



Developing Next-Generation Engineered TCR-T Cells with CRISPR

Keystone Symposium: Engineering the Genome

Birgit Schultes, Ph.D. | February 10, 2020

Disclosure: Employee of Intellia Therapeutics, Inc.

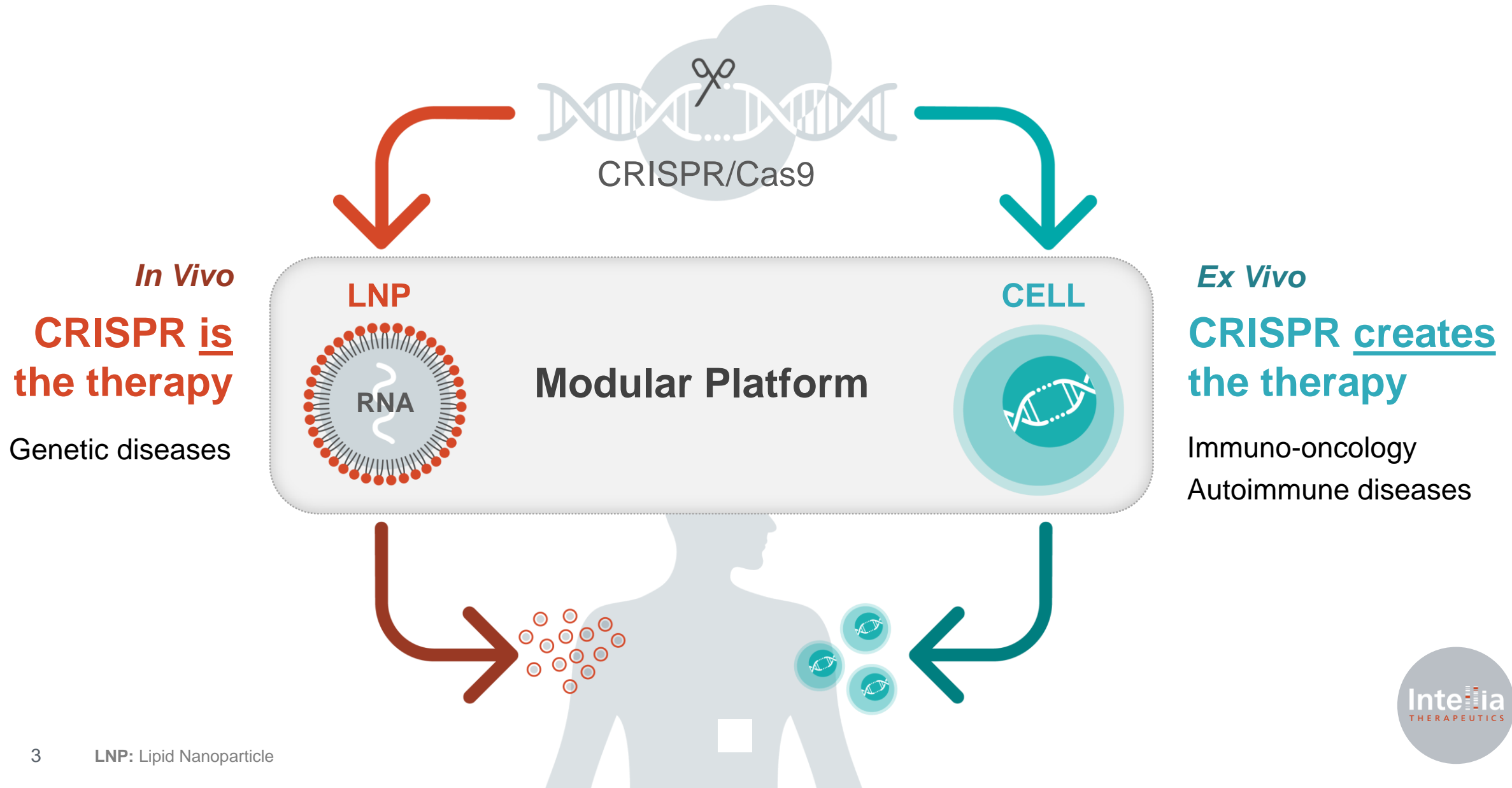
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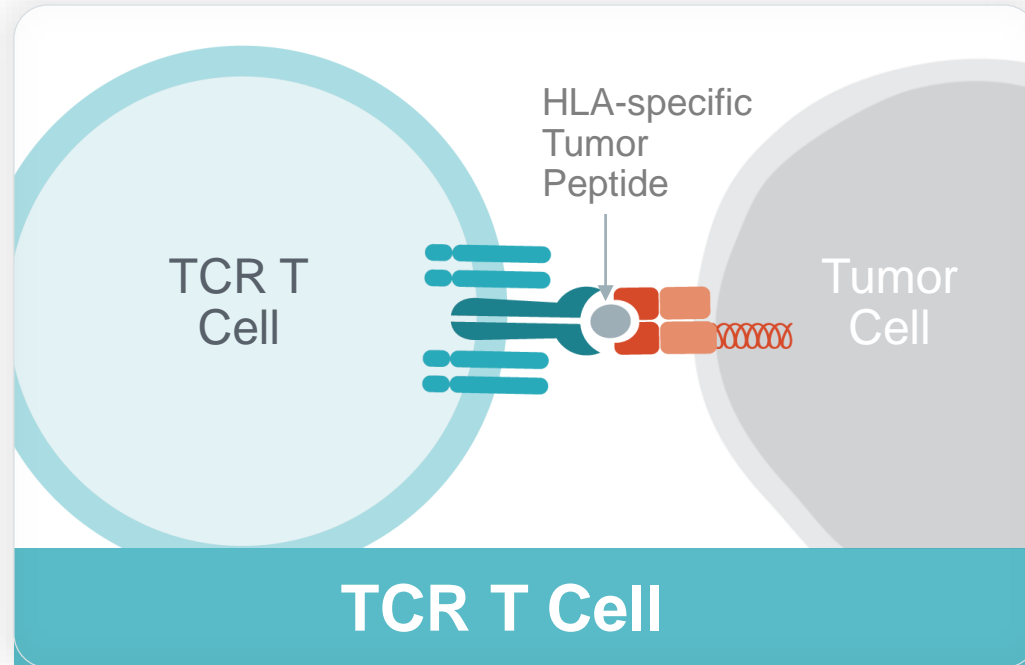
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Intellia Therapeutics is a Full-Spectrum Genome Editing Company



Intellia is Employing TCR Adoptive T Cell Therapy for a Broad Range of Cancers

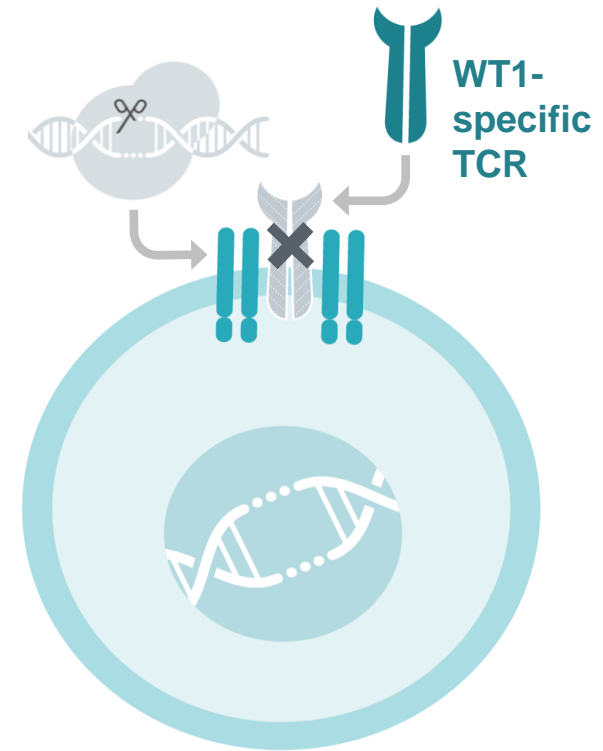


- Recognizes both surface and intracellular tumor antigens
- Physiological activation prevents premature exhaustion
- Natural TCR selection prevents reactivity against healthy tissues
- Preliminary clinical data suggest that TCR T cells show less cytokine release syndrome than CAR-T cells

Our Goal: Engineer T Cell Therapies While Preserving Normal Cell Physiology

CRISPR-Based Engineering

- Enables **precise** gene KO and insertion
- **Replaces** endogenous with therapeutic TCR^{1,2}:
 - Enhances TCR expression and function
 - Reduces mispairing and GvHD risk
 - Improves cell drug quality and potency



Improving Safety and Efficacy Over
Current Engineered Cell Therapy Approaches

PRECISE • POTENT • PERSISTENT

KO: Knockout

GvHD: Graft-Vs-Host Disease

¹Provasi, Genovese et al., Nature Medicine 2012

²Mastaglio et al., Blood 2017

Acute Myeloid Leukemia (AML) is Intellia's Initial Cell Therapy Indication



Cancer of the blood and bone marrow that is **rapidly fatal without immediate treatment**, and is the most common type of acute leukemia in adults¹

~20K

New cases in the U.S. in 2018¹

>40K

New cases in the 7MM² in 2018¹

<30%

5-year overall survival¹

NTLA-5001 in development for AML

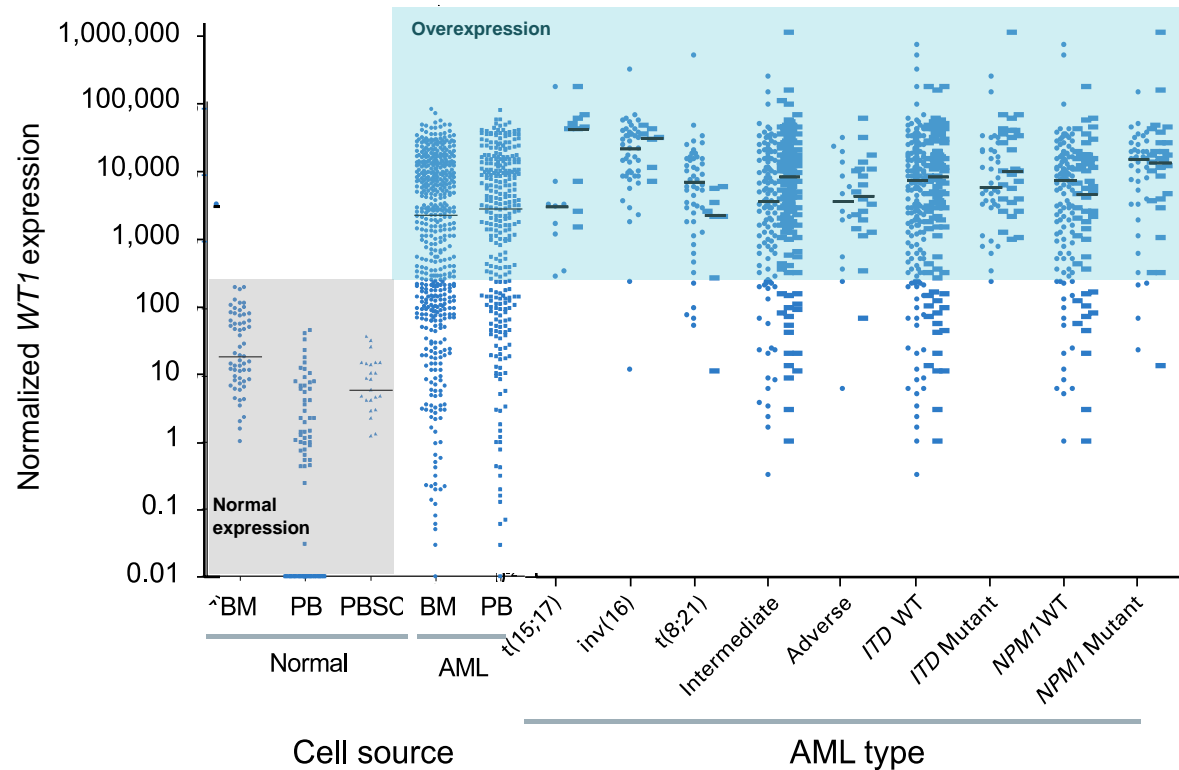
Engineer WT1-specific T cells capable of specifically killing AML blasts



¹ NIH SEER Cancer Stat Facts: Leukemia – Acute Myeloid Leukemia (AML)

² GlobalData EpiCast Report: Acute Myeloid Leukemia July 2017, 7MM: Seven Major Markets (includes U.S.)

