Gene Therapy for α-Thalassemia: Towards an In Utero Therapeutic Approach

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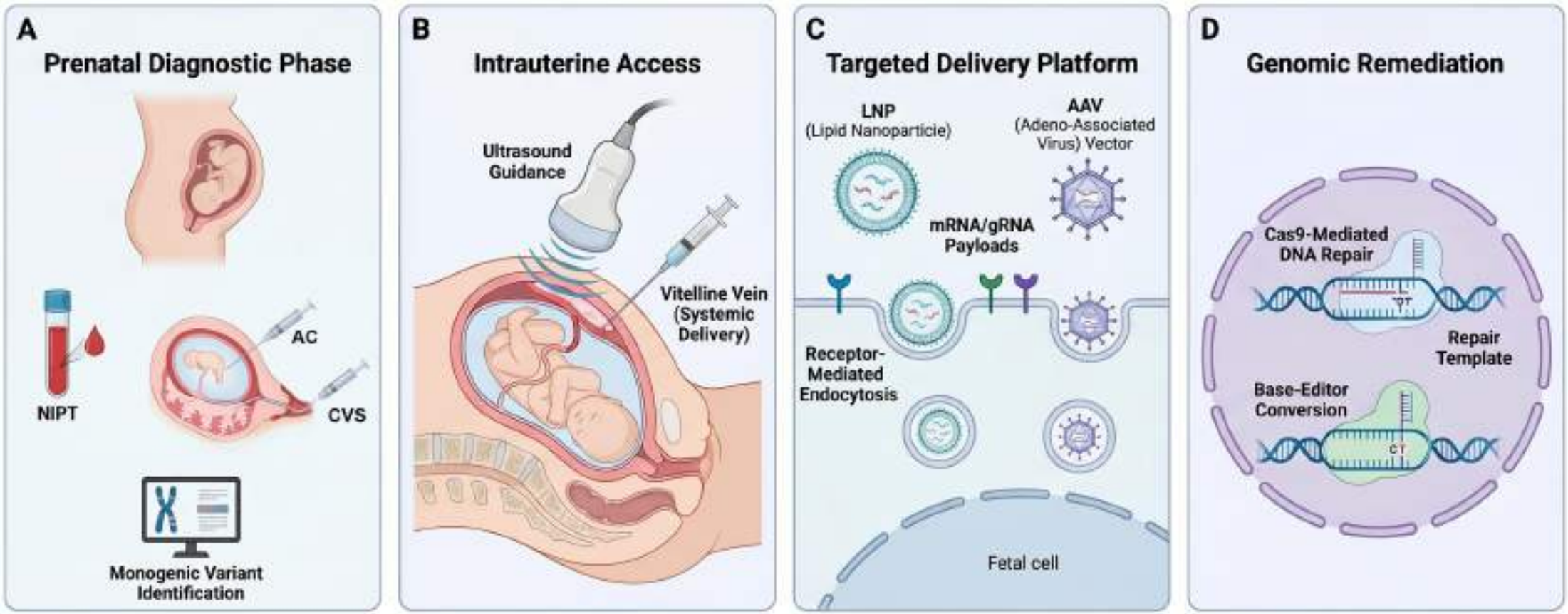
In utero genome editing (IUGE)

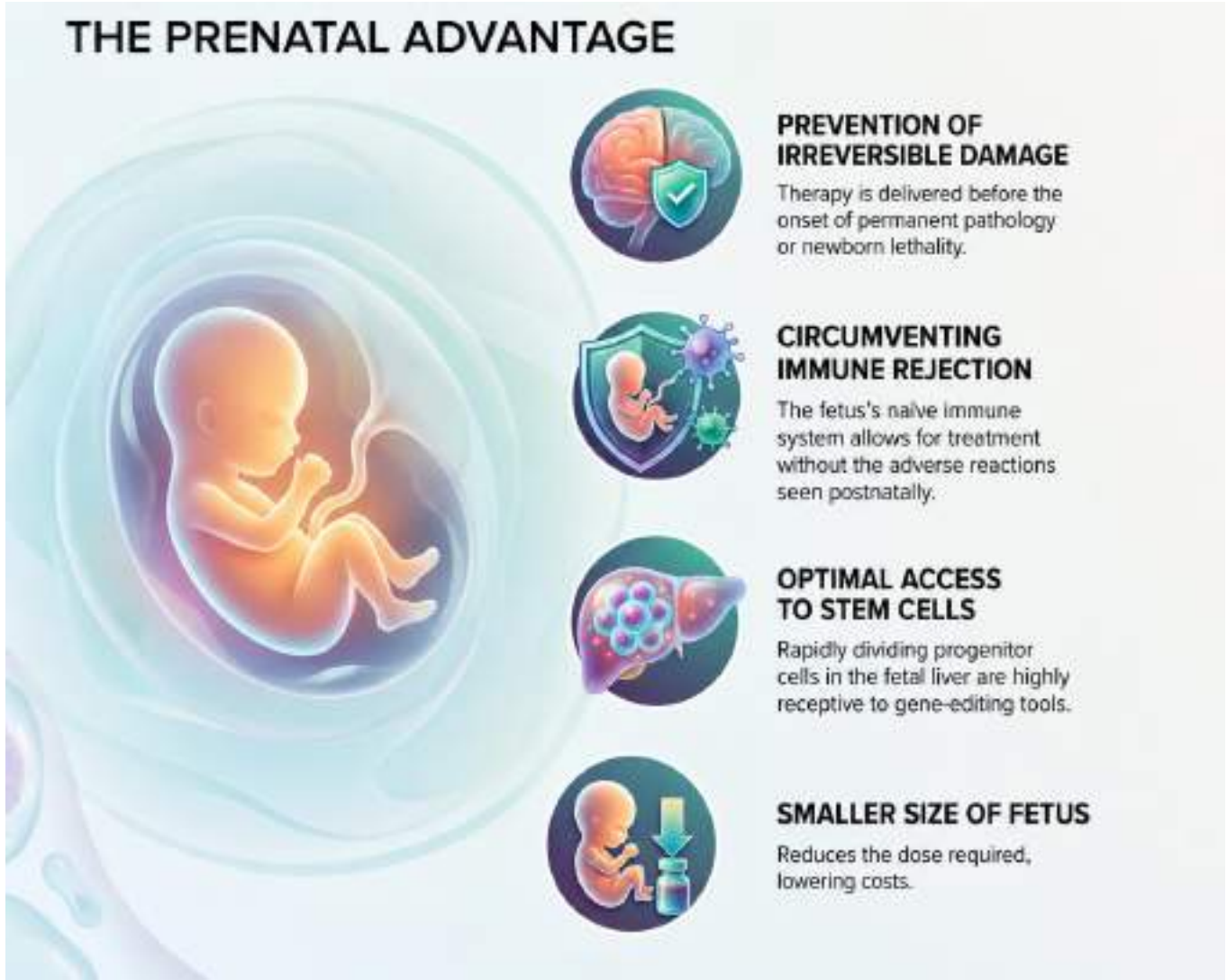
- *In utero genome editing (IUGE) is defined as the site-specific molecular correction of pathogenic variants within the developing fetus.*

- *Constitutes a paradigmatic evolution in molecular medicine, transitioning from reactive postnatal symptom management to proactive prenatal genetic correction or even prevention.*

Outline of steps involved in IUGE



Rationale for Fetal Gene Therapy



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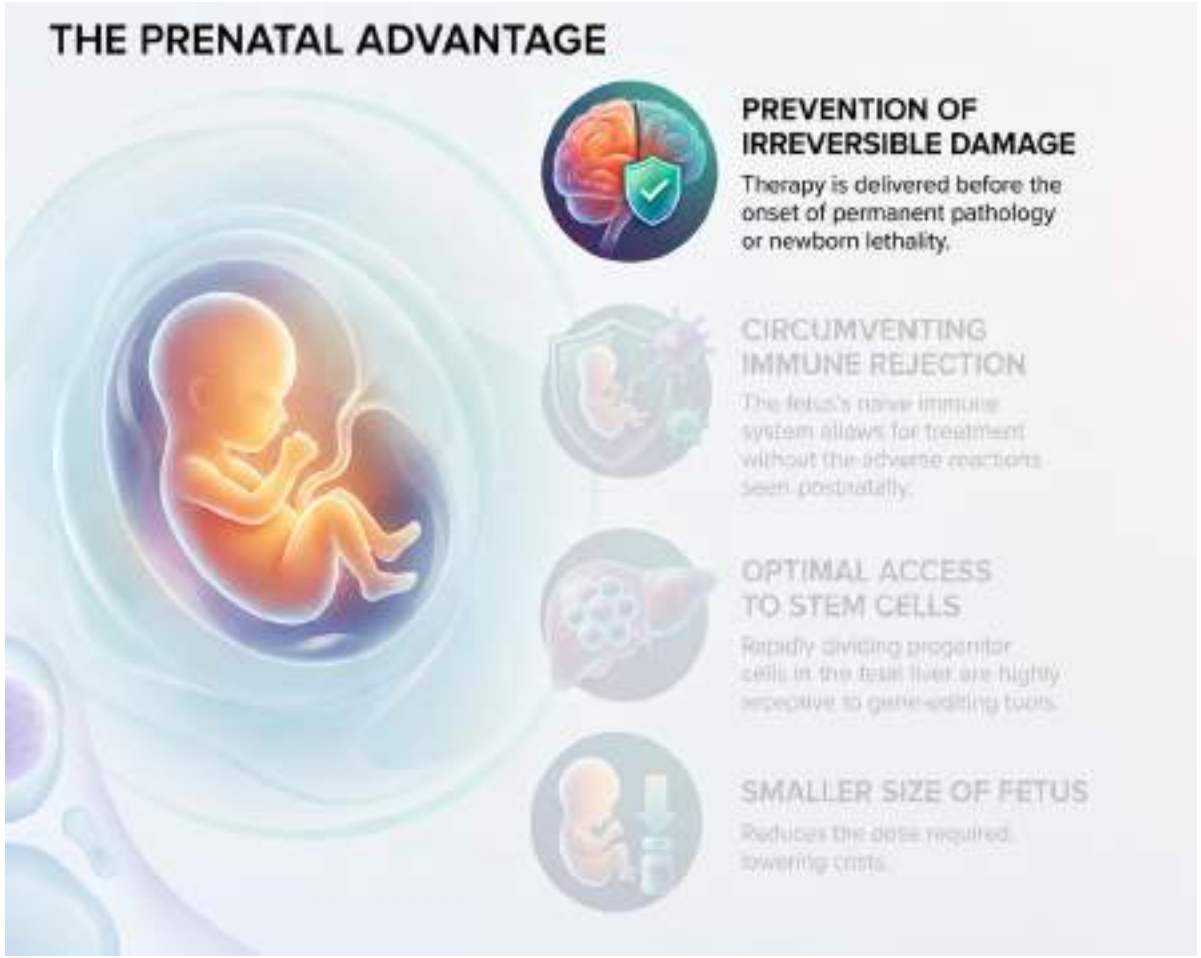
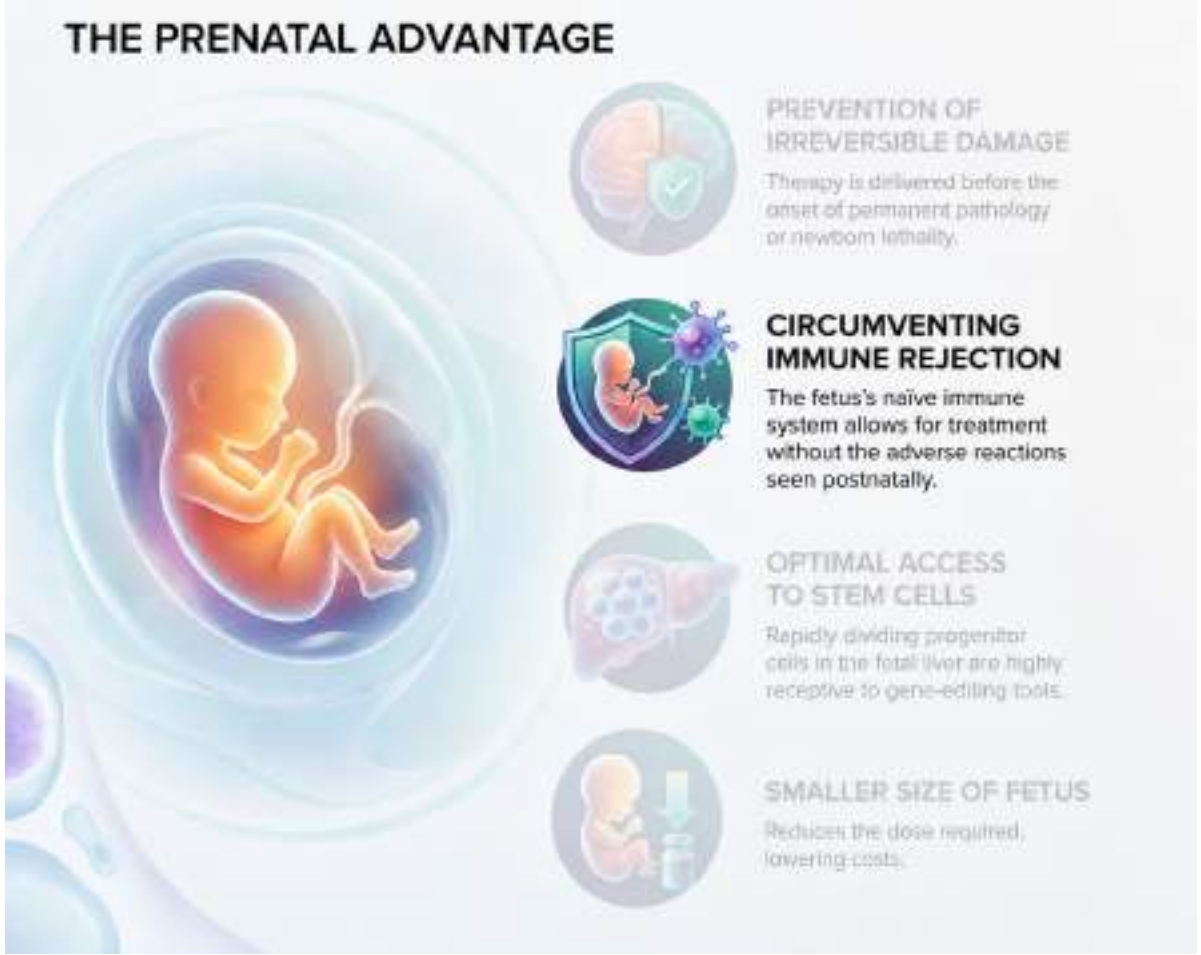


Early intervention to prevent irreversible damage: Enables therapeutic outcomes during early development for disorders with time-sensitive pathophysiology and severe life-threatening prenatal consequences.

Early intervention to prevent irreversible damage

- *IUGE makes sense in diseases with severe clinical prognosis and **early-gestational phenotypic onset**. Ideal candidates are monogenic disorders that cause significant morbidity or lethality prior to birth, or those for which existing enzyme replacement therapies (ERT) are rendered ineffective by immune rejection or the inability to cross protected physiological compartments if applied postnatally.*
- *This preventative logic is particularly relevant for disorders such as α-thalassemia major, lysosomal storage disorders, and severe neurodevelopmental pathologies, where pathogenic cascades may begin in fetal life and postnatal intervention may occur only after irreversible tissue injury has already been established*

Rationale for Fetal Gene Therapy



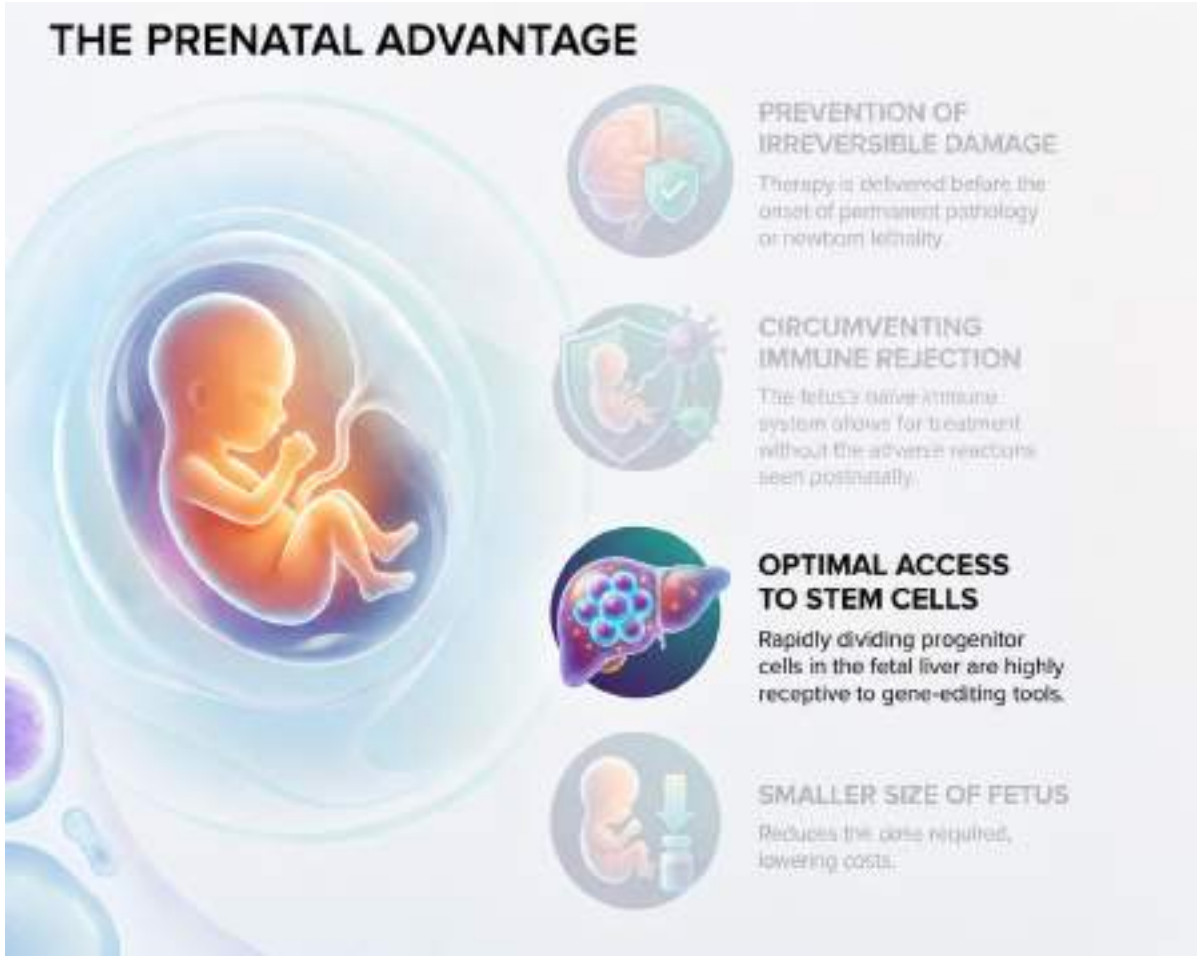
Fetal immune tolerance:
Reduces the risk of severe immune response while inducing long-term tolerance to exogenous proteins.

Advantages of the fetal immune system:

- The unique characteristics of the fetal immune system offer significant advantages, including immunologic tolerance to novel antigens, reduced risk of immune-mediated rejection, and the potential for long-term tolerance induction. The fetal immune system has evolved to be immature and must not attack maternal antigens that cross the placenta.

- In animal models of hemophilia B, in utero delivery of adeno-associated virus (AAV) vectors achieves sustained transgene expression without triggering the production of neutralizing antibodies, offering a significant advantage over postnatal therapies.
- Patients who receive postnatal treatment for hemophilia B often develop inhibitory antibodies against clotting factor IX, complicating management and reducing long-term efficacy.
- This immunological advantage may also be beneficial for disorders such as lysosomal storage diseases since immune responses to enzyme replacement therapy in patients without endogenous protein can limit efficacy and require long-term immunosuppression.

Rationale for Fetal Gene Therapy

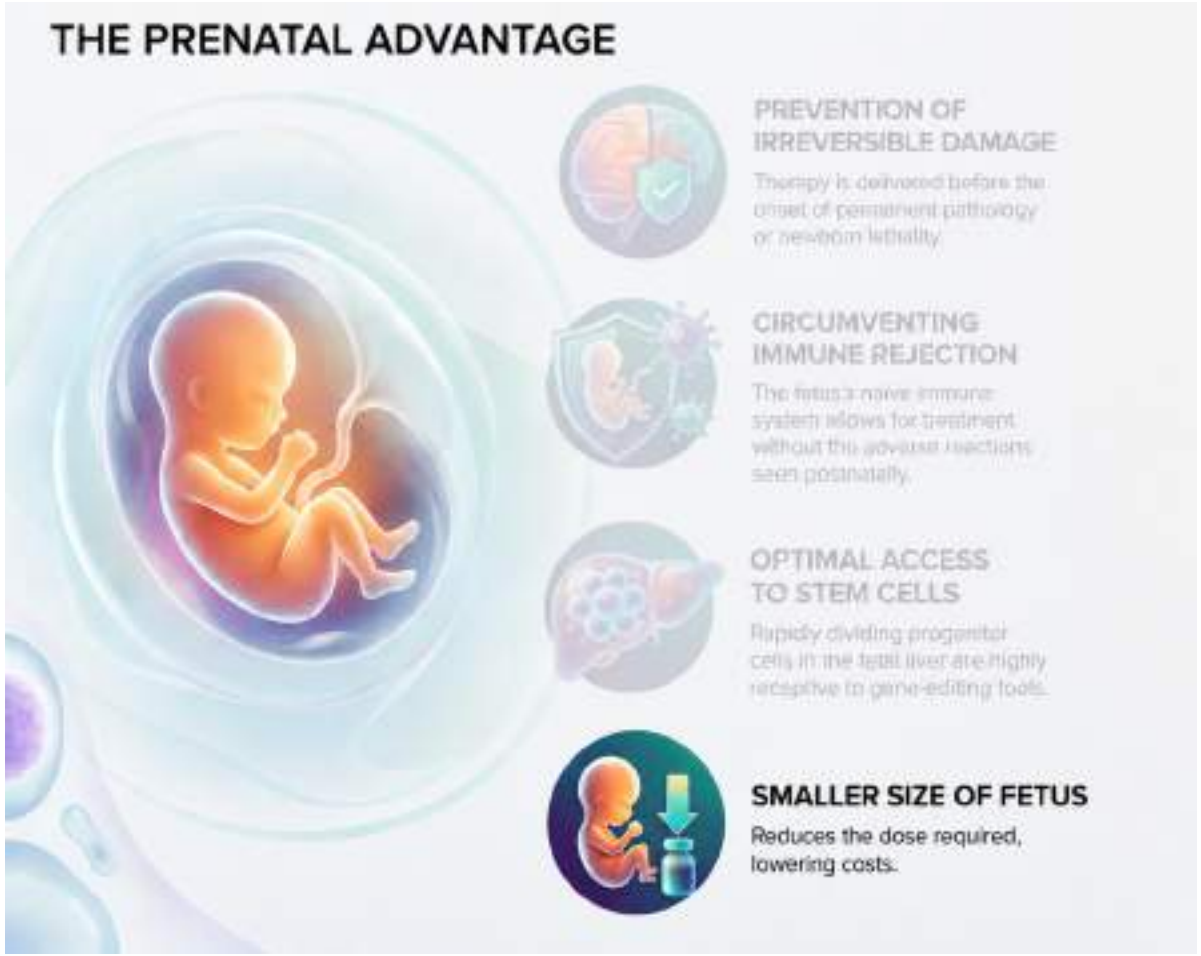


Fetal biodistribution and tissues accessibility: Allows widespread biodistribution and targeting of critical tissue due to developmental factors such as blood-brain barrier permeability.

Fetal biodistribution and tissues accessibility

- The proliferative and less differentiated state of certain cell types in the fetus enhances the potential for widespread distribution of genetic therapies, enabling systemic correction in multi-organ conditions.
- The presence of **accessible and rapidly expanding stem and progenitor cell populations** ensures that genomic modifications are stochastically and hierarchically propagated
- Additionally, some tissues, such as hematopoietic cells and the brain, may be more accessible for targeting during the prenatal period due to the ectopic location of hematopoietic progenitors and the increased permeability of the blood-brain barrier during fetal development. Prenatal systemic injection of AAV9 leads to superior neuronal accessibility compared with postnatal delivery, representing a better opportunity to target many pediatric neurological diseases.

Rationale for Fetal Gene Therapy



Smaller size of the fetus: Reduces the dose required, lowering costs.

Smaller size of the fetus:

- The smaller size of the fetus requires more modest amounts of the therapeutic agent, simplifying manufacturing. [the **high effective dose-to-mass ratio** achievable in the 100- to 500-g fetus]

- Central to the systemic viability of these platforms is their capacity to drastically reduce the economic and logistical barriers associated with advanced therapies.
- Because the mid-gestational fetus possesses a physical mass several orders of magnitude smaller than a neonate (e.g., 300 g at 20 weeks versus 3.5 kg at term), the absolute quantity of good manufacturing practice (GMP)-grade materials required for a therapeutic dose is reduced by approximately 10- to 40-fold
- This reduction in manufacturing burden may significantly attenuate the cost of goods, potentially democratizing access to genetic remediation in low- and middle-income regions where the burden of hemoglobinopathies is most acute and if a cost-based model of treatment pricing is applied

Challenges of fetal gene therapy

1. Technical complexity: Requires precise targeting through invasive procedures (e.g., intravascular or intracerebral injections), advanced imaging techniques and skilled expertise for fetal injections.

2. Vector limitations: Currently, viral vectors carry risks of immunogenicity and insertional mutagenesis while non-viral vectors have lower transfection efficiency.

3. Maternal risks: Complications of invasive procedures (infection, bleeding, preterm labor), maternal immune responses and maternal gene integration or editing.

4. Risks to the fetus: Invasive procedures complications (bleeding, infection, fetal demise), oncogenic events (rapidly dividing fetal tissues may be more vulnerable) and germline modifications.

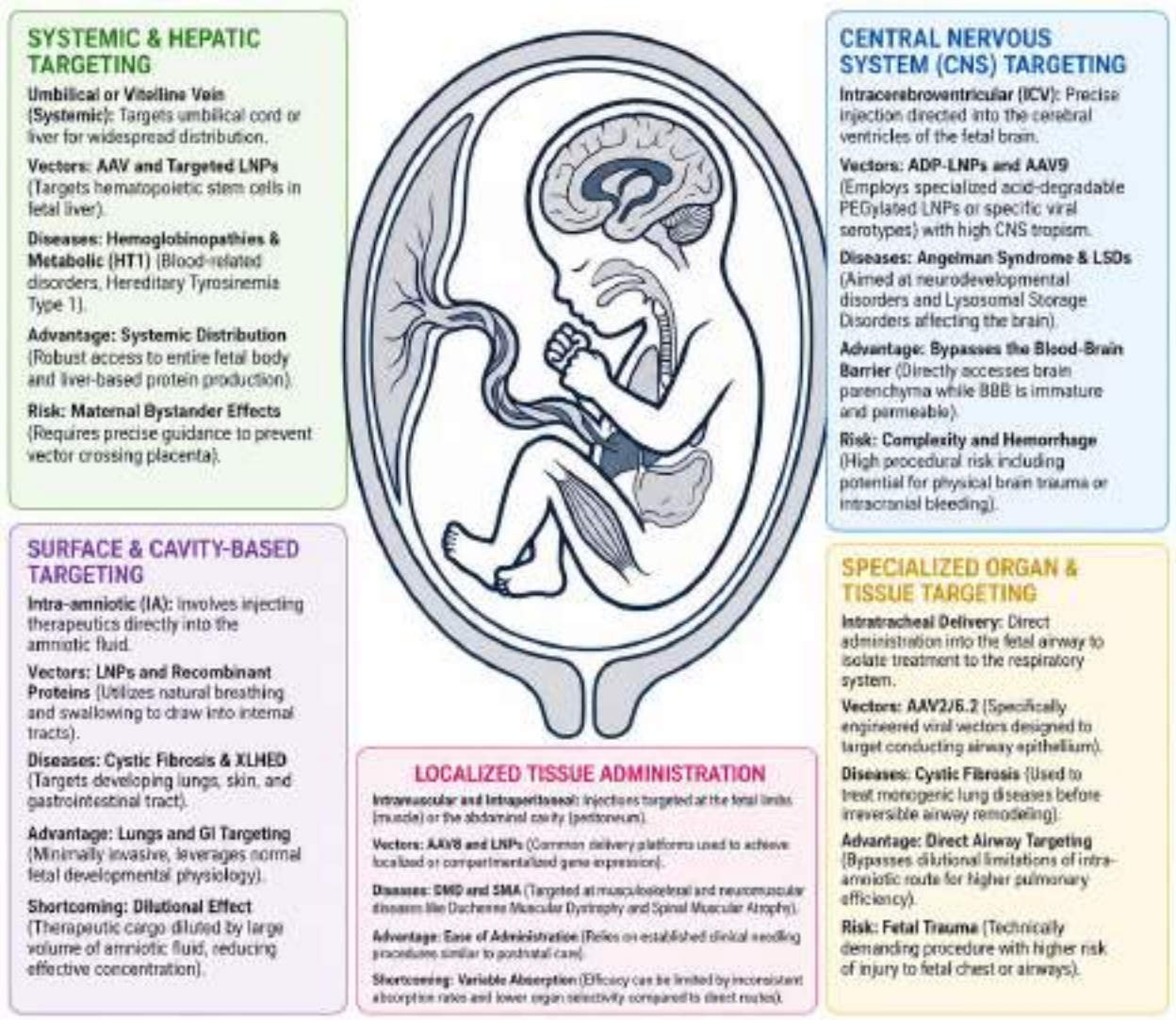
5. Ethical considerations: Raises questions about consent for fetal interventions, equity in access to this approach, and concerns about germline modifications.

Technical complexity: Routes of Administration

- The delivery of gene therapy vectors during prenatal development requires precise targeting to help minimize off-target effects and maximize therapeutic efficacy.
- Delivery methods, such as intra-amniotic, intraperitoneal, intravascular, or intracerebral injections, can be technically challenging and require advanced imaging techniques and highly skilled individuals.

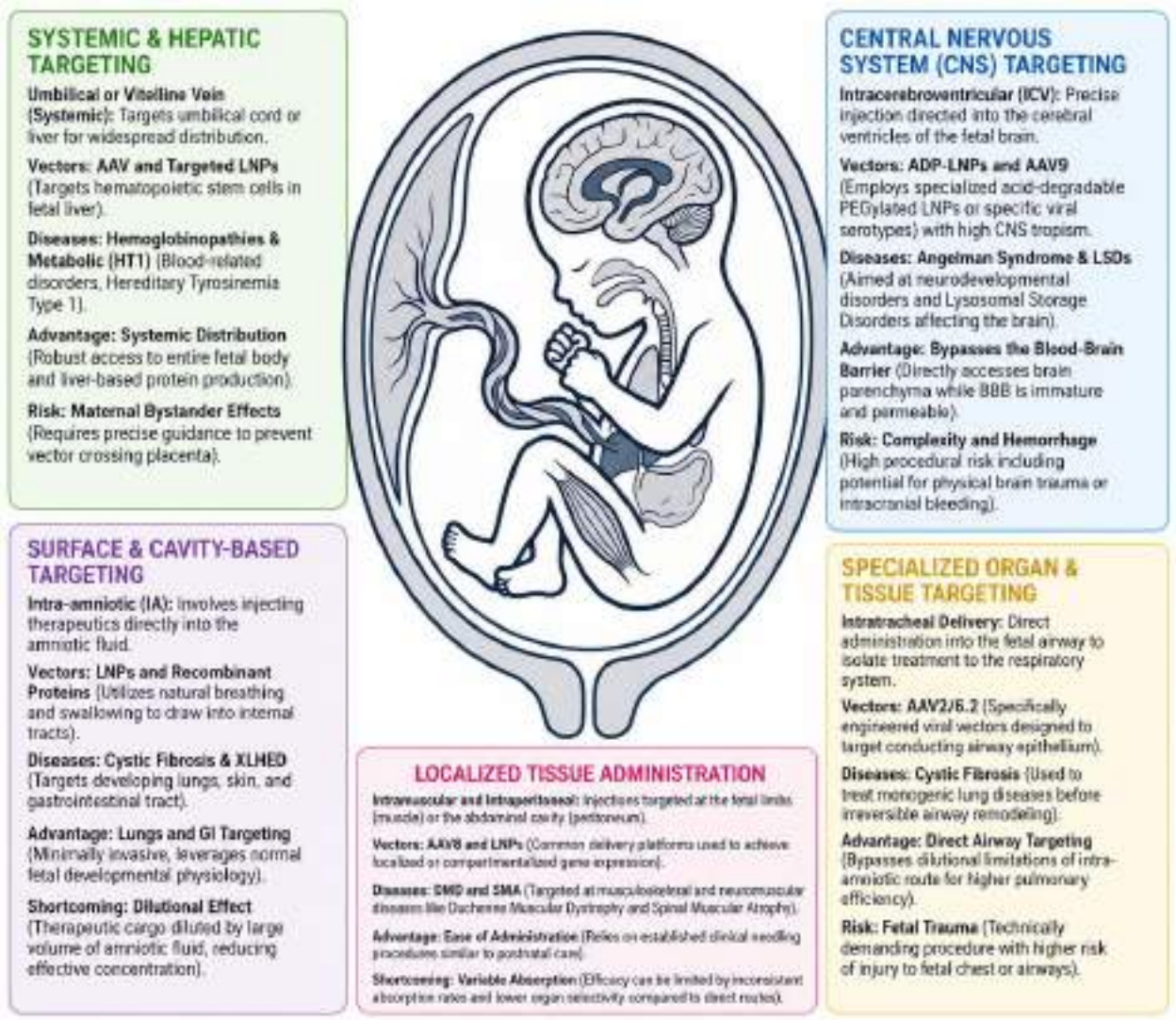
Technical complexity

Routes of administration



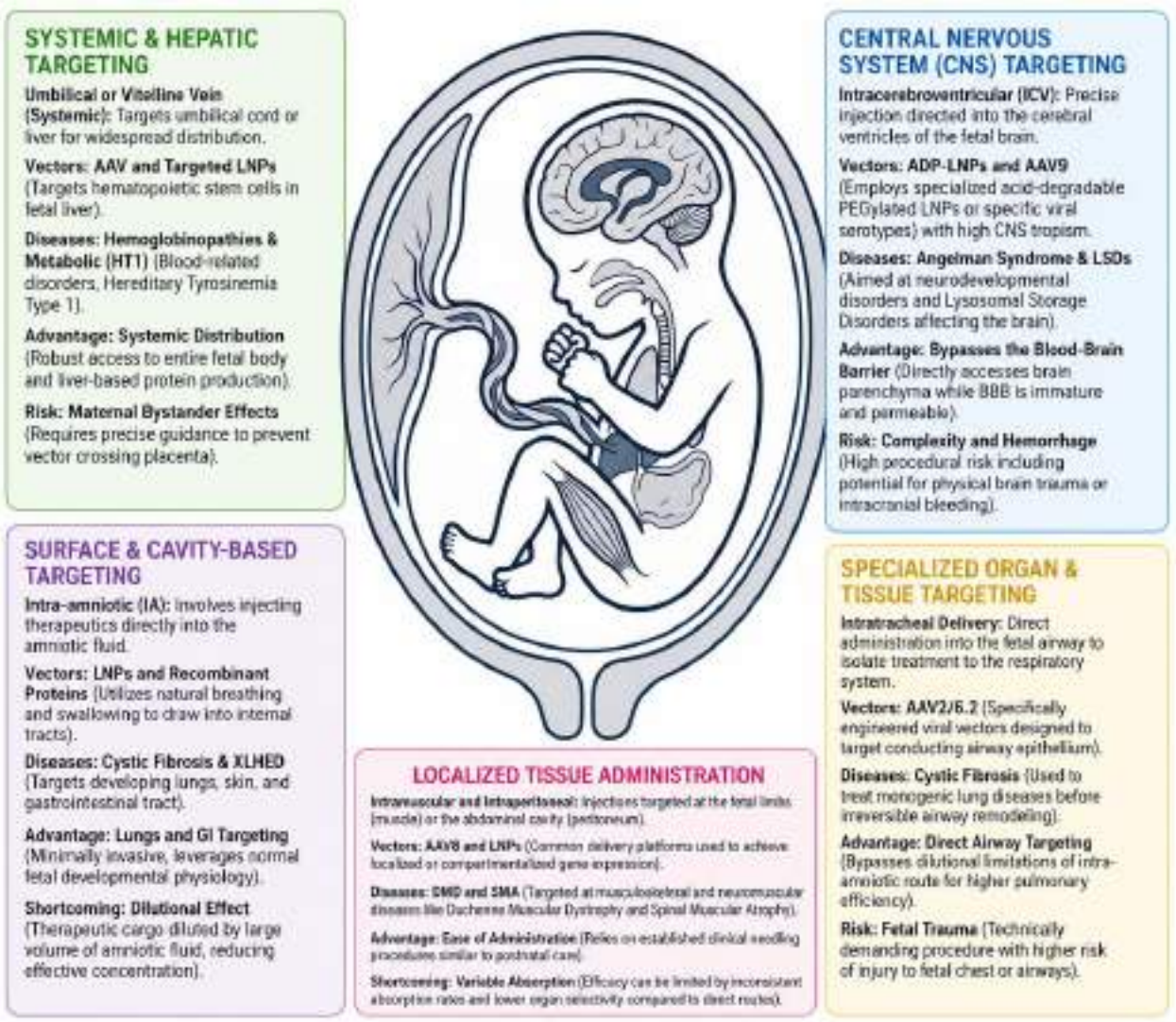
Systemic Vitelline/Umbilical Injection: This route maximizes biodistribution to the fetal liver and the hematopoietic stem cell (HSC) niche, enabling systemic treatment of metabolic and hematologic disorders

Routes of administration



Intra-amniotic (IA) Delivery: By leveraging the fetus's natural swallowing and breathing of the amniotic fluid, this modality enables organ-specific targeting of the **pulmonary epithelium** and gastrointestinal tract.

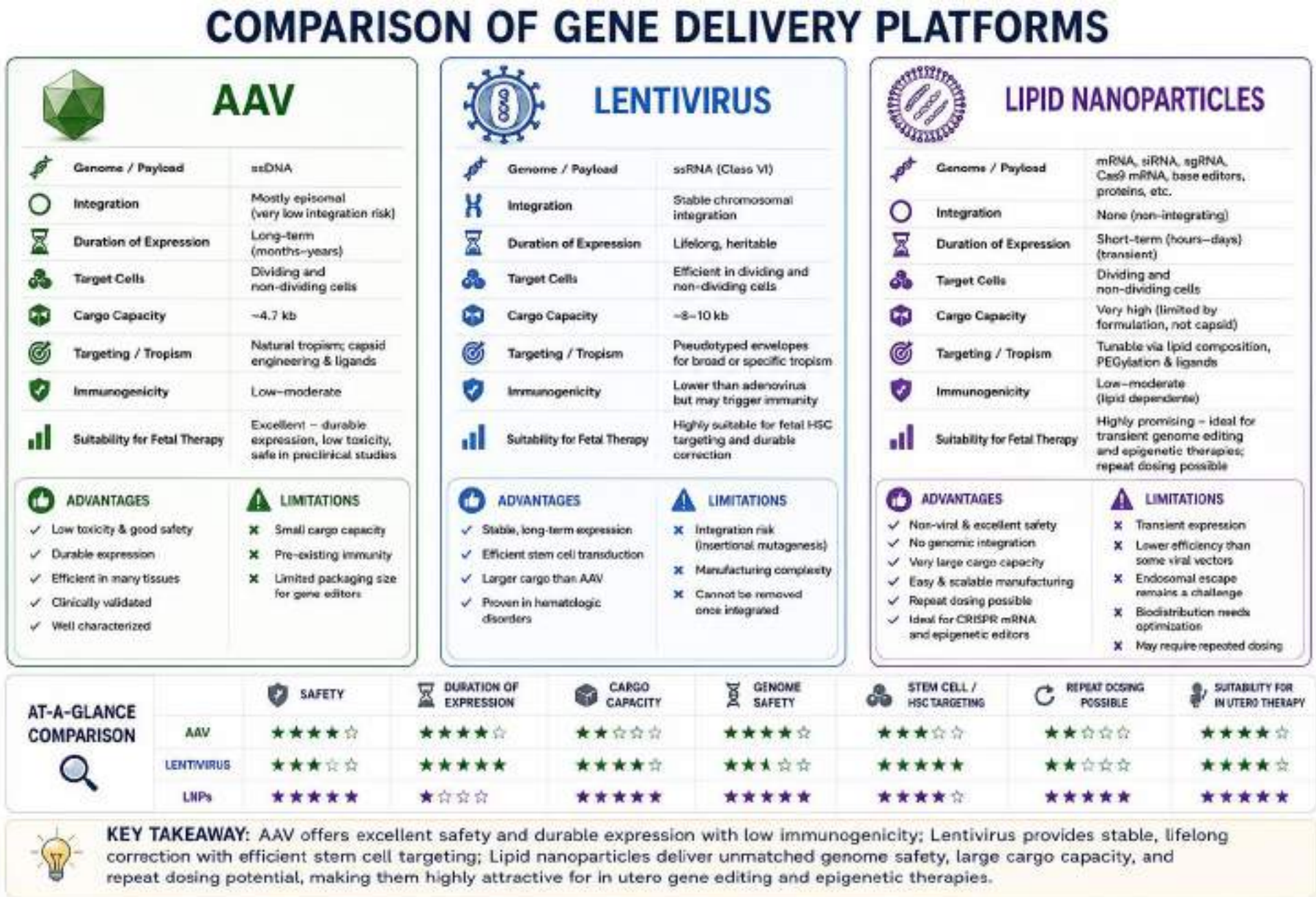
Routes of administration



Intracerebroventricular (ICV) Delivery: Direct access to the brain ventricles allows for the widespread transfection of **neural stem/progenitor cells (NSPCs)**, bypassing the blood-brain barrier to treat neurogenetic pathologies.

Too risky for fetal gene therapy?

Vector limitations



MATERNAL RISKS

Key maternal considerations in fetal gene therapy

1. PROCEDURE-RELATED RISKS

Fetal gene therapy requires invasive procedures that carry risks for the mother. These include potential complications such as infection of the uterus, bleeding, preterm labor, and risk to the mother's health.

2. SURGICAL RISKS

The mother may have to undergo emergency procedures such as caesarean section secondary to fetal complications resulting from the gene therapy procedure – fetal hemorrhage, fetal bradycardia, premature rupture of the membranes, infection, and preterm birth.

3. IMMUNOLOGIC RESPONSES

Maternal immunologic responses to the therapeutic vector or the introduced transgenic protein can compromise the efficiency of gene transfer to the fetus and potentially affect maternal health.

4. RISK OF UNINTENDED MATERNAL GENE INTEGRATION / EDITING

There is a minimal but important risk of unintended maternal gene integration or editing due to low doses of therapeutic agents that may reach the mother directly and cross biological barriers to the fetus.

KEY TAKEAWAY

The risk–benefit balance of fetal gene therapy must be carefully weighed, particularly because the intervention is intended to benefit the fetus and not harm the unaffected mother. A thorough assessment of maternal safety, including immunologic, genetic, and obstetric risks, is critical in the development and clinical implementation of these therapies.

Risks to the fetus

- Fetal gene therapy can result in fetal injury

- The rapidly dividing fetal tissues are particularly susceptible to oncogenic events following gene therapy due to off target editing.

- Risk of altering the germline raising ethical and safety concerns

Risks to the fetus

- As an invasive procedure, fetal gene therapy can result in fetal injury, hemorrhage, infection due to breaching, preterm birth, or even fetal death.
- The rapidly dividing fetal tissues may render the fetus particularly susceptible to oncogenic events following gene therapy due to off target editing. The use of integrating vectors, such as lentiviral vectors, which may activate proto-oncogenes or disrupt tumor suppressor genes, carrying a risk of insertional mutagenesis, potentially leading to cancer.

- Prenatal gene therapies risk altering the germline if therapeutic DNA integrates into germ cells, raising ethical and safety concerns about passing genetic modifications (on or off target) to future generations. [a risk that necessitates the development of transient delivery systems like mRNA-loaded LNPs. Germline gene modification is strictly prohibited and is not an objective of fetal gene therapy.]

Consent for fetal interventions

- Obtaining informed consent for fetal gene therapy presents the challenge that the fetus cannot provide consent, and the decision is made by the parents. Parents will have to weigh the **potential benefits to the fetus** against **risks to both the fetus and the mother**, likely with some **uncertainty** about long-term outcomes.
- **Informed consent** is complicated by the potential for "therapeutic misconception," where parents may overestimate the benefits while underestimating the risks of a nascent technology

- However, these ethical considerations are not unique to fetal gene therapy and have been discussed in the context of other fetal interventions such as fetal surgery. Nevertheless, consenting for fetal gene therapy raises specific concerns including the risk of germline transmission that extend beyond the patient for whom consent is sought.

Equity and accessibility concerns

- Fetal gene therapy is likely to be an expensive and resource consuming intervention, raising concerns about equitable access. There is a risk that this approach will be available only to populations or countries with advanced healthcare systems, exacerbating health disparities. Stakeholders and policymakers must ensure that fetal gene therapy, if proven effective, will be accessible to underserved populations.

ETHICAL CONSIDERATIONS

Fetal gene therapy raises several ethical challenges that must be carefully addressed, in addition to regulatory considerations.

1. MATERNAL–FETAL SAFETY AND RISK–BENEFIT BALANCE

- The intervention must benefit the fetus without exposing the mother to undue risk.
- Comprehensive assessment of maternal safety (immunologic, genetic, obstetric) is essential.

2. GERM LINE MODIFICATION

- Integration into germ cells could lead to heritable changes.
- Germline modification is strictly prohibited and not an objective of fetal gene therapy.

3. INFORMED CONSENT

- Obtaining truly informed consent from parents is complex and challenging.
- Must include discussion of unknowns, long-term risks, and alternatives.

4. OFF-TARGET AND LONG-TERM RISKS

- Off-target edits and insertional mutagenesis may cause oncogenic events or other harms.
- Long-term follow-up and monitoring are ethically imperative.

5. EQUITY AND ACCESS

- Ensure fair access and avoid exacerbating healthcare disparities.
- Prevent the creation of 'genetic privilege'.

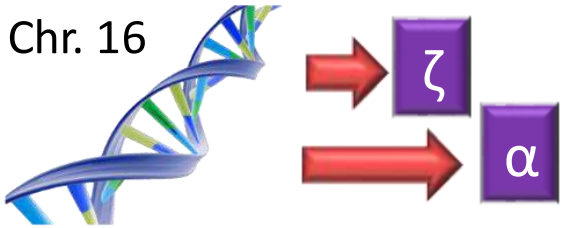
6. REGULATORY OVERSIGHT AND TRANSPARENCY

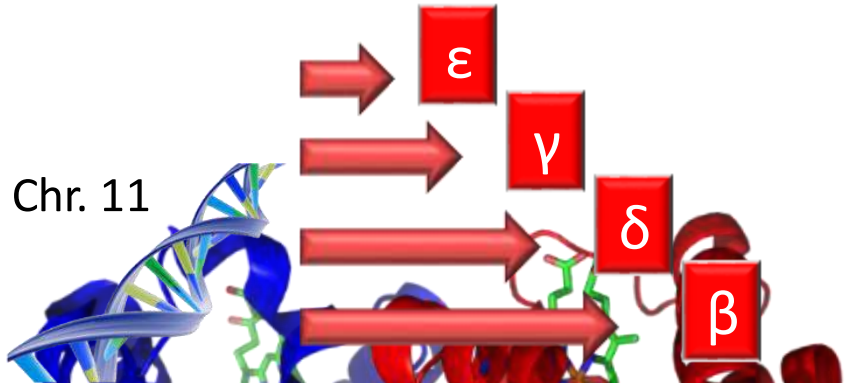
- Rigorous oversight, transparent reporting, and adherence to international guidelines are essential.
- Alignment with frameworks (e.g., WHO, ICH S12) is critical.

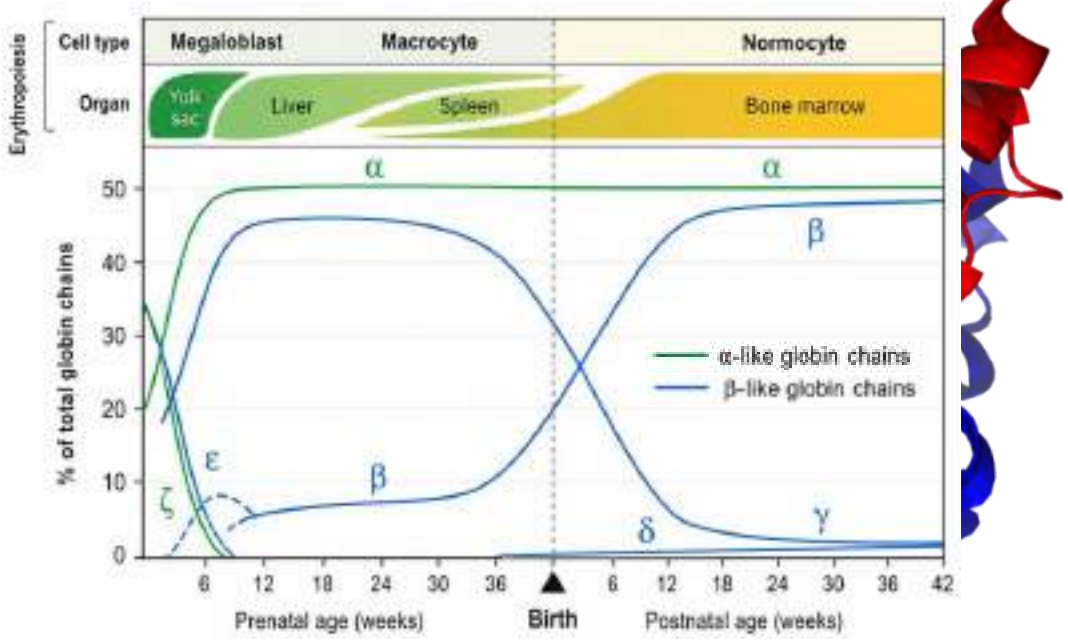
Ethical success requires a commitment to safety, transparency and the well-being of both mother and child, with the ultimate goal of preventing severe disease before birth.

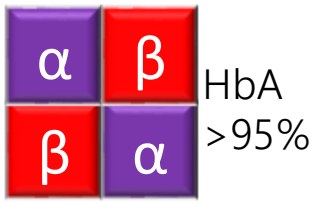
Hemoglobin

- O_2 and CO_2 transport from and to the lungs
- Two loci for H.s. globin genes
  - Embryonic: ζ & ε
  - Fetal: α & γ
  - Adult: α & δ/β
- Hemoglobin, a tetrameric metalloprotein
  - 2 α-like globin chains
  - 2 β-like globin chains
  - 4 heme molecules with central $Fe^{2+/3+}$
- Adult expression of human hemoglobins









HbA
>95%

HbA2
<3.5%

HbF
<1.0%

Hemoglobin

- Adult expression of human hemoglobins

HbA
>95%

HbA2
<3.5%

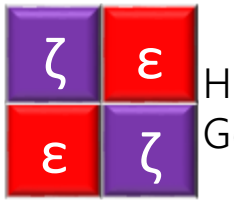
HbF
<1.0%

- Hemoglobins at birth

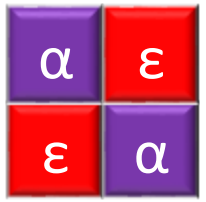
HbA
>25%

HbF
<75%

- Embryonic hemoglobins

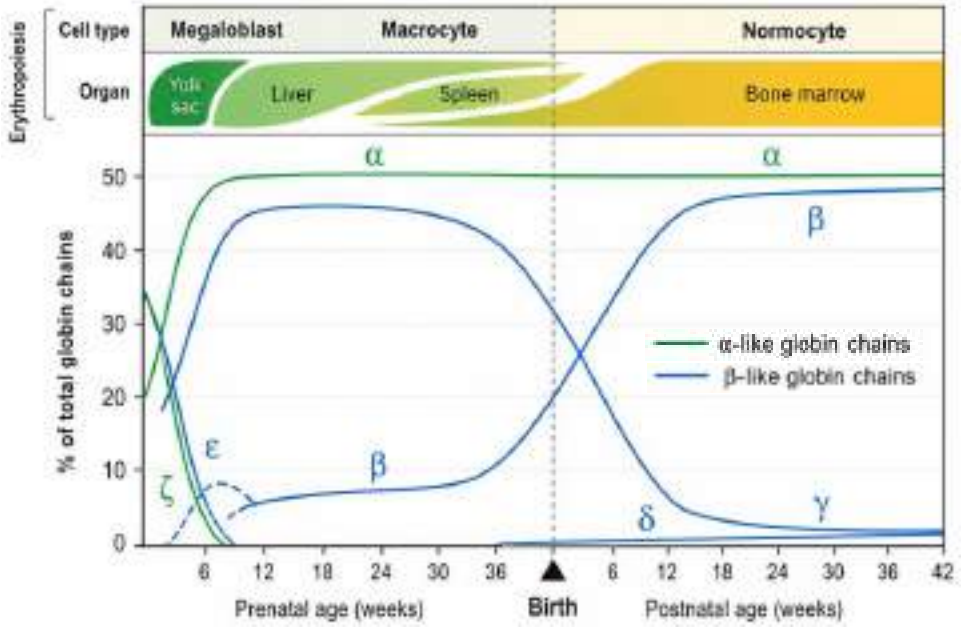


Hb
Gower 1

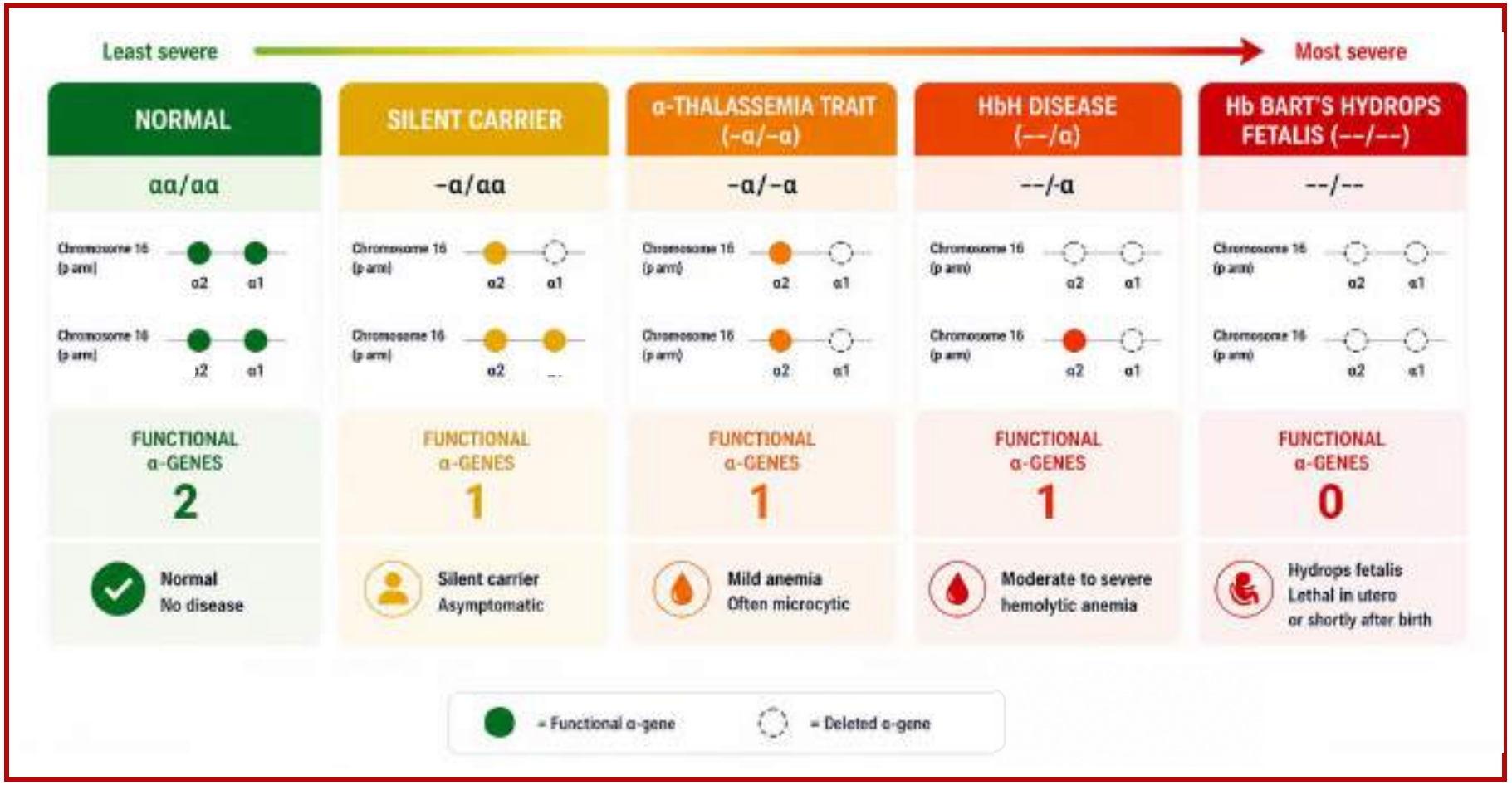


Hb
Gower 2

Hb
Portland 1



Alpha thalassemia genotypes & phenotypes



Gene Therapy for hemoglobinopathies (β-thal and SCD)

FDA-approved gene therapies

ZYNTEGLO®
(betibeglogene autotemcel)

Indication:
Transfusion-dependent β-thalassemia

FDA approved
August 2022

CASGEVY®
(exa-cel)

Indication:
Sickle cell disease (SCD) and β-thalassemia

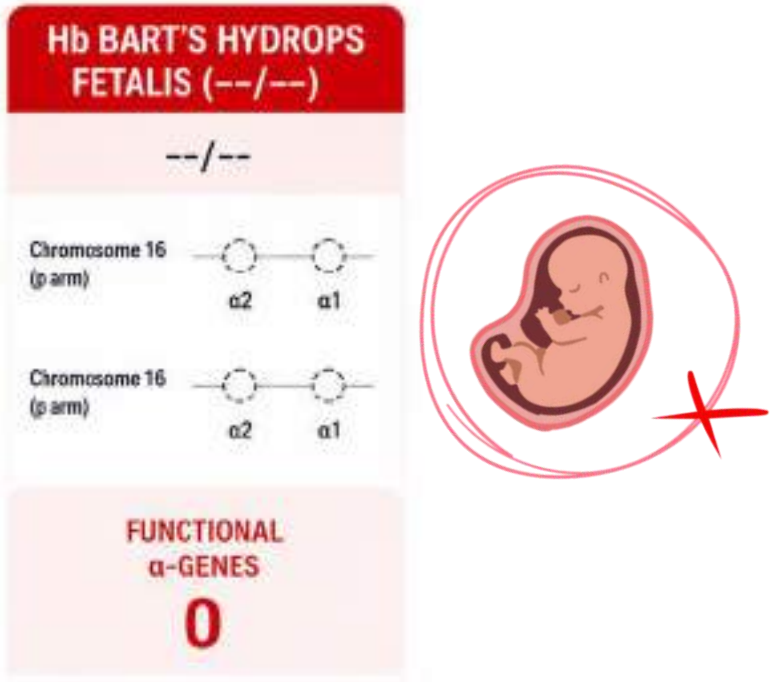
FDA approved
December 2023

Real impact for patients

- Reduces or eliminates the need for lifelong transfusions
- Restores healthy hemoglobin production
- Improves quality of life and physical functioning
- Durable, long-term clinical benefit observed

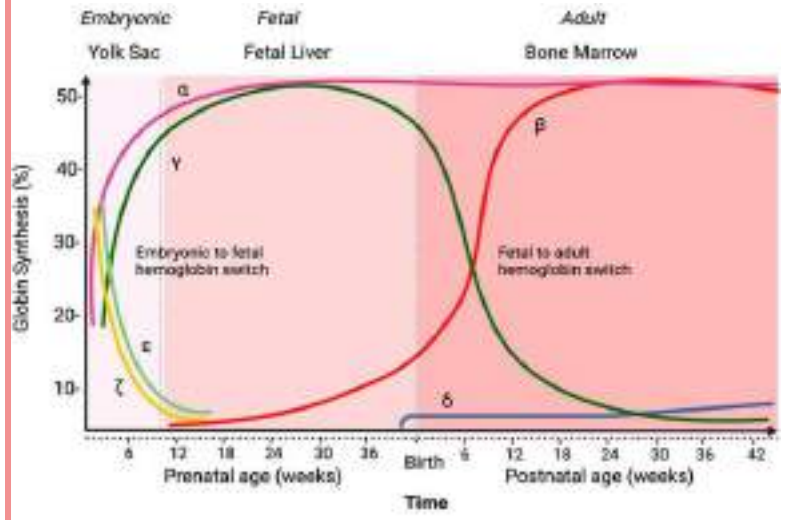
These landmark approvals mark a **new era in treatment** for hemoglobin disorders and pave the way for **future advances** in α-thalassemia.

Gene Therapy for α-thalassemia



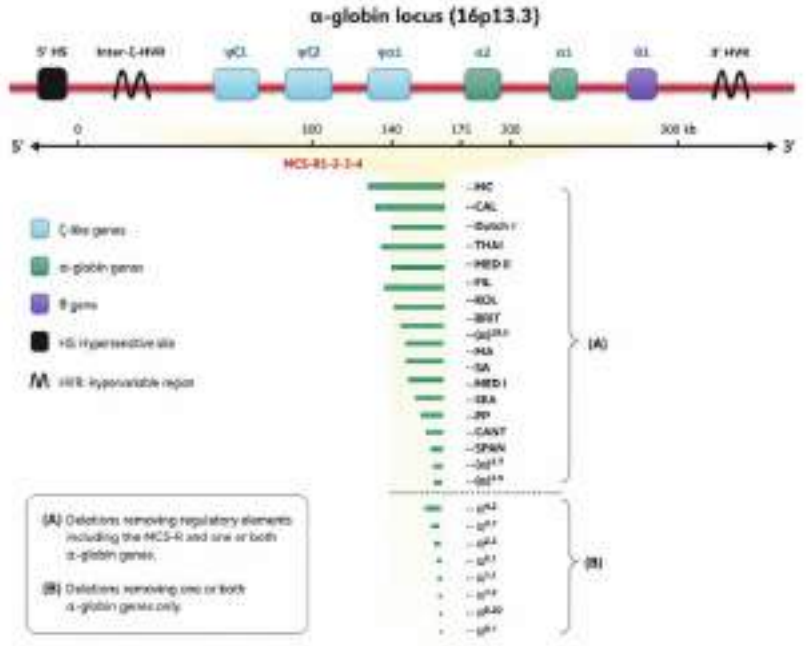
α-thalassemia major is lethal

No representative cell systems exist



Silencing of ζ-globin is stronger than γ-globin

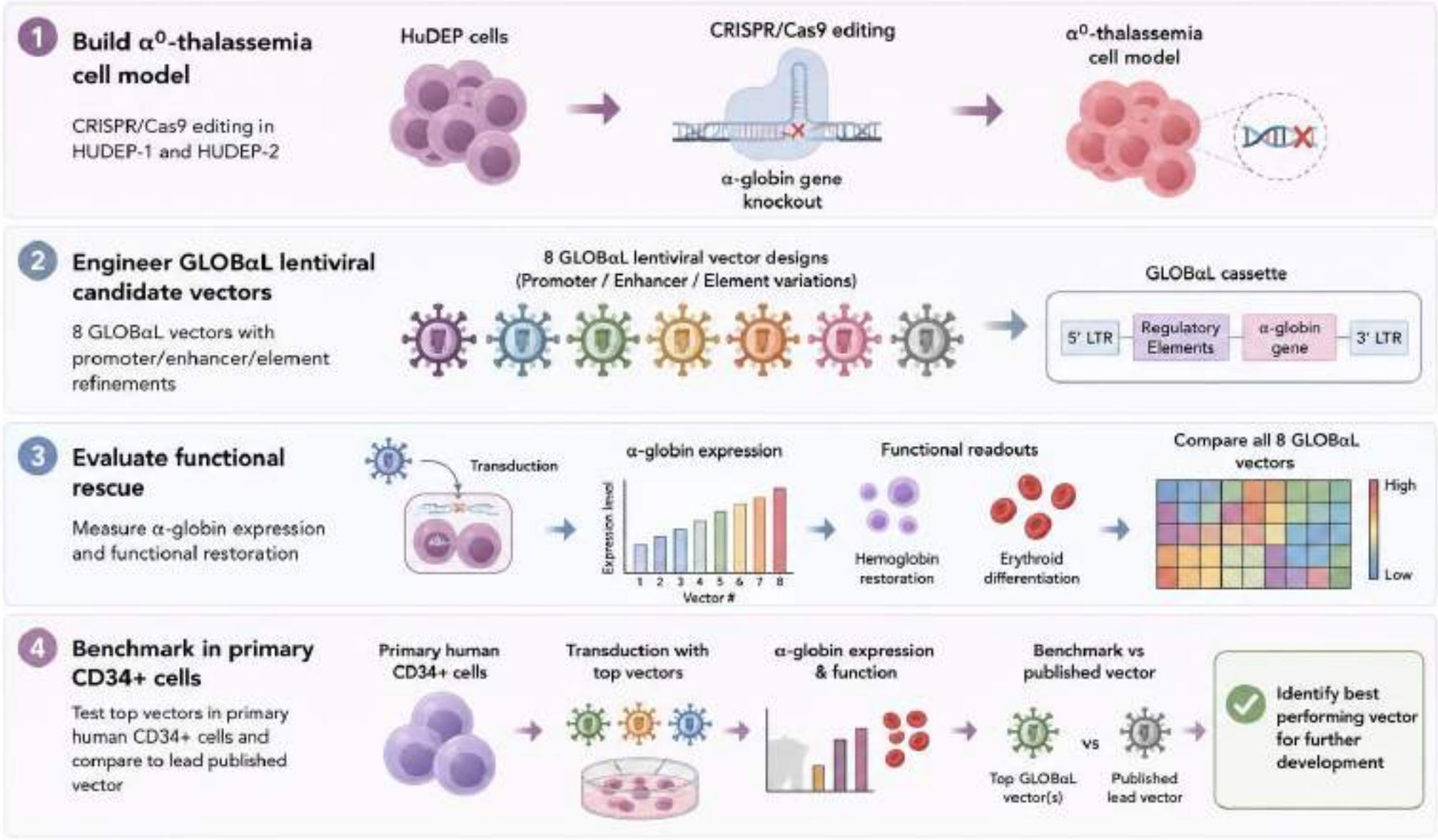
ζ-β tetramers are not naturally occurring



α-thalassemia is caused by large deletions

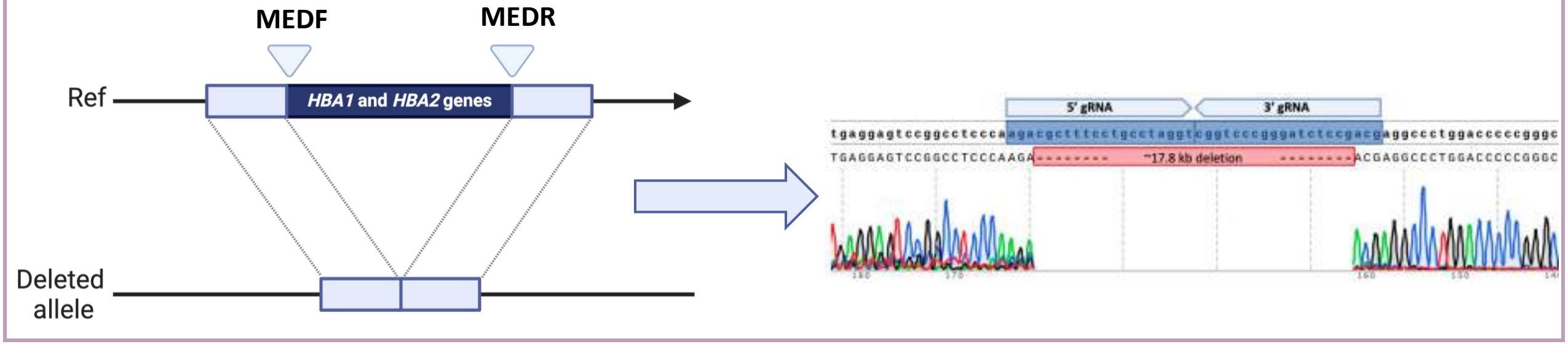
Genome editing and HDR are likely inefficient as treatment

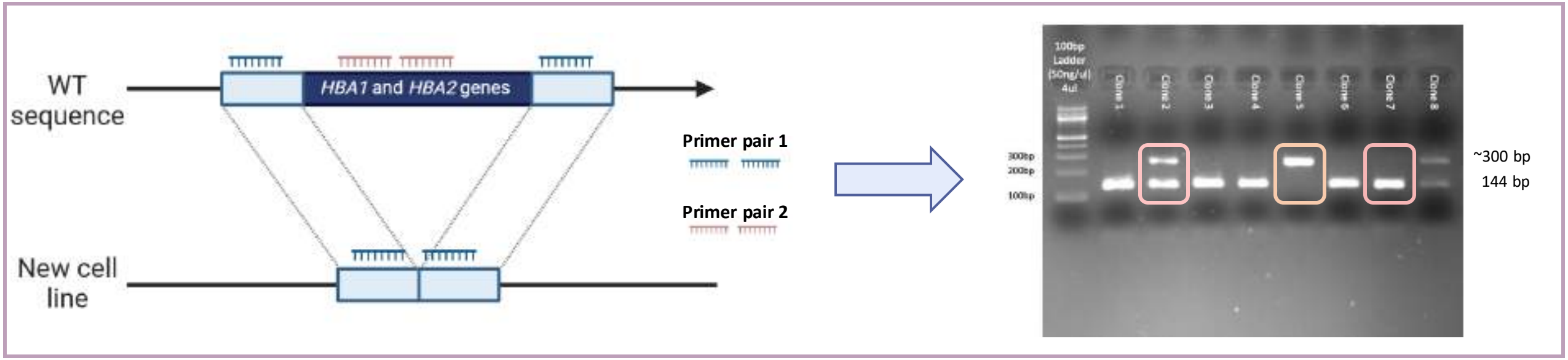
Primary aim: Develop and evaluate optimized lentiviral vectors to restore α-globin expression in α-thalassemia



Outcome: Identify and validate the best performing GLOBαL lentiviral vector(s) for α-globin gene therapy.

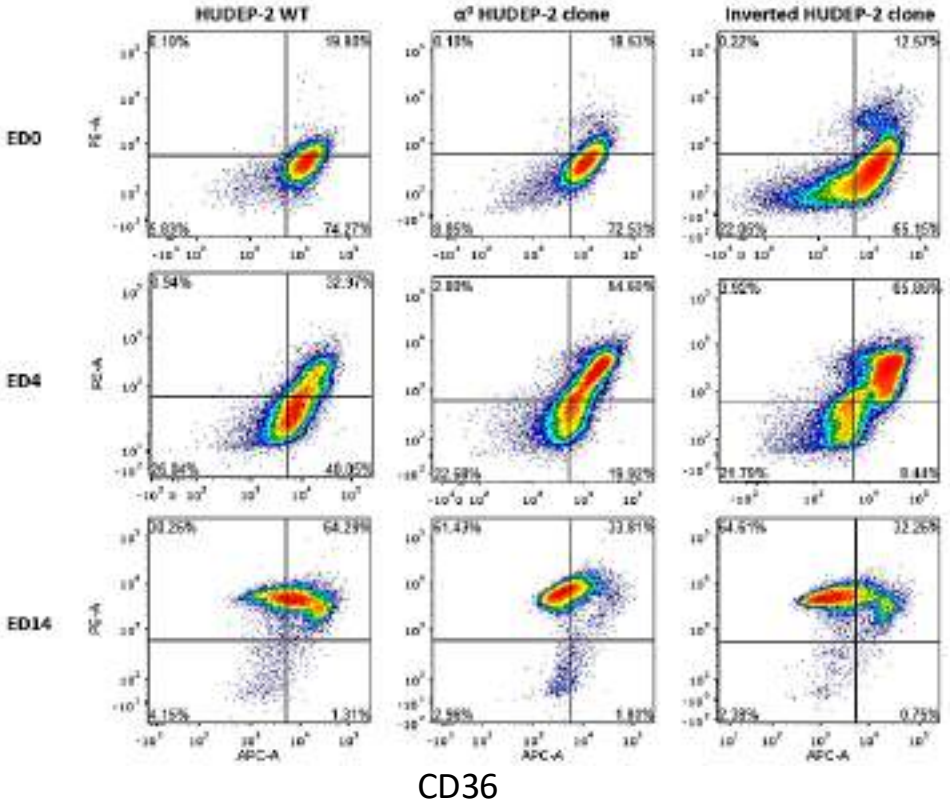
HBA1/HBA2 region was successfully deleted in bulk populations of HUDEP-2 (and HUDEP-1) cells

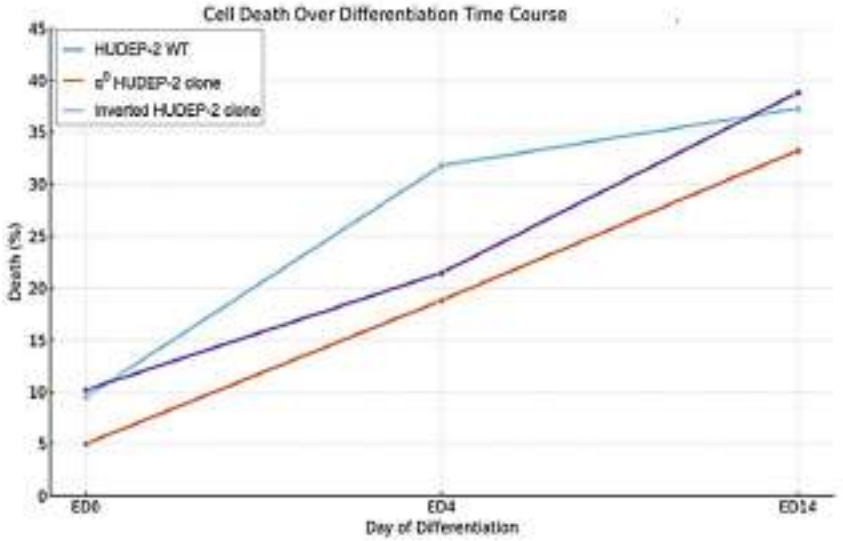




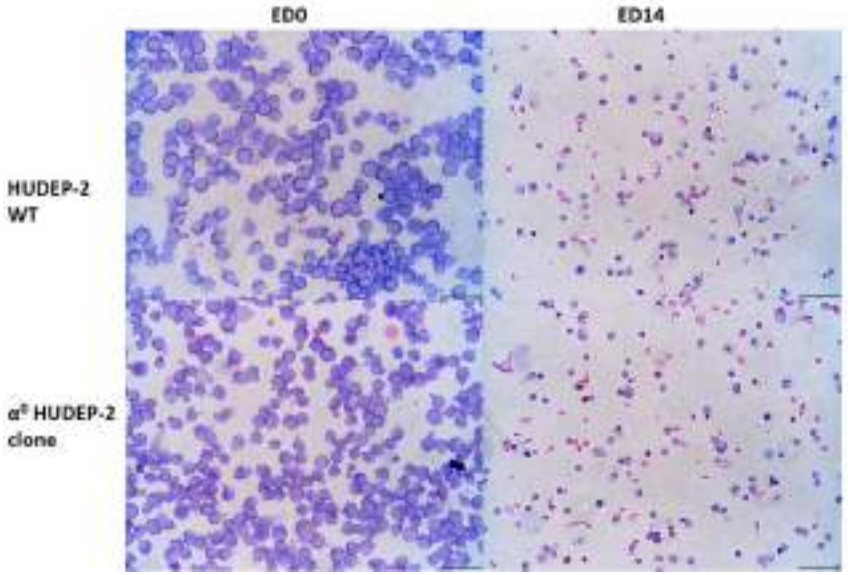
Unaffected erythroid differentiation capacity in HUDEP-2 α0-thalassemia clonal cell population

All cells exhibited sustained differentiation through all time points





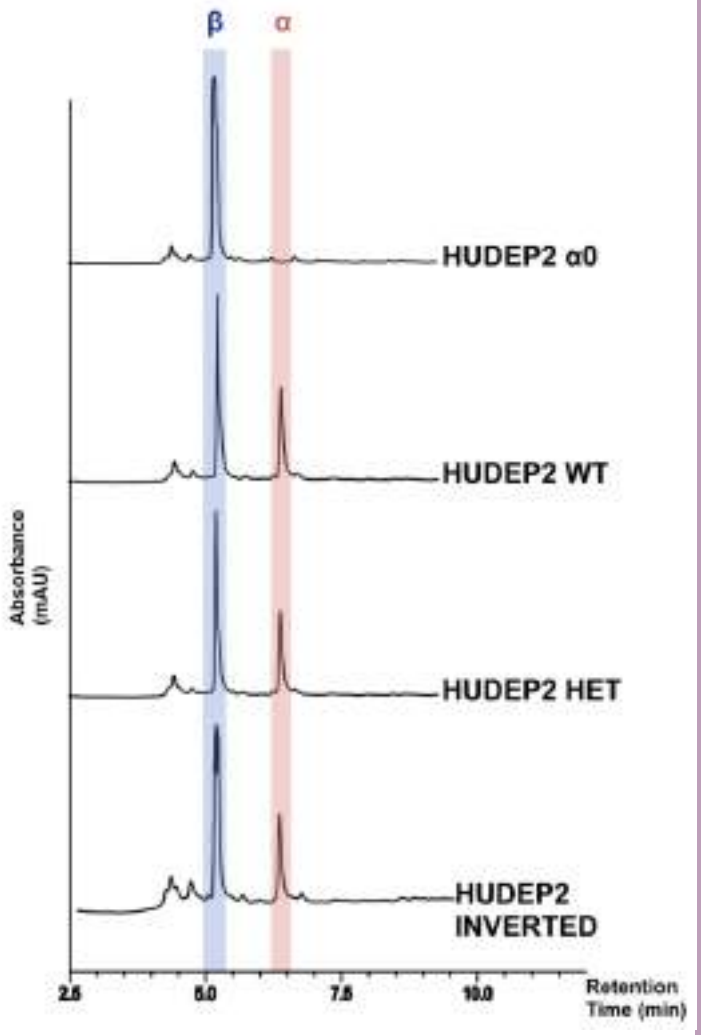
Increased apoptosis during differentiation in all cell lines



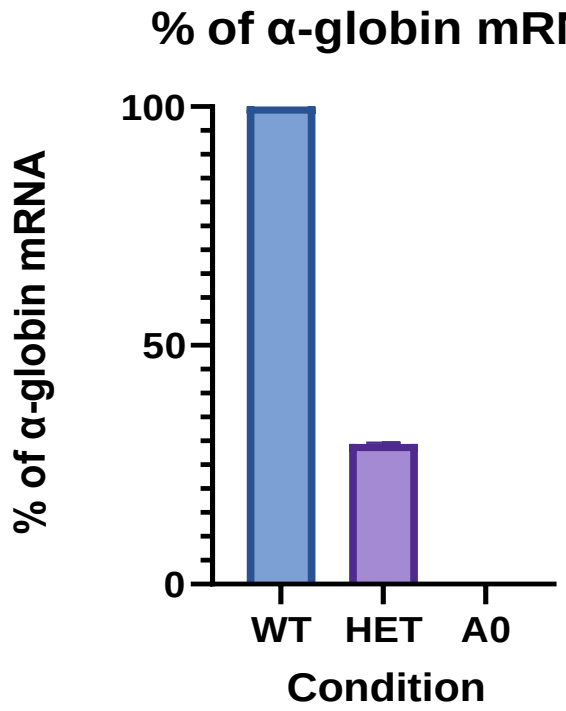
No impairment in morphological progression in α^0 HUDEP-2 cells

No α-globin expressed in the α0-thalassemia biallelic clonal cell line both at the RNA and protein level

RP-HPLC



RT-qPCR

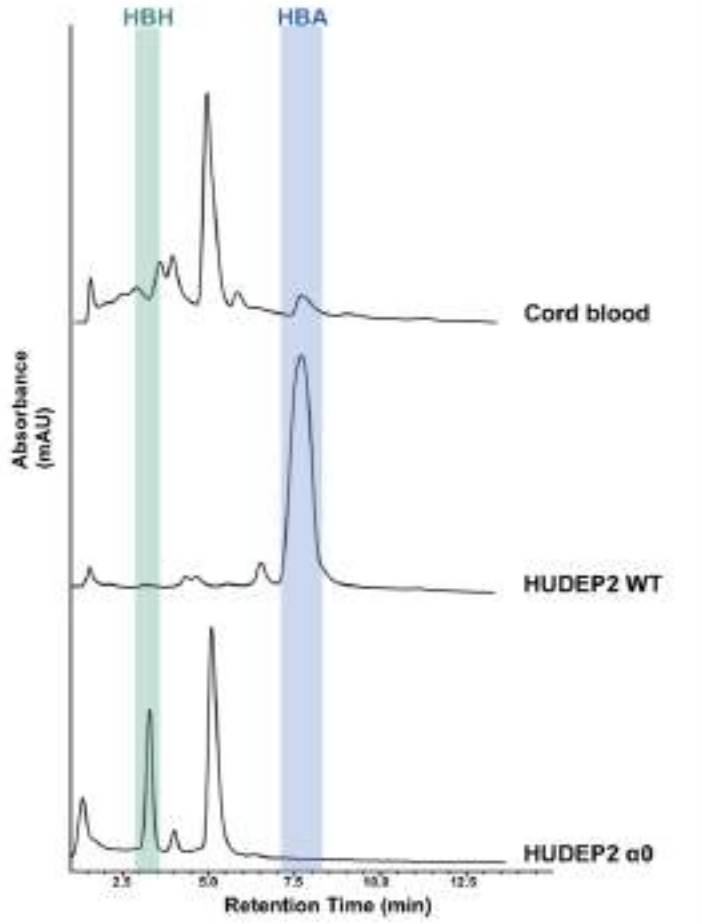


No α-globin or HbA in α^0 HUDEP-2 clone

Reduced α-globin in biallelic inverted HUDEP-2 clones

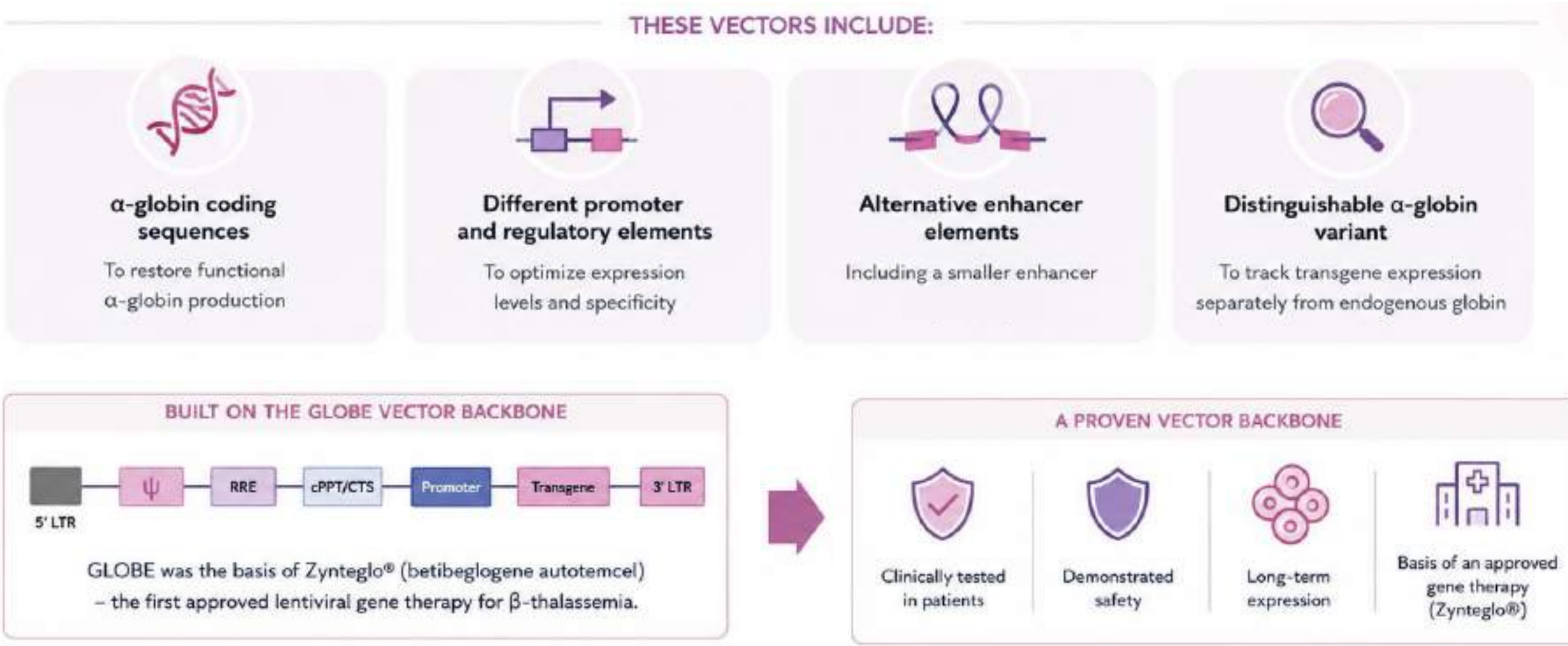
A peak that resembles that of HbH is identified in the α^0 HUDEP-2 clone

CE-HPLC

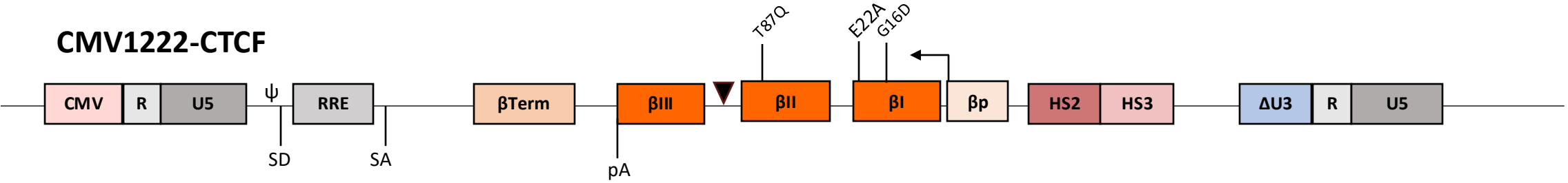


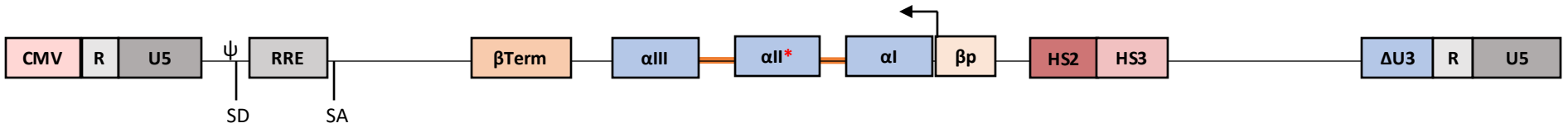
Engineer GLOBE-derived lentiviral vectors for α-globin gene delivery (GLOBαL)

We designed and developed a panel of lentiviral vectors, which we call GLOBαL vectors, to integrate an exogenous α-globin gene into the genome and restore its expression

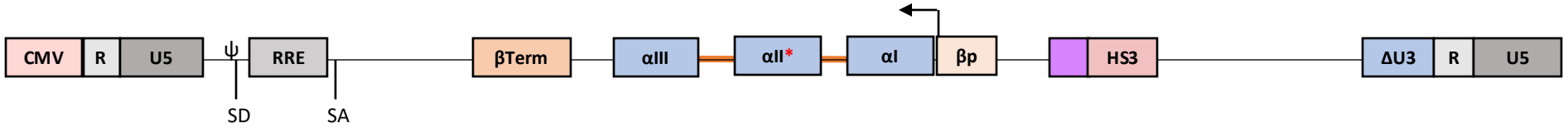


GLOBαL vector structure: An α-Globin Derivative of the (Milan) GLOBE Vector





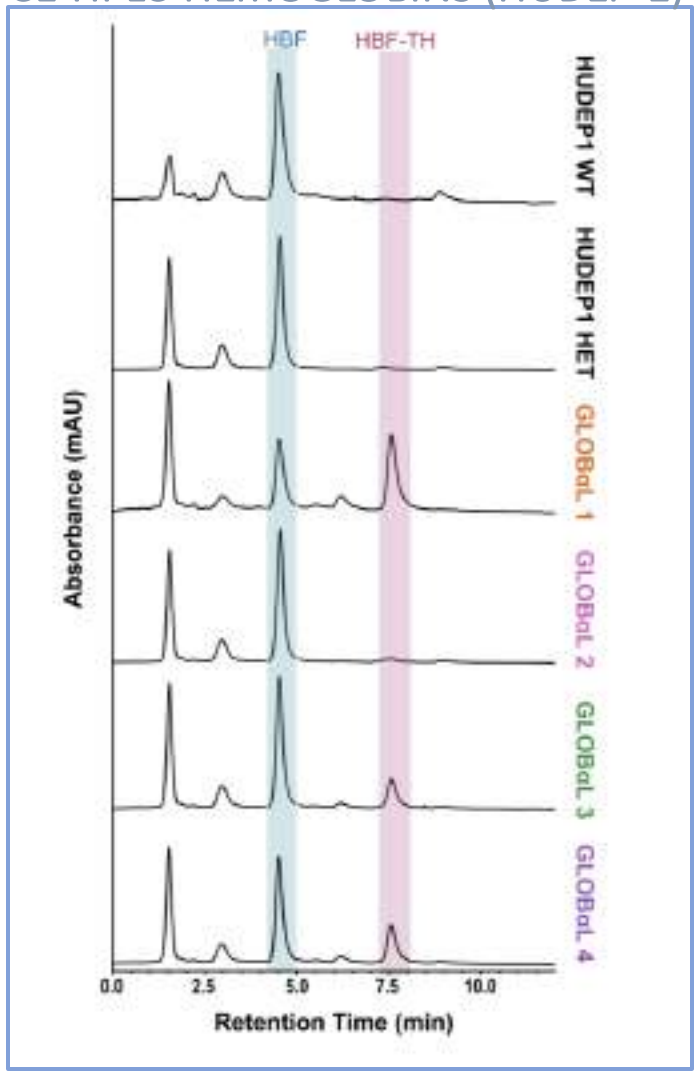
General structure of GLOBαL 1-4



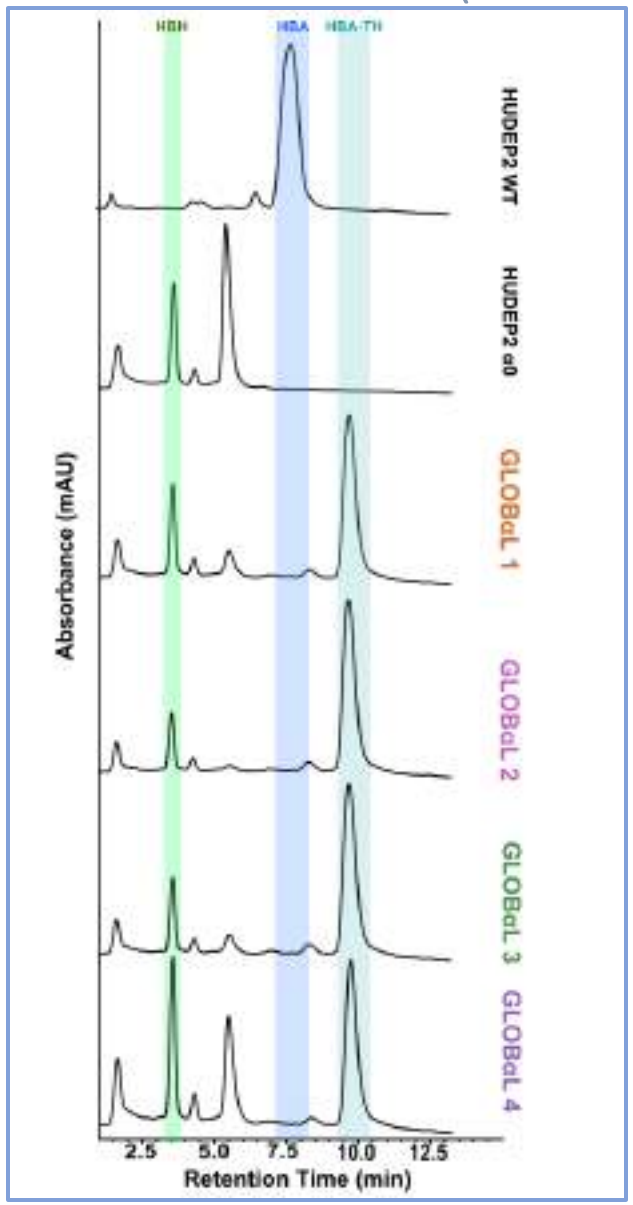
General structure of GLOBαL 5-8

All GLOBαL vectors show α-globin expression in adult and fetal-like cells lines

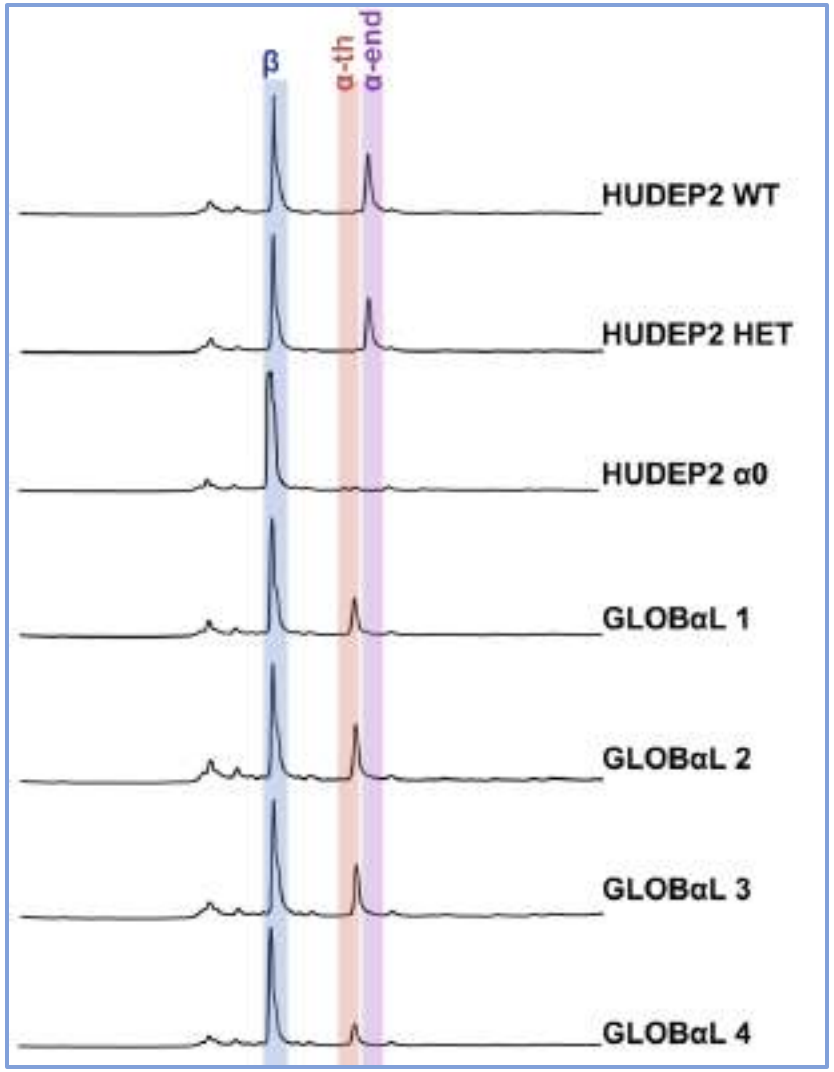
CE-HPLC-HEMOGLOBINS (HUDEP-1)



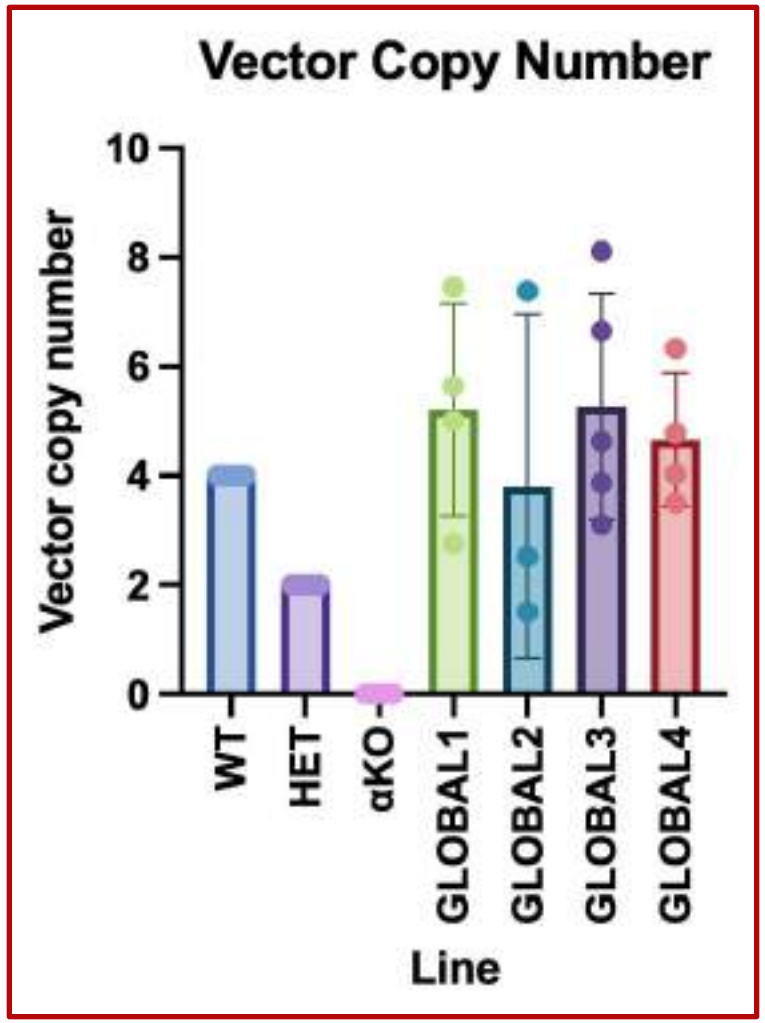
CE-HPLC-HEMOGLOBINS (HUDEP-2)

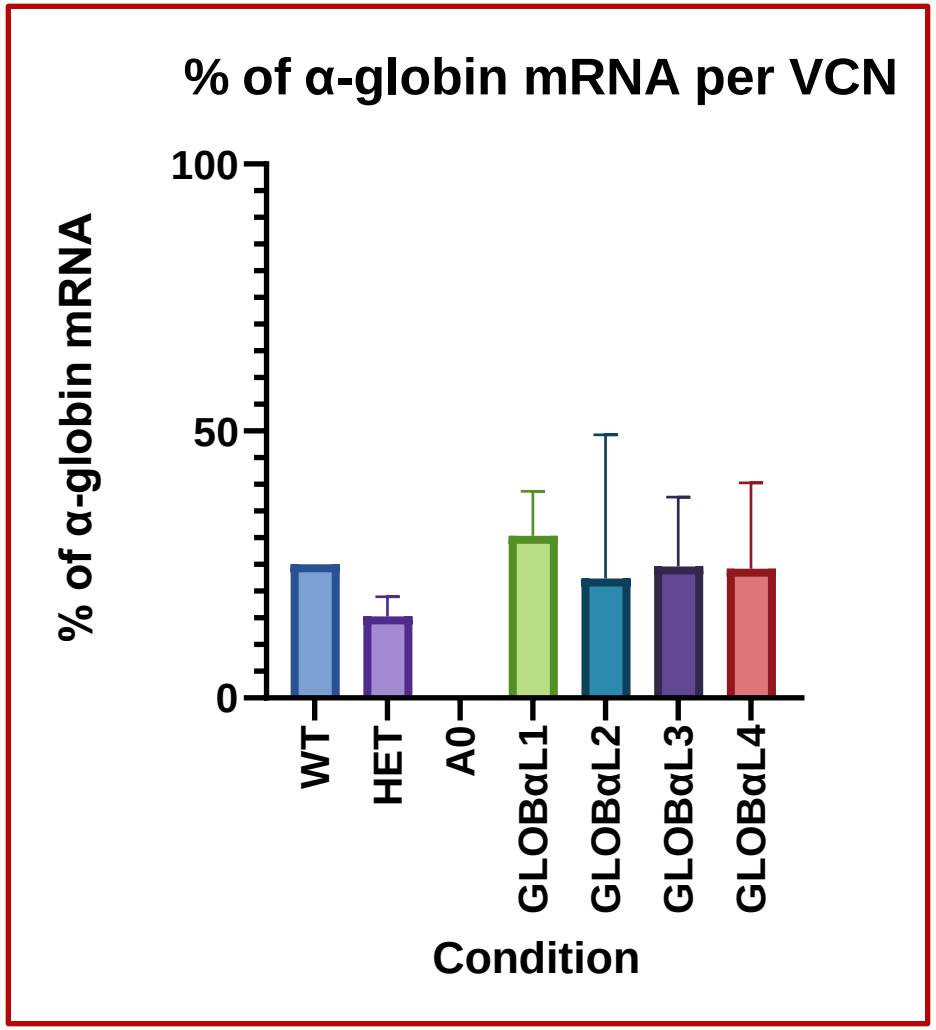


RP-HPLC-GLOBINS (HUDEP-2)

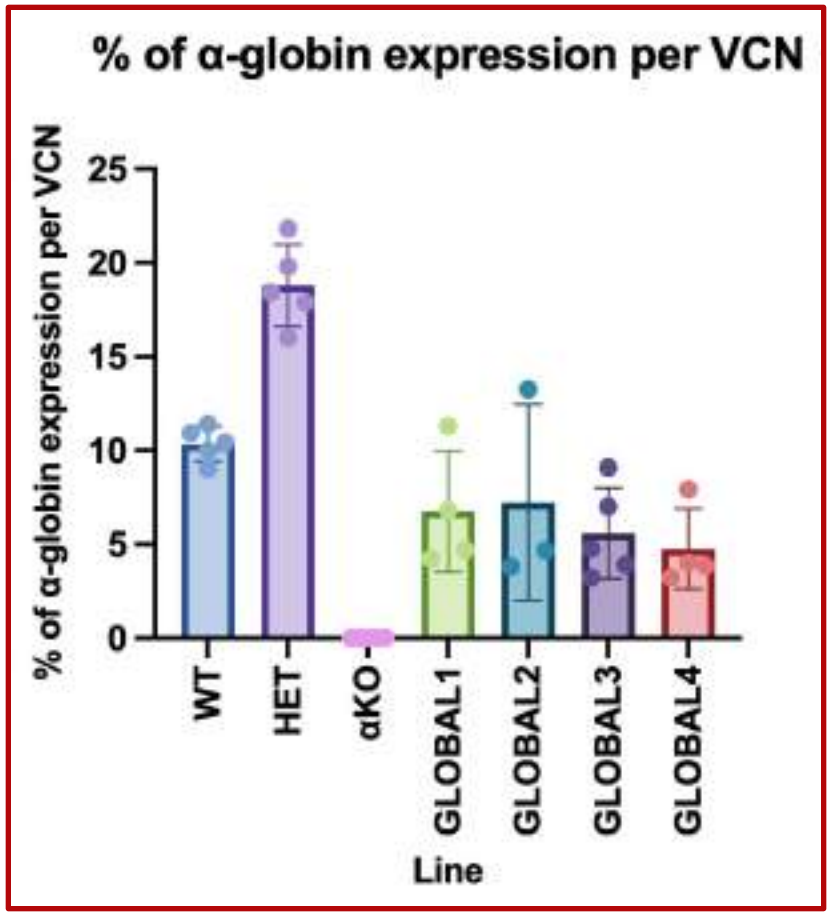


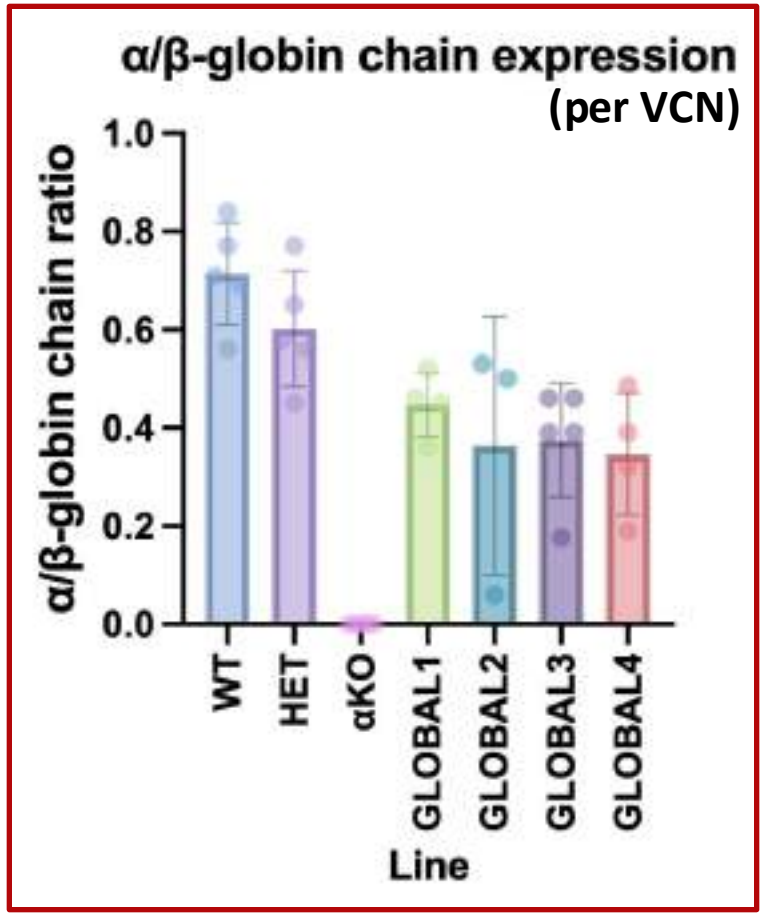
All GLOBαL vectors drive exogenous alpha globin expression in bulk-transduced HUDEP-2 α0 cells



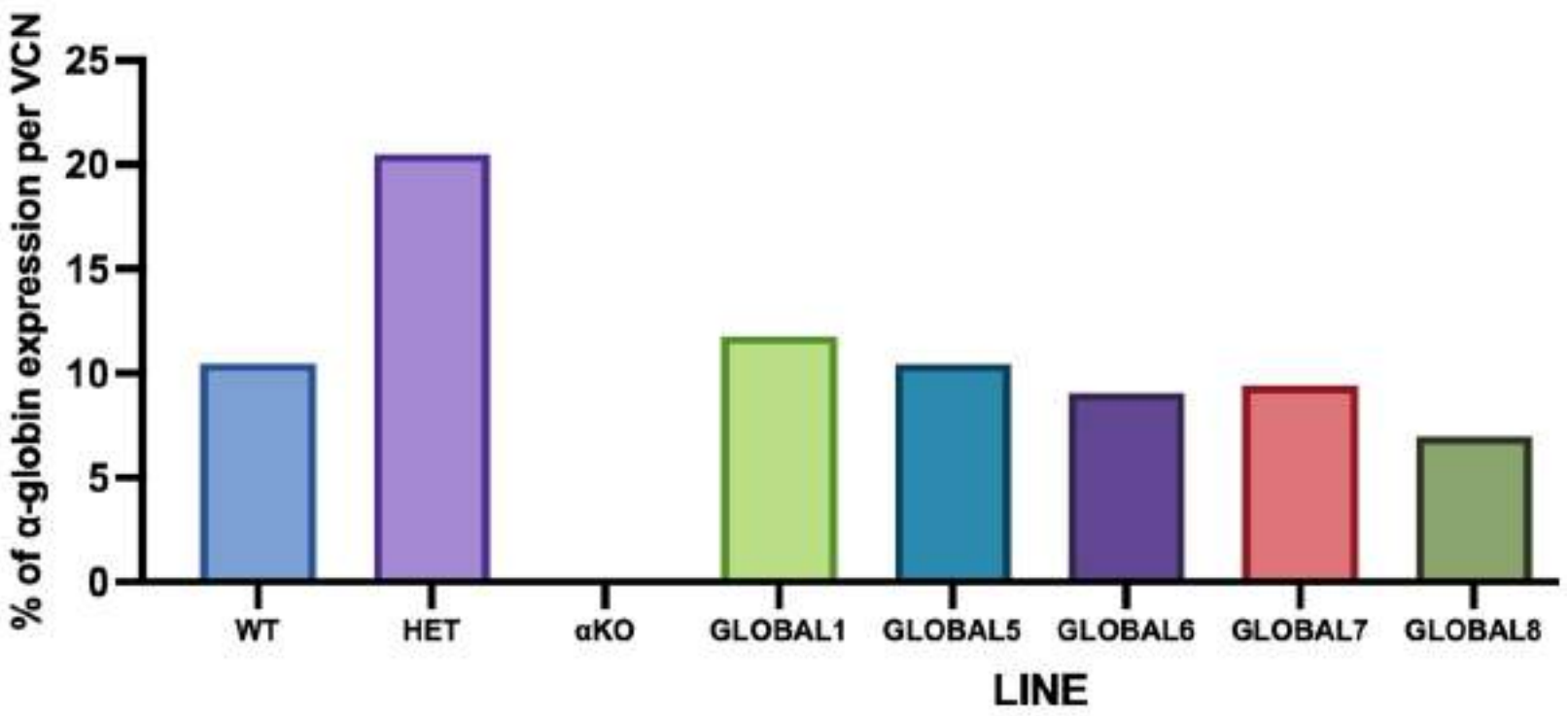


All GLOBαL vectors drive exogenous alpha globin expression in bulk-transduced HUDEP-2 α0 cells





Comparison of α-globin expression vectors in HUDEP2 α0 cells



Discussion

Engineered and assessed clonal α^0-thalassemia adult (HUDEP-2) model cell lines

Detected the benign α-globin transgene in adult and fetal erythroid cells

Generated a panel of 8 lentiviral GLOBαL constructs

Compared levels of exogenous α-globin expression.

Future Aims

- Isolate and characterize HUDEP-1 α^0-thalassemia biallelic clones
- Functionally test and compare the 8 lentiviral vectors and published control in HUDEP-1 α^0-thalassemia clones
- Pre-clinical validation of the lead vectors in human CD34+ cells from HbH disease patients (α-/--)
- In utero evaluation in alpha^0 thalassemic mice (intrahepatic lentiviral injections)

THANK YOU

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Ms. Charis Constantinou (PhD candidate)
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