



ISBMT

Indian Society for Blood & Marrow Transplantation

BMT MASTERCLASS

ORGANISED
BY ISBMT

2025

13th & 14th December 2025

**'Experiences in my HSCT Journey' –
Alkylators in Conditioning – HSCT to
Gene Therapy!**

Alok Srivastava

Haematology Research Unit, St John's Research
Institute &

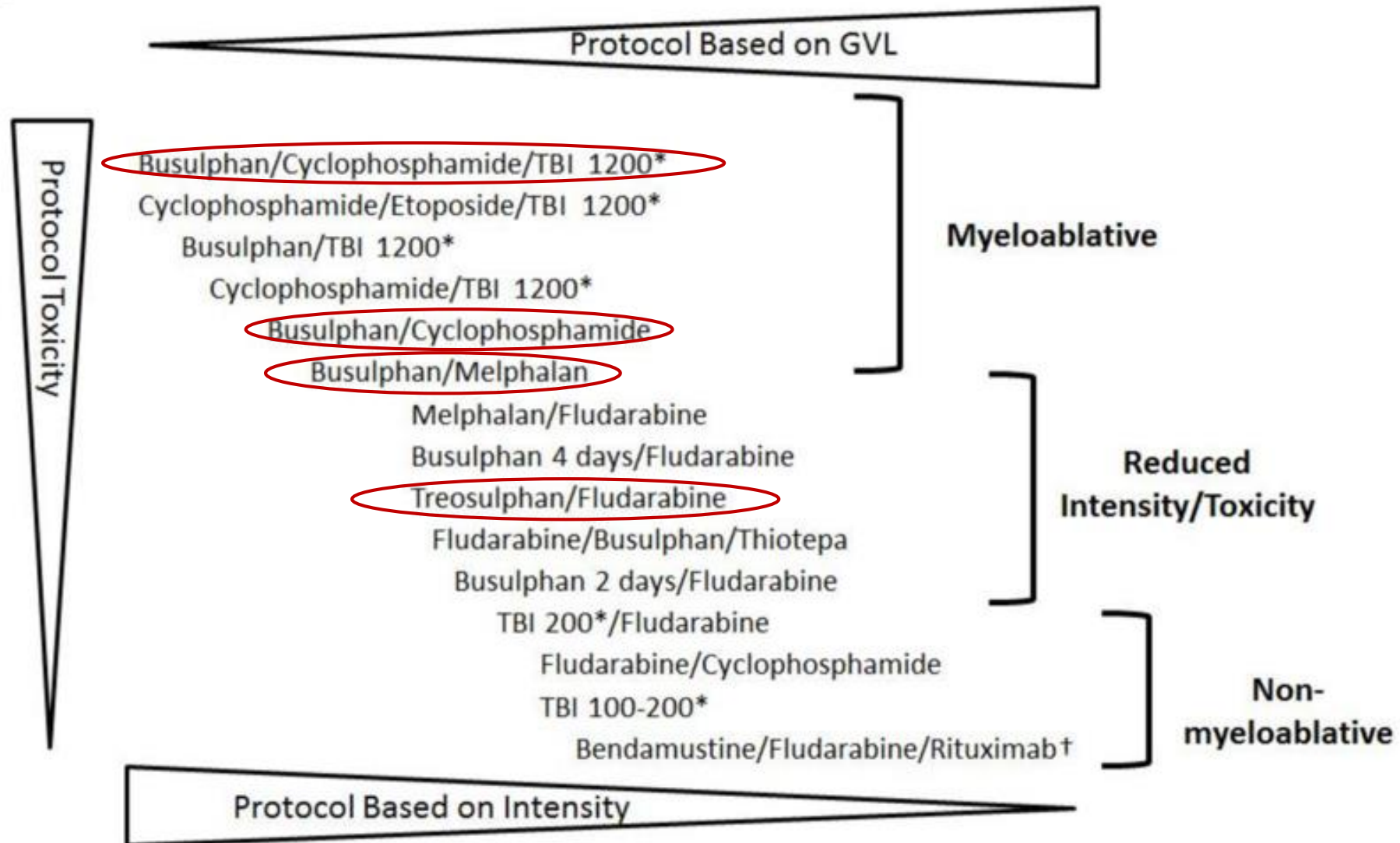
Dept. of Haematology, St. John's Medical
College Hospital



