Ex vivo applications of Cas12a

Richard A Morgan

Keystone Symposia,

Engineering the Genome/Emerging Cellular Therapies

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Disclosures

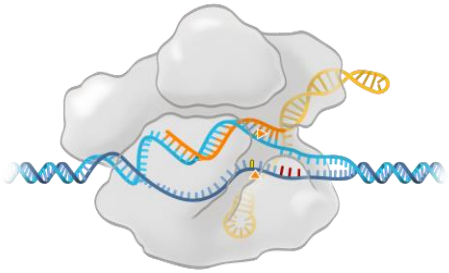
- Rick Morgan is a stockowner of and employee at Editas Medicine, Inc.

Cas12a: The Other CRISPR Nuclease

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

SpCas9

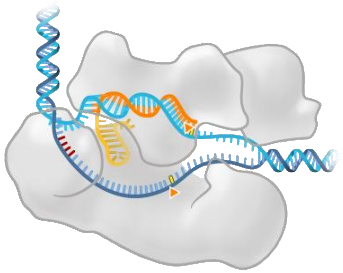


Targets G-rich PAMs

Separate crRNA and trRNA that can be linked (~100 nt)

Predominantly blunt DNA cut or 1 nt overhang

AsCas12a



Targets T- and C-rich PAMs

Naturally occurring ~40 nt single guide RNA

5’ staggered DNA cut with 4 nt overhangs

Cas12a is Highly Specific Primarily Due to Intrinsic DNA Target Engagement Mechanism that is Distinct from SpCas9

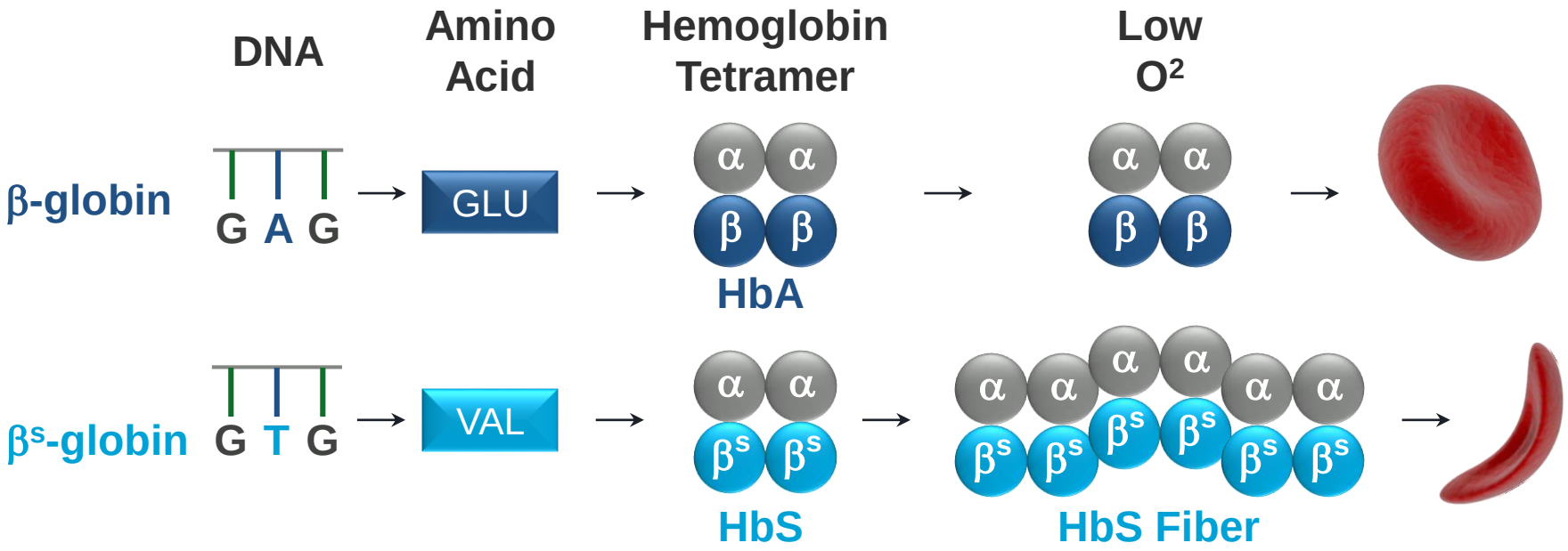
Zuris et al. Molecular Therapy Vol 27 No 4S1 April 2019

Strohkendl et al. Mol Cell 2018, vol 71, 816-824
Swarts et al. Biochem Soc Trans 2019, vol 47, 1499-1510

Cas12a Applications for Engineered Cell Medicines:

Sickle Cell Disease
NK Cell Therapy for Cancer

Etiology of Sickle Cell Disease

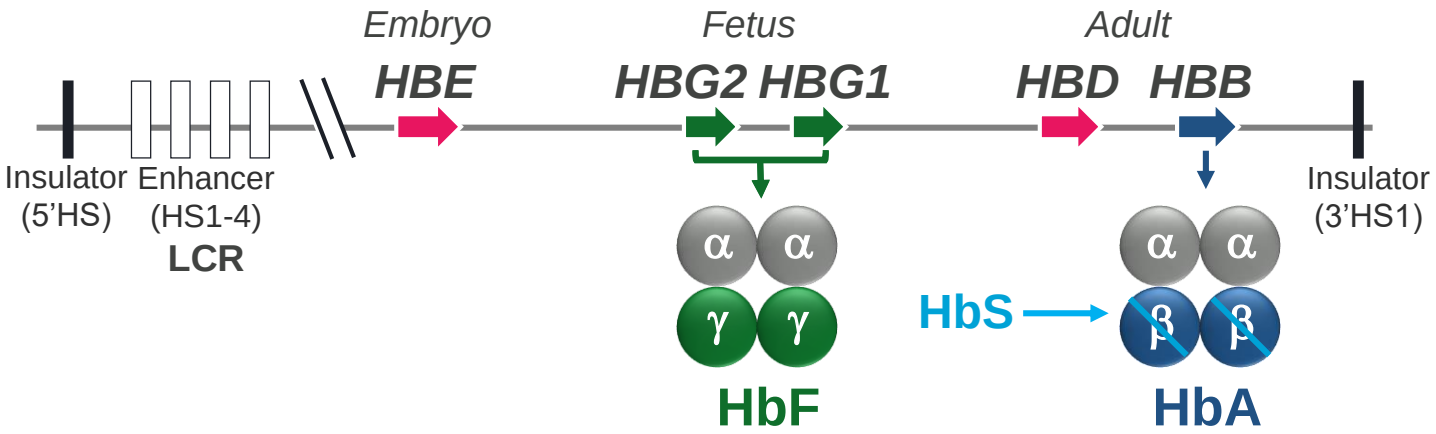


- Sickle cell disease (SCD) is caused by a single mutation E6V of the β-globin chain, leading to polymerization of hemoglobin (Hb) and formation of sickle hemoglobin (HbS) fibers when deoxygenated.
- Symptoms include anemia, acute chest syndrome, pain crises, and an array of other complications.
- Patients suffer significant morbidity and early mortality.

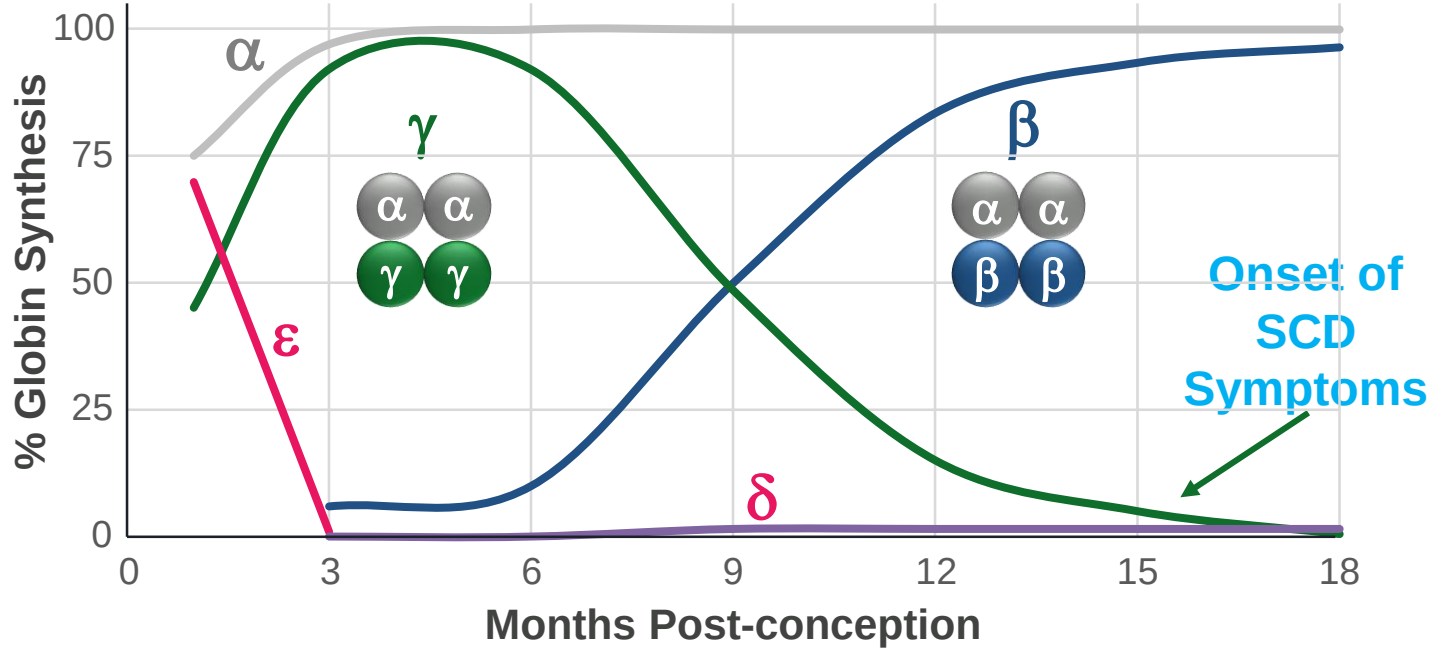
Bender MA. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, Amemiya A, editors. Gene Reviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2018.

