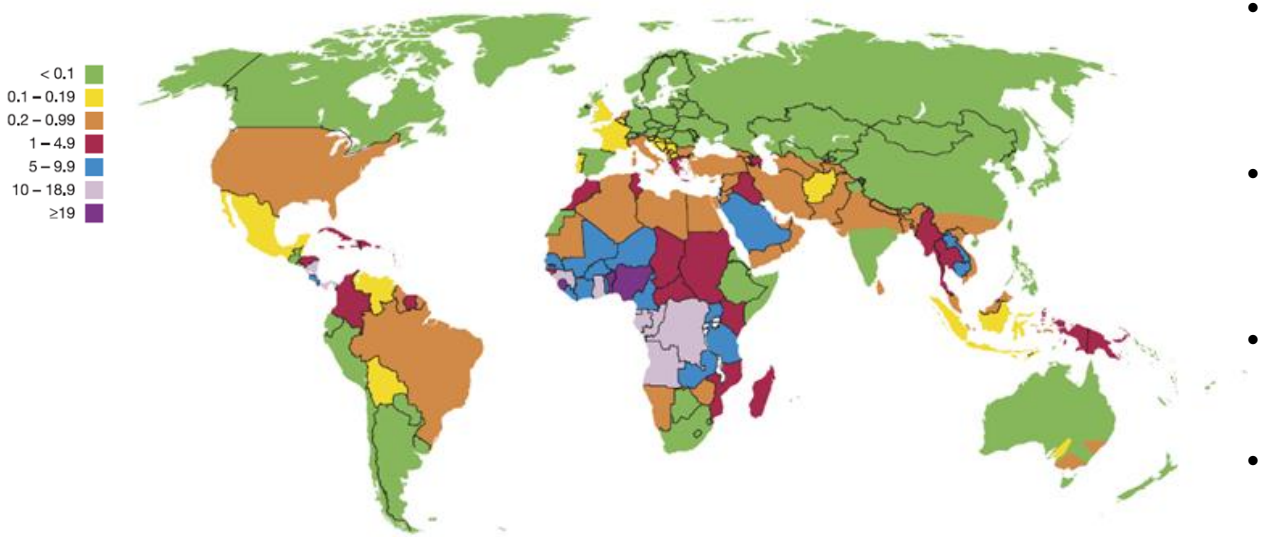
Gene therapy in beta-haemoglobinopathies

Pr Michaela Semeraro, MD PhD
Centre d'Investigations Cliniques, INSERM CIC1419
AP-HP.Centre – Université de Paris-Cité
UMR 1343 Pharmacologie et évaluation des thérapeutiques chez l'enfant et la femme enceinte

The hemoglobinopathies

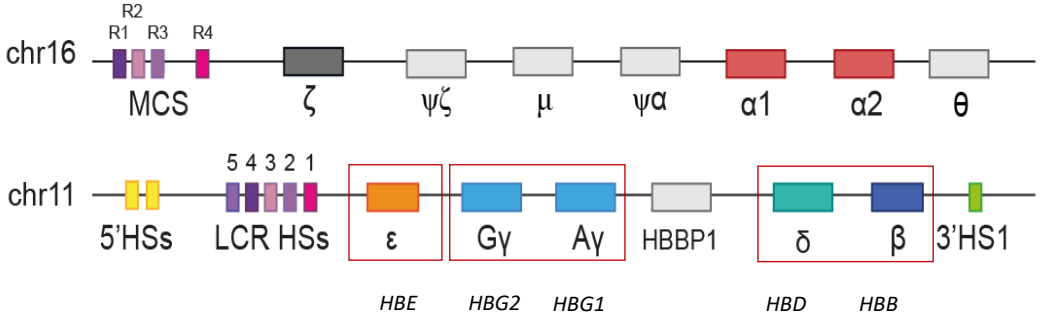


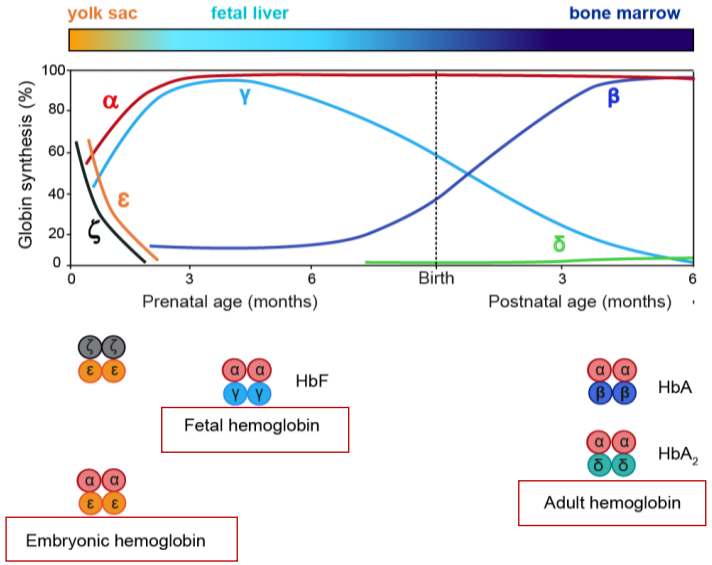
Births with a pathological Hb disorder per 1,000 live births

- **>350,000 children are born each year with a severe inherited Hb disorder**
- **~ 80% of affected children are born in low or middle income countries**
- **3.4% of deaths in children aged under 5 years**
- **>9 million carriers become pregnant annually**
- **5.2% of the world population carries a significant variant**

WHO, June 2008; Piel FB,
Hematol Oncol Clin N Am, 2016

Organization AND regulation of globin genes

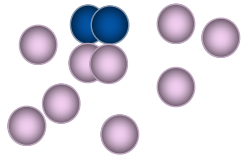




12-16 g/dl Hb

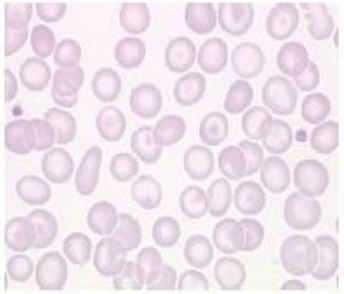
Beta-thalassemia

Reduced or absent synthesis of β-globin chain (α-globin precipitates)



Intramedullary death of red blood cell precursors

Anemia

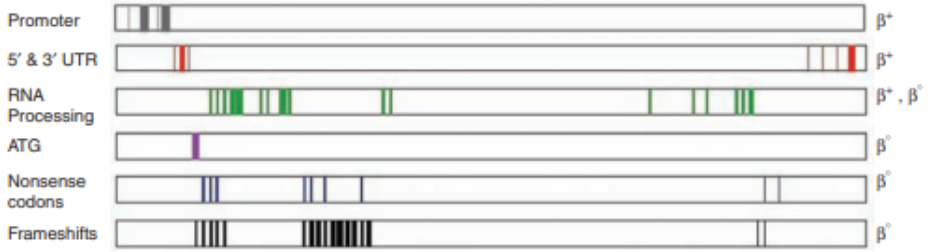


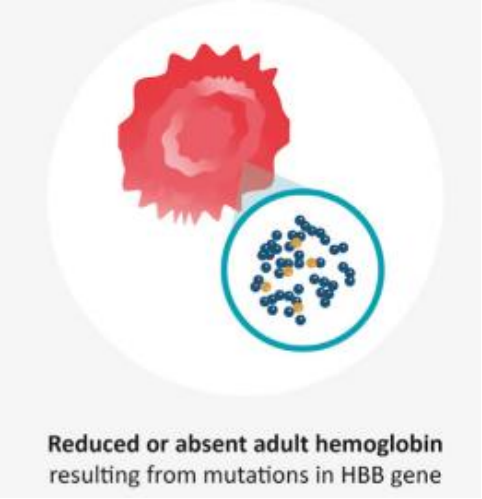
Anémie hypochrome microcytaire

Schematic representation of the type and distribution of β-thalassemia mutations

more than 350 mutations reported to date

=> Imbalance in α-/non-α-globin chains



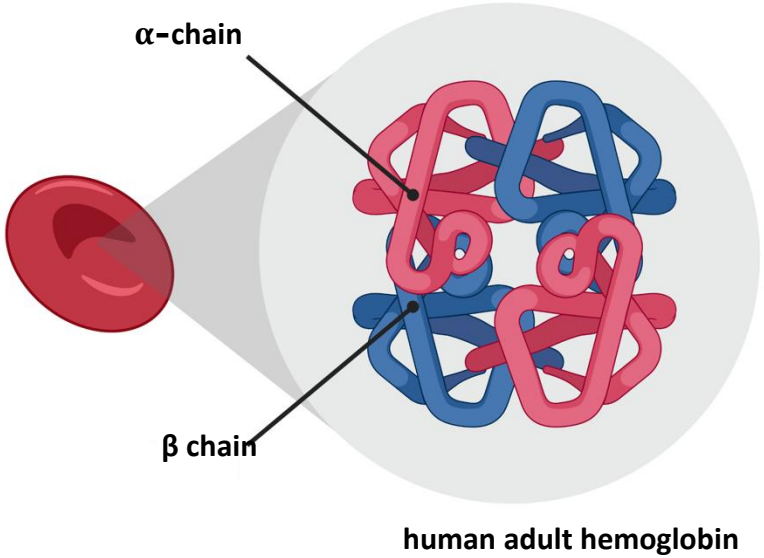


Reduced or absent adult hemoglobin resulting from mutations in HBB gene

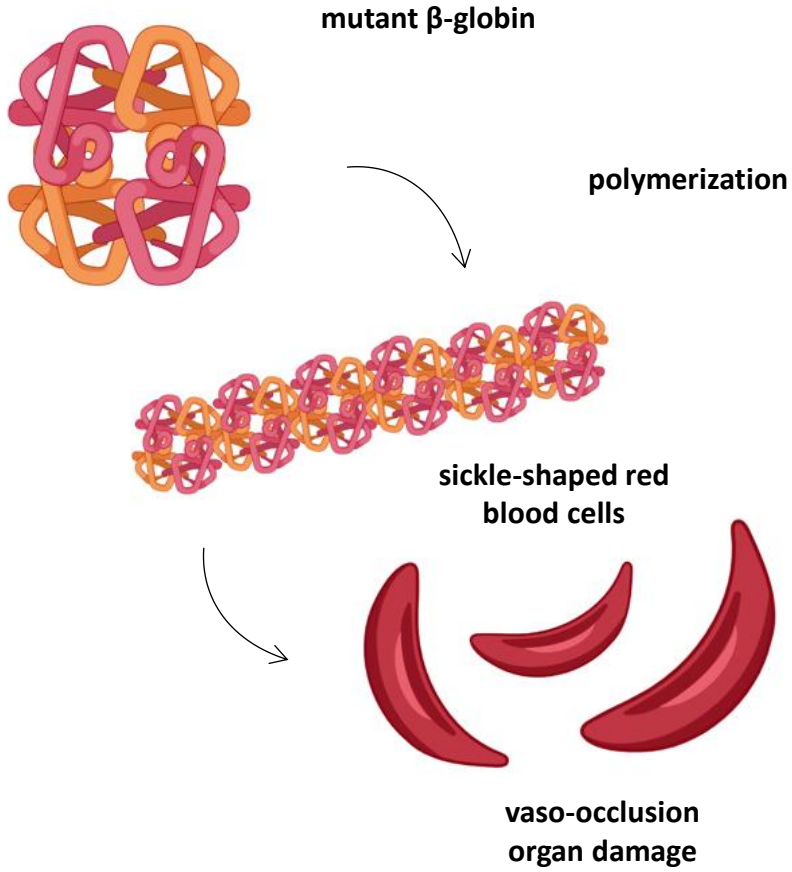
Sickle cell disease (SCD)

Genetic blood disorder caused by 1 mutation in the β-globin gene

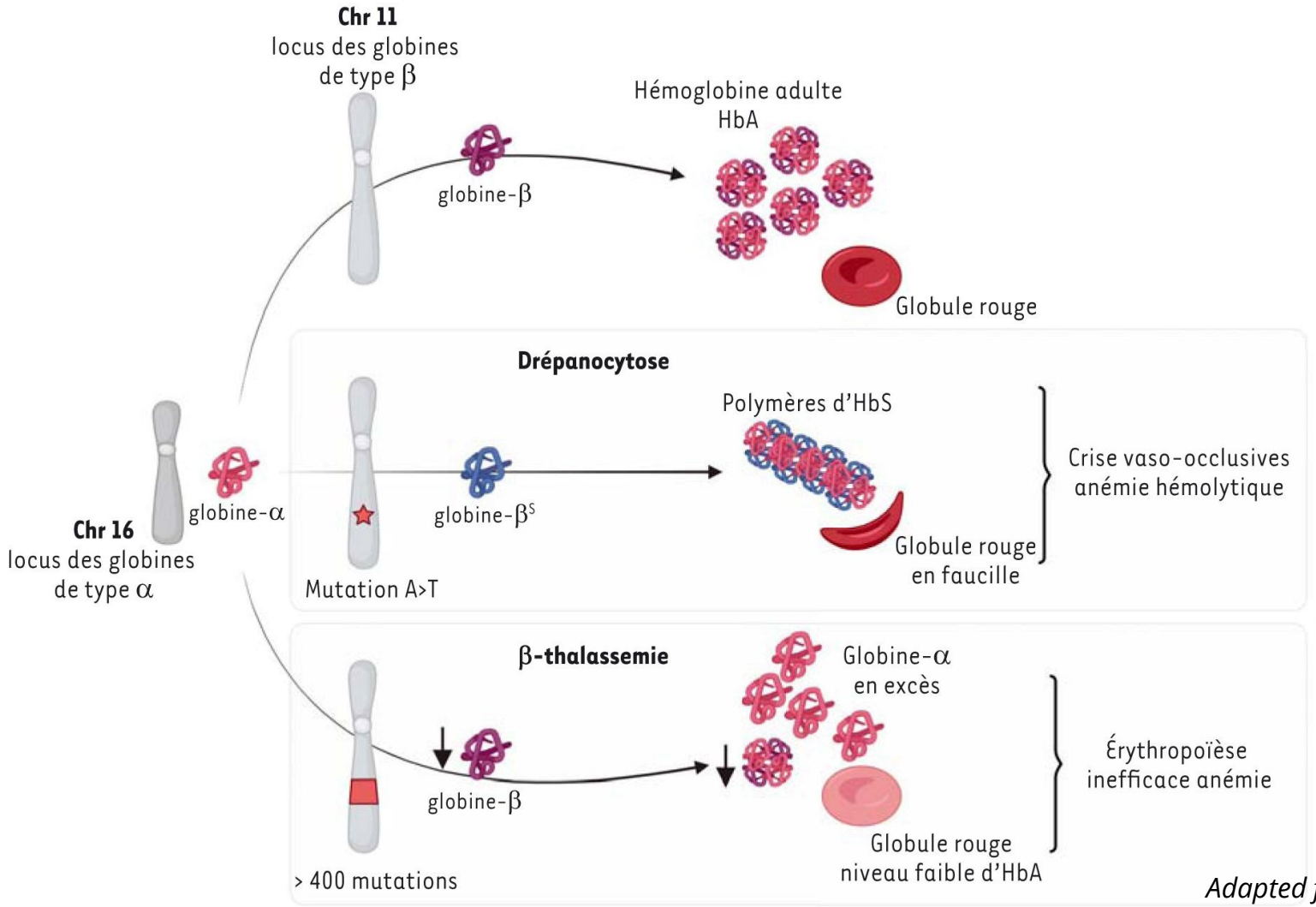
Point mutation (c.20A>T) in HBB gene
leading to the formation of βS-globin chain (Glu=>Val substitution)



human adult hemoglobin



Known molecular defect=> cutting-edge for the biomedical research in gene therapy (GT)