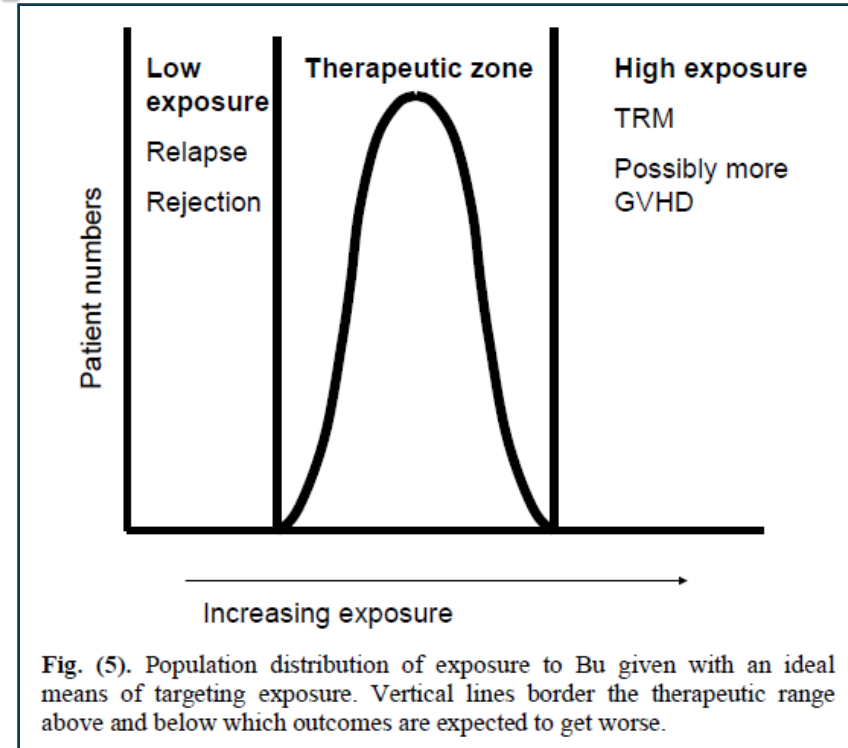
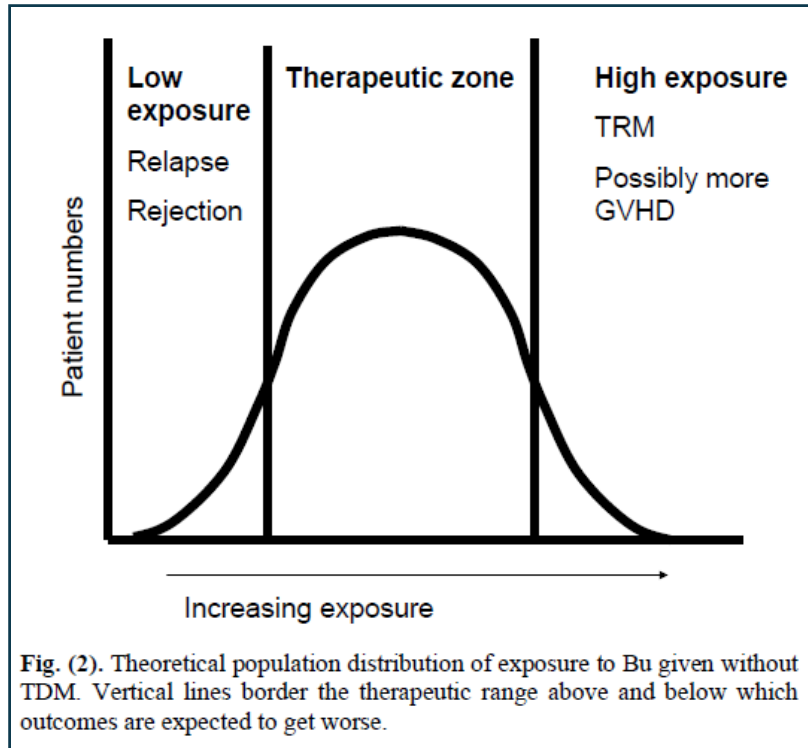
St. John's

National Academy of
Health Sciences

Conditioning Therapy Intensity



Conditioning Therapy in Stem Cell Transplantation: What is required?