Wilms' Tumor 1 (WT1) is Highly Over-Expressed in AML, Making it an Attractive TCR Antigen



Cilloni et al., J Clin Oncol, 2009

- Identified as top cancer antigen by NCI¹
- Plays key role in oncogenicity and is expressed in AML progenitor cells
- Intracellular protein expressed across different AML subtypes, independent of driver mutation, and maintained in relapsing disease
- Clinical activity and safety demonstrated with WT1 vaccines and T cell therapy in AML^{2,3}

¹Cheever et al. Clin Cancer Res, 2009

²DeStasi et al. Front Immunol. 2015

³Chapuis et al. Nature Med. 2019

Path to NTLA-5001, Our WT1-TCR T Cell Development Candidate



1. Identify High-Affinity WT1-TCR

- Natural TCR from healthy volunteers
- Specific and potent for WT1 tumor antigen
- HLA-A*02:01 restricted



2. Engineer T Cell Product with CRISPR/Cas9

- Eliminate endogenous TCR to prevent mispairing
- In locus insertion of therapeutic WT1-TCR



3. Characterize T Cell Product

- TCR avidity
- Polyfunctionality and anti-tumor reactivity
- TCR specificity and safety

High Potency WT1-Specific TCRs Identified from Healthy Donor T Cells

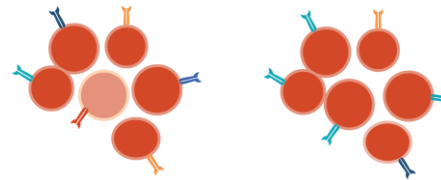
Pool of 141 peptides
Length: 15-mers*



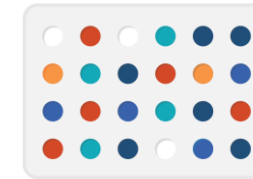
1 In vitro stimulation
with WT1 peptide pool



2 Evaluation of the
Expansion of WT1-specific
T lymphocytes

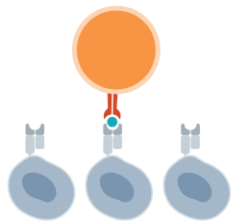


3 Identification of the
immunogenic epitope

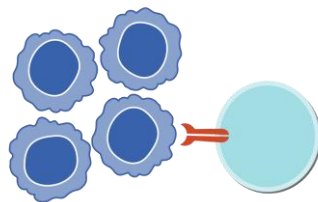


Chiara Bonini

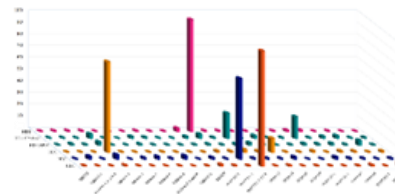
4 Determination of
HLA restriction



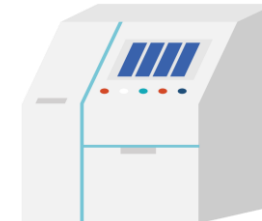
5 Assessment of target
cell recognition



6 Evaluation of the clonality
of the TCR repertoire



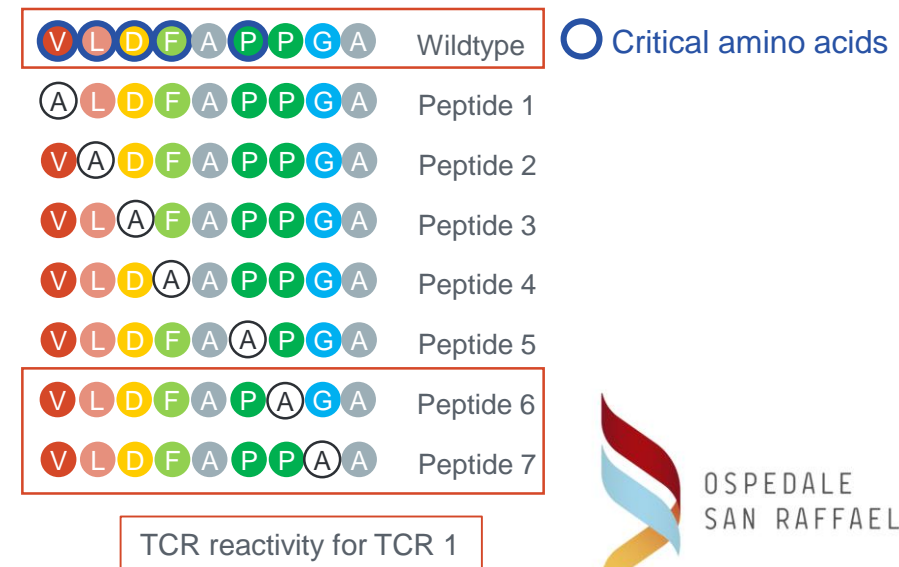
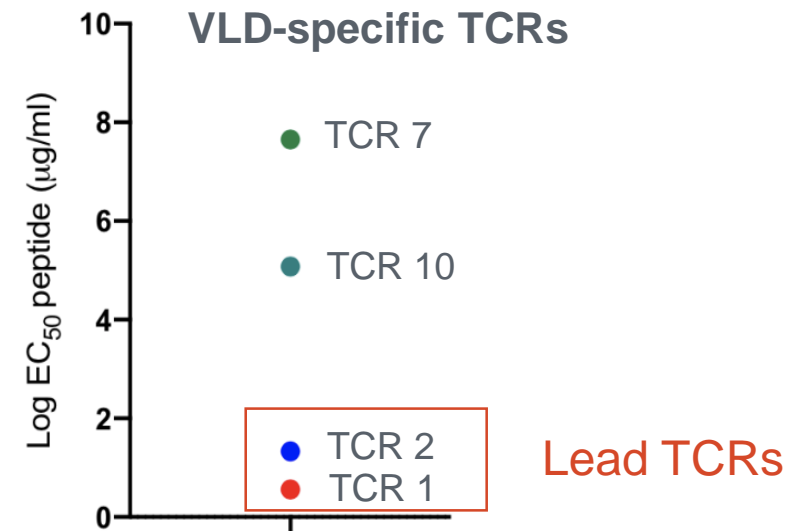
7 High-throughput sequencing
of the TCR repertoire



Eliana Ruggiero

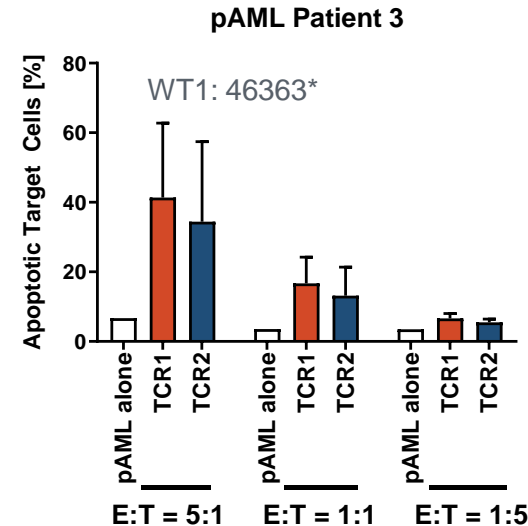
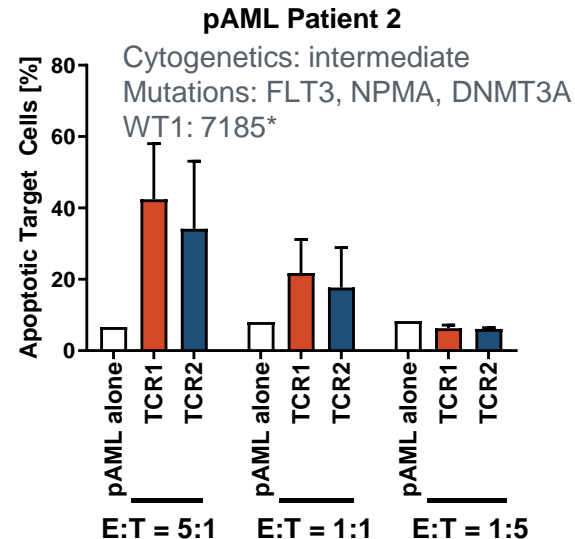
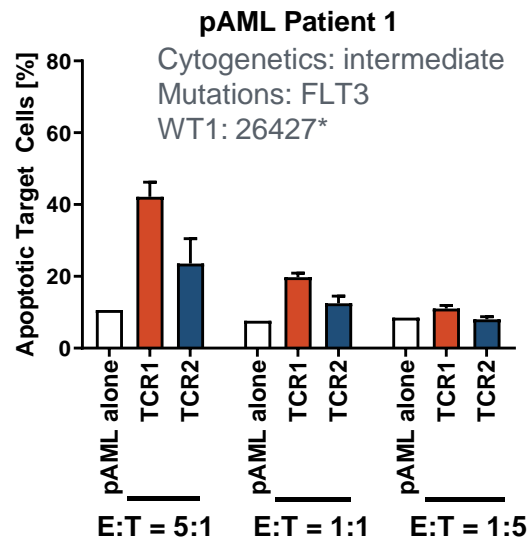
OSR Collaborators Identified Potent and Specific WT1-TCRs

- Seven HLA-A*02:01 restricted TCRs identified to two distinct immunogenic epitopes (outside of well-described RMF epitope)
- Two lead TCRs selected based on avidity and potency assays
- Binding epitopes identified and characterized by alanine scanning
- Lead TCRs recognize VLDFAPPGA epitope
 - Epitope is presented by tumor- and immuno-proteasome
- TCR-binding motif not found in human proteome outside of WT1

