

SLEEK: A Method for Highly Efficient Knock-in and Expression of Transgene Cargos for Next-Generation Cell-Based Medicines

American Society for Gene and Cell Therapy Annual Meeting 2022

John Zuris

Associate Director, Editing Technologies

editas MEDICINE

May 18, 2022

Disclosure

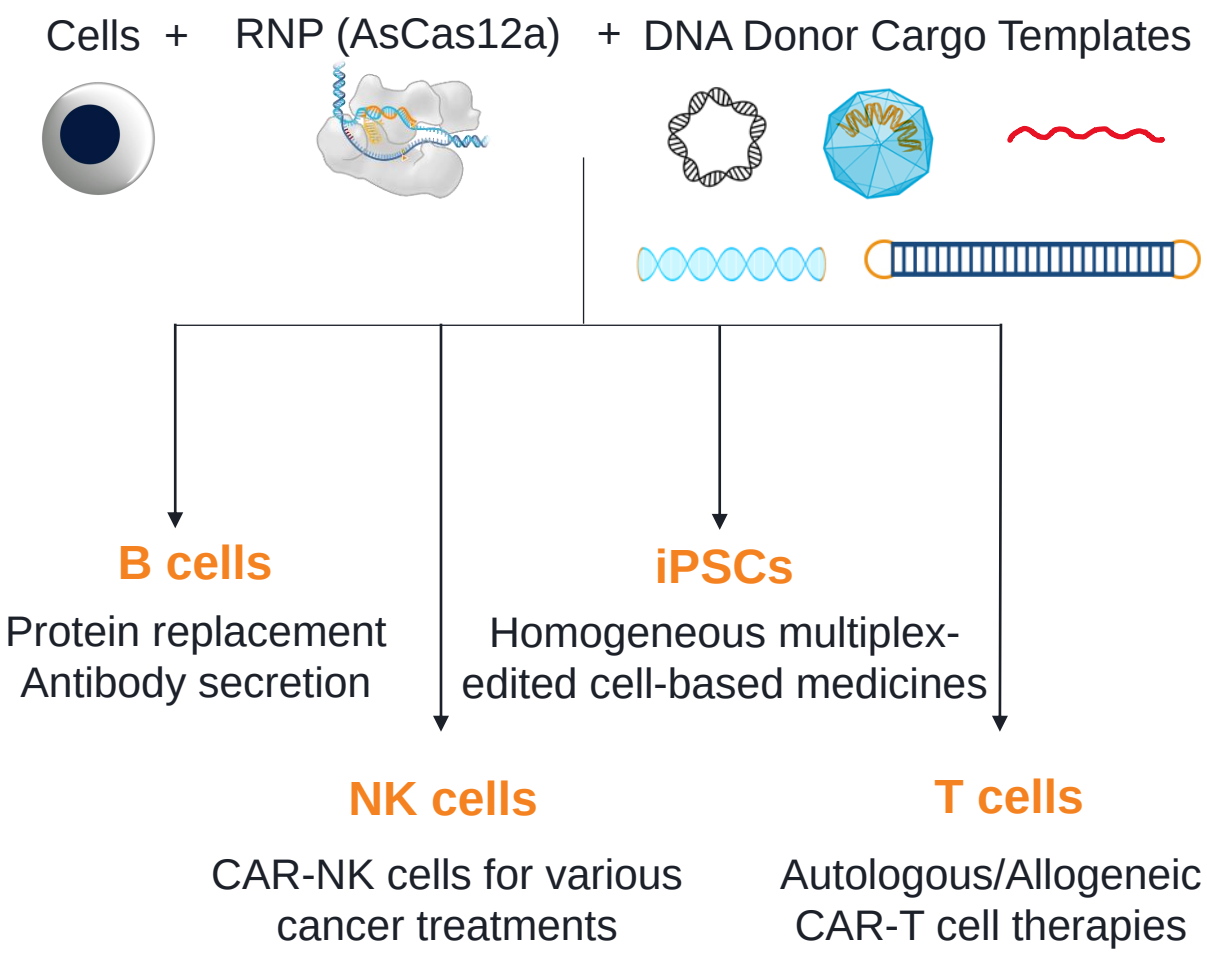
I am an employee and shareholder of Editas Medicine

Our Goal was to Develop an Editing Technology That Could Fundamentally Improve the Generation of Cell-Based Medicines

SLEEK™ GENE EDITING

SeLection by Essential-gene Exon Knock-In

- Enables >95% knock-in efficiency
- High-level, tunable cargo expression
- Homogeneous editing
- Efficient multicistronic cargos
- Simplifies iPSC clone selection process
- Robust, lineage-independent, expression of functional cargo in iPSCs



CAR: chimeric antigen receptor; Cas: CRISPR-associated protein; iNK: IPSC-derived natural killer cell; iPSC: induced pluripotent stem cell; NK: natural killer; RNP: ribonucleoprotein; TME: tumor microenvironment.

The Specificity Case for Selecting AsCas12a Over SpCas9

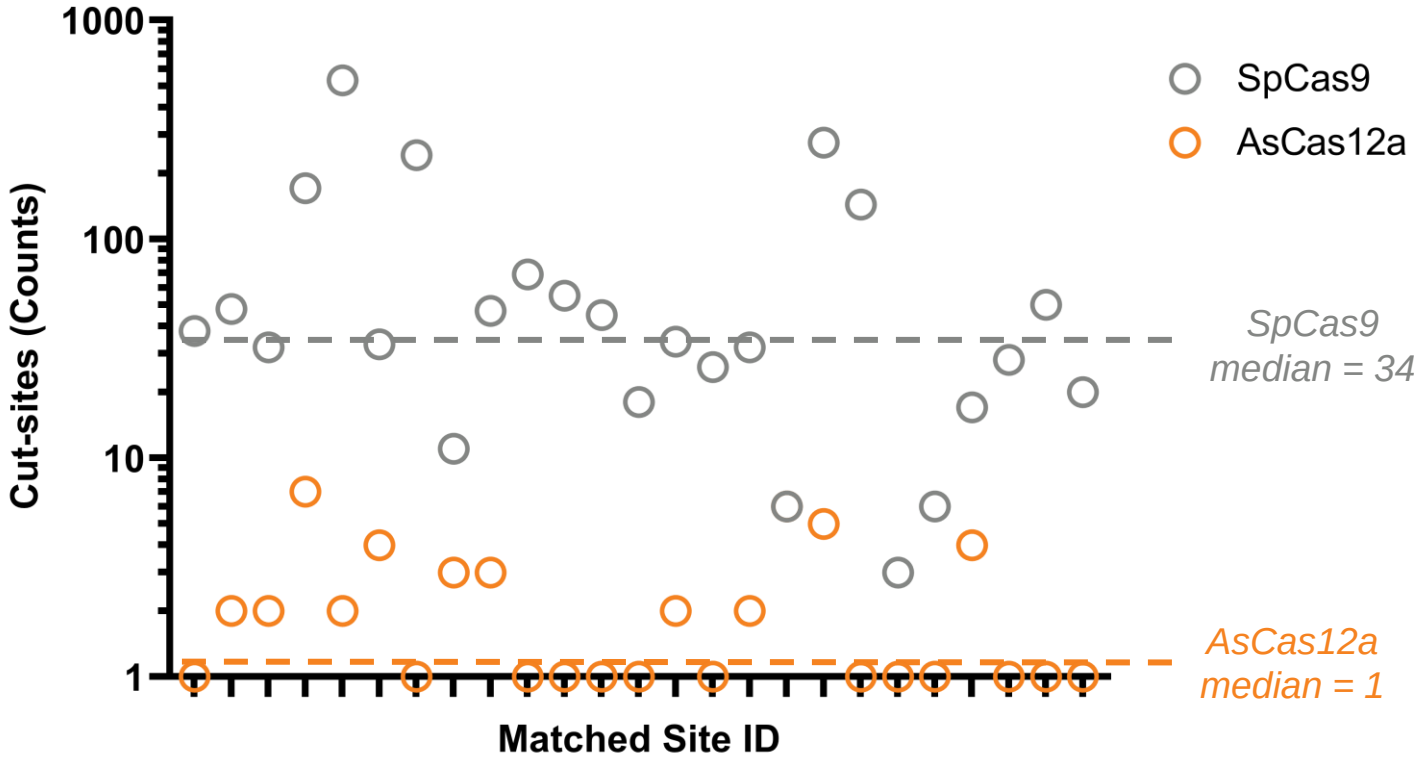
Experimental Design to Assess Specificity

- Assessed using Digenome-Seq—a fully reconstituted cutting and WGS detection assay
- Assayed 25 randomly selected “matched sites” in the genome, and run at saturating RNP concentration and time

Matched Target Site (20-Ns):
TTTVNNNNNNNNNNNNNNNNNNNN**NGG**

AsCas12a PAM **SpCas9 PAM**

Digenome-Seq with 25 Matched Genomic Sites



Gotta et al. Cold Spring Harbor 2019

AsCas12a is 10-100x More Specific Than SpCas9

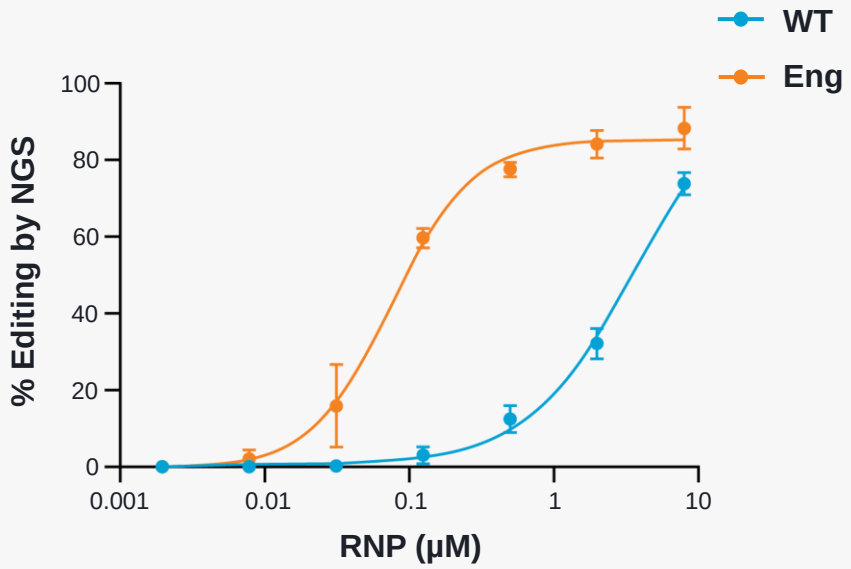
RNP: ribonucleoprotein; PAM: protospacer adjacent motif; Cas: CRISPR-associated protein; WGS: whole genome sequencing.