* **Myeloablative** (=High toxicity) vs **Non-myeloablative** (=Reduced intensity = lower toxicity)
(TBI / Busulfan / Melphalan) (Lower dose alkylators & Fludarabine / Cyclophosphamide)

* Reduced intensity (non-myeloablative) vs Reduced toxicity (myeloablative)?

Busulphan and melphalan prior to autologous bone marrow transplantation

A Srivastava¹, K F Bradstock, J Szer, L de Bortoli, D J Gottlieb

¹ Department of Haematology Westmead Hospital, New South Wales, Australia.

*13 males & 11 females, aged 27-53 years (median 39.5 years) received oral busulphan 1 mg/kg q6 h on days -6 to -3, followed by i.v. melphalan 140 mg/m² on day -2 and infusion of cryopreserved haemopoietic cells on day 0

*The major toxicity seen was gastrointestinal with nausea, vomiting and diarrhoea in 17 patients and severe mucositis in 22. There was no evidence of cardiotoxicity, nephrotoxicity, haemorrhagic cystitis or clinical signs of hepatic veno-occlusive disease.

*Of the group of 13 lymphomas, overall and relapse-free actuarial survival at 36 months was 64% and 58%, respectively,

Randomized trial of two different conditioning regimens for bone marrow transplantation in thalassemia – the role of busulfan pharmacokinetics in determining outcome

Bone Marrow Transplantation (2005) 36, 839–845

M Chandy¹, P Balasubramanian¹, SV Ramachandran¹, V Mathews¹, B George¹, D Dennison², R Krishnamoorthy³ and A Srivastava¹

1. REGIMEN A [Bu600] – busulfan 600 mg/m² given as four divided doses over 4 days and cyclophosphamide 200 mg/kg given over 4 days (50 mg/kg/day i.v over 1 h).
2. REGIMEN B [Bu16] – busulfan 16 mg/kg as 1 mg/kg/dose four times daily × 4 days, cyclophosphamide 200 mg/kg given over 4 days (50 mg/kg/day i.v over 1 h) and ALG (Pasteur Merieux) 30 mg/kg/day for 3 days.

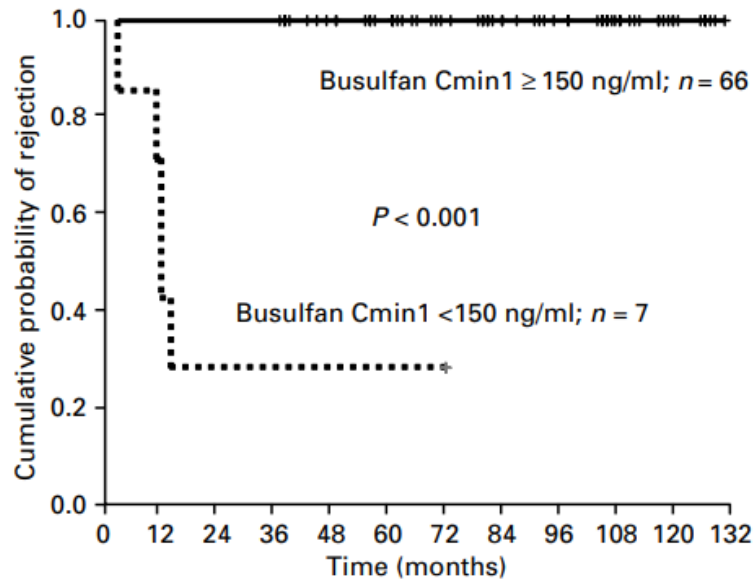


Figure 1 Probability of graft rejection depending on busulfan C_{min-1} .