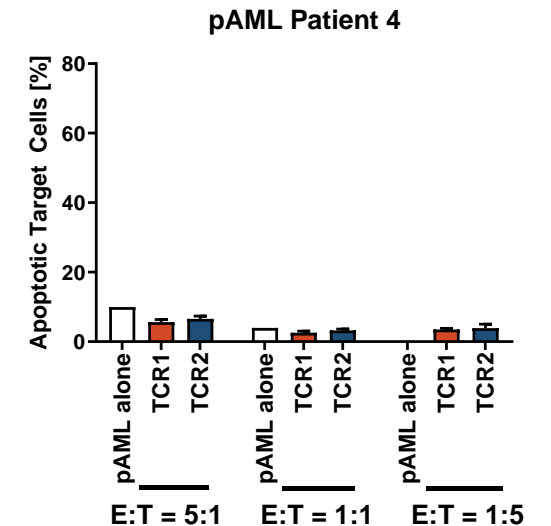


Specific Recognition and Killing of Primary AML Blasts Presenting WT1 Epitope

HLA-A*02:01 Positive



HLA-A*02:01 Negative (Control)



Mean + SD for cytotoxicity of T cell preparations from 3 healthy donors, 6 h apoptosis assay

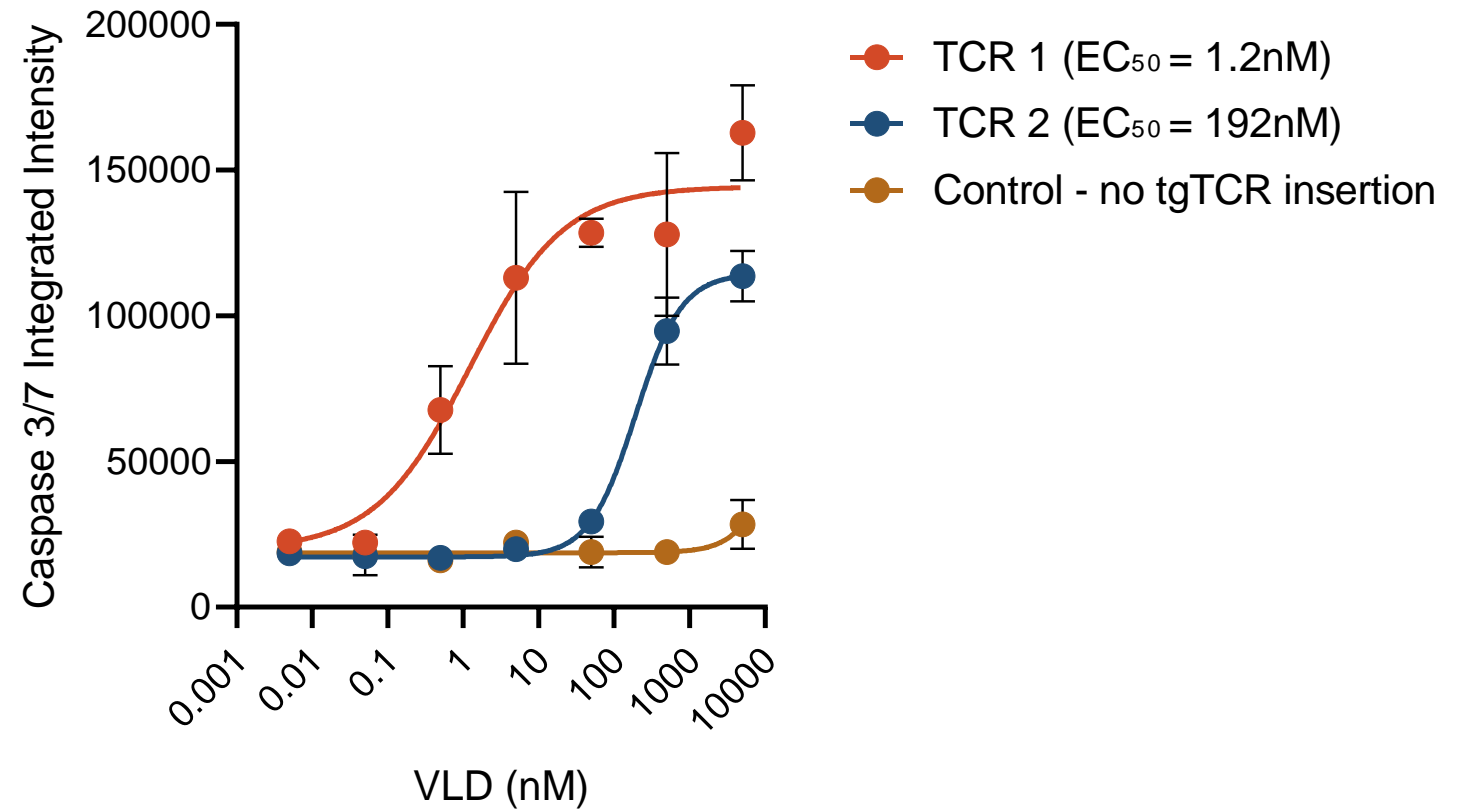
*WT1 measured by qPCR

Dose-dependent and HLA-specific cytotoxicity of edited T cells against primary AML blasts overexpressing WT1 across three patient samples

Lead WT1-TCR T Cells Kill Target Cells with High Avidity

**TCR 1 shows low
nM avidity**

**TCR 1 selected as
lead candidate**



Cytotoxicity of edited T cells against T2 cells pulsed with WT1-derived VLD peptide

Path to NTLA-5001, Our WT1-TCR T Cell Development Candidate



1. Identify High-Affinity WT1-TCR

- Natural TCR from healthy volunteers
- Specific and potent for WT1 tumor antigen
- HLA-A*02:01 restricted



2. Engineer T Cell Product with CRISPR/Cas9

- Eliminate endogenous TCR to prevent mispairing
- In locus insertion of therapeutic WT1-TCR

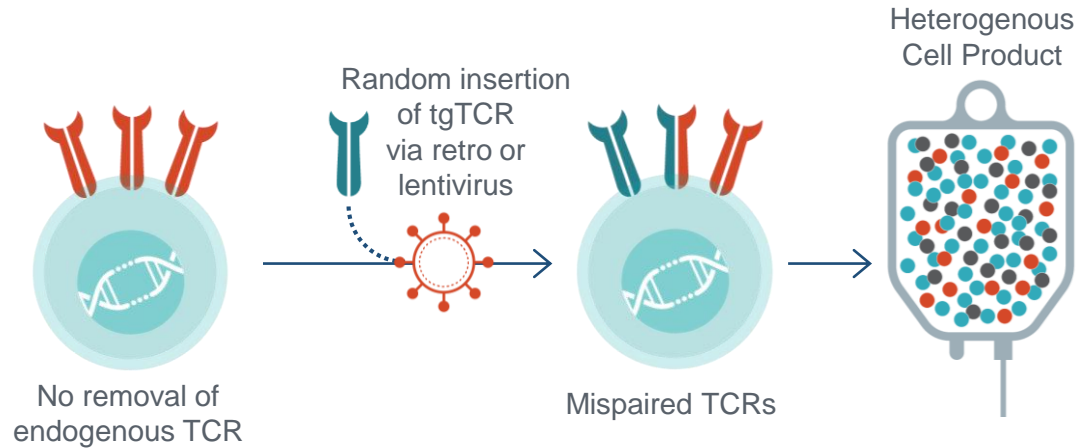


3. Characterize T Cell Product

- TCR avidity
- Polyfunctionality and anti-tumor reactivity
- TCR specificity and safety

CRISPR Engineering Overcomes Key Challenges of Traditional TCR Approaches

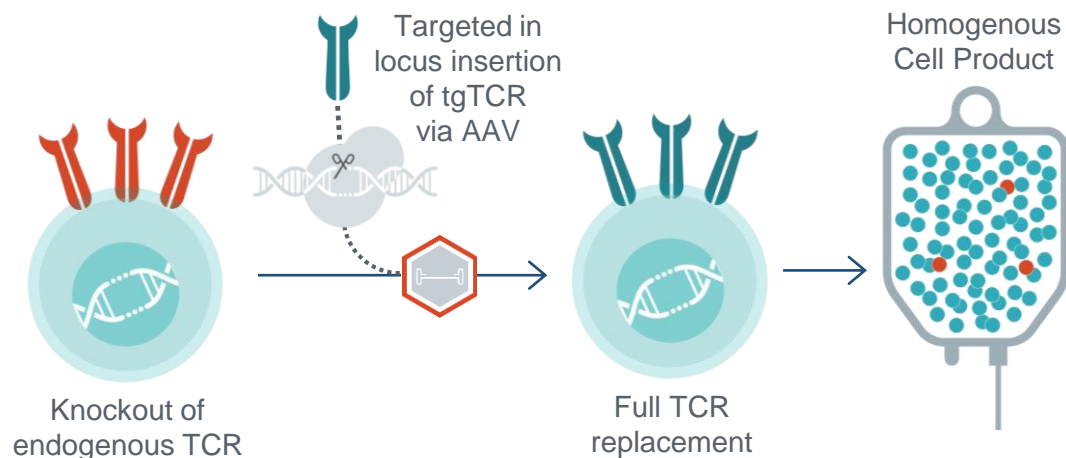
Traditional tgTCR addition



Key Challenges

- Random insertion of tgTCRs creates mutagenesis risk
- Mixed expression of endogenous and tgTCR
- Mispaired TCRs have unpredictable specificities and pose GvHD risk
- Lower tgTCR expression per T cell leads to reduced efficacy

