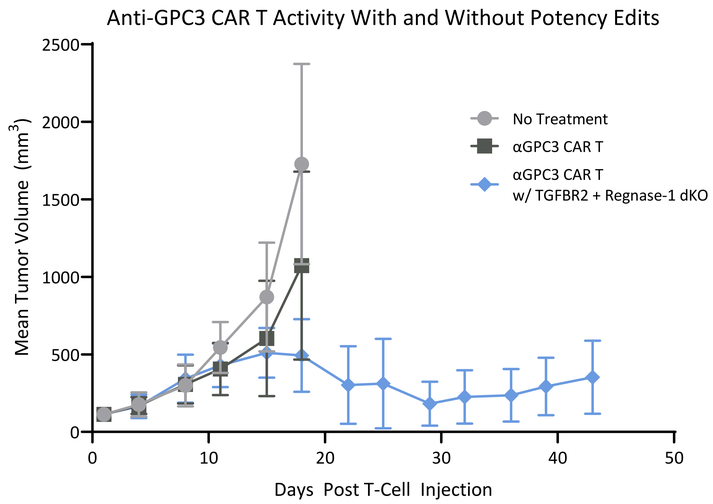
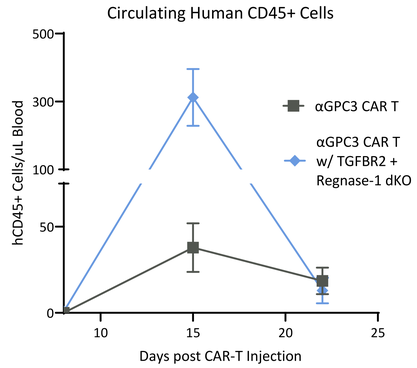


Figure 6: Potency Edits Significantly Enhance Anti-GPC3 Anti-tumor Efficacy *In Vivo*



- NSG mice were inoculated with the HCC-derived cell line Hep3B, and the resulting tumors were allowed to grow to a median size of 150mm^3. Mice were then dosed with 4e6 CAR+ cells after which tumor volumes were measured biweekly.

Figure 7: TGFβR2 and Regnase-1 dKO Improves CAR T Cell Proliferation



- Circulating CAR T cells were monitored using flow cytometry.
- CAR T cells containing the potency edits expanded nearly 10-fold higher *in vivo* than those without the potency edits

Conclusions

- CRISPR-edited anti-GPC3 CAR T cell therapies are a promising potential option for the treatment of HCC.
- We have shown that our allogeneic anti-GPC3 CAR T cells containing the HN3 single domain binder have significant activity both *in vitro* and *in vivo* against HCC-derived cell lines
- Potency edits enhance CAR T activity and provide deeper resistance to the suppressive tumor microenvironment.
- Disruption of the endogenous TGFBR2 gene via CRISPR shows superior attenuation of TGF-β signaling relative to a dominant negative TGFBR construct.

References: Moek, K., Fehrmann. R., Van der Vegt, B., de Vries, E., de Groot, D., Glypican 3 Overexpression across a Broad Spectrum of Tumor Types Discovered with Functional Genomic mRNA Profiling of a Large Cancer Database *Am J. Pathol.* 2018 Sep;188(9):1973-1981. doi: 10.1016/j.ajpath.2018.05.014; Feng, M., Gao, W., Wang, R., Chen, W., Man, Y., Figg, W., Wang, X., , Dimitrov, D., and Ho, M., Therapeutically targeting glypican-3 via a conformation-specific single-domain antibody in hepatocellular carcinoma, *Proc Natl Acad Sci U S A.* 2013 Mar 19; 110(12) doi: 10.1073/pnas.1217868110