NATIONAL INSTITUTES OF HEALTH

Novel and **Ex**ceptional **T**echnology and **R**esearch **A**dvisory **C**ommittee (NExTRAC)

Genome Editing

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Potential Conflicts of Interest

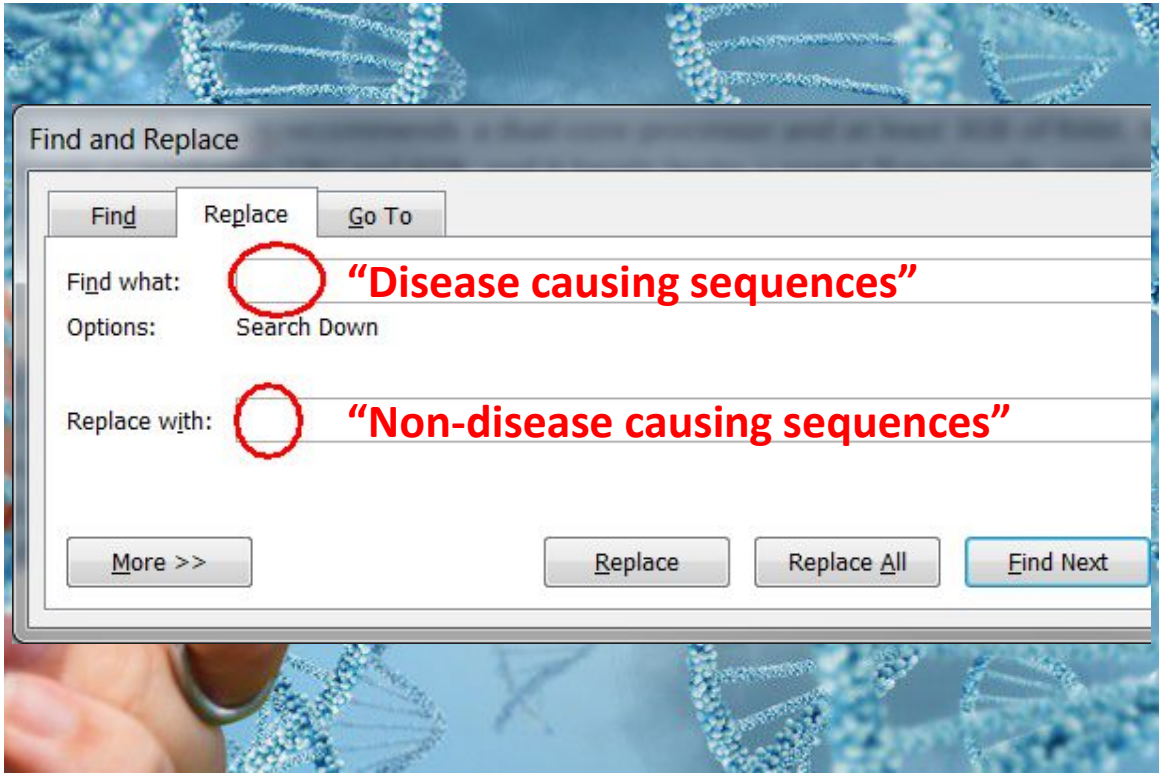
CRISPR Therapeutics: Equity and SAB
Allogene Therapeutics: Equity and SAB

Managed through Stanford in accordance with their conflict of interests policy.