CRISPR tgTCR replacement

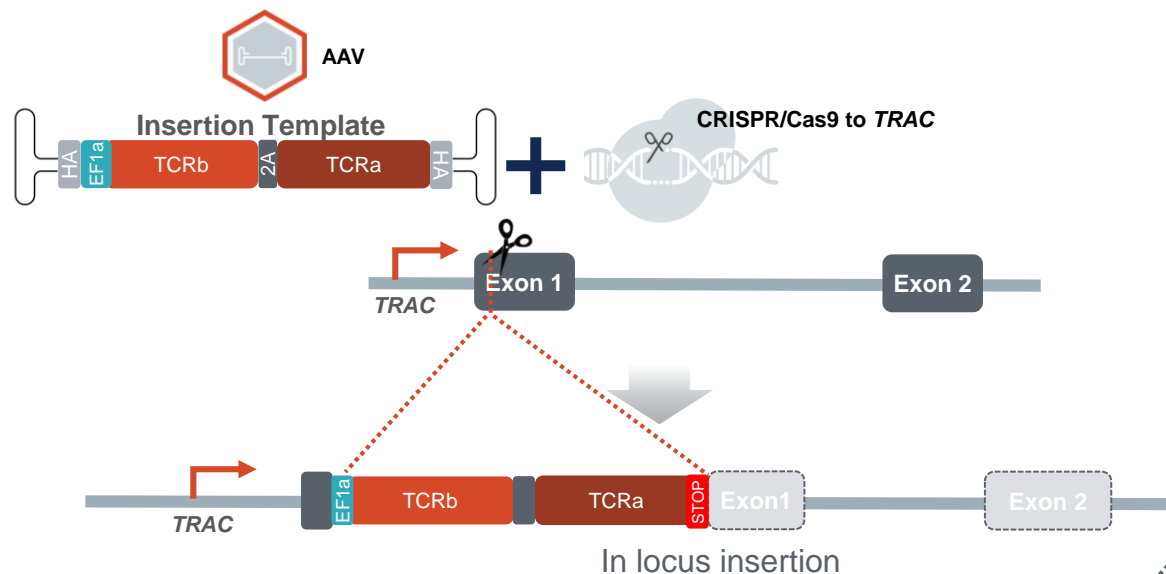


Our Solution

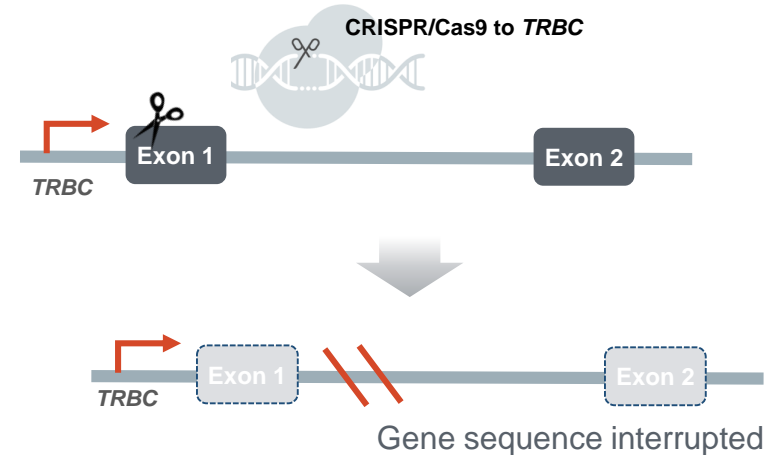
- Precise replacement of endogenous TCR with tgTCR
- No insertional mutagenesis risk
- Reduces TCR mispairing and GvHD risk
- High tgTCR expression per T cell leads to a more efficacious cell product

CRISPR/Cas9 Engineering of tgTCR T Cell Therapies

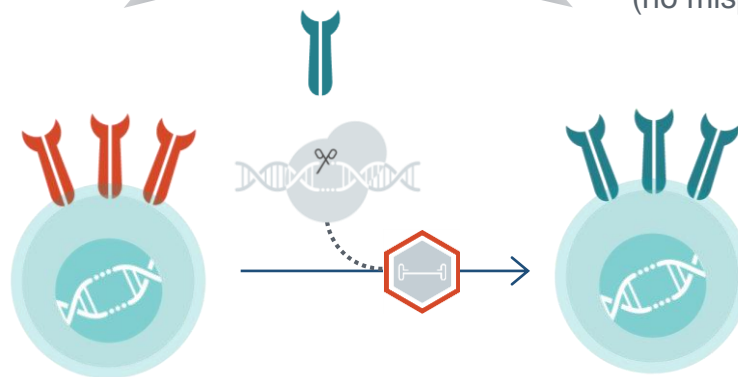
Insertion of tgTCR $\alpha\beta$ chains at the *TRAC* locus



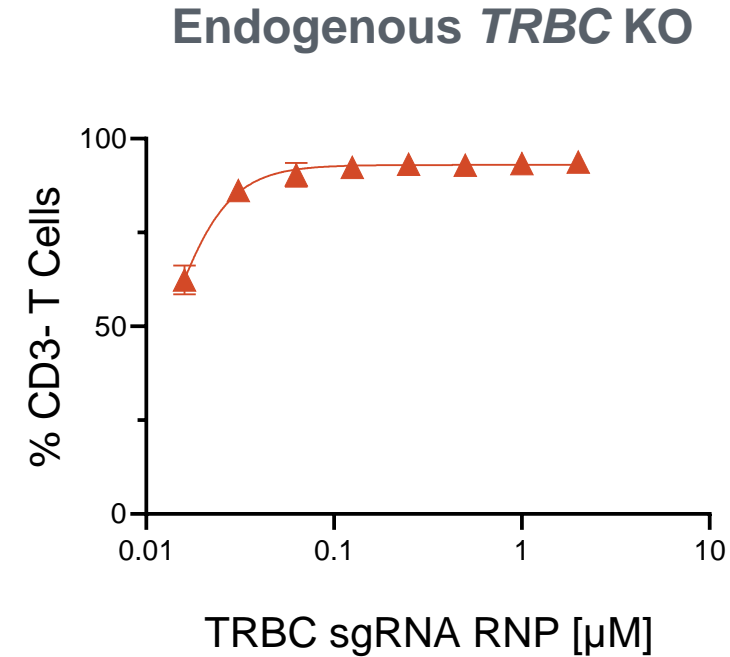
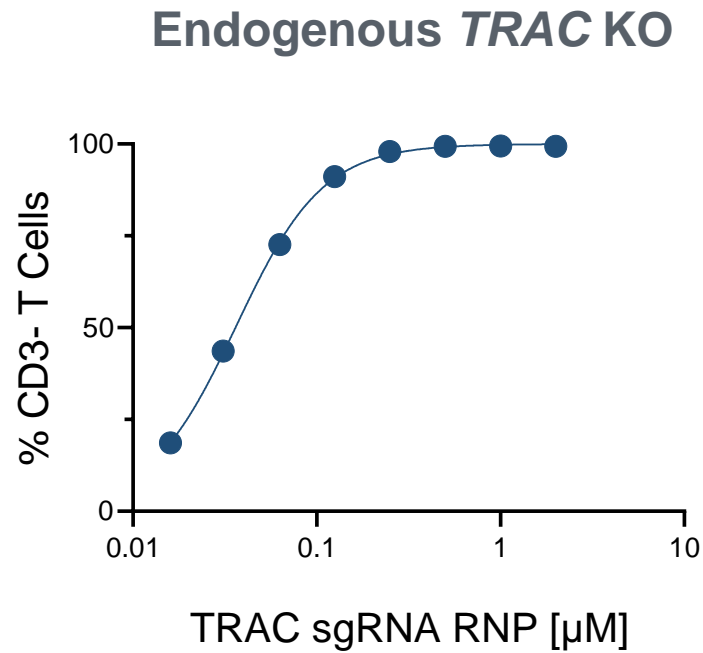
CRISPR/Cas9 site-specific KO of *TRBC*



No expression of endogenous TCR
(no mispairing)

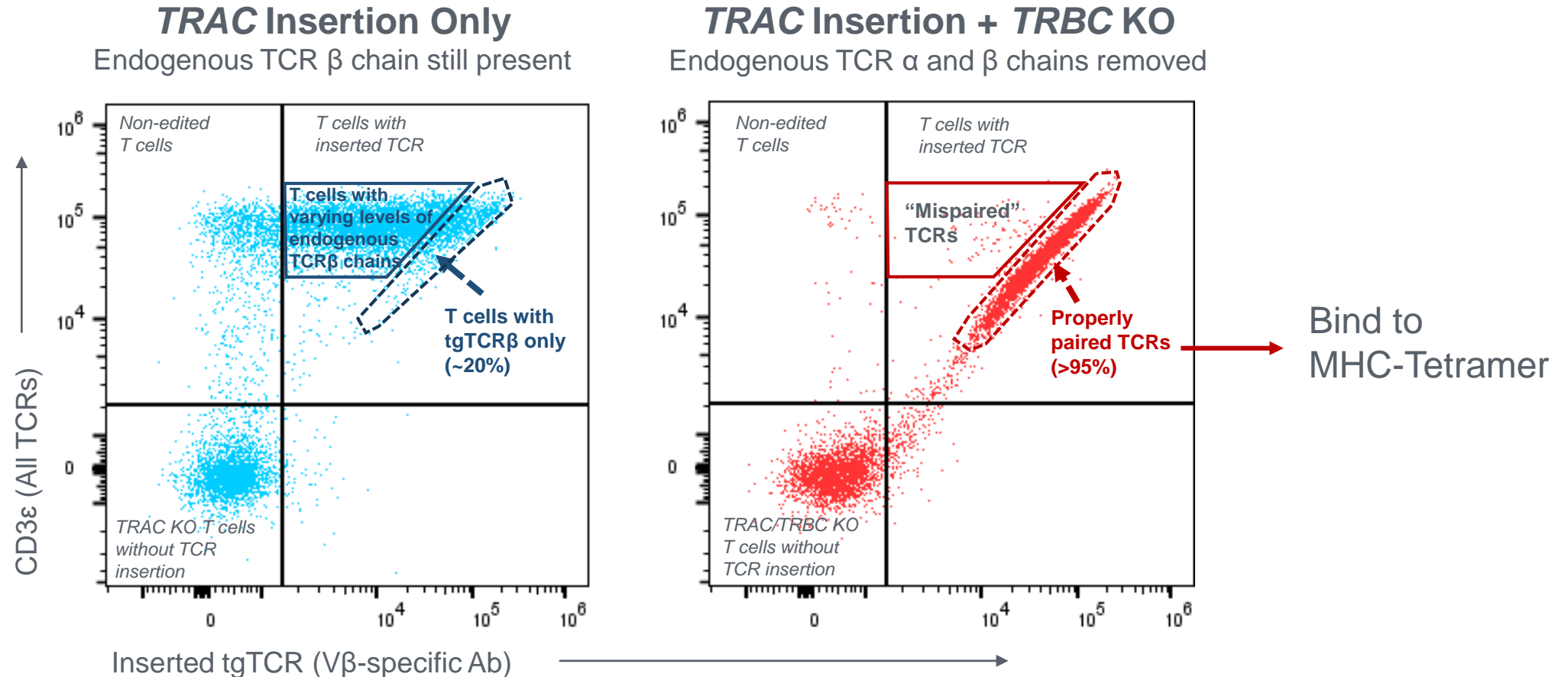


Potent and Specific sgRNAs Identified for *TRAC* and *TRBC*



sgRNAs show potent *TRAC* and *TRBC* KO with >98% KO of endogenous TCR
Off-target analyses demonstrate specificity of sgRNAs

