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Allogeneic anti-PTK7 CAR-T cells for the treatment of solid tumors

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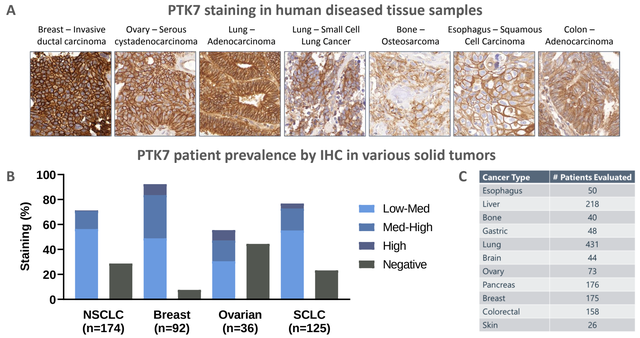
Abstract

Protein Tyrosine Kinase 7 (PTK7), also known as colon carcinoma kinase 4 (CCK-4), is a highly conserved catalytically inactive tyrosine kinase with inherent signal transduction activity. PTK7 is a member of the Wnt signaling pathway and thought to function in cell proliferation, adhesion, migration and apoptosis. PTK7 has been shown to be highly expressed in certain cancer types including colon, breast, lung, pancreatic, renal and ovarian. Downregulation of PTK7 by shRNA has been shown to reduce tumor growth and metastases development in xenograft mouse models. Furthermore, high PTK7 expression in triple negative breast cancer and NSCLC patients has been linked to poor prognosis. PTK7 therefore appears to be an attractive target for solid cancer therapeutic intervention and has been targeted clinically with an Antibody Drug Conjugate (PF-06647020). However, the protein is also expressed to a lesser degree in some normal adult tissues, particularly in the stromal cells of the ovary, placenta, uterus and lung. We have developed a human/murine cross-reactive anti-PTK7 CAR construct and assessed the ability of allogeneic anti-PTK7 CAR-T cells bearing that construct to target cancer and elicit toxicity in mice. These anti-PTK7 CAR-T cells were shown to be efficacious *in vitro* and in immunocompromised mouse xenograft models of breast, lung, colon, pancreatic and ovarian cancer. However, consistent in all xenograft models studied, a drop in body weight was observed shortly after injection from which all mice rapidly recovered to above baseline. Latent toxicity that was more variable amongst the xenograft experiments was also observed. Understanding and developing appropriate mitigation strategies to address the observed toxicities may be required to develop further a safe and efficacious allogeneic anti-PTK7 CAR-T cell therapy for solid tumor cancers.

Figure 1: PTK7 has high expression in a variety of solid human cancers



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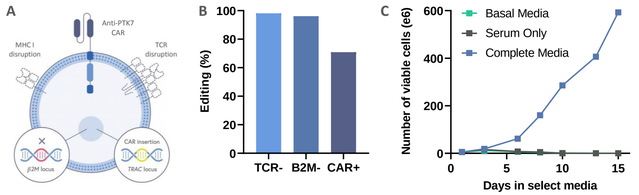
Cancer Type	# Patients Evaluated
Esophagus	50
Liver	218
Bone	40
Gastric	48
Lung	431
Brain	44
Ovary	73
Pancreas	176
Breast	175
Colorectal	158
Skin	26

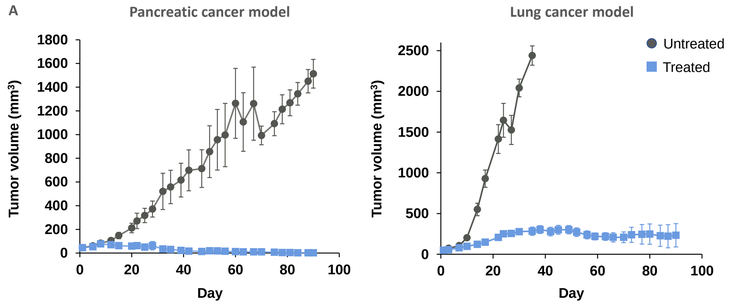
(A) FFPE human diseased tissue microarrays show high PTK7 expression when stained with a mouse monoclonal anti-human PTK7 antibody. **(B)** Scoring numerous patient samples for intensity of staining and percentage of section stained reveals that >50% of patient samples from breast, ovarian and lung cancers have some PTK7 expression. **(C)** Total number of patients evaluated based on tumor cell types.