Harnessing Natural Anti-sickling Hemoglobin to Treat Sickle Cell Disease

β-globin locus

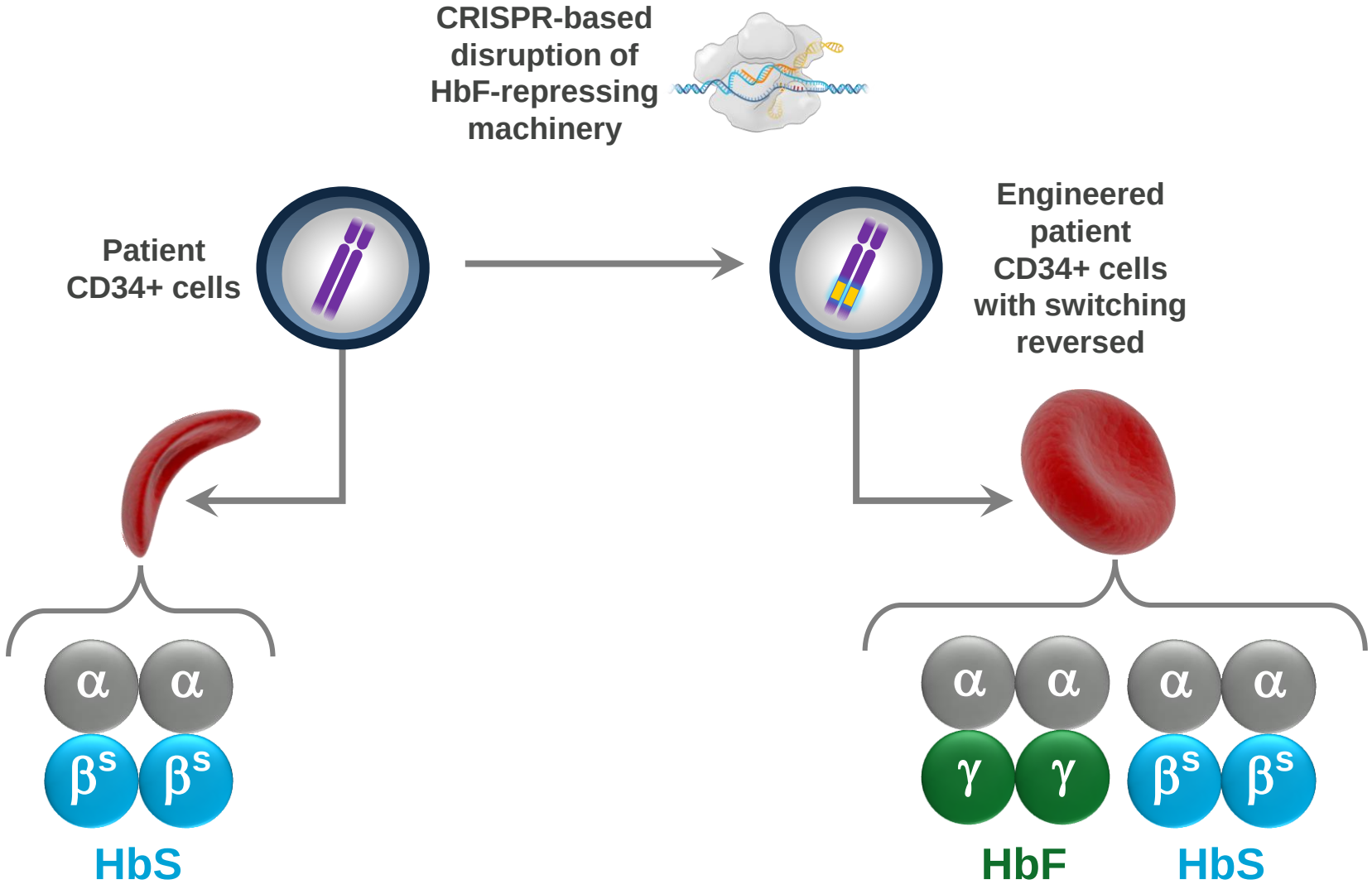


Globin switch



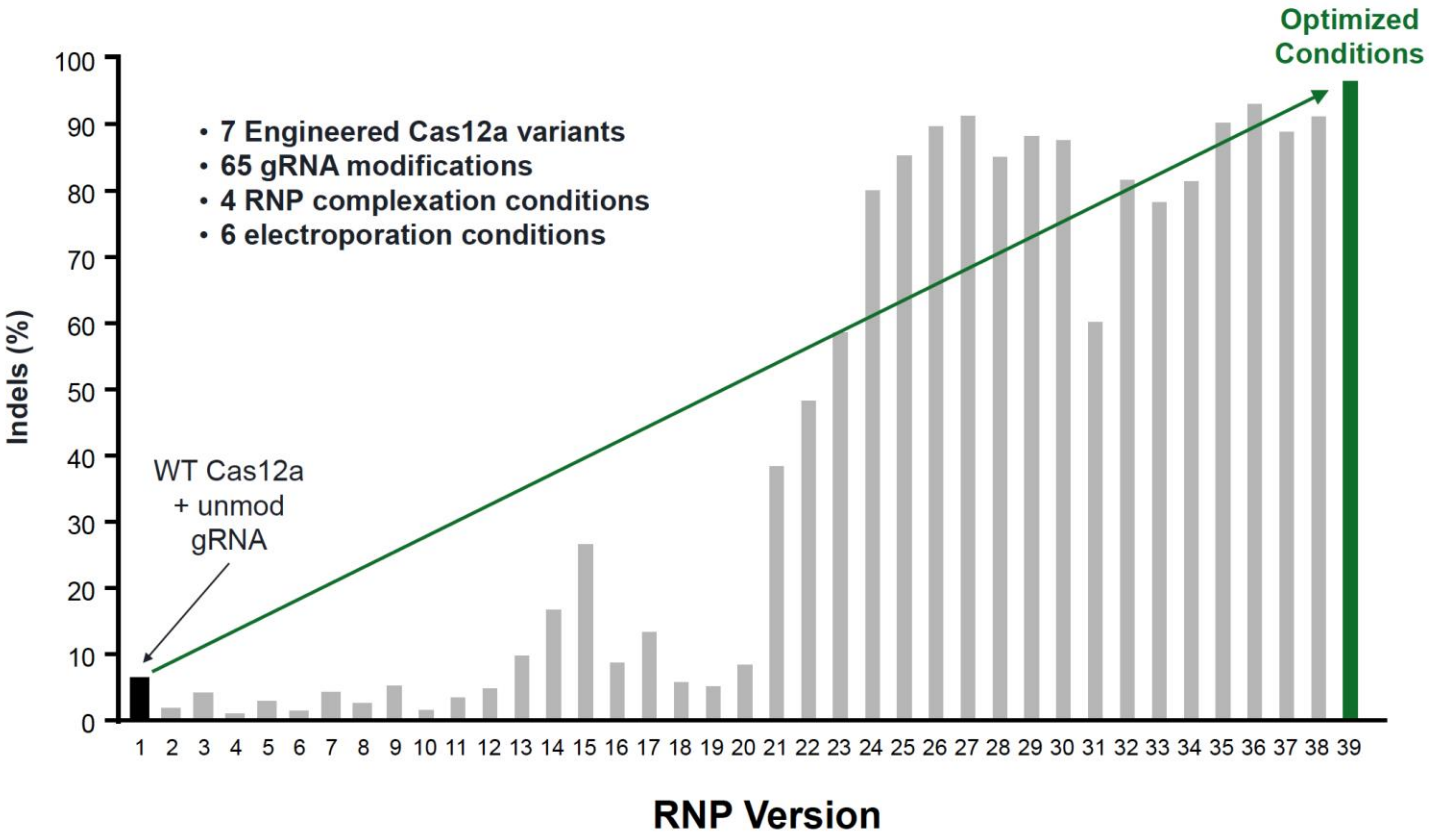
Adapted from Canver and Orkin, Blood, 2016

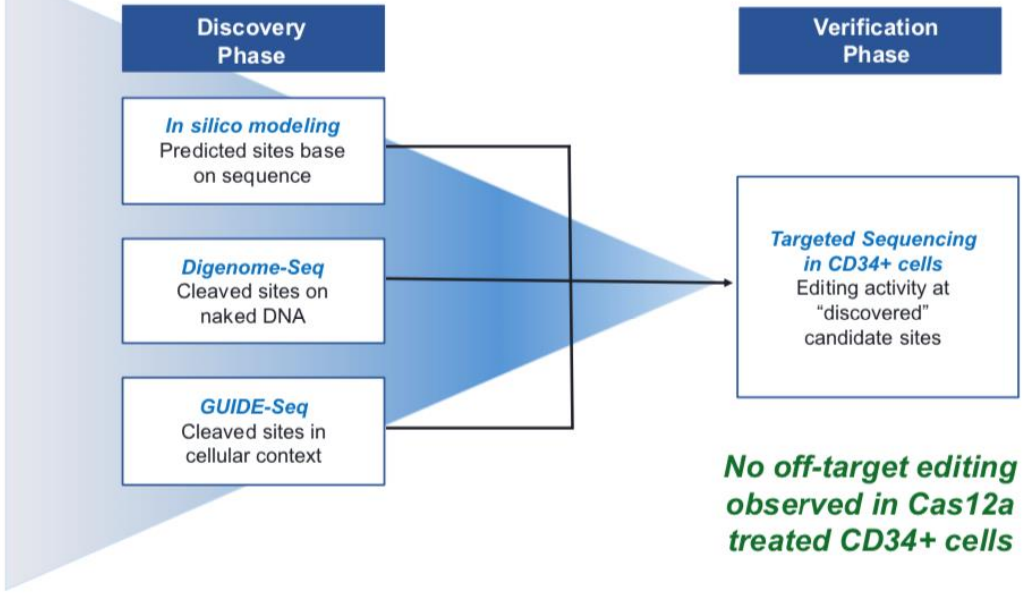
Genome Editing to Reverse Hemoglobin Switching for Treating Sickle Cell Disease



From - Chang et al. Annual Meeting of the American Society of Hematology, San Diego, CA., December 2, 2018

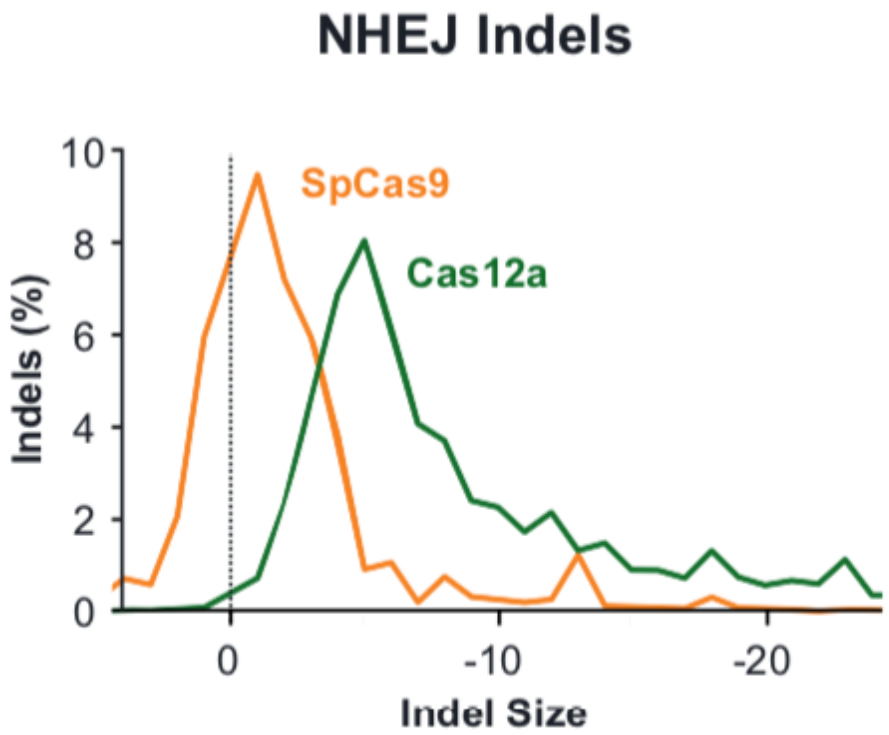
Optimized CRISPR Cas12a-based Editing at the HBG Distal CCAAT box Region – EDIT-301

