Non-viral targeted knock-in of a KRAS G12D specific TCR, CD8α/β, and chimeric cytokine receptor in the *TRAC* locus outperforms lentiviral-based engineering of T cells

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Abstract #1223

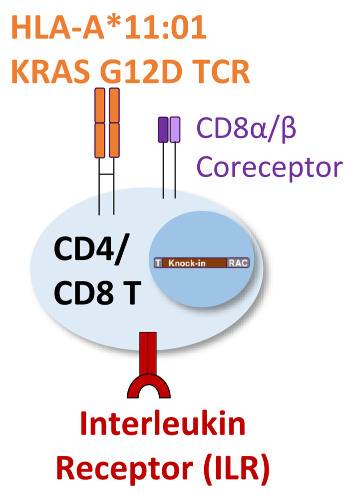
Abstract

T cells engineered with T cell receptors (TCRs) enable targeting of clonally-expressed oncogenic driver mutations and have potential to induce durable responses in patients with solid tumors. Viral vectors, including lentiviral vectors (LVV), have been a standard modality to deliver transgenes for T cell therapies but are severely limiting in their manufacturing time, cost, and cargo size. In contrast, non-viral targeted gene knock-in (KI) overcomes these limitations, substantially reducing manufacturing complexity. Here, we compare primary human T cells engineered using LVV or KI processes to express a TCR recognizing a KRAS G12D mutant peptide presented on HLA-A*11:01, the CD8α/β coreceptor, and a chimeric interleukin receptor (ILR). We developed and optimized a non-viral manufacturing process that uses a novel CRISPR-Cas12a system to knock-in the transgene cassette within the *TRAC* locus and to simultaneously knock out the endogenous TCR[1]. Our non-viral KI platform edits primary T cells with high efficiency, and we show that KI engineered TCR T cells performed equivalently or better in functional assays relative to LVV-engineered cells. Together, these data support the utility of a non-viral gene KI approach and its planned incorporation into clinical development.

Overview of Transgenes

Autologous primary human CD4+ and CD8+ T cells were engineered to express:

- A high avidity HLA-A*11:01-restricted TCR specific for the *KRAS G12D* mutation,
- CD8 α/β co-receptor that drives a coordinated CD8/CD4 T cell response by allowing for CD4 stimulation that promotes CD8+ T cell functional persistence,
- Interleukin Receptor, ILR, a fusion protein that promotes anti-tumor activity through increased T cell proliferation and survival.



Gene Editing

We used MG29-1 nuclease from Metagenomi, a Type V CRISPR-Cas system[1] to knockout the endogenous TCR[2] and drive transgene integration via homologous recombination

TCR KO improves TCR T cell functionality

HuCCT1 cells expressing endogenous A11-G12D were co-cultured with transduced and TCR-edited CD4/CD8 T cells. T cells were rechallenged with fresh tumor cells (↑). KO of the *TRAC/TRBC* improved killing of tumor cells.