For more on Cas12a superior specificity to SpCas9, see: Kim et al. *Nat Biotech* 2016; Kleinstiver et al. *Nat Biotech* 2016; Kim et al. *Nat Methods* 2017; Strohkendl et al. *Mol Cell* 2018; Zhang et al. *Nat Comms* 2021.

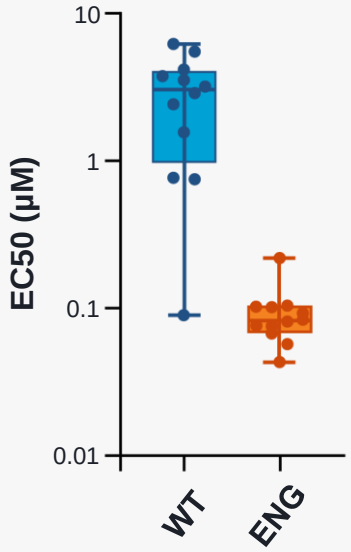
Engineered AsCas12a Shows Robust Efficiency and Potency

T cells

AsCas12a Engineering Results in a 2-Log Potency Improvement



All Guides Screened are Highly Active with Eng. AsCas12a



Engineered AsCas12a PAM Variants are Also Available, Further Expanding Target Space

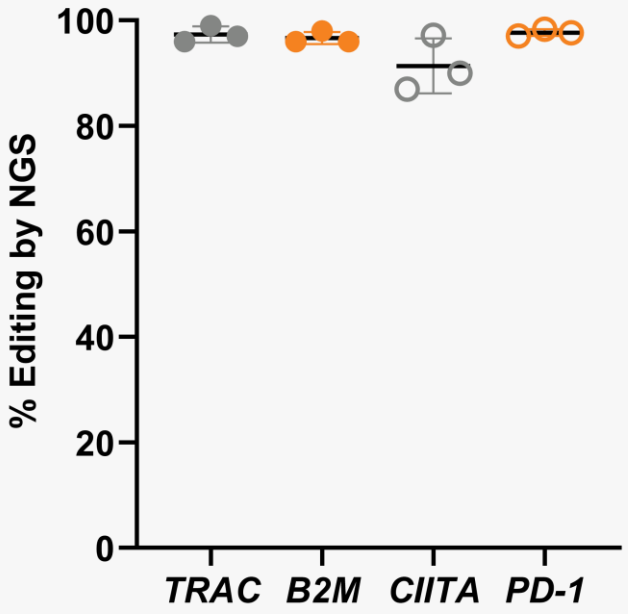
Cas: CRISPR-associated protein; Eng: engineered; NGS: next generation sequencing; RNP: ribonucleoprotein; WT: wildtype.

Zhang, Zuris et al. *Nat Commun.* 2021

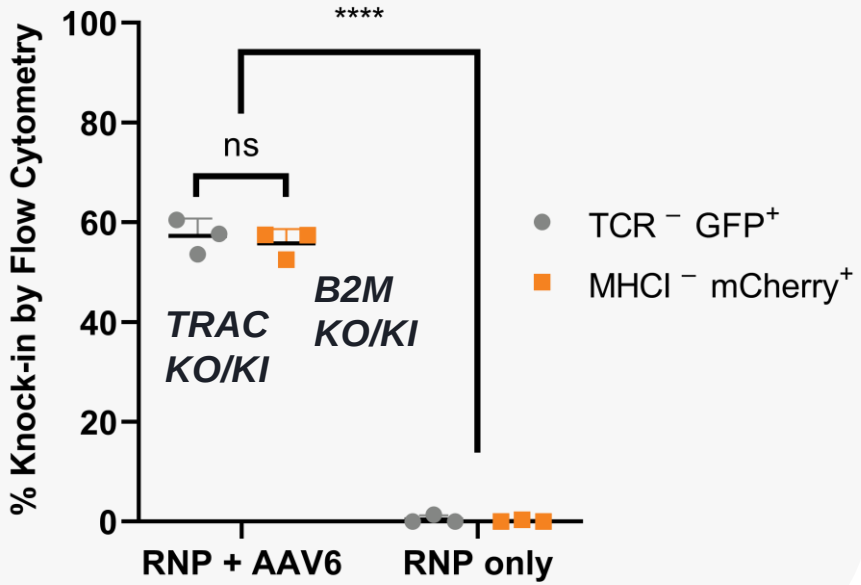
Despite Major Progress, Efficient Knock-in Remains a Challenge

T cells

Robust Multiplexed Gene KO

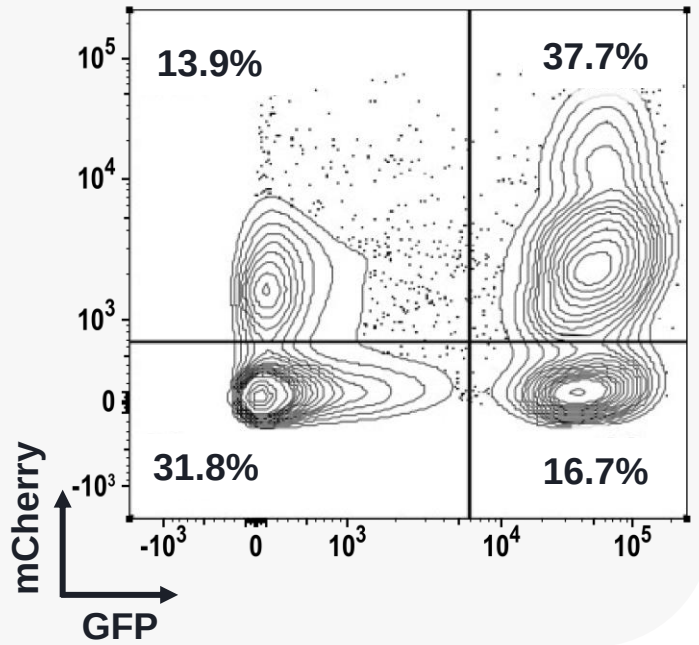


Transgene Knock-in with AAV6



Dual Knock-in with AAV6

TRAC KO / GFP KI and B2M KO / mCherry KI



Impressive KO Results Near 100%, Single Knock-in ~60%, Double Knock-in ~40%

AAV: adeno-associated virus type 6; B2M; beta-2 macroglobulin; CIITA: class II major histocompatibility complex transactivator; GFP: green fluorescent protein; KI: knock-in; KO: knock-out; NGS: next generation sequencing; MHC: major histocompatibility complex; PD: programed cell death protein; RNP: ribonucleoprotein; TRAC: T-cell receptor α constant

What if We Could Overcome This Knock-in Challenge?

SLEEK: SeLection by Essential-gene Exon Knock-in

Desired Capability

- Selection for knock-in over indel edits
- High-level constitutive expression of cargo(s)

Key Criteria

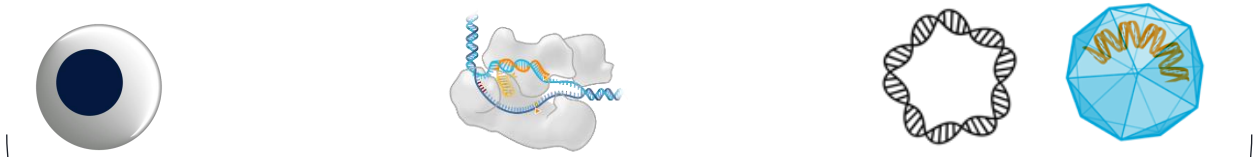
- Indels are lethal
- Editing (NHEJ) rates must be high
- High-level constitutive promoter

Editing Overview

Cells of interest

RNP (Engineered AsCas12a)

DNA Donor Template Example: Plasmid or AAV6



Electroporate and Recover for ~7 Days

Editing Outcomes

Unedited

Normal essential protein
No effect on cell health

Positive Selection

Indel (NHEJ)