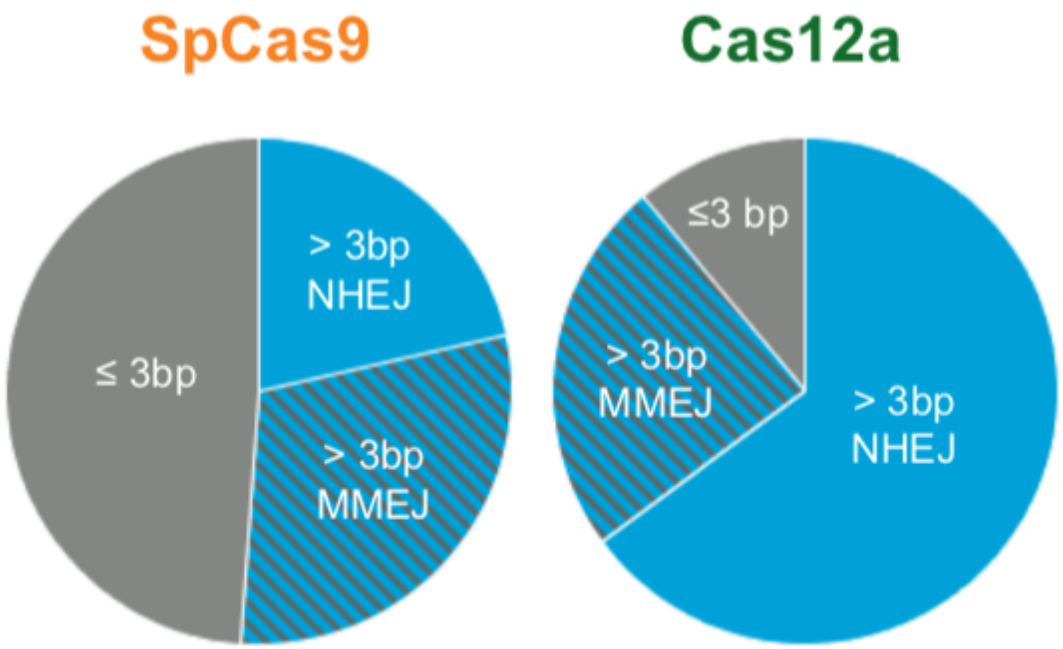




From – De Dreuzy et al. Annual Meeting of the American Society of Hematology, Orlando. FL, December 9, 2019

Cas12a Showed Improved Editing in HSCs with Desired Indel Size and Specificity

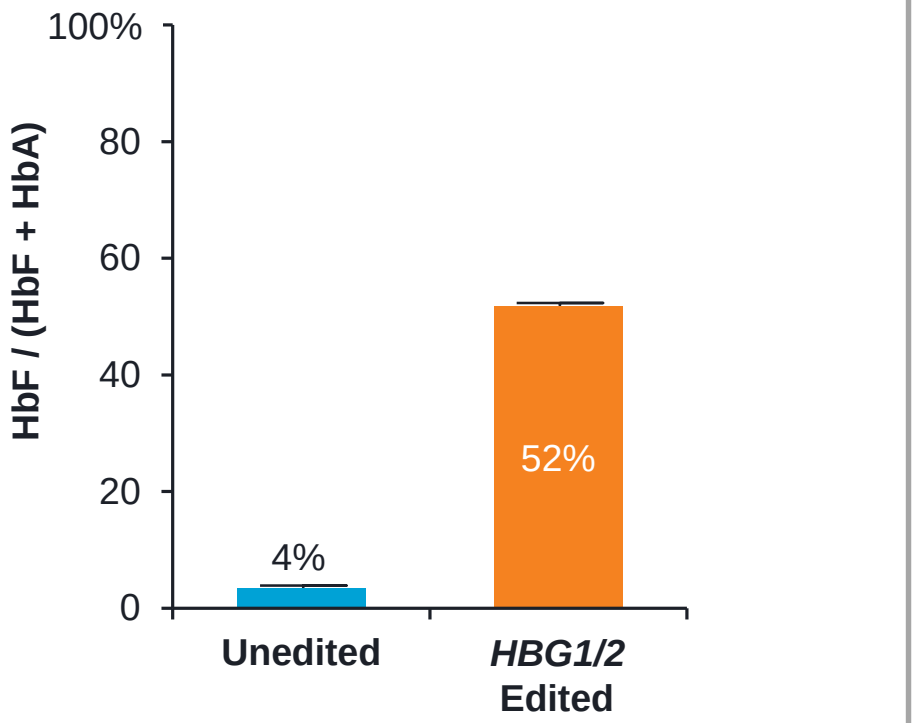




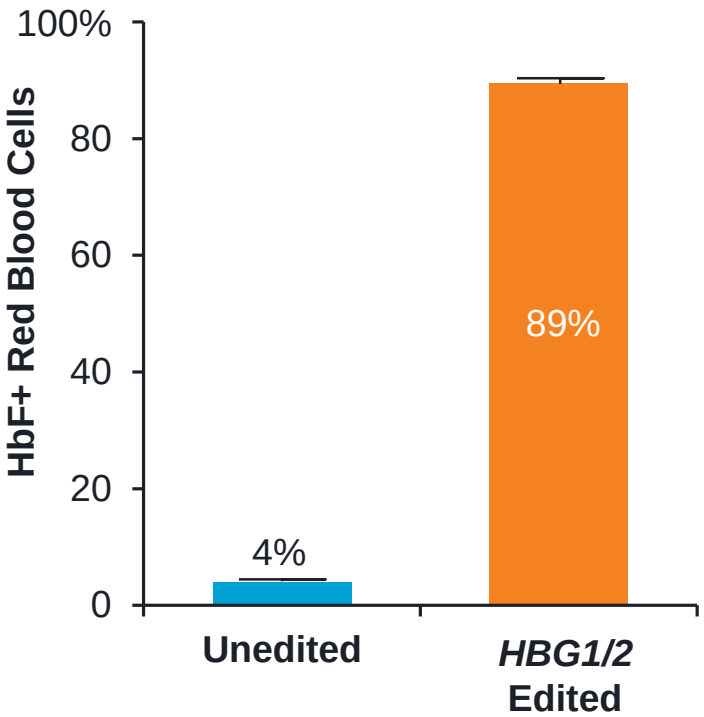
From – De Dreuzy et al. Annual Meeting of the American Society of Hematology, Orlando. FL, December 9, 2019

Editing *HBG1/2* Induces Robust Fetal Hemoglobin *In Vivo*

Robust HbF in human CD34+ cells *in vivo*



High pan-cellular human HbF in red blood cells



n = 5 healthy human donors in NBSGW mice at 16 weeks

HbF: fetal hemoglobin; HbA: adult hemoglobin

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

Cas12a Editing for SCD

Cas12a produced more long term HbF inducing indels than SpCas9 at the HBG distal CCAAT box region.

Greater than 90% indels were achieved after optimization of electroporation conditions and selection of best performing Cas12a variant and gRNA modifications

EDIT-301 had no detectable off target editing and contained highly edited long term HSCs (> 90 % indels) that engrafted mice with high polyclonality and no lineage skewing.

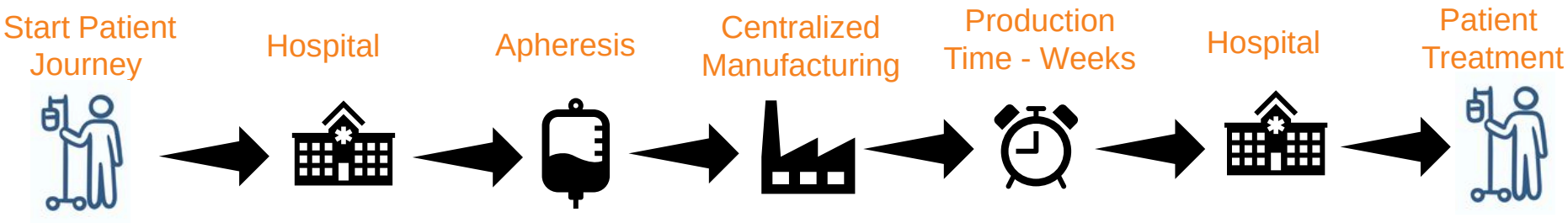
50% HbF levels were observed in vivo with pancellular distribution.