Figure 2: Allogeneic anti-PTK7 CAR-T cells are generated efficiently using CRISPR/Cas9



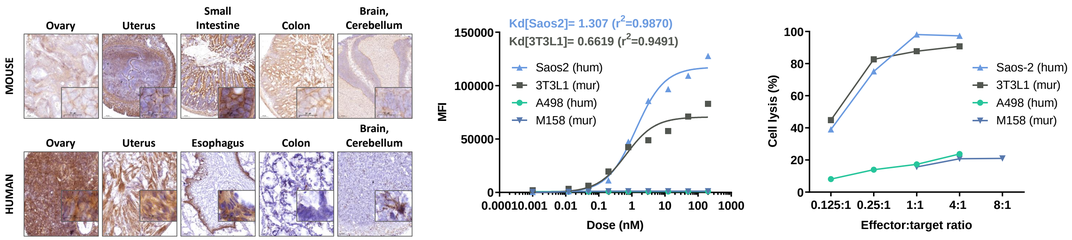
(A) CRISPR/Cas9 genome editing of T cells from healthy donors is used to generate allogeneic anti-PTK7 CAR-T cells. To prevent GvHD, TCR expression is ablated by site-specific integration of an antigen-specific CAR construct into the *TRAC* locus by homology-directed repair after using CRISPR/Cas9 to introduce the double strand break. To enhance persistence of allogeneic cells, MHC I expression is eliminated by disrupting the *β2M* gene. **(B)** Multiplexed CRISPR/Cas9 editing results in near-complete elimination of TCR and MHC I surface expression, as well as high CAR expression, as measured by flow cytometry. **(C)** Anti-PTK7 CAR-T cells remain dependent on cytokines for growth and survival following multi-editing, suggesting no oncogenic transformation has occurred.

Figure 3: Anti-PTK7 CAR-T cells show efficacy across a variety of *in vivo* xenograft models, but appear to induce acute and latent toxicity



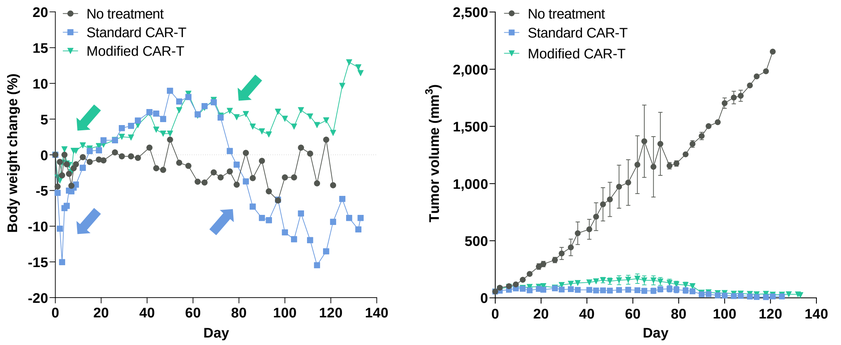
(A) Anti-PTK7 CAR-T cells show efficacy against various solid tumor xenograft models, including the pancreatic (Hs766T) and lung (NCI-H1975) cancer models shown here. Hs766T or NCI-H1975 tumor cells were injected subcutaneously into NOG mice and CAR-T cells were dosed once mean tumor size had reached approximately 50mm^3 (n=5 each group). **(B)** In all xenograft models, transient acute toxicity (indicated by a drop in body weight) was observed 5-7 days following CAR-T cell injection, as shown in the representative body weight plot for the Hs766T model (blue arrow at left). All mice rapidly recovered the lost body weight. Some models, including the Hs766T model, also showed latent toxicity starting after 55 days on study (blue arrow at right).

Figure 4: On-target/off-tumor toxicity is visible in mice because anti-PTK7 CAR-T cells exhibit murine crossreactivity



(A) PTK7 expression was evaluated by IHC on frozen mouse and human normal tissue panels (FDA Standard) using a custom recombinant monoclonal biotinylated antibody specific to the PTK7 CAR construct. **(B)** Murine and human cell lines were compared for binding affinity to the custom recombinant mAb. **(C)** High PTK7 expressing murine and human cell lines were compared in a 24-hour *in vitro* cytotoxicity assay. The murine PTK7 expression and crossreactivity observed makes mice a useful model for understanding the potential toxicity of anti-PTK7 CAR-T cells in humans.

Figure 5: A "modified" anti-PTK7 CAR mitigates on-target/off-tumor toxicity *in vivo* while maintaining efficacy



A "modified" anti-PTK7 CAR construct was designed to mitigate the toxicity observed in mouse *in vivo* studies. This modified CAR enables spatial control of CAR-T cells within the tumor microenvironment (TME). CAR-T cells bearing the modified CAR were evaluated in a mouse xenograft tumor study. **(A)** As shown in this Hs766T pancreatic carcinoma xenograft model in NOG mice (n=5 each group), modified anti-PTK7 CAR-T cells do not produce the acute and latent toxicity observed as loss in body weight with standard CAR-T cells. **(B)** In the same study, modified CAR-T cells still reduced tumor burden to a similar extent as standard CAR-T cells. Both CAR-T products were dosed at 1x10^7 CAR$^+$ T cells.

Conclusions from Preclinical Studies

- PTK7 is highly expressed in many different solid tumor cancer types and to a lesser extent in some normal tissues
- Anti-PTK7 CAR-T cells are efficacious *in vivo* against a variety of solid tumor cell lines
- Acute and latent toxicity *in vivo*, observed as loss of body weight, occurs due to species crossreactivity of the anti-PTK7 CAR-T cells
- We developed a mitigation strategy to address the on-target/off-tumor toxicity through spatial control of modified CAR-T cells within the TME
- Modified anti-PTK7 CAR-T cells successfully mitigate both the acute and latent toxicity while maintaining high efficacy *in vivo*

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