Inactivates essential protein
Lethal to cells

Negative Selection

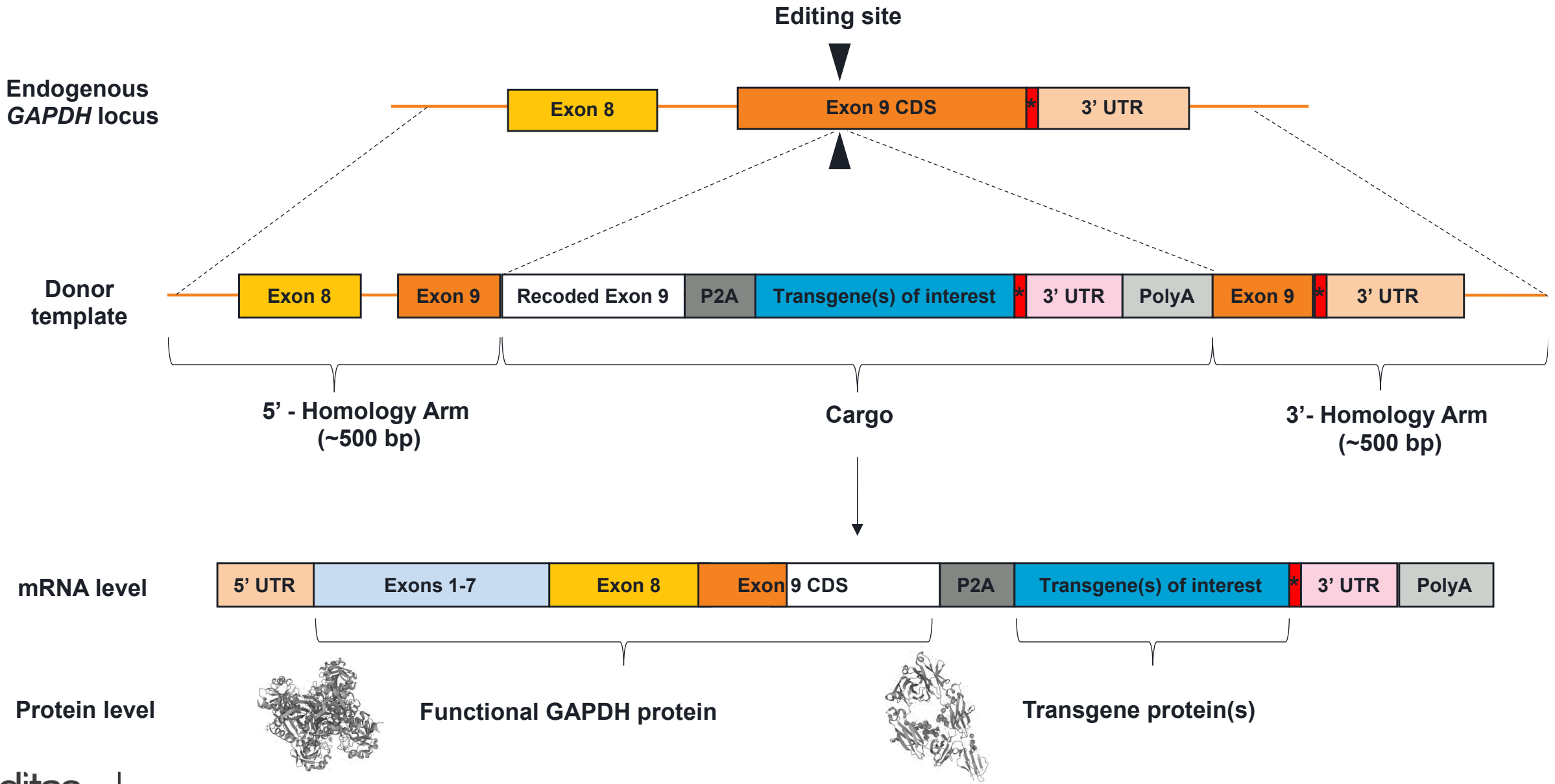
Repair (HDR)

Normal essential protein
No effect on cell health

Positive Selection

Cas: CRISPR-associated protein; HDR: homology directed recombination; RNP: ribonucleoprotein; NHEJ: non-homologous end joining.

Molecular Design of SLEEK Knock-in Construct

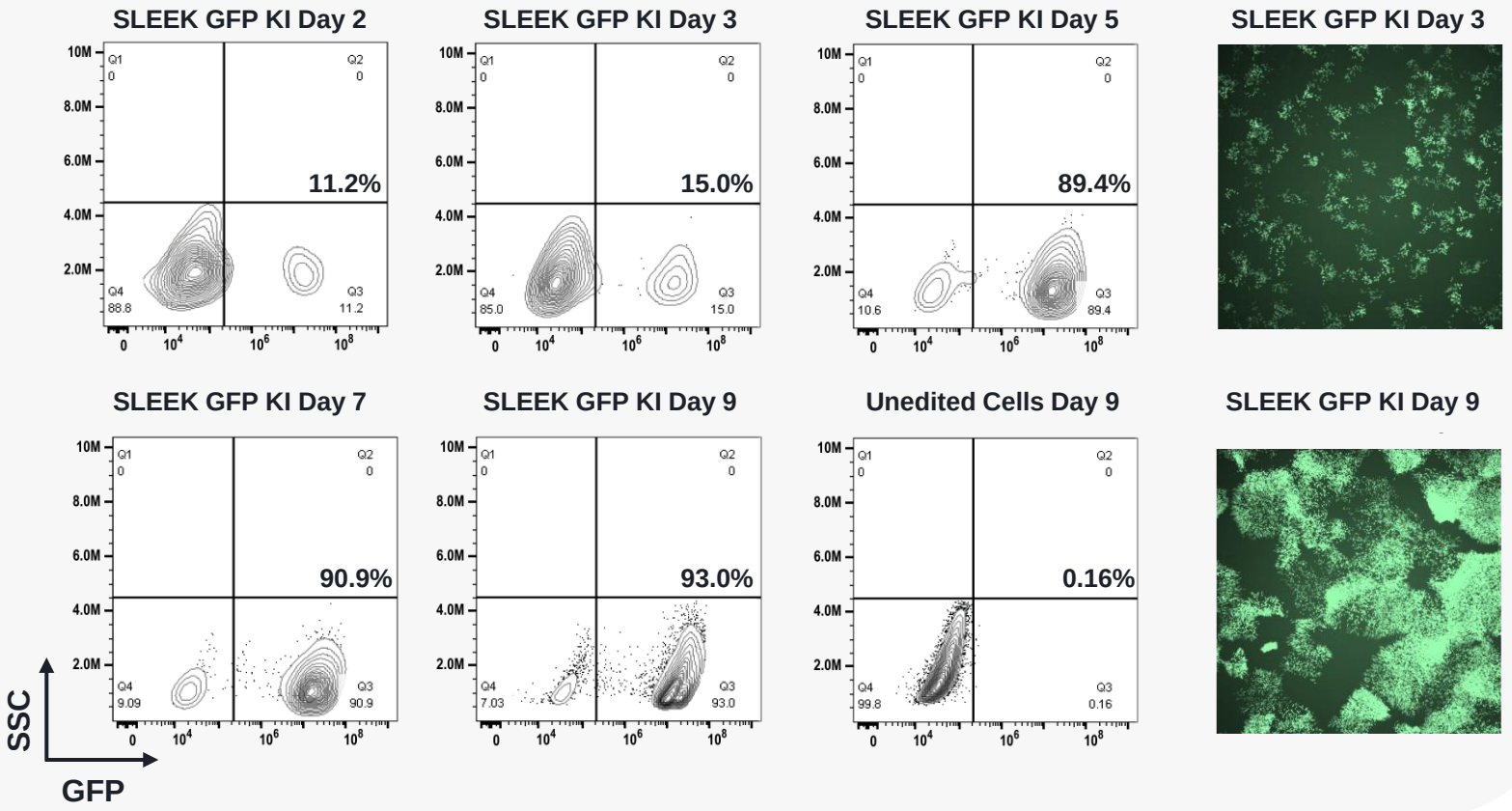


CDS: coding DNA sequence; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; mRNA: messenger RNA; P2A: peptide 2A; UTR: untranslated region.

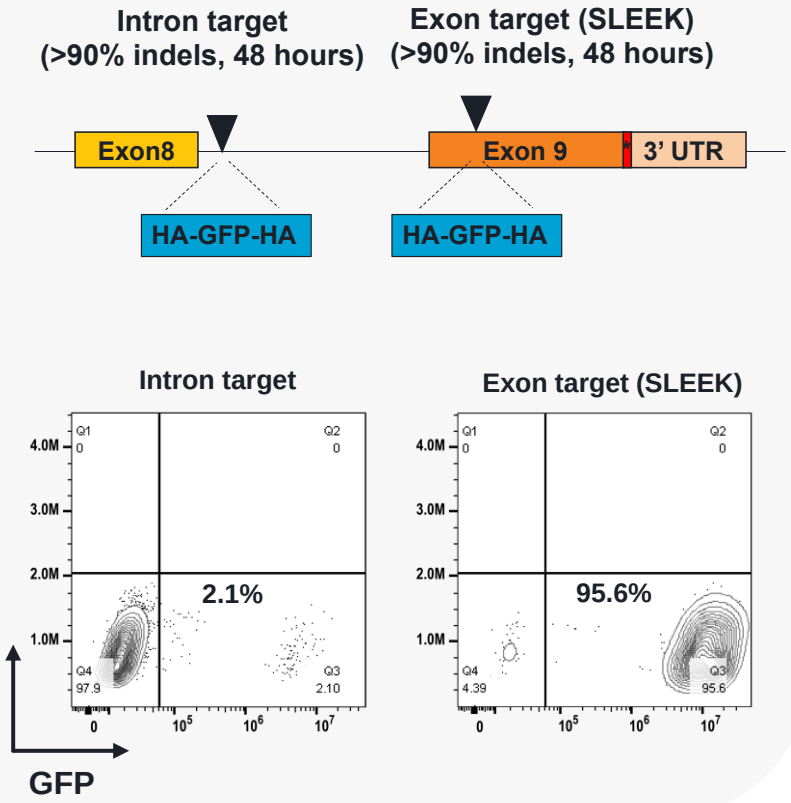
Reduction to Practice of SLEEK Technology

iPSCs

Selection of Knock-in Edit Over KO Edit



Validation of SLEEK Hypothesis

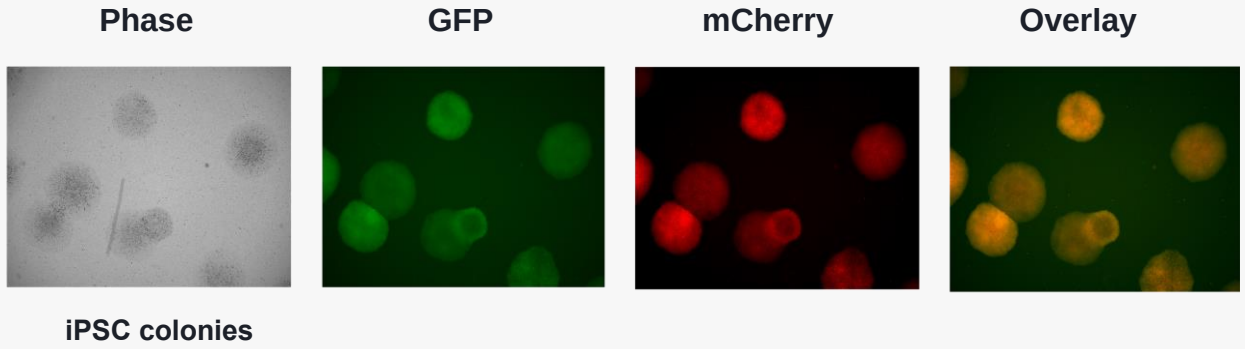


HA: homology arm; GFP: green fluorescent protein; iPSC: induced pluripotent stem cell; KI: knock-in; KO: knock-out; SSC: side scatter; UTR: untranslated region.

