Poster # 1691

Novel CRISPR-associated gene editing systems enable efficient and specific TRAC/TRBC knockout triggering robust expression and sensitivity of a T cell receptor specific for mutant KRAS G12D

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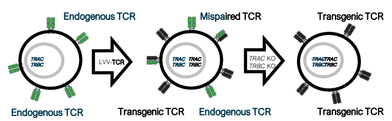
Abstract

T cells engineered with T cell receptors (TCRs) recognizing epitopes derived from intracellular oncogenic drivers like mutant KRAS, the most frequently altered gene in human cancers, have the potential to induce durable responses in patients with solid tumors. Competition for CD3 proteins and potential of mispairing between transgenic and endogenous TCRs can lead to decreased surface expression of the transgenic TCR, and thus diminished sensitivity[1]. Here, we employed a novel CRISPR-associated nuclease[2,3] to genetically inactivate endogenous TCRs with high efficiency in primary human T cells. Off-target analysis of editing did not identify any *bona fide* off-targets. *TRAC*/*TRBC* knockout (KO) improved expression of the transgenic TCR and improved the sensitivity of the engineered T cells. Finally, we achieved efficient non-viral targeted integration of transgene into primary T cells. Our data supports the utility of editing of *TRAC*/*TRBC* genes and nonviral transgene integration in clinical development of TCR-engineered T cell therapy for treating KRAS-mutant solid tumors.



Knockout of endogenous TCR could
- eliminate mispairing,
- improve functional expression of transgenic TCRs and
- improve signaling by freeing the available CD3 pool.

Gene editing (GE)

MG29-1 from Metagenomi, is a Type V CRISPR-Cas system identified from sequencing of environmental samples. The nuclease typically recognizes a 5' 4 bp PAM sequence (TTTN) and uses a 20 bp spacer sequence. Lead gRNA sequences targeting the *TRAC* and *TRBC* loci were identified through screening across multiple gRNA candidates for activity in 293 T cells and primary T cells.

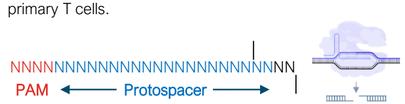


Fig 1: Activity of MG29-1 at *TRAC*/*TRBC*

CD4/CD8 T cells electroporated with gRNAs targeting *TRAC* and *TRBC* genes were assessed for expression of CD3 on cell surface and indels at on-target genomic loci. High editing efficiency was achieved for MG29-1 at both *TRAC* and *TRBC* loci. Activity was comparable to CRISPR/Cas9 system.

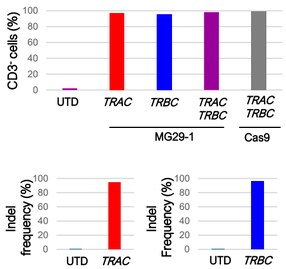


Fig 2: Off-target analysis of GE (*in silico*)

590 computationally predicted potential off-targets (OTs) were selected across *TRAC* and *TRBC* gRNAs that had up to 6 mismatches. Indel activity was evaluated at each of these potential OTs in primary T cells treated with the *TRAC* and *TRBC* gRNAs. High on-target editing efficiency was observed but no off-target activity was detected above background beyond the quantification limit of the assay (0.05%).

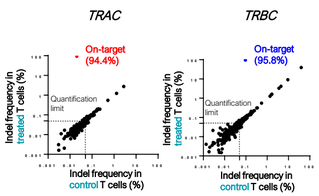


Fig 3: Off-target analysis of GE (*in vitro*)

Oligo-capture analysis was performed in primary T cells to further evaluate the specificity of these nuclease/gRNA combinations. A few potential OTs were identified each of which had at least 100-fold fewer barcodes than the target sites and had multiple mismatches (>9) to the target site suggesting the low likelihood of these OTs being true positives.

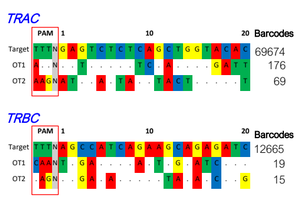


Fig 4: Translocation analysis

Frequencies of potential translocation outcomes between the *TRAC* and *TRBC* loci were evaluated in edited primary T cells using dPCR and karyotyping. Editing T cells with *TRAC*/*TRBC* gRNAs resulted in a low frequency of translocation events. No enrichment of translocation events was observed over culture time.

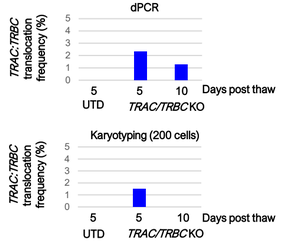
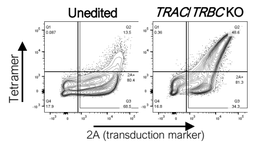


Fig 5: Improved binding to tetramer

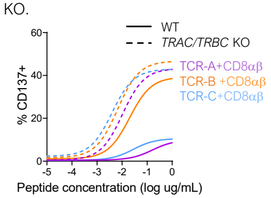
Primary CD4/CD8 T cells were transduced via lentiviral vector (LVV) with TCRs specific for KRAS HLA-A11-G12D and CD8αβ and electroporated with *TRAC*/*TRBC* gRNAs. Endogenous TCR KO resulted in improved tetramer binding.



