Highly Efficient Multi-Gene Knockout and Transgene Knock-in using CRISPR-Cas12a in Induced Pluripotent Stem Cells for the Generation of Engineered Cell Immunotherapies

JA Zuris, CM Margulies, R Viswanathan, JN Edelstein, R Pattali, KW Gareau, SN Scott, S Lele, AC Wilson, JI Moon, N Dilmac, AE Friedland, RA Morgan

John Zuris, Ph.D.

May 15th, 2020

Disclosure

- John Zuris is an employee and shareholder at Editas Medicine

Building a Genomic Medicine Leader

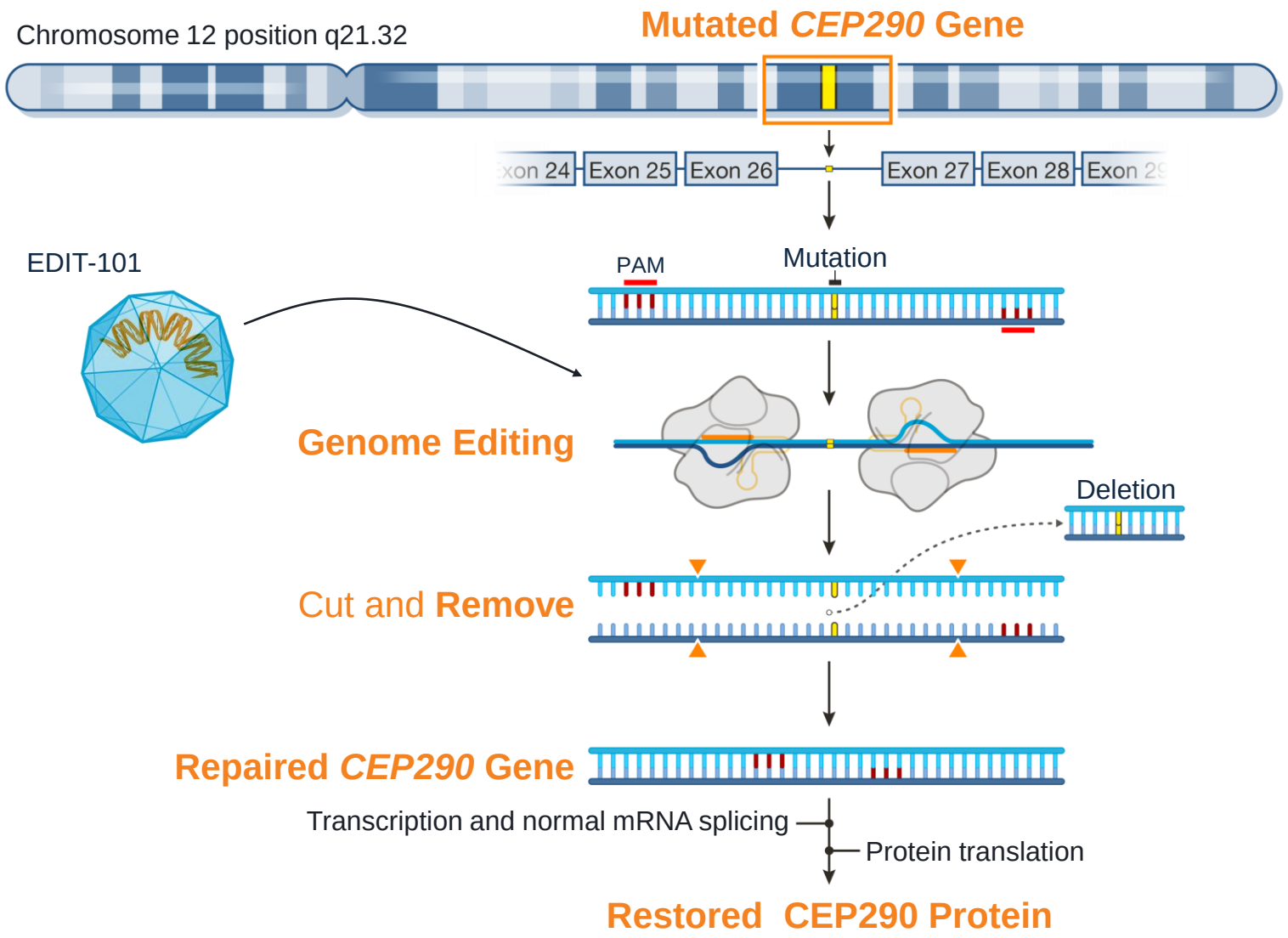
CRISPR Gene Editing to Develop Differentiated, Transformational Medicines for High Unmet Need

In Vivo CRISPR Medicines	Engineered Cell Medicines
Leverage AAV-mediated editing with SaCas9 into additional therapeutic areas	Develop best-in-class medicines for hemoglobinopathies using Cas12a and solid tumors using iPSC-derived cells

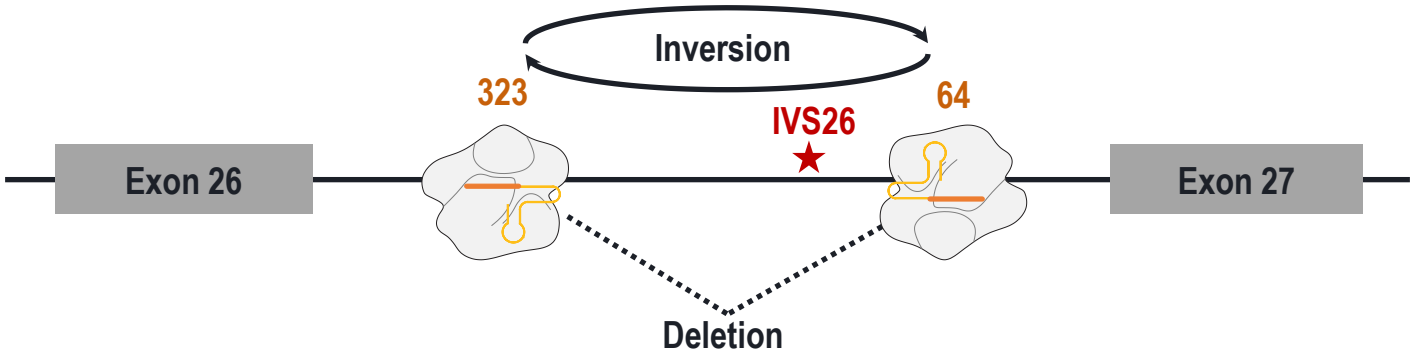
Maintain Best-in-class Platform & Intellectual Property, and Advance Organizational Excellence

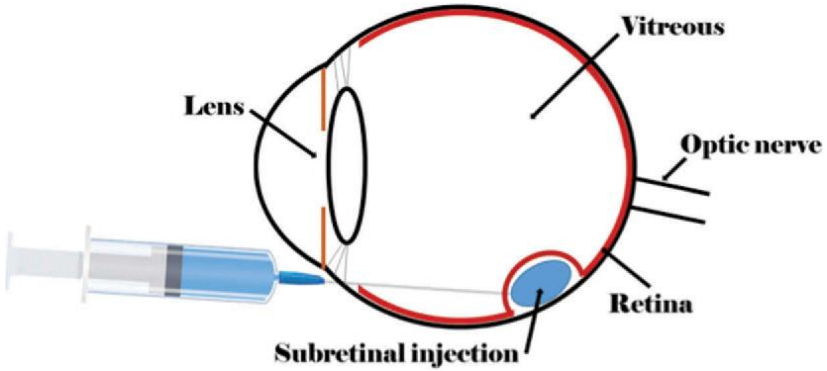
CRISPR: clustered regularly interspaced short palindromic repeat; SaCas9: *Staphylococcus aureus* CRISPR-associated protein 9; Cas12a: CRISPR-associated protein 12a; iPSC: induced pluripotent stem cell

First in human CRISPR-Cas Gene Editing: EDIT-101 for LCA10

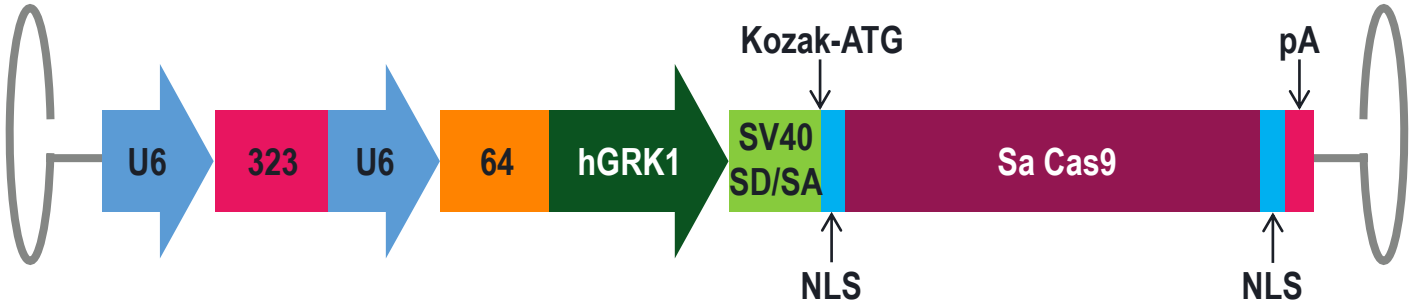


AGN-151587 (EDIT-101): SaCas9 and 2 gRNAs in AAV to Correct LCA10-IVS26 Mutation





First patient dosed at Oregon Health & Science University (OHSU) Casey Eye Institute, in February 2020