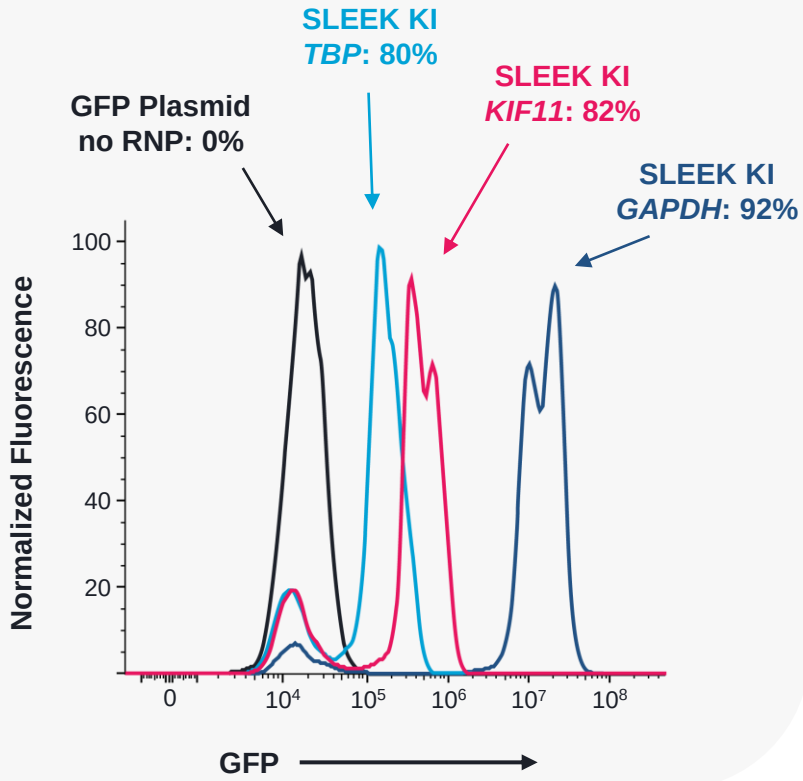
Multicistronic Knock-in and Tunable Expression With SLEEK

iPSCs

Multicistronic KI of GFP and mCherry Transgene Cargos



Tunable Cargo Expression by KI at Different Genes



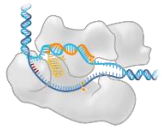
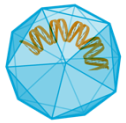
CDS: coding DNA sequence; GFP: green fluorescent protein; KIF11: kinesin family member 11; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; KI: knock-in; P2A: peptide 2A; RNP: ribonucleoprotein; TBP: TATA binding protein; UTR: untranslated region.

SLEEK is More Efficient and Potent Than *TRAC* KI Gold Standard

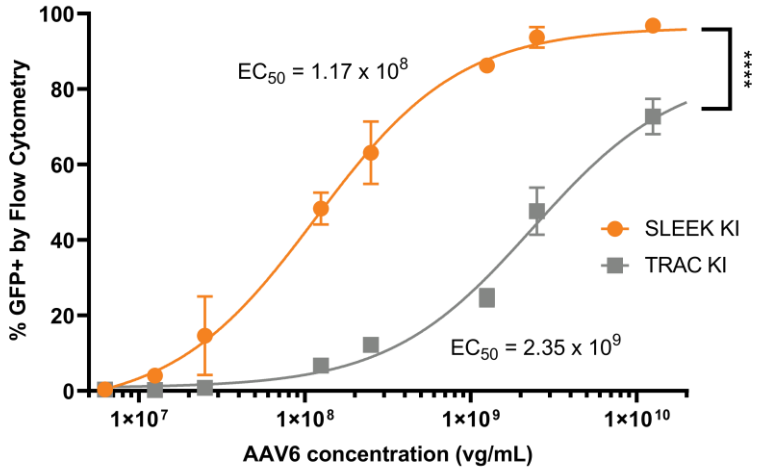
T cells

Experimental Schematic

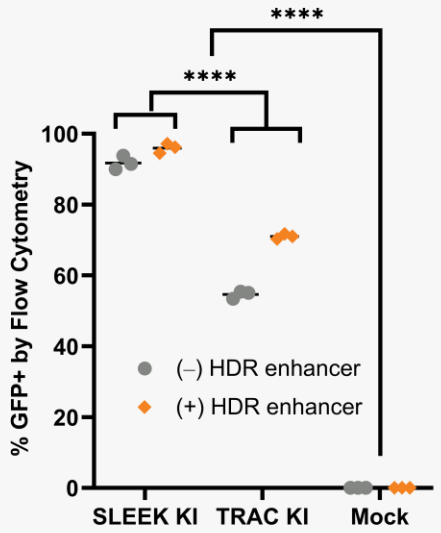
T cells + TRAC or SLEEK RNPs + AAV6 GFP (TRAC or SLEEK)

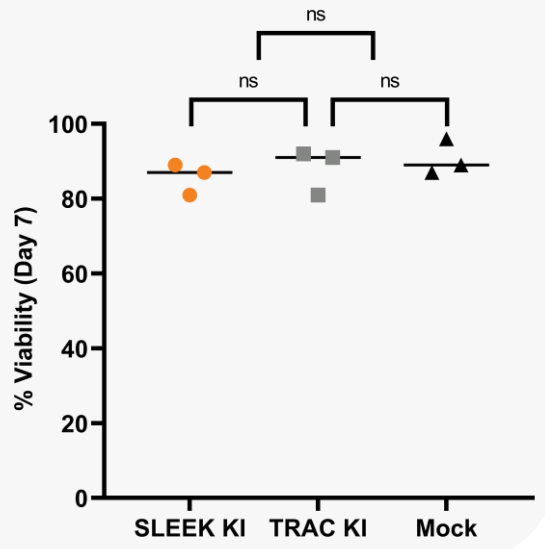
→ KI Efficiency, Viability, Expansion



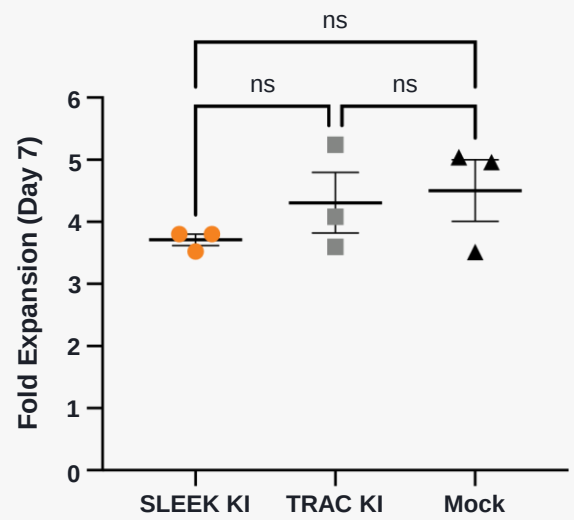
KI Efficiency



Viability



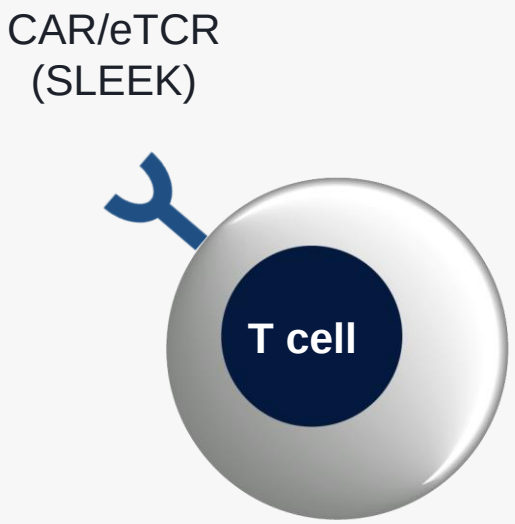
Expansion



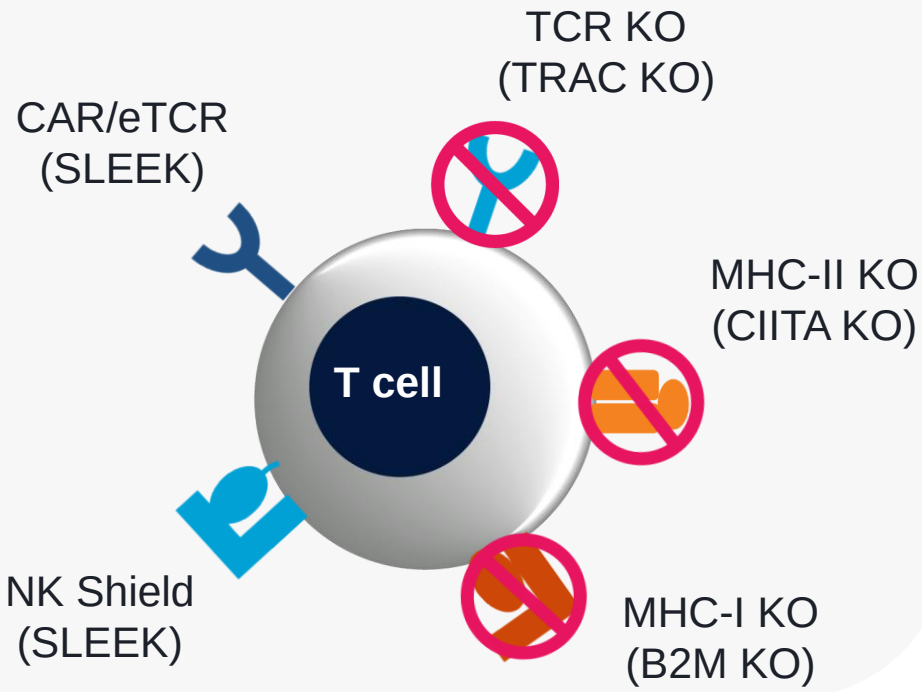
AAV6: adeno-associated virus type 6; GFP: green fluorescent protein; HDR: homology-directed repair; KI: knock-in; ns: not significant; RNP: ribonucleoprotein; TRAC: T-cell receptor α constant.