KRAS A11.G12D TCR	Tetramer MFI	
	Unedited	KO
TCR-A	1197	3214
TCR-B	7629	14250
TCR-C	369	2505

Fig 6: Enhanced functional avidity

CD137 (T cell activation marker) expression on transduced and edited T cells co-cultured with HLA-A11 KRAS G12D mutant peptide demonstrates enhanced reactivity upon *TRAC*/*TRBC* KO.



KRAS A11.G12D TCR	EC50 (nM)	
	Unedited	KO
TCR-A	178.9	14.4
TCR-B	22.2	7.3
TCR-C	31.1	4.4

Fig 7: Improvement in cell killing (*in vitro*)

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

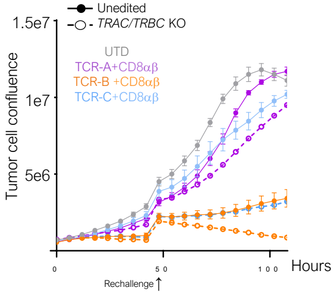


Fig 8: *In vivo* activity of edited TCR T cells

1e7 CD4/CD8 TCR-T transduced with A11-G12D TCR-B, CD8αβ, & interleukin receptor (ILR) or FAS-41BB with *TRAC*/*TRBC* KO were intravenously administered 9 days after subcutaneous inoculation of HuCCT1 cells. Inclusion of ILR or FAS-41BB improved anti-tumor response. ILR led to durable complete responses in mice.

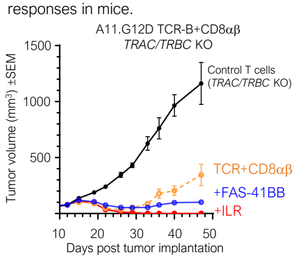


Fig 9: Non-viral transgene insertion

MG29-1 nuclease and gRNA was utilized to integrate non-virally delivered transgene cassette at *TRAC* locus via HDR. High knock-in (KI) efficiency of the transgene was achieved with both research grade and cGMP compatible equipment.

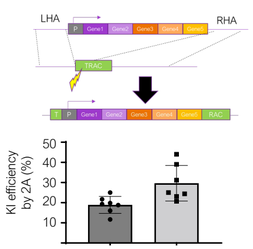
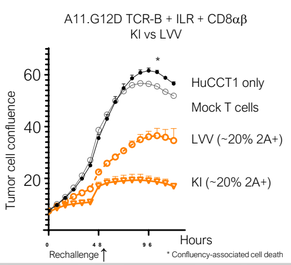
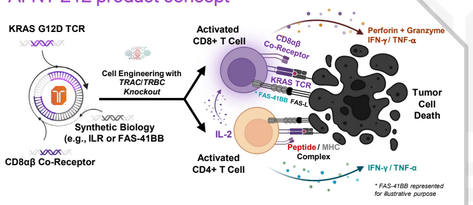


Fig 10: Cytotoxicity of KI cells

HuCCT1 cells were co-cultured with CD4/CD8 T cells engineered via LVV or targeted knock-in. T cells were rechallenged with fresh tumor cells at 48 hr. T cells engineered via non-viral knock-in showed improved tumor cell killing.



AFNT-212 product concept



- CD4/CD8 T cells transduced with high potency TCR that specifically recognize KRAS G12D presented by HLA-A11:01
- CD8 α/β co-receptor drives a coordinated CD8/CD4 T cell response by allowing for CD4 stimulation that promotes CD8+ T cell functional persistence
- *TRAC*/*TRBC* KO improves TCR expression and functional avidity
- ILR fusion protein improves proliferation and survival in tumor
- FAS-41BB[4] switch receptor improves proliferation and efficacy in response to FAS ligand on tumor cells

Summary

- MG29-1 nuclease and *TRAC*/*TRBC* gRNA combinations achieved high KO efficiency in primary human CD4/CD8 T cells and showed high specificity for the on-target sites
- Endogenous *TRAC*/*TRBC* KO improved functionality of T cells as assessed via tetramer binding, activation and *in vitro* and *in vivo* cytotoxicity against a tumor cell line that endogenously expresses HLA-A11 and mutant G12D KRAS
- ILR and FAS-41BB improved *in vivo* efficacy of TCR⁺ T cells
- Achieved high transgenesis efficiency through non-viral knock-in that resulted in functional TCR⁺ T cells
- AFNT-212 program poised to enter clinical testing in 2024

References

1. Schober *et al.*, Orthotopic replacement of T-cell receptor α- and β-chains with preservation of near-physiological T-cell function, Nature Biomed. Engg., 2019.
2. Goltsman *et al.*, Novel Type V-A CRISPR effectors are active nucleases with expanded targeting capabilities, CRISPR J., 2020.
3. Lamothe *et al.*, Novel CRISPR-associated gene-editing systems discovered in metagenomic samples enable efficient & specific genome engineering. *CRISPR J.* 2023 (In press)
4. Oda, S. K. et al. A Fas-4-1BB fusion protein converts a death to a pro-survival signal and enhances T cell therapy. J Exp Med 217 (2020)

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May 16-19, 2023

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