THRIVE™ Non-Viral Targeted Transgene Knock-In Platform Generates Specific And Potent TCR T Cell Products For The Treatment Of Solid Cancers

Poster # 836

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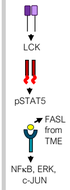
Abstract

Selective and homogenous expression of oncogenic driver mutations such as KRAS-G12D and TP53-R175H make them ideal targets for cancer therapies. Adoptive T cell therapies with engineered T cell receptors (TCRs) recognizing mutated driver proteins have led to durable responses in patients with solid tumors. Lentiviral vector (LVV)-based T cell manufacturing suffers from limited cargo size, heterogeneous transgene expression, high manufacturing cost and complexity, and the potential for oncogenesis due to random genomic insertions. To overcome these challenges, we have developed THRIVE™, a non-viral, gene-editing-based T cell manufacturing platform that achieves high rates of targeted insertion of multi-parameter constructs. THRIVE-engineered TCR T cells expressing transgenic TCR, CD8α/β coreceptor and synthetic fusion proteins demonstrated improved cytotoxicity as compared to LVV-engineered T cells both *in vitro* during tumor cell challenges and *in vivo* after single administration in mice harboring tumor xenografts. The non-viral knock-in platform also enables robust expansion of engineered T cells achieving anticipated dose levels for clinical trials. These data support the clinical readiness and advancement of the THRIVE platform for engineering TCR T cell therapy for the treatment of advanced solid malignancies.

Fig. 1: Armored TCR T Cell Therapy



The wildtype CD8 **(CD8α/β)** coreceptor potentiates signal 1 (TCR) and may trigger a coordinated CD4+ and CD8+ T cell immune response against cancer.

An Interleukin Receptor, **ILR**, that may provide constitutive signal 3 and promote anti-tumor activity through increased T cell proliferation and survival.

FASBB, FAS receptor fused with 4-1BB signaling domain, provides signal 2 upon FASL binding and may help with memory development, proliferation, metabolism, & survival.

Autologous primary **CD4+ and CD8+** TCR T cell therapies

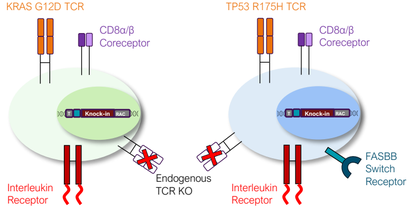
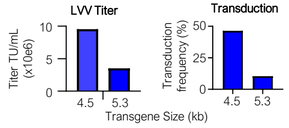
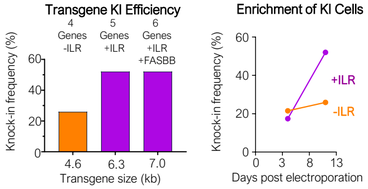


Fig. 2: Non-Viral Knock-In (KI) Platform Achieves High Integration Frequency of Multi-Transgene Constructs

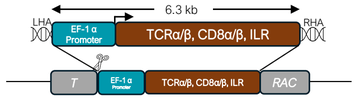
Lentiviral (LVV) delivery is limited by cargo size. Larger constructs result in lower vector titers and transduction efficiency.



Optimized non-viral KI platform achieves high transgene integration efficiency with multi-gene constructs. ILR armoring enables enrichment of KI cells.



Non-viral targeted KI within the *TRAC* gene using MG29-1, a CRISPR-Cas12a system. Transgene KI also disrupts the *TRAC* gene that enhances expression of the transgenic TCR and improves functional avidity of TCR T cells.



KRAS-G12D TCR T cells engineered via KI show superior tumor control *in vivo* over LVV engineered cells.

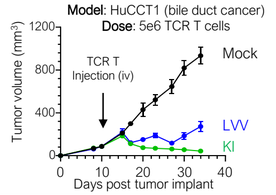
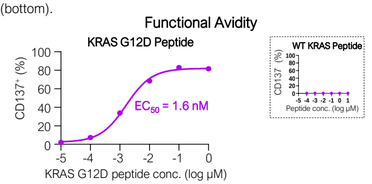
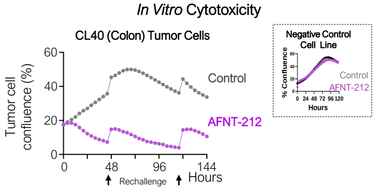


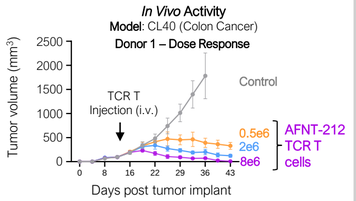
Fig. 3: Activity of Non-Viral KI Engineered AFNT-212 KRAS-G12D TCR T Cells

AFNT-212 TCR-T cells show high functional avidity and specificity to the KRAS G12D peptide (top) and controlled tumor cell growth *in vitro* even after tumor rechallenges (bottom).