Fig 1: Lentiviral delivery is limited by cargo size

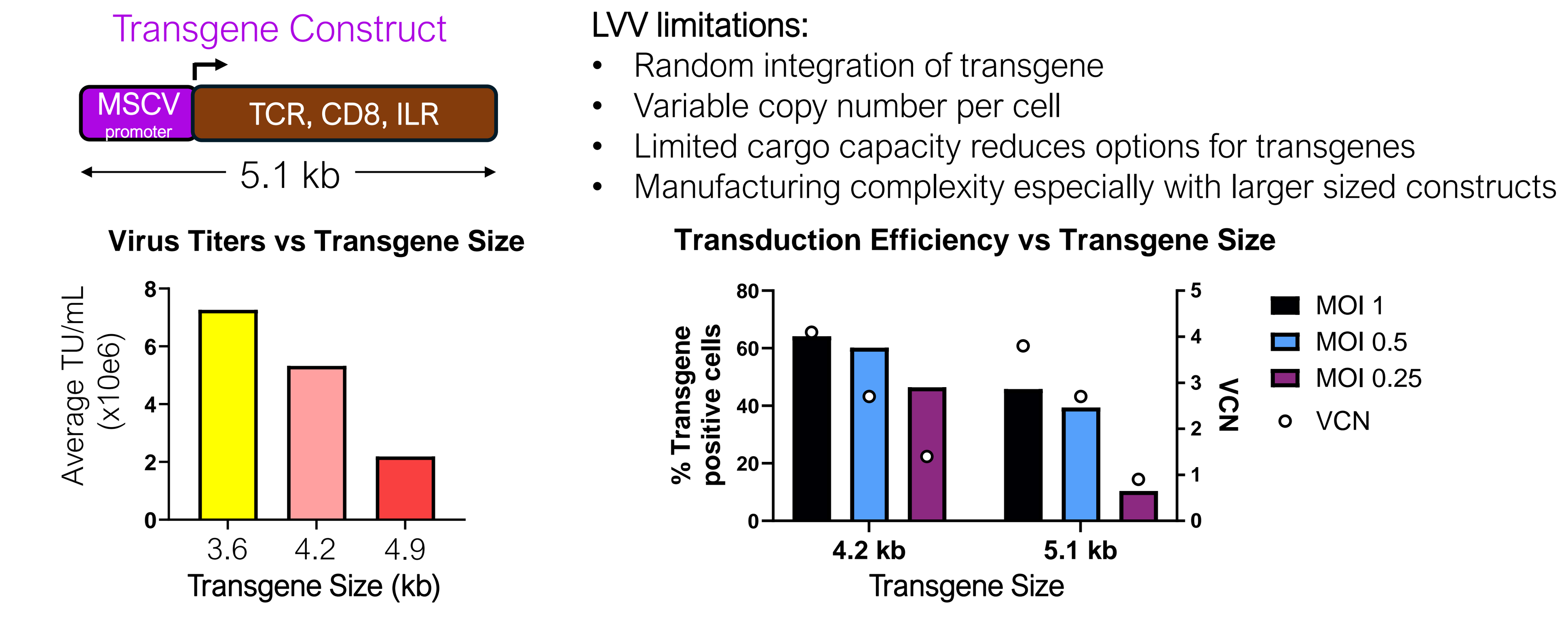
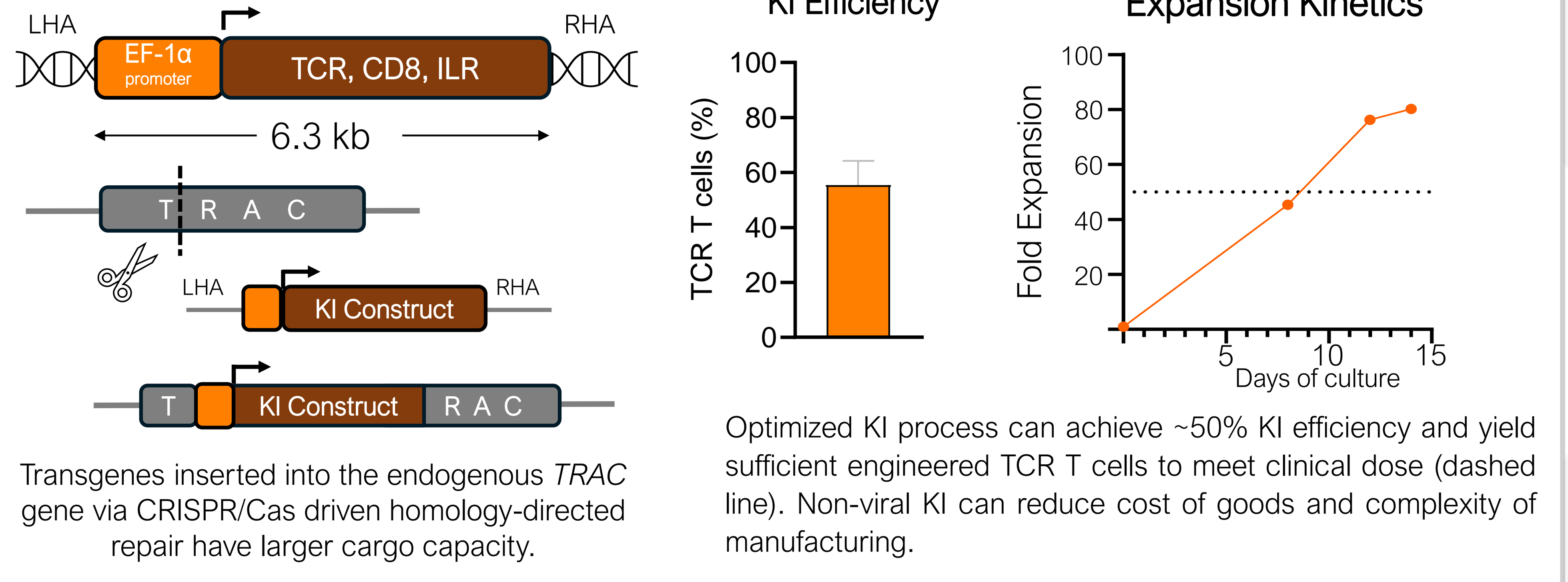