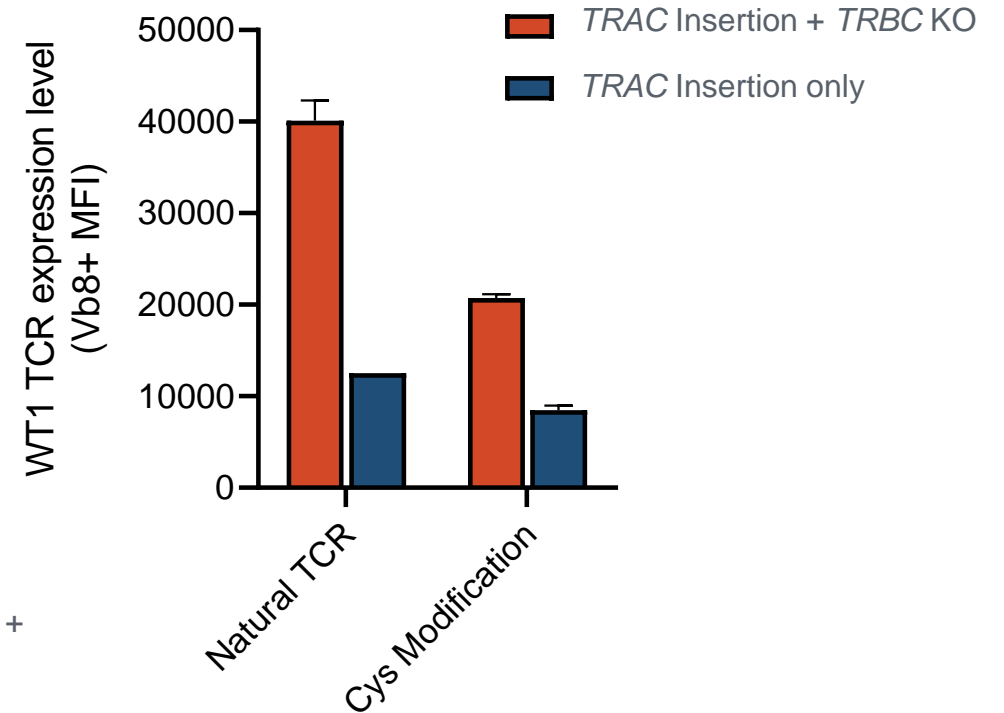
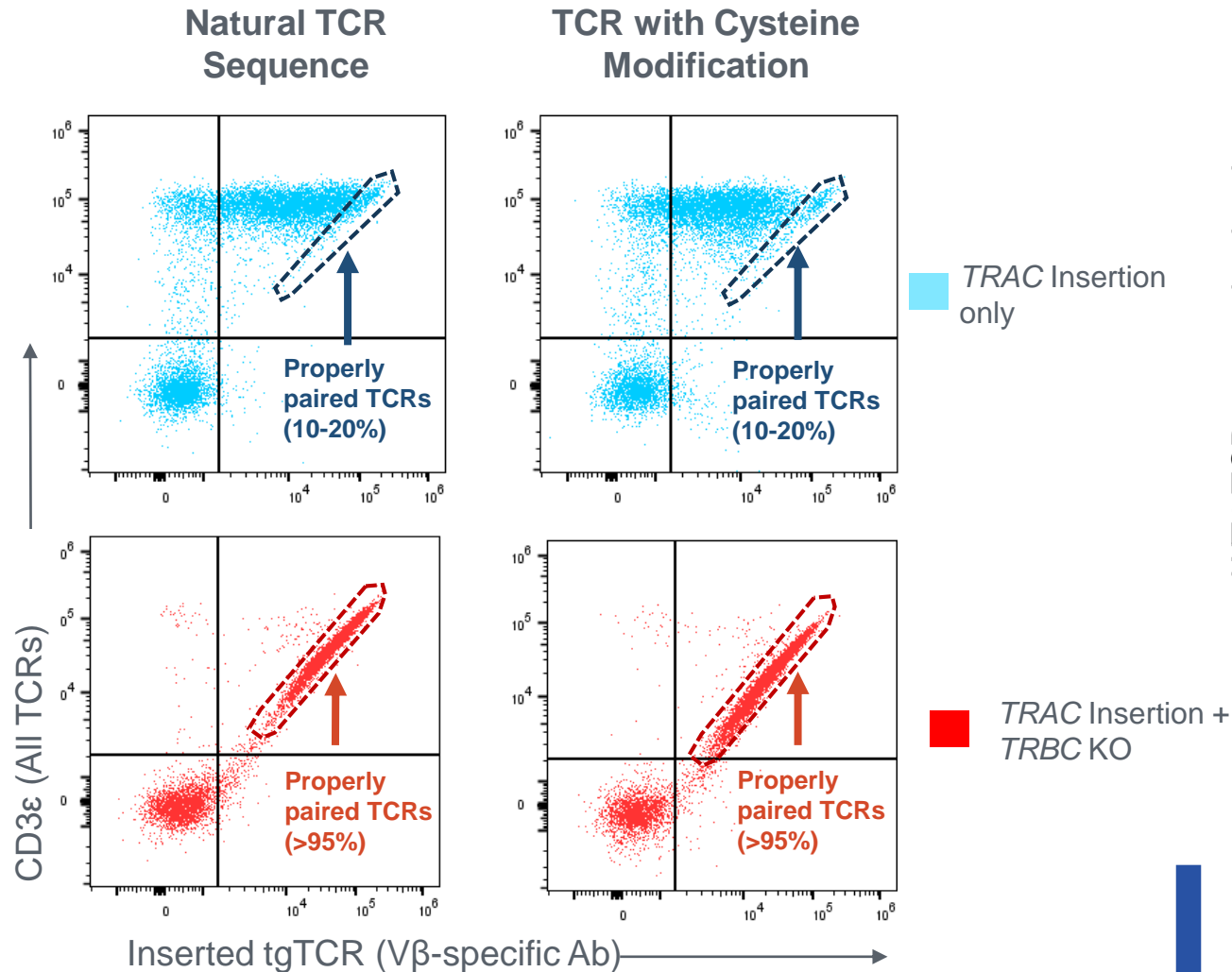
Eliminating Endogenous TCR Results in Homogeneous T Cell Product with >95% of tgTCR Chain Pairs



Comparable insertion rates of tgTCR (65-80% CD3+) with or without TRBC KO

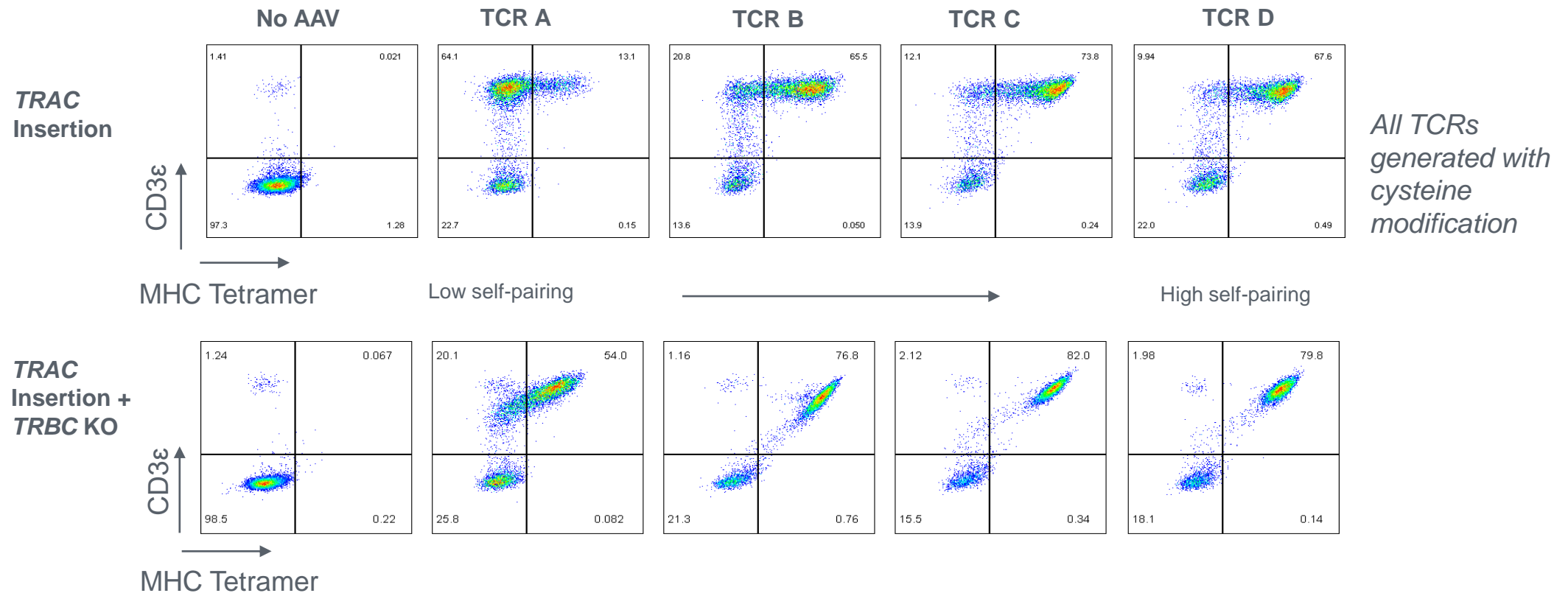
Expression of the properly paired tgTCR is greatly increased with TRBC KO

TCR α and β Chain Cysteine Modifications Do Not Prevent Mispairing



Cysteine modification does not solve the mispairing problem and lowers TCR expression per cell

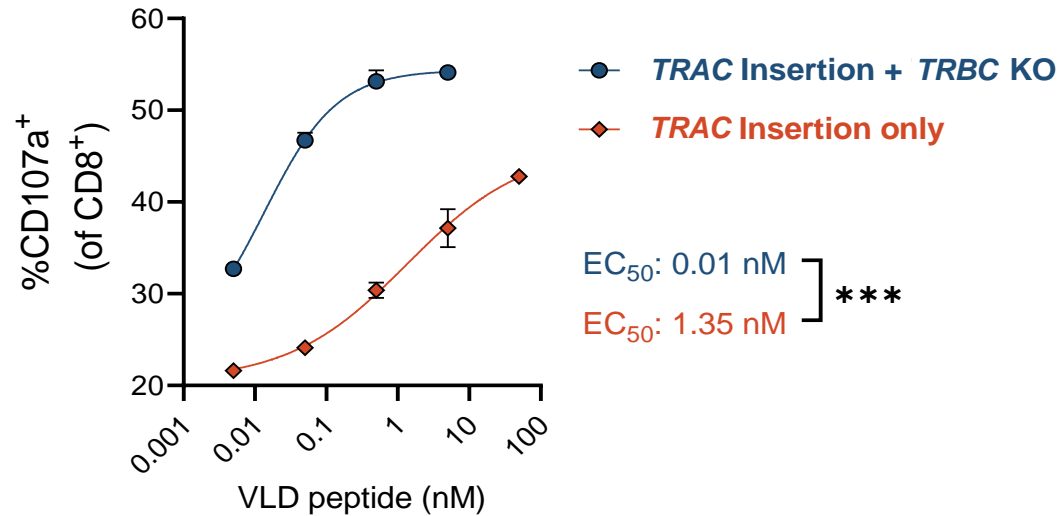
High and Uniform tgTCR Expression Results from Full KO of Endogenous TCR