In vivo treatment of CL40 tumor bearing NSG mice with AFNT-212 cells resulted in dose-dependent robust responses (top) and maintained complete durable responses in 5/5 treated mice until end of study (bottom).



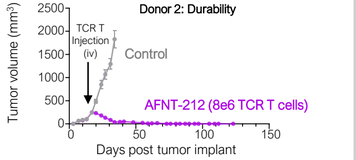
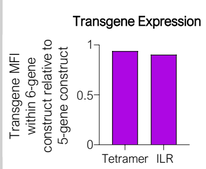
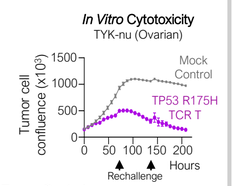


Fig. 4: TP53-R175H TCR T Cells Show Robust Tumor Control

KI engineered TP53-R175H TCR T cells show robust expression of transgenes. Genetically-engineered primary CD4+/CD8+ TCR T cells control TYK-nu tumor cells, an endogenously expressing HLA-A*02:01 TP53-R175H line, both *in vitro* and *in vivo* in a NSG mouse model.





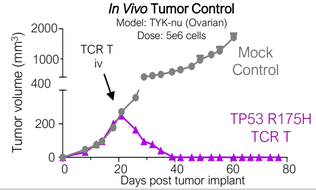
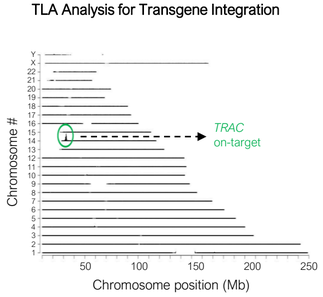


Fig. 5: Safety of Gene Editing Reagents

Target locus amplification (TLA) analysis showed transgene integration only within the desired *TRAC* locus. No off-target transgene integration was observed demonstrating specificity of GE reagents (MG29-1 and TRAC gRNA).



Potential off-target sites for GE reagents identified using *in silico* prediction and oligo-capture method were assayed for insertions and deletions in gene-edited T cells using a targeted sequencing assay. None of the potential off-targets showed significant activity.

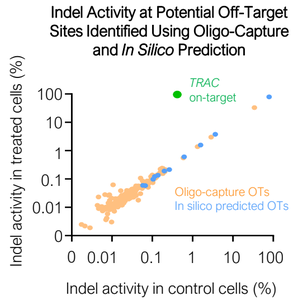
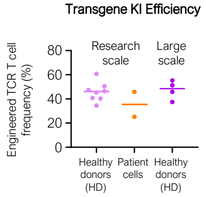
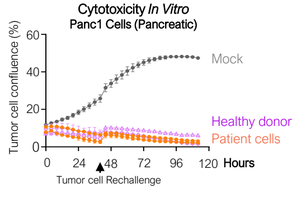
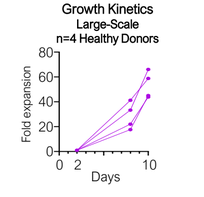


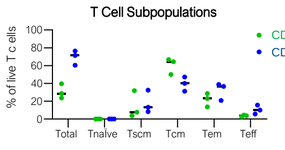
Fig. 6: THRIVE Platform Enables Robust and Scalable Manufacturing of TCR T Cells

Non-viral THRIVE platform achieves high transgene knock-in efficiency in T cells from healthy and patient starting material in both research-scale and large-scale process. Engineered AFNT-212 T cells demonstrated robust proliferation and reached relevant cell yields for clinical application. Patient cells showed tumor control similar to healthy donor cells. AFNT-212 cells show high prevalence of central memory T cells and minimal expression of exhaustion markers.

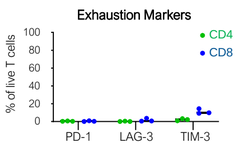








	CD95	CCR7	CD45 RO
Tnalve	-	+	-
Tscm	+	+	-
Tcm	+	+	+
Tem	+	-	+
Teff	+	-	-



Summary

- Non-viral knock-in using MG29-1 nuclease and TRAC gRNA achieves efficient targeted insertion of multi-transgene cassettes of up to 7 kb in size in CD4+/CD8+ primary T cells.
- KI engineered TCR T cells show superior anti-tumor activity compared to cells engineered with LVV.
- ILR armoring enables enrichment of engineered TCR T cells.
- AFNT-212 (KRAS-G12D TCR T) and TP53-R175H TCR T cells engineered via non-viral KI show robust *in vitro* cytotoxicity and exceptional anti-tumor activity *in vivo* achieving durable complete responses.
- Gene editing reagents show low risk of genotoxicity.
- THRIVE™, Non-viral KI platform can achieve high transgene integration efficiency and cell growth to yield relevant numbers of engineered TCR-T cells for clinical application.
- TP53-R175H program is poised to enter preclinical IND-enabling studies in 2024, and AFNT-212 program is poised to enter clinical testing in 2024.

Memorial Sloan Kettering Cancer Center

Fred Hutch Cancer Center

Metagenomi

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