Allogeneic CAR T Cells Targeting LIV1 for the Treatment of Breast Cancer

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Introduction

- Allogeneic CAR T cells represent an "off-the-shelf" investigative treatment option that can address the unmet need for immediately available, potentially curative cancer therapies
- HER2 represents an important target for breast cancer treatment. Several approved breast cancer therapies target HER2; however, limitations include the lower prevalence of HER2 overexpression (20% of all breast cancer cases) and well-characterized respiratory and cardiac toxicities
- Triple negative breast cancer (TNBC) is the clinical subtype with the poorest prognosis and accounts for ~15% of all breast cancer cases. TNBC does not express estrogen receptor (ER), progesterone receptor (PR) or HER2
- LIV1 is a zinc transporter protein with high prevalence in breast cancer and limited expression in normal tissues, reducing the risk of off-target toxicity
- LIV1 has been explored clinically as a target via the antibody drug conjugate (ADC) ladiratuzumab vedotin

Figure 1: CRISPR/Cas9 gene-edited allogeneic CAR T chassis

- Our gene-edited allogeneic CAR T manufacturing process begins with collection of T cells from a healthy donor, followed by electroporation of Cas9 ribonucleoprotein to make the following edits:
  - **TCR KO** to minimize the risk of GvHD
  - **β2M KO** to eliminate MHC class I expression and mitigate host T-cell-mediated clearance of CAR T cells
  - **CAR KI** via precise insertion of CAR transgene into the TRAC locus (using an AAV template)

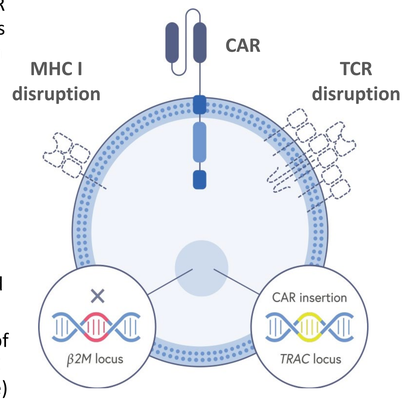


Figure 2: LIV1 is expressed in all clinical subtypes of breast cancer

- A breast cancer tumor microarray consisting of 140 unique tissues was stained by immunohistochemistry (IHC) to evaluate LIV1 expression
- LIV1 was detected in all clinical subtypes of breast cancer
- 68% of the tissues stained were LIV1+, consistent with Sussman, *et al*. (2014)

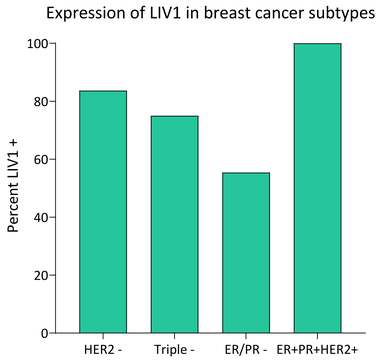
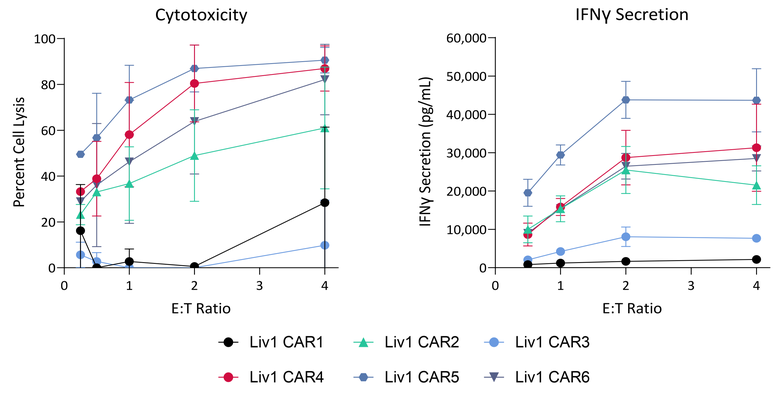


Figure 3: Cytotoxicity of anti-LIV1 CAR constructs correlates with IFNγ secretion



- Anti-LIV1 CAR constructs with varying scFv sequence and costimulatory domain were screened. The six constructs shown above were selected for further testing
- Four of the six anti-LIV1 CAR constructs exhibited a potent cytotoxic response to the HR+HER2- MCF-7 breast cancer cell line in a 24-hour *in vitro* cytotoxicity assay
- IFNγ secretion by anti-LIV1 CAR T cells co-cultured with MCF-7 cells correlated with cytotoxicity

Figure 4: Anti-LIV1 CAR T cells exhibit potent activity against a TNBC cell line

- Three anti-LIV1 CAR constructs were selected for further testing against the MDA-MB-231 TNBC cell line in a 24-hour *in vitro* cytotoxicity assay
- CAR5 and CAR6 demonstrated the strongest cytotoxicity