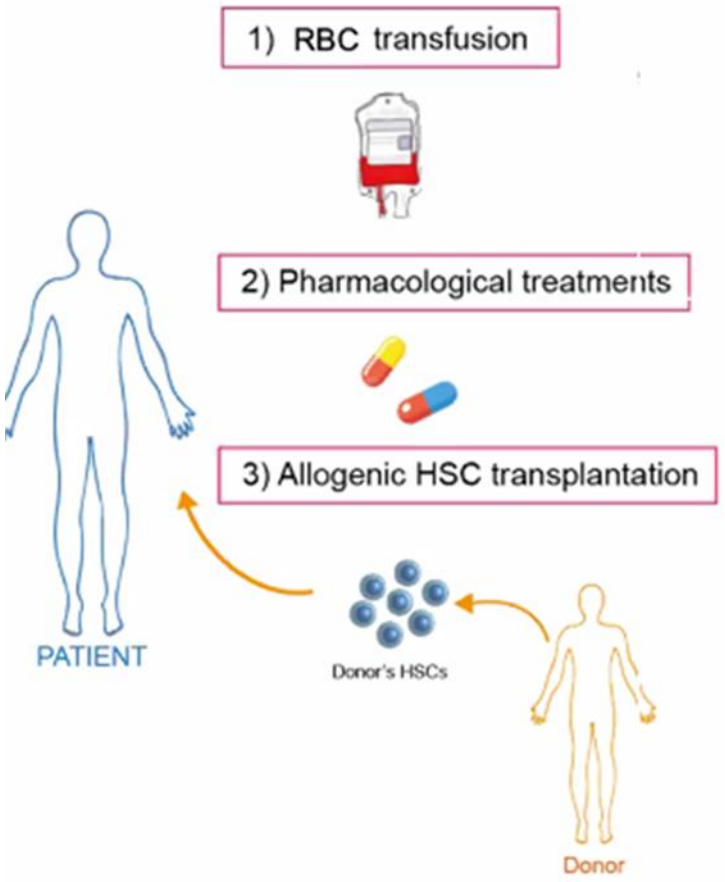


Adapted from Brusson M et al 2025 Med Sci

Are beta-hemoglobinopathies considered as an unmet medical need?

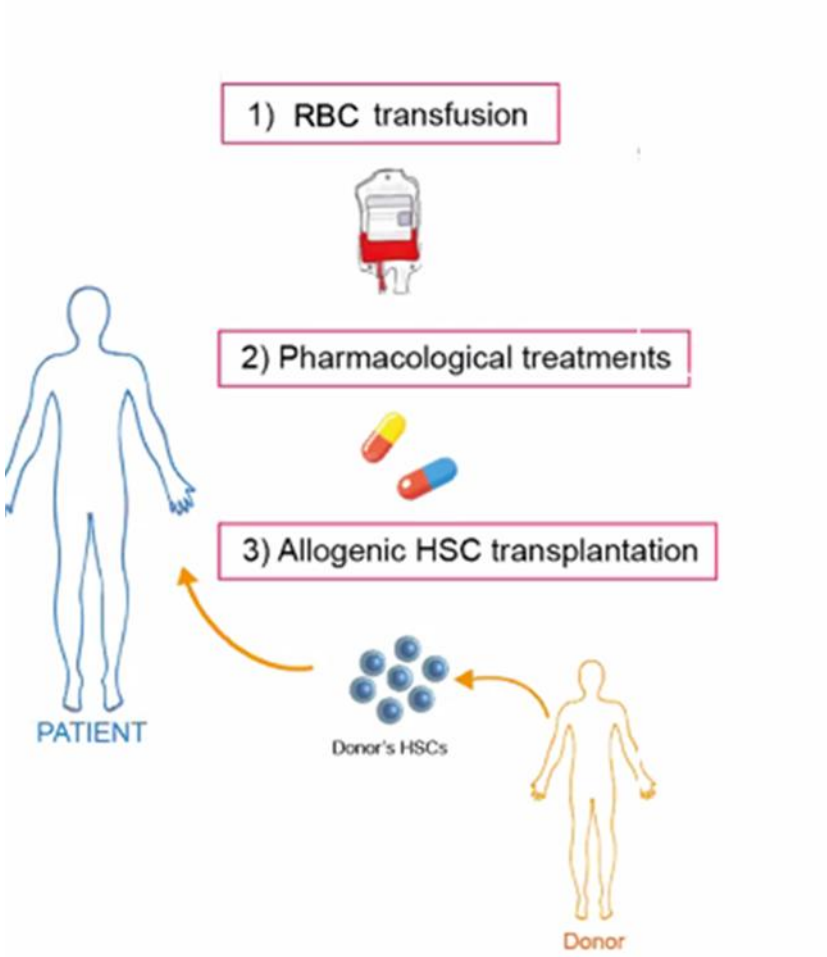
Are beta-hemoglobinopathies considered as an unmet medical need?

LIMITED CURATIVE TREATMENTS



- The only curative treatment is allogeneic hematopoietic stem cell (HSC) transplantation
- More severe cases++++
- Only ~10% of SCD patients have a histo-compatible sibling donor
    - Haplo-identical allograft: mortality 14% and cumulative risk acute + chronic GVH 18%.

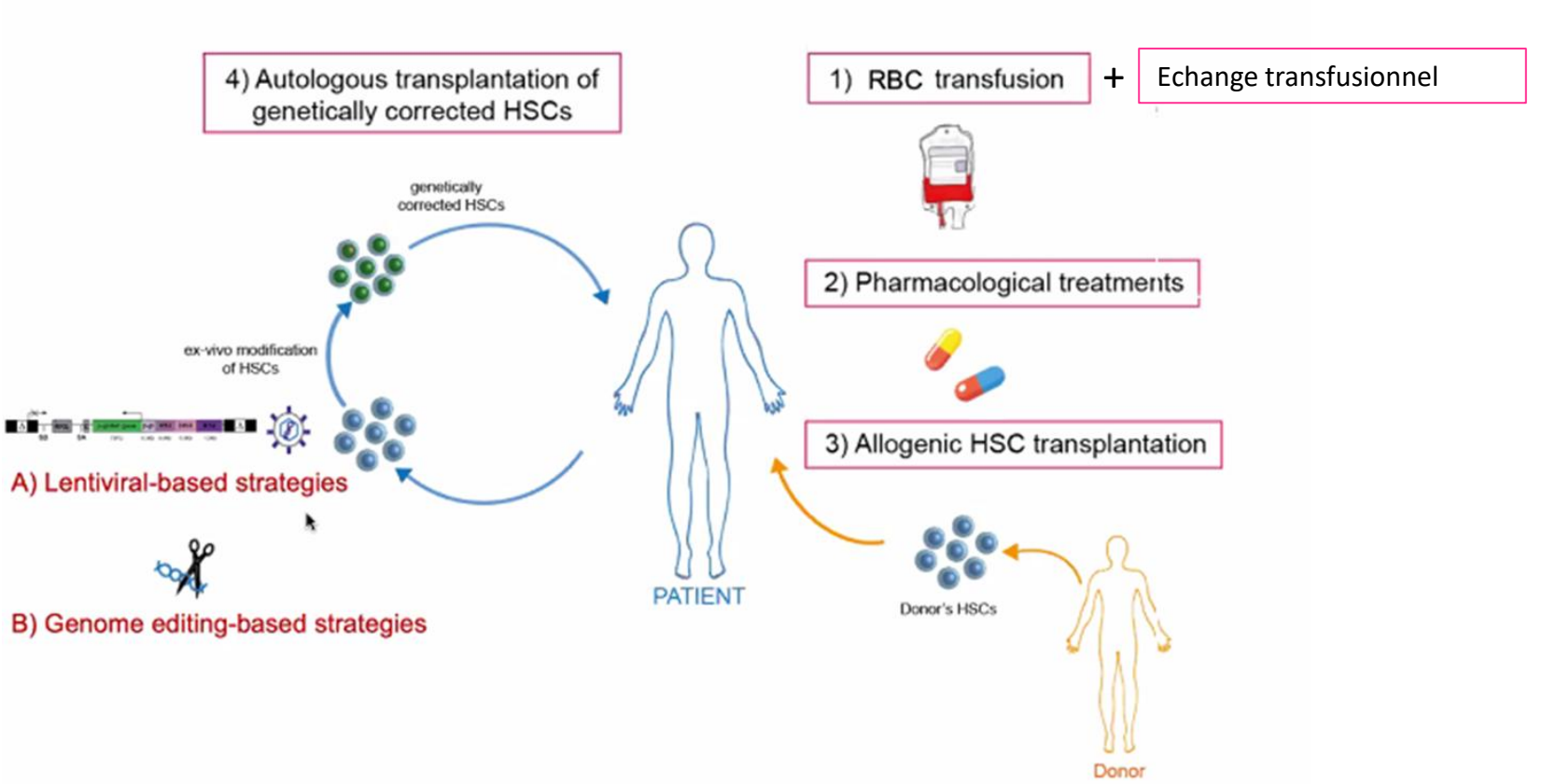
Are beta-hemoglobinopathies considered as an unmet medical need?



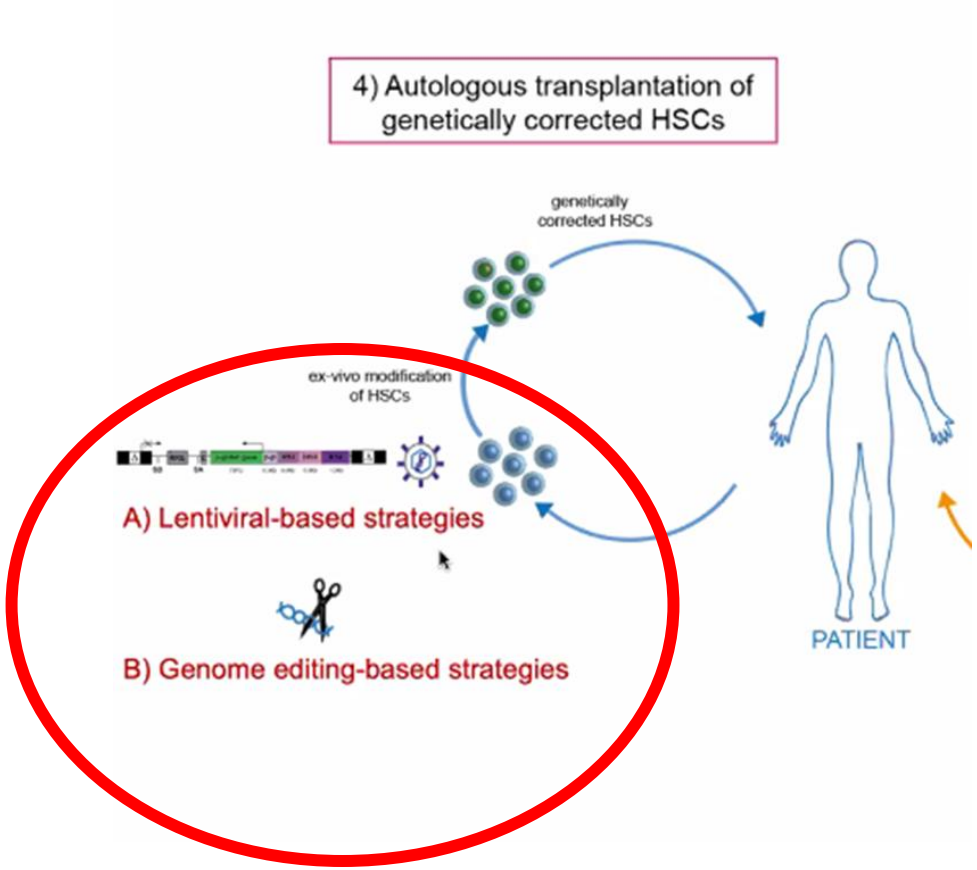
- **Chronic disease burden:** Patients require life-long management (e.g., transfusions, chelation, pain control).
- **Impact on quality of life:** High morbidity and reduced life expectancy in severe cases.
- **Healthcare disparities:** Often affects populations with limited access to advanced care.