Many Opportunities to Use SLEEK to Generate Highly Homogeneous Engineered T Cell and NK Cell Medicines

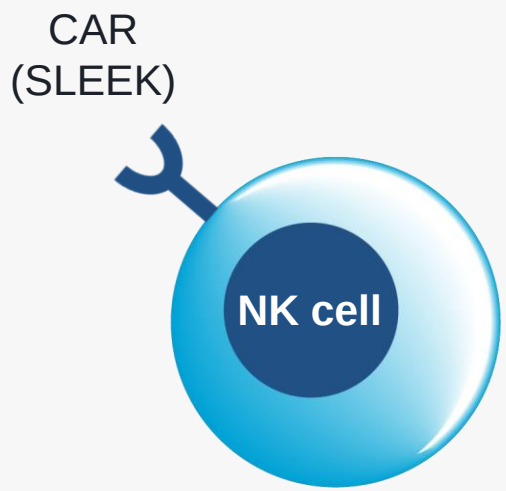
Autologous Engineered T cell



Allogeneic Healthy Donor Engineered T Cell



Allogeneic Healthy Donor Engineered NK Cell



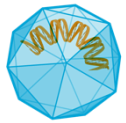
B2M: beta-2 macroglobulin; CAR: chimeric antigen receptors; CIITA: class II major histocompatibility complex transactivator; KI: knock-in; KO: knock-out; MHC: major histocompatibility complex; NK: natural killer; TCR: T-cell receptor.

SLEEK Enables the Generation of Multi-Edited Cell-Based Medicines

T cells

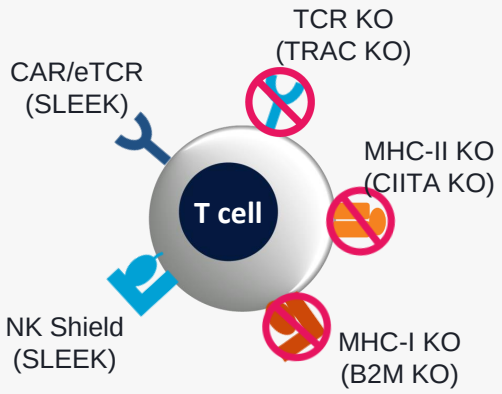
Experimental Schematic

T cells + TRAC, B2M, CIITA and SLEEK RNPs + AAV6 (CD19 CAR)



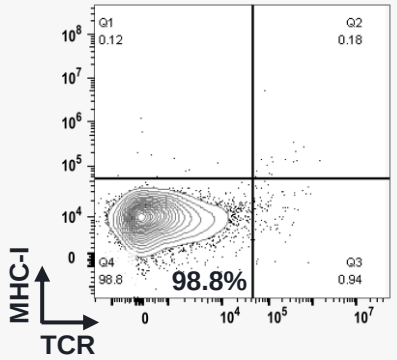
→ Flow Cytometry
Tumor Killing Assay

Allogeneic Healthy Donor Applications

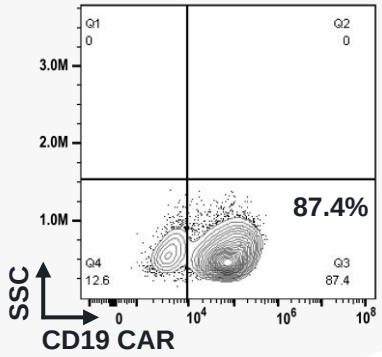


Validation of KO/KI

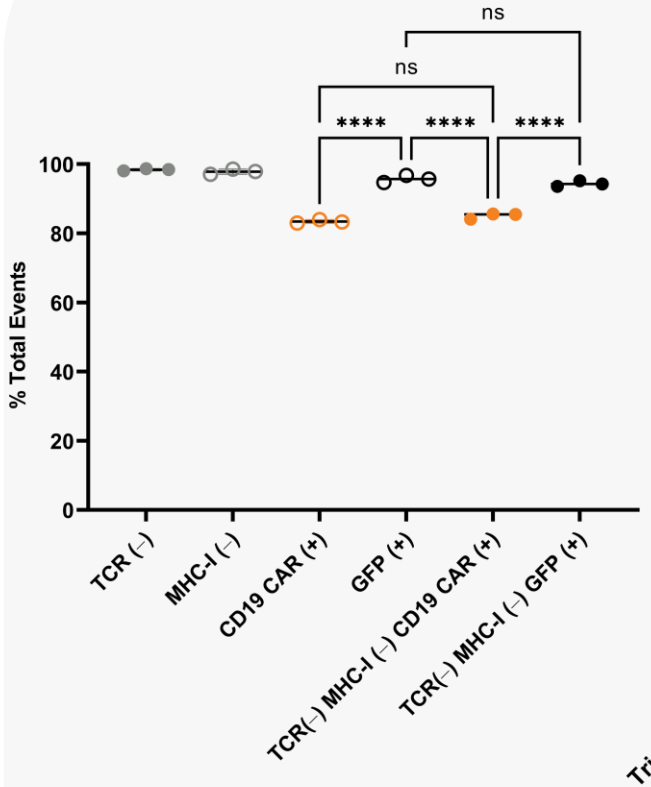
MHC-I and TCR KO



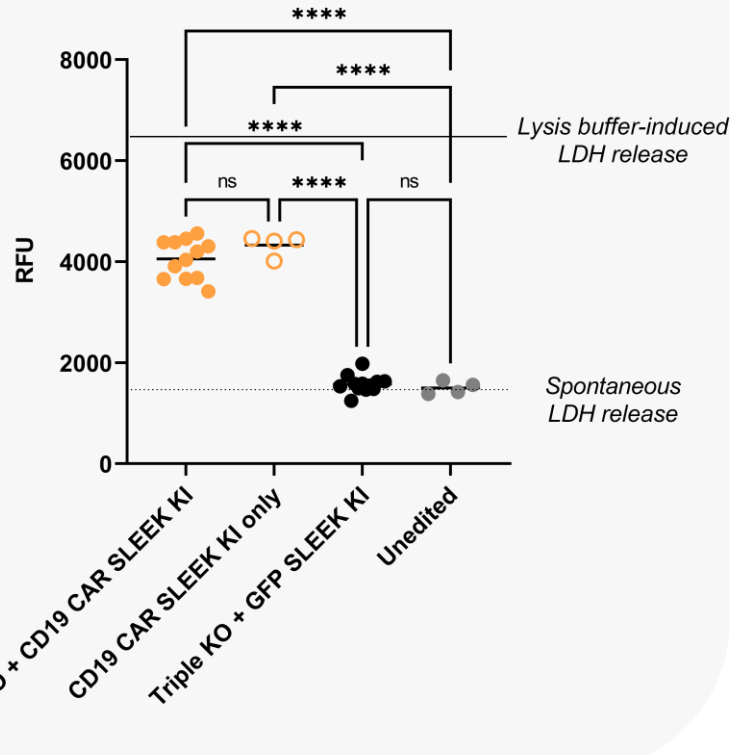
CD19 CAR SLEEK KI



KI Efficiency



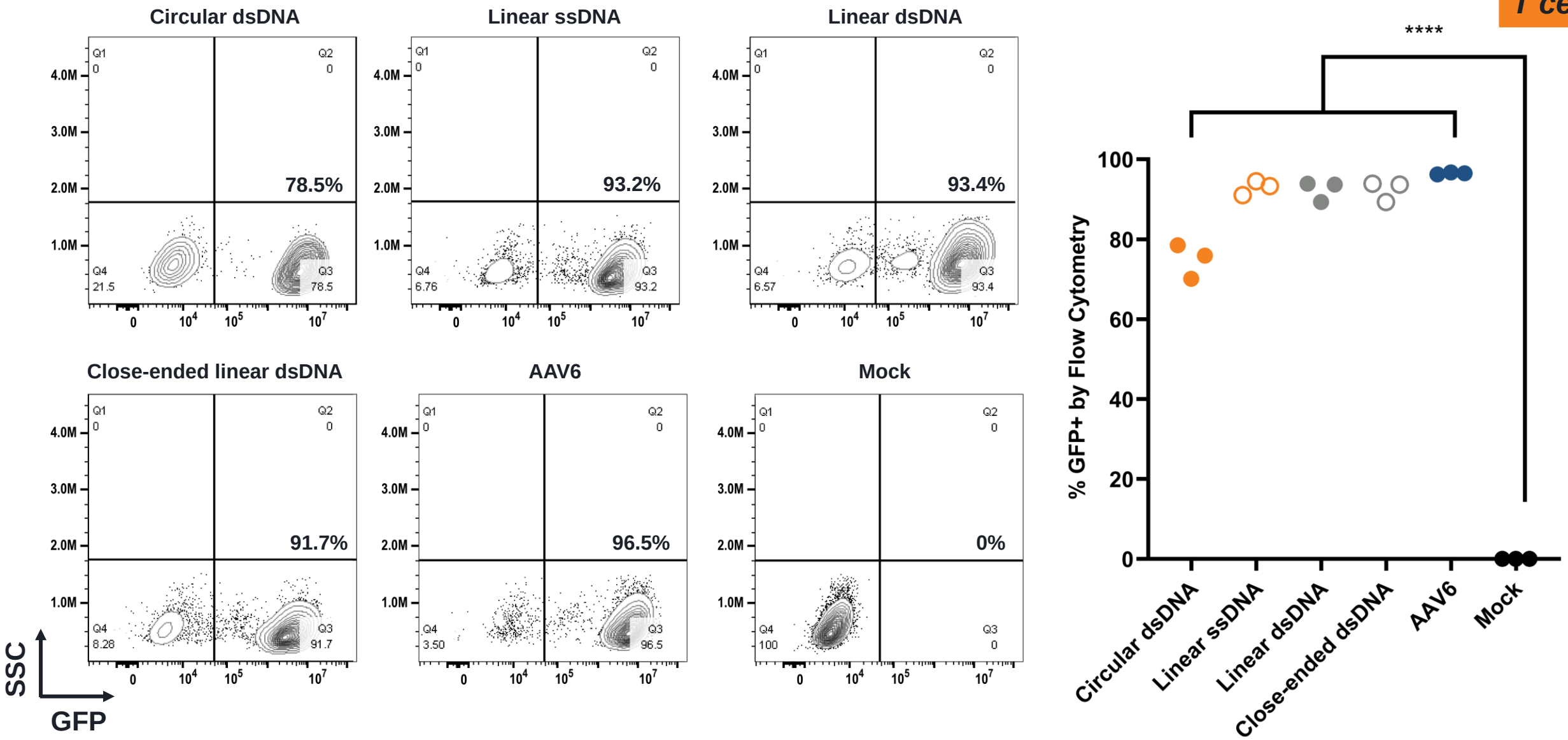
Tumor Killing



AAV6: adeno-associated virus type 6; B2M; beta-2 macroglobulin; CAR: chimeric antigen receptors; CIITA: class II major histocompatibility complex transactivator; CD: cluster of differentiation; GFP; green fluorescent protein; KI: knock-in; KO: knock-out; MHC: major histocompatibility complex; NK: natural killer; SSC: side scatter; TRAC: T-cell receptor α constant; TCR: T-cell receptor.