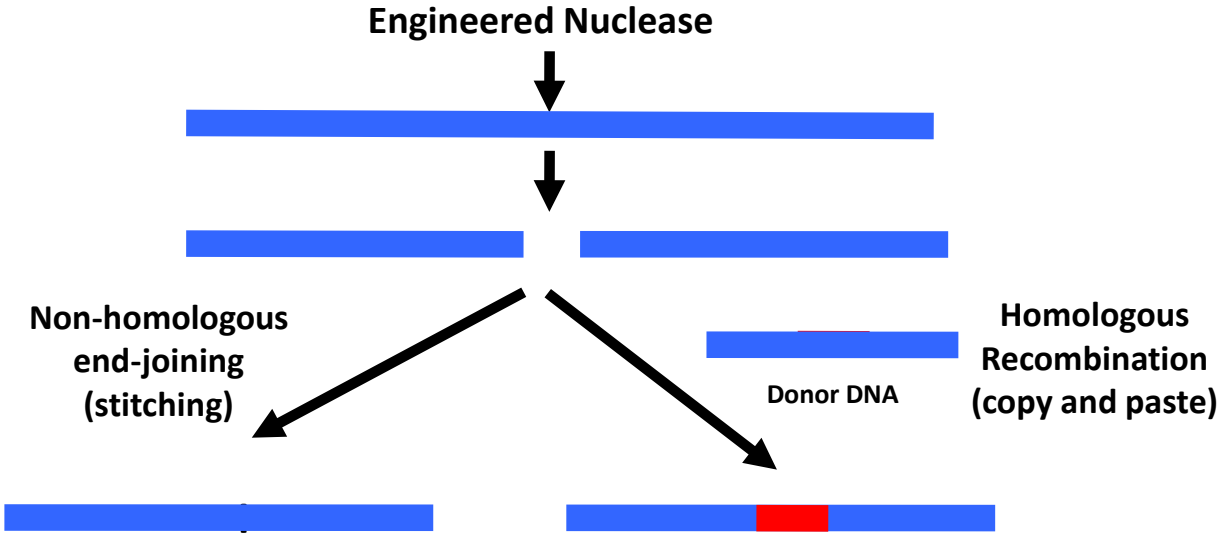
Outline of Talk

1. What is genome genome editing?
2. How do you do genome editing?
3. Why are people excited about it?
4. What are some issues that we need to think about?

Genome Editing is changing the DNA sequence of a cell with up to single nucleotide precision



Nuclease Based Strategies are the Most Well-Developed Methods of Genome Editing



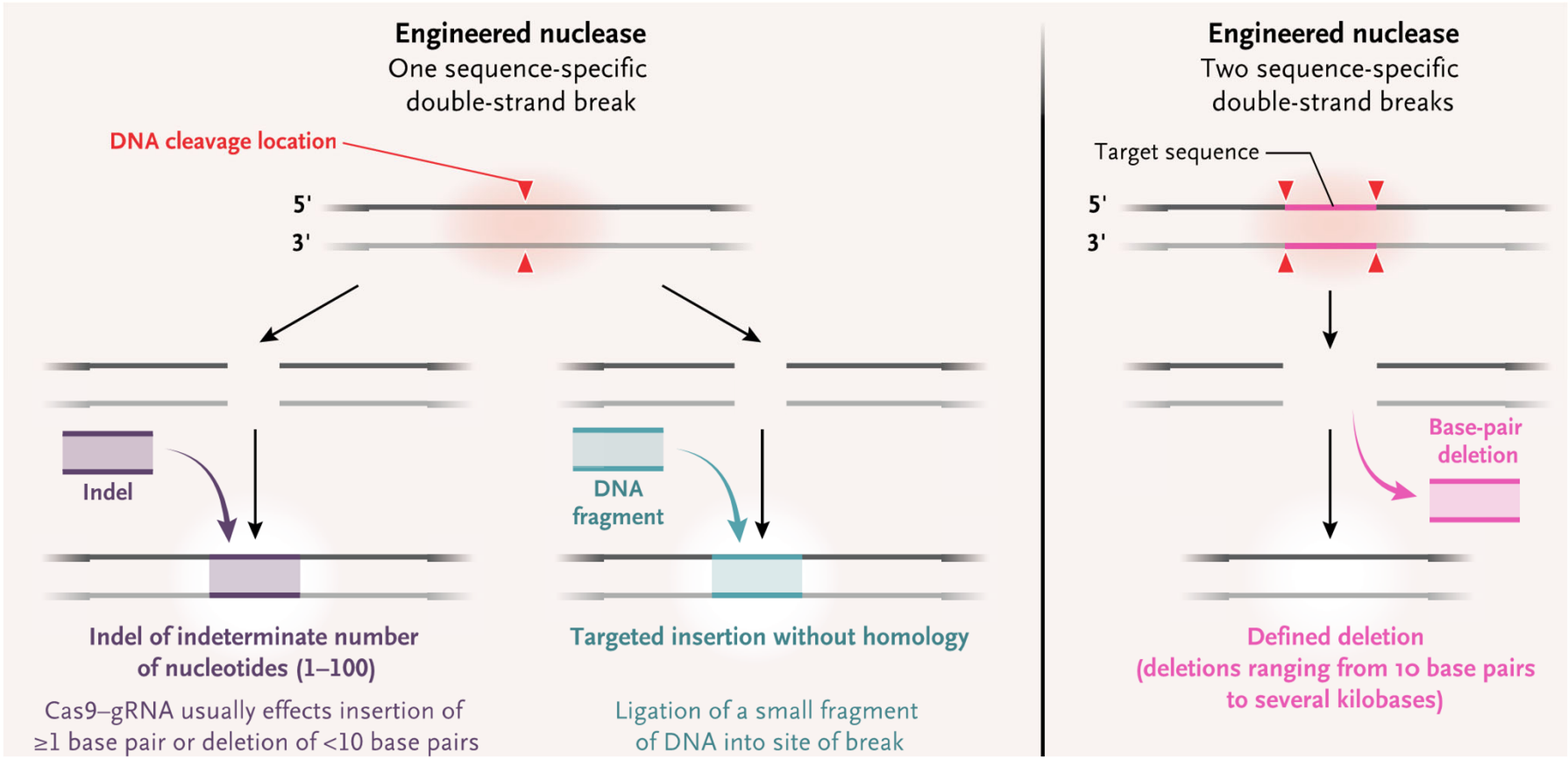
Precise Spatial Modification
(DSB stimulates process by >10^{10})

Precise Spatial AND Nucleotide Modification of Genome
(DSB stimulates process by >10^{5})

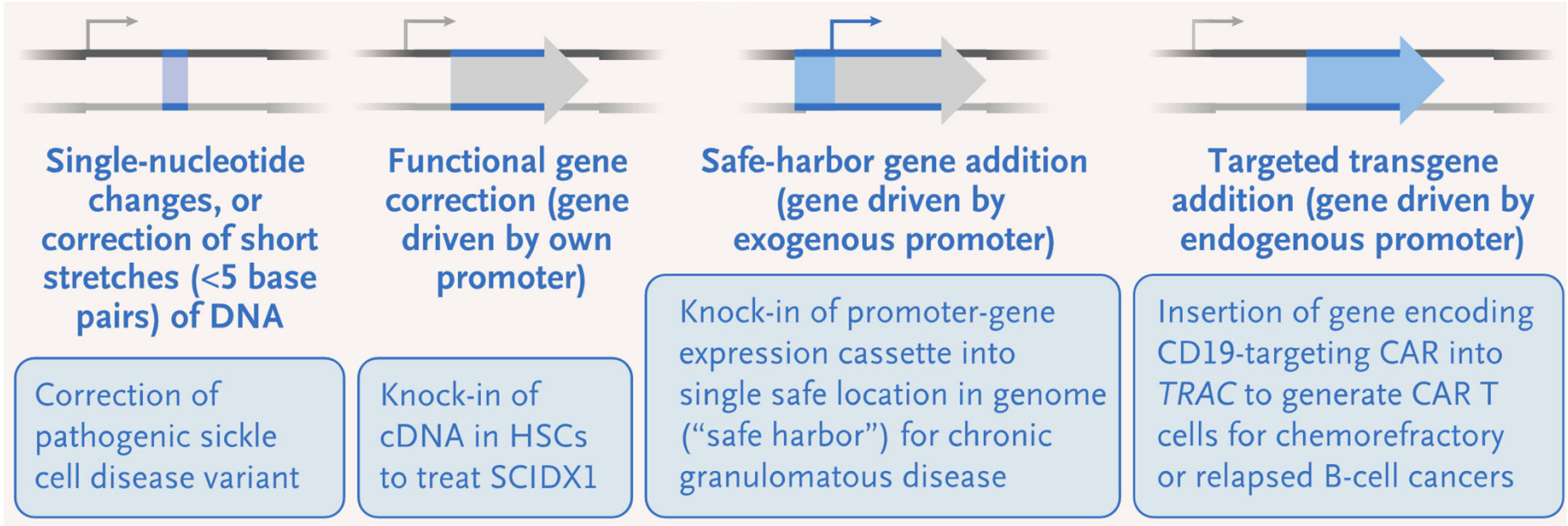
Method to Break Things

Method to Fix/Add Things

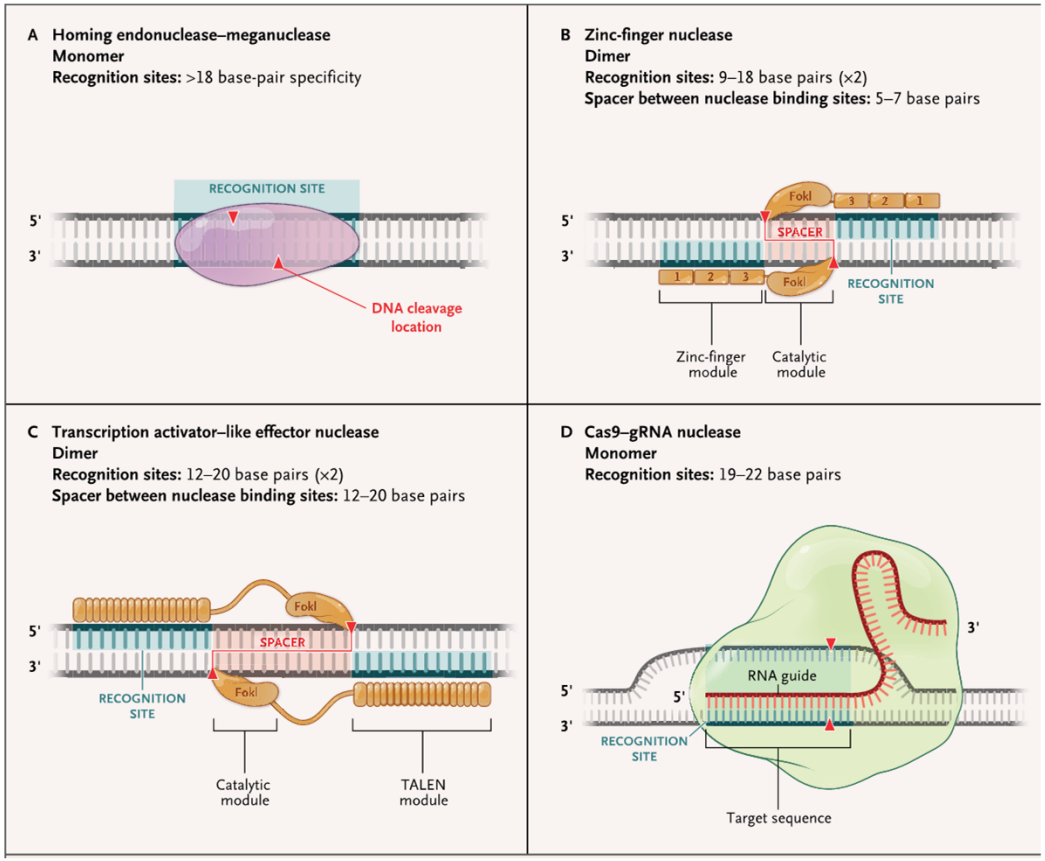
Multiple Uses of NHEJ Based Editing



Multiple Uses of Homologous Recombination Based Editing



A Variety of Engineered Nuclease Options



Porteus "A New Class of Medicines through DNA Editing" NEJM (2019)

Non-nuclease based genome editing strategies

(in different stages of development)

- AAV targeted integration without a break (Russell)
- Peptide Nucleic Acids (Glazer)
- Base Editing (Liu)
- Targeted Recombinases (Barbas, Gersbach)
- Targeted Transposases (Sternberg)
- Prime Editing (Liu)

Why is genome editing so exciting?

- **Tremendously powerful and easy to use research tool**
  - **Knock-outs, knock-in, screens, lineage tracking…**
  - **With Cas9 based systems, works essentially in every biologic system**
- **Accelerate development of improved agricultural products**
  - **Evaluated differently in the US and Europe as a GMO**
- **Could potentially improve the health of humans using precision genetic engineering**

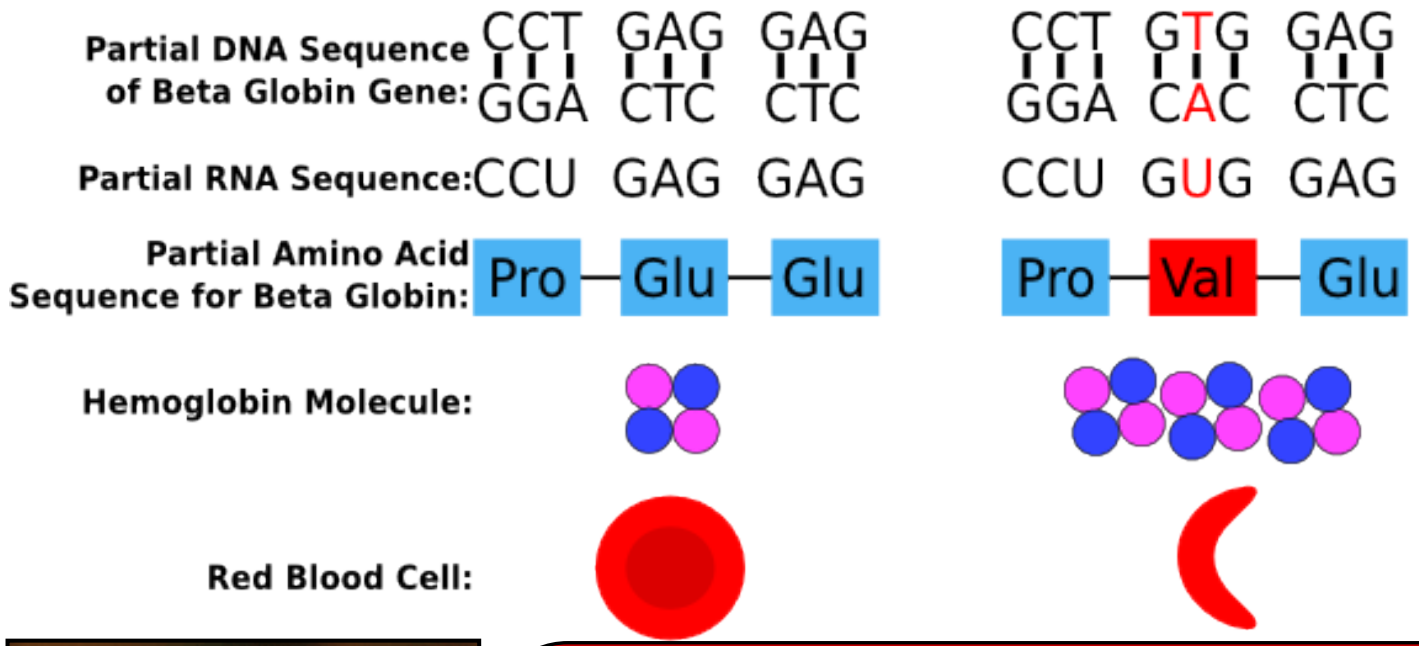
Monogenic Diseases Permeate Medicine
(6,000-10,000 such diseases)
(Patients: ~30 million in USA, ~350 million worldwide)

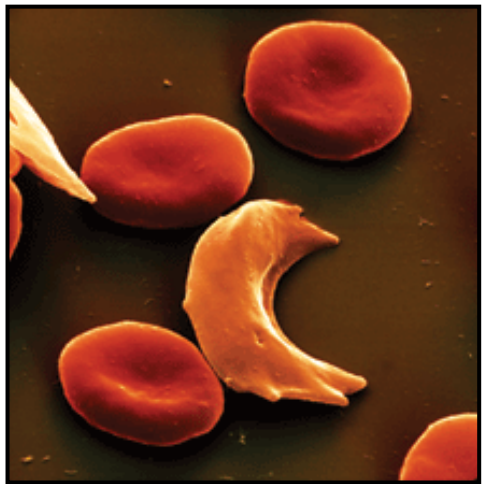
Hematology: Sickle Cell Disease/Thalassemia
Hematology: Hemophilia
Pulmonary: *Cystic Fibrosis*
Immunology: Primary Immunodeficiencies (e.g. Severe Combined Immunodeficiency (SCID)), MSMD, IPEX
Cardiology: Familial Hypercholesterolemia
Dermatology: Epidermolysis bullosa
Genetics: Muscular Dystrophy, MPS I, Gaucher
Neurology: Huntington's Disease, Myotonic Dystrophy, NGLY1 deficiency
Oncology: *RUNX1*-FPD, BRCA1, BRCA2, NF

.

Each patient affects a larger community of people (echoes of the disease) + life years saved

Example 1: Ex Vivo Correction of a Disease Causing Variant (sickle cell disease)

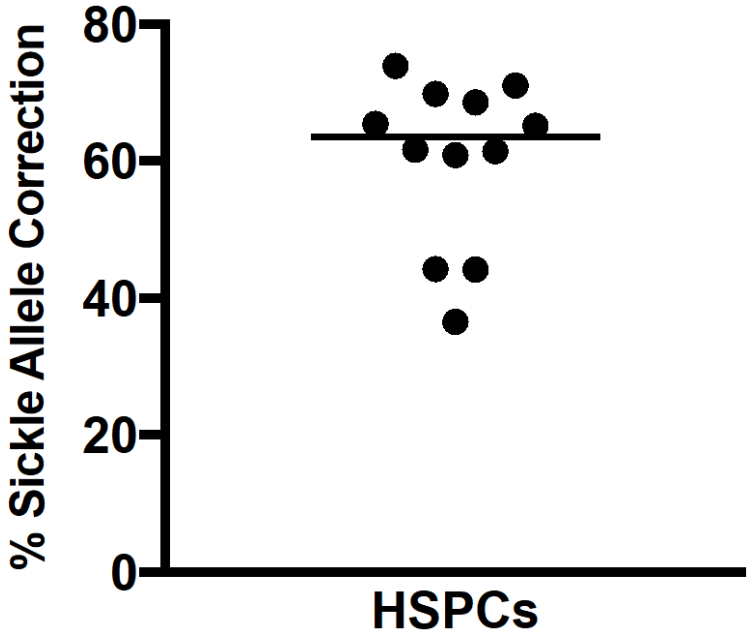




Median Lifespan

United States: mid-40s
(though taking medicine for pain >3 times/week)
(neurocognitive damage starts occurring in first years of life)

Africa: 5-8 years old



Example 2: In Vivo Amelioration of Duchenne's Muscular Dystrophy

GENE EDITING

In vivo gene editing in dystrophic mouse muscle and muscle stem cells

Mohammadsharif Tabebordbar,[1,2]* Kexian Zhu,[1,3]* Jason K. W. Cheng,[1] Wei Leong Chew,[2,4] Jeffrey J. Widrick,[5] Winston X. Yan,[6,7] Claire Maesner,[1] Elizabeth Y. Wu,[1]† Ru Xiao,[8] F. Ann Ran,[6,7] Le Cong,[6,7] Feng Zhang,[6,7] Luk H. Vandenberghe,[8] George M. Church,[4] Amy J. Wagers[1]‡

GENE EDITING

In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy

Christopher E. Nelson,[1,2] Chady H. Hakim,[3] David G. Ousterout,[1,2] Pratiksha I. Thakore,[1,2] Eirik A. Moreb,[1,2] Ruth M. Castellanos Rivera,[4] Sarina Madhavan,[1,2] Xiufang Pan,[3] F. Ann Ran,[5,6] Winston X. Yan,[5,7,8] Aravind Asokan,[4] Feng Zhang,[5,9,10,11] Dongsheng Duan,[3,12] Charles A. Gersbach[1,2,13]*

GENE EDITING

Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy

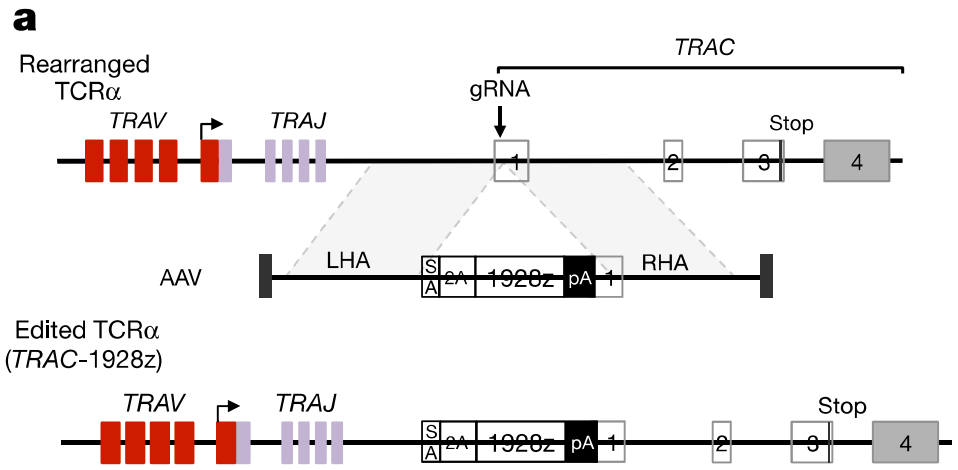
Chengzu Long,[1,2,3]* Leonela Amoasii,[1,2,3]* Alex A. Mireault,[1,2,3] John R. McAnally,[1,2,3] Hui Li,[1,2,3] Efrain Sanchez-Ortiz,[1,2,3] Samadrita Bhattacharyya,[1,2,3] John M. Shelton,[4] Rhonda Bassel-Duby,[1,2,3] Eric N. Olson[1,2,3]†

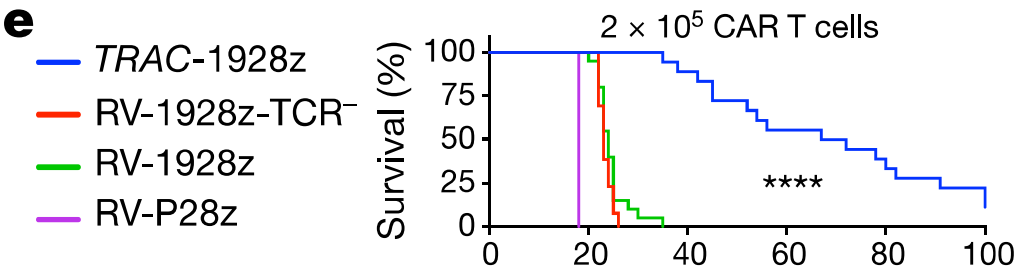
Example 3: Potentially improving anti-cancer based cell therapies

(combining genome editing with synthetic biology)

Targeting a CAR to the *TRAC* locus with CRISPR/Cas9 enhances tumour rejection

Justin Eyquem[1]*, Jorge Mansilla-Soto[1]*, Theodoros Giavridis[1], Sjoukje J. C. van der Stegen[1], Mohamad Hamieh[1], Kristen M. Cunanan[2], Ashlesha Odak[1], Mithat Gönen[2] & Michel Sadelain[1]





Genome Editing Moving Forward

1. **Technical issues of specificity (how to measure, how to evaluate, how to minimize) are being solved by the healthy mechanisms of biomedical research.**

2. **In human genome editing, what applications should and should not be developed?**

3. **Equity and Justice: How to assure that all people who might benefit from these innovative therapies will have access to them?**
   - **How to assure that we do not start thinking of human beings as objects to be engineered/manipulated?**

The Bioethical 2x2 Matrix

Somatic Cell Editing for Disease/Prevention of Disease	**Germline/Heritable Editing For Disease/Prevention of Disease**
Somatic Cell Editing for Enhancement	**Germline/Heritable Editing for Enhancement**

Challenge of Getting High Tech Therapies to All Patients in the World

Hematopoietic Stem Cells Harvested from Patient

x 10^6 CD34/kg

x 10^6 CD34/kg

Autologous

into Patient

8-10 mg/kg)

Ideal: >5 x 10^6 CD34/kg

At

Minimum: 5% Gene Correction

Ideal: >10% Gene Correction

with

No off-target mutations/rearrangements above background and no signs of functional toxicity

GMP Grade AAV6 (LCGM)

GMP Grade Electroporation (Lonza)

History of Medicine:
Innovative Medicines Result in New Cures

Sanitation/Clean Water

Antisepsis/Anesthesia

Vaccines

Small Molecules

Biologics
(Enzymes and Antibodies)

These modalities remain foundational in medicine. In fact, we still need to figure out how to distribute to more people in the world (equity and justice)

Cell and Gene Medicines

Microbiome Manipulation

"Living Drugs"
Different PK/PD
Migrate, Divide, Respond…