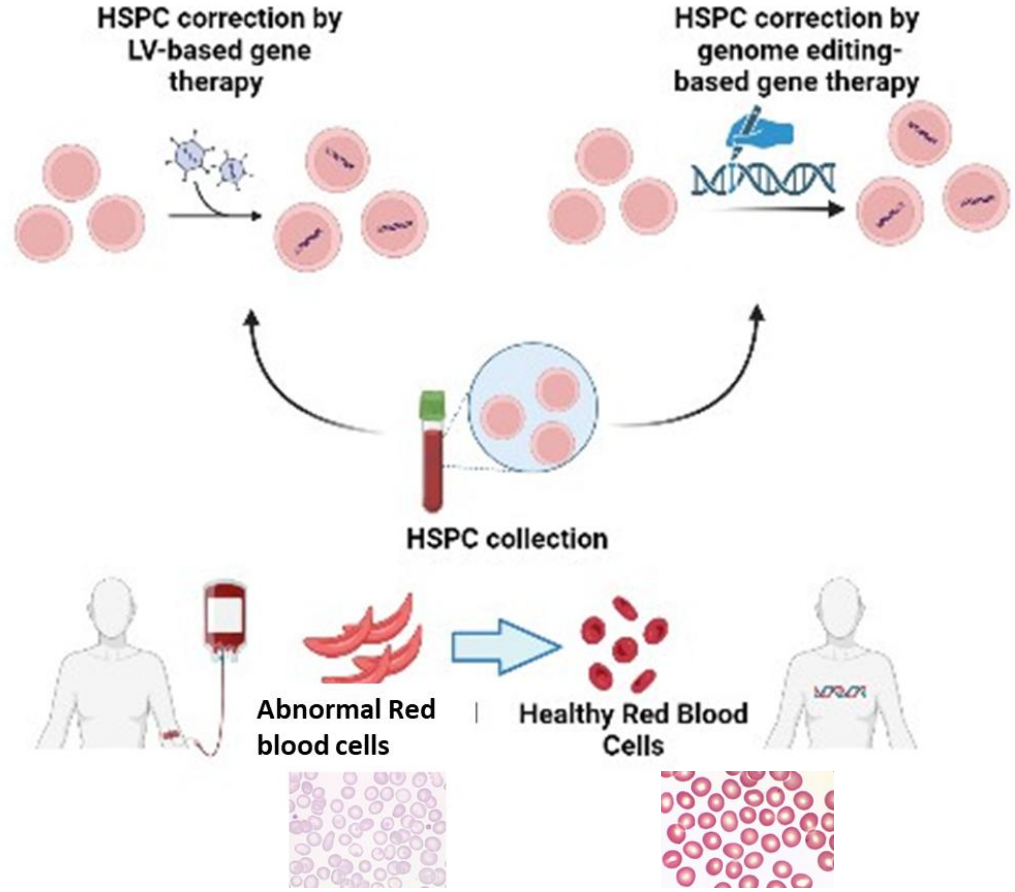
Beta-hemoglobinopathies : gene therapy options

Transplantation of autologous hematopoietic stem/progenitor cells (HSC), genetically corrected *ex vivo*, represents a promising therapeutic option for patients without a compatible donor

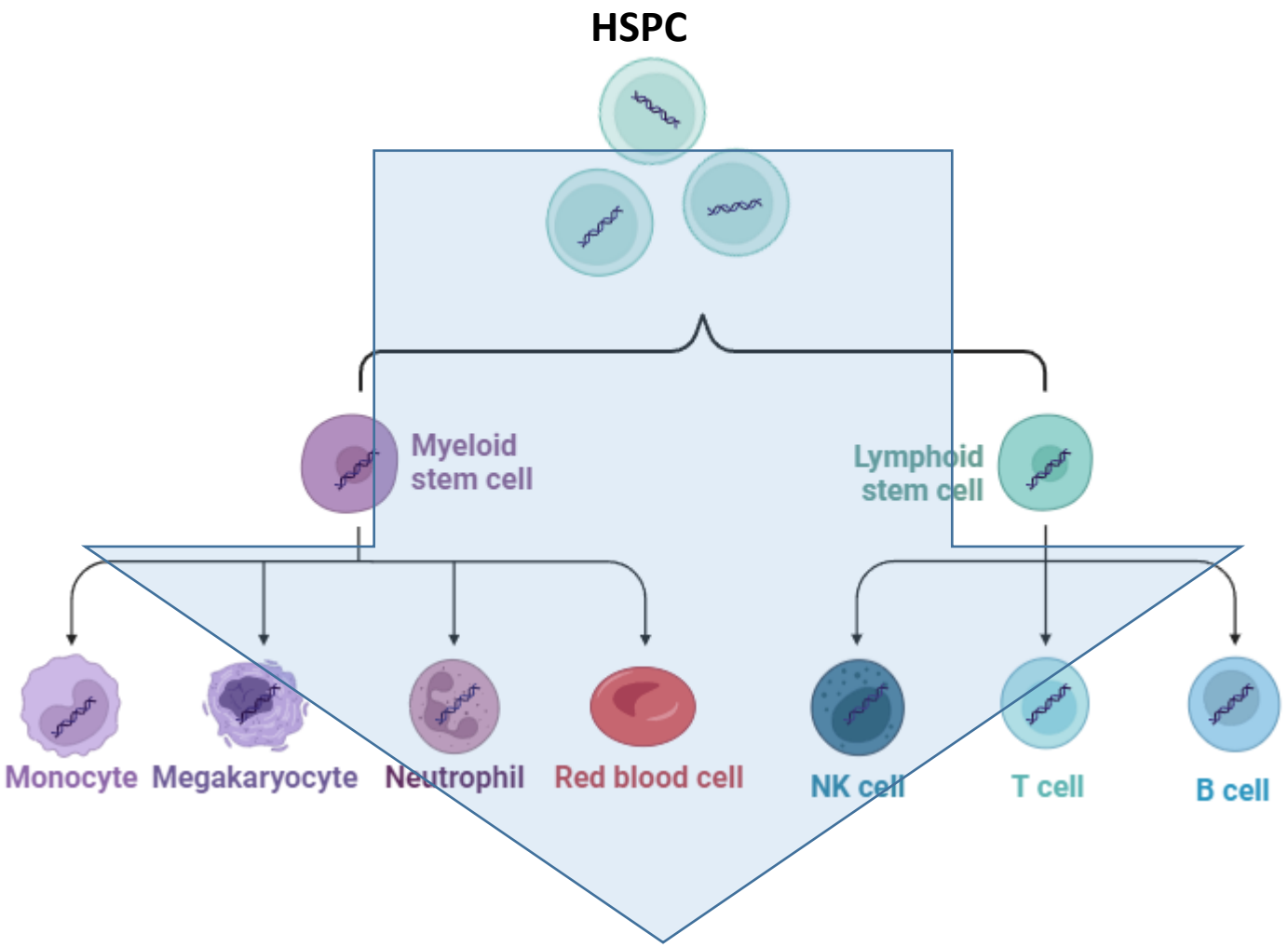


Beta-hemoglobinopathies: HSCT and gene therapy



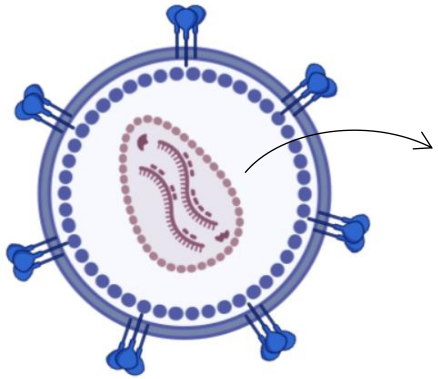


Ex vivo manufacturing
Potentially infinite applications



Gene therapy strategies for β-Hbpathies

Based on lentiviral vectors



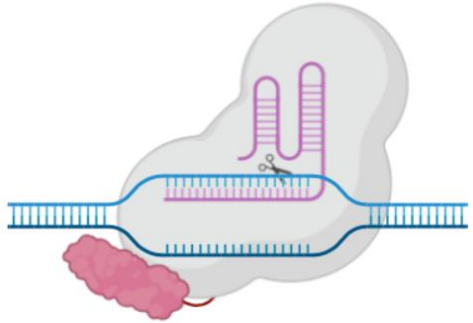
delivering a therapeutic gene that integrates in the genome

Lentiviral vector

- Viral gene therapy benefits from well-established techniques and efficient delivery systems

It faces challenges such as immunogenicity and insertional mutagenesis.

Based on genome editing



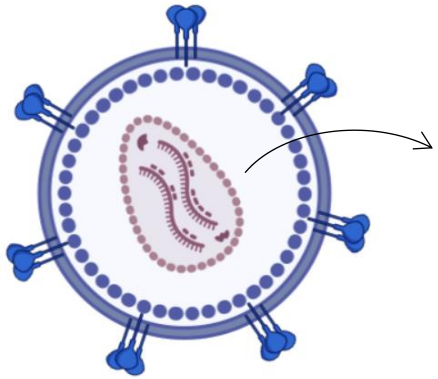
CRISPR-Cas base editor

- Targeted endogenous globin gene modification
- High-level physiological expression

Delivery challenges, off-target effects, and technical complexity

Gene therapy strategies for β-Hbpathies

Based on lentiviral vectors



delivering a therapeutic gene that integrates in the genome

Lentiviral vector

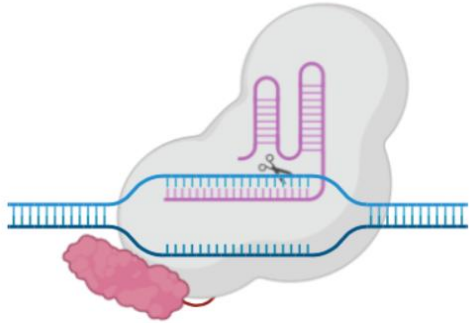
TDT (n=44)

Beti-cel (LV BB305): Phase III (n=22)
GLOBE LV: Phase I/II (n=18)
TNS9.3.55: Phase I (n=4)

SCD (n=42)

Lentiglobin (LV BB305): Phase I/II/III (n=9+35)
DREPAGLOBE: Phase I/II (n=4)
ARU-1801 (LV-HbFG16D): Phase I/II (n=4)

Based on genome editing



CRISPR-Cas base editor

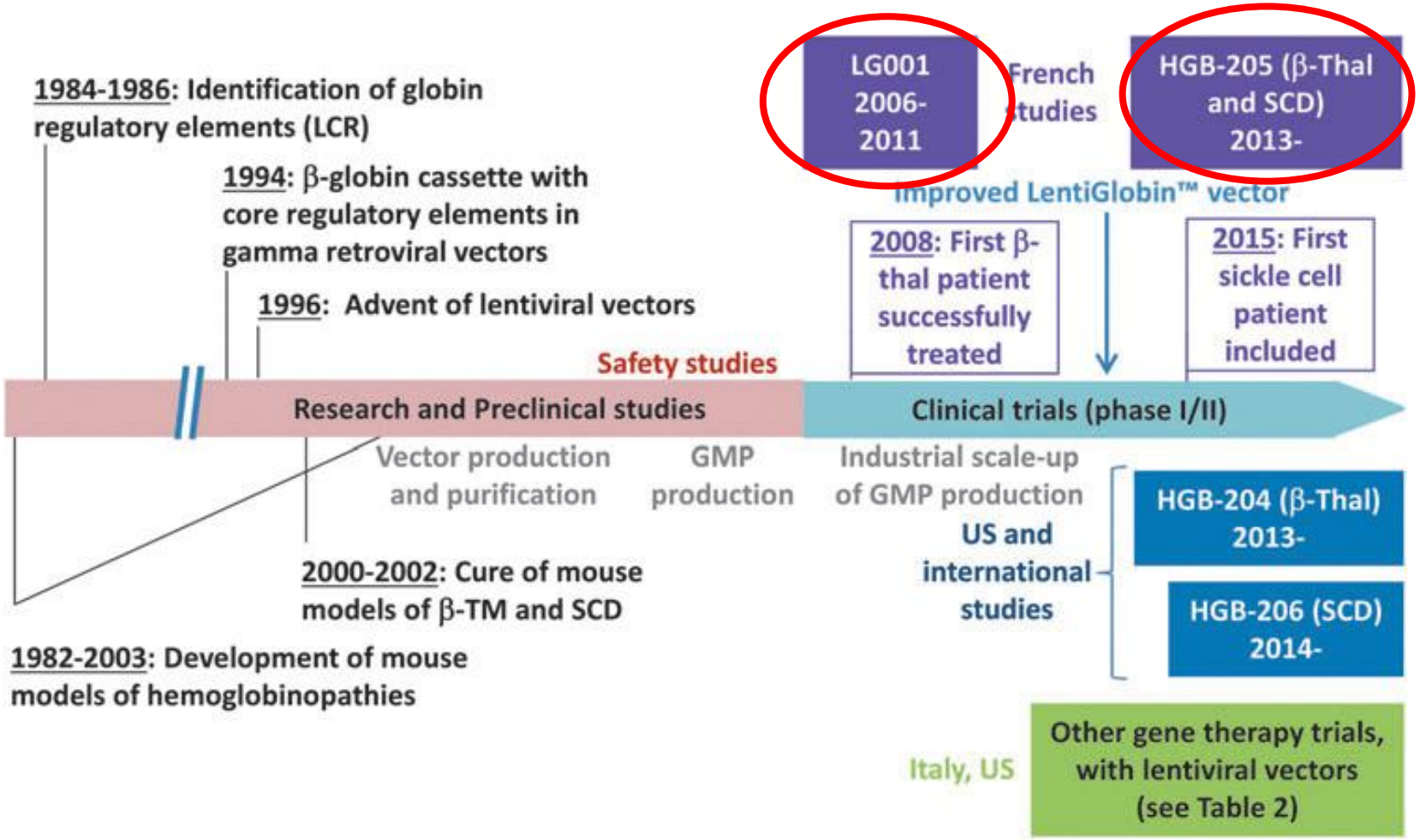
TDT (n=48)

- **THALES (ZFN disrupt BCL11a): Phase I/II (n=4)**
- **Exacel (CRISPR Cas9): Phase I/II/III (n=52)**

SCD (n=48)

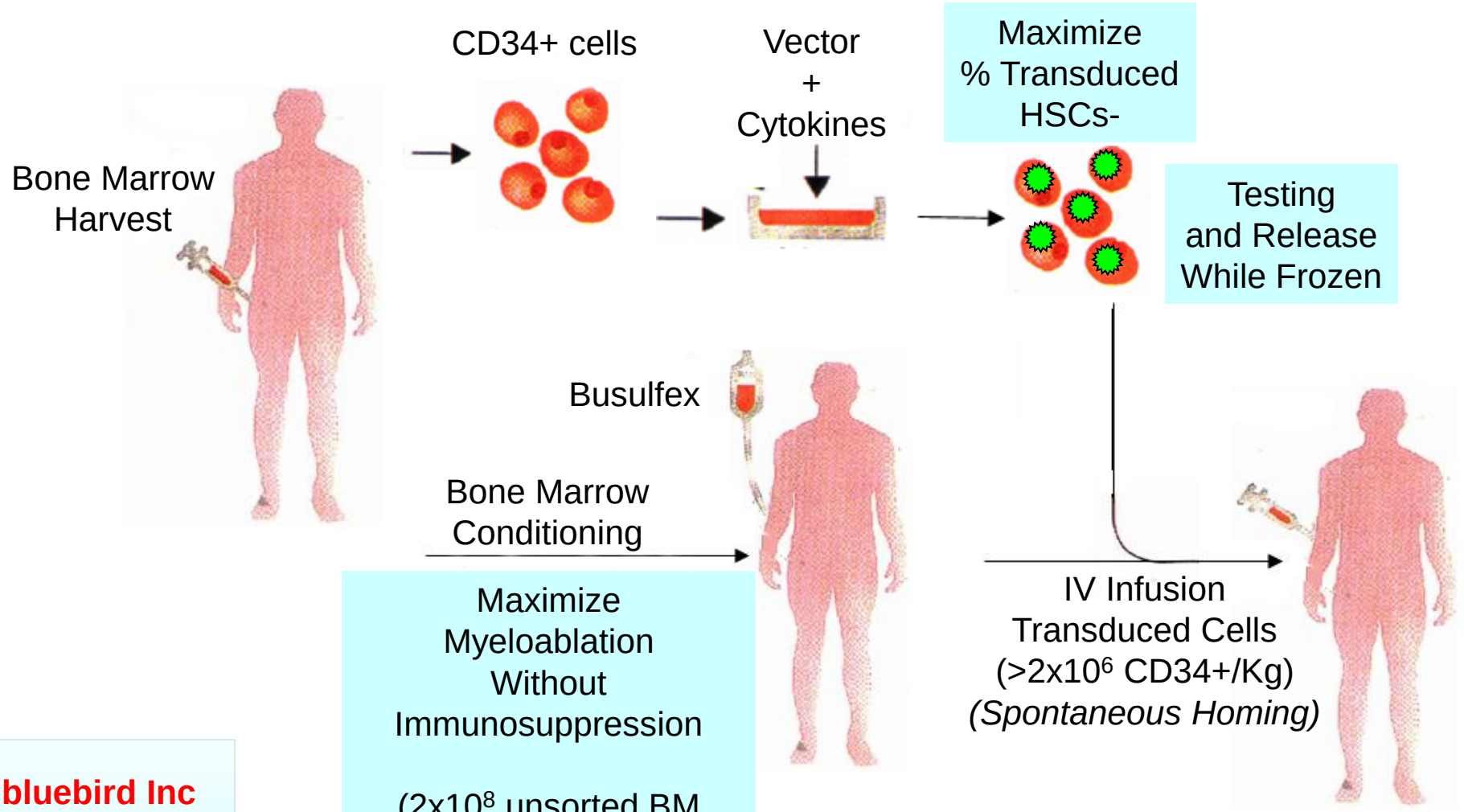
- **BIVV003 (ZFN): Phase I/II (n=4)**
- **Nula Cel (CRISPR-Cas9): Phase I/II, pause (n=?)**
- **Exa-cel (CRISPR-Cas9): Phase I/II/III (n=44)**