SLEEK is Similarly Efficient With Non-Viral DNA Templates

T cells

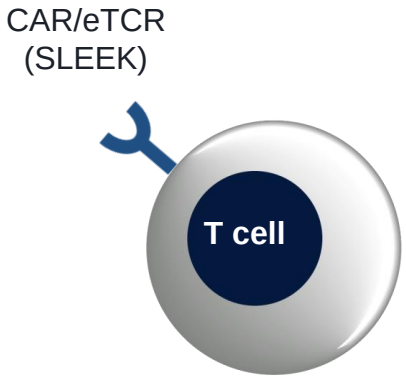


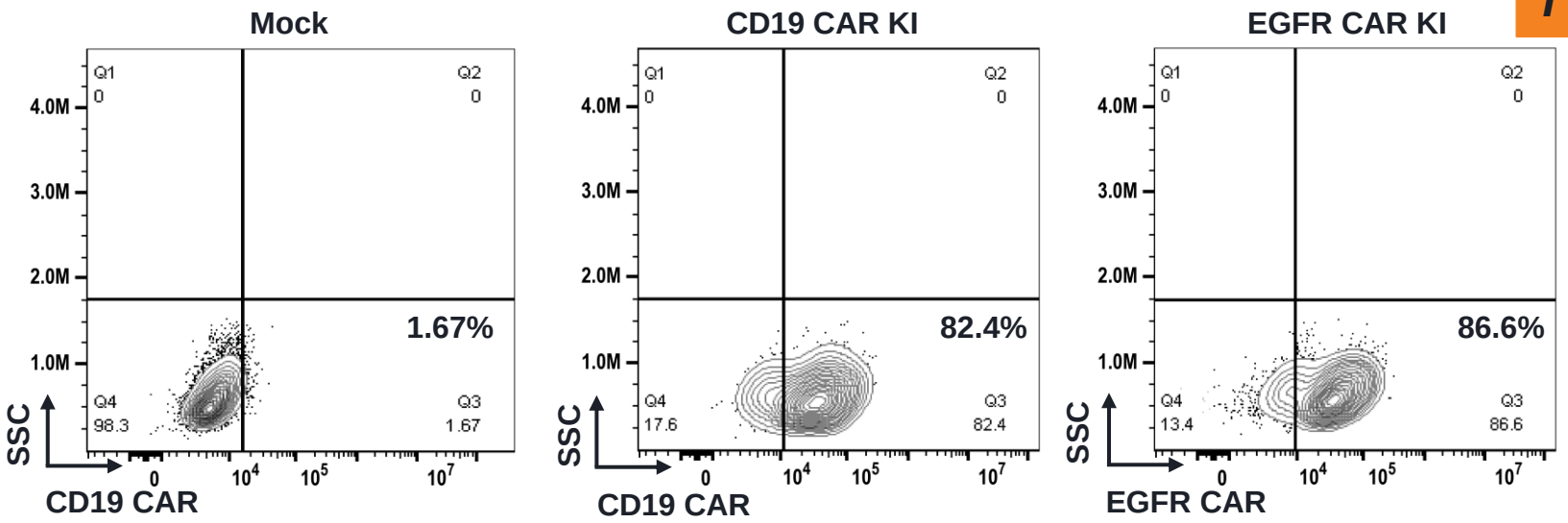
AAV6: adeno-associated virus type 6; dsDNA: double stranded deoxyribonucleic acid; GFP: green fluorescent protein; SSC: side scatter; ssDNA: single stranded deoxyribonucleic acid.

SLEEK KI of Functional Cargos With Non-Viral DNA Templates

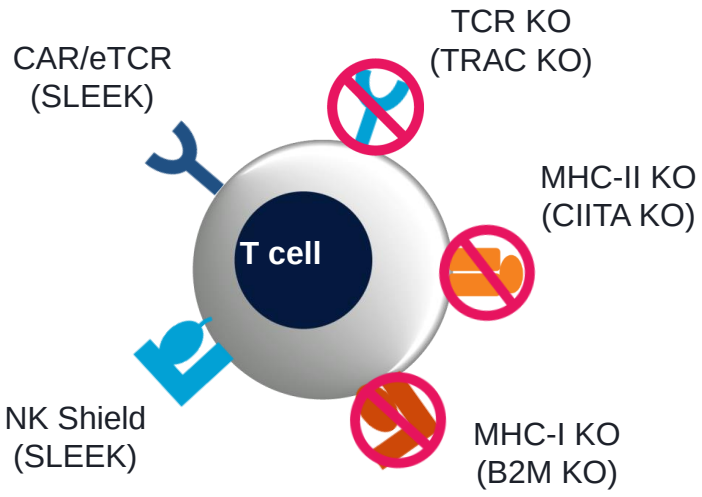
T cells

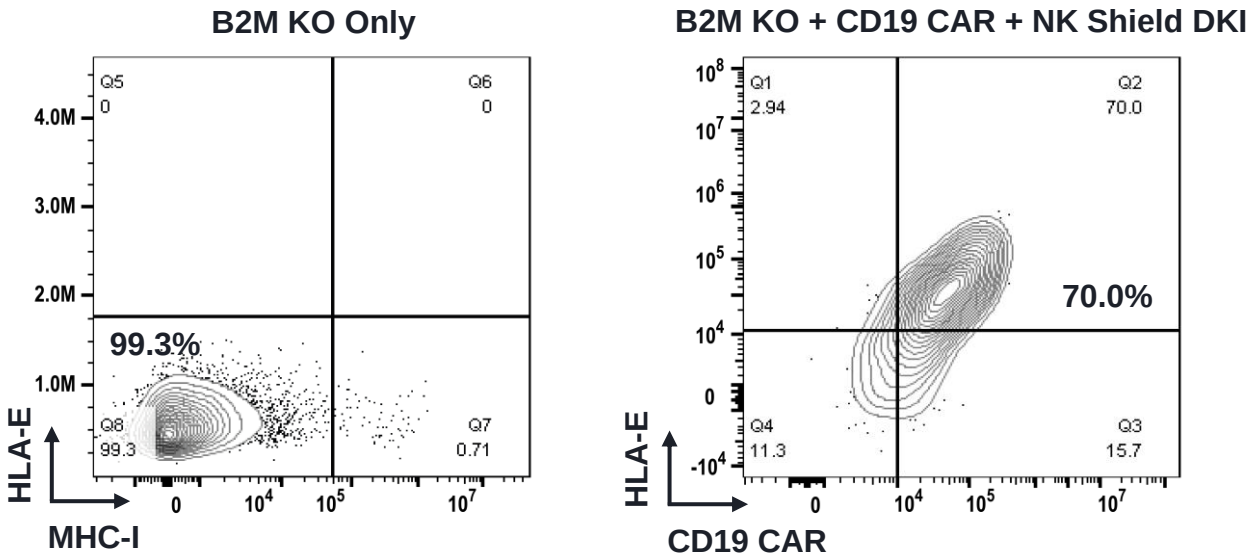
Autologous





Allogeneic

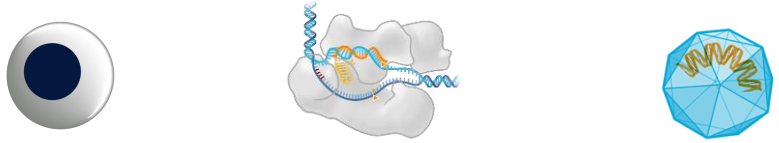




B2M; beta-2 macroglobulin; CAR: chimeric antigen receptors; CD: cluster of differentiation; DKI: double knock-in; EGFR: epidermal growth factor receptor; HLA-E: human leukocyte antigen E; GFP; green fluorescent protein; KI: knock-in; KO: knock-out; MHC: major histocompatibility complex; NK: natural killer; SSC: side scatter; TCR: T-cell receptor.