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There Are Many Nucleases to Choose from but for *Ex Vivo* Gene Editing Using AsCas12a Offers Several Potential Advantages to SpCas9

Editas general suite of nucleases

Variant	PAM	Frequency (bp)
SpCas9	NGG	1 in 8
SaCas9	NNGRRT	1 in 32
SaCas9 KKH	NNNRRT	1 in 8
AsCas12a	TTTV	1 in 43
AsCas12a RR	TYCV/CCCC	1 in 18
AsCas12a RVR	TATV	1 in 43
LbCas12a	TTTV	1 in 43

PAM sites are not limiting for general KO and large transgene KI applications

Small ~40 nt Cas12a guide is advantageous for manufacturing and sequence fidelity

AsCas12a is a more specific nuclease compared to SpCas9

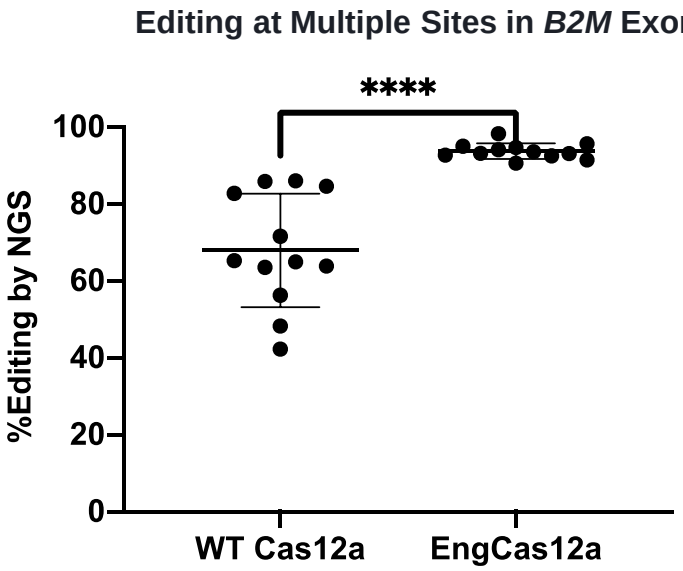
Matched Target Site (20-Ns):
TTTVNNNNNNNNNNNNNNNNNNNNGGRRT

From - Gotta et al. Genome Engineering: Frontiers of CRISPR/Cas Cold Spring Harbor, NY October 10, 2019

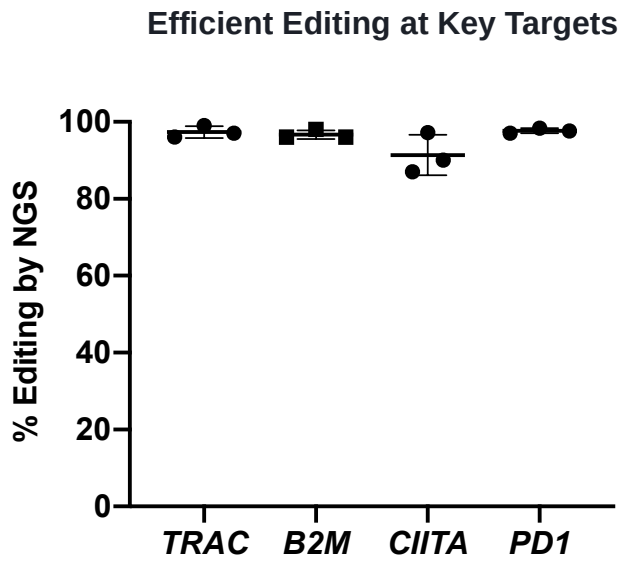
References on AsCas12a target specificity:
Kim et al. Nat Biotech 2016
Strohkendl et al. Mol Cell 2018
Swarts et al. Biochem Soc Trans 2019

Engineered Cas12a (EngCas12a) Robustly Edits at Higher Efficiency than WT Cas12a and is Capable of Efficient Knock-in in T Cells