Table 3 Analysis of outcome in relation to busulfan dose

	Bu 600/Cy 200		Bu 16/Cy 200/ALG		P-value
	n	(%)	n	(%)	
Overall survival	32	(68)	34	(47)	0.822
Disease-free survival	32	(68)	30	(64)	0.828
Follow-up (months) median	63	1–124	52	1–124	0.376
Rejection	2	(4)	4	(9)	0.677**
Mortality	15	(32)	13	(28)	0.652*
<i>Outcome by class</i>					
Class II	n = 21		n = 22		
Overall survival	17	(81)	19	(86)	0.698
Disease-free survival	17	(81)	19	(86)	0.698
Rejection	—	—	—	—	—
Mortality	4	(19)	3	(14)	0.631
Class III	n = 26		n = 25		
Overall survival	15	(58)	15	(60)	1.000
Disease-free survival	15	(58)	11	(44)	0.406
Rejection	2	(10)	4	(22)	0.302
Mortality	9	(35)	10	(40)	0.691

2-16 fold IIV in PK parameters on oral busulfan

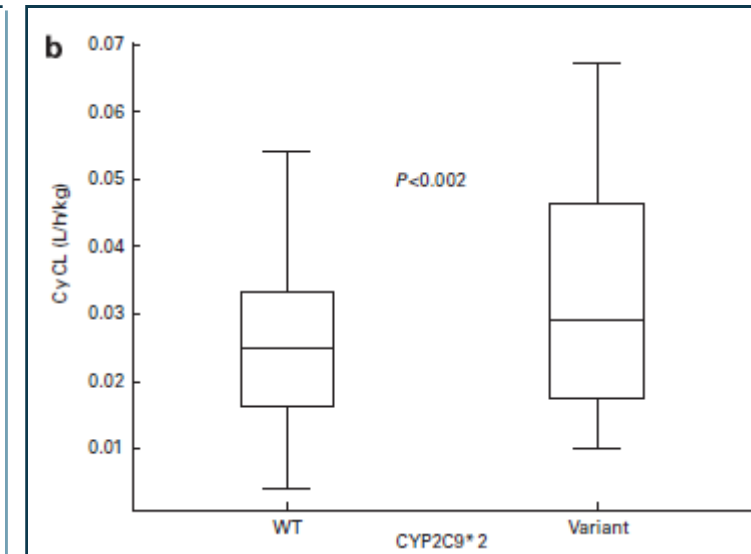
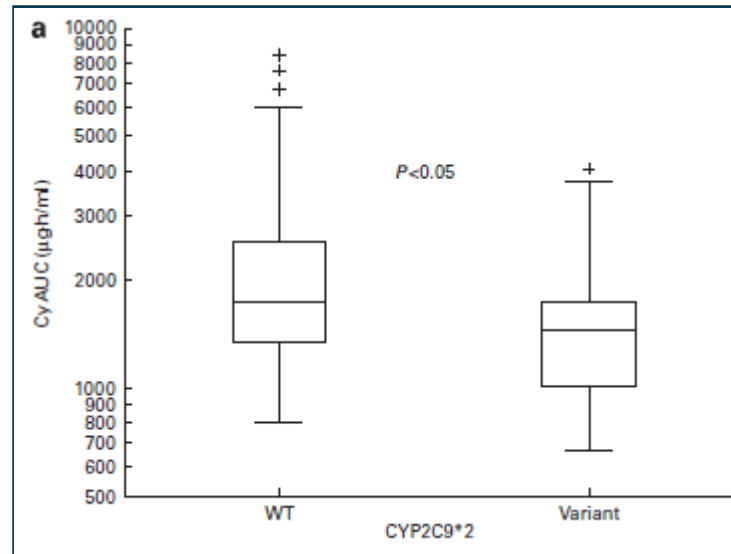
Population pharmacokinetics of cyclophosphamide in patients with thalassemia major undergoing HSCT

