Scientific Advancements in Gene Therapies: Opportunities for Global Regulatory Convergence

Hybrid Public Workshop

September 4, 2024

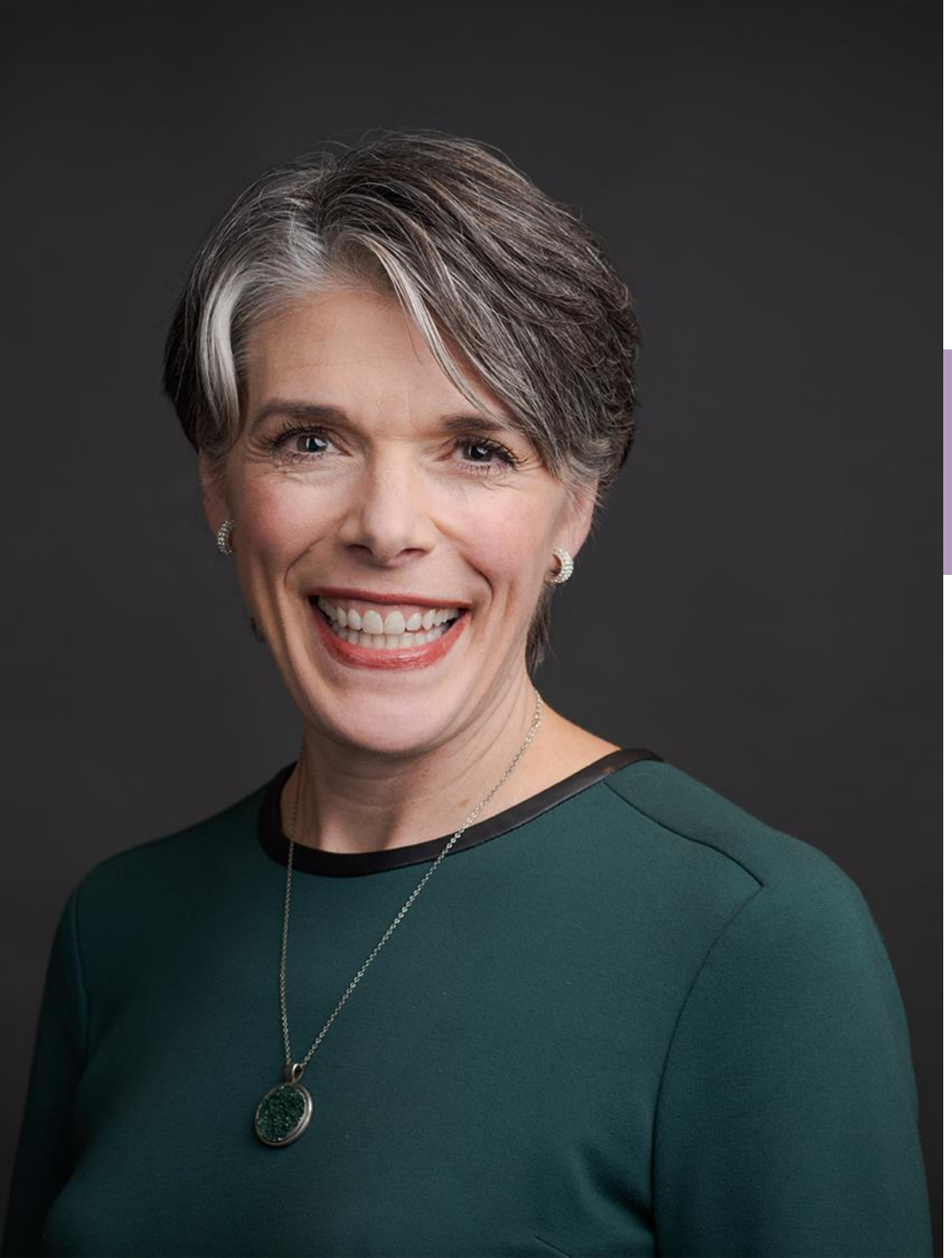
10am-4pm *(eastern)*

The public meeting will begin shortly

In collaboration with

BILL & MELINDA GATES *foundation*

REAGAN-UDALL FOUNDATION FOR THE FDA



REAGAN-UDALL FOUNDATION FOR THE FDA

Welcome

Susan C. Winckler, RPh, Esq.

CEO, Reagan-Udall Foundation for the FDA

Hybrid Meeting

REAGAN-UDALL FOUNDATION FOR THE FDA

Joining online:
Microphone and video will remain off during the meeting
Share your questions using the Zoom Q&A function

Joining in-person:
Please write your questions on the index cards provided

This public meeting is being recorded
The slides, transcript, and video will be available at www.ReaganUdall.org

Today's Agenda (Eastern Time)

REAGAN-UDALL FOUNDATION FOR THE FDA

10am	Welcome
10:05am	Opening Remarks
10:20am	Session 1: The Current State of Gene Therapy
11:35am	Session 2: Panel Discussion
12:15pm	Lunch
1pm	Session 3: The Next Generation of Gene Therapies
2pm	Break
2:10pm	Session 4: Regulators' Perspective
2:55pm	Session 5: How do we prepare for the next generation of gene therapy, as industry, regulators, and a health care system?
3:55pm	Closing Remarks & Adjourn

REAGAN-UDALL FOUNDATION FOR THE FDA

Opening Remarks

Peter Marks, MD, PhD

Director

Center for Biologics Evaluation and Research

U.S. Food and Drug Administration

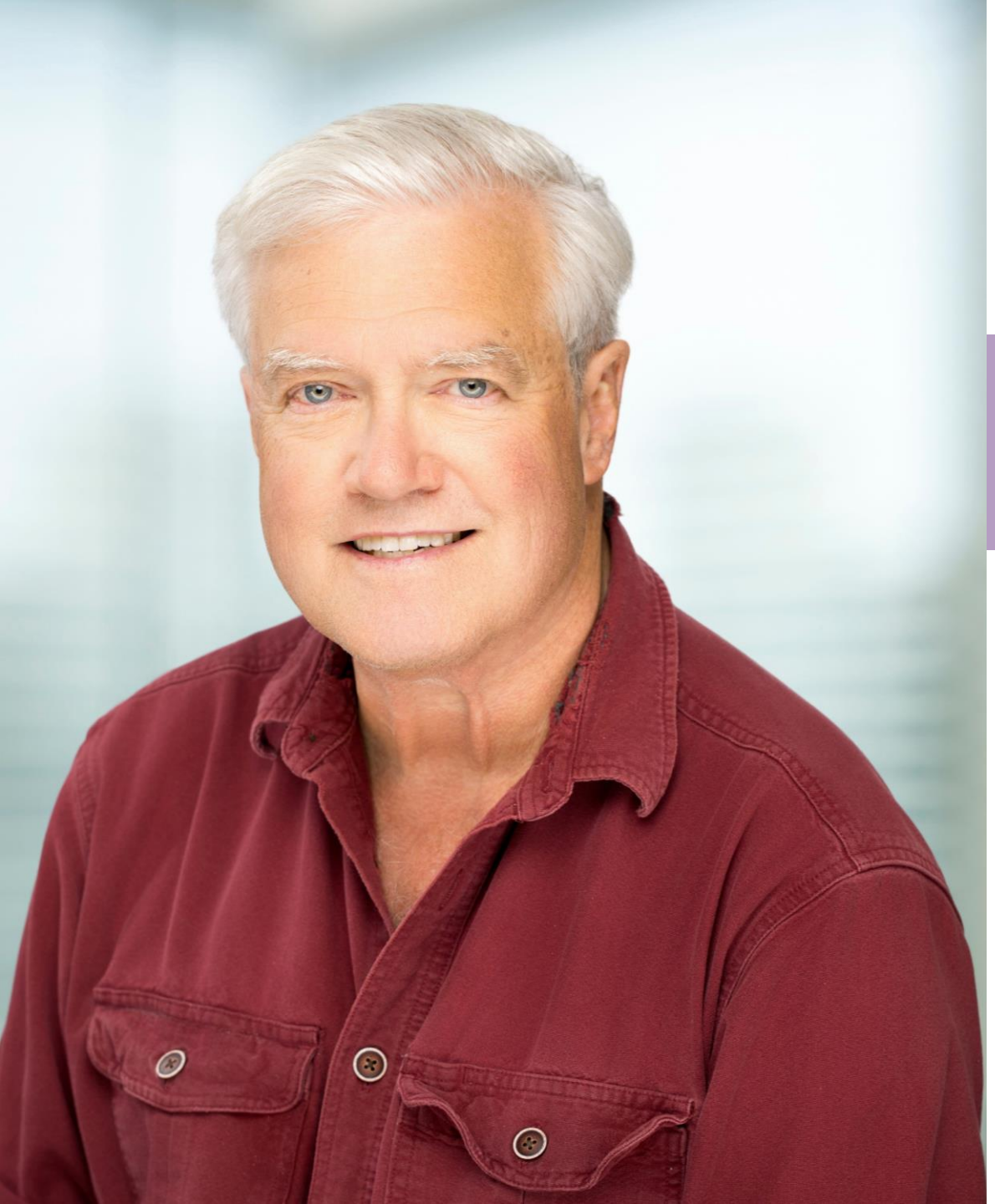
REAGAN-UDALL FOUNDATION FOR THE FDA

Opening Remarks

Julie Makani, MD, PhD

Muhimbili University of Health and Allied Sciences (Tanzania)

Tanzania High Commission to the UK



REAGAN-UDALL FOUNDATION FOR THE FDA

Opening Remarks

Mike McCune, MD, PhD

Bill & Melinda Gates Foundation

Session 1: The Current State of Gene Therapy

REAGAN-UDALL FOUNDATION FOR THE FDA

- **David Williams, MD,** Harvard Medical School
- **Eric Karikari-Boateng, MS,** Food and Drugs Authority (Ghana)
- **Kwasi Nyarko, PhD,** WHO Regional Office for Africa (WHO-AFRO)
- **Maneesha Inamdar, PhD,** Institute for Stem Cell Science and Regenerative Medicine

Scientific Advancements in Gene Therapies: Opportunities for Global Regulatory Convergence

The Current State of Gene Therapy

David A. Williams, MD

Reagan-Udall Foundation for the FDA
September 4, 2024

Disclosures

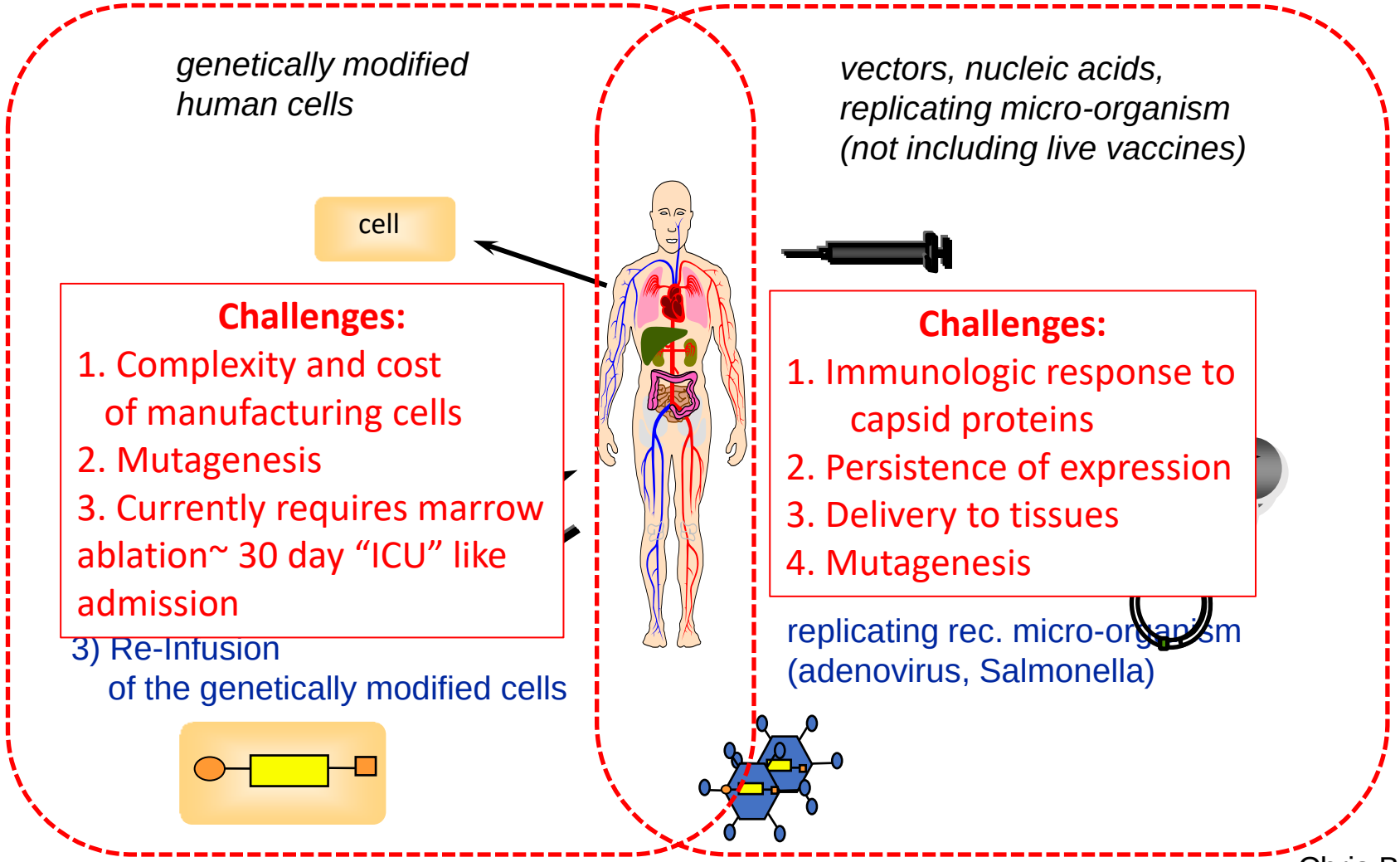
updated 8/18/2022 and covers past 2 years

- Bluebird bio provided GMP vector for SCD clinical trial.
- Orchard Therapeutics provided GMP vector for X-SCID clinical trial.
- Steering Committee, Novartis ETB115E2201 (eltrombopeg in pediatric aplastic anemia). Advisory fees donated to NAPAAC.
- Insertion Site Analysis Advisory Board, bluebird bio; Scientific consultant for FDA Advisory Committee on Eli-Cel and Beti-Cel BLA applications and presentations. (ended)
- Scientific Advisory Board, Beam Therapeutics (ended).
- Chief Scientific Chair, Emerging Therapy Solutions. (ended)
- Scientific Advisory Board, Skyline Therapeutics (formerly Geneception) (ended).
- Insertion Site Advisory Board, Biomarin (ended).
- Verve Therapeutics, consultant.
- Monte Rosa Therapeutics, consultant.
- Tessera Therapeutics, consultant.

Lecture outline

- Overview of gene therapy (GT)
- FDA approved products
- Two short “vignettes” of success in Sickle Cell Disease
- GT in Low- and Middle-Income Countries (LMIC)
- Institutional infrastructure based on success at Boston Children’s Hospital

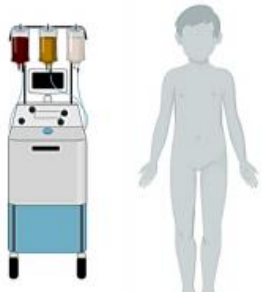
Gene Therapy Medicinal Products



Chris Baum with modifications

Ex Vivo gene therapy targeting blood diseases: Lentivirus or Gene Editing

HSC collection
Mobilization



Myeloablative conditioning

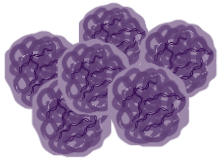
Manufactured cell product infusion

Transduced HSCs and progenitors engraft and differentiate into various blood cells

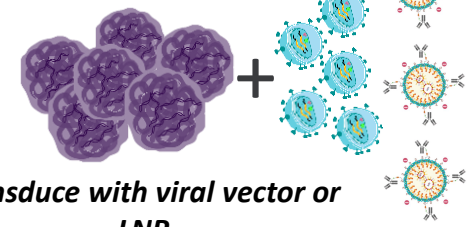
2-year follow-up

Long-term follow-up study
15 years total follow-up

HSC gene transfer



Select CD34+ cells



Transduce with viral vector or LNP

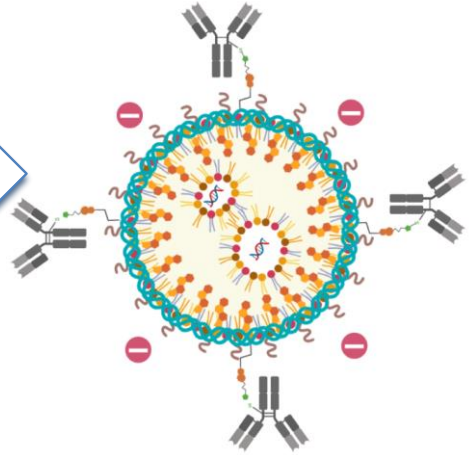
Cryopreserve, test, release

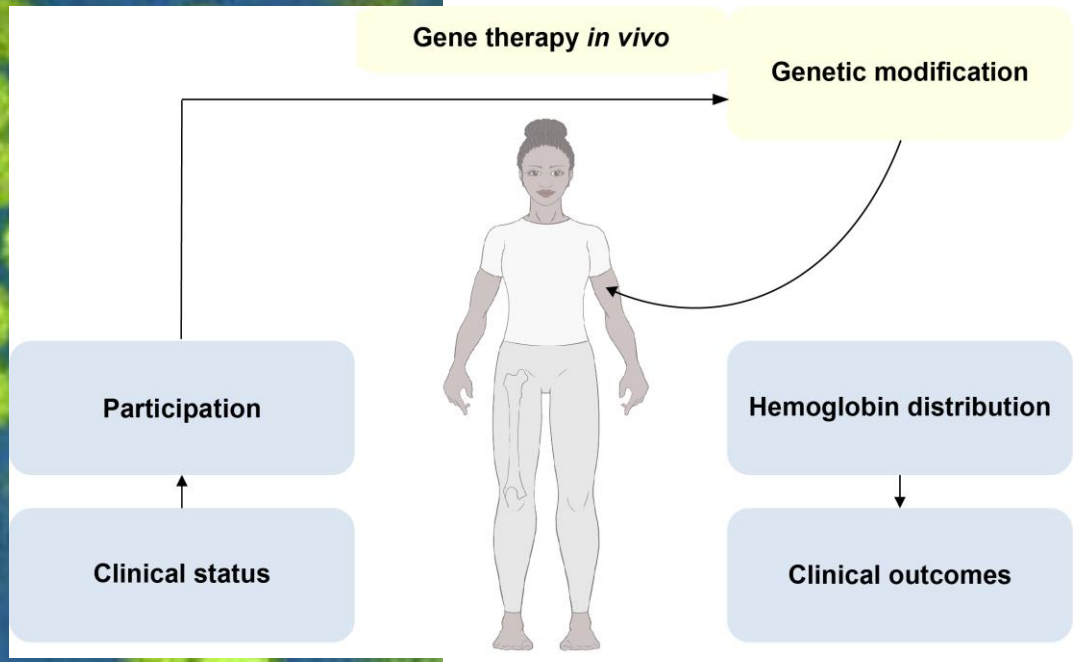
In vivo gene delivery: Adenovirus, Adeno-associated Virus (AAV) or lipid nanoparticle (LNPs)

Adenovirus
(childhood cold)
1999 Jesse
Gelsinger

Adeno Associated
Virus
(non-pathogenic)
Good safety profile
Long-term expression

“Decorated”
lipid
nanoparticle





FDA approved Gene Therapies

Product Name	Indication	Eligibility	Type of Gene Therapy
Zolgensma	Spinal Muscle Atrophy	Pediatric up to 2yrs of age	In vivo
Luxturna	Inherited Retinal Disease	Pediatric Adult	In vivo
Skysona	Adrenoleukodystrophy	Pediatric : ages 4-17 yrs	Ex vivo – gene addition
Zynteglo	Transfusion dependent Thalassemia	Pediatric and adult	Ex vivo – gene addition
Hemegenix	Hemophilia B	18 and older	In vivo
Elevidys	Duchenne's Muscular Dystrophy	4 years and older	In vivo
Roctavian	Hemophilia A	18 years and older	In vivo
Casgevy	Sickle Cell Disease\Transfusion dependent Thalassemia	12 years and older	Ex vivo – gene editing
Lyfgenia	Sickle Cell Disease	12 years and older	Ex vivo – gene addition
Lenmeldy	Metachromatic leukodystrophy (MLD)	Pediatric	Ex vivo – gene addition

Colleen Dansereau, BCH GT Program

FDA approved CAR T Cell Therapies

Product Name	Indication	Eligibility
Kymriah	Relapsed/Refractory B cell Acute Lymphoblastic Leukemia (ALL) Relapsed/Refractory B cell Non - Hodgkin's Lymphoma (NHL)	Pediatric & Young Adult Adult
Yescarta	B cell Non- Hodgkin's Lymphoma (NHL) Follicular Lymphoma	Adult Adult
Tecartus	Mantle Cell Lymphoma (MCL) B cell Non- Hodgkin's Lymphoma (NHL)	Adult
Breyanzi	B cell Non- Hodgkin's Lymphoma (NHL)	Adult
Abecma	Multiple Myeloma	Adult
Carvykti	Multiple Myeloma	Adult

Pipeline 2024 Gene Therapies

Product Name	Indication	Company/Sponsor	Type of Gene Therapy	Regulatory Status
Kresladi	Leukocyte Adhesion Deficiency -1	Rocket Pharma	Ex vivo	Complete Response Letter
Upstaza	aromatic L–amino acid decarboxylase (AADC) deficiency	PTC Therapeutics	In vivo	BLA accepted PDUFA – 11/13/2024

Colleen Dansereau, BCH GT Program

In vivo genetic products: administration

AAVs are delivered frozen and stored in freezers in pharmacy, they are ordered for each patient.

Pharmacy thaws and draws up in biosafety cabinet. The time from thaw to administration varies per product but usually 1-2 hrs so careful coordination between pharmacy and care team is required.

Due to price and institutional risk, we do not store stock of these but order for each patient, delivery times between 2 days to 2 weeks depending on product.

Most of these are IV administration, except Luxturna (retinal). Premedication with steroids and tapered steroid course for 30-60 days is the norm post infusion, as is scheduled LFT monitoring.

Mostly outpatient (Zolgensma, Roctavian, Elevidys) administered in infusion center

Outpatient 6-8 hr stay, we don't thaw the product for the patient until they are on site. Infusion is 1-3 hrs. depending on product. Post infusion observation time is 3-6 hrs. Luxturna done in outpatient day surgery setting.

Ex vivo genetic products: administration

Delivered after autologous collection, cell manufacturing in GMP laboratory and release criteria are met-> usually 60-90 days.

Patient admitted to Stem Cell Transplant (ICU-like setting) and given ‘conditioning’ (currently chemotherapy dosage to completely ablate bone marrow).

Product thawed in Cell Therapy facility and delivered to floor for infusion.

Most patients admitted for ~4 weeks to unit then followed closely after discharge since still immunocompromised.

FDA approved Gene Therapies

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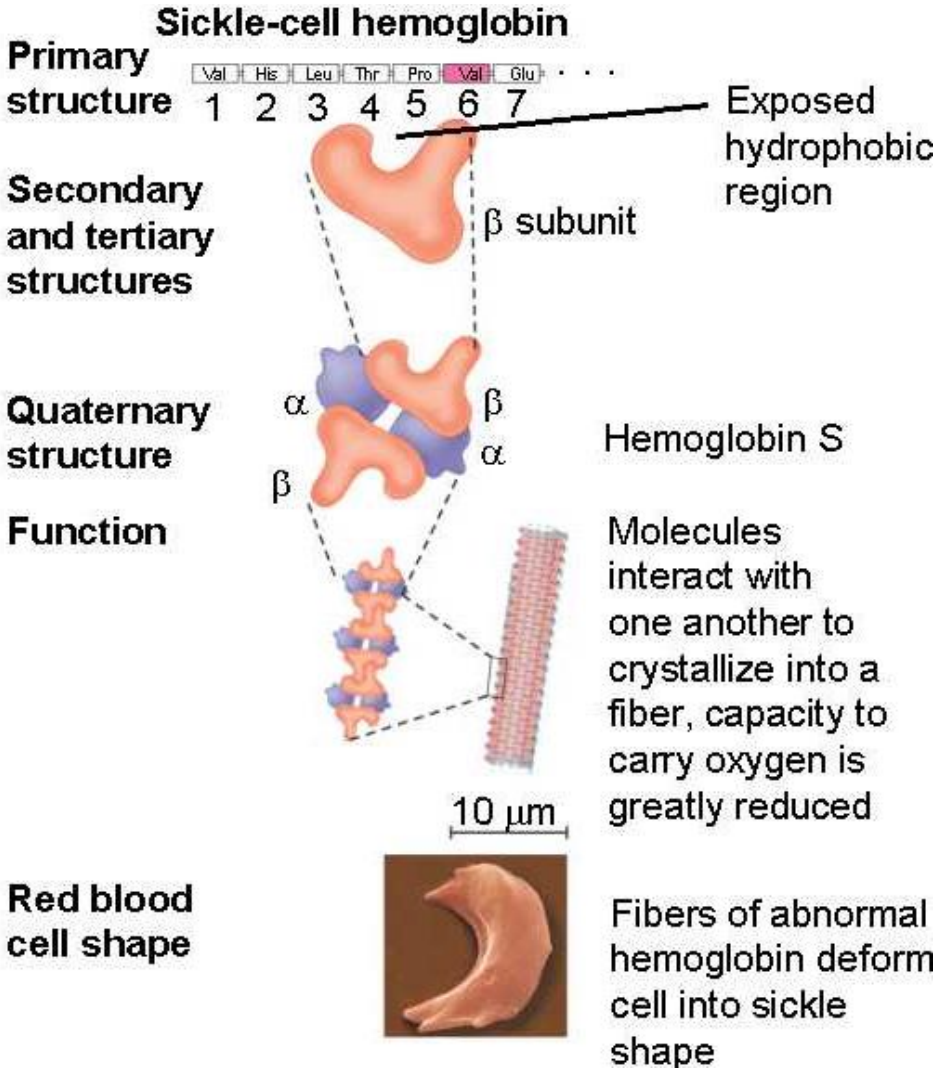
Sickle Cell Disease



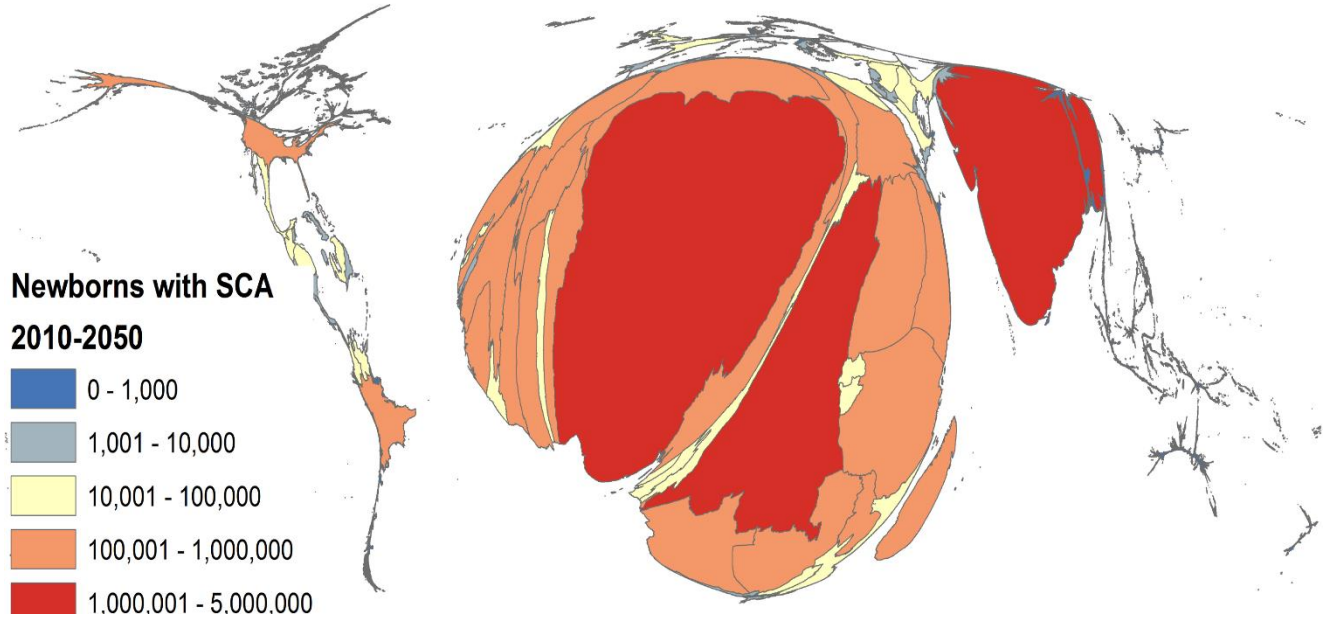
Clinical Manifestations

Chronic hemolytic anemia
Vaso-occlusion: **Pain**

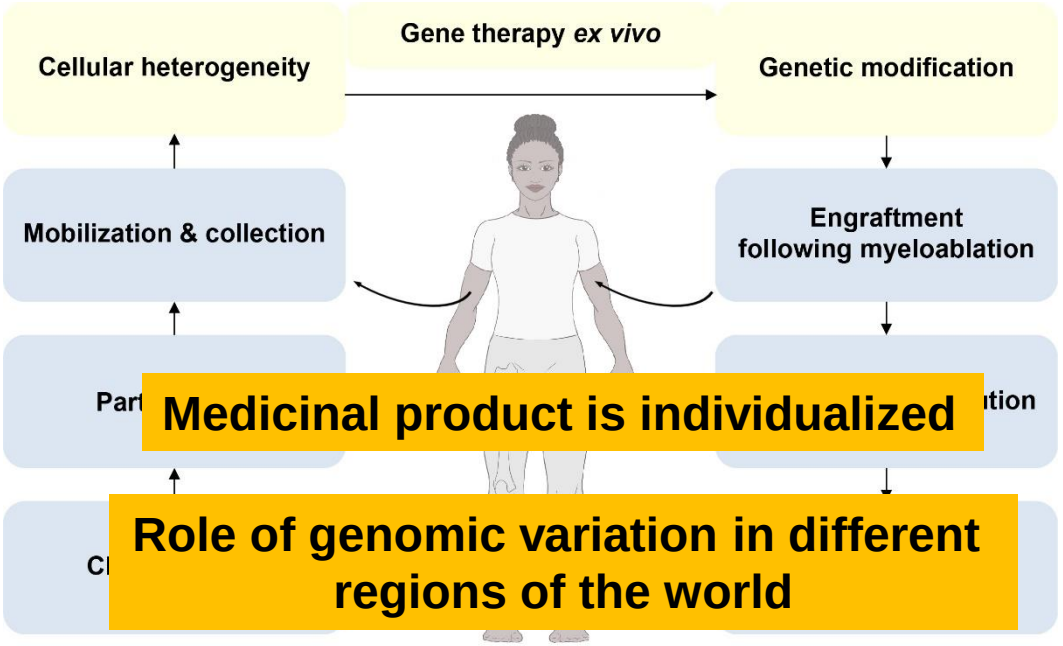
Acute and chronic

Vascular: End organ damage due to ischemia
Stroke, lung, hypertension, renal disease, avascular necrosis, stasis ulcers

Sickle cell disease: world-wide burden is not approachable using ex vivo gene therapy



Piel *et al*. PLOS Med (2013) 10:1.



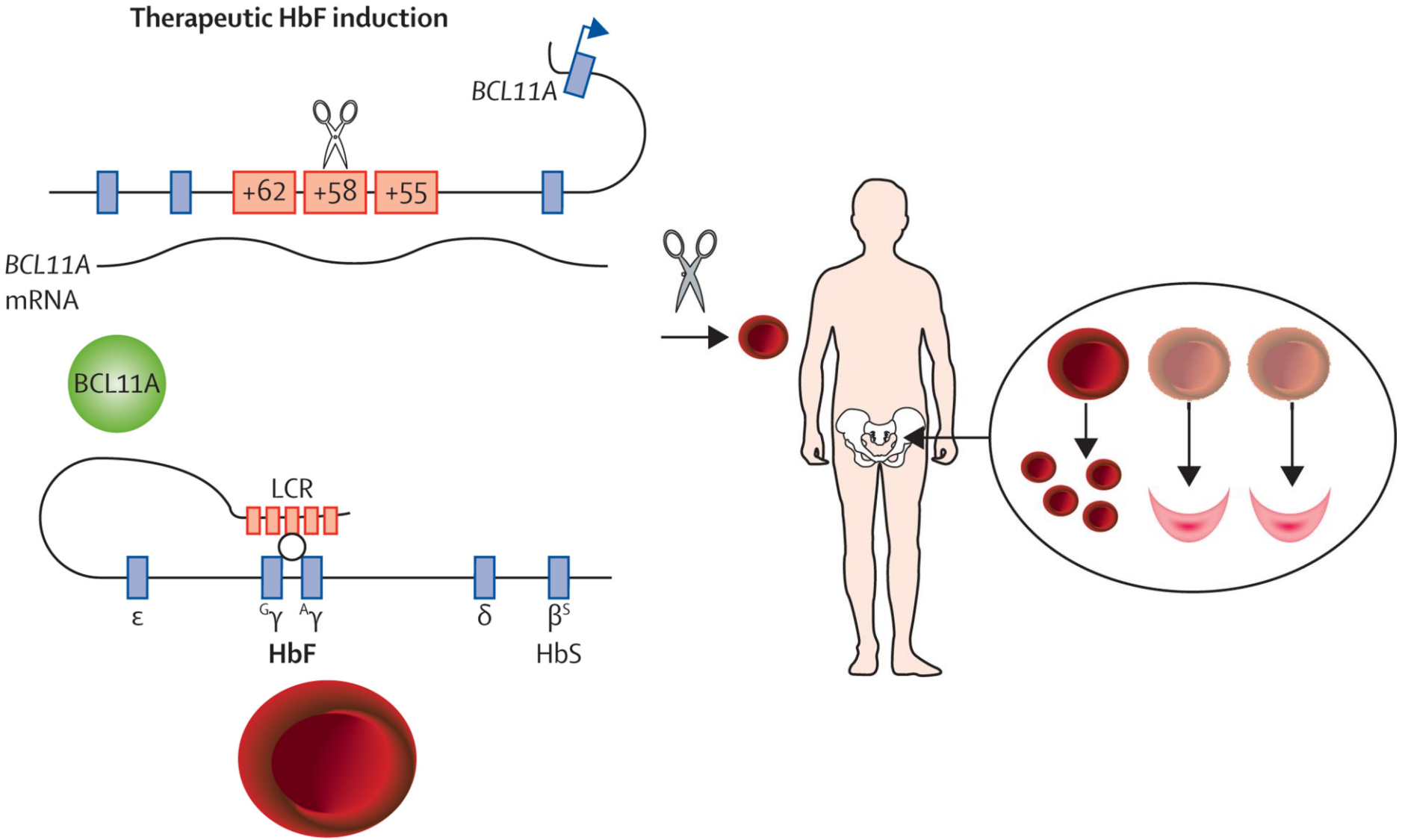
Rosanwo and Bauer. Mol Ther (2021) 29:3163.

Dana-Farber Cancer Institute | Boston Children's

Dana-Farber/Boston Children's Cancer and Blood Disorders Center

HARVARD MEDICAL SCHOOL TEACHING HOSPITAL

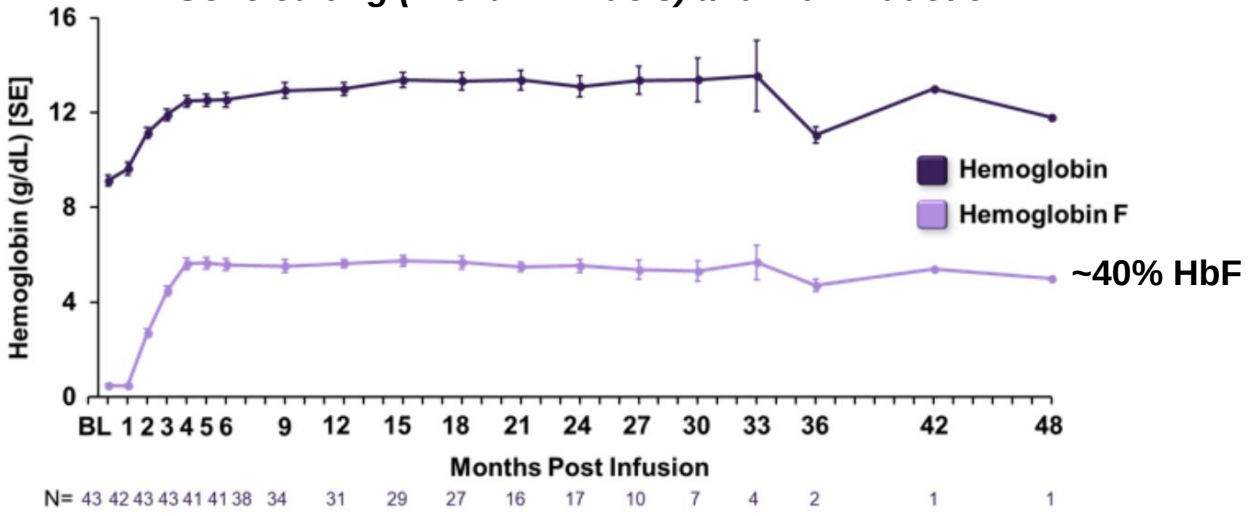
Therapeutic vision: Ex vivo gene editing-> Casgevy®



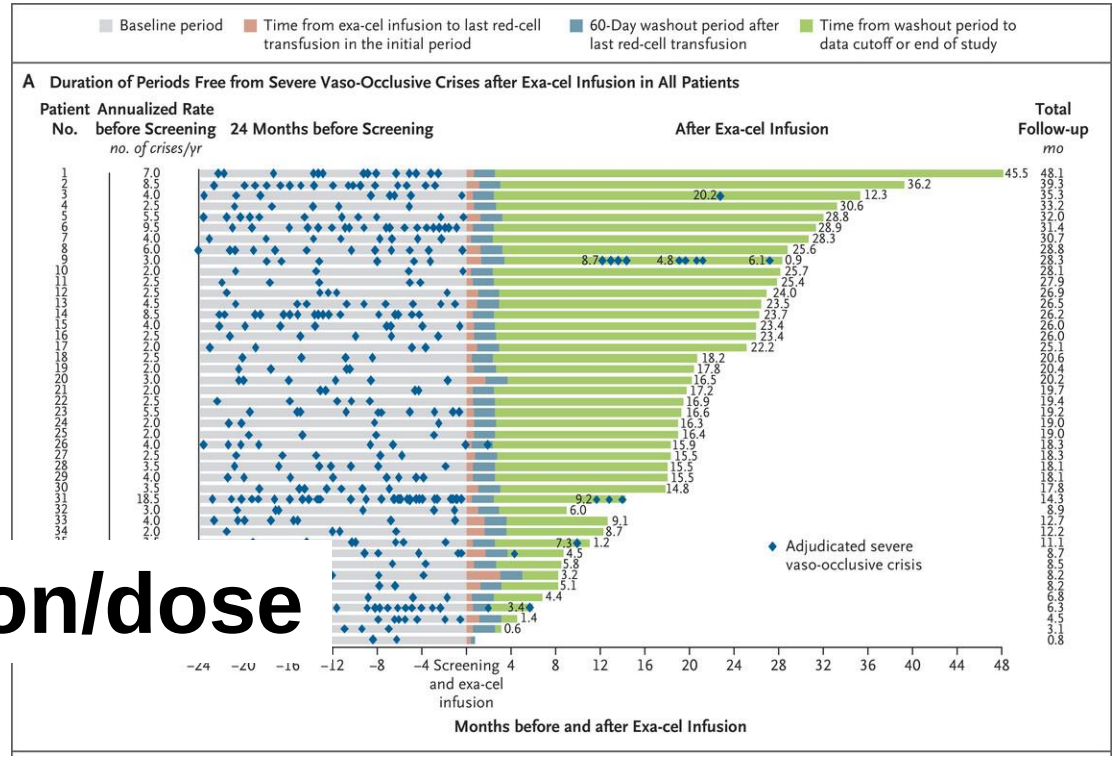
Lettre and Bauer. Lancet (2016) 387:2554. Wu *et al.* Nat Med (2019) 25:776.

Pivotal trial in editing for HbF induction in SCD

Gene editing (~75% PB indels) and HbF induction



Vaso-occlusive crisis reduction



Costs: $2.2 million/dose

Hemolysis reduction

Visit	
Baseline **mean (SD), range***	
Month 6 **mean (SD), range**	140.4 (59.2) 33.6, 293.9 n = 29
Month 12 **mean (SD), range**	141.4 (66.5) 66.2, 413.3 n = 29
Month 24 **mean (SD), range**	152.2 (44.9) 79.9, 273.1 n = 16

----------INDICATIONS AND USAGE----------

CASGEVY is an autologous genome edited hematopoietic stem cell-based gene therapy indicated for the treatment of sickle cell disease (SCD) in patients 12 years and older with recurrent vaso-occlusive crises (VOCs). (1)

Patients treated with Exa-cel (Casgevy) show substantially reduced hemolysis and vaso-occlusive episodes.
Still they have some residual hemolysis and 6 out of 43 had vaso-occlusive episodes after therapy.
Impact on SCD organ function insidious deterioration and long-term outcomes remain unknown.

Frangoul *et al.* NEJM (2024); FDA.com 10/31/2023 Ad Com Meeting Briefing Document (Sponsor); Package Insert 12/8/2023.

FDA approved Gene Therapies

Product Name	Indication	Eligibility	Type of Gene Therapy
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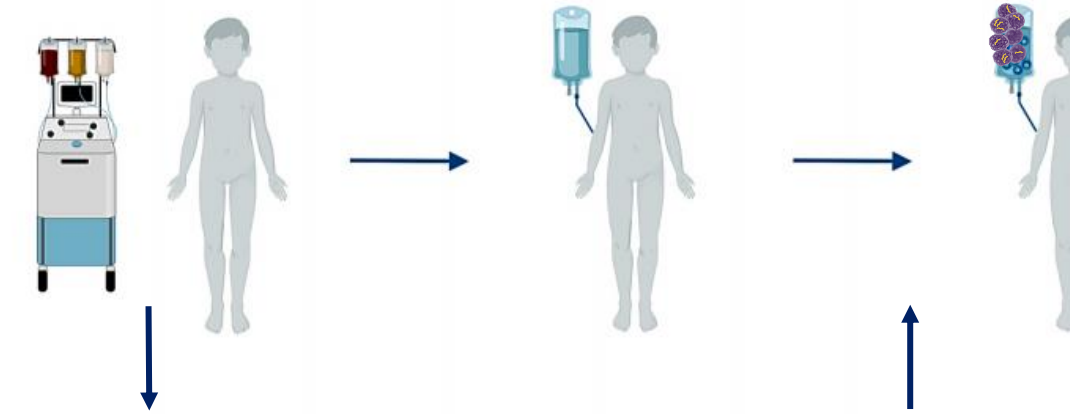
Ex Vivo gene therapy targeting blood diseases: Lentivirus vector LentiD

HSC collection
Mobilization

Myeloablative conditioning

Manufactured cell product infusion



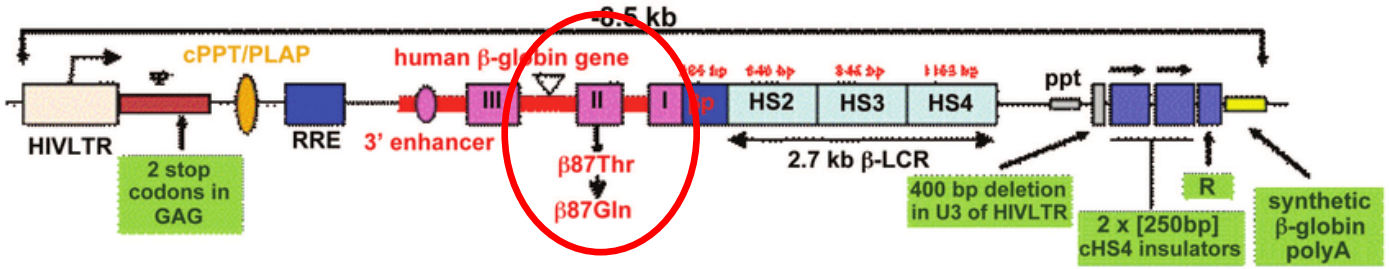
Transduced HSCs and progenitors engraft and differentiate into various blood cells

2-year follow-up

Long-term follow-up study
15 years total follow-up

LentiD vector

Vector cDNA designed to inhibit HbS polymers



Pivotal LVV: Hemoglobin addition in SCD-> Lyfgenia®

Original Article

Biologic and Clinical Efficacy of LentiGlobin for Sickle Cell Disease

Julie Kanter, M.D., Mark C. Walters, M.D., Lakshmanan Krishnamurti, M.D., Markus Y. Mapara, M.D., Ph.D., Janet L. Kwiatkowski, M.D., M.S.C.E., Stacey Rifkin-Zenenberg, D.O., Banu Aygun, M.D., Kimberly A. Kasow, D.O., Francis J. Pierciey, Jr., M.Sc., Melissa Bonner, Ph.D., Alex Miller, B.Sc., Xinyan Zhang, Ph.D., Jessie Lynch, M.S., Dennis Kim, M.D., M.P.H., Jean-Antoine Ribeil, M.D., Ph.D., Mohammed Asmal, M.D., Ph.D., Sunita Goyal, M.D., Alexis A. Thompson, M.D., M.P.H., and John F. Tisdale, M.D.

N Engl J Med
Volume 386(7):617-628
February 17, 2022

The NEW ENGLAND JOURNAL of MEDICINE

Changes in the Rate of Vaso-Occlusive Events before and after LentiGlobin Infusion (Lyfgenia®*)

Price: $3.4 million

*FDA required ‘Black Box’ warning due to leukemias in early cohort of trial. Scientific evidence suggests not related to vector insertion.

The NEW ENGLAND JOURNAL of MEDICINE

How are patients monitored after commercial drug treatment?

Post marketing follow-up is product dependent: examples

1. A 15 yr 150 patient f/u study with:
•Baseline bone marrow biopsy (to be performed pre-infusion at a time point per treating HCP discretion)
•Blood specimens q6M first 3 years (CBC, ISA, VCN) and then annually for 15 years

2. A postmarketing, prospective, multi-center, observational study, to assess and characterize the risk of secondary malignancies after treatment and to assess the long-term safety of the GT product. The study will include 250 patients with sickle cell disease, and each enrolled patient will be followed for 15 years after product administration. The study design will include monitoring (at pre-specified intervals) for clonal expansion with adequate testing strategies.

•baseline bone marrow aspirates that included histopathology, karyotype, FISH, and RHP.
•**Year 1- D100, M6, M12**: CBC, pb blood smear, ISA, VCN, reticulocytes
•**Years 2-10- q4M**: CBC, pb blood smear, ISA, VCN, reticulocytes
•**Years 11-15**- **q6M** :CBC, pb blood smear, ISA, VCN, reticulocytes

Post marketing follow-up is product dependent: examples

3. A post-marketing, prospective, observational, study to assess and characterize the risk of secondary malignancies, and long-term safety following treatment with GT. This study will enroll a minimum of 17 subjects. The enrolled patients will be followed for 15 years after product administration."

Post marketing follow-up is product dependent: examples

POSTMARKETING REQUIREMENTS UNDER SECTION 505(o)

Section 505(o) of the Federal Food, Drug, and Cosmetic Act (FDCA) authorizes FDA to require holders of approved drug and biological product applications to conduct postmarketing studies and clinical trials for certain purposes, if FDA makes certain findings required by the statute (section 505(o)(3)(A), 21 U.S.C. 355(o)(3)(A)).

We have determined that an analysis of spontaneous postmarketing adverse events reported under section 505(k)(1) of the FDCA will not be sufficient to identify an unexpected serious risk of secondary malignancies and off-target effects following genome editing after administration of exagamglogene autotemcel.

Furthermore, the pharmacovigilance system that FDA is required to maintain under section 505(k)(3) of the FDCA is not sufficient to assess this serious risk.

Therefore, based on appropriate scientific data, we have determined that you are required to conduct the following studies:

2. Conduct studies to comprehensively assess and screen for the impact of sequence heterogeneity on the risk of off-target editing in the patient population for exagamglogene autotemcel. Specifically,

    i. Perform a new in silico off-target analysis using publicly available databases/datasets to allow for inclusion of more variants. Specifically, perform the analysis using all variants with at least 0.5%

    allele frequency in at least one of the five continental groups (Africa, Europe, East Asia, South Asia, and the Americas).

    ii. Perform confirmatory testing, as appropriate and feasible, of all the off-target loci nominated from the new in silico analysis from (i) as well as those that were not accounted for in the previous study using appropriate samples harboring variants.

    a. Screen for the presence of all previously identified variants (e.g., CPS1) as well as any variants identified in study (i) and (ii) in the patients treated in Studies 121, 111, 141, 151, 161, and 171.

    b. For patients with a confirmed variant(s), assess for indels and chromosomal changes at each respective locus in appropriate samples.

Role of genomic variation in different regions of the world?

https://www.fda.gov/vaccines-blood-biologics/casgevy

Boston Children's

What is the status of gene therapy in Low- and Middle-Income Countries (LMIC)?

ONLINE COVER: Getting on the Gene Therapy Bus to Remission. Gene and cell therapies may enable remission or even cure for intractable diseases such as sickle cell disease, hemophilia, cancers, and HIV. However, the high costs and complexity of these new treatments have meant limited access for patients, particularly those in low- and middle-income countries. The cover image depicts many patients queueing up to get on the gene therapy bus, with only a few able to board. The current gap in access to these transformative therapies is discussed in two special articles. A Review by Doxzen *et al.* analyzes progress and roadblocks to implementation of gene and cell therapies in six countries with a high disease burden. A Viewpoint by Olayiwola *et al.* highlights the critical importance of engaging patients in all steps of cell and gene therapy development.

Credit: Moses Supercharger/JABASA HIV Artseum, Kampala, Uganda (Concept); John Mary Kyambadde, Vanessa Nannyonjo, and Moses Katabira (Artists)

What is the status of gene therapy in Low- and Middle-Income Countries (LMIC)?

- Estimated >500 phase 1-3 gene therapy trials in progress
- There are ~300 gene therapy trials in genetic diseases
- None in Africa, India or Brazil
- Since 1994, 62 clinical trials using some kind of gene therapy to treat HIV infection have been registered
- The number of gene & cell therapy trials for HIV which have taken place in Africa: none

Gene therapies development: slow progress and promising prospect. Hanna E, et al. J Mark Access Health Policy, 2017

What are the barriers to development and implementation of GT trials in LMIC?

- Health care facilities and health care delivery
  - ➢Current approaches require intensive inpatient treatment
- Manufacturing
  - ➢Complex and individualized product manufacturing then shipping, storage and administration
- Regulatory
  - ➢Lack of harmonization across regulatory jurisdictions
- Costs
  - ➢Prohibitive even in high income countries and focused on upfront, short-term costs and reimbursement
- Community acceptance

What are the barriers to development and implementation of GT trials in LMIC?

- **Health care facilities and health care delivery**
  - Lack of equitable access to health care
  - Rural populations vs tertiary care centers

➢Could develop “hub and spoke” structure with distribution of care according to expertise, facilities and work force

Modified from Doxzen *et al.*, *Sci. Transl. Med.* **16**, eadn1902 (2024) 8 May 2024

What are the barriers to development and implementation of GT trials in LMIC?

- **Manufacturing**
  - Global shortage of facilities, including in HIC
  - LMIC may need short term outsourcing with plan to develop capabilities in long-term via local R & D
  - Alternative is affordable licensing (eg Caring Cross) or Point of Care Manufacturing

Modified from Doxzen *et al.*, *Sci. Transl. Med.* **16**, eadn1902 (2024) 8 May 2024

What are the barriers to development and implementation of GT trials in LMIC?

- **Finances**
  - May require collaborative approach with philanthropy and government funding (eg Gates Foundation)
  - Will require priority setting by local authorities
    - High-cost individual curative therapies vs broad provision of less costly treatments for common conditions
    - Overall cost burden of chronic disease complications are considerations but allocation of resources often based on short-term costs
  - Point of Care manufacturing may significantly reduce overall costs
  - Investment in local manufacturing to reduce long-term costs

Modified from Doxzen *et al.*, *Sci. Transl. Med.* **16**, eadn1902 (2024) 8 May 2024

What are the barriers to development and implementation of GT trials in LMIC?

- **Regulatory**
  - Opportunity for regional harmonization (eg Africa Medicines Agency)
  - Potential for “continent” level agreements
  - Ideal would-be international framework for regulatory oversight
  - Local manufacturing could help simply (eg N=1 in UK)

Global

- Develop harmonized regulatory requirements and processes
- ? Central global review process adopted by all countries
- Provide education around best practices in carrying out and oversight of GT trials

Modified from Doxzen *et al.*, *Sci. Transl. Med.* **16**, eadn1902 (2024) 8 May 2024

What are the barriers to development and implementation of GT trials in LMIC?

- **Community buy in**
  - Early involvement of community groups in planning and implementing trials (eg Joint Adherent Brothers and Sisters Against AIDs; National Alliance of Sickle Cell Organizations (India)
  - Focus on community education

Modified from Doxzen *et al.*, *Sci. Transl. Med.* **16**, eadn1902 (2024) 8 May 2024

What does the future hold: A child anywhere in the world with a fatal disease can access genetic therapies

- Development and implementation of “gene therapy in a vial” for in vivo delivery> One shot, single dosage therapy.
- Development and implementation of less toxic conditioning and/or *in vivo* selection for corrected cells.
- Development of critical infrastructure to allow equitable access to healthcare across rural populations and under-resourced health care.
- Development of funding models that recognize long-term savings in resource and patient suffering.

Initiate Institutional Planning

- Identify a Clinical Physician Lead/ Product Champion
- Draft the patient workflow or model for care delivery
- Establish a stakeholder group – all touchpoints for patient should be represented
- Nominate a single point of contact as liaison between institution and company/sponsor

Colleen Dansereau, BCH GT Program

Planning Model

Education

- Clinical Staff – sponsor trainings, SOP development
- Nonclinical Staff – develop content appropriate product education
- Patient \Families– treatment education, patient journey
- Payor Community- product education, delivery model vs SOC

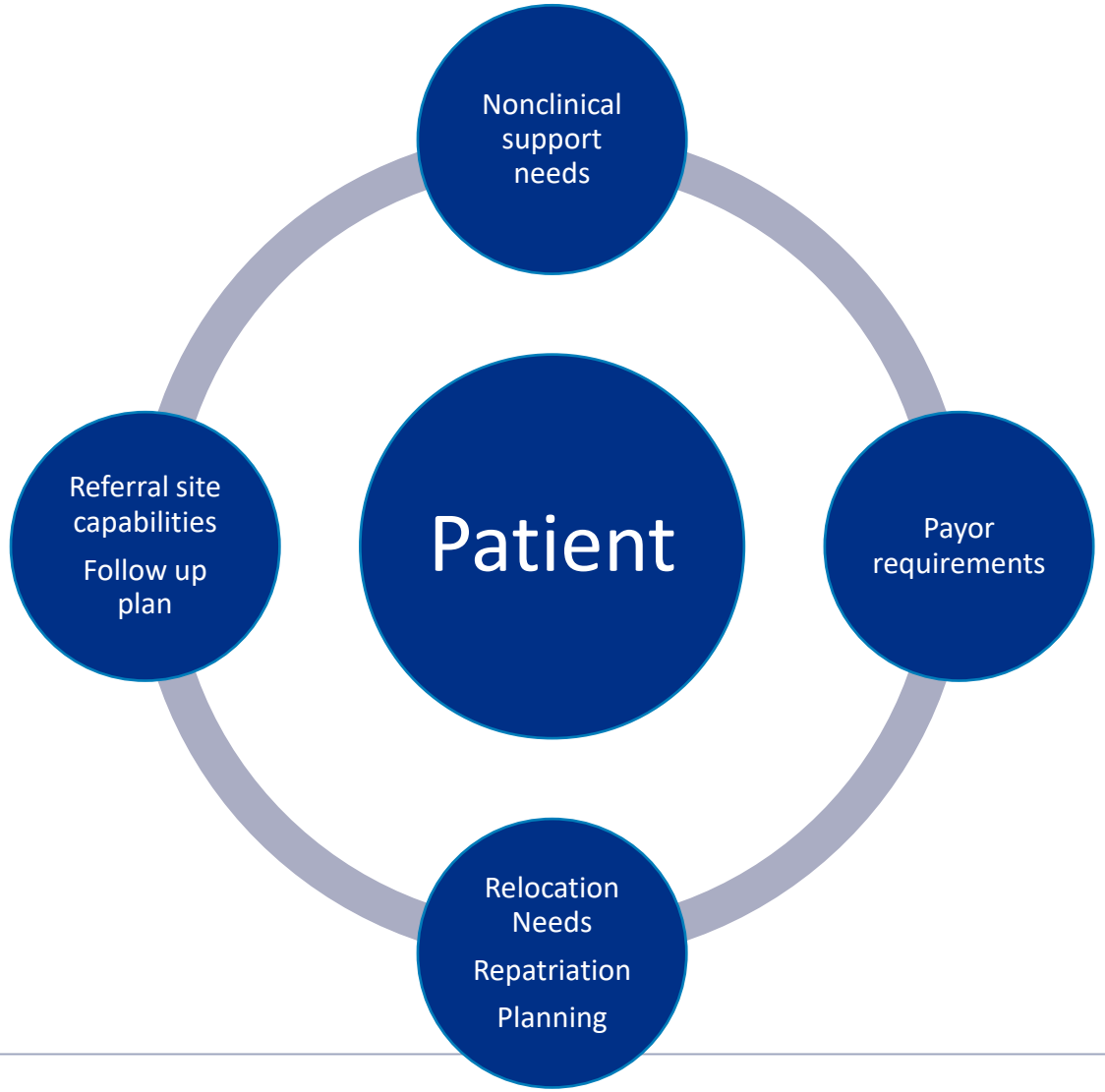
Communication

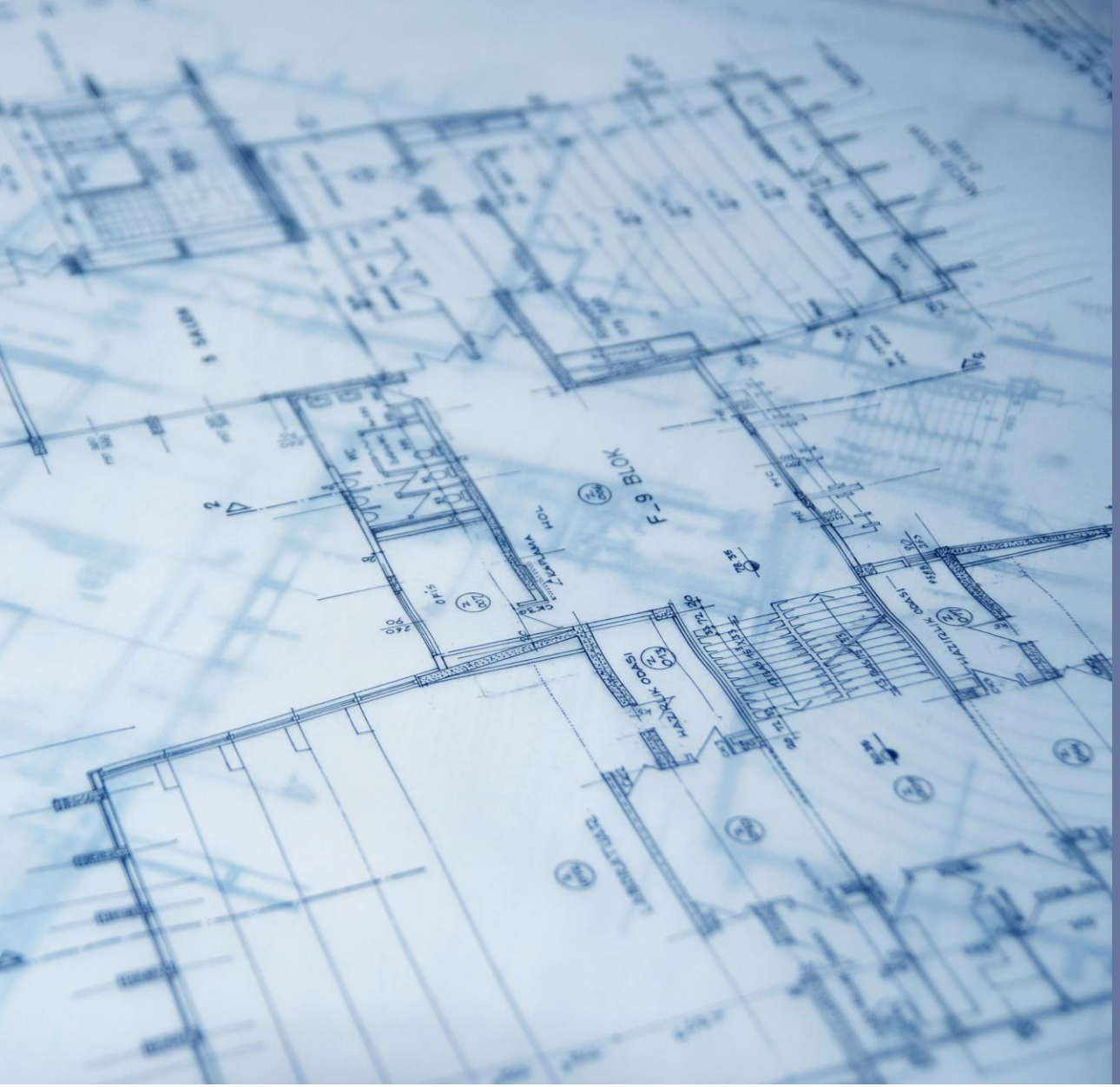
- Establish single point of contact
- Organize regularly scheduled multidisciplinary meetings
- Create DL
- Use marketing and communication groups
- Ongoing continuous quality improvement

Operations

- Assessment of end-to-end care delivery model
- Each stakeholder conduct assessment and identify needs in their area
- Combine product delivery needs with current institutional state to identify gaps
- Create an implementation plan and checklist
- Conduct Mock Runs

Patient Access Considerations





Design a Blueprint for the Future

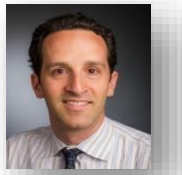
1. **Organize your institution**
   - Establish a core team to manage product entry and adoption
   - Create processes that are treatment focused instead of disease based

2. **Standardize where possible**
   - Assessment guides, Implementation plans and Checklists
   - Standard contract language
   - Letters of Medical Necessity & Treatment consents
   - Education materials

3. **Establish relationships with key institutional contacts**
   - Legal
   - Disease Center Leaders
   - Patient Care /Clinical Operations
   - Financial Services/Payor Contracting Group/Government Affairs

Gene Therapy Program @ BCH

Leadership


D. Bauer
Director


C. Duncan
Medical
Director-


C. Dansereau
Sr. Director
Operations

Clinical Research Team


B. Kerwin
CRC


E. Morris
CRC


O. Silva
Program Coord


A. Federico,
Research NP


M. O'Donnell
Research RN

Clinical Investigators


S.Prockop
HSCT


M.Heeney
Heme


E. Esrick
Heme


L.Silverman
Oncology


W. Al-Hertani
Genetics


S.Baumeister
HSCT


S. Croteau
Heme


L. Lehmann
HSCT


P. Ghosh
Neurology


A.Uluer
Pulm/CF


S. Emani
Cardiology

Translational Program Leaders


J. Manis
Transfusion


W. Tann
Genetics


B. Darras
Neurology


O.Bodamer
Genetics


A. Fulton
Ophthalmology


H. Dorkin
Pulm/CF


S. Stone
Neurosurgery


P.Genovese


C. Brendel


M.Armant


D.Pellin

Key Partners

TransLab - BCH

Connell & O'Reilly Cell Manufacturing Core Facility (DFCI)

Institutional Center for Translational and Clinical Research (ICCTR)

Clinical Translational Study Unit (CTSU)/Experimenal Therapeutics Unit

Collaborating Partners

BIOMARIN
PTC THERAPEUTICS
SAREPTA THERAPEUTICS
bluebirdbio
Beam THERAPEUTICS
avexis
Spark THERAPEUTICS
Orchard therapeutics
ALERION
Sangamo THERAPEUTICS
agtc
NOVARTIS

FOOD AND DRUGS AUTHORITY
FDA
GHANA
Your Well-being, Our Priority

REGULATION OF CELL AND GENE THERAPY

IN LOW- AND MIDDLE- INCOME COUNTRIES (LMICs)
THE CASE OF AFRICA

PRESENTED BY : ERIC KARIKARI-BOATENG

OUTLINE:

- Definition of Cell and Gene Therapy
  - US-FDA
  - EMA
  - WHO
- Regulation of Cell and Gene Therapy Products (Current Situation in LMICs with reference to Africa)

OUTLINE

- The perspective of LMICs for acceptance of CGTs
- Way forward
- Conclusion

Definition

- **Gene therapy** is a technique that modifies a person's genes to treat or cure disease.
- Human gene therapy seeks to modify or manipulate the expression of a gene or to alter the biological properties of living cells for therapeutic use. (**US- FDA**)

- **Advanced Therapy Medicinal Product** (**ATMP**)

A medicine for human use that is based on genes, cells, or tissue engineering (**EMA**).

Definition

- **WHO**

Advanced therapy medicinal product (ATMP): any cell or gene therapy product or tissue engineered product that has been substantially manipulated and/or performs a different function in the recipient than in the donor. (**WHO, report on consideration in developing regulatory framework for HCTs and ATMPs, 2023**)

Definition

- **WHO**

Cell therapy product: a product composed of human nucleated cells intended for replacement or reconstitution, and/or for the treatment or prevention of human diseases or physiological conditions, through the pharmacological, immunological or metabolic action of its cells or tissues.(**WHO, report on consideration in developing regulatory framework for HCTs and ATMPs, 2023**)

Definition

WORLD ECONOMIC FORUM (WEF)

- The use of genetic material to treat or prevent disease, involving the introduction of a genetic sequence into cells in vivo or ex vivo

Cell and Gene Therapy (CGT)

- **Mechanism of Cell and Gene Therapy**

Generally, the mechanisms of Cell and Gene therapy includes;

Replacing a disease-causing gene with a healthy copy of the gene

Inactivating a disease-causing gene that is not functioning properly

Inserting a new or modified gene into the body to help treat a disease

This new class of medicine have the potency of curing genetic, infectious and malignant diseases.

They present a one-time approach of treatment for a long-term value.

Regulation of Cell and Gene Therapy (CGT)

- In mid-2022, there were more than 2,000 gene therapies in development, from early-stage research to late-stage clinical testing. The focus is spread across dozens of therapeutic areas, including cancer, neurological, blood, immunological, and cardiovascular diseases.
- How many of these therapies are found on the continent?
- How many trials in these novel therapy take place in LMICs?

Regulation of Cell and Gene Therapy (CGTs)

- Clinical research remains in High Income Countries (HICs) whilst LMICs carry nearly 90% of disease burden.
  (WEF report on Accelerating Global Access to Gene Therapies, 2022)

Data On Clinical Trials (1991-May,2018)

source: clinicaltrials.gov

Region	Number of Studies
World	2,74,049
Africa	7,192
Central America	2,651
East Asia	29,006
Japan	5,028
Europe	77,473
Middle East	11,037
North America	1,23,470
North Asia	4,801
Pacifica	6,648
South America	9,037
South Asia	4,133
Southeast Asia	5,498

Regulation of Cell and Gene Therapy (CGTs)

- In August 2022, there were approximately 1,000 open gene therapy clinical trials (including CAR-T) globally, yet fewer than 5% were recruiting in LMICs (not including China), with only four trials in Africa. (**WEF report on Accelerating Global Access to Gene Therapies, 2022).**

Regulation of Cell and Gene Therapy (CGTs)

Current state and understanding of CGTs in LMICs

- Regulation is at an infantile stage in majority of the NRAs in the LMICs. Only six (6) Africa NRAs have attained WHO Maturity Level 3.
- Absence of guidance / guidelines specific for Cell and Gene therapy.

- Products from Cell and Gene Therapy may be regulated as Biologics.

(WHO consideration in Developing a Regulatory Framework for Human cells and Tissues and for Advanced Therapy Medicinal Products, muscat Oman, 2024)

- Lack of expertise in assessing applications in this critical area in most of the countries on the continent.

The perspective of LMICs for Acceptance of CGTs

There is the need for Cell and Gene therapy in LIMCs. Since biological and genetic diversity varies widely across populations, countries cannot rely solely on gene therapies developed and tested abroad.

CASE STUDY

Uganda	Tanzania	South Africa	Thailand	India
– HIV prevalence 5.5% in adults (0.7% global average)[1]	– ~11,000 babies born annually with sickle cell disease[3]	– 6.7% prevalence of HBV[4]	– 3–9% prevalence of beta thalassaemia among newborns[7]	– More than 1 million new cases of cancer are diagnosed every year (accounting for ~8% of the world's cancer patients)[8]
– >15,000 babies born annually with sickle cell disease (~300,000 globally)[2]		– 7.2 million people living with HIV[5]		
		– 24.6/100,000 males born with haemophilia A[6]		

CASE STUDY

- Five LMICs (Uganda, Tanzania, South Africa, Thailand and India) were examined in a case study to identify essential areas for capacity building to support long term development and delivery of CGTs in LMICs

- These countries from the LMICs class are actively pursuing gene therapies, targeting a broad spectrum of diseases (HIV, HBV, sickle cell disease, beta thalassaemia, haemophilia and some oncology diseases).

Way forward

1. Development of Regulatory guidelines (Guidance document) for ethic commitees and NRAs
2. Need for appropriate patient and public education on the various aspects of cell and gene therapies.
3. High quality studies exploring patient and public opinions and experiences of cell and gene therapy are required.
4. Building of capacity of ethics committees in clinical trial authorization for Cell and Gene therapy.
5. Regulatory capacity building including the provision of training and any other means of support to LMICs in strengthening regulatory systems and staff.
6. Development of international, regional, and national guidelines on regulation of CGTPs for a range of topics including good tissue practices; good manufacturing practices; tissue traceability etc.

Way forward

7. Technical assistance for review of CGTP applications through Clinical Trial Authorization.

through marketing authorization, post-licensure monitoring and long-term follow up

8. Practice of regulatory reliance on decisions of more advanced NRAs and/or WHO

9. Funding exploratory gene therapy R&D appropriate for LMICs infrastructure.

10. Community engagement from the beginning of R&D to improve accessibility, affordability and acceptability

Way forward

- Encouragement of clinical trials in LMICs through building site infrastructure and training investigators

Conclusion

- From the discussions above, there is clearly the need for Cell and Gene therapy in LMICs and steps must be taken to ensure accessibility since they represent the population with highest disease burden with respect to some diseases.

THANK YOU

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www.fdaghana.gov.gh

Ending disease in Africa

Strategic approach to developing capacity and providing regulatory oversight for gene therapy clinical trials in Africa

Dr Kwasi Nyarko
AVAREF Coordinator
WHO Regional Office for Africa

Washington, Sept 4, 2024

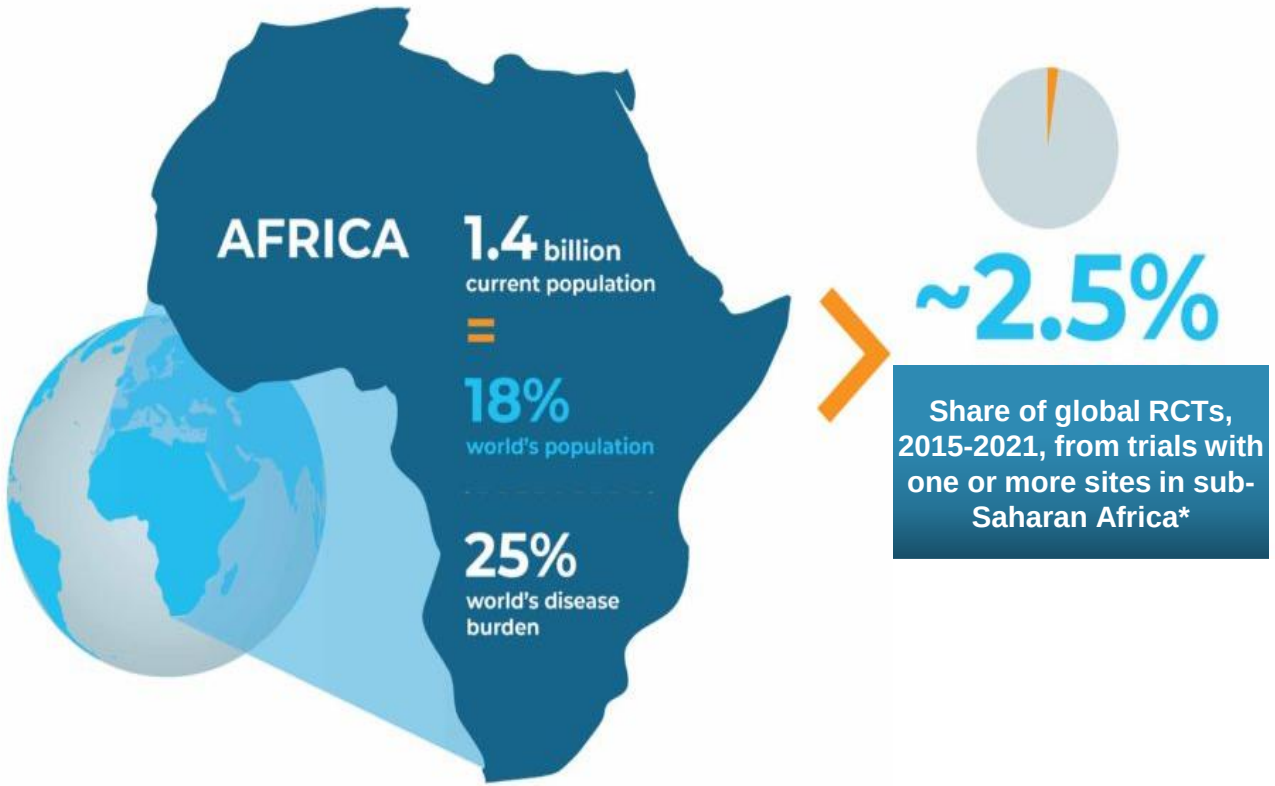
World Health Organization
African Region

UHC/UCN
Universal Health Coverage/Communicable and noncommunicable Diseases

Five points to be covered…

- INTRODUCTION & CONTEXT
- EMERGING CLINICAL TRIAL ECOSYSTEM IN AFRICA
- DEVELOPING REGULATORY CAPACITY FOR CLINICAL TRIALS OVERSIGHT IN AFRICA
- A STRATEGIC APPROACH FOR GENE THERAPY CLINICAL TRIALS IN AFRICA
- THANK YOU

Clinical Trials in Africa



- 30 million < 5 years suffer from vaccine-preventable diseases (VPDs) annually in Africa
- Continent produces less than 2% of its vaccines
- Deficit in African generated data for most medicines
- Majority of genetic diversity in the world exists within Africa
- Emerging Environment initiatives such as African Medicines Regulatory Harmonization (AMRH)/African Medicines Agency (AMA), Continental Free Trade Zone
- The global clinical trials market size was valued at USD 49.8 billion in 2022 and is expected to expand at a compound annual growth rate (CAGR) of 5.8% from 2023 to 2030

AFRICA ….

- Africa is the second-largest and second-most populous continent after Asia with a population of at about 1.5 billion people, about 20% of the world's population
- Youngest population with a median age of about 19 years
- At least 3000 distinct nations with the greatest genetic diversity
- Africa Continental Free Trade Zone – a single marketplace with a population of 1.4 billion estimated to be 2.5 billion by 2050
- African Medicines Agency, a continental regulatory agency, will tremendously influence the future of clinical trials in Africa

Overview of the African Vaccines Regulatory Forum (AVAREF)

A Vision of an African population with timely access to safe and efficacious medical products of assured quality

Established as an informal network 17 years ago by WHO

Uses a network approach to build technical/scientific expertise, competence, and skills required to support regulatory decision making

Capacity building and training in member countries for both NRAs and NECs including clinical trial optimization exercises…

Collaborating effectively with several partners and stakeholders including AU agencies such as AUDA-NEPAD, US-FDA, EMA, Paul-Ehrlich…

AVAREF Objectives

- To increase the efficiency and quality of reviews and inspections
- To promote Patient Safety
- To increase the timeliness and transparency of regulatory decisions for all interventional trials conducted in Africa
- To accelerate the African Medicines Regulatory Harmonization (AMRH) process, linking all Regional Economic Communities (RECs)
- To stimulate Innovation and Research in Africa
- To enhance emergency preparedness on the continent, in RECs, and in individual countries

AVAREF-BCG Survey Results: Level of Preparedness for Clinical Trials

Processes and Tools

- Many do not have documented processes
- Processes are often **not streamlined** for efficiency
  - 60% of reviews conducted **sequentially**
  - High **reliance on standing committees** which may meet monthly

Organisation and Governance

- Most countries have NRAs and NECs
- Regulatory decisions made by NRAs or NECs or both, MoH
- Majority have dedicated units for CTAs
- **Limited collaboration between NRA & NEC**

Human Resources

- NRAs **have limited capacity allocated to CTA review**
- Small pool of evaluators
  - **Average of ~2 FTEs for review of clinical trial review**
  - **NECs have an average of ~4 FTEs**
- L**ow volume of applications**: only 6 countries declared receiving more than 1 application per month

Digital Infrastructure

- ~93% of NRAs and ~84% of NECs have requested support in digitally enabling their processes

Overall Assessment

- 4 High: 23 Medium: 4 Low
- Low/Medium
- 28 - Human resources
  - 27 - Digital infrastructure
  - 23 - Processes and harmonisation
  - 22 - Org & governance

Human Resources – Expert Evaluators a Critical bottleneck

The Current Needs of the Clinical Trial Ecosystem

A thriving ecosystem for clinical trials in Africa

- Low numbers of clinical trials: limited phase I, few First In Africa (FIA) studies, Complex trials designs

Strategic Approach to Human Resource Capacity Building

- Lack of expertise and experienced evaluators (reviewers) in NRAs
- Insufficient training and/or development opportunities

Effective and efficient processes

- Non optimization of processes for managing and reviewing clinical trial dossiers
- Need of digital infrastructure

Opportunities for effective collaboration

- Need for collaboration amongst the several initiatives
- Limitation on Reliance mechanisms

A model for operationalization of AMA which builds on the gains made by initiatives such as AVAREF and support for continued involvement of interested and affected stakeholders and partners

Challenges Identified by NRAs/AVAREF

- Low Clinical Trial Activity (Quantity, Quality, Diversity) levels relative to international counterparts.
  - Low numbers of clinical trials (limited phase I, few First In Africa (FIA) Studies, Complex trials designs (Adaptive designs, CHIM studies).
- Inconsistent time to final regulatory decisions after benefit-risk assessments made at joint review sessions. Overall time to regulatory decision still below that of international counterparts
- Limitations on Technical expertise, digital infrastructure, and other resources impedes a thriving clinical trial ecosystem. Inexperienced Evaluators (Reviewers).
- Reliance mechanisms that are not the best fit for Continent.

Maturity Levels of Member States for Clinical Trials

Majority of countries (n=32) are on ML1

- 27 were based on self-benchmarking
- 5 were benchmarked by WHO: Burundi, Malawi, Mozambique, South Sudan, Uganda

- **Three countries achieved ML2**
  - **Ethiopia and Kenya (WHO benchmarked),**
  - **The Gambia (self-benchmarked)**

- **Six countries achieved ML3 based on WHO benchmarking: Ghana, Nigeria, Rwanda, and Tanzania, Zimbabwe**

- **CAR, Eritrea, Mauritius, Lesotho, Sao Tome & Principe are yet to be benchmarked**

State of Regulatory Oversight for the Clinical Trials in Africa

- Over the past 18 years, Africa has seen marked increases in regulatory capacity for medicines
- A similar increase in harmonization of regulatory and ethics processes for oversight
- Reliance mechanisms have been essential in the progress to date
- This success is limited in nature when placed in the larger context.

Sickle Cell Disease in Africa

- Sickle Cell Disease (SCD) in Africa
  - 66% of the 120 million people living with SCD worldwide live in Africa.
  - Around 1,000 new babies are born every day with a high risk of SCD, making it the most widespread genetic disorder in Africa region.
  - SCD is most prevalent in Africa, affecting about 800 out of every 100,000 people.
  - 6.4% of under-five mortality in Africa is attributed to SCD.
  - 50-80% of infants in Africa born with SCD die before the age of 5 years.
  - 38,403 deaths from sickle cell disease in 2019, a 26% increase since 2000.
- Sickle cell disease (SCD) needs urgent attention. In many countries, there are no or limited newborn screening programs.
- African countries can improve SCD management and control through a comprehensive SCD management approach focusing on prevention, screening, and management strategies.
- Access to advanced therapies such as cell, tissue, and gene therapies will change the management of SCD

AVAREF 4 Reinforcing Pillars of Action

Excellence in Capacity Building

- Training Regulatory Reviewers – Fundamental & Specialized
- Training for Ethics Reviewers
  Synchronization of Ethics reviews with regulatory reviews
- Multi-Country Joint Review of Clinical Trial Applications (CTAs)
  Digital Platforms
- Regulatory Harmonization – Tools, Guidelines, Framework development

Partnerships and Alliances for Results

- African Union Organizations
  Africa CDC, AUDA-NEPAD
- Competent International Regulatory Agencies and Institutions
- Donors, Development Partners such as BMGF, Wellcome Trust, CEPI, etc.

Trusted Expertise - Specialized and/or Technical Support

- Emergency Registration of medicines such as Covid-19 Vaccines, Ebola Vaccines, Malaria Vaccines, etc.
- Rosters of Expertise to support NRAs, NECs, and realization of African Medicines Agency
- Technical Committees for Clinical Trials

Life Cycle Approach

- Regulatory and Ethics Involved in all aspects of Innovation cycle from design, research, commercialization, regulation, product registration, post authorizations
- Clinical Trial Sites
- Vaccine Manufacturing

AVAREF Service Offerings for Industry

Clinical Trial Scientific Advice

1

AVAREF Secretariat convenes experts from African NRAs, ECs, and independent experts to provide advice to Applicants.

Clinical Trial Reviews

2

AVAREF Secretariat convenes African NRAs & ECs and coordinates timely and efficient review of clinical trial applications

Facilitated Registrations (including during emergencies)

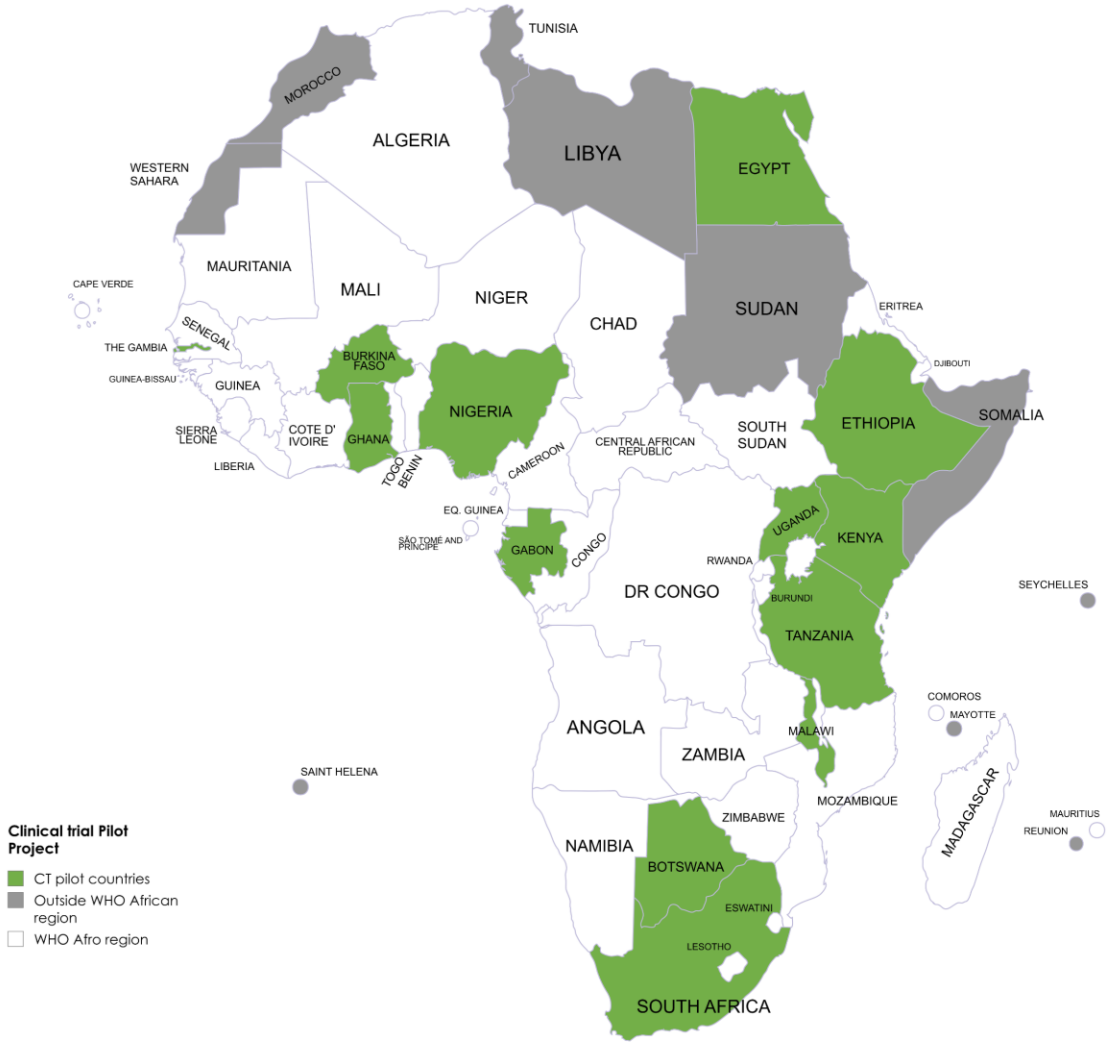
3

AVAREF Secretariat convenes African NRAs & ECs and coordinates timely and efficient review of data for registration of a medical product

CLINICAL TRIALS IN AFRICA – A NEW VISION

- The volume of Clinical Trials in Africa is increasing and this will continue in the coming years
- The Operationalized African Medicines Agency (AMA) will be ready for the influx of these trials
- AVAREF Plus Clinical Trial Pilot is designed to address commonly anticipated challenges (Irritants) experienced by Product Developers
  - Predictability and Consistency
    - Data/Information Requirements for regulatory and/or ethics approval
    - Timelines to decision making
    - Streamlined Processes and/or Procedures
  - High Quality Scientific Advice, Regulatory Decisions
- AVAREF Plus Pilot Designed to Reinforce Capacity for NRAs, NECs, Ecosystem
  - Regulatory Strengthening, Harmonization, and Excellence
  - Pandemic Preparedness
  - Reviewer Development Programs
  - Access to wide range of expertise within the network
  - Access to Expert Specialist Support

AVAREF PLUS Reliance Oversight Clinical Trials Pilot Project



13 Member Countries designated for the Clinical Trial Pilot project based on:

- **Maturity level**
- **Number of Clinical Trials**
- **Involved in AU3S**
- **Regional and linguistic representation**

CONSIDERATIONS TOWARDS A STRATEGIC APPROACH FOR REGULATORY OVERSIGHT

- Africa - 3000 Separate Nations with the most Genetic Diversity
- Africa Continental Free Trade Zone – a single marketplace with a population of 1.4 billion estimated to be 2.5 billion by 2050
- Regulatory Reliance Network consisting of 14 selected African Countries would enhance regulatory harmonization, excellence, and convergence
- Opportunities for convergence of ideas and initiatives for transformation

A STRATEGIC APPROACH FOR GENE THERAPY CLINICAL TRIALS IN AFRICA

- Towards A Thriving Ecosystem for Gene Therapy Clinical Trials in Africa
  - Support Clinical Researchers, Investigators, and Research Institutions
  - Support for Clinical Trial Sites
  - Support Capacity building for Regulatory and Ethics Oversight for Clinical Trials

- Regulatory and Ethics Capacity Building supporting Gene therapy Clinical Trials leveraging AVAREF initiatives such as the Reliance network of member states
  - Include African Sites in Clinical Trials for Gene Therapy
  - Training of Reviewers within NRAs for Assessment of clinical trial applications for gene therapy studies
  - Engagement and Involvement of African Institutions, Researchers, Investigators, Regulators, and Communities in gene therapy clinical trials
  - Support for regulatory reliance network for advanced therapies including clinical trials

Gene Therapy Clinical trials in Africa to facilitate addressing unique health challenges and enhancing global medical research diversity

Enhance Global Health Outcomes
Research in Africa is crucial for developing treatment for diseases prevalent in this region and can lead to interventions that benefit communities worldwide.

Build Research Capacity Locally

Investing in capacity development empowers local researchers, strengthens healthcare systems, and fosters sustainable research practices.

Enhance evidence-based policy- and decision making

Data from clinical research can inform policies for better healthcare decision-making in diverse environments.

Equity

Ensures all populations benefit from advancements in vaccines, diagnostics and treatments

Genetic Equity – for diverse genetics of the continent to be included in research with genetic implications

Engagement and Acceptance

Active involvement of African Regulatory Agencies, Clinical Trial Sites, Scientists, Investigators, Communities, and Patients will enhance ultimate acceptance of resulting therapies and overall buy-in

Strengthening Harmonization, Convergence and Excellence

Enhanced regulatory and ethics capacity will support a thriving ecosystem to support development of innovative, transformative therapies

THANK YOU

AVAREF
African Vaccine Regulatory Forum

For Questions, Suggestions and Comments, contact:
Dr. Kwasi Nyarko
Email: nyarkok@who.int

UHC/UCN
Universal Health Coverage/Communicable and noncommunicable Diseases

World Health Organization
African Region

ethical considerations for gene therapies *in LMICs*

MANEESHA S INAMDAR

Director, Institute for Stem Cell Science and Regenerative Medicine (inStem), Bangalore, India
Professor, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India

Ethical considerations

Informed choice

Access

HOPE AND FEAR

Genome editing technologies should be supported as they promise to fulfil an "unmet need".

Technologies could be used for enhancement rather than treating serious disease -how should these boundaries be set?

What is an unmet need?

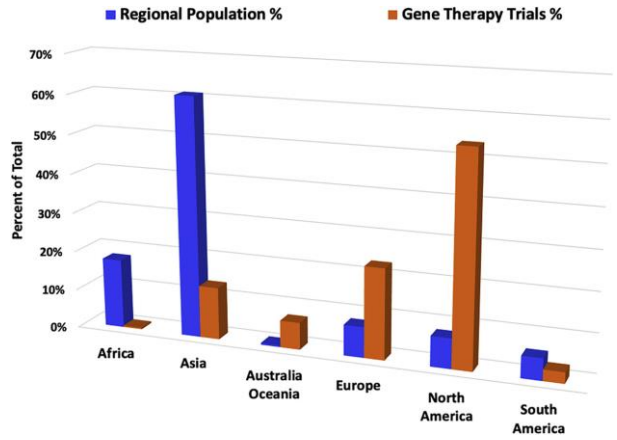
Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity.

Principles, Constitution of WHO

8 of the top 10 most populated countries are LMIC

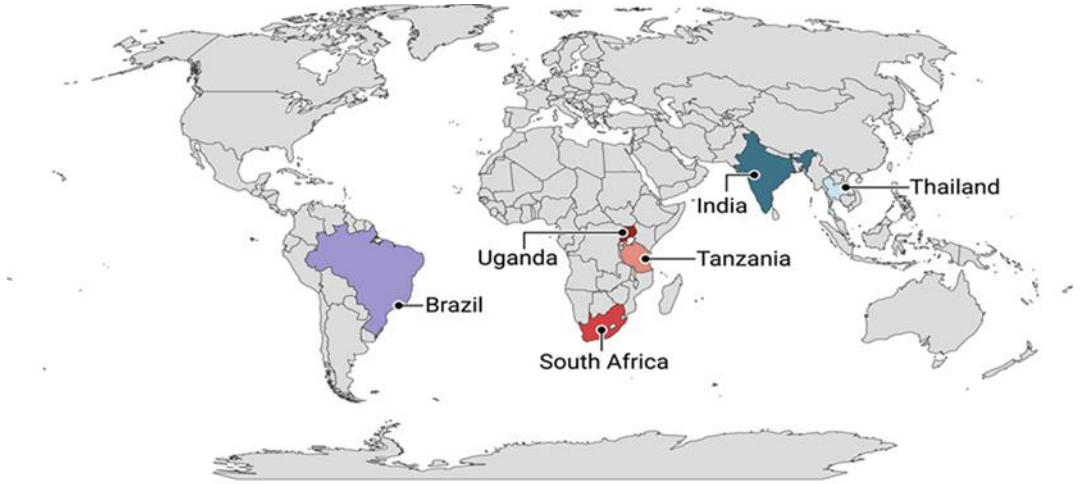
They make up about a third of the world's population

Global gene therapy trial distribution



Where is the unmet need?

Discrepancy in global disease burden and trial sites



Brazil
Population 215.3 million

Hemophilia: 13,618 people living with hemophilia

Number of new cases per year:
Leukemia: 11,859
Non-Hodgkin's lymphoma: 11,093
Multiple myeloma: 5757
Hodgkin lymphoma: 2667

India
Population 1.4 billion

Number of new cases per year:
Leukemia: 49,883
Non-Hodgkin's lymphoma: 39,736
Multiple myeloma: 16,526
Hodgkin lymphoma: 9611

Thailand
Population 71.7 million

β-Thalassemia: 6480 infants born each year with severe β-thalassemia

Uganda
Population 47.3 million

HIV: 1.4 million adults ≥15 years old living with HIV

South Africa
Population 59.9 million

Hemophilia: 2404 people living with hemophilia

Tanzania
Population 65.5 million

SCD: 11,000 infants born each year with SCD

Source: World Population Review

Cornetta K, et. al Gene therapy access: Global challenges, opportunities, and views from Brazil, South Africa, and India. Mol Ther. 2022 Jun 1;30(6):2122-2129

Kevin W. Doxzen et al. The translational gap for gene therapies in low- and middle-income countries. Sci. Transl. Med.16, eadn1902 (2024)

Approved Gene Therapies

Loopholes

- off-target and other unwanted events
- efficiency varies
- *ex vivo*
- *in vivo*

- √ *Scientific feasibility*
- √ *Ethical acceptability*
- √ *Robust oversight*
- √ *Benefit to Society*

What about in LMICs?

"The Committee also recognised that relatively few countries have established an appropriate translational pathway for somatic treatments involving human genome editing, with robust regulation and oversight to ensure patient safety and public confidence."

WHO Expert Advisory Committee on Developing Global Standards for Governance and Oversight of Human Genome Editing

REPORT OF THE SECOND MEETING

Science & Society

Science, Ethics, governance and policy aspects-

- must consider the needs and views of those who will receive the therapy.

WHAT DOES CELL AND GENE THERAPY MEAN TO US?

- Will treat people with genetic disorders.
- May eradicate disease.
- Is it safe?
- Will it change the nature of what it means to be human?

  - **Don’t know/ Don’t understand/….. Not my problem.**

“Roti, kapda, makaan”

Is genome editing a greater cause for worry?

Hydroxyurea- low cost treatment

Health hazards

https://justenergy.com/wp-content/uploads/2019/01/AirPollution-1.jpg

https://regencyhealthcare.in/wp-content/uploads/2019/12/2_Dec.png

https://www.painscale.com/article/how-are-common-side-effects-and-rare-side-effects-defined

https://sigmaearth.com/wp-content/uploads/2023/04/c-users-admin-downloads-minimalist-colorful-organ-7.png

Who is driving the technology?

Who will use the technology?

- Technology is new and rapidly evolving
- Long term impact not completely understood

- Emerging GT are initiated in resource rich country settings and then introduced in low- and middle- income countries (LMICs).
- Could further promote inequities.

- Mechanisms for engagement and governance in LMICs will differ, given the diverse values, beliefs, social and cultural norms and governance systems.
- Could promote *medical tourism* for unsafe or untested interventions in regions where regulation is weak/absent.

- Exploitation by fraudulent use of technology- *ethics dumping* in countries with limited resources and regulatory oversight.

Issues surrounding consent to somatic GT in LMIC populations

Informed choice:

- Competence
- Disclosure
- Understanding
- Voluntariness
- Authorization/Refusal

Who will decide?

- *Informed choice* (consent or refusal) process is complex.
- Statutory age of consent to treatment or being involved in a research varies considerably between countries [1].
- Patients usually appear with family and friends.
- Explanations, technical terms/descriptions need to be in a local language and multilingual.

[1]Regmi, P. R., Aryal, N., Kurmi, O., Pant, P. R., van Teijlingen, E., & Wasti, S. P. (2017). Informed consent in health research: Challenges and barriers in low-and middle-income countries with specific reference to Nepal. *Developing world bioethics*, 17(2), 84-89.

Clinician/ researcher perspective

Informed choice:

Disclosure

Understanding

What/how much information to disclose?

- Likely to be completely subjective; Limited comprehension of informed choice among clinical trial participants.
- Lack of genetic literacy among patient and caregivers resulted in suspicions about access and success of GT [1].

How much did the participant understand?

- Core individual values and religious beliefs against GT [2].
- Acceptance to GT among public is directly related with the seriousness of the condition [3].
- Use of alternative medicines, may have contraindicative effects. May not be understood *by developers of technology.*.

[1]*Salgado R, Moore H, Martens JWM, et al. (2017).Societal challenges of precision medicine: bringing order to chaos. Eur J Cancer 84:325–34.*

[2]*Cornetta K, Brown CG. Balancing personalized medicine and personalized care. Acad Med 2013;88:309–13.*

[3]*Robillard JM, Roskams-Edris D, Kuzeljevic B, et al. Prevailing public perceptions of the ethics of gene therapy. Hum Gene Ther 2014;25:740–6*

Consent and Withdrawal from Trial

Informed choice:

Voluntariness

Authorization/Refusal

- Comprehension of study information and design varies among participants in both high- and low-income settings [1].
- Are they contributing to knowledge production or getting access to a treatment?
- LMIC participants less likely than those in developed countries to say they can refuse participation in, or withdraw from, a trial [1].
- Agreeing to the trail may be the only way to access the therapy.

[1]*Mandava, A., Pace, C., Campbell, B., Emanuel, E., & Grady, C. (2012). The quality of informed consent: mapping the landscape. A review of empirical data from developing and developed countries. Journal of medical ethics, 38(6), 356-365. doi: 10.1136/medethics-2011-100178*

[2]*Cornetta K, Brown CG. Balancing personalized medicine and personalized care. Acad Med 2013;88:309–13.*

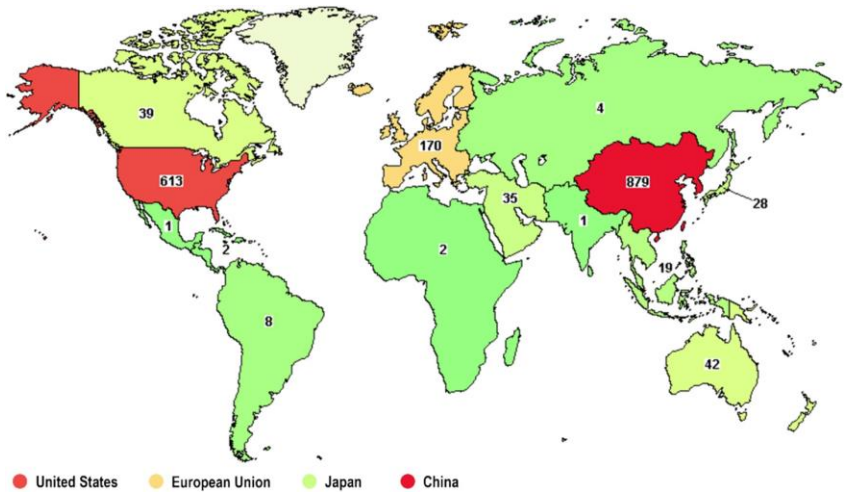
Challenges of conducting clinical trials in LMIC populations

A point to note is that India has a lot of patients, so usually trials have an oversubscription of participants.

- Religious beliefs and traditional practices influence enrollment and sample collection processes.
- A major problem is follow-up / non-compliance.
- Unlike in Western nations, since insurance is not available/used often, non-compliance is inconsequential to patient.
- Thus, studies are difficult to do and usually, trials need to over enroll such that they can account for dropouts.

Ethical issues surrounding access to GT

Clinical trial status of CAR-T cell products worldwide, accessed on April 27, 2024



Concentrated in wealthier regions

Cost of GT is prohibitive.

3,12,91,977- 31,12,41,917 INR

(0.37 to 3.7 million USD)

30-300 times the annual income

Cornetta K, et. al Gene therapy access: Global challenges, opportunities, and views from Brazil, South Africa, and India. Mol Ther. 2022 Jun 1;30(6):2122-2129

Devi, S. K., et. al (2024). Regulatory landscape and challenges in CAR-T cell therapy development in the US, EU, Japan, and India. European Journal of Pharmaceutics and Biopharmaceutics, 114361.

issues surrounding access

- Insurance coverage for genetic conditions and testing is still a work in progress.
- Low-cost government supported insurance does not have rare disease coverage.
- Where therapies are tested and where they are available to patients can differ— approval rate in slower in less wealthy regions.
- Limited infrastructure and capability for storage and recordkeeping in medical facilities– makes long-term follow-up and care difficult.

- Launched in April 2024, developed by IIT Bombay, Tata Memorial Centre and ImmunoACT
- Commercially approved in India for B-lymphomas and B-Acute Lymphoblastic Leukaemia where one or more lines of treatment have failed
- Treatment cost : ₹ 0.4-0.45 crores (US counterpart costs ₹3- 4 crores)

Developing GT for Sickle Cell Anemia

- 8 collaborative institutes including hospitals. Led by **Institute for Genomics and Integrative Biology**. and **Narayana Nethralaya Foundation**
- Funded by the Ministry of Tribal Affairs and the Department of Science and Technology
- *ex vivo* and *In vivo* Gene Therapy approaches

Developing GT for β-Haemoglobinopathies

- base editing and prime editing
- Lentiviral gene therapy - knocking down BCL11A
- CRISPR-Cas9 mediated HSPC gene therapy – for HIV gene

Centre for Stem Cell Research, a unit of inStem, Bengaluru
at Christian Medical College, Vellore

Phase I/II first in human clinical trial in India- First gene therapy for a genetic disorder, Hemophilia A in India

Approved by CDSCO

Lentiviral mediated haematopoietic stem cell-vector based Gene Therapy of Hemophilia A

ii. Novel AAV3-FIX Padua vector based clinical trial for GT of Hemophilia B - Developed a novel HSCs based lentiviral vector mediated gene therapy product for Hemophilia A;

ethical issues surrounding access

Its not just about money

- Limited genetic and clinical workforce that can be applied for personalized medicine.
- De-prioritizing personalized therapy perpetuates existing disparities in scientific and technical capabilities.
- Broad adoption of uniform ethics processes for vulnerable populations varies; people may have more faith on the medical practitioner and may be exploited.
- Lack of uniform diagnostic and treatment paradigms.

Public Engagement and Empowerment

Important to have wide dissemination of information, transparency and responsible stewardship of science

The Way Forward

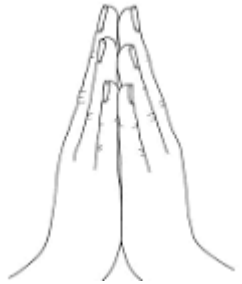
- Even though the technology may be safe from the perspective of science and medicine, opinions of society must be considered.
- Outreach, education and engagement activities should aim to empower people so that they can make an informed and understood choice.
- Most LMICs lack the resources and capacity to do this. Must reduce dependency.
- Capacity building - "…skew subsequent deliberations within LMICs by the force of precedent, despite the potentially very different local circumstances and worldviews".

Global Forum for Bioethics in Research (GFBR) 2019 booklet

Ethics, governance and policy considerations of gene therapy

- Based on current scientific knowledge – (yet nimble).
- In harmony with global action - (yet alert to local needs).
- Must consider differences in ethical views and values, social priorities, culture - within and across nations.
- Must be applicable in multiple contexts – foster changes in behavior of those doing the research.

acknowledgements



- In consultation with and inputs from:
  Dr. Arkasubhra Ghosh, Narayana Nethralaya Foundation
  Dr. Françoise Baylis, Dalhousie University
  - Additional inputs:
  Dr. Joy Zhang

- Literature survey and slides:
  Dr. Sabuj Bhattacharya, iBRIC-inStem
  Dr. Ketan Thorat, iBRIC-inStem
  Dr. Arkasubhra Ghosh, Narayana Nethralaya Foundation

https://image.slidesharecdn.com

Session 2: Panel Discussion





- **Jimi Olaghere,** Gene Therapy Recipient
- **David Williams, MD,** Harvard Medical School
- **Eric Karikari-Boateng, MS,** Food and Drugs Authority (Ghana)
- **Kwasi Nyarko, PhD,** WHO Regional Office for Africa (WHO-AFRO)
- **Maneesha Inamdar, PhD,** Institute for Stem Cell and Regenerative Medicine

Lunch

The meeting will resume at 1 pm ET

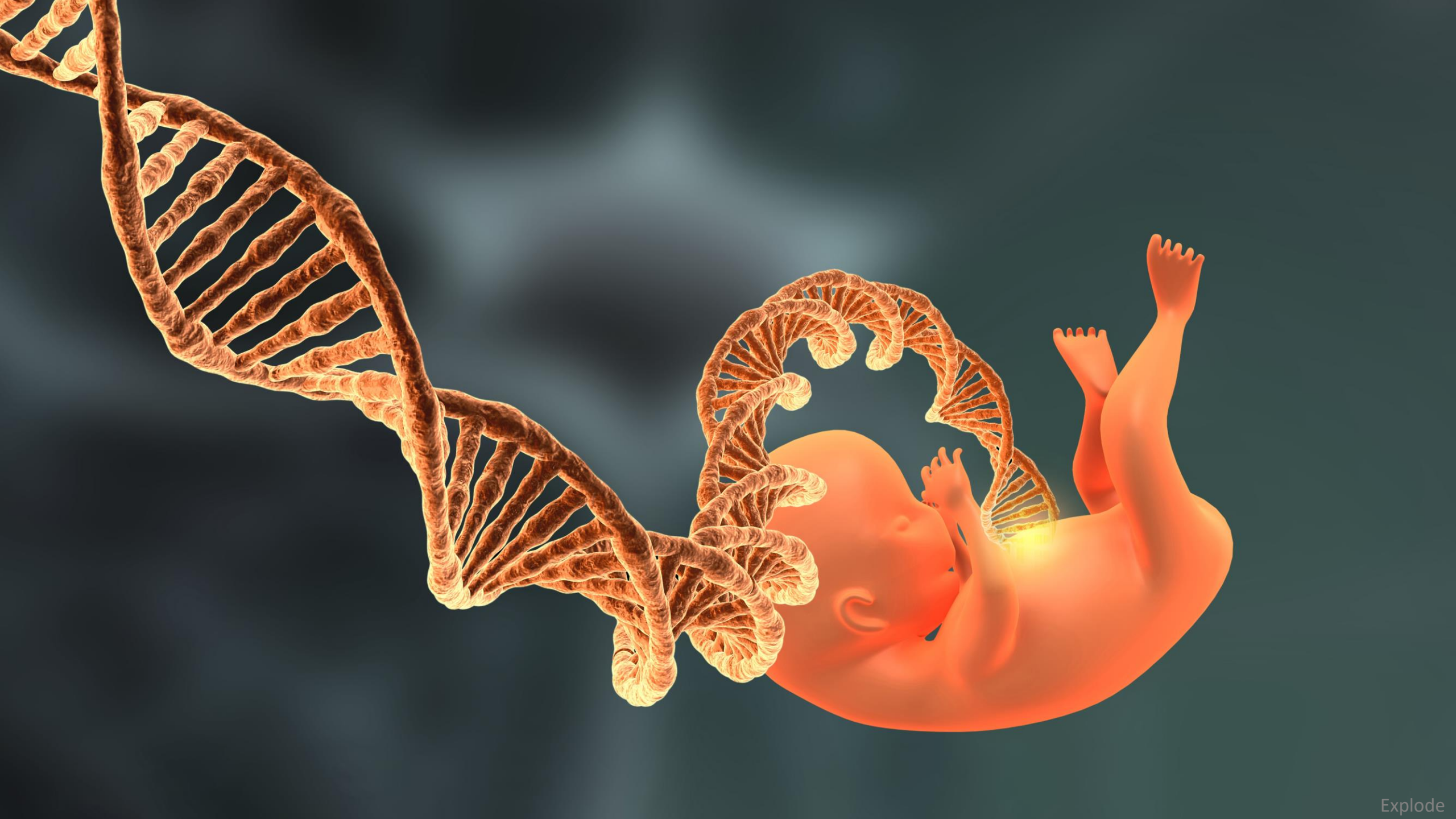
REAGAN-UDALL FOUNDATION FOR THE FDA

Session 3: The Next Generation of Gene Therapies

REAGAN-UDALL FOUNDATION FOR THE FDA




- **David Liu, PhD,** Broad Institute of MIT and Harvard
- **Tony Ho, MD,** Pivotal Lifesciences
- **John Tisdale, MD,** National Heart, Lung, and Blood Institute (NIH)
- **Hildegard Büning, PhD,** Hannover Medical School



Explode

…TGGGCGGAC…

↓

…TGGGTGGAC…

Progeria

…CCTGAGGAG…

↓

…CCTGTGGAG…

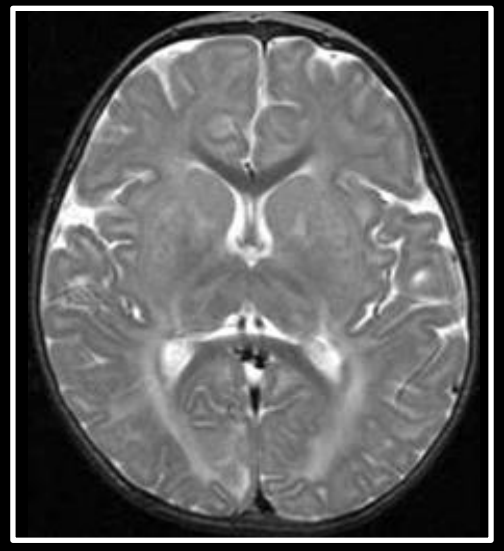
Sickle-cell disease

…CATCTTTGG…

↓

…CATTGG…

Cystic fibrosis

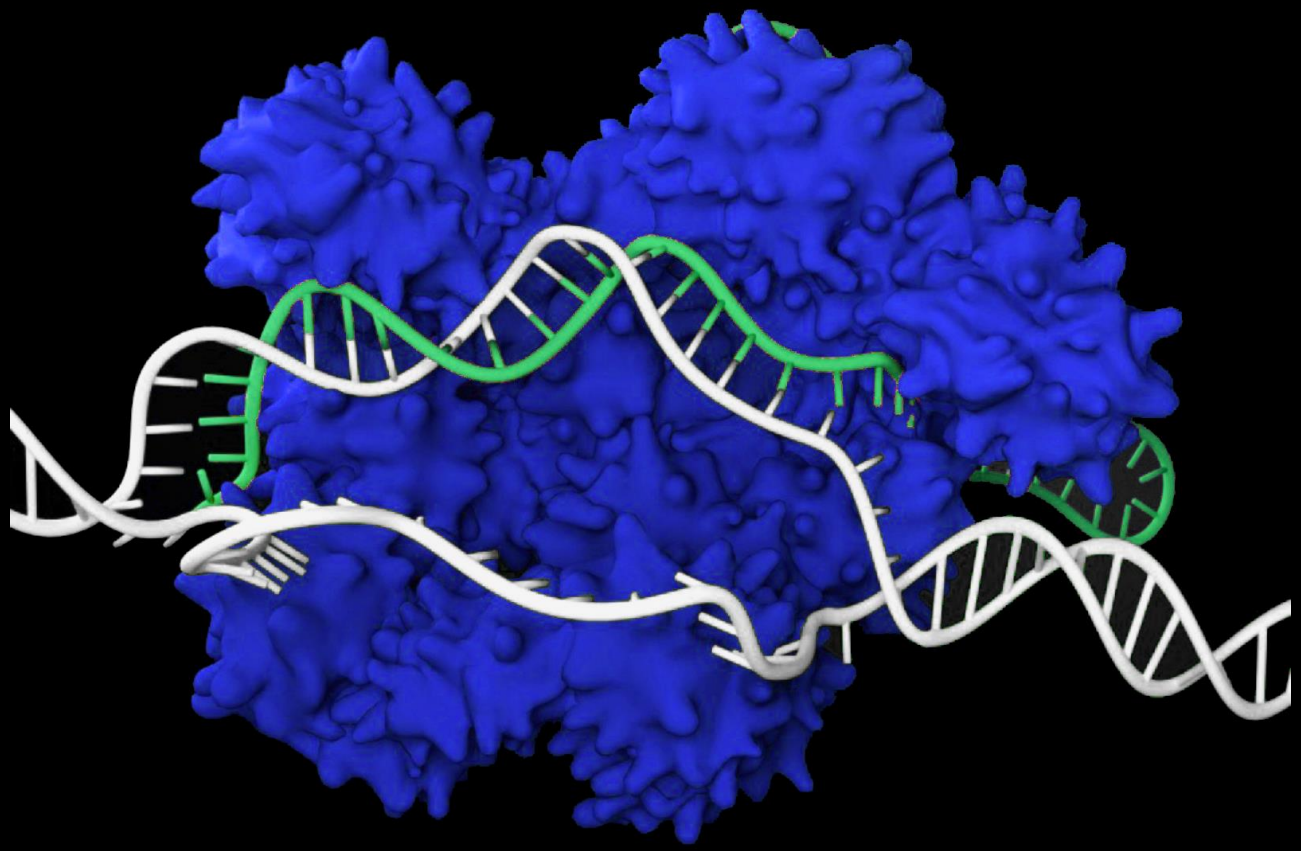


…ATCCTA…

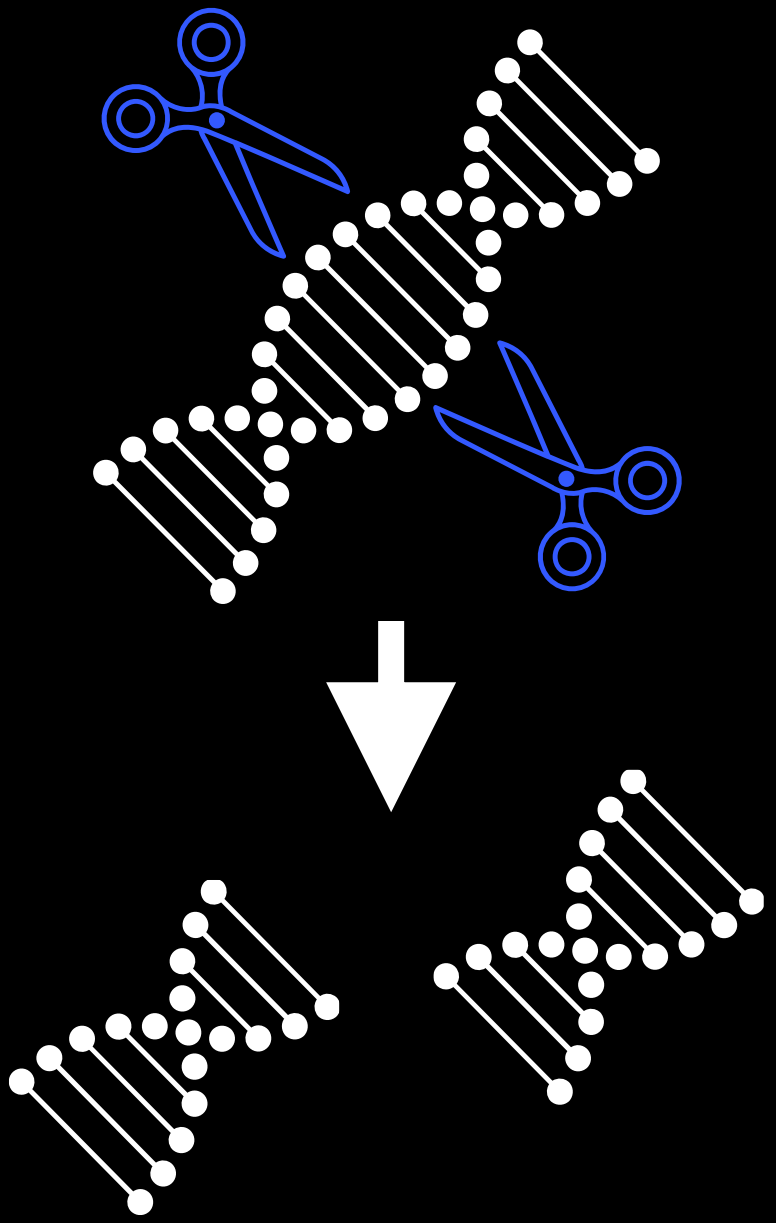
↓

…ATCTATCCTA…

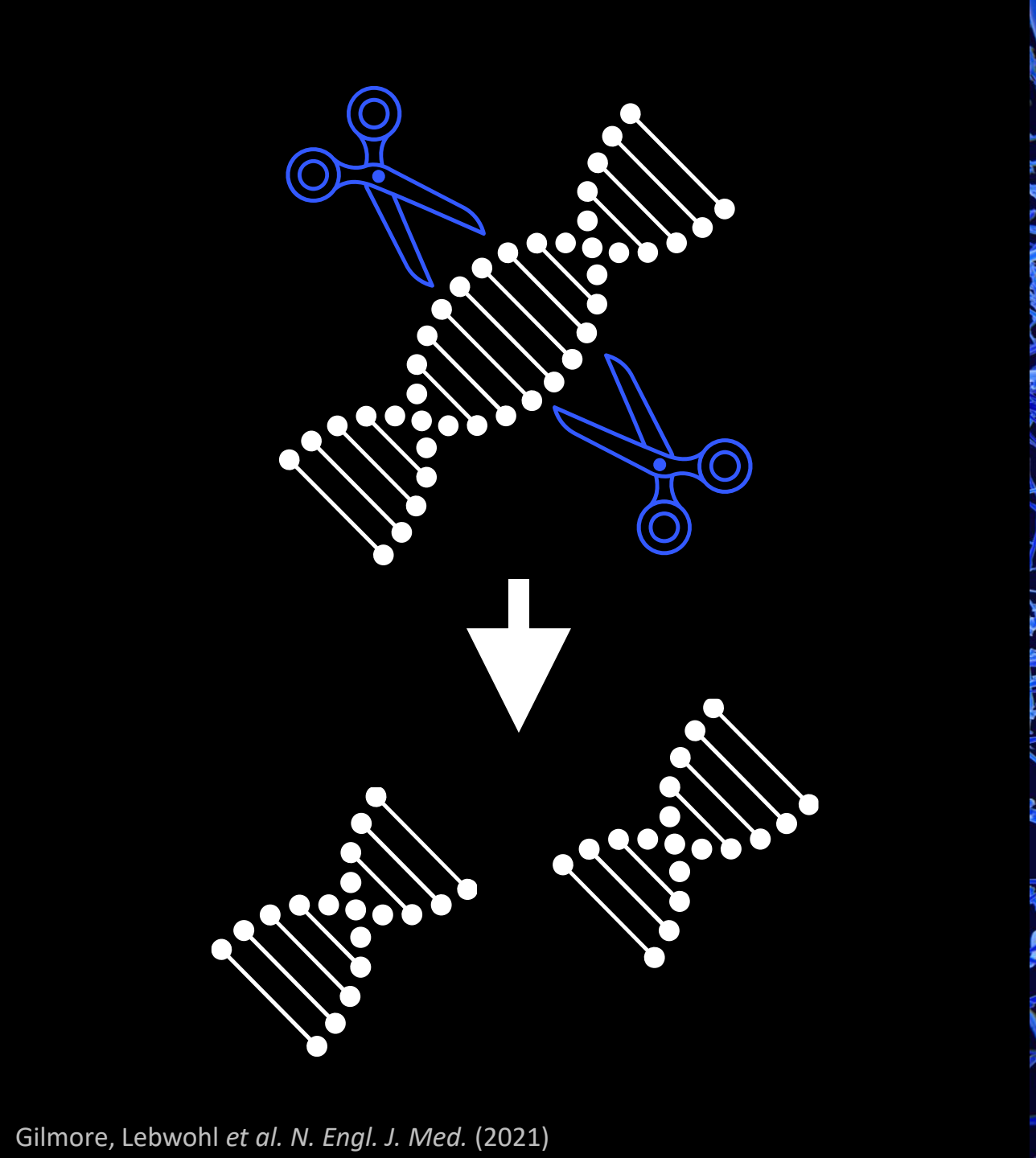
Tay-Sachs disease

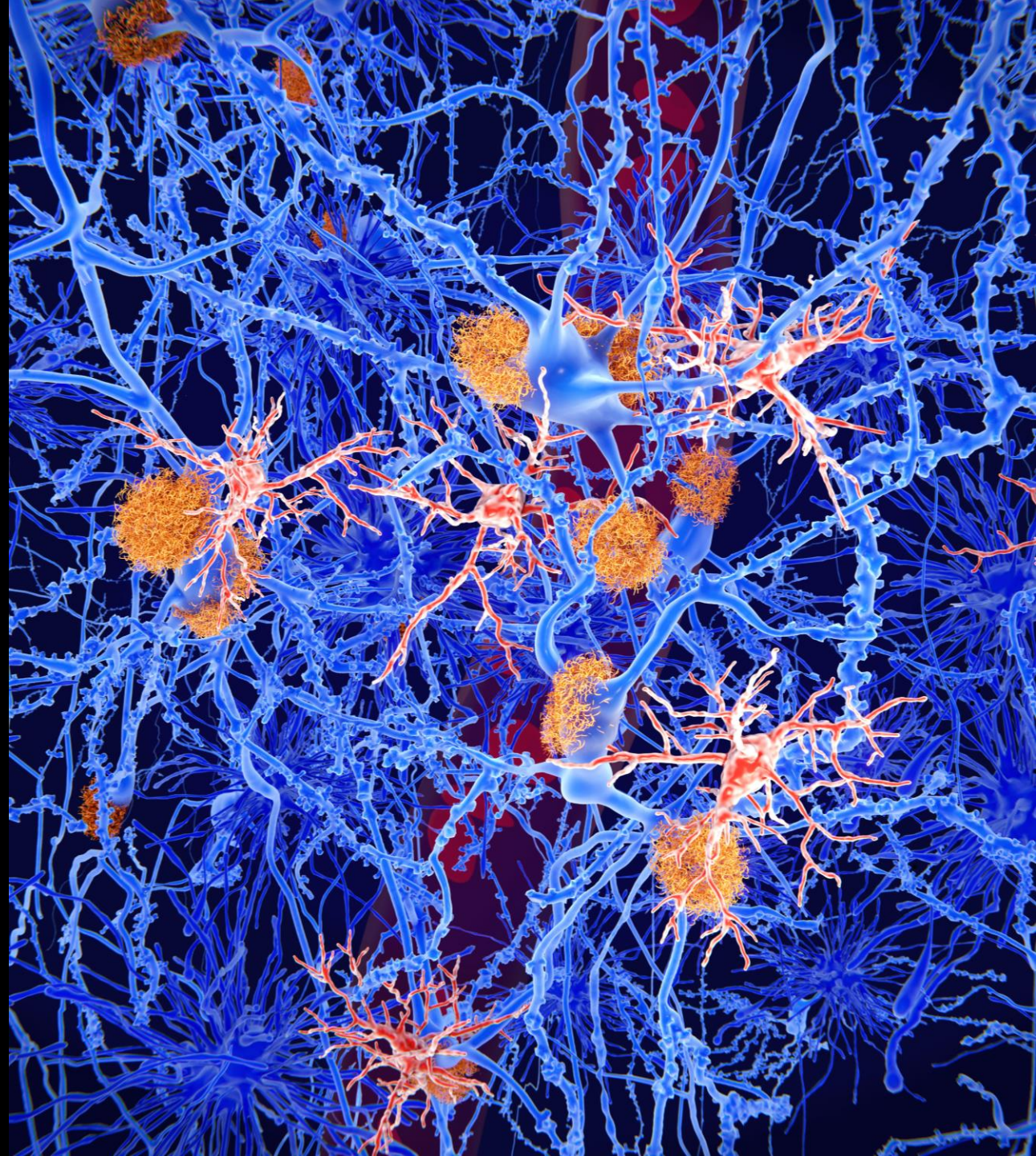


CRISPR-Cas9



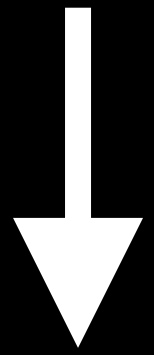
CRISPR-Cas9 gene editing: pioneering work of Charpentier, Church, Doudna, Siksnys, Zhang, and others





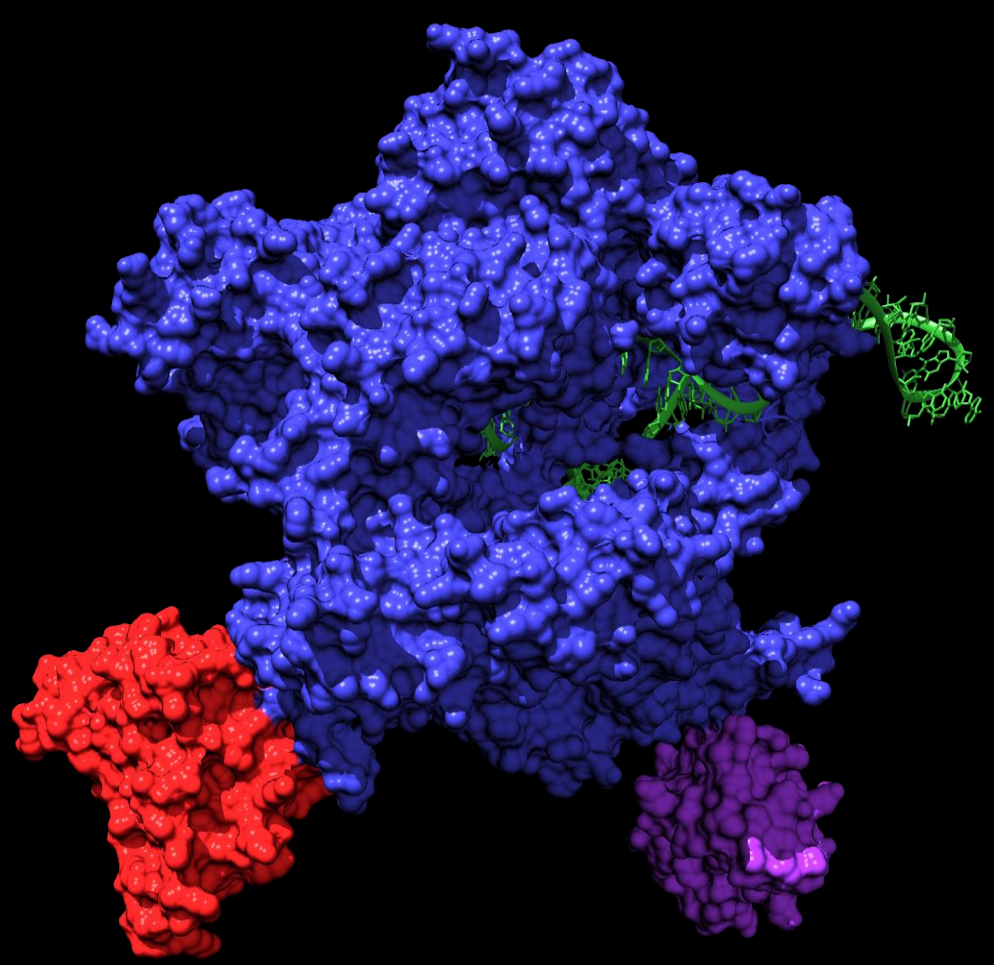
Gilmore, Lebwohl *et al. N. Engl. J. Med.* (2021)

...TGGGTGGAC... Progeria

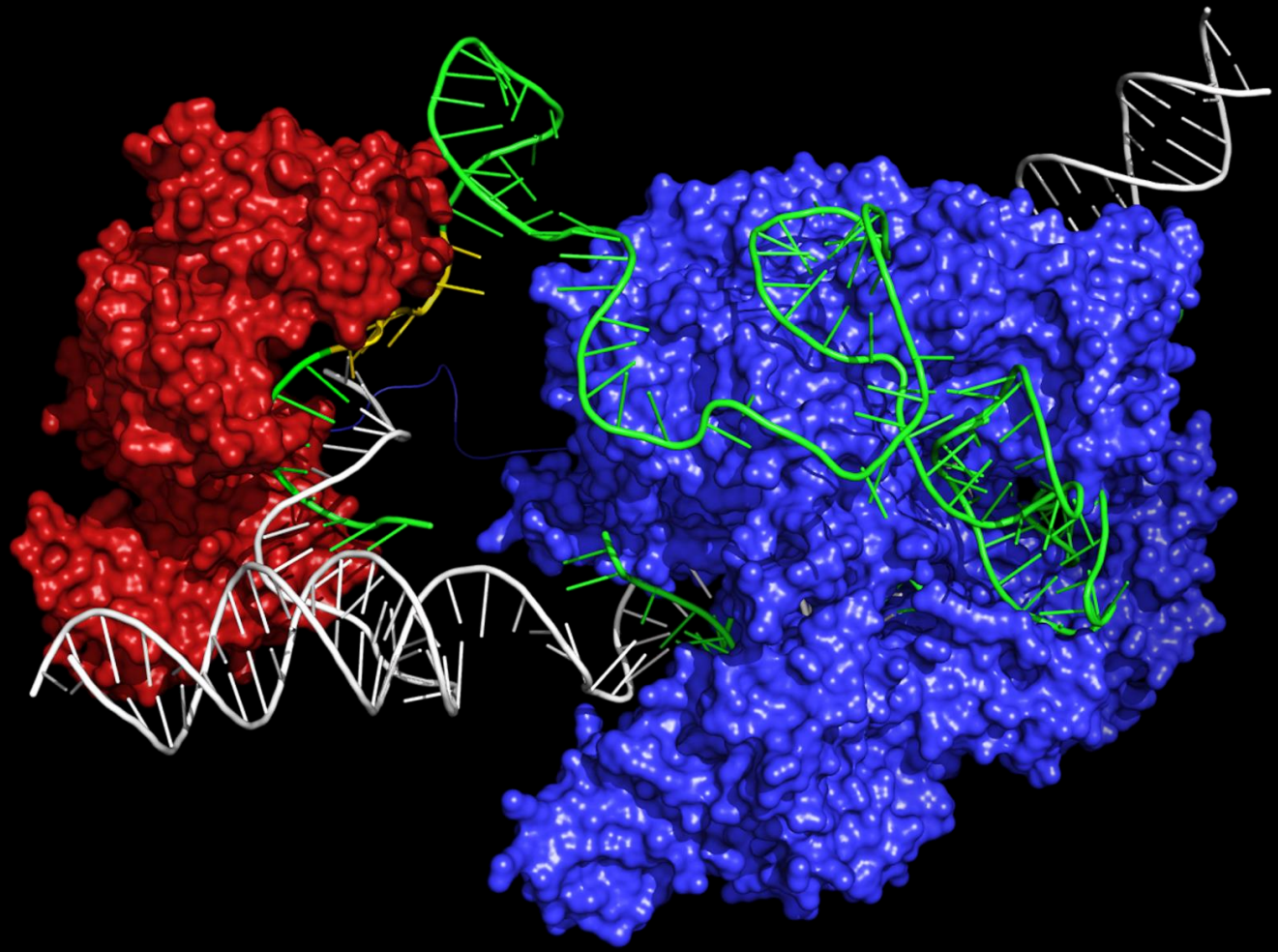


?

...TGGGCGGAC... Normal



Base editor

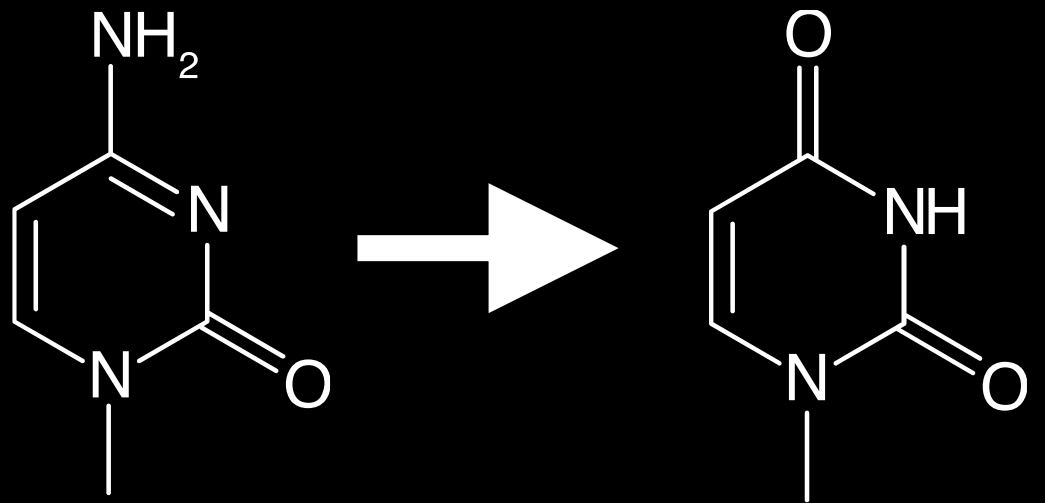


Prime editor

Komor, Liu *et al. Nature* **533**, 420 (2016); Gaudelli, Liu *et al. Nature* **551**, 464 (2017)
Anzalone, Liu *et al. Nature* **576**, 149 (2019)

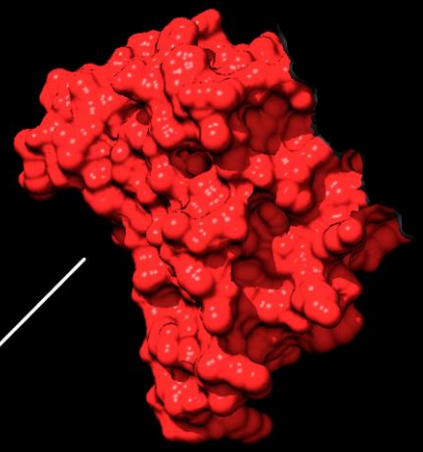
From: David Liu
Date: November 1, 2013 at 4:27:43 PM
To: Alexis Komor

What might be even more interesting is TALE- or Cas9-programmed DNA editors. If you could program a specific A--> G, for example, at precisely one site in the human genome with enzyme-like efficiency and no stochasticity, I think you could really transform genome engineering and possibly human therapeutics.



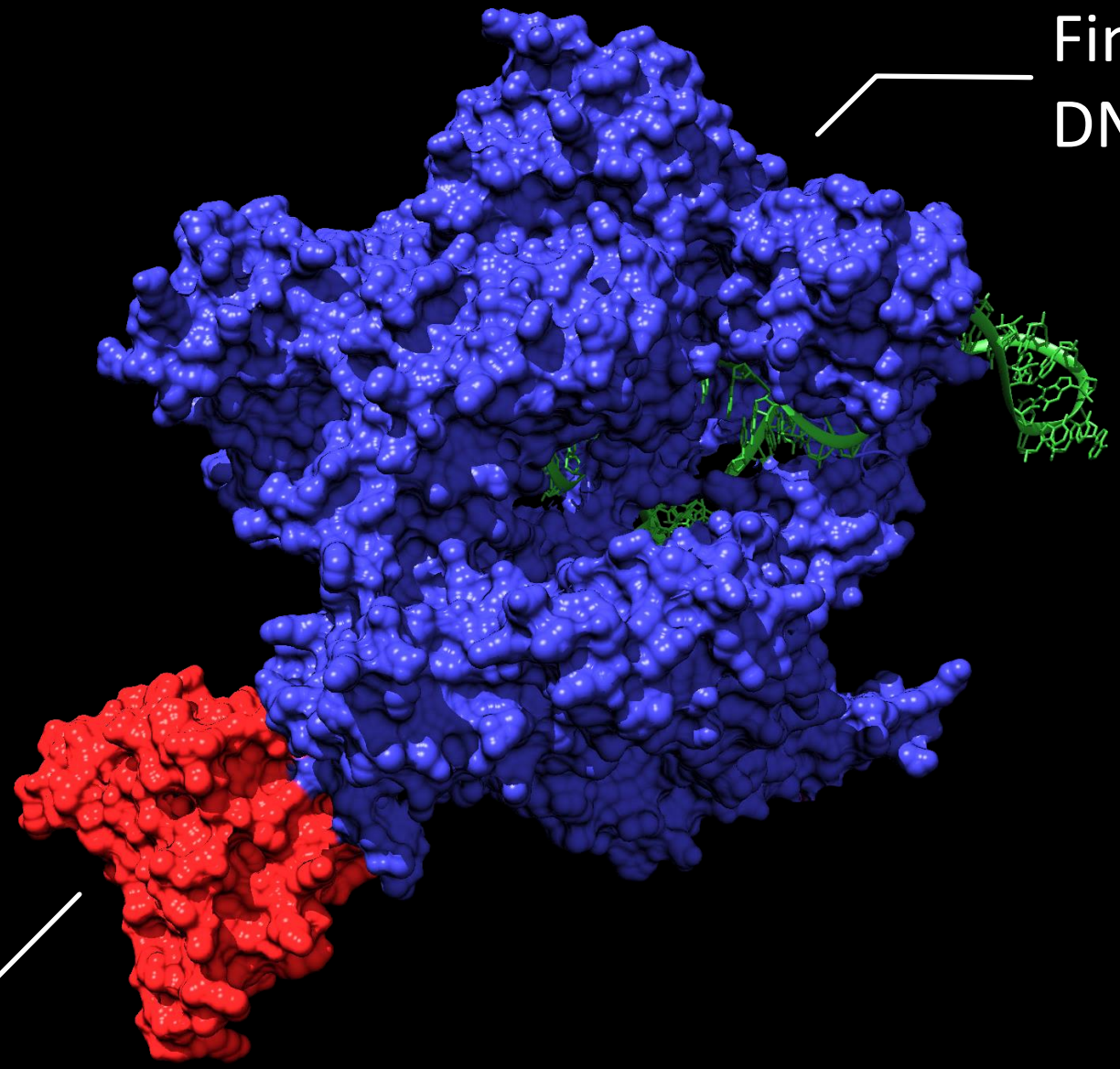
C

“T”

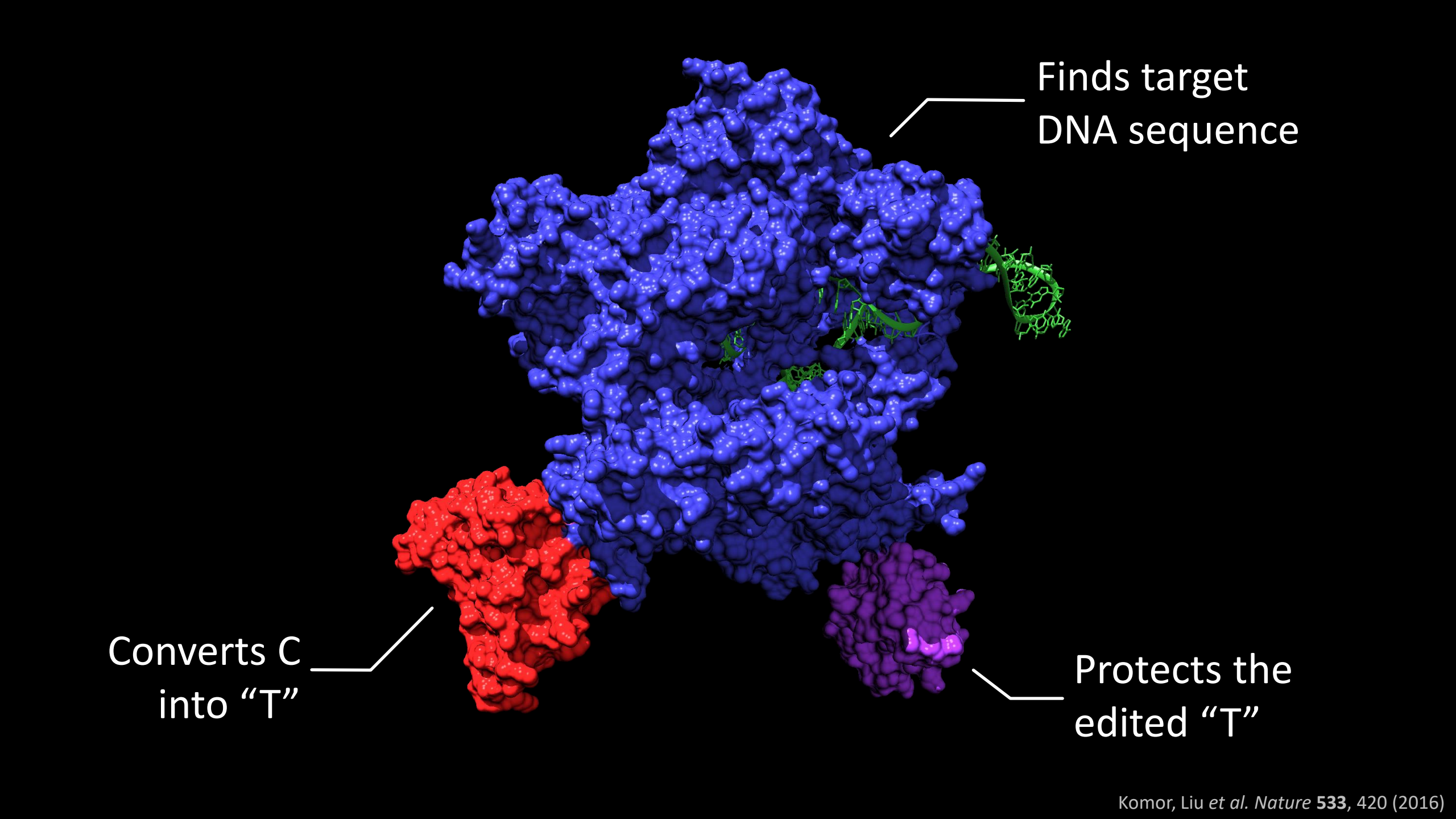


Converts C into “T”

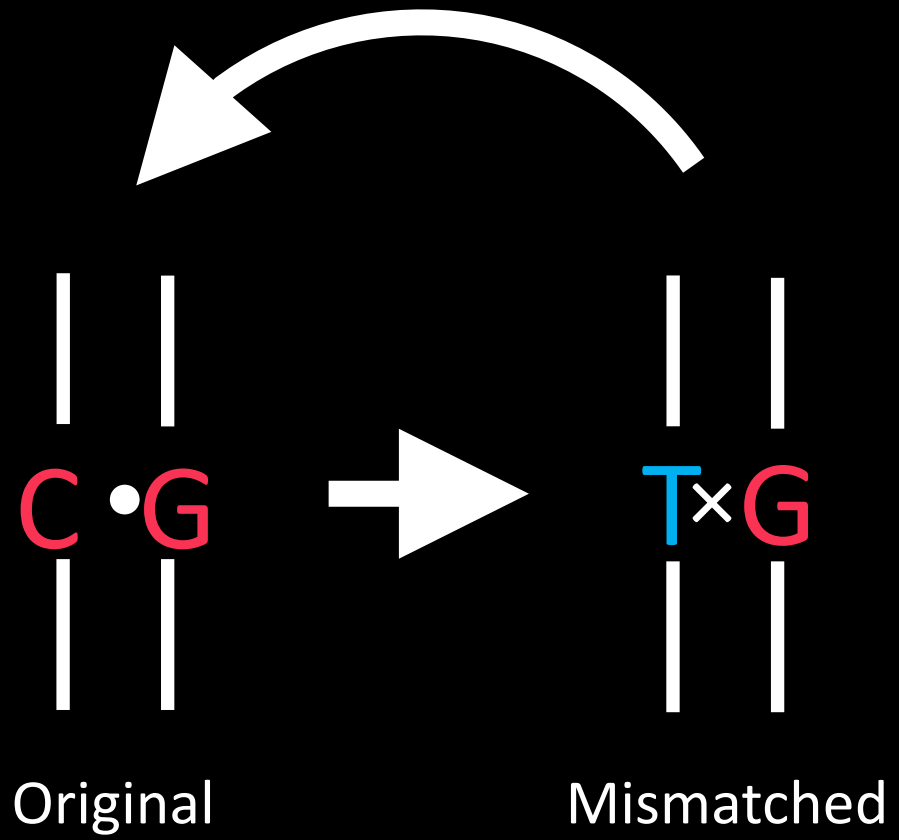
Komor, Liu *et al. Nature* **533**, 420 (2016)



Komor, Liu *et al. Nature* **533**, 420 (2016)



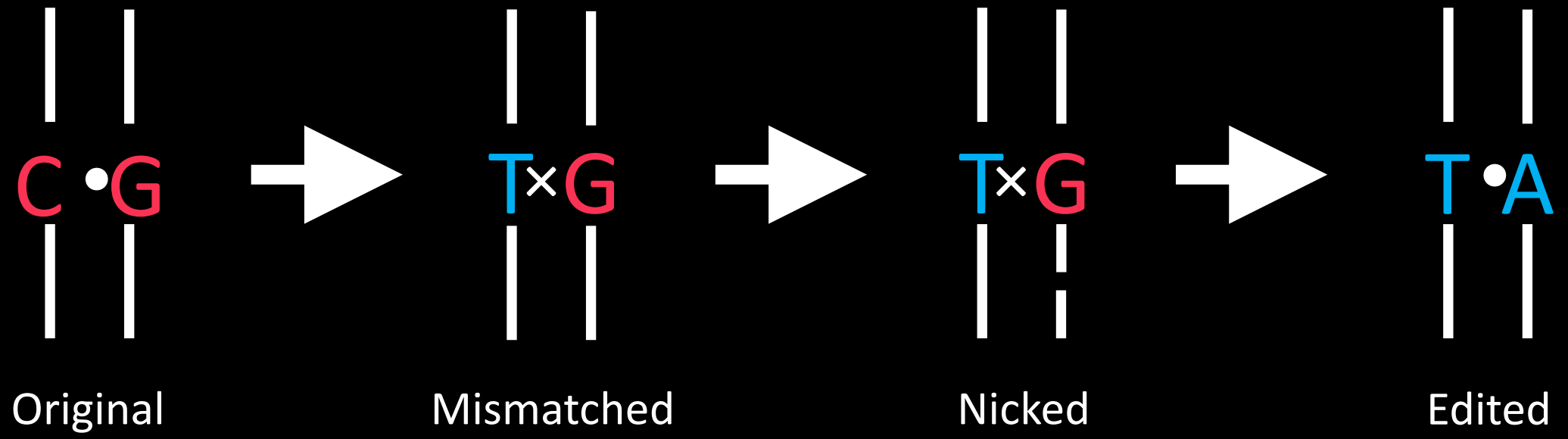
Komor, Liu *et al. Nature* **533**, 420 (2016)



C pairs with G

T pairs with A

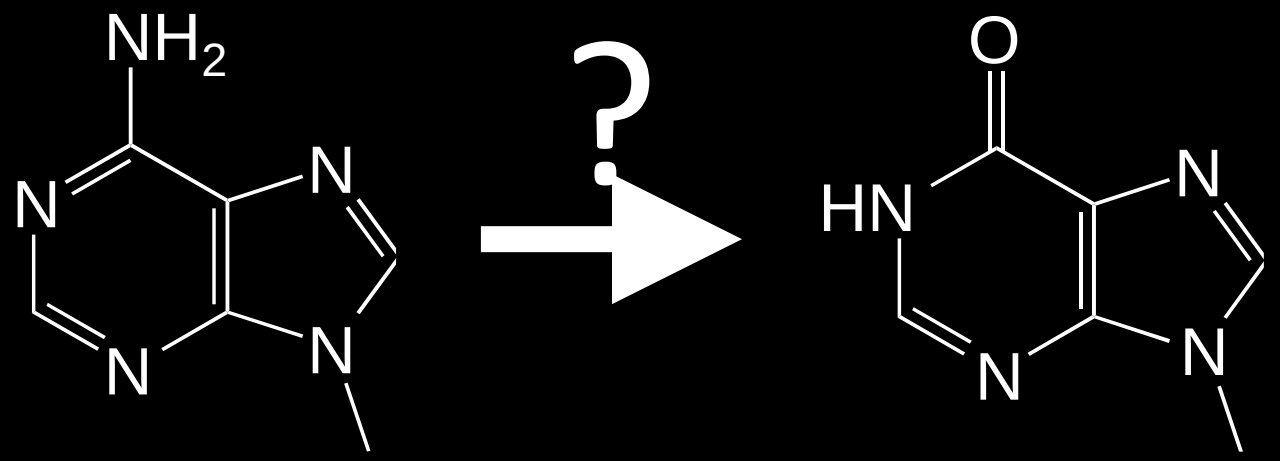
Komor, Liu *et al. Nature* **533**, 420 (2016)



C pairs with G

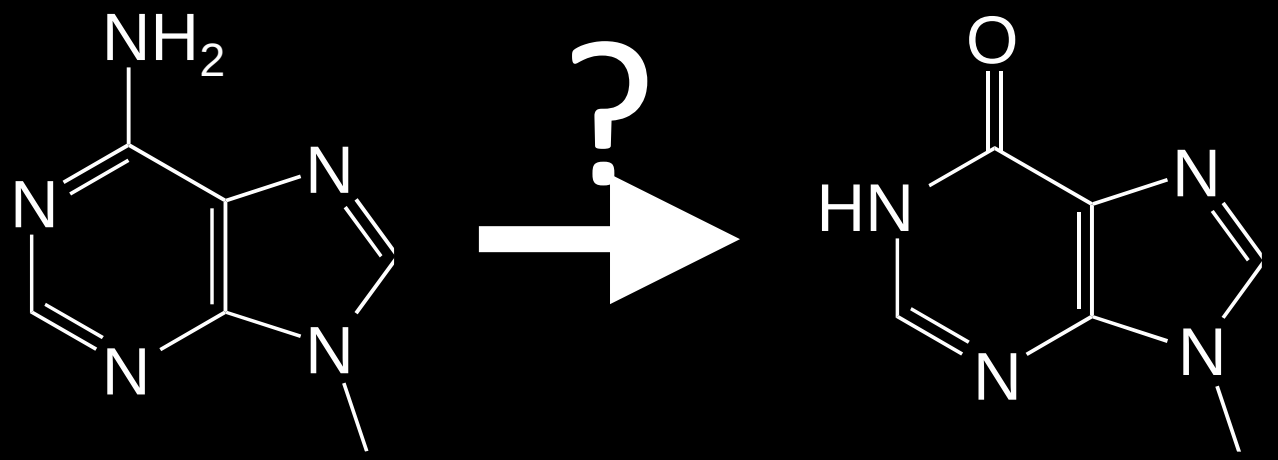
T pairs with A

Komor, Liu *et al. Nature* **533**, 420 (2016)



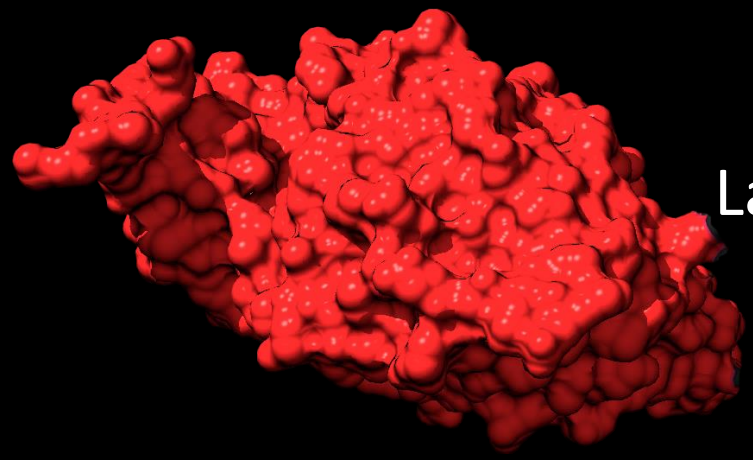
A

“G”



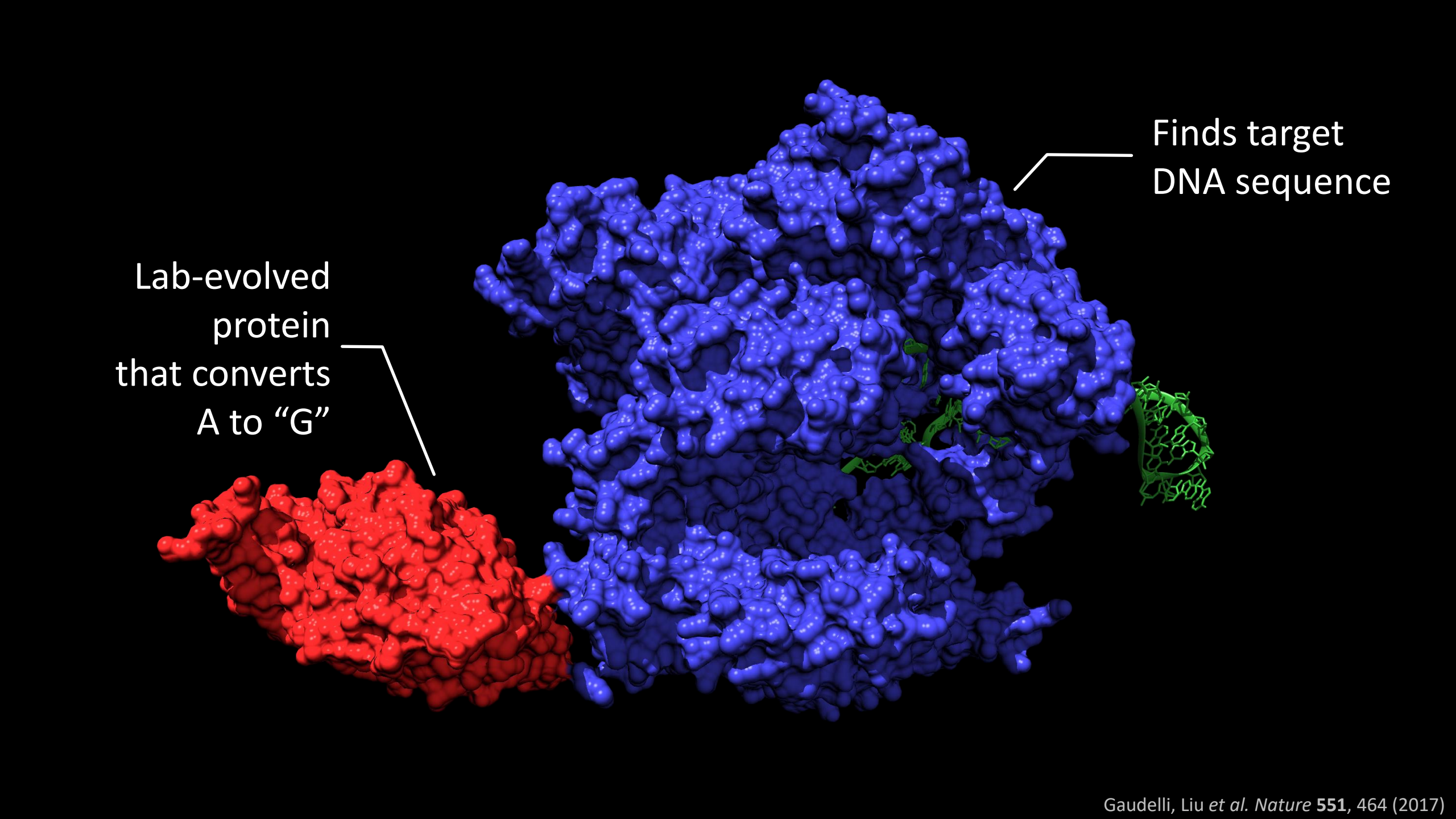
A

“G”

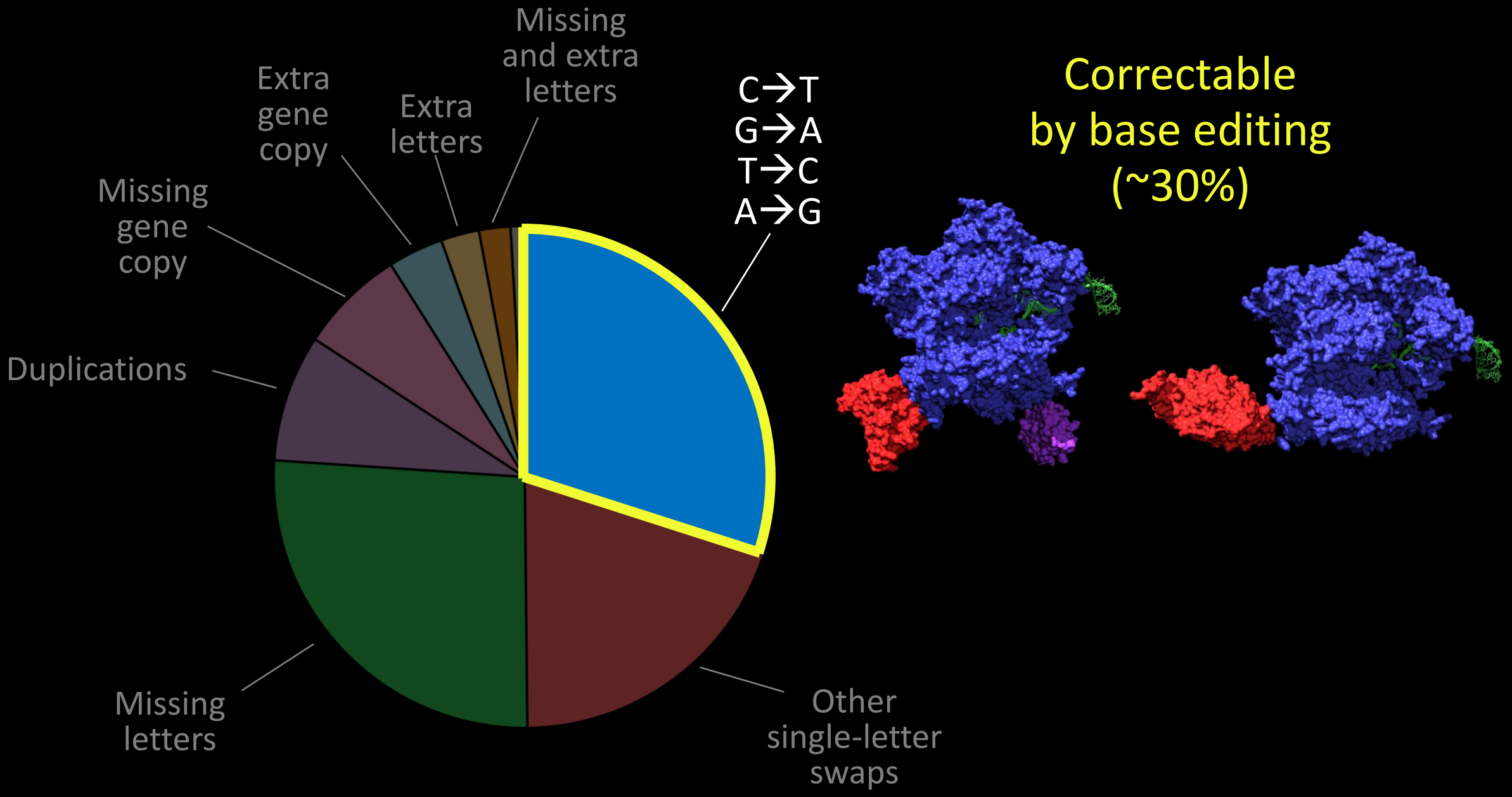


Lab-evolved protein that converts A to “G” in DNA

Gaudelli, Liu *et al. Nature* **551**, 464 (2017)



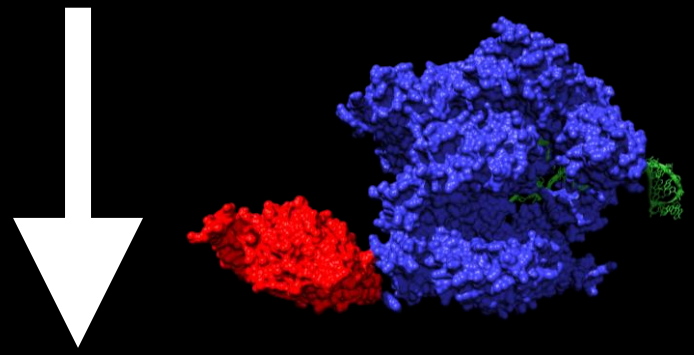
Gaudelli, Liu *et al. Nature* **551**, 464 (2017)



Landrum et al., *Nucl. Acids Res.* 44, D862 (2016), accessed July, 2019

...TGGG**T**GGAC...

Progeria



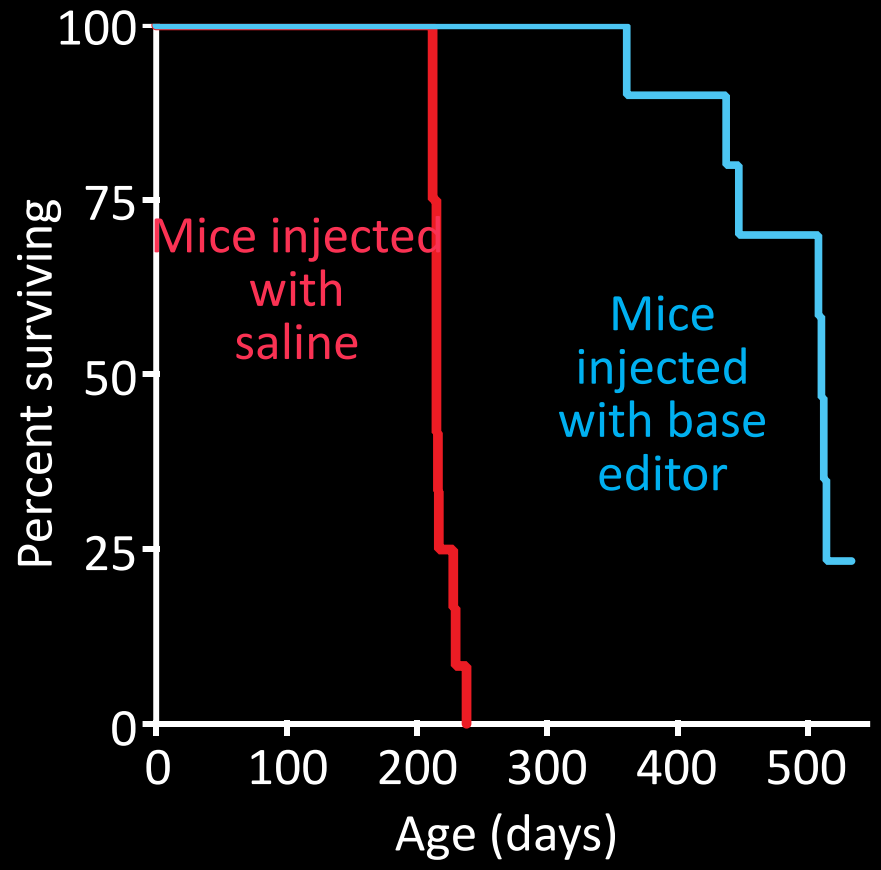
...TGGG**C**GGAC...

Normal

Koblan, Erdos, Collins, Brown, Liu *et al. Nature* 589, 608 (2021)

Untreated progeria mouse
7 months old

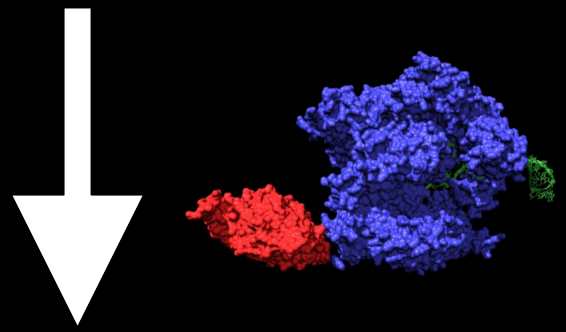
Base editor-treated progeria mice
11 months old



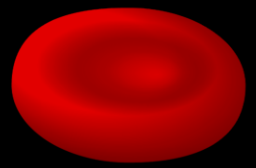
Koblan, Erdos, Collins, Brown, Liu *et al. Nature* 589, 608 (2021)

...ACTCCTGTGGAGAAG...

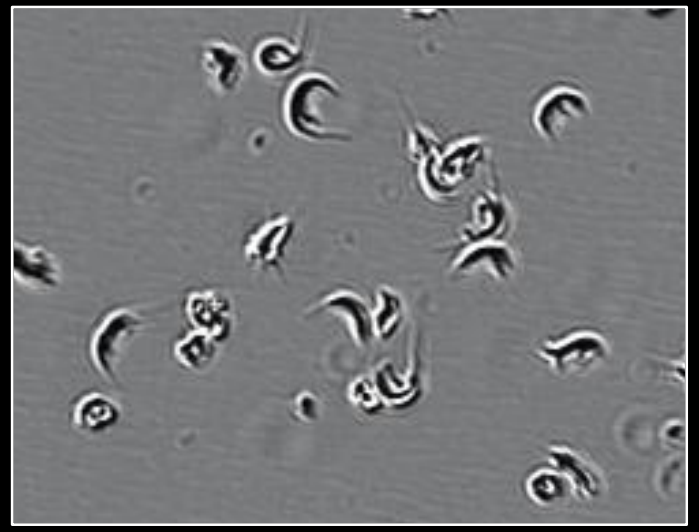
Sickle-cell hemoglobin

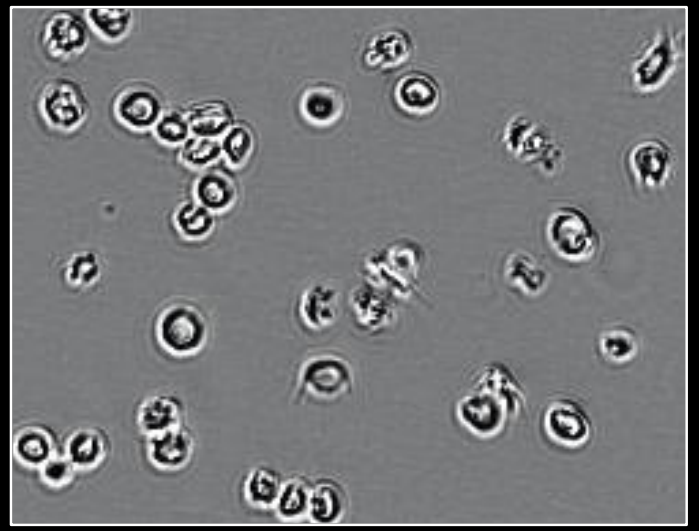


...ACTCCTGCGGAGAAG...

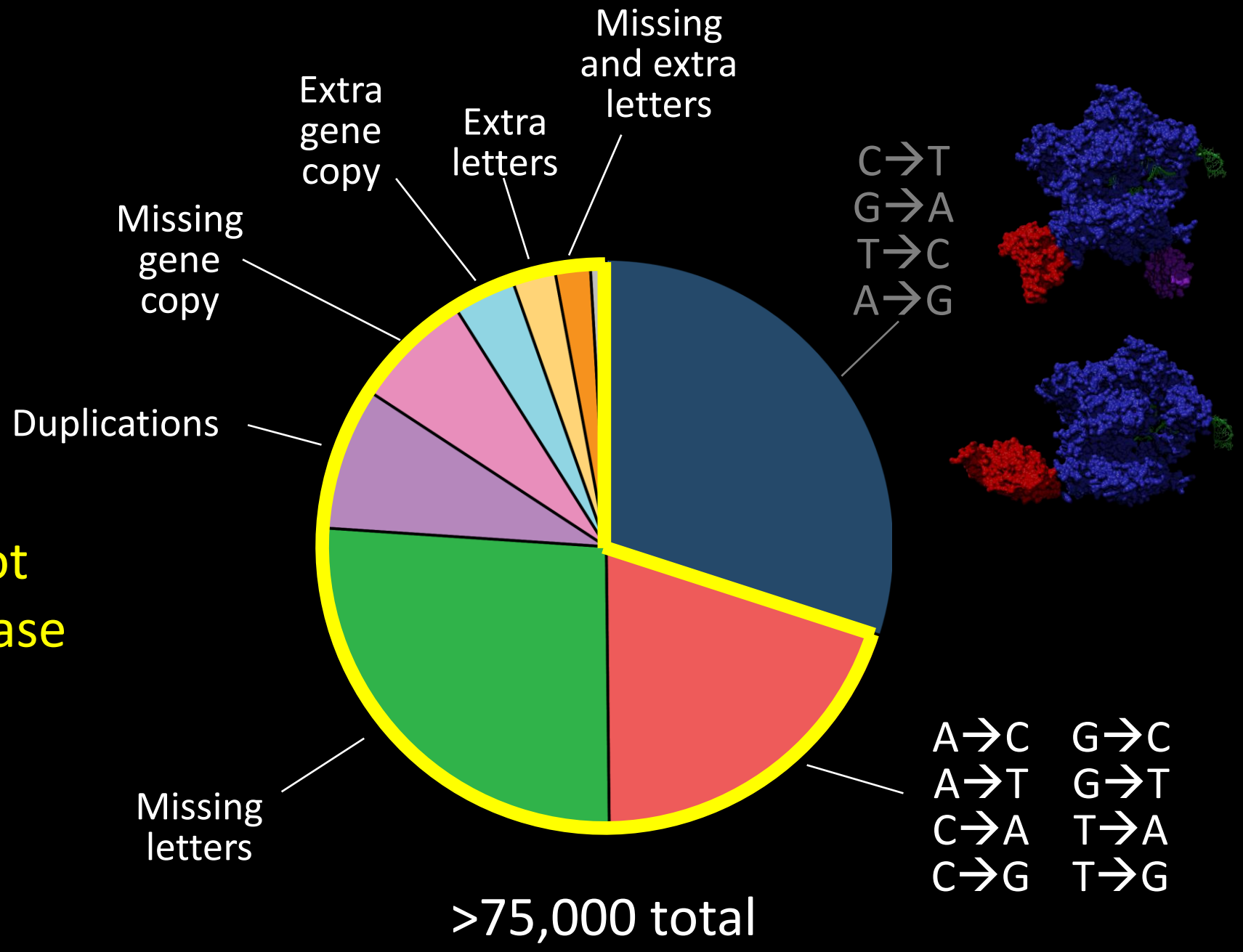


Benign hemoglobin



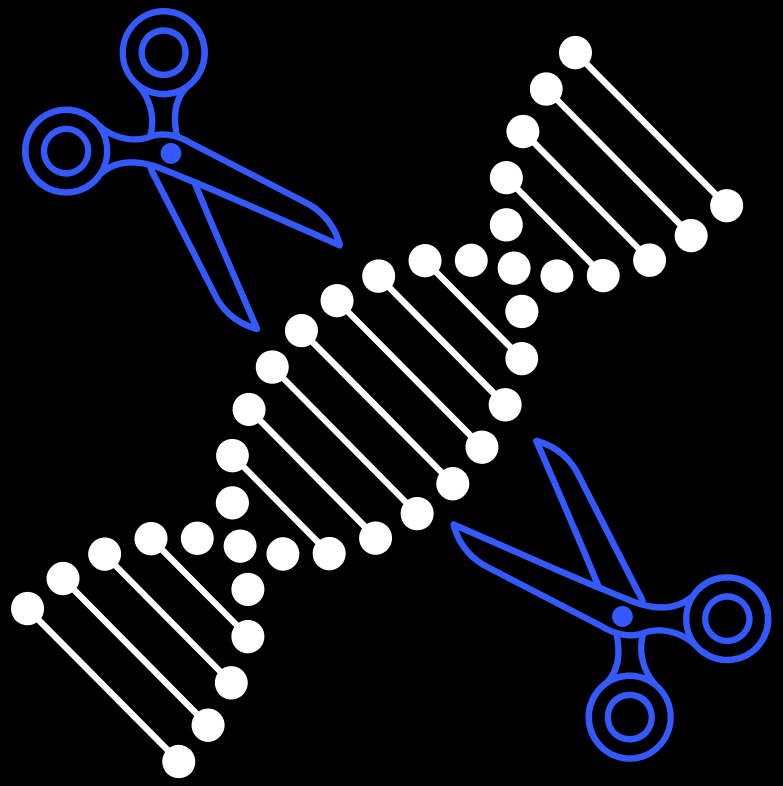


Newby, Yen, Woodard, Mayuranathan, Weiss, Liu *et al. Nature* **595**, 295 (2021)

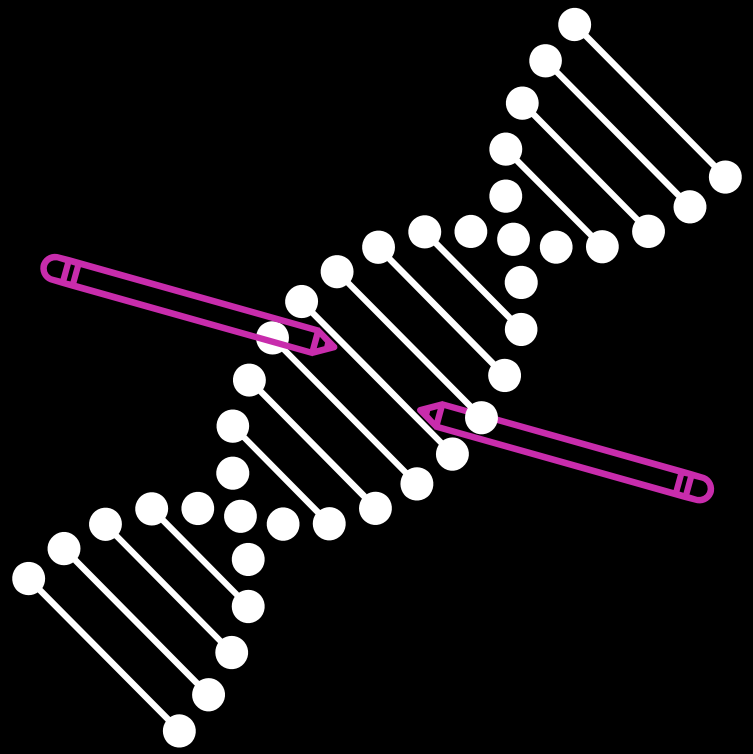


Misspellings not correctable by base editing: ~70%

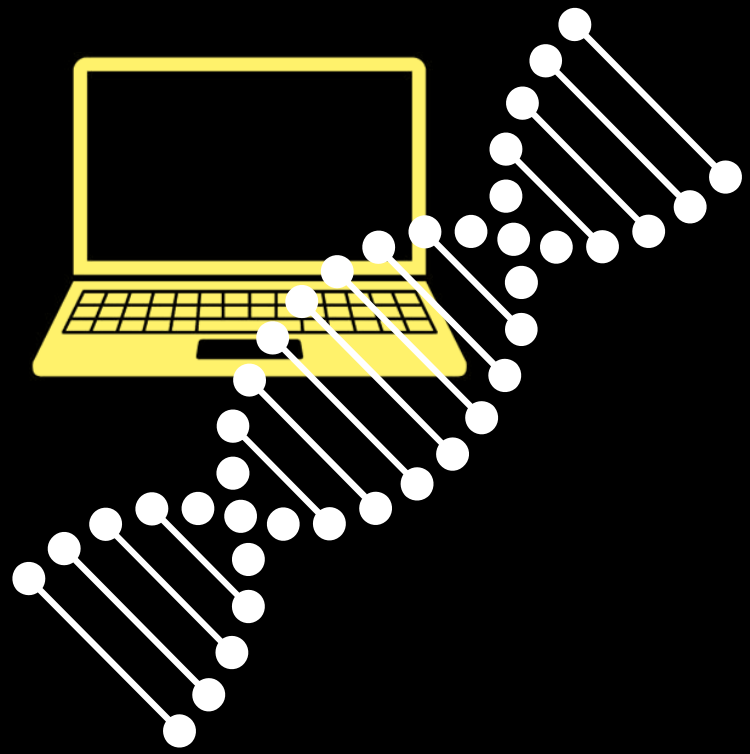
Landrum et al., *Nucl. Acids Res.* 44, D862 (2016)



CRISPR-Cas9

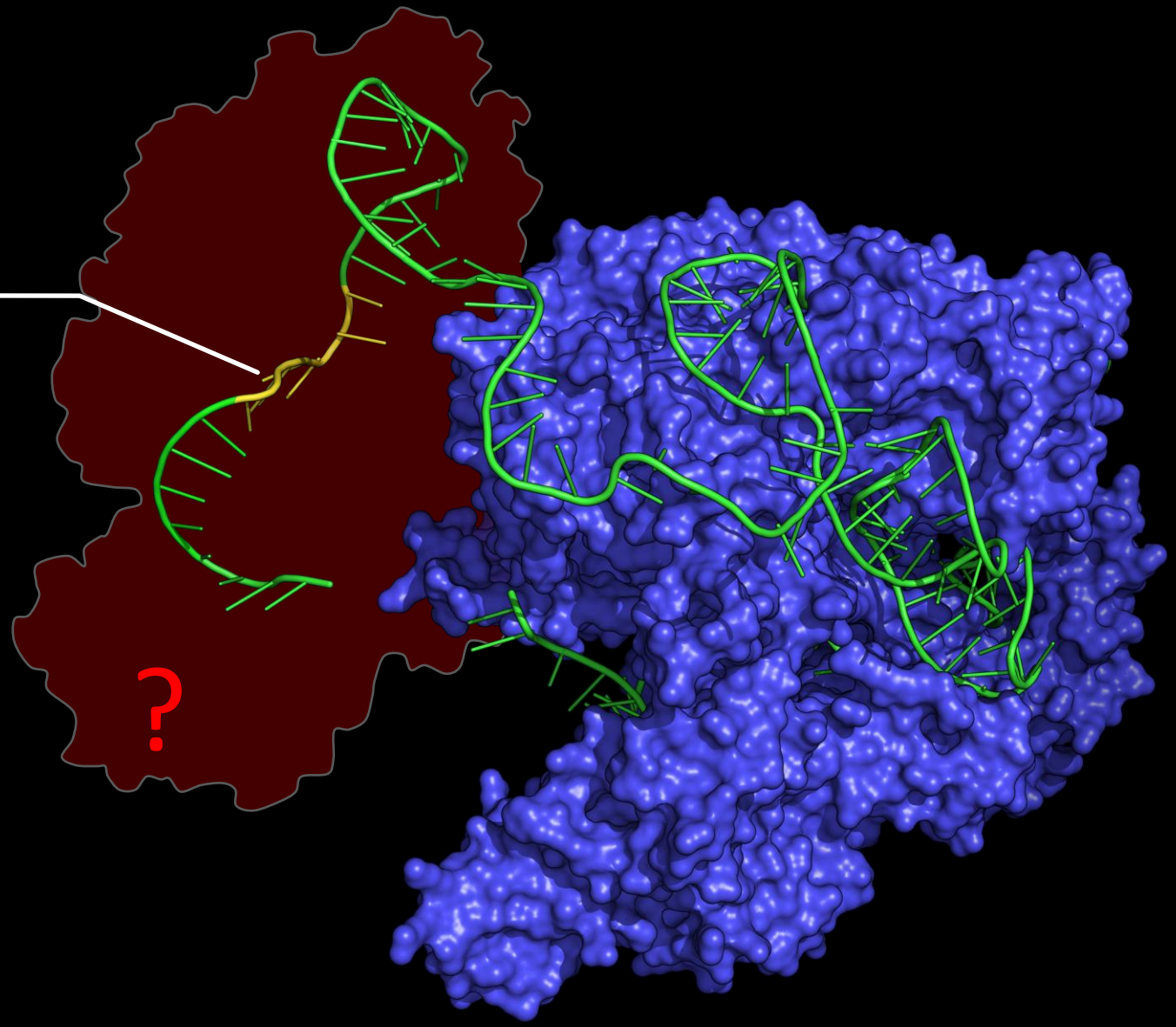


Base editors



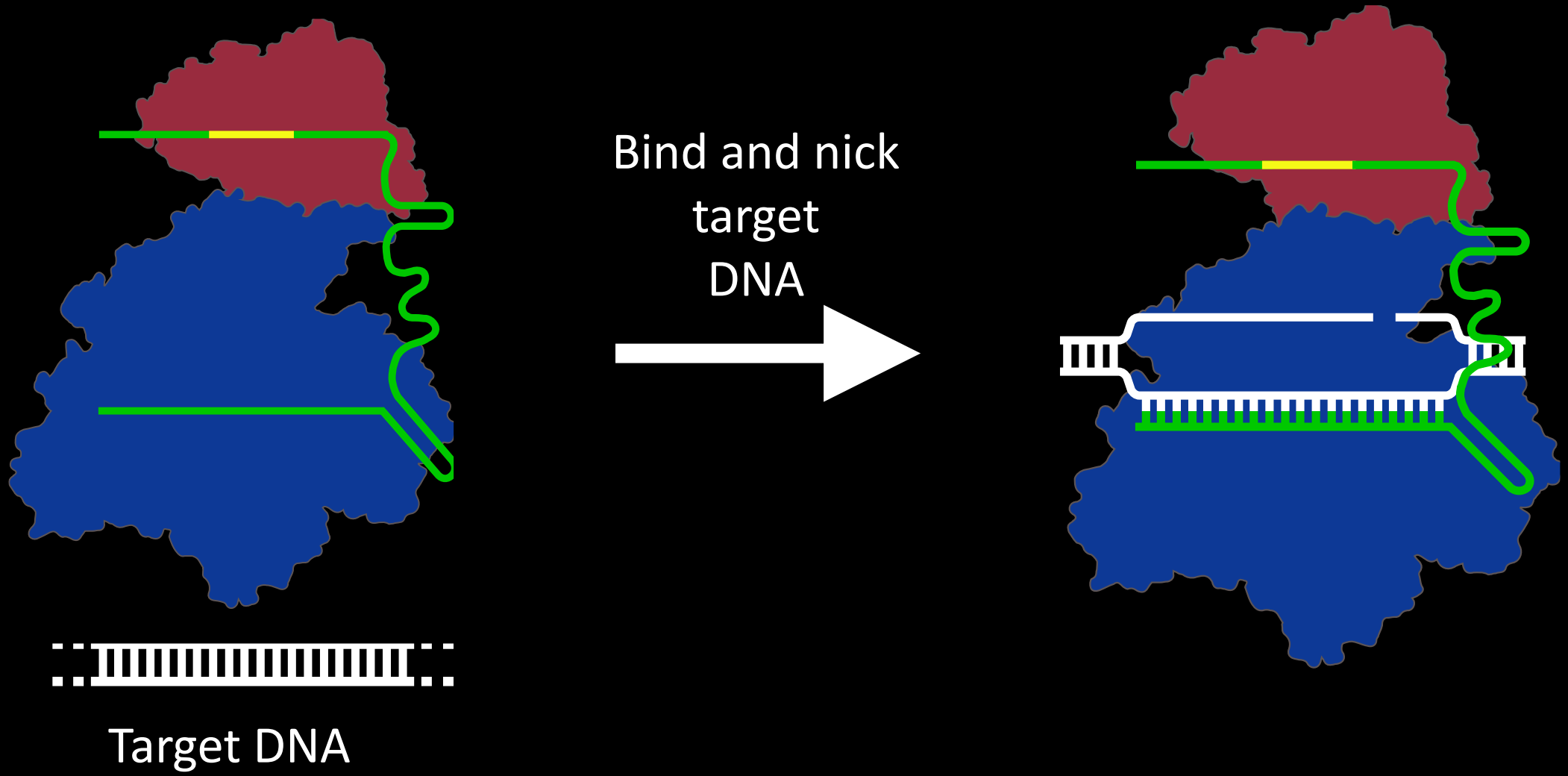
???

Specifies where to edit AND encodes the corrected DNA sequence



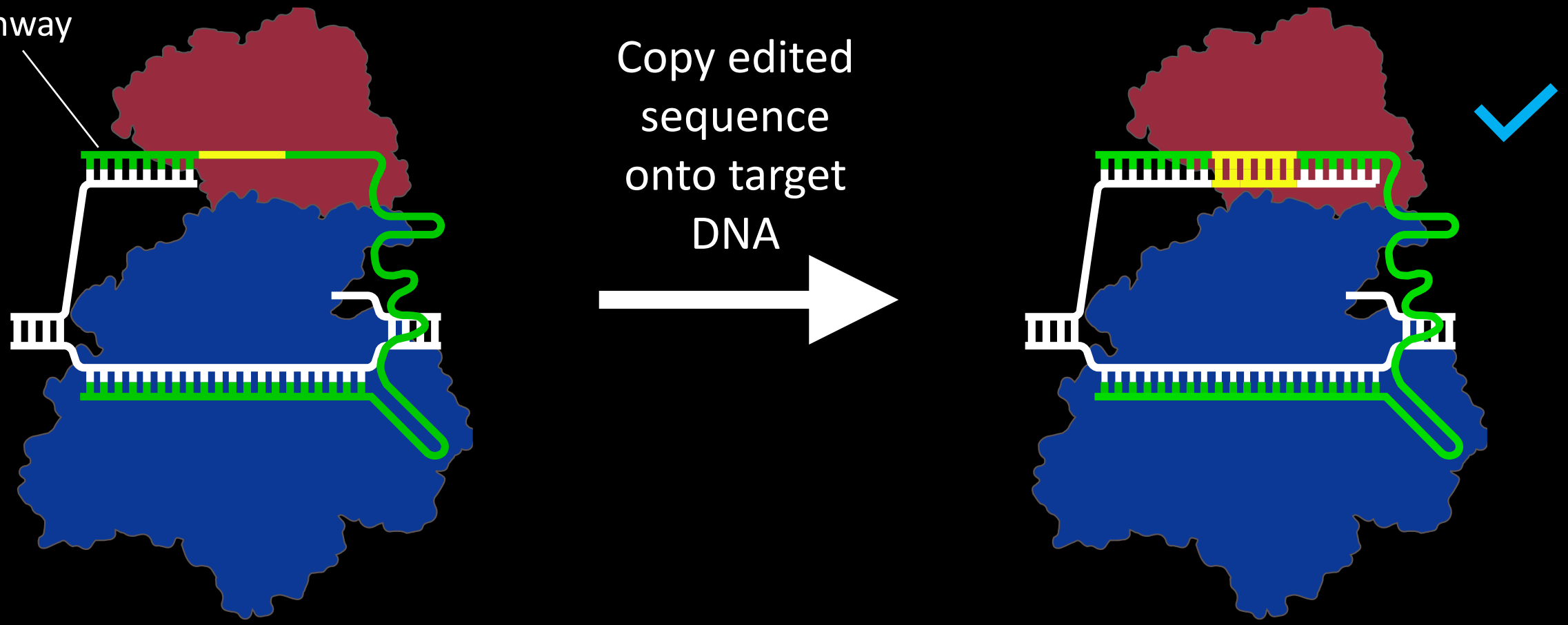
Anzalone, Liu *et al. Nature* **576**, 149 (2019)

1. Can we copy RNA sequence into a target DNA site?



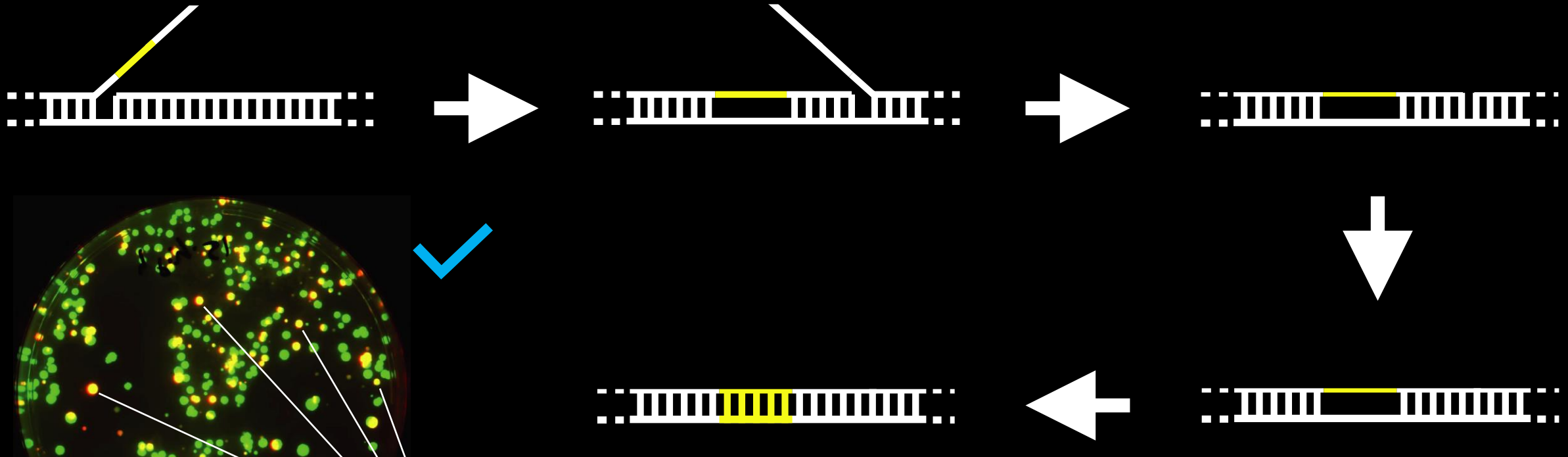
Anzalone, Liu *et al. Nature* **576**, 149 (2019)

1. Can we copy RNA sequence into a target DNA site?



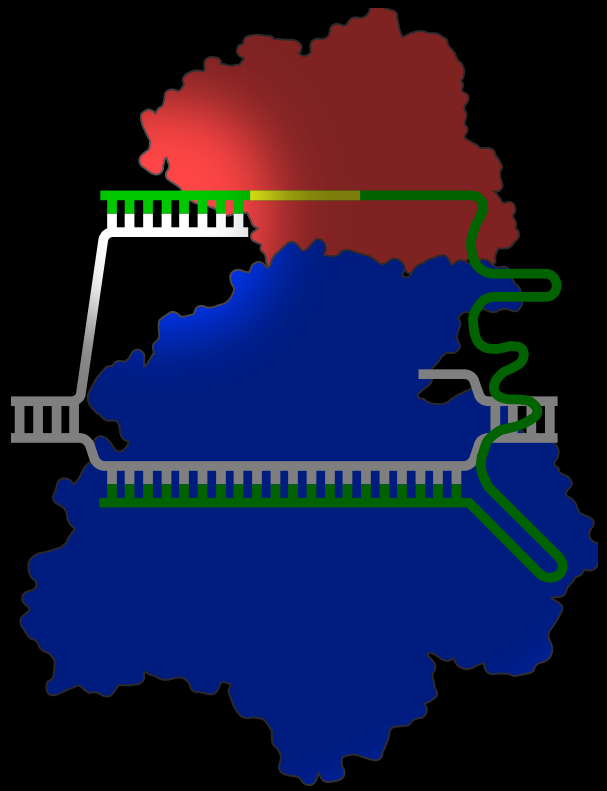
Anzalone, Liu *et al. Nature* **576**, 149 (2019)

2. What happens to the flap of edited DNA in a cell?

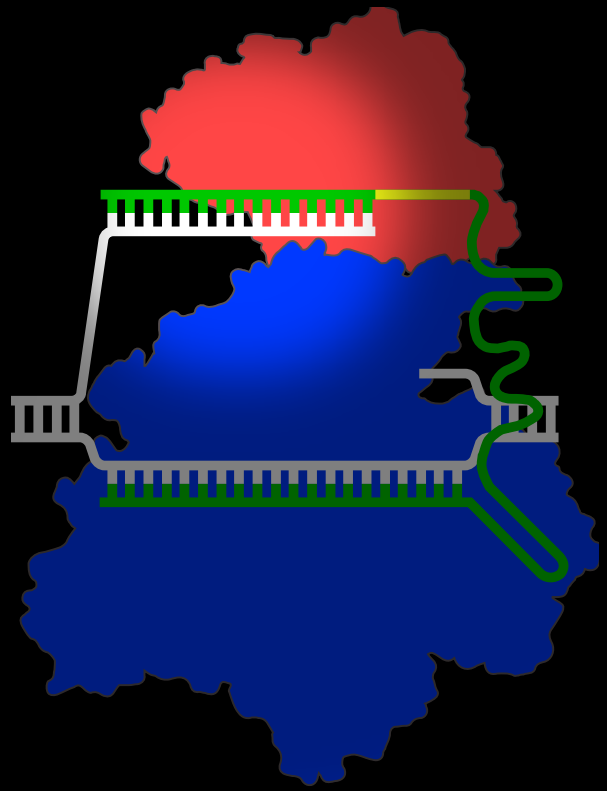


Anzalone, Liu *et al. Nature* **576**, 149 (2019)

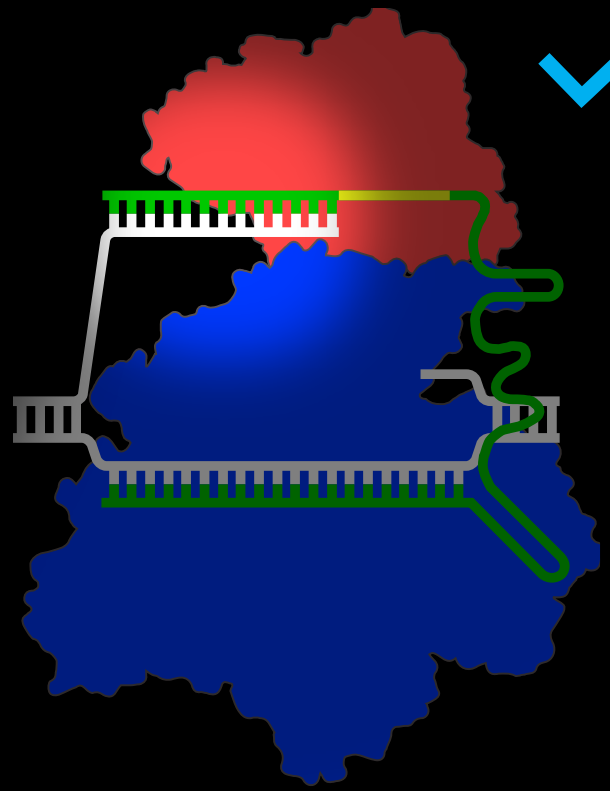
3. How can prime editing work in human cells?



Runway too short



Runway too long

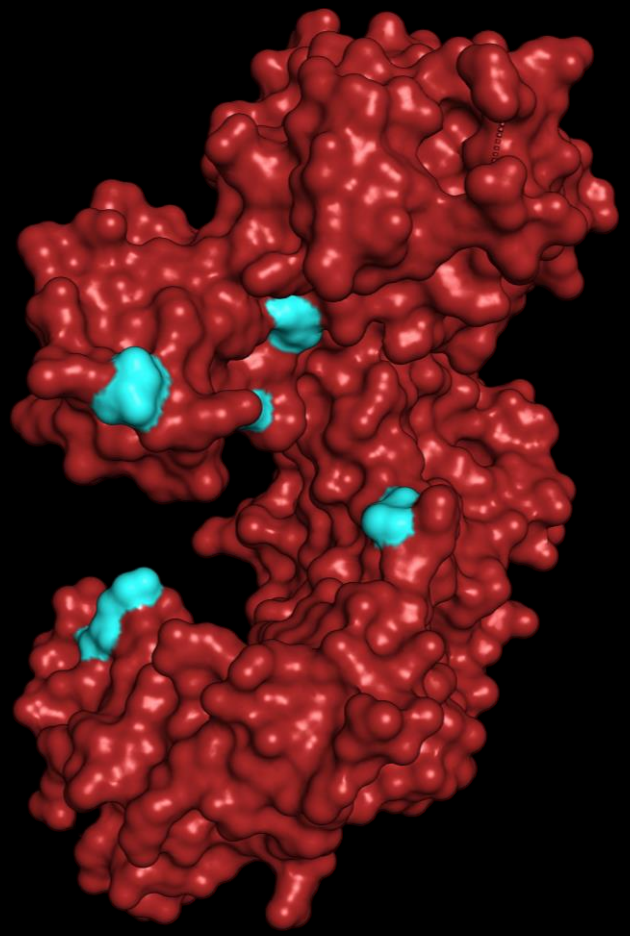


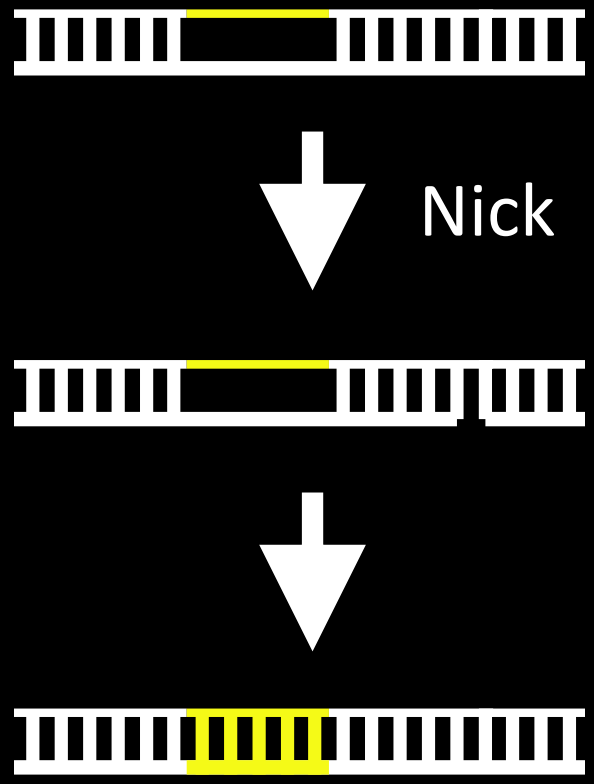
Runway just right

Anzalone, Liu *et al. Nature* **576**, 149 (2019)

4. Can prime editing in human cells be efficient?

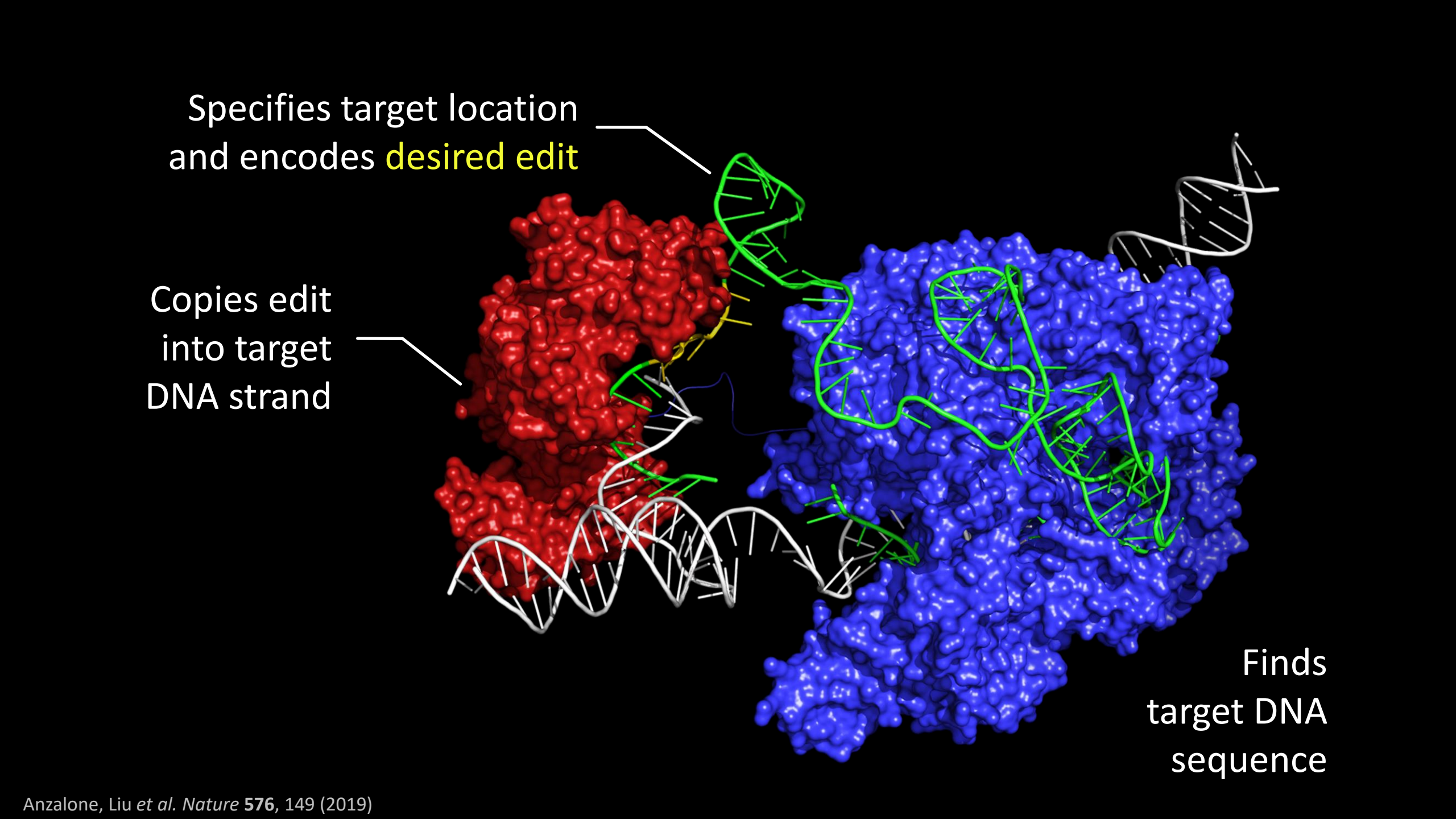
~1% → ~10% editing





~10% → >30% editing

Anzalone, Liu *et al. Nature* **576**, 149 (2019)



Anzalone, Liu *et al. Nature* **576**, 149 (2019)


CCGTATATCTATCCTATGGCC


AGGAACCTTGTCCCGTCCCCAGAAACAT
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TGCTG
CCGTATATCTATCCTATGGCC
CACATA
TCAGCCAGACACAATCATACAGGTGTGG
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CACAGGATGGGAGGCAGGAAGGTCTGG
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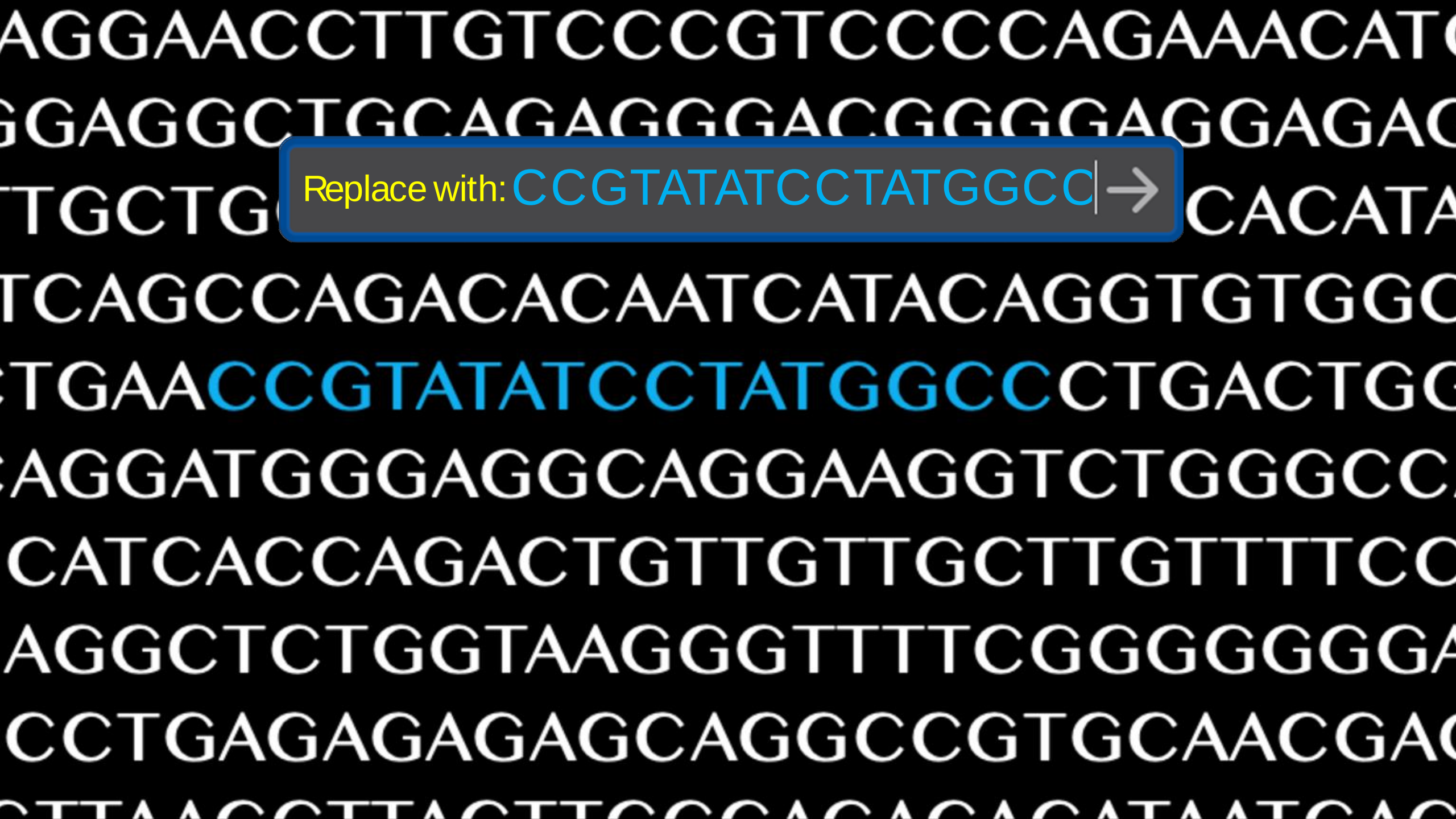
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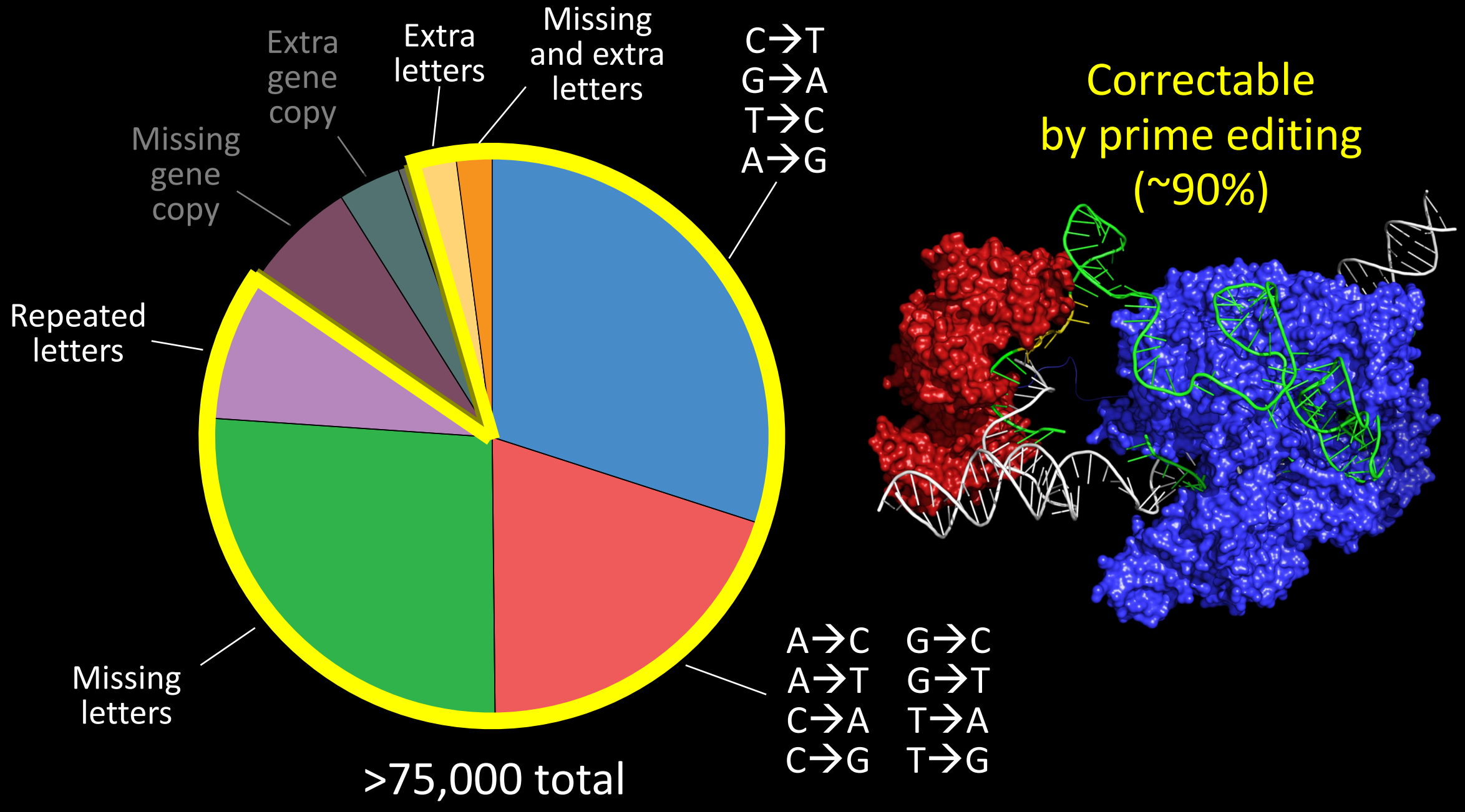

Landrum et al., *Nucl. Acids Res.* 44, D862 (2016)

nature

Article

Search-and-replace genome editing without double-strand breaks or donor DNA

https://doi.org/10.1038/s41586-019-1711-4

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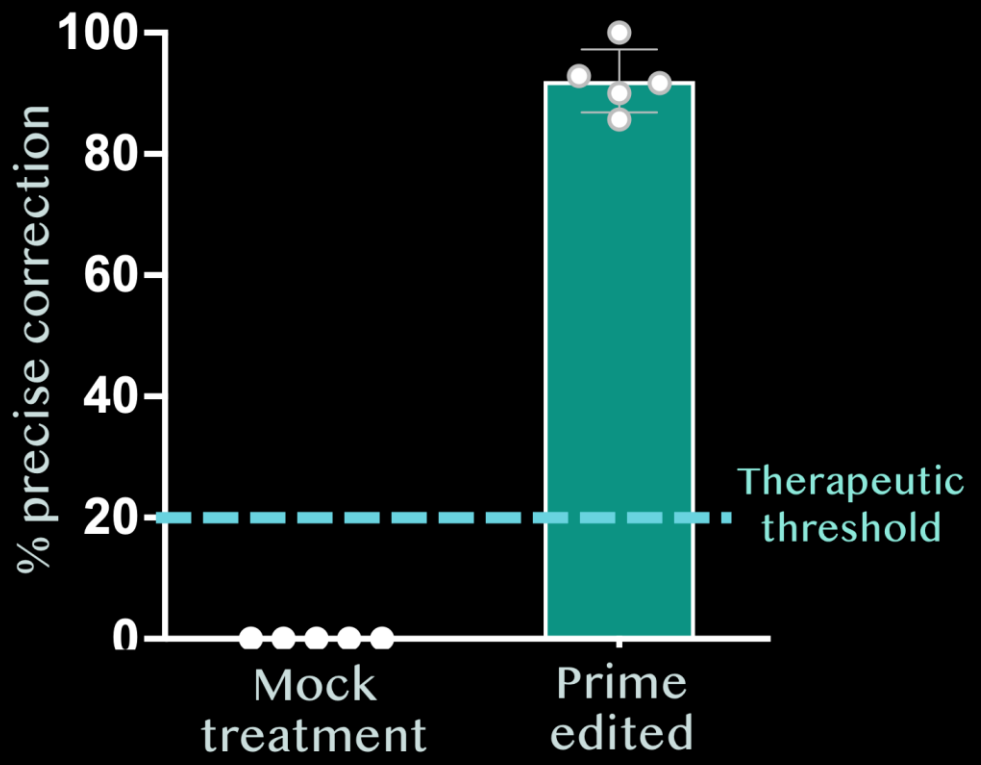
Andrew V. Anzalone[1,2,3], Peyton B. Randolph[1,2,3], Jessie R. Davis[1,2,3], Alexander A. Sousa[1,2,3], Luke W. Koblan[1,2,3], Jonathan M. Levy[1,2,3], Peter J. Chen[1,2,3], Christopher Wilson[1,2,3], Gregory A. Newby[1,2,3], Aditya Raguram[1,2,3] & David R. Liu[1,2,3]*

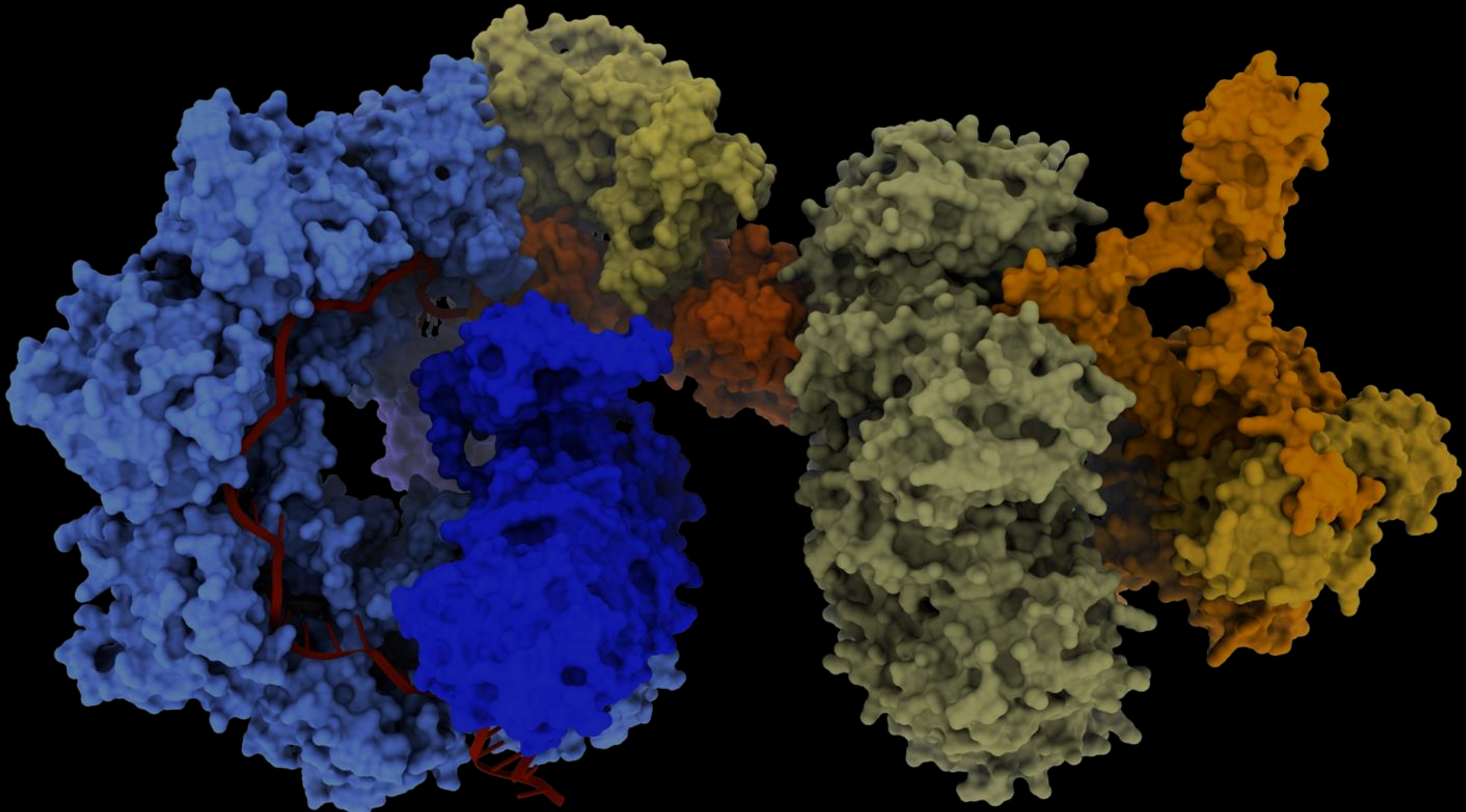
Most genetic variants that contribute to disease[1] are challenging to correct efficiently and without excess byproducts[2–5]. Here we describe prime editing, a versatile and precise genome editing method that directly writes new genetic information into a specified DNA site using a catalytically impaired Cas9 endonuclease fused to an engineered reverse transcriptase, programmed with a prime editing guide RNA (pegRNA) that both specifies the target site and encodes the desired edit. We performed more than 175 edits in human cells, including targeted insertions, deletions, and all 12 types of point mutation, without requiring double-strand breaks or donor DNA templates. We used prime editing in human cells to correct, efficiently and with few byproducts, the primary genetic causes of sickle cell disease (requiring a transversion in *HBB*) and Tay–Sachs disease (requiring a deletion in *HEXA*); to install a protective transversion in *PRNP*; and to insert various tags and epitopes precisely into target loci. Four human cell lines and primary post-mitotic mouse cortical neurons support prime editing with varying efficiencies. Prime editing shows higher or similar efficiency and fewer byproducts than homology-directed repair, has complementary strengths and weaknesses compared to base editing, and induces much lower off-target editing than Cas9 nuclease at known Cas9 off-target sites. Prime editing substantially expands the scope and capabilities of genome editing, and in principle could correct up to 89% of known genetic variants associated with human diseases.

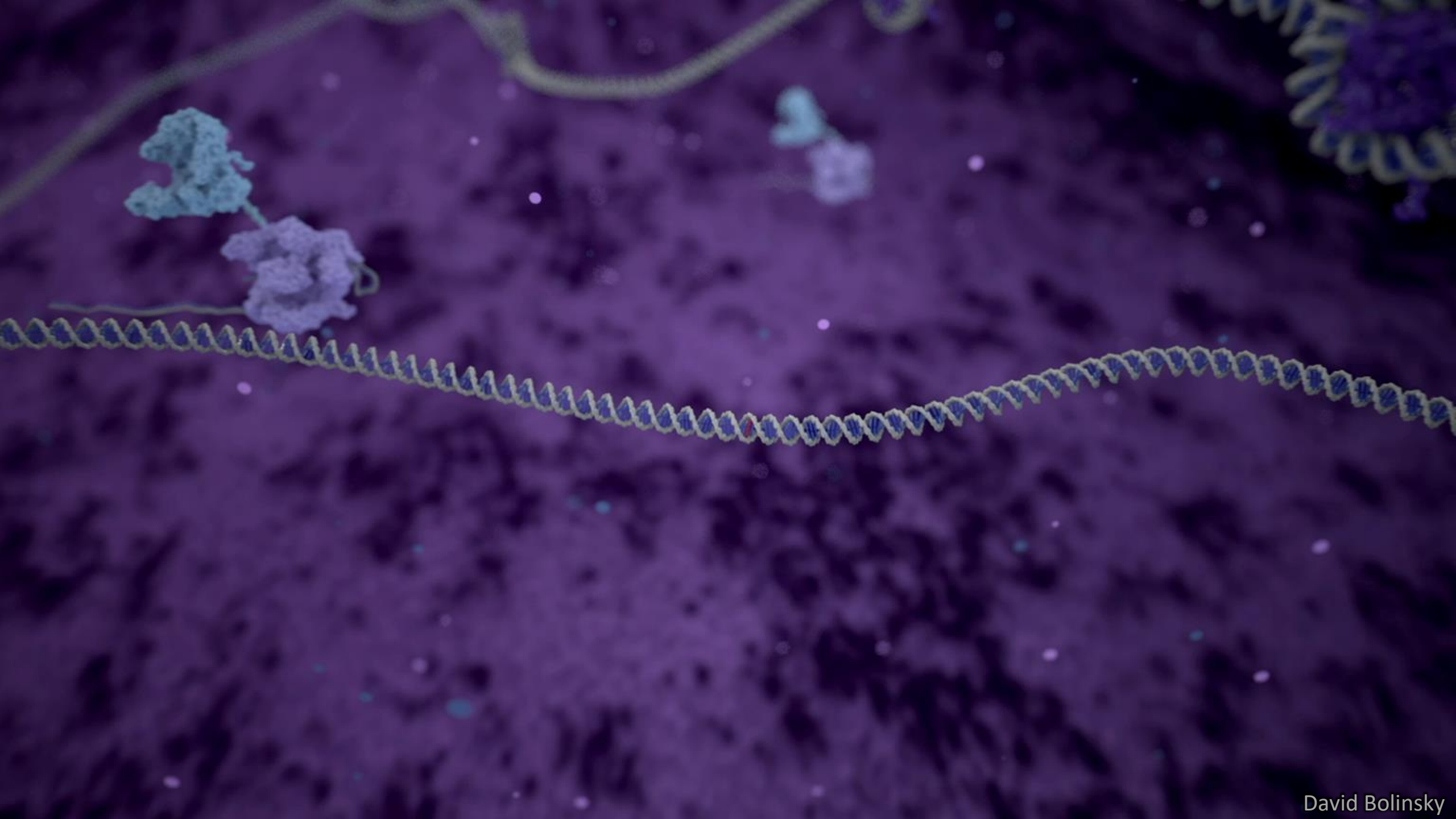

ESB Professional

Candidate	Target	Status
BEAM-101	*HBG* for SCD & b-thalassemia	Phase 1/2 US
TvT (UCL/GOSH Qasim lab)	Multiplex-edited CAR-T for T-cell leukemia	Phase 1 UK **readouts**
VERVE-101 VERVE-102	*PCSK9* for familial heart disease	Phase 1b NZ US UK CA **readouts**
BEAM-201	Multiplex-edited CAR-T ALL/AML	Phase 1/2 US
CARAML (UCL/GOSH Qasim lab)	Multiplex-edited CAR-T for AML	Phase 1 UK
BRL-103 (BioRay Labs)	*HBG* for b-thalassemia	Phase 1/2 CN
CS-101 (CorrectSeq)	*HBG* for b-thalassemia	Phase 1 CN **readouts**
BEAM-302	*AAT* for alpha-1 antitrypsin deficiency	Phase 1/2 UK
U.S. NIAID	*CYBB* for chronic granulomatous disease	Phase 1/2 US

Prime-edited bone marrow cells after engraftment in mice






David Bolinsky


vchal

Next Generation Precision Medicine

Scientific, Regulatory, and Global Access Challenges

Focus on Sickle Cell Disease & Beta Thalassemia

Tony Ho, MD

Large Unmet Medical Need: Sickle Cell Disease (SCD) and Beta Thalassemia

Blood disorders caused by mutations in the β-globin gene

Sickle cell Normal cell β-thalassemia

Significant worldwide burden

ANNUAL BIRTHS

300k Sickle cell disease[1]

60k β-thalassemia[2,3]

People living with the disease

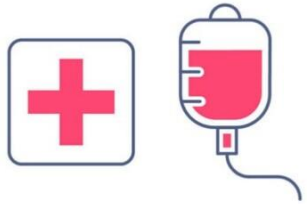
7.7M

Sickle cell Disease

High morbidity and mortality

Anemia and transfusions Pain Early death

Heavy burden of patient care



Frequent transfusions & hospitalizations

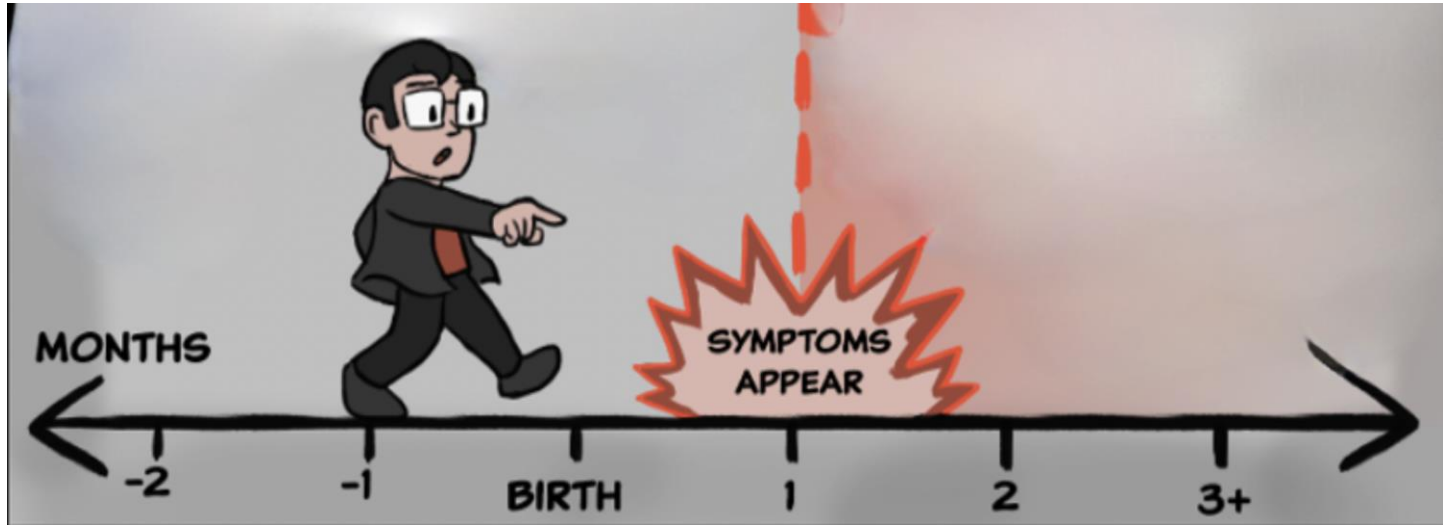
1.7M

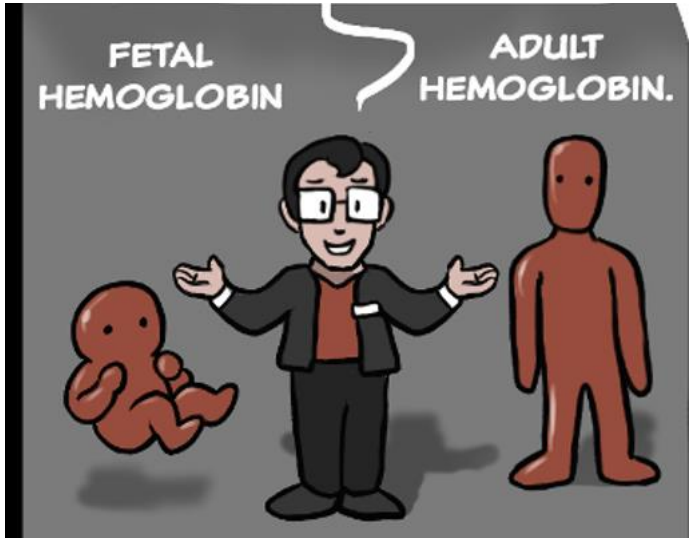
Thalassemia

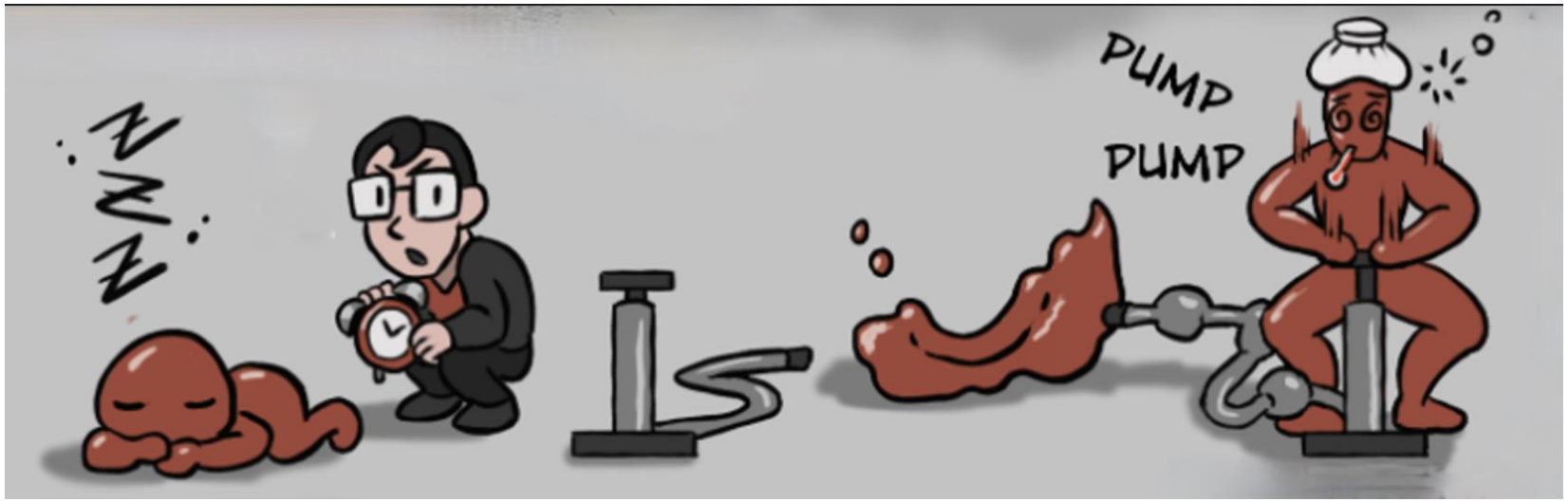
The Hallmark of SCD: Pain

- Pain is prevalent
- Most pain events are managed at home
- Approximately 230,000 emergency room visits and 70,000 hospitalizations per year in the U.S.
- $2 billion estimated costs of emergency room visits and hospitalizations
  - $1.7 million on total lifetime medical costs per person

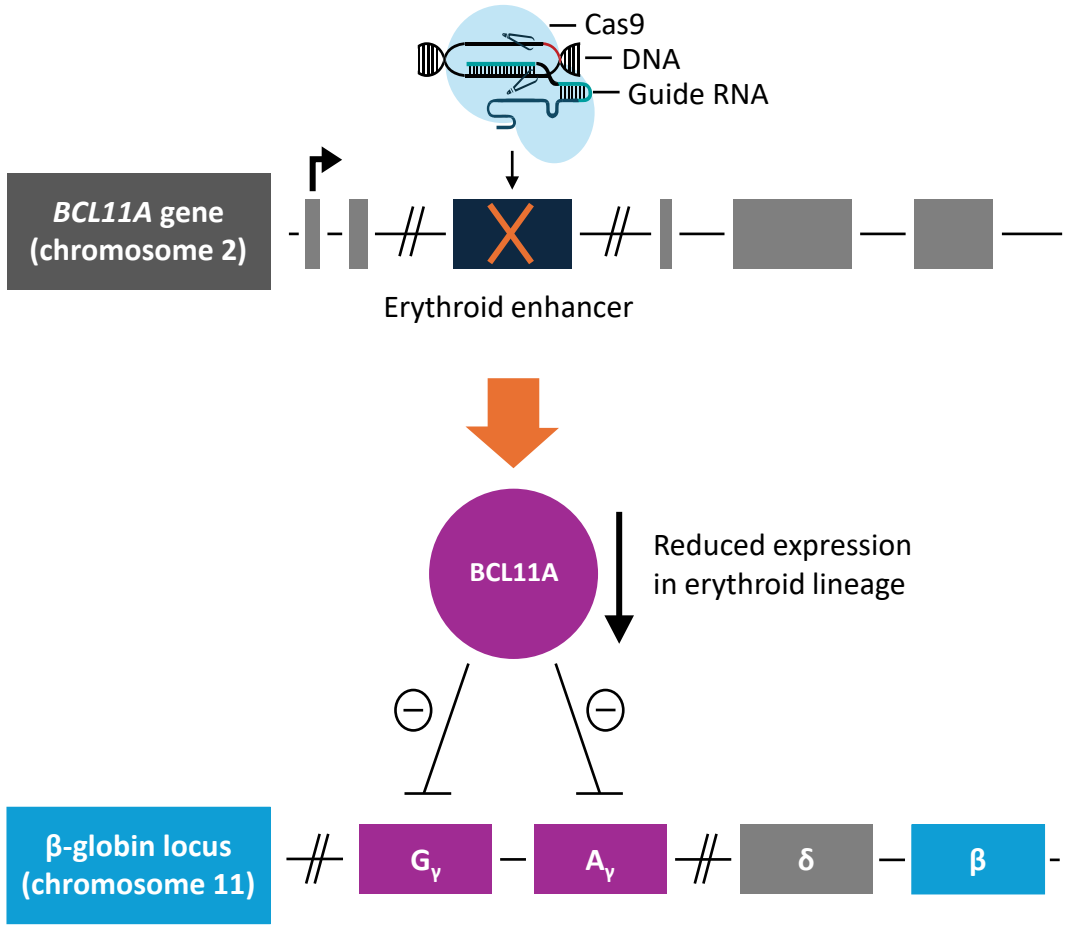
SCD and Beta Thalassemia are Diseases of Adult Hemoglobin







CRISPR-Cas9-Mediated Editing of *BCL11A* Increases HbF Levels[1]



- Naturally occurring genetic polymorphisms in BCL11A are associated with elevated HbF and decreased severity of TDT and SCD2-4
- BCL11A suppresses expression of HbF
- Editing of BCL11A results in reactivation of γ-globin expression and formation of HbF (a2g2) in mouse models
- CTX001 is produced using *ex vivo* editing of the erythroid enhancer region of BCL11A in CD34+ HSPCs and reduces erythroid-specific expression of BCL11A
- Infusion of CTX001 leads to an increase in HbF levels in erythroid cells *in vivo*

HbF: fetal hemoglobin; HSPCs: hematopoietic stem progenitor cells; SCD: sickle cell disease; TDT: transfusion-dependent β-thalassemia.

1. Figure modified from Canver MC, Orkin SH. *Blood*. 2016;127:2536-2545; 2. Murray N, et al. *Br J Haematol*. 1988;69:89-92; 3. Conley CL, et al. *Blood*. 1963;21:261-281; 4. Bank A. *Blood*. 2006;107:435-443.

casgevy®
(exagamglogene autotemcel)
suspension for IV infusion

World's First Approved CRISPR Therapy

"This is major for me and my family. Two years without me being in the hospital? Wow. We just can't believe it. But we're so grateful."

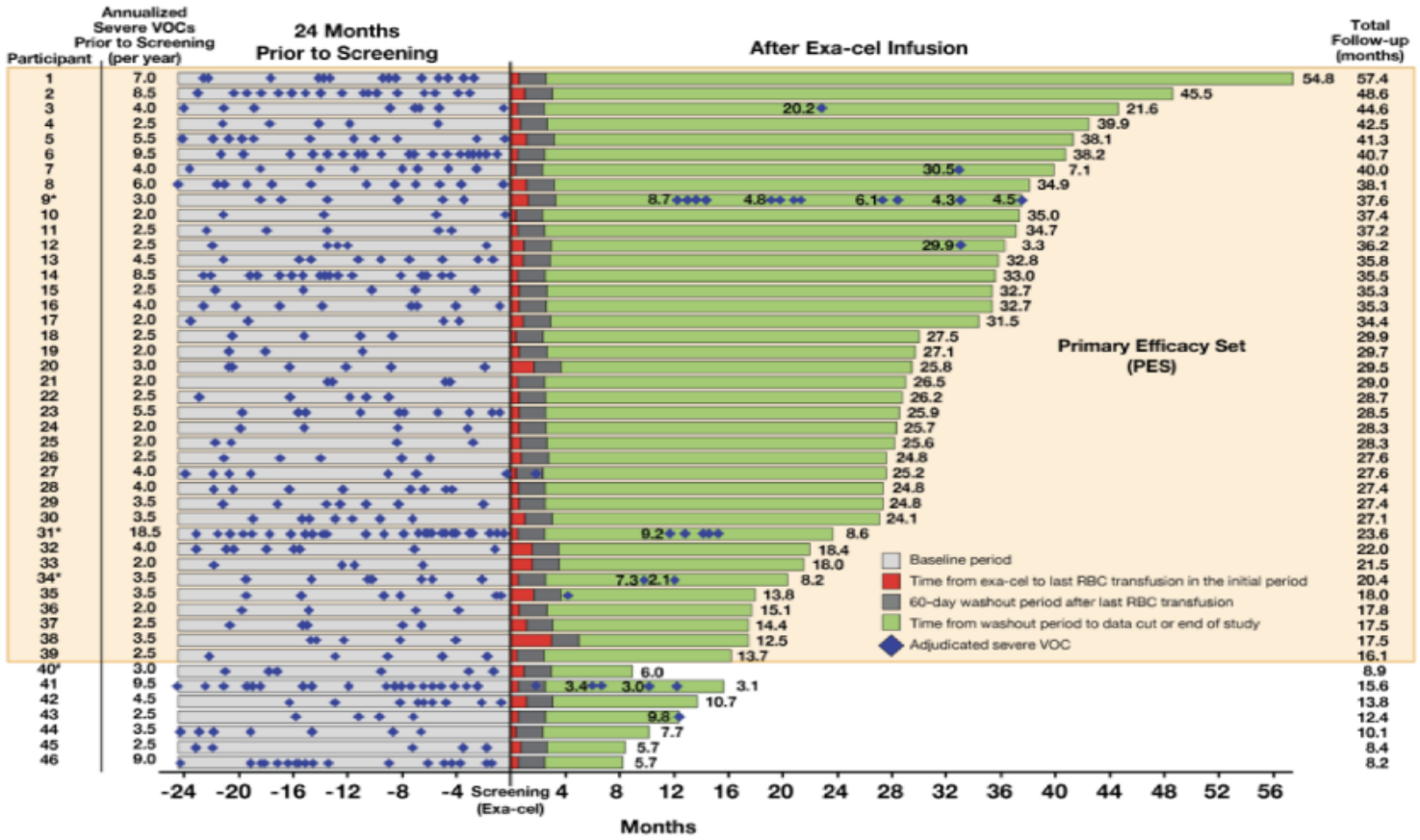
"I finally get to live a normal life and be happy. It's unbelievable."

NPR, DECEMBER 31, 2021

casgevy®
(exagamglogene autotemcel)
suspension for IV infusion

World's First Approved CRISPR Therapy

SCD: VOC-free and no in-patient hospitalizations for VOCs achieved out to 54.8 months



Frangoul et al. EHA 202

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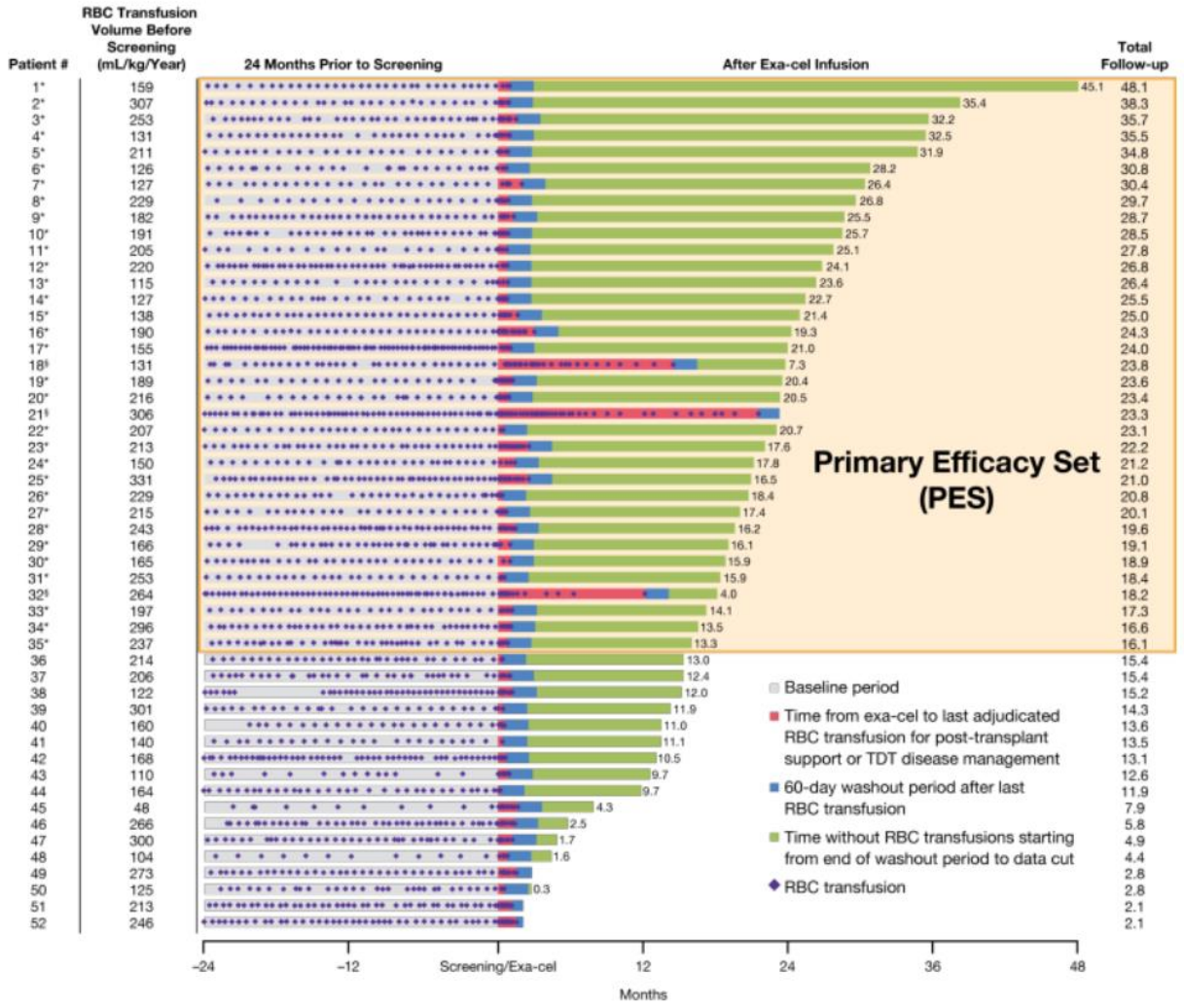
World's First Approved CRISPR Therapy

TDT: Transfusion independence achieved out to 45 months



Locatelli et al. NEJM 2024

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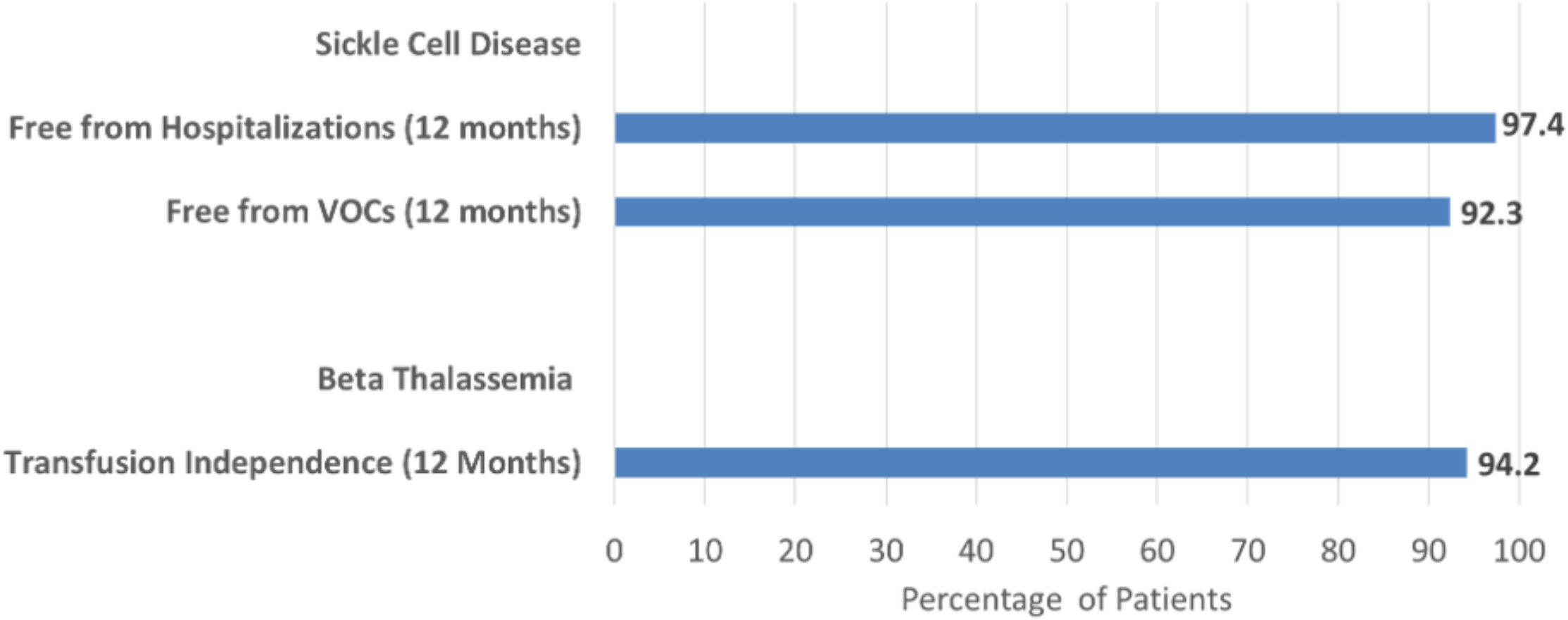
World's First Approved CRISPR Therapy
Key Clinical Trial Results



VOC = Vaso-Occlusive Events

Frangoul et al EHA 2024

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(exagamglogene autotemcel)
suspension for IV infusion

World's First Approved CRISPR Therapy

MHRA

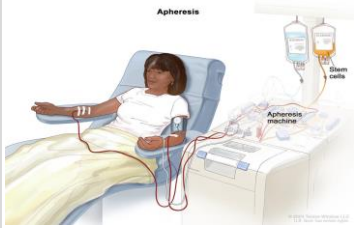
FDA U.S. FOOD & DRUG ADMINISTRATION

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Approval based on Phase 1 trial

Ex Vivo Gene Editing Global Access Limitations

CASGEVY®



Process

- Complex *ex vivo* 3-month process
- Specialized facilities to edit cells, significant access, and cost barriers

Requirements

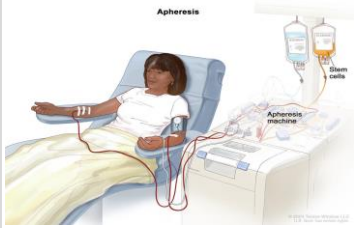
- Myeloablation conditioning
  - Cancer, fertility risks
  - Immune-compromised for weeks to months

Cost

- $2.2M for Casgevy alone
- Plus $100-150K in hospitalization and other associated costs

Ex Vivo Gene Editing Global Access Limitations

CASGEVY®



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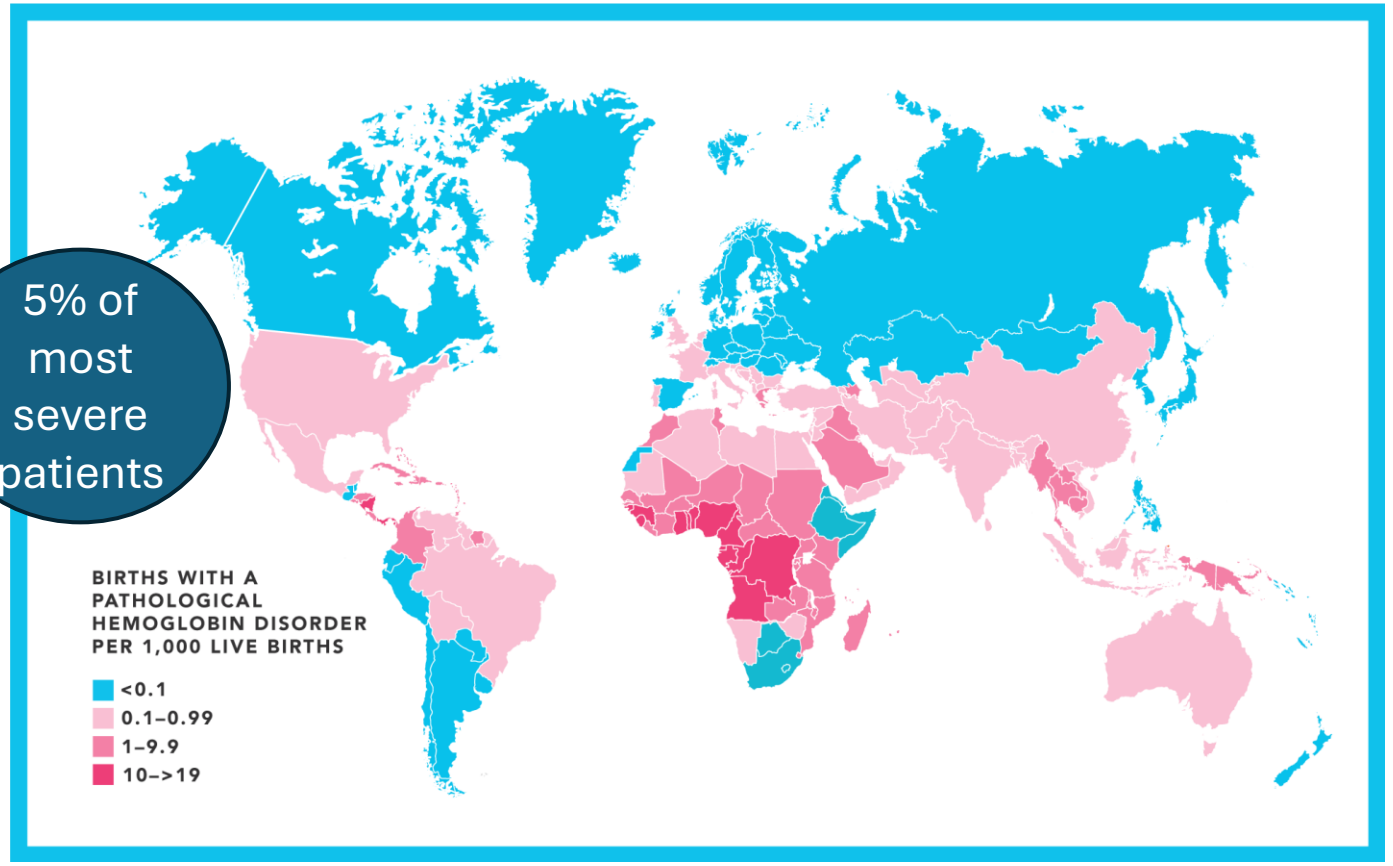
- Myeloablation conditioning
  - Cancer, fertility risks
  - Immune-compromised for weeks to months

Cost

- $2.2M for Casgevy alone
- Plus $100-150K in hospitalization and other associated costs

>10 million people worldwide

5% of most severe patients



casgevy® (exagamglogene autotemcel) suspension for IV infusion

World's First Approved CRISPR Therapy

Gray has relatives who are still struggling with sickle cell.

"I hope this will be available to everyone who needs it."

"It's horrible knowing that something is out there that can cure your disease, but you can't access it."

NPR, DECEMBER 31, 2021

Next Frontier for Achieving a Functional Cure with Global Access

Future: *In Vivo* Editing

Process

- “Drug-in-a-Bottle”, curative, single IV injection, administered in an outpatient setting
- Low COGS and no hospitalization costs
- Overcomes logistical, cost, and safety barriers

Requirements

- Myeloablation not required

Ideal *In Vivo* Gene Delivery Vehicles

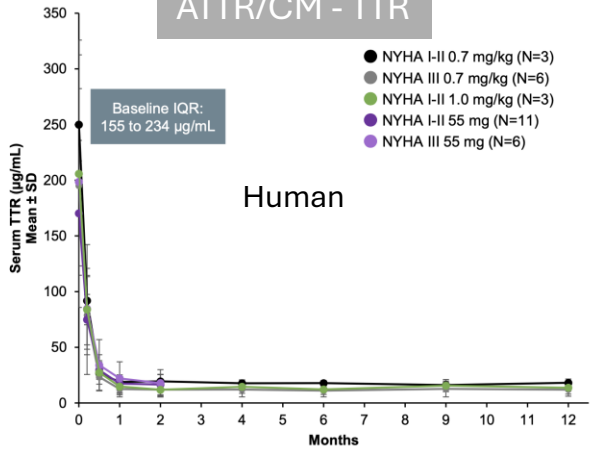
Ultimate Precision Medicine

- Only deliver to the cell of interest
- Only biologically active in the cell of interest
- Transient expression of the editing machinery
- Non-immunogenic, low-toxicity
- Can dose repeatedly if need it

In Vivo Hepatocyte Editing Using LNP – Currently in Human Clinical Trials

Intellia THERAPEUTICS

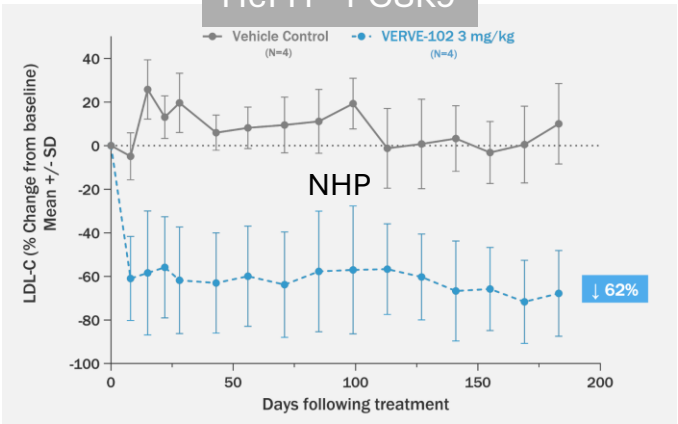
ATTR/CM - TTR



Status: Phase 3

verve THERAPEUTICS

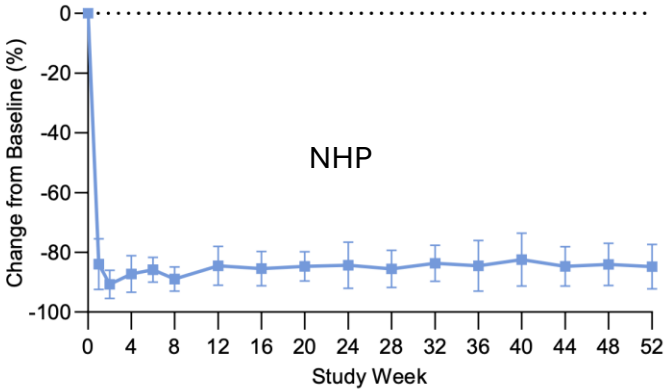
HeFH – PCSK9



Status: Phase 1

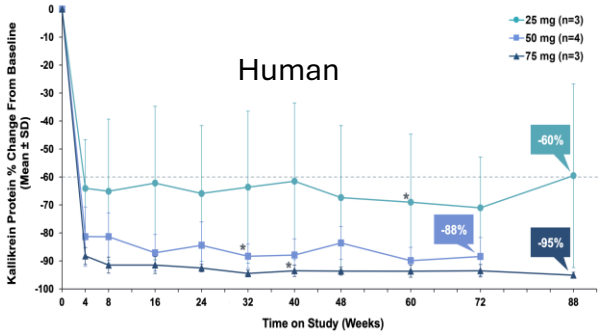
CRISPR THERAPEUTICS

ASCVD - ANGPTL3



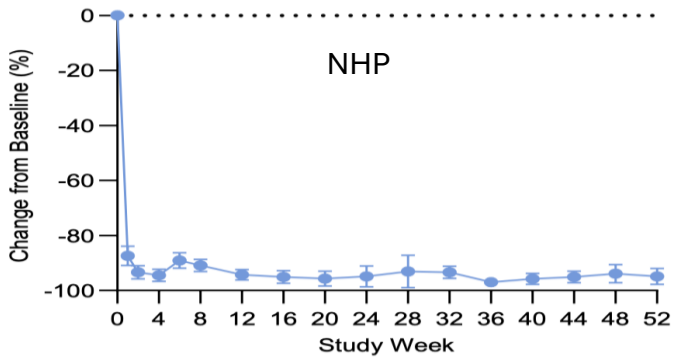
Status: Phase 1

HAE - KLKB1



Status: Phase 2

ASCVD – Lp(a)



Status: Phase 1

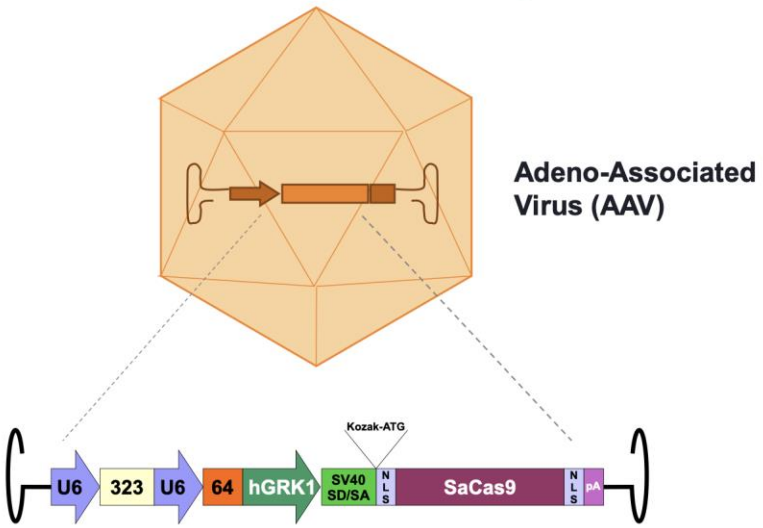
In Vivo Editing via Viral Vectors

LCA10– CEP290

AAV5 encoding two gRNAs and SaCas9 delivered subretinally as a single administration

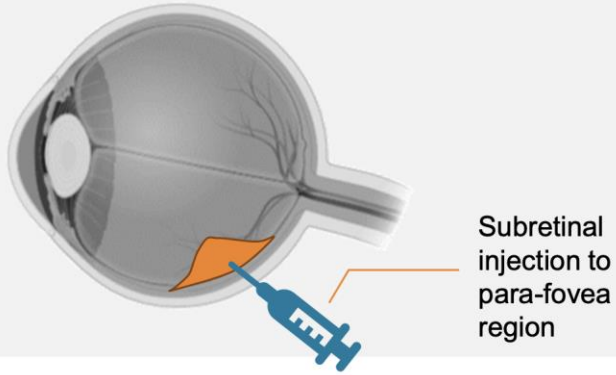
EDIT-101

CRISPR-Cas9 gene editing



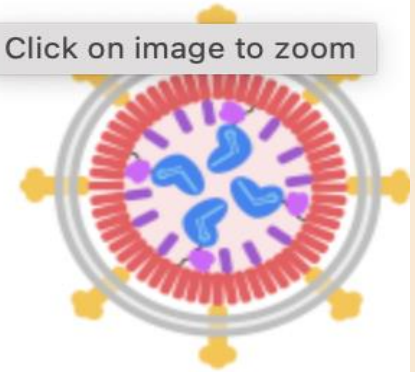
EDIT-101 specifically targets the part of the retina where viable photoreceptors are found

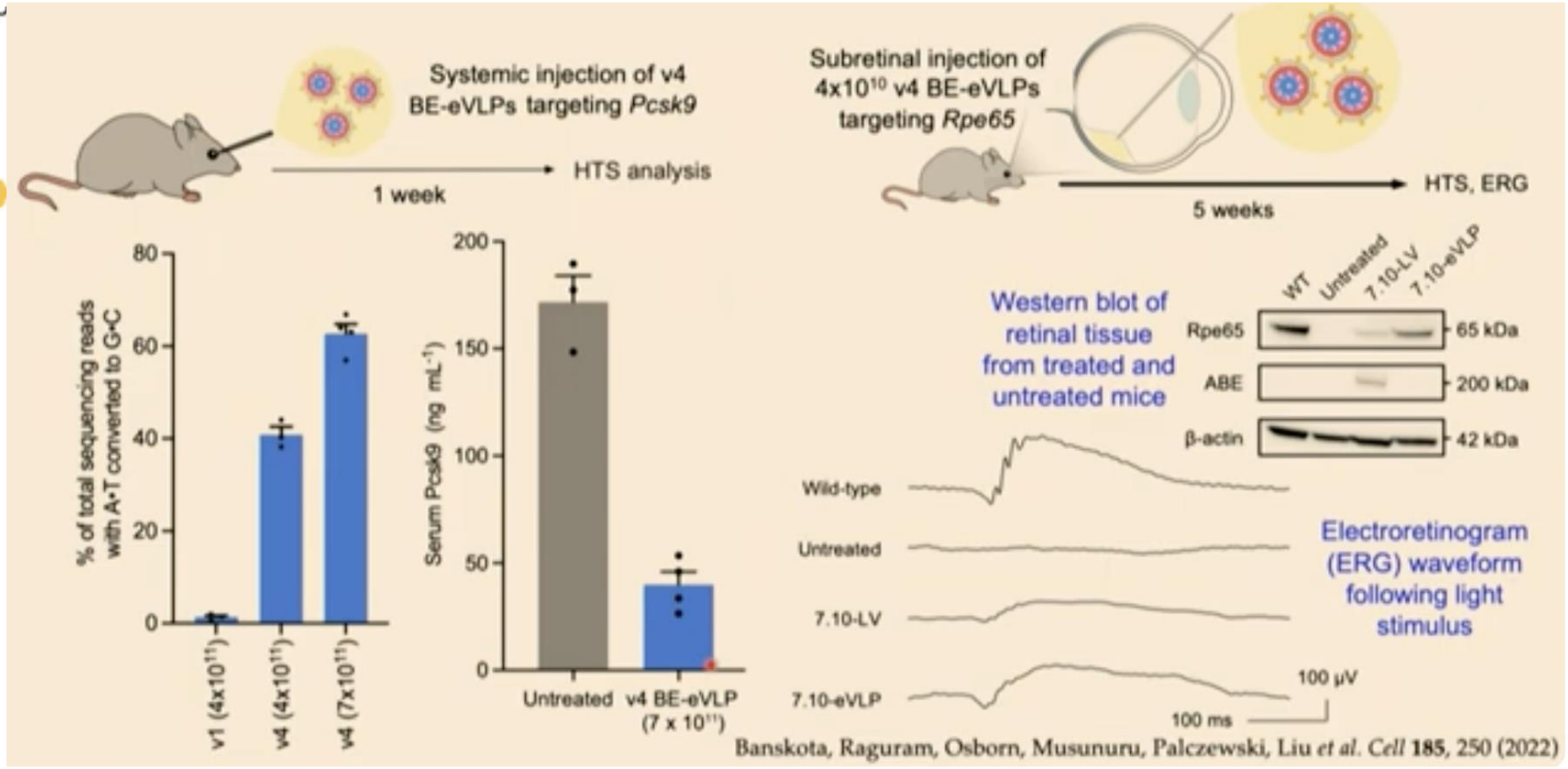
- **Photoreceptor-tropic** AAV5 vector
- **Highly specific** Guide RNAs
- **Restricted** Cas9 expression in **Photoreceptor Cells**
- **Local delivery to subretinal space** limits the risk of biodistribution outside of the eye



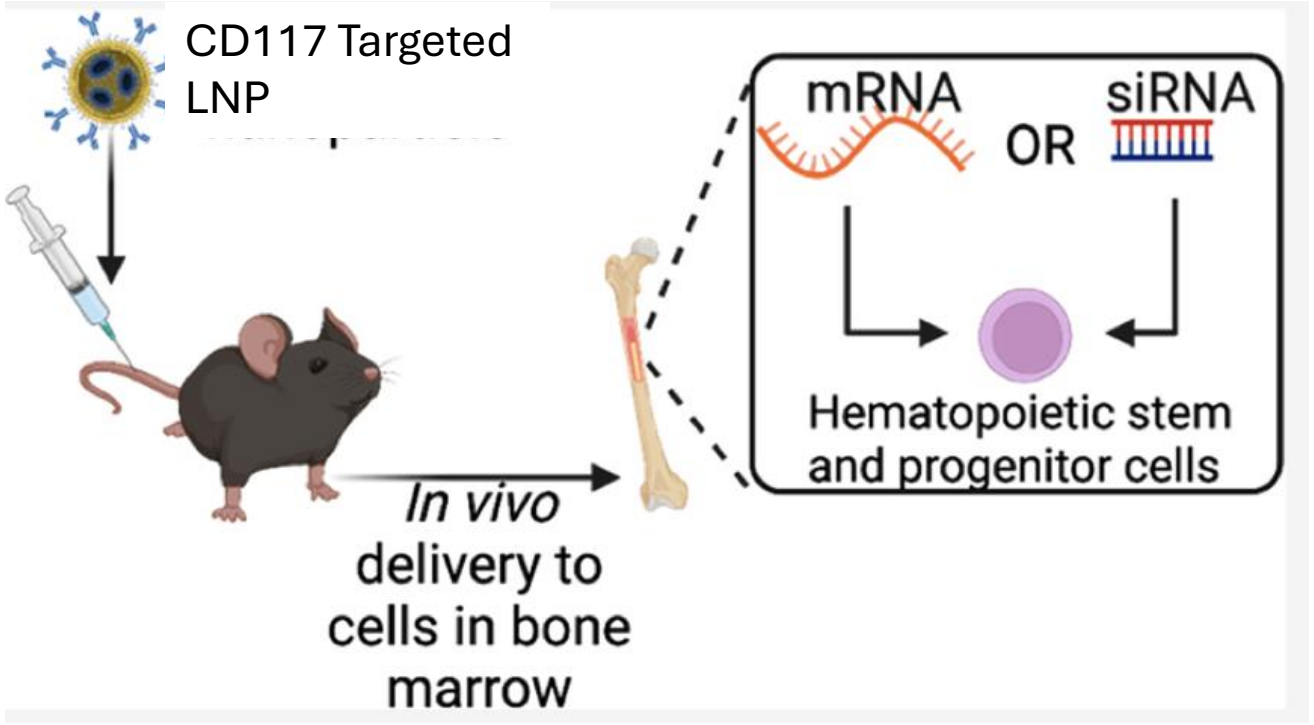
Quest to Deliver an *In Vivo* Gene Editing Approach Using Non-Viral Vectors Outside of the Liver

Viral-Like Particles

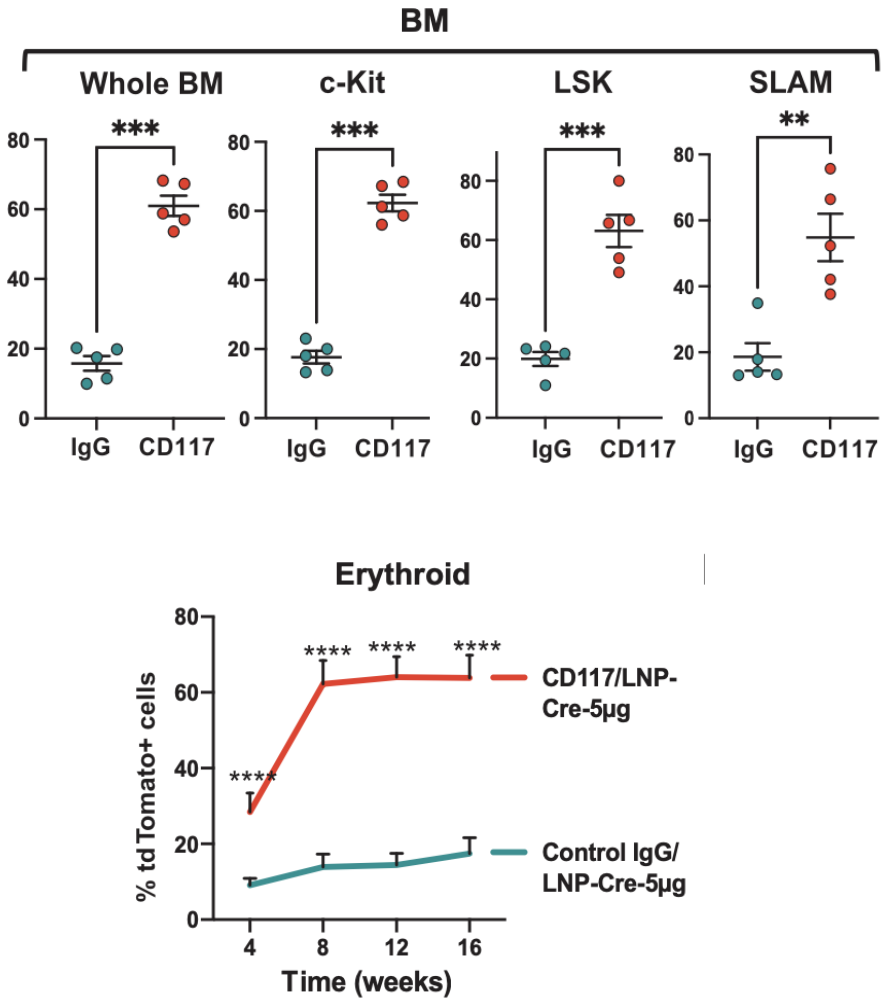




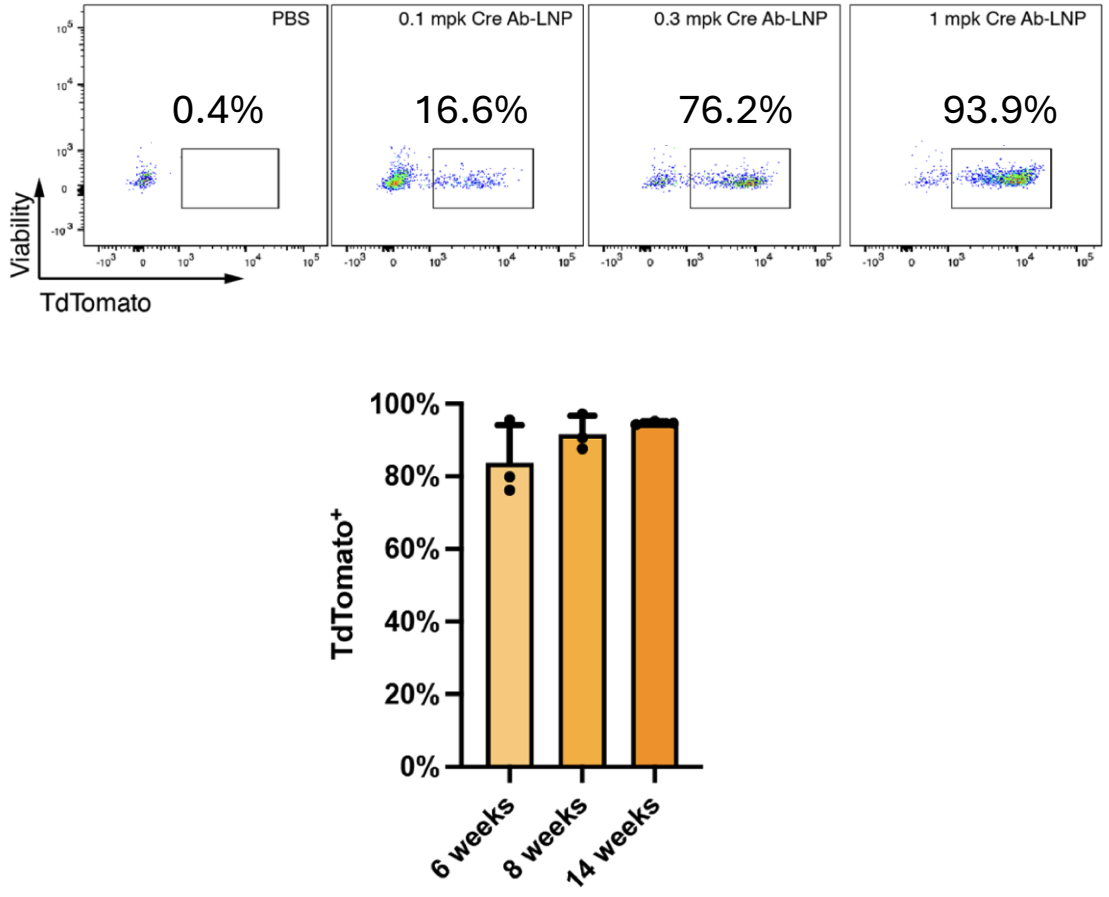
Targeted LNP Approach To Edit Hematopoietic Stem Cells



Targeted LNP Approach To Edit Hematopoietic Stem Cells



Breda et al., Science 381, 436–443 (2023)



Shi, Toyonaga, Anderson, Nano Lett. 2023, 23, 2938−2944

Era of Precision Medicine – Maximized Benefit to Risk of Therapy

Operate only on the diseased tissues

Treat every cell in the body

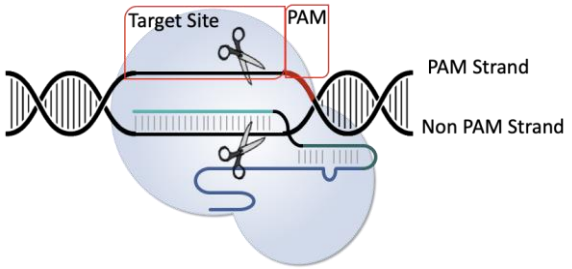
NaturesPharm

Only deliver the drug to the brain

Treated every cell in the body
Increased benefit to risk by targeting differences in sensitivity between tumor and normal cells

Target Antigens preferentially over-expressed in tumor

Molecular Scalpel



Ex vivo – only edit the cell of interest

In vivo - combined with delivery and cell specific promoter – edit only the cell of interest

Challenges for *In Vivo* Gene Editing

Scientific/Development

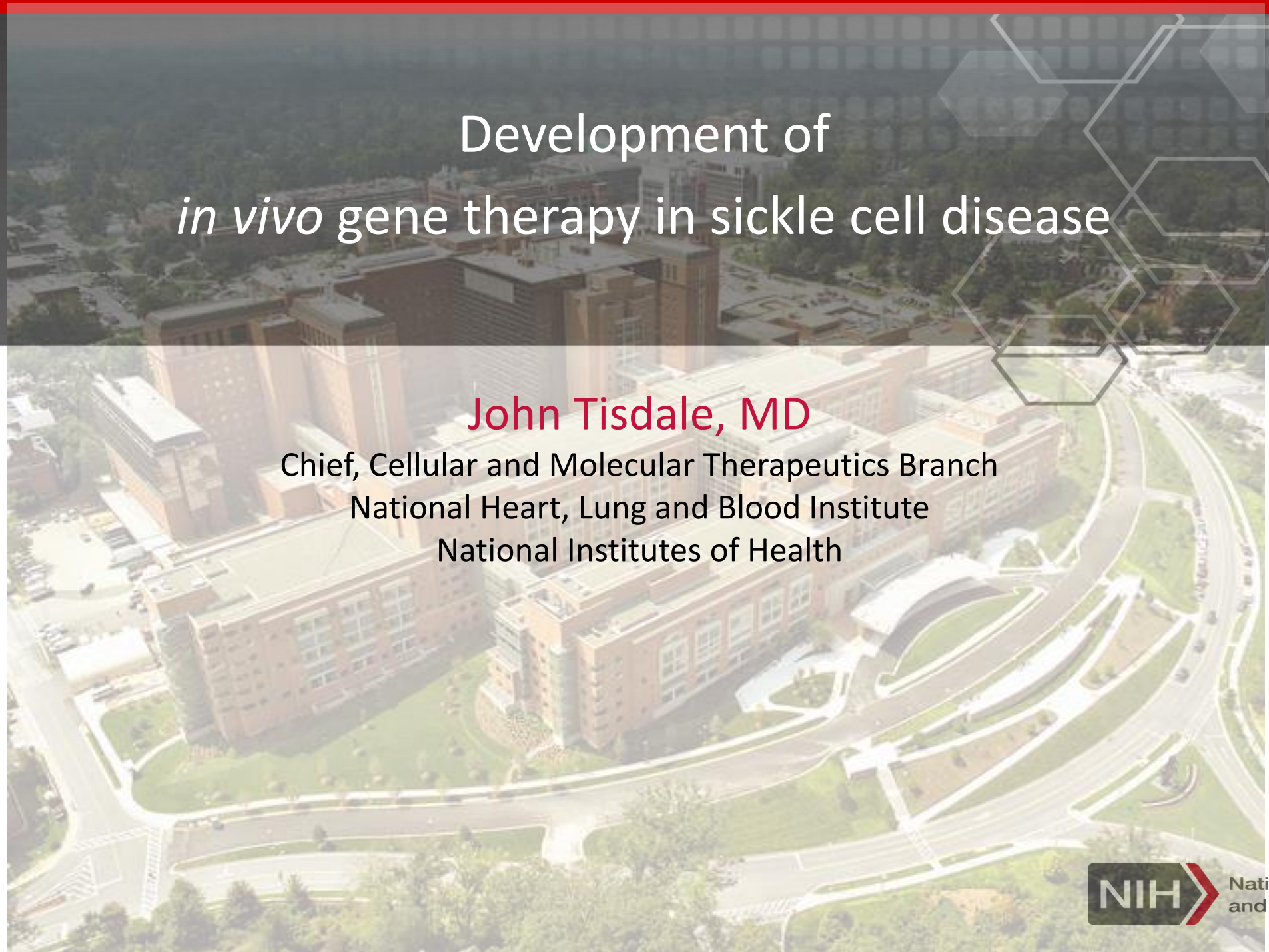
- Low immunogenicity
- Low toxicity
- Sufficient editing of the target cells
- Off target editing
- On target, off target cell editing
- Germline editing

Regulatory/Access

- Benefit and risk evaluation for one-time *in vivo* gene editing treatments
- Framework for early approval (e.g. Potential Phase 1 approval)
- Long term follow up
- Global Regulatory approval
- Access
  - In developed countries
  - In developing countries
  - In least developed countries

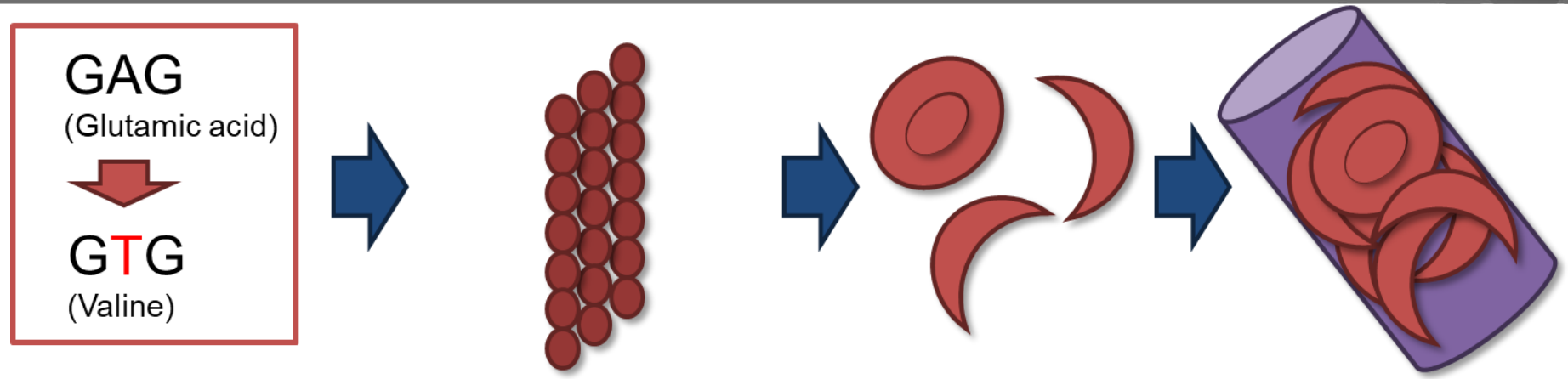
Development of *in vivo* gene therapy in sickle cell disease

John Tisdale, MD
Chief, Cellular and Molecular Therapeutics Branch
National Heart, Lung and Blood Institute
National Institutes of Health



National Heart, Lung, and Blood Institute

Sickle cell disease: a single-gene disorder



β-globin gene mutation

20A>T mutation
1/12 African Americans

Hemoglobin S polymerization

Upon deoxygenation

Sickling red blood cells

Vessel occlusion

Recurrent pain
Anemia
Multi-organ damage
Early mortality

HSC transplantation
Gene therapy

Hydroxyurea

Painkiller

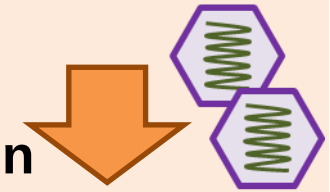
In vivo HSC-targeted gene addition/editing therapy

Ex vivo gene addition/editing

Cell processing center

Harvest

Hematopoietic stem cells (HSCs)



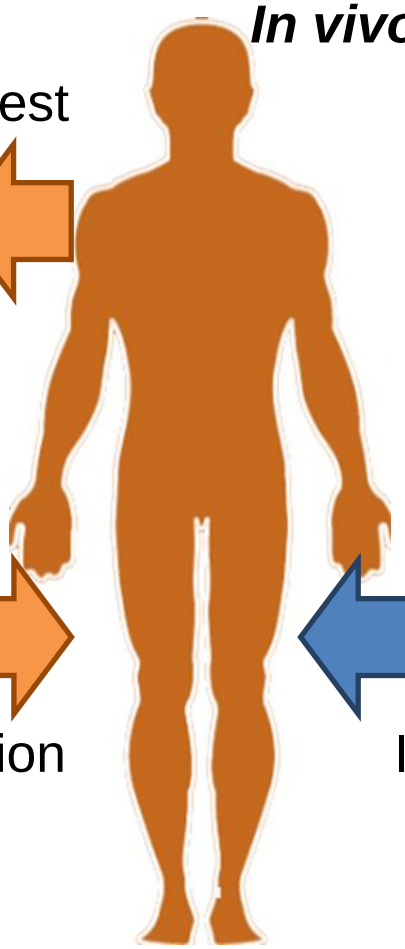
β-globin **gene addition** with a lentiviral vector

or

Gene editing with an engineered nuclease

Transplantation

- **Efficacy and safety confirmed**
- Hospitalization needed
- **A cell processing center needed**
- High cost

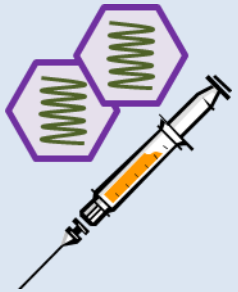


Patients with blood disorders

In vivo gene addition/editing

HSC-targeted gene delivery system

- Viral vectors
- Nanoparticles



Injection

- Under development
- Simple method
- **Walk-in basis**
- **Low cost**

Lentiviral vector gene therapy becomes a reality for sickle cell disease

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DOI: 10.1002/ajh.26741

AJH WILEY

RESEARCH ARTICLE

Lovo-cel gene therapy for sickle cell disease: Treatment process evolution and outcomes in the initial groups of the HGB-206 study

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Abstract

lovo-cel (bb1111; LentiGlobin for sickle cell disease [SCD]) gene therapy (GT) comprises autologous transplantation of hematopoietic stem and progenitor cells transduced with the BB305 lentiviral vector encoding a modified β-globin gene ($\beta^{A\text{-}T87Q}$) to produce anti-sickling hemoglobin (HbA^{T87Q}). The efficacy and safety of lovo-cel for SCD are being evaluated in the ongoing phase 1/2 HGB-206 study (ClinicalTrials.gov: NCT02140554). The treatment process evolved over time, using learnings from outcomes in the initial patients to optimize lovo-cel's benefit–risk profile. Following modest expression of HbA^{T87Q} in the initial patients (Group A, $n = 7$), alterations were made to the treatment process for patients subsequently enrolled in Group B ($n = 2$, patients B1 and B2), including improvements to cell collection and lovo-cel manufacturing. After 6 months, median Group A peripheral blood vector copy number (≥0.08 c/dg) and HbA^{T87Q} levels (≥0.46 g/dL) were inadequate for substantial clinical effect but stable and sustained over 5.5 years; both markedly improved in Group B (patient B1: ≥0.53 c/dg and ≥2.69 g/dL; patient B2: ≥2.14 c/dg and ≥6.40 g/dL, respectively) and generated improved biologic and clinical efficacy in Group B, including higher total hemoglobin and decreased hemolysis. The safety of the lovo-cel for SCD treatment regimen largely reflected the known side effects of HSPC collection, busulfan conditioning regimen, and underlying SCD; acute myeloid leukemia was observed in two patients in Group A and deemed unlikely related to insertional oncogenesis. Changes made during development of the lovo-cel treatment process were associated with improved outcomes and provide lessons for future SCD GT studies.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Biologic and Clinical Efficacy of LentiGlobin for Sickle Cell Disease

J. Kanter, M.C. Walters, L. Krishnamurti, M.Y. Mapara, J.L. Kwiatkowski, S. Rifkin-Zenenberg, B. Aygun, K.A. Kasow, F.J. Pierciey, Jr., M. Bonner, A. Miller, X. Zhang, J. Lynch, D. Kim, J.-A. Ribeil, M. Asmal, S. Goyal, A.A. Thompson, and J.F. Tisdale

ABSTRACT

BACKGROUND
Sickle cell disease is characterized by the painful recurrence of vaso-occlusive events. Gene therapy with the use of LentiGlobin for sickle cell disease (bb1111; lovotibeglogene autotemcel) consists of autologous transplantation of hematopoietic stem and progenitor cells transduced with the BB305 lentiviral vector encoding a modified β-globin gene, which produces an antisickling hemoglobin, HbA^{T87Q}.

METHODS
In this ongoing phase 1–2 study, we optimized the treatment process in the initial 7 patients in Group A and 2 patients in Group B with sickle cell disease. Group C was established for the pivotal evaluation of LentiGlobin for sickle cell disease, and we adopted a more stringent inclusion criterion that required a minimum of four severe vaso-occlusive events in the 24 months before enrollment. In this unprespecified interim analysis, we evaluated the safety and efficacy of LentiGlobin in 35 patients enrolled in Group C. Included in this analysis was the number of severe vaso-occlusive events after LentiGlobin infusion among patients with at least four vaso-occlusive events in the 24 months before enrollment and with at least 6 months of follow-up.

RESULTS
As of February 2021, cell collection had been initiated in 43 patients in Group C; 35 received a LentiGlobin infusion, with a median follow-up of 17.3 months (range, 3.7 to 37.6). Engraftment occurred in all 35 patients. The median total hemoglobin level increased from 8.5 g per deciliter at baseline to 11 g or more per deciliter from 6 months through 36 months after infusion. HbA^{T87Q} contributed at least 40% of total hemoglobin and was distributed across a mean (±SD) of 85±8% of red cells. Hemolysis markers were reduced. Among the 25 patients who could be evaluated, all had resolution of severe vaso-occlusive events, as compared with a median of 3.5 events per year (range, 2.0 to 13.5) in the 24 months before enrollment. Three patients had a nonserious adverse event related or possibly related to LentiGlobin that resolved within 1 week after onset. No cases of hematologic cancer were observed during up to 37.6 months of follow-up.

CONCLUSIONS
One-time treatment with LentiGlobin resulted in sustained production of HbA^{T87Q} in most red cells, leading to reduced hemolysis and complete resolution of severe vaso-occlusive events. (Funded by Bluebird Bio; HGB-206 ClinicalTrials.gov number, NCT02140554.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Dr. Tisdale can be contacted at johntis@mail.nih.gov or at the Cellular and Molecular Therapeutics Branch NHLBI–NIDDK, National Institutes of Health, Bethesda, MD 20814.

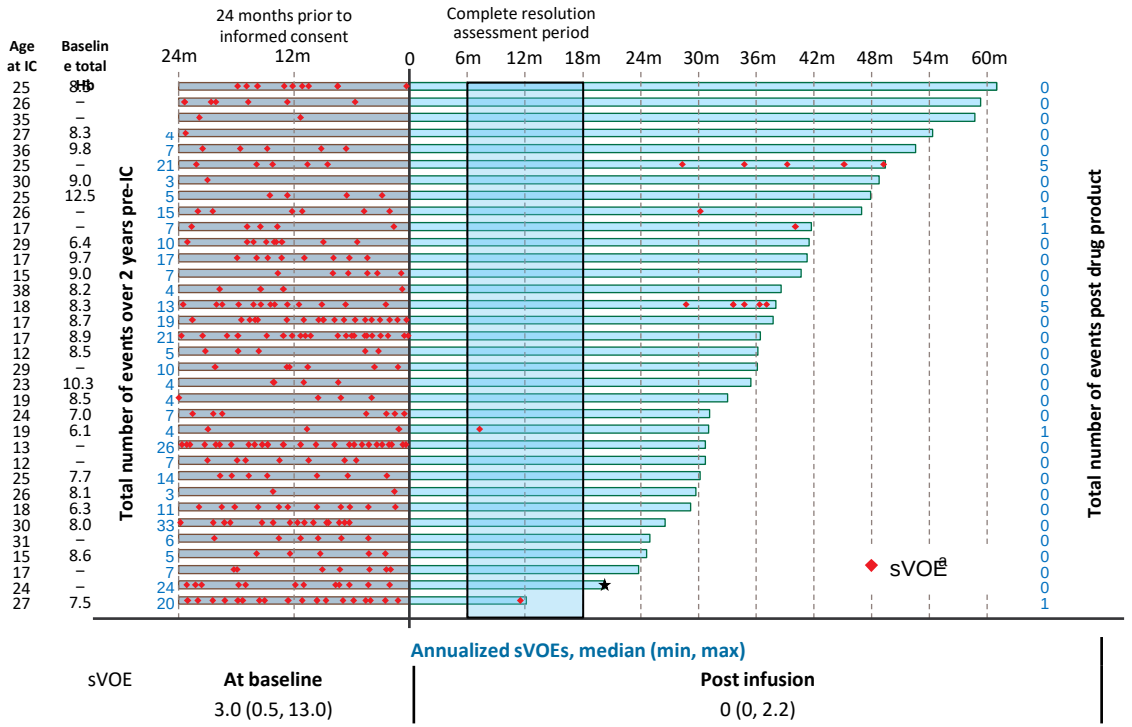
Drs. Kanter and Walters contributed equally to this article.

This article was published on December 12, 2021, at NEJM.org.

DOI: 10.1056/NEJMoa2117175

NIH National Heart, Lung, and Blood Institute

Updated results of the HGB206 and HGB210 studies: 94% (32/34) achieved complete resolution of severe VOEs



sVOE Resolution

- **94.1%** (32/34; 95% CI, 80.3-99.3) of patients experienced **complete resolution of sVOEs**[b]
  (sVOE defined as: A VOE requiring ≥24-hour hospital or ER observation unit visit or ≥2 visits to a day unit or ER over a 72-hour period, with both visits requiring intravenous treatment)

Hospital Admissions & Days

- **85.3%** (29/34) of patients had no VOE[c]-related hospital admissions from 6 months post infusion to last follow-up

- Among patients with VOEs post lovo-cel infusion, annualized median (min, max):
  - **Hospital admissions** were reduced from **2.5** (1, 13) to **0.41** (0, 2)
  - **Hospital days** were reduced from **15.75** (3.5, 136.0) to **2.20** (0.0, 25.4)

★ Death, due to significant baseline SCD-related cardiopulmonary disease; not considered related to lovo-cel.
An Independent Event Adjudication Committee confirmed VOEs met protocol criteria. [a]Defined as a VOE requiring ≥24-hour hospital or emergency room (ER) observation unit visit or at least 2 visits to a day unit or ER over a 72-hour period, with both visits requiring intravenous treatment; all VOEs of priapism were also considered sVOEs. [b]Maintained for a median of XX months (min, max). [c]Any of the following: acute episodes of pain with no medically determined cause other than a vaso-occlusion lasting 2 hours and requiring care at a medical facility; acute chest syndrome requiring oxygen treatment and/or blood transfusion; acute hepatic sequestration; acute splenic sequestration; or acute priapism lasting 2 hours and requiring care at a medical facility.

Hb, hemoglobin; IC, informed consent; SCD, sickle cell disease; sVOE, severe vaso-occlusive event; VOE, vaso-occlusive event.

Population: Evaluable for VOE-CR and sVOE-CR

Data as of Feb 13, 2023

National Heart, Lung, and Blood Institute

A topic in 2022 ASGCT : *in vivo* HSC gene therapy

Francis Collins, MD, PhD
NIH director (until 2021)

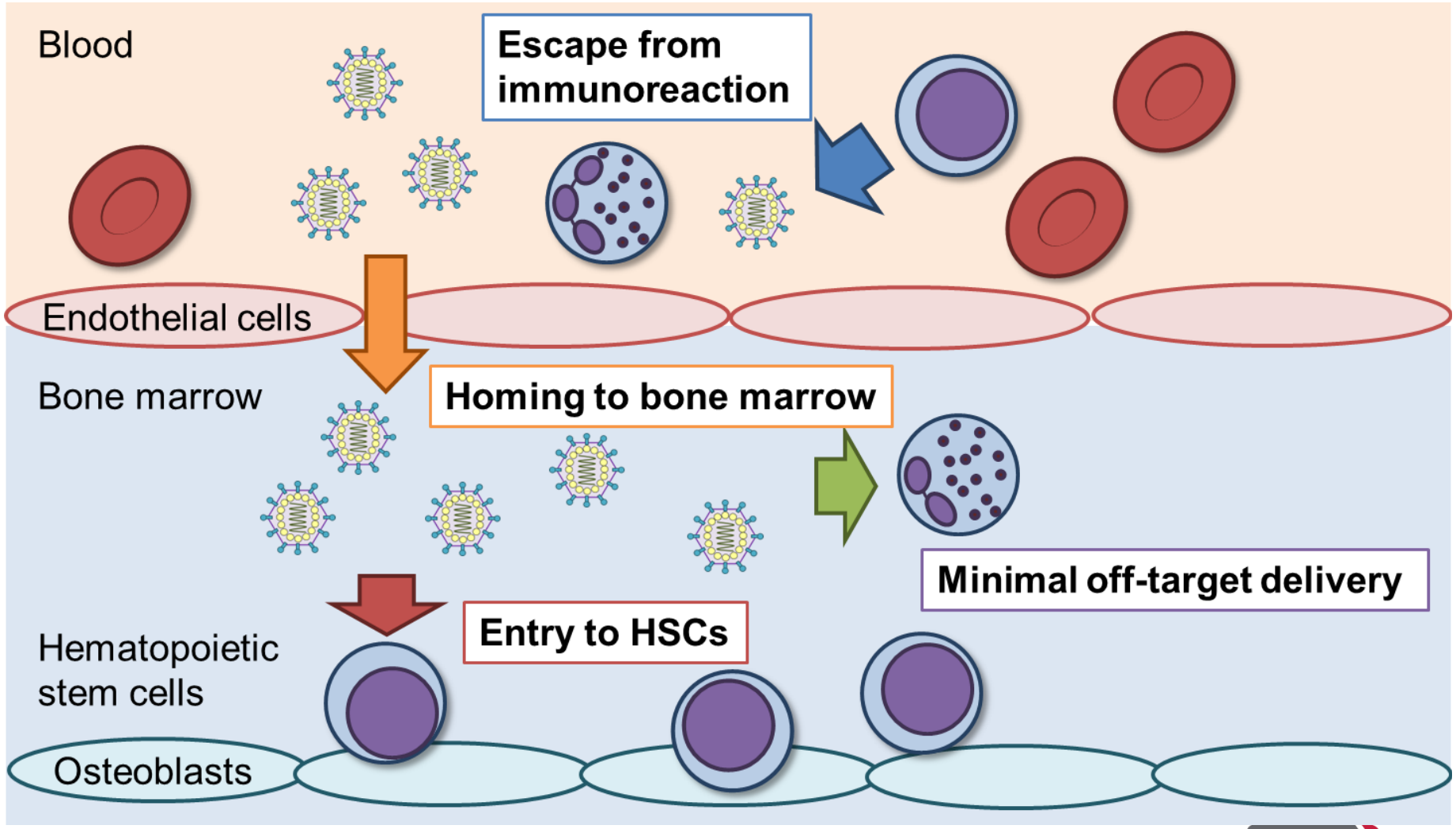
- Why *in vivo*?
  - Not feasible to remove the affected tissue for most genetic diseases
  - Current ex vivo protocols (such as SCD) are complex, risky, and expensive
- Why gene editing?
  - Not limited to gene replacement
  - Can be done by a single infusion

Clearly, ex vivo therapy for 100,000 SCD patients is out of the question, even before acknowledging that most of the patients are spread across Africa and India. "We have to come up with a strategy" to help these patients, Collins said.

In a collaboration with the Bill and Melinda Gates Foundation, NIH is mounting an effort for a one-shot SCD cure that could be administered in a low-resource setting. Ambitiously, Collins said his team thought, "let's cure HIV at the same time."

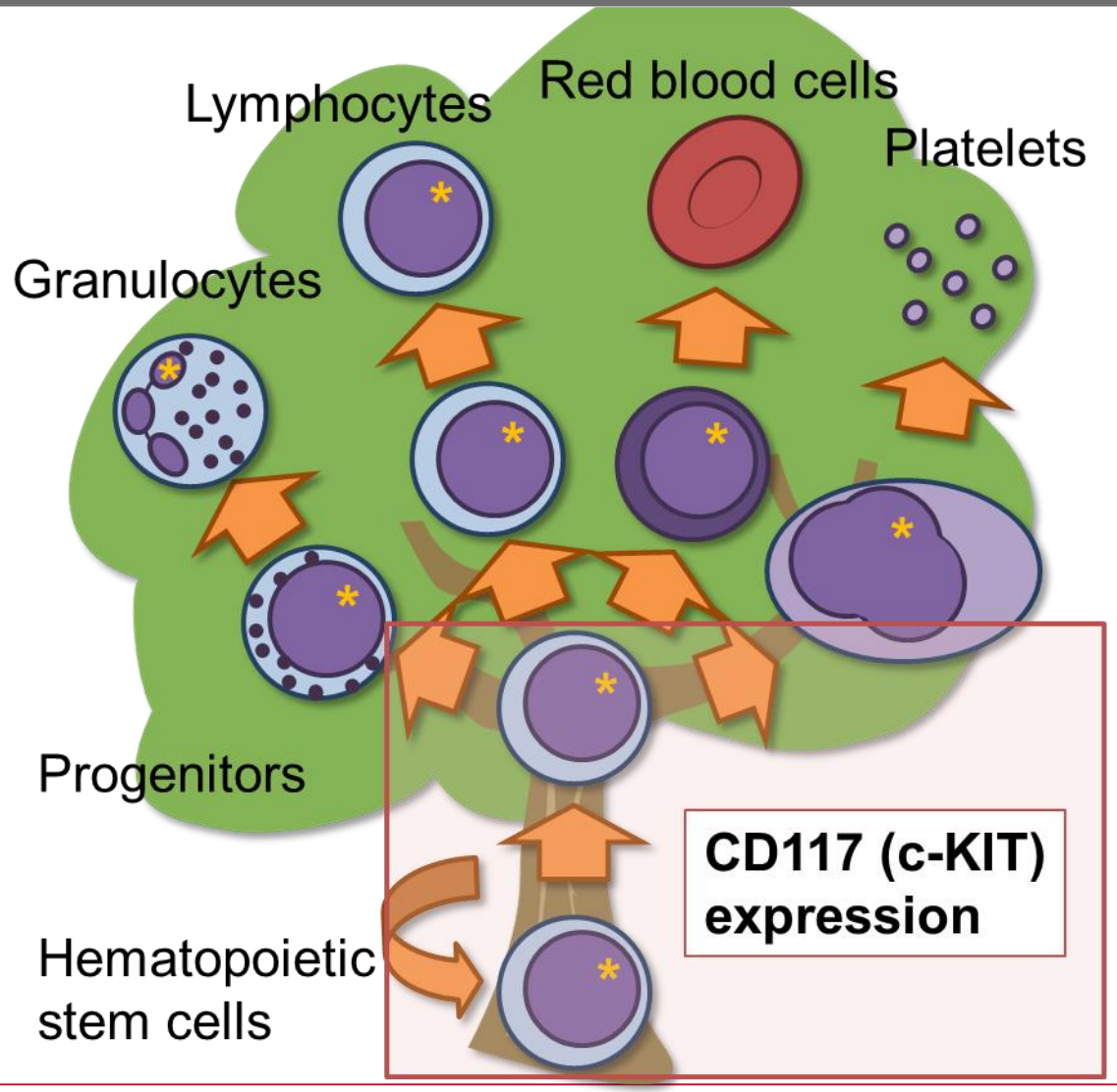
Genetic Engineering & Biotechnology News. 2022

Barriers for a targeted gene delivery to HSCs



Cruz LJ. Front Genome Ed. 2022

CD117 (c-KIT) is an ideal target for *in vivo* delivery to HSCs

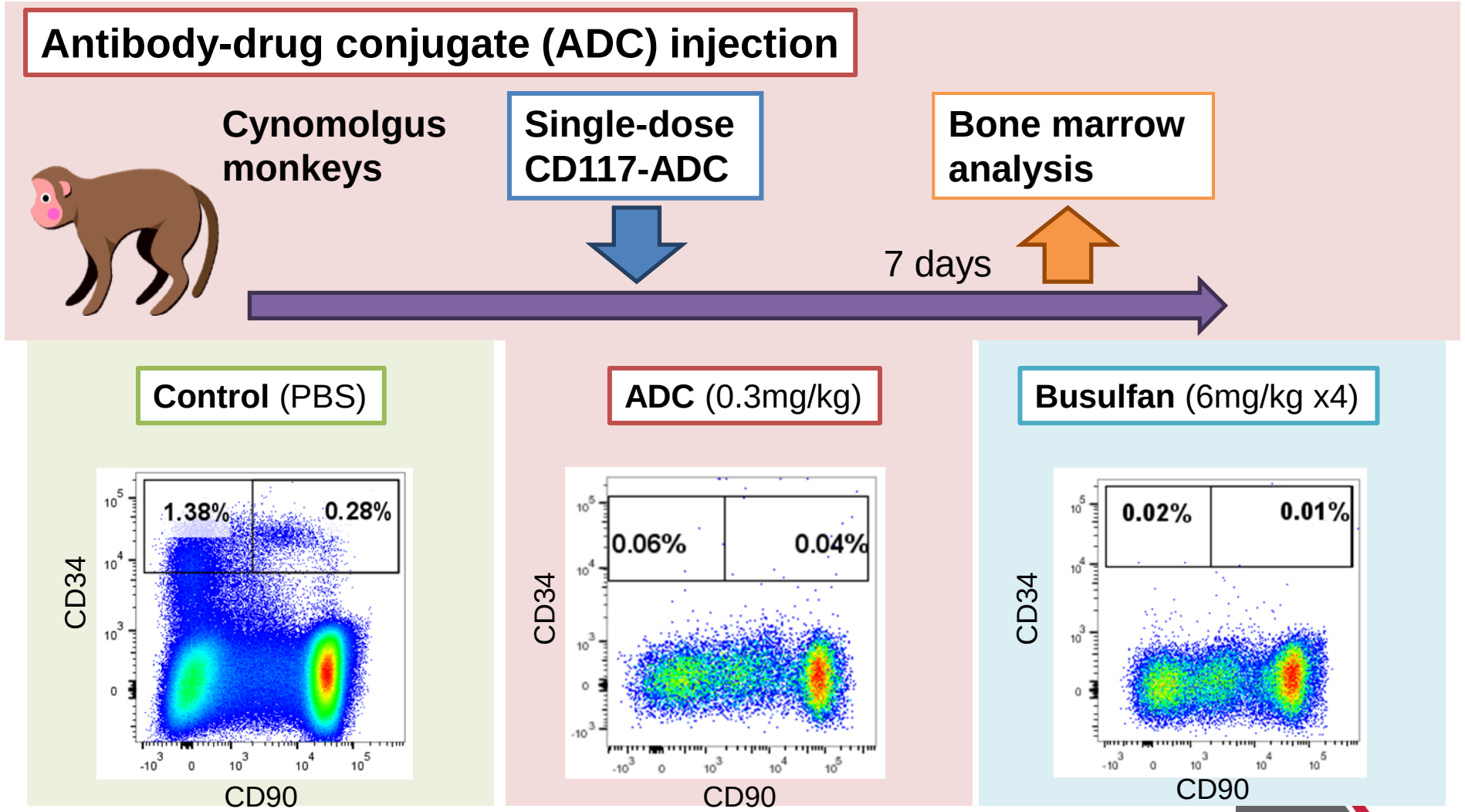


Hematopoietic stem cells (HSCs)

- Produce all types of blood cells for the life of a patient
- A one-time cure of SCD allowed by a gene repair in HSCs

CD117 expression is limited to stem and progenitor cells

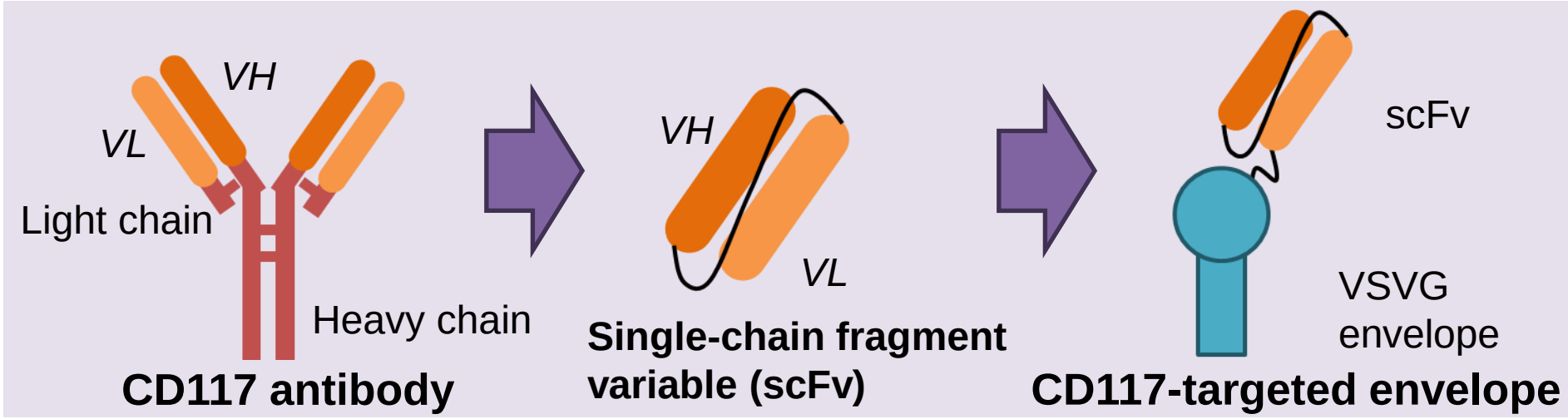
HSC-targeted delivery with antibody-drug conjugate (ADC)

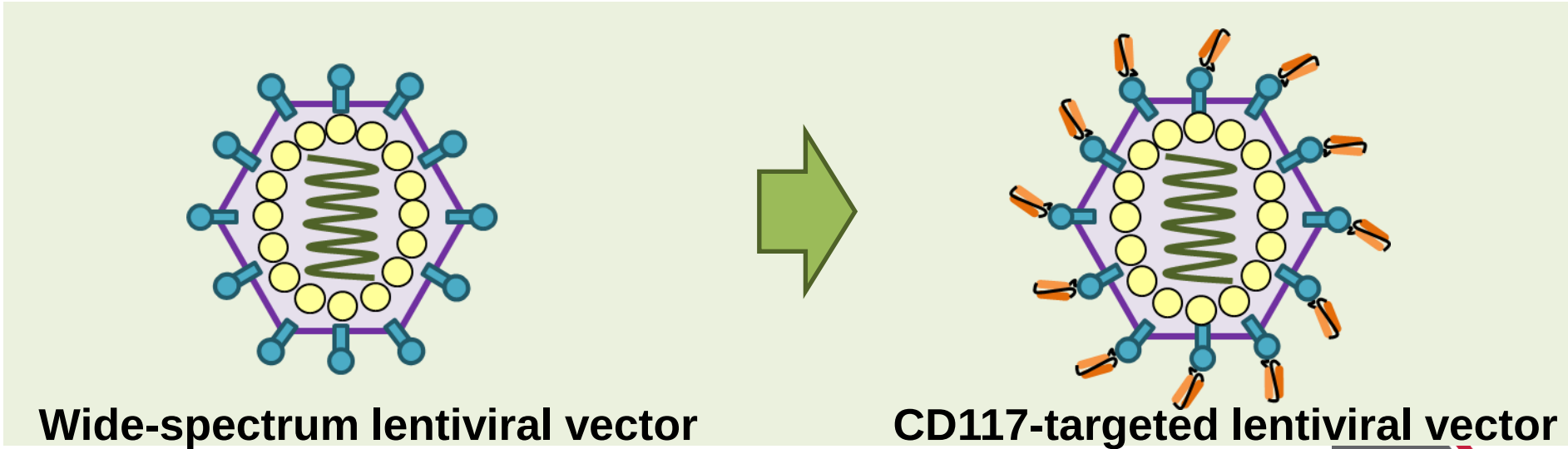


Uchida N. Nat Commun. 2023

NIH National Heart, Lung, and Blood Institute

Lentiviral vectors with a CD117-targeted scFv envelope

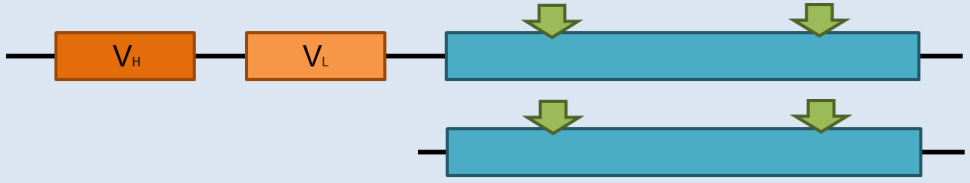




Optimization of scFv to improve CD117-targeted vectors after phage display screening

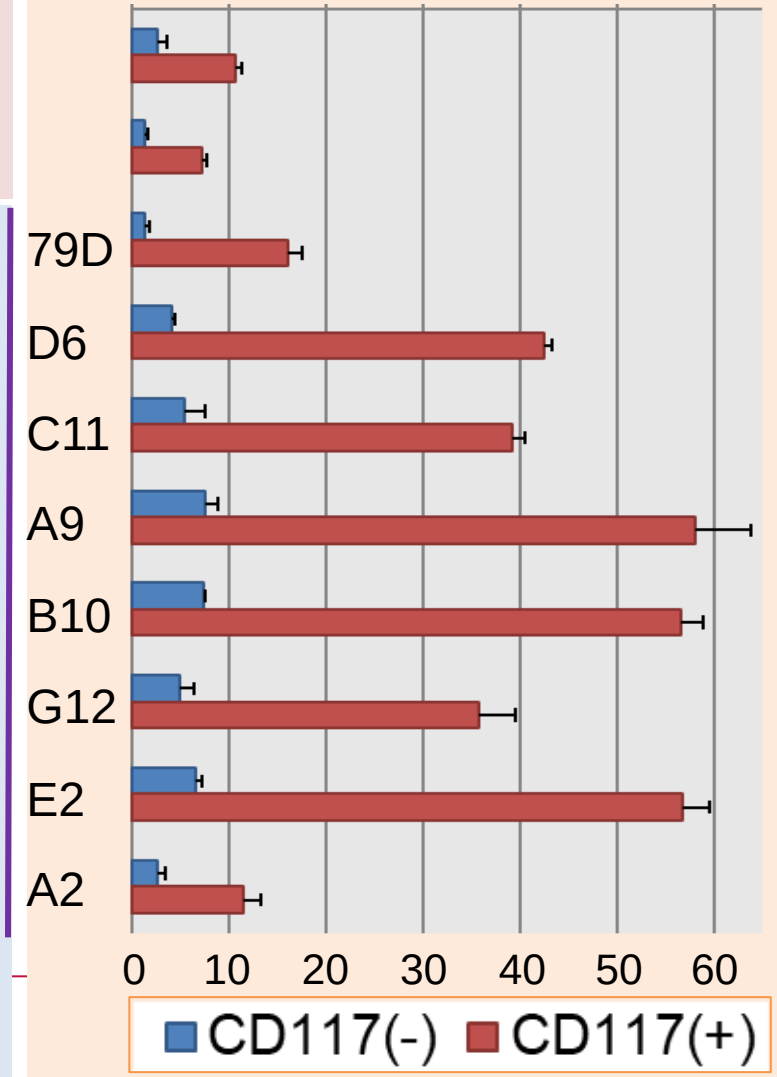
Non-targeted envelope

Targeted envelope



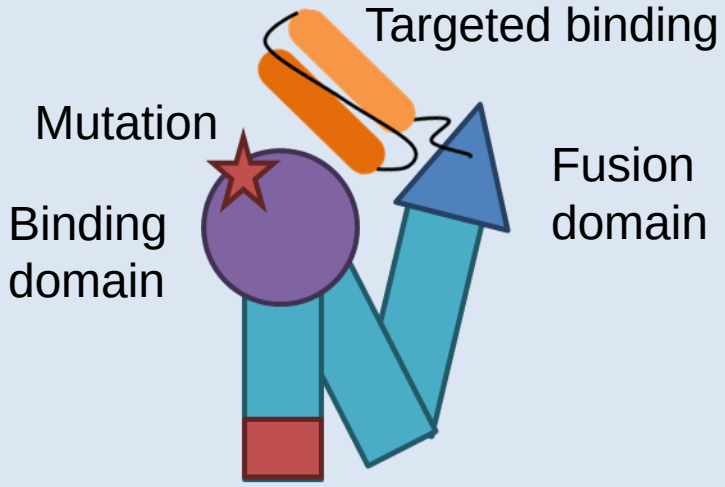
Mix of scFv-VSVGmut and VSVGmut

%GFP in CD117(+)/(-) cells



Further optimize the targeted envelope design

One-protein envelope

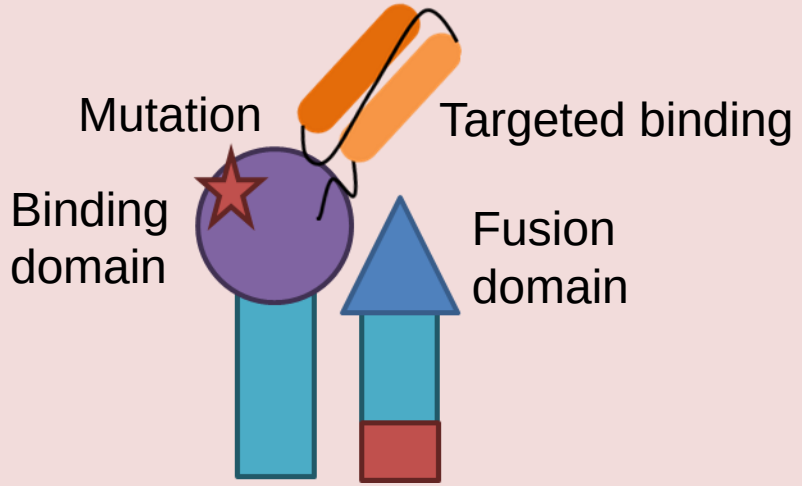


VSVG envelope

- Non-functional: The scFv/peptide attached to the fusion domain
- High titer: Adapted to the HIV-1 system

1. Retroviral envelopes (Amph, RD114, GaLV)
2. VSVG envelope
3. Cocal envelope

Two-protein envelope



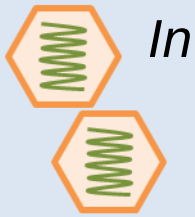
Measles envelope

- Functional: The scFv/peptide attached to the binding domain
- Low titer: Need to adapt to the HIV-1 system

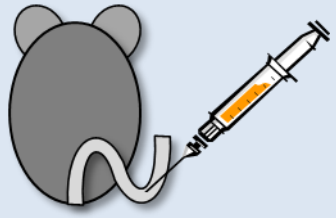
1. Measles envelope
2. Sindbis envelope

In vivo HSC-targeted gene delivery with LNPs in mice

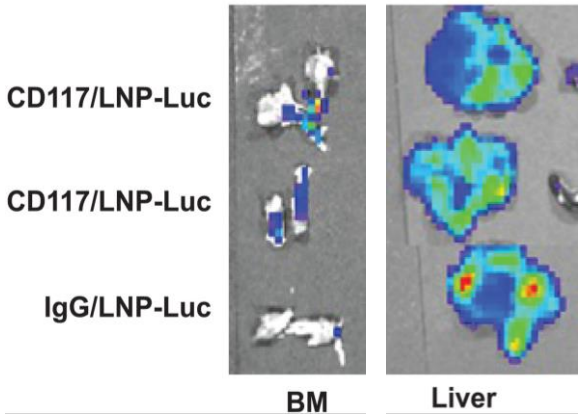
CD117-targeted lipid nanoparticles (LNPs) to deliver Luc or Cre RNA

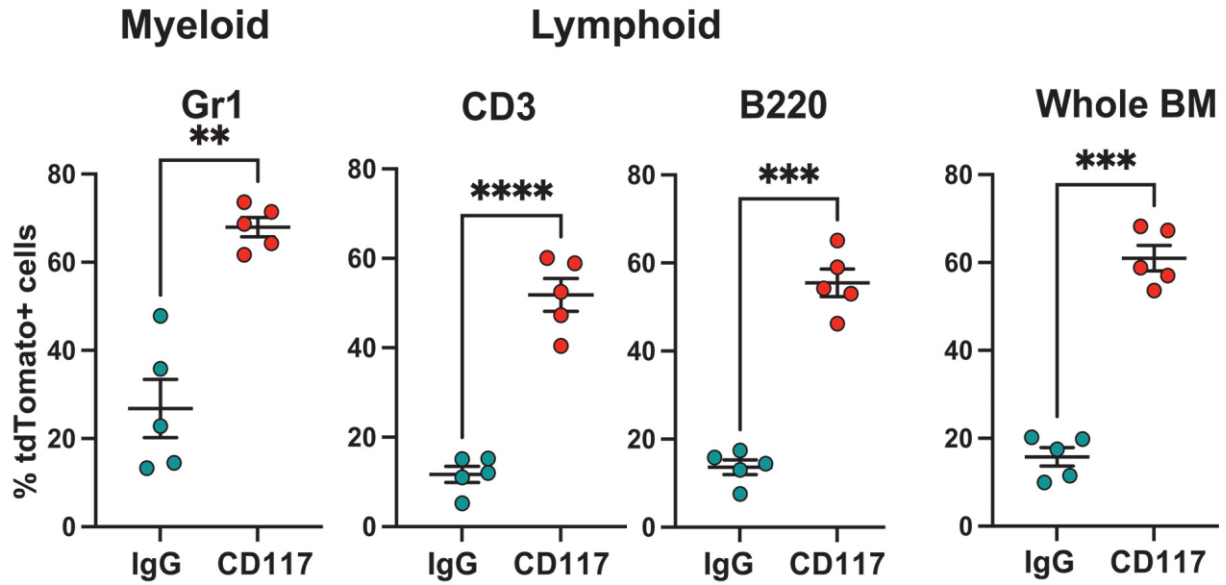


In vivo injection



Cre-responsive tdTomato reporter mice

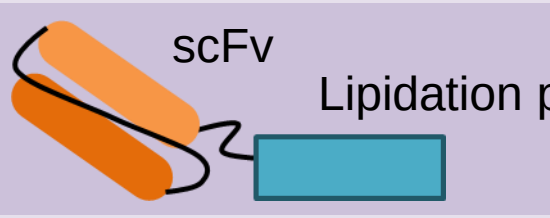


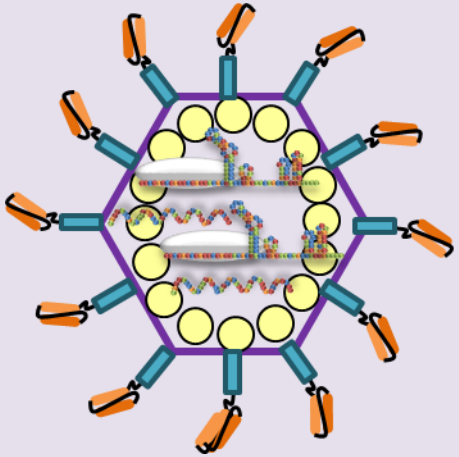


Systemic injection of CD117-targeted LNPs resulted in ~60% tdTomato activation in blood cells and bone marrow cells in mice.

Switch from scFv to targeted peptides for stable lipid nanoparticles manufacturing

Ionizable lipid nanoparticles with CD117-scFv

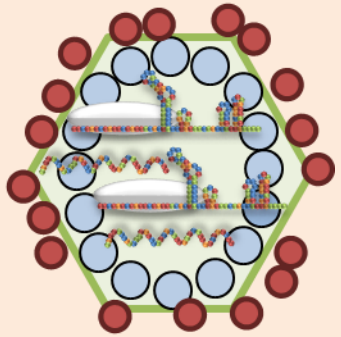




Difficult for stable generation

Ionizable lipid nanoparticles with CD117-targeted peptides

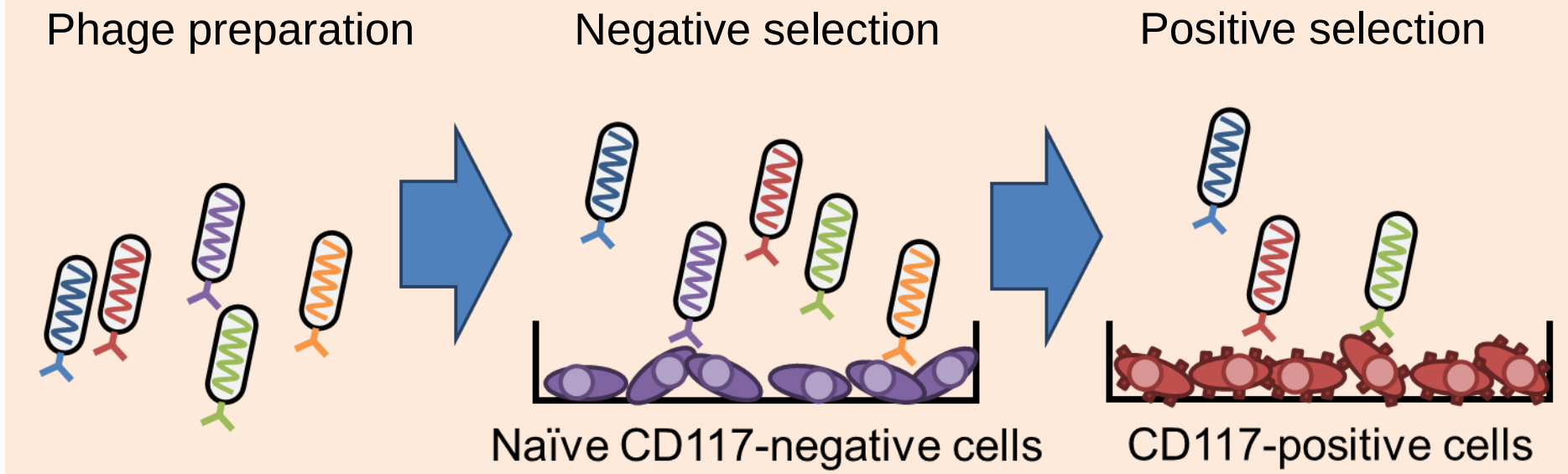
CD117-targeted peptide



Possible for stable generation

Kedmi R. Nat Nanotechnol. 2018

Phage display screening with different media



Generate a peptide library

Remove non-specific peptides

Select CD117-targeted peptides

10%, 20%, 70%, and 90% human plasma

Selection media (binding phages) and Cell lysate (internalized phages)

CD117-targeted phage display screening with human plasma

10%Plasma		2. 20%Plasma		3. 70%Plasma		4. 90%Plasma	
Extra-cellular	Intra-cellular	Extra-cellular	Intra-cellular	Extra-cellular	Intra-cellular	Extra-cellular	Intra-cellular
1. Mix	1. P16	1. Rand	1. Mix	1. P10	1. P14	1. P13	1. P15
2. Mix	2. Rand	2. Rand	2. Mix	2. N/A	2. P14	2. P15	2. P15
3. P12	3. Rand	3. Del	3. Rand	3. P10	3. P14	3. P13	3. P15
4. P9	4. P16	4. P13	4. N/A	4. P10	4. Rand	4. P15	4. P15
5. Mix	5. P16	5. Del	5. P12	5. P10	5. P14	5. P15	5. P15
6. P9	6. P16	6. Del	6. P13	6. P10	6. P14	6. P13	6. P15
7. P12	7. P16	7. Del	7. Del	7. P10	7. P14	7. P15	7. P15
8. P13	8. P16	8. Del	8. Rand	8. P10	8. P14	8. P15	8. P15
9. Mix	9. P15	9. N/A	9. Mix	9. P11	9. P14	9. P13	9. P15
10. P9	10. Rand	10. Rand	10. Rand	10. P11	10. P14	10. P15	10. P15
11. Mix	11. Mix	11. Rand	11. P12	11. P11	11. P14	11. P15	11. P15
12. P9	12. P16			12. P11	12. P14	12. P15	12. P15

- Human plasma concentration doesn't affect peptide binding to CD117.
- CD117-internalized peptides can be found in extracellular samples.

More efficient transduction in CD117+ cells with targeted vectors

Non-targeted envelope

VSVG

VSVGmut

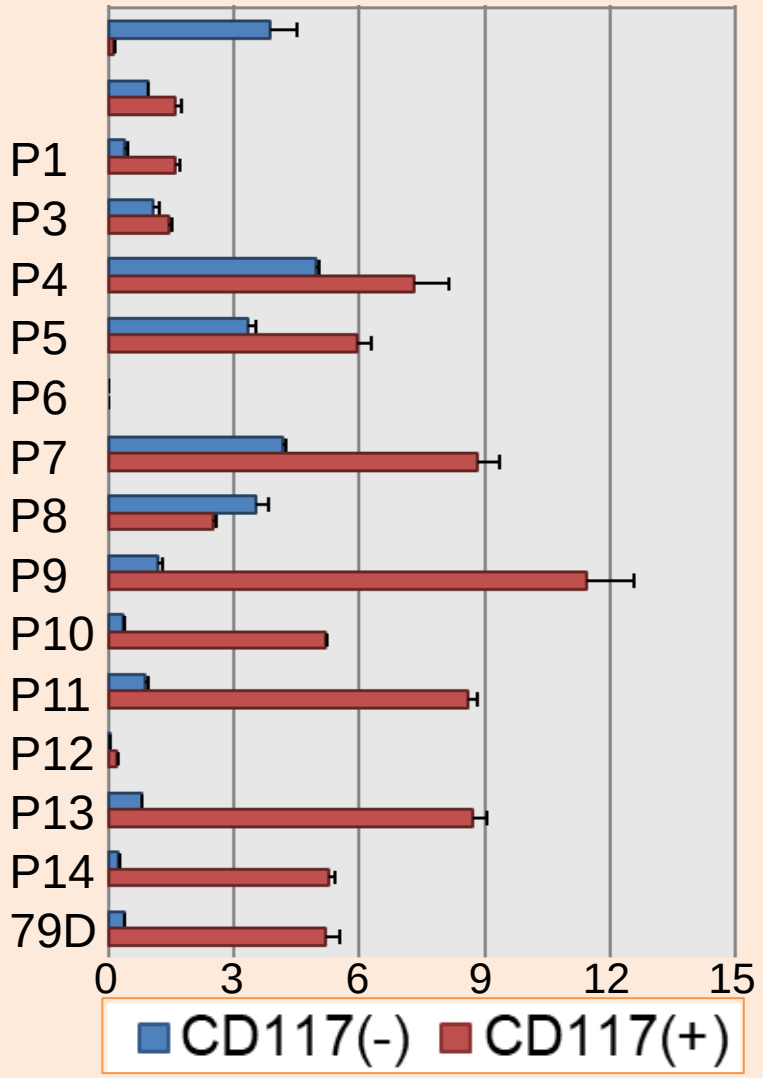
Targeted envelope

Peptide-VSVGmut

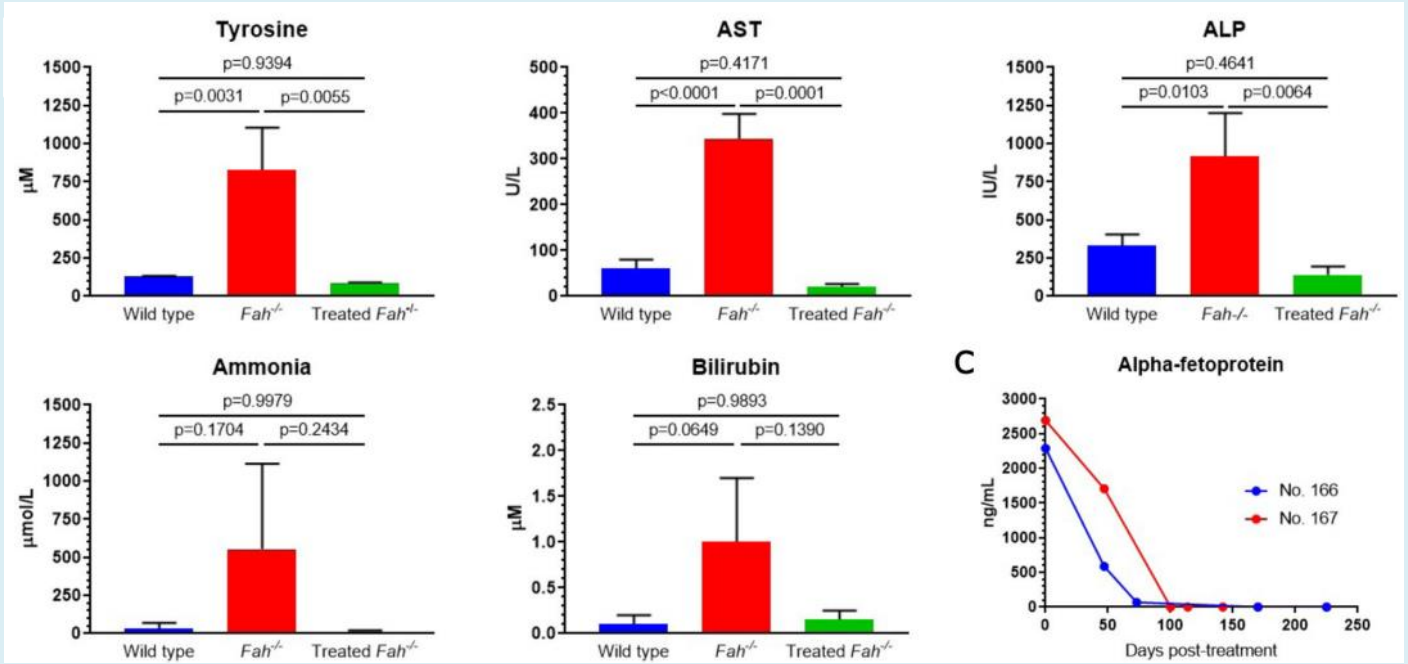
V_H V_L

Mix of scFv-VSVGmut and VSVGmut

%GFP in CD117(+)/(-) cells

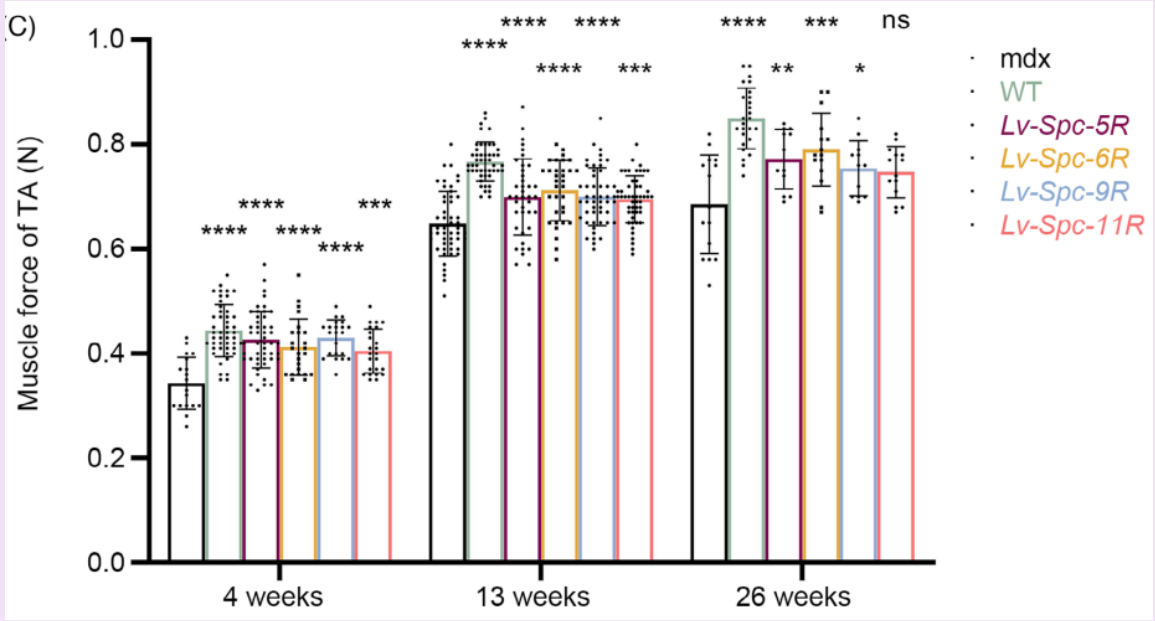


Use of Lentiviral Vectors to Treat Hereditary Tyrosinemia Type 1 *In Vivo*



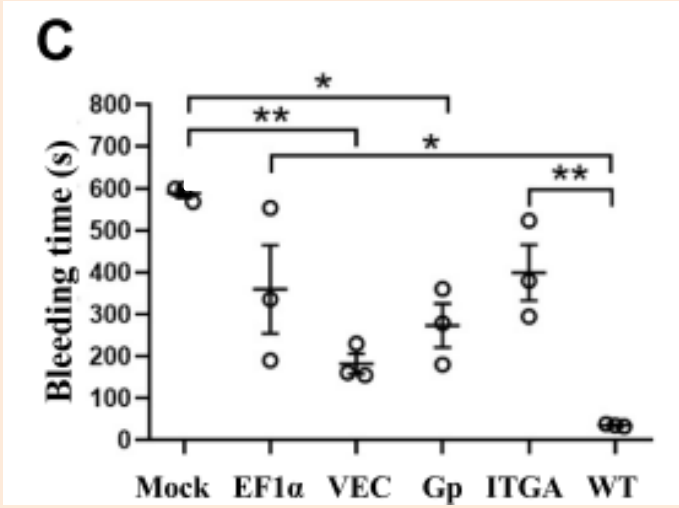
Nicolas *et al*. Nat Comm, 2022

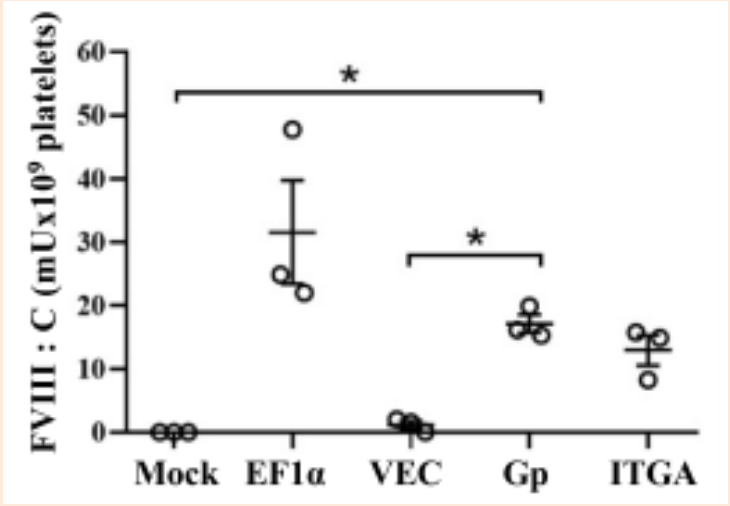
Improvement of Pull Force in Duchenne Muscular Dystrophy Mouse Model Post *In Vivo* Lentiviral Treatment

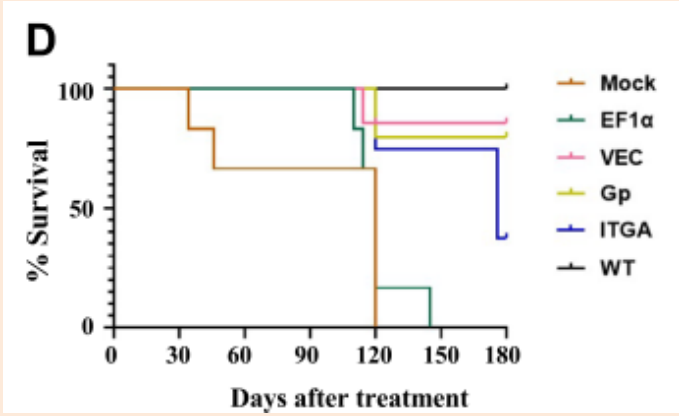


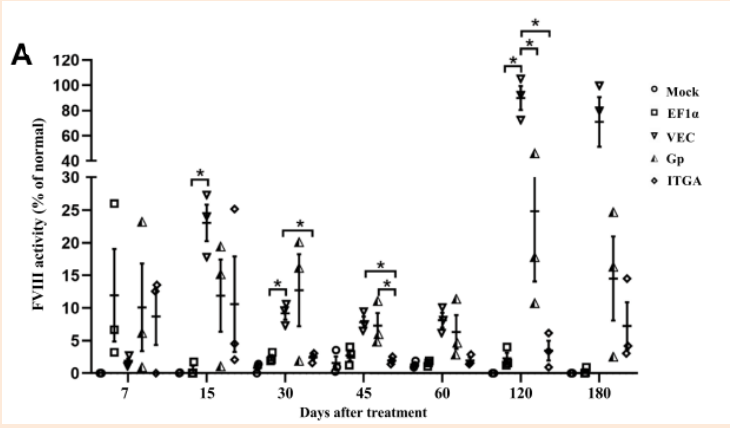
Wang *et al*., MedComm, 2024

In Vivo Lentiviral Delivery for the Treatment of Hemophilia A



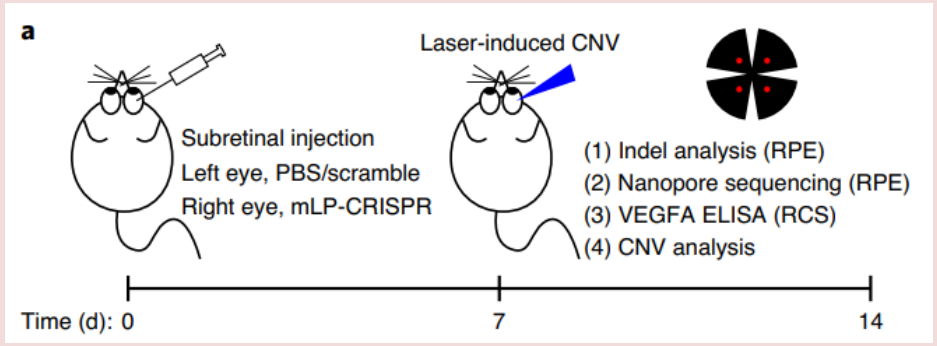


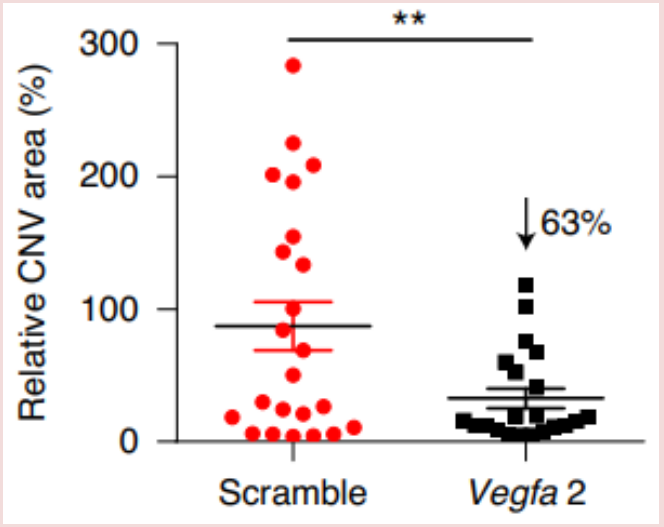


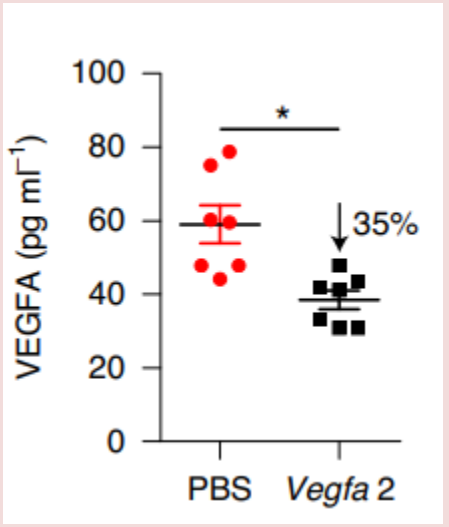


Gong *et al*., Mol Med, 2023

Subretinal Injection Lentiviral Vectors to Deliver CRISPR-Cas9 Machinery *In Vivo* to Treat Wet Age-related Macular Degeneration

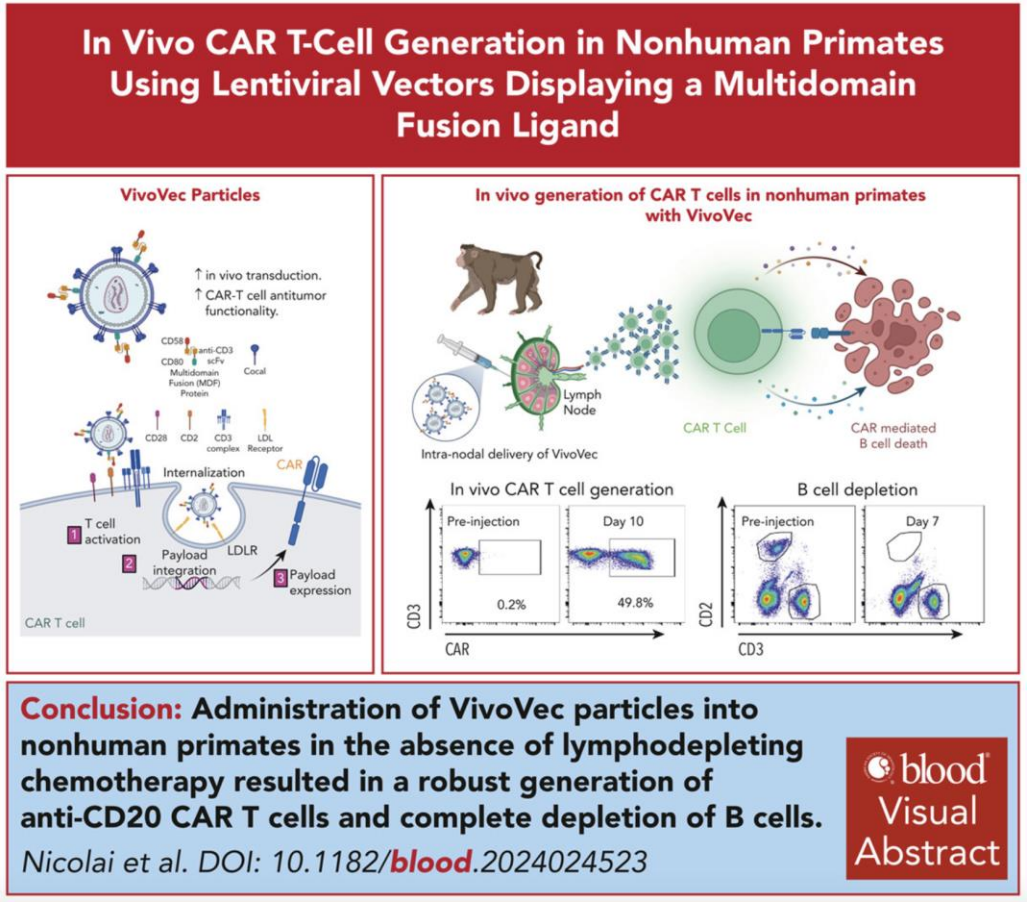


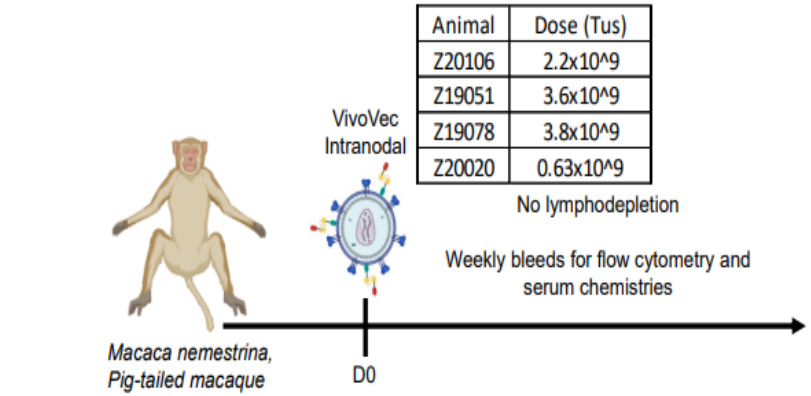




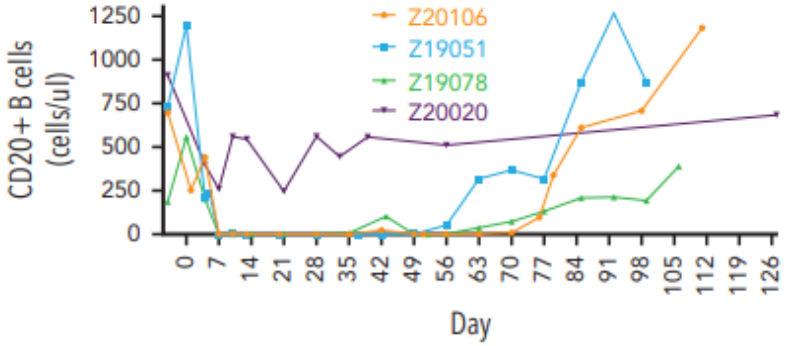
Ling *et al*., Nat Biomed Eng 2021

In Vivo CAR-T Cell Generation with VVPs





Animal	Dose (Tus)
Z20106	2.2x10^9
Z19051	3.6x10^9
Z19078	3.8x10^9
Z20020	0.63x10^9



Nicolai *et al*. Blood, 2024

How many cells do we have to reach?

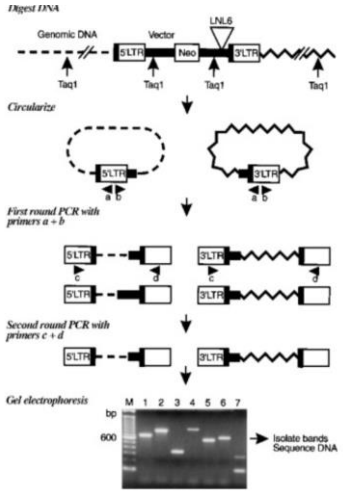
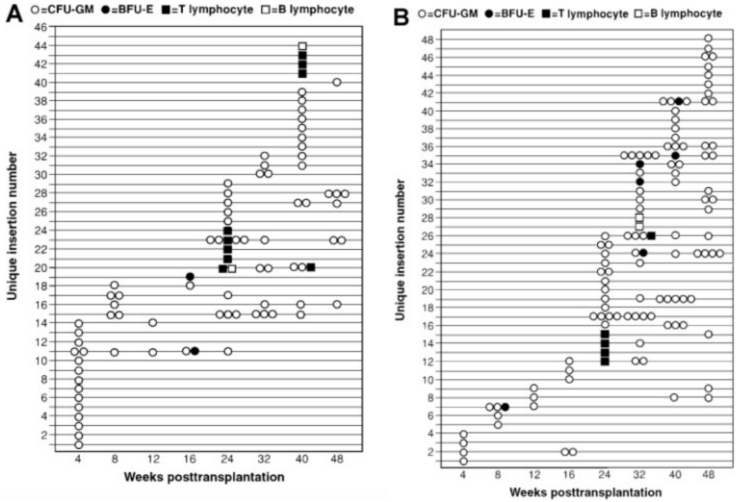


Table 2. Genomic flanking sequences found in more than one lineage

Unique insertion number/source	MoMuLV	Flanking genomic DNA
11/animal 1, GM-CFU	3'LTR cgggggtctttca	CATGCAGCATGTATCAAAAT
11/animal 1, BFU-E	3'LTR cgggggcctttcx	CATGCAGCATGTATCAAAAT
20/animal 1, GM-CFU	3'LTR cgggggtctttca	TCAAGAAGCTAAATATTATC
20/animal 1, T cells	3'LTR cgggggtctttca	TCAAGAAGCTAAATATTATC
20/animal 1, B cells	3'LTR cgggggtctttca	TCAAGAAGCTAAATATTATC
23/animal 1, T cells	3'LTR cgggggtctttca	AAACCACATAAATATACAGA
23/animal 1, GM-CFU	3'LTR cgggggtctttca	AAACCACATAAATATACAGA
7/animal 2, GM-CFU	3'LTR cgggggtctttca	AACACTGAGGAGACTTCAGC
7/animal 2, BFU-E	3'LTR cgggggtctttca	AACACTGAGGAGACTTCAGC
12/animal 2, GM-CFU	3'LTR cgggggtctttca	TATAAAGTATAATTGTCCTA
12/animal 2, T cells	3'LTR cgggggtctttca	TATAAAGTATAATTGTCCTA
14/animal 2, GM-CFU	3'LTR cgggggtctttca	GAACAAGTCACTTTGGGAGG
14/animal 2, T cells	3'LTR cgggggtctttca	GAACAAGTCACTTTGGGAGG
15/animal 2, GM-CFU	3'LTR cgggggtctttca	AAACTAAATATATTAGATAG
15/animal 2, T cells	3'LTR cgggggtctttca	AAACTAAATATATTAGATAG
24/animal 2, GM-CFU	3'LTR cgggggtctttca	TACATGGCAAGTGCCCGCCT

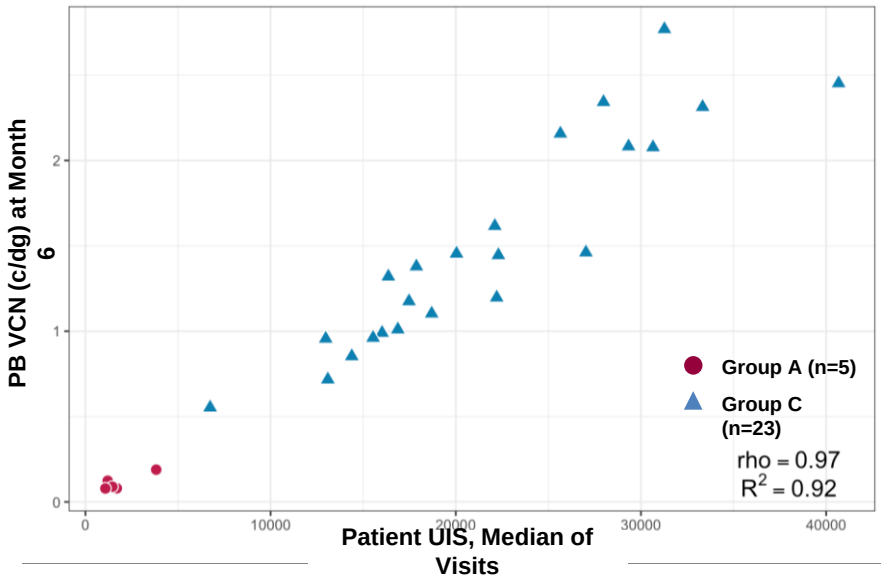


- Mathematical models for capture and release estimate a range of 5 to 44 clones in animal 1 and 8 to 60 clones in animal 2 contributed to hematopoiesis in the first year
- Starting CD34 cell number was 20 million and that these represent 1% of the bone marrow
- 5 cells per 10^7 bone marrow mononuclear cells contributed to hematopoiesis in the first year
- 3-4 liters of bone marrow in a 75kg human at 4-5 e 10^9 MNCs per L equaling 20 e 10^9
- This translates into around 100,000 HSCs per human

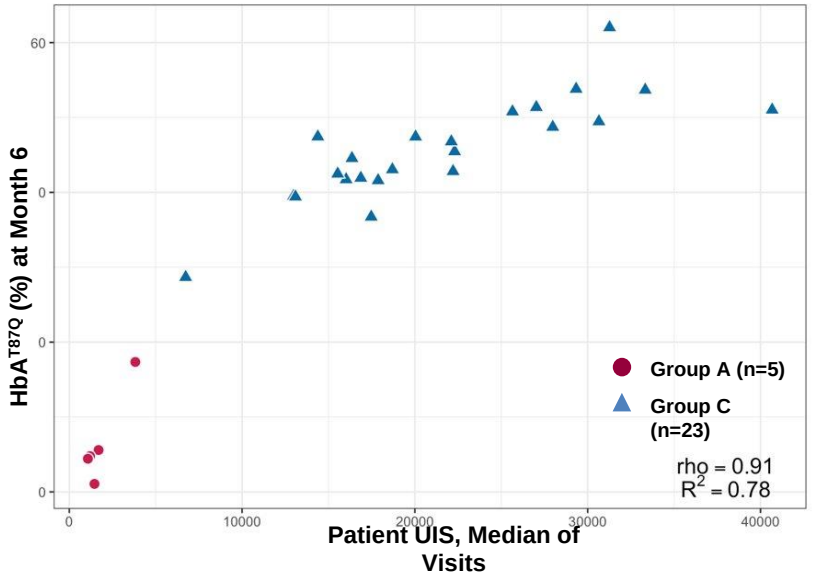
Kim, *et al.*, Blood, 2000

HGB-206 Groups A and C: Median unique insertion sites (UIS) correlate with PB VCN and HbA^{T87Q} at Month 6 post-LentiGlobin infusion

Median UIS vs PB VCN at Month 6



Median UIS vs HbA^{T87Q} % at Month 6



- Median UIS (as assessed by ISA with S-EPTS/LM-PCR) detected per visit for each patient and aggregated for all visits

c/dg, copies per diploid genome; HbA^{T87Q}, Hb with modified β-globin gene (β^{A-T87Q}); ISA, integration site analysis; PB, peripheral blood; S-EPTS/LM-PCR, shearing extension primer tag selection ligation-mediated polymerase chain reaction; UIS, unique insertion sites; VCN, vector copy number.

National Heart, Lung, and Blood Institute

Data as of 17 February 2021

Acknowledgements

John Tisdale Lab at NIH

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Julia Ball
Robert Donahue
Matthew Hsieh
Selami Demirci
Bjorg Gudmundsdottir
All other members

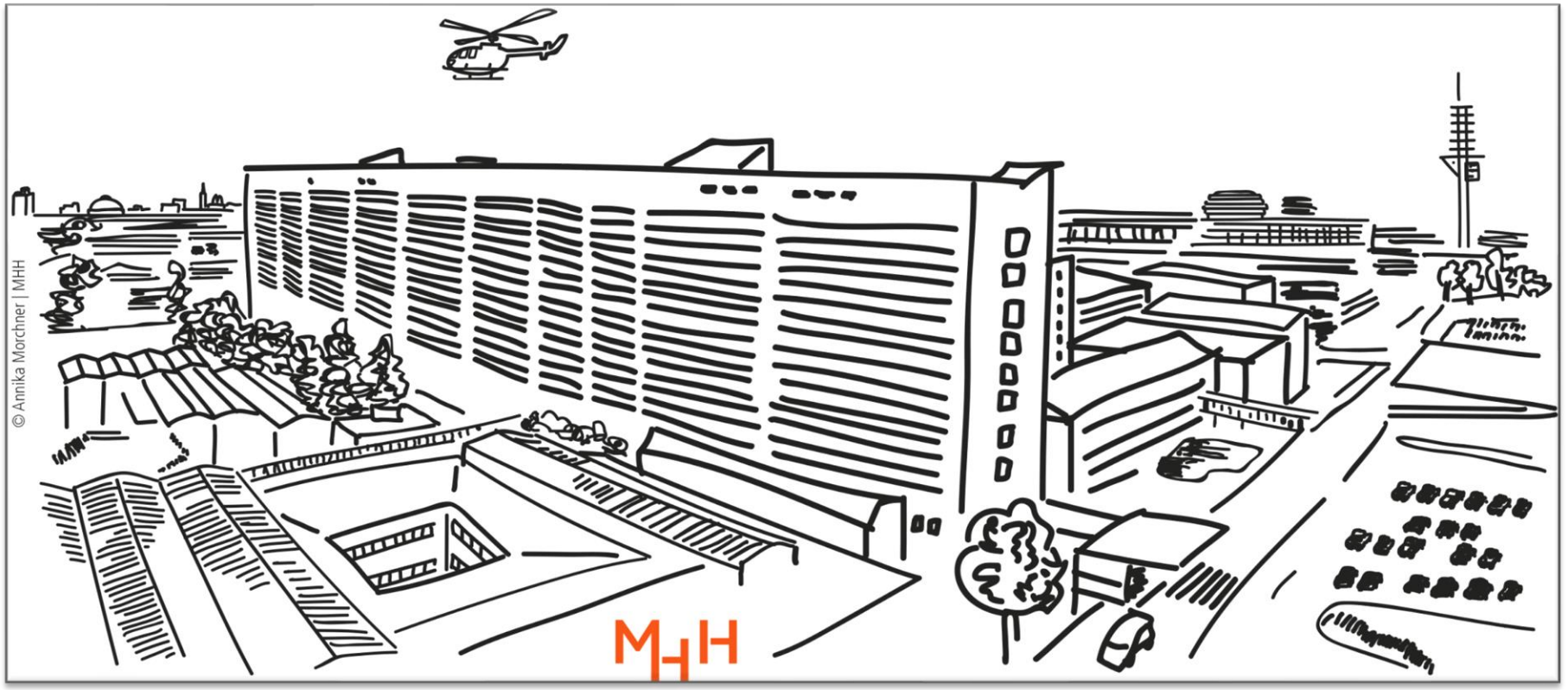
Takashi Okada Lab at University of Tokyo

- **NHLBI / NIDDK**
- **Bill & Melinda Gates Foundation**
- **Japan Agency for Medical Research and Development**
- **Japan Society for the Promotion of Science**

onal Heart, Lung, Blood Institute

Adeno-associated virus (AAV) and adenoviral (AdV) vectors for *in vivo* gene therapy

Hildegard Büning



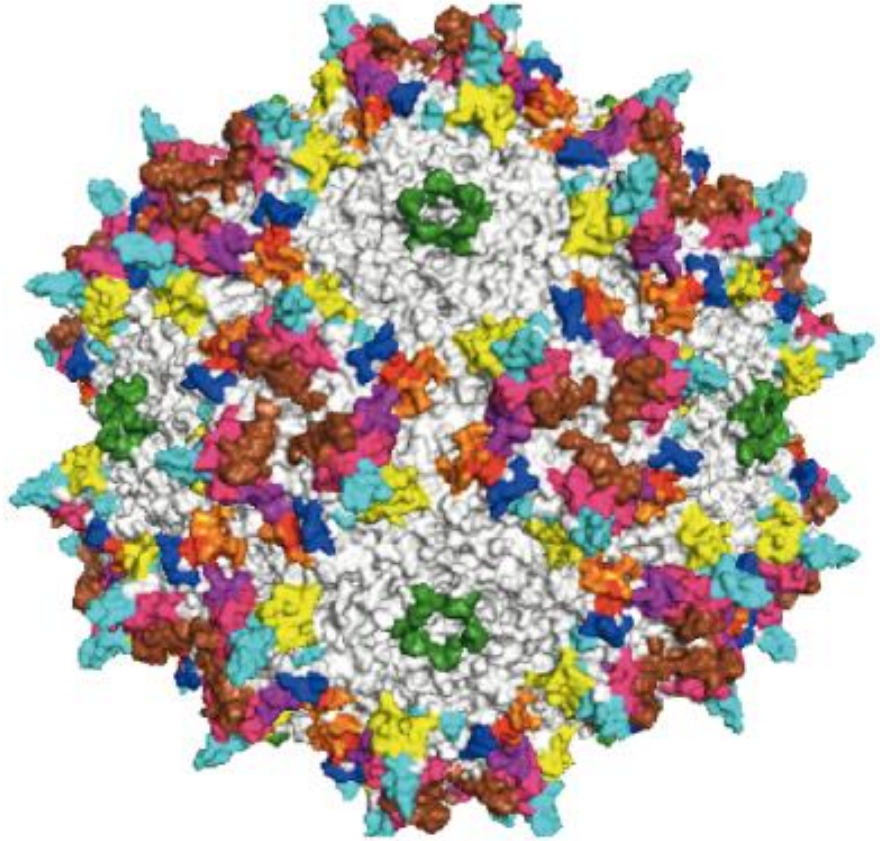
Scientific Advancements in Gene Therapies: Opportunities for Global Regulatory Convergence

Institute of Experimental Hematology
Hannover Medical School
buening.hildegard@mh-hannover.de

Adeno-associated virus (AAV)
Family *Parvoviridae*, genus *Dependoparvovirus*

portfolio of serotypes and variants

broad tropism



non-enveloped protein capsid
(Ø 20-25 nm)

single-stranded DNA genome
(~ 4.7 kb)

capsid image: Kotterman et al. Nat. Rev. Gen. 2014

The AAV vector system

Alipogene tiparvovec (® Glybera) – **AAV1** – 2012-2017*	**Lipoprotein lipase deficiency**
Voretigen Neparvovec (® Luxturna) – **AAV2** – 2017*, **	**Inherited retinal dystrophy**
Onasemnogen-Abeparvovec (® Zolgensma) – **AAV9** – 2019*, **	**Spinal muscular atrophy**
Eladocagene Exuparvovec (® Upstaza) – **AAV2** – 2022*	**Aromatic L-amino acid decarboxylase deficiency (AADC)**
Valoctocogen Roxaparvovec (® Roctavian) – **AAV5** – 2022*, **	**Haemophilia A**
Etranacogen Dezaparvovec (® Hemgenix) – **AAV5** – 2023*, **	**Haemophilia B**
fidanacogene elaparvovec-dzkt (® BEQVEZ) – **AAVRh74**- 2024**	
delandistrogene moxeparvovec-rokl (® ELEVIDY) - **AAVRh74** – 2023**	**Duchenne Muscular Dystrophy**

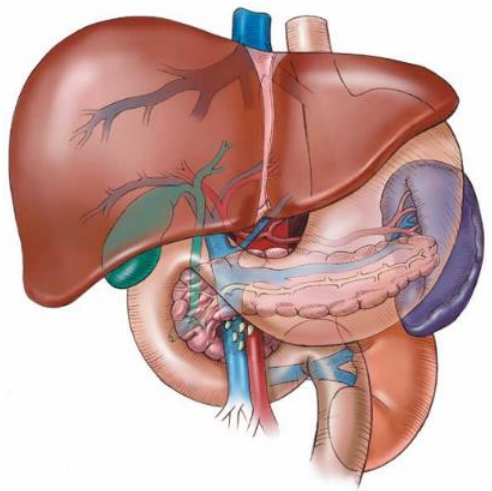
* European Medicines Agency; ** U.S. Food & Drug Administration; ATPM in green = intravenous administration

Challenges of 1st generation AAV vectors when applied intravenously

low efficacy

human body:
3.7x10e13 cells*

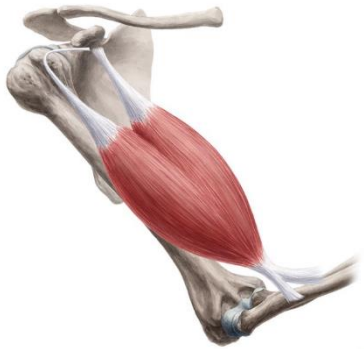
5×10^{11} to 6×10^{13} AAV vector particles / kg body weight



modified from slide kindly provided by Ian Alexander (University of Sydney; *https://commonfund.nih.gov/HuBMAP

Challenges of 1st generation AAV vectors when applied intravenously

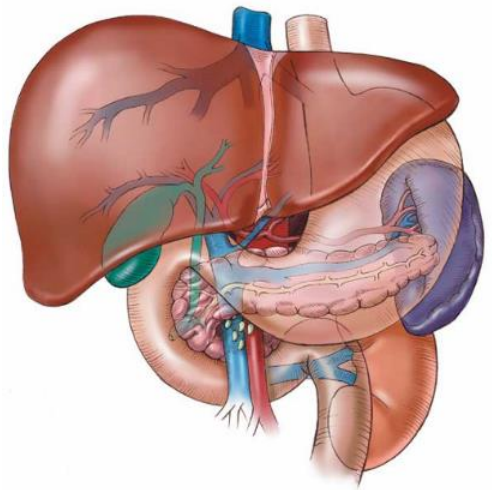
loss of vector particles in "off-target" tissue



$\geq 10^{14}$ vg/kg

5×10^{11} to 6×10^{13} AAV vector particles (vg) / kg body weight

low efficacy



Challenges of 1st generation AAV vectors when applied intravenously

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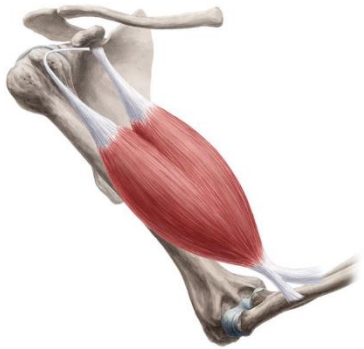

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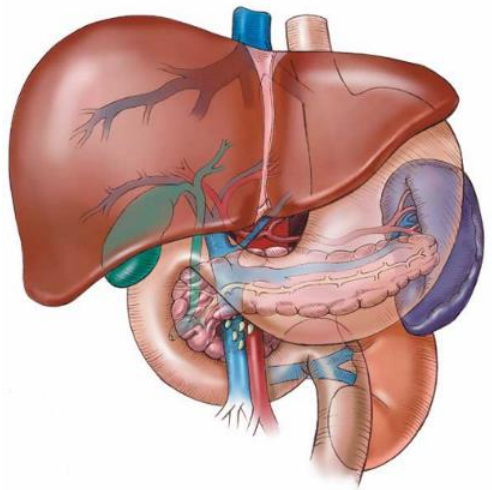


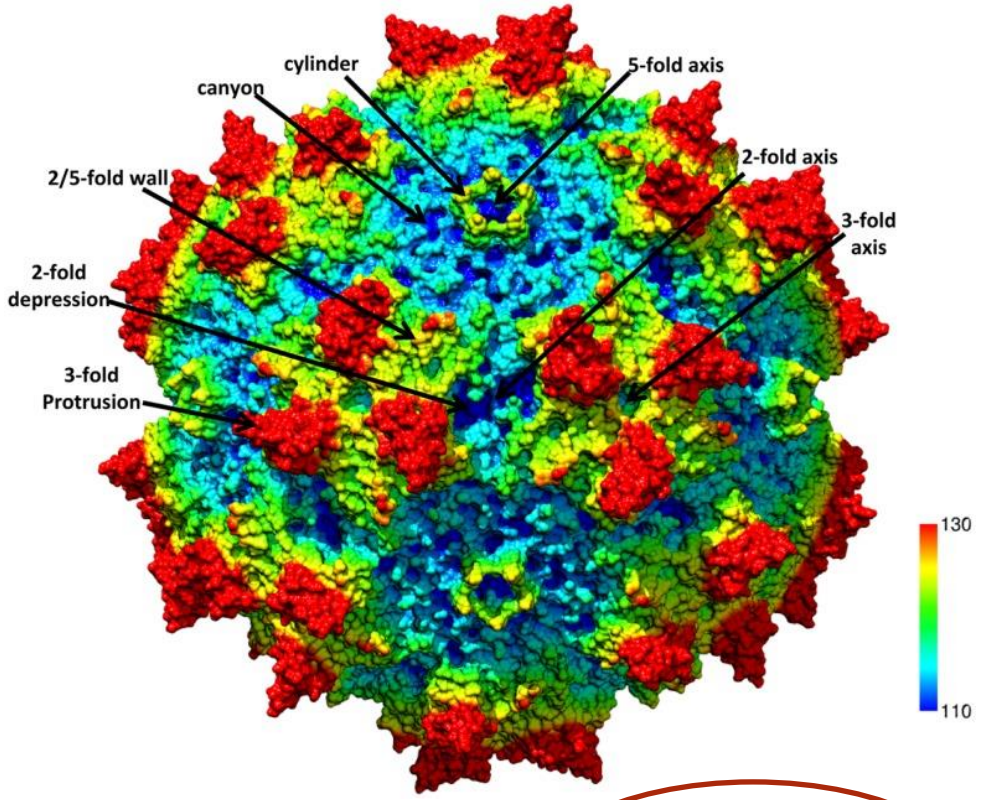
host immune system

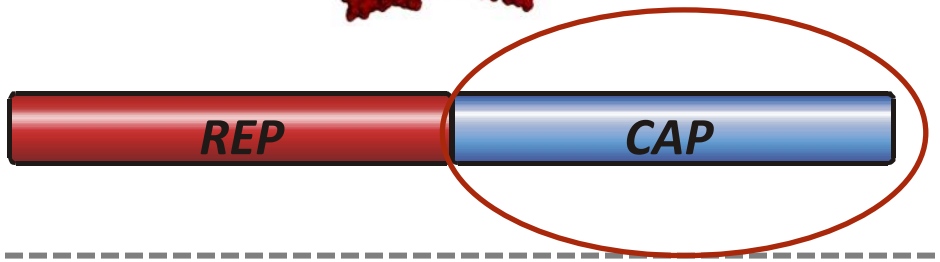
modified from slide kindly provided by Ian Alexander (University of Sydney;

Improve the host-vector interaction by capsid engineering

Rational design

- **Genetic insertion:**
  - Receptor-binding ligands
  - Nanobodies
  - Design ankyrin repeat proteins (DARPin)
  - Amino acid substitutions





capsid image: Tseng et al., 2014

Improve the host-vector interaction by capsid engineering

Rational design

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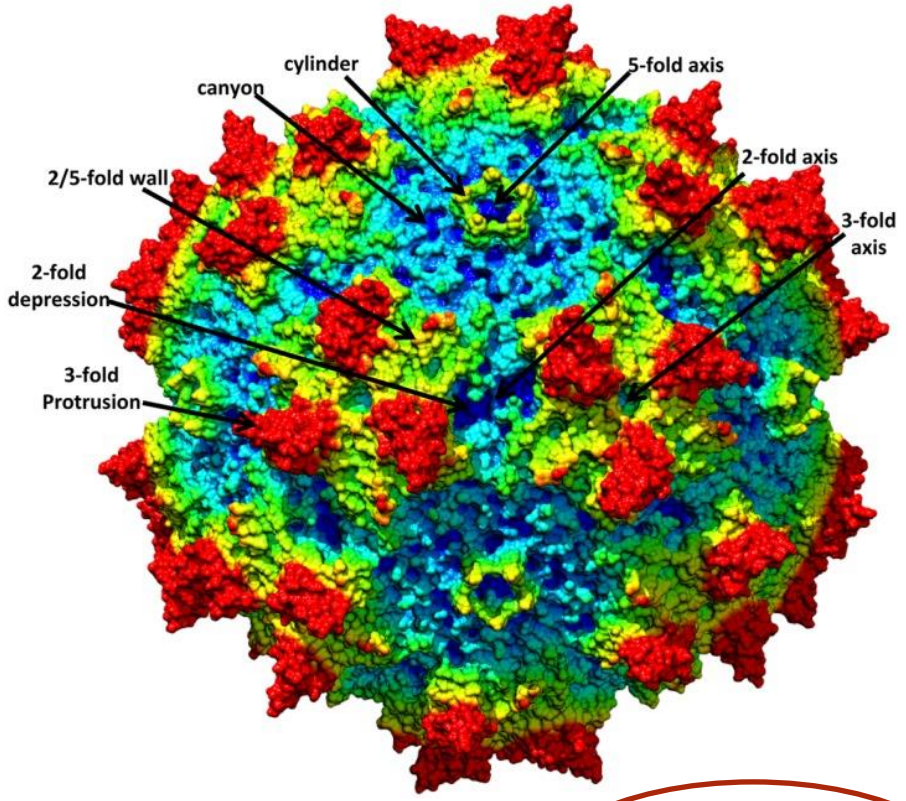
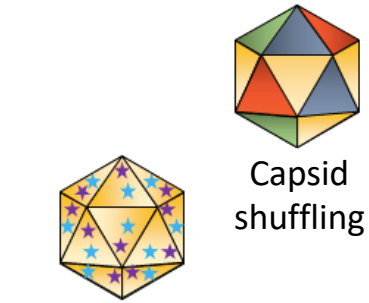
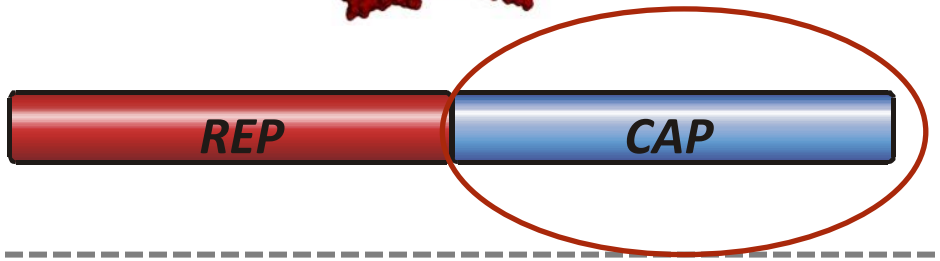
  - Amino acid substitutions
- **Non-genetic insertion:**
  - Single-chain antibodies
  - DARPins





”directed” evolution



capsid image: Tseng et al., 2014; schematic scheme of library capsids: Büning et al., Curr Opin Pharmacol 2015

Improve the host-vector interaction by capsid engineering

Rational design

- **Genetic insertion:**
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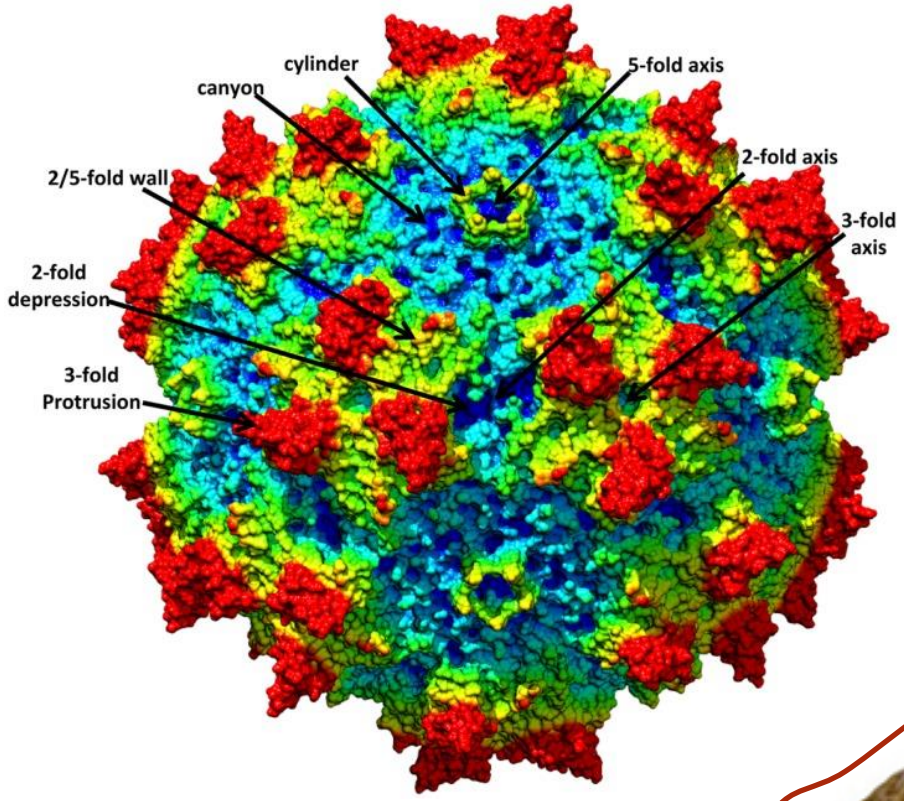
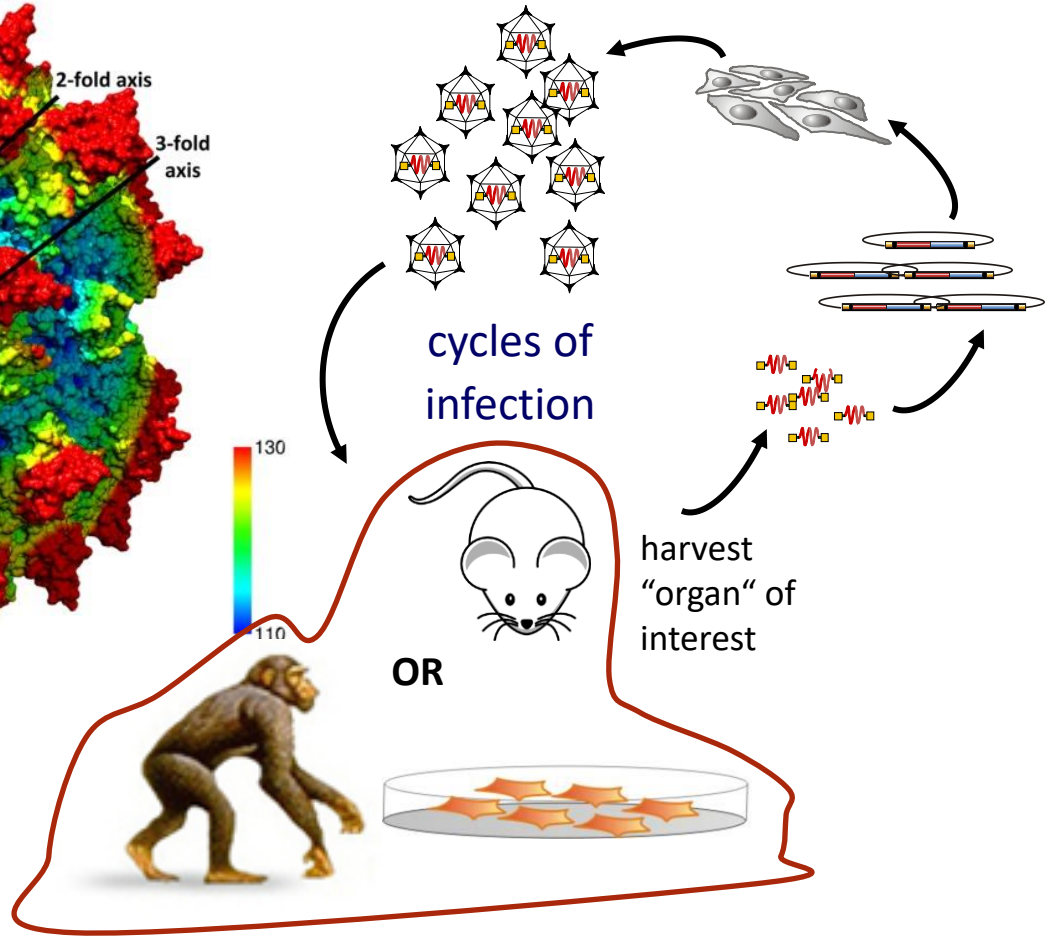


”directed” evolution

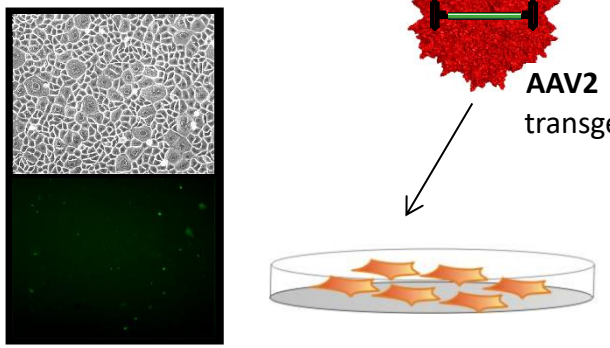


capsid image: Tseng et al., 2014

Few examples of increased efficacy

images from
Sallach et al., Mol Ther. 2014

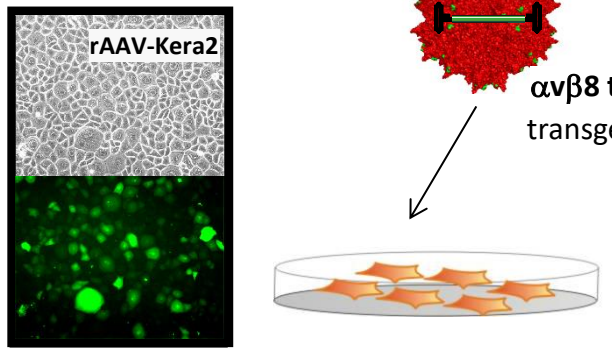
low transduction efficiency



AAV2
transgene: eGFP

1er human keratinocytes

high transduction efficiency



αvβ8 targeted AAV
transgene: eGFP

1er human keratinocytes

Molecular Therapy
Methods & Clinical Development
Review

2019

Capsid Modifications for Targeting and Improving the Efficacy of AAV Vectors

Hildegard Büning[1,2] and Arun Srivastava[3]

[1]Institute of Experimental Hematology, Hannover Medical School, Hannover, Germany; [2]REBIRTH Cluster of Excellence, Hannover Medical School, Hannover, Germany; [3]Division of Cellular and Molecular Therapy, Departments of Pediatrics and Molecular Genetics & Microbiology, Powell Gene Therapy Center, University of Florida College of Medicine, Gainesville, FL, USA

Molecular Therapy
Methods & Clinical Development
Original Article

2023

Novel AAV variants with improved tropism for human Schwann cells

Matthieu Drouyer,[1] Tak-Ho Chu,[2] Elodie Labit,[3] Florencia Haase,[1] Renina Gale Navarro,[1] Deborah Nazareth,[1] Nicole Rosin,[3] Jessica Merjane,[1] Suzanne Scott,[1] Marti Cabanes-Creus,[1] Adrian Westhaus,[1] Erhua Zhu,[4] Rajiv Midha,[2] Ian E. Alexander,[4,5] Jeff Biernaskie,[2,3,6] Samantha L. Ginn,[4,9] and Leszek Lisowski[1,7,8,9]

Received: 9 August 2018
Accepted: 5 February 2019
Published online: 06 March 2019

SCIENTIFIC REPORTS

2019 OPEN

Vector uncoating limits adeno-associated viral vector-mediated transduction of human dendritic cells and vector immunogenicity

Axel Rossi[1,2], Léa Dupaty[3], Ludovic Aillot[1,8], Liang Zhang[4], Célia Gallien[1], Michael Hallek[5], Margarete Odenthal[6], Sahil Adriouch[3], Anna Salvetti[1,8] & Hildegard Büning[2,4,7]

Vol. 82, No. 12

PNAS

2008

Next generation of adeno-associated virus 2 vectors: Point mutations in tyrosines lead to high-efficiency transduction at lower doses

Li Zhong*†‡, Baozheng Li*, Cathryn S. Mah*†, Lakshmanan Govindasamy†§, Mavis Agbandje-McKenna†§, Mario Coop
Roland W. Herzog*†¶, Irene Zolotukhin*, Kenneth H. Warrington, Jr.*†, Kirsten A. Weigel-Van Aken*†‡¶,
Jacqueline A. Hobbs*†‖, Sergei Zolotukhin*†¶, Nicholas Muzyczka†¶, and Arun Srivastava*†‡§**

*Division of Cellular and Molecular Therapy, Department of Pediatrics, †Powell Gene Therapy Center and Genetics Institute, ‡Shands Cancer Center, and Departments of §Biochemistry and Molecular Biology, ¶Molecular Genetics and Microbiology, and ‖Psychiatry, University of Florida College of Medicine, Gainesville, FL 32610

JOURNAL OF VIROLOGY, June 2008, p. 5887–5911
0022-538X/08/$08.00+0 doi:10.1128/JVI.00254-08

2008

In Vitro and In Vivo Gene Therapy Vector Evolution via Multispecies Interbreeding and Retargeting of Adeno-Associated Viruses▽†

Dirk Grimm,[1]‡ Joyce S. Lee,[1] Lora Wang,[1] Tushar Desai,[2] Bassel Akache,[1] Theresa A. Storm,[1] and Mark A. Kay[1]*

Departments of Pediatrics and Genetics, 300 Pasteur Drive,[1] and Department of Biochemistry, 279 Campus Drive,[2] School of Medicine, Stanford University, Stanford, California 94305

Received 4 February 2008/Accepted 2 April 2008

Reduction of Vector Dose:
⇒ Antigenic load
⇒ Production costs

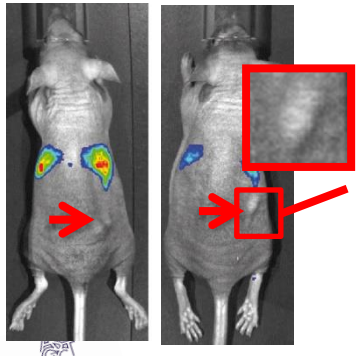
Few examples of re-directed tropism

AAV2

transgene: luciferase

tumor

off-target transduction



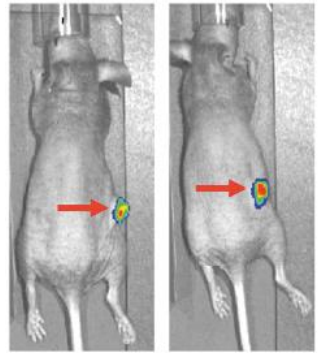
Xenograft tumor model

Her2/neu-directed AAV

transgene: luciferase

tumor

Precise *in vivo* targeting



Xenograft tumor model

images from
Münch et al., Mol Ther. 2013, Münch et al., Nat. Commun. 2015

2024

Molecular Therapy
Original Article

Development of CNS tropic AAV1-like variants with reduced liver-targeting following systemic administration in mice

Matthieu Drouyer,[1,6] Jessica Merjane,[1,6] Deborah Nazareth,[1] Maddison Knight,[1] Suzanne Scott,[1] Sophia Li,[1] Samantha L. Ginn,[2] Erhua Zhu,[2] Ian E. Alexander,[2,3] and Leszek Lisowski[1,4,5]

Hepatology, 2022

ORIGINAL ARTICLE

Adeno-associated virus serotype 2 capsid variants for improved liver-directed gene therapy

Nadja Meumann[1,2] | Marti Cabanes-Creus[3] | Moritz Ertelt[4,5] | Renina Gale Navarro[3] | Julie Lucifora[6] | Qinggong Yuan[7,8] | Karin Nien-Huber[9] | Ahmed Abdelrahman[9] | Xuan-Khang Vu[1] | Liang Zhang[2,10] | Ann-Christin Franke[1] | Christian Schmithals[11] | Albrecht Piiper[11] | Annabelle Vogt[12] | Maria Gonzalez-Carmona[12] | Jochen T. Frueh[13] | Evelyn Ullrich[13] | Philip Meuleman[14] | Steven R. Talbot[15] | Margarete Odenthal[2,10] | Erhard Seifried[9] | Clara T. Schoeder[4] | Joachim Schwäble[9] | Michael Ott[7,8]

2022

Molecular Therapy
Original Article

AAV capsid engineering identified two novel variants with improved in vivo tropism for cardiomyocytes

Laura Rode,[1,6] Christian Bär,[1,2,5,6] Sonja Groß,[1] Axel Rossi,[3] Nadja Meumann,[3] Janika Viereck,[1] Na Ke Xiao,[1] Isabelle Riedel,[1] Anika Gietz,[1] Karina Zimmer,[1] Margarete Odenthal,[4] Hildegard Büning, and Thomas Thum[1,2,5]

2022

nature neuroscience

RESOURCE

https://doi.org/10.1038/s41593-021-00969-4

Check for updates

AAV capsid variants with brain-wide transgene expression and decreased liver targeting after intravenous delivery in mouse and marmoset

David Goertsen[1,12], Nicholas C. Flytzanis[1,12], Nick Goeden[1,12], Miguel R. Chuapoco[1], Bradley Cummins[2], Yijing Chen[3], Yingying Fan[3], Qiangge Zhang[4,5], Jitendra Sharma[4,6,7,8], Chang Liping Wang[3], Guoping Feng[4,5], Yu Chen[9,11], Nancy Y. Ip[9,10,11], James Pickel[2]

2021

Cell

CellPress
OPEN ACCESS

Article

Directed evolution of a family of AAV capsid variants enabling potent muscle-directed gene delivery across species

Mohammadsharif Tabebordbar,[1,13,14,*] Kim A. Lagerborg,[1,2,13] Alexandra Stanton,[1,3] Emily M. King,[1] Simon Ye,[1,4] Liana Tellez,[1] Allison Krunnfusz,[1] Sahar Tavakoli,[5,6,7,8] Jeffrey J. Widrick,[9] Kathleen A. Messemer,[5,6,7] Emily C. Troiano,[9] Behzad Moghadaszadeh,[9] Bryan L. Peacker,[5,6,7] Krystynne A. Leacock,[5,6,7] Naftali Horwitz,[5,6,7,11] Alan H. Beggs,[1,9] Amy J. Wagers,[5,6,7,11,*] and Pardis C. Sabeti[1,10,12,*]

[1]Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA

2024

Molecular Therapy
Original Article

Cell specific in vivo gene delivery with DART-AAVs targeted to CD8

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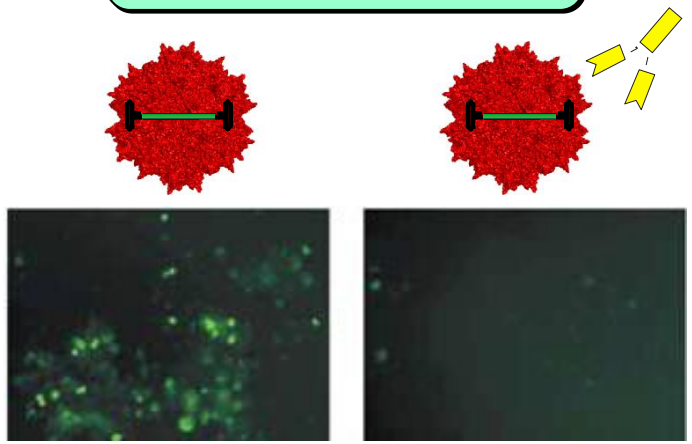
Reduction of Vector Dose & Reduction/Avoidance of off-target transduction:

⇒ Antigenic load
⇒ Production costs
⇒ safety

Few examples of escaping pre-existing humoral responses

Pre-existing immunity

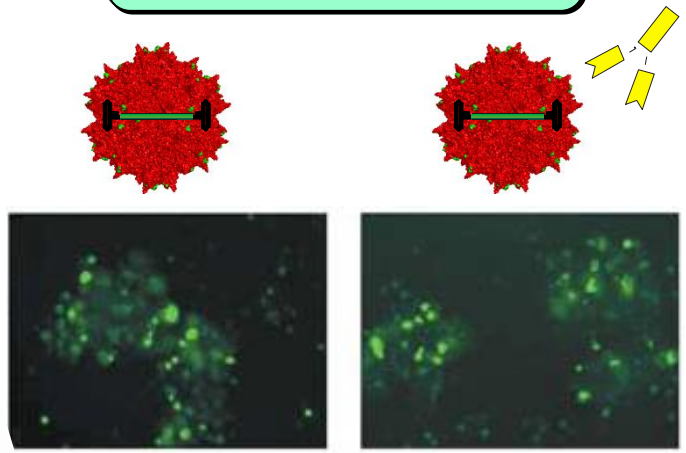
rAAV2
transgene: eGFP



Leukemia cell line: Mec1

Escape pre-existing immunity

rAAV2-MecA
transgene: eGFP



Leukemia cell line: Mec1

images from Huttner et al., Gene Ther. 2003

2009

PNAS

A myocardium tropic adeno-associated virus (AAV) evolved by DNA shuffling and in vivo selection

Lin Yang[a], Jiangang Jiang[a], Lauren M. Drouin[b], Mavis Agbandje-Mckenna[b], Chunlian Chen[a], Chunping Qiao[a], Dongqiuye Pu[a], Xiaoyun Hu[c], Da-Zhi Wang[c], Juan Li[a], and Xiao Xiao[a,1]

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Communicated by Yuet Wai Kan, University of California, San Francisco School of Medicine, San Francisco, CA, January 4, 2009 (received for review September 4, 2008)

2006

nature biotechnology

Directed evolution of adeno-associated virus yields enhanced gene delivery vectors

Narendra Maheshri[1], James T Koerber[1], Brian K Kaspar[2] & David V Schaffer[1]

Adeno-associated viral vectors are highly safe and efficient gene delivery vehicles. However, numerous challenges in vector design remain, including neutralizing antibody responses, tissue transport and infection of resistant cell types. Changes must be made to the viral capsid to overcome these problems; however, very often insufficient information is available for rational design of improvements. We therefore applied a directed evolution approach involving the generation of large mutant capsid libraries and selection of adeno-associated virus (AAV) 2 variants with enhanced properties. High-throughput selection processes were designed to isolate mutants within the library with altered affinities for heparin or the ability to evade antibody neutralization and deliver genes more efficiently than wild-type capsid in the presence of anti-AAV serum. This approach, which can be extended to additional gene delivery challenges and serotypes, directs viral evolution to generate 'designer' gene delivery vectors with specified, enhanced properties.

nature biotechnology

GENE THERAPY

Blood 2013

Engineered AAV vector minimizes in vivo targeting of transduced hepatocytes by capsid-specific CD8$^+$ T cells

Ashley T. Martino,[1] Etiena Basner-Tschakarjan,[2] David M. Markusic,[3] Jonathan D. Finn,[2] Christian Hinderer,[2] Shangzhen Zhou,[2] David A. Ostrov,[4] Arun Srivastava,[3] Hildegund C. J. Ertl,[5] Cox Terhorst,[6] Katherine A. High,[2,7,8] Federico Mingozzi,[7] and Roland W. Herzog[3]

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Optimization of stealth adeno-associated virus vectors by randomization of immunogenic epitopes

Stephan Maersch [a], Anke Huber [a], Hildegard Büning [a,b], Michael Hallek [a,b], Luca Perabo [a,*]

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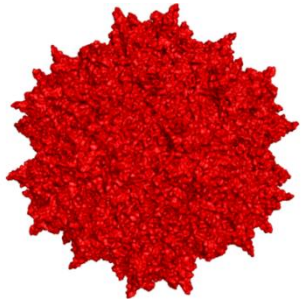
Transduction despite pre-existing immunity:

⇒ Possibility to include seropositive patients

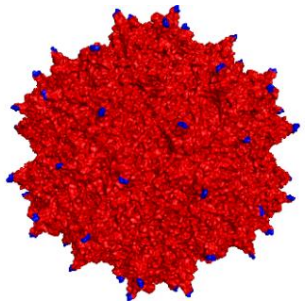
⇒ Repeated administration

Example of decreasing *de novo* immune responses



AAV2

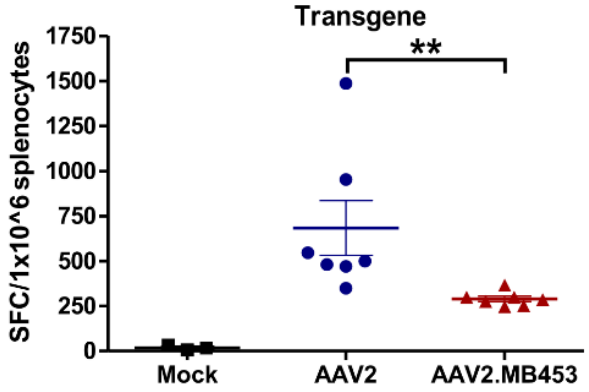
versus



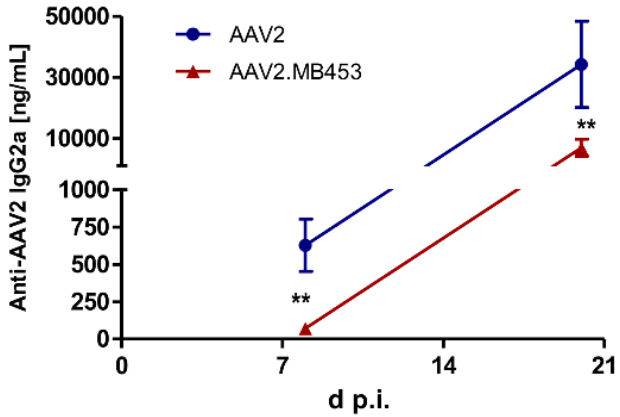
AAV2-MB453

intravenous

Reduced CD8⁺ T cell responses

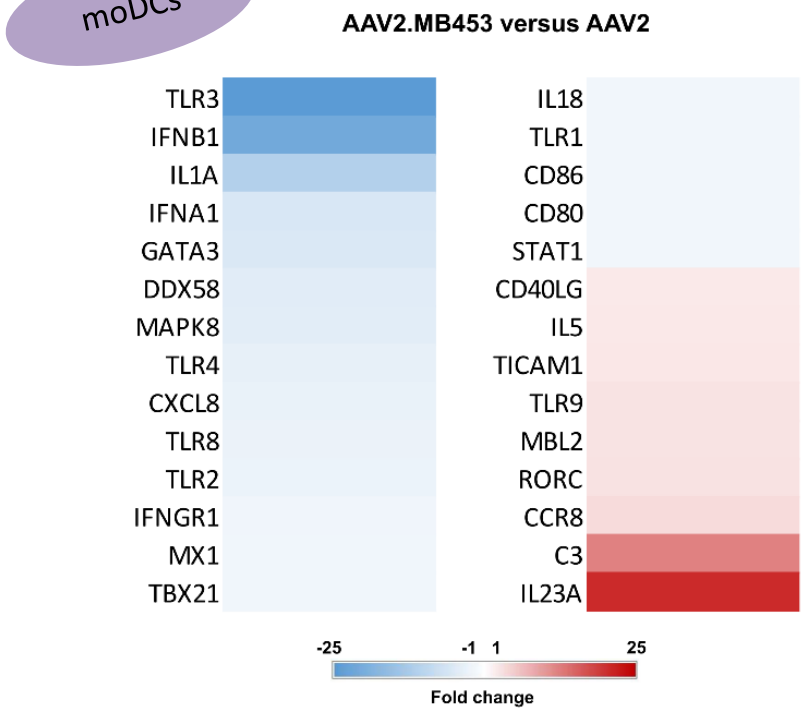


Reduced humoral responses



Reduced mRNA levels of cytokines and chemokines

moDCs

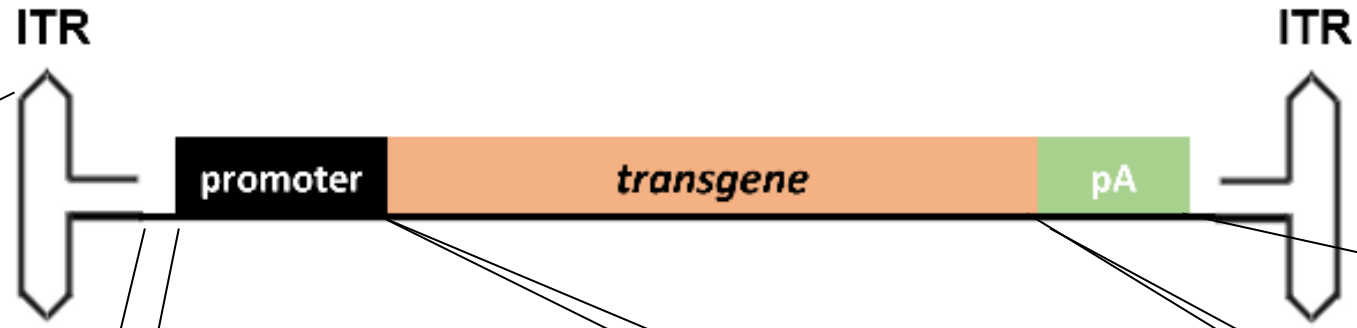


Modulation of host immune response:

⇒ safety ↑

images from Bentler et al., MTMCD 2023; moDCs= monocytes derived dendritic cells

Improve the host-vector interaction by vector genome engineering



Self-complementary AAV vector genome configuration
⇒ increased transgene expression
⇒ overcoming limitation of second strand synthesis

Depletion of CpG content of ITRs
⇒ decreased innate immune response

Optimal Promoter design (size/strength/cell type selectivity/...)
⇒ tailored expression level
⇒ lower immunogenicity
⇒ impact on duration

Codon optimization
⇒ increased transgene expression

Depletion of CpG content
⇒ decreased innate immune response

Transgene design
⇒ decrease in size
⇒ reduced immune response

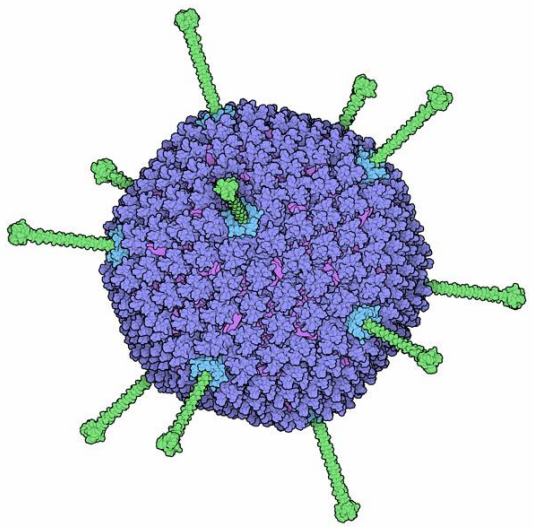
Optimized poly A sequence
⇒ size reduction
⇒ improved mRNA stability

Addition of " regulatory elements"
=> avoidance of off-target expression (miRNA target sites)
⇒ improved mRNA stability (e.g., WPRE)

Beyond AAV… example of AdV engineering

portfolio of serotypes and variants

broad tropism

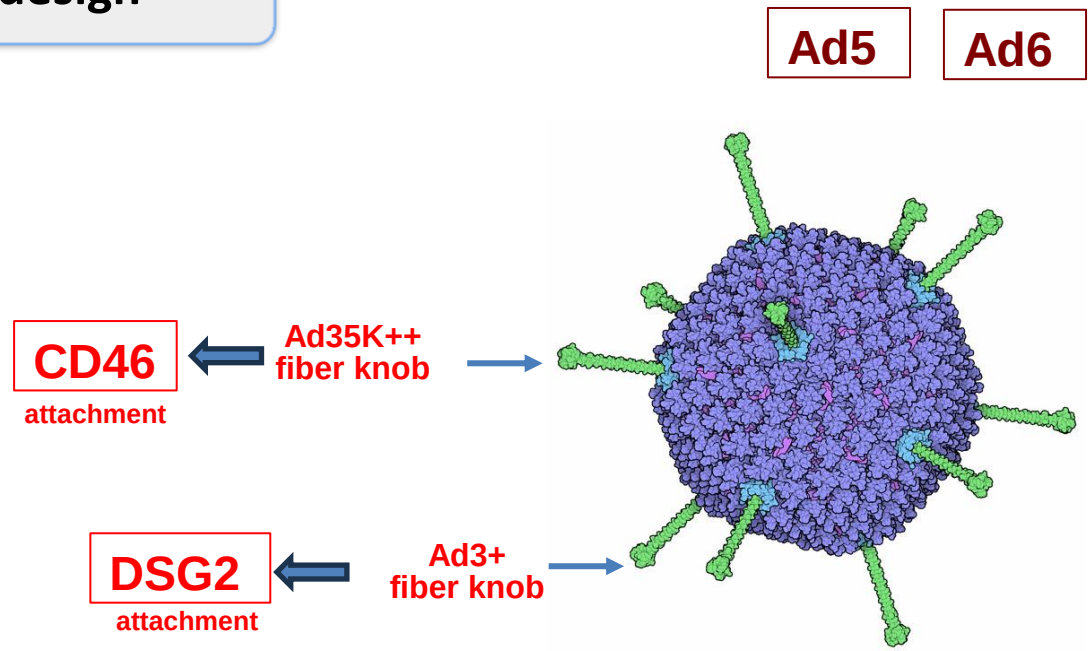


non-enveloped protein capsid
(Ø 70-90 nm)

double-stranded DNA genome
(~ 35 kb)

Beyond AAV… example of AdV engineering

Rational design

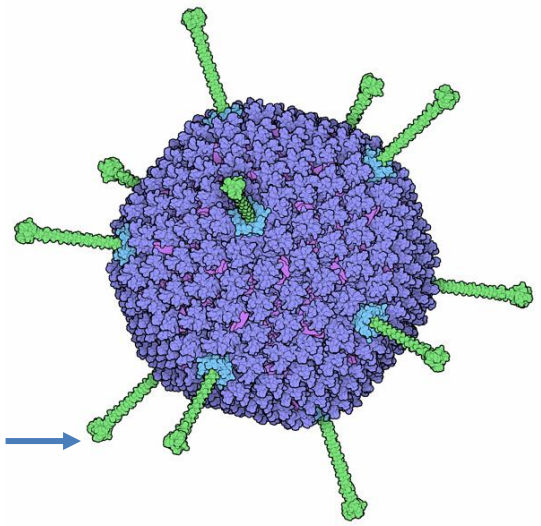


- **Fiber:**
  - Hybrid Fibers
  - Replace RGD loop by targeting peptide (e.g. SIKVAV)*
- **Hexon:**
  - Liver de-targeting mutation

Richter et al. Blood 2016, Wang et al., Blood Adv 2022, Wang et al. MTMCD 2023, *Yao et al., Mol Ther. 2024

Beyond AAV… example of AdV engineering

Ad5 **Ad6**

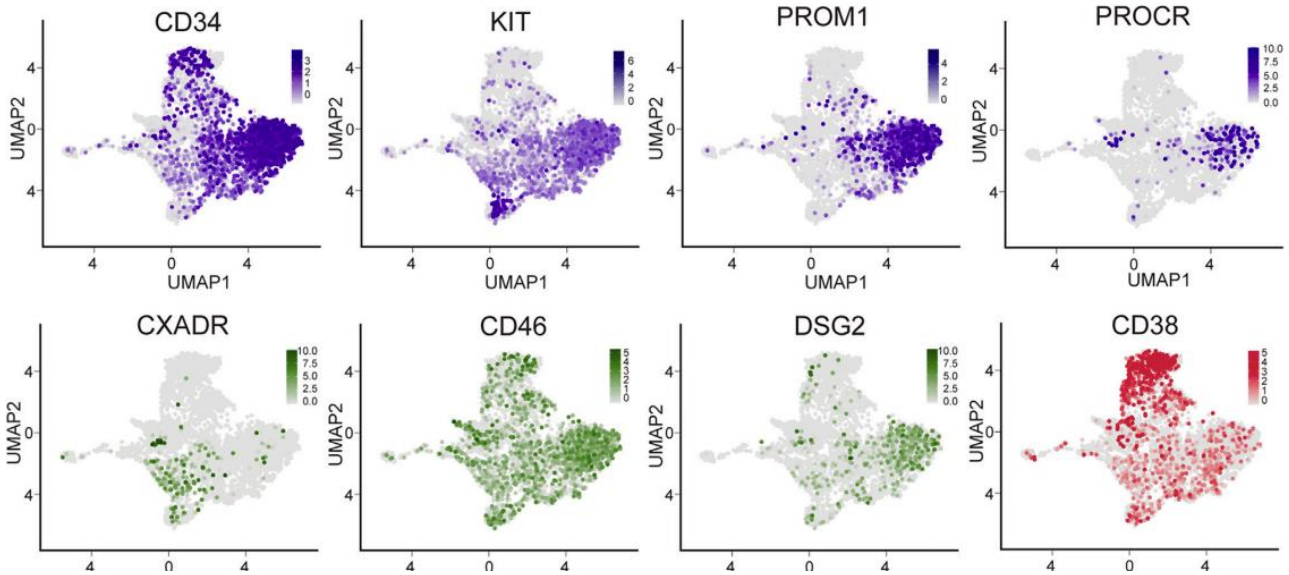


CD46 ⟸ Ad35K++ fiber knob →
attachment

DSG2 ⟸ Ad3+ fiber knob →
attachment

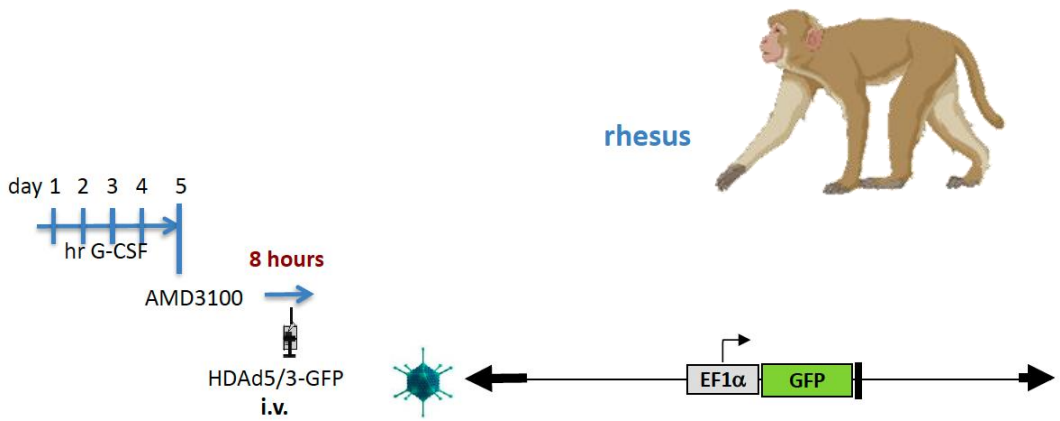
Receptors expressed on HSPC

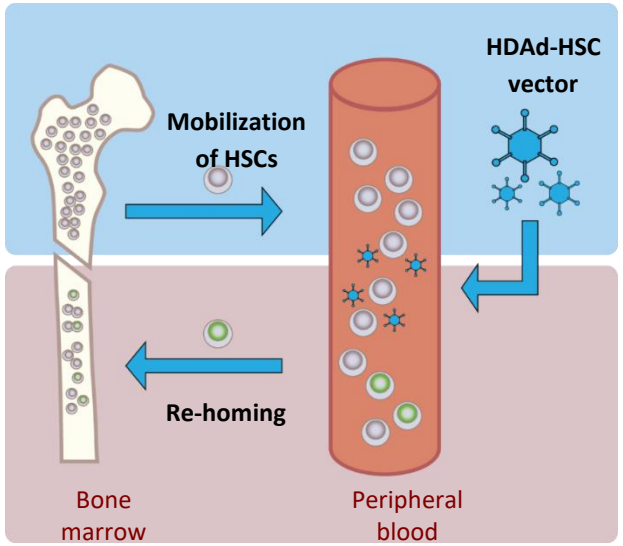
Single cell RNA-seq*

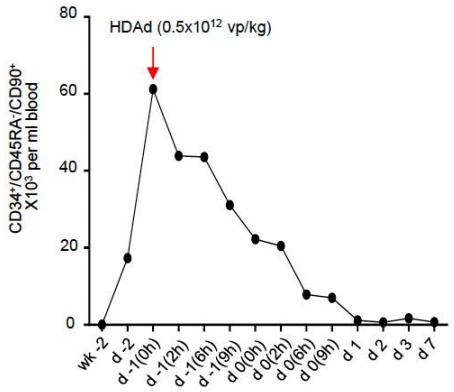


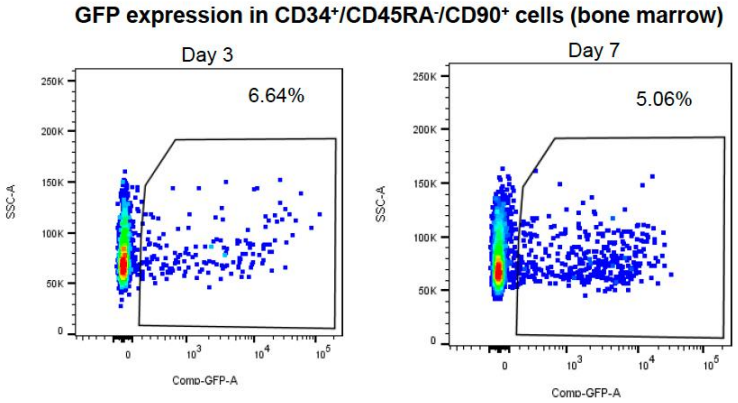
slide modified from slide kindly provided by Andre Lieber (University of Washington); *Yao et al., Mol Ther. 2024

Engineered AdV vectors can be used to transduce HSPCs *in vivo*



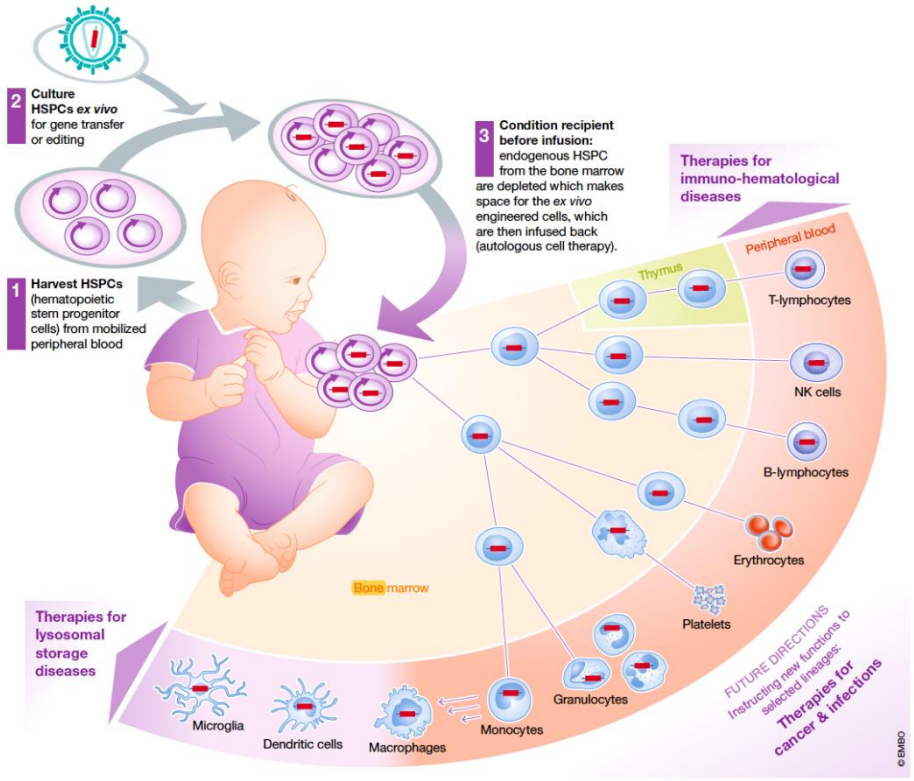




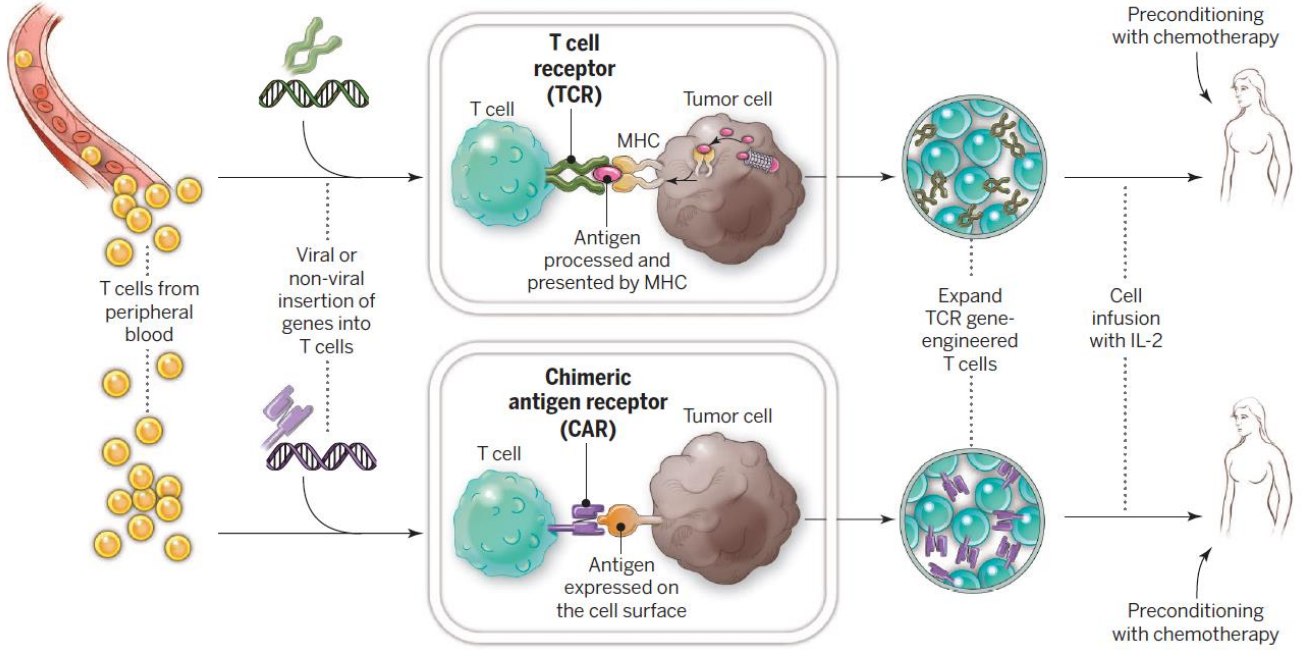


Ex vivo Gene Therapy

HSPCs



T Lymphocytes



Images from Naldini EMBO Mol Med 2019 & Rosenberg et al., Science 2015 ; HSPCs = hematopoetic stem and progenitor cells

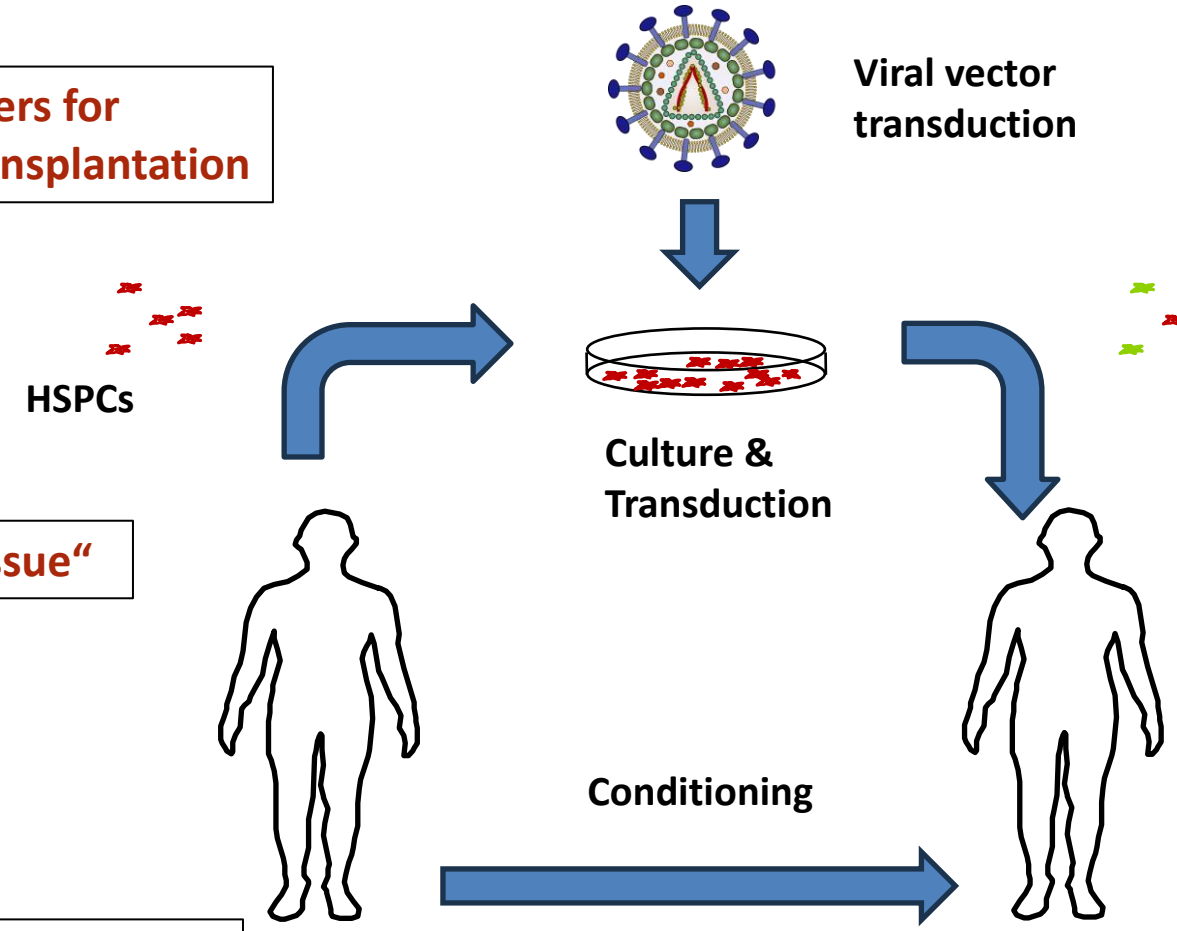
Challenges of *ex vivo* Gene Therapy Approaches

Specialized centers for harvesting HSPCs & transplantation

"Time-to-Treatment issue"

Treatment costs

A true personalized medicine ("no off-the-shelf)



Negative impact on viability, pluripotency, and engraftment activity through *ex vivo* culture

Genotoxicity through integrating viral vectors

Cell stress through *ex vivo* transduction

Conditioning might cause morbidity or mortality

Challenges of *ex vivo* Gene Therapy Approaches – Moving towards *in vivo* Gene Therapy as possible solution

***Ex vivo* Gene Therapy**		***In vivo* Gene Therapy**
specialized centers for harvesting HSPCs & transplantation	→	not required
treatment costs	→	lower treatment costs*
"Time-to-Treatment issue"	→	shortening of "Time-to-Treatment"
a true personalized medicine ("no off-the-shelf")	→	"off-the-shelf " product
conditioning might cause morbidity or mortality	→	no conditioning required
negative impact on viability, pluripotency, and engraftment activity through *ex vivo* culture	→	no *ex vivo* culturing; presumably moving towards transduction within the bone marrow*
genotoxicity through integrating viral vectors	→	gene editing instead of vector integration*
cell stress through *ex vivo* transduction	→	lower cell stress when transduction occurs *in vivo*
vector transfer avoided	→	higher antigenic load
no issue regarding pre-existing anti-vector immunity	→	Issue of pre-existing humonal immunity
no risk of off-target transduction	→	risk of off-target transduction

*Already addressed by **vector engineering**

BREAK

The meeting will resume at 2:10 pm ET

REAGAN-UDALL FOUNDATION FOR THE FDA

Session 4: Regulators' Perspective

- **Sol Ruiz, PhD,** Spanish Medicines Agency (AEMPS)
- **Peter Marks, MD, PhD,** Center for Biologics Evaluation and Research, U.S. Food and Drug Administration
- **Eric Karikari-Boateng, MS,** Food and Drugs Authority (Ghana)

Session 5: How do we prepare for the next generation of gene therapy, as industry, regulators, and a health care system?

REAGAN-UDALL FOUNDATION FOR THE FDA

- **Hildegard Büning, PhD,** Hannover Medical School
- **Cecelia Calhoun, MD, MPHS, MBA,** Yale University School of Medicine
- **Jeremy Farrar, MD, PhD,** World Health Organization
- **Julie Makani, MD, PhD,** Muhimbili University of Health and Allied Sciences
- **Peter Marks, MD, PhD,** Center for Biologics Evaluation and Research, U.S. Food and Drug Administration
- **Kwasi Nyarko, PhD,** WHO Regional Office for Africa (WHO-AFRO)
- **Jimi Olaghere,** Gene Therapy Recipient

REAGAN-UDALL

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FOR THE FDA

Scientific Advancements in Gene Therapies: Opportunities for Global Regulatory Convergence

Hybrid Public Workshop

September 4, 2024

10am-4pm *(eastern)*

Thank You for Joining Us!

Meeting materials will be posted on our website: www.reaganudall.org

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