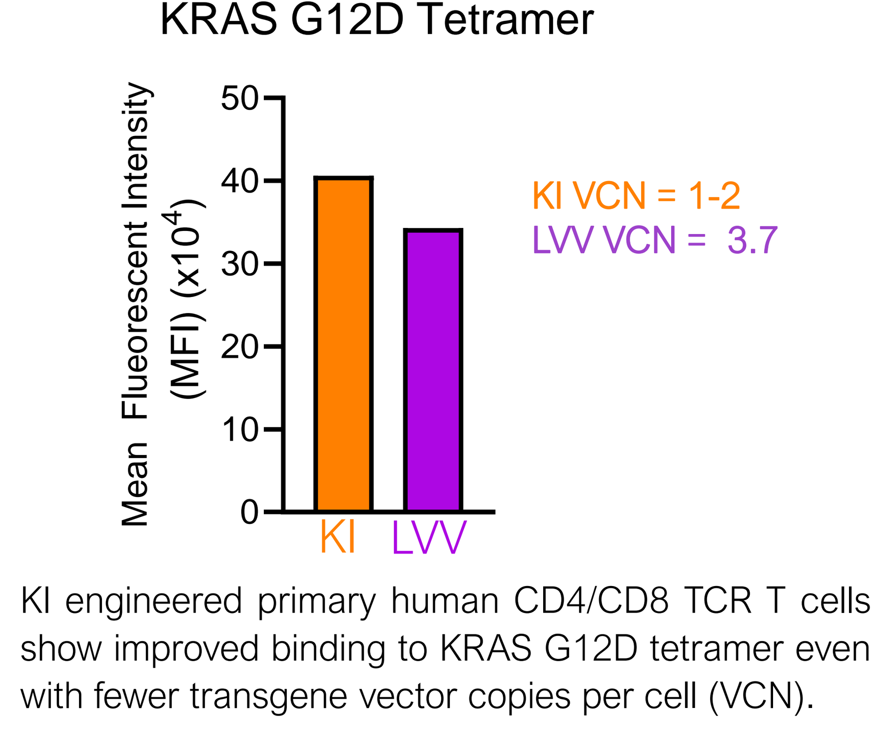
Fig 2: Non-viral Knock-in can achieve high transgene integration frequency even with large transgenes



Transgenes inserted into the endogenous *TRAC* gene via CRISPR/Cas driven homology-directed repair have larger cargo capacity.