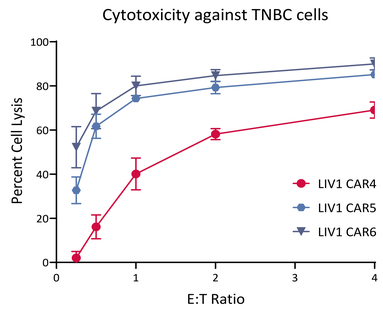
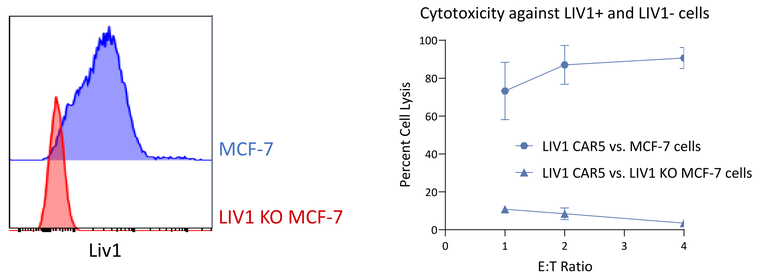
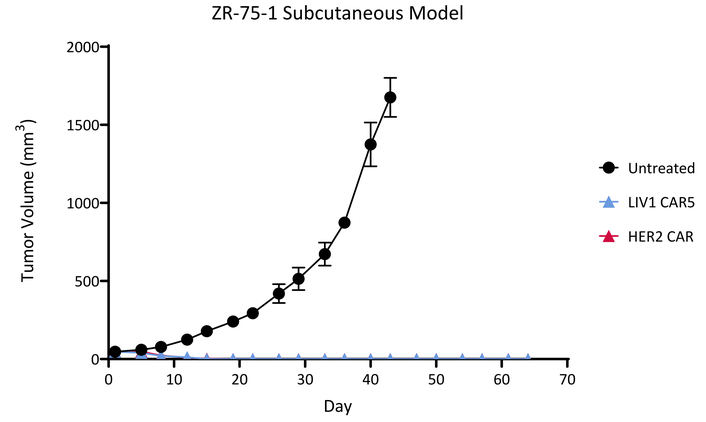


Figure 5: Cytotoxicity of anti-LIV1 CAR T cells is dependent on LIV1 expression



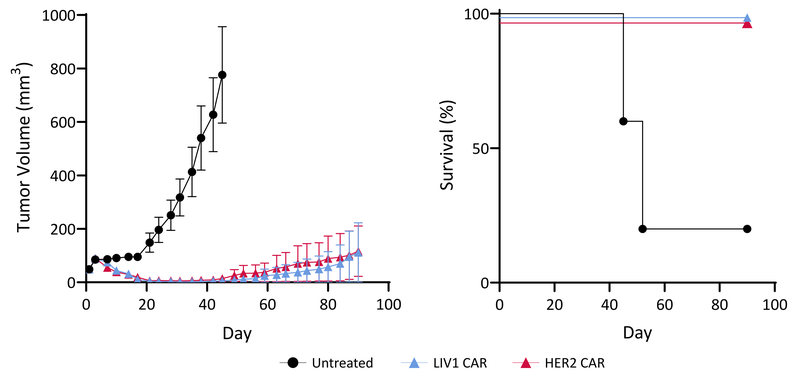
- A LIV1-negative MCF-7 cell line was developed using CRISPR/Cas9 to knock out LIV1 and validated by flow cytometry
- Anti-LIV1 CAR T cells show potent activity against LIV1-expressing MCF-7 cells but not LIV1-negative MCF-7 cells, demonstrating the specificity of the anti-LIV1 CAR

Figure 6: Anti-LIV1 and HER2 CAR T cells exhibit potent activity in an HR+HER2+ *in vivo* subcutaneous model



- Both anti-LIV1 and anti-HER2 CAR T cells demonstrate control of ZR-75-1 subcutaneous tumors. Mice were dosed at 2e7 CAR+ T cells
- All mice treated had no detectable tumor remaining

Figure 7: Anti-LIV1 and HER2 CAR T cells exhibit potent anti-tumor activity in an *in vivo* mammary fat pad model



- Both anti-LIV1 and anti-HER2 CAR T cells demonstrate control of HR+HER2+ ZR-75-1 mammary fat pad tumors (left panel). Anti-LIV1 CAR T cells eliminated tumor in all but one mouse. Mice were dosed at 2e7 CAR+ T cells
- Mice treated with both types of CAR T cells show improved survival (right panel). Lines at 100% are staggered for visual convenience

Conclusions

- LIV1 represents a promising target for CAR T cell therapy to treat breast cancer
- LIV1 is widely expressed across all clinical subtypes of breast cancer, with limited expression on normal tissue
- We generated anti-LIV1 CAR T cells that exhibit potent activity *in vitro* and *in vivo* against multiple breast cancer cell lines and that can traffic to the mammary fat pads of mice
- Our next-generation investigative CAR T programs build upon our core allogeneic CAR T chassis by incorporating KO of Regnase-1 and TGFBR2. These edits could enhance the potency of anti-LIV1 CAR T cells even further

References: Sussman et al., Mol Cancer Ther. 2014 Dec;13(12):2991-3000. SGN–LIV1A: A Novel Antibody–Drug Conjugate Targeting LIV-1 for the Treatment of Metastatic Breast Cancer.