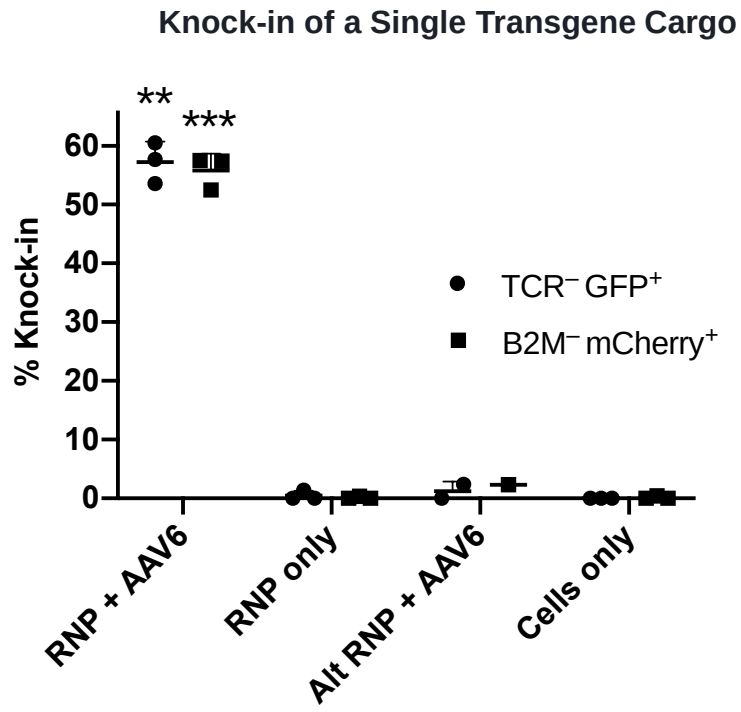
EngCas12a has superior activity



High knock-out across loci



High AAV6-mediated knock-in

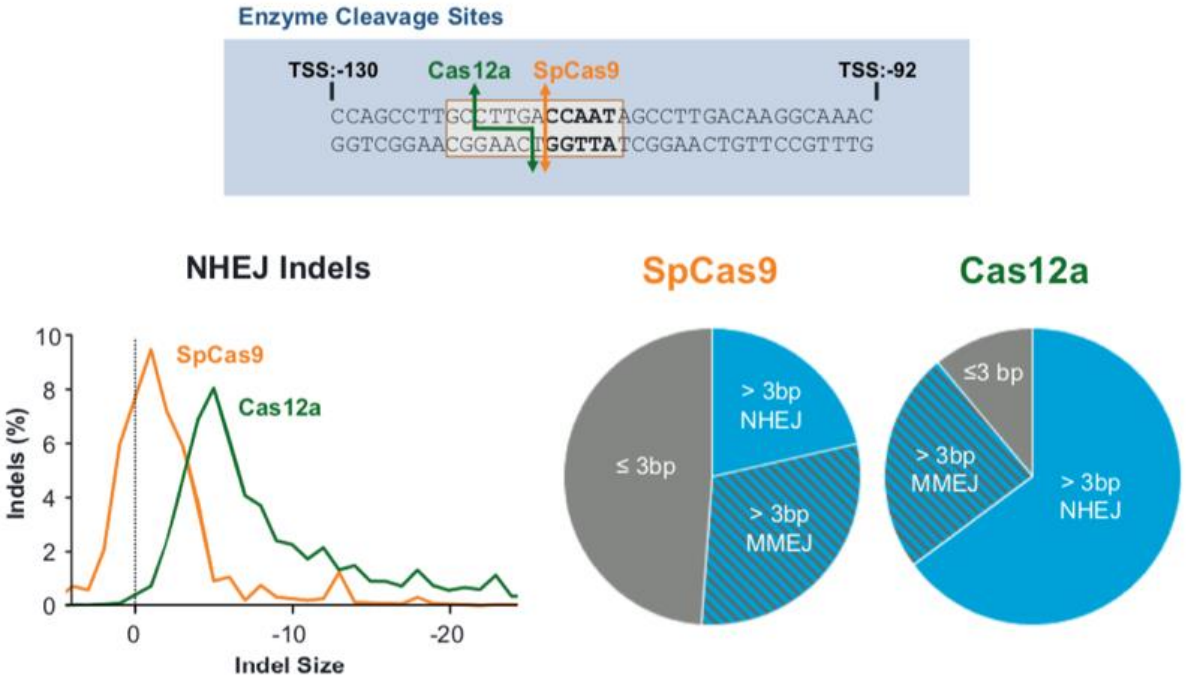


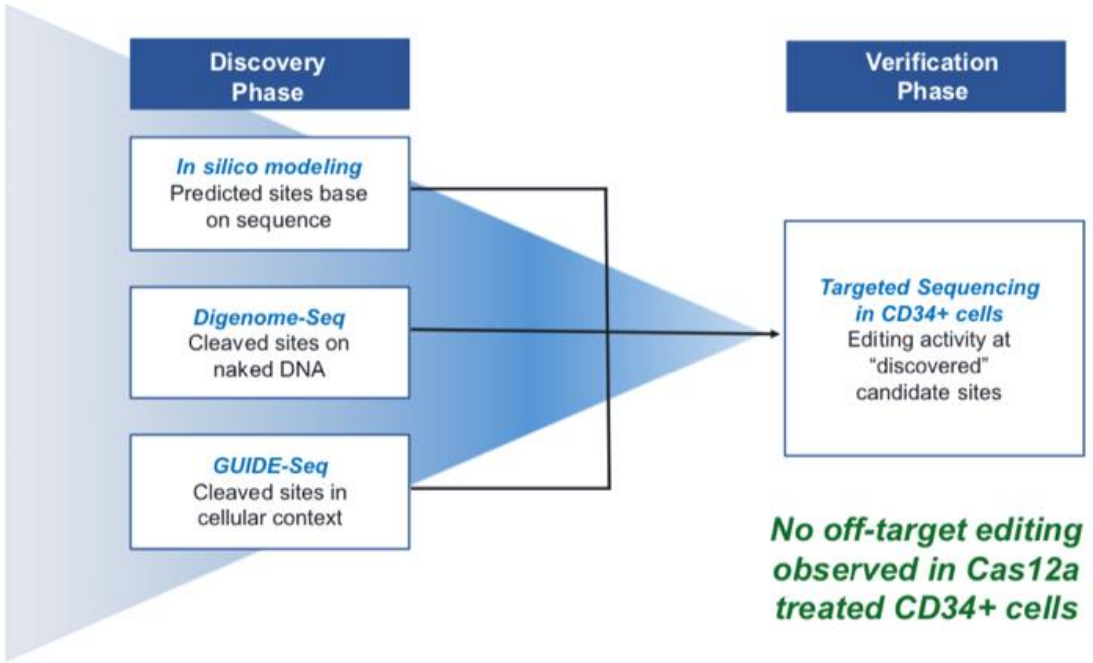
Based on AsCas12a *Ultra* nuclease from IDT

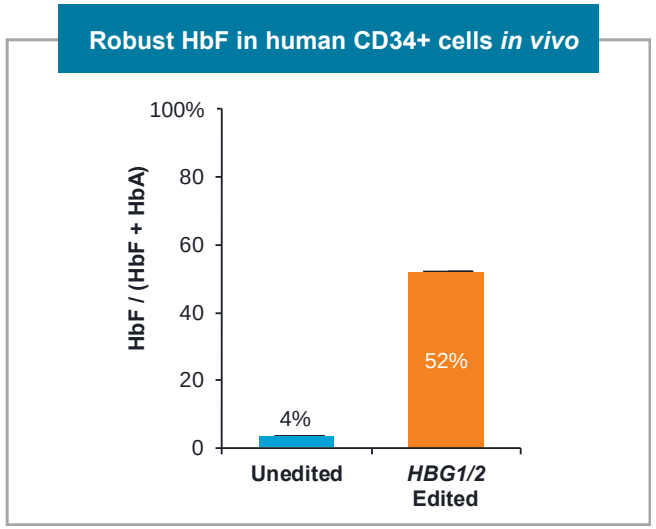
IDT INTEGRATED DNA TECHNOLOGIES

Up to 40% double knock-in

EngCas12a is Used in EDIT-301, an Experimental Medicine for Sickle Cell Disease







High specificity of WT nuclease retained in EngCas12a

Have since verified this attribute in many other cell types

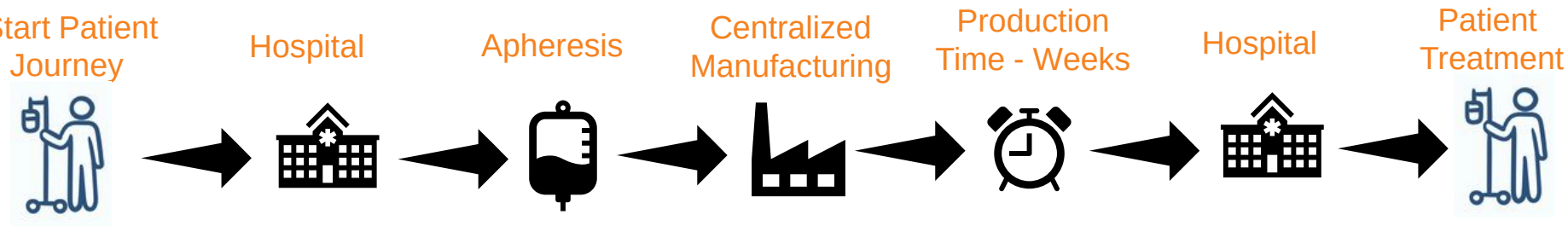
>90% editing in HSCs with EngCas12a

>50% HbF levels that persist after engraftment

From – De Dreuzy et al. Annual Meeting of the American Society of Hematology, Orlando. FL, December 9, 2019

The Case for Developing an Allogeneic Medicine for Oncology

Autologous

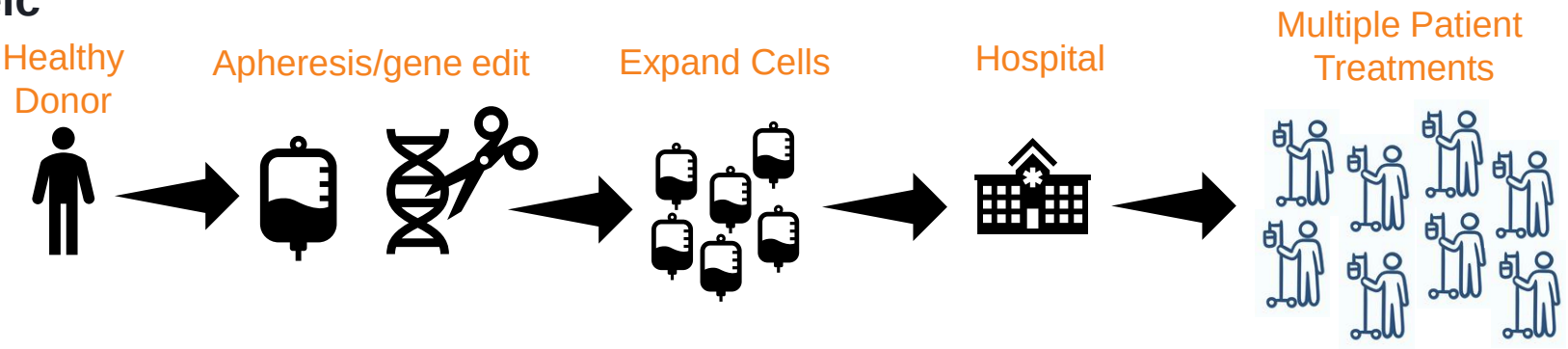


COGs

$$$

Scope

Allogeneic



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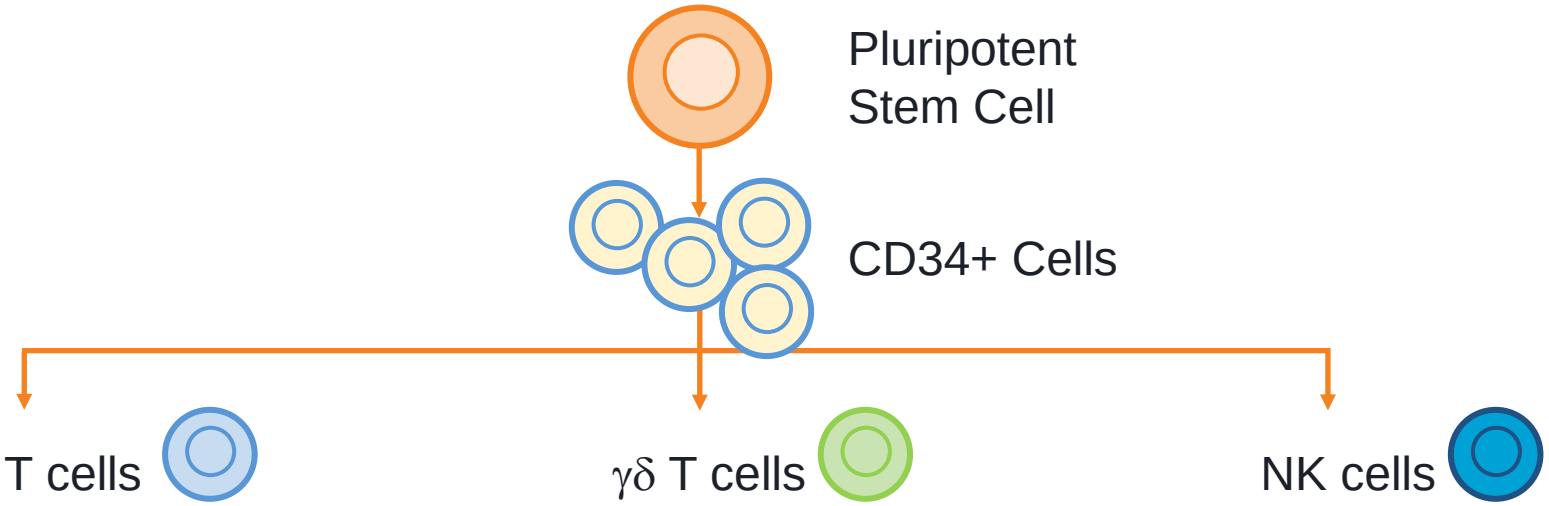
Potential Cell Types for Allogeneic Cell Medicines for Cancer