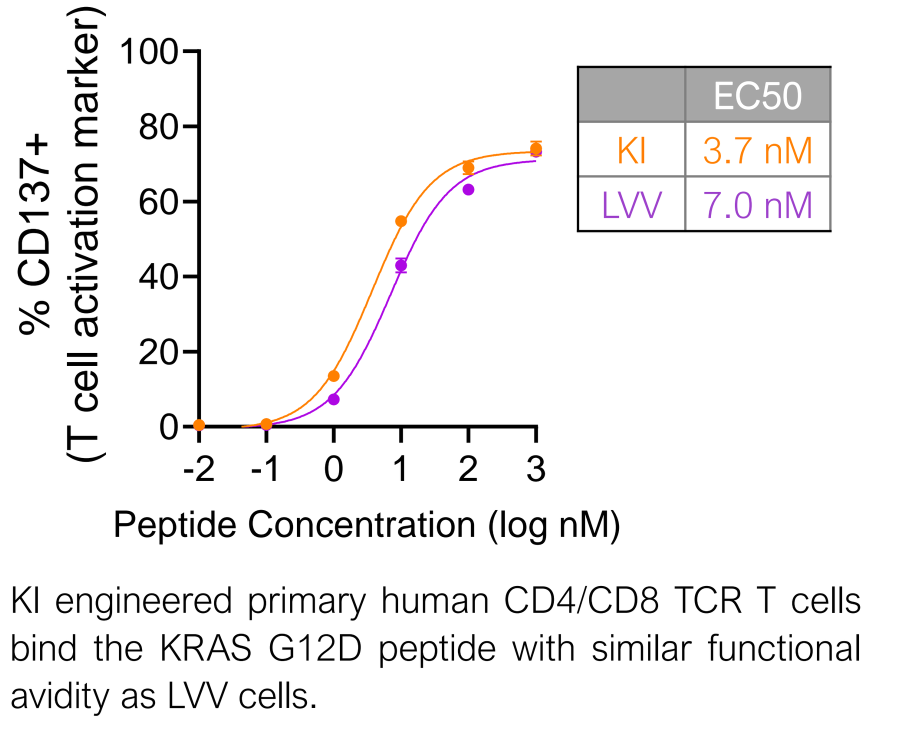
Optimized KI process can achieve ~50% KI efficiency and yield sufficient engineered TCR T cells to meet clinical dose (dashed line). Non-viral KI can reduce cost of goods and complexity of manufacturing.

Fig 3: KI TCR T cells show improved tetramer binding



KI engineered primary human CD4/CD8 TCR T cells show improved binding to KRAS G12D tetramer even with fewer transgene vector copies per cell (VCN).

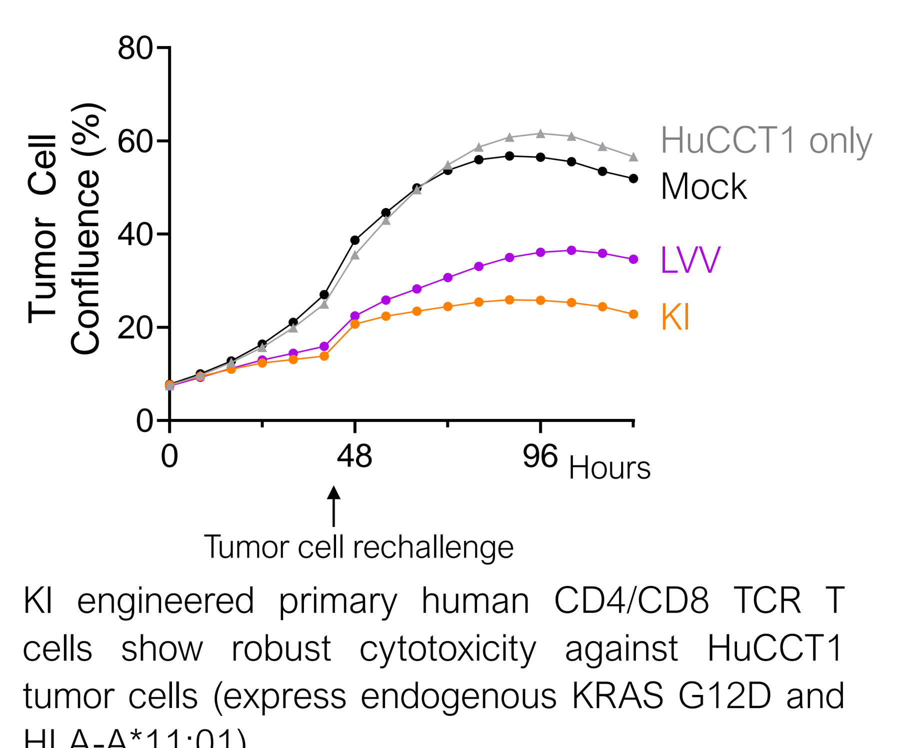
Fig 4: KI TCR T cells show high functional avidity to the KRAS G12D peptide