EDIT-301 program, heading toward IND in 2020

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

Oncology Cell Therapy Development

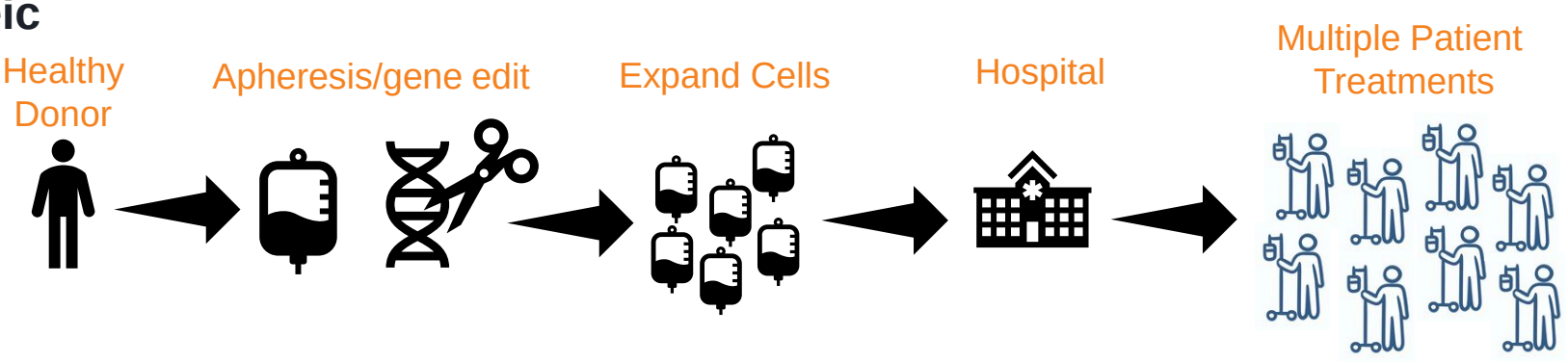
Autologous



COGs $$$

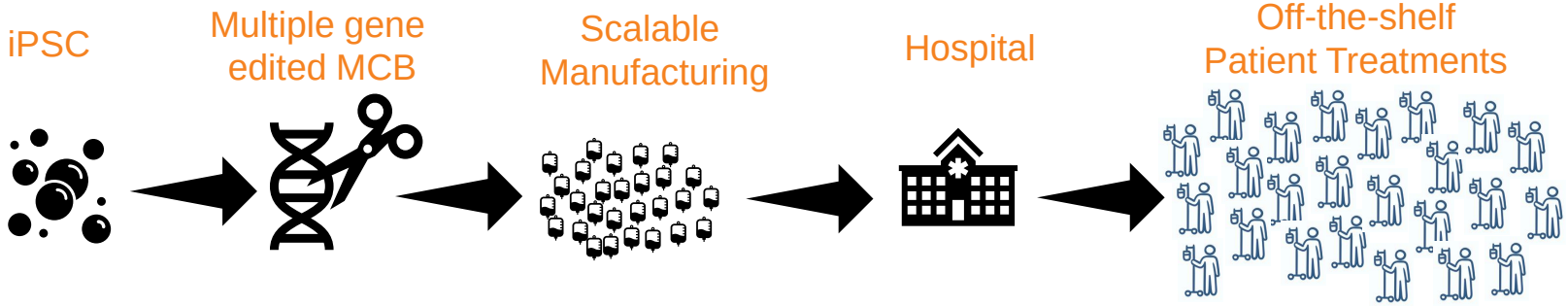
Scope

Allogeneic



$$

Off-the-shelf



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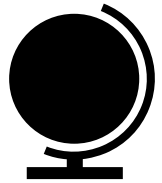
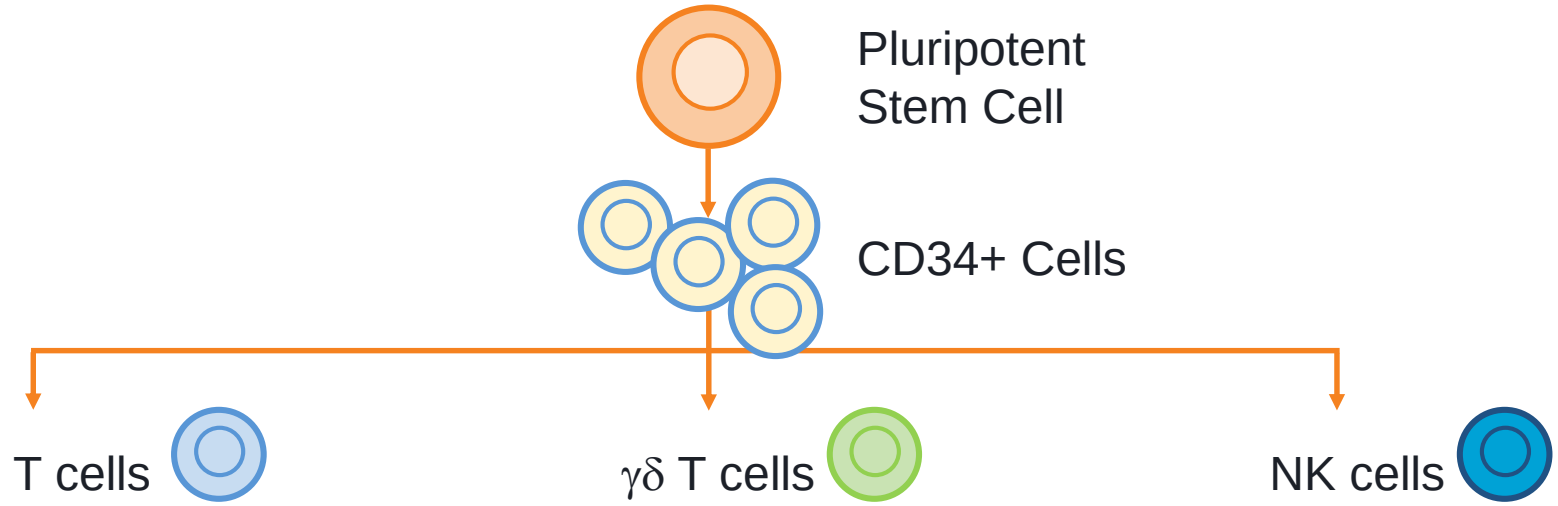


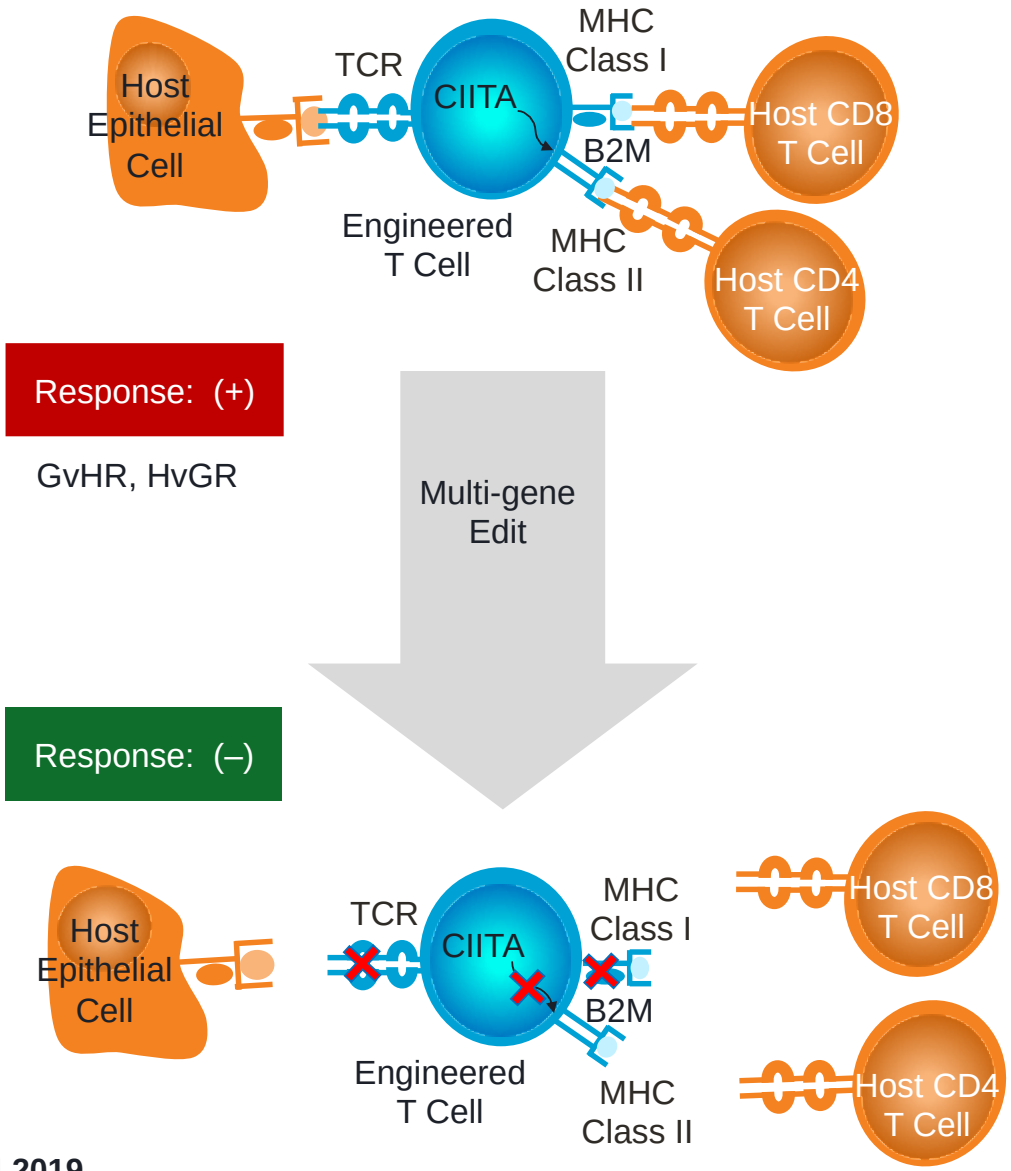
Potential Cell Types for Allogeneic Cell Medicines