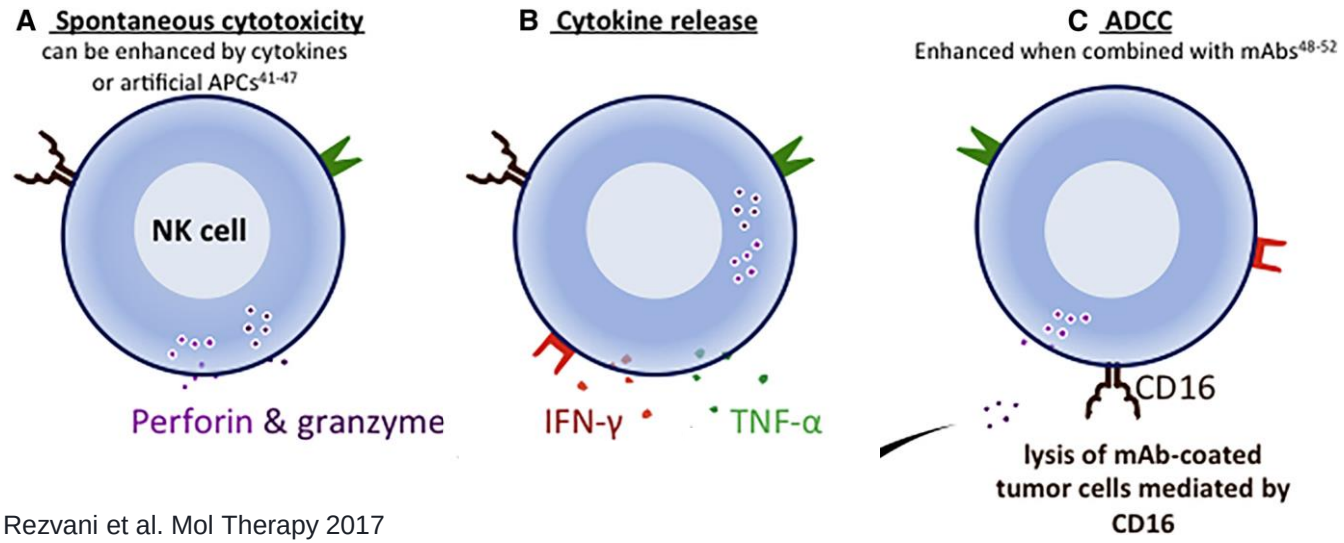
	ADAPTIVE	INNATE	
Immune System			
Tumor Recognition	• αβ T cell receptor • CAR	• γδ T cell receptor • Innate receptors • CAR	• Innate receptors • Antibody-directed • CAR
Graft-vs-Host Risk	Higher	Lower	

CAR: chimeric antigen receptor

Potential Advantages of NK Cell Therapy for Oncology

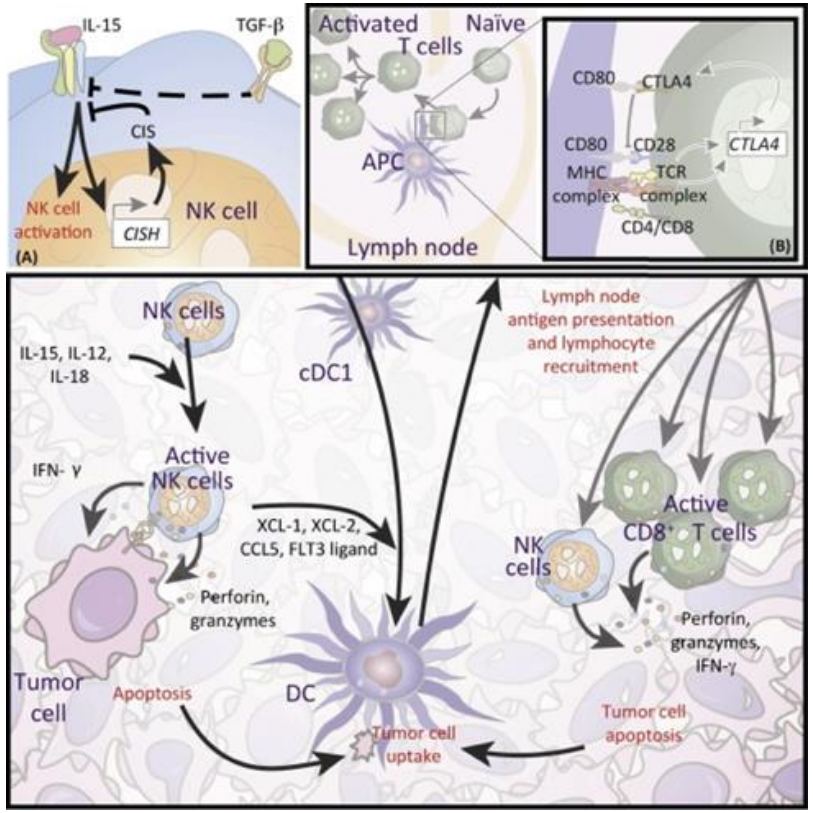
Multiple tumor-recognition mechanisms; potential to combine with therapeutic antibodies to enhance ADCC

- Loss of MHC
- NKG2D
- NCRs
- CD16, etc…



Rezvani et al. Mol Therapy 2017

NK cells induce adaptive immune responses against tumors



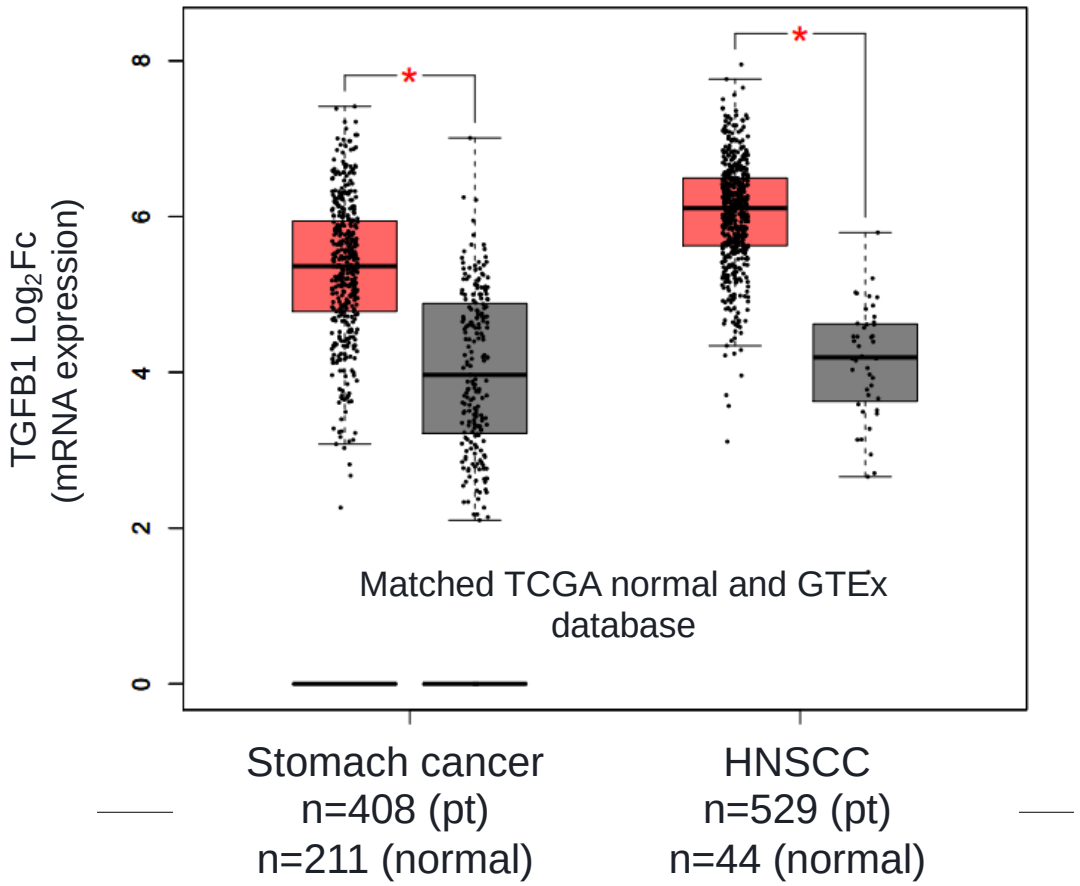
Souza-Fonseca-Guimaraes, et al., Trends in Immunology, 2019, Vol. 40, 142-158

From - Morgan et al. Keystone Emerging Cellular Therapies: Cancer and Beyond/Engineering the Genome February 10 Banff, Alberta 2020