LV GTfor Hbpathies: not a brand new history



O. Negre et al, Human Gene Therapy 2016

HGB-205 TRIAL



Sponsor bio bluebird Inc

HGB-205: the follow-up of B-thal patients

Clinical biological complete remission for 4 patients with homozygous β thalassemia TDT

nature medicine

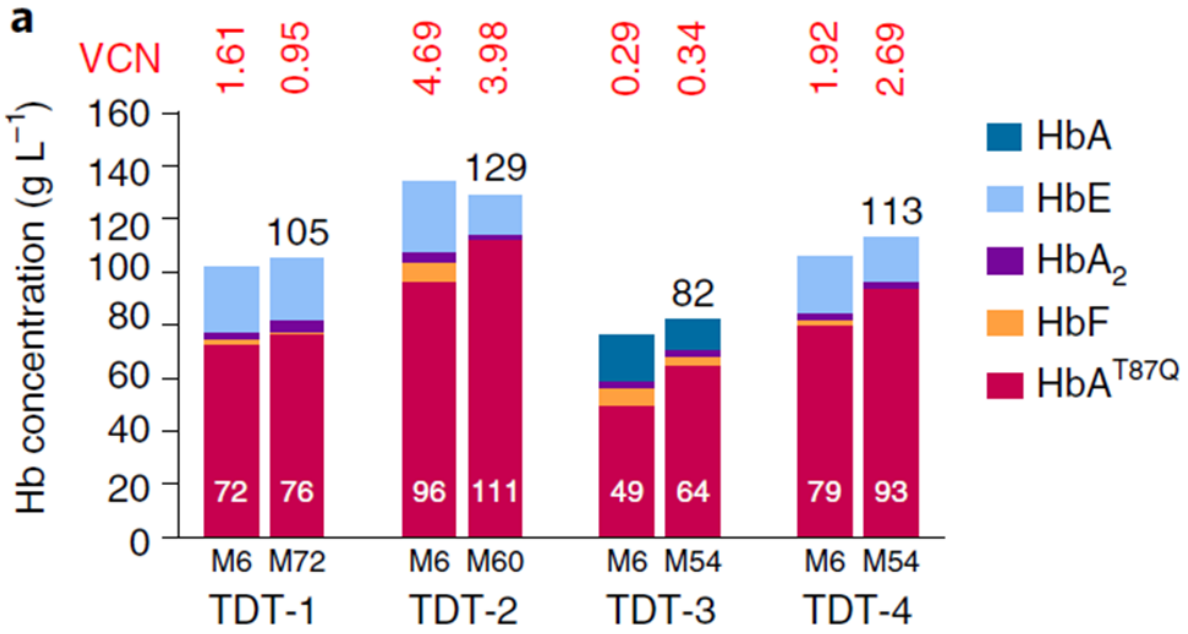
ARTICLES

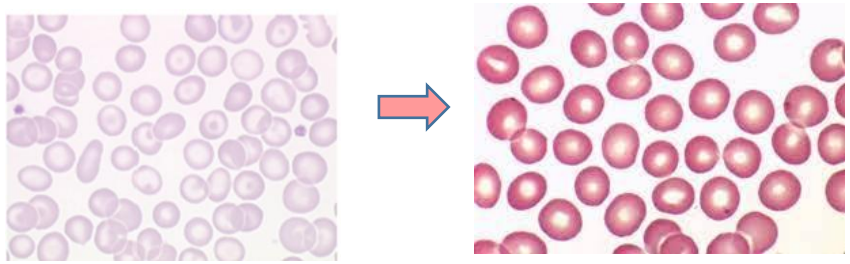
https://doi.org/10.1038/s41591-021-01650-w

Check for updates

Long-term outcomes of lentiviral gene therapy for the β-hemoglobinopathies: the HGB-205 trial

Elisa Magrin[1,2,26], Michaela Semeraro[3,4,26], Nicolas Hebert[5,6,26], Laure Joseph[1], Alessandra Magnani[1,2], Anne Chalumeau[7], Aurélie Gabrion[1,2], Cécile Roudaut[1,2], Jouda Marouene[3], Francois Lefrere[1], Jean-Sebastien Diana[1], Adeline Denis[7], Bénédicte Neven[8], Isabelle Funck-Brentano[8], Olivier Negre[9,10,27], Sylvain Renolleau[11], Valentine Brousse[12], Laurent Kiger[5], Fabien Touzot[1,2], Catherine Poirot[13,14], Philippe Bourget[15], Wassim El Nemer[16], Stéphane Blanche[8], Jean-Marc Tréluyer[3,4], Mohammed Asmal[10,27], Courtney Walls[10], Yves Beuzard[5,9], Manfred Schmidt[17], Salima Hacein-Bey-Abina[1,2], Vahid Asnafi[18], Isabelle Guichard[19], Maryline Poirée[20], Fabrice Monpoux[21], Philippe Touraine[22], Chantal Brouzes[23], Mariane de Montalembert[12], Emmanuel Payen[9], Emmanuelle Six[7], Jean-Antoine Ribeil[1,2,10,27], Annarita Miccio[7,28], Pablo Bartolucci[5,6,28], Philippe Leboulch[9,24,28] ✉ and Marina Cavazzana[4,7,25,28] ✉





HGB-205: the follow-up of SCD patients

Long-term complete remission for 2/3 patients with homozygous sickle cell disease

nature medicine

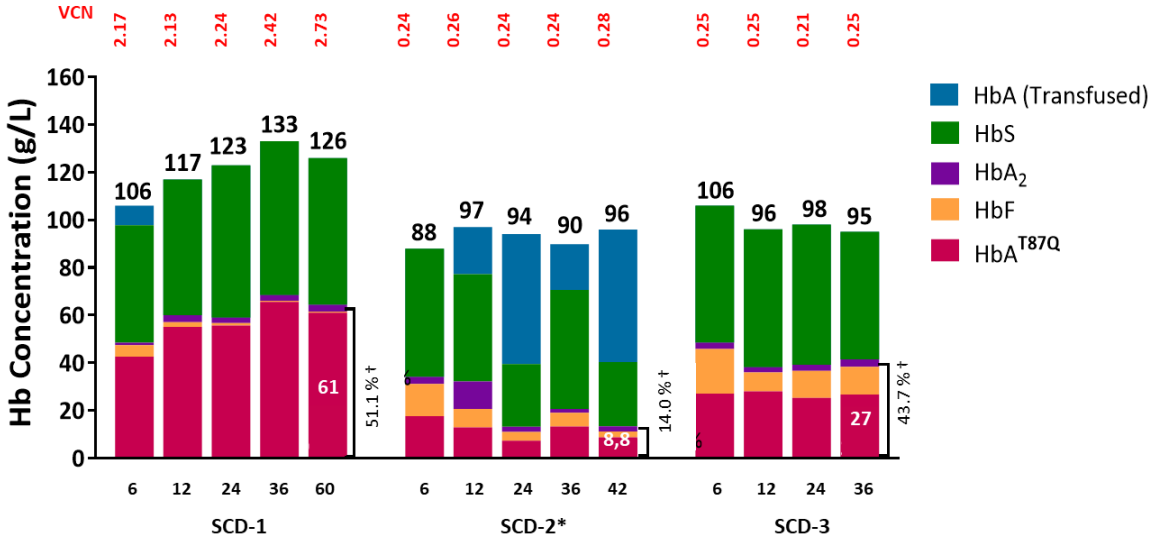
ARTICLES

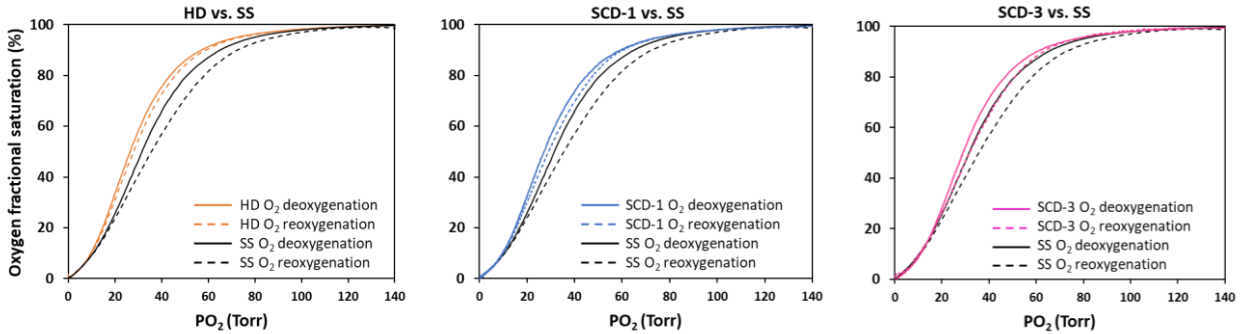
https://doi.org/10.1038/s41591-021-01650-w

Check for updates

Long-term outcomes of lentiviral gene therapy for the β-hemoglobinopathies: the HGB-205 trial

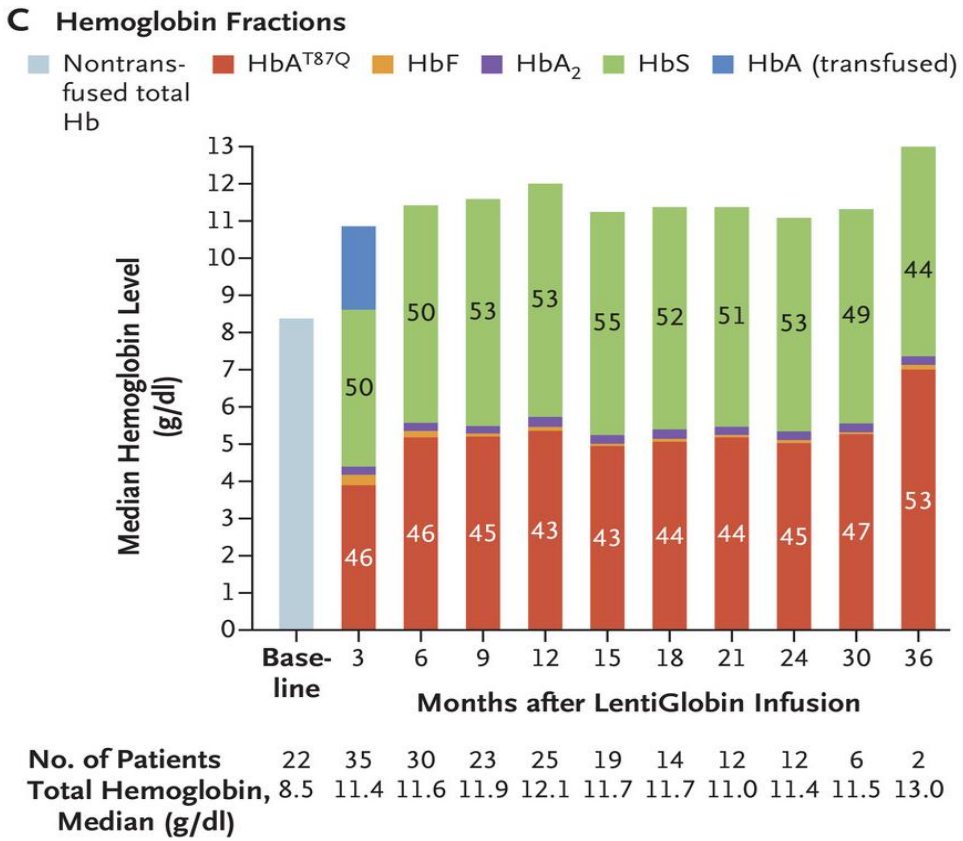
Elisa Magrin[1,2,26], Michaela Semeraro[3,4,26], Nicolas Hebert[5,6,26], Laure Joseph[1], Alessandra Magnani[1,2], Anne Chalumeau[7], Aurélie Gabrion[1,2], Cécile Roudaut[1,2], Jouda Marouene[3], Francois Lefrere[1], Jean-Sebastien Diana[1], Adeline Denis[7], Bénédicte Neven[8], Isabelle Funck-Brentano[8], Olivier Negre[9,10,27], Sylvain Renolleau[11], Valentine Brousse[12], Laurent Kiger[5], Fabien Touzot[1,2], Catherine Poirot[13,14], Philippe Bourget[15], Wassim El Nemer[16], Stéphane Blanche[8], Jean-Marc Tréluyer[3,4], Mohammed Asmal[10,27], Courtney Walls[10], Yves Beuzard[5,9], Manfred Schmidt[17], Salima Hacein-Bey-Abina[1,2], Vahid Asnafi[18], Isabelle Guichard[19], Maryline Poirée[20], Fabrice Monpoux[21], Philippe Touraine[22], Chantal Brouzes[23], Mariane de Montalembert[12], Emmanuel Payen[9], Emmanuelle Six[7], Jean-Antoine Ribeil[1,2,10,27], Annarita Miccio[7,28], Pablo Bartolucci[5,6,28], Philippe Leboulch[9,24,28] ✉ and Marina Cavazzana[4,7,25,28] ✉