	EC50
KI	3.7 nM
LVV	7.0 nM

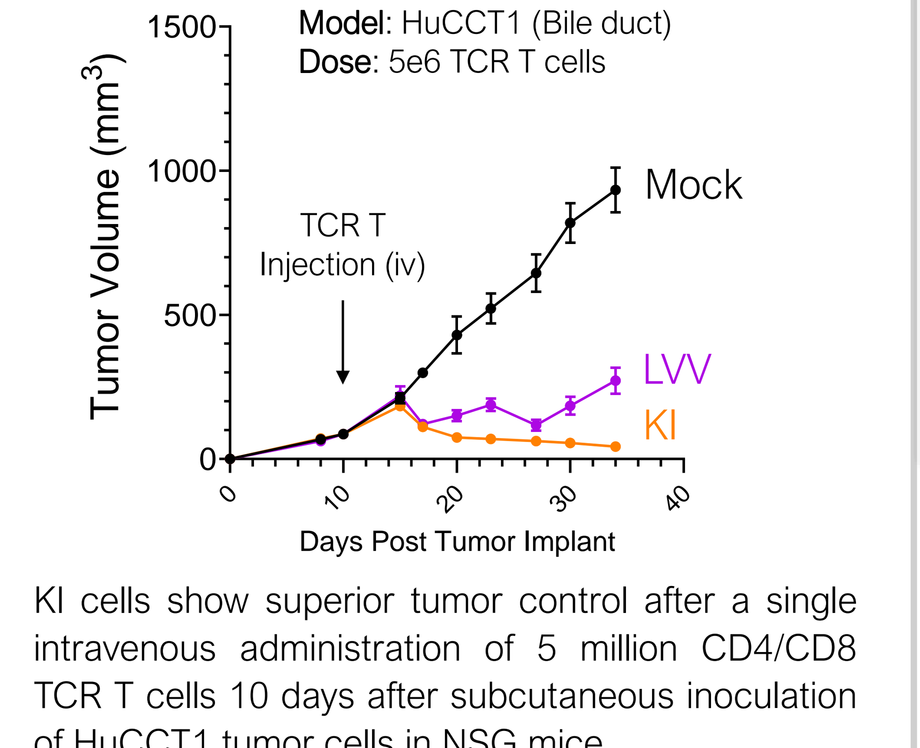
KI engineered primary human CD4/CD8 TCR T cells bind the KRAS G12D peptide with similar functional avidity as LVV cells.