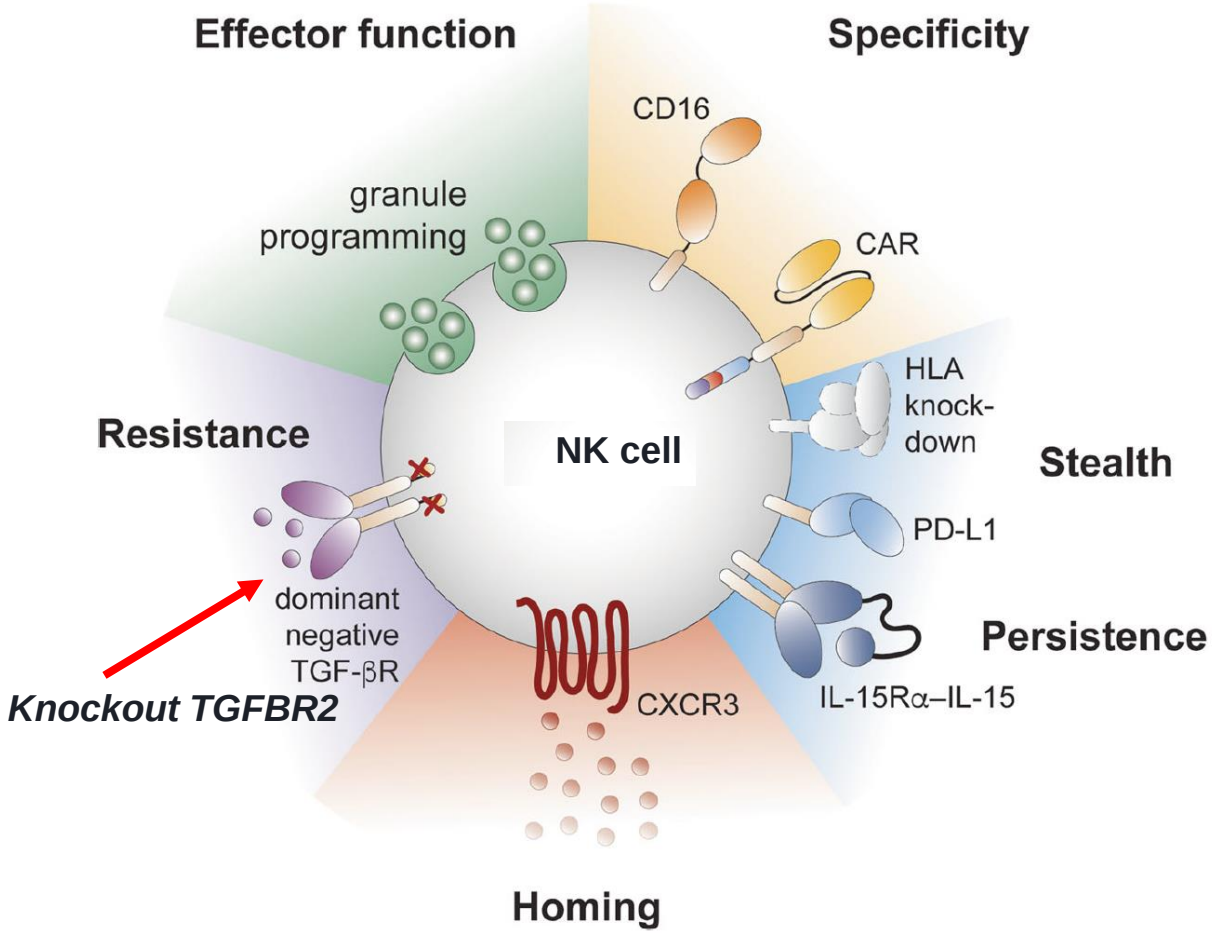
To date, no evidence of GvHD in patients treated with allogeneic NK cells, unlike off-the-shelf T cells

Case for Knocking out the TGFBR2 Protein on the Surface of NK Cells: TGF-β is a Major NK Cell Suppressive Cytokine

TGF-β is Overexpressed in Many Solid Tumors



Souza-Fonseca-Guimaraes, et al., Trends in Immunol, 2019, Vol. 40, 142-158



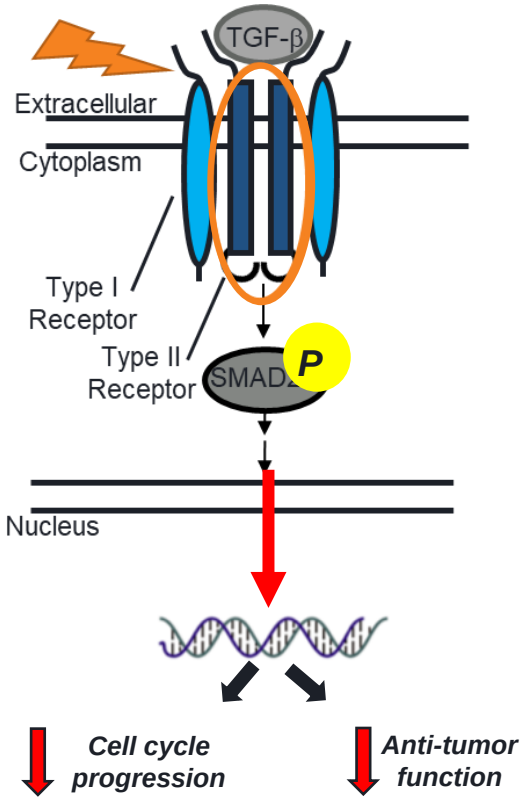
Modified from Saetersmoen et al. Seminars Immunopathology 2019

From - Morgan et al. Keystone Emerging Cellular Therapies: Cancer and Beyond/Engineering the Genome February 10 Banff, Alberta 2020

Efficient KO of *TGFBR2* in Healthy Donor NK Cells with EngCas12a Leads to Expected Loss of TGF-β Signaling