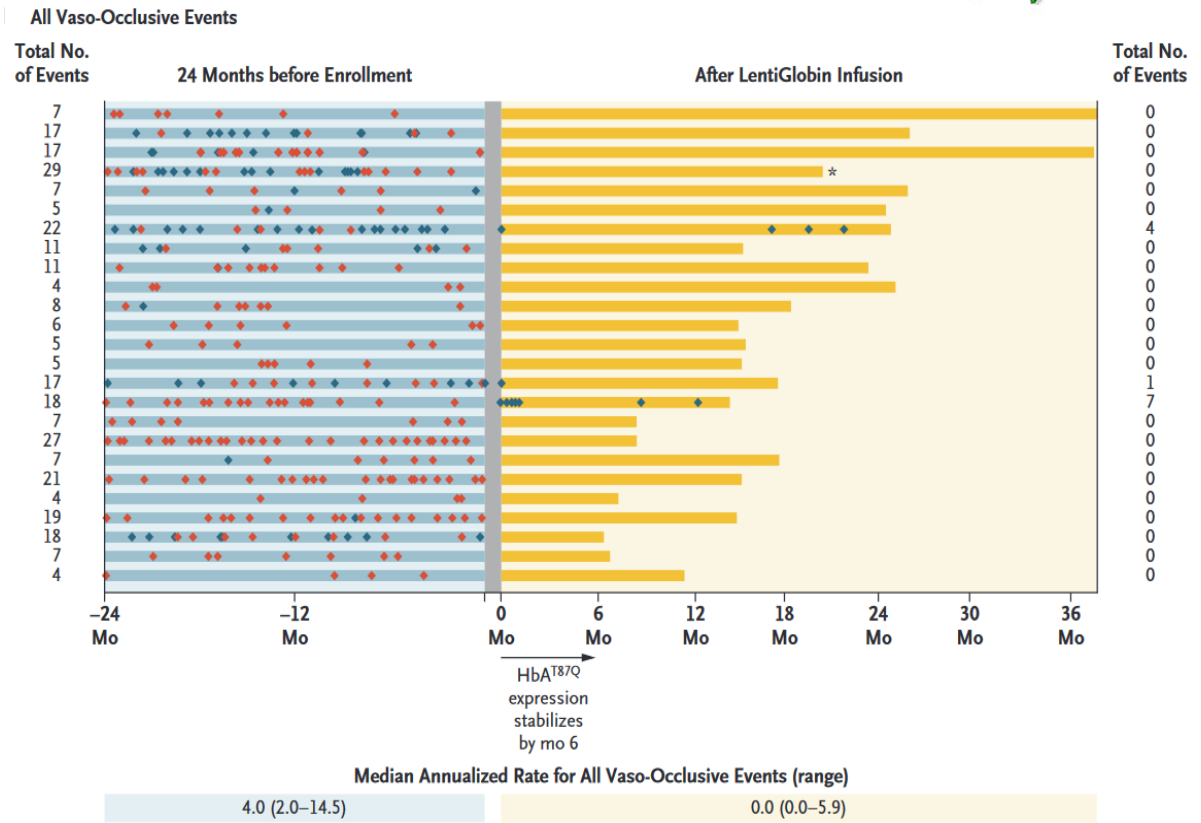




From phase 3 (HGB-206) to FDA approval

HGB-206 Group C: Median HbS ≤ 50% post LentiGlobin treatment





Decreased Hemolysis to normal value

J. Kanter et al. 2022 NEJM

Genotoxicity in gene addition strategy

- No insertional oncogenesis events in TDT
- 2 patients with SCD were diagnosed with AML

independent of insertional oncogenesis

Acute Myeloid Leukemia Case after Gene Therapy for Sickle Cell Disease

Authors: Sunita Goyal, M.D., John Tisdale, M.D., Manfred Schmidt, Ph.D., Julie Kanter, M.D., Jennifer Jaroscak, M.D., Dustin Whitney, Ph.D., Hans Bitter, Ph.D., +9, and Melissa Bonner, Ph.D. Author Info & Affiliations

Published December 12, 2021 | N Engl J Med 2022;386:138-147 | DOI: 10.1056/NEJMoa2109167 | VOL. 386 NO. 2

blood

ISSUES FIRST EDITION ABSTRACTS COLLECTIONS

PERSPECTIVE | SEPTEMBER 16, 2021

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

Clinical Trials & Observations

Richard J. Jones, Michael R. DeBaun

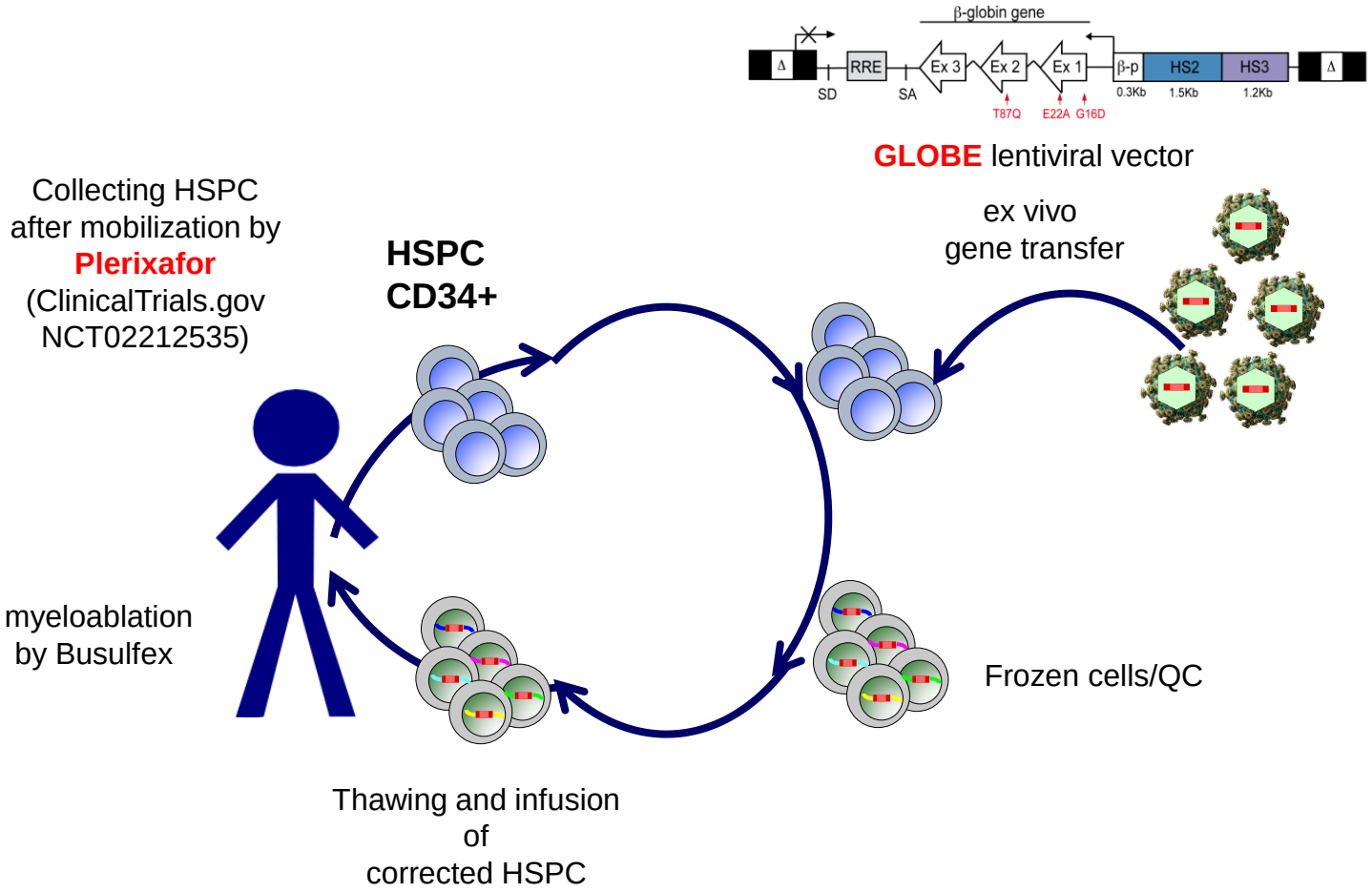
From phase 3 (HGB-206) to FDA approval

zynteglo™
(autologous CD34+ cells encoding $\beta^{A\text{-}T87Q}$-globin gene)

Zynteglo (betibeglogene autotemcel), a gene therapy for **transfusion-dependent beta-thalassemia (TDT)**,
Cost: High (over €1 million)

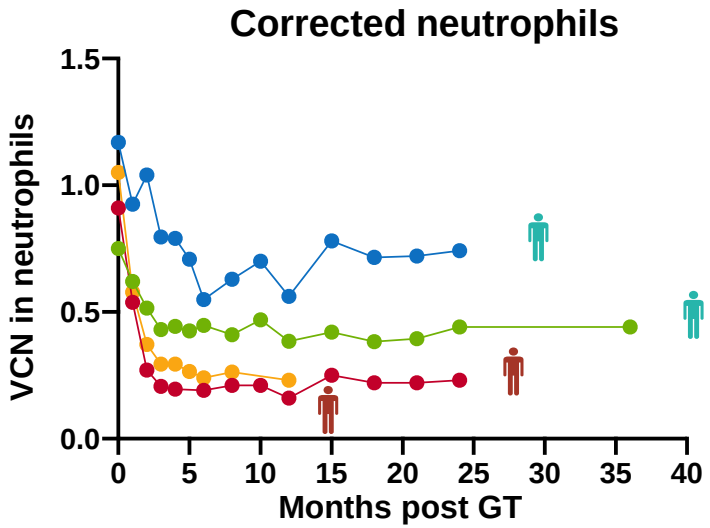
Lyfgenia (lovotibeglogene autotemcel), a gene therapy for **sickle cell disease (SCD)**, is **approved and available** in the **United States**
Cost: ~$3.1 million in the U.S.
EMA (Europe): Approval status still pending

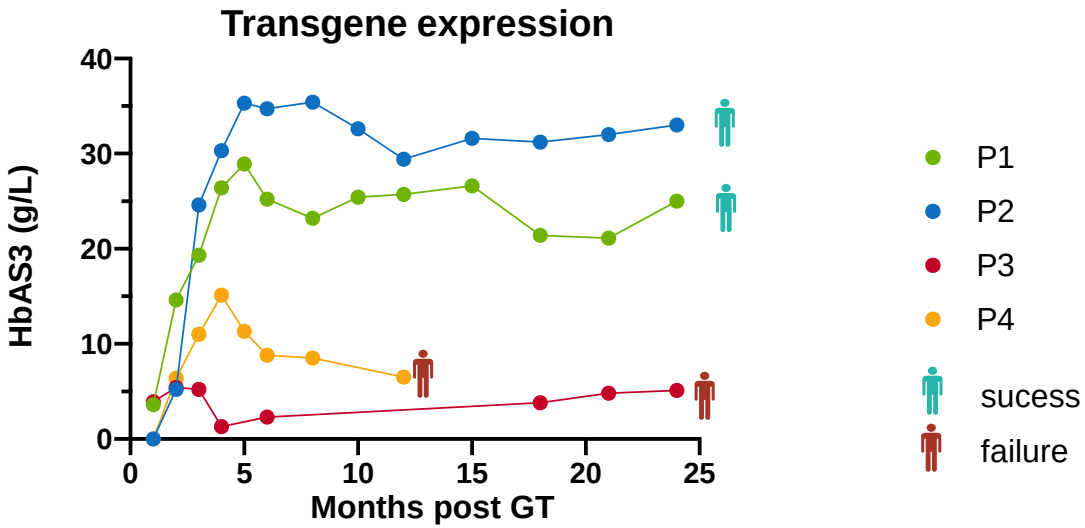
The Drepaglobe protocol (NCT03964792)



The Drepaglobe protocol: follow-up

	P1	P2	P3	P4
Patients				
Age at GT (years)	19	29	34	15
Sex	M	M	M	F
Follow up post GT (months)	33	25	21	8
Drug product				
HSPCs infused (x10^6/kg)	8.10	6.62	5.97	6.39
VCN in drug product	0.75	1.17	0.91	1.05
VCN in neutrophils at the last FU	0.41 (M27)	0.72 (M21)	0.22 (M18)	0.24 (M6)