Fig 5: KI TCR T cells show robust cytotoxicity *in vitro*



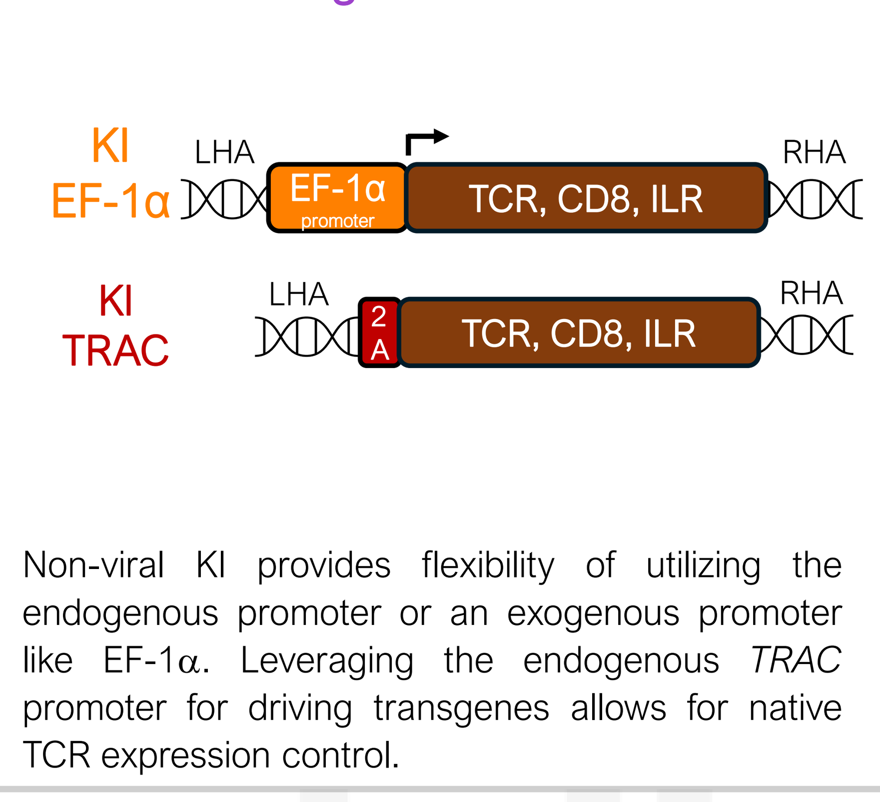
KI engineered primary human CD4/CD8 TCR T cells show robust cytotoxicity against HuCCT1 tumor cells (express endogenous KRAS G12D and HLA-A*11:01).

Fig 6: KI cell show superior anti-tumor activity in a xenograft model *in vivo*



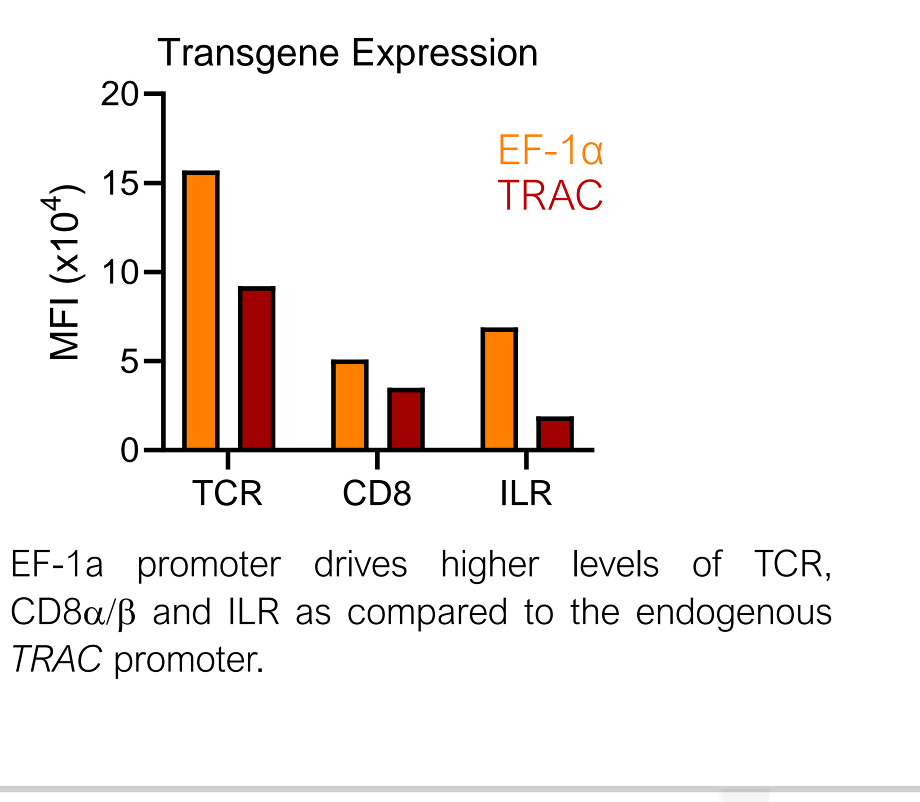
KI cells show superior tumor control after a single intravenous administration of 5 million CD4/CD8 TCR T cells 10 days after subcutaneous inoculation of HuCCT1 tumor cells in NSG mice.