	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	

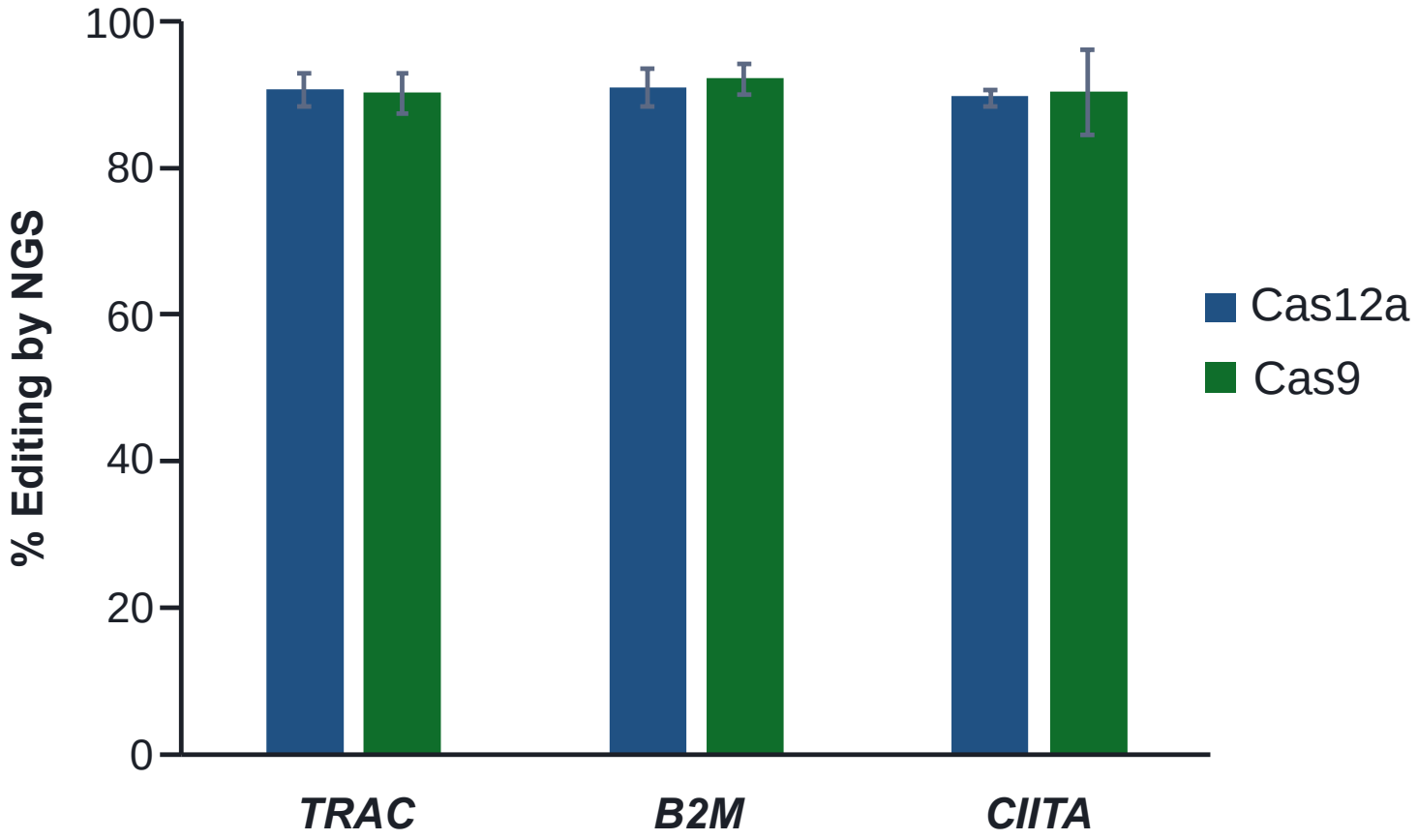
CAR: chimeric antigen receptor

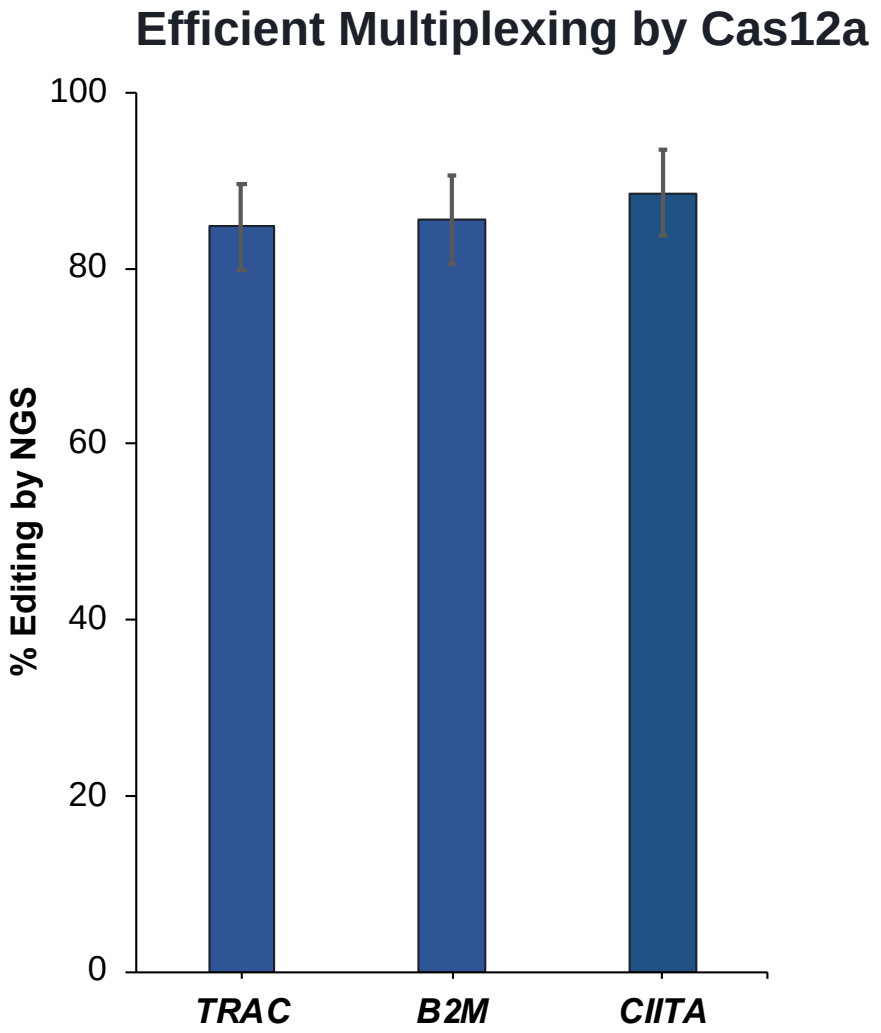
Multiple Gene Edits Required for the Generation of an "Off-the-shelf" Allogeneic T Cell Products



Zuris et al. Molecular Therapy Vol 27 No 4S1 April 2019

Making an Allo T Cell: Cas12a Comparable to SpCas9





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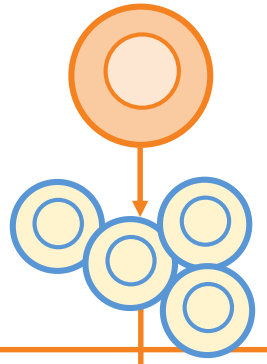
Potential Cell Types for Allogeneic Cell Medicines