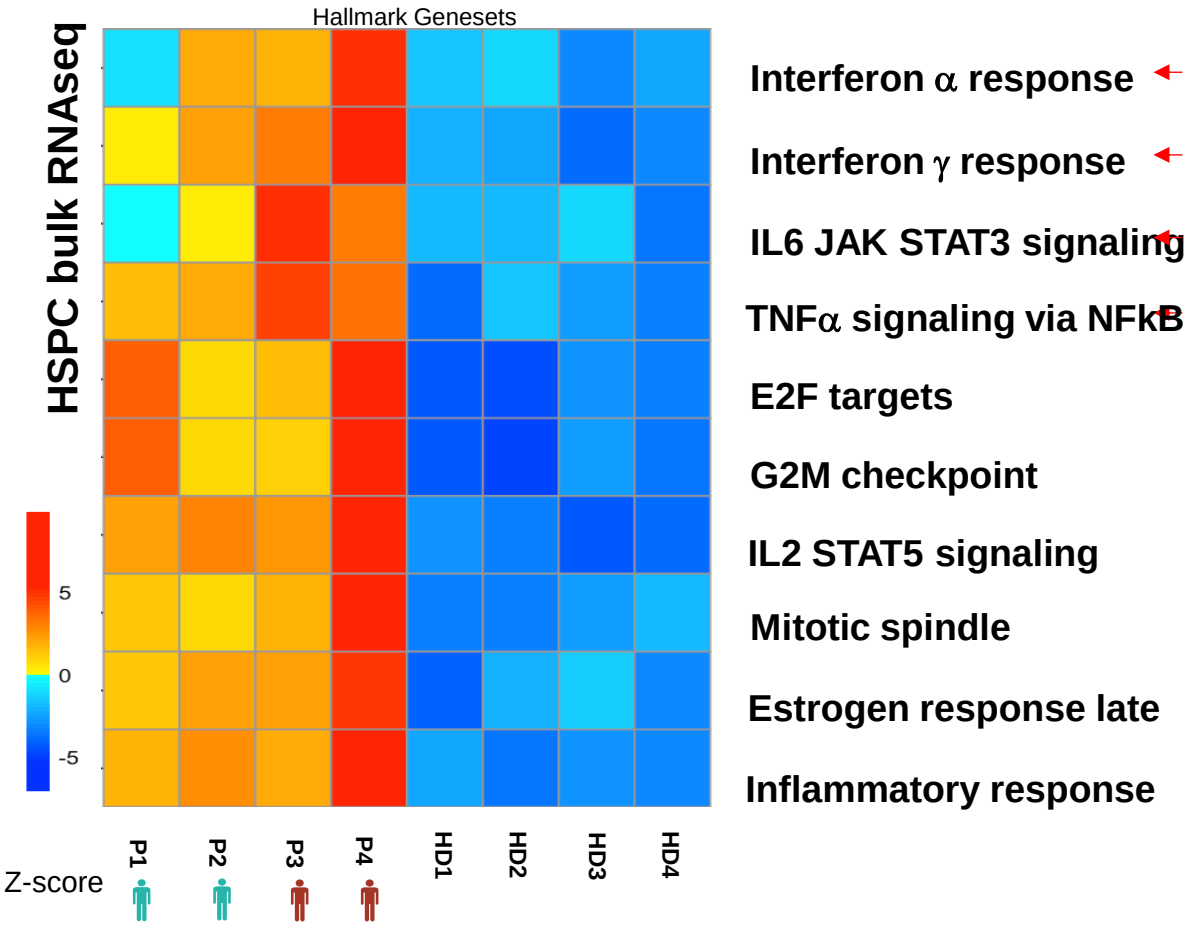


➢ **Interindividual variability in term of engraftment in SCD patients**

Sobrino et al Nat Comm 2025

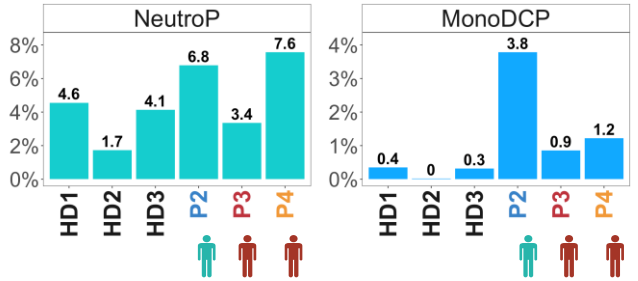
Determinants of HSC engraftment efficacy

HSPC inflammation

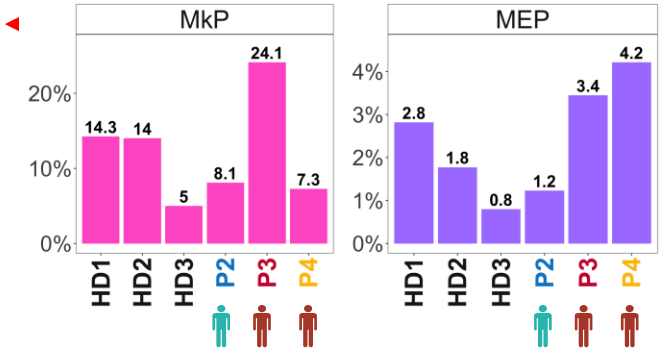


HSC lineage bias

P2 and P4 : Myeloid bias



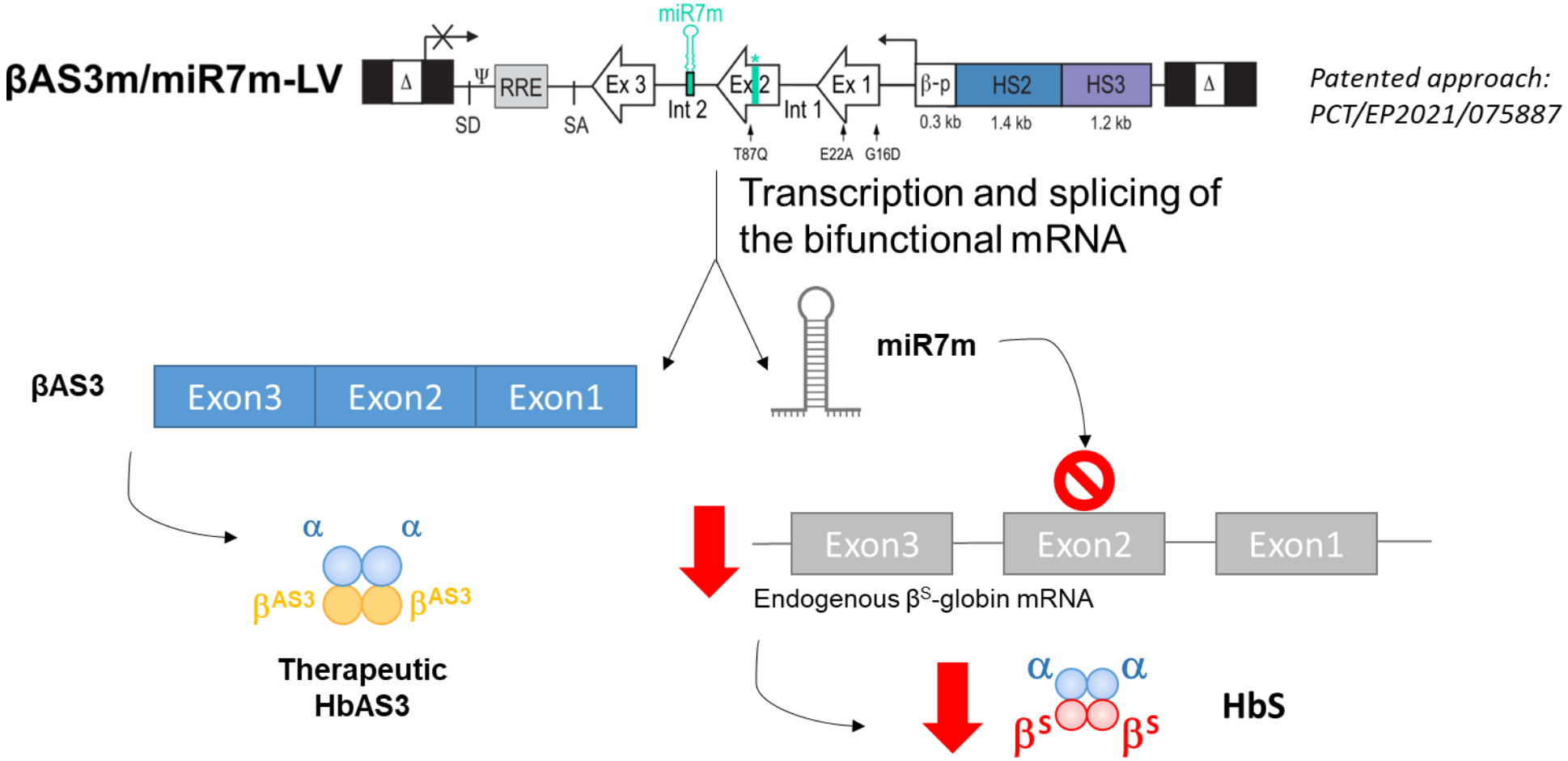
P3 : Erythro/ Megakaryocytic bias



✓ **HSC engraftment efficacy depends on their infused number, inflammatory state and lineage bias**

Sobrino et al Nat Comm 2025

Bifunctional LV meets the efficacy requirements: gene addition and gene silencing



The bifunctional βAS3m/miR7m LV downregulates β^S-globin expression and increases βAS3 incorporation in Hb and ameliorates red blood cell sickling

M Brusson et al Mol Therapy 2023

Lessons from the gene addition clinical trials

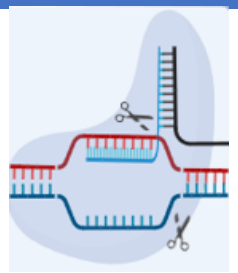
- **Myeloablative conditioning** is needed to maximize CD34+ engraftment of lentiviral-transduced cells

- **Low toxicity profile (OS>98%):**
  - 90 patients treated by lentiviral gene addition strategy
  - Low transplant comorbidities
  - 2 AML in SCD, not related to insertion

- **Heterogenous results in SCD gene therapy** linked to the diseased hematopoiesis

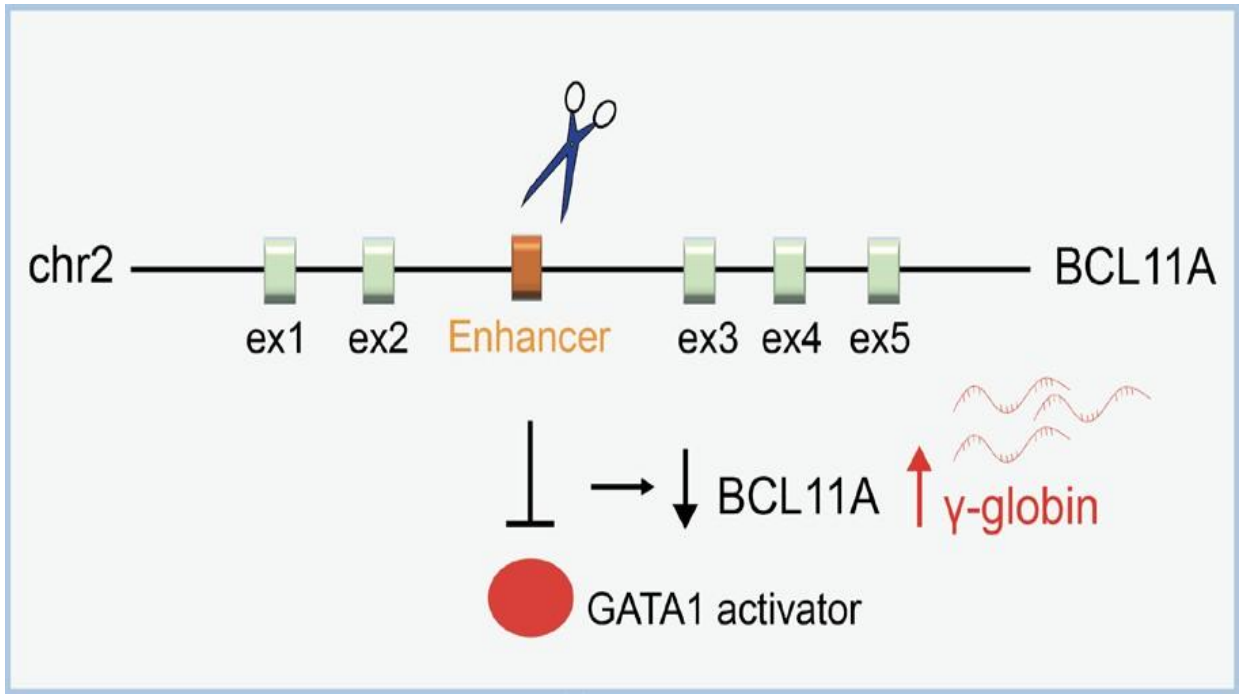
- **Perspectives:** Targeted anti-inflammatory treatment to prevent HSCs bias, bifunctional LV strategy

Gene disruption Nuclease-mediated strategy for γ-globin reactivation



γ-globin reactivation

KO of the γ-globin repressor BCL11A

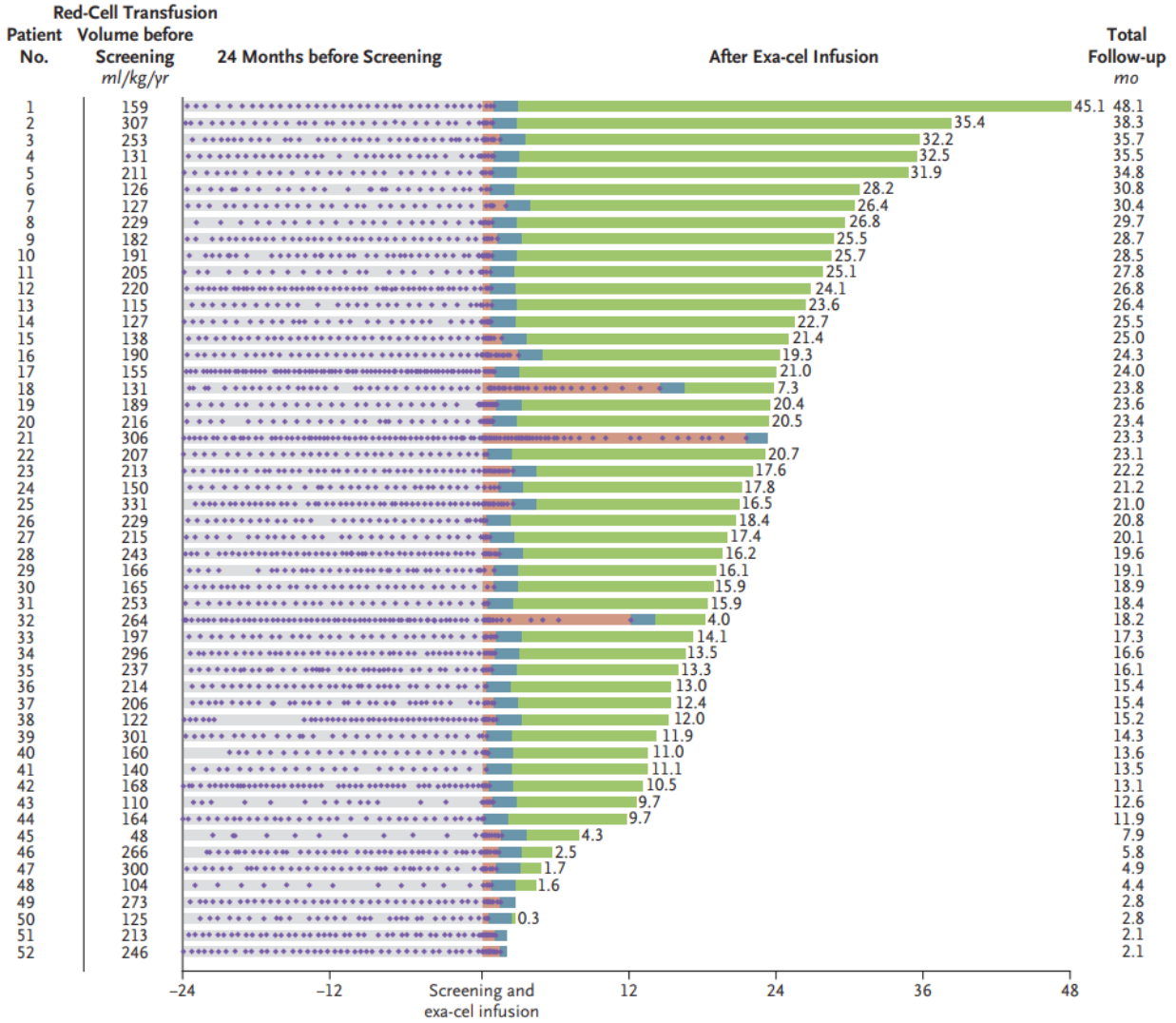


Canver, Nature, 2015

Pros: *efficient in HSCs*
no need for corrective dDNA
Cons: *DSB-induced toxicity*

Transcription factors such as *BCL11A, ZBTB7A and MYB* are among the key regulators of HbF expression. Decreasing the expression of these genes has been shown to reactivate HbF expression.