Fig 7: EF-1α and Promoter-less construct designs for KI



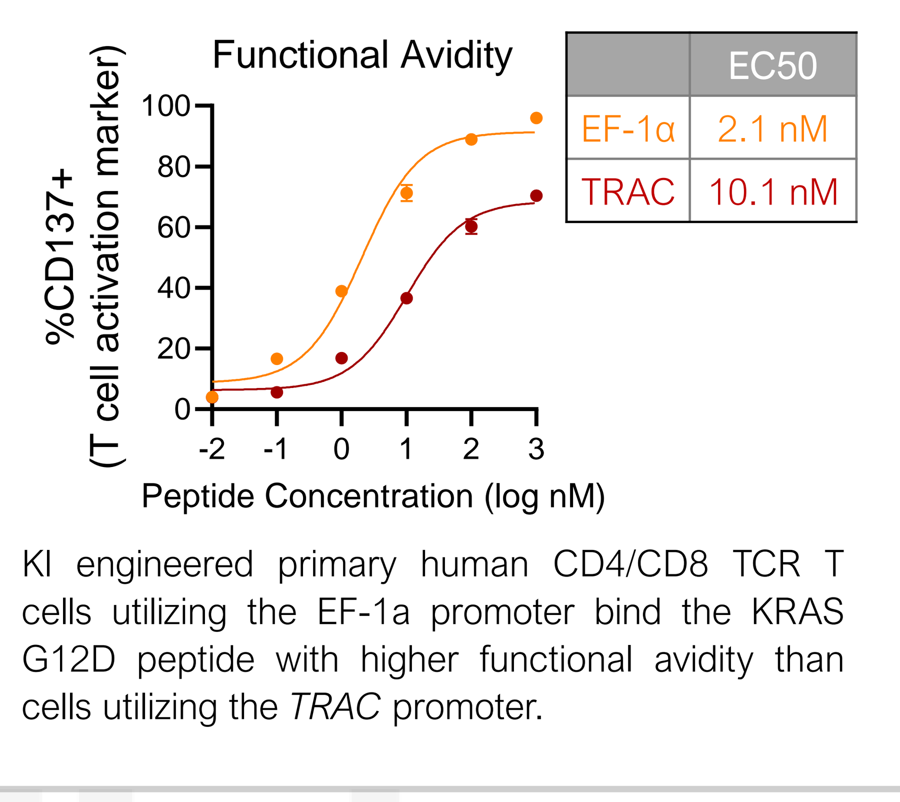
Non-viral KI provides flexibility of utilizing the endogenous promoter or an exogenous promoter like EF-1α. Leveraging the endogenous *TRAC* promoter for driving transgenes allows for native TCR expression control.

Fig 8: EF-1α promoter drives stronger transgene expression



EF-1a promoter drives higher levels of TCR, CD8α/β and ILR as compared to the endogenous *TRAC* promoter.