SLEEK Achieves Best-in-Class Knock-in Rates Across Cell Types

Cells + RNP (AsCas12a) + AAV6 (Cargo)



B cells

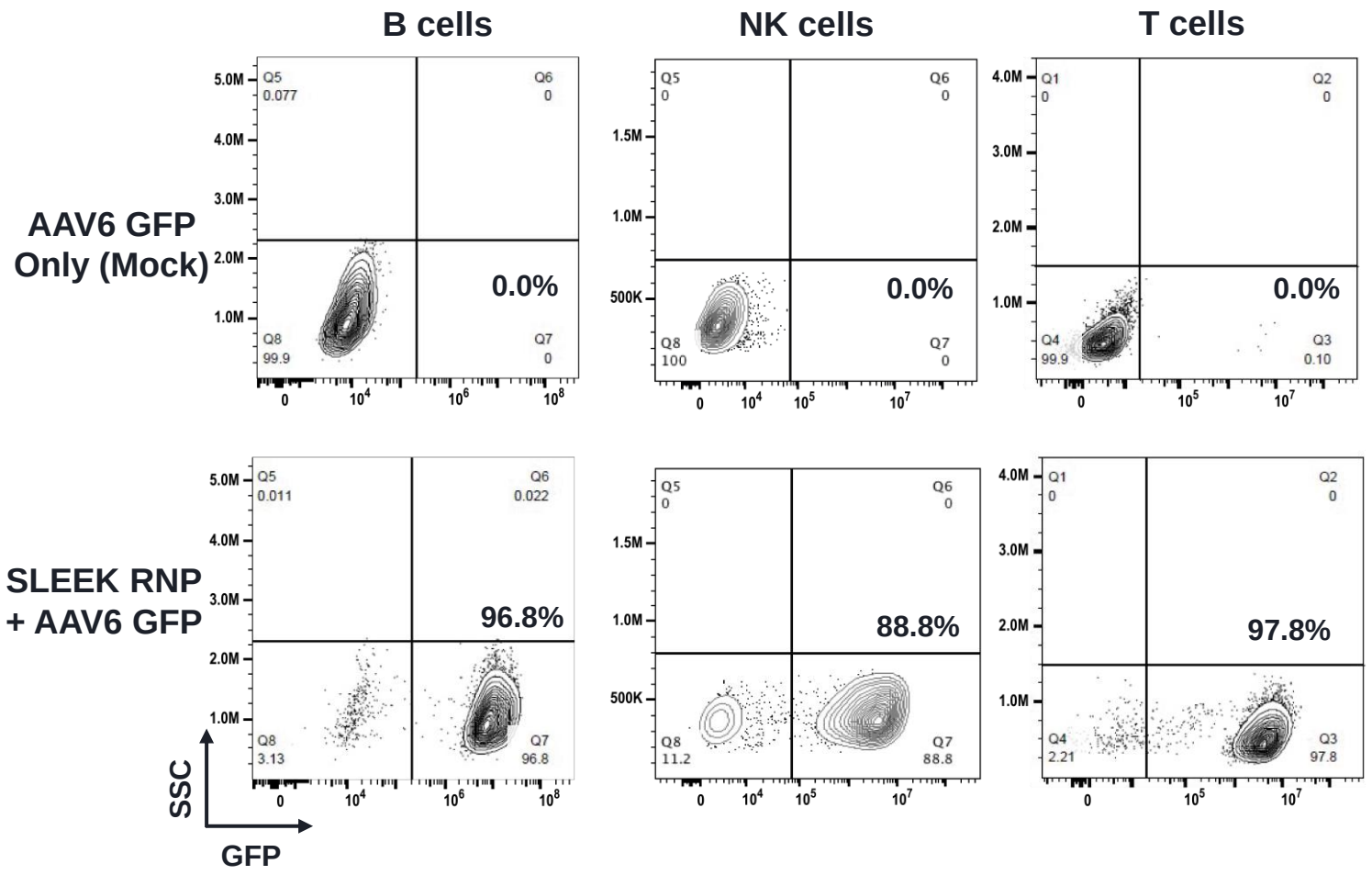
Protein replacement
Antibody secretion

T cells

Autologous/Allogeneic
CAR-T cell therapy

NK cells

CAR-NK cells for various cancer treatments

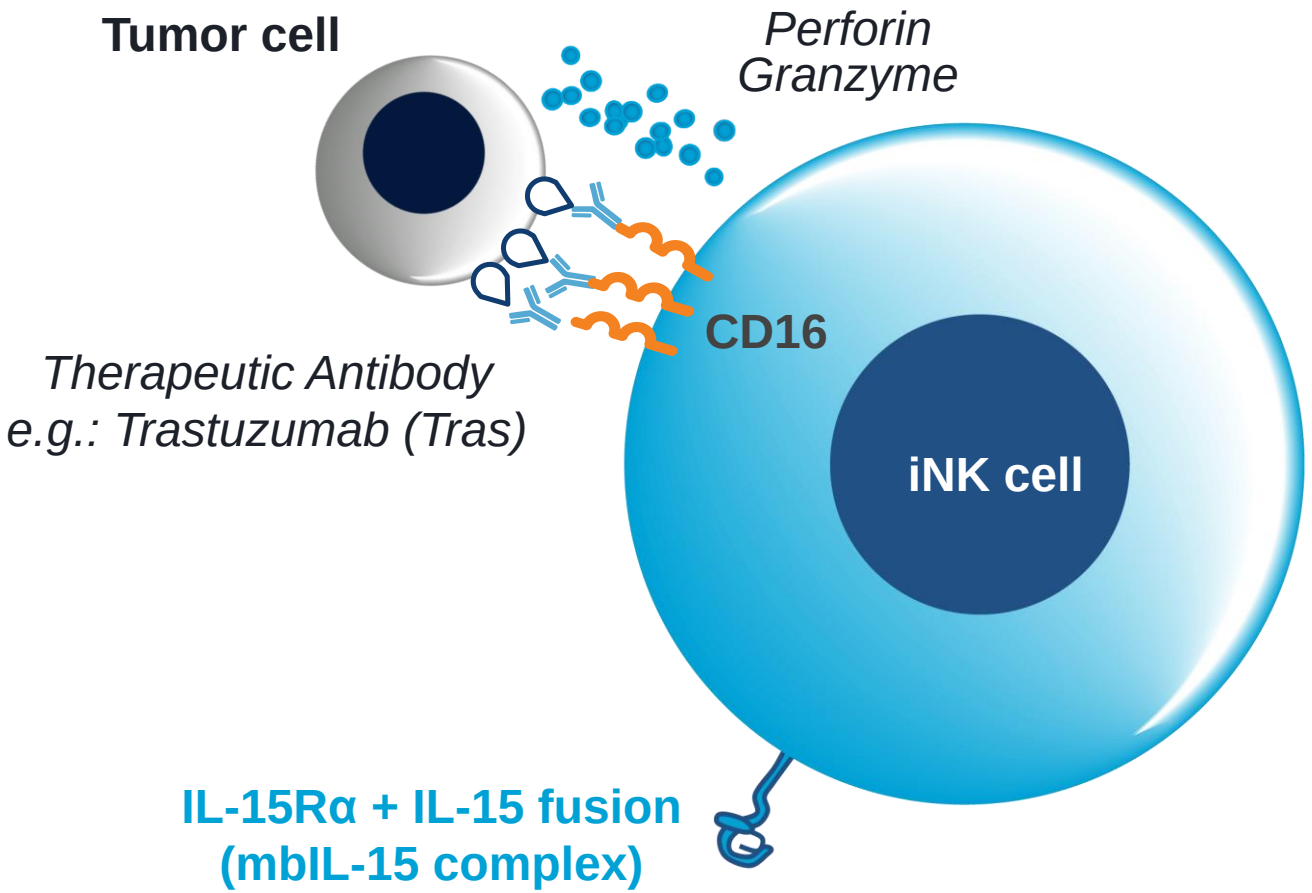


SLEEK KI Efficiency Approaches 100% Which Improves Product Purity of Edited Cell Medicines

AAV6: adeno-associated virus type 6; Cas: CRISPR-associated protein; CAR: chimeric antigen receptors; GFP; green fluorescent protein; KI: knock-in; NK: natural killer; RNP: ribonucleoprotein; SSC: side scatter.

Engineering an iNK Cell With Enhanced Functions Using SLEEK

iNK cells



Enhance Antibody-Dependent Cellular Cytotoxicity (ADCC)

Constitutive overexpression of **CD16** through **CD16** knock-in

Enhance survival and/or expansion of iNK cells

Increased **IL-15** signaling through knock-in of **mbIL-15** complex

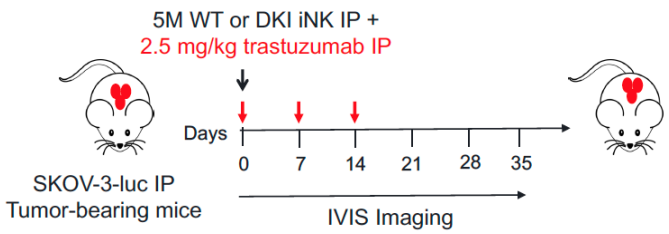
Generation of an Edited iNK Cell Through SLEEK Double Knock-in of CD16 and mbIL-15 (SLEEK DKI)

ADCC: Antibody-Dependent cellular cytotoxicity; CD: cluster of differentiation; iNK: IPSC-derived natural killer cell; IL-15Rα: interleukin-15 receptor α; mbiL-15: membrane-bound IL-15; NK: natural killer.

SLEEK DKI iNKs Administered in Combination With Trastuzumab Induced Significant to Complete Tumor Clearance in Mice

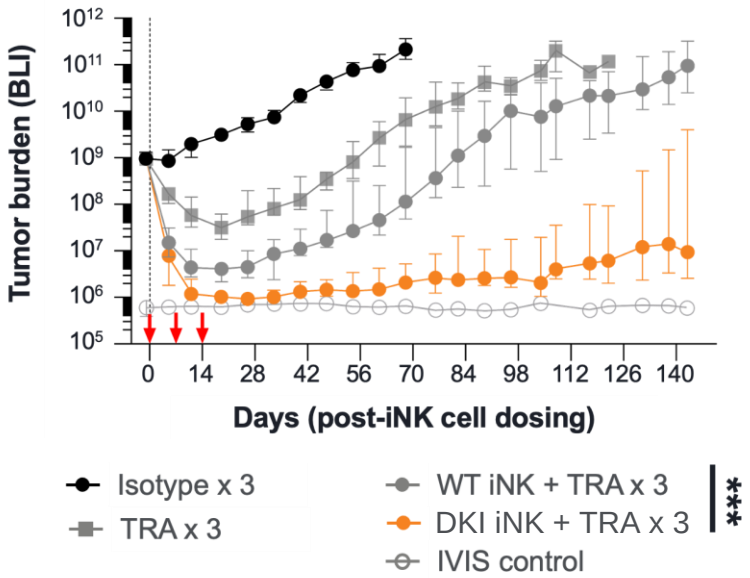
iNK cells

Overview of in vivo study to assess SLEEK DKI iNK

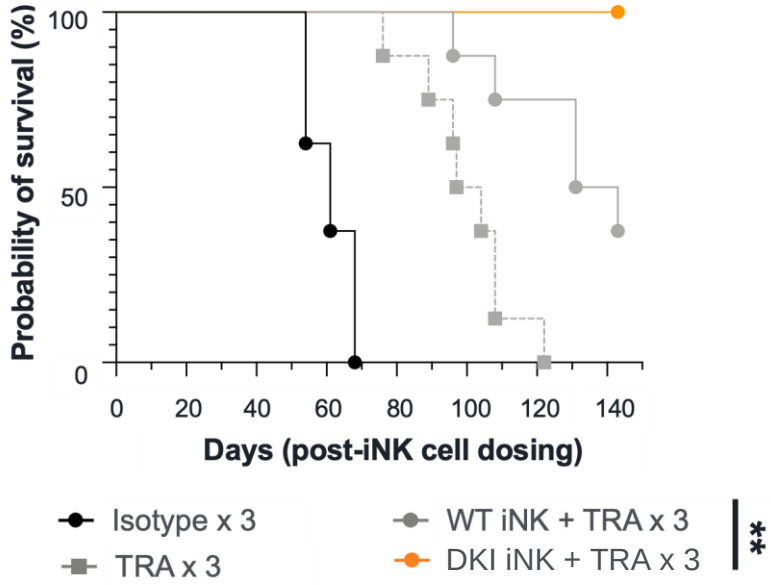


Visit poster #1320 for abstract 1106 on May 18th!