Exacel for Transfusion-Dependent β-Thalassemia



31 beta0/beta0 like

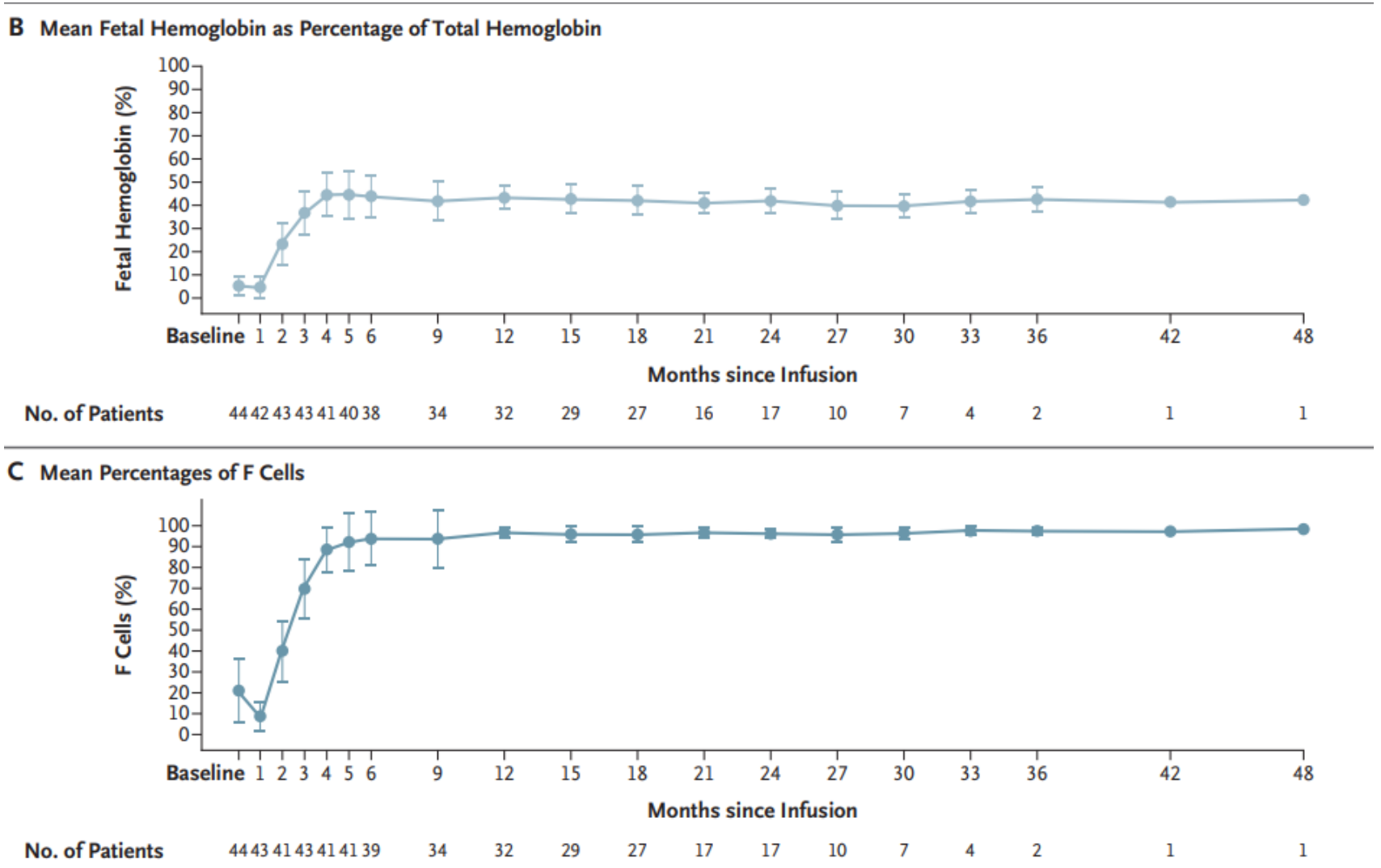
21 non beta0/beta

Transfusion independence in 91%

F Locatelli et al NEJM 2024

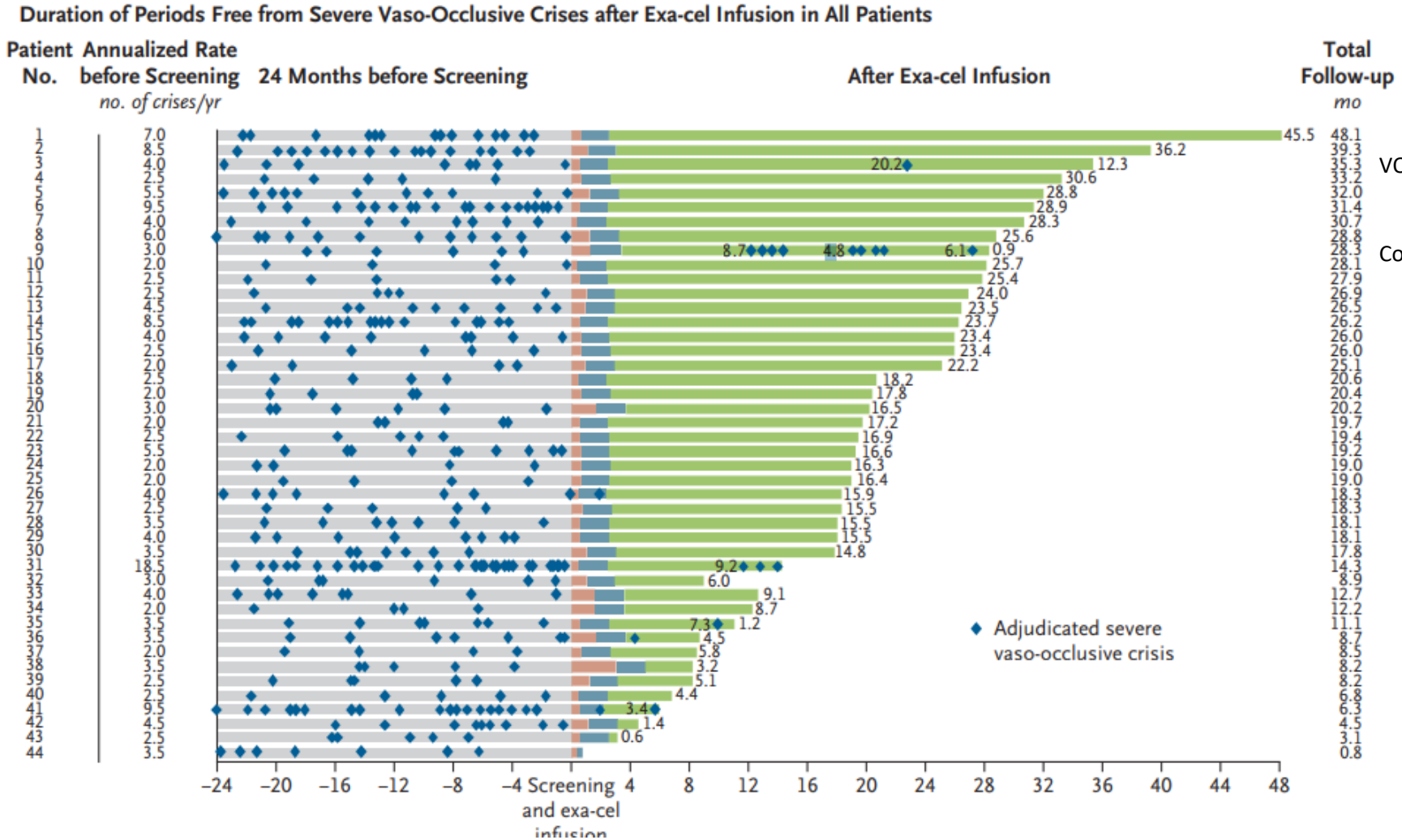
Patients With SCD Had Clinically Meaningful Increases in HbF (>20%) that Occurred Early and Were Sustained Over Time

44 SCD patient had completed myeloablative busulfan conditioning and received exacel



(Frangoul, New Engl Med 2024, Locatelli ASH2023)

SCD: Participants who achieved freedom from VOC(VF12) Maintained VOC-free From 13.1 months to 36.5 months

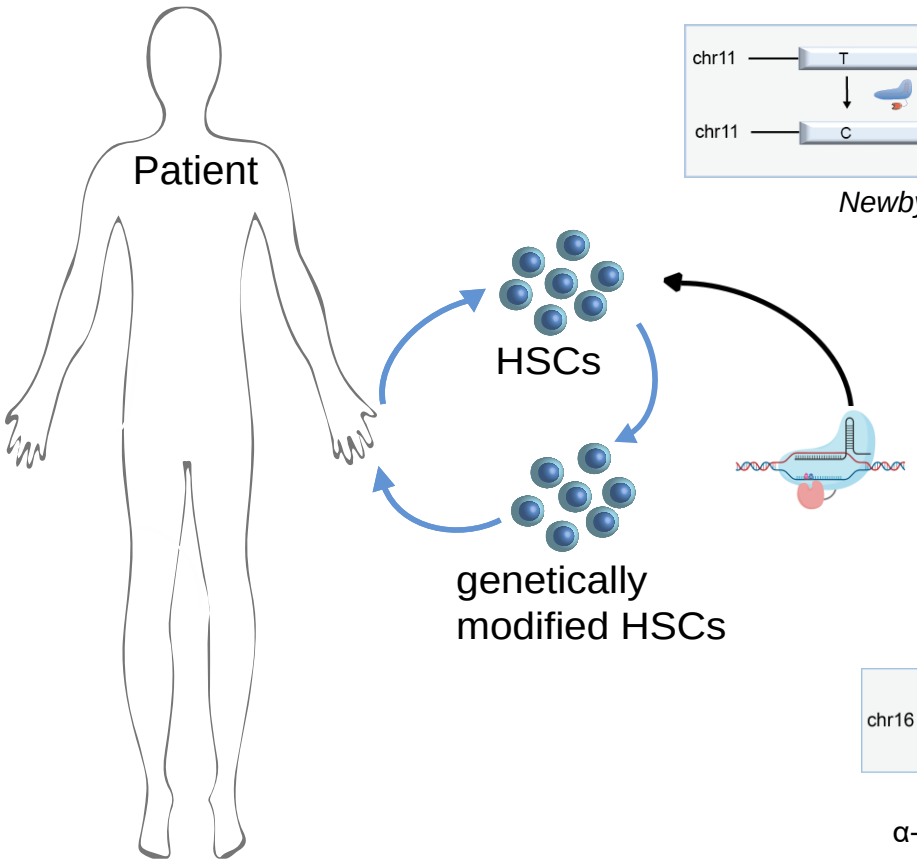


VOC in setting of PB19 infection

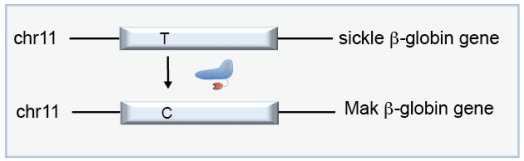
Complex commorbidities and chronic pain

(Frangoul, New Engl Med 2024, Locatelli ASH2023)

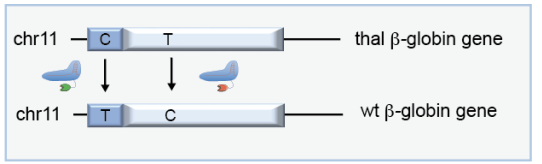
Therapeutic approaches for β-hemoglobinopathies: gene editing



Gene correction

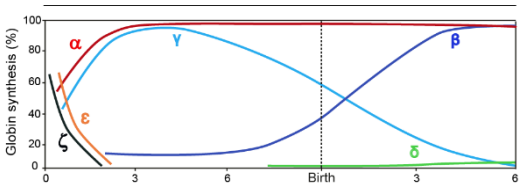


Newby, Nature, 2021



Liang, Protein Cell, 2017; Gehrke, Nat. Biotechnol., 2018; Zeng, Nat. Med., 2020 ; Hardouin, under revision

γ-globin reactivation



α-globin downregulation

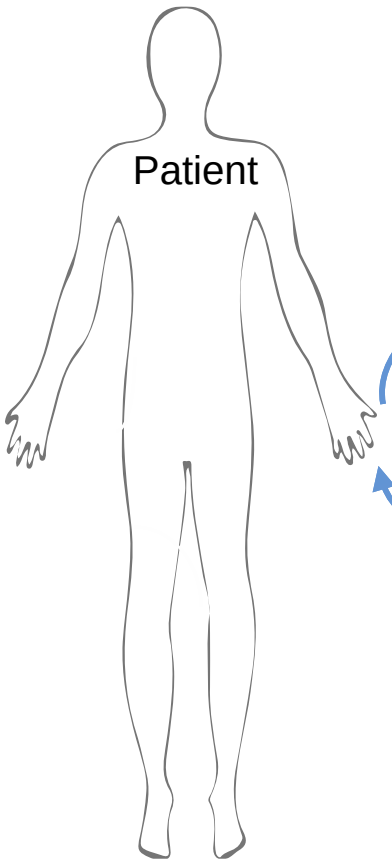
α-globin downregulation:

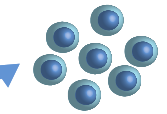
- prevents α-precipitation in β-thalassemic patients
- reduces hemolysis and anemia in SCD
- restores balance between α-globin and β-globin

VERTEX

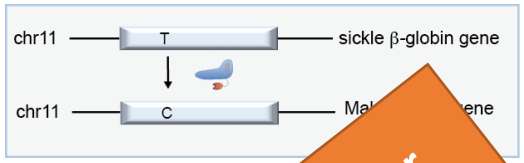
Vertex Announces Authorization of the First CRISPR/Cas9 Gene-Edited Therapy, CASGEVY™

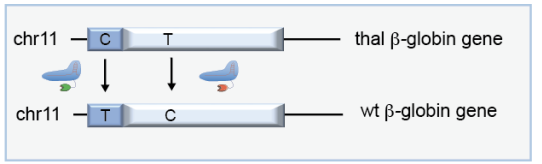
Therapeutic approaches for β-hemoglobinopathies: gene editing





Gene correction

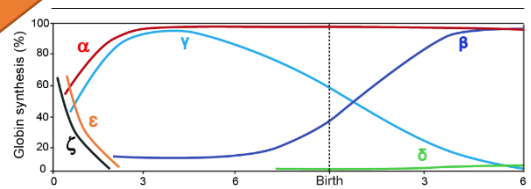




Liang, Protein Cell, 2017; Gehrke, Nat. Biotechnol., 2018; Zeng, Nat. Med., 2020 ; Hardouin, under revision

Prix de 2,2 millions de dollars par patient

globin reactivation



α-globin downregulation

α-globin downregulation:

- prevents α-precipitation in β-thalassemic patients
- reduces hemolysis and anemia in SCD
- restores balance between α-globin and β-globin

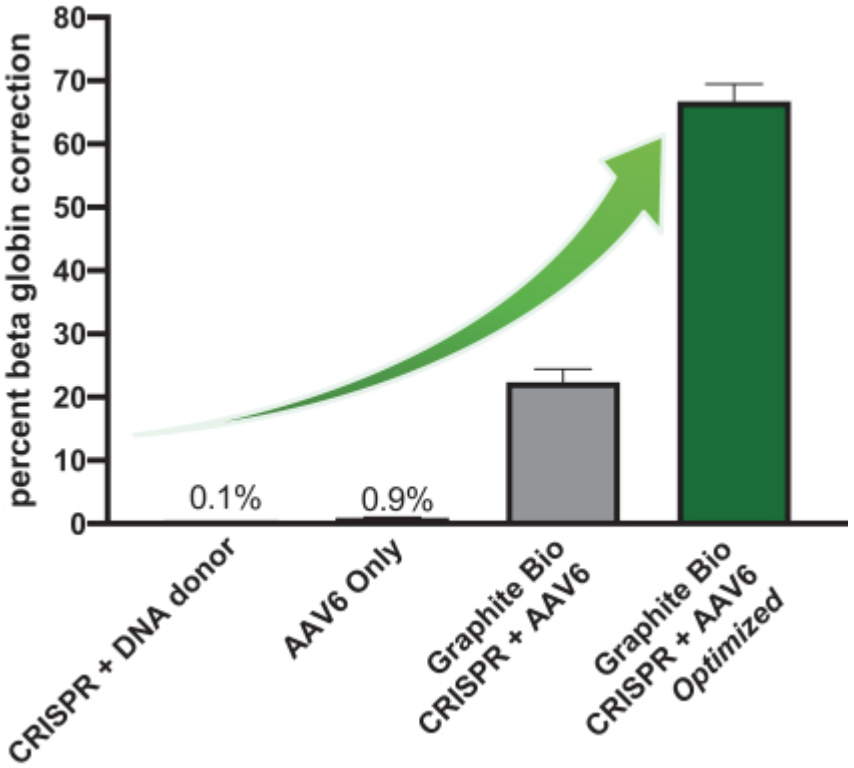
VERTEX

Vertex Announces Authorization of the First CRISPR/Cas9 Gene-Edited Therapy, CASGEVY™

Therapeutic approaches for β-hemoglobinopathies: GRAPHITE TRIAL

The **Graphite Bio (CEDAR) trial** used an **ex vivo** gene editing approach

- **Therapy:** Nula-cel (formerly GPH101), a CRISPR-based gene editing therapy designed to correct the sickle cell mutation in the β-globin gene (HBB).
- **Trial:** Phase 1/2 CEDAR study, aimed at evaluating safety, engraftment, gene correction, and hemoglobin restoration in up to 15 patients with severe SCD.
- **Mechanism:** Ex vivo editing of patient-derived hematopoietic stem cells using CRISPR-Cas9 and an AAV6-delivered DNA repair template to restore adult hemoglobin (HbA) production.
- In late 2022, the first patient dosed experienced **prolonged pancytopenia** (persistently low blood cell counts), necessitating ongoing transfusions and growth factor support.
- **Discontinued development**



Gene Therapy for β-hemoglobinopathies : Conclusions

Gene therapy is an Alternative to cure

HSCT remains the first option to cure beta hemoglobinopathies

- less than 20% of eligible patients matched related donor
- Alternative transplant (USP, Haplo): high complication rates

Phase I/II phase III gene addition strategy and gene disruption of BCL11A

Good safety profile

Gene Therapy for β-hemoglobinopathies : Conclusions

Gene therapy is an Alternative to cure

HSCT remains the first option to cure beta hemoglobinopathies

- less than 20% of eligible patients matched related donor
- Alternative transplant (USP, Haplo): high complication rates

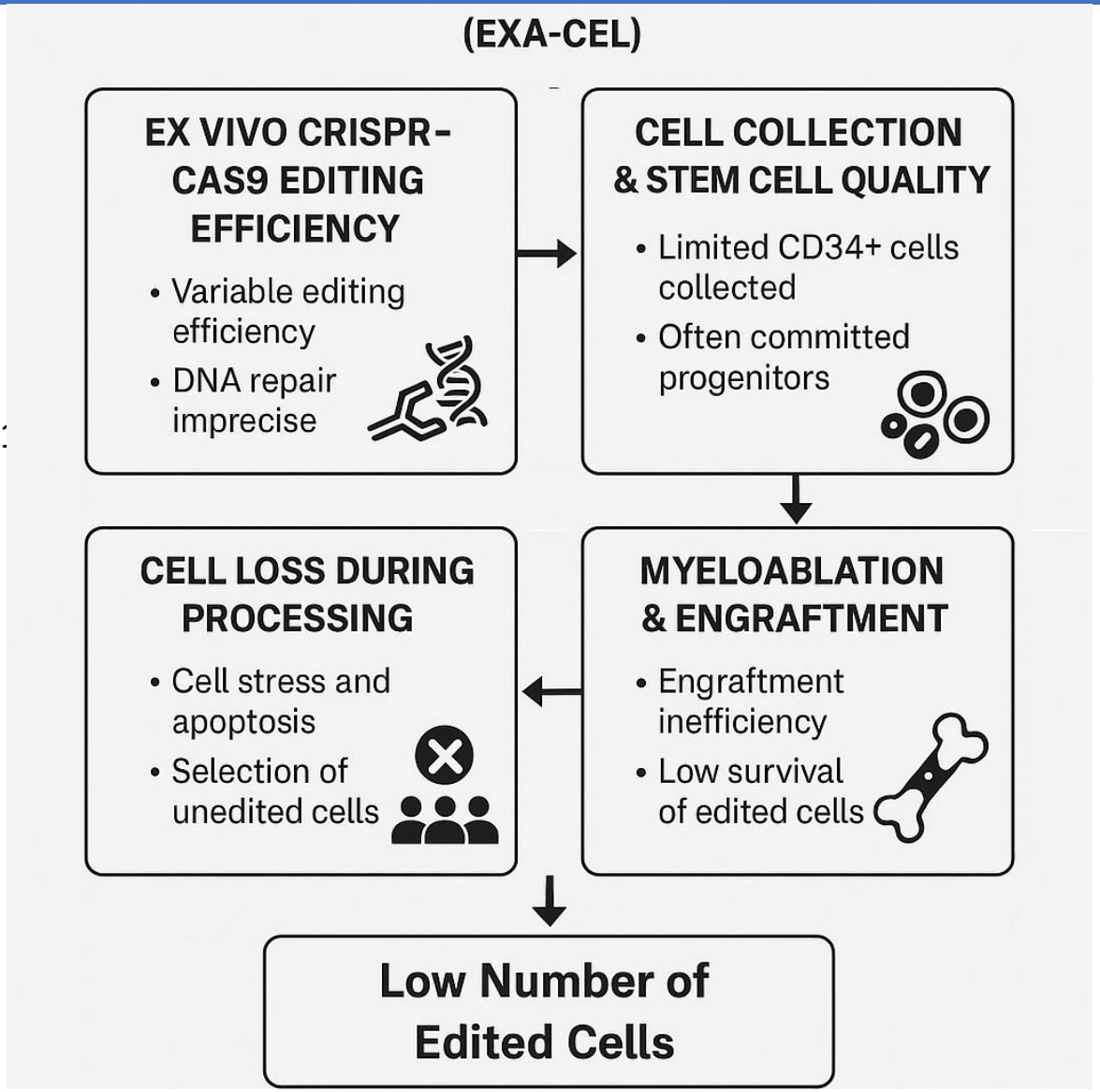
Phase I/II phase III gene addition strategy and gene disruption of BCL1

Good safety profile

Specific issues linked to autologous setting:

- Inflammation
- Clonal hematopoiesis

Specific issues linked to GE:



Gene therapy remains minimally used because of its **exorbitant cost and complexity to produce**

Combining gene therapy with next-generation treatments for beta hemoglobinopathies

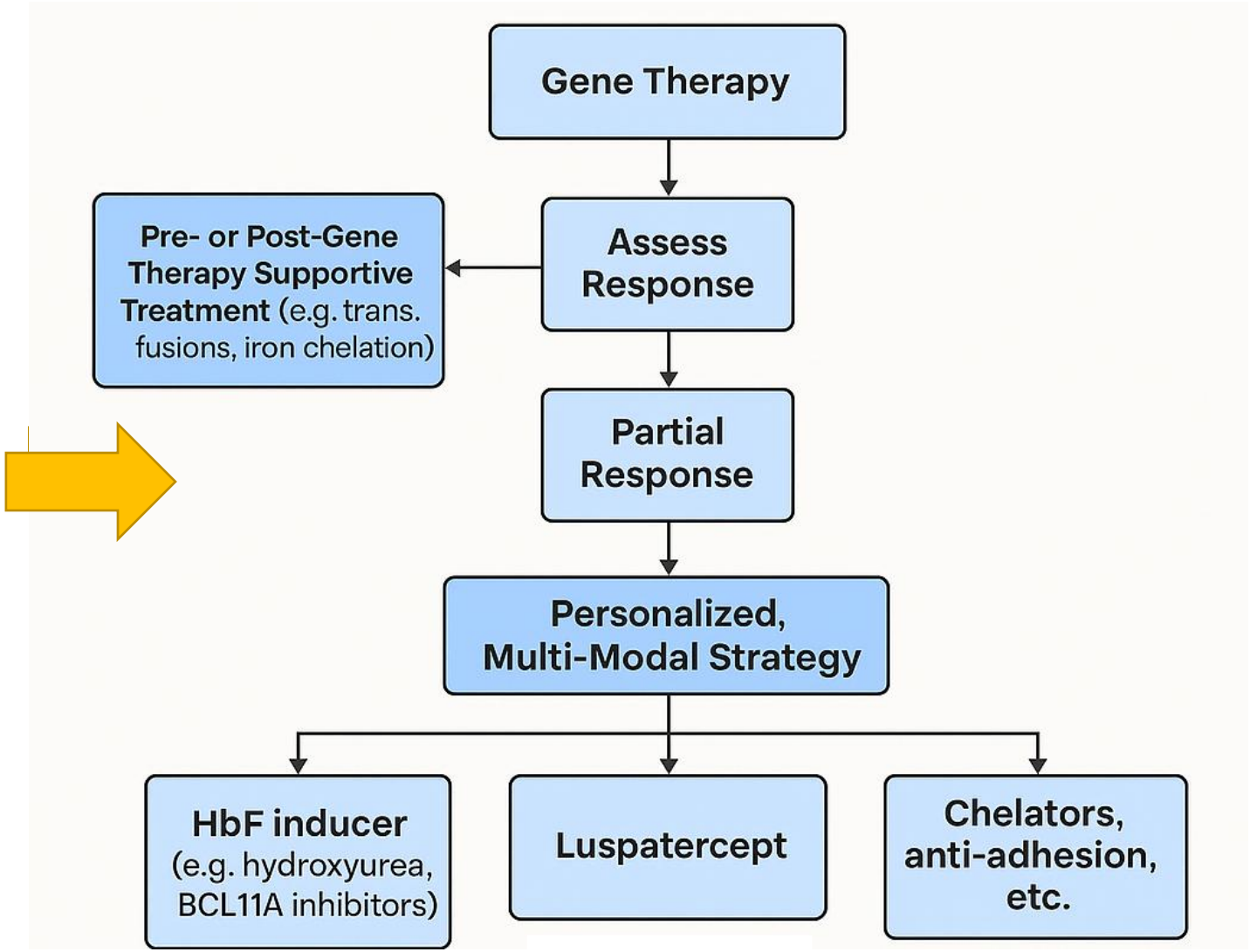
Next-Generation Treatment Strategies for Beta Hemoglobinopathies

Emerging Strategy	Approaches
Pharmacological Reactivation of Fetal Hemoglobin (HbF)	Hydroxyurea, BCL11A inhibitors, Decitabine analogs
Ineffective Erythropoiesis Modulation	Luspatercept, Sotatercept
Anti-adhesion and Anti-inflammatory Therapies	Crizanlizumab, Voxelotor
Iron Metabolism Modulation (Early-stage)	Improved iron chelators, Hepcidin mimetics or modulators
mRNA and Protein-Based Therapies	Synthetic mRNA or protein delivery
Cell Therapy (Non-genetic)	Allogeneic HSCT with reduced intensity conditioning or haploidentical donors

Combining gene therapy with next-generation treatments for beta hemoglobinopathies

Next-Generation Treatment Strategies for Beta Hemoglobinopathies

Emerging Strategy	Approaches
Pharmacological Reactivation of Fetal Hemoglobin (HbF)	Hydroxyurea, BCL11A inhibitors, Decitabine analogs
Ineffective Erythropoiesis Modulation	Luspatercept, Sotatercept
Anti-adhesion and Anti-inflammatory Therapies	Crizanlizumab, Voxelotor
Iron Metabolism Modulation (Early-stage)	Improved iron chelators, Hepcidin mimetics or modulators
mRNA and Protein-Based Therapies	Synthetic mRNA or protein delivery
Cell Therapy (Non-genetic)	Allogeneic HSCT with reduced intensity conditioning or haploidentical donors



Thank you for your attention

Pr Marina Cavazzana

Aknowledgements

Center of Clinical Investigation CIC1419 (Pr Tréluyer)-Hôp Necker: J Marouene, E Cano

Centre d'investigation Clinique spécialisé en Biothérapie (CIC-BT) Pr **M Cavazzana-** Hôp Necker: L Joseph, E Magrin, JS Diana, S.Manceau, E. Schwartz, C. Roudaut, A. Gabrion, C. Tuchmann-Durand

Chromatin and gene regulation during development's lab **A. Miccio, E. Six, S. Sobrino**, A. Chalumeau, T. Felix, M. Brusson

Imagine Collaborators A. Corsia, O. Hermine A. Corsia T. Trovati

URP 7323 – Pharmacologie et évaluation des thérapeutiques chez la femme enceinte et l'enfant -UPC

Most importantly, we wish to thank all the patients and their families