P Balasubramanian^{1,4}, S Desire^{1,4,5}, JC Panetta^{2,4}, KM Lakshmi¹, V Mathews¹, B George¹, A Viswabandya¹, M Chandy^{1,6}, R Krishnamoorthy³ and A Srivastava¹

Table 1. Population estimated secondary parameters estimated using the two-stage approach

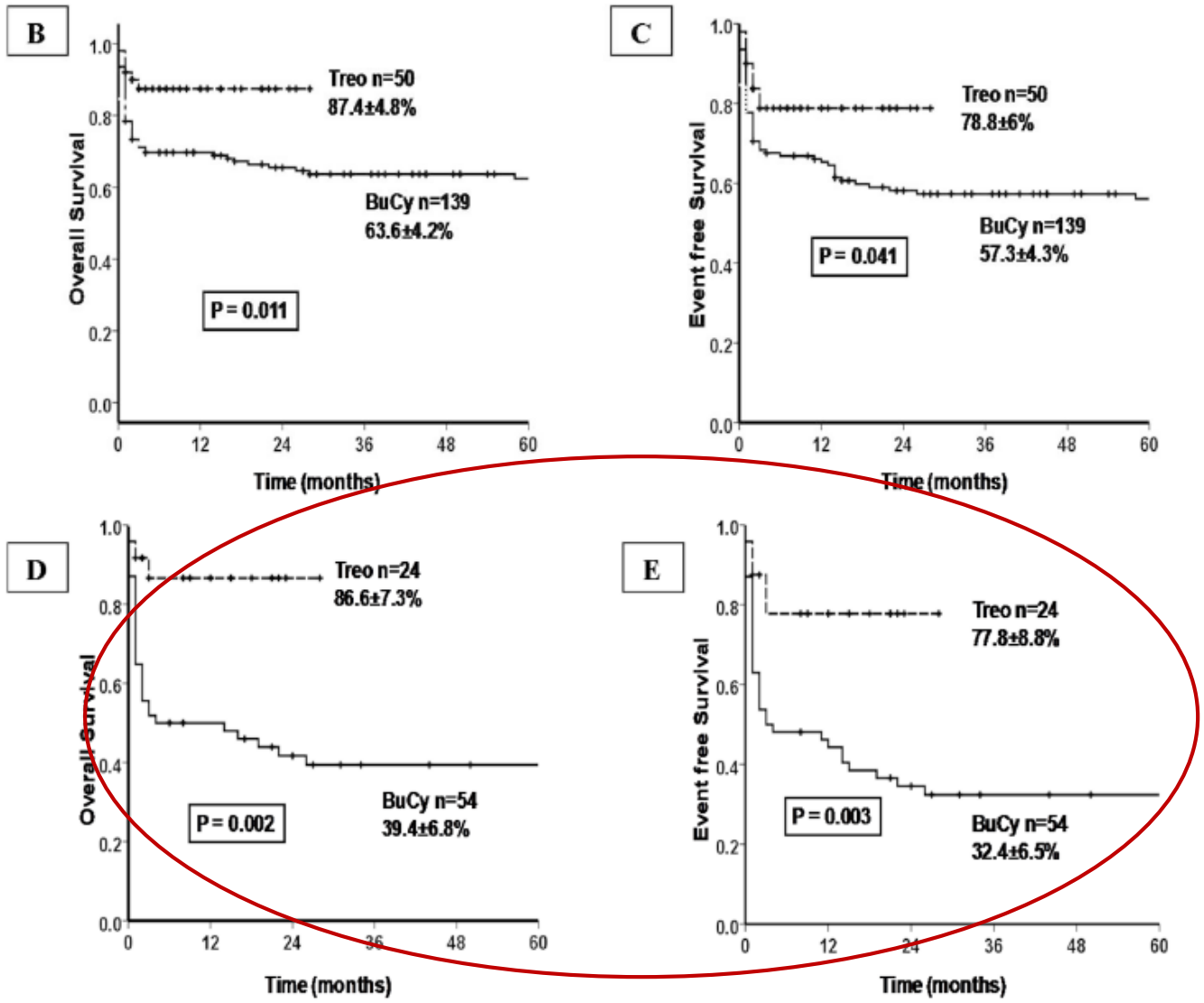
	Population mean (range)	IIV (CV%)	IOV (CV%)
CY AUC ($\mu\text{g h/mL}$)	1858.8 (866-9445)	51	62
CY C_{max} ($\mu\text{g/mL}$)	637.6 (292-2664)	41	48
CY C_{min} ($\mu\text{g/mL}$)	6.7 (0.06-121)	92	103
CY $t_{1/2}$ (h)	1.59 (0.54-4.2)	24	35
HCY AUC ($\mu\text{g h/mL}$)	5200 (953-22573)	45	24
HCY C_{max} ($\mu\text{g/mL}$)	1700 (250-6542)	49	33
HCY C_{min} ($\mu\text{g/mL}$)	0.019 (0.41-216)	114	58
HCY $t_{1/2}$ (h)	0.12 (0.02-0.19)	17	12
HCY AUC/CY AUC	2.8×10^{-3} (4.1×10^{-4} - 1.6×10^{-2})	40	55