During the course of the 144-day experiment, 6/8 DKI iNK-treated mice had no detectable tumor



On day 144, 100% of DKI iNK-treated mice were alive, compared with <50% of WT iNK-treated mice



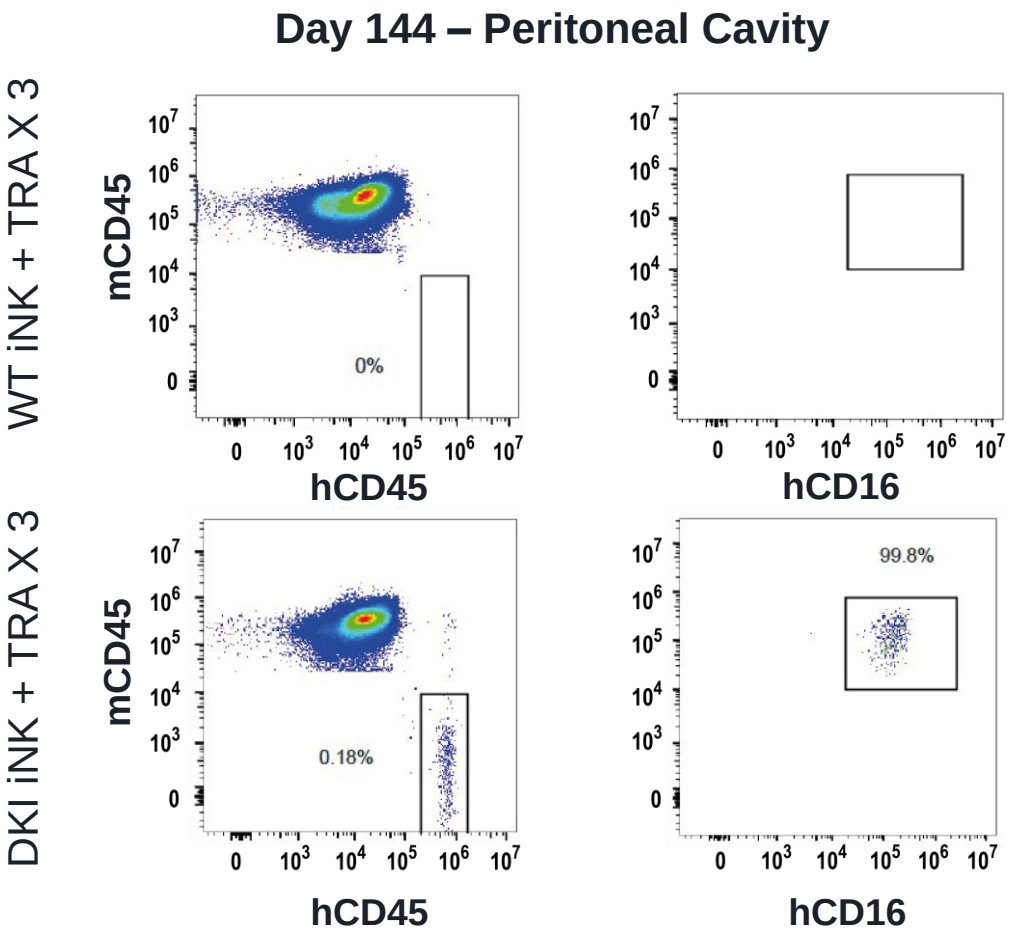
Strong Tumor Clearance Attributed to Robust Expression of CD16 Cargo by SLEEK

DKI: double knock-in; iNK: IPSC-derived natural killer cell; IP: intraperitoneal; Iso: isotype; IVIS: in vivo imaging system; NK: natural killer; SKOV-3-luc: luciferase-expressing SKOV-3 cell line; TRA: trastuzumab; WT: wild-type.

Gerew et al. ASH, 2021; Allen, Khan et al. AACR, 2022.

SLEEK DKI iNKs Show Prolonged *In Vivo* Persistence Beyond 144 Days

iNK cells



- SLEEK iNKs continued to express high levels of CD16 up to Day 144 post-dosing
- No exogenous cytokine support needed

Impressive Persistence in iNKs From Robust Expression of mbIL-15 Cargo by SLEEK

CD: cluster of differentiation; iNK: IPSC-derived natural killer cell; hCD: human CD; mCD: mouse CD; NK: natural killer; TRA: trastuzumab; WT: wild-type.

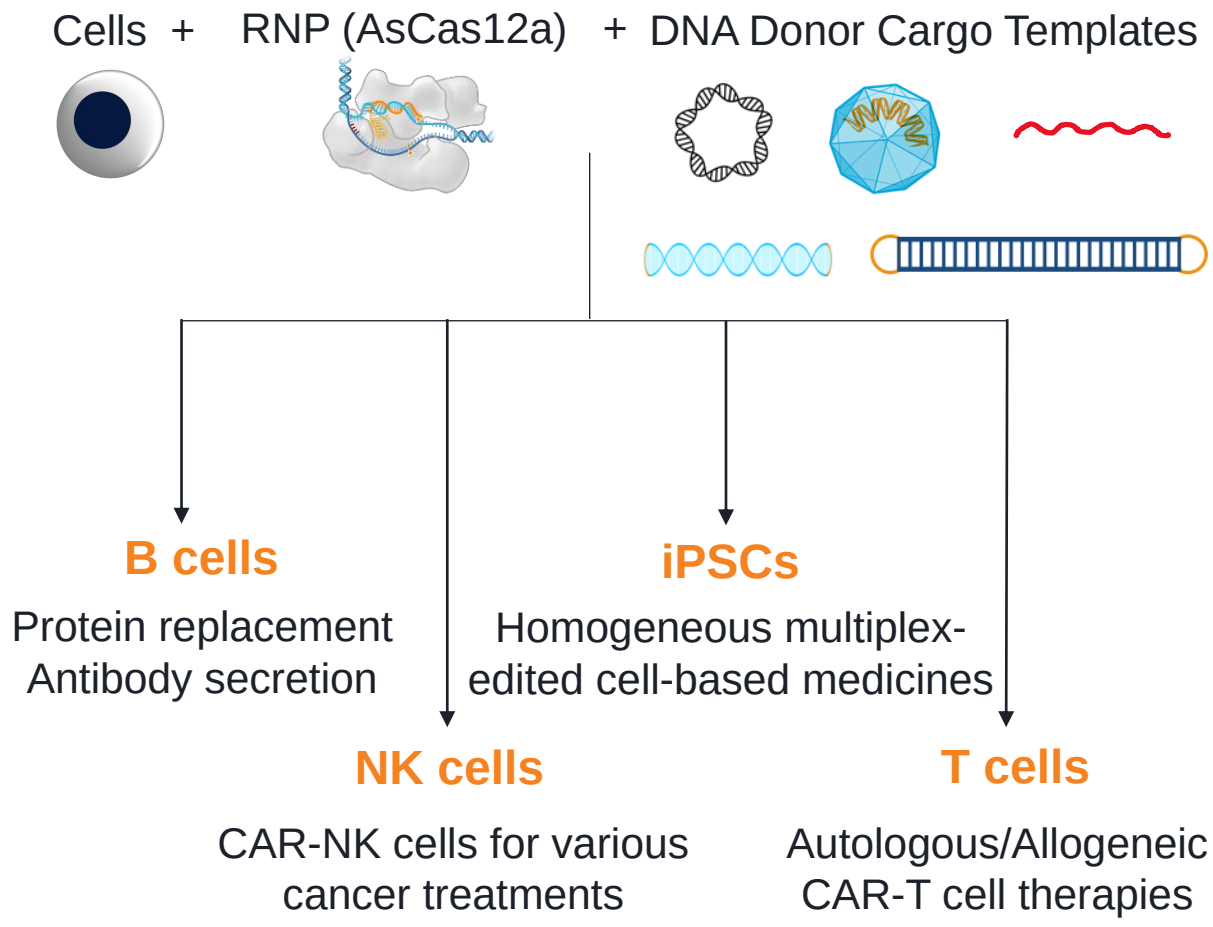
Allen, Khan et al. AACR, 2022.

Our Goal was to Develop an Editing Technology that Could Fundamentally Improve the Generation of Cell-Based Medicines

SLEEK™ GENE EDITING

Se**L**ection by **E**ssential-gene **E**xon **K**nock-In

- Enables >95% knock-in efficiency
- High-level, tunable cargo expression
- Homogeneous editing
- Efficient multicistronic cargos
- Simplifies iPSC clone selection process
- Robust, lineage-independent, expression of functional cargo in iPSCs



We Believe SLEEK Fundamentally Improves the Generation and Clinical Potential of Cell-based Medicines

See other Editas oral and poster presentations including our iNK abstract: #1106, poster May 18th

CAR: chimeric antigen receptor; Cas: CRISPR-associated protein; iNK: IPSC-derived natural killer cell; iPSC: induced pluripotent stem cell; NK: natural killer; RNP: ribonucleoprotein; TME: tumor microenvironment.