Pluripotent Stem Cell

CD34+ Cells

αβ T cells

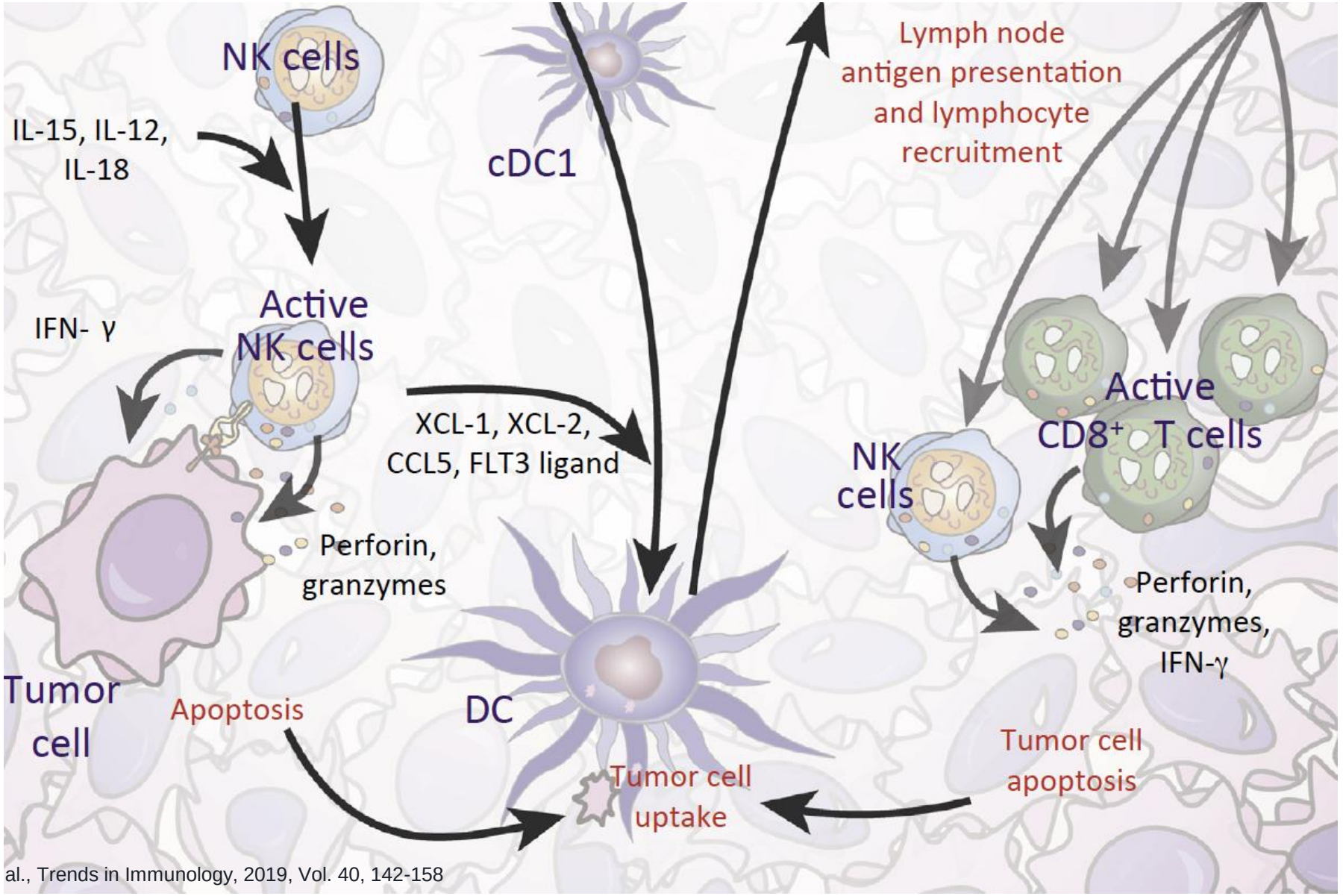
γδ T cells

NK cells

	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	

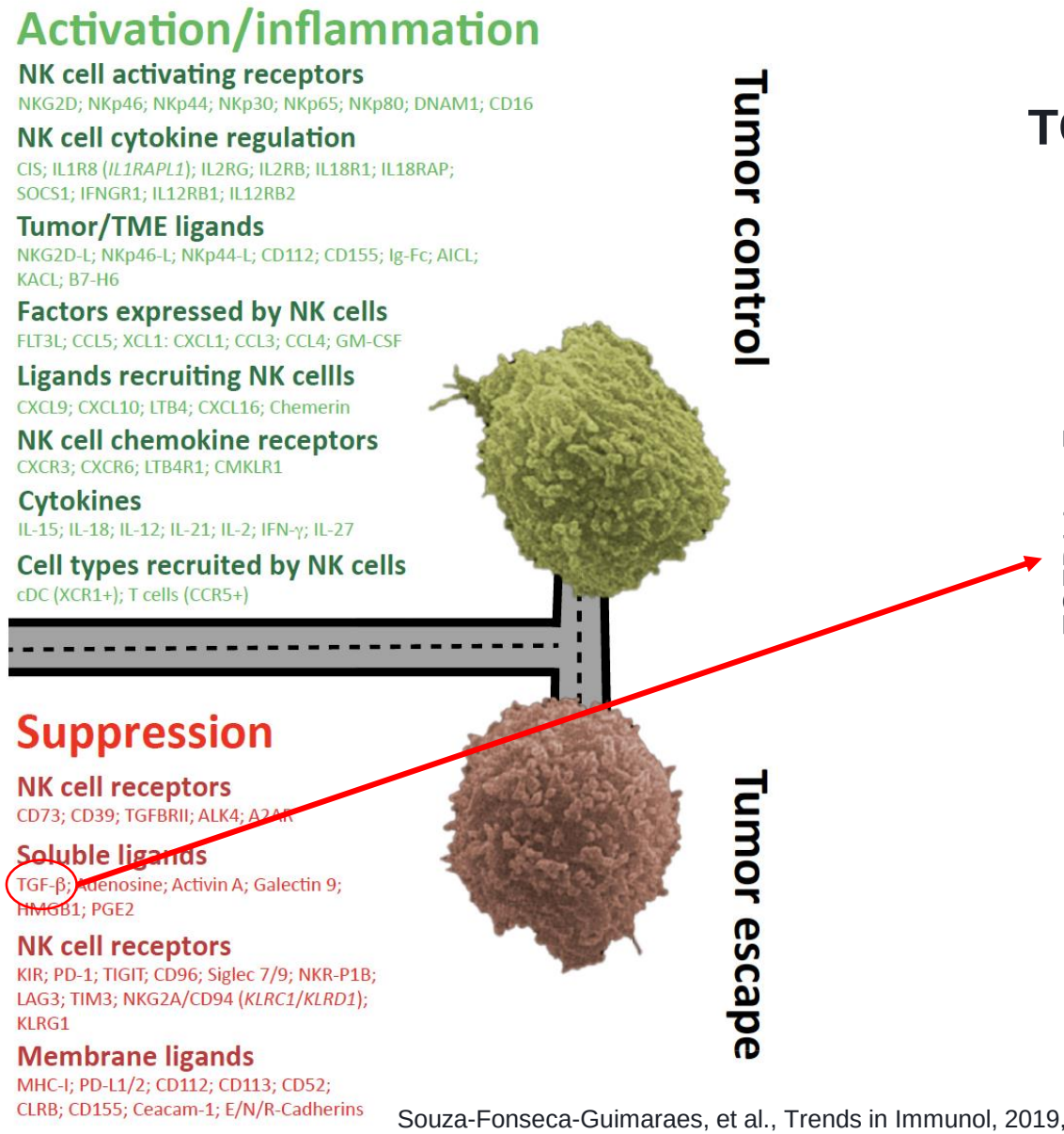
CAR: chimeric antigen receptor

NK Cells Can Both Directly Kill Tumors and Stimulate the Endogenous Immune System

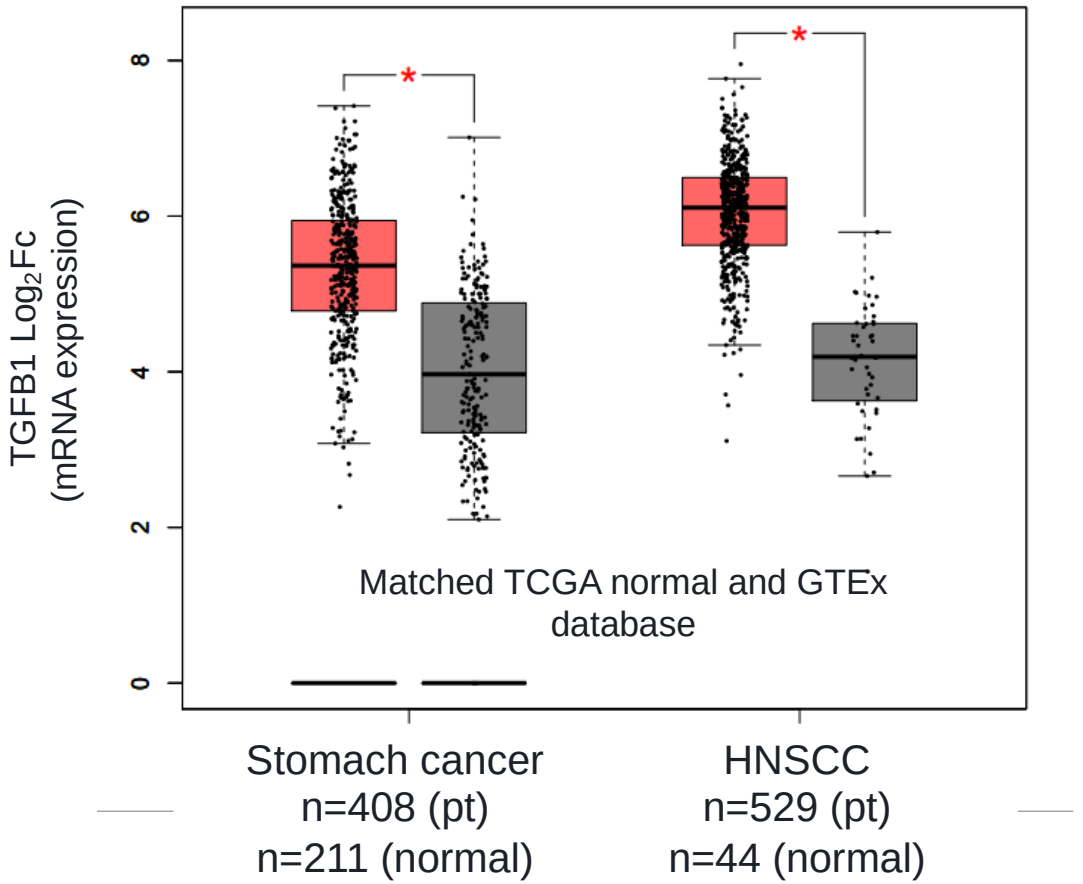


Souza-Fonseca-Guimaraes, et al., Trends in Immunology, 2019, Vol. 40, 142-158

TGF-β is a Major NK Cell Suppressive Cytokine



TGF-β is Overexpressed in Many Solid Tumors

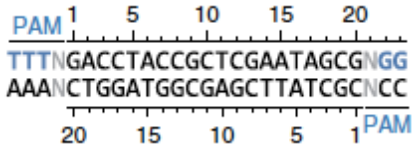


Souza-Fonseca-Guimaraes, et al., Trends in Immunol, 2019, Vol. 40, 142-158

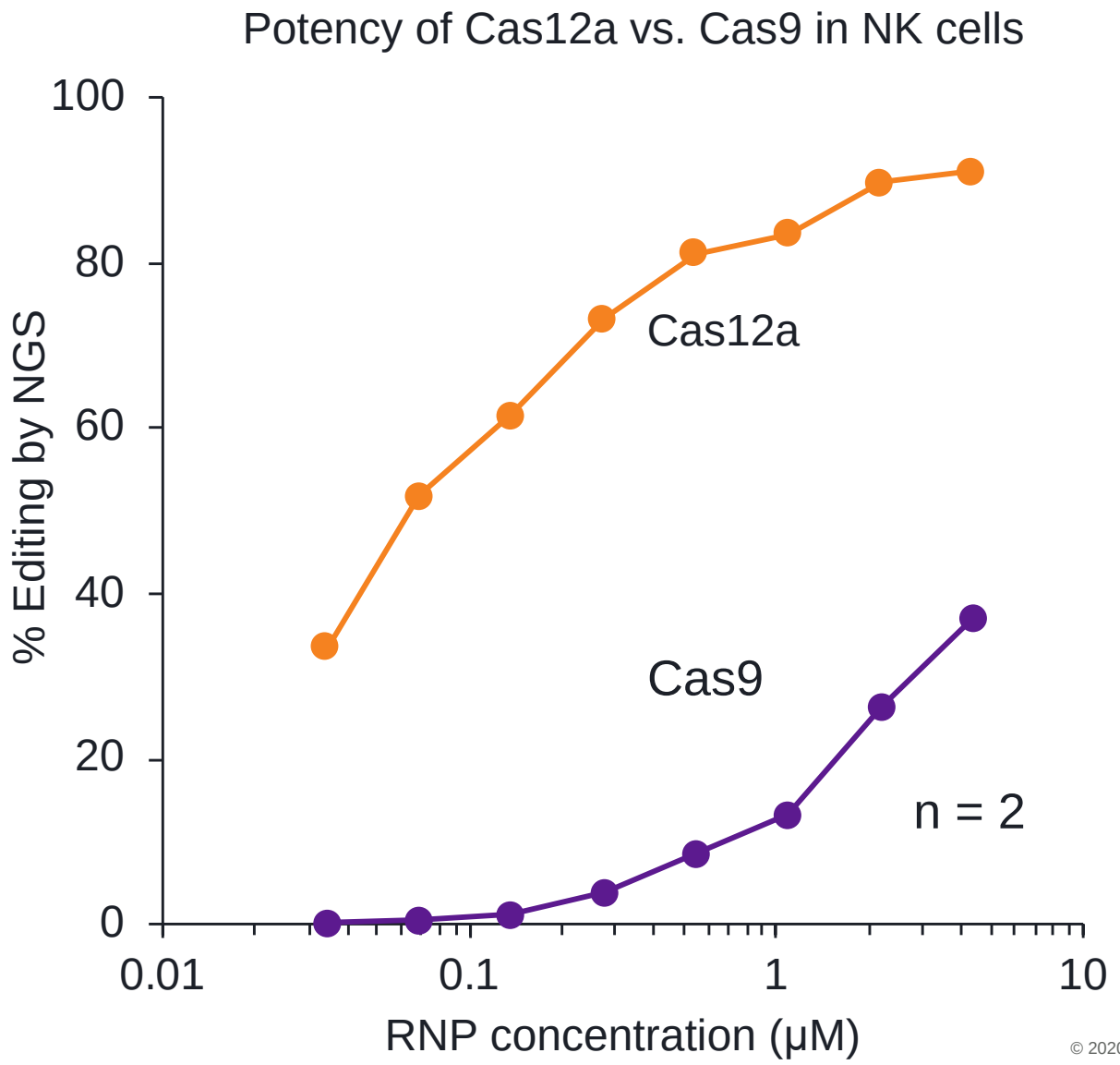
Potency Comparison of Engineered Cas12a and SpCas9 in NK Cells at the Same Target Site in the Genome