Abbreviations: AUC = area under the concentration vs time curve; C_{max} = maximum concentration; CV% = coefficient of variation; HCY = hydroxy CY; IIV = inter-individual variability; IOV = inter-occasion variability.



- *17-114% IIV and 12-103% IOV in CY and HCY PK parameters were observed
- *Body Wt and age were the main covariates
- *CYP2C9*2 explained a significant portion of the IIV in the clearance

Improved Clinical Outcomes of High Risk β Thalassemia Major Patients Undergoing a HLA Matched Related Allogeneic Stem Cell Transplant with a Treosulfan Based Conditioning Regimen and Peripheral Blood Stem Cell Grafts



Allogeneic hematopoietic stem cell transplantation in thalassemia major: results of a reduced-toxicity conditioning regimen based on the use of treosulfan

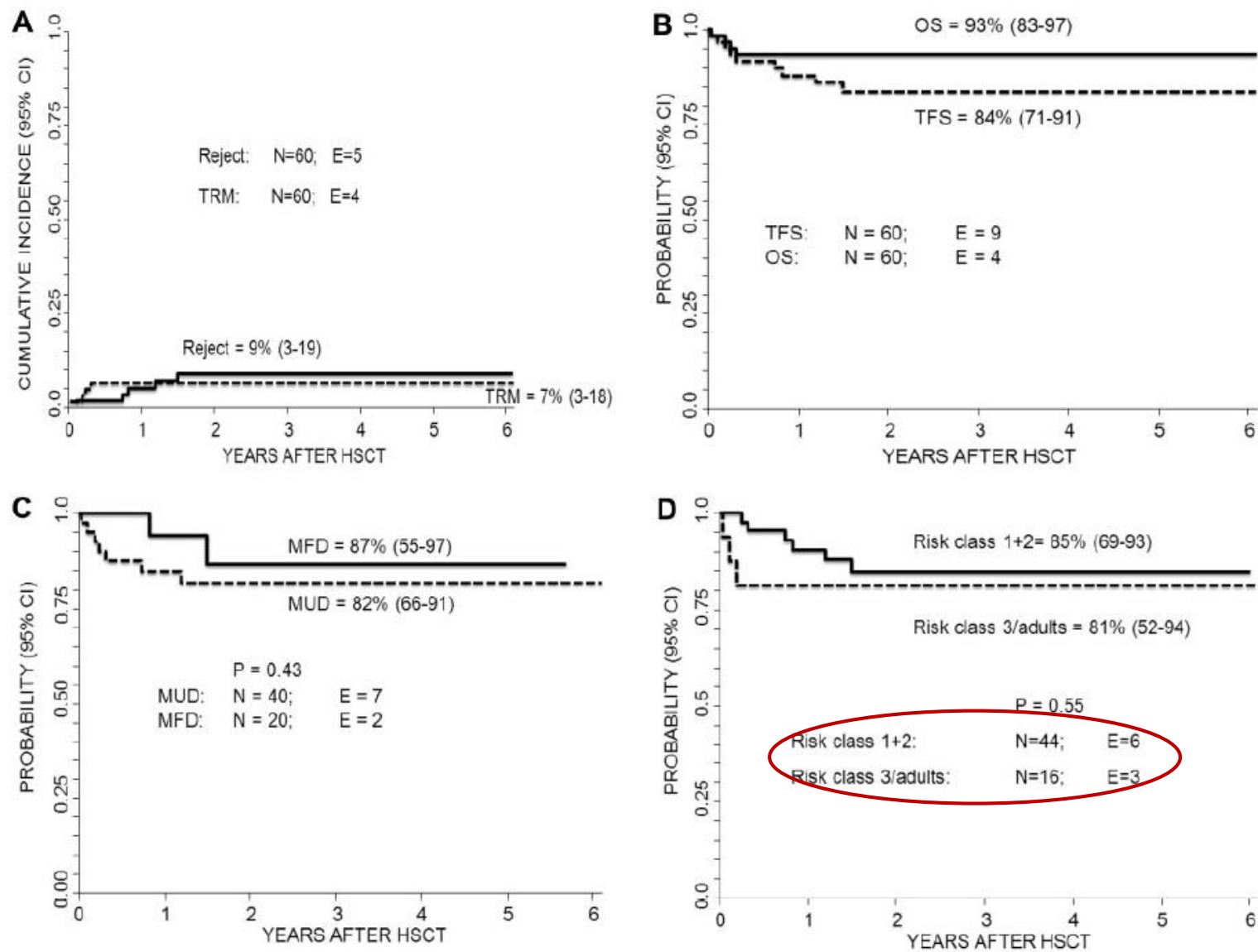
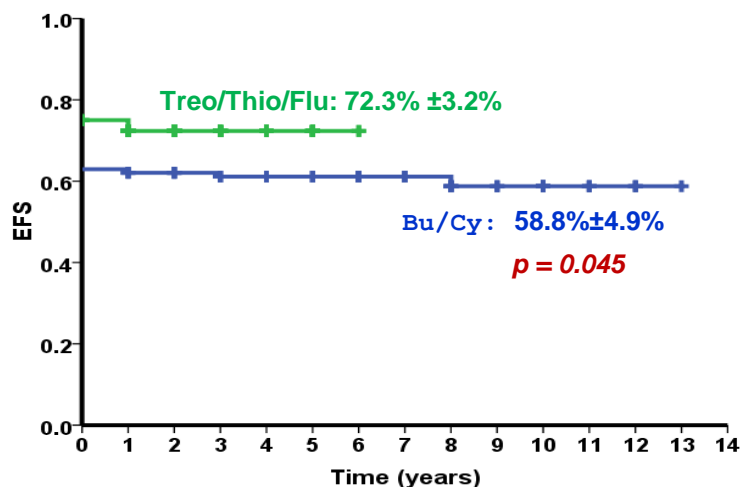
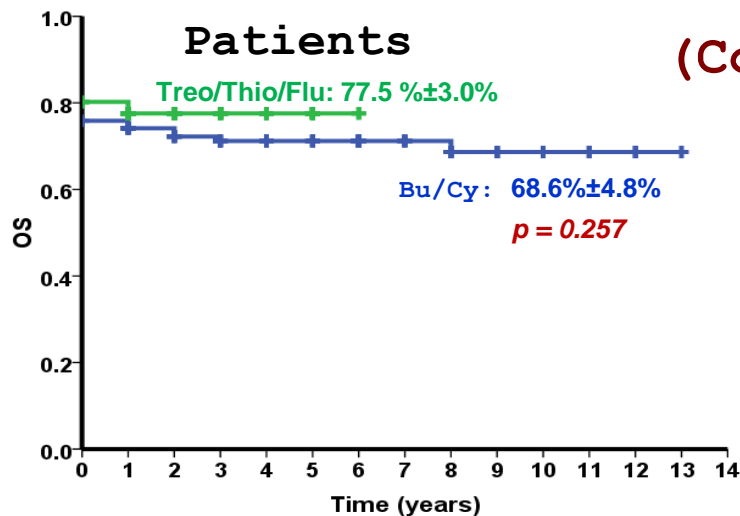