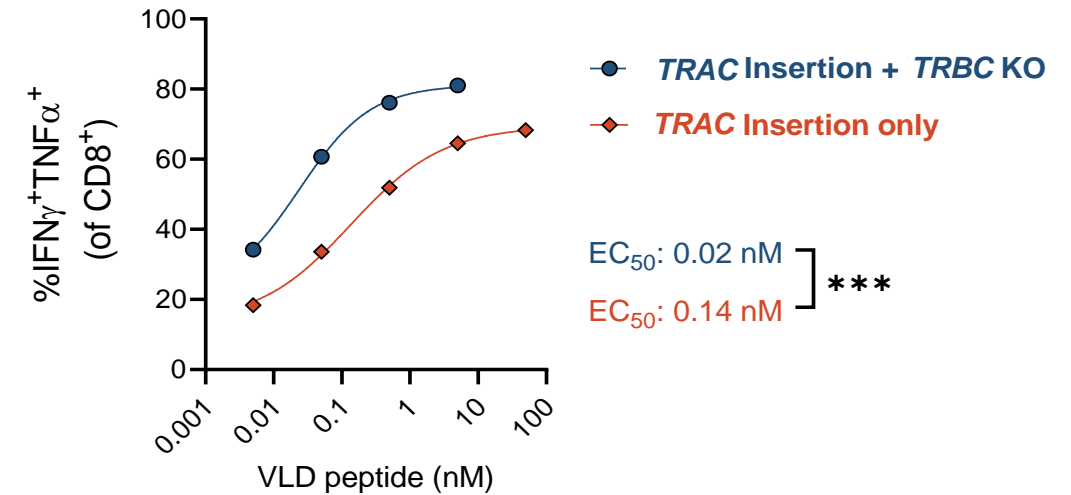
Extent of mispairing is TCR-dependent but detectable in all tested TCRs

Elimination of Endogenous TCR Significantly Improves T Cell Product Target Recognition and Activation

T Cell Degranulation After Peptide Stimulation



T Cell Cytokine Release After Peptide Stimulation

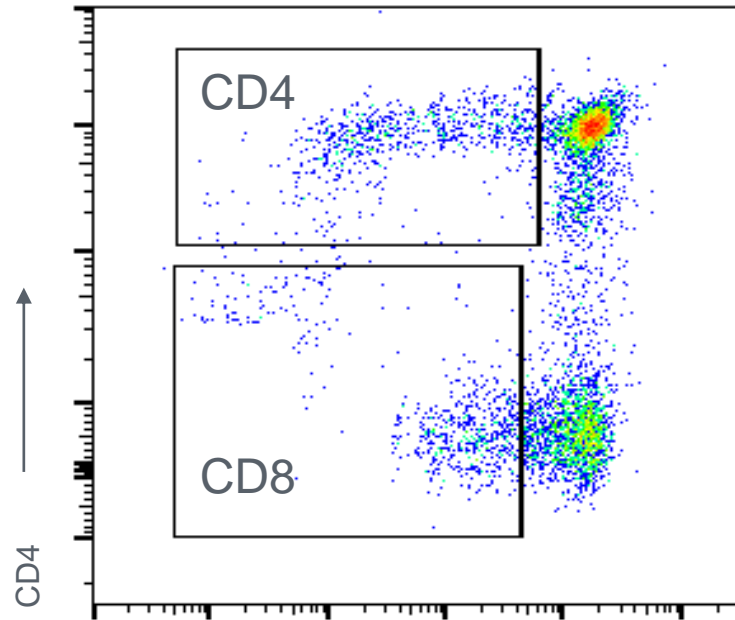


**Increased cytotoxicity and cytokine secretion
both observed following peptide stimulation**

Endogenous *TRAC* and *TRBC* KO Reduces Off-Target Reactivity

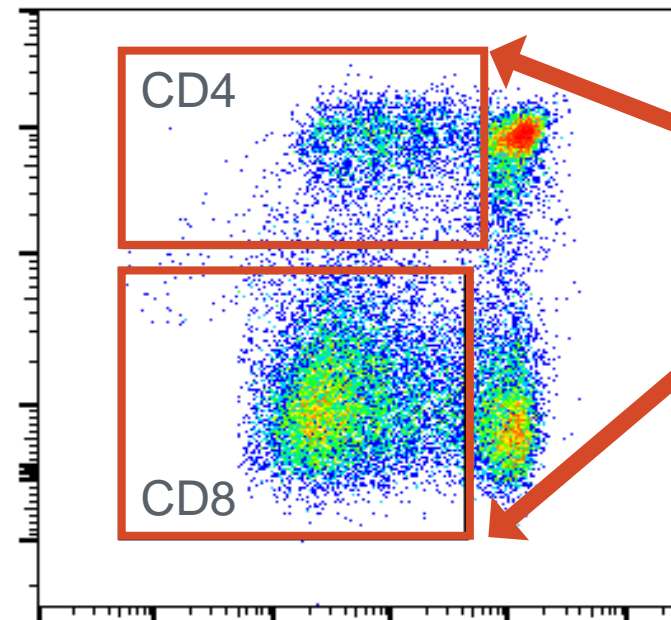
HLA Mismatched Target Cells in Mixed Lymphocyte Reaction Assay

tgTCR *TRAC* Insertion + *TRBC* KO



Dilution of Cell Trace Violet in dividing cells

tgTCR *TRAC* Insertion Only

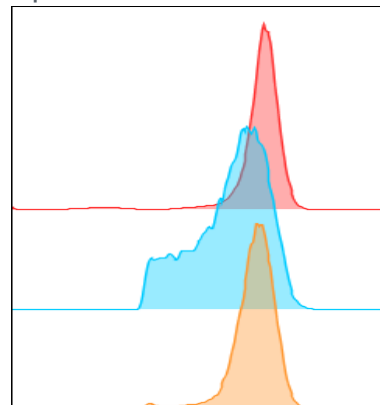
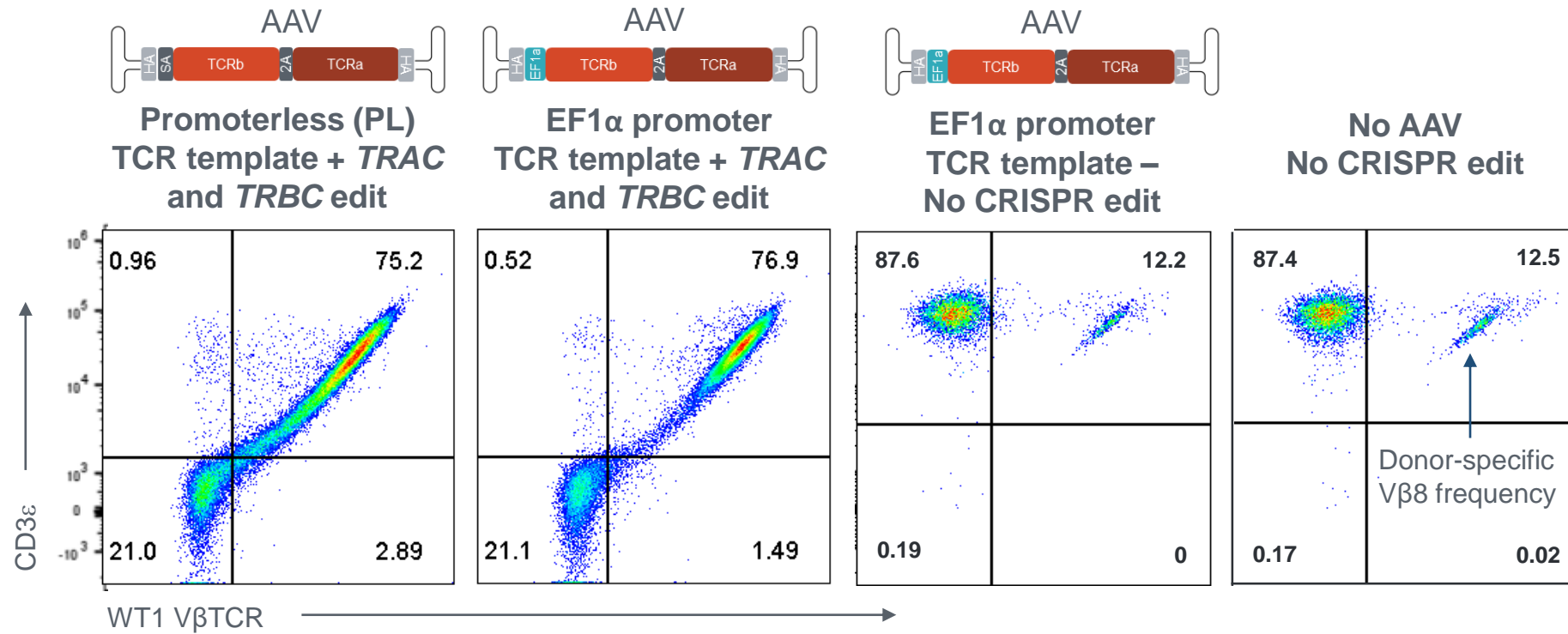


Unwanted cell proliferation

Dilution of Cell Trace Violet in dividing cells

Without both *TRAC* and *TRBC* KO, mispaired TCRs lead to strong alloreactivity of T cells

EF1 α Promoter Drives Higher and More Uniform Expression of tgTCR than *TRAC* Promoter



Unmanipulated T cells

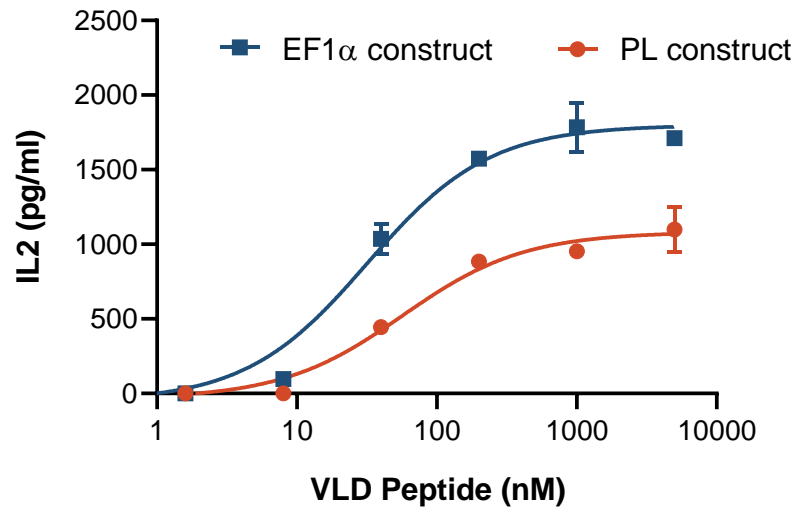
TCR driven off *TRAC* promoter

TCR driven off EF1 α promoter

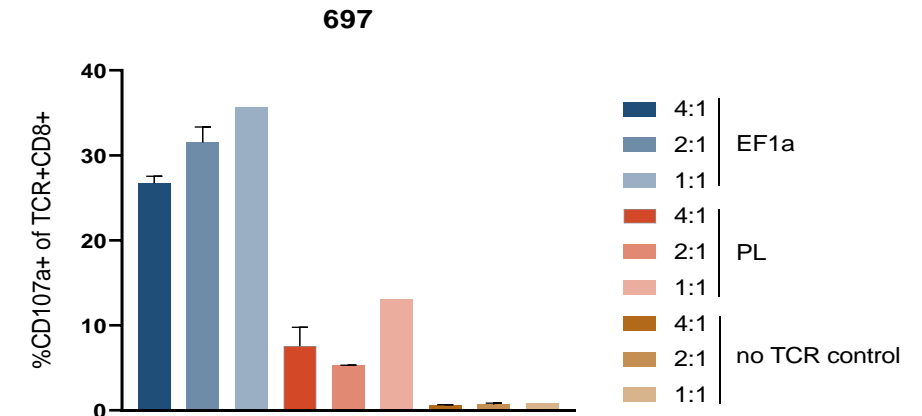
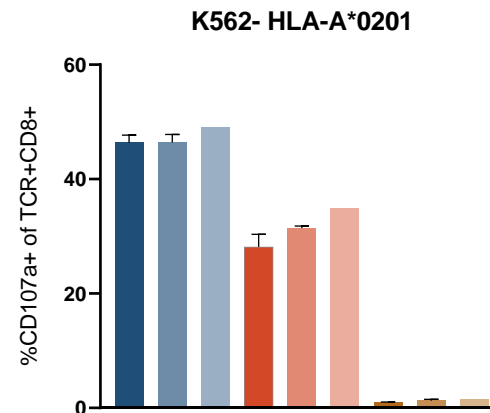
Endogenous *TRAC* promoter drives lower expression of TCR α and β chains
EF1 α promoter provides near physiological expression of the tgTCR construct
No episomal expression in final product