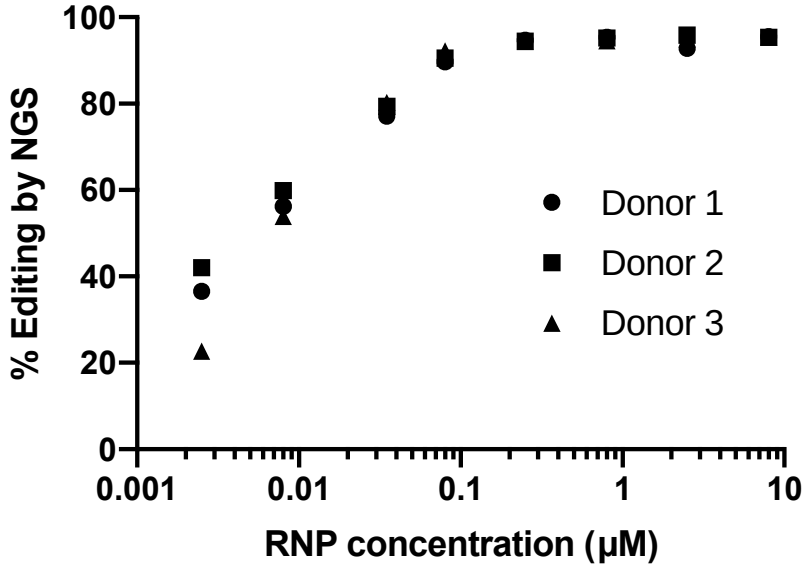
Rationale

NK cell



Editing

Editing at *TGFBR2* in Three Different Donors

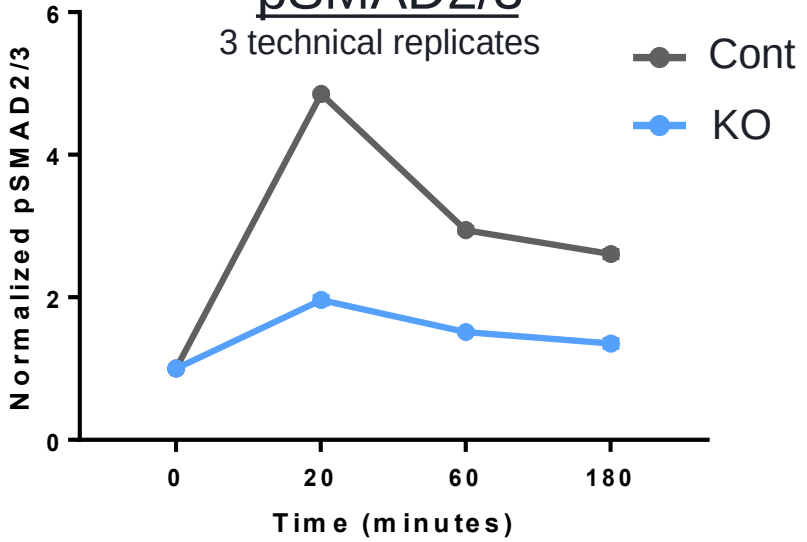


Biology

KO NK cells, minimal pSMAD2/3 in presence of TGF-β

pSMAD2/3

3 technical replicates

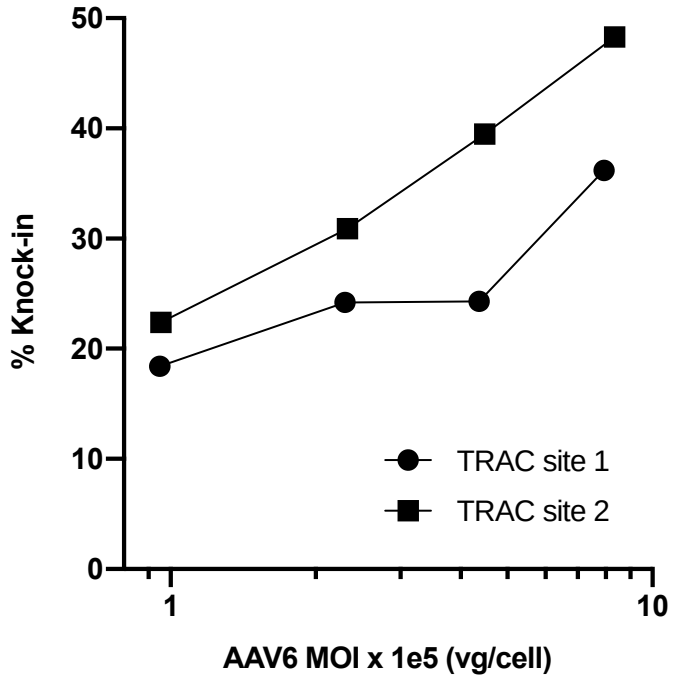


Stimulation with 10ng/mL TGF-β

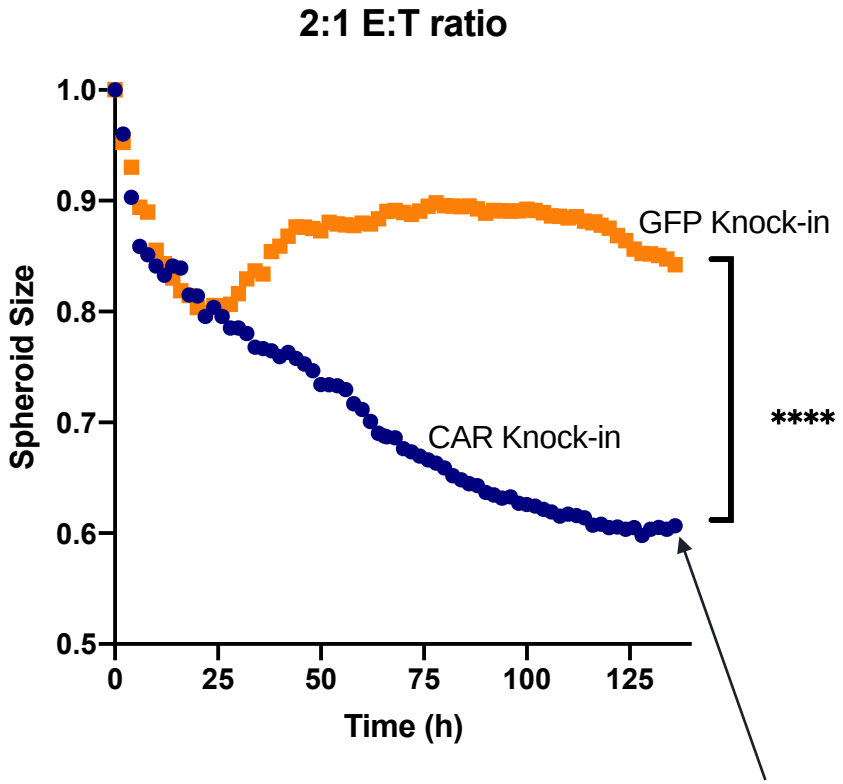
From - Morgan et al. Keystone Emerging Cellular Therapies: Cancer and Beyond/Engineering the Genome February 10 Banff, Alberta 2020

Efficient Transgene Knock-in with EngCas12a in Healthy Donor NK Cells Enables Testing of New Biology

50% knock-in with EngCas12a



Tumor-targeting CAR knock-in enhances killing

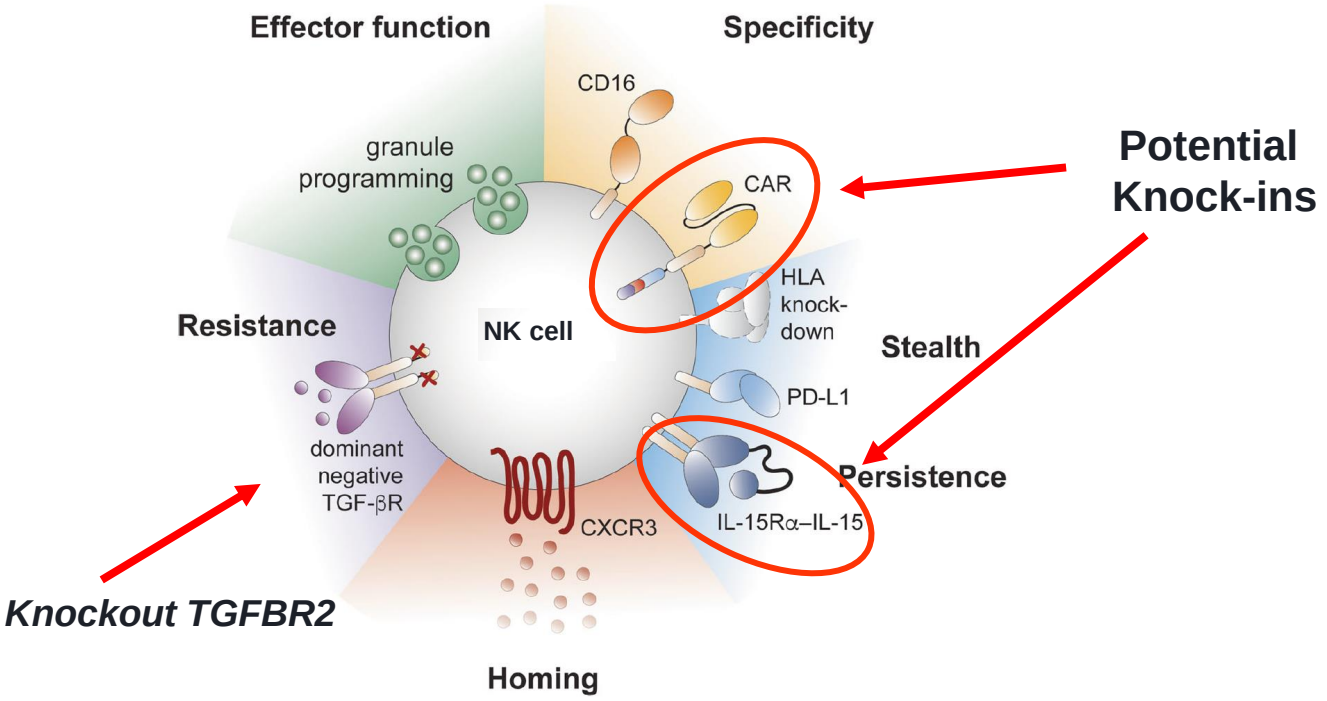


Enhanced tumor killing with CAR

EngCas12a Can Overcome the Editing Challenges in Creating Highly Engineered Cells for Targeting Solid Tumors

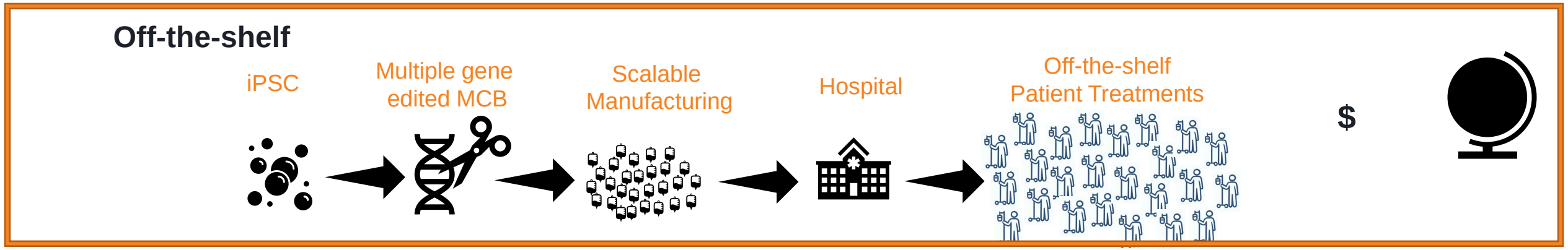
Summary of what we achieved with EngCas12a platform for potentially making medicines for oncology:

- EngCas12a retains intrinsic AsCas12a specificity advantage over SpCas9
- EngCas12a can robustly edit targets across cell types, including functional KO of TGFBR2 in NK cells
- EngCas12a can efficiently knock-in transgene cargos into T cells and NK cells