Figure 1. Outcomes of the study population. (A) Cumulative incidence of transplantation-related mortality (TRM) and graft rejection (Reject). (B) Five-year Kaplan-Meier estimate of overall survival (OS) and TFS for the whole cohort of patients. (C) Five-year Kaplan-Meier estimate of TFS according to the type of donor used (MFD indicates matched family donor; and MUD, matched unrelated donor). (D) Five-year Kaplan-Meier estimate of TFS according to the patient's class of risk.

Outcome of Allogeneic Stem Cell Transplantation for Thalassemia Major in India

Comparison by Conditioning Regimen: Bu/Cy vs Treo/Flu/Thiotepa (2000

All Patients - 2014) (n=593)
(Combined data from 6 HSCT centres)

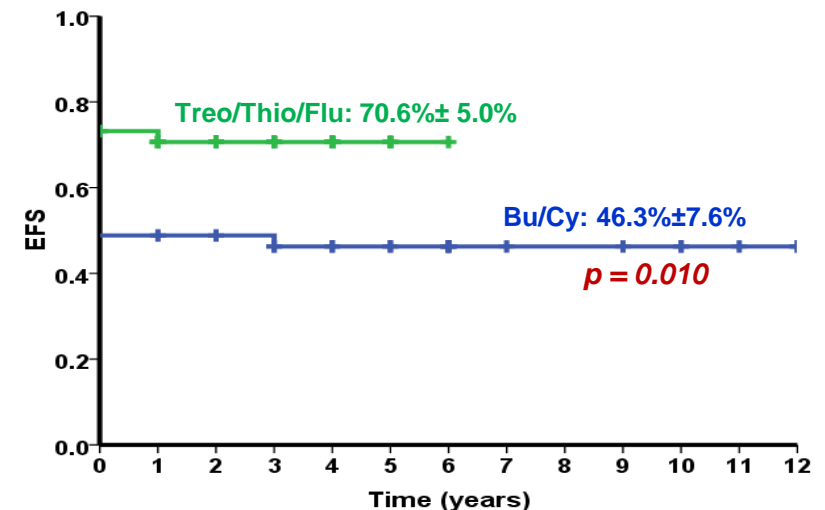
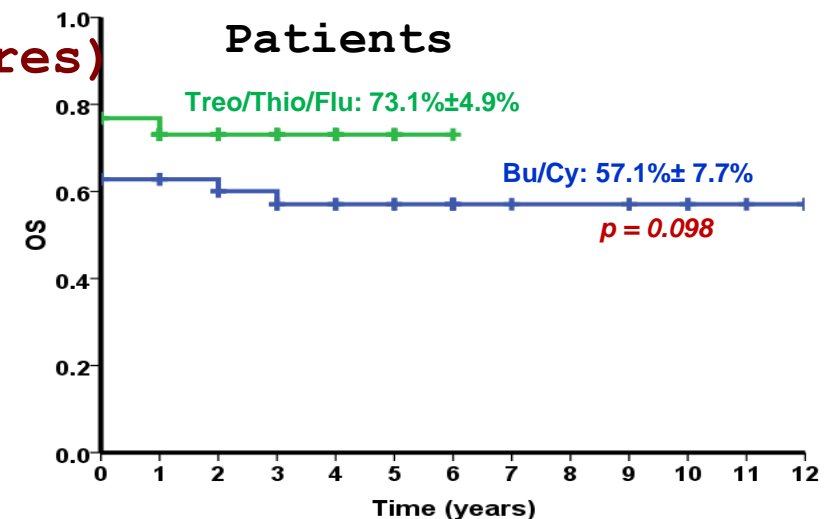


patient characteristics & conditioning regimen