Superior Cytokine Production and Tumor Cell Killing with EF1 α Promoter

T Cell Cytokine Release After T2-Peptide Stimulation



T Cell Cytotoxicity Against WT1-Expressing Leukemia Cell Lines



Higher TCR expression with EF1 α promoter allows for:

- Lower peptide threshold
- Higher cytokine production
- Better tumor cell killing at naturally presented WT1 peptide levels

Path to NTLA-5001, Our WT1-TCR T Cell Development Candidate



1. Identify High-Affinity WT1-TCR

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2. Engineer T Cell Product with CRISPR/Cas9

- Eliminate endogenous TCR to prevent mispairing
- In locus insertion of therapeutic WT1-TCR

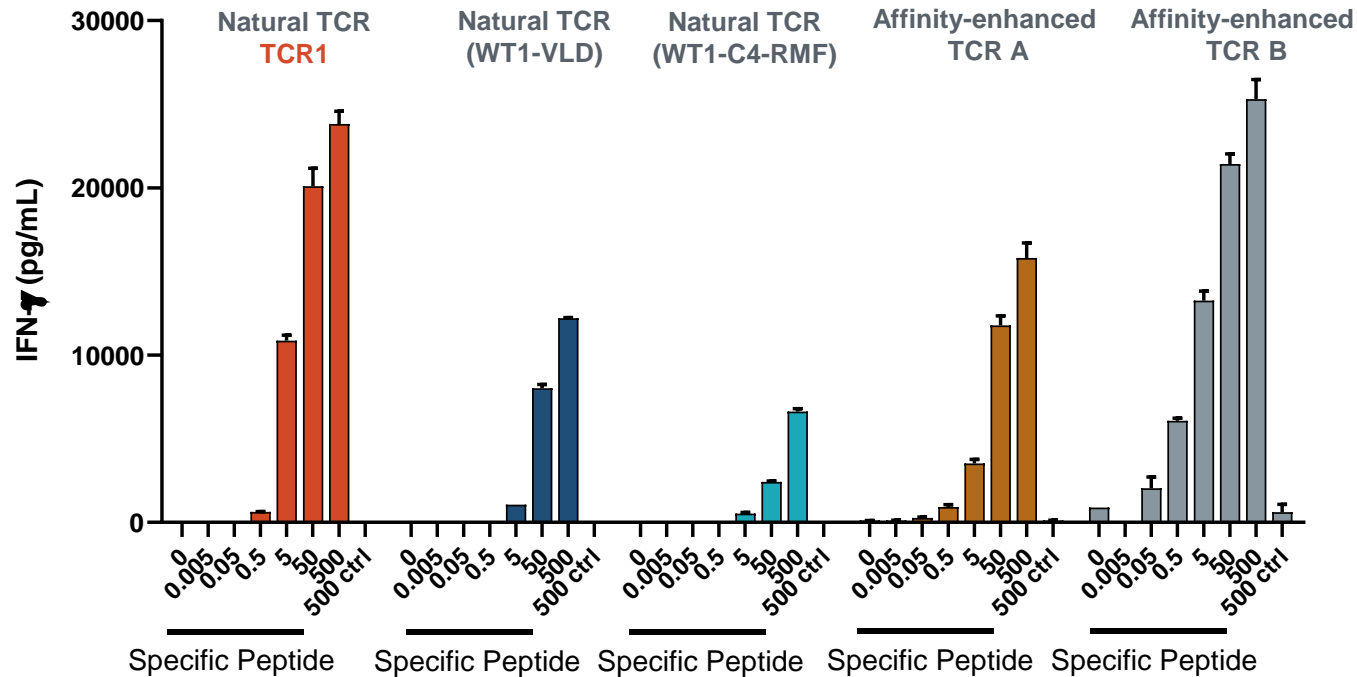


3. Characterize T Cell Product

- TCR avidity
- Polyfunctionality and anti-tumor reactivity
- TCR specificity and safety

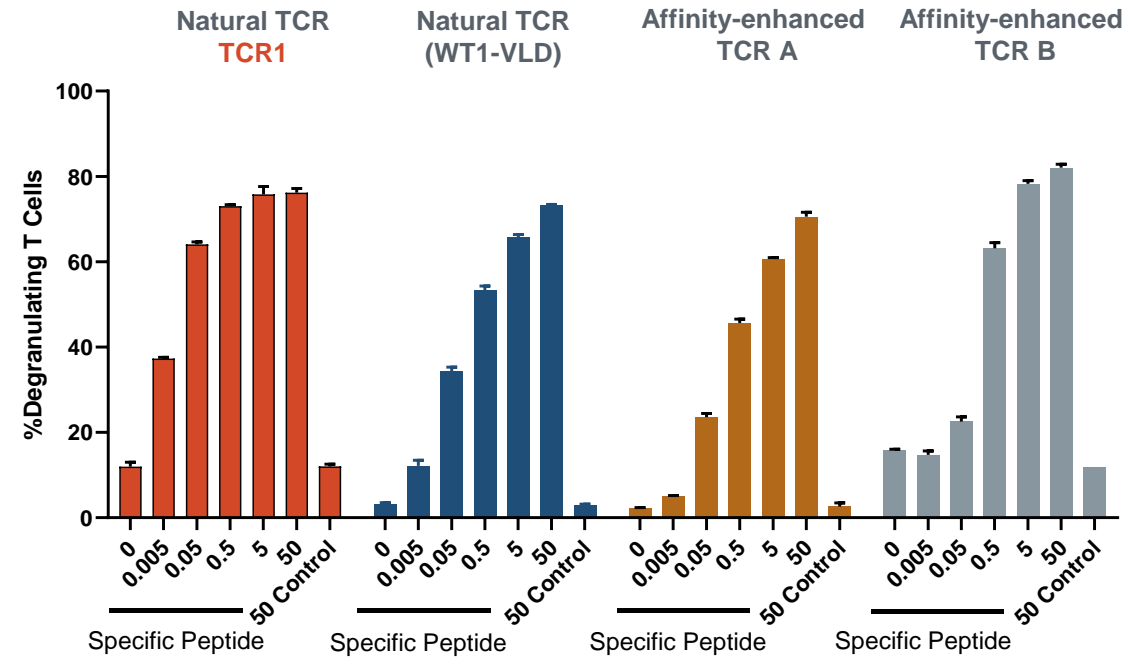
Lead TCR Shows Superior Target Recognition *In Vitro* vs. Other Natural WT1-Specific TCRs, Comparable to Affinity-Enhanced TCRs

T Cell Cytokine Release After Peptide Stimulation



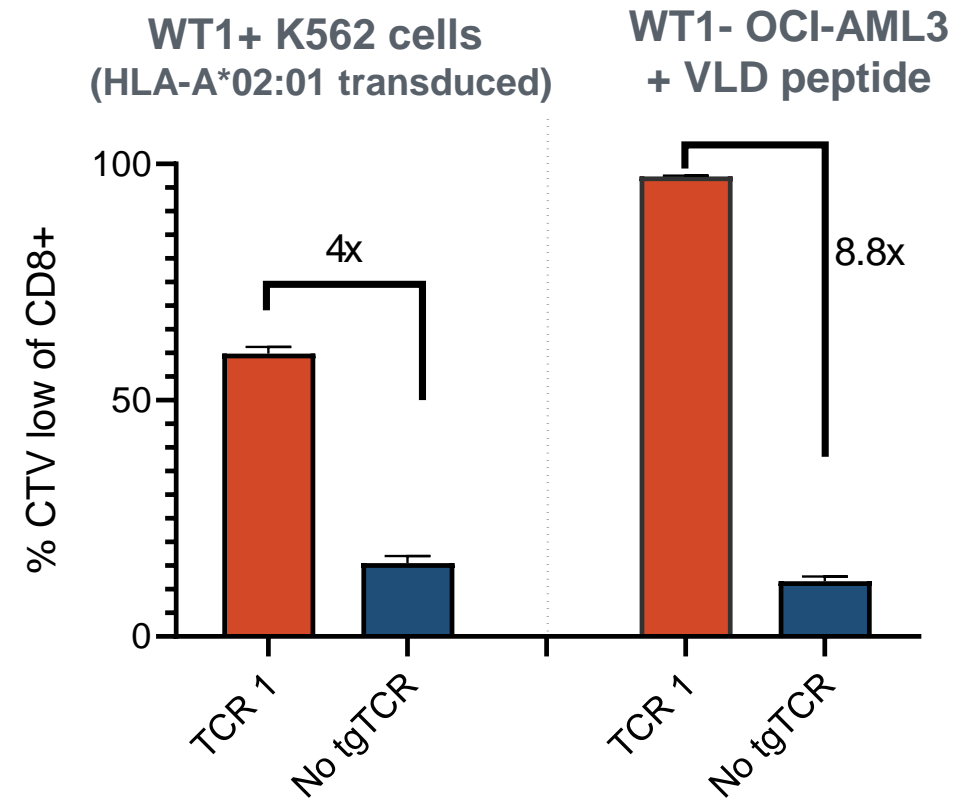
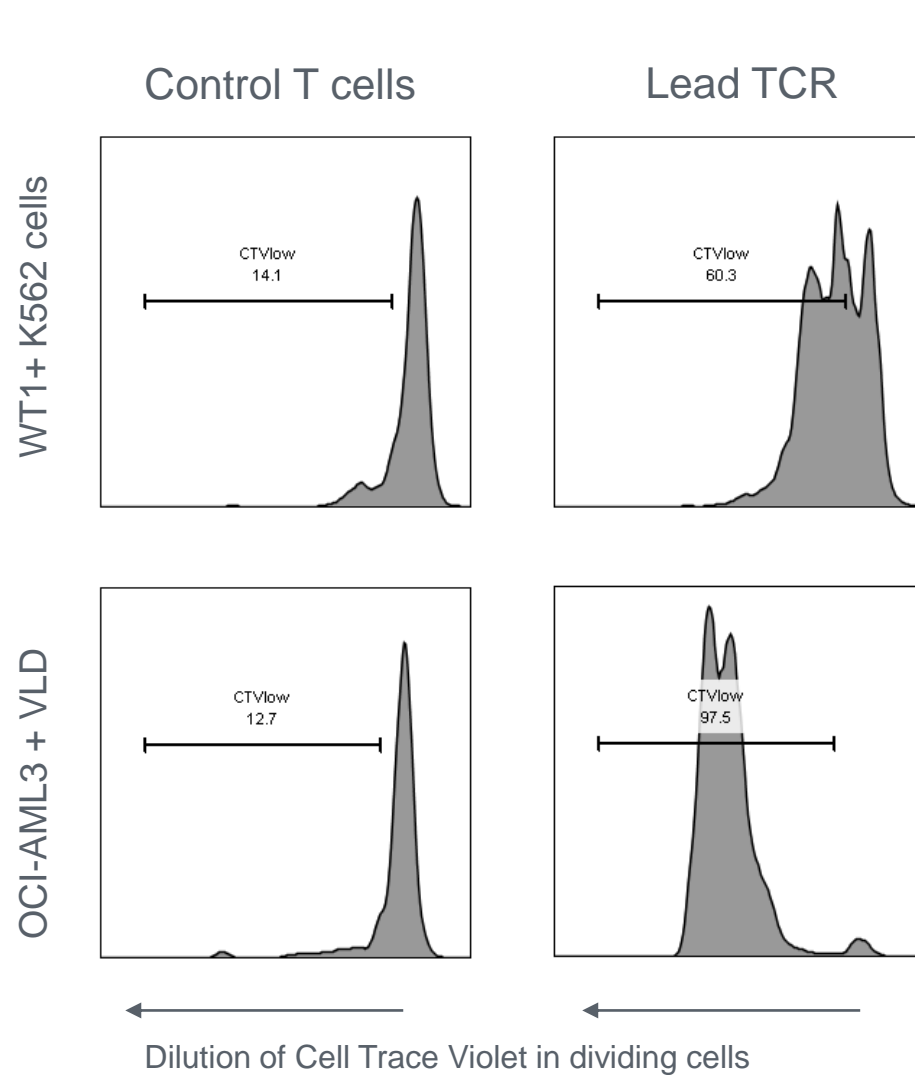
Lead TCR Avidity:
 $EC_{50} = 6.3$ nM