Modified from Saetersmoen et al. Seminars Immunopathology 2019

The Case for Developing an iPSC-Derived Medicine for Oncology



Our process:

iPSC → Edited clonal iPSC line → iNK cells

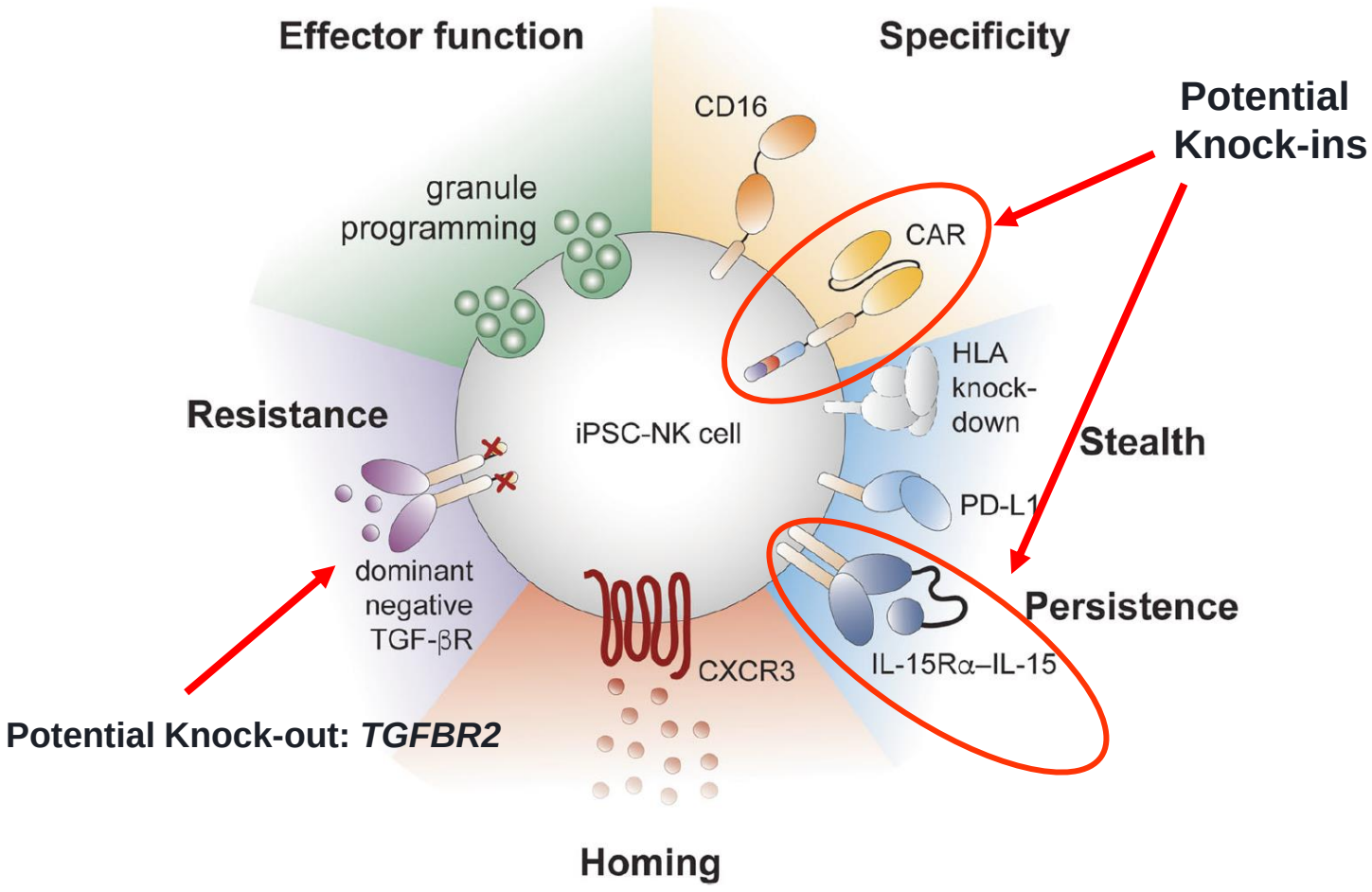
Editing + clonal identification

Differentiate

High editing efficiency with engineered Cas12a Genome characterization builds on *in vivo* editing capabilities	Infinitely renewable Defined genome	Developing a proprietary, scalable differentiation method	Highly engineered line Low COGs Off-the-shelf

iPSC platform allows for highly edited cells

Knock-in of Many Transgenes Poses a Major Manufacturing Challenge

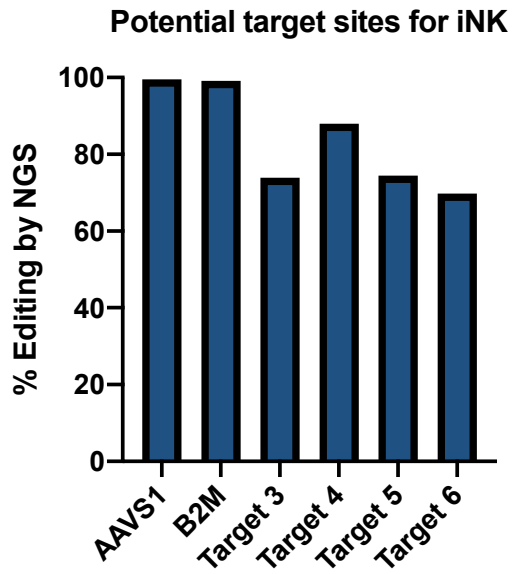


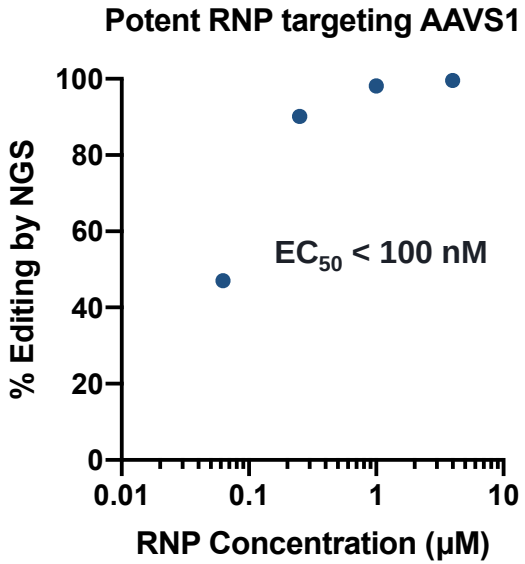
First key step in our iPSC editing platform:

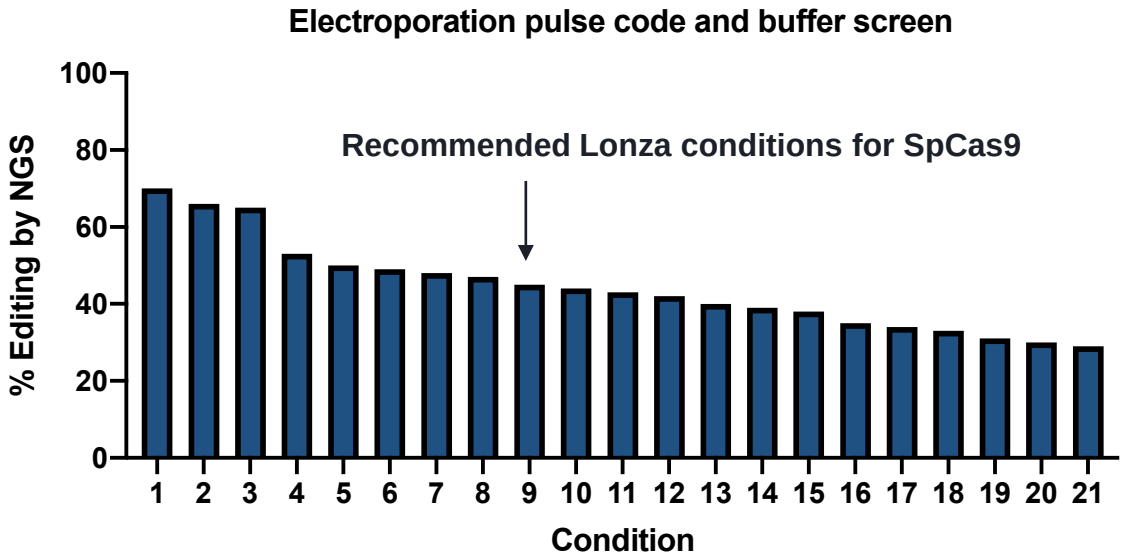
Demonstrate highly efficient dsDNA break occurrence (i.e. knock-out) in order to maximize opportunity knock-in efficiency

Saetersmoen et al. Seminars Immunopathology 2019

Highly Efficient Gene Editing in iPSCs Using EngCas12a







Additional advances to our iPSC editing platform:

- Made up to four edits at once and developed assays to assess which edit combinations show most promise
- Developed method to edit cells in a consistent manner so that editing and differentiation results are consistent
- Developed method to detect the possible infrequent occurrence of a translocation between two on-target edits

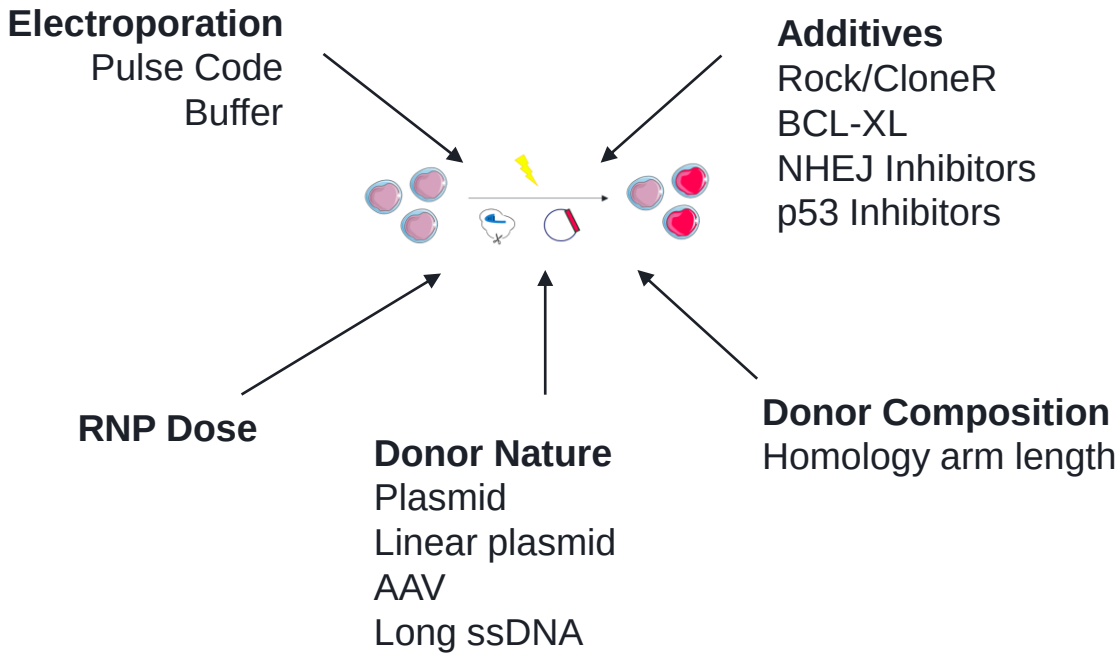
Transgene Knock-in in an iPSC Requires Additional Considerations

Factors to consider while making a highly-edited iPSC-derived NK cell

Attributes	Donor type		
	AAV	Plasmid	ssODN
Efficient transgene insertion	High	Low	Moderate
Toxicity when delivered to cells	Moderate	High	Moderate
Off-target integration events	Low	Moderate	Low
Delivery of large cargos	Moderate	**HIGH**	Low
Manufacturing challenge	High	**LOW**	High

Optimization is needed for plasmid-based transgene knock-in in iPSCs

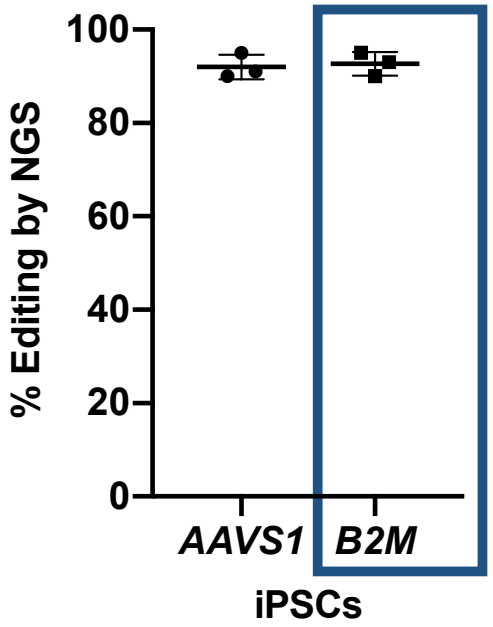
Several parameters that could be tested:



Transgene Knock-in Efficiency Using Plasmid Approaches 20% in iPSCs

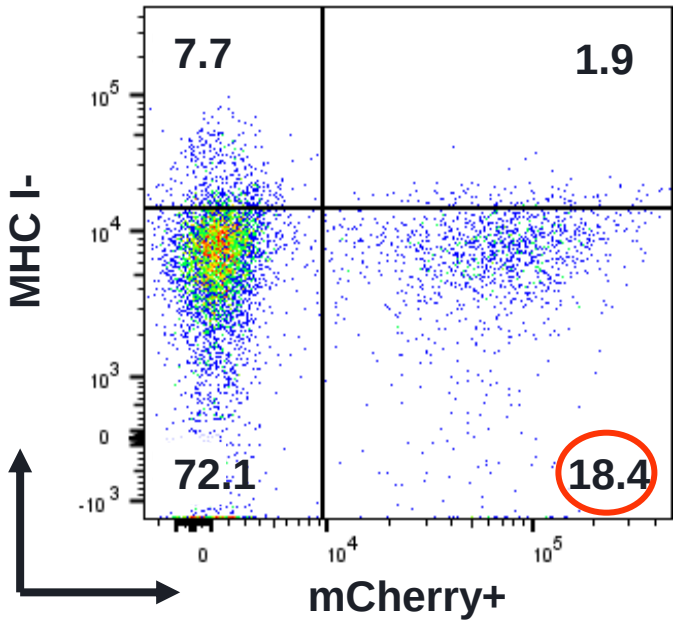
Efficient Editing at Potential Targets

Editing with EngCas12a

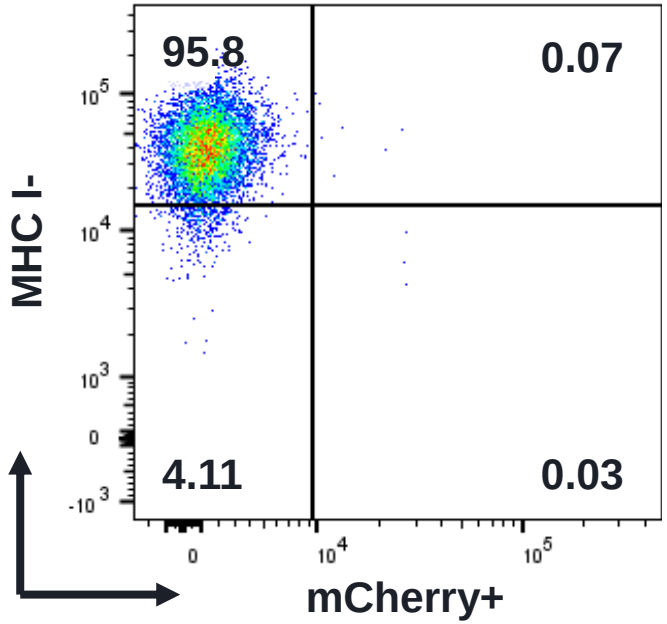


Efficient Knock-in of Transgene Cargo

RNP + mCherry plasmid



mCherry plasmid only



This knock-in efficiency should greatly reduce the number of iPSC clones that need to be analyzed

We Demonstrate that Edited iPSCs Can be Differentiated into Functional iPSC-Derived NK cells (iNKs) with Enhanced Tumor Killing Activity

Process of going from an edited iPSC to a differentiated iNK cell

iPSC → Edited clonal iPSC line → iNK cells

Editing + clonal identification

- High editing efficiency with engineered Cas12a
- Genome characterization builds on *in vivo* editing capabilities

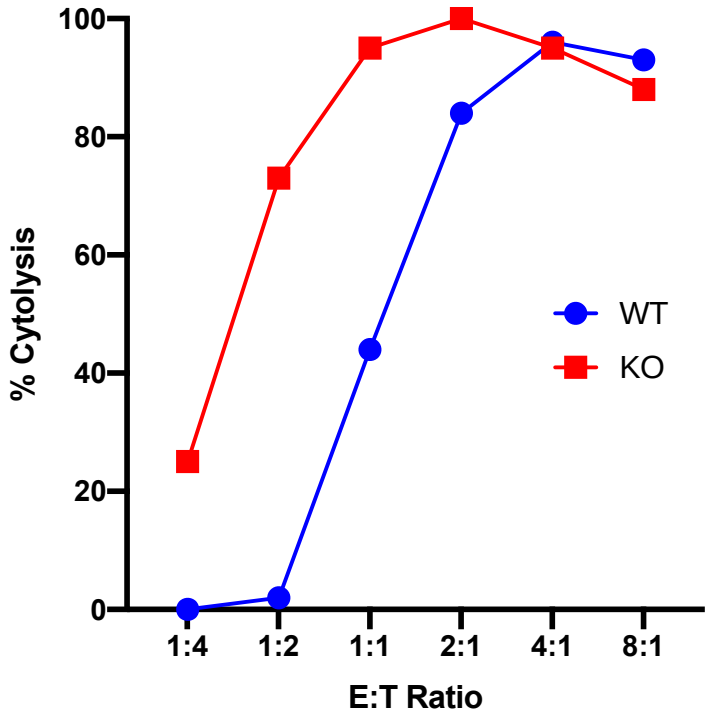
- Infinitely renewable
- Defined genome

Differentiate

- Developing a proprietary, scalable differentiation method

- Highly engineered line
- Low COGs
- Off-the-shelf

iNK killing of SKOV3 cells



Summary

ENGCAS12A AS A POWERFUL GENE EDITING PLATFORM	▪ EngCas12a is more potent than Wild Type Cas12a but retains its strongly superior specificity compared to SpCas9 ▪ EngCas12a is capable of highly efficient KO and transgene KI across cell types
WHY NK CELLS FOR CANCER?	▪ Allogeneic NK cells are an effective cancer cell therapy without evidence of Graft vs Host Disease ▪ Using EngCas12a we obtain robust editing to reduce TGF-β impact on NK cells
BUILDING A PLATFORM TO MAKE HIGHLY ENGINEERED IPSC-DERIVED NK CELLS	▪ iPSCs offer the potential to create Off-the-Shelf therapies at low costs ▪ Using the best nuclease possible can increase chances of finding clones with the desired edit and inserted construct ▪ Edited iPSCs can differentiate into iNKs with enhanced tumor killing ability

Acknowledgements

Editas Colleagues

iNK Program Team
Sickle Program Team
Healthy Donor NK Program Team
Discovery Biology Team
Lead Discovery Team
Biological Development Team
Boulder Chemistry Team

Partners

Allergan
Bristol-Myers Squibb
BlueRock Therapeutics
Sandhill Therapeutics
Integrated DNA Technologies
GenEdit