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CHALLENGES AND PROSPECTS IN ADVANCED CELL THERAPIES

Stem cell collection: open problems and desired solutions

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Disclosures

In the last two years I have had the following relationships, including financial ones with subjects with commercial interests in the field of healthcare:

- Grant/Research support: Miltenyi Biotec, Sanofi Genzyme, Therakos
- Speaker bureau: BMS Celgene, Johnson & Johnson, Kite Gilead, Miltenyi Biotec, Therakos, Terumo, Vertex
- Travel support: Johnson & Johnson, Pierre Fabre, Kite Gilead, Therakos

FDA / EMA approved HSC gene therapies

Brand (INN)	Disease	Mechanism	Approval	Key Result
Casgevy (Exagamglogen-autotemcel; exa-cel)	SCD + TDT	CRISPR/Cas9 BCL11A enhancer ↑ HbF	FDA Dec 2023 (SCD) / Jan 2024 (TDT)	29/31 SCD pts: 0 VOCs 32/35 TDT pts: transfusion-independent
Lyfgenia (Lovotibeglogen-autotemcel; lovo-cel)	SCD	LVV βAT87Q-globin gene addition	FDA Dec 2023	88% complete resolution of VOEs at 6–18 months
Zynteglo (Betibeglogen-autotemcel; beti-cel)	β-Thalassemia	LVV βAT87Q-globin Gene addition	EMA 2019 / FDA Aug 2022	89% transfusion-independent at 2 yrs
Skysona (Elivaldogene autotemcel; eli-cel)	Cerebral ALD (boys, early)	LVV ABCD1 gene addition	FDA Sep 2022	91% free from major functional disability at 24 months
Libmeldy (Atidarsagen-autotemcel; ati-cel)	MLD (pediatric)	LVV ARSA (Arylsulfatase) gene Addition	EMA Dec 2020 (not FDA)	Pre-symptomatic pts: near-normal motor/ cognitive function
Strimvelis (ari-cel)	ADA-SCID (pediatric)	γ-retroviral vector ADA gene addition	EMA May 2016 (not FDA)	100% survival at 3 yrs; immune reconstitution
Lenmeldy (atidarsagene autotemcel)	MLD (pediatric)	LVV ARSA gene addition	FDA Mar 2024	First FDA-approved for MLD; $4.25M; world's costliest drug

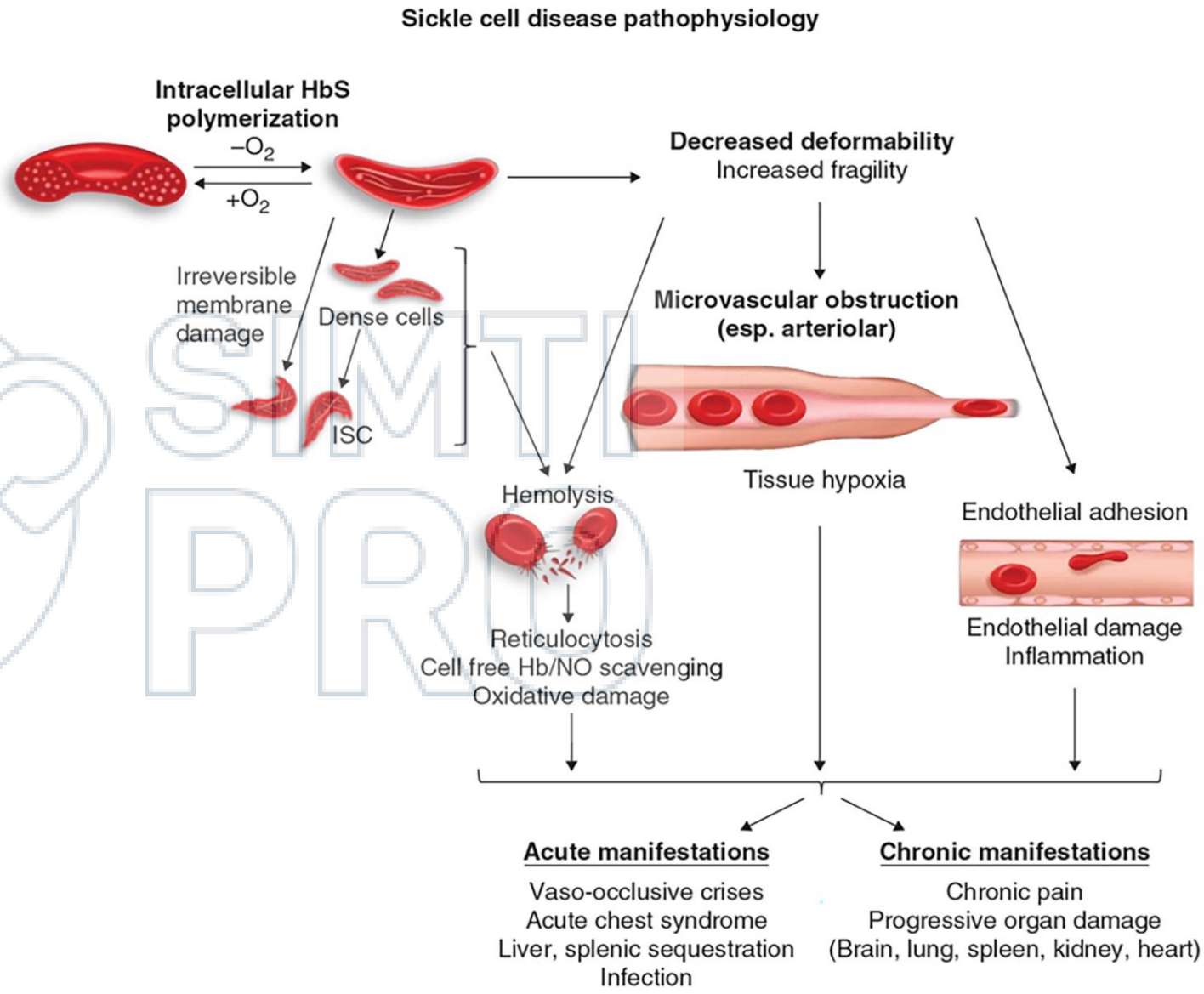
Sickle cell disease & transfusion dependent ß-thalassemia

- Sickle cell disease (SCD) and transfusion-dependent β-thalassemia (TDT) are inherited blood disorders caused by pathogenic variants of the β-globin gene.
- Historically, allogeneic hematopoietic stem cell transplantation (HSCT) from HLA–matched donors has been the only curative option, but most patients with SCD or TDT lack HLA-matched donors.
- Gene therapy–based ASCT for the treatment of SCD and TDT entails a complex patient journey and requires the careful implementation of numerous policies and procedures.

ASCT=autologous stem cell transplantation

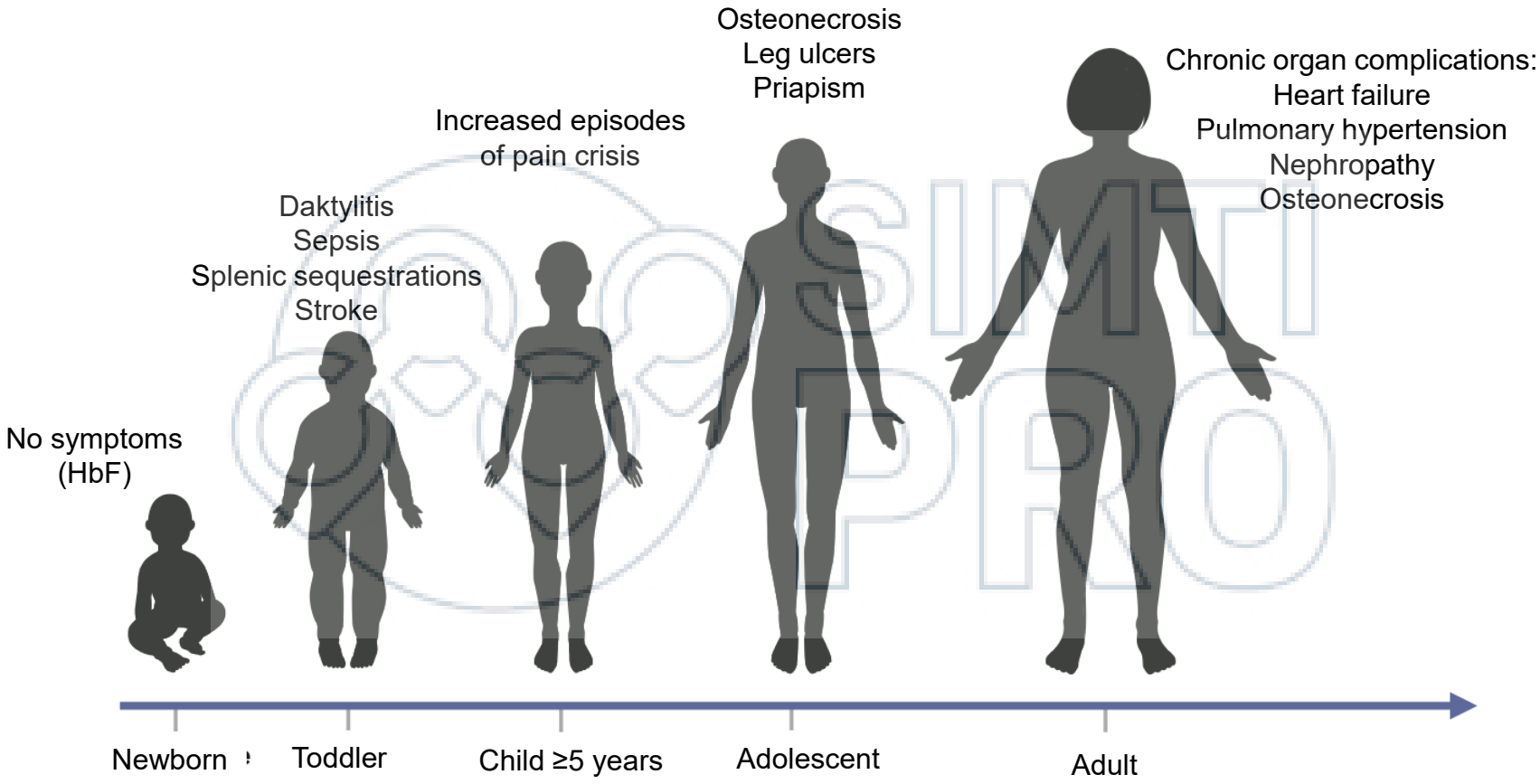
Pathophysiology of SCD

- Low oxygen → polymerization, dense cells & irreversibly sickeld cells
- Decreased deformability
- Microvascular obstructions
- Hemolysis
- Endothelial adhesion
- Acute and chronic manifestations



Alan N. Schechter, Br J Haematol. 2025;206:842–845

SCD symptoms vary depending on age



With courtesy from Julia Riedl

The HSC gene therapy workflow

Patient Selection	HSC Mobilization	Apheresis Collection	Ex Vivo Manufacturing	Myeloablative Conditioning	Infusion & Engraftment
Diagnosis confirmed No matched donor Fit for myeloablation	G-CSF ± Plerixafor (SCD: Plerixafor only) 2–3 hrs before apheresis	CD34+ target: **20×10⁶/kg** (for Exa-cel) **≥12×10⁶/kg** (Zynteglo) **≥5×10⁶/kg** (others)	LVV transduction or CRISPR editing **~5–6 months** (Casgevy) **≥3×10⁶/kg** (Casgevy)	Busulfan ×4 days PK-guided dosing VOD prophylaxis	≥48h post-busulfan IV autologous product Monitor engraftment

Backup HSC collection (≥2×10⁶/kg CD34+ cryopreserved) is mandatory before myeloablative conditioning is initiated in all current protocols.

Gene therapy summary (SCD)

- All current gene therapy protocols require ex vivo modification of autologous HSCs.
- Several SCD-related problems impair HSC collection:
  - Stressed and damaged bone marrow
  - Potential cytotoxicity by hydroxyurea
  - Inability to use G-CSF → can cause severe vaso-occlusive events

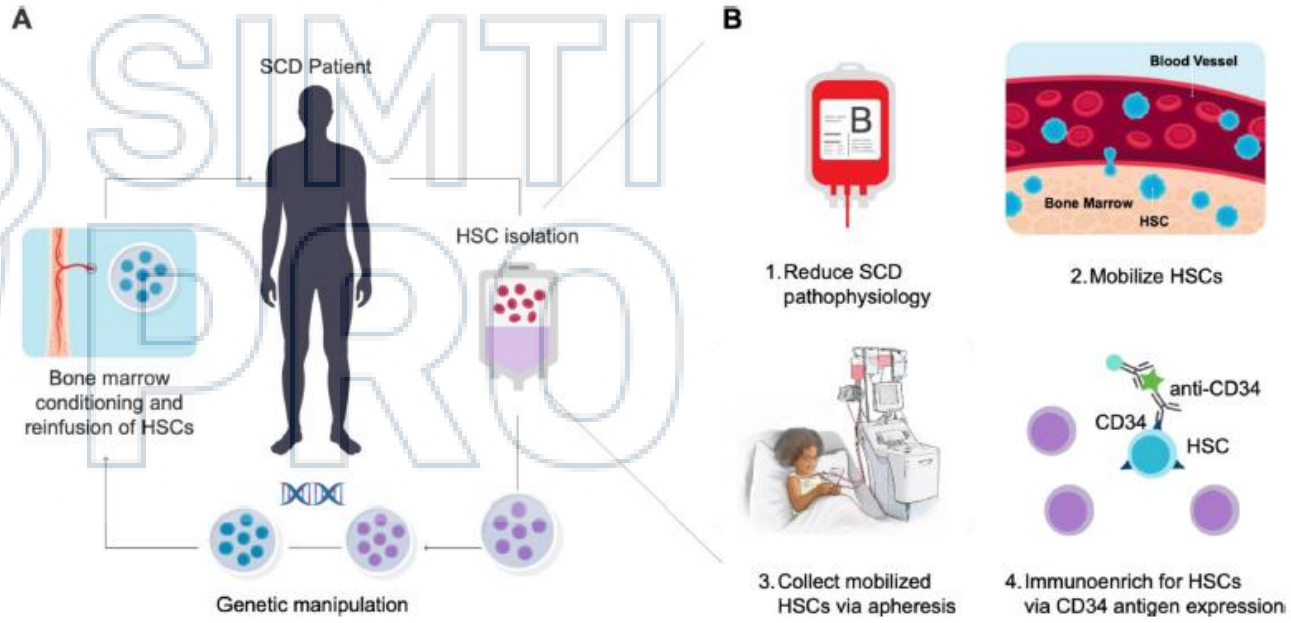


Figure 1. Overview of gene therapy for sickle cell disease (SCD).
A. Major steps in all current SCD gene therapies. B. Important steps in hematopoietic stem cell (HSC) collection. Optimization of each step has contributed to recent successes in SCD gene therapy.

Leonard and Weiss, Curr Opin Hematol 2024

Gene Addition vs. Gene Editing

Gene Addition – Lentiviral Vector (LVV)

- CD34+ HSCs transduced *ex vivo* with LVV carrying therapeutic gene
- Semi-random integration into genome (SIN design reduces oncogenesis risk)
- Permanent — silences endogenous abnormal gene; expresses functional copy
- Approved products: Zynteglo (β-globin), Lyfgenia (βAT87Q-globin), Skysona (ABCD1), Libmeldy/Lenmeldy (ARSA), Strimvelis (ADA)
- Risk: Insertional mutagenesis — MDS/AML observed with Skysona (3 cases); Lyfgenia black box for hematologic malignancy under investigation

Gene Editing – CRISPR/Cas9

- Cas9 RNP electroporated into mobilized CD34+ HSCs, no viral vector
- Disrupts erythroid enhancer of BCL11A → reactivates fetal hemoglobin (HbF)
- HbF replaces defective HbS (SCD) or absent HbA (TDT) — functionally curative
- In pivotal trials: >75% edited alleles maintained at follow-up
- No exogenous DNA insertion → theoretically lower insertional mutagenesis risk
- Emerging approaches: base editing (Beam), prime editing (Tessera), in vivo LNP delivery to HSCs (preclinical 2025)

Comparison of SCD Trial Transfusion Regimens

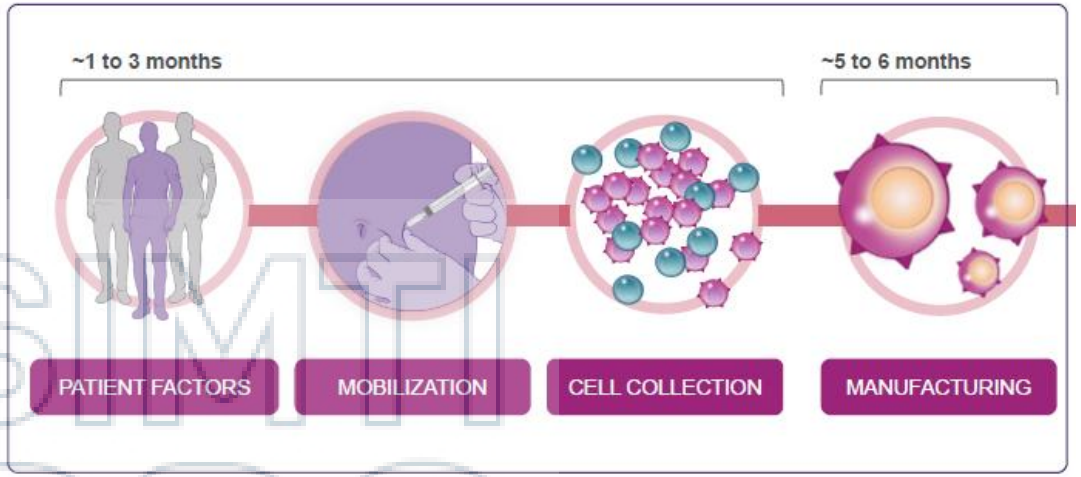
	Vertex	Beam	BBB	GRASP/NHLBI
Pre-mobilization Transfusion (reduction of HbS)	8 weeks	6 weeks	8 weeks	12 weeks
Pre-collection exchange	<7 days of collection HbS <30%	<7 days of collection HbS <20%	<7 days of collection HbS <30%	<4 days of collection HbS <30%
Collection	3 days up to 3 cycles	4 days up to 4 cycles	3 days up to 3 cycles	3 days up to 3 cycles
Cell Target	$20x10^6$ for product $2x10^6$ backup Product – 6-9 months	$18x10^6$ for product $2x10^6$ backup Product –3-4 months	$20x10^6$ for product $1.5x10^6$ backup Product – 2-4 months	$15x10^6$ for product $2x10^6$ backup Product – 7 weeks
Pre-conditioning exchange	<7 days of Chemotherapy	<7 days of Chemotherapy	<7 days of Chemotherapy	<7 days of Chemotherapy

Please refer to individual sponsors for updated specific milestones!

exa-cel, exagamglogene autotemcel

The SCD patient and cell journey (exa-cel)

4 elements contribute to the success rate of delivering drug product back to a patient.



exa-cel, exagamglogene autotemcel

Optimizing apheresis for SCD patient populations is key

- Novel cell therapies require sufficient cells to generate the Gene Therapy (GT) Product usually appr. **5-15x** or more the number of HPCs for a conventional ASCT
  - To compensate intrinsic loss during manufacturing: e.g. transduced vs electroporated
  - Stem cell purification

ASCT=autologous stem cell transplantation

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- Clinical outcome *likely* improved with larger cell dose at transplant
  - Selective advantage of infused SCD GT product is not defined (yet)

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  - Stem cell purification
- Clinical outcome *likely* improved with larger cell dose at transplant
  - Selective advantage of infused SCD GT product is not defined (yet)
- Usually at least 2 days of collection to obtain sufficient cell numbers needed
  - 2 days of collection to pool for GT product and backup necessary **BUT** multiple cycles are common
  - Cell manufacturing and logistics drives cell dose outcomes
  - Multiple mobilizations & collections, especially in children challenging (CVC, hospital admission)

ASCT=autologous stem cell transplantation

Overall preparatory transfusion regimen

- RBC transfusion/red cell exchange to stabilize and improve VOEs while SCD drugs are paused (6-12 weeks before apheresis)
- *Theoretically* reduce erythroid hyperplasia and medullary cavity inflammation **AND** reduce end organ damage to patients prior to mobilization
- Promote suppression of stress erythropoiesis which may aid in improving outcomes of stem cell collection

VOE=veno-occlusive events

Real life collection limitations of GT for SCD

The patient journey for gene therapy can take up to a year!

CLIMB trial

CRISP/Cas9 technology

Exa-cel	SCD (n=46)	TDT (n=56)
1 cycle %	33	79
2 cycles or fewer %	65	95
3 cycles or fewer %	80	-

exa-cel, exagamglogene autotemcel

BEACON trial

Table S3. Number of Mobilization Cycles for Patients.

Number of mobilization cycles, n (%)	Total N=44
1 cycle	15 (34.1)
2 cycles	15 (34.1)
3 cycles	7 (15.9)
4 cycles	2 (4.5)
5 cycles	3 (6.8)
6 cycles	2 (4.5)

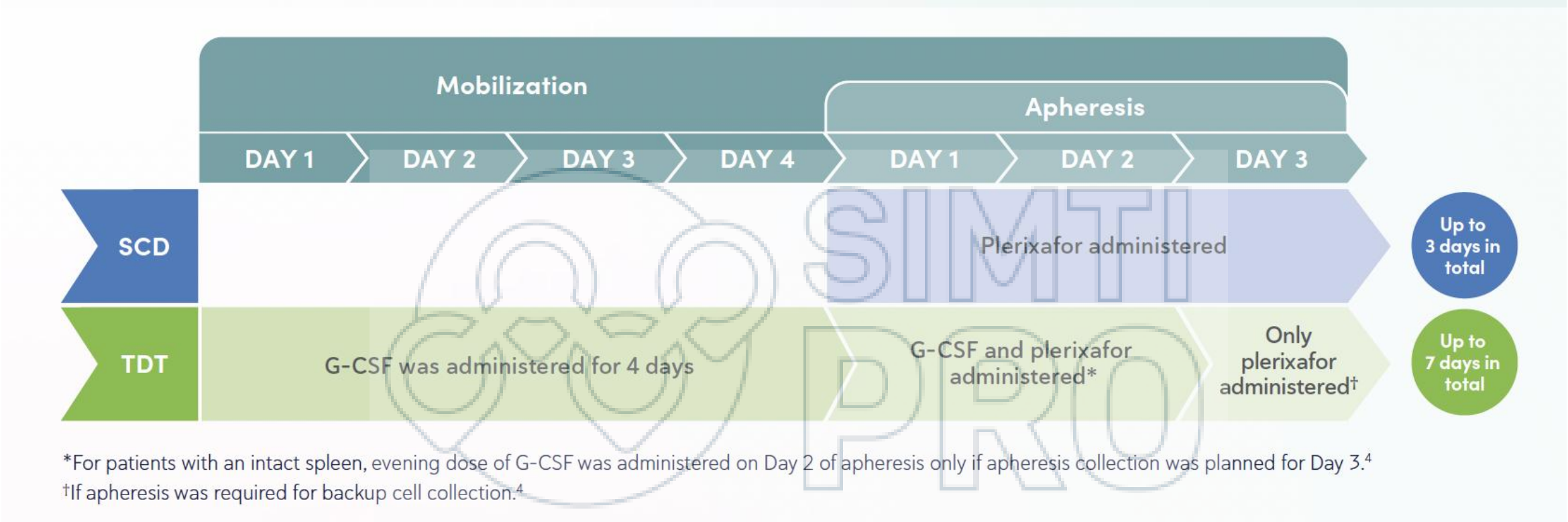
Note: Data shown are from the Full Analysis Set

Base Editing of *HBG1* and *HBG2* Promoters for Sickle Cell Disease

A.O. Gupta,[1] A. Sharma,[2] H. Frangoul,[3,4] J. Kanter,[5] M.Y. Mapara,[6] J. Dalal,[7] A. Alavi,[8] J.J. Jaroscak,[9] E. Ayala,[10] J.F. DiPersio,[11] E.D. Ziga,[12] M. Eapen,[13] S. Rifkin-Zenenberg,[14] A.C. Minella,[15] Y. Chen,[15] S. Chesler,[15] S. Ambati,[15] T.S. Bowman,[15] B. Habtemariam,[15] M. Joseney-Antoine,[15] P.S. Chockalingam,[15] L. Lin,[15] S. Goyal,[15] A. Simon,[15] A.A. Thompson,[16] and M.M. Heeney,[17] for the BEACON Investigators*

Median of 1 cycle/3 collections

Mobilization

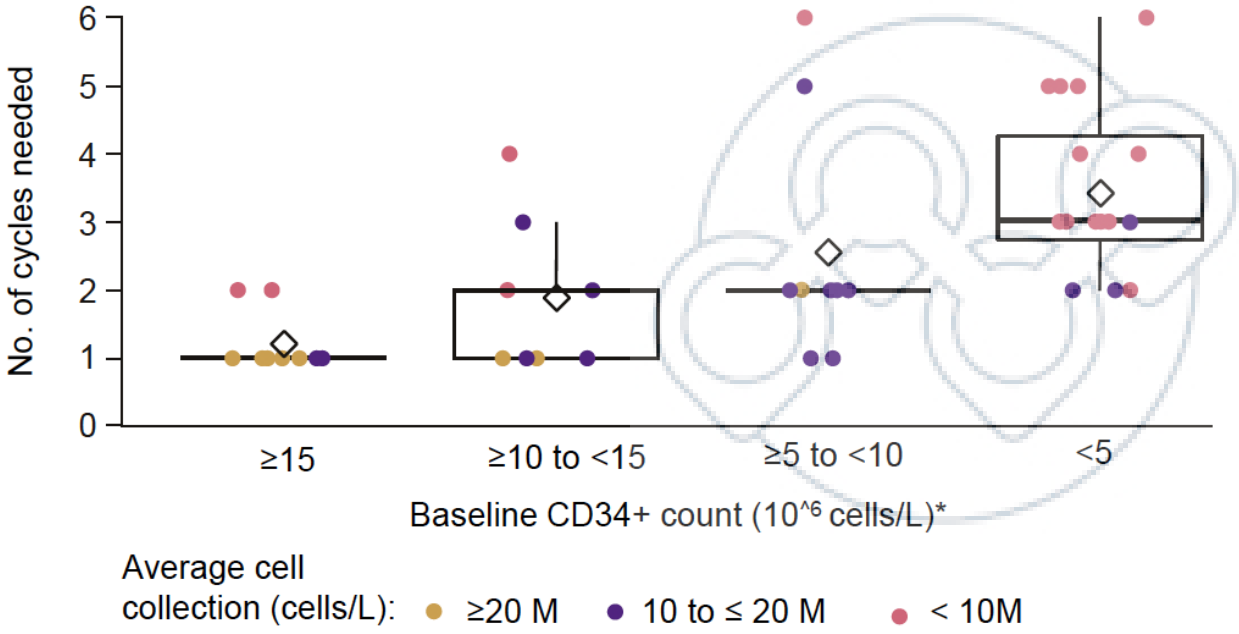


Up to **3 consecutive days** of cell collection for SCD

Unmodified **backup** (at least $2x10^6$ CD34+ cells/kg) must also be collected

Re-mobilization after rest period of >2-4 weeks (other suggestions: 4-8 weeks; Frangoul et al TCT 2025)

Influence on baseline CD34+ counts on making the dose



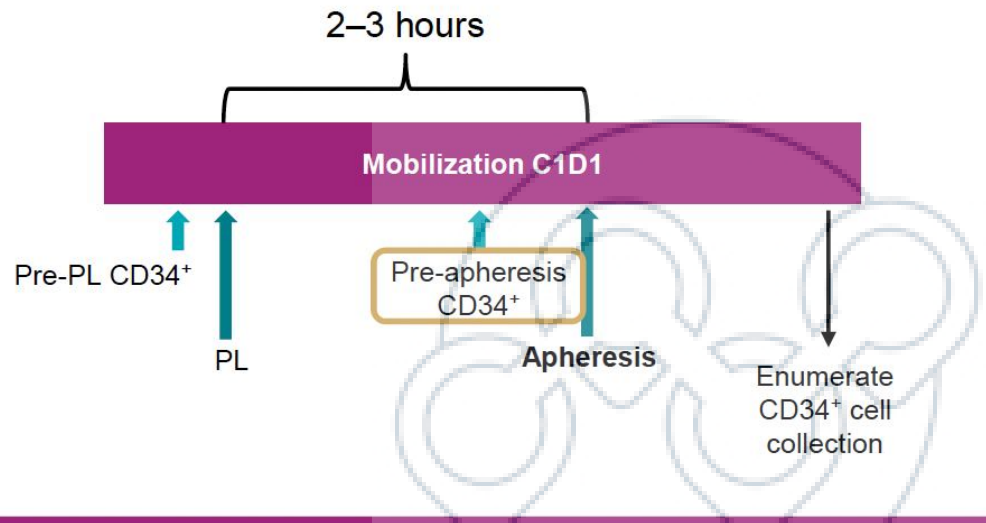
Mean (SD)	Baseline CD34+ cell count (10^6 cells/L)			
	≥15	≥10 to <15	≥5 to <10	<5
Cycles needed	1.2 (0.4)	1.9 (1.1)	2.6 (1.7)	3.4 (1.3)

Higher baseline counts → fewer collection cycles

HEME Cell Collection Deck 2026

Pre-apheresis CD34+ PB count vs cell collection

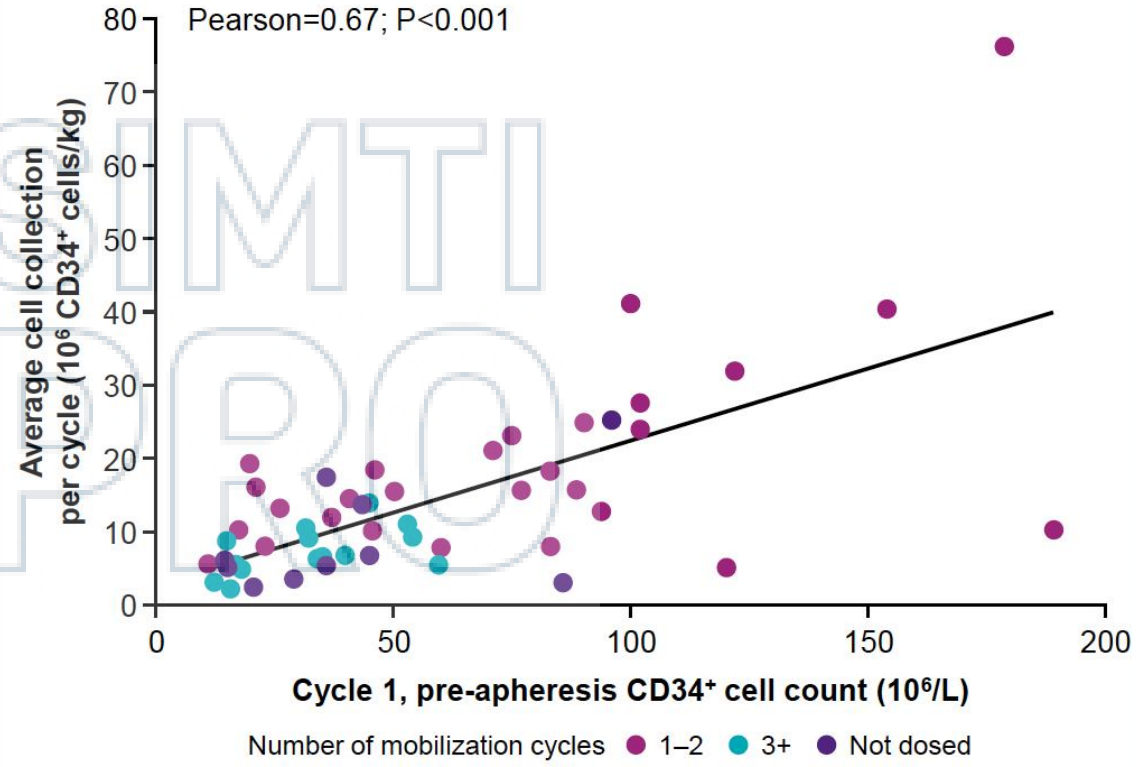
1



Large correlation* between Cycle 1 pre-apheresis (2 hours post-plerixafor) peripheral blood CD34+ count and average CD34+ cell collection per cycle[2]

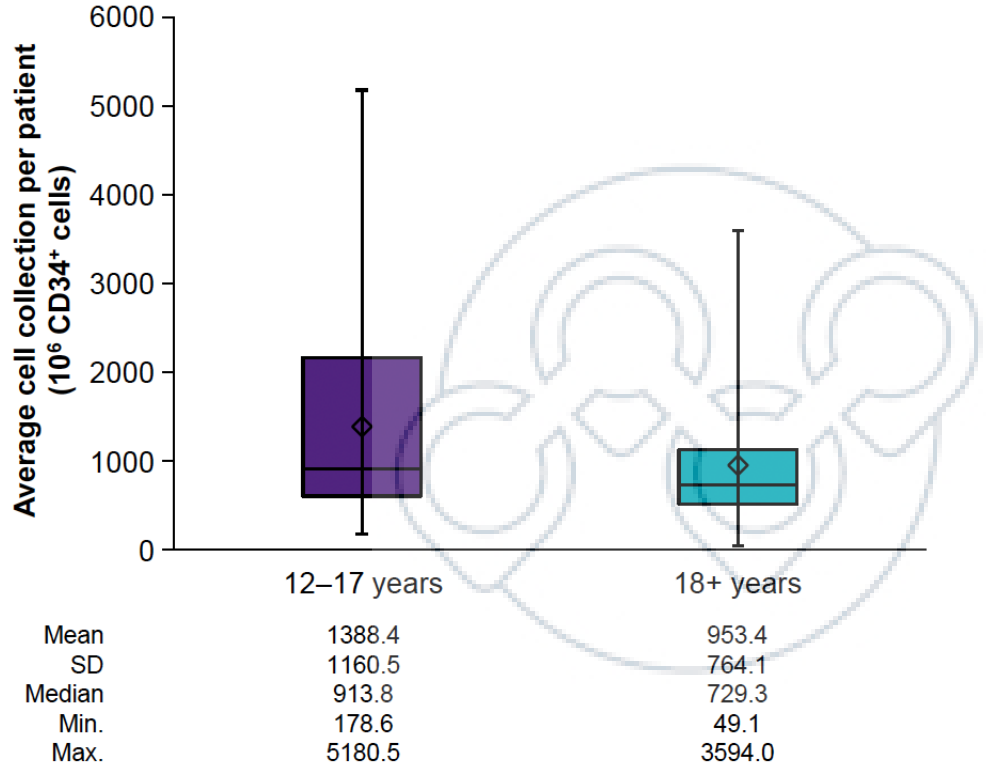
*The strength of the correlation is considered to be small, medium, and large in magnitude when r = 0.10, r = 0.30, and r = 0.50, respectively.[2,3]

2



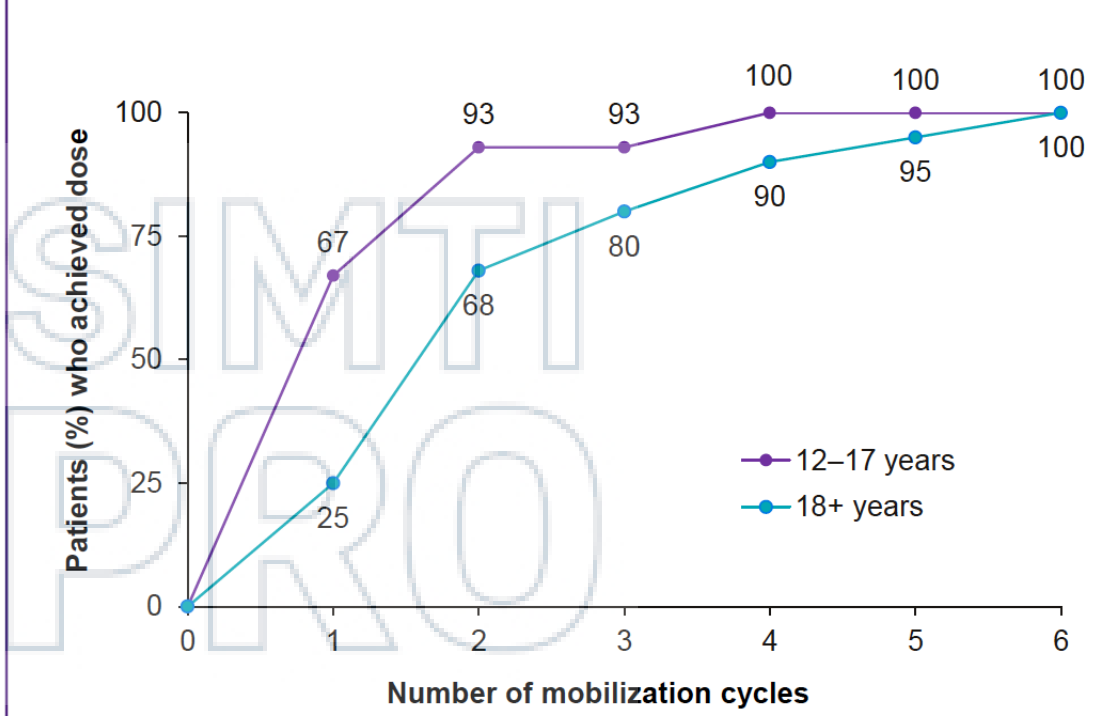
HEME Cell Collection Deck 2026

Impact of age on cell collection



	12–17 years	18+ years
Mean	1388.4	953.4
SD	1160.5	764.1
Median	913.8	729.3
Min.	178.6	49.1
Max.	5180.5	3594.0

Mean and median CD34+ cell collection/patient was higher in adolescents than adults



67% of adolescent and 25% of adult patients with SCD achieved the minimum dose in 1 collection cycle

HEME Cell Collection Deck 2026

Combination of plerixafor and G-CSF in SCD

- G-CSF is generally contraindicated due to the risk of triggering VOEs, ACS, multi-organ failure, and even death, particularly in the presence of high HbS levels
- G-CSF at a dose of 5-10 µg/kg (not ≥300µg), no VoE

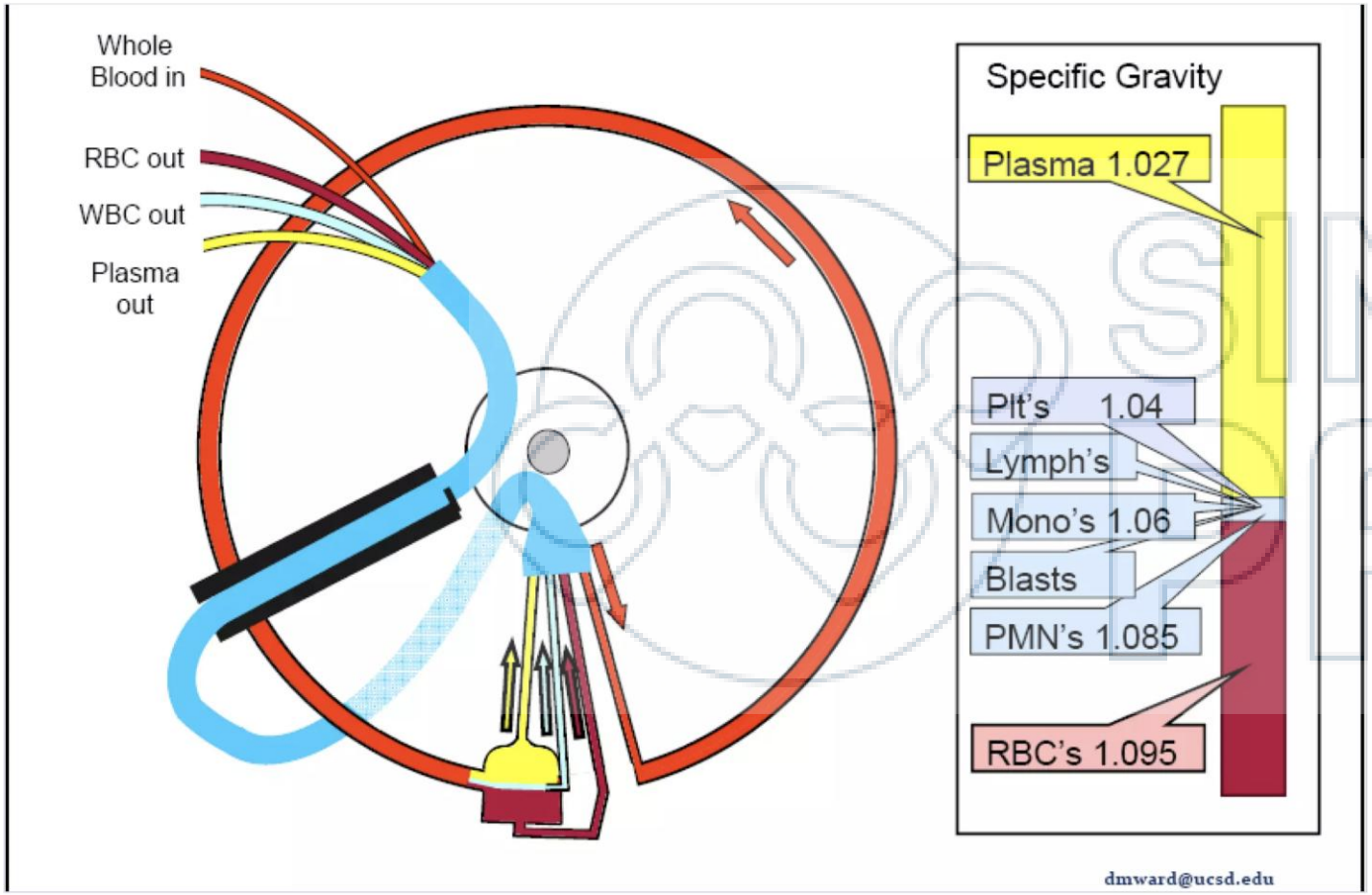
Patient	Cycle 1 mobilization	Cycle 1 CD34/µl	Cycle 1 CD34$10^6$/kg	Cycle 2 and 3 mobilization	Cycle 2 CD34/µl	Cycle 2 CD34 10^6/kg	Cycle 3 CD34/µl	Cycle 3 CD34 10^6/kg
29 yrs, male	PLX	51	16.2	PLX+G-CSF	162	30.16	-	-
23 yrs, male	PLX	26	7.9	PLX+G-CSF	264	73.3	-	-
21 yrs, male	PLX	56	20.5	PLX+G-CSF	36	9.32	105	33.1
32 yrs, female*	PLX	111	19.31	PLX+G-CSF	38	6	84	6.59
21 yrs, female	PLX	31	5.76	PLX+G-CSF	162	31.73	-	-

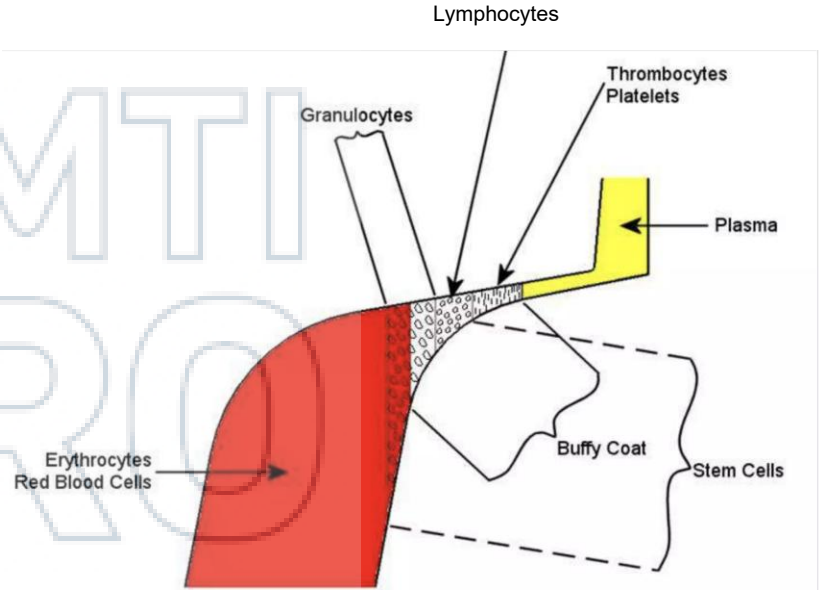
*4th cycle: CD34 142/µL ➜ 30.6x10^6/kg CD34+

ACS=acute chest syndrome, VOE=veno-occlusive events

Arcioni et al., Am J Hematol 2026

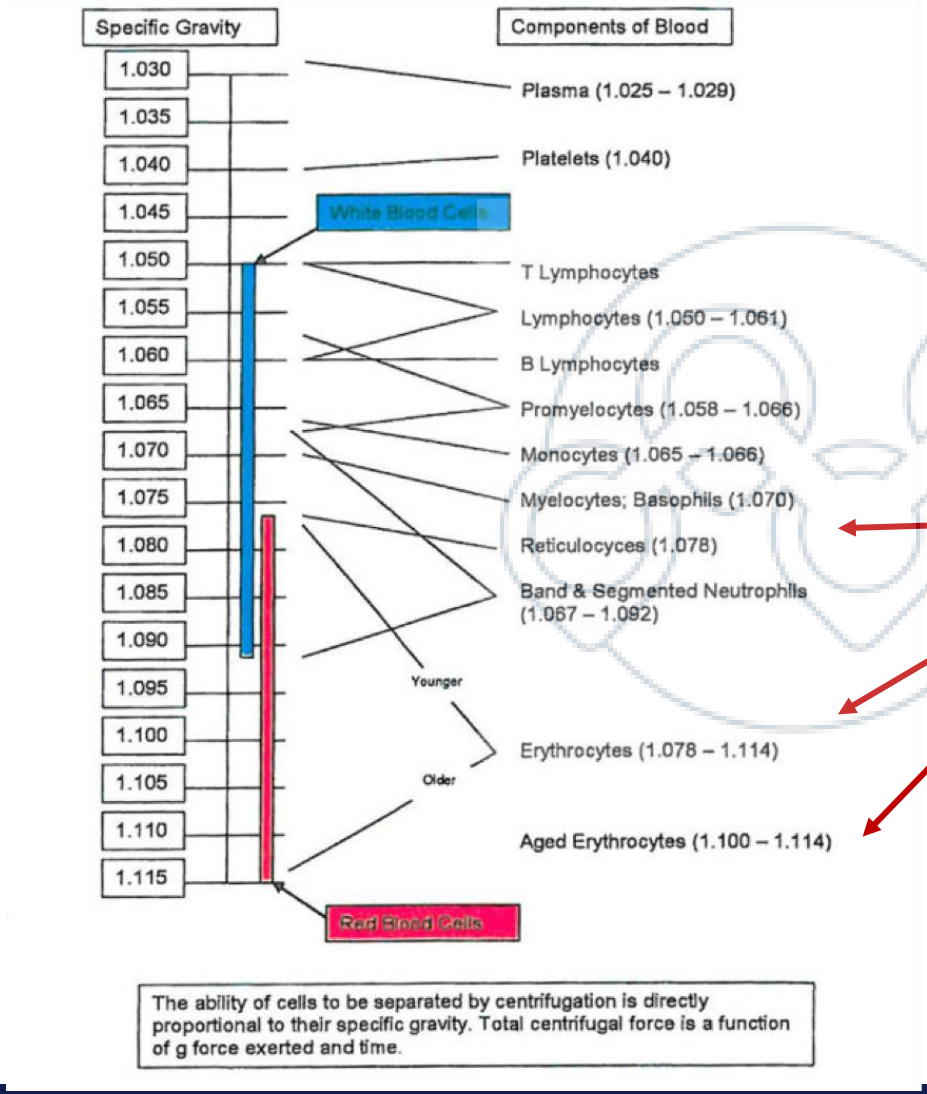
Apheresis instrument





With courtesy of John Manis, Boston

Density alters the collection interface



RBCs have different densities during lifespan

With courtesy of John Manis, Boston

Optimizing HPC apheresis in SCD

- Spectra Optia (Terumo BCT), cMNC platform
- Increase Inlet/AC ratio from 12 (standard) to 8-12
- Prophylactic Ca supplementation i.v.
- Lower the CP to target a deep buffy coat collection to the second darkest colour (medium CP) or the darkest colour (low CP)
- Consider to add heparin to ACD-A
- If clots are visible also consider ASS
- Calculate the blood volume to be processed by using the institutional collection efficiency CE

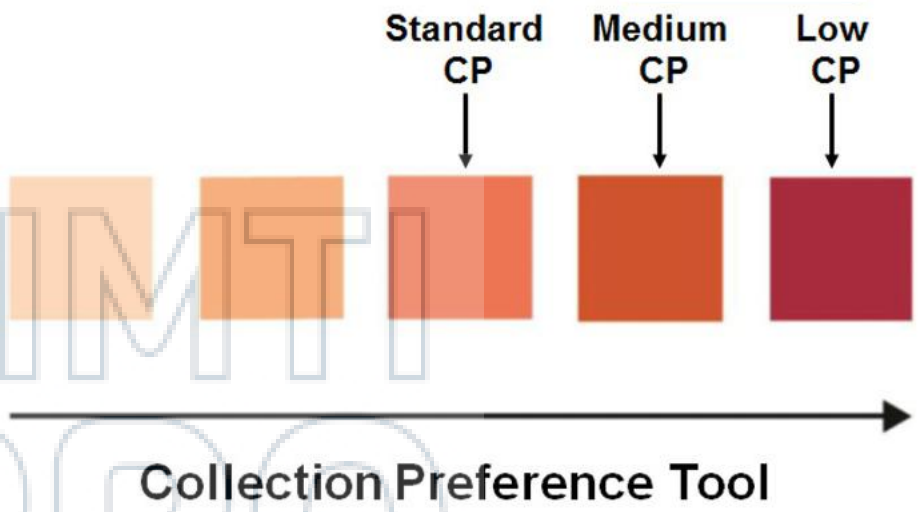


FIGURE 1 Collection depth within the buffy coat is controlled by collection preference (CP). For standard HSPC collection, CP is set to match the colour of the product to the third darkest colour on the collection preference tool (standard CP). To target a deep buffy coat collection, CP is adjusted to match the colour of the product to the second-darkest colour (medium CP) or to the darkest colour (low CP, with CP 15–20) on the collection preference tool. [Colour figure can be viewed at wileyonlinelibrary.com]

CP=Collection preference

Sharma et al, Br J Haematol. 2022

Optimizing HPC apheresis in SCD

TABLE 1 Patient laboratory and autologous HSPC collection results

	Total ($n = 43$)	Low CP ($n = 19$)	Medium CP ($n = 24$)	p-Value
Pre-collection patient laboratory studies				
WBC ($\times 10^9$/l)	20.92 (15.27–29.37)	17.11 (14.40–20.63)	29.07 (20.92–31.38)	<0.001
Hct (%)	29.0 (26.9–30.5)	29.5 (27.6–30.9)	28.4 (26.0–30.4)	0.60
Platelet ($\times 10^9$/l)	204 (138–272)	161 (130–265)	222 (161–295)	0.09
HbS (%)	27.7 (21.9–30.3)	27.7 (21.9–30.3)	26.9 (21.6–30.6)	0.78
CD34 (/µl)	49 (26–87)	34 (25–60)	59 (27–131)	0.044
HSPC apheresis collection				
Whole blood processed per procedure (l)	17.42 (15.23–20.67)	17.74 (16.77–18.00)	16.15 (13.78–22.81)	0.89
TBV per procedure	3.8 (2.9–4.0)	3.9 (3.7–4.0)	3.3 (2.7–4.0)	0.19
HSPC yield ($\times 10^6$/kg CD34+ cells)	4.05 (2.11–8.04)	5.51 (3.45–9.93)	2.74 (1.68–6.75)	0.41
CE1 (%)	39.54 (30.73–80.58)	82.23 (44.43–96.54)	35.79 (24.78–39.61)	0.016
CE2 (%)	33.12 (25.05–64.86)	73.10 (44.43–81.02)	25.24 (19.35–31.12)	<0.001
HSPC products				
Hct (%)	5.5 (4.3–7.0)	6.4 (5.5–7.7)	4.7 (4.2–6.1)	0.009
Granulocyte (% of total nucleated cells)	3.0 (1.7–5.0)	4.0 (3.0–5.0)	1.8 (1.0–2.7)	0.022
Platelet ($\times 10^9$/l)	940 (720–1247)	807 (673–985)	1135 (727–1532)	0.10

Blood volume to be processed

$$(CE2\%) = \frac{collected\ cell\ number}{cells{-}preapheresis\ in\ peripheral\ blood\ \ x\ \ processed\ blood\ volume}\ x\ 100$$

- For a prospective study: ≥75% of patients should exceed the calculated mean of the retrospective analyses.

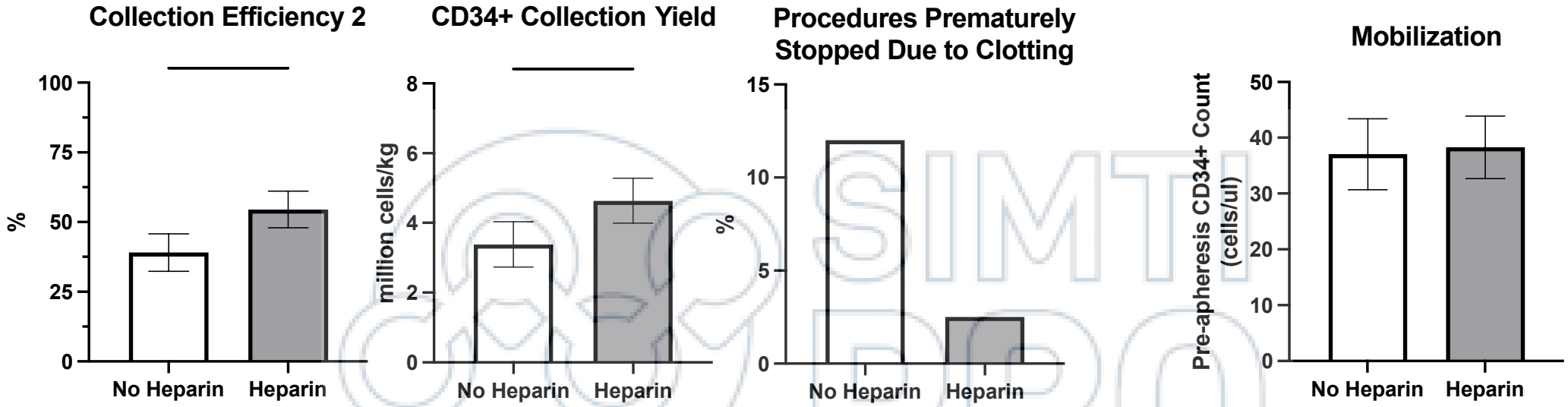
$$Process\ volume\ (L) = \frac{target\ cell\ dose\ [6/kg] \times 100{,}000}{45 \times CD34\ in\ peripheral\ blood\ [10^6/L]}$$

Blood volume to be processed

Datum:		
P-Nummer:		
Apherese Nr.:	2	(die wievielte Pherese)
Noch benötigte Zellzahl:	4	X10E6/kg
CD 34+ precount:	72,8	Zellen/ul
Gewicht des Patienten:	80	kg
GBV des Patienten:	5,319	L
(berechnet mit Optia)		
Nach Formel berechnetes Blutvolumen:	10,84	L
mit 15% Aufschlag:	12,47	L
Dies ist das	2,04	fache des GBV
Ergebnis: Bitte	**12,47**	**L einstellen**

Dr. Stephanie Winter

Addition of heparin improved apheresis outcomes



Influence of glycosaminoglycan-derived oligosaccharides on SDF-1 dependent chemotactic effect on PBPC: Recruitment significant more often with heparin (68% vs 25% of pts)

No bleeding events or heparin related side effects occurred

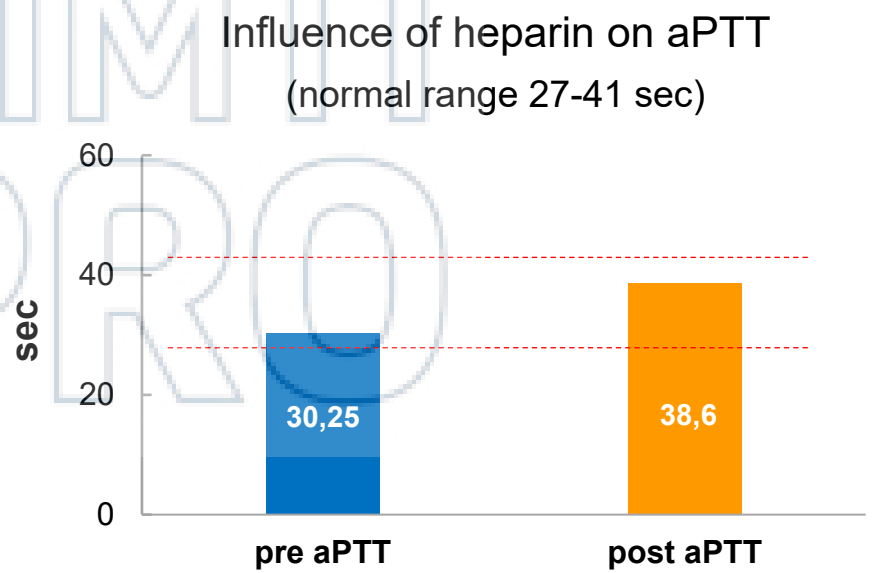
Subjective improvement in interface stability

With courtesy of John Manis, Boston

Addition of heparin and aPTT (Vienna)

PBSC collection with Spectra Optia cMNC platform

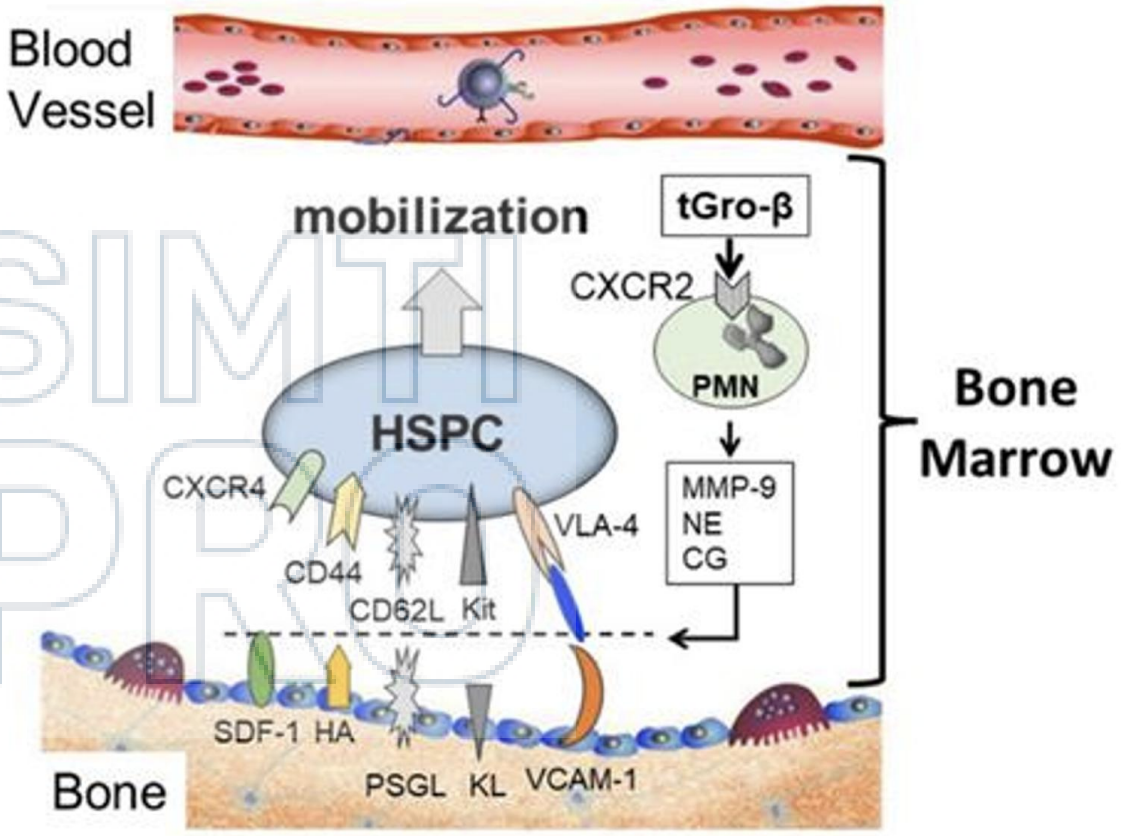
- ACD-A 1:12 if ≤3x TBV processed
- ACD-A + heparin if >3-x TBV processed
  - 750mL Citrate + 4.500 IU heparin
  - Inlet/AC ratio to 1:22



TBV=total blood volume

Novel options: Motixafortide (Aphexda)

- CXCR4 antagonist approved in the US for MM
- CD34+ cell counts peak 16 hrs postdose, decline slightly thereafter
- In studies remained elevated above baseline at the last observation (i.e. 24-hrs postdose)
- High affinity and >72 hour receptor occupancy allows 1 dose to last for 2 consecutive days of apheresis



Motixafortide in US trials, early data encouraging

TBV=total blood volume

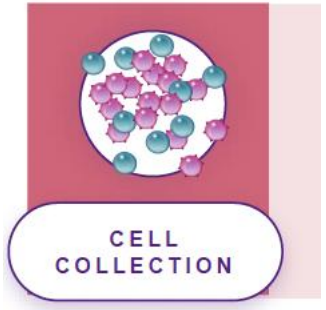
Summary

Disease severity, age, baseline and pre-apheresis CD34+ cell count correlate with CD34+ collection outcomes in SCD.

Patient factors can predict manufacturing success as more cells collected increases the likelihood of making a full dose in fewer cycles.

Real-world data confirm that observations from clinical trials with mobilization are more challenging in SCD than TDT.

Higher numbers of CD34+ collected increases probability of achieving dose. Therefore prospective studies are warranted to validate observations with G-CSF in SD and other novel approaches.



In RWE to date, 48% of SCD & 88% of TDT patients achieved a dose of exa-cel in 1–2 collection cycles.

Consistent communication between the clinical facility and apheresis unit is important to optimize the patient journey with the aim to achieve successful GT product manufacturing.

Thank you for your attention!