PRINCIPLES AND APPLICATIONS OF GENE THERAPY-CLINICAL TRIALS

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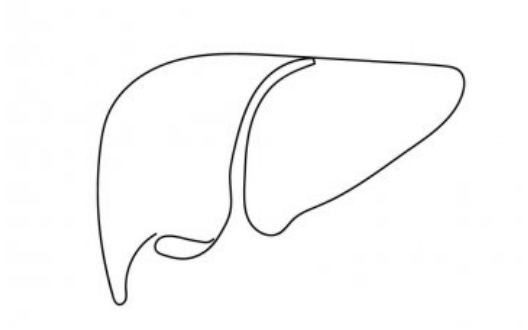
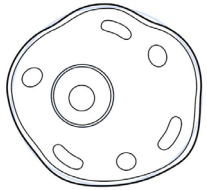
ARISTOTLE UNIVERSITY OF THESSALONIKI

ADVANCE THERAPY MEDICINAL PRODUCTS (ATMPs)

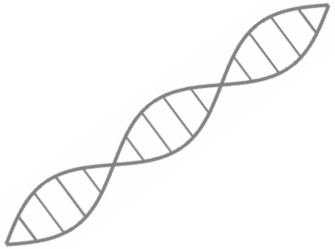
- Biomedicinal products for human use that are based on genes, tissues or cells, offering groundbreaking new opportunities for the treatment of disease and injury
- Can be classified into three main types



Tissue Engineered Products (TEP)



Somatic Cell Therapy Medicinal Products (sCTMP)



Gene Therapy Medicinal Products (GTMP)

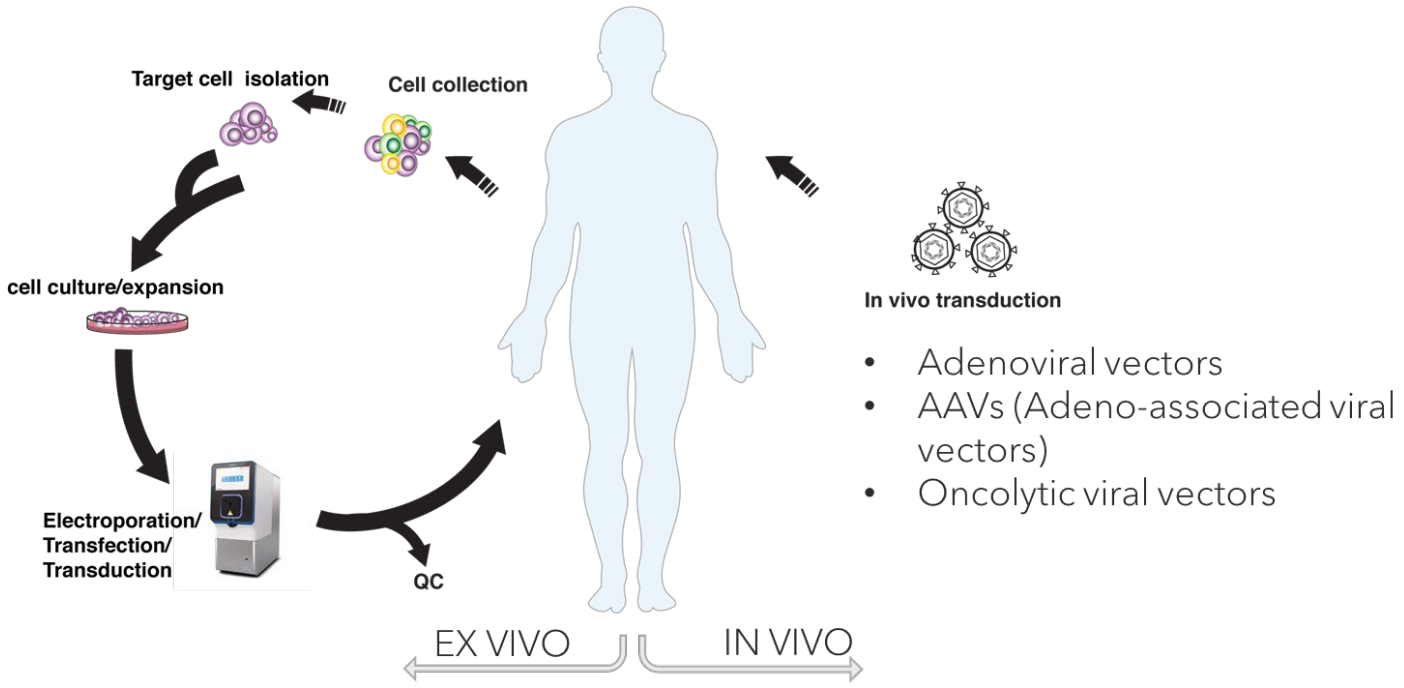
GENE THERAPY PRODUCTS

Gene Therapy Medicinal Products (GTMP)

- Genes that lead to a therapeutic, prophylactic or diagnostic effect. They work by inserting 'recombinant' genes into the body, usually to treat a variety of diseases, including genetic disorders, cancer or long-term diseases. A recombinant gene is a stretch of DNA that is created in the laboratory, bringing together DNA from different sources.

BASIC PRINCIPLES OF GENE THERAPY

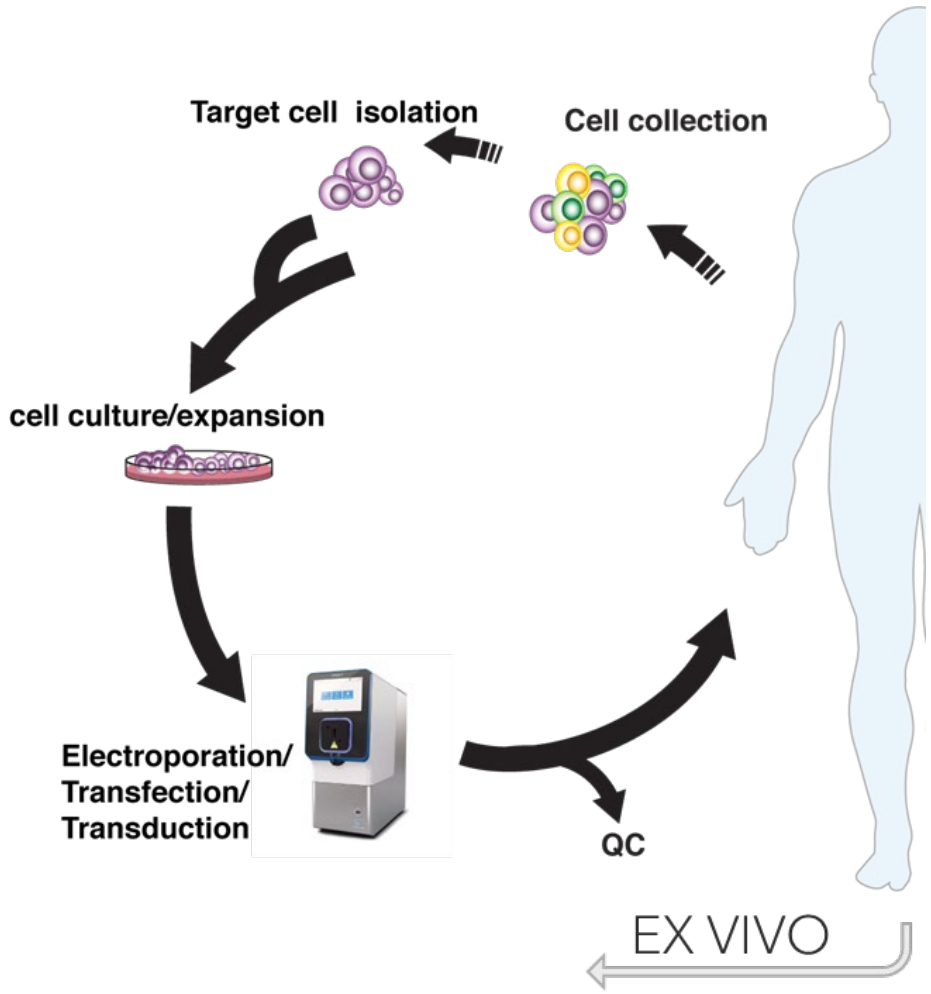
- Gene therapy is a novel treatment method which utilizes genes or short oligonucleotide sequences as therapeutic molecules, instead of conventional drug compounds.
- This technique is widely used to treat those defective genes which contribute to disease development.
- Gene therapy involves the introduction of one or more foreign genes into an organism to treat hereditary or acquired genetic defects.
- In gene therapy, DNA encoding a therapeutic protein is packaged within a "vector", which transports the DNA inside cells within or outside the body.



EX VIVO GENE THERAPY

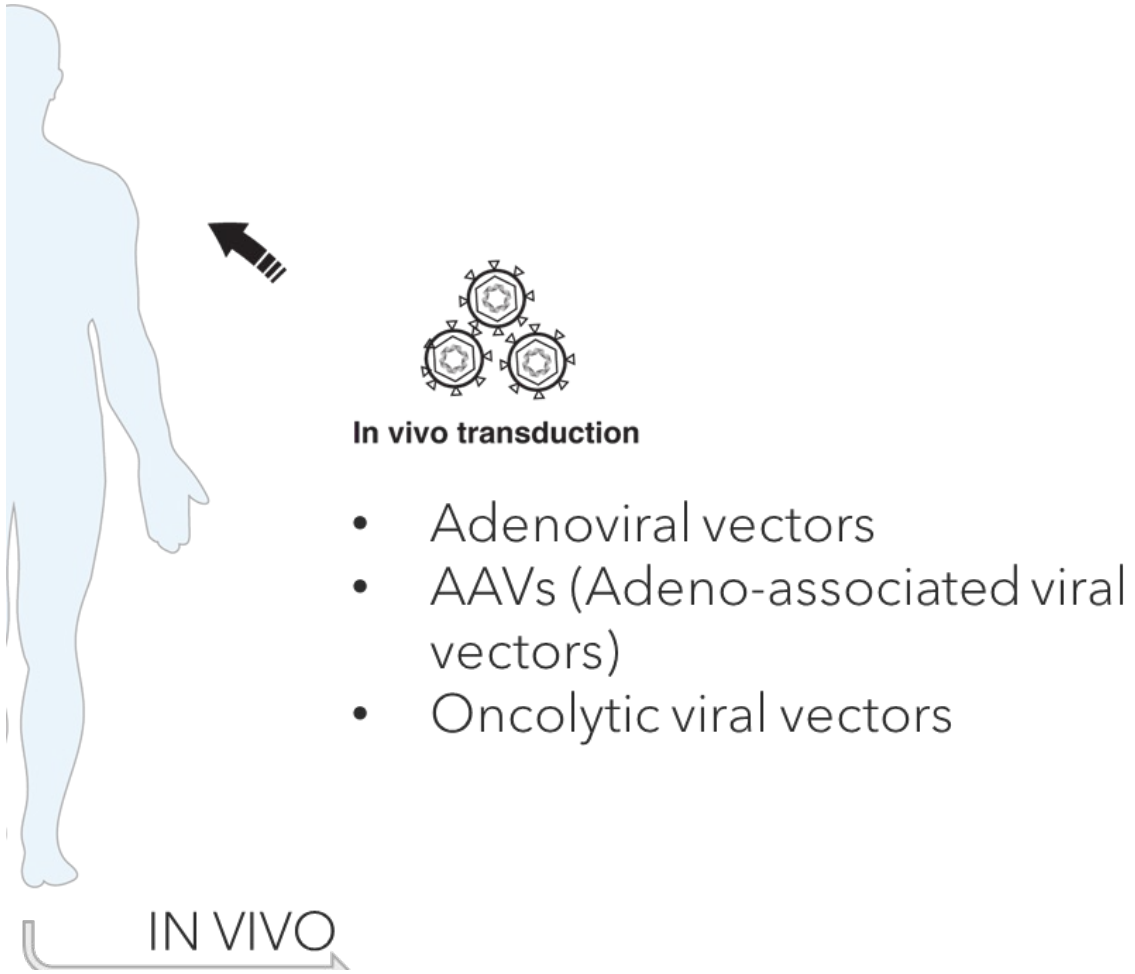
- This approach can be applied to the tissues like hematopoietic cells and skin cells which can be removed from the body, genetically corrected outside the body and reintroduced into the patient body where they become engrafted and survive for a long period of time.

- Genes are transferred to the cells grown in culture.
- Modified cells are selected, multiplied and then introduced into the patient.
- The use of autologous cells avoids immune system rejection of the introduced cells.



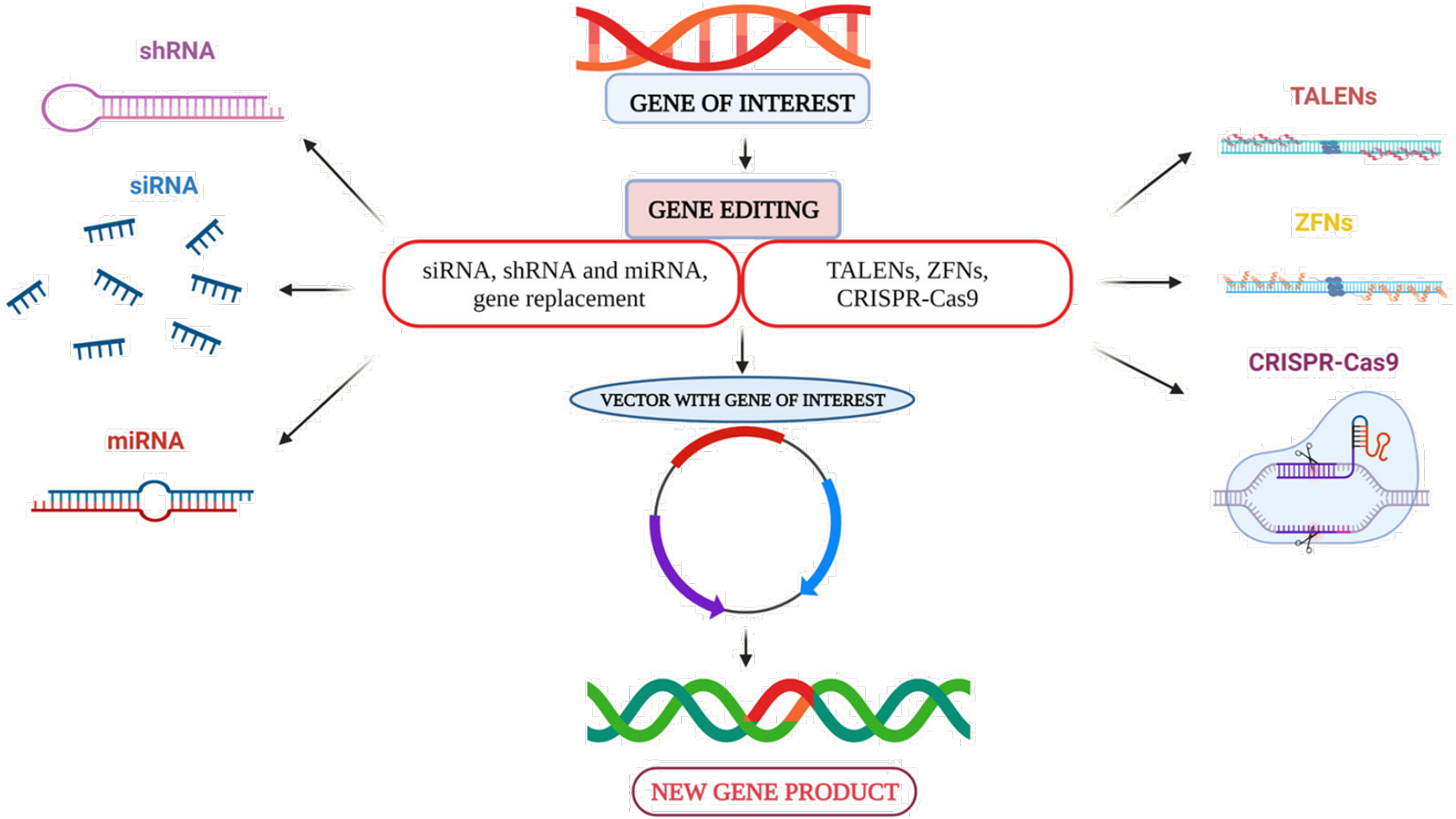
IN VIVO GENE THERAPY

- Transfer of desired genes directly into the tissues of the patient.
- This is done in case of tissues whose individual cells cannot be cultured in vitro in sufficient numbers (like brain cells) and/or where re-implantation of the cultured cells in the patient is not efficient.
- The efficiency of gene transfer and expression determines the success of this approach, because of the lack of selection and amplification of cells which take up and express the foreign gene.



Differences Between in vivo and ex vivo Gene Delivery Systems	
In vivo	Ex vivo
Technically simple	Technically complex
No requirement of specialized infrastructure	Requirement of specialized infrastructure
Vectors introduced directly	No vectors introduced directly
QC not possible	QC possible
Less invacive	More invasive
More immunogenic	Less immunogenic

WHAT CAN GENE THERAPY DO?

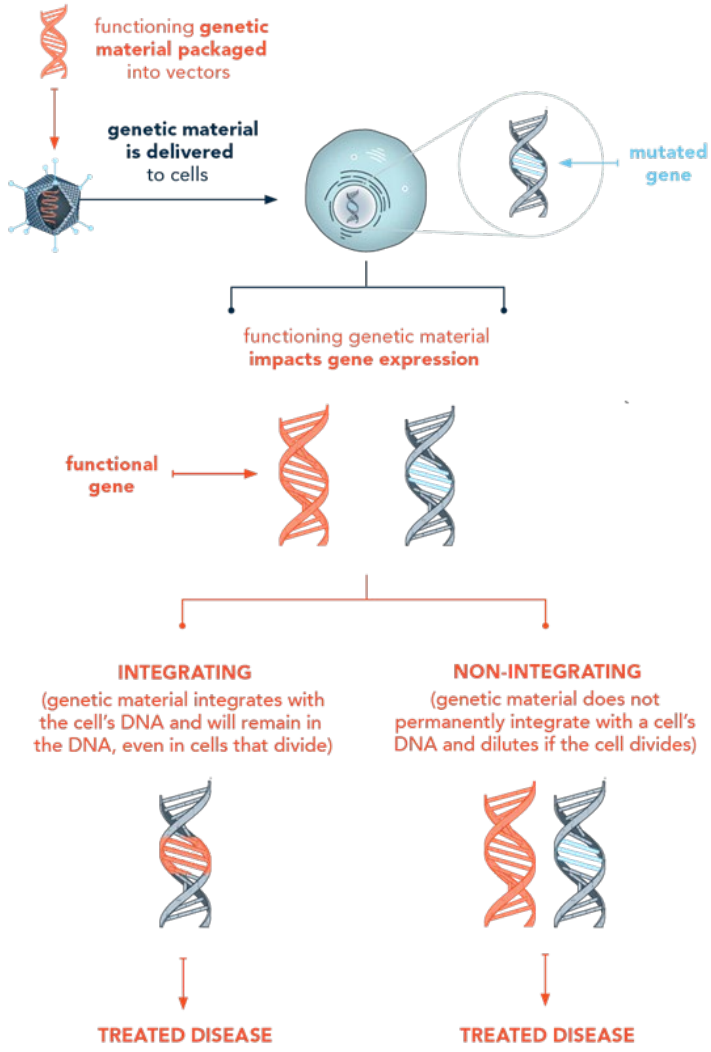


GENE ADDITION

Gene addition is probably the most common gene therapy technique being explored for monogenic diseases.
This usually involves the delivery of functional copies of a gene (transgene) into a person's cells by a vector.

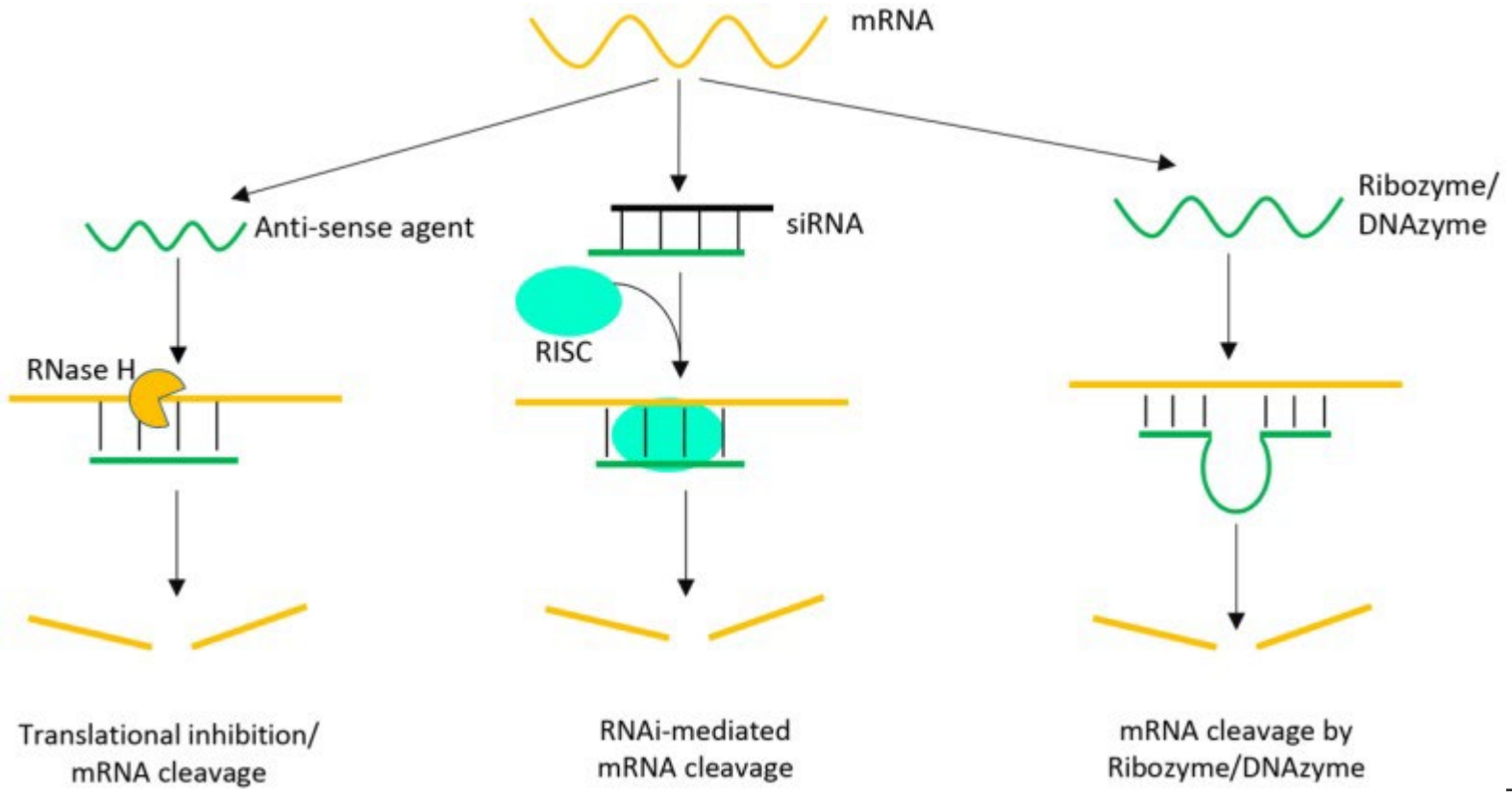
Vectors deliver the functional gene to the patient's cells, either in vivo or ex vivo.

Once inside the cell, the transgene provides the cell with instructions that lead to the production of functional proteins. With gene addition therapy, the mutated gene does not need to be replaced or removed. This provides the cell with the instructions that lead to the production of functional genes, while not needing to replace or remove the mutated gene.



TARGETED INHIBITION OF GENE EXPRESSION

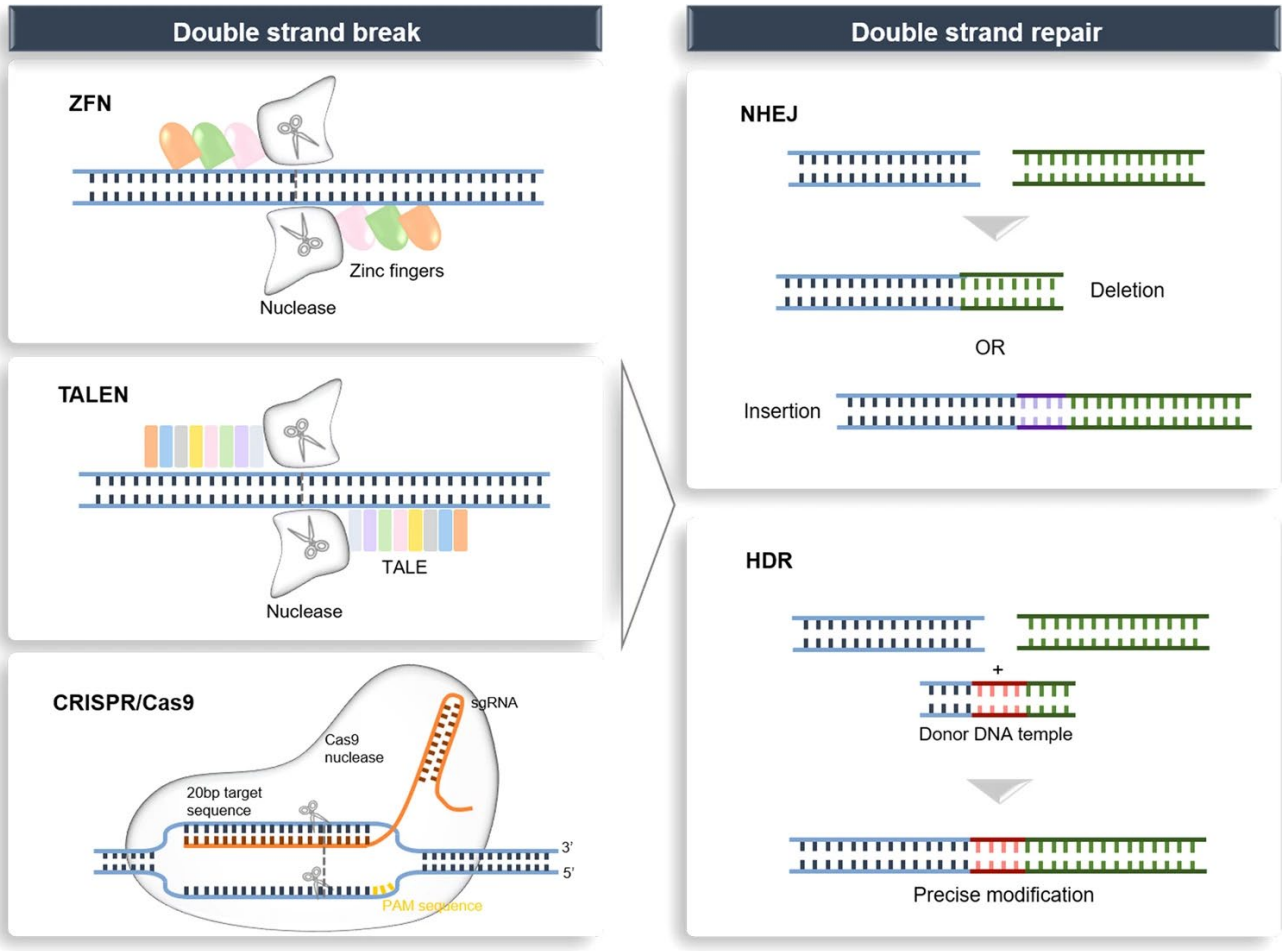
This approach aims to block the expression of any diseased gene or a new gene expressing a protein which is harmful for a cell or regulate gene regulators.



-Antisense ODN or gene
-RNA interference
-DNAzyme

Theranostics. 2017; 7(4): 1010–1025.

TARGETED GENOMIC MODIFICATION



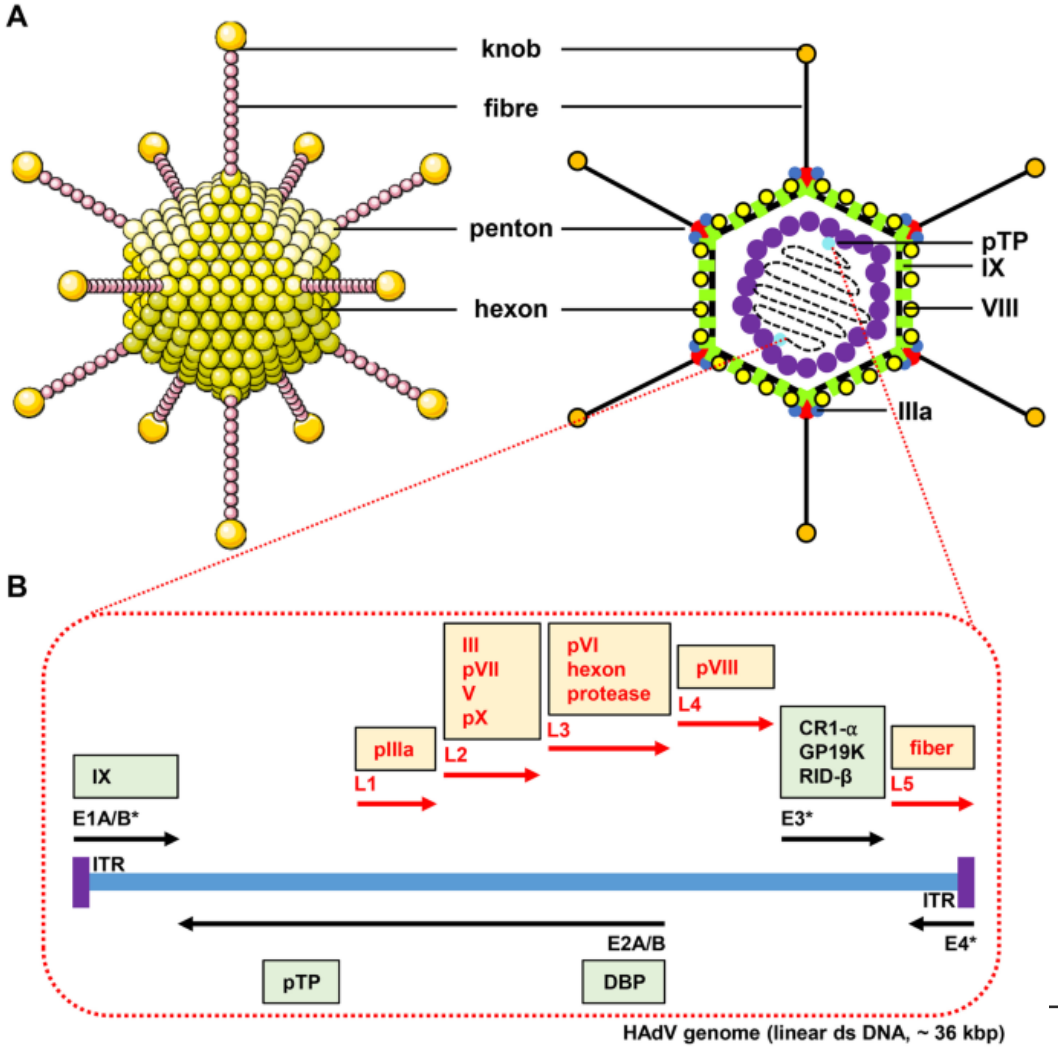
- Gene correction
- Gene addition in safe harbor loci
- Gene deactivation targeting coding sequences
- Gene deactivation targeting regulatory sequences
- Gene reactivation targeting cis-acting elements
- Gene reactivation targeting trans-acting elements
- Gene replacement

Combination of all the above

https://doi.org/10.1038/s41392-019-0089-y

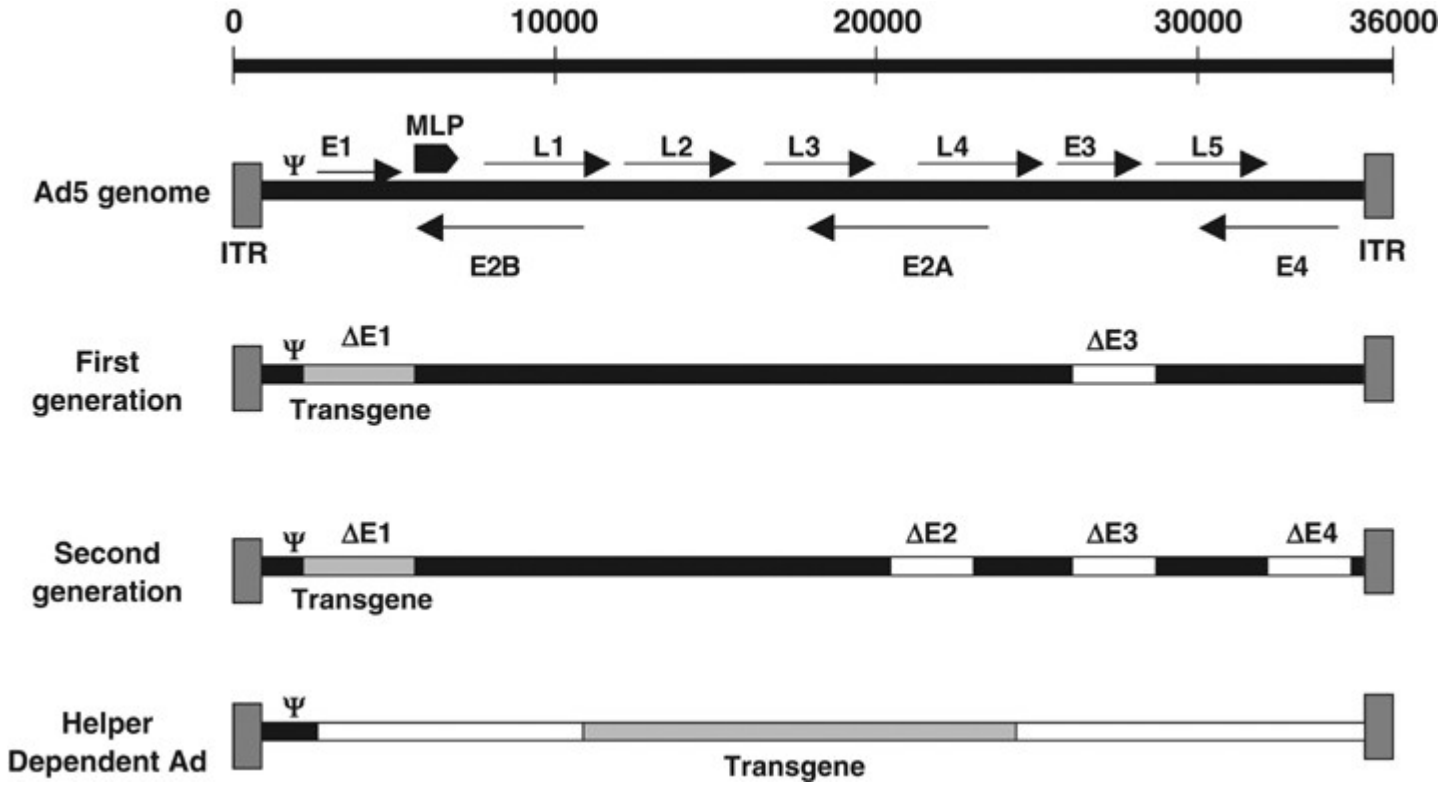
DELIVERY MEANS

ADENOVIRUS



- Adenoviral virions are non-enveloped icosahedral-shaped capsids ranging from 70 to 90 nm in diameter.
- Each capsid encompasses a total of 252 proteins (240 trimeric hexons, 12 pentameric penton bases, and 12 trimeric fibre proteins).
- The capsid contains linear double-stranded (ds) DNA ranging from 26 to 46 kb.
- The Ad genome is divided into 4 early (E) and 5 late (L) transcriptional units.
    - Early transcriptional units encode non-structural proteins which regulate Ad DNA replication and host cell metabolism.
    - Late transcriptional units encode structural proteins which form the Ad virion.

ADENOVIRUS



Helper-dependent or gutless Ads:

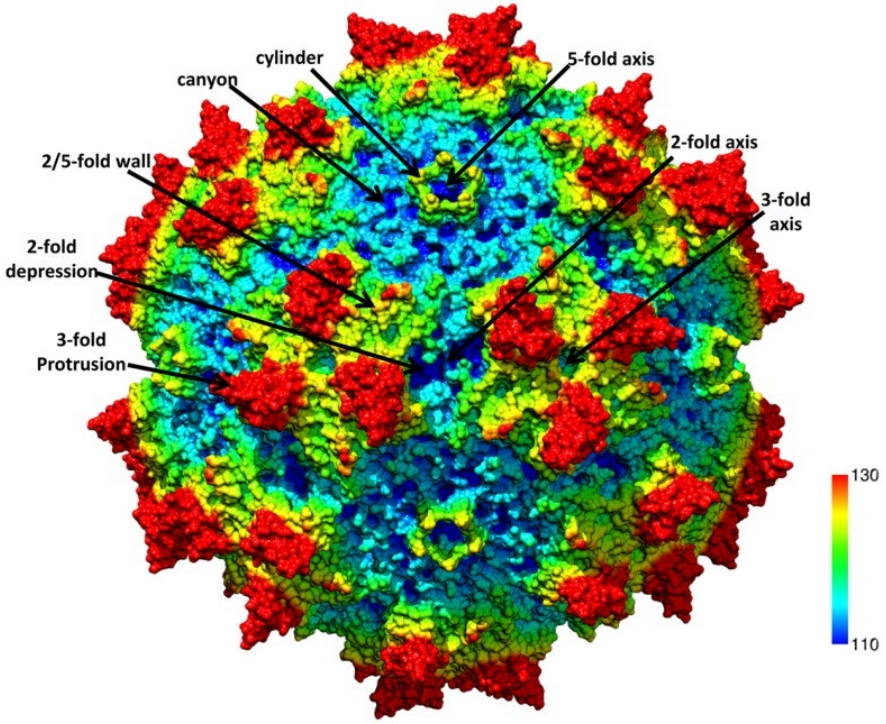
very attractive for gene therapy because of the highly reduced in vivo immune response while maintaining high transduction efficiency and tropism.

Nowadays, gutless adenovirus is administered in different organs, such as the liver, muscle or the central nervous system achieving high-level and long-term transgene expression in rodents and primates.

However, as devoid of all viral coding regions, gutless vectors require viral **proteins supplied in trans by a helper virus**.

https://doi.org/10.1038/sj.gt.3302612

ADENO-ASSOCIATED VIRUS (AAV)

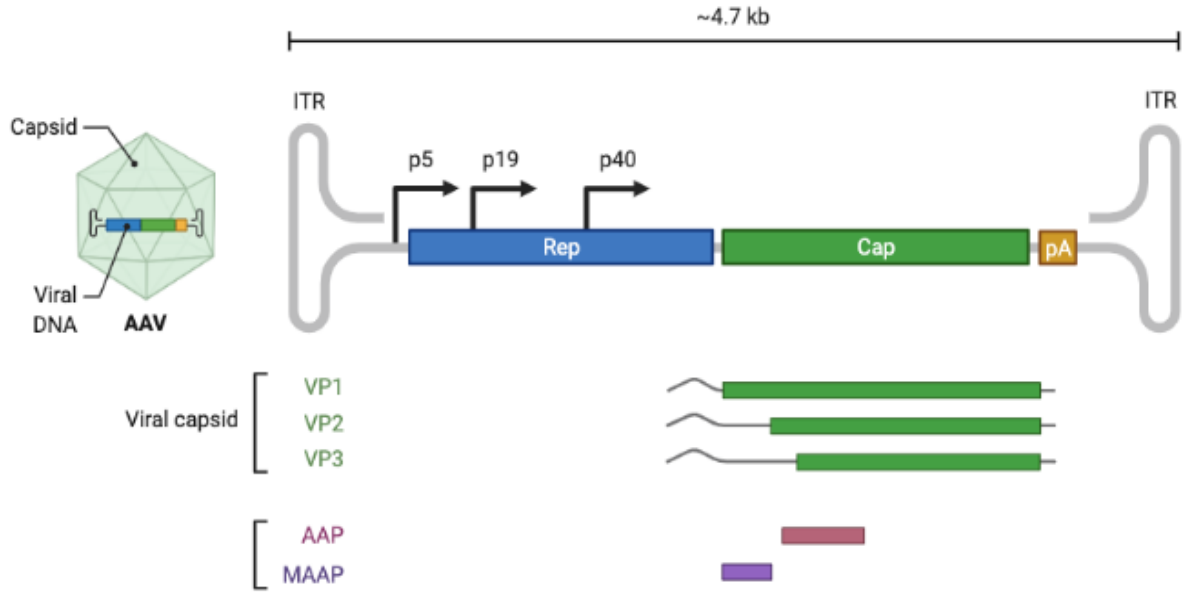


- Adeno-associated viruses (AAVs) are small viruses able to infect humans and other primate species, however, are **not pathogenic**.
- They belong to the genus Dependoparvovirus, which in turn belongs to the family Parvoviridae.
- They are small (approximately 26 nm in diameter) replication-defective, nonenveloped viruses and have linear single-stranded DNA (ssDNA) genome of approximately 4.7 kilobases (kb).

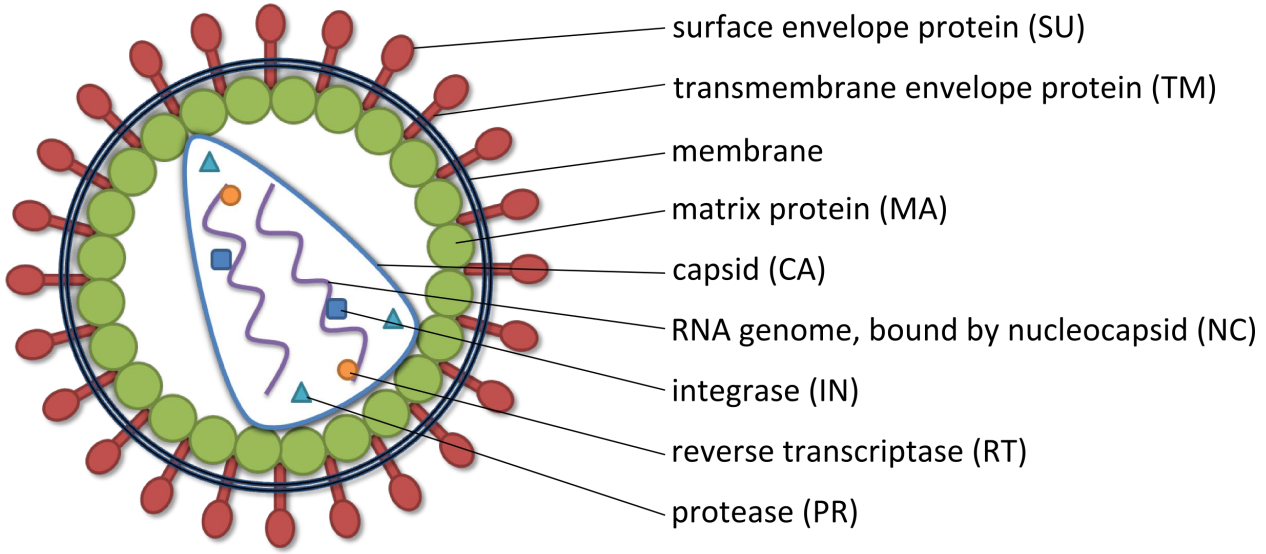
Its life cycle is dependent on the presence of a helper virus, such as AdV

ADENO-ASSOCIATED VIRUS (AAV)

AAV has a linear single-stranded DNA (ssDNA) genome of approximately **4.7-kilobases (kb)**, with two 145 nucleotide-long inverted terminal repeats (**ITR**) at the termini.
ITRs are repeated sequences that self-complement: provide **stability** to each end of the genome, play a key role in **integration**, are involved in **loading** of the genome into the AAV capsid particle, act as **promoters**.
The virus does not encode a polymerase and therefore relies on cellular polymerases for genome replication.
The ITRs flank the two viral genes – rep (replication) and cap (capsid), encoding non-structural and structural proteins, respectively. For gene therapy approaches, rep is only used during the AAV production stage.



RETROVIRUS

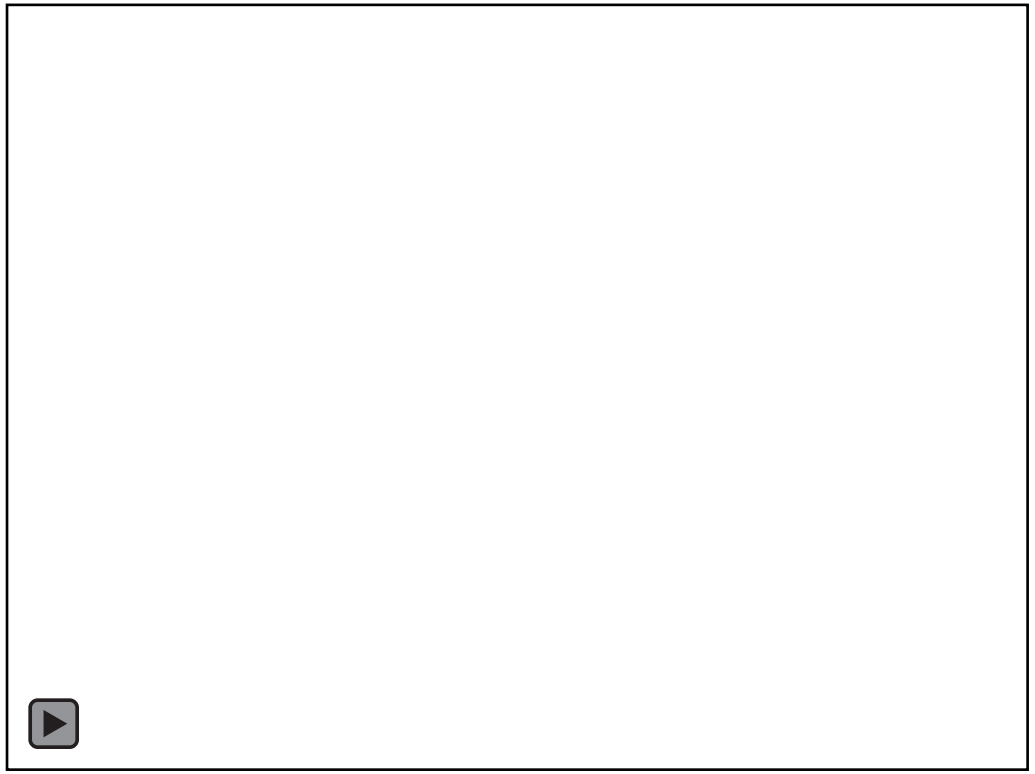


Retroviruses, consist of enveloped particles about 100 nm in diameter.

The main virion components are:

Envelope: composed of lipids (obtained from the host) as well as glycoprotein encoded by the env gene. three distinct functions: protection from the extracellular environment, enabling the retrovirus to enter/exit host cells through endosomal membrane trafficking, and the ability to directly enter cells by fusing with their membranes.
RNA: consists of two identical single-stranded RNA molecules 7–10 kilobases in length. The two molecules are present as a dimer, formed by base pairing between complementary sequences.
Proteins: consisting of gag proteins, protease (PR), pol proteins, and env proteins.

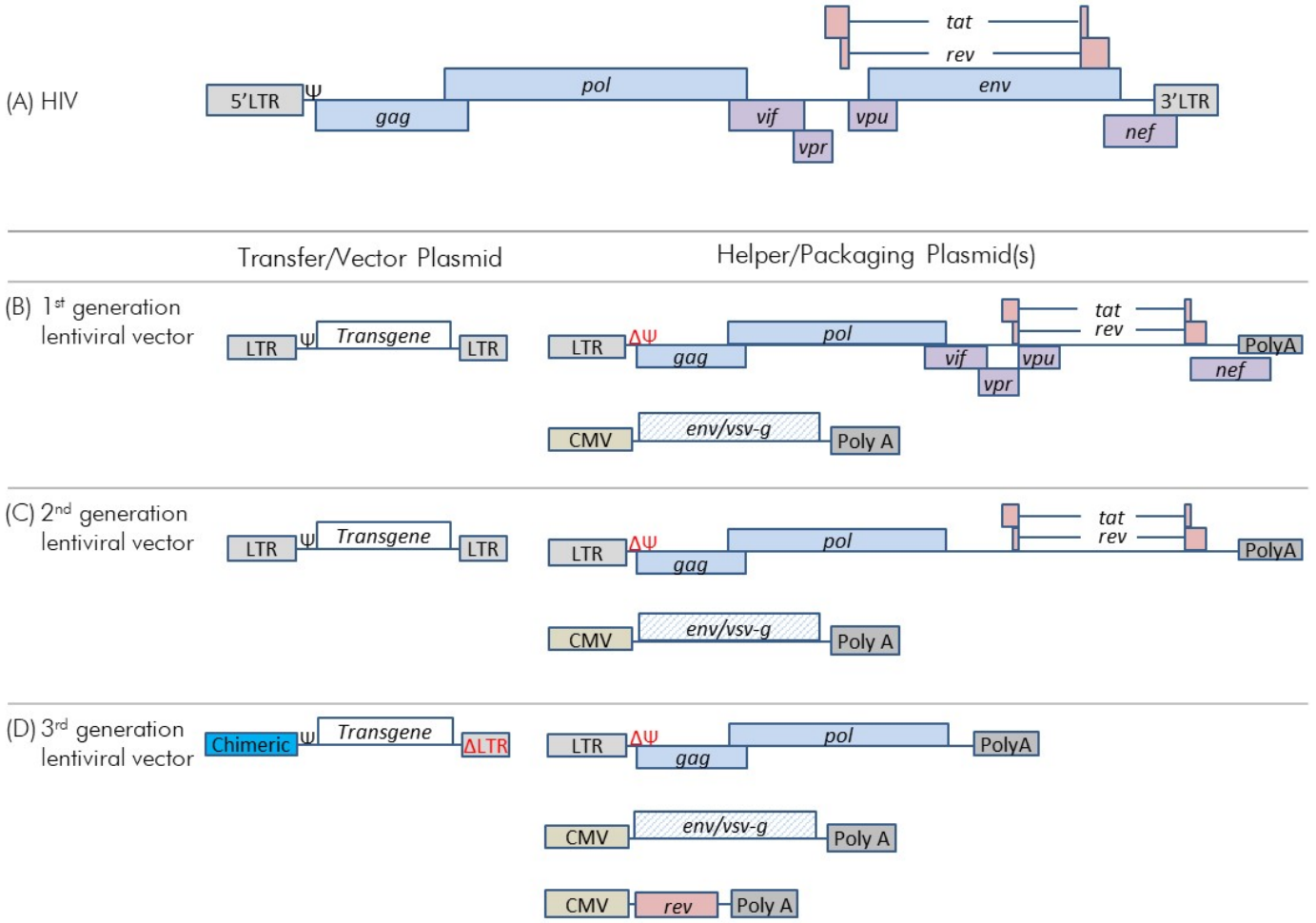
RETROVIRUS



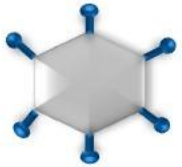
After invading a host cell's cytoplasm, the virus uses its own **reverse transcriptase** to produce DNA from its RNA genome, the reverse of the usual pattern, thus retro (backwards).
The new DNA is then incorporated into the host cell genome by an **integrase** enzyme, at which point the retroviral DNA is referred to as a provirus.
The host cell then treats the viral DNA as part of its own genome, transcribing and translating the viral genes along with the cell's own genes, producing the proteins required to assemble new copies of the virus.

LENTIVIRUS

- Lentivirus is a genus of retroviruses that cause chronic and deadly diseases characterized by long incubation periods, in humans and other mammalian species (e.g. HIV).
- Lentiviruses can integrate a significant amount of viral complementary DNA into the DNA of the host cell and can efficiently infect nondividing cells, so they are one of the most efficient methods of gene delivery.
- They can become endogenous, integrating their genome into the host germline genome, so that the virus is henceforth inherited by the host's descendants.



GT VECTOR COMPARISON




	ADENOVIRUS	AAV	γ-RETROVIRUS	LENTIVIRUS
SIZE	~90-100 nm	~25 nm	~80-100 nm	~80-100 nm
GENOME	dsDNA	ssDNA	ssRNA	ssRNA
PACKAGING CAPACITY	~8 kb – 36 kb	~4.7 kb	10 kb	8 kb
TRANSDUCTION	Dividing and non-dividing cells	Dividing and non-dividing cells	Dividing cells	Dividing and non-dividing cells
TRANSDUCTION EFFICIENCY	High	Moderate	Moderate	Moderate
INTEGRATION	Non-integrating	Non-integrating	Integrating	Integrating
EXPRESSION	Transient	Transient or stable	Stable	Stable
BIOSAFETY LEVEL	BSL-2	BSL-1	BSL-2	BSL-2
IMMUNOGENICITY	High	Low	Moderate-High	Moderate-High
GENE THERAPY STRATEGY	*In vivo*	*In vivo*	*Ex vivo*	*Ex vivo*

Drawbacks:

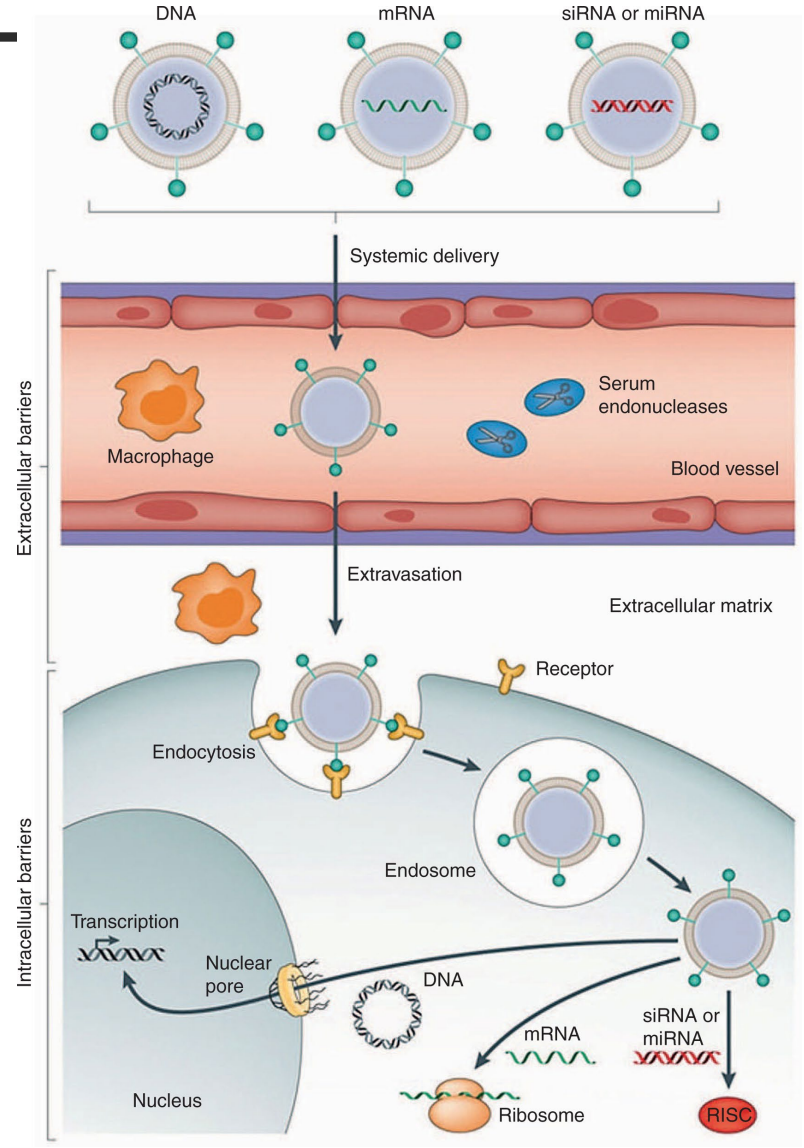
- Insertional genotoxicity
- Immune destruction of genetically modified cells
- Immune reactions towards in vivo viral-administration

OTHER GT VEHICLES
NANOPARTICLES

Many types of nanoparticles have been evaluated as gene carriers, which include:

- lipid-based nanoparticles,
- polymer-based nanoparticles,
- inorganic nanoparticles.

!The most important challenges are encapsulation efficiency, stability of nanoparticles, degradation in blood circulation, endocytosis by target cells, endosomal escape, delivery efficiency, and toxicity of pharmacology.



GENE THERAPY'S INFANCY

3 March 1972, Volume 175, Number 4025

SCIENCE

Gene Therapy for Human Genetic Disease?

Proposals for genetic manipulation in humans raise difficult scientific and ethical problems.

Theodore Friedmann and Richard Roblin

"…In our view, gene therapy may ameliorate some human genetic diseases in the future….For the foreseeable future however, we oppose any further attempts at gene therapy…because

(i) Our understanding of gene regulation and genetic recombination is inadequate

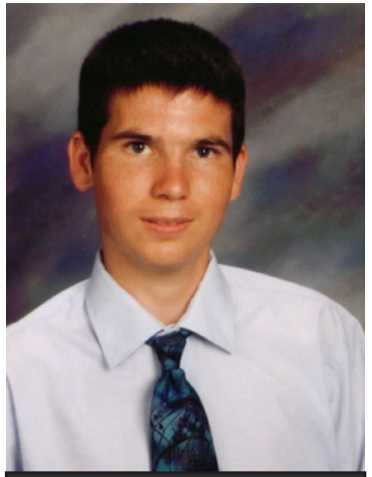
(ii) Our understanding of the details of the relation between the molecular defect and the disease state is rudimentary for all genetic diseases

(iii) We have no information on the short-range and long-term side effects of gene therapy…

FIRST STEPS

CLINICAL TRIALS

Gene Therapy Death Prompts Review of Adenovirus Vector



Jesse Gelsinger
1981 –1999

In the hot zone. James Wilson faced 2 days of questioning by colleagues and government advisers over the death of an 18-year-old patient.

GT RENAISSANCE

Science
AAAS

Gene Therapy of Human Severe Combined Immunodeficiency (SCID)-X1 Disease

Marina Cavazzana-Calvo *et al.*

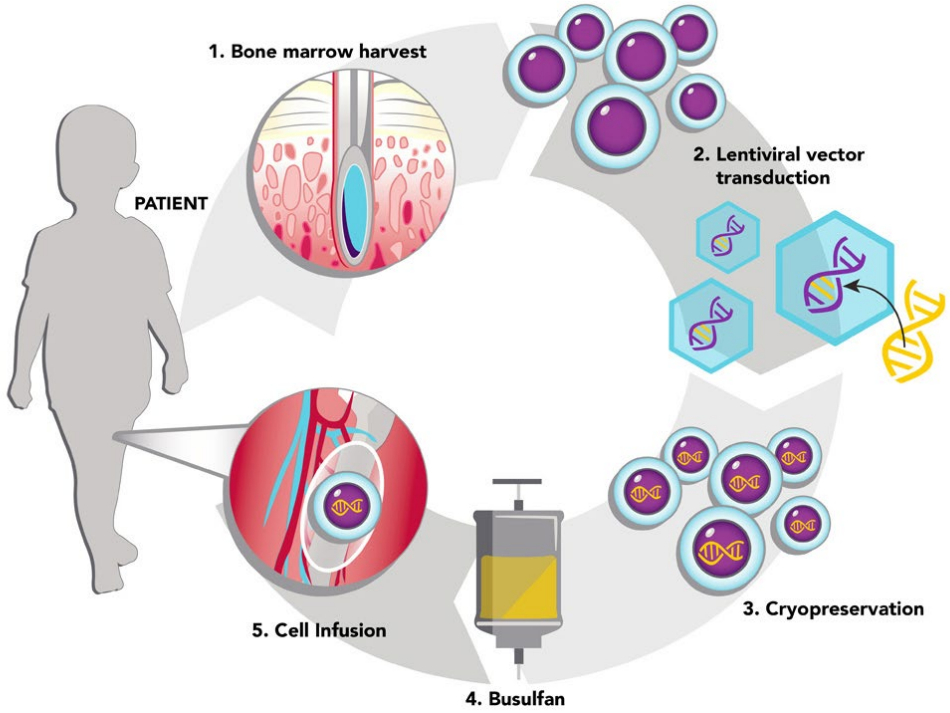
Science **288**, 669 (2000);
DOI: 10.1126/science.288.5466.669

David Phillip Vetter, 1971-1984

Severe combined immunodeficiency (SCID) is a group of rare disorders caused by mutations in different genes involved in the development and function of infection-fighting immune cells. Infants with SCID appear healthy at birth but are highly susceptible to severe infections.

EX VIVO GENE THERAPY FOR SCID

- Cells of interest harvested from the patient
- Cells modified by viral transduction ex vivo
- Patient receives myeloablation
- Cells are returned back to the patient



The NEW ENGLAND JOURNAL of MEDICINE

SPECIALTIES TOPICS MULTIMEDIA CURRENT ISSUE LEARNING/CME AUTHOR CENTER PUBLICATIONS

ORIGINAL ARTICLE

Autologous Ex Vivo Lentiviral Gene Therapy for Adenosine Deaminase Deficiency

Authors: Donald B. Kohn, M.D., Claire Booth, M.B., B.S., Kit L. Shaw, Ph.D., Jinhua Xu-Bayford, D.I.P., Elizabeth Garabedian, R.N., Valentina Trevisan, M.D., Denise A. Carbonaro-Sarracino, Ph.D., +45, and H. Bobby Gaspar, M.B.,

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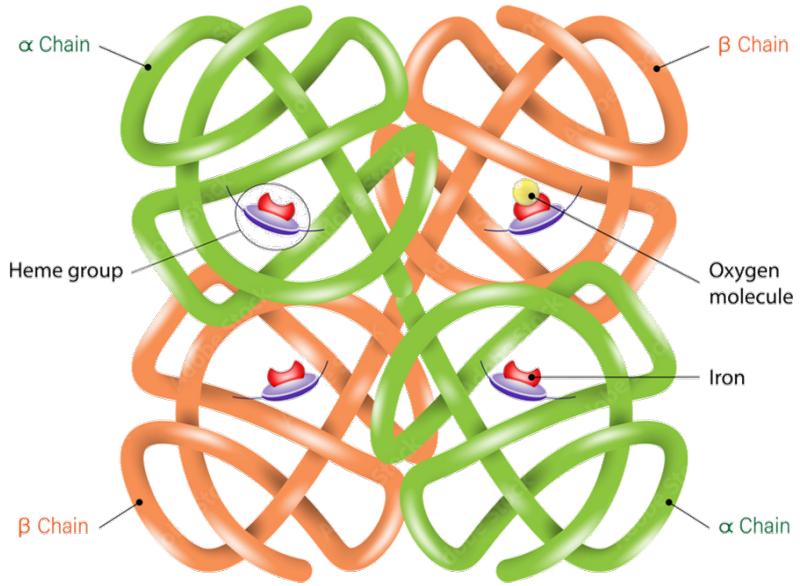
EMA APPROVED

GENE THERAPIES FOR MONOGENIC DISEASES

THE PARADIGM OF β-HEMOGLOBINOPATHIES

HEMOGLOBIN



- Hemoglobin is an iron-rich protein in red blood cells.
- Oxygen entering the lungs attaches to hemoglobin in the blood, which carries it to tissues in the body.
- When someone has insufficient red blood cells or the ones they have do not work properly, the body does not have enough of the oxygen it needs to function. This condition is anemia.

HEMOGLOBINOPATHIES

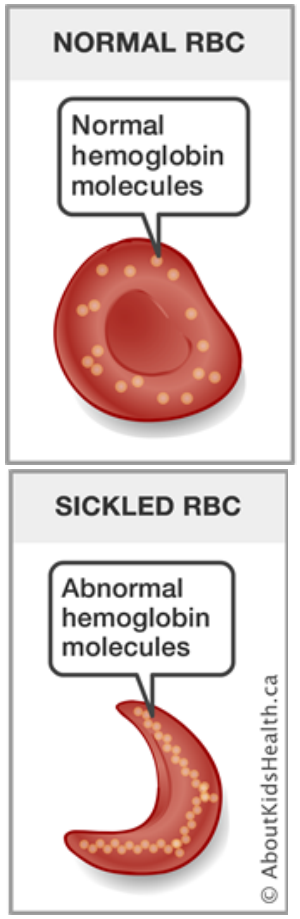
Sickle cell disease (SCD)

GGA CTC CTC
CCT GAG GAG

Pro | Glu | Glu → HbA

GGA CAC CTC
CCT GTG GAG

Pro | Val | Glu → HbS



HEMOGLOBINOPATHIES

β-thalassemia major

α-globin

β-globin

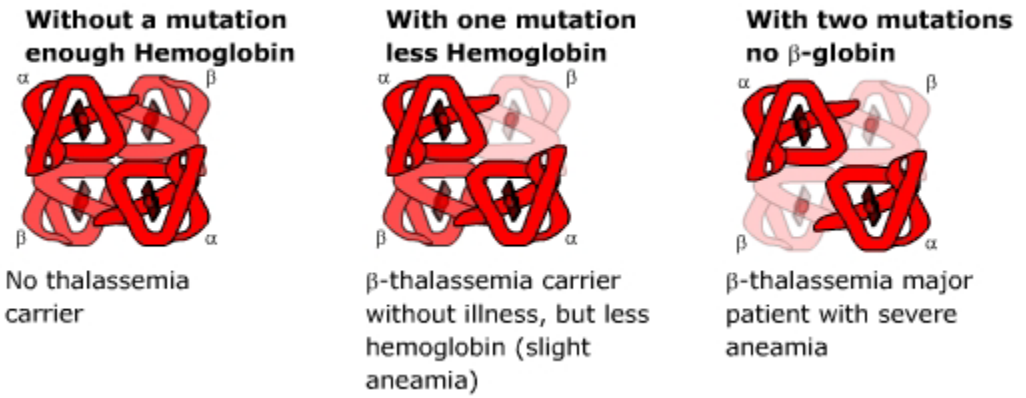
>300 mutations

HbA

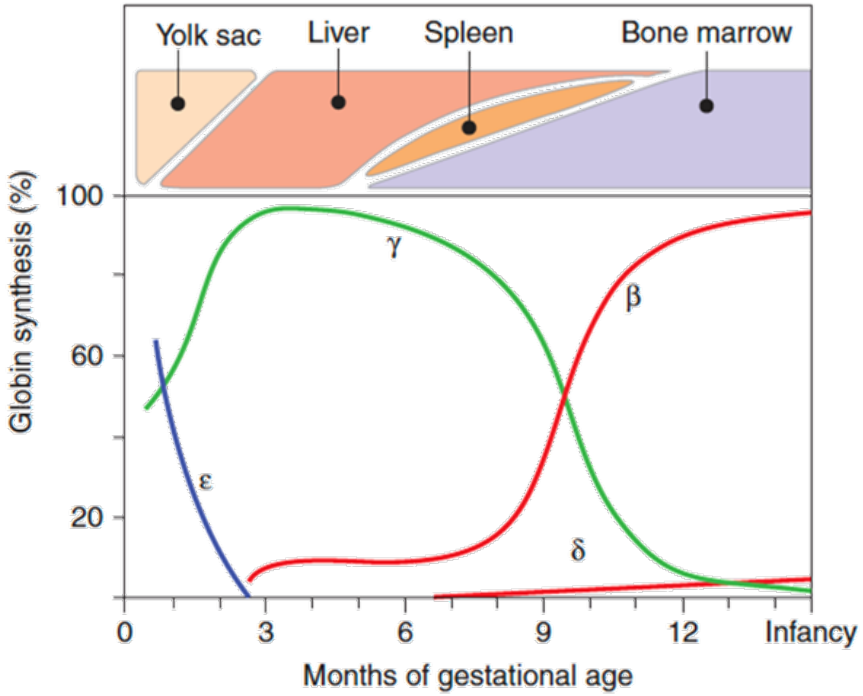
Free α-globin chains

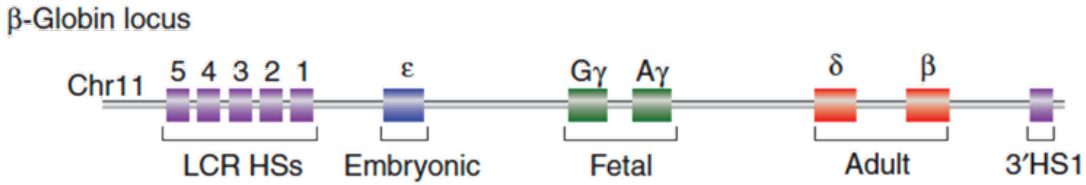
- Hemolytic anemia
- Bone marrow expansion
- Bone deformities
- Iron accumulation

A MATTER OF QUANTITY

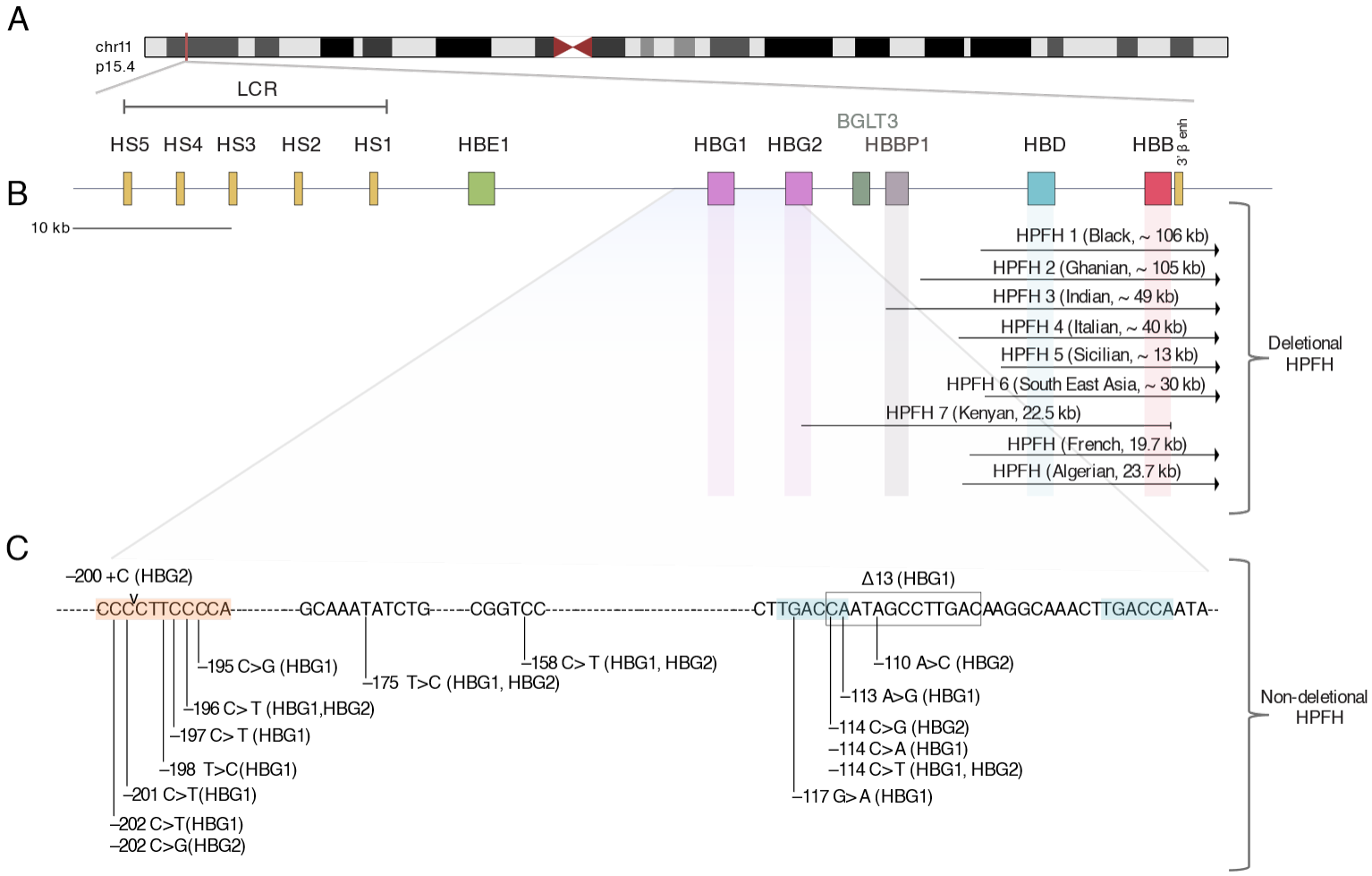


GLOBIN SWITCHING





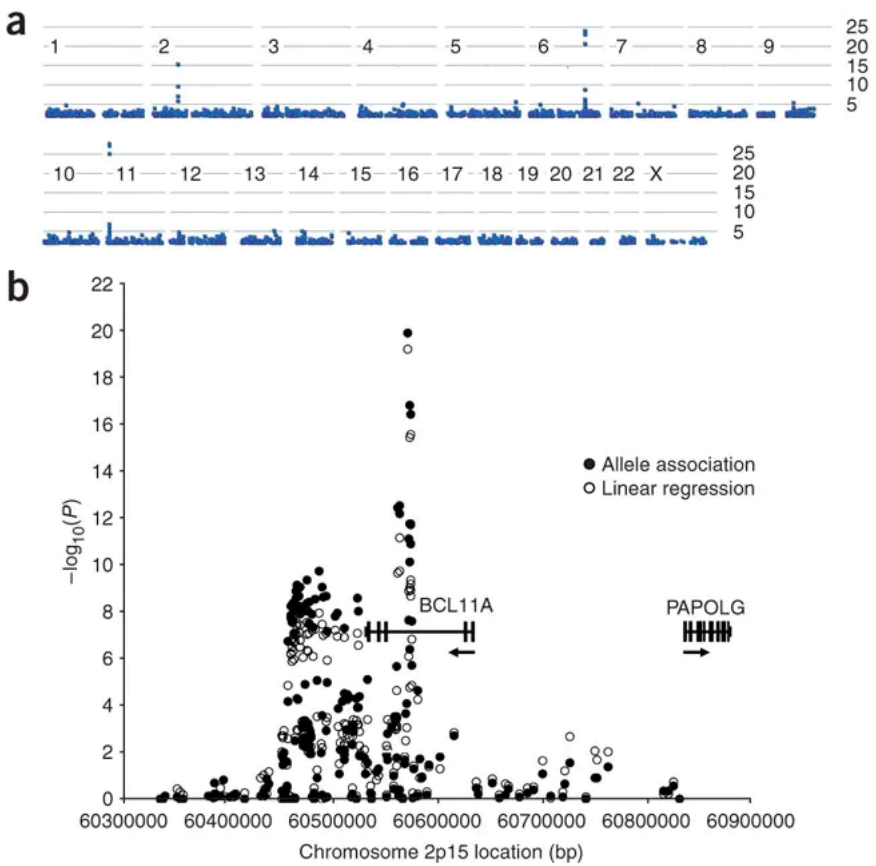
GLOBIN SWITCHING

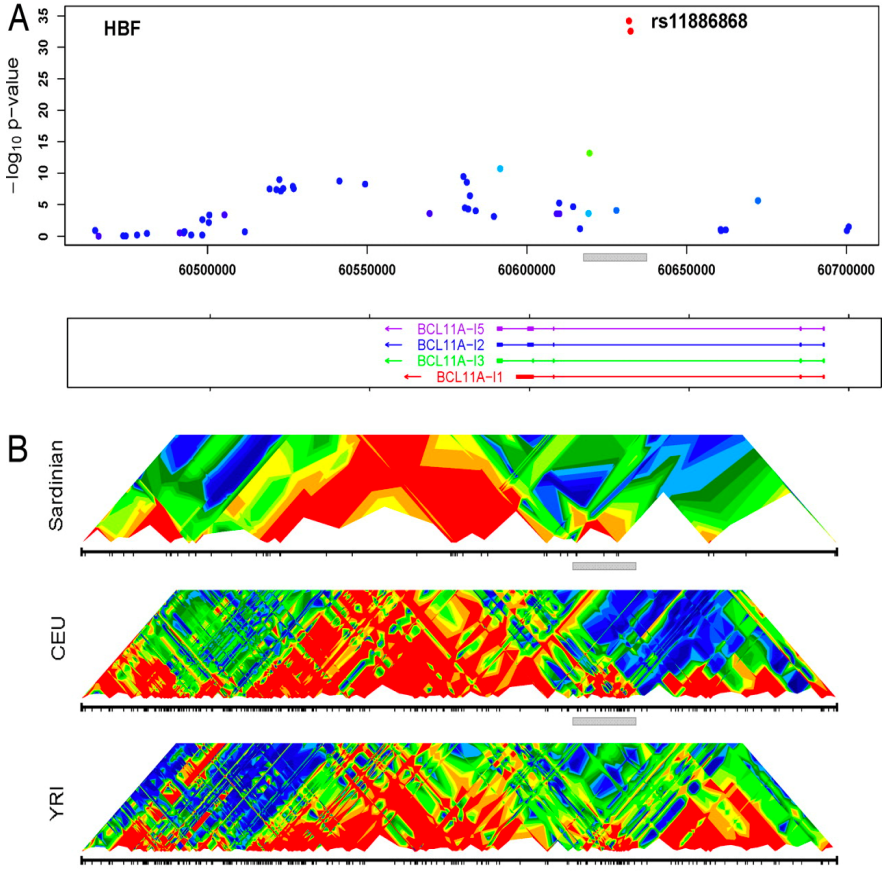


Paschoudi et al, JoMS 2023

HBF REGULATORS

BCL11a



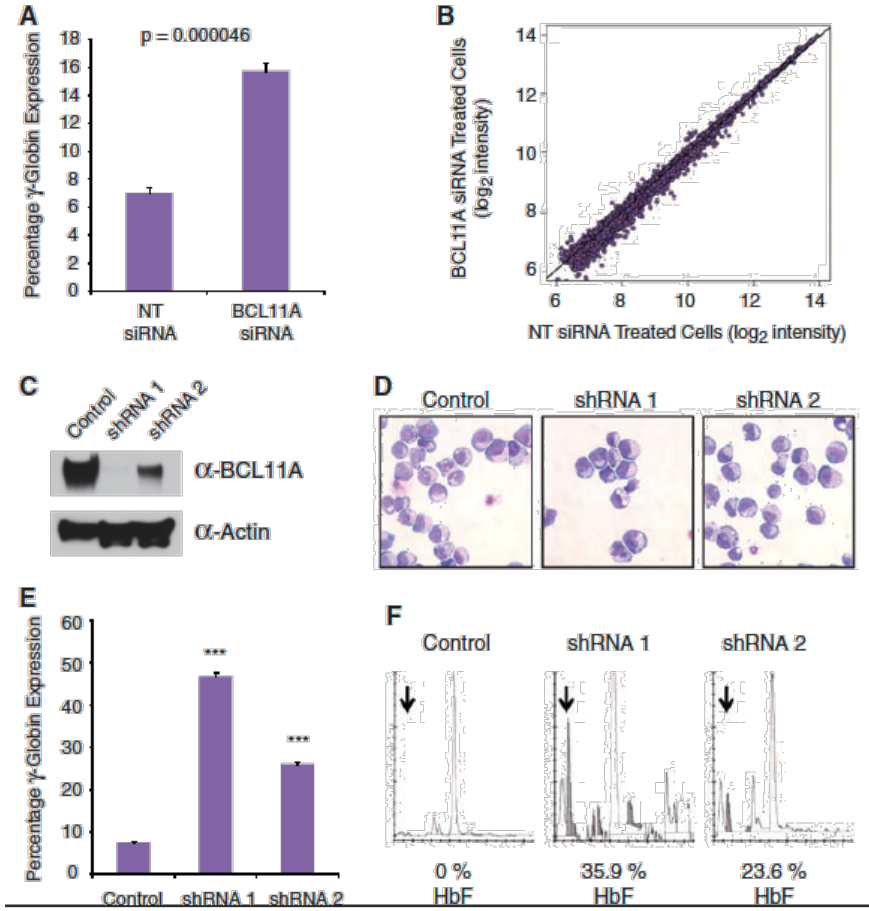


Menzel S. et al. , Nat genetics 2007

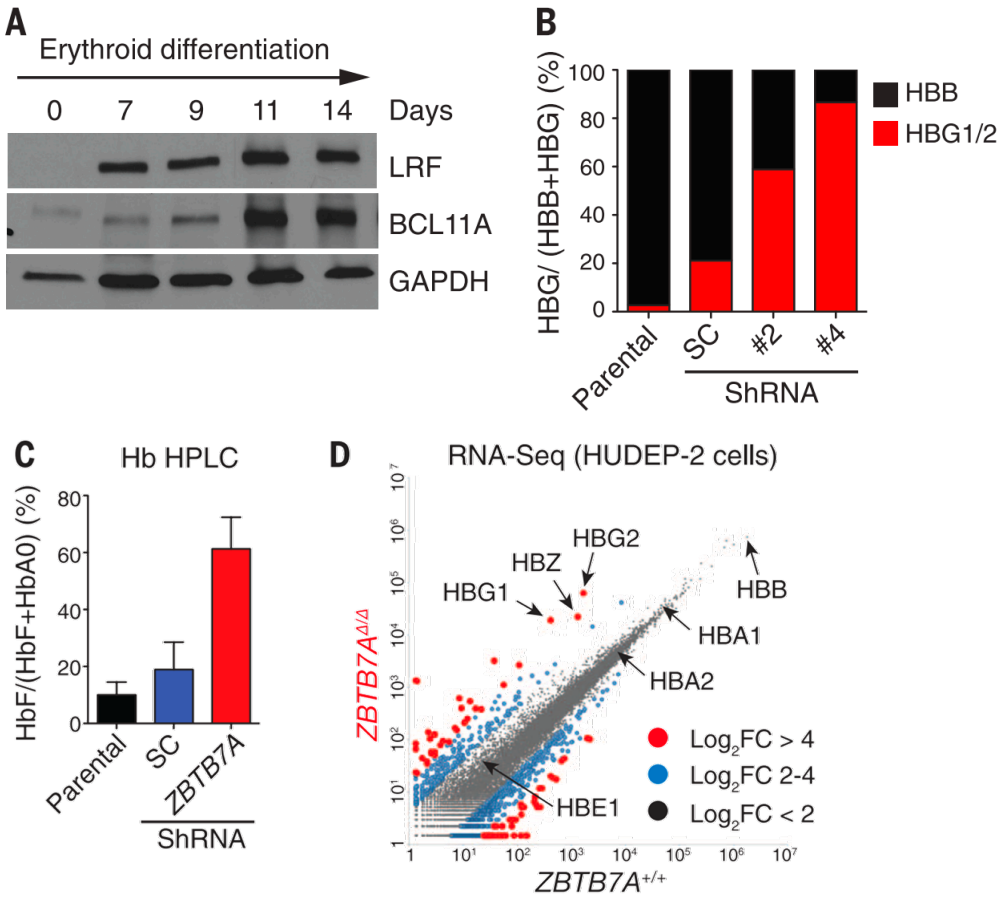
Uda et al. ,PNAS 2008

HBF REGULATORS

BCL11a



LRF/ZBTB7



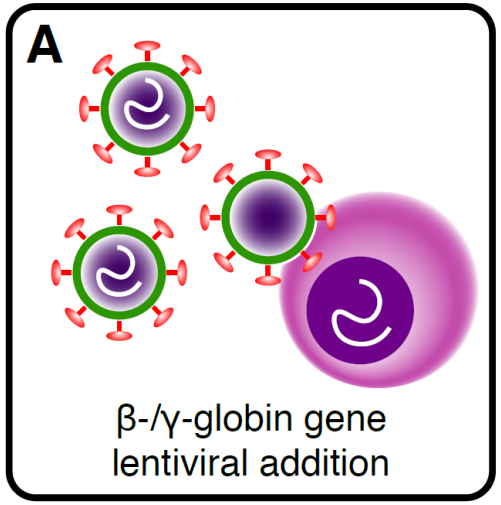
Sankaran VG et. al. Science, 2008

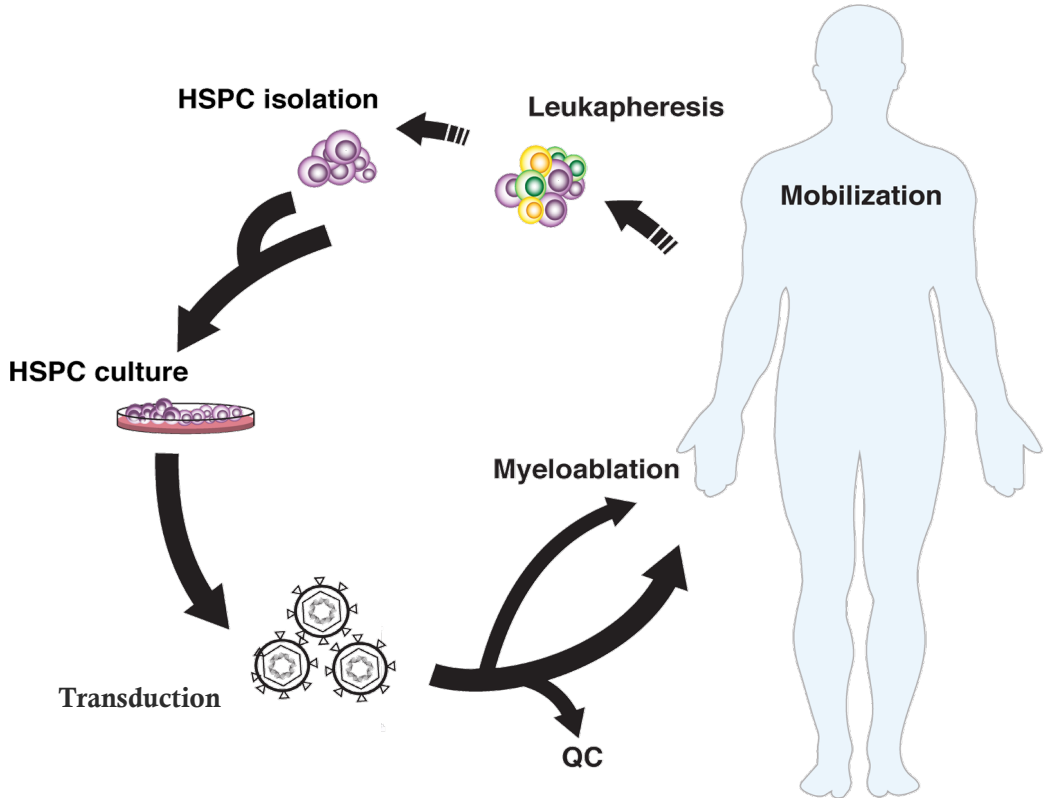
Masuda T et. al. Science, 2016

VIRAL GENE THERAPY FOR β-HEMOGLOBINOPATHIES

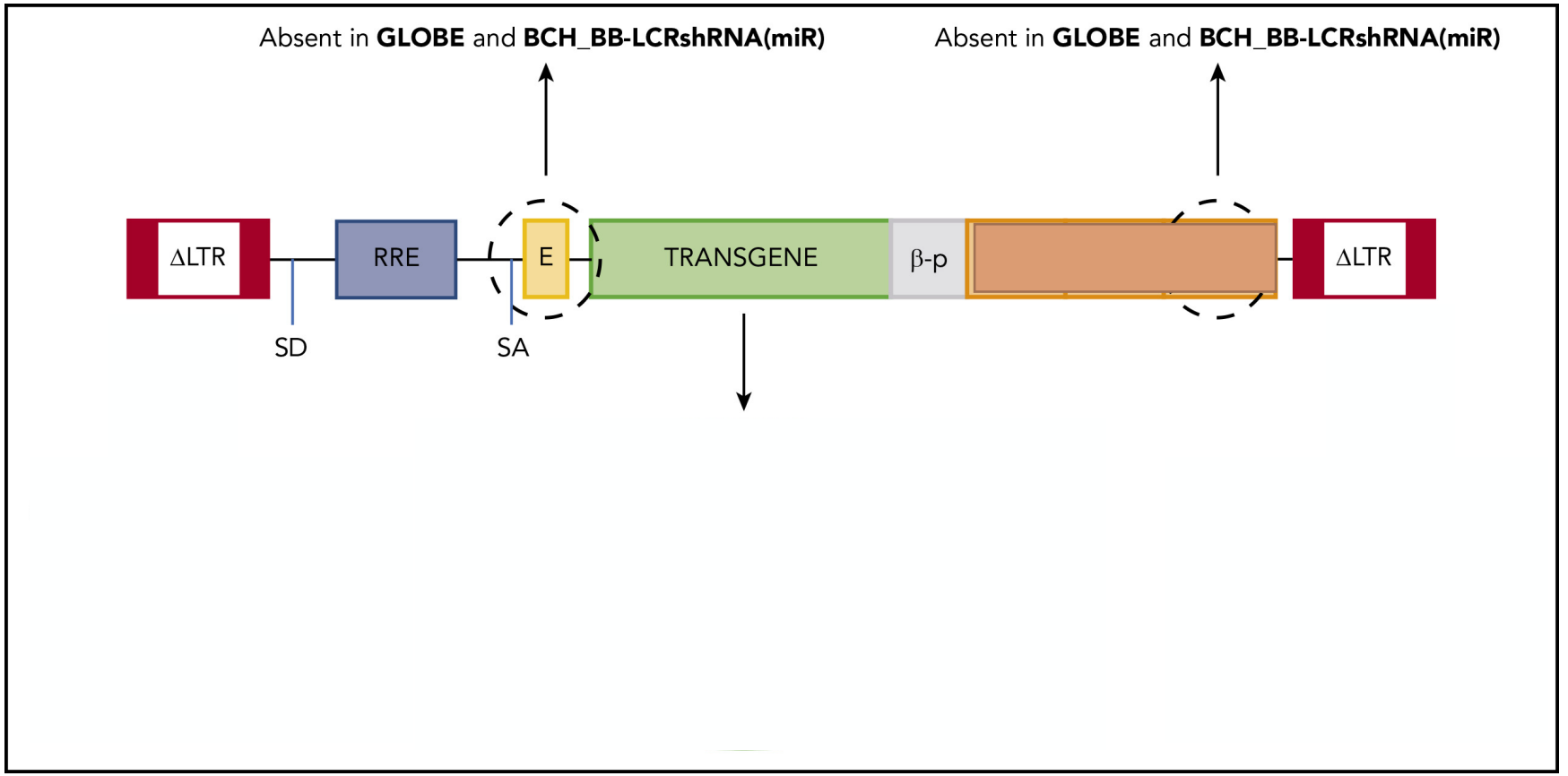
GENE ADDITION IN B-HEMOGLOBINOPATHIES

One type of gene modification- Viral mediated



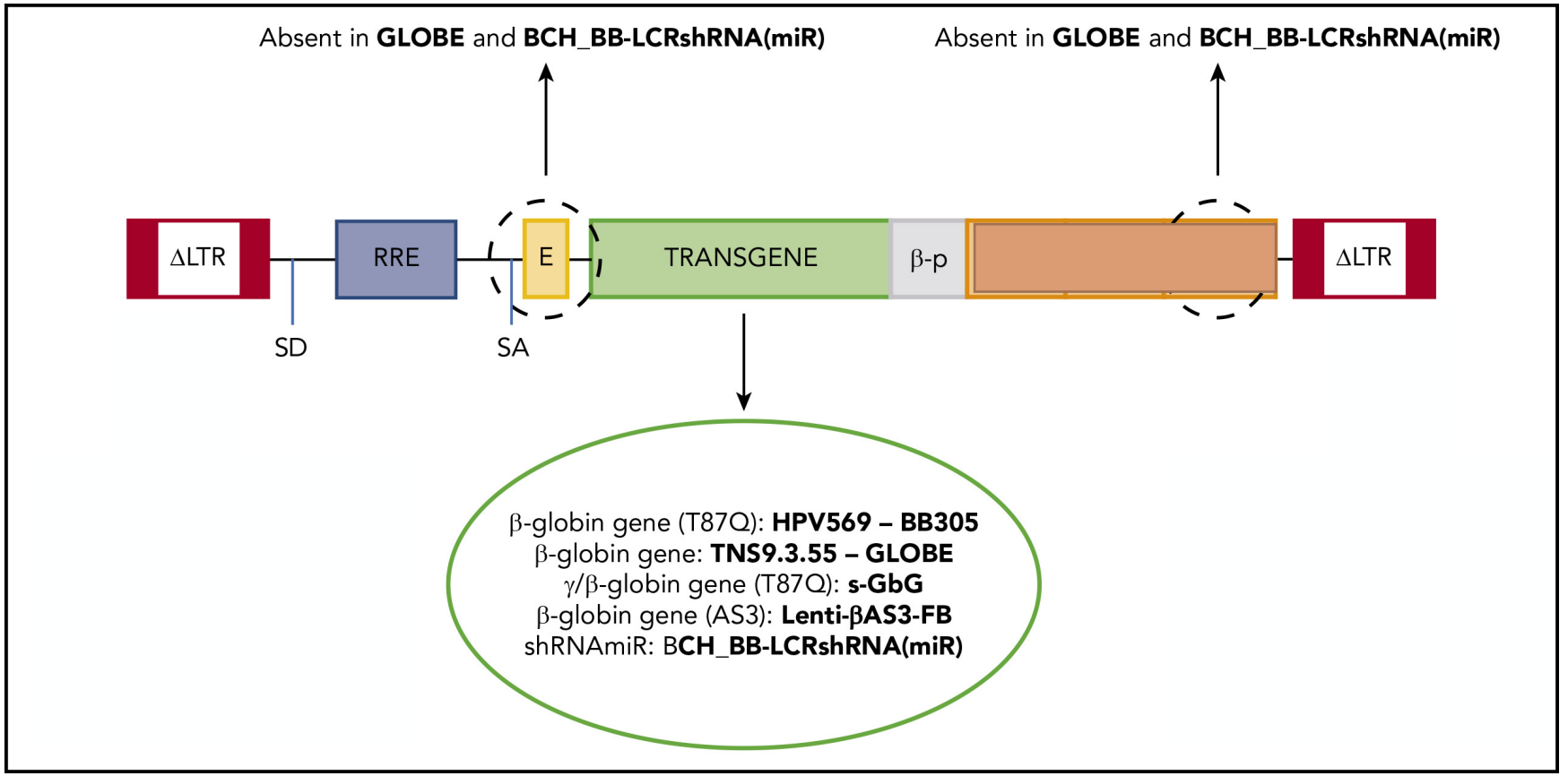


VIRAL VECTOR VARIATIONS



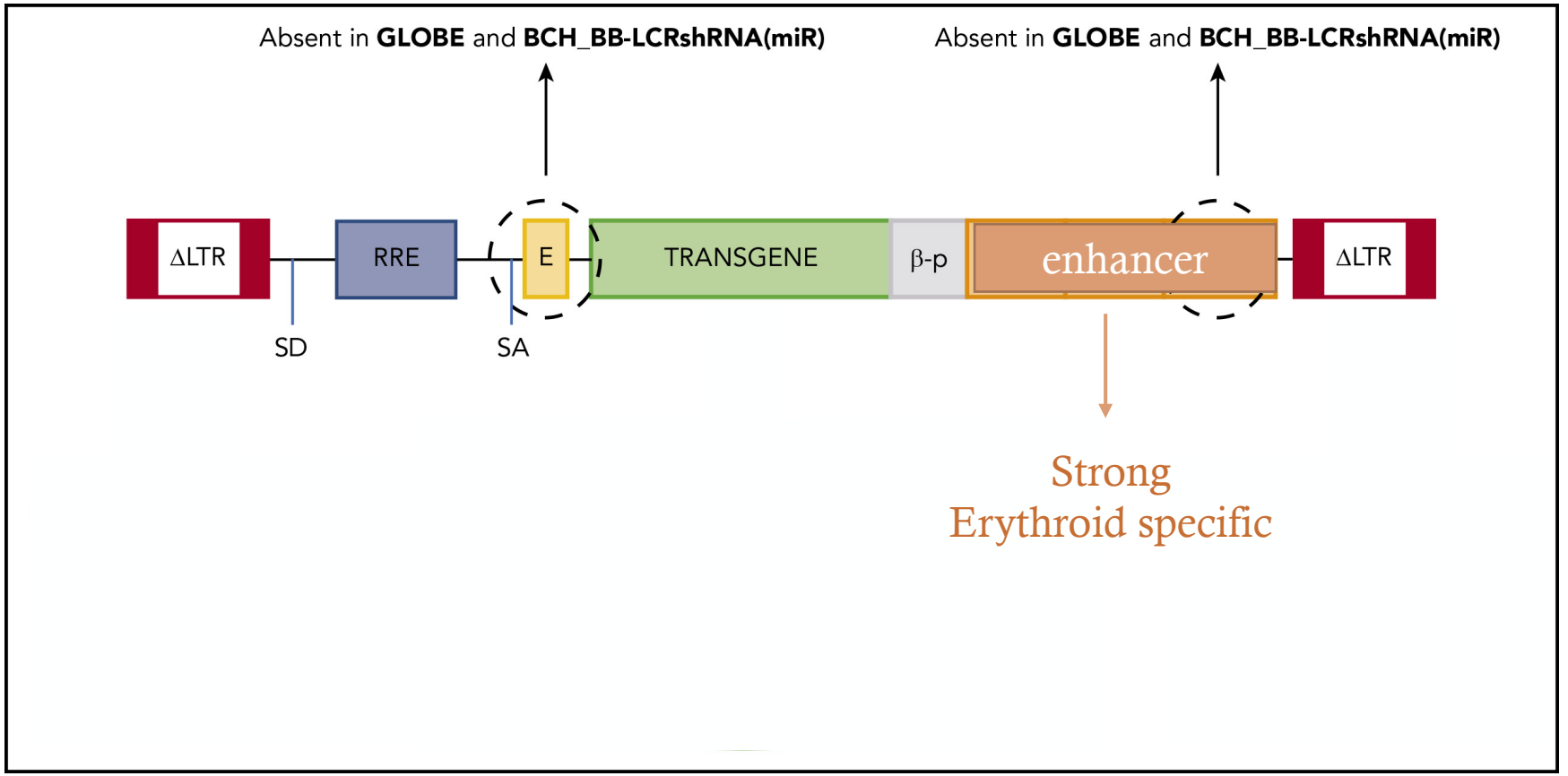
MAGRIN et al. Blood 2019

VIRAL VECTOR VARIATIONS



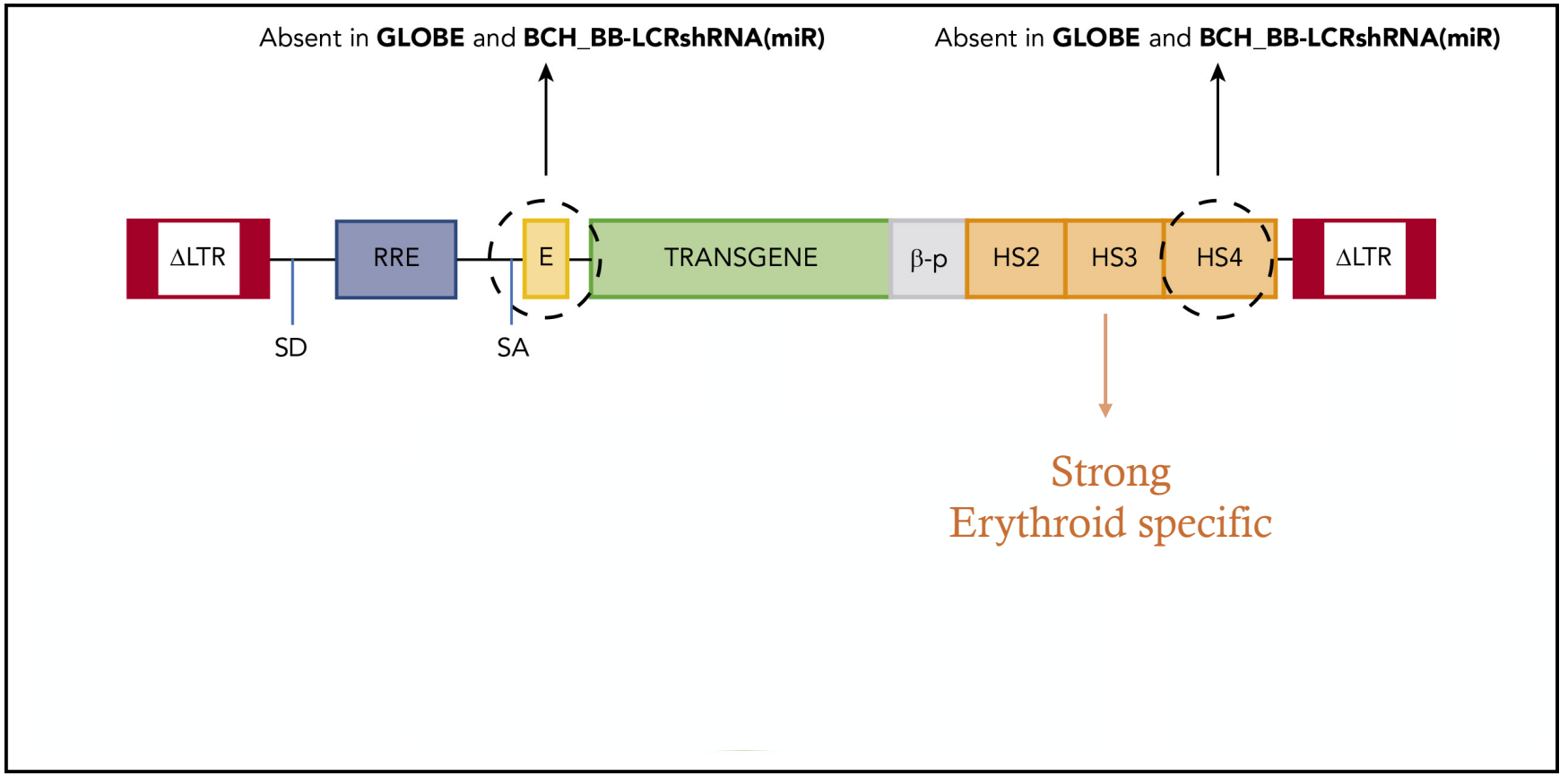
MAGRIN et al. Blood 2019

VIRAL VECTOR VARIATIONS



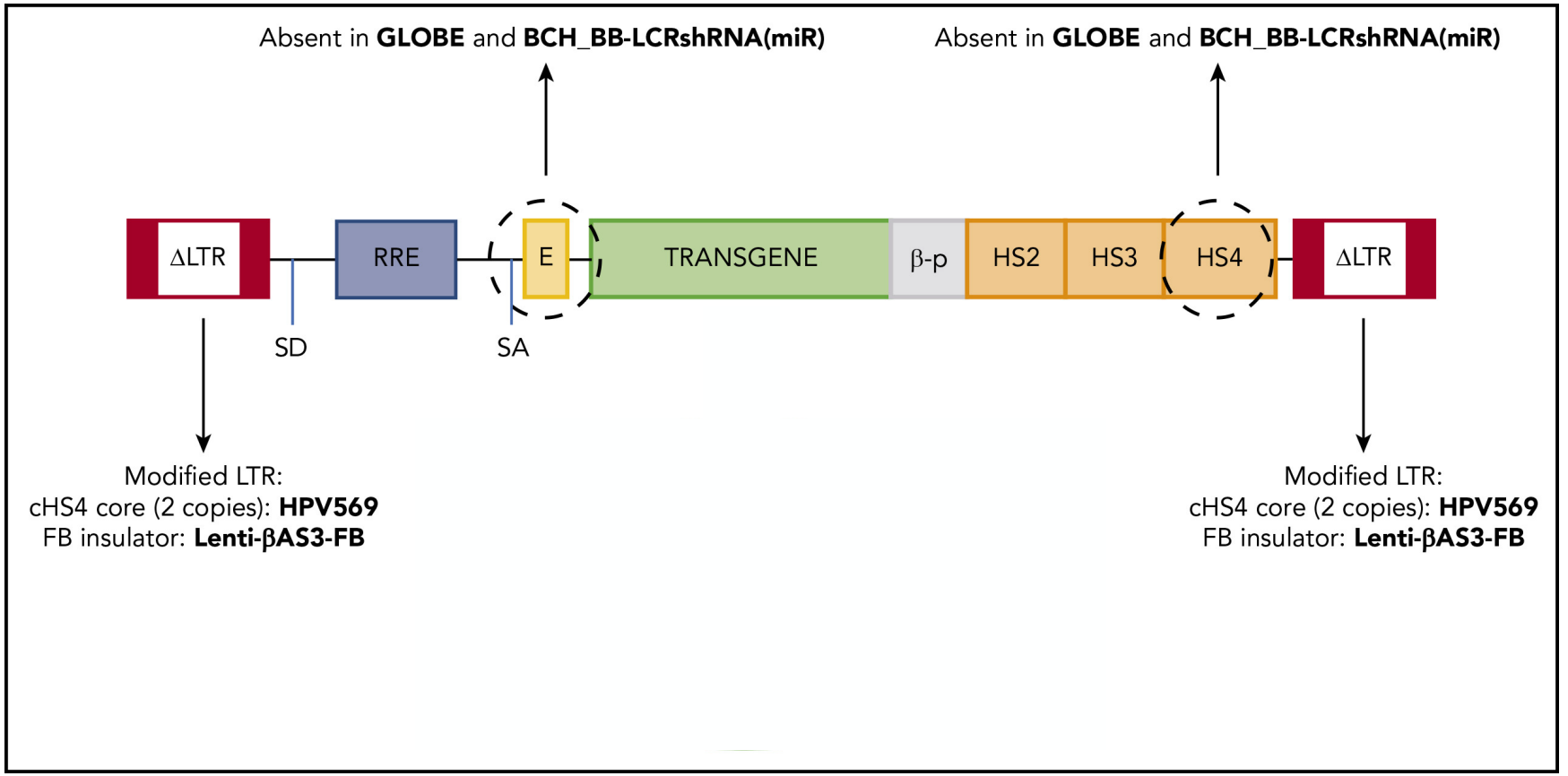
MAGRIN et al. Blood 2019

VIRAL VECTOR VARIATIONS



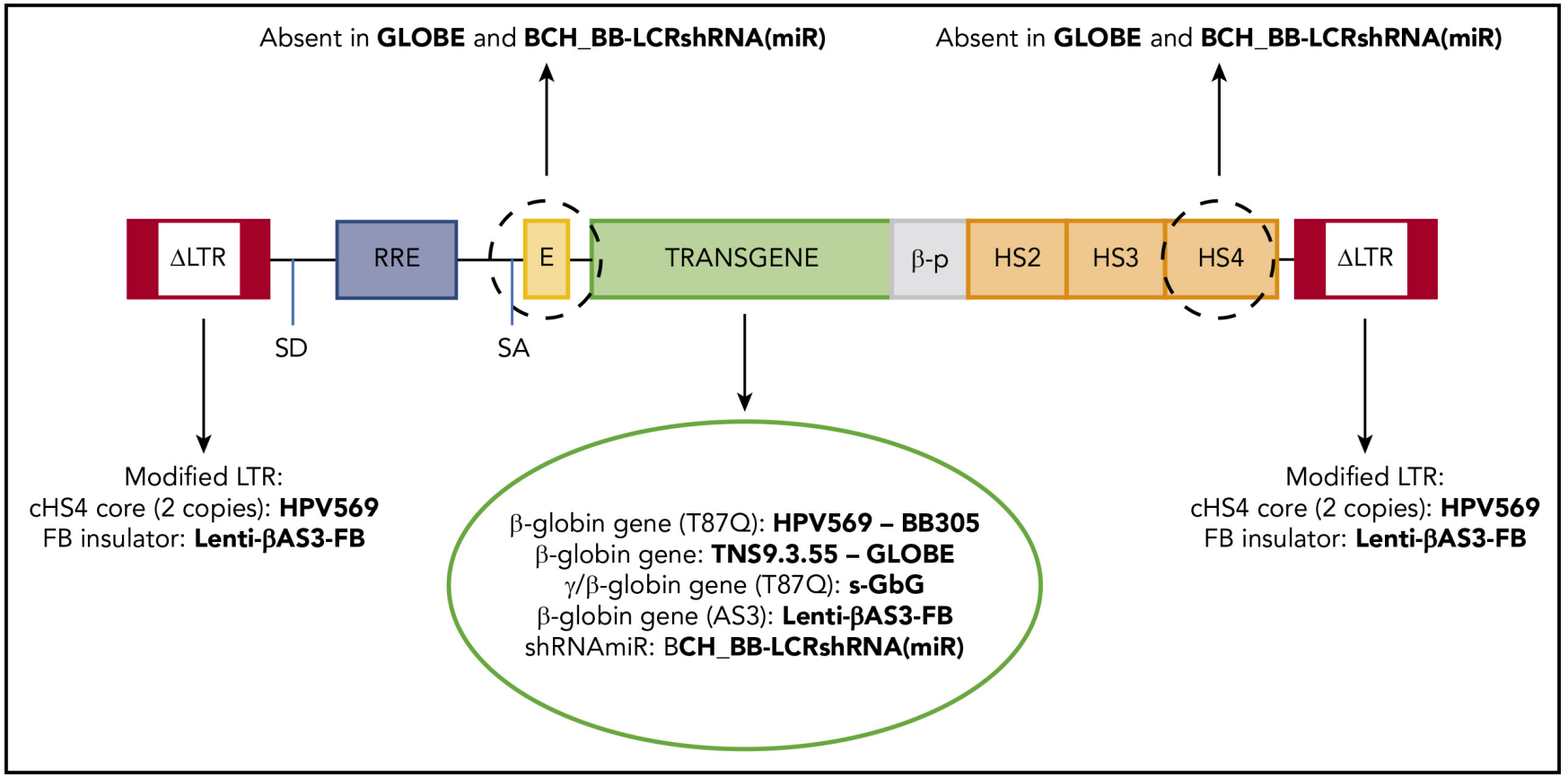
MAGRIN et al. Blood 2019

VIRAL VECTOR VARIATIONS



MAGRIN et al. Blood 2019

VIRAL VECTOR VARIATIONS



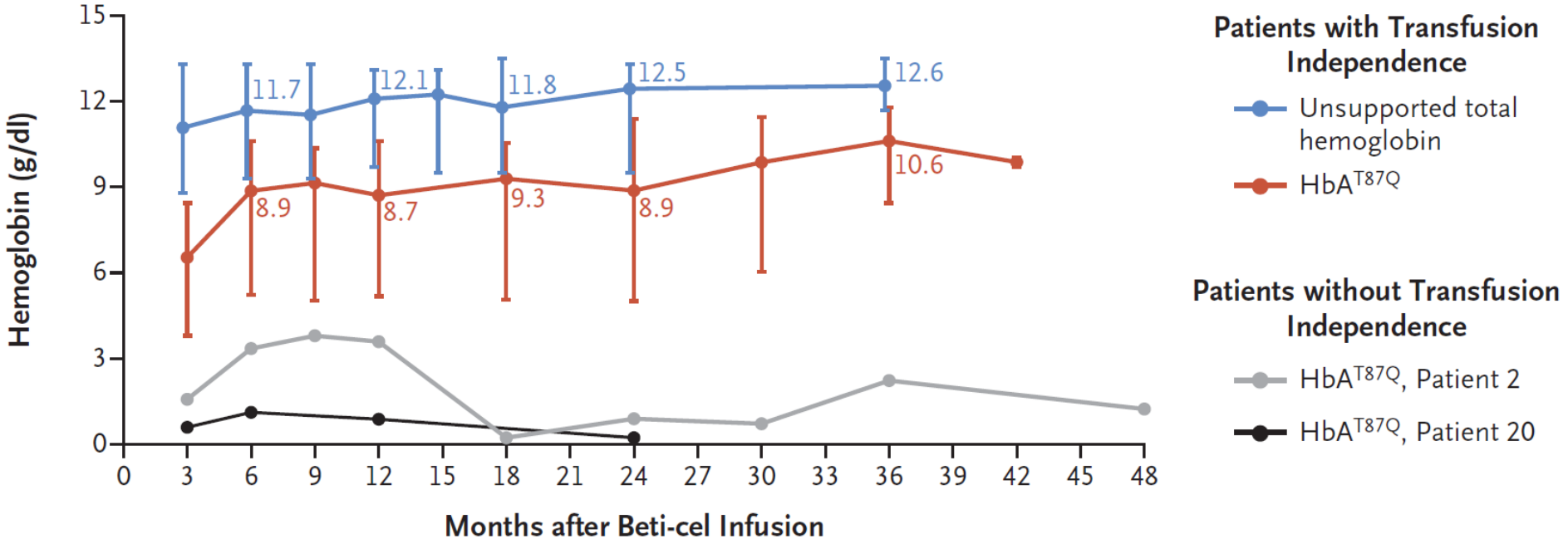
MAGRIN et al. Blood 2019

GT CLINICAL TRIALS

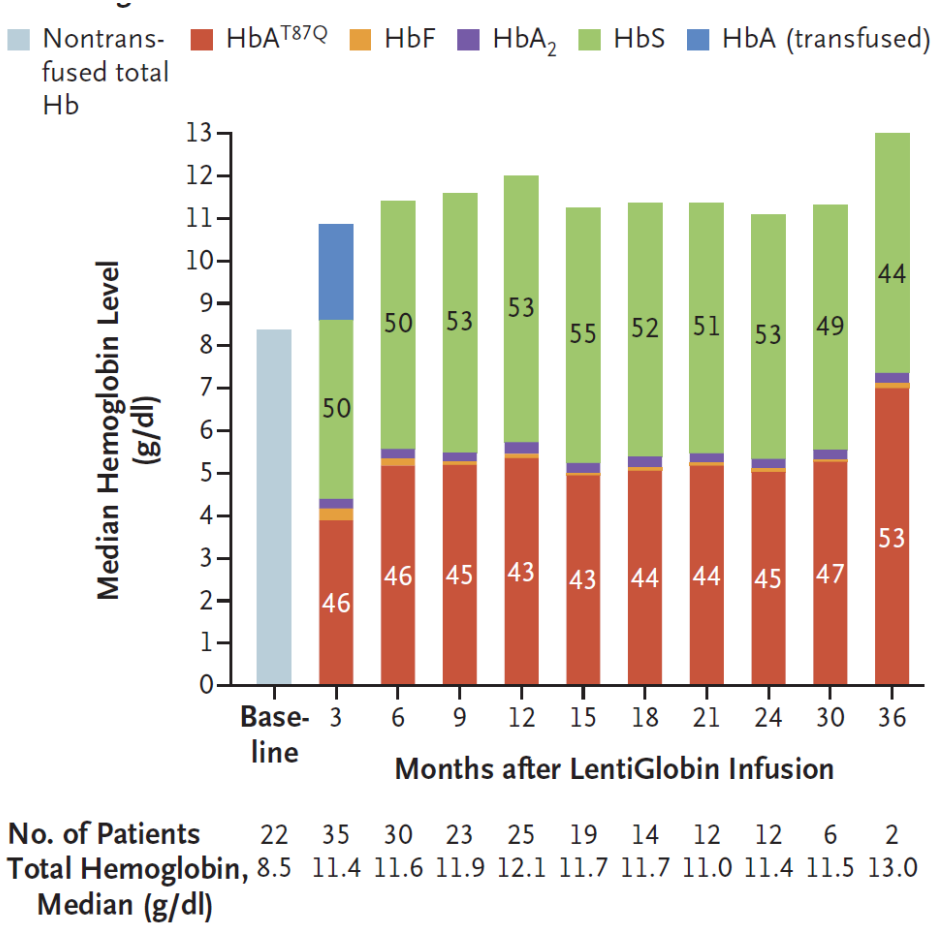
The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 FEBRUARY 3, 2022 VOL. 386 NO. 5

Betibeglogene Autotemcel Gene Therapy for Non–β^0/β^0 Genotype β-Thalassemia



GT CLINICAL TRIALS



The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 FEBRUARY 17, 2022 VOL. 386 NO. 7

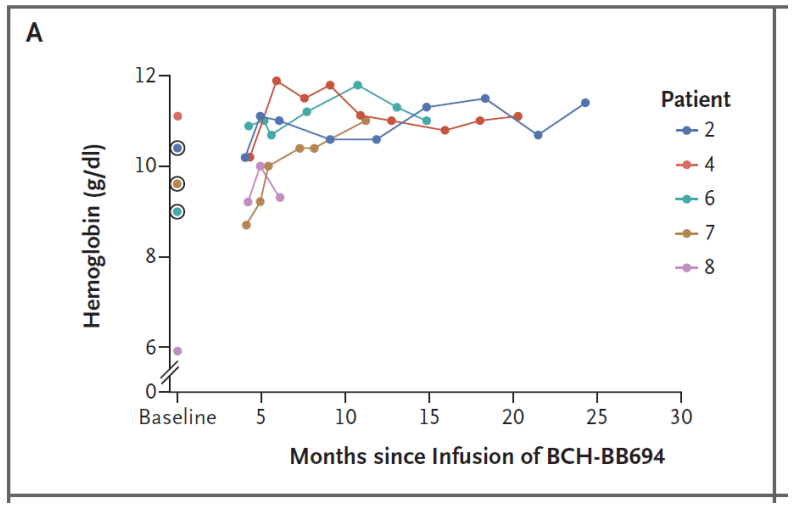
Biologic and Clinical Efficacy of LentiGlobin for Sickle Cell Disease

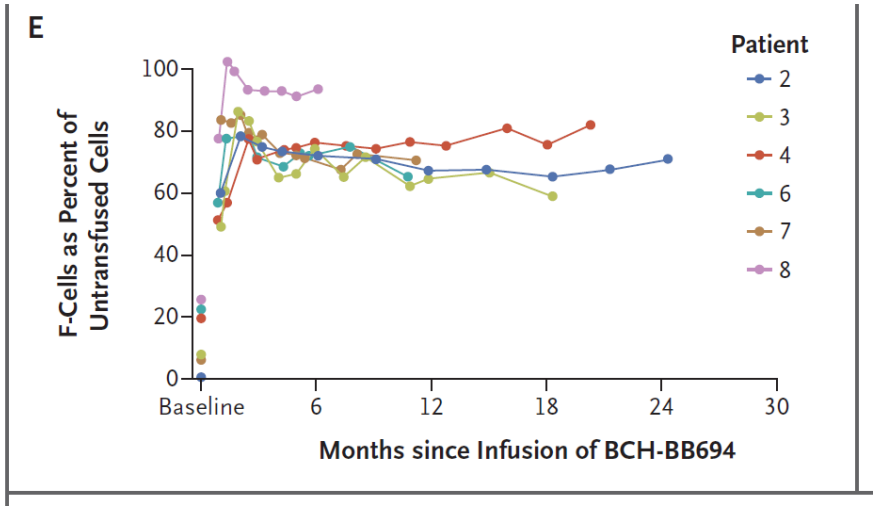
GT CLINICAL TRIALS

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JANUARY 21, 2021 VOL. 384 NO. 3

Post-Transcriptional Genetic Silencing of *BCL11A* to Treat Sickle Cell Disease





LV GT LIMITATIONS

01

B-globin vectors difficult to package and transduce HSCs

02

Low titers

03

High VCN necessary for therapeutic effect

04

Risk of hematologic malignancies due to random integration

GENOME EDITING AS A THERAPEUTIC TOOL

- Mutations in >4,800 of the 25,000 annotated genes in the human genome, have already been linked to disease phenotypes
- Disease linked mutations are located in both coding and noncoding regions of the genome

OMIM Morbid Map Scorecard (Updated April 2nd, 2024) :

Total number of phenotypes* for which the molecular basis is known	7,512
Total number of genes with phenotype-causing mutation	4,899

* Phenotypes include (1) single-gene mendelian disorders and traits; (2) susceptibilities to cancer and complex disease (e.g., BRCA1 and familial breast-ovarian cancer susceptibility, 113705.0001, and CFH and macular degeneration, 134370.0008); (3) variations that lead to abnormal but benign laboratory test values ("nondiseases") and blood groups (e.g., lactate dehydrogenase B deficiency, 150100.0001 and ABO blood group system, 110300.0001); and (4) select somatic cell genetic disease (e.g., GNAS and McCune-Albright syndrome, 139320.0008 and IDH1 and glioblastoma multiforme, 147700.0001.)

GENOME EDITING AS A THERAPEUTIC TOOL

- Genome editing provides the possibility of removing or correcting deleterious mutations
- Altering the genome can be used as a therapeutic approach for both monogenic and non-monogenic diseases such as cardiovascular disease, HIV, Alzheimer disease and hemoglobinopathies.

Dissected OMIM Morbid Map Scorecard (Updated May 20th, 2022) :

Class of phenotype	Phenotype	Gene *
Single gene disorders and traits	6,109	4,273
Susceptibility to complex disease or infection	690	501
"Nondiseases"	153	120
Somatic cell genetic disease	233	130

*Some genes may be counted more than once because mutations in a gene may cause more than one phenotype and the phenotypes may be of different classes (e.g., activating somatic BRAF mutation underlying cancer, 164757.0001. and germline BRAF mutation in Noonan syndrome, 164757.0022.)

GENOME EDITING IN B-HEMOGLOBINOPATHIES

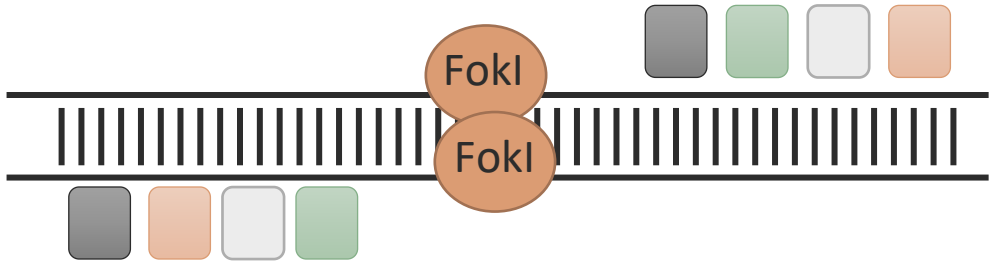
100+One types of gene modification

- Gene correction
- Gene addition in safe harbor loci
- Gene deactivation targeting coding sequences
- Gene deactivation targeting regulatory sequences
- Gene reactivation targeting cis-acting elements
- Gene reactivation targeting trans-acting elements
- Gene replacement

Combination of all the above

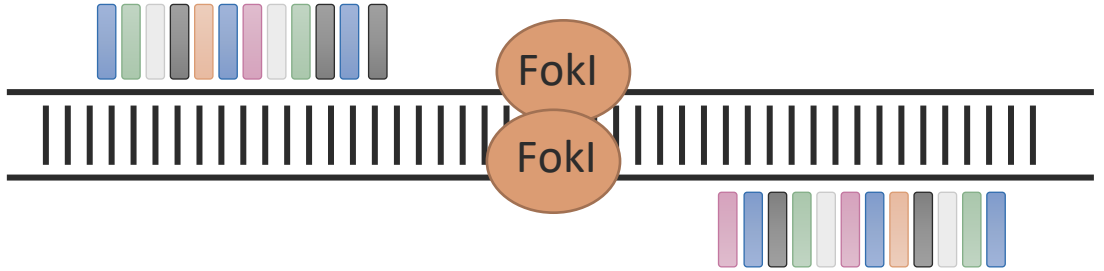
GENOME EDITING TOOLS: FIRST GENERATION OF CUSTOM DESIGNED NUCLEASES

ZFNs



2003

TALENs

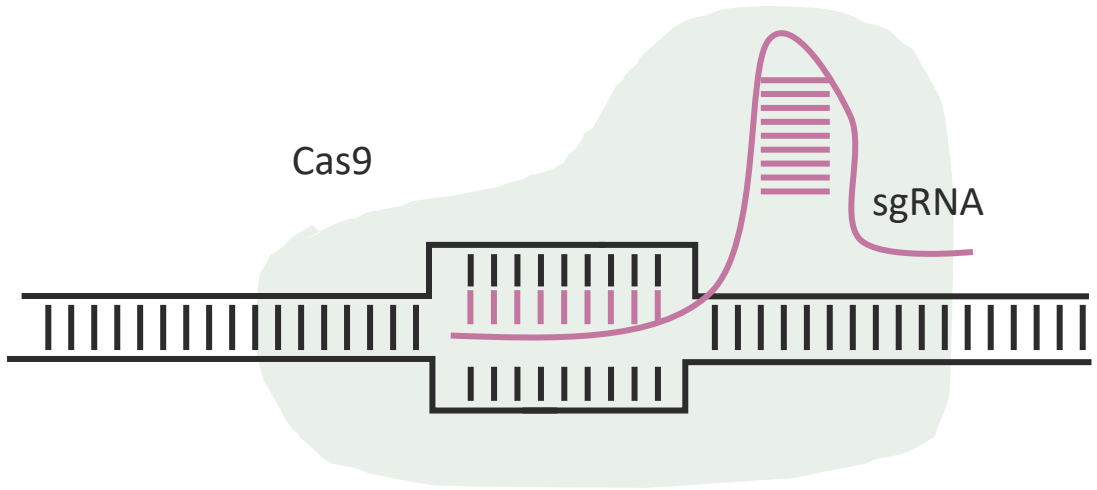


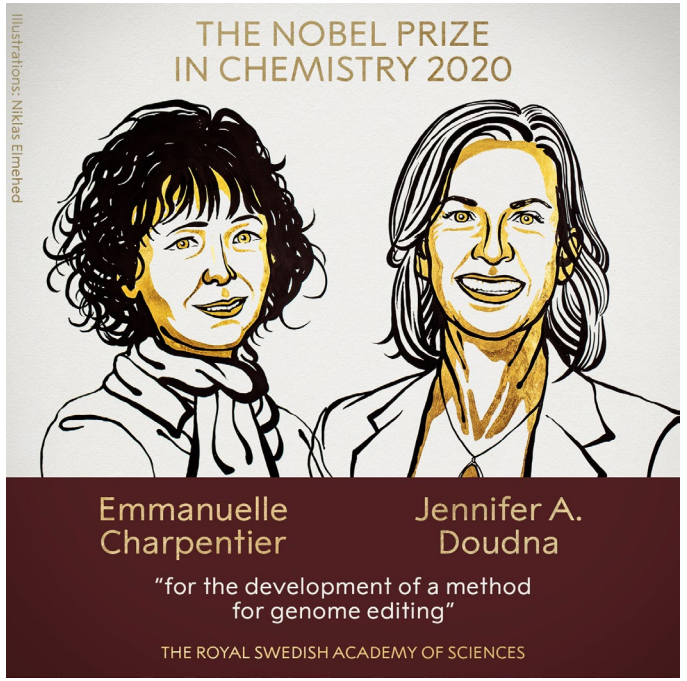
2009

Sangamo THERAPEUTICS

cellectis EDITING LIFE

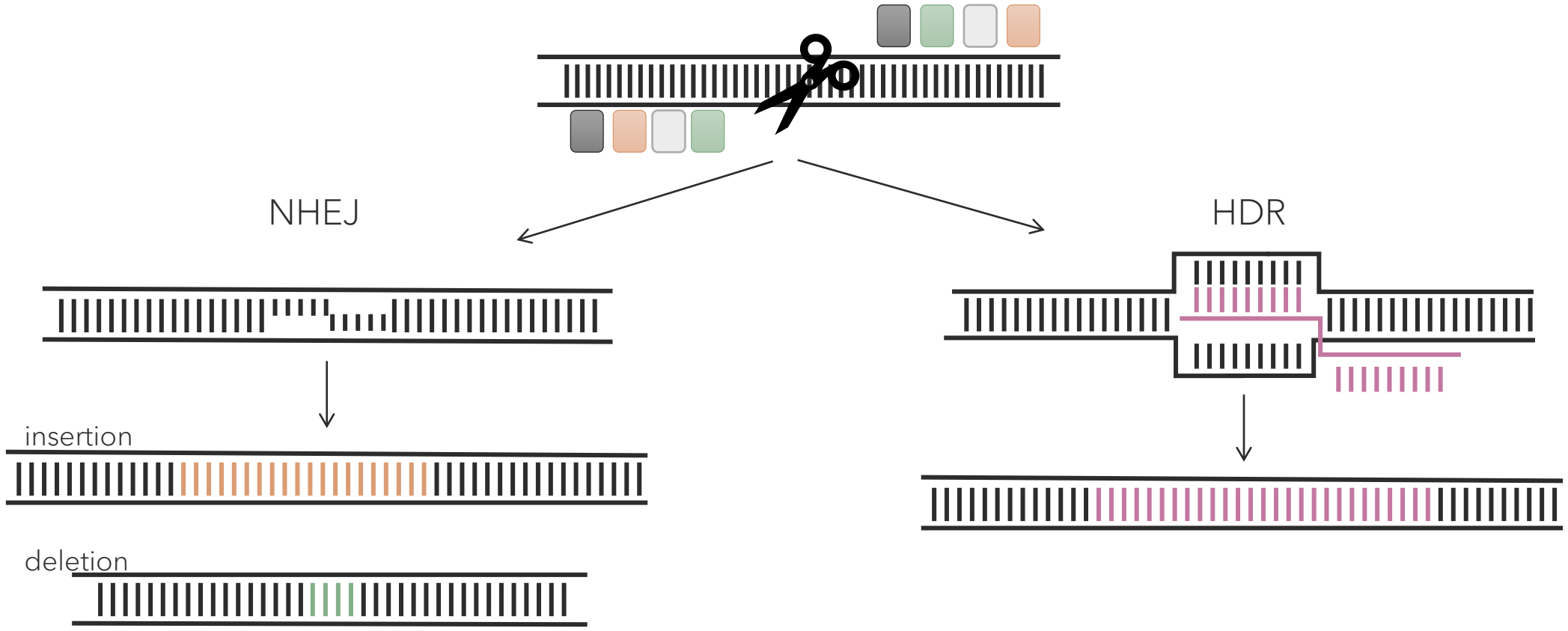
THE REVOLUTIONARY CRISPR/CAS9 SYSTEM





2012

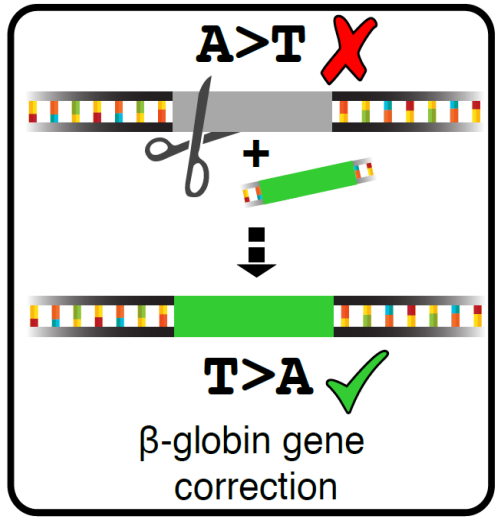
GENOME EDITING



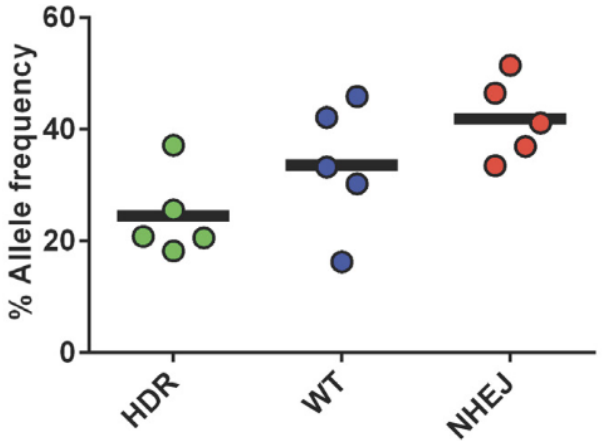
EX VIVO GENOME EDITING

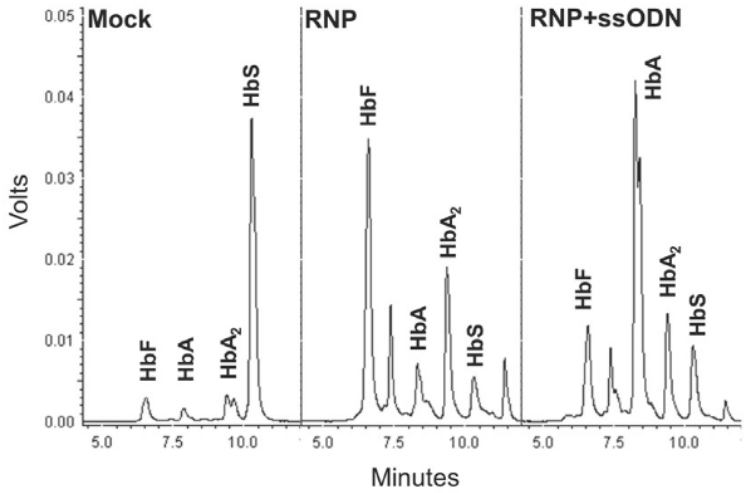
Beta hemoglobinopathies as a disease model

GENOTYPE CORRECTION



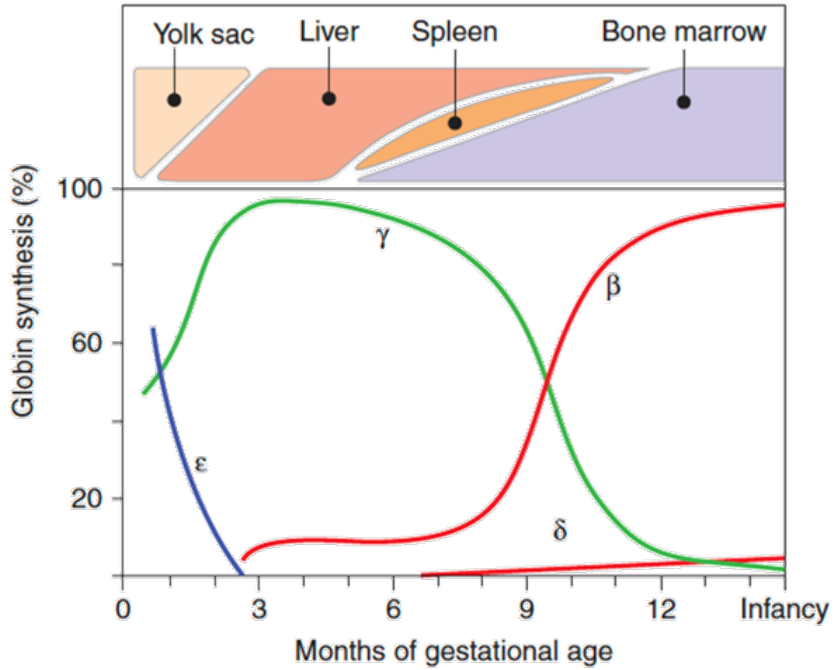
ssODN
AAV
IDLV

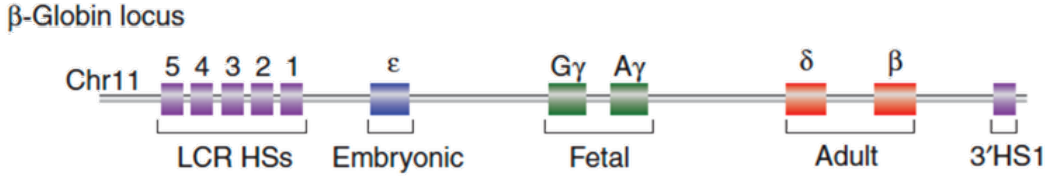




SH. Park et al. Nucleic Acids Research, 2019

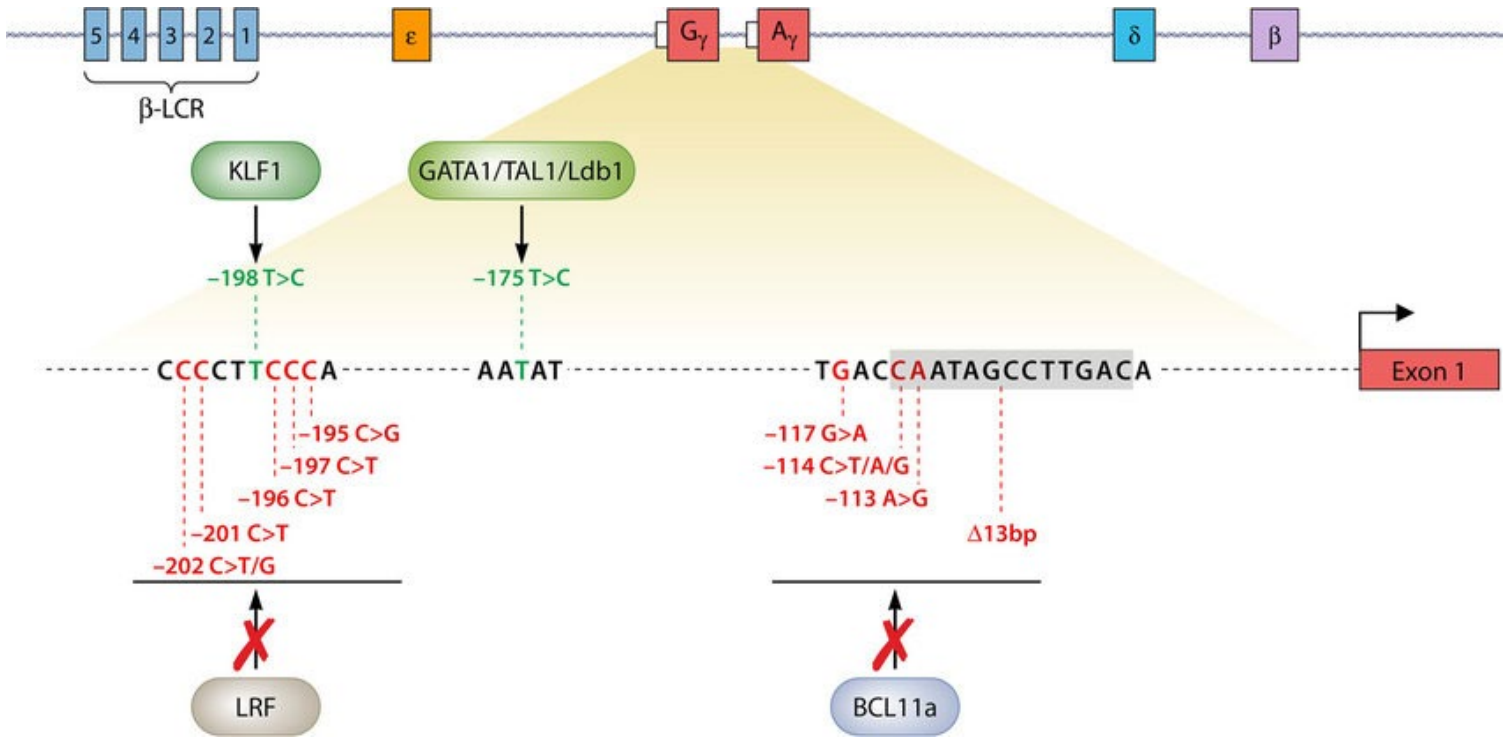
GLOBIN SWITCHING





Manipulating the globin switch to achieve high level expression of fetal hemoglobin

GENOME EDITING APPROACHES TO ACHIEVE AN HPFH PHENOTYPE

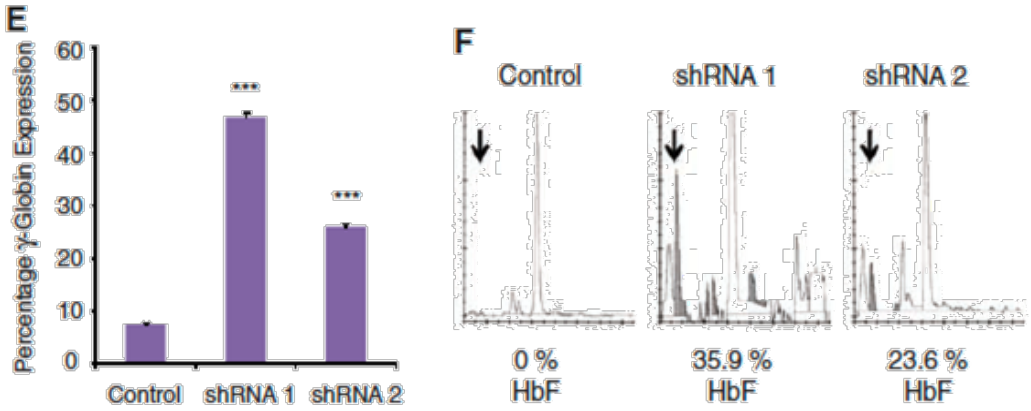


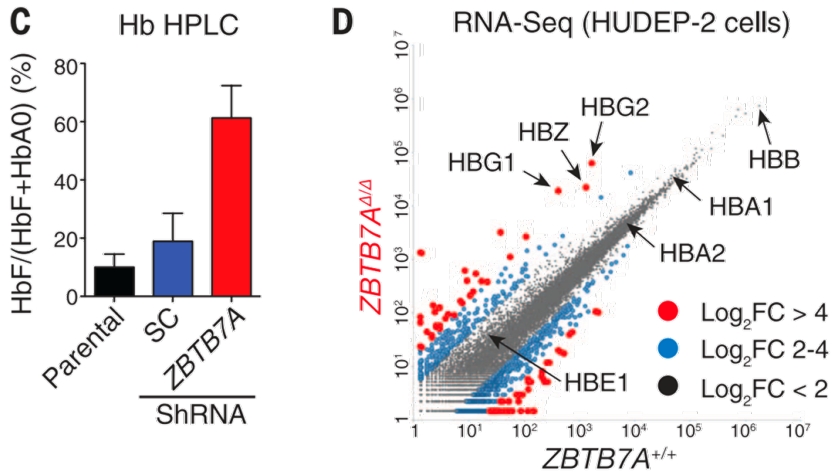
HBF REGULATORS

BCL11A

Gene KO in HSCs:
not applicable

LRF/ZBTB7A

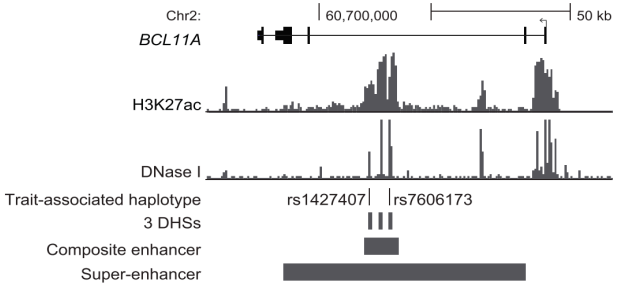


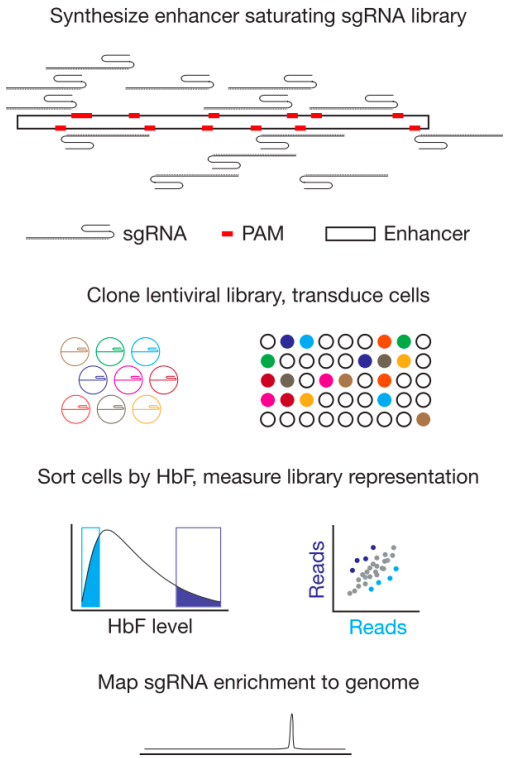


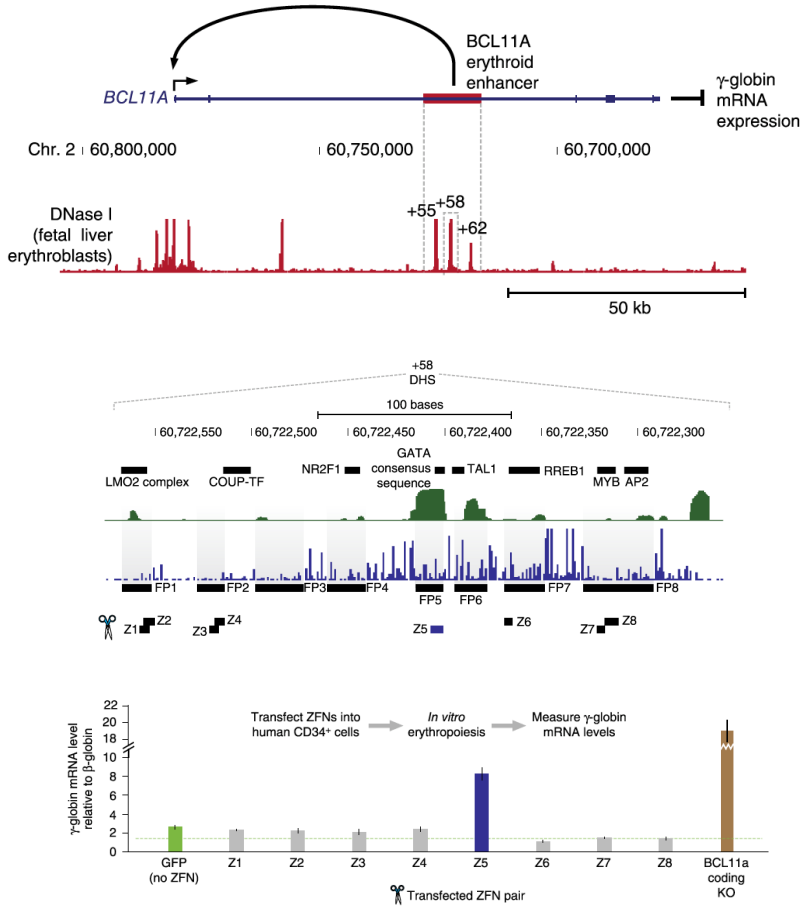
Sankaran VG et. al. Science, 2008

Masuda T et. al. Science, 2016

THE ERYTHROID ENHANCER OF BCL11A



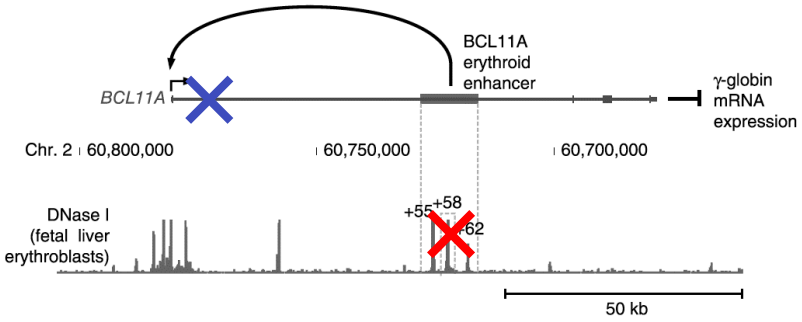


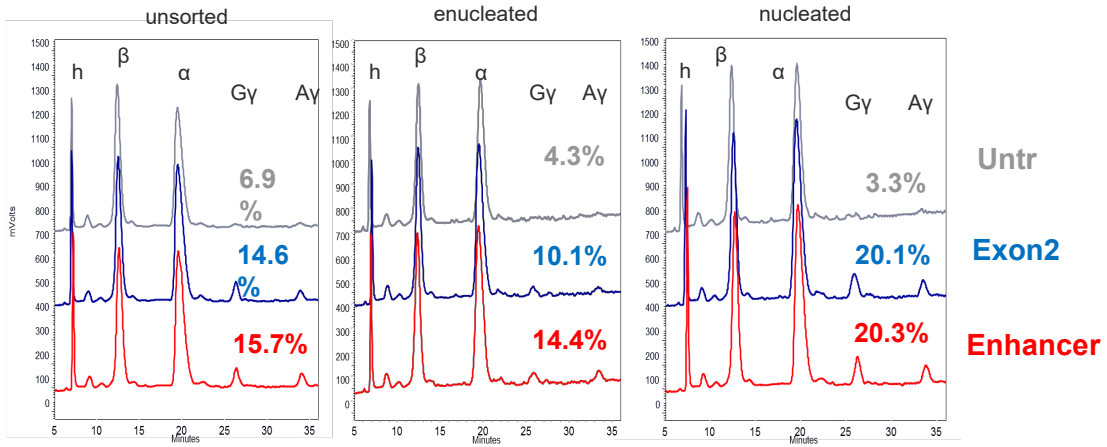


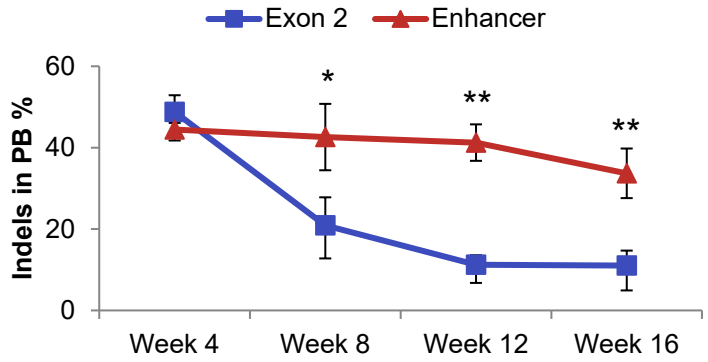
Canver MC. et al, Nature 2015

Vierstra J. et al, Nat. Methods 2015

ZFN BCL11A CODING VS ENHANCER KO

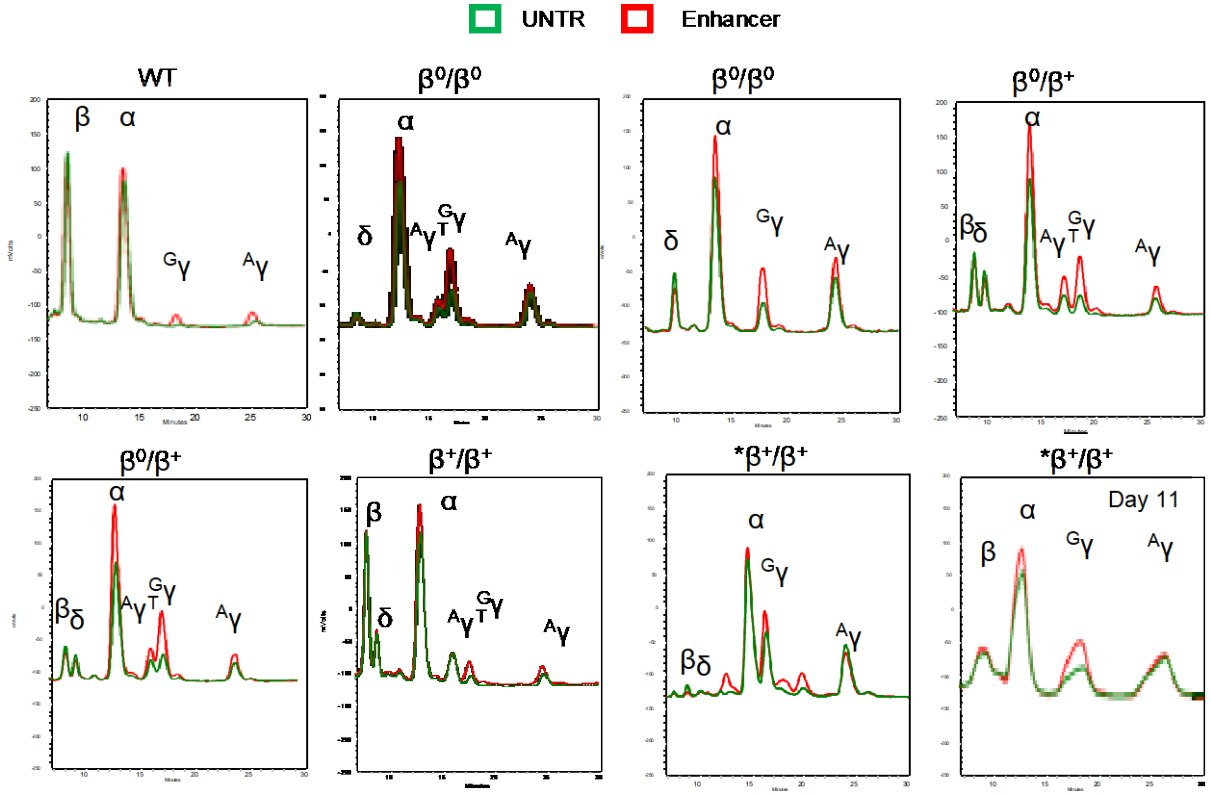


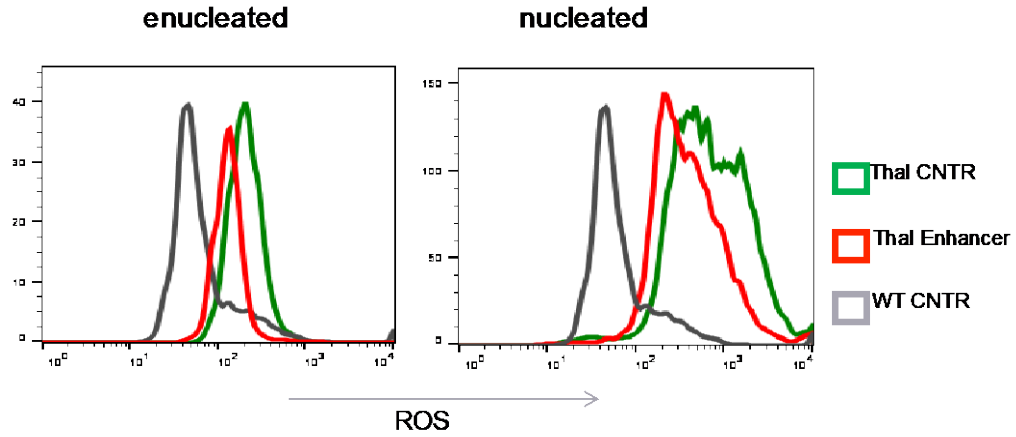


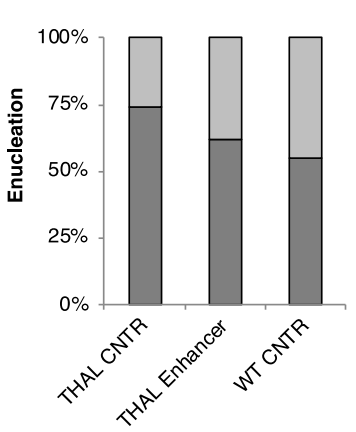


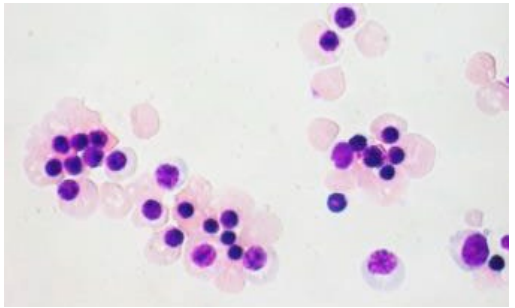
Psatha et al., Mol Ther Methods Clin Dev. 2018

EFFECT OF eBCL11A KO IN THALASSEMIC ERYTHROID CELLS

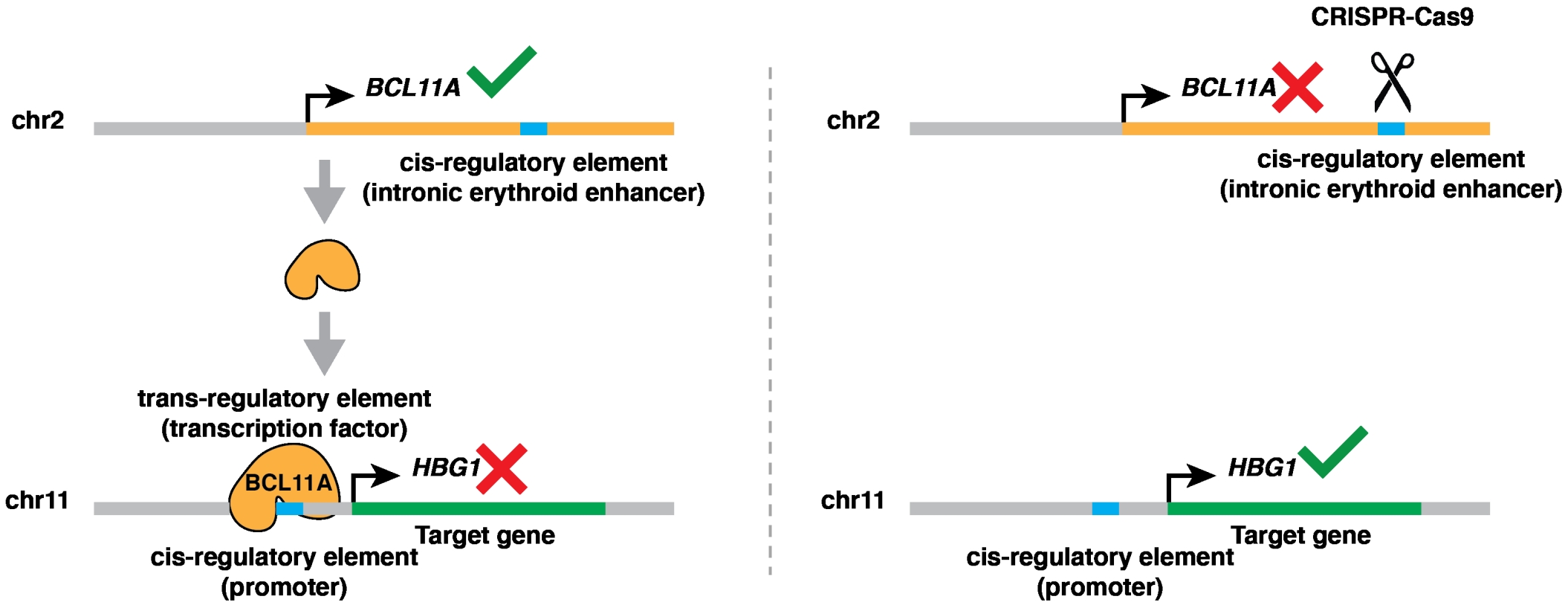








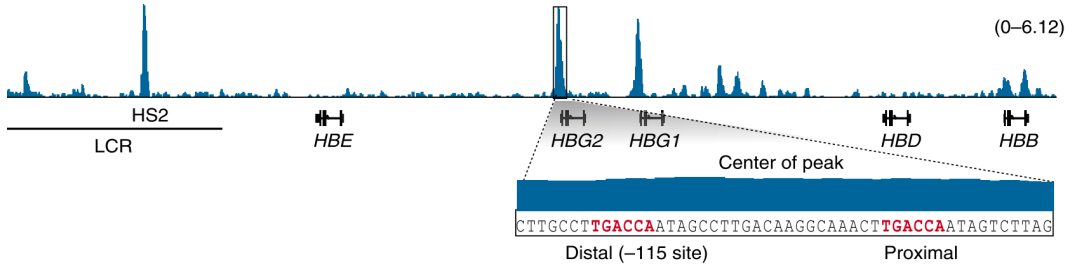
Psatha et al., Mol Ther Methods Clin Dev. 2018


chr2
BCL11A
cis-regulatory element
(intronic erythroid enhancer)
trans-regulatory element
(transcription factor)
BCL11A
chr11
HBG1
Target gene
cis-regulatory element
(promoter)
CRISPR-Cas9
chr2
BCL11A
cis-regulatory element
(intronic erythroid enhancer)
chr11
HBG1
Target gene
cis-regulatory element
(promoter)

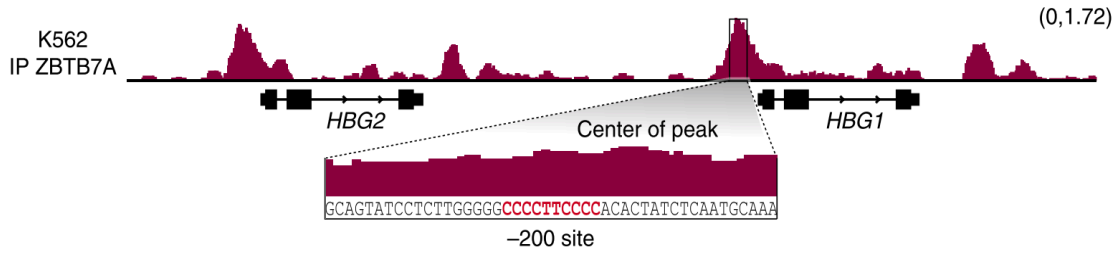
GAMMA GLOBIN PROMOTER: ANOTHER ERYTHROID TARGET

- Introduction of HPFH mutations
- Disruption of TF-binding sites

Chip-Seq for BCL11A

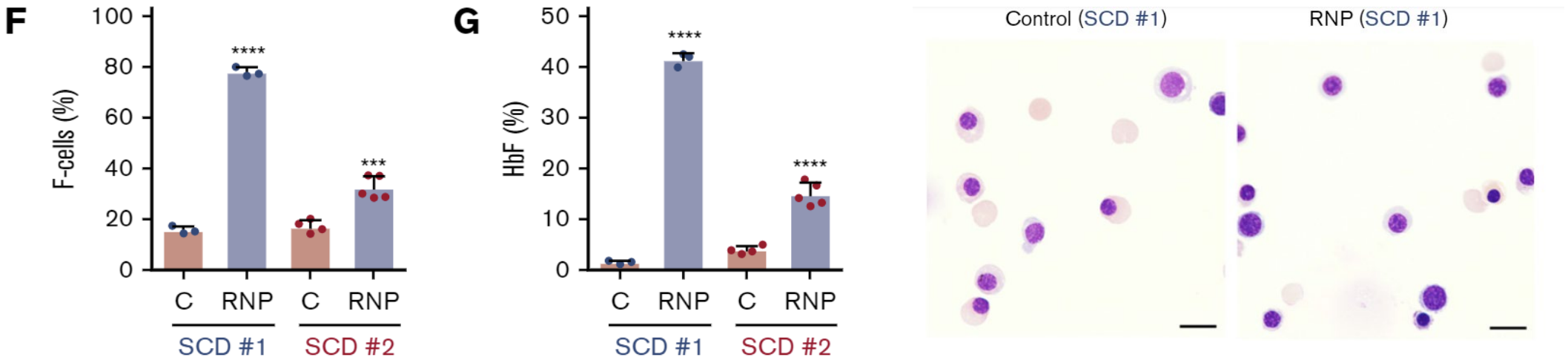


Chip-Seq for LRF/ZBTB7a



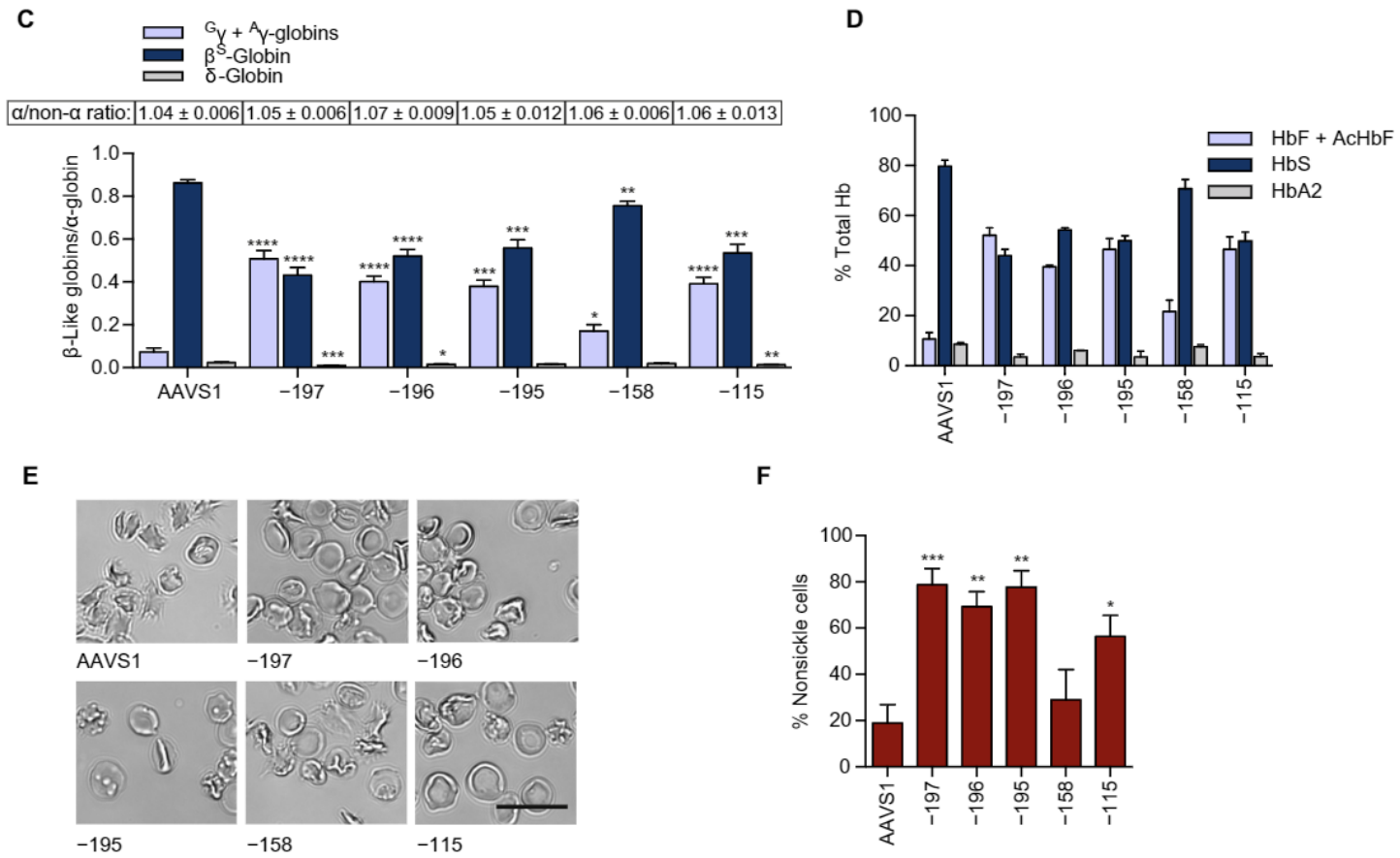
Martyn G.E. et al, Nat. Genetics 2018
Liu N. et al, Cell 2018

HBG-115 (BCL11A BS) EDITING IN SDC ERYTHROID CELLS



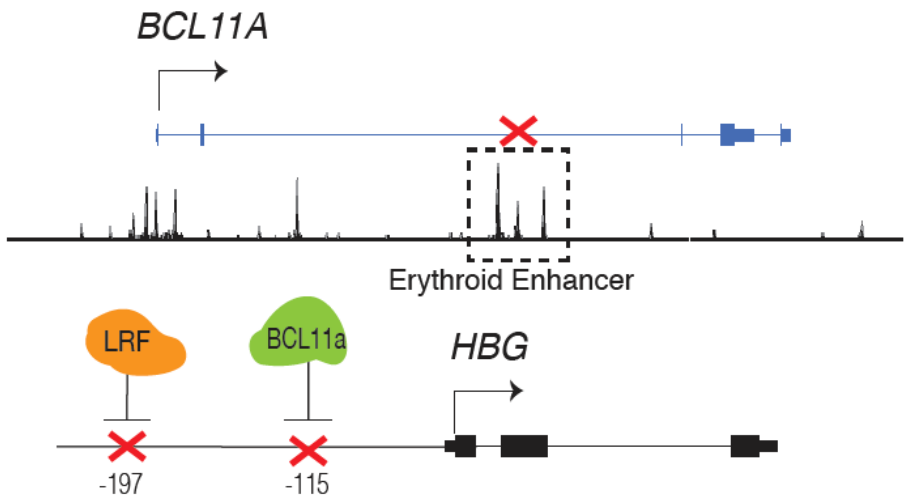
J.-Y.M., P.A.D., T.M et al. Blood Advances 2019

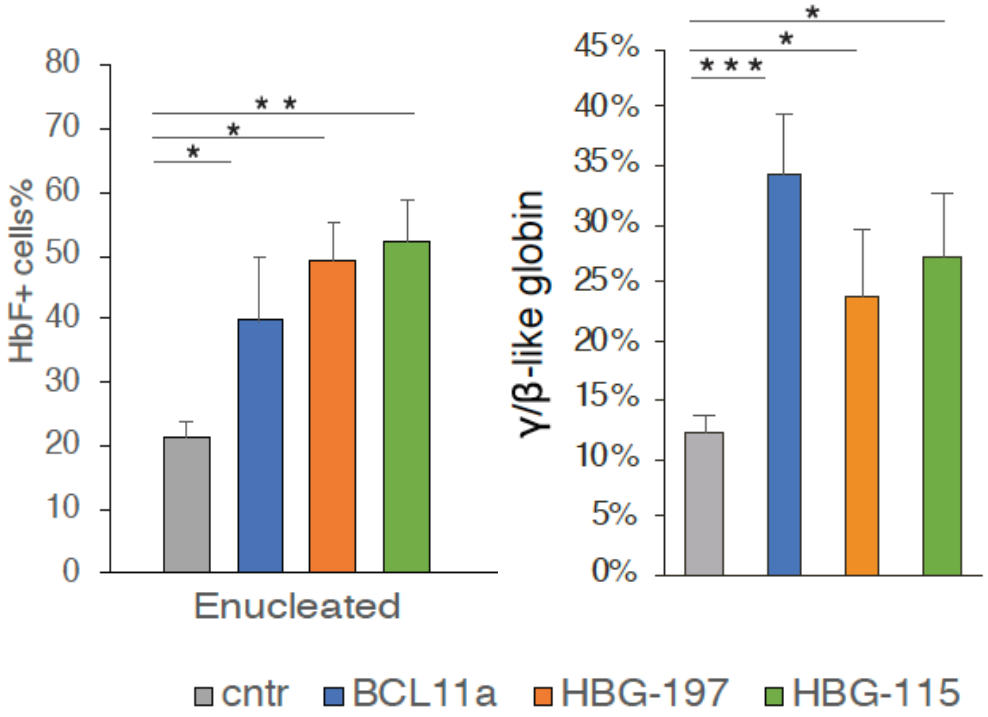
HBG-196 (LRF BS) EDITING IN SDC ERYTHROID CELLS



Weber et al., Sci. Adv. 2020

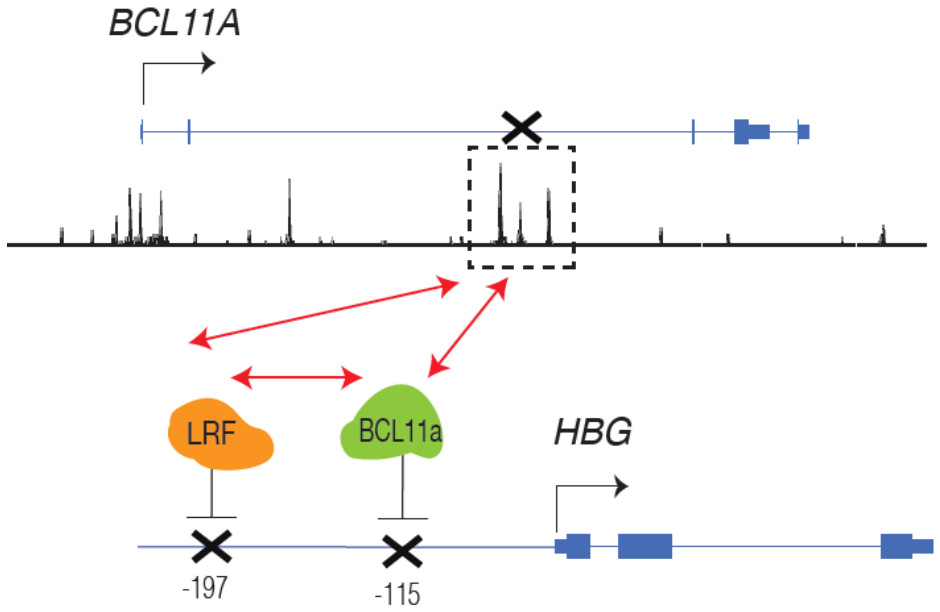
COMPARISON OF THE CLINICALLY APPLICABLE MUTATIONS

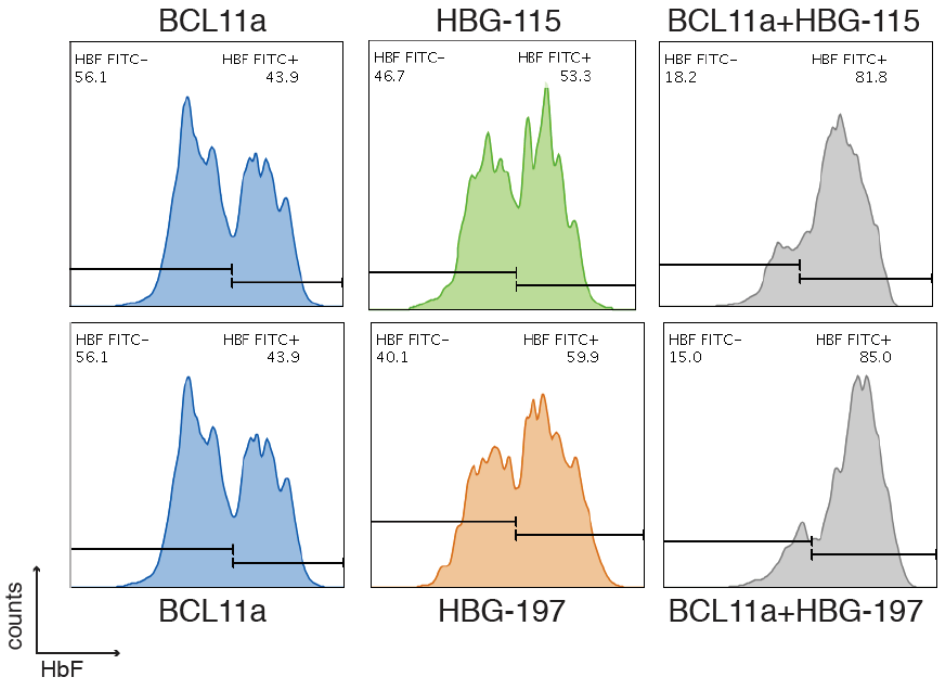




Psatha, Georgakopoulou et al. Blood, 2021

MULTIPLEX MUTAGENESIS IN HUMAN HSCs





Psatha, Georgakopoulou et al. Blood, 2021

Genome Editing Clinical Trials in the Hemoglobinopathies with IND Applications Received by the U.S. FDA

Indication	Goal	Nuclease/target	Sponsor, collaborator	Clinical trial ID, reference	# Subjects dosed	Notes, references
SCD	Elevate HbF	Cas9/BCL11A enhancer	Vertex Pharmaceuticals, CRISPR Therapeutics	NCT03745287	4	[19]
TDT	Elevate HbF	Cas9/BCL11A enhancer	Vertex Pharmaceuticals, CRISPR Therapeutics	NCT03655678	6	[19]
SCD	Elevate HbF	ZFN/BCL11A enhancer	Sangamo Therapeutics, Sanofi	NCT03653247	—	[20,38,39]
TDT	Elevate HbF	ZFN/BCL11A enhancer	Sangamo Therapeutics, Sanofi	NCT03432364	4	[20,38,39]
SCD	Elevate HbF	Cas9/HBG1/2 promoter	Editas Medicine	—	—	IND submitted 12/9/2020
TDT	Elevate HbF	Cas9/HBG1/2 promoter		—	—	Guided to IND submission in 2021
SCD	Elevate HbF	Cas9/not disclosed	Intellia Therapeutics, Novartis	—	—	Novartis has not disclosed precise strategy
TDT	Elevate HbF	Cas9/not disclosed	Intellia Therapeutics, Novartis	—	—	Novartis has not disclosed precise strategy
SCD	Repair HbS mutation	Cas9 HBB correction	Graphite Bio	—	—	Developed and taken to IND by M. Porteus (Stanford) and then transferred to Graphite[36]
SCD	Repair HbS mutation	Cas9 HBB correction	UCSF Benioffs, UCLA, IGI	—	—	Developed at the IGI, UCSF, and UCLA, [37] taken to IND Nov 2020 by same team

PHARMA

Vertex, CRISPR's gene-editing therapy Casgevy wins early FDA nod to treat beta thalassemia

By **Kevin Dunleavy** · Jan 16, 2024 3:40pm

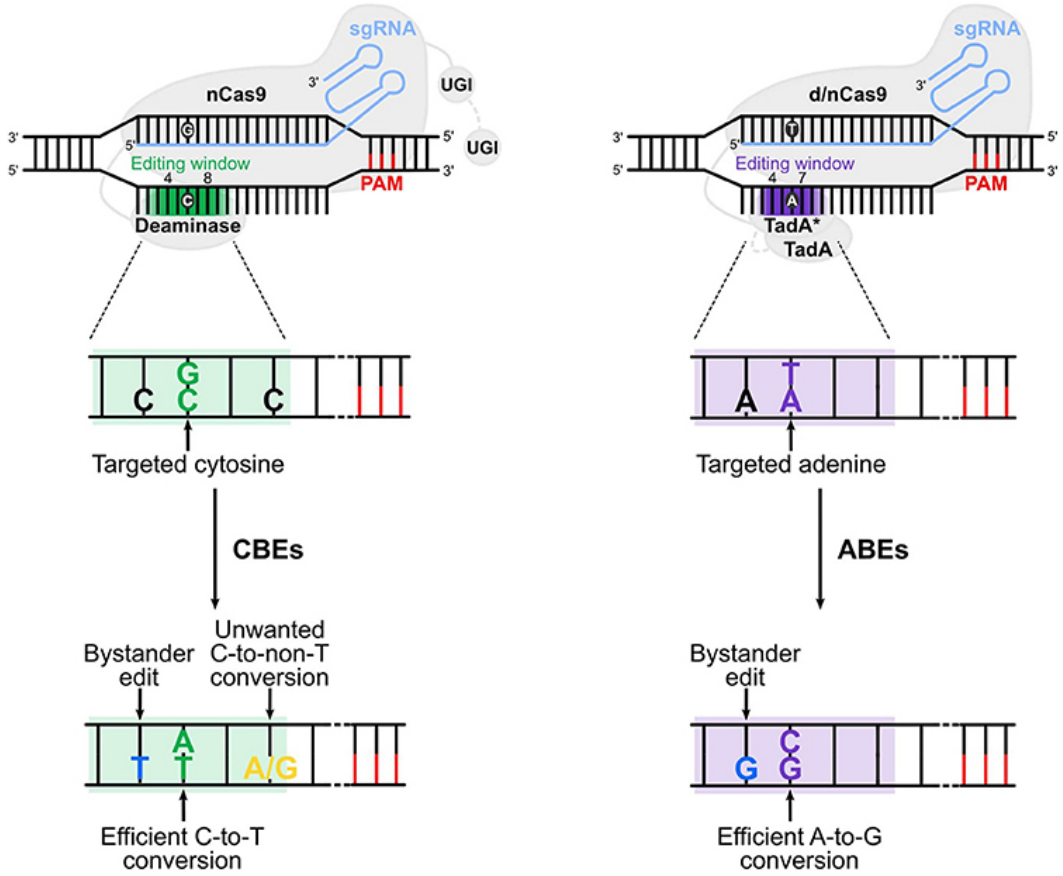
CRISPR | Vertex Pharmaceuticals | FDA | Casgevy (exa-cel)

Vertex-CRISPR's Casgevy Gets Positive EMA Panel Opinion, Approval Decision in Q1 2024

Published: Dec 15, 2023 | By Kate Goodwin

BASE EDITING

precise point mutations without DSBs



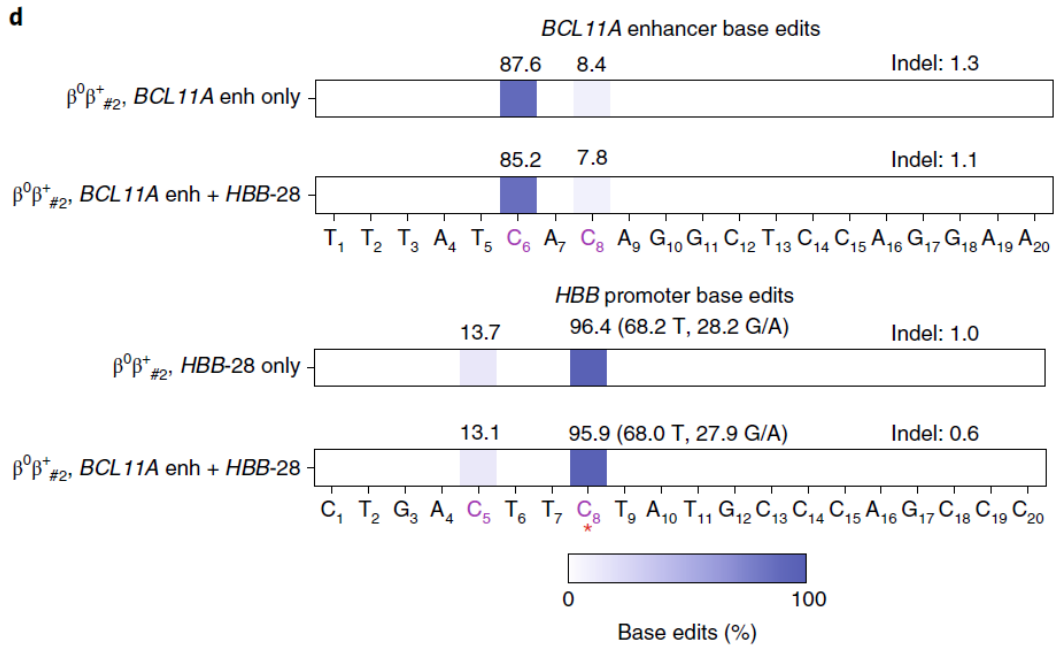
Cytosine base editors (CBEs):
nCas9 +deaminase +UGI= C:G → T:A

Adenine base editors (ABEs):
d/nCas9 +2xTadA, = A:T → G:C

Reviewed by Antoniou et al. Front. Genome Ed.,2021

CBE TO CORRECT BETA-THALASSEMIA

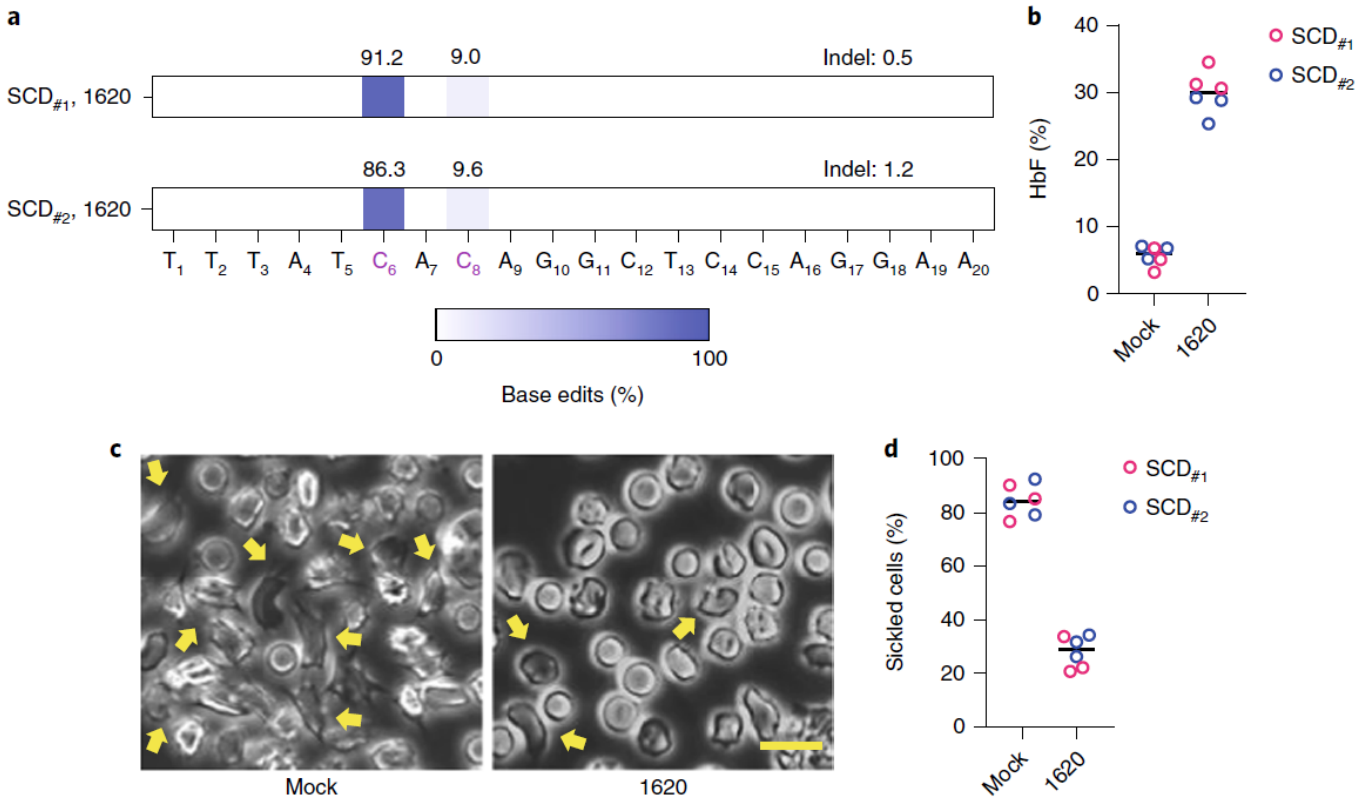
the HBB-28 mutation +eBCL11a



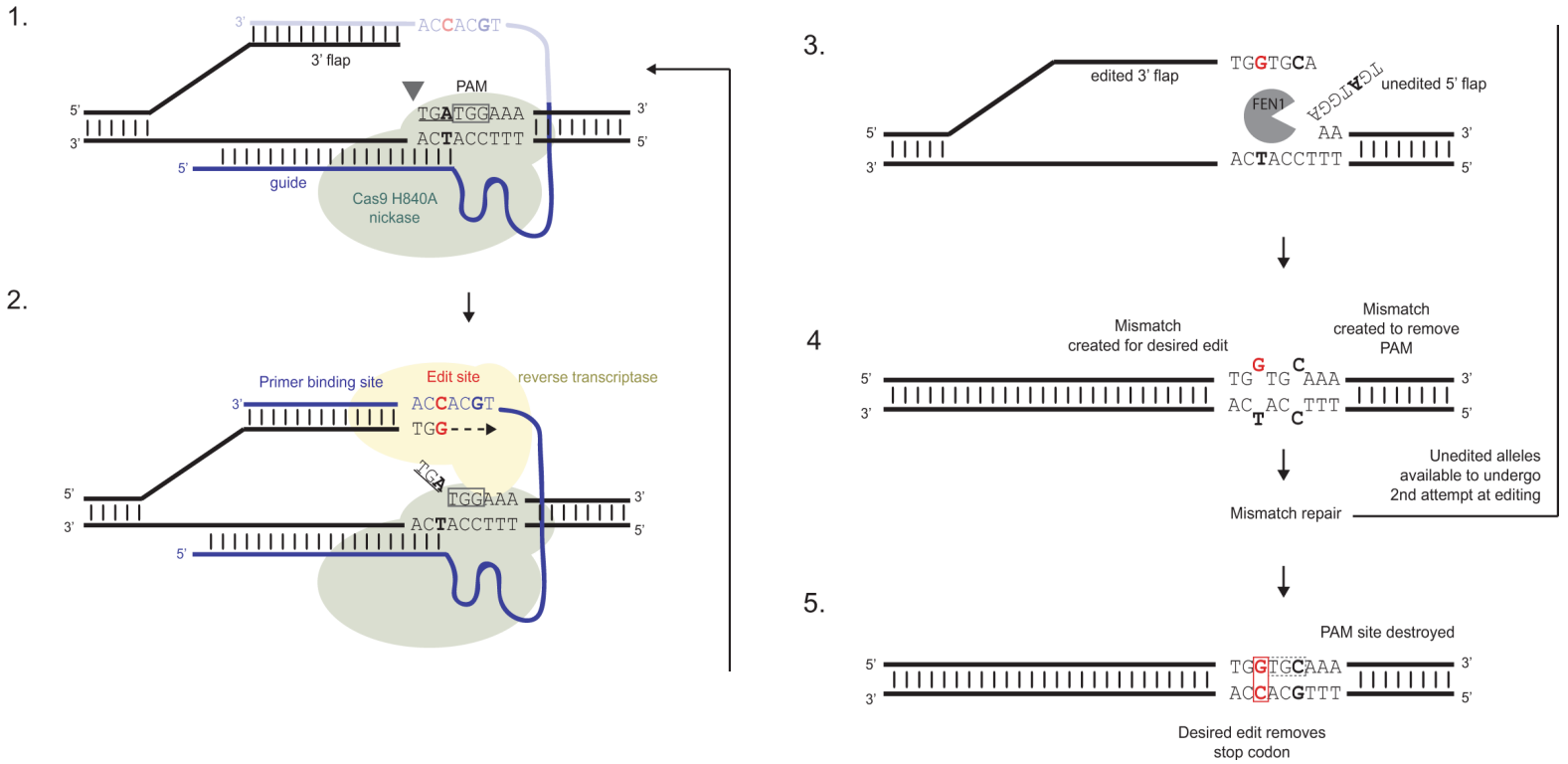
Zeng, J.. et al. Nat Med 2020

CBE TO CORRECT SCD

A3A (N57Q)-BE3



PRIME EDITING SEARCH-AND-REPLACE GENOME EDITING



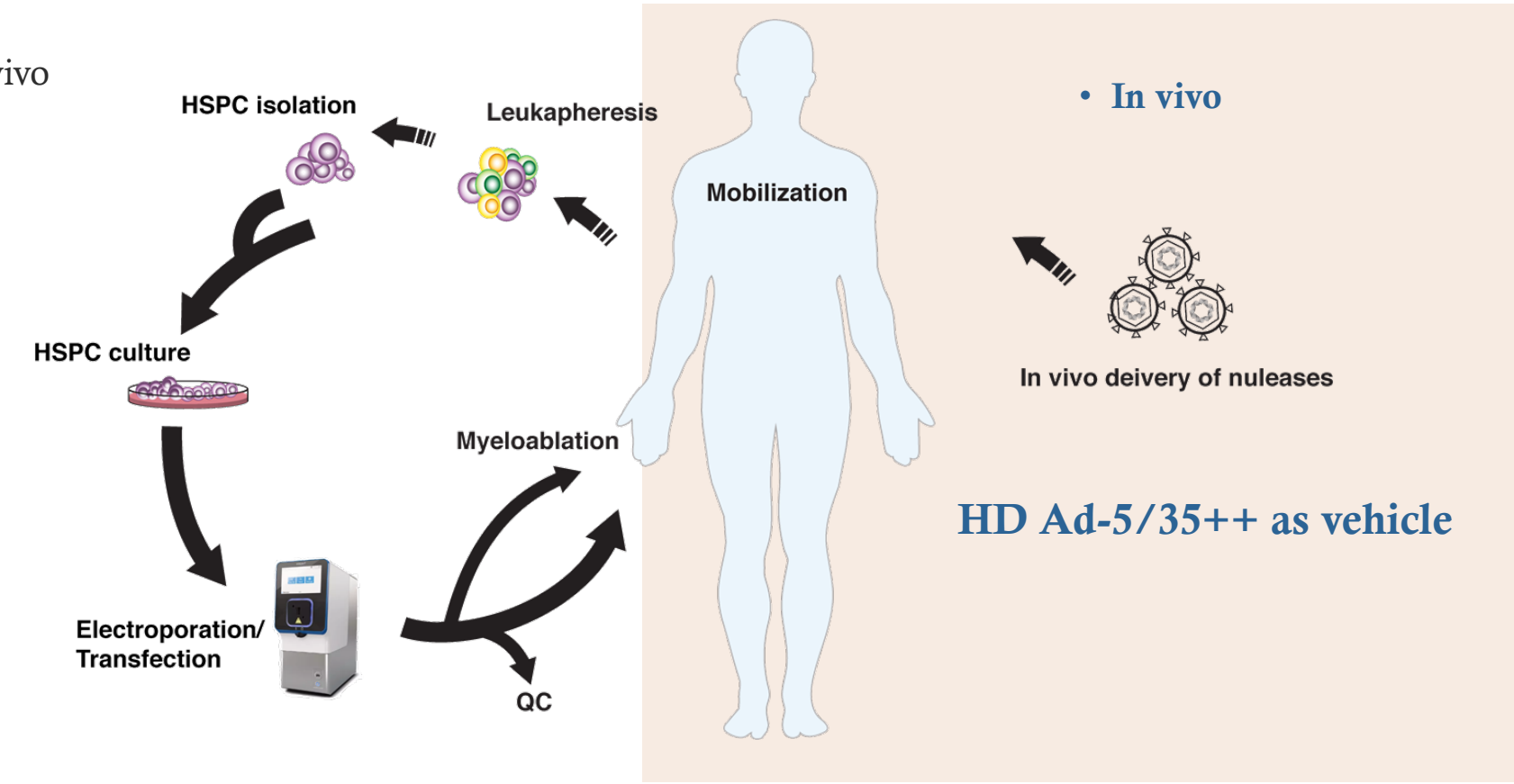
- All 12 types of point mutations
- Insertions (of up to 44 bp)
- Deletions (of up to 80 bp)
- Combination of modifications

Reviewed by J. Scholefield & P.T. Harrison Gene Therapy 2021

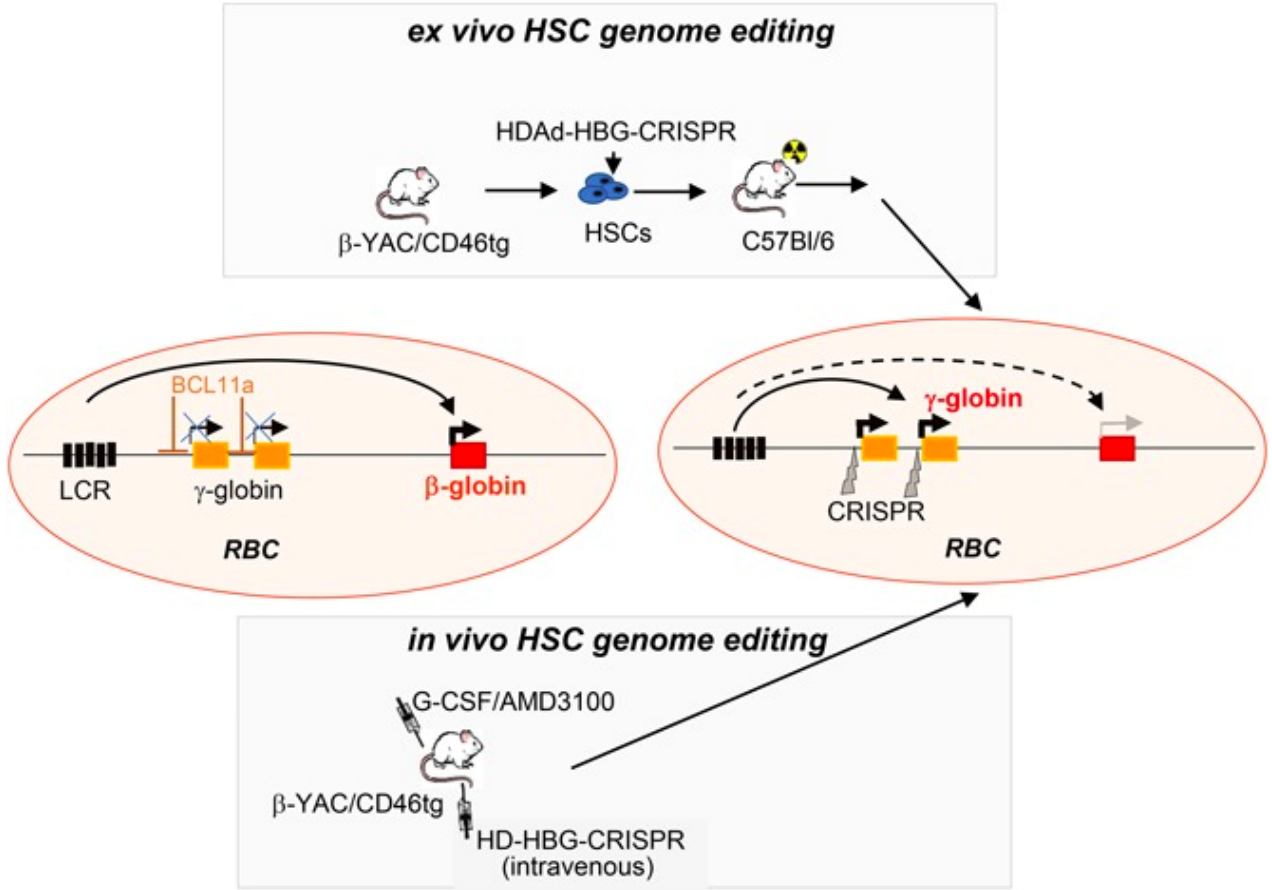
IN VIVO GENOME EDITING

IN VIVO MODIFICATION OF HSCs

- Ex vivo



IN VIVO HSC GENOME EDITING IN β-YAC MICE



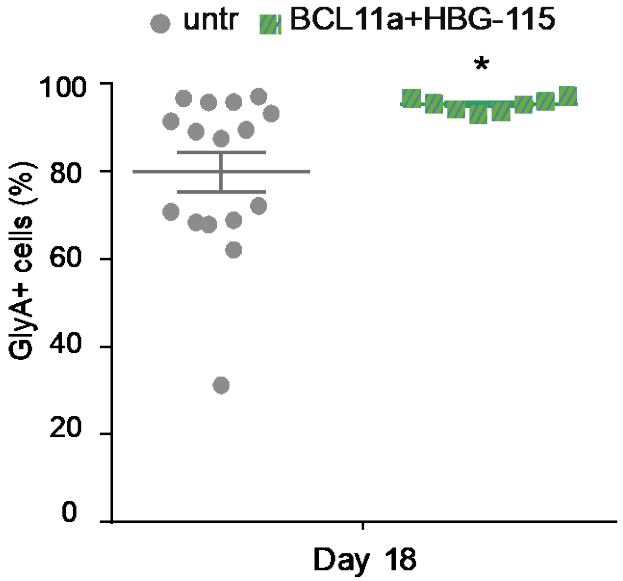
Li C. & Psatha N. et al, Blood 2018

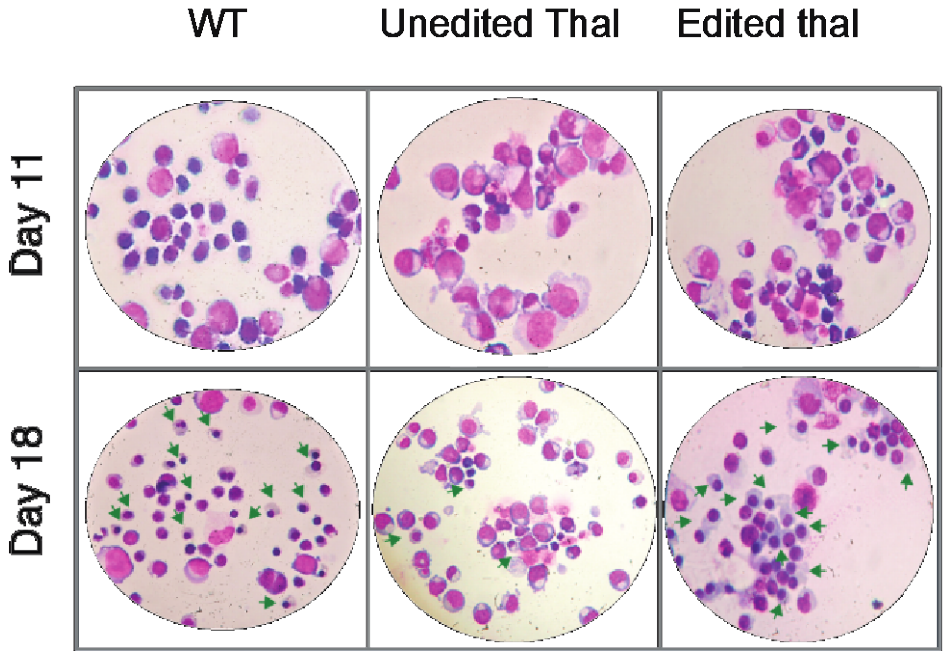
A NOVEL HD AD-5/35++ VECTOR FOR MULTIPLEX MUTAGENESIS OF HUMAN HSCs

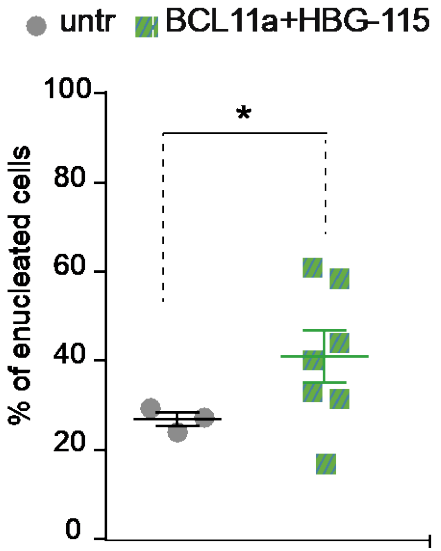
Patient	Genotype	Phenotype	Mobilization scheme
P01	CD39/IVSI-110	β^0/β^+	G-CSF+Plerixafor
P02	CD39/CD39	β^0/β^0	G-CSF
P03	IVSI-110/IVSI-110	$\beta^{+/}\beta^+$	Plerixafor

Psatha et al. Blood, 2021

IMPROVED ERYTHROPOIESIS AFTER DOUBLE EDITING

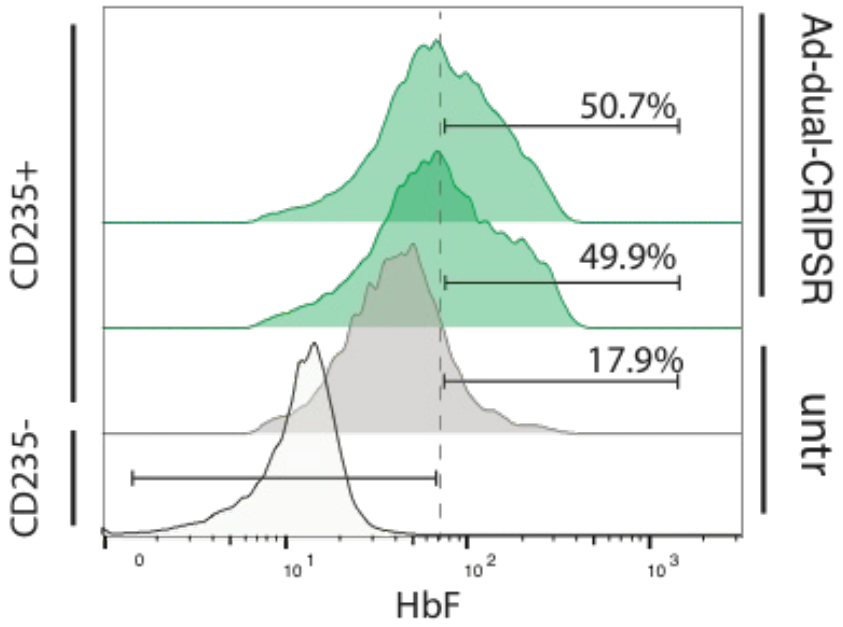


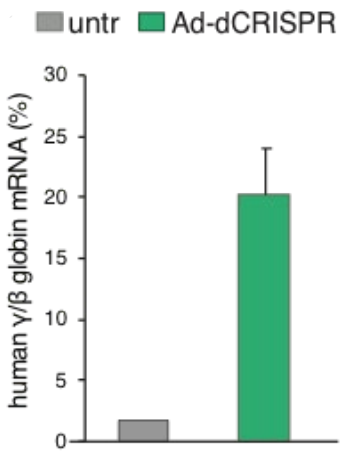


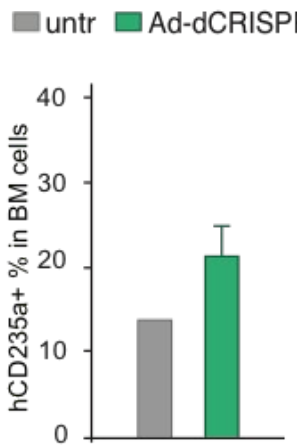


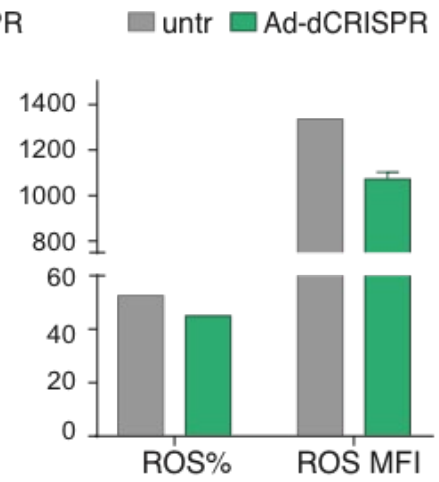
Psatha, Georgakopoulou et al. Blood, 2021

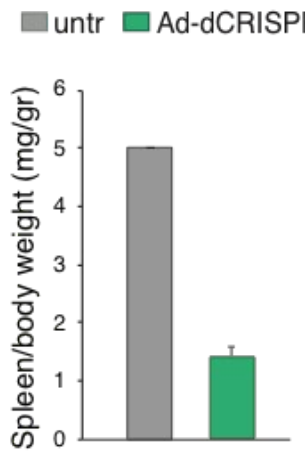
PHENOTYPE CORRECTION





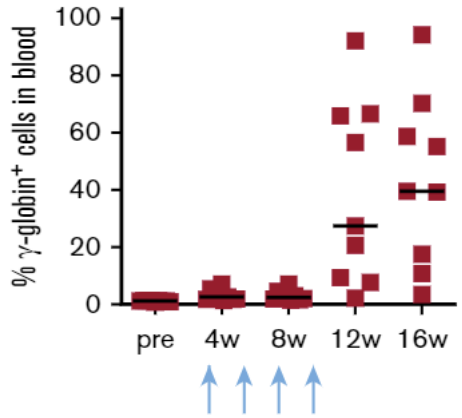


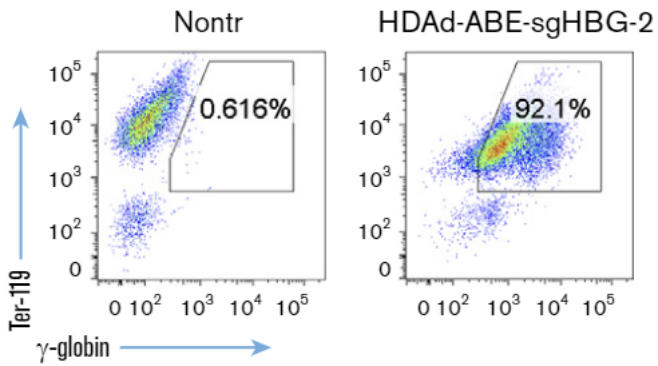


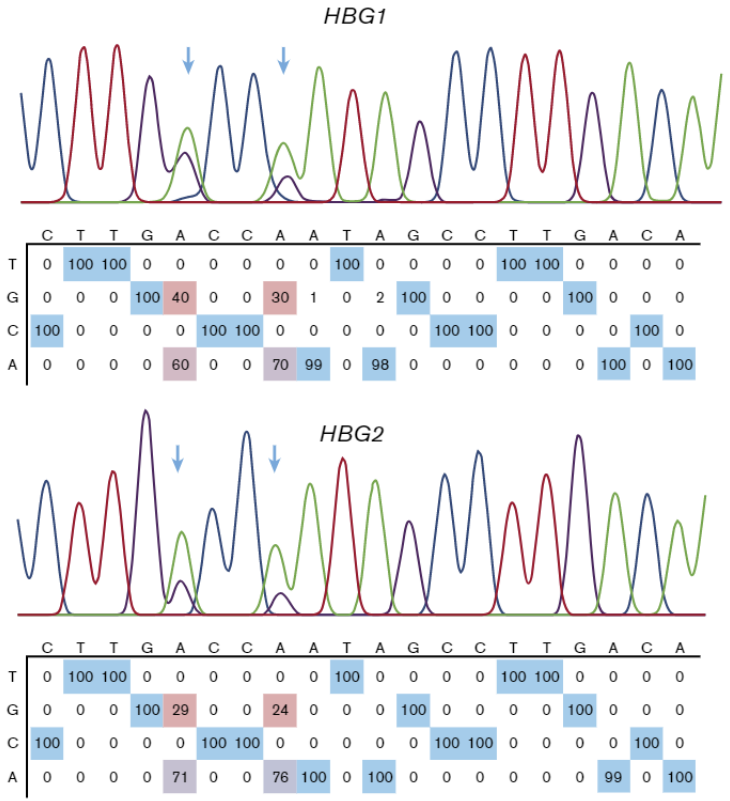


Psatha, Georgakopoulou et al. Blood, 2021

HDAD5/35++ MEDIATED IN VIVO BASE EDITING







HBG-113

Li et al. Blood Advances, 2021

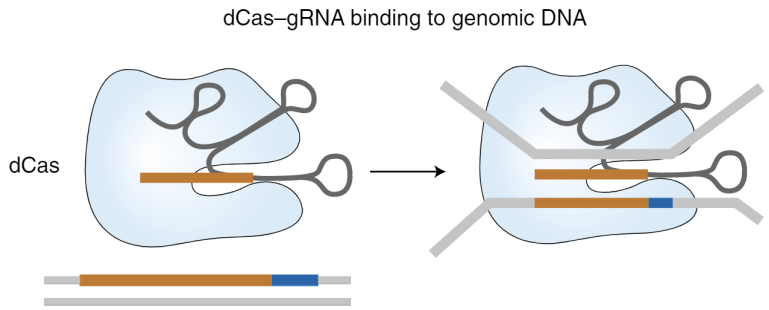
GENOME EDITING RISKS AND DRAWBACKS

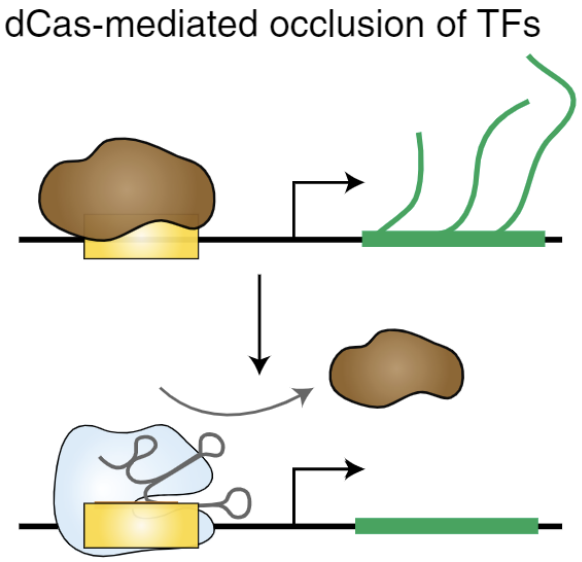
- Unintended edits or “off-target” effects
- Introduction of unwanted mutations
- Chromosomal instability and genomic translocations
- DSB mediated genotoxicity

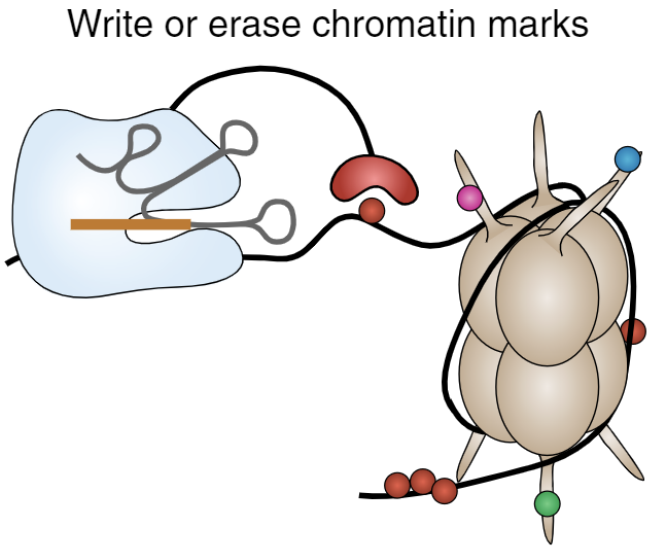
EPIGENOME EDITING

Editing beyond the genome

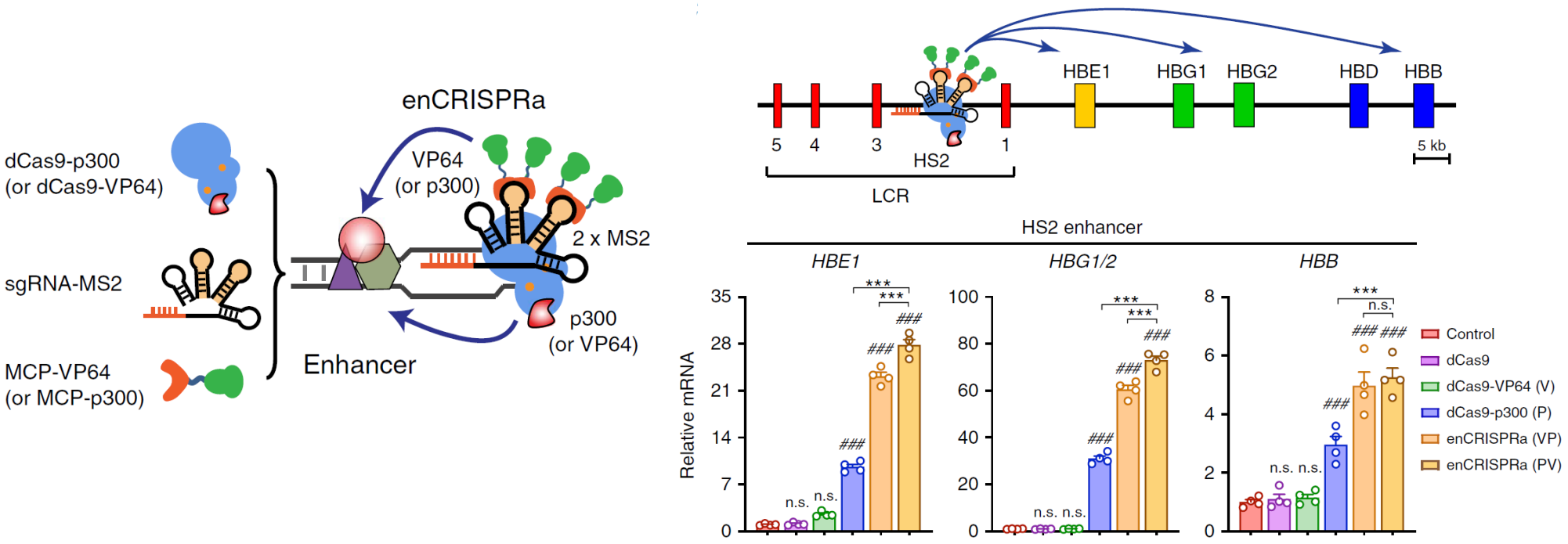
EPIGENOME EDITING





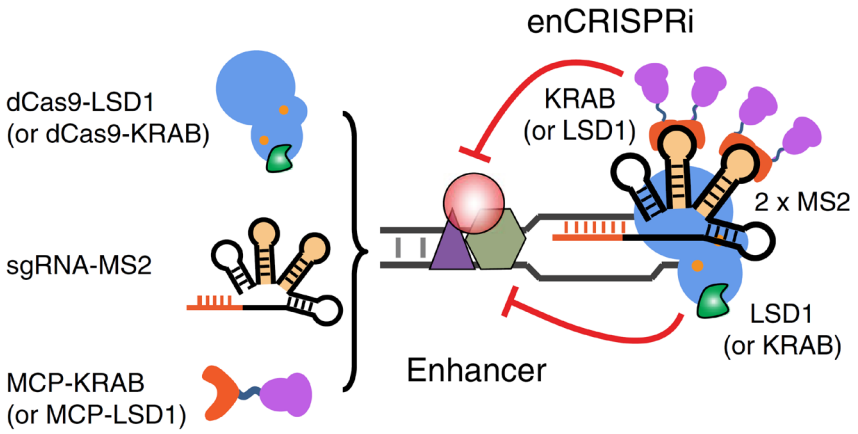


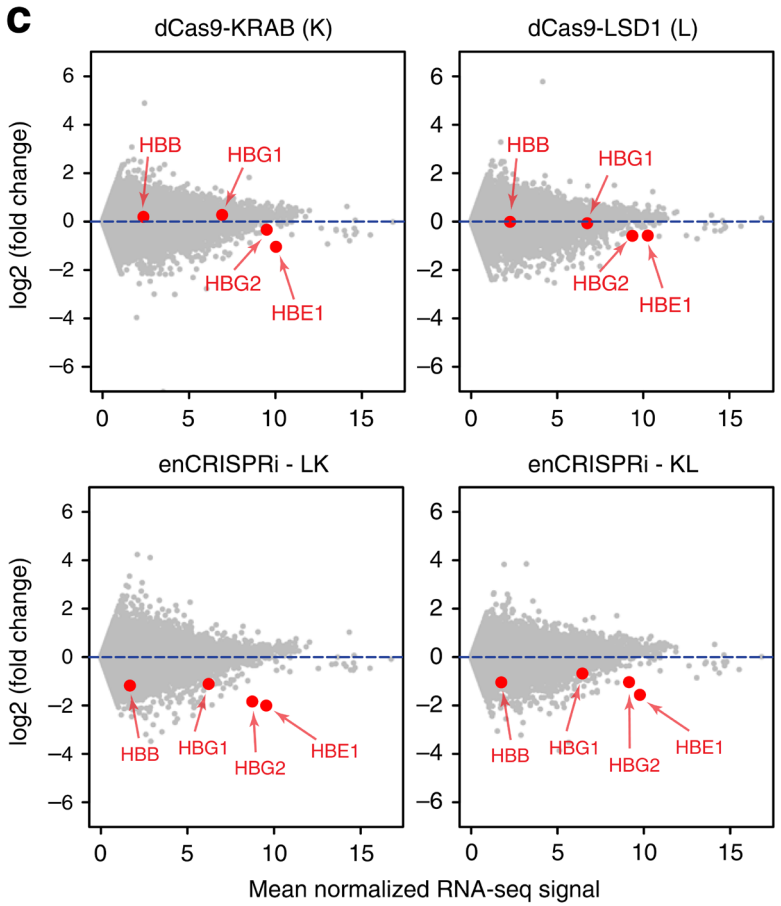
EPIGENOME EDITING TARGETING THE BETA GLOBIN LOCUS



K. Li et al, Nat Commun, 2020

EPIGENOME EDITING TARGETING THE BETA GLOBIN LOCUS





K. Li et al, Nat Commun, 2020

COST OF GT

If you google “most expensive drugs”…

10. Luxturna

- Cost: $$850,000 per one-time dose
- Manufacturer: Spark Therapeutics
- Use: Biallelic RPE65-Mediated Inherited Retinal Disease
- FDA Approval Date: December 19, 2017

5. Zolgensma

- Cost: $2.1 million per one-time dose
- Manufacturer: Novartis
- Use: Spinal Muscular Atrophy
- FDA Approval Date: May 24, 2019

4. Zynteglo

- Cost: $2.8 million per one-time dose
- Manufacturer: Novartis
- Use: Beta-thalassemia
- FDA Approval Date: September 16, 2022

COST OF GT

If you google “most expensive drugs”…

1. Lenmeldy

- Cost: $4.25 million per one time treatment
- Manufacturer: Orchard Therapeutics
- Use: Metachromatic leukodystrophy (MLD)
- FDA Approval Date: March 18, 2024

2. ~~1.~~ Hemgenix

- Cost: $3.5 million per one-time dose
- Manufacturer: CSL Behring
- Use: Hemophilia B
- FDA Approval Date: November 22, 2022

3. ~~2.~~ Elevidys

- Cost: $3.2 million per one-time dose
- Manufacturer: Sarepta Therapeutics
- Use: Duchenne Muscular Dystrophy (DMD)
- FDA Approval Date: June 22, 2023

4 ~~3.~~ Skysona

- Cost: $3 million per one-time dose
- Manufacturer: bluebird bio, Inc.
- Use: Cerebral Adrenoleukodystrophy (CALD)
- FDA Approval Date: September 16, 2022

Thank you