T Cell Degranulation After Peptide Stimulation



Lead TCR Avidity:
 $EC_{50} = 0.013$ nM

Lead TCR T Cells Proliferate in Response to WT1⁺ Tumor Cells

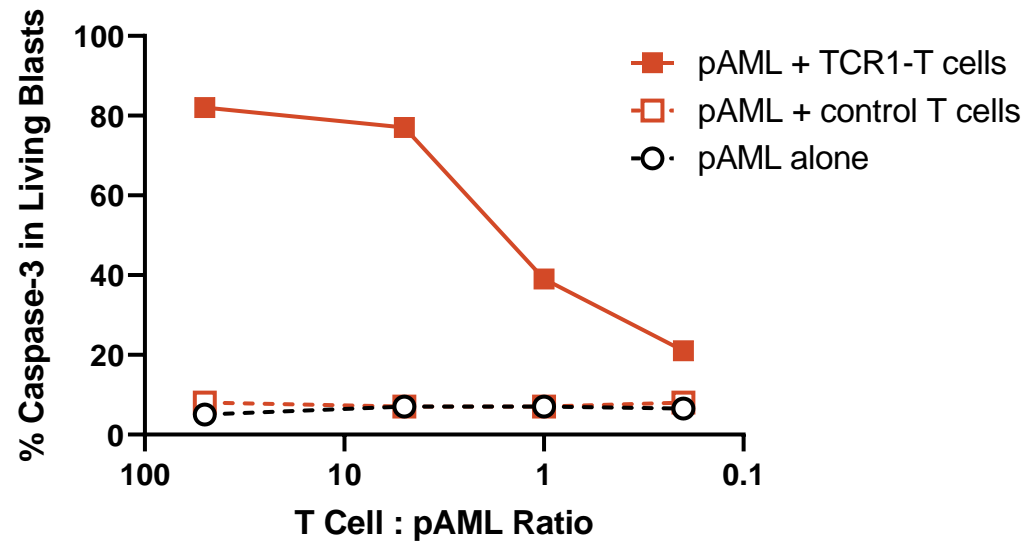


Engineered T cells proliferate in response to:

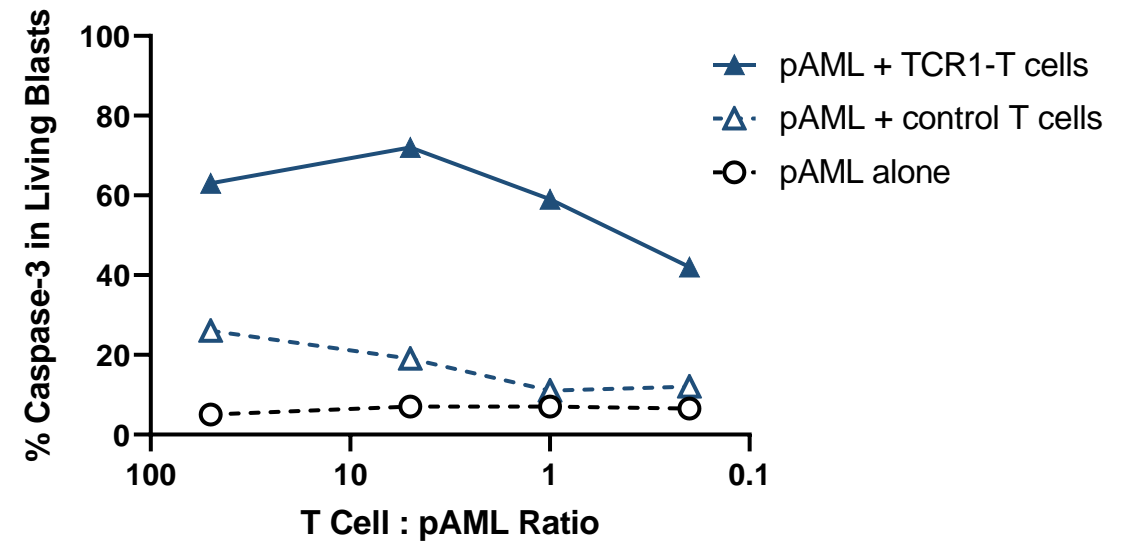
- Naturally processed WT1 peptide (K562-A2.1)
- WT1 peptide-pulsed tumor cells (OCI-AML3)

Lead TCR T Cells Kill Primary AML Blasts

pAML Patient 5
T cell donor 2

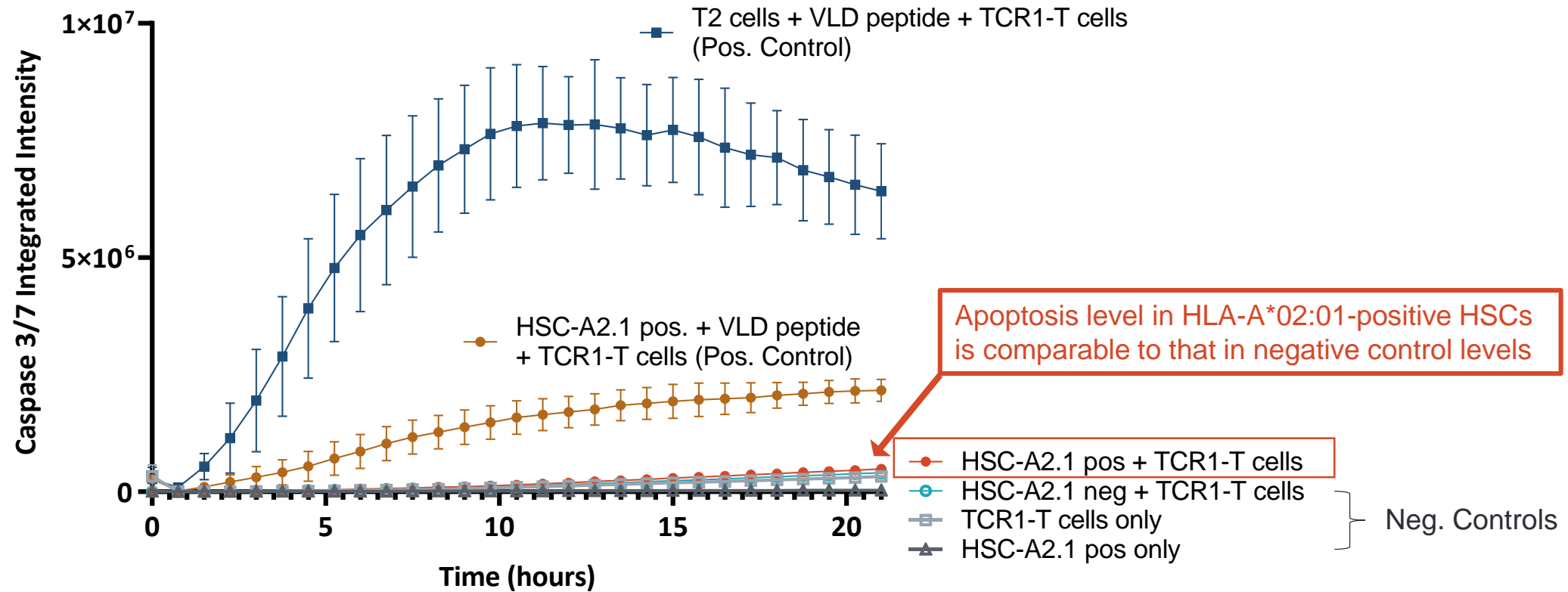


pAML Patient 5
T cell donor 4



Cytogenetics: intermediate
Mutations: FLT3, NPMA, DNMT3A
6 h apoptosis assay

Lead TCR Shows No Cytotoxicity Against Bone Marrow CD34+ Cells Expressing Normal Levels of WT1



Mean + SD for Incucyte apoptosis assay, CD34+ bone marrow cells (HLA-A*0201 positive and negative) ± TCR 1 T cell preparations from 3 donors

Multiple Workstreams to Advance Cell Therapy Efficacy in Solid Tumors



Allogeneic Cell Source

- Edit determinants of allo-reactivity to achieve persistence in presence of T and NK cells



Functional Modulation

- Knockout and/or knock-in (insertion) of key receptors to modulate T cell functionality



Solid Tumor Efficacy

- CRISPR screening to unravel targetable key regulators of T cell fitness in different tumor microenvironments

Key Takeaways

- NTLA-5001 nominated as WT1-TCR-directed development candidate
- Consistent high-level editing efficacy
 - >98% KO of endogenous TCRs
 - >70% in locus insertion of tgTCRs
- TCR shows nanomolar avidity
- Specific and potent killing of WT1-positive AML blasts
- No detectable bone marrow cell toxicity
- Expect to submit an Investigational New Drug (IND) application in 1H 2021
- AML program provides foundation for expansion into solid tumors



Acknowledgements



Cell Therapy Team
(2019 AML World Awareness Day)



- **Chiara Bonini**
- **Eliana Ruggiero**
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- Claudia Politano
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- Lorena Stasi
- Barbara Camisa
- Beatrice Cianciotti
- Alessia Potenza
- Francesco Manfredi
- Paola Vella
- **Fabio Ciceri**



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