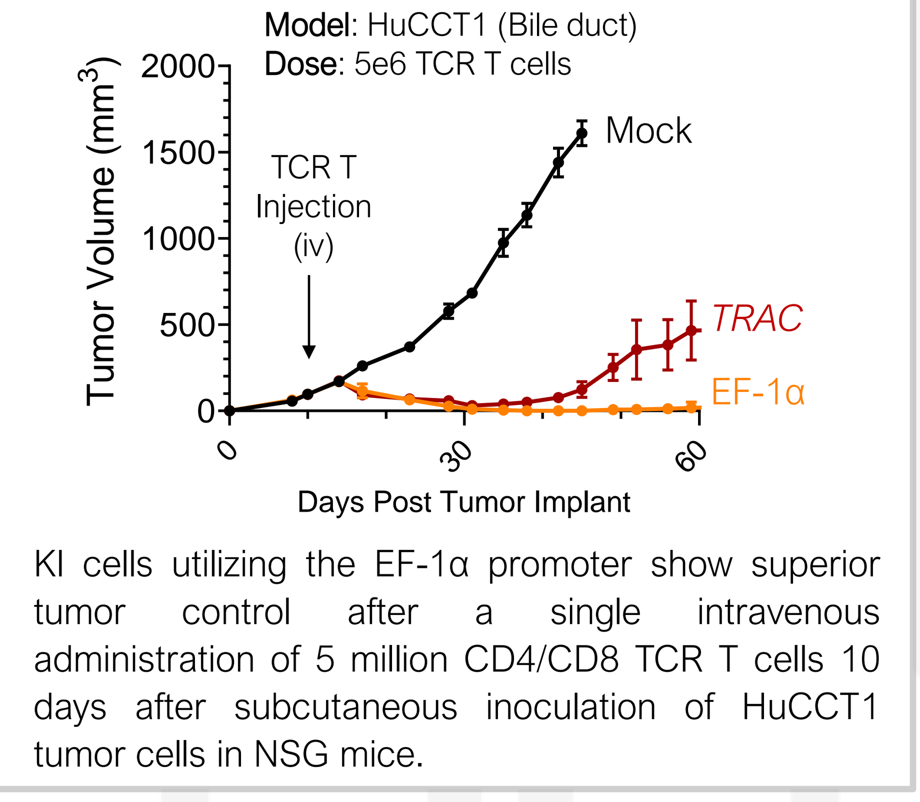
Fig 9: EF-1α promoter resulted in higher functional avidity



	EC50
EF-1α	2.1 nM
TRAC	10.1 nM

KI engineered primary human CD4/CD8 TCR T cells utilizing the EF-1a promoter bind the KRAS G12D peptide with higher functional avidity than cells utilizing the *TRAC* promoter.

Fig 10: EF-1α promoter drives superior anti-tumor activity in an *in vivo* model



KI cells utilizing the EF-1α promoter show superior tumor control after a single intravenous administration of 5 million CD4/CD8 TCR T cells 10 days after subcutaneous inoculation of HuCCT1 tumor cells in NSG mice.

Summary

- Non-viral targeted gene knock-in achieved high KI efficiency and cell expansion in primary CD4/CD8 T cells.
- KI engineered cells show higher transgene expression and superior *in vitro* and *in vivo* activity compared to cells engineered with LVV despite a lower transgene copy number per cell
- EF-1α promoter drives higher transgene expression than the endogenous *TRAC* promoter leading to increased avidity and functionality of KI cells
- The KI process has been established for the **AFNT-212 product poised to enter clinical testing in 2024.**

References

1. Goltsman, D.S.A. et al. Novel Type V-A CRISPR Effectors Are Active Nucleases with Expanded Targeting Capabilities, CRISPR J., 2020.
2. Schober, K. et al. Orthotopic replacement of T-cell receptor α- and β-chains with preservation of near-physiological T-cell function, Nature Biomed. Eng., 2019.

See SITC Abstract #355 for AFNT-212 product overview

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