Control target site for comparing SpCas9 and AsCas12a

MS5



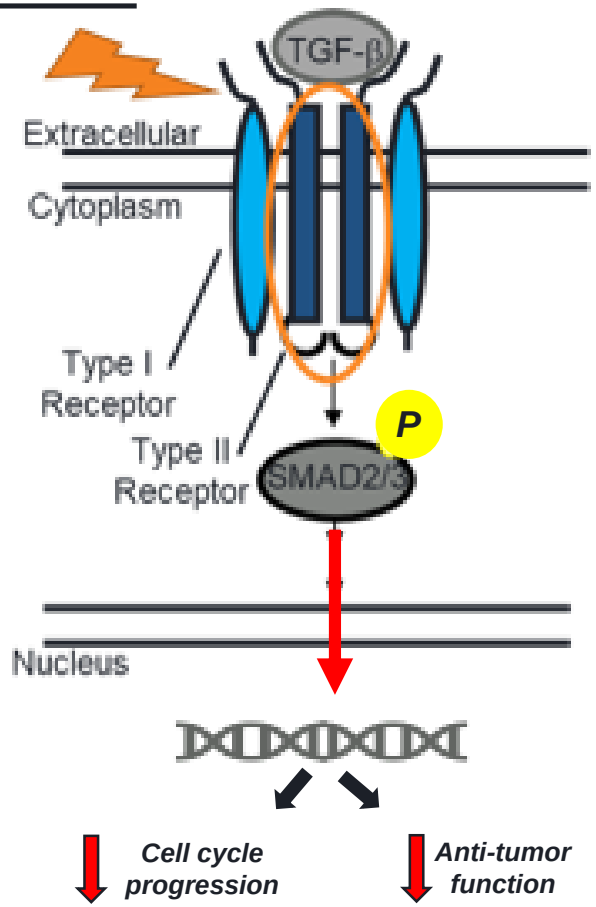
MS5 chosen from Kleinstiver et al. Nat Biotech 2016 vol 34, 869-874



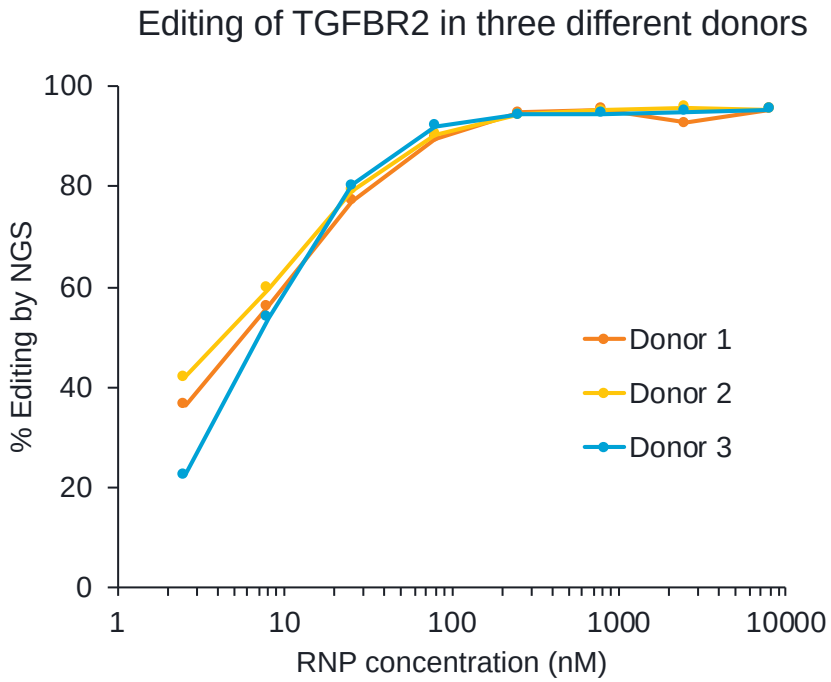
Robust Gene Editing at TGFBR2 in Healthy Donor NK Cells with an Engineered Cas12a

Rationale

NK cell

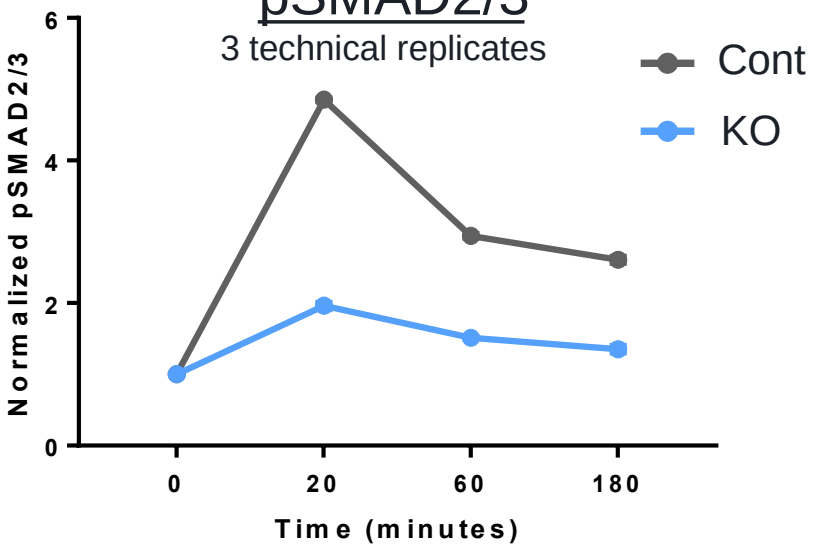


Editing



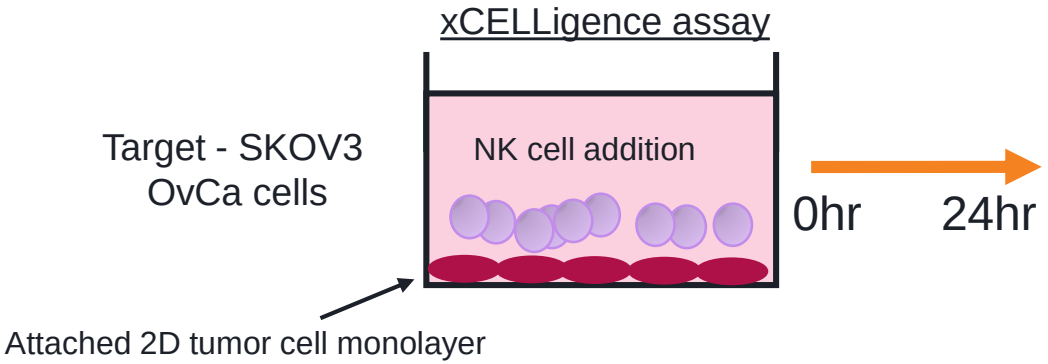
Biology

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



Stimulation with 10ng/mL TGF-β

TGFBR2 Knockout (KO) NK Cells Showed Superior Effector Function than Unedited Control NK Cells

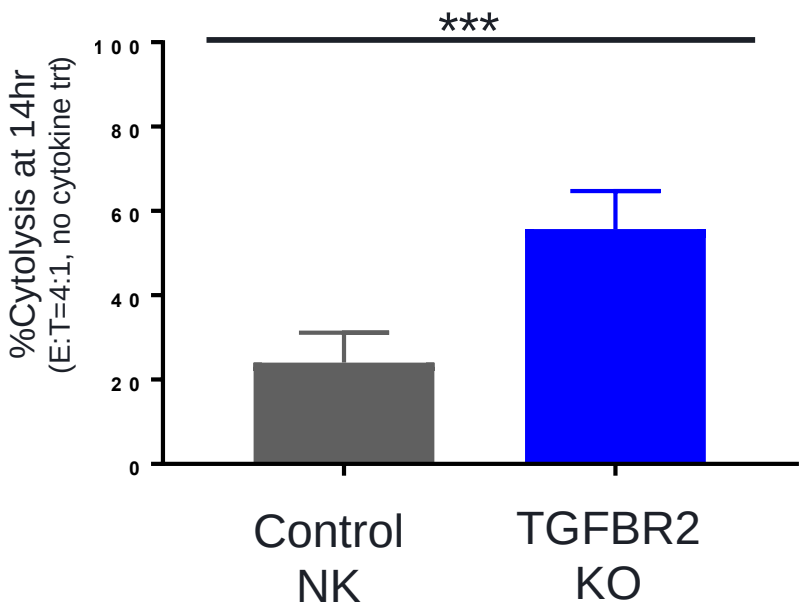


Assay readouts

- % Cytolysis by xCELLigence
- IFNγ production by FACS

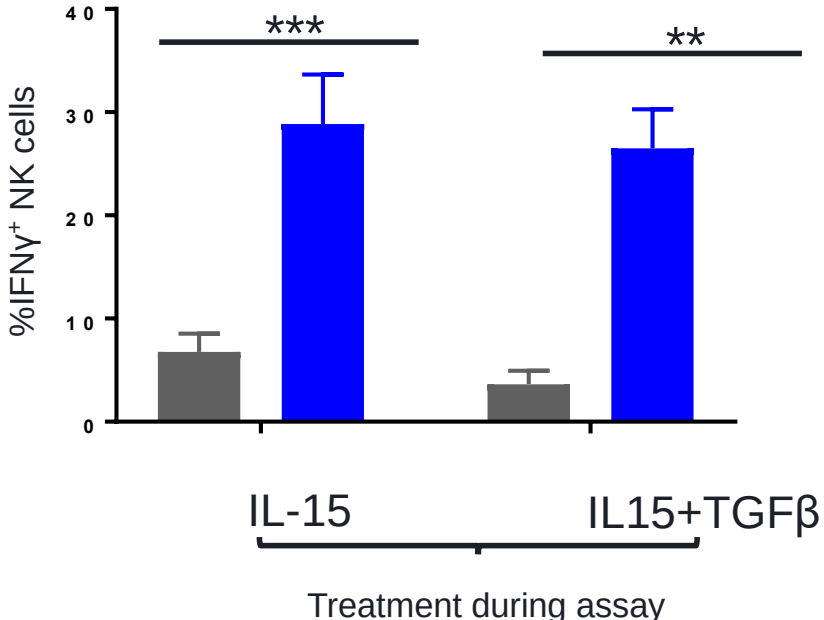
% Cytolysis

Average of 3 donors, 5 independent experiments

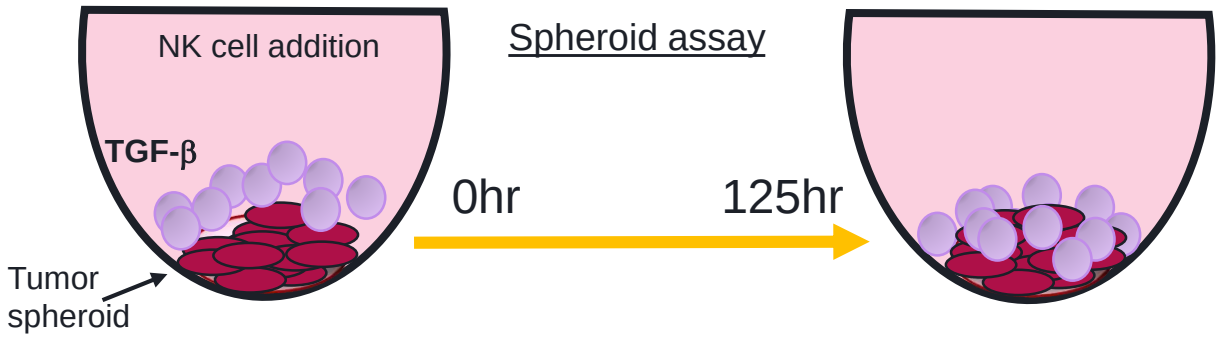


%IFNγ+NK cells at 24hr

Average of 3 donors



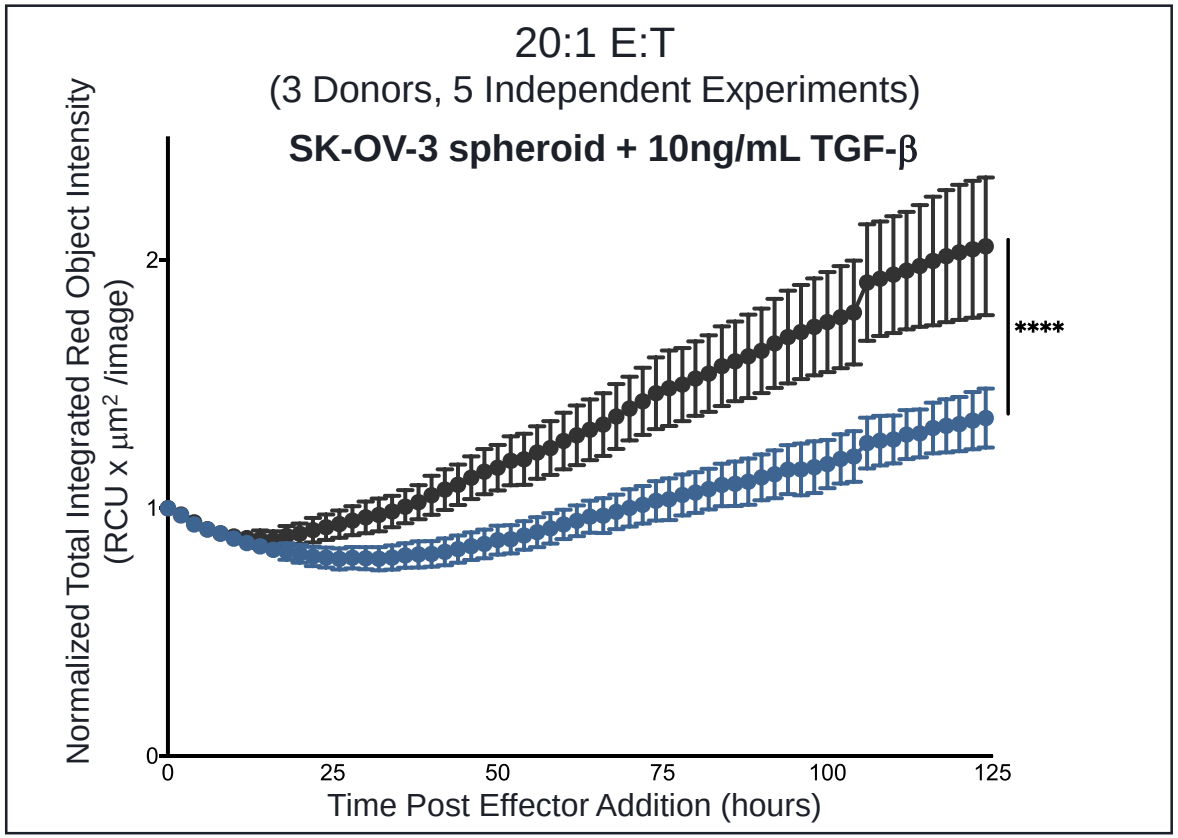
TGFBR2 KO Exerted Superior Control of Tumor Spheroids for >125hrs in the Presence of TGF-β

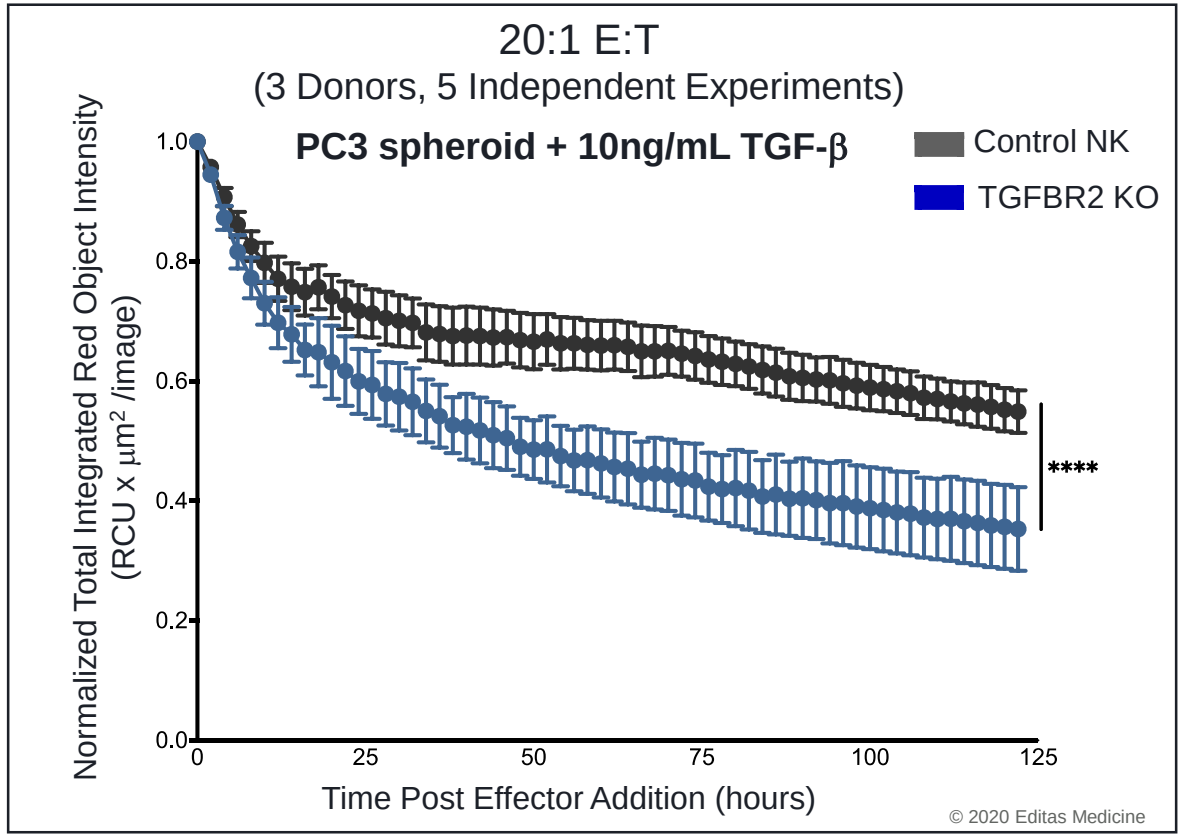


Assay readouts

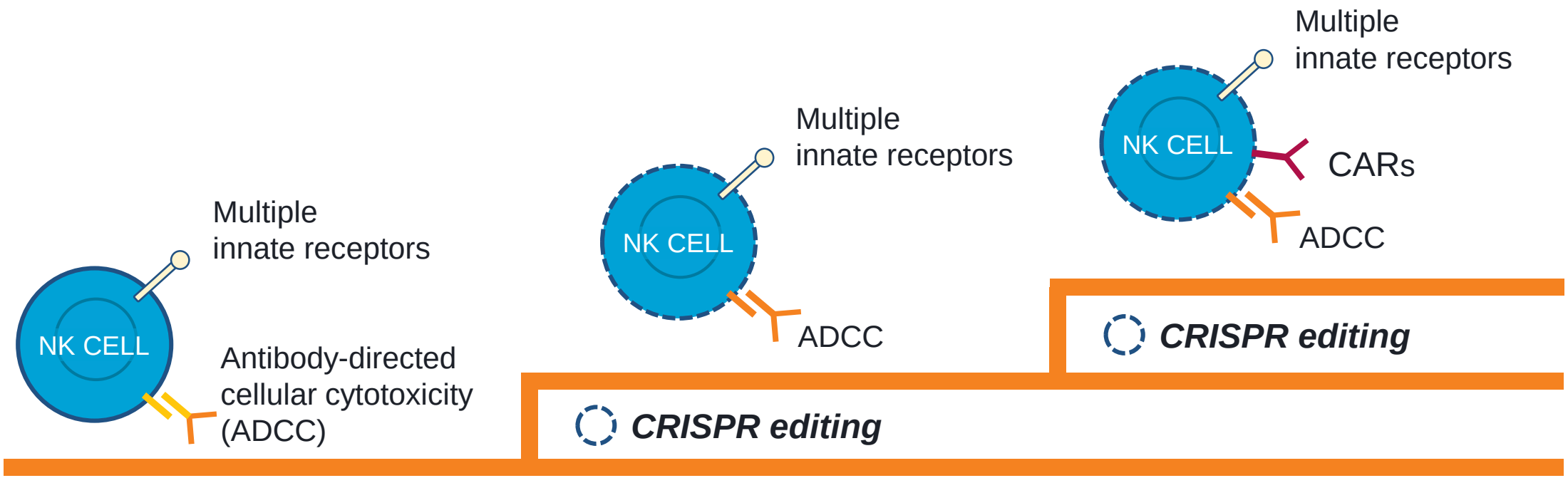
- Changes in spheroid size over time (killing by NK cells)

(measurement of red object intensity)





NK Therapeutic Strategy for Winning in Solid Tumors



Improved ADCC, persistence, and tumor micro-environment (TME) resistance

Improved ADCC, persistence, and additional TME resistance

Improved recognition of tumor cells lacking T cell antigens for PD-1 nonresponding tumors with innate receptors and CARs

Cas12a Editing for NK Cell-based Therapies

The next generation of allo and off-the-shelf cell therapies for cancer will require robust and specific gene editing.

Cas12a produces efficient and specific gene editing, comparable or superior to SpCas9 in both primary T cells and NK cells.

Greater than 90% editing obtained targeting the TGFBR2 gene in primary human NK Cells.

TGFBR2 editing NK cells demonstrated superior effector cell function in both short and long-term cell killing assay.

Gene edited healthy donor NK program progressing to IND-enabling studies

Acknowledgements

Sickle Cell Disease Team

KaiHsin Chang, Edouard de Dreuzy, Jack Heath

Healthy Donor NK Cell Team

Karrie Wong, Chris Borges, John Zuris

Editas

Discovery, Development, Chemistry, and Operations

Partners

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