Il patient journey della terapia genica nei pazienti affetti da emoglobinopatie

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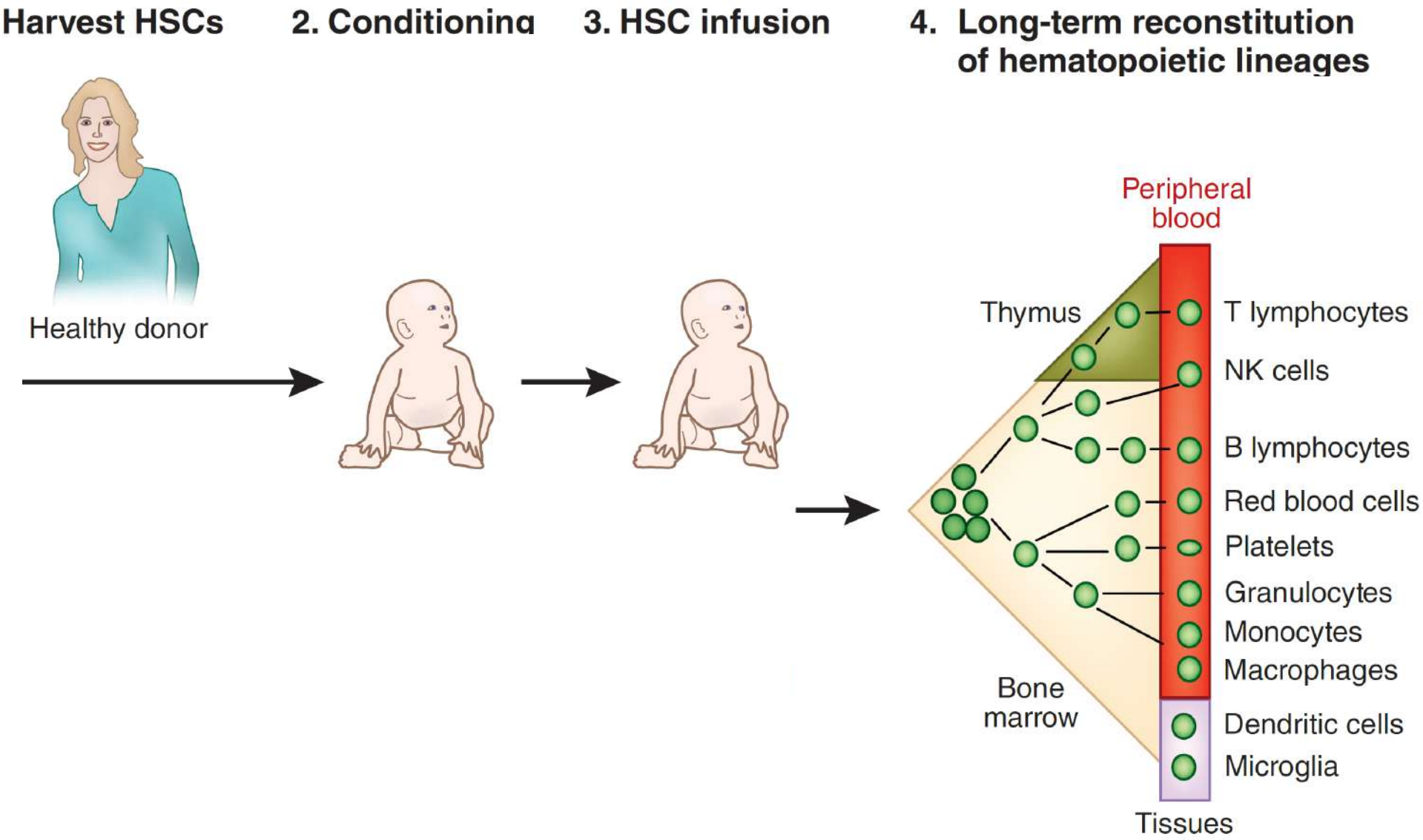
Disclosures of Franco Locatelli

Name of Company	Research Support	Employee	Consultant	Stockholder	Speaker's Bureau	Advisory Board	Other
Miltenyi					X		
Amgen					X	X	
Novartis					X	X	
BMS					X		
GILEAD					X		
Sanofi						X	
SOBI					X		
Vertex						X	

Potentially Curative Treatments in Patients With Hemoglobinopathies

Allogeneic hematopoietic cell transplantation

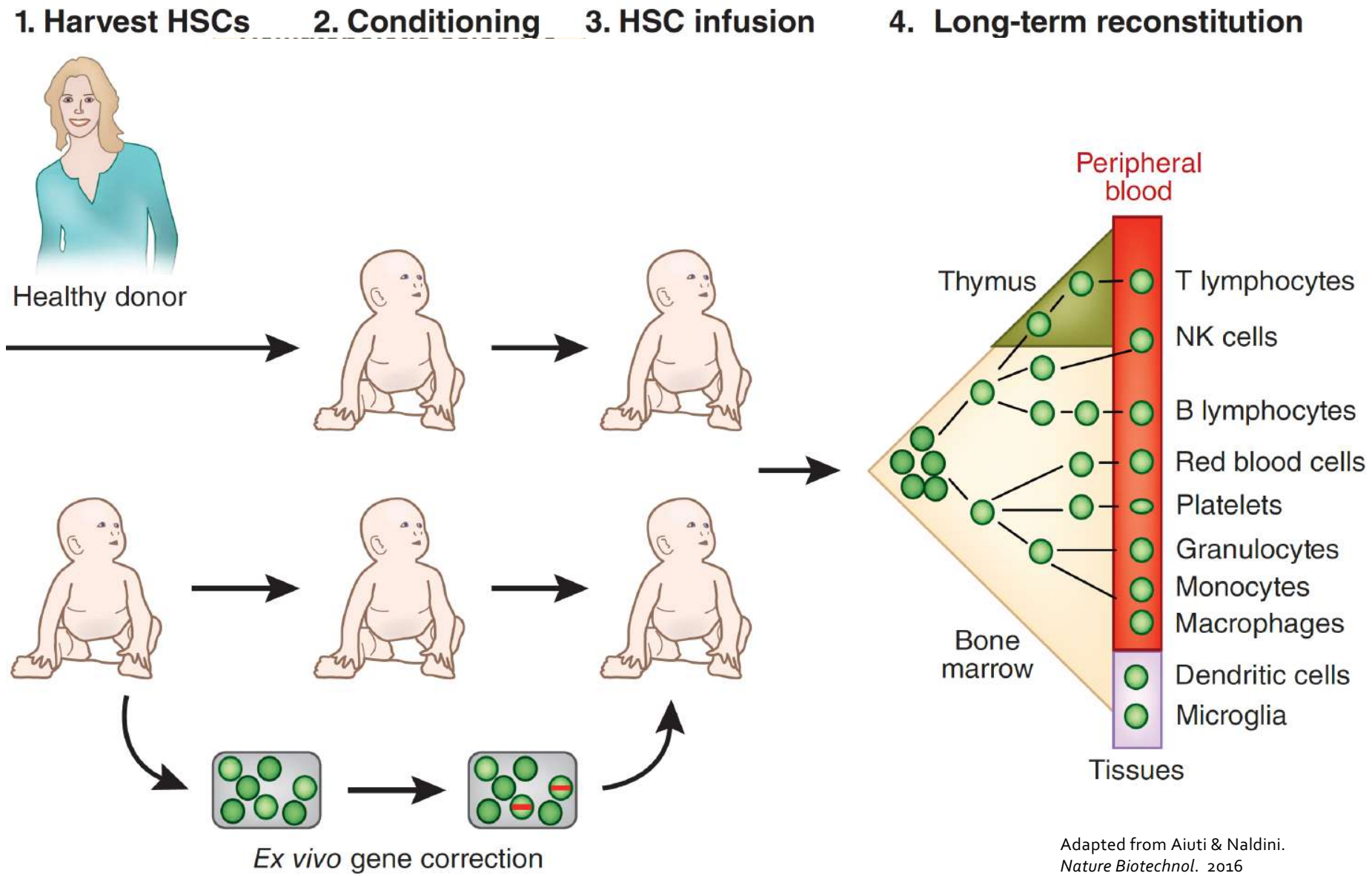


Adapted from Aiuti & Naldini. Nature Biotechnol. 2016

Potentially Curative Treatments in Patients With Hemoglobinopathies

Allogeneic hematopoietic cell transplantation

Gene Therapy



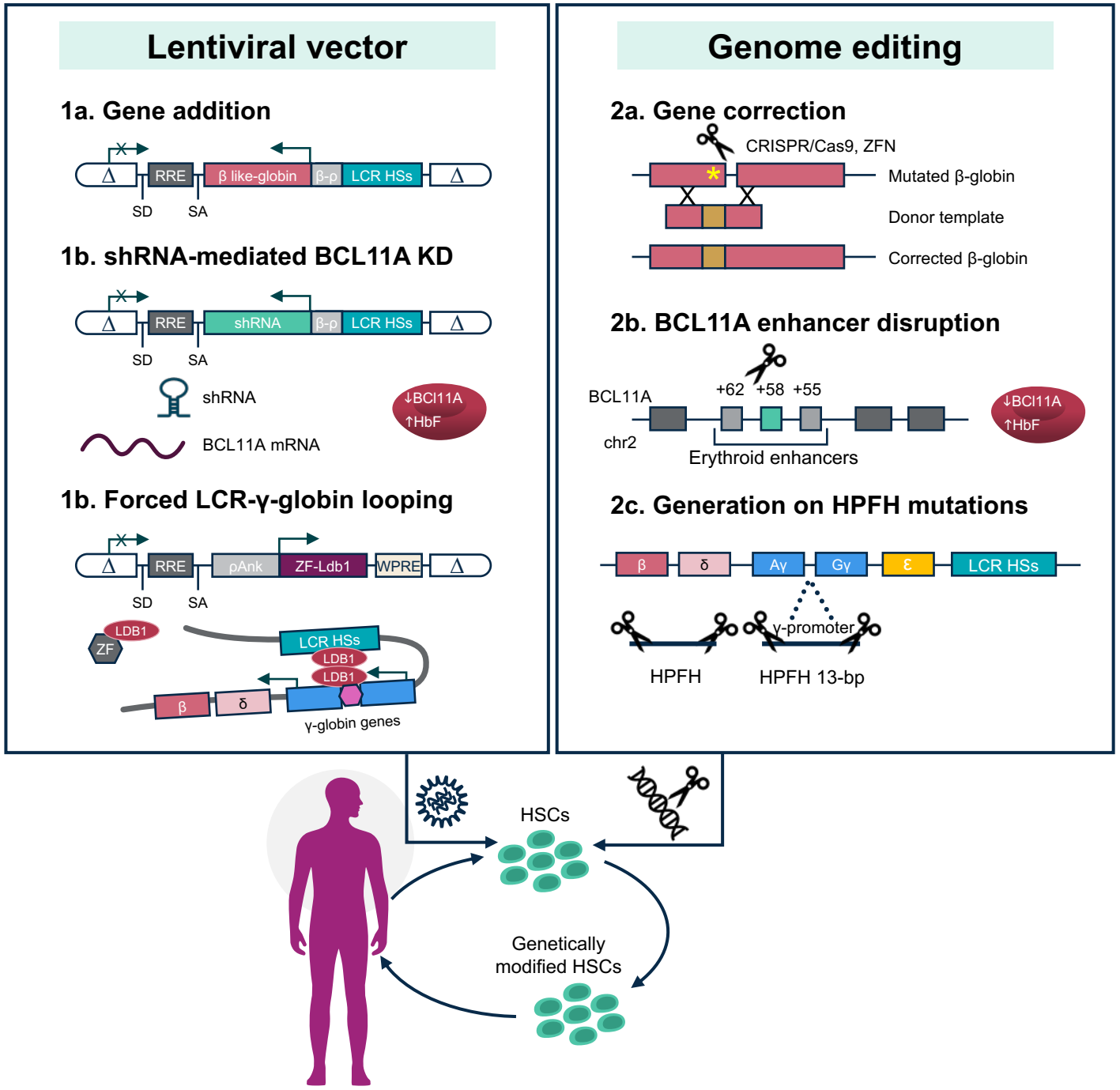
Adapted from Aiuti & Naldini. *Nature Biotechnol*. 2016

The paradigmatic model of gene therapy for haemoglobinopathies

More than one option

Gene therapy

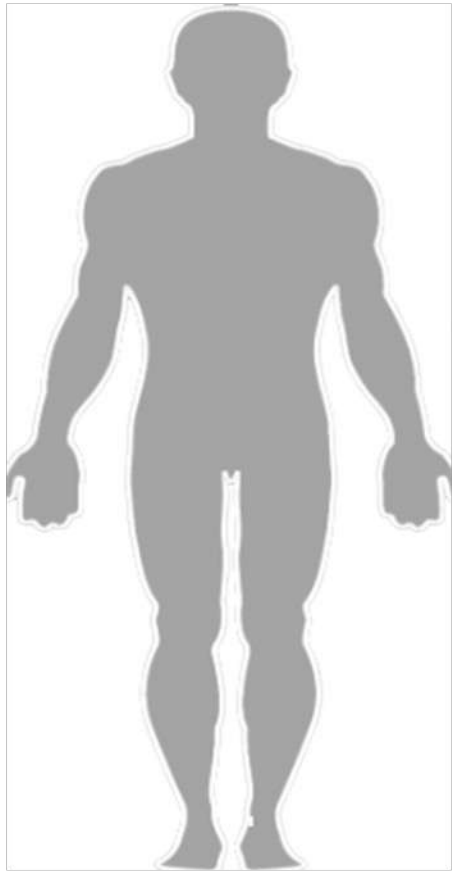
Genome editing



HPFH, hereditary persistence of foetal haemoglobin; HSC, haematopoietic stem cell; LCR, locus control region; mRNA, messenger ribonucleic acid; shRNA, short hairpin ribonucleic acid; ZFN, zinc-finger nucleases.

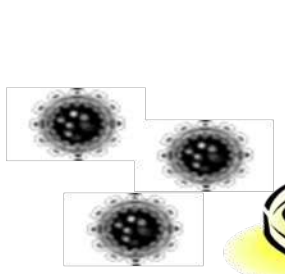
Cavazzana M, et al. *Mol Therapy*. 2017;25(5):1142–154.

Autologous Stem Cells Are the Basis of All Current Gene Therapy and Genome Editing Trials

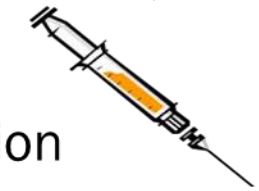


Harvest

Patients' own bone marrow stem cells



Use different gene therapy techniques to introduce or increase production of healthy ɣ- or β-globin genes from stem cells



Transplantation back to patients

SCD/Thalassemia patients

- Patients serve as their own donor
- Available for all patients
- No need for immunosuppression
- No risk of GVHD

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Exagamglogene Autotemcel for Transfusion-Dependent β-Thalassemia

F. Locatelli, P. Lang, D. Wall, R. Meisel, S. Corbacioglu, A.M. Li, J. de la Fuente, A.J. Shah, B. Carpenter, J.L. Kwiatkowski, M. Mapara, R.I. Liem, M.D. Cappellini, M. Algeri, A. Kattamis, S. Sheth, S. Grupp, R. Handgretinger, P. Kohli, D. Shi, L. Ross, Y. Bobruff, C. Simard, L. Zhang, P.K. Morrow, W.E. Hobbs, and H. Frangoul, for the CLIMB THAL-111 Study Group*

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Exagamglogene Autotemcel for Severe Sickle Cell Disease

H. Frangoul, F. Locatelli, A. Sharma, M. Bhatia, M. Mapara, L. Molinari, D. Wall, R.I. Liem, P. Telfer, A.J. Shah, M. Cavazzana, S. Corbacioglu, D. Rondelli, R. Meisel, L. Dedeken, S. Lobitz, M. de Montalembert, M.H. Steinberg, M.C. Walters, M.J. Eckrich, S. Imren, L. Bower, C. Simard, W. Zhou, F. Xuan, P.K. Morrow, W.E. Hobbs, and S.A. Grupp, for the CLIMB SCD-121 Study Group*

Gene editing approaches: approval by regulatory agencies

Both FDA, at the end of 2023, and EMA, at the beginning of 2024, have approved the gene editing appoach aimed at re-activating the synthesis of HbF in patients above the age of 12 years in patients with:

- **TDT, including β^0/β^0 genotypes, defined as a history of ≥100 mL/kg/year or ≥10 units/year of packed RBC transfusions in the previous 2 years;**
- **Severe SCD and a history of ≥2 severe VOCs per year in the previous 2 years**

Key Differences between allogeneic HSCT and Exa-cel

Curative approaches for haemoglobinopathies include two essential types: allogeneic HSCT and autologous Gene Therapy[1,2]

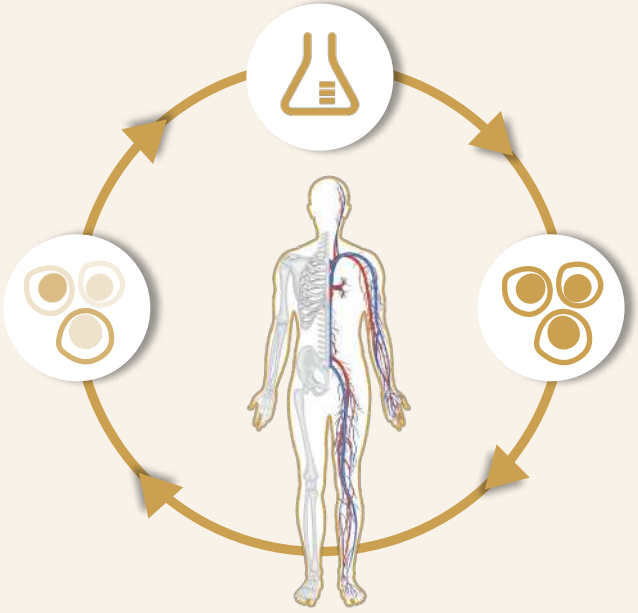
Allogeneic HSCT[1]

Involves the collection of HSCs from a healthy donor and infusion of donor HSCs into the patient

Once a suitable donor is identified, the transplantation process can proceed relatively quickly. However, finding an unrelated matched donor can be challenging and time-consuming.[4a,b]

Exa-cel (and autologous Gene Therapy in general)[1,3]

Involves the collection of HSCs from a patient's own body, with or without modification and/or expansion of HSCs, and subsequent infusion of HSCs back into the patient



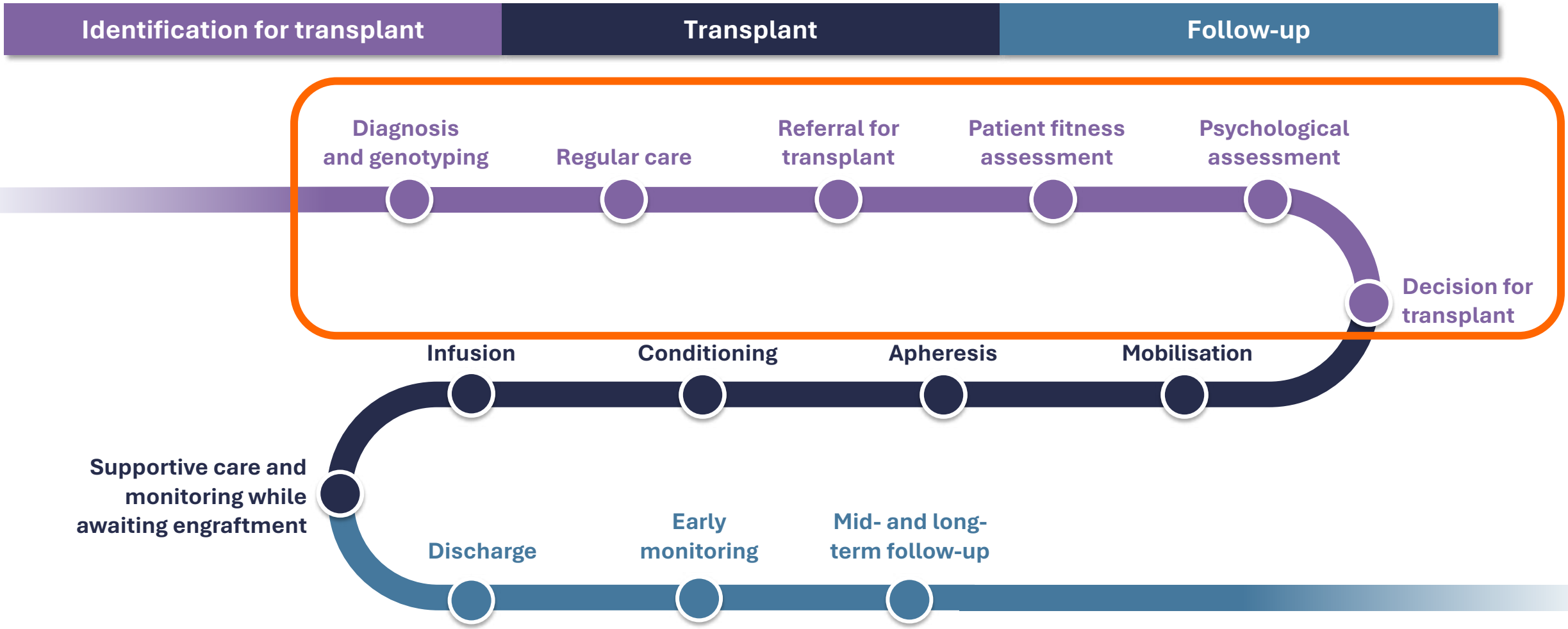
Requires time for preparation. The process of harvesting and manufacturing the patient's cells before re-infusion can take several months, especially if multiple collection cycles are needed.[5]

HSC, haematopoietic stem cell; HSCT, haematopoietic stem cell transplantation.

1. Singh A, et al. *Cancer Res*. 2016;76(22):6445-51; 2. Ali N, et al. *Stem Cells Transl Med*. 2015;4(8):873-7; 3. Epah J, Schäfer R. *Gene Ther*. 2021;28(9):528-41; 4. Gragert L, et al. N Engl J Med. 2014;371(4):339-348; 5. Frangoul H, et al. Transplant Cell Ther. 2025 Mar 7:S2666-6367(25)01061-9. doi: 10.1016/j.jtct.2025.02.025. Epub ahead of print

a)Data are specific to HSCT but not SCD or TDT; b)Percentages were obtained from a US adult-donor registry and reflect the likelihood of identifying an adult donor with an 8/8 HLA match (based on patient race and ethnicity

The journey towards the cure in haemoglobinopathies



Frangoul H, et al. Transplant Cell Ther. 2025 Mar 7:S2666-6367(25)01061-9. doi: 10.1016/j.jtct.2025.02.025. Epub ahead of print.

The impact of age on HSCT outcomes for SCD and TDT

Allogeneic HSCT

- In large registry studies, **younger patients had better outcomes** than older patients[1–3]
- Optimal age for HSCT:
  - **SCD**: ≤14 years[1,2]
  - **TDT**: ≤14–15 years[3]
- Recent data on **adult patients** transplanted from **haploidentical donors** report **excellent outcomes** in adults with **SCD**[4] and **acceptable outcomes** in adults with **TDT**[5]

Exa-cel

- Most patients given the treatment are **adolescents** (≥12 years) **and adults**[6,7]; pediatric trials are ongoing.
- **Upper age limit for enrolment in registration studies is 35 years**[6,7]
- **No differences in outcomes between adolescents and adults** in the largest cohorts reported so far[6-8]
- No reported differences in main safety and efficacy outcomes according to genotype, transfusion burden, iron overload status[6,7]
- Number of treated patients is still **limited**[6,7]

HSCT, haemopoietic stem cell transplantation; SCD, sickle cell disease; TDT, transfusion-dependent β-thalassaemia.

1. Baronciani D, et al. *Bone Marrow Transplant*. 2016;51(4):536–41; 2. Li C, et al. *Blood Adv*. 2019;3(17):2562–70; 3. Eapen M, et al. *Lancet Haematol*. 2019;6(11):e585–96; 4. Kassim AA, et al. *Blood*. 2024;143(25):2654–65; 5. Anurathapan U, et al. *Biol Blood Marrow Transplant*. 2020;26(6):1106–12; 6. Frangoul H, et al. N *Engl J Med*. 2024;390(18):1649–62; 7. Locatelli F, et al. *N Engl J Med*. 2024;390(18):1663–76; Locatelli F, et al. Blood (2024) 144 (Supplement 1): 512: https://doi.org/10.1182/blood-2024-205779

Eligibility checklist to aid physicians with patient selection

- Fit for **myeloablative autologous stem cell transplant** on the judgement of a transplant physician with experience in transplantation for haemoglobinopathies[1]
- In the CLIMB-111 study, assessments were made to confirm the absence of the following post-workup investigations:[2]
  - A known and available fully matched **HLA-related donor**
  - Prior **allogeneic HSCT**
  - Baseline estimated glomerular filtration rate **<60 mL/min/1.73 m^2**
  - Patients with associated **α-thalassaemia** and **>1 alpha deletion** or **alpha multiplications**
  - Patients with **sickle cell β-thalassaemia variant**
  - Clinically significant and active **bacterial**, **viral**, **fungal**, or **parasitic** infection
  - White blood cell count **<3 × 10^9/L** or platelet count **<50 × 10^9/L** not related to hypersplenism

For SCD

- One and two **alpha gene deletion** is an inclusion[3]

HLA, human leukocyte antigen; HSCT, haematopoietic stem cell transplantation; L, litre; SCD, sickle cell disease.

1. Vertex. CASGEVY® (exagamglogene autotemcel) SmPC. Available at: https://www.ema.europa.eu/en/documents/product-information/casgevy-epar-product-information_en.pdf. Last updated 3 December 2024. Accessed May 2025;
2. ClinicalTrials.gov. NCT03655678. Accessed May 2025; 3. Frangoul H, et al. *N Engl J Med*. 2021;384(3)(Suppl):252–60.

A methodical approach is key to determining patient eligibility for exa-cel treatment[1]

A checklist can help collate all the necessary information for submission

Several factors must be considered when referring a patient to the NHP

NHP MDT referral form for exa-cel treatment in TDT:[1]

Do you want to refer this patient for exa-cel gene therapy ☐ Yes, ☐ No
If 'Yes' continue competing this form below if 'No' stop here.

Please fill in the below if the patient is being referred for exa-cel gene therapy

Question/Patient has the following:	Answer
Documented homozygous β-thalassaemia (including β^0/β^0, β^0/β^0-like, or non–β^0/β^0-like genotype) or compound heterozygous β-thalassaemia including Haemoglobin E/ β-thalassaemia and a history of at least 100 mL/kg/year or 10 units/year of packed RBC transfusions in the prior 2 years.	☐ Yes, ☐ No
Karnofsky performance status of ≥80% for patients ≥16 years of age OR Lansky performance status of ≥80% for patients <16 years of age.	☐ Yes, ☐ No
Known and available fully matched HLA related donor	☐ Yes, ☐ No
Prior allogeneic HSCT	☐ Yes, ☐ No
Patients with associated α-thalassaemia with >1 alpha deletion or alpha multiplications. Patients with sickle cell β-thalassaemia variant	☐ Yes, ☐ No
Clinically significant and active bacterial, viral, fungal, or parasitic infection as determined by the attending physician	☐ Yes, ☐ No
White blood cell count $<3\times10^9$/L or platelet count $<50\times10^9$/L not related to hypersplenism	☐ Yes, ☐ No
History of a significant bleeding disorder	☐ Yes, ☐ No
Any prior or current malignancy or myeloproliferative disorder or a significant immunodeficiency disorder	☐ Yes, ☐ No
Advanced liver disease defined as: Aspartate transaminase (AST), alanine transaminase (ALT) >3 × the upper limit of normal (ULN), or conjugated bilirubin value >2.5 × ULN, or: a) Baseline prothrombin time (International Normalized Ratio; INR) >1.5 × ULN, or b) History of cirrhosis or any evidence of bridging fibrosis on a prior liver biopsy, if available c) Patients with active hepatitis infection d) Patients with history of chronic hepatitis infection are also excluded unless liver biopsy within 3 months prior to or at screening shows no evidence of bridging fibrosis or cirrhosis e) Liver iron content (LIC) ≥15 mg Fe/g dry weight on R2 or T2* MRI of liver unless liver biopsy within three months prior to or at screening shows no evidence of bridging fibrosis or cirrhosis	☐ Yes, ☐ No a) ☐ Yes, ☐ No b) ☐ Yes, ☐ No c) ☐ Yes ☐ No d) ☐ Yes, ☐ No e) ☐ Yes, ☐ No
A cardiac T2* <10ms by MRI or left ventricular ejection fraction (LVEF) <45% by echocardiogram	☐ Yes, ☐ No
Baseline estimated glomerular filtration rate <60 mL/min/1.73 m2	☐ Yes, ☐ No, ☐ Pending
Diffusion capacity of the lungs for carbon monoxide (TLCO) <50% of predicted (corrected for haemoglobin and/or alveolar volume)	☐ Yes, ☐ No, ☐ Pending

AST, aspartate transaminase; ALT, alanine transaminase; HSCT, haematopoietic stem cell transplantation; HLA, human leukocyte antigen; INR, international normalised ratio; LIC, liver iron content; LVEF, left ventricular ejection fraction; MDT, multidisciplinary team; MRI, Magnetic Resonance Imaging; NHP, National Haemoglobinopathy Panel; RBC, red blood cell; T2*, transverse relaxation time; TDT, transfusion-dependent β-thalassaemia; TLCO, transfer factor for carbon monoxide; ULN, upper limit of normal.

1. Thalassaemia Gene Therapy MDT Referral Form. National Haemoglobinopathy Panel, NHS. https://www.nationalhaempanel-nhs.net/the-mdt. Accessed May 2025.

Baseline pre-transplant evaluations and recommendations

		SCD		TDT	
ORGAN/SYSTEM	**TEST**	**Allogeneic HSCT**	**Exa-cel**	**Allogeneic HSCT**	**Exa-cel**
Infectious disease screening	Infectious disease markers	++ (+++ CMV serology)	++	++ (+++ CMV serology)	++
	Check for MDR bacteria colonisation	++	++	++	++
Haematology	HLA antibody screen	+++ (risk of GF if DSA)	+ (may predict PLT transfusion refractoriness)	+++ (risk of GF if DSA)	+ (may predict PLT transfusion refractoriness)
	Extended RBC phenotyping	+++	+++	++	++
	Quantification of RBC transfusion requirements	+++ (rare phenotypes)	+++ (rare phenotypes)	++	++
	HbS%, with simple or exchange transfusion to <30% prior to conditioning	+++	+++	No	no
	Hypertransfusion regimen • (How long? Which pre-transfusional Hb value?)	No (see above)	No (see above)	++ (ineffective erythropoiesis suppression)	++ (ineffective erythropoiesis suppression)
Lung	Pulmonary function tests	++	++	++	++
Heart	ECG, Echocardiogram	++	++	++	++
Kidney	Urinalysis, Urine albumin-to-creatinine ratio, GFR	+++	+++	++	++
Nervous system	Brain MRI/MRA Transcranial doppler	+++	+++	Not routinely recommended	Not routinely recommended

CMV, cytomegalovirus; DSA, donor specific anti-human leukocyte antigens; ECG, electrocardiogram; GF, graft failure; GFR, glomerular filtration rate; GT, gene therapy; HbS, haemoglobin S; HSCT, haemopoietic stem cell transplantation; MDR, multi-drug resistant; MRI, magnetic resonance imaging; MRA, magnetic resonance angiography; PLT, platelet; RBC, red blood cell; SCD, sickle cell disease; TDT, transfusion-dependent β-thalassaemia.

Iron Overload Assessment

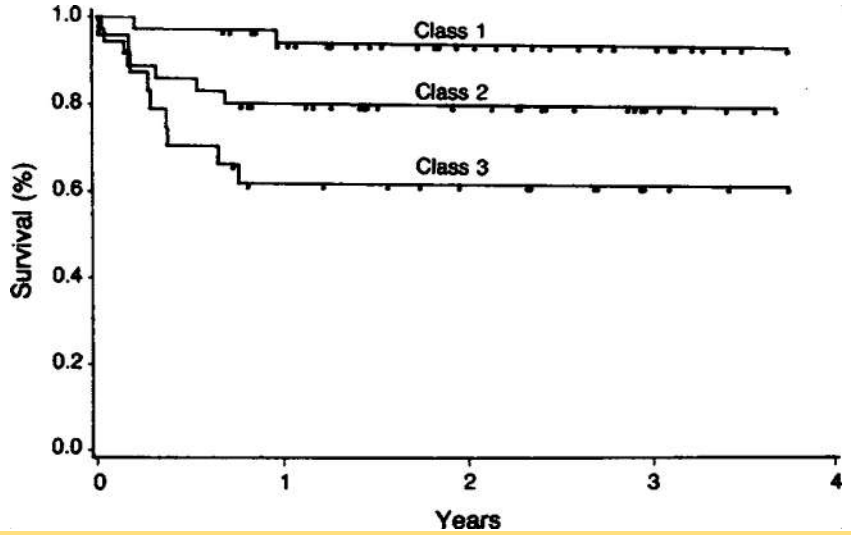
	SCD		TDT	
TEST	**Allogeneic HSCT**	**Exa-cel**	**Allogeneic HSCT**	**Exa-cel**
Ferritin	+ (inaccurate indicator)	+ (inaccurate indicator)	+ (inaccurate indicator)	+ (inaccurate indicator)
Heart-Liver MRI	Based on transfusion burden	Based on transfusion burden	+++	+++
Liver biopsy	Based on LIC	Based on LIC	Based on LIC	Based on LIC

Pesaro Criteria[1]

- Quality of chelation therapy before transplantation (regular versus not regular)
- Hepatomegaly (liver edge palpable more than 2 cm below the costal margin)
- Any degree of liver fibrosis at pre-transplant hepatic biopsy examination

Limits:

a) mostly qualitative;
b) only validated in allogenic setting;
c) not validated in patients >16 years;
d) not validated in recent years (≈35 years ago).



Pesaro Criteria provided the **proof of concept that prolonged exposure to iron toxicity in TDT** is a relevant cause of oxidative damage to human tissues and **increases the risk of transplant-related complications and toxicity**[2]

- **No validated pre-transplant cut-off values (LIC or ferritin)** consistently associated with HSCT outcomes.[3]

  Elevated pre-HSCT LIC or serum ferritin in children have failed to demonstrate a consistent association with inferior transplant outcomes in published reports.

LIC: Liver iron concentration; TDT, transfusion-dependent β-thalassemia.

1) Lucarelli G, et al. *N Engl J Med* 1990; 322:417–21. 2) Angelucci E, et al. *Am J Hematol*. 2017;92(5):411-413. 3) Gibson N, et al. *ASH Annual meeting 2023 and 2024*.

Iron overload and Gene Therapy (TDT only)

Iron Overload in autologous Gene Therapy clinical trials[1-3]

Enrollment criteria for TDT: LIC <15 mg/g dw (if greater, biopsy to exclude bridging fibrosis or cirrhosis) AND Cardiac T2* ≥10 msec

Baseline Values	Lowest-risk values	Median values	Highest-risk values
Ferritin	260 ng/mL[2]	1,287–1,975 ng/mL[1-3]	10,021 ng/mL[1]
LIC	1 mg/g dw[1]	3.5–5.3 mg/g dw[1-3]	41 mg/g dw[1]
Heart T2*	75 msec[3]	34–37 msec[1-3]	12.4 msec[2]

No reported differences in main safety and efficacy outcomes (including VOD risk) according to iron overload status[1-3]

Italian Society of Thalassemias and Hemoglobinopathies (SITE) - European Hematology Association (EHA) recommendations for autologous Gene Therapy in TDT[4]

- **Patients with significant iron accumulation (LIC >7 mg/g dw) should have a "suspended indication"** for autologous gene-therapy until values return to acceptable limits (LIC <7 mg/g dw).
- **If LIC ≥15 mg/g dw, liver biopsy should be performed**. If the liver biopsy demonstrates bridging fibrosis, cirrhosis, or active hepatitis, gene therapy with myeloablative conditioning is not appropriate.
- Gene Therapy with **myeloablative conditioning is not appropriate for patients with TDT who have evidence of severely elevated iron in the heart** (i.e., patients with cardiac T2* <10 ms by MRI).

LIC, liver iron concentration TDT, transfusion-dependent β-thalassemia.

1) Locatelli F, et al. *N Engl J Med* 2022;386:415–427; 2) Locatelli F, et al. *N Engl J Med*. 2024;390(18):1663-1676; 3) Kwiatkowski JL, et al. *Lancet* 2024; 404(10468):2175-2186 4) Baronciani D, et al. *Hemasphere*. 2021;5(5):e555;

Key concepts in iron overload management before Gene Therapy

- Both **the magnitude of iron overload and the duration of exposure to toxic iron are critical** determinants of iron-related tissue damage[1]
- **Regular and life-long chelation therapy is crucial** for successful outcomes in order to consistently suppress tissue reactive iron species and prevent tissue damage[2]
- **Evidence supporting the benefit of intensive pre-transplant chelation in poorly chelated patients is limited** (with the greatest potential advantage expected in younger patients who have sufficient time to reduce iron burden and allow for tissue repair)[1]
- In TDT, **a cardiac T2* value below 20 msec is already associated with an increased risk of cardiac morbidity** - long before reaching the critical threshold of 10 msec[3,4]
- Preclinical evidence that **iron overload impairs HPSCs function** through iron-induced reactive oxygen species[5]
  - Potentially relevant issue for HPSCs mobilization/collection/manufacturing

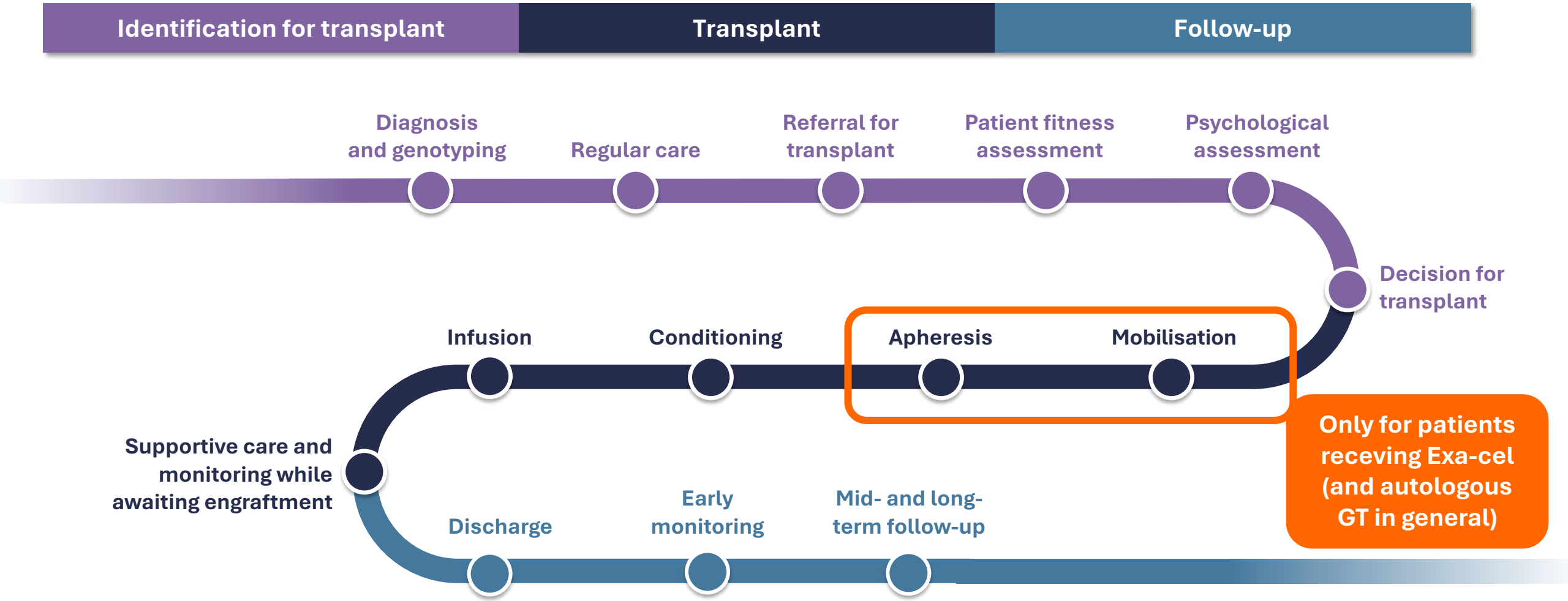
Suggested Management Approach for subjects with LIC >7 and <15 mg/g dw and/or T2*<20 and ≥ 10 msec

- At least 6 months of intensive chelation therapy before GT targeting **LIC <7 mg/g dw** and cardiac **T2*>20 msec**
- If target values are not achieved despite optimal efforts, consider proceeding with GT based on clinical judgment/additional exams

GT: Gene Therapy HPSCs: hematopoietic stem and progenitor cells; TDT: transfusion-dependent β-thalassemia.

1) Angelucci E. *Blood* 2025;145(4):372-382. 2) Coates TD, et al *Blood* 2025;145(4):359-371. 3) Kirk P, et al. *Circulation* 2009;120(20):1961-1968. 4) Pennell DJ, et al. *Circulation* 2013;128(3):281-308; 5) Jin X, et al. *Haematologica*. 2018;103(10):1627-1634.

The journey towards the cure in haemoglobinopathies



Frangoul H, et al. Transplant Cell Ther. 2025 Mar 7:S2666-6367(25)01061-9. doi: 10.1016/j.jtct.2025.02.025. Epub ahead of print.
GT, gene therapy.

Pre-mobilisation phase for exa-cel

TDT[1]

- Patients hyper-transfused to achieve a goal of pre-transfusion Hb ≥11 g/dL before mobilisation starts
  - Enrich for bona fide haematopoietic stem cells in the harvested CD34+ cell compartment by suppressing erythroid lineage expansion and skewing that is seen in β-thalassaemia[2]
- *Iron chelation therapy must be stopped at least 7 days prior to myeloablative conditioning*

SCD[1]

- Treatment with HU and other disease-modifying agents should be discontinued 8 weeks before the planned start of mobilization.
- **RBC exchange or simple transfusions** before the planned start of mobilization*. *For how long?*
- **Goal:** maintain HbS level of <30% of total Hb while keeping total Hb concentration ≤11 g/dL

  *(not always easy…)**
  - Reduce stress erythropoiesis and stabilize the bone marrow before mobilisation[3,5]
  - Enrich for bona fide haematopoietic stem cells similar to TDT[4]
  - Reduce the risk of complications during mobilization and apheresis[4]

* *To be repeated before conditioning*

CD, cluster of differentiation; Hb, haemoglobin; HbS, haemoglobin S; HSCT, haematopoietic stem-cell transplantation; HU, hydroxyurea; RBC, red blood cell; SCD, sickle cell disease; TDT, transfusion-dependent β-thalassaemia.

1. Casgevy : EPAR - Product information; 2. Thompson AA, et al. *N Engl J Med*. 2018;378(16):1479–93; 3. Uchida N, et al. *Hum Gene Ther Clin Dev*. 2017;28(3):136–44; 4. Hsieh MM, and Tisdale JF. *Haematologica*. 2018;103(5):749–50; 5. Leonard A, et al. *Br J Haematol*. 2019;186(2):286–99.

Mobilisation and apheresis

Exa-cel

TDT[1]

G-CSF

- Given at the dose of 5 µg/kg for 5 to 6 days
- Approximately Q12h dosing for patients with a spleen (once daily dosing for splenectomized subjects)

PLERIXAFOR:

- Single dose of 0.24 mg/kg 2–3 hours before planned aphereris

SCD[1]

PLERIXAFOR ALONE:

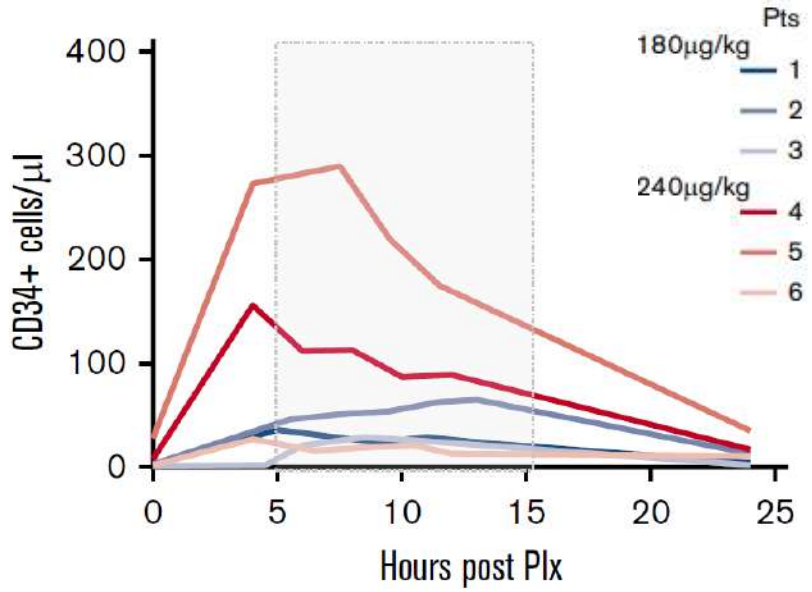
- Single dose of 0.24 mg/kg 2–3 hours before planned aphereris

	TDT[2]	SCD[3]
Number of mobilization cycles, median (range)	1.0 (1, 4)	2.0 (1, 6)
Exa-cel dose: 10^6 x $CD34^+$ cells/kg, mean (range)	7.4 (3.0, 19.7)	4.0 (2.9, 14.4)

Allogeneic HSCT for TDT/SCD

- **No need for mobilisation and apheresis for the recipient**
- Backup collection is recommended before conditioning[4,5] (++ when alternative donors are employed)

Peak of mobilisation of CD34+cells in SCD appears to be much earlier than in healthy donors[a 6,7]



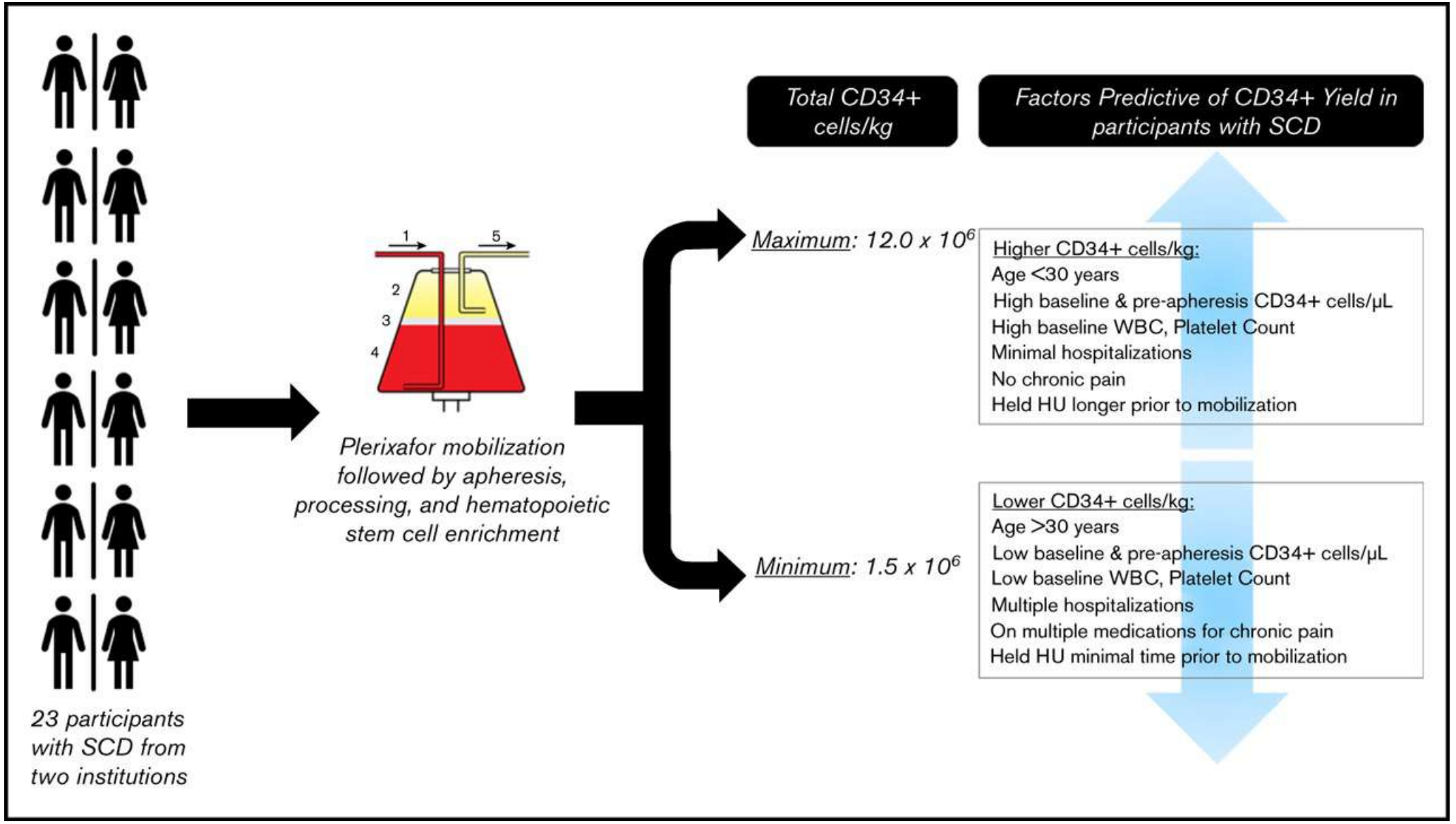
Cells were obtained immediately before mobilisation and after Plx administration during apheresis at the indicated time intervals. The shaded grey rectangle represents the period of apheresis collection.

[a]N=23 adult patients received a single dose of plerixafor (tested at lower than standard dose [180 µg/kg] and standard dose [240 µg/kg]) followed by CD34+ cell monitoring in PB and apheresis collection.

G-CSF, granulocyte colony stimulating factor; HSCT, haematopoietic stem-cell transplantation; PB, peripheral blood; Plx, plerixafor; Q12h, every 12 hours; SCD, sickle cell disease; TDT, transfusion-dependent β-thalassaemia.

1. Casgevy : EPAR - Product information; 2. Locatelli F, et al. *N Engl J Med*. 2024;390(18):1663–76; 3. Frangoul H, et al. *N Engl J Med*. 2024;390(18):1649–62; 4. Fu H, et al. Blood 2010; 116 (21): 4524; 5. Algeri M, et al. Hematol Oncol Clin North Am. 2023:413-432; 6. Esrick EB, et al. *Blood Adv*. 2018;2(19):2505–12; 7. Lagresle-Peyrou, et al. *Haematologica*. 2018;103(5):778–86.

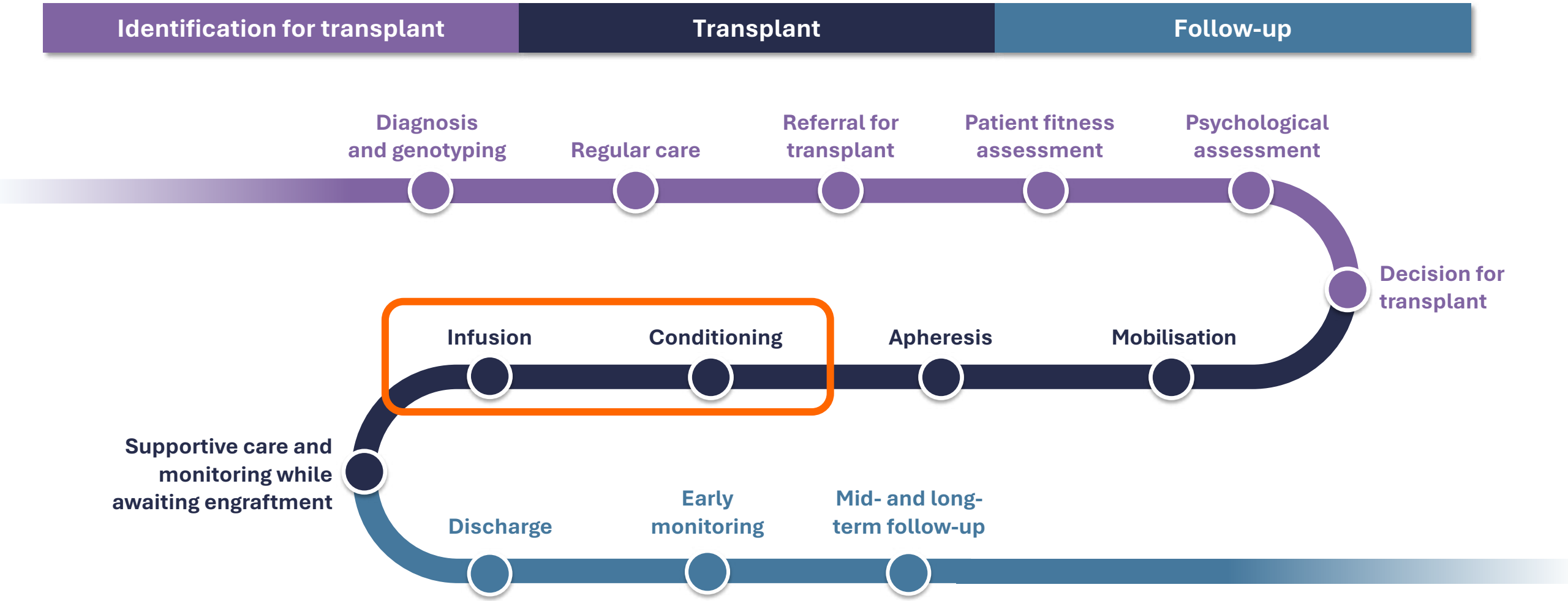
Factors impacting the efficiency of CD34+ collection in SCD patients[a]



a) Data non derived from Exa-cel treated patients

Leonard A, et al. *Blood Adv.* 2021;5(9):2403–11

The journey towards the cure in haemoglobinopathies



Frangoul H, et al. Transplant Cell Ther. 2025 Mar 7:S2666-6367(25)01061-9. doi: 10.1016/j.jtct.2025.02.025. Epub ahead of print.

Conditioning regimens

Allogeneic HSCT

Myeloablation (++TDT) **AND immune-ablation**
involving serotherapy or lympholytic cytotoxic agents

- First studies conducted with busulfan[1]
- Treosulfan–thiotepa: reduced toxicity, trend towards increased risk of secondary GF/autologous reconstitution[2]

SCD: MAC, NMA and RIC[3]

- MAC: generally used in younger patients
- NMA/RIC protocols: generally used in adults, less toxicity but increased risk GF/mixed chimerism

Exa-cel[4,5]

Myeloablation ONLY
(required to avoid dilution of the therapeutic effect[3])

Myeloablative busulfan

- **Target cumulative busulfan exposure of 90 mg*h/L (range 80 to 100 mg*h/L) over 4 days**

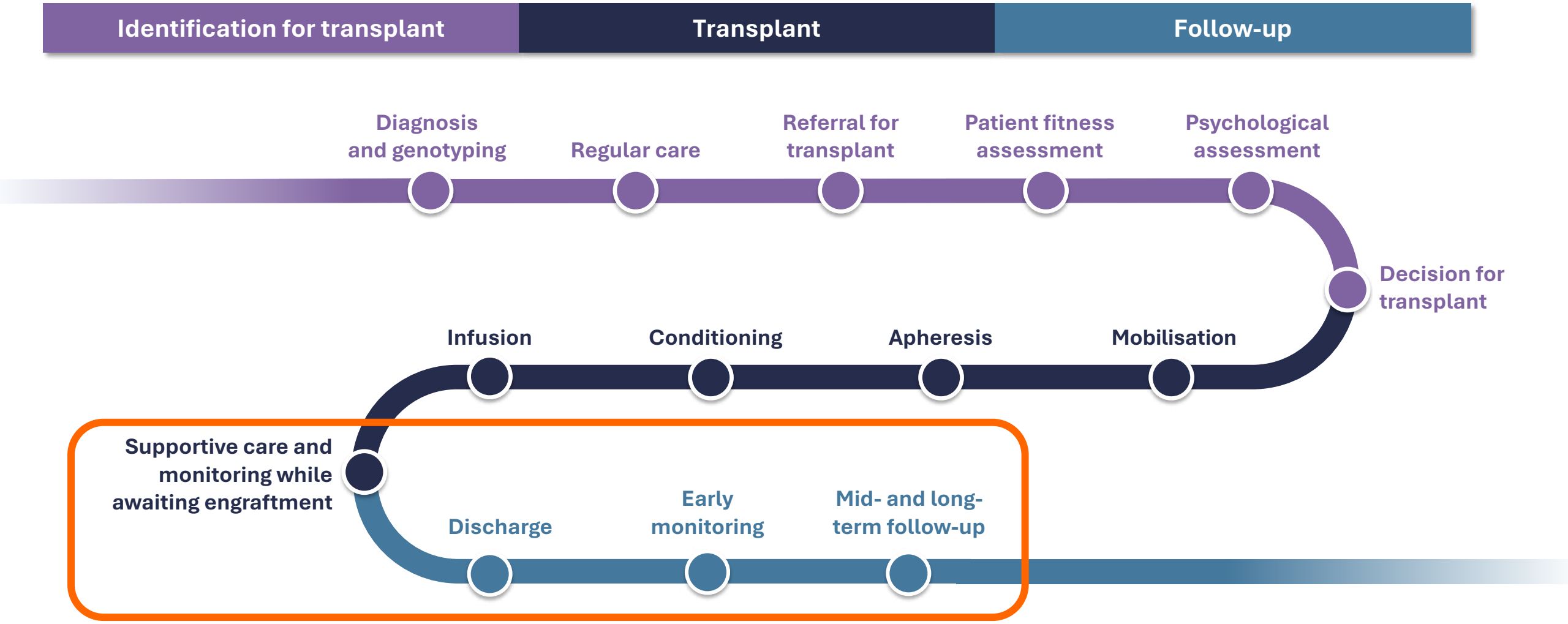
Busulfan Q6h or Q24h?[6,7]

- **Q6h recommended always and even more if busulfan dose cannot be adjusted on Day 2 based on availability of PK results**
- **Higher peak levels associated with single daily doses have been associated with a high risk of VOD**
- **Consider placing an additional peripheral line for blood sampling during PK analysis to avoid possible cross-contamination**

GF, graft failure; GT, gene therapy; HSCT, haematopoietic stem-cell transplantation; PK, pharmacokinetics; Q6h, every 6 hours; Q24h, every 24 hours; RIC, remote ischaemic conditioning; SCD, sickle cell disease; TDM, therapeutic drug monitoring; TDT, transfusion-dependent β-thalassaemia; TI, transfusion independence; VOD, veno-occlusive disease.

1. Algeri M, et al. *Hematol Oncol Clin North Am*. 2023:413-432; 2. Lüftinger R. *Ann Hematol*. 2022;101(3):655–65; 3. Stenger EO, et al. Blood. 2019;134(25):2249–60.; 4. Locatelli F, et al. N Engl J Med. 2024;390(18):1663–76; 5. Frangoul H, et al. N Engl J Med. 2024;390(18):1649–62; 6. Sharma A, et al. *Blood* 2024; 144(26):2693-2705; 7. Philippe M, et al. *Bone Marrow Transplant*. 2019; 54(3):448-457.

The journey towards the cure in haemoglobinopathies



Frangoul H, et al. Transplant Cell Ther. 2025 Mar 7:S2666-6367(25)01061-9. doi: 10.1016/j.jtct.2025.02.025. Epub ahead of print.

Duration of bone marrow aplasia

Allogeneic HSCT

MAC: the patient is already aplastic during graft infusion or may become aplastic shortly after[a]

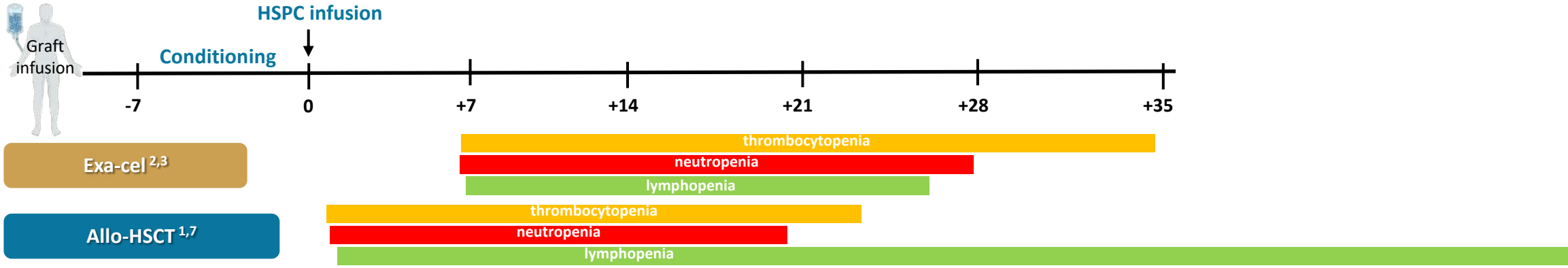
- Median time to neutrophil engraftment: 15–25 days[1]
- Median time to platelet engraftment: 20–30 days[1]
- The patient also has deep lymphopenia; immune reconstitution may take several months (or years . . .)[1,4]

Exa-cel

MAC with busulfan alone: the aplastic phase usually begins after 7–10 days after graft infusion

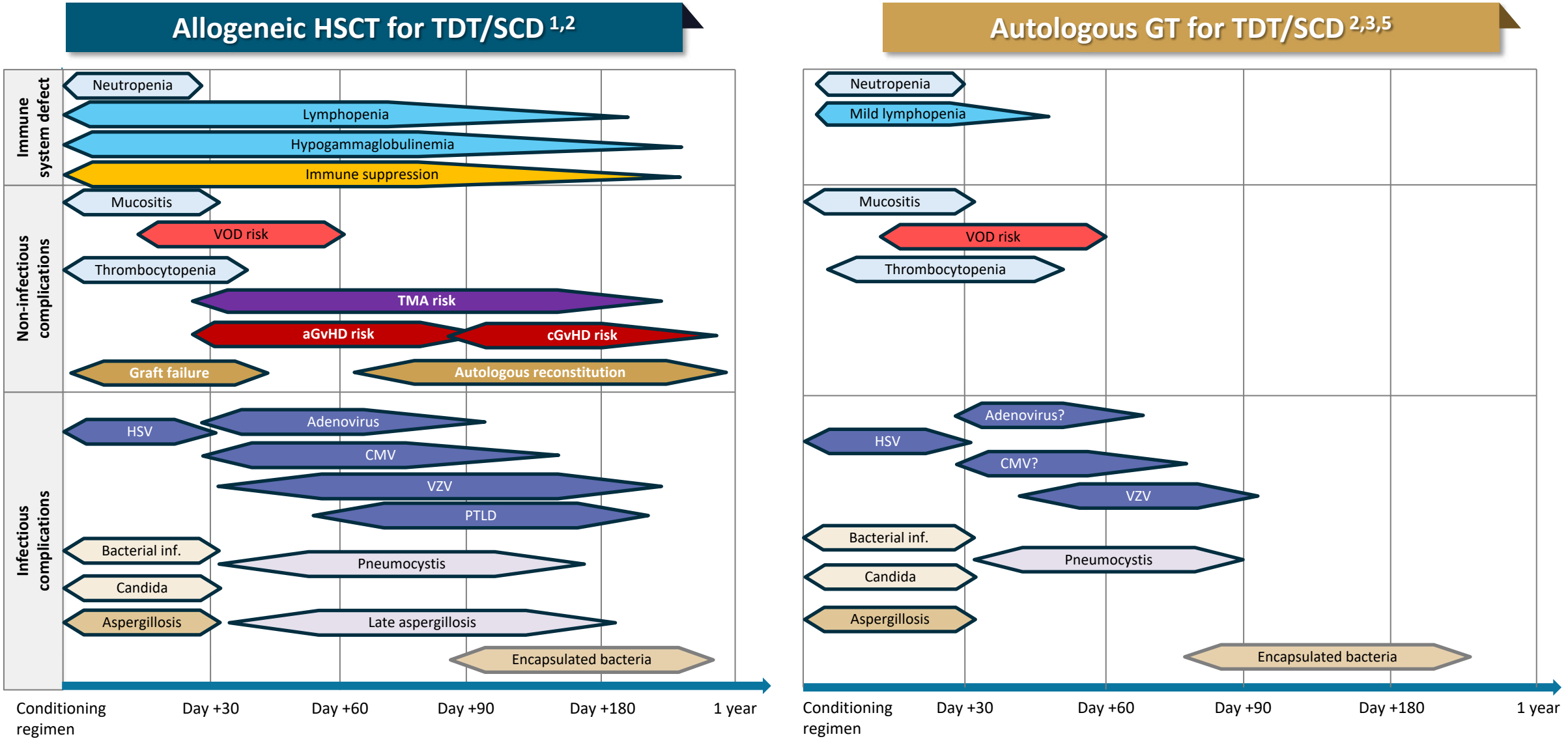
- Median time to neutrophil engraftment: 27-29 days[2,3]
- Median time to platelet engraftment: 35–44 days[2,3]
- Lymphopenia following single agent busulfan is rare and usually mild

Faster neutrophil and platelet recovery in case of splenectomy – with no impact on main outcome measures[2]



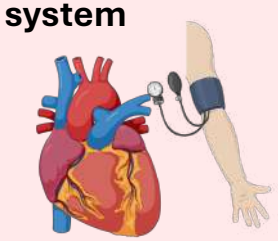
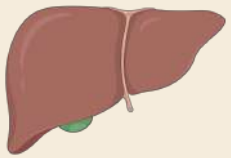
[a]Depending on graft source, time of transplant and conditioning agent.

1. Sureda A, et al., eds. *The EBMT Handbook: Hematopoietic Cell Transplantation and Cellular Therapies*; Springer International Publishing: Cham, Switzerland, 2024:725–735; 2. Locatelli F, et al. *N Engl J Med*. 2024;390(18):1663–76. DOI: 10.1016/S0140-6736(24)01884-1; 3. Frangoul H, et al. *N Engl J Med.* 2024;390(18):1649–62. DOI: 10.1056/NEJMoa2309676; 4. Kepenekli E, et al. *J Pediatr Inf.* 2019;13(3):e97–e102.



aGvHD, acute graft-versus-host disease; cGvHD, chronic graft-versus-host disease; CMV, cytomegalovirus; HSCT, haematopoietic stem-cell transplantation; HSV, herpes simplex virus; inf, infection; PTLD, post-transplant lymphoproliferative disorder; SCD, sickle cell disease; TDT, transfusion-dependent β-thalassaemia; TMA, thrombotic microangiopathy; VOD, veno-occlusive disease; VZV, varicella zoster virus. 1. PDQ Pediatric Treatment Editorial Board. *Pediatric Hematopoietic Stem Cell Transplant and Cellular Therapy for Cancer (PDQ®): Health Professional Version.* 13 June 2024. In: PDQ Cancer Information Summaries [Internet]. Bethesda (MD): National Cancer Institute (US); 2002. Last accessed 19 March 2025; 2. Frangoul H, et al. *Transplant Cell Ther*. 2025:S2666-6367(25)01061-9; 3. Tomblyn M, et al. *Biol Blood Marrow Transplant*. 2009;15(10):1143–238. Erratum in: *Biol Blood Marrow Transplant*. 2010;16(2):294; 4. Hutt D. Engraftment, Graft Failure, and Rejection. 2017. In: Kenyon M, Babic A, editors. *The European Blood and Marrow Transplantation Textbook for Nurses: Under the Auspices of EBMT* [Internet]. Cham (CH): Springer; 2018. Chapter 13; 5. Walters M, et al. *Biology of Blood and Marrow Transplantation.* 2020;26(3):S38–9:1083-8791.

Key early post-transplant toxicities and preventive measures

	Toxicity	Preventive measures
Cardiovascular system 	• **Increased susceptibility to hypertension** due to neurologic (SCD) and renal function impairment (SCD/TDT) as well as medications (Allo-HSCT)[1] • Disease specific cardiovascular comorbidities[2,3]	• **Prevention/treatment of hypertension (BP within 10th percentile of baseline and 90th percentile for age/sex)[1,7,15]** • **Regular echocardiogram evaluation and low threshold for re-evaluation, particularly if pre-existing pulmonary hypertension, suboptimal baseline EF (<55%) or moderate-to-mild cardiac iron overload (T2* >10 and <20 ms)[3–5,15]**
Central nervous system 	• Higher risk of **neurologic complications** in SCD (ischaemic/haemorrhagic stroke)[1,7] • Risk of **PRES** in SCD/TDT given allo-HSCT[8]	• **Prevention/treatment of hypertension (BP within 10th percentile of baseline and 90th percentile for age/sex)[1,7]** • **Maintain platelets values > 50,000/µL[1,7]** • **Avoid Hb <8 and >11 g/dL until acute toxicities have resolved[1]** • **Prevention of CNI-induced hypomagnesemia (allo-HSCT)[1]**
Liver 	• **Oxidative stress** early after HSCT[8] • Risk of hepatic **VOD**[10–12] • Risk factors: iron overload/liver fibrosis, hepatitis, busulfan-based MAC, systemic vasculopathy (SCD)[10–14]	• **Antioxidants supplementation early after HSCT[16] (benefit unclear)** • **Ursodeoxycholic acid as prophylaxis[6]** • **Defibrotide prophylaxis in selected, high-risk, cases[6]** • **Liver stiffness measurement by transient elastography for early diagnosis[17]**

Icons provided by speaker. BP, blood pressure; CNI, calcineurin inhibitor; EF, ejection fraction; Hb, haemoglobin; HSCT, haemopoietic stem cell transplantation; MAC, myeloablative conditioning; PRES, posterior reversible encephalopathy syndrome; SCD, sickle cell disease; TDT, transfusion-dependent β-thalassaemia; VOD, veno-occlusive disease.

1. Stenger EO, et al. *Blood*. 2019;134(25):2249–60; 2. Vasbinder A, et al. *JACC CardioOncol*. 2023;5(6): 821–32; 3. Hayek SS, et al. *Circulation*. 2024;149(16):e1113–e1127; 4. Triadyaksa P, et al. *J Magn Reson Imaging*. 2020;52(5):1340–51;
5. Locatelli F and Algeri M. Gene manipulation. In: Taher AT, et al., eds. *Guidelines for the management of transfusion-dependent β-thalassaemia*. Nicosia, Cyprus: Thalassaemia International Federation; 2025;
6. Sharma A, et al. *Blood* 2024;144(26):2693–705; 7. Walters MC, et al. *Blood*. 1995;85(4):879–84; 8. Gaziev J, et al. *Biol Blood Marrow Transplant*. 2017;23(9):1531–40; 9. Chi M, et al. *Oxid Med Cell Longev*. 2023;2023:3532756; 10. Corbacioglu S, et al. *Biol Blood Marrow Transplant*. 2019;25(7):1271–80; 11. Faraci M, et al. *Biol Blood Marrow Transplant*. 2019;25(2):313–20; 12. Dalle JH, et al. *Biol Blood Marrow Transplant*. 2016;22(3):400–9; 13. Lynch K, et al. *Mediterr J Hematol Infect Dis*. 2023;15(1):e2023060; 14. Corbacioglu S, et al. *Bone Marrow Transplant*. 2018;53(2):138–45; 15. Stenger EO, et al. *Blood*. 2019;134(25):2249–60; 16. Fuji S, et al. *Biol Blood Marrow Transplant*. 2015;21(10):1707-13; 17. Colecchia A, et al. *Biol Blood Marrow Transplant*. 2019;25(5):995-1003.

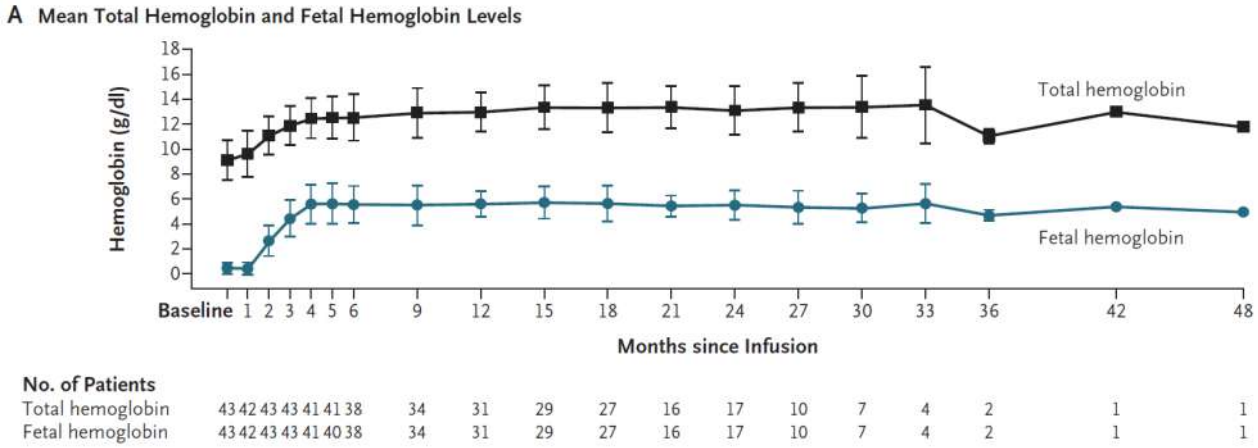
Differences in engraftment monitoring

Allogeneic HSCT

- Engraftment is monitored with **chimerism**[1]
- Donor chimerism as low as 10–20% on whole blood has been associated with stable donor-derived erythropoiesis, but there is not yet a consensus definition on the level of **mixed chimerism** that defines a successful cure[2,3]
- Myeloid chimerism is a helpful indicator but does not fully describe post-HSCT phenotypes[2,3]
- **Chimerism stability is important!**[2,3]
- In SCD, **monitoring of Hb fractions can be useful**
  - Rising HbS, particularly >50%, suggests impending autologous recovery[2,3]
- **Thalassaemia recurrence is possible even >30 years** after HSCT[4]

Exa-cel

- Chimerism is not applicable (autologous cells)[1]
- **Monitoring of Hb fractions can be useful**[5]
- In SCD, **HbS continues to be present**[6]
- Late reconstitution of pathological erythropoiesis and **disease recurrence is unlikely**[7]



Frangoul H, et al. N Engl J Med. 2024;390(18):1649–62

Hb, haemoglobin; HbS, haemoglobin S; HSC, haematopoietic stem cell; HSCT, haemopoietic stem cell transplantation; SCD, sickle cell disease.

1. Sureda A, et al., eds. *The EBMT Handbook: Hematopoietic Cell Transplantation and Cellular Therapies*; Springer International Publishing: Cham, Switzerland, 2024:725–735; 2. Krishnamurti L. *Front Pediatr.* 2021;8:551170; 3. Stenger EO, et al. *Blood.* 2019;134(25):2249–60; 4. NHS England Evidence Review. February 2023. https://www.england.nhs.uk/wp-content/uploads/2023/11/2120-evidence-review-allo-hsct.pdf. Last accessed 25 March 2025; 5. Lutfi F, et al. *Hematol Oncol Stem Cell Ther.* 2020;13(1):23-31; 6. Frangoul H, et al. N *Engl J Med*. 2024;390(18):1649–62; 7. Locatelli F, et al. *Mol Ther.* 2024;32(5):1202-1218.

Follow-up recommendations and return to normal life

	Allogeneic HSCT	Exa-cel
Safety considerations	• **Significant complications** (GvHD, infections, poor graft function and other immune-mediated complications)[1] • Risk of re-admission, intensive monitoring and supportive care[1] • **Late effects** increasingly analysed and detailed[1]	• **Most AEs occur in the first 3 months:** AEs may occur in response to exposure to GT, MAC and autologous HSCT[2,3] • **Limited information on re-admission, supportive care and late effects**[2,3]
 CVL removal	• Usually **not earlier than 3 months** after HSCT • Can be delayed by complications[4]	• Once stable **neutrophil/platelet engraftment** is observed and **no regular transfusions** are required[6]
 Return to school/work	• Lack of universal consensus • Usually **not recommended before 6 months and only once the patient is off immune suppression** (may take longer in cases with complications or for patients working with children, sick people or animals)[5,7,9]	• Lack of universal consensus • In principle, **possible 3 months after HSCT** (on the basis of ANC and ALC)[5,7] • Majority of participants are expected to resume school/work by 6 months[7]
 Follow-up	• **Need to be regularly followed by the transplanter** for at least 1 year after HSCT[5-6] • Close monitoring in the first weeks/months. The schedule can be eased if there are no complications and GvHD does not occur (monitor at least every 3–4 months until 2 years after HSCT)1,[5] • Comprehensive long-term follow-up[1,10]	• After engraftment and discharge, patients should be seen by the transplanter once in a month for the first 6 months, then every 3 months until 1 year after HSCT[6] • Periodic access to the transplant facility is recommended, but **monitoring can also be shared with local haematologists after 3 months**[6] • **Yearly blood counts up to 15 years after infusion** • Comprehensive long-term follow-up (as for allogeneic HSCT)[11]

AE, adverse event; ALC, absolute lymphocyte count; ANC, absolute neutrophil count; CVL, central venous line; GvHD, graft-versus-host disease; HSCT, haemopoietic stem cell transplantation; SCD, sickle cell disease; MAC, myeloablative conditioning. 1. Sureda A, et al., eds. *The EBMT Handbook: Hematopoietic Cell Transplantation and Cellular Therapies*; Springer International Publishing: Cham, Switzerland, 2024:725–735; 2. Locatelli F, et al. N Engl J Med. 2024;390(18):1663–76; 3. Frangoul H, et al. N Engl J Med. 2024;390(18):1649–62; 4. Panse J, et al. *Ann Hematol*. 2022;101(10):2317-2324. ; 5. Bhatt NS, et al. *Bone Marrow Transplant*. 2024;59(5): 653–9; 6. Locatelli F and Algeri M. Gene manipulation. In: Taher AT, et al., eds. *Guidelines for the management of transfusion-dependent β-thalassaemia.* Nicosia, Cyprus: Thalassaemia International Federation; 2025; 7. Salit RB, et al. *Biol Blood Marrow Transplant*. 2020;26(8):1520–6; 8. Bhatt NS, et al. *Transplant Cell Ther*. 2021;27(8):679.e1–679.e8; 9. Stenger EO, et al. *Blood*. 2019;134(25):2249–60; 10. Shenoy S, et al. *Biol Blood Marrow Transplant*. 2018;24(7):1313–21; 11. Sharma A, et al. *Blood*. 2024;144(26):2693–705;

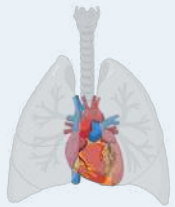
Main recommendations for post-transplant immunisation

Allogeneic HSCT	Exa-cel
• **Comprehensive re-immunisation schedule** is recommended[1,2]	• **Individuals may retain immunity** from most previous vaccinations[1,4]
• **Early (from 3–6 months) pneumococcal re-immunisation**[1] (+++ patients with SCD and/or functional or surgical asplenia)[5]	• **Serological evaluations may guide** 'catch-up' or 'booster' vaccinations against organisms for **which seroprotective immunity is lacking**[4]
• Consider yearly influenza and SARS-CoV-2 vaccinations 3 months after therapy[1,2]	• **Consider full re-immunisation for encapsulated organisms** (start pneumococcal conjugate vaccines already at 3 months)[5]
	• Consider yearly influenza and SARS-CoV-2 vaccinations 3 months after therapy[1,2]

HSC, haematopoietic stem cell; HSCT, haemopoietic stem cell transplantation; SCD, sickle cell disease.

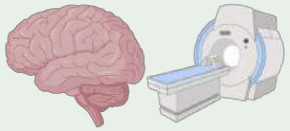
1. Tomblyn M, et al. *Biol Blood Marrow Transplant*. 2009;15(10):1143–238. Erratum in: *Biol Blood Marrow Transplant*. 2010;16(2):294; 2. John TD, et al. *Cytotherapy*. 2024;26(7):660–71;
3. Ullmann AJ, et al. *Ann Hematol*. 2016;95(9):1435–55; 4. Sharma A, et al. *Blood*. 2024;144(26):2693–705; 5. Stenger EO, et al. *Blood*. 2019;134(25):2249–60.

Key considerations for long-term management



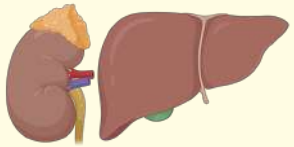
Heart and lungs[1]

- Pulmonary function tests (with DLCO) at 3, 6 and 12 months and then yearly for at least 5 years – yearly echocardiogram (adjust the monitoring schedule in case of complications, such as cGvHD or toxicity)[1]
- Promote vaccinations against respiratory pathogens (e.g., Sars-CoV-2, seasonal influenza)[1,2–4]



Nervous system assessments[1-3]

- Regular neurocognitive evaluations (include QoL if possible)
- Brain MRI at 1 and 2 years after HSCT in SCD

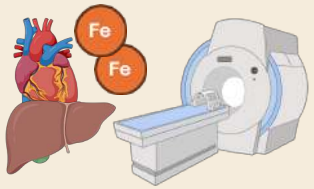


Kidney and hepatobiliary system[1]

- Assess liver function tests every month through 1 year after HSCT, every 3 months in year 2, and then as clinically indicated[1]
- Continue ursodeoxycholic acid and perform yearly hepatobiliary ultrasound for cholestatic disease not resolved after HSCT[1,6]
- Monitor renal function regularly until CNI and other nephrotoxic therapy is discontinued, then at least yearly (include proteinuria)[1]

Endocrine system[1]

- Track growth and pubertal development every 6 months in children and adolescents
- HbA1c, thyroid and sexual hormones (+AMH in females) at least yearly after HSCT
- Vitamin D and bone mineral density evaluation at least yearly after HSCT



Iron overload monitoring and iron removal therapy after HSCT[1,3,7]

- Assess iron load with serum ferritin and transferrin saturation every 3–6 months commencing 6 months after HSCT until normal
- Cardiac and liver MRI for monitoring every 6 months (plus chelation, if needed) after HSCT until iron overload has normalised (TDT and SCD with history of chronic transfusions and elevated ferritin/transferrin saturation)
- Post-transplant iron removal therapy (phlebotomy or chelation) until normalization of iron deposits (LIC <5 mg/g - serum ferritin <500 ng/mL)[7]

Icons provided by speaker. AMH, anti-müllerian hormone; cGvHD, chronic graft-versus-host disease; CNI, calcineurin inhibitor; DLCO, diffusing capacity of the lungs for carbon monoxide; HbA1c, haemoglobin A1c; HSCT, haematopoietic stem cell transplantion; MRI, magnetic resonance imaging; QoL, quality of life; SCD, sickle cell disease; TDT, transfusion-dependent β-thalassaemia.

1. Shenoy S, et al. *Biol Blood Marrow Transplant*. 2018;24(7):1313–21; 2. Sharma A, et al. *Blood*. 2024;144(26):2693–705; 3. Stenger EO, et al. *Blood*. 2019;134(25):2249–60; 3; 4. Tomblyn M, et al.*Biol Blood Marrow Transplant*. 2009;15(10):1143–238; 54. Erratum in: Biol Blood Marrow Transplant. 2010;16(2):294; 5. John TD, et al. *Cytotherapy*. 2024; 26(7):660–71; 6. McDonald GB. *Hepatology*. 2010;51(4):1450–60; 7. Angelucci E. *Blood*. 2025;145(4):372–82;

Take-home messages

Genome editing approaches can advance the treatment of haemoglobinopathies by offering a potential functional cure, without the need for donors.

While genome editing and allogeneic stem cell transplantation share some similarities, they differ significantly in mechanism of action, risk profile, and clinical application. These distinctions directly impact post-transplant monitoring and management.

Pre and post-transplant management frameworks for Exa-cel cannot be directly extrapolated from allogeneic transplantation experiences due to fundamental differences in treatment dynamics.

Differences between Exa-cel and allogeneic stem cell transplantation include:

- Patient selection
- Stem cell collection
- Acute complications
- Short- and long-term monitoring intensity
- Functional recovery

When dealing with late complications and long-term monitoring, data on Exa-cel remain limited.
Until dedicated guidelines are established, adherence to allogeneic transplantation recommendations is advisable.

Special thanks to:
Mattia Algeri, MD