GENES AND GENE THERAPY

Lucio Luzzatto
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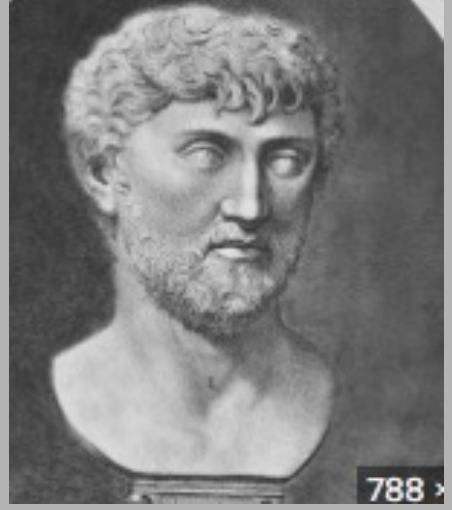
University of Florence, Firenze, ITALY

Symposium:
INNOVATIVE THERAPIES IN HEMATOLOGY
Avellino, 31 marzo 2023

THE DAWN OF CONTEMPORARY GENETICS

	Flower color	Pea shape	Pea color	Pod color	Pod shape	Plant height	Flower position
DOMINANT	Purple	Round	Yellow	Green	Inflated	Tall	Axial
RECESSIVE	White	Wrinkled	Green	Yellow	Constricted	Short	

Gregor Johann **Mendel** (Hynčice, 20 July **1822** – Brno, 6 January 1884)



Ercolano (96 a.C.)-
Roma (53 d.C.)

Poſſon' anc' alle volte a gli Avi loro
Nascer ſimili i figli, e de' Proavi
Rinovar le ſembianze, e ciò ſuccede
Perchè ſpeſſo miſchiati in molti modi
Celano i Genitor molti principj
Nel proprio corpo, che di mano in mano
Dalla ſtirpe diſceſi ; i Padri a' Padri
Danno, e quindi è che Venere produce
Con diverſi fortuna aſpetti varj,
E de' noſtri Antenati i volti imita
I moti i geſti le parole e il pelo:

DI TITO LUCREZIO CARO

DELLA NATURA DELLE COSE

LIBRI SEI.

TRADOTTI

DA ALESSANDRO MARCHETTI

LETTORE DI FILOSOFIA E MATTEMATICHE
NELL' UNIVERSITA' DI PISA
ET
ACCADEMICO DELLA CRUSCA.

PRIMA EDIZIONE.

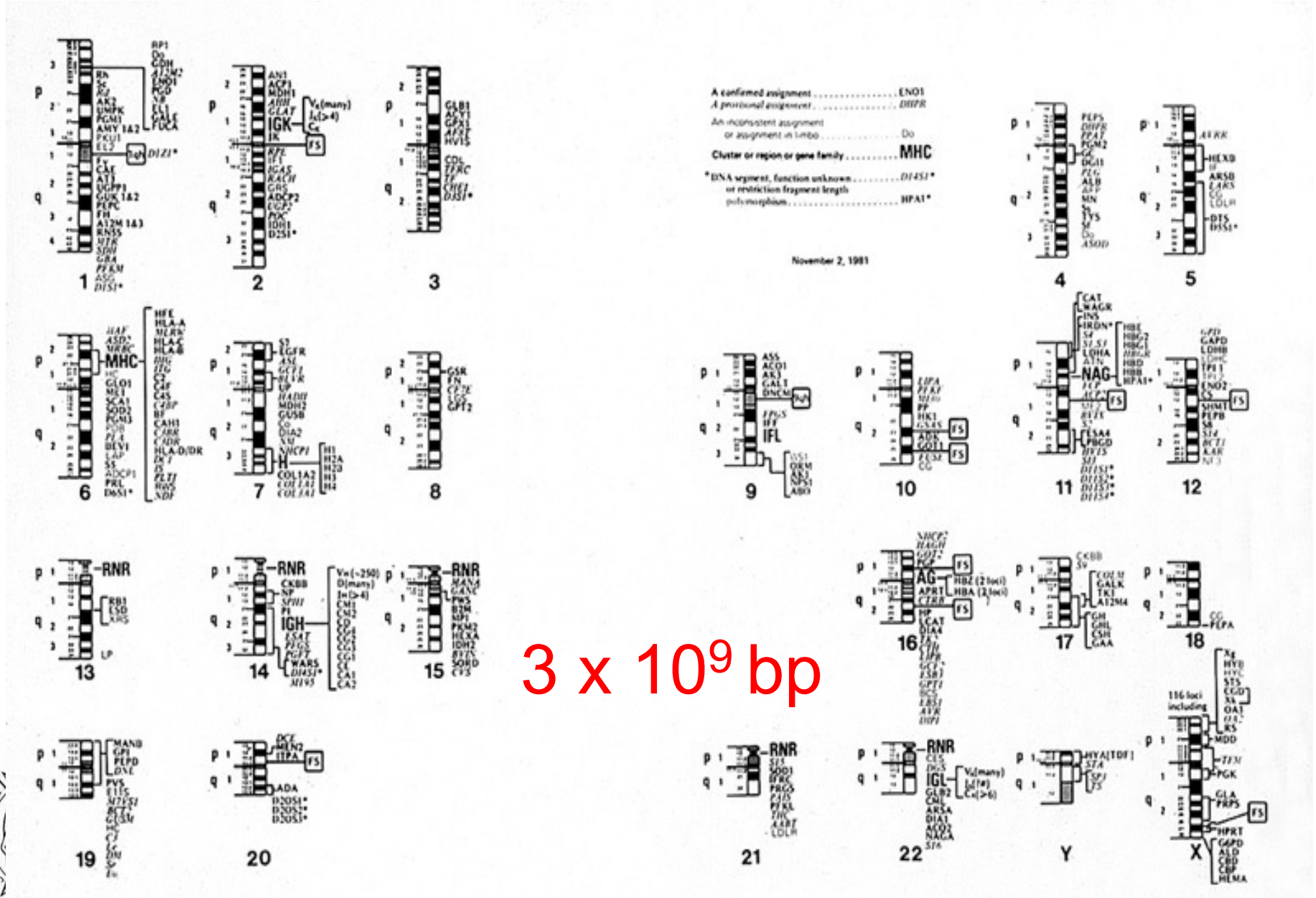
LONDRA.

Per GIOVANNI PICKARD MDCCXVII.

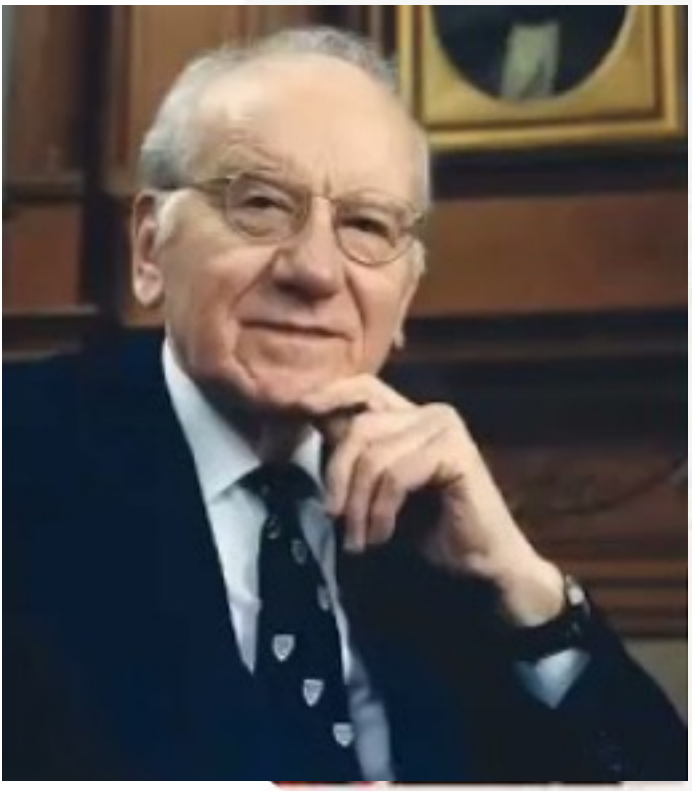
Definition of GENE ca. 1966

- Unit of inheritance
- Unit of mutation
- Functional unit (*gene expression*)

THE HUMAN GENOME



Victor McKusick

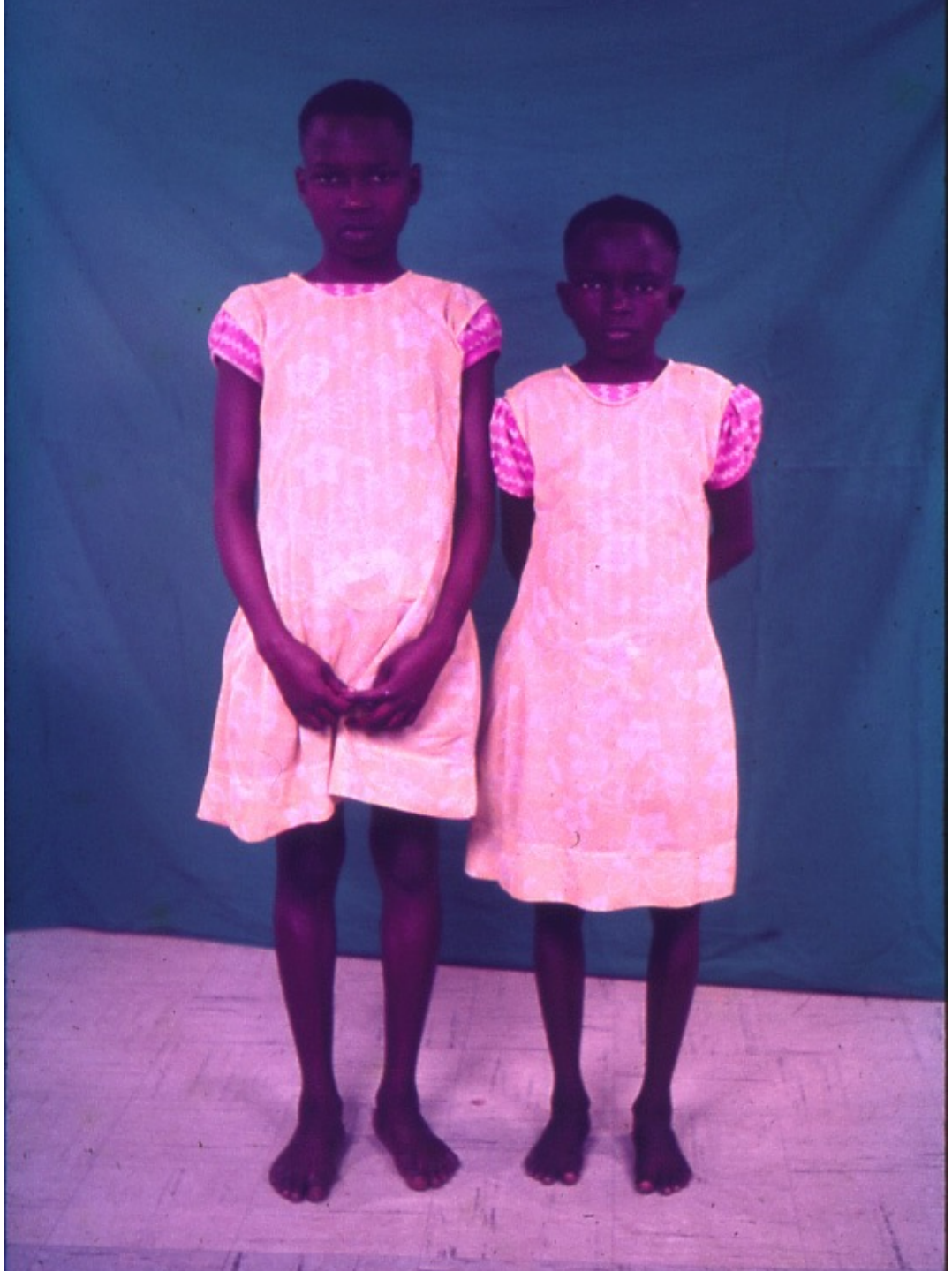


Mendelian Inheritance in Man (MIM)

Twelve print editions of MIM, the first published in 1966 and the most recent published in 1998

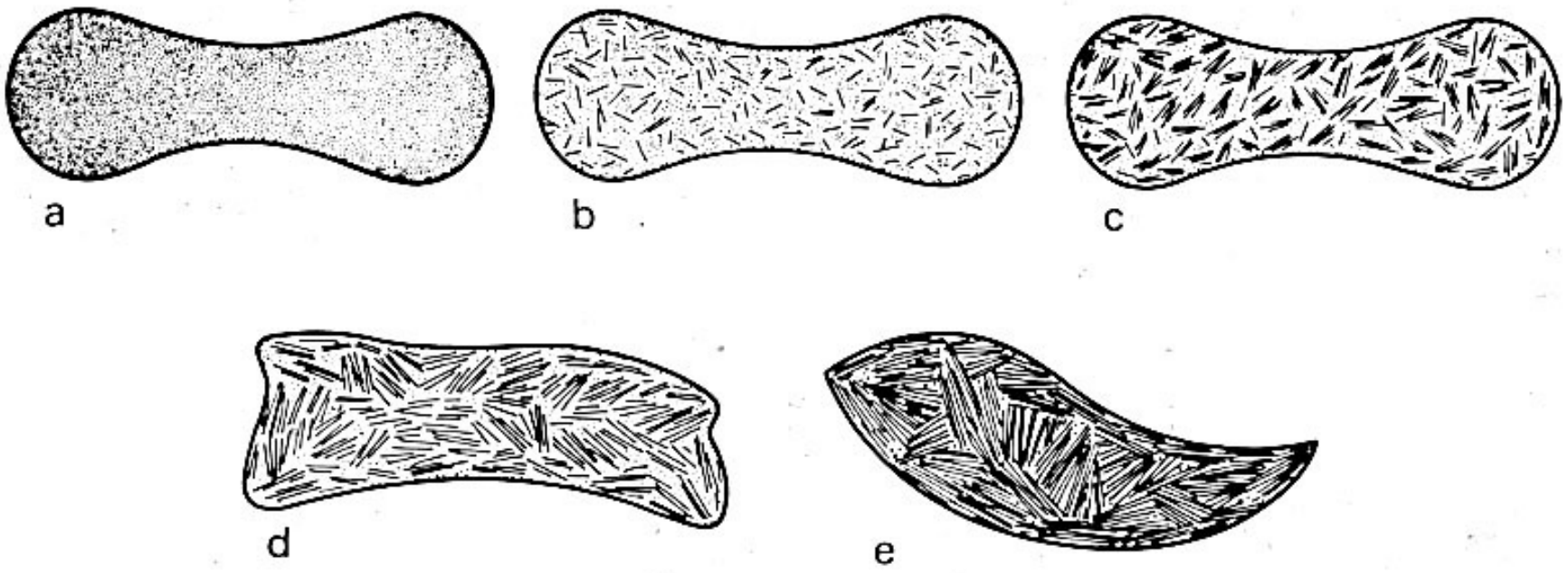
‘Classic” Mendelian Diseases

- *Dominant*: Huntington Disease
- *Recessive:* Cystic Fibrosis
- *X-linked:* Hemophilia

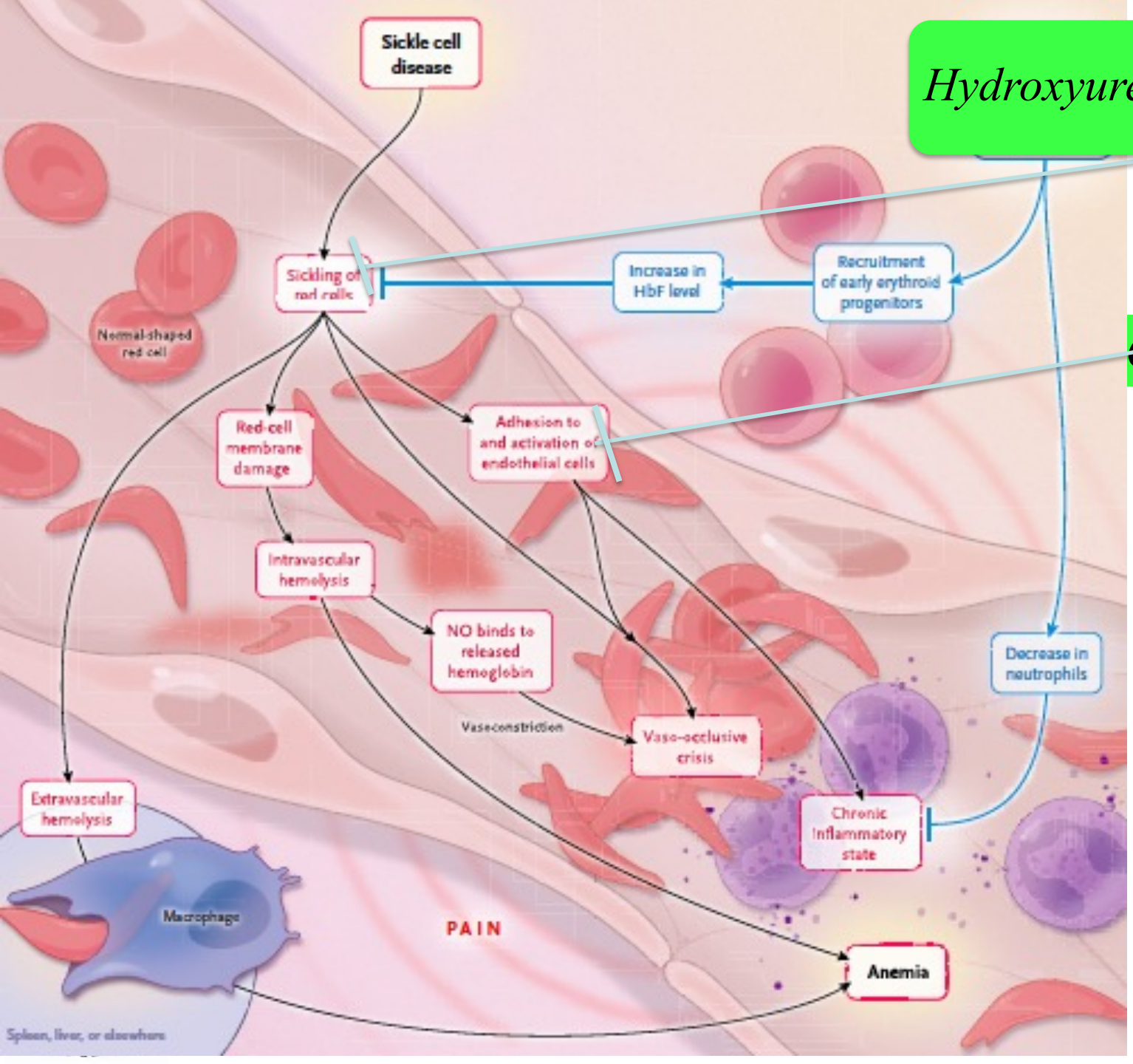


Twins aged 9
Homozygous
sickle cell
anaemia
(Courtesy
of parents
Ibadan, Nigeria)
1974

Polymerization of deoxy-Hb S is a fast reaction; sickling of a red cellis a slower gradual process



From Noguchi and Schechter (1981)



Hydroxyurea

Voxelotor

Crizanlizumab

Modified from Luzzatto & Makani *NEJM* **380**,187, 2019)

DIFFERENT POINTS OF VIEW ON MENDELIAN INHERITANCE

Genotype	*'Sickling test'*	*Hb electro-phoresis*	*Clinical picture*
HBB/HBB (AA)	Negative	A	Normal
HBB^{E6V}/HBB (AS)	**Positive**	A + S	Normal
HBB^{E6V}/HBB^{E6V} (SS)	**Positive**	S	**Severe haemolytic anaemia**

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Classification of HBB^{E6V} mutant gene			

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Classification of HBB^{E6V} mutant gene	**Dominant**		

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Classification of HBB^{E6V} mutant gene	**Dominant**	Co-dominant	

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Classification of HBB^{E6V} mutant gene	**Dominant**	Co-dominant	**Recessive**

DIFFERENT POINTS OF VIEW ON MENDELIAN INHERITANCE

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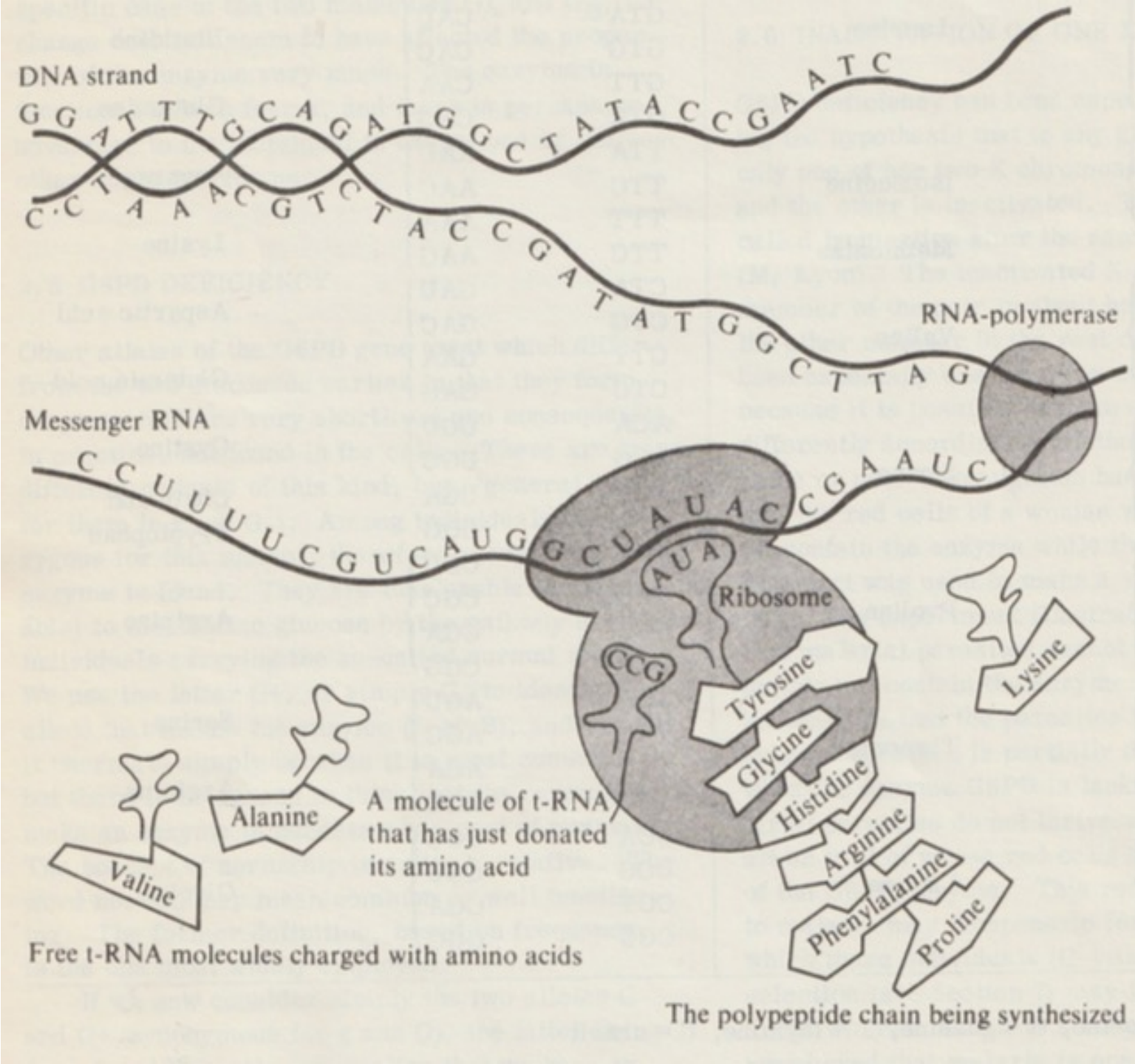
Classification of HBB^{E6V} mutant gene	**Dominant**	Co-dominant	**Recessive**

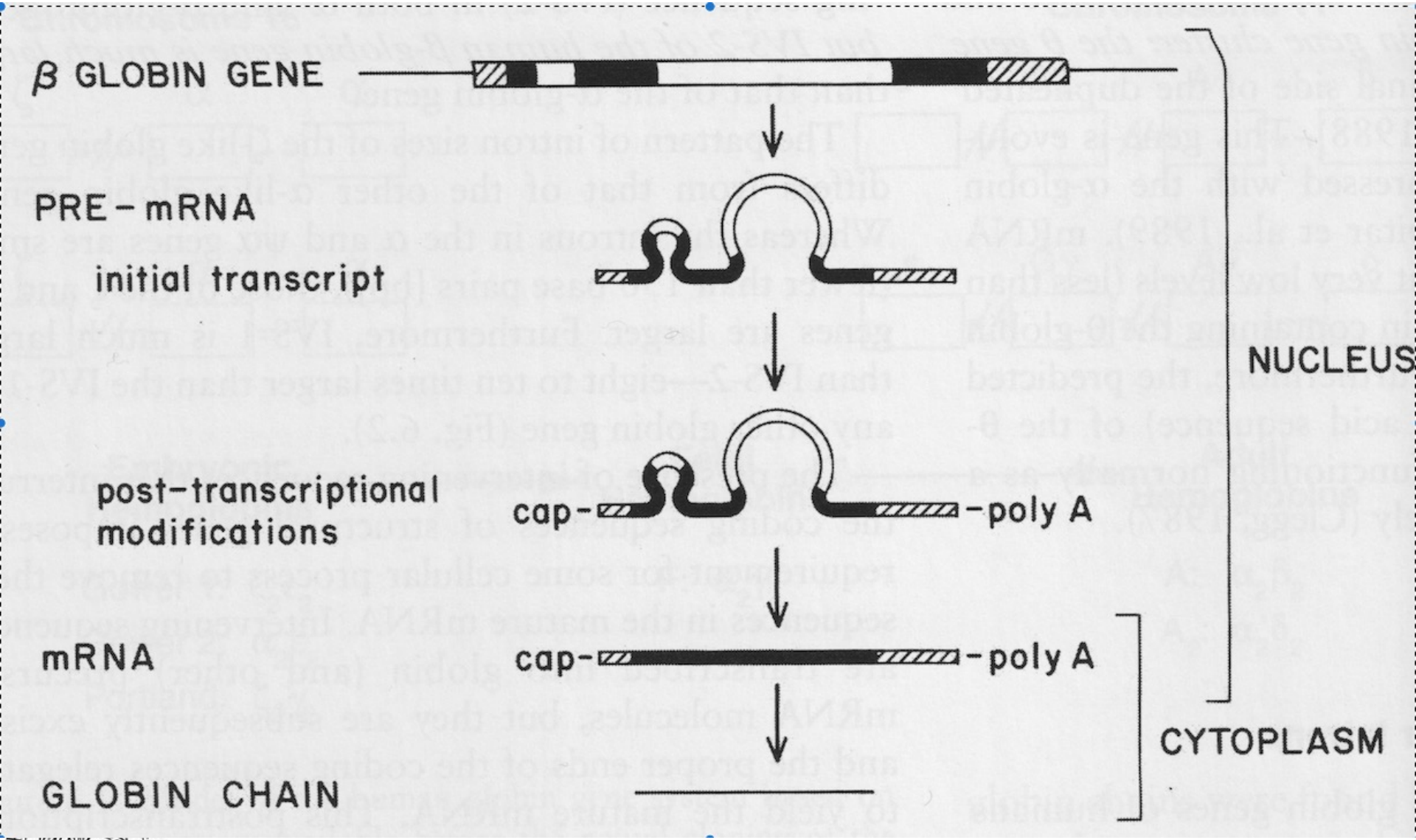
The genotype of a person is an absolute entity; the phenotype depends on what you are looking at.

A DIAGRAM OF GENE EXPRESSION



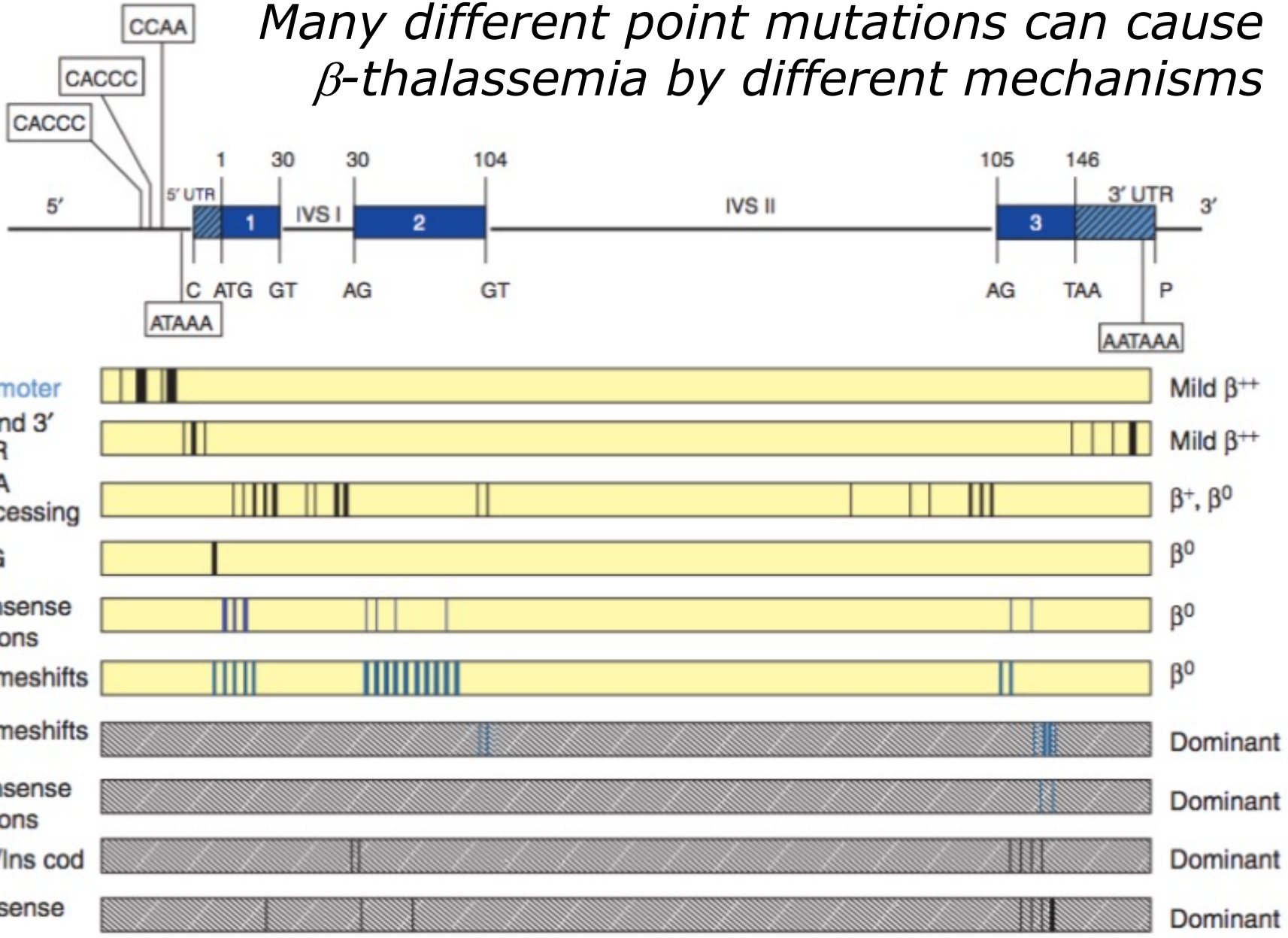
(From L.L Cavalli-Sforza *Elements of Human Genetics, 1969)*

A critical step between transcription and translation: maturation/splicing of the primary transcript



(From Bunn & Forget, 1982)

Many different point mutations can cause β-thalassemia by different mechanisms



(From Swee Lay Thein *Cold Spring Harb Perspective Med*, 2013}

MISGUIDED ENTHUSIASM FOR GENE THERAPY

Washington Post. 1980 Oct 8:A1, A15.
Doctor tried gene therapy on two humans.
Jacobs P.
PMID: 11646108 [PubMed - indexed for MEDLINE

Two patient with severe β-thalassaemia had bone marrow radiation; followed by intra-marrow injection of a plasmid with a β-globin cDNA insertion

'Classic" Mendelian Diseases

- *Dominant*: Huntington Disease[1]
- *Recessive:* Cystic Fibrosis[2]
- *X-linked:* Hemophilia[3]

[1] HD is caused by gain of function mutations of *HTT*, that confer toxic properties to the huntingtin protein

[2] CF is caused by loss of function mutations of *CFTR*, encoding *a* chloride channel

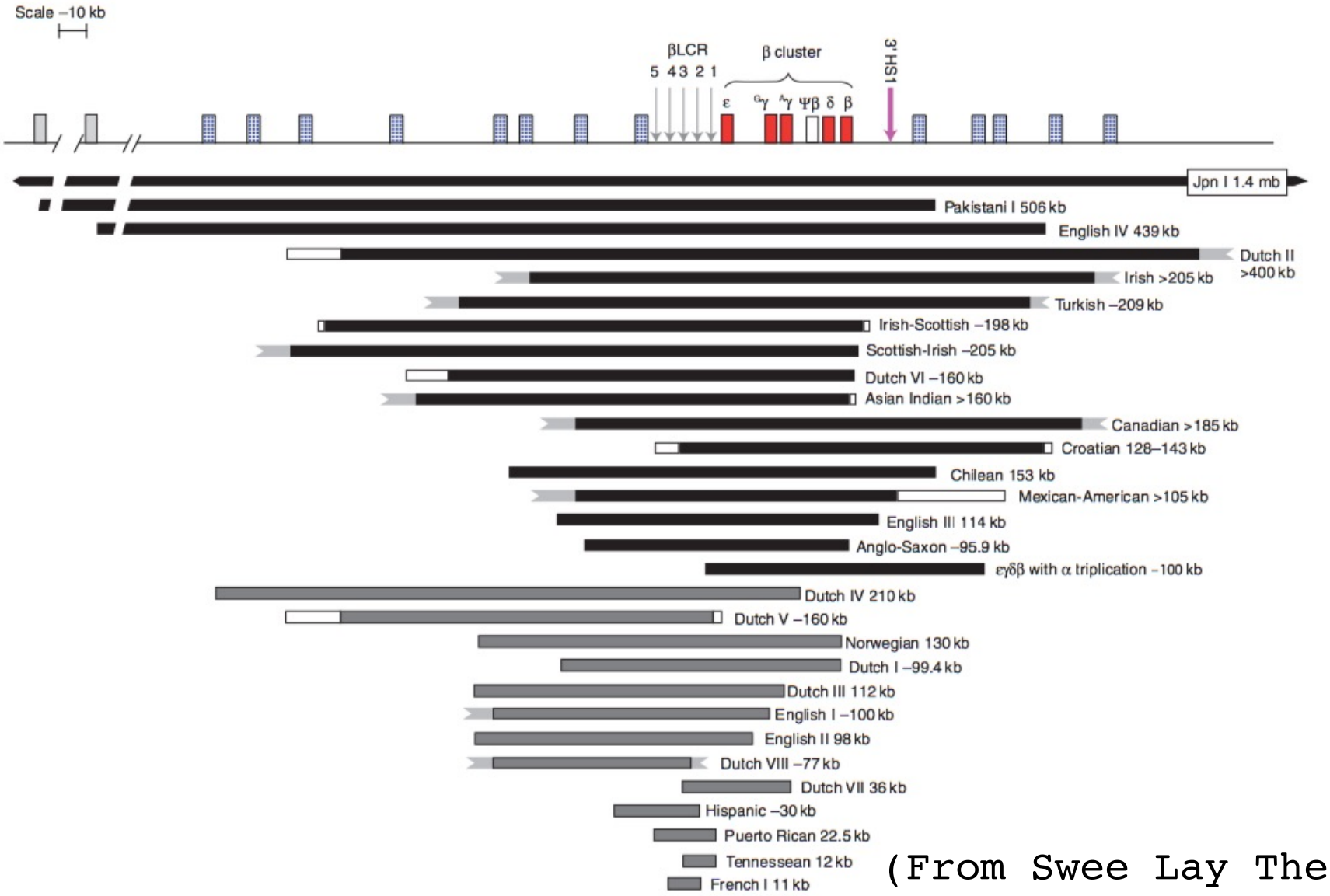
[3] Hemophilia A is caused by loss of function mutations of *F8,* encoding coagulation factor VIII

A protocol for gene therapy of an inherited blood disease

1. Clone gene
2. Insert in vector
3. Transfer into haematopoietic stem cells (HSC)
4. Obtain appropriate expression in progeny of HSC

(LL, in F Boiron & O Cohen-Hagenauer, *Symposium on gene transfer*, Paris, 1982)

Large deletions can cause β-thalassemia and other related syndromes



(From Swee Lay Thein *Cold Spring Harb Perspective Med*, 2013}

DISCOVERY OF AN *ENHANCER*

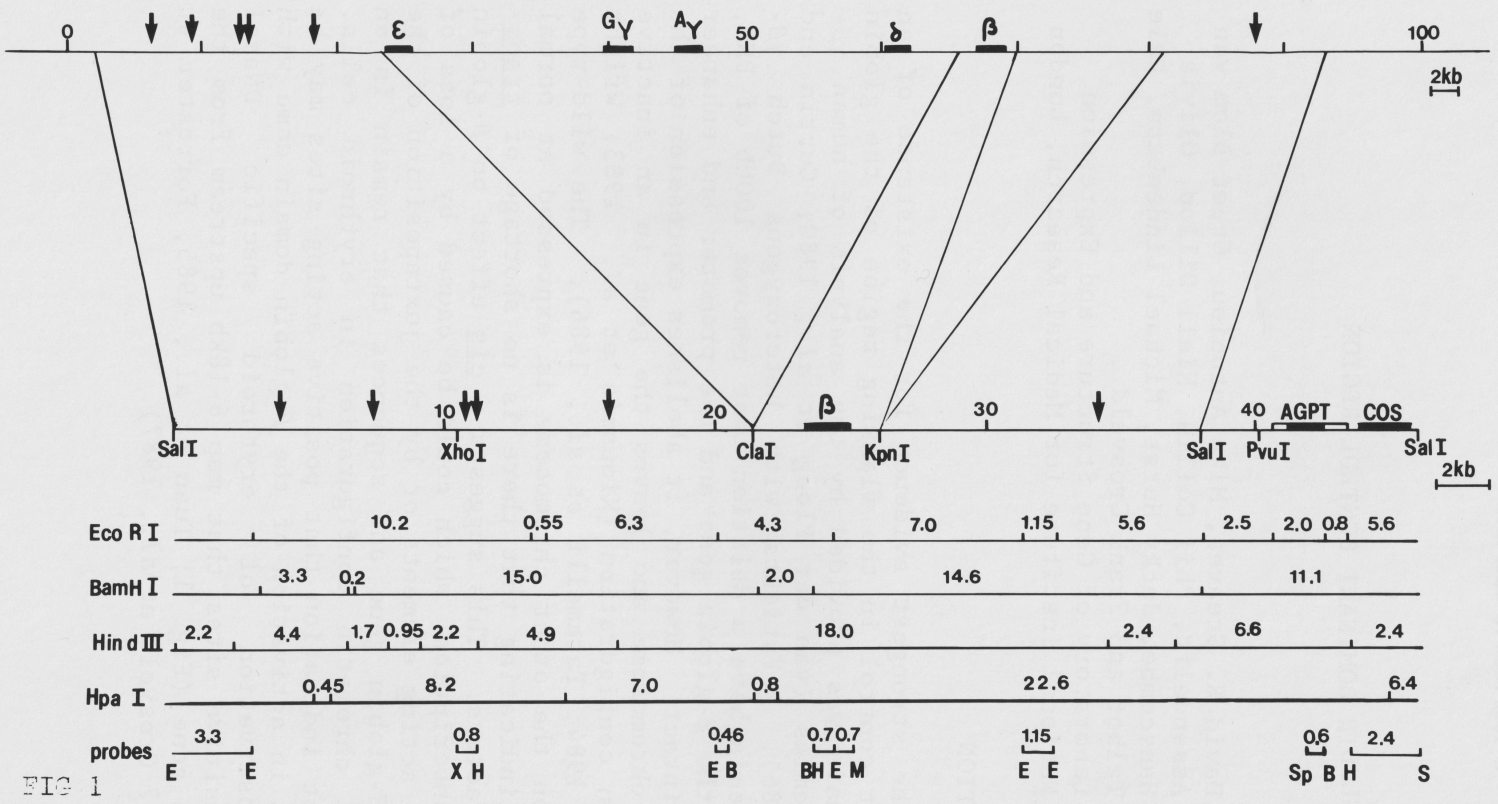


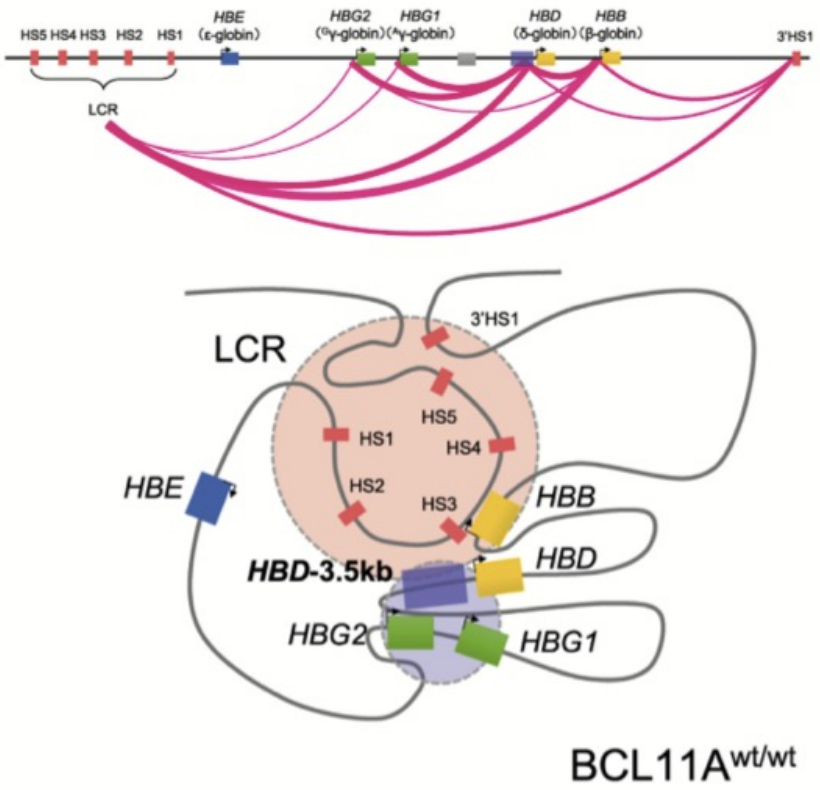
FIG 1

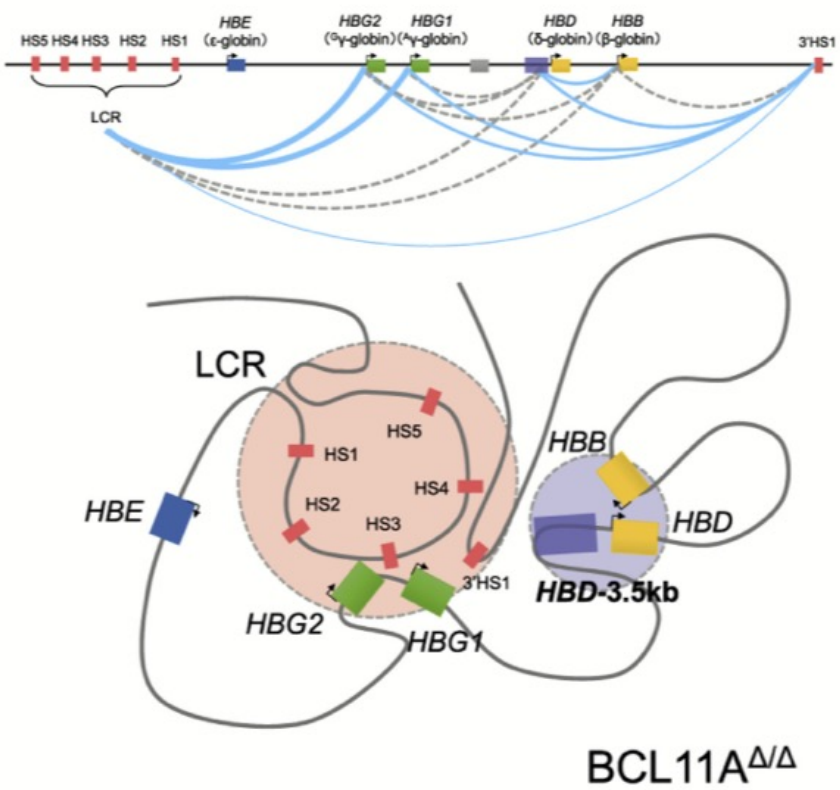
The human β-globin gene locus.

The human β-globin domain with all the functional genes is illustrated at the top. The β-globin minilocus leading to full expression of the β-globin gene in transgenic mice and MEL cells (Grosveld et al., 1987; Blom van Assendelft, 1988) is shown at the bottom; arrows indicate DNaseI super hypersensitive sites; sizes are in kilobases.

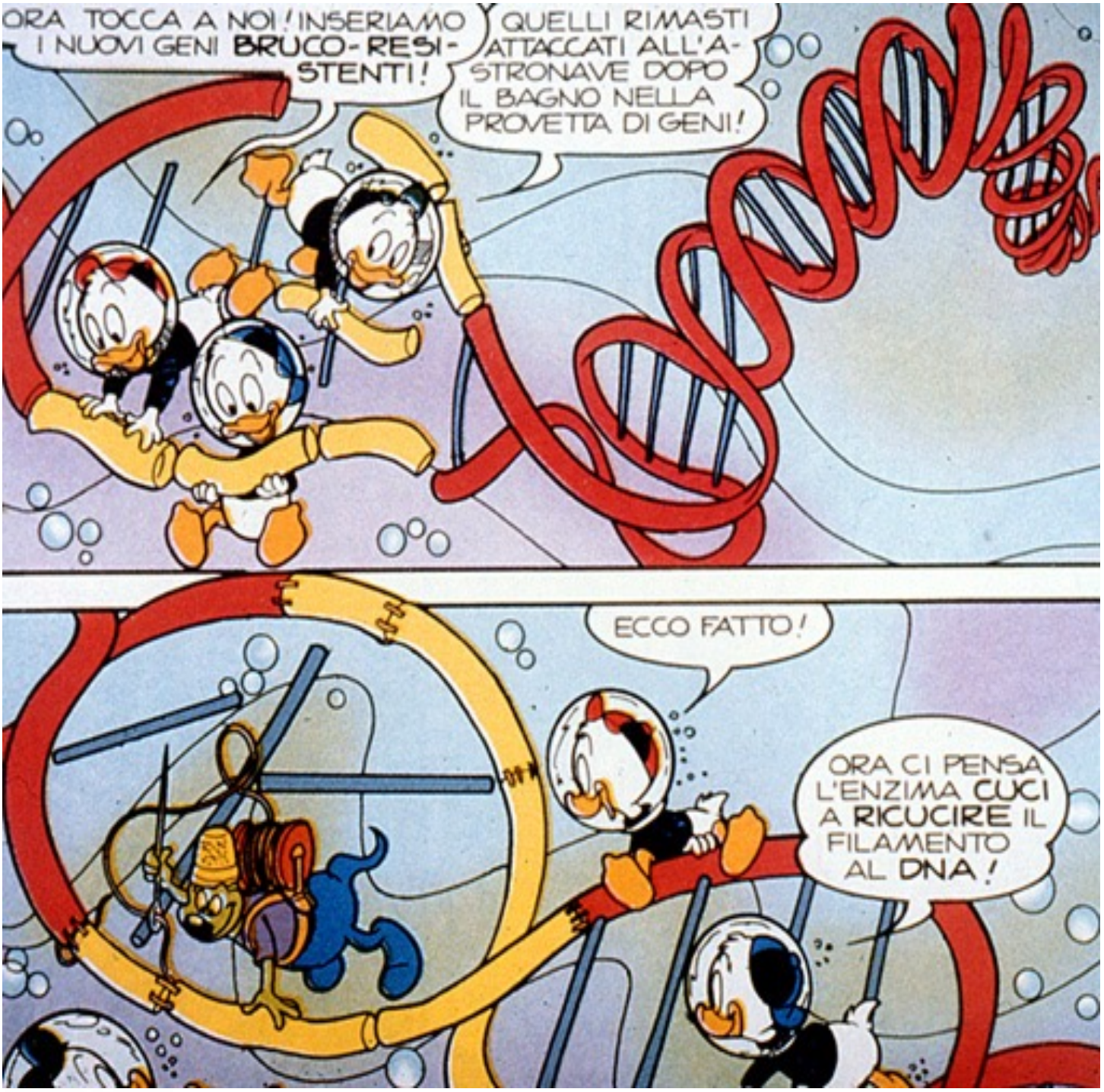
(From Frank Grosveld et al., *Hemoglobin Switch* Meeting, 1990)

MAJOR ROLE OF *BCL11A* IN THE HUMAN HEMOGLOBIN FETAL TO ADULT SWITCH





(From Shen et al., *Nat Commun* **12:**4991,2021)

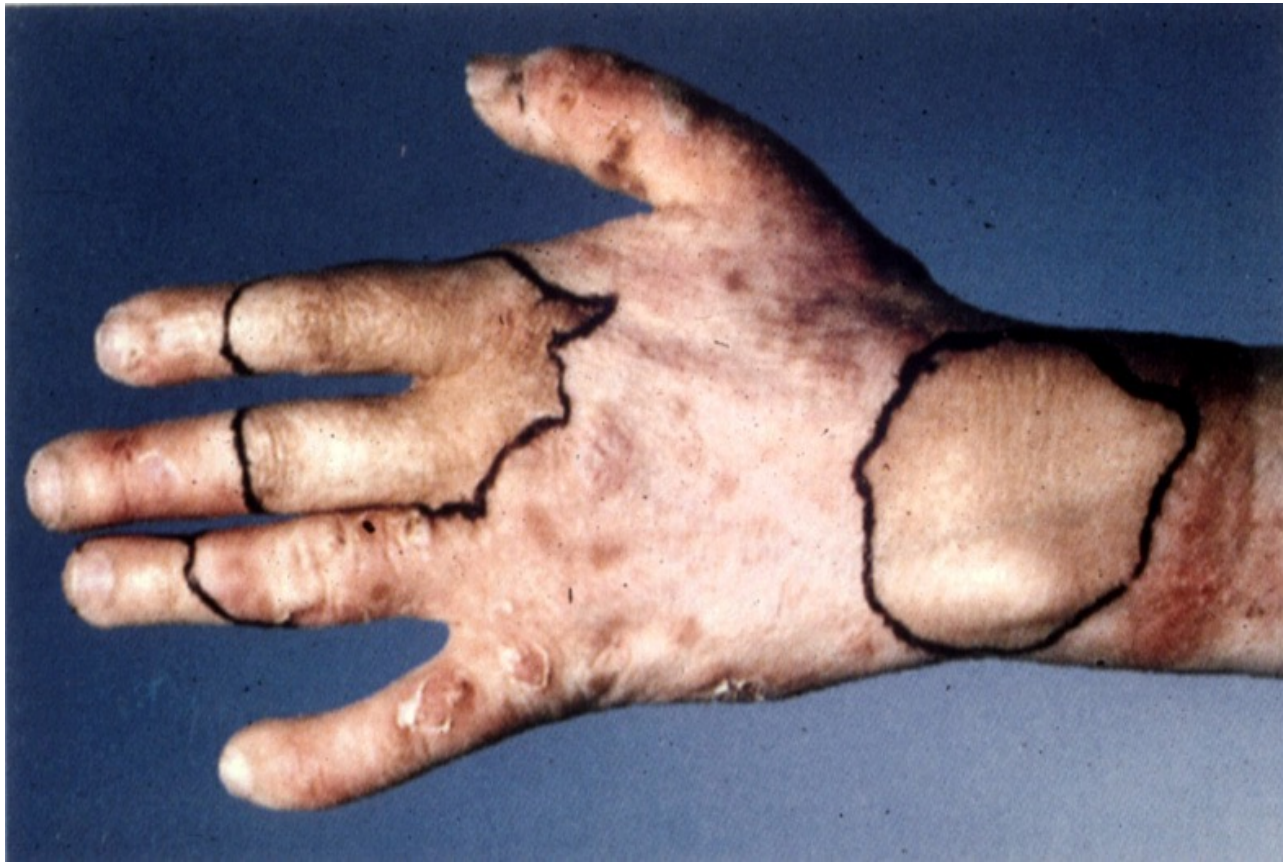


Genetic engineering comes into its own when it is reflected in children cartoons

From *TOPOLINO*, 1990

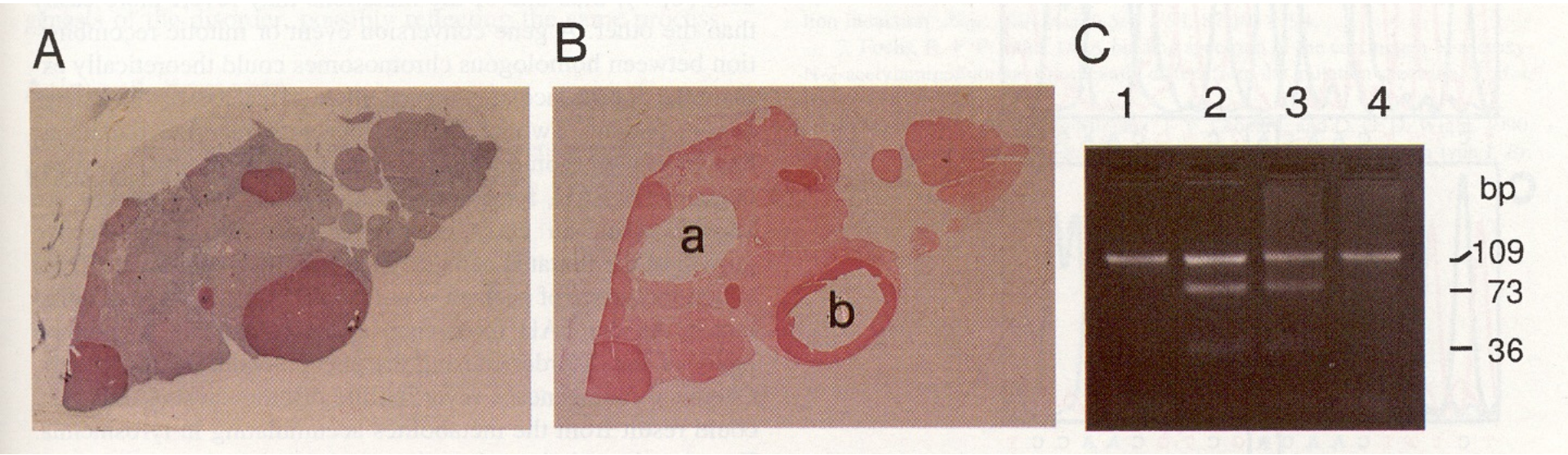
ATROPHIC BENIGN EPIDERMOLYSIS BULLOSA

Areas of normal skin result from mosaicism for a revertant of one of the two COL17A1 *mutant alleles*



(*From* Jonkman *et al.*, *Cell* **88**:543,1997)

SELF-CORRECTION BY BACK MUTATION OF FUMARYLACETOACETASE DEFICIENCY IN THE LIVER OF A PATIENT WITH TYROSINEMIA TYPE I



(*From* Kvittingen *et al.*, *JCI* **94**:1657,1994)

Somatic mutations can mitigate or correct human disease

- Back-mutation in Tyrosinemia type I (Evittingen et al 1994)
- Intragenic recombination in Bloom's syndrome (Ellis et al 1995)
- Back-mutation in ADA deficiency SCID (Hirschhorn et al 1996)
- Revertant mosaicism in *Epidermolysis bullosa* (Jonkman et al 1997)

Seminars in
HEMATOLOGY

VOL 35, NO 2, APRIL 1998

FROM THE GENETIC BASIS OF BLOOD DISORDERS TO GENE TRANSFER FOR THE PURPOSE OF GENE THERAPY

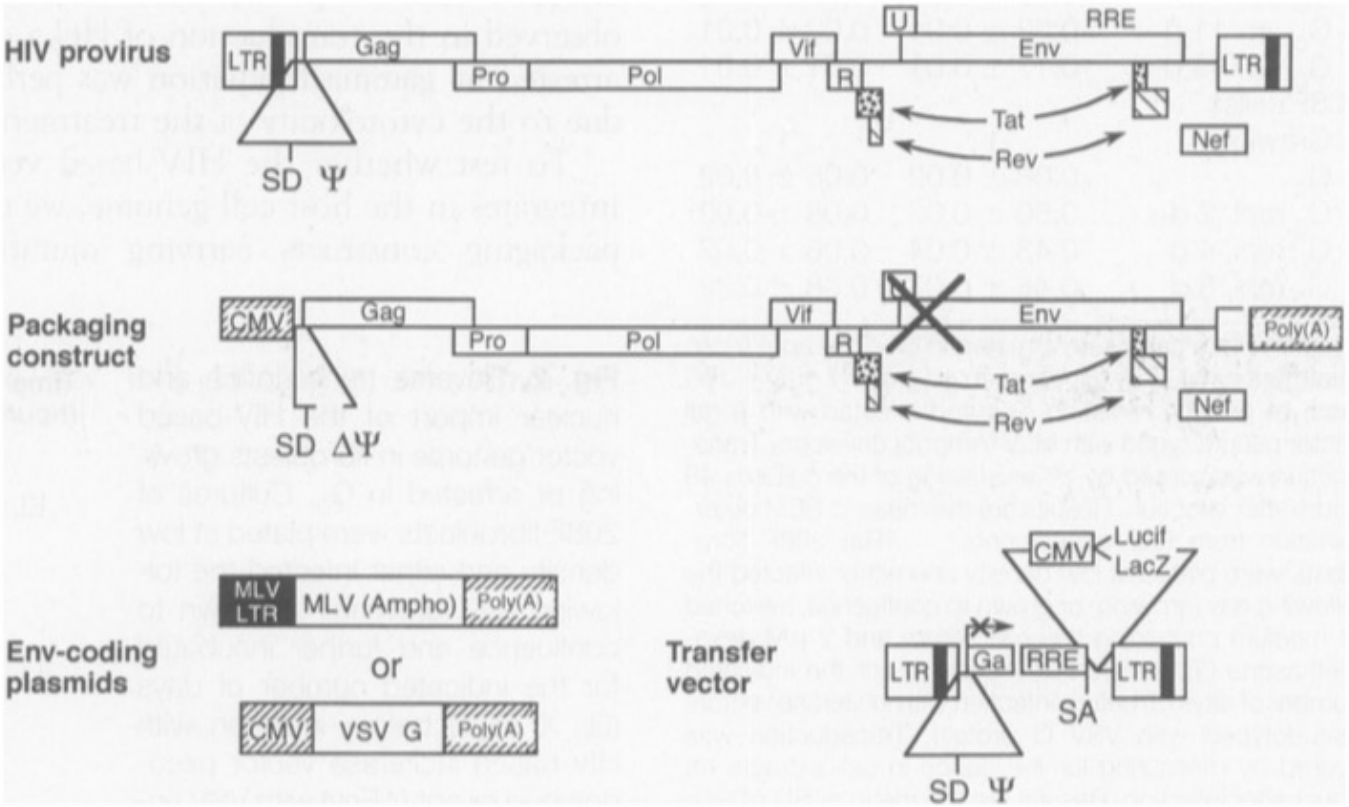
Lucio Luzzatto, MD

Guest Editor

In Vivo Gene Delivery and Stable Transduction of Nondividing Cells by a Lentiviral Vector

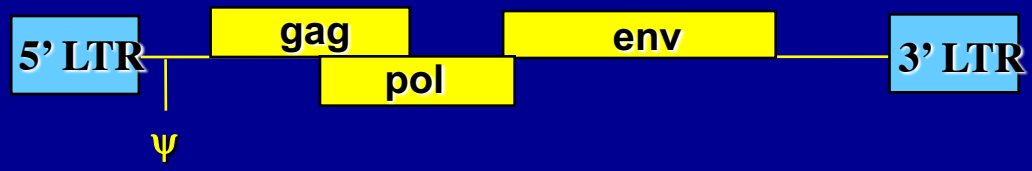
Luigi Naldini, Ulrike Blömer, Philippe Gallay, Daniel Ory,
Richard Mulligan, Fred H. Gage, Inder M. Verma,* Didier Trono

SCIENCE • VOL. 272 • 12 APRIL 1996

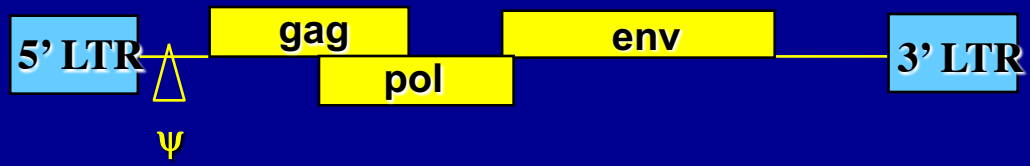


Retroviral Vector System

Moloney Leukemia Virus

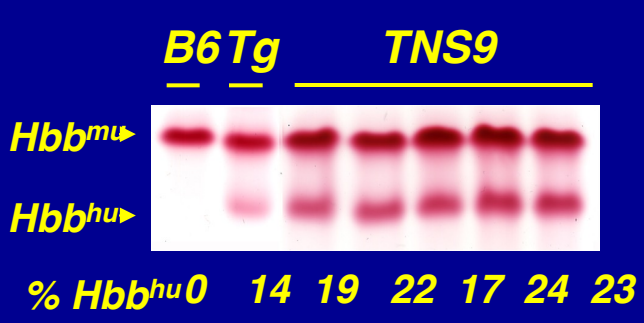


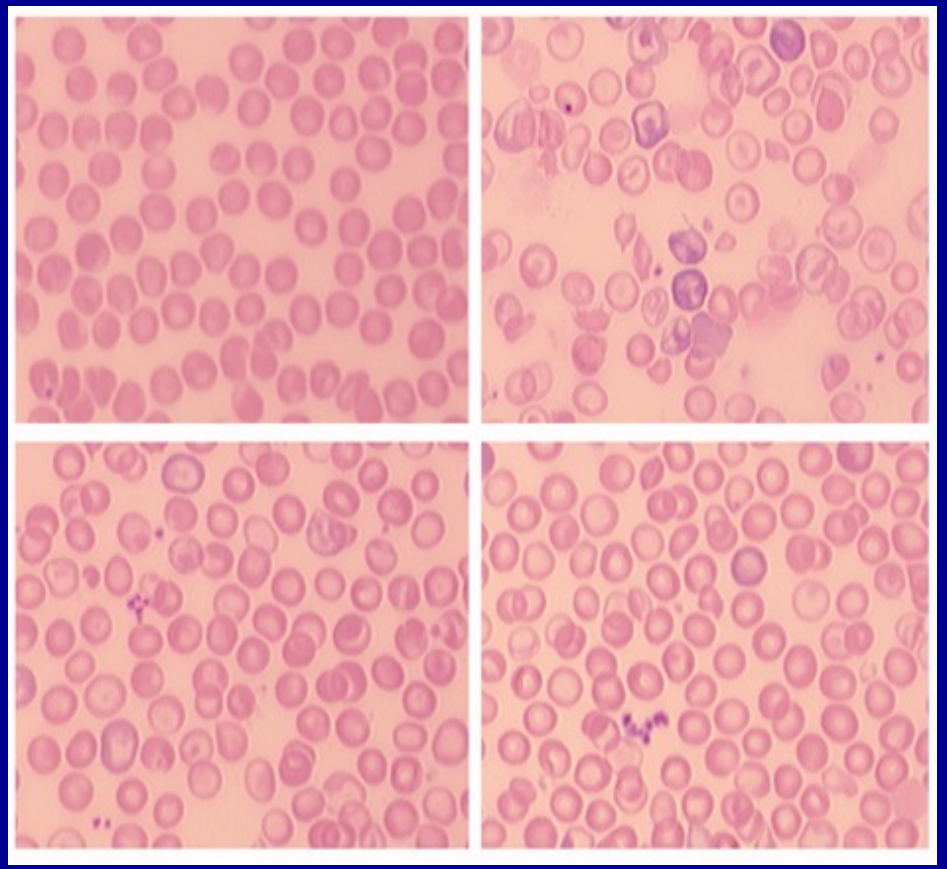
Helper genome



Transfer Vector: pSFG

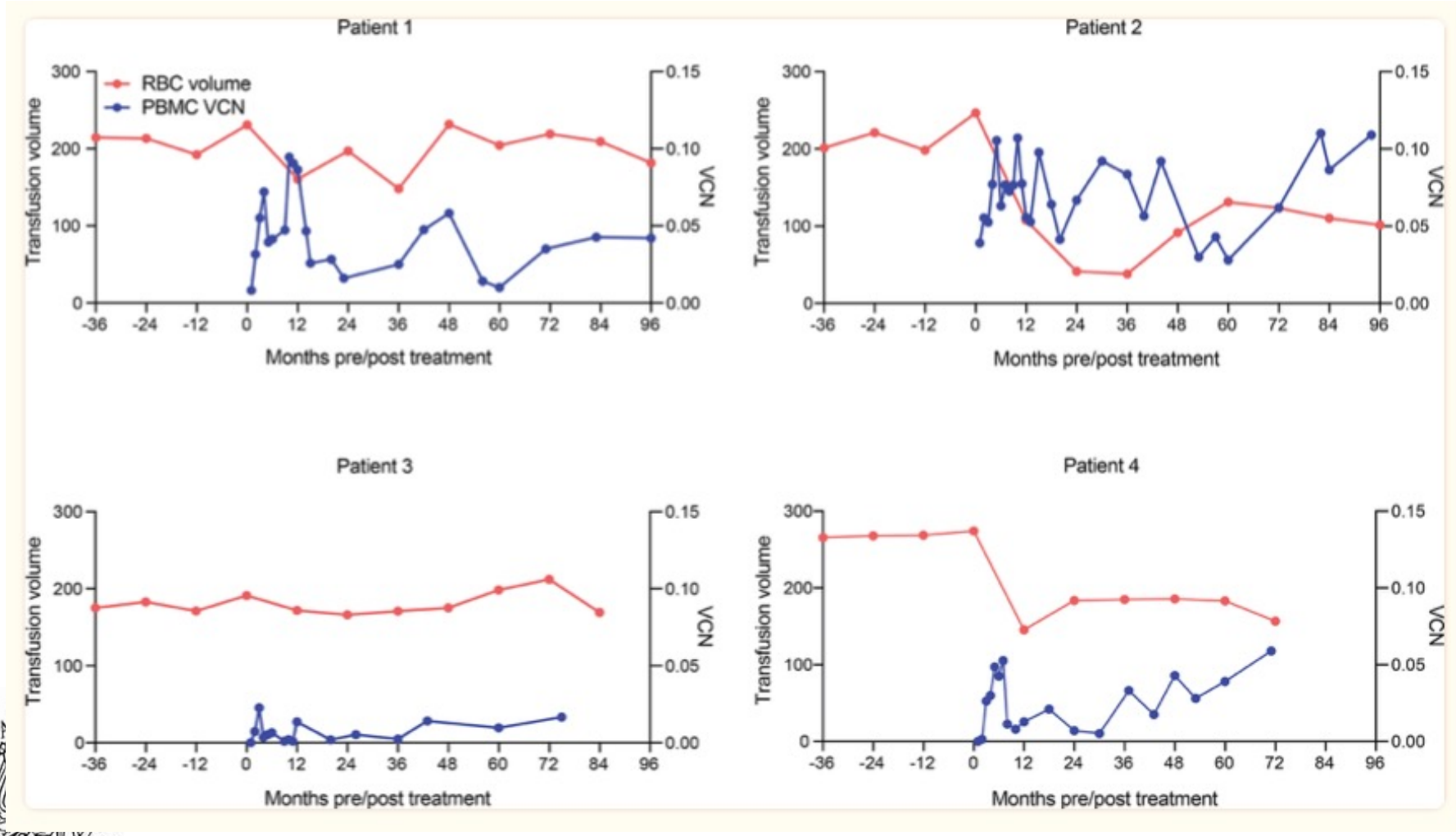
High levels of human β-globin expression result in correction of abnormal red cell morphology in HBB^{th3}/+ *bone marrow chimeras*





(From May *et al*, *Nature* **408**:*82*,2000)

Lentiviral-mediated β-globin gene transfer (vector TNS-9*) after non-myelo-ablative conditioning provides substantial reduction in transfusion requirement in patients with severe β-thalassemia*



(From Boulad et al., *Nat Medicine* **28:**63,2022)

Correction of ADA-SCID by Stem Cell Gene Therapy Combined with Nonmyeloablative Conditioning

Alessandro Aiuti,[1] Shimon Slavin,[2] Memet Aker,[2] Francesca Ficara,[1] Sara Deola,[1] Alessandra Mortellaro,[1] Shoshana Morecki,[2] Grazia Andolfi,[1] Antonella Tabucchi,[3] Filippo Carlucci,[3] Enrico Marinello,[3] Federica Cattaneo,[1] Sergio Vai,[1] Paolo Servida,[4] Roberto Miniero,[5] Maria Grazia Roncarolo,[1,6] * Claudio Bordignon[1,6]*†

28 JUNE 2002 VOL 296 SCIENCE

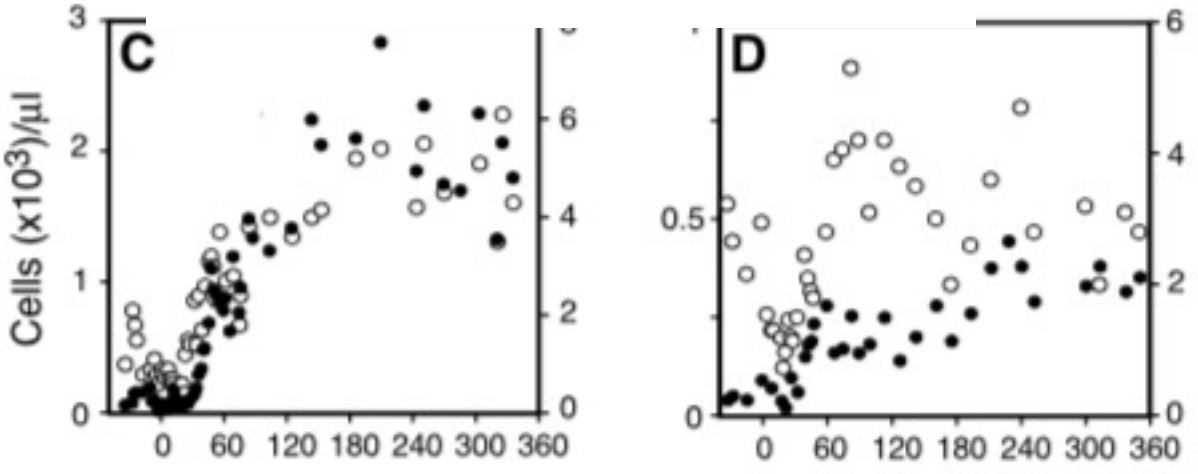


Table 1 | Gene therapies targeting inborn errors of immunity

Gene therapy	Disease	Defective cells	Selective advantage of corrected cells	Need for specific gene regulation?	Status
Gene addition	SCID-X1	T cells, NK cells	+++	No	Successful clinical trial
	ADA SCID	T cells, B cells, NK cells	+++	No	Successful clinical trial[a]
	RAG1 SCID	T cells, B cells	++	Unclear	Clinical trial
	RAG2 SCID	T cells, B cells	++	Unclear	Preclinical
	Artemis SCID	T cells, B cells	++	No	Clinical trial
	Wiskott–Aldrich syndrome	T cells, B cells, DCs, platelets	+	No	Successful clinical trial
	CGD, gp91phox deficiency	Phagocytes	–	Unclear	Successful clinical trial
	CGD, p47phox deficiency	Phagocytes	–	Unclear	Clinical trial
	Leukocyte adhesion deficiency	Phagocytes	–	No	Successful clinical trial

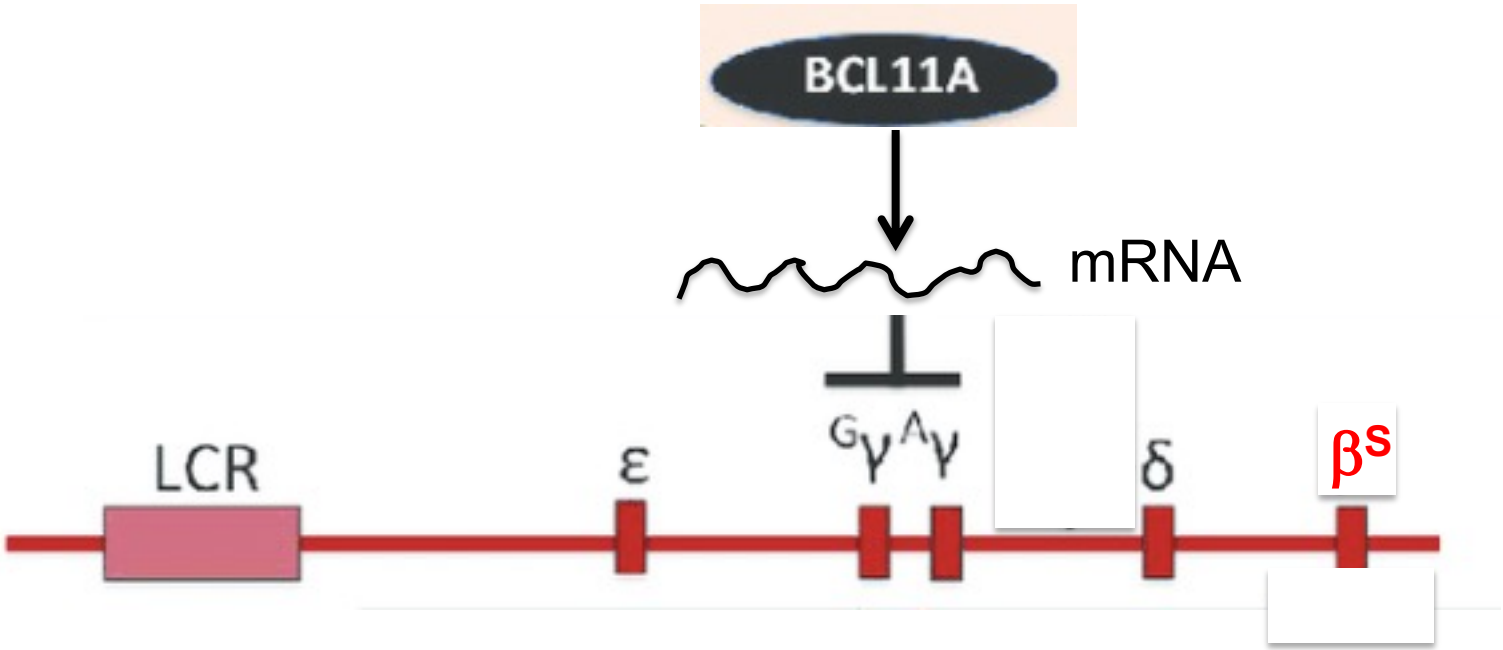
(From Alain Fischer *Nature Revs Immunol* 2022)

MODALITIES OF GENE THERAPY

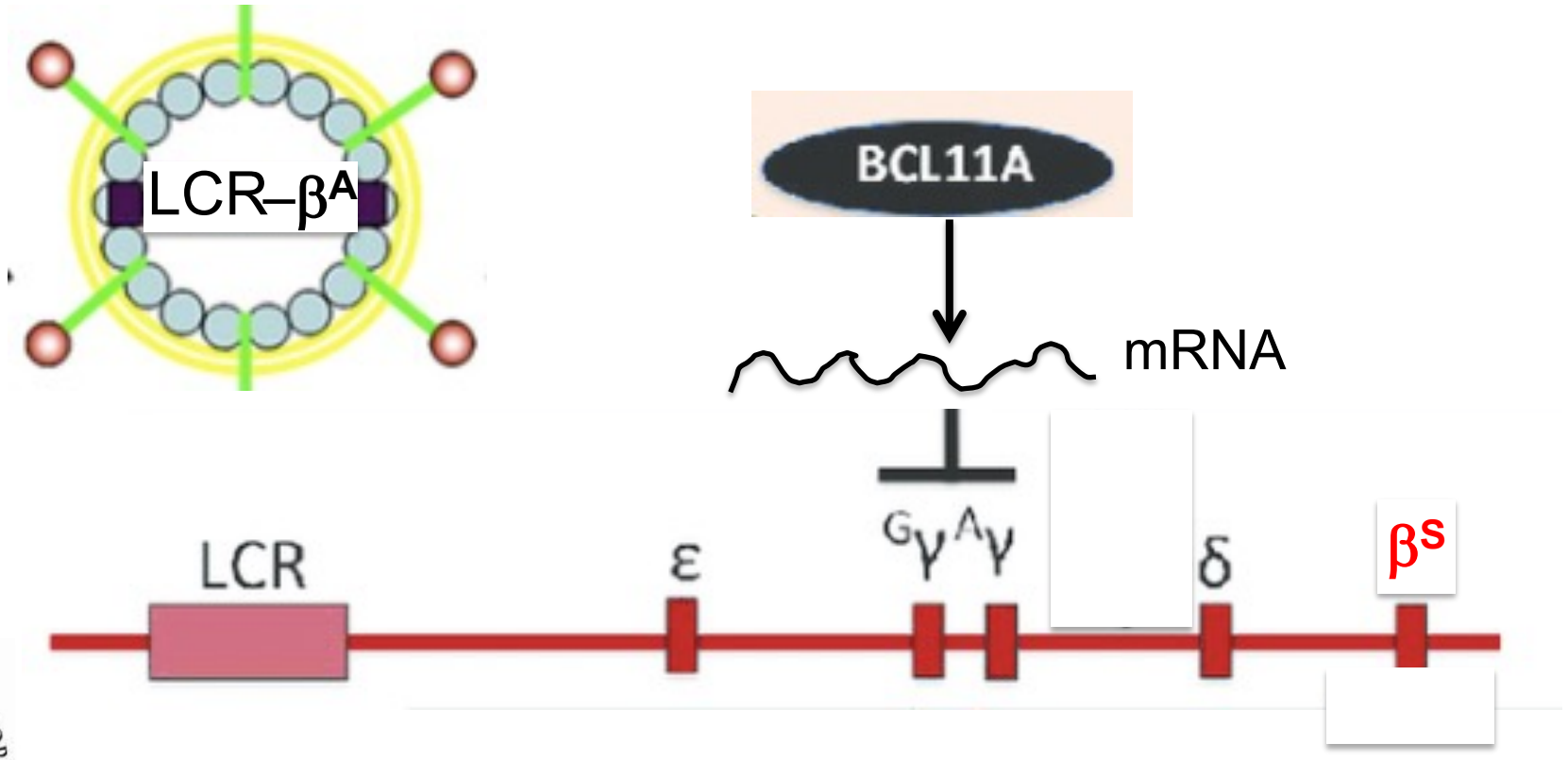
- Lentiviral insertion into genome
- AAV episomal insertion
- Gene editing
- *Ex vivo* gene therapy
- *In vivo* gene therapt


would you say you had a dominant or recessive character
gene therapy

Simplified diagram of the β-globin gene cluster in a patient with homozygous sickle cell anaemia

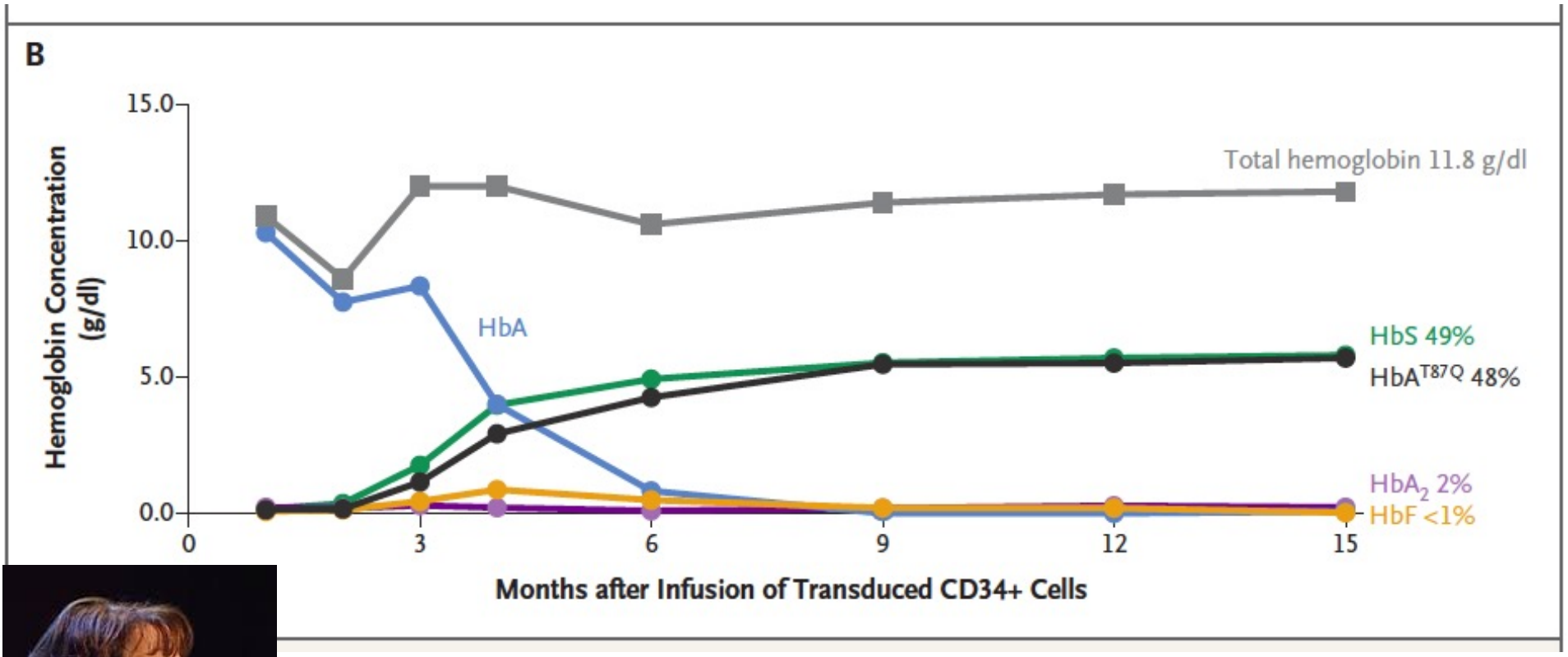


A lentiviral vector inserts
into the genome of hematopoietic stem cells
a β^A globin gene with appropriate regulators and insulators



(As in Ribeil et al., *NEJM* **376:**848,2017)

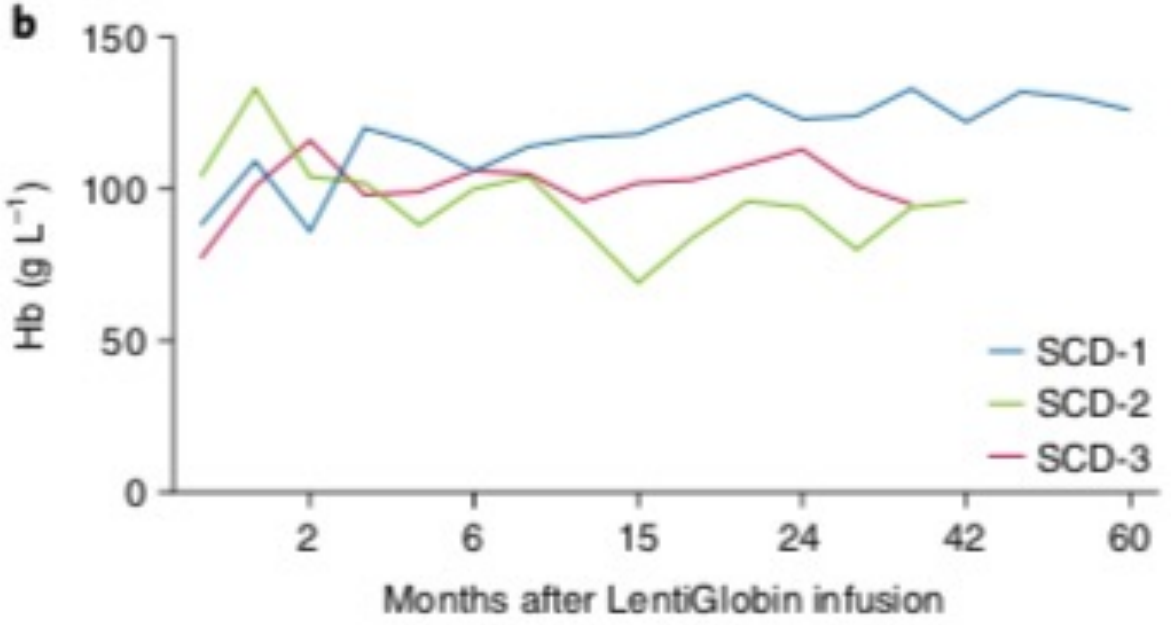
SUCCESFUL GENE THERAPY
IN A PATIENT WITH SICKLE CELL ANAEMIA

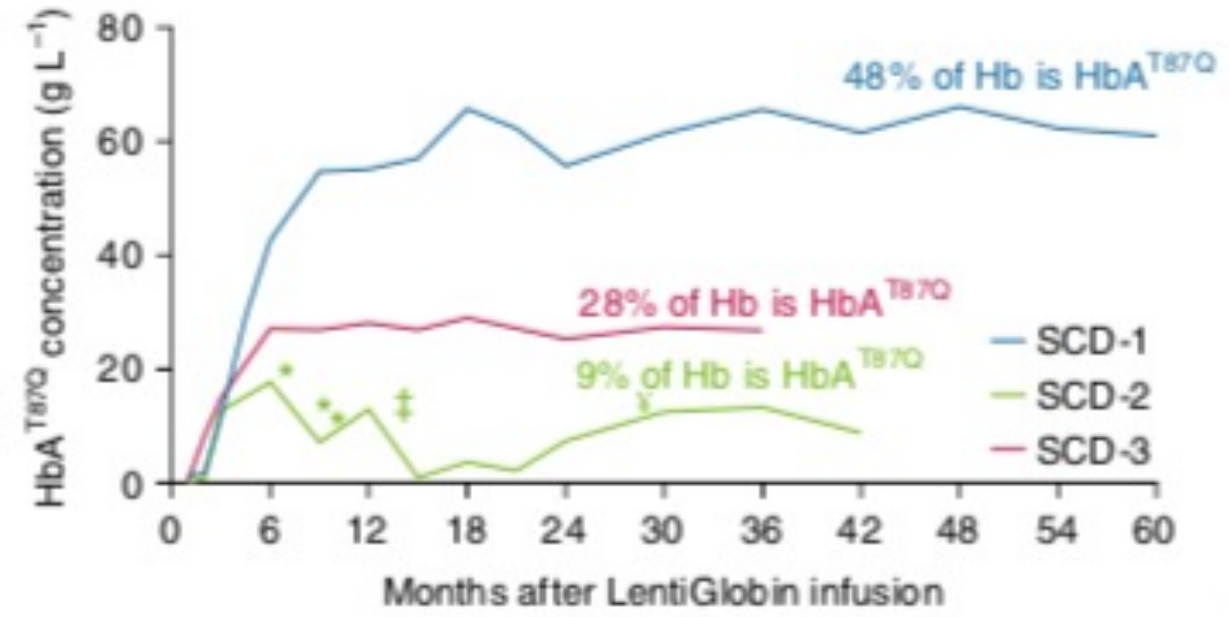


Courtesy of ***Marina Cavazzana-Calvo***

(Ribeil et al, NEJM **376:**848,2017)

HEMATOLOGIC COURSE OF 3 PATIENTS WITH SICKLE CELL DISEASE AFTER LENTIVIRAL-MEDIATED GENE THERAPY

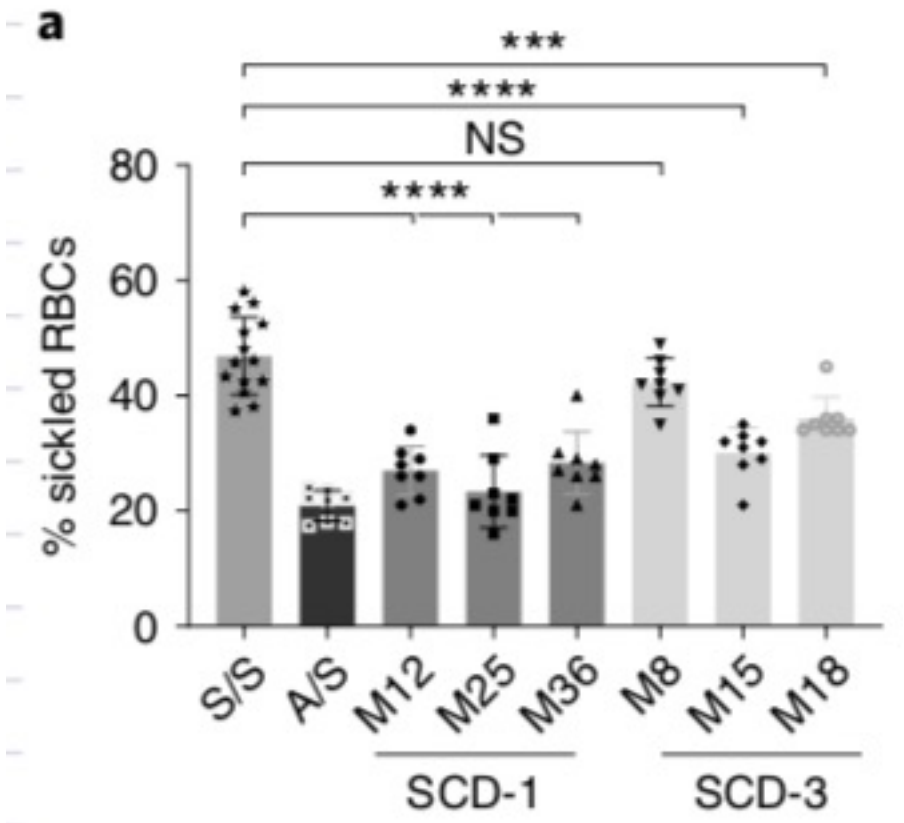




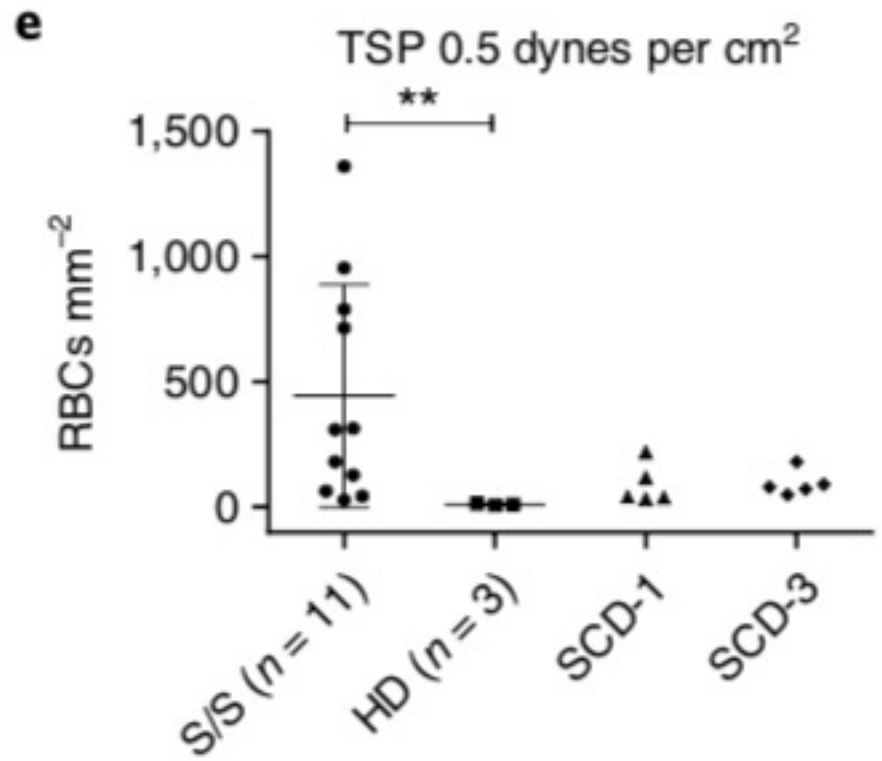
(From Magrin et al., *Nature Medicine* **28:**71,2022)

Amelioration of pathophysiology of sickle cell disease after gene therapy

SICKLING

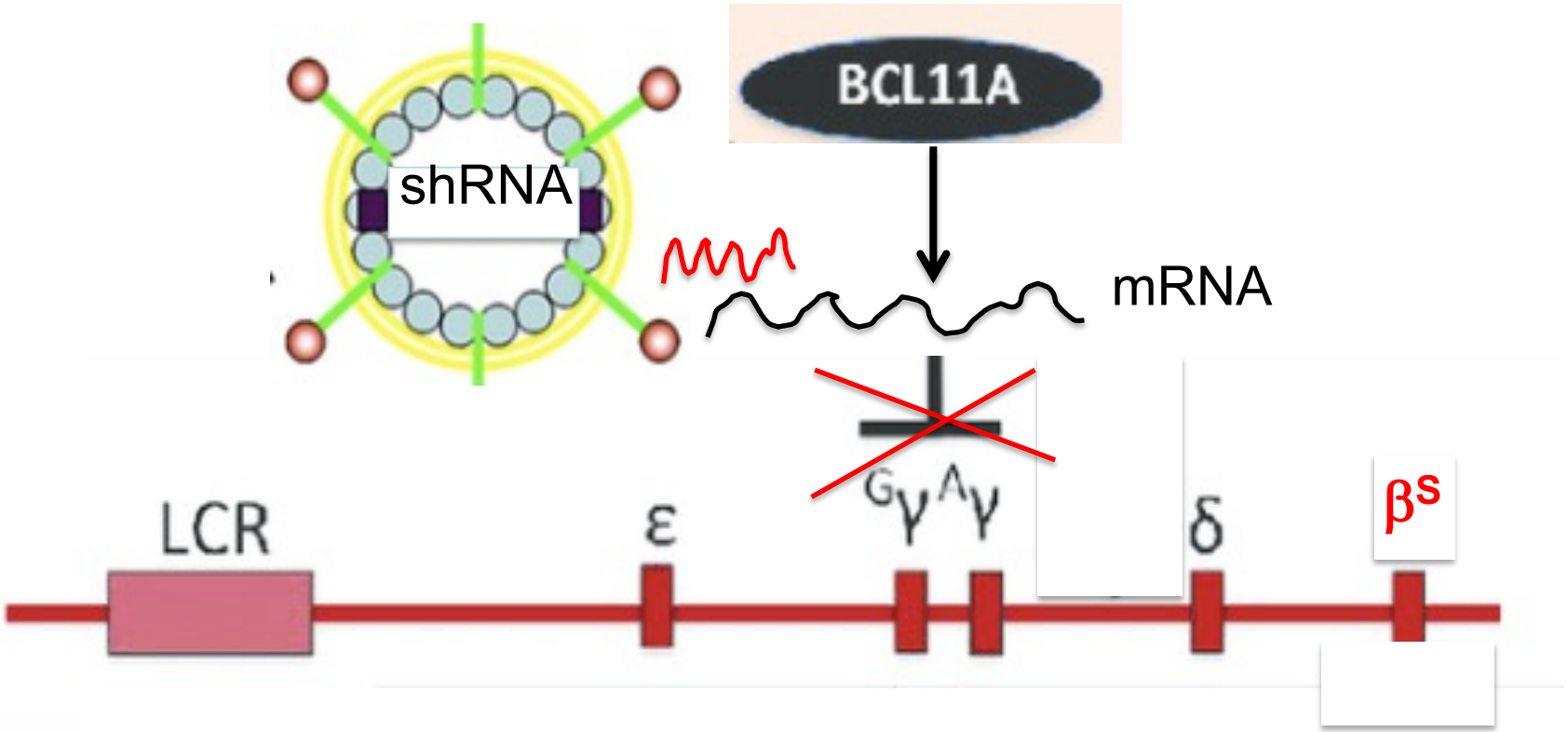


ADHESION TO THROMBOSPONDIN-COATED SURFACE



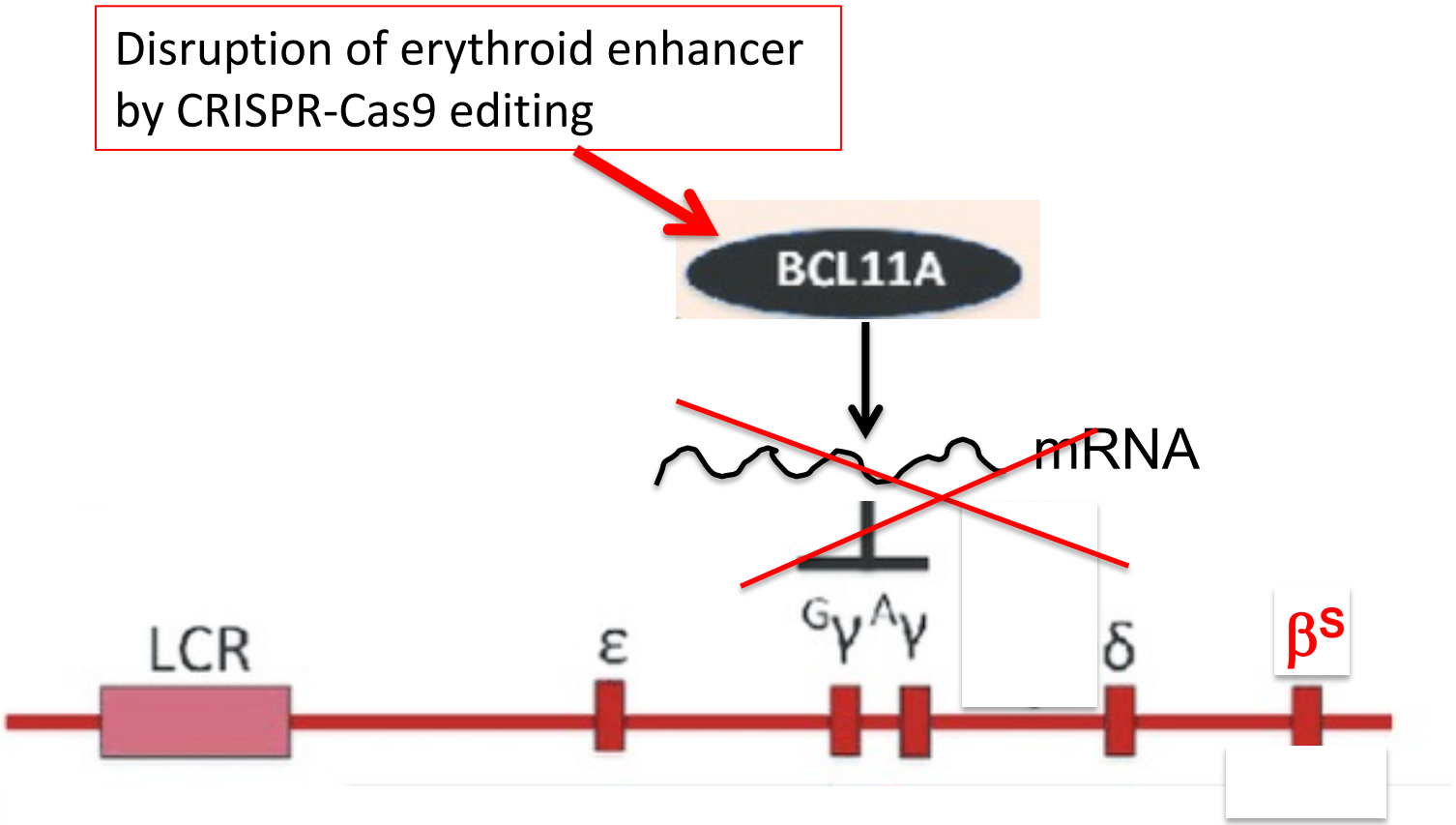
(From Magrin et al., *Nature Medicine* **28:**71,2022)

A lentiviral vector inserts into the genome of hematopoietic stem cells a short inhibitory RNA that prevents translation of BCL11A, *thus de-repressing the* γ *globin genes*



(As in Esrick et al., *NEJM* **384:**205,2021)

Lipid NanoParticles convey to hematopoietic stem cells a guide RNA that targets BCL11A *that is then disrupted by Cas9 nuclease, with consequent de-repression of the γ globin genes*

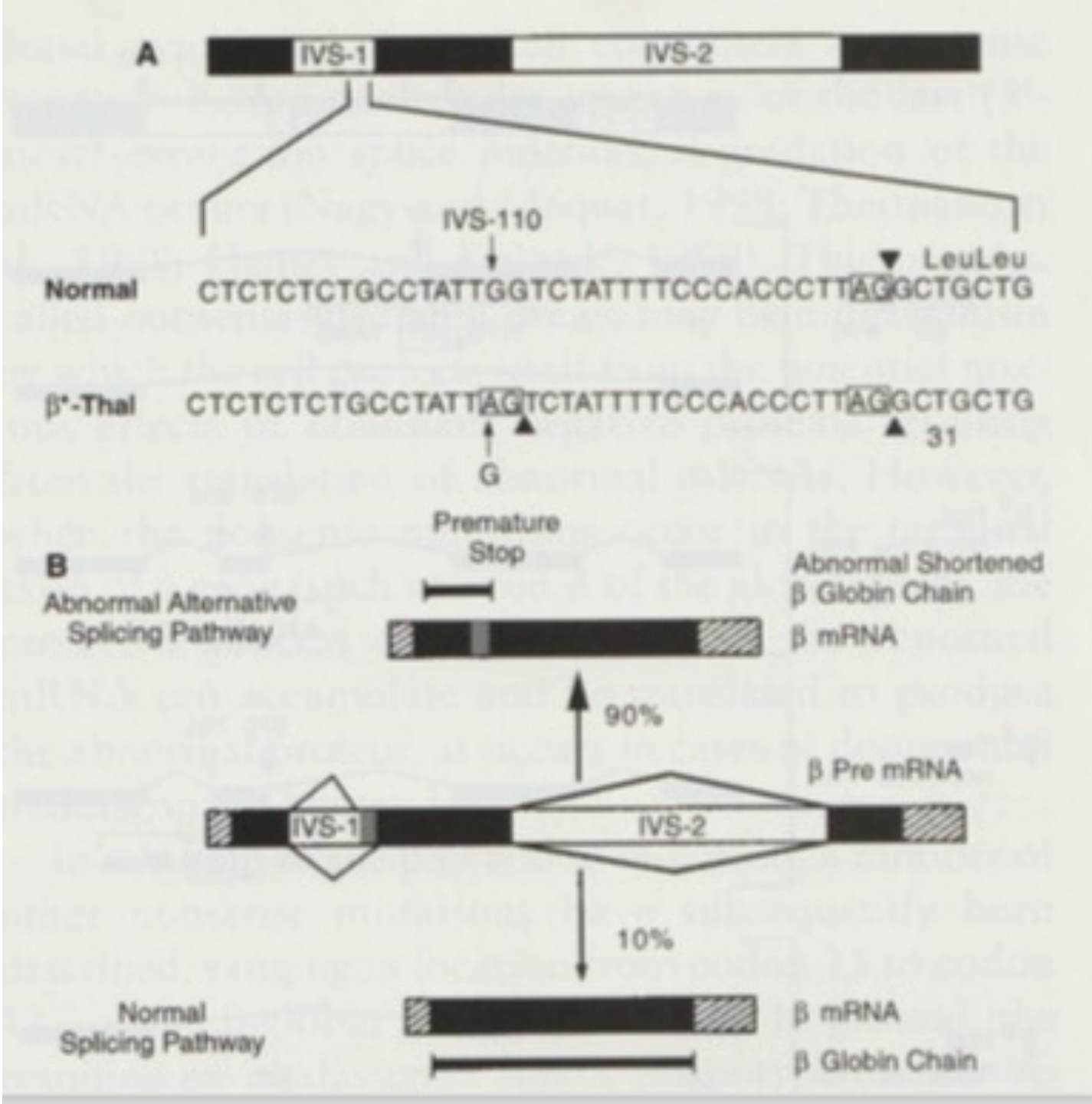


(As in Frangoul et al., *NEJM* **384:**252,2021)

SCD: Gene Therapy *versus* HSCT

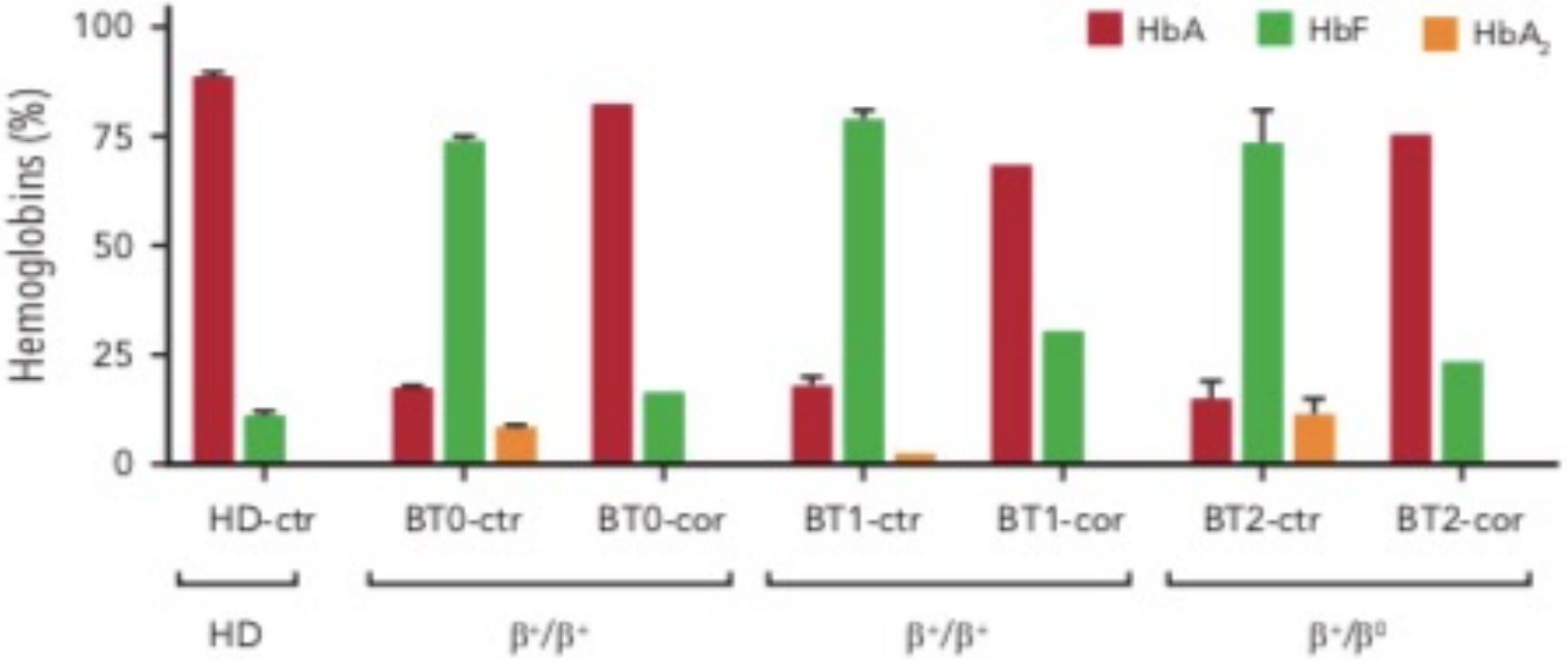
	HSCT (BMT)	*Gene Therapy*
'Conditioning'	Myelo-ablative (moderate to heavy)	Myelo-ablative (mild to heavy)
Successful therapeutic outcome	Replacement of SS cells with donor cells (AA or AS)	SS cells converted to AS cells; or marked increase in Hb F
Frequent occurrence	Mixed donor chimerism (MDC): >30% OK	?
Phenocopy of AS heterozygote	No	**Yes, potentially**
GVHD	Frequent	**No**

An intronic point mutation can cause severe β-thalassemia

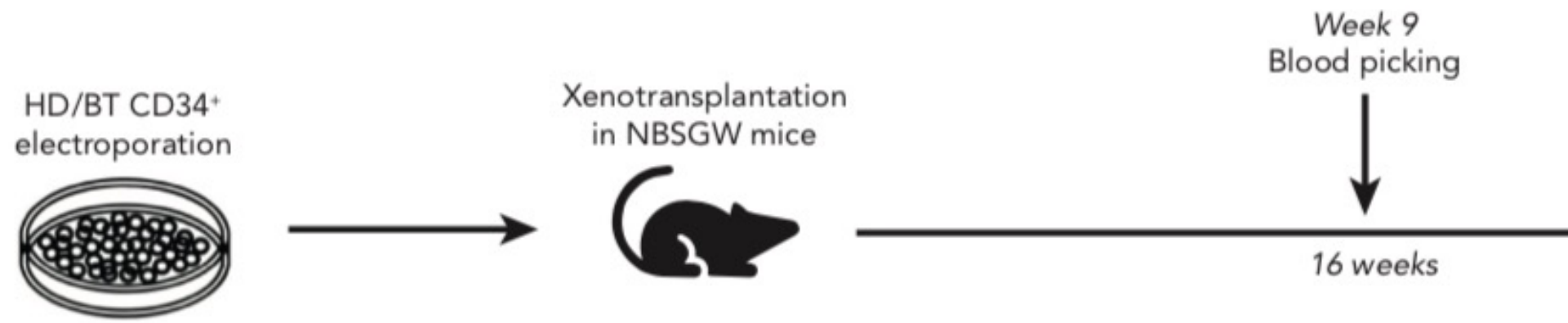


(From Busslinger, Moschonas & Flavell, *Cell* **27:**289,1981)

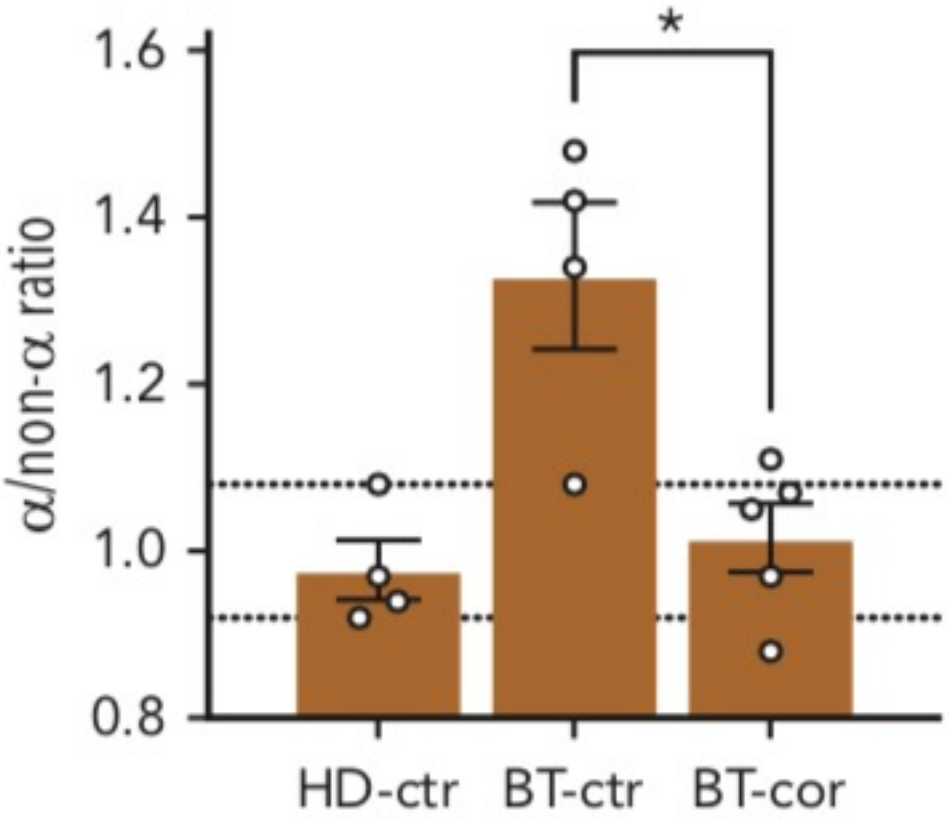
IN VITRO CORRECTION BY BASE EDITING OF THE SEVERE β-THALASSEMIA SPLICING MUTATION *IVS I-110*



(From Hardouin et al., *Blood* **141**,1169,2023)

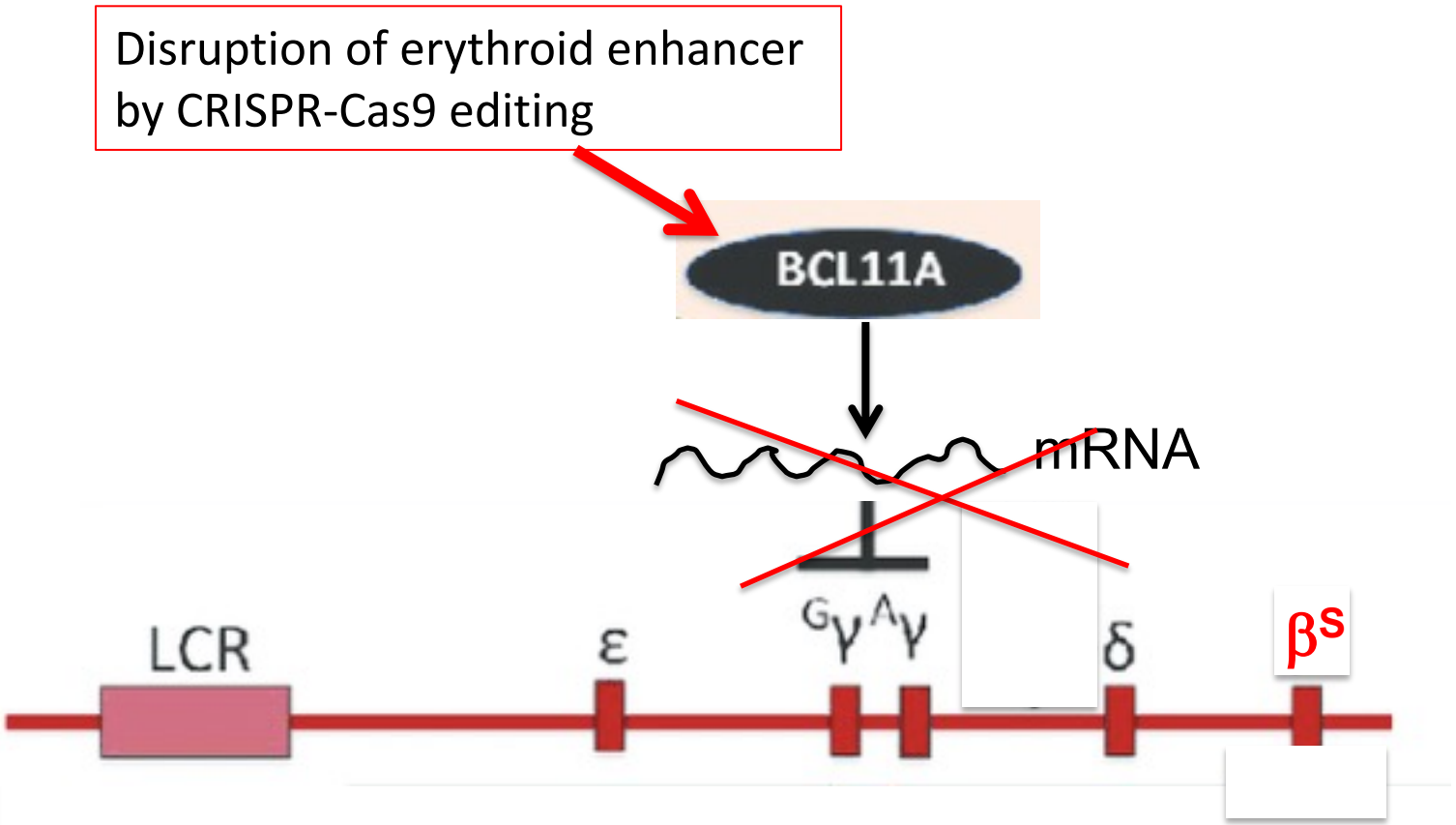


Base-edited HSC from a patient with severe β-thalassemia provide normal α/β globin biosynthetic ratio after xeno-transplantation into immuno-deficient mice



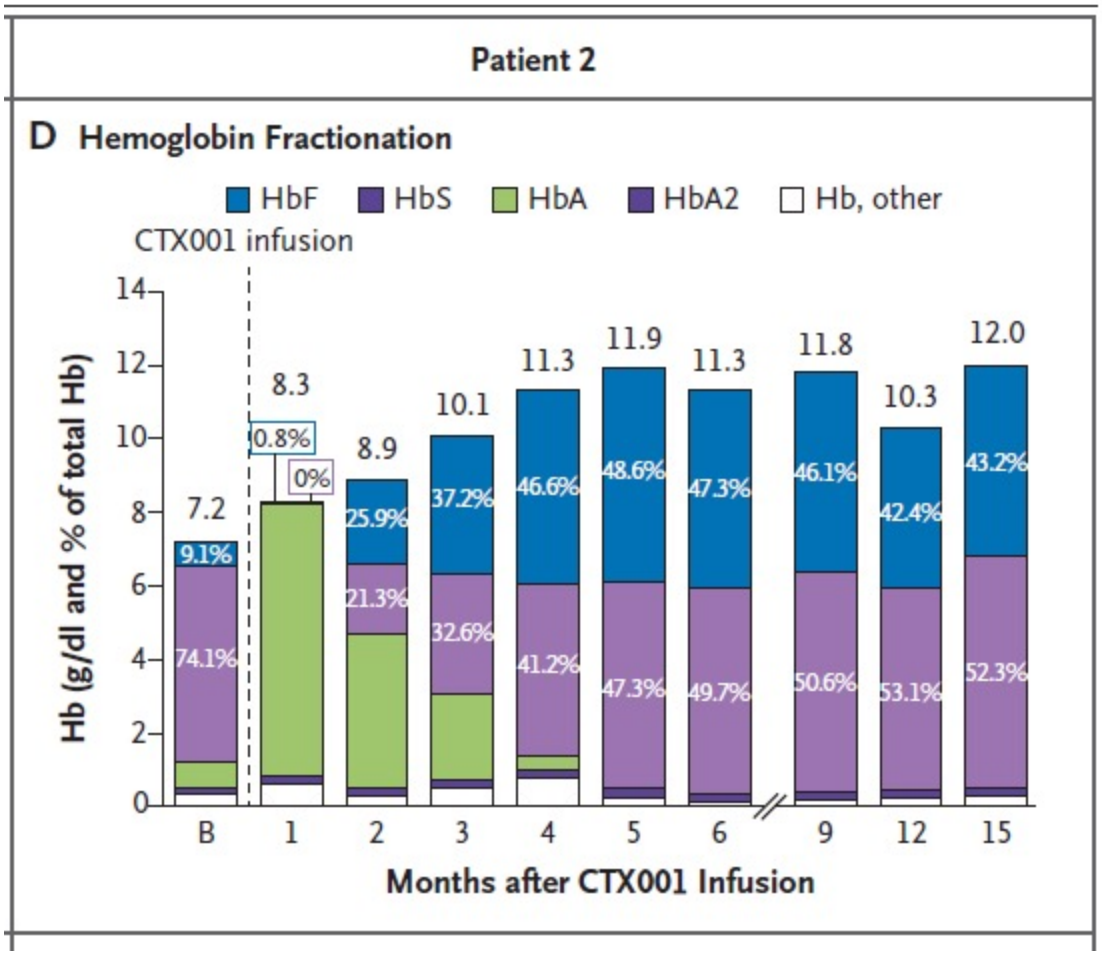
(From Hardouin et al., *Blood* **141**,1169,2023)

Lipid NanoParticles convey to hematopoietic stem cells a guide RNA that targets BCL11A *that is then disruoted by Cas9 nuclease, with consequent de-repression of the γ globin genes*



(As in Frangoul et al., *NEJM* **384:**252,2021)

CRISPR-mediated inactivation of BCL11A *causes impressive increase in Hb F in a patient with severe SCD*



(From Frangoul et al.,*NEJM* **384:**252,2021)

In March 2021 betibeglogene autotemcel, licensed in 2019 by FDA and EMA, was temporarily suspended because:

- 2 patients developed MDS
- 1 patient developed AML

- Insertional mutagenesis?
- Role of ‘conditioning regimen’?
- Pre-existing somatic mutations?

Table 1. Age, sex, race/ethnicity, and time-adjusted SIRs for selected cancers among patients with SCD, California, 1988-2014

	Observed cases	Expected cases	SIR	95% CI
All cancers	115	143.70	0.80	(0.66-0.96)
Solid tumor	76	123.25	0.62	(0.49-0.77)
Breast	16	29.73	0.54	(0.31-0.87)
Respiratory	16	13.13	1.22	(0.70-1.98)
Digestive system	16	22.18	0.72	(0.41-1.17)
Urinary system	8	6.06	1.32	(0.57-2.60)
Female genital	5	11.63	0.43	(0.14-1.00)
Male genital	6	16.71	0.36	(0.13-0.78)
Hematologic tumors	31	18.03	1.72	(1.17-2.44)
Lymphoma	15	10.38	1.45	(0.81-2.38)
Leukemia	12	5.17	2.32	(1.20-4.05)
ALL	3	1.64	1.83	(0.38-5.35)
CLL	3	0.62	4.83	(1.00-14.11)
AML	6	1.67	3.59	(1.32-7.82)

(From Brunson *et al.*, *Blood* **130:**1597,2017)

blood

Perspective

Leukemia after gene therapy for sickle cell disease: insertional mutagenesis, busulfan, both, or neither

Richard J. Jones[1,*] and Michael R. DeBaun[2,*]

[1]Sidney Kimmel Cancer Center at Johns Hopkins, Johns Hopkins University, Baltimore, MD; and [2]Vanderbilt-Meharry Sickle Cell Disease Center of Excellence, Vanderbilt University Medical Center, Nashville, TN

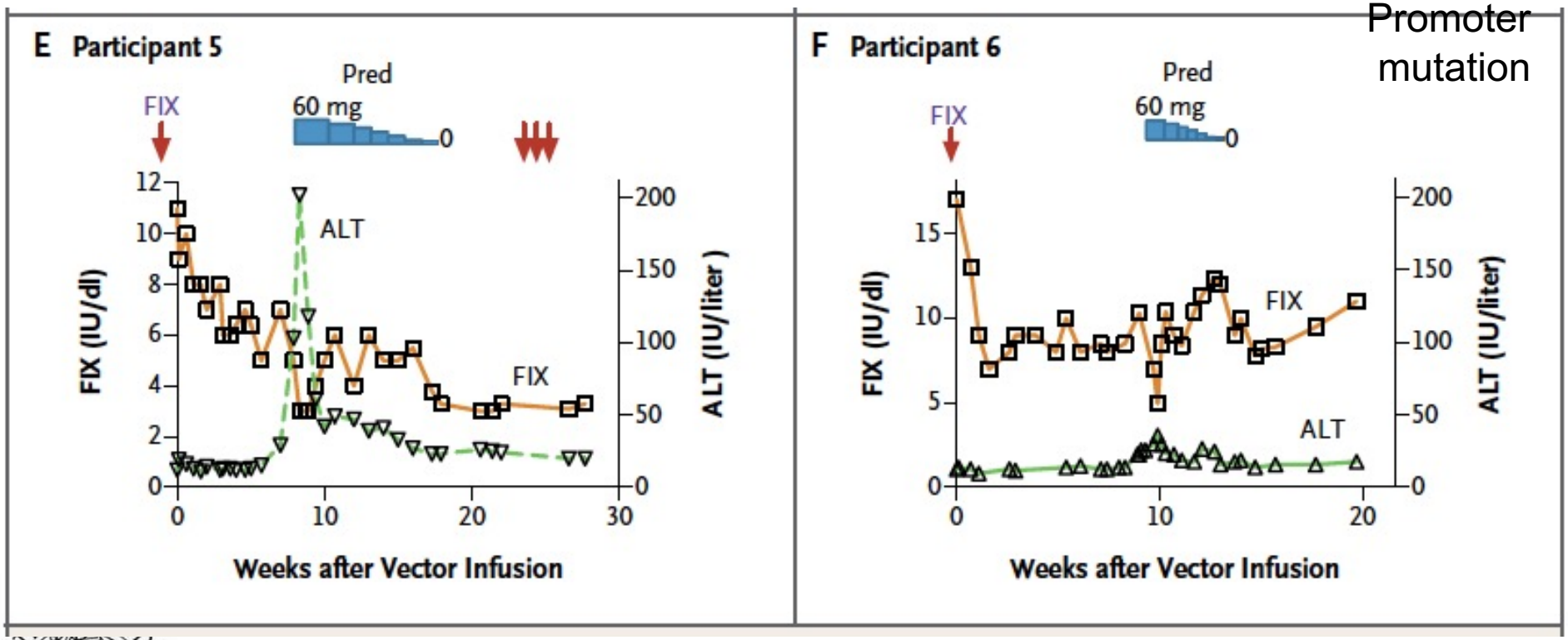
The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 DECEMBER 22, 2011 VOL. 365 NO. 25

Amit C Nathwani et al.

F9

Promoter mutation



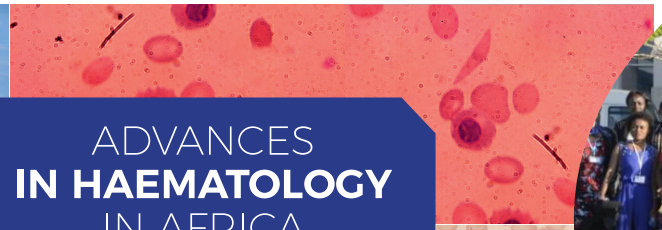
Intravenous infusion of Factor IX AAV vector corrects severe hemophilia B

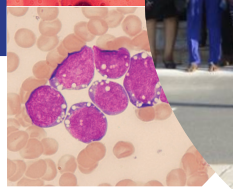
Table 1. Genome editing and gene therapy clinical trials in SCD as of March 2021

Goal	Nuclease/target	Sponsor, collaborator	Clinical trial ID	Estimated participants
Elevate HbF	CTX001/BCL11A	Vertex Pharmaceuticals Incorporated, CRISPR Therapeutics	NCT03745287	45
Elevate HbF	Plerixafor/BCL11A	Bioverativ, a Sanofi company	NCT03653247	8
Elevate HbF	OTQ923 or HIX763/ BCL11A	Novartis Pharmaceuticals	NCT04443907	30
Goal	**Viral vector**	**Sponsor, collaborator**	**Clinical trial ID**	**Estimated participants**
Repair HbS mutation	Lenti/G-βAS3-FB lentiviral Vector	California Institute for Regenerative Medicine	NCT02247843	6
Elevate HbF	ARU-1801	Aruvant Sciences GmbH	NCT02186418	10
Repair HbS mutation	GLOBE1 lentiviral vector expressing the βAS3 globin gene	Assistance Publique–Hôpitaux de Paris	NCT03964792	10
Repair HbS mutation	LentiGlobin BB305 lentiviral vector	bluebird bio	NCT04293185	35
Repair HbS mutation	Lentiviral vector encoding the normal β-globin gene	Memorial Sloan Kettering Cancer Center, Sanofi	NCT02193191	39
Repair HbS mutation	LentiGlobin BB305 lentiviral vector	bluebird bio	NCT02140554	50
Elevate HbF	Lentiviral vector containing a short hairpin RNA targeting BCL11A	Boston Children's Hospital	NCT03282656	15

(From Jones & DeBaun, Blood **138:**942,2021)

AUGUST 13th - 16th, 2018





ADVANCES IN HAEMATOLOGY IN AFRICA

MUHIMBILI UNIVERSITY HOSPITAL
Dar-es-Salaam, TANZANIA

ORGANIZED BY
Muhimbili National Hospital (MNH)
Muhimbili University of Health and Allied Sciences (MUHAS)

PROMOTED BY
Fondazione Internazionale Menarini

Day 1 / **Sickle cell anaemia**
Day 2 / **Haematological Malignancies**
Day 3 / **Red Cell Disorders**
Day 4 / **Haemostasis, Lab Haematology, Blood Transfusion**

COURSE DIRECTORS:

Julie Makani and Lucio Luzzatto
Professors of Haematology
Muhimbili University of Health and Allied Sciences
Dar-es-Salaam, Tanzania

INVITED SPEAKERS:

N. Bazuaye, Nigeria
F. Caligaris-Cappio, Italy
C. Camaschella, Italy
M. Cavazzana, France
M. Cazzola, Italy
E. Costa, Italy
E. Gluckman, France
E. Kaale, Tanzania
A. Karadimitris, UK
M. Laffan, UK
M. Lyimo, Tanzania
P. Magesa, Tanzania
A. Magesa, Tanzania
A. Makubi, Tanzania
P. Manseru, Tanzania
T. Mughal, USA
E. Mwaikambo, Tanzania
B. Ngasia, Congo
S. Nkya, Tanzania
S. Ofori-Acquah, Ghana
F. Olopade, Nigeria
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S. Rwezaula, Tanzania
M. Sadelain, USA
P. Scanlan, Tanzania
A. Schuh, UK
E. Tebuka, Tanzania
V. Tubman, USA
S. Uyoga, Kenya
R. Ware, USA

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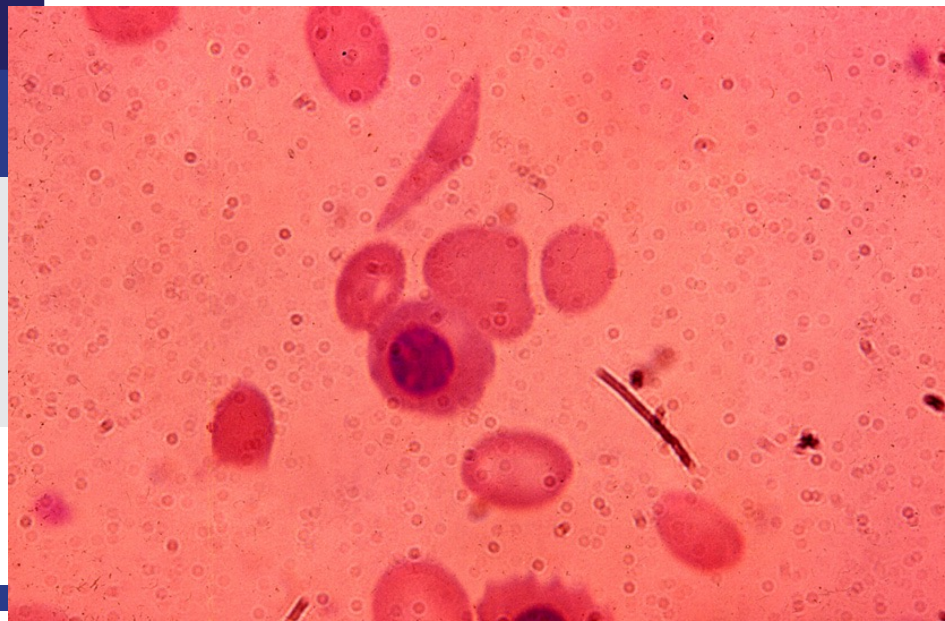

American Journal of Hematology AJH

Amer J Hematol
97:1505,2022

EDITORIAL

Blood diseases in Africa: Redressing unjust disparities is an urgent unmet need

Julie Makani ✉, Marina Cavazzana, Kalpna Gupta, Obiageli Nnodu, Isaac Odame, Leon Tshilolo, Russell Ware, Lucio Luzzatto ✉

- **Adding SCD**
  to the triad of conditions (HIV, tuberculosis, malaria) for which cost of treatment is born by the Global Fund.
- **BMT solidarity programme:**
  for every BMT (HSCT) procedure in Europe/US, 0.1% of the expense could be deposited into a fund to support BMT in accredited centers in Africa.
- **Rare Disease treatment matching programm**e:
  for every patient treated with a super-expensive drug (e.g. eculizumab) reimbursed by NHS/insurance, the manufacturer offers the drug to one patient with the same disease in Africa.

THANKS to:

Ibadan

Olugbemiro SODEINDE
Yinka FALUSI
Emanuel NWACHUKU
Olaniyi BABALOLA
Ulrich BIENZLE

Napoli

Bruno ROTOLI
Salvatore FORMISANO

London

Ted GORDON-SMITH
Frank GROSVELD
Tony PINCHING

New York

David ARATEN
Farid BOULAD
Michel SADELAIN
Stefano RIVELLA

Dar-es-Salaam

Julie MAKANI
Furaini TLUWAY
Fredrick LUONGO
Florence URIO
Bruno MMBANDO
Mwashungi ALLY