Preclinical development of safe and effective T cell receptors specific for mutant KRAS G12D peptide

Loïc Vincent, Ph.D.
Chief Scientific Officer
Affini-T Therapeutics
Watertown, MA & Seattle, WA

Disclosure Information

Loïc Vincent, PhD

I have the following relevant financial relationships to disclose:

- Employee of Affini-T Therapeutics
- Consultant for bit.bio
- Stockholder in Affini-T Therapeutics, Alaya.bio, Takeda

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Targeting oncogenic driver mutations like KRAS strikes at the core of tumor biology

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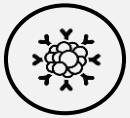
Cancer cells are dependent on oncogenic drivers	KRAS mutations are present in 30% of all solid tumors	Targeting KRAS has been clinically de-risked by approved G12C therapies	Affini-T TCRs have high specificity for KRAS and other oncogenic drivers
			
Oncogenic driver mutations initiate and maintain cancer growth, and are present in each tumor cell	KRAS represents the most frequently mutated oncogene in difficult-to-treat solid tumors	Recent drug approvals demonstrate single agent activity but need improved duration of response	Affini-T leverages TCRs to attack only cancer cells, utilizing synthetic biology to enhance persistence
Minimizes tumor heterogeneity and escape mechanisms	Provides impact for a high unmet medical need	Robust R&D interest for drugs targeting KRAS	Therapeutic modality with clinical PoC

Identification and characterization of HLA-A*11:01 KRAS G12D TCRs

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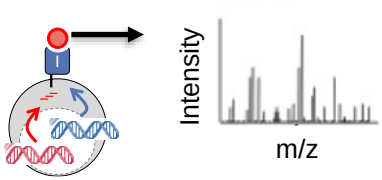
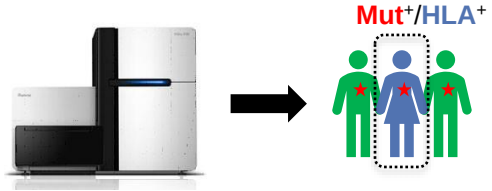
Christopher A. Klebanoff, MD, Medical Oncologist and Laboratory Head, Memorial Sloan Kettering Cancer Center (MSK), New York, NY

- Immunogenicity and therapeutic targeting of recurrently mutated ‘public’ neoantigens
- SY18 - Dharma Master Jiantai Symposium in Targeted Therapy: Cellular Therapies for Cancer
- **April 18, 2023, 12:30-2:00pm (Tangerine Ballroom 2 - WF2)**

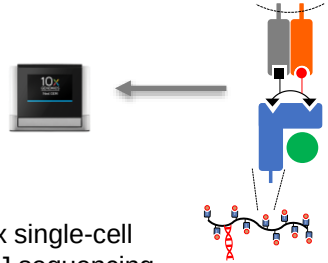
i) Immuno-peptidomic screen → ii) Public NeoAg biotrust → iii) TCR retrieval → iv) TCR validation



a) Recurrent **driver mutations**
b) Prevalent **HLA-I** alleles

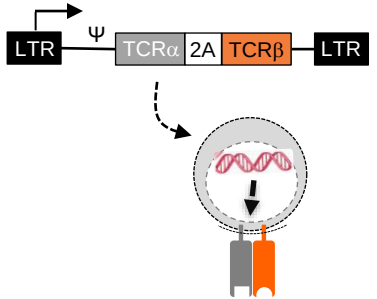


MSK-IMPACT + DARWIN



10x single-cell V(D)J sequencing

DNA barcoded dextramer



AFNT-212: A11 KRAS G12D TCR engineered CD4+ and CD8+ T cells with synthetic receptor for durability

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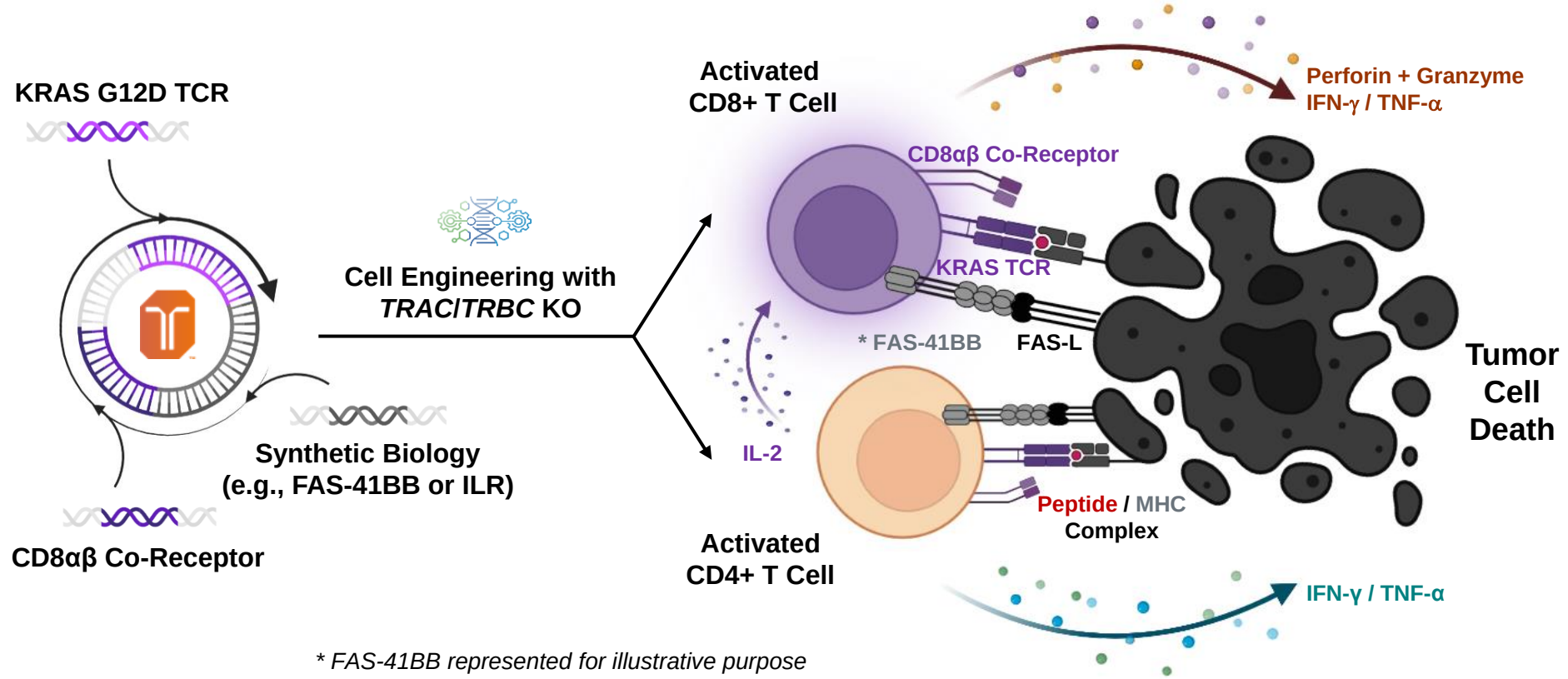

** FAS-41BB represented for illustrative purpose*

KO = Knockout

230102EA

Knockout of endogenous *TRAC* and *TRBC* could enhance functional avidity of CD4+ and CD8+ TCR-T cells

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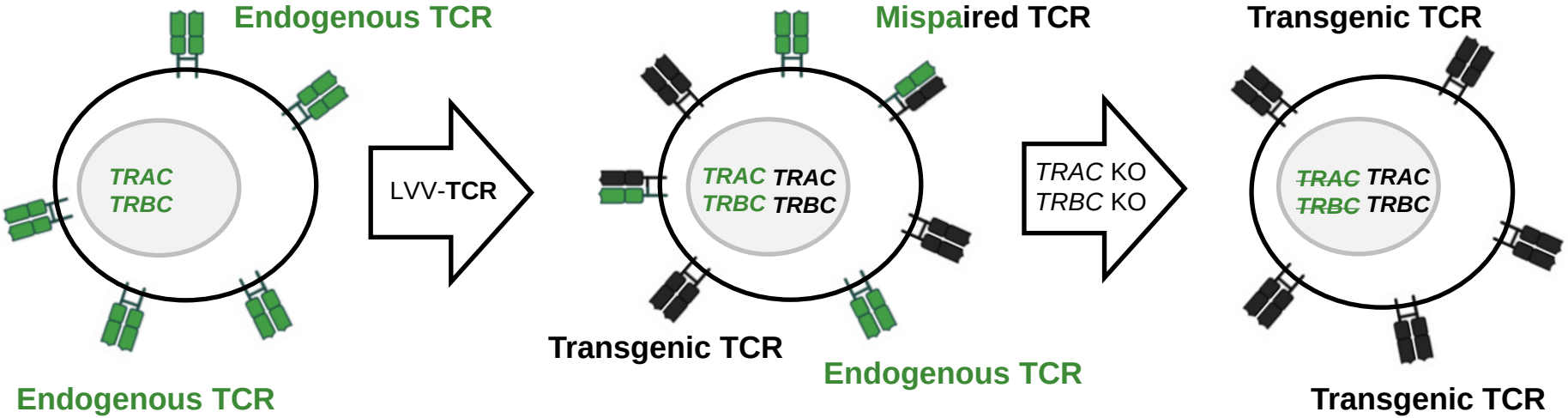

- Knockout of endogenous TRAC/TRBC (Metagenomi Type V CRISPR/Cas) could
  - eliminate mispairing,
  - improve functional expression of transgenic TCRs and
  - improve signaling by freeing the available CD3 pool.

CD4+ and CD8+ TCR-engineered T cells with knockout of endogenous *TRAC* and *TRBC* genes show enhanced activation in presence of mutant KRAS G12D peptide

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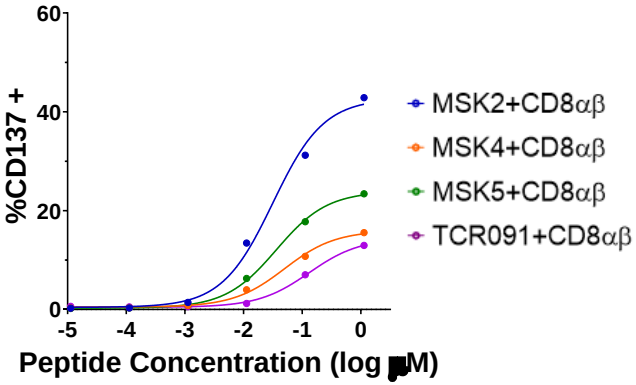
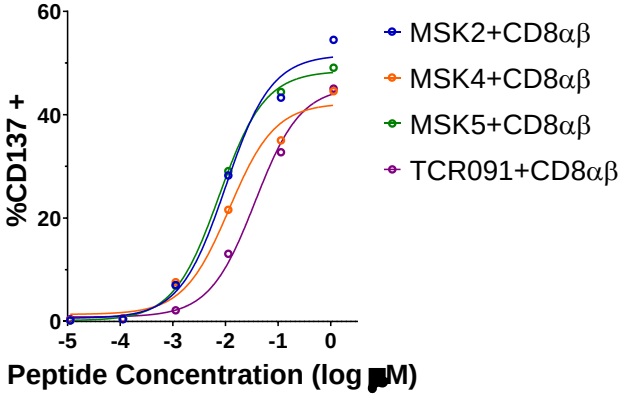
T Cell Activation

WT *TRAC*/*TRBC*



T Cell Activation

***TRAC*/*TRBC* dKO**



EC50 (nM)

Construct	WT	dKO
MSK2	31.6	10.1
MSK4	49.8	11.8
MSK5	36.3	7.6
TCR091	131.3	36.5

dKO improved EC50 by 3-5 folds over WT

- Primary CD4+ and CD8+ T cells were transduced with indicated lentiviruses, electroporated with *TRAC* and *TRBC* targeting RNPs, expanded for 10 days and treated with mutant KRAS G12D peptide; Activation of TCR-T cells was assessed via CD137 expression
- Primary CD4+ and CD8+ TCR-T cells with knockout of endogenous *TRAC*/*TRBC* genes showed enhanced activation upon stimulation with index peptide

dKO = Double Knockout of TRAC and TRBC

KRAS G12D TCR-T cells with TRAC/TRBC knockout exhibit robust *in vitro* cancer killing in repeat tumor challenge assay

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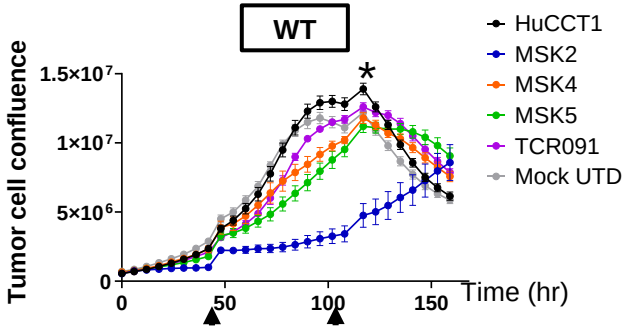
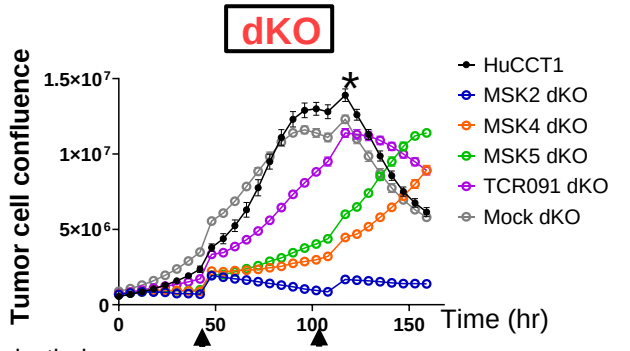
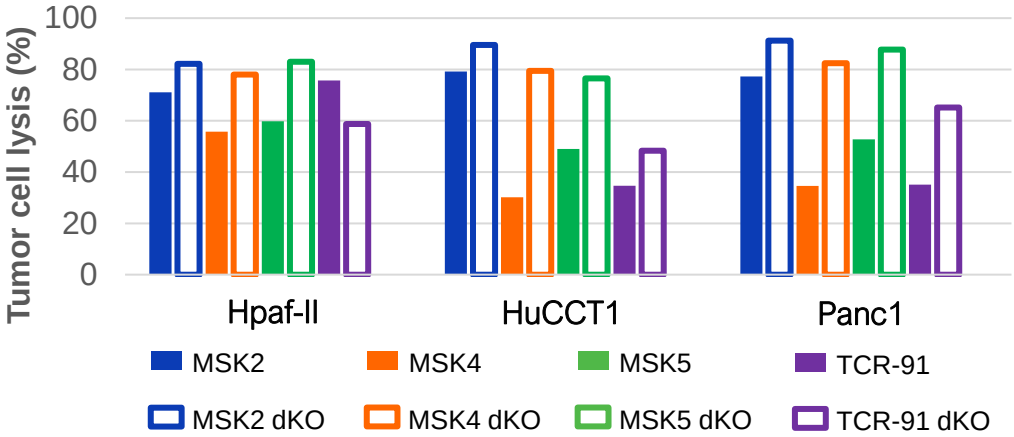
Tumor Cell killing HuCCT1 cells (3:1 E:T)





* Cell death due to confluency

Tumor Cell Lysis Efficiency



- Engineered CD4+ and CD8+ TCR-T cells were assessed for function in a tumor cell killing assay across three G12D presenting cell lines
- CD4+ and CD8+ TCR-T cells with knockout of endogenous TRAC/TRBC genes showed robust killing with rechallenge modelling chronic exposure to tumor

KRAS G12D TCRs to mutant neoepitope are highly selective with low potential for cross-reactivity

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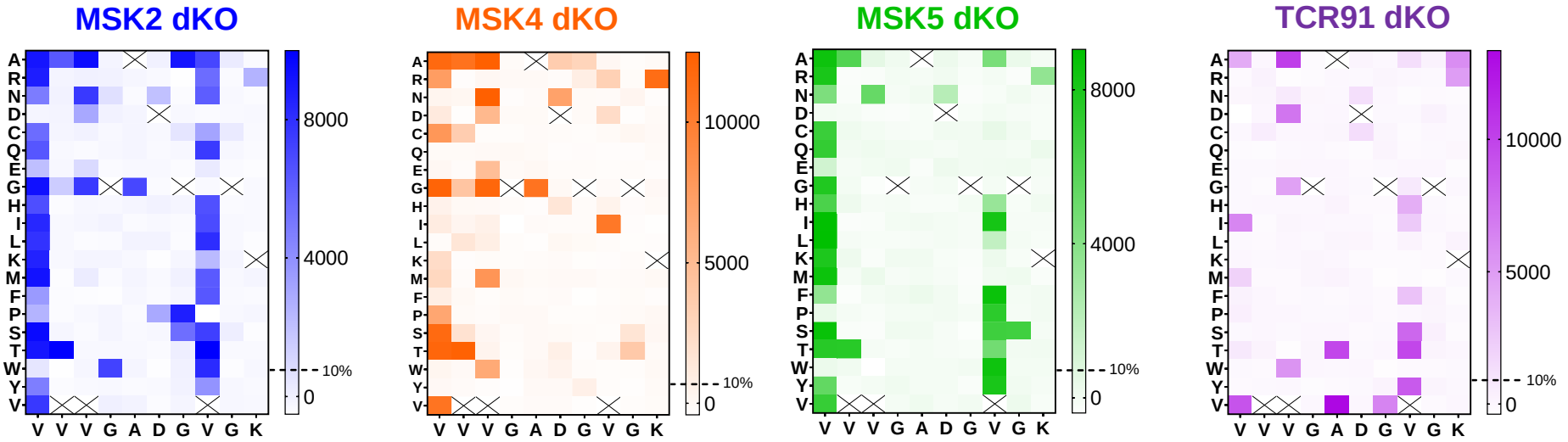

TCR	# Potential Off-Target Peptides
MSK2	21
MSK4	9
MSK5	1
TCR91	0

- CD4+/CD8+ dKO KRAS G12D TCR-T cells were stimulated with KRAS G12D or mutant peptides in which the cognate amino acid was sequentially changed to all possible 19 amino acids; T cell activity was assessed via secreted IFNγ levels
- No/few potential off-targets were identified for G12D TCRs

KRAS G12D TCR-T cells with TRAC/TRBC knockout demonstrate robust preclinical anti-tumor activity *in vivo*

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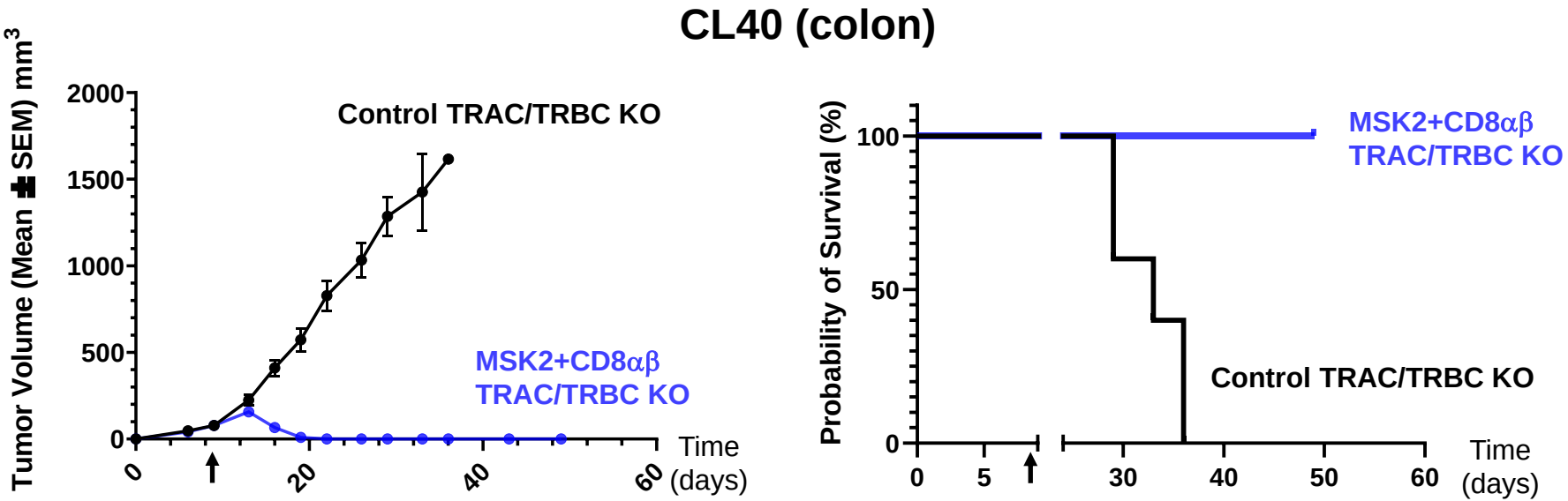

- NSG mice randomized after SC tumor implantation (5 mice/group)
- Dose: single IV administration of $10x10^6$ KRAS G12D TCR CD4+ and CD8+ T cells (1:1 ratio) on day 9

- CD4+/CD8+ dKO KRAS G12D TCR-T cell therapy induced 100% Complete Responses and 100% Overall Survival

Engineering strategies to overcome the various barriers in solid tumors, restricting efficacy of cell therapies

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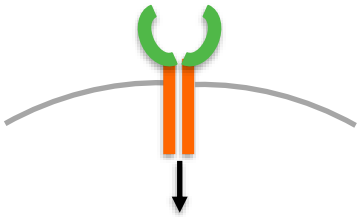
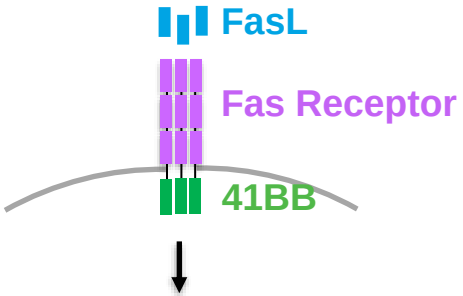
Interleukin Receptor (ILR)



pSTAT5

- Signal 3 promotes proliferation
- Increases proliferation, survival in tumor and chemokine receptor expression
- No cytokine independent survival

FAS-41BB



NFκB/ERK/c-Jun

- Signal 2 upon FASL binding
- Enhances proliferation upon FASL engagement
- Increases survival in the tumor, provides costimulatory signal, promotes metabolism to support T cell activation and memory development

CD4+ and CD8+ KRAS G12D TCR-T cells equipped with chimeric ILR show enhanced proliferation and tumor cell killing *in vitro*

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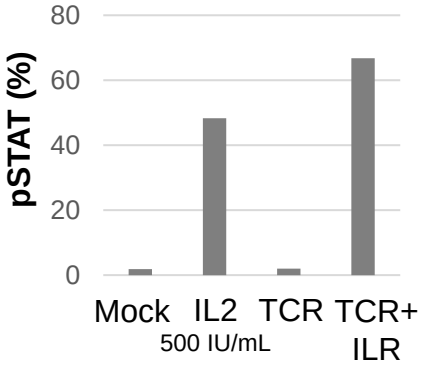
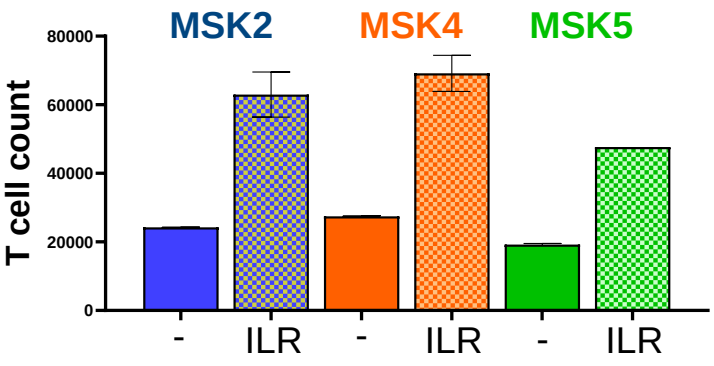
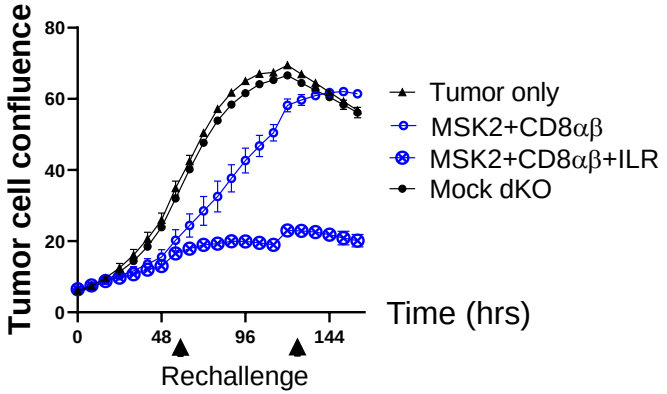
pSTAT5



T Cell Proliferation in Response to HuCCT1 Tumor Cells



Tumor Cell Killing HuCCT1 8:1 E:T



- CD4+ and CD8+ T cells were engineered with KRAS G12D TCR, CD8αβ, TRAC/TRBC KO and ILR
- Engineered T cells were assessed for activation of pSTAT, proliferation in response to tumor cells and for killing of tumor cells upon persistent exposure from multiple rechallenges
- Inclusion of ILR enhanced potency of engineered KRAS G12D TCR CD4+ and CD8+ T cells

pSTAT = Phosphorylated STAT

cGMP compatible scale-up process for non-viral KI enables efficient genetic engineering of TCR-T cells

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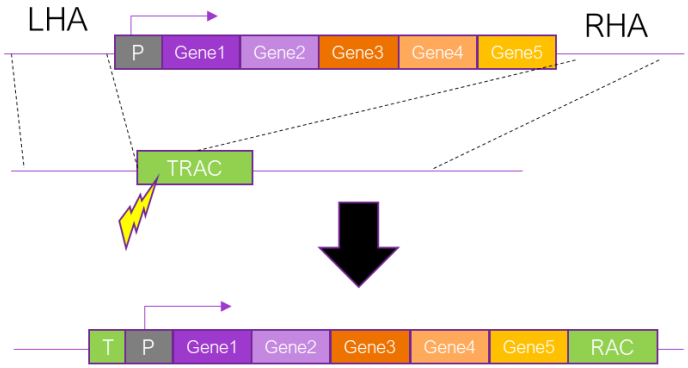
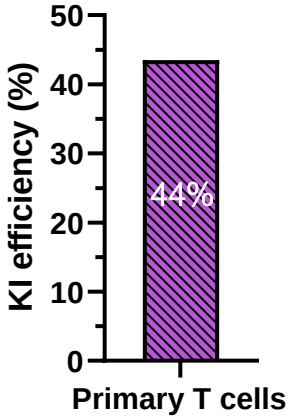
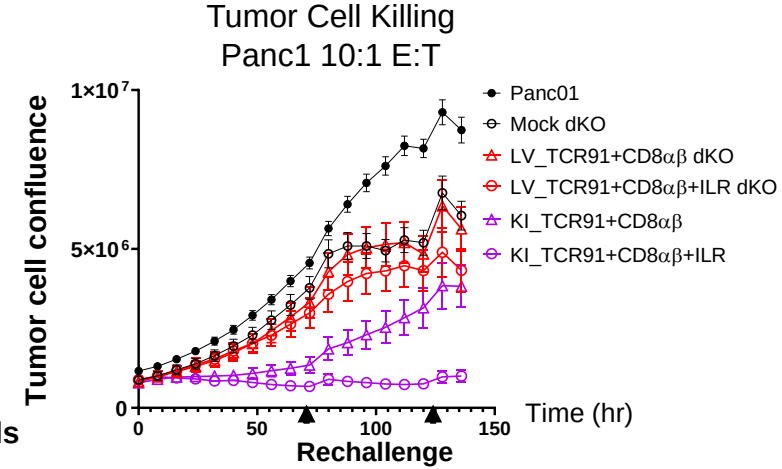
Non-Viral Targeted Transgene Integration



KI Efficiency and Functionality in T Cells





- Key advantages over LVV mediated delivery
  - Enables larger cargo size
  - Consistent targeting into a desired locus with defined copy number and expression of transgenes
  - Reduced manufacturing complexity and cost
  - Can enable innovative master clinical trial designs

- KI yielded up to 44% integration efficiency in primary CD4+ and CD8+ T cells
- KI cells outperformed LVV transduced cells in the rechallenge assay modelling chronic exposure to tumor

KI = Knockin

Summary of findings

- CD4+ and CD8+ T cells engineered with specific mutant KRAS G12D TCRs showed robust anti-tumor activity *in vitro* and *in vivo*.
- Knockout of endogenous *TRAC* and *TRBC* genes enhanced avidity and cytotoxicity of engineered KRAS G12D-specific TCR-T cells.
- Incorporation of synthetic biology engineering such as a chimeric ILR in engineered KRAS G12D TCR-T cells enhanced proliferation of engineered T cells in response to tumor cells and cytotoxicity against the tumor cells.
- Non-viral KI transgene integration enabled efficient genetic engineering of KRAS G12D TCR-T cells.
- **AFNT-212 targeting of KRAS G12D+ tumors in HLA-A*11:01+ patients is poised to enter clinical testing in 2024.**

Acknowledgements

affini

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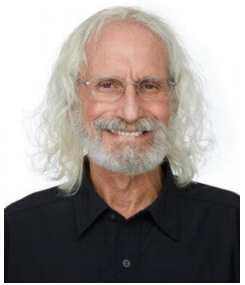
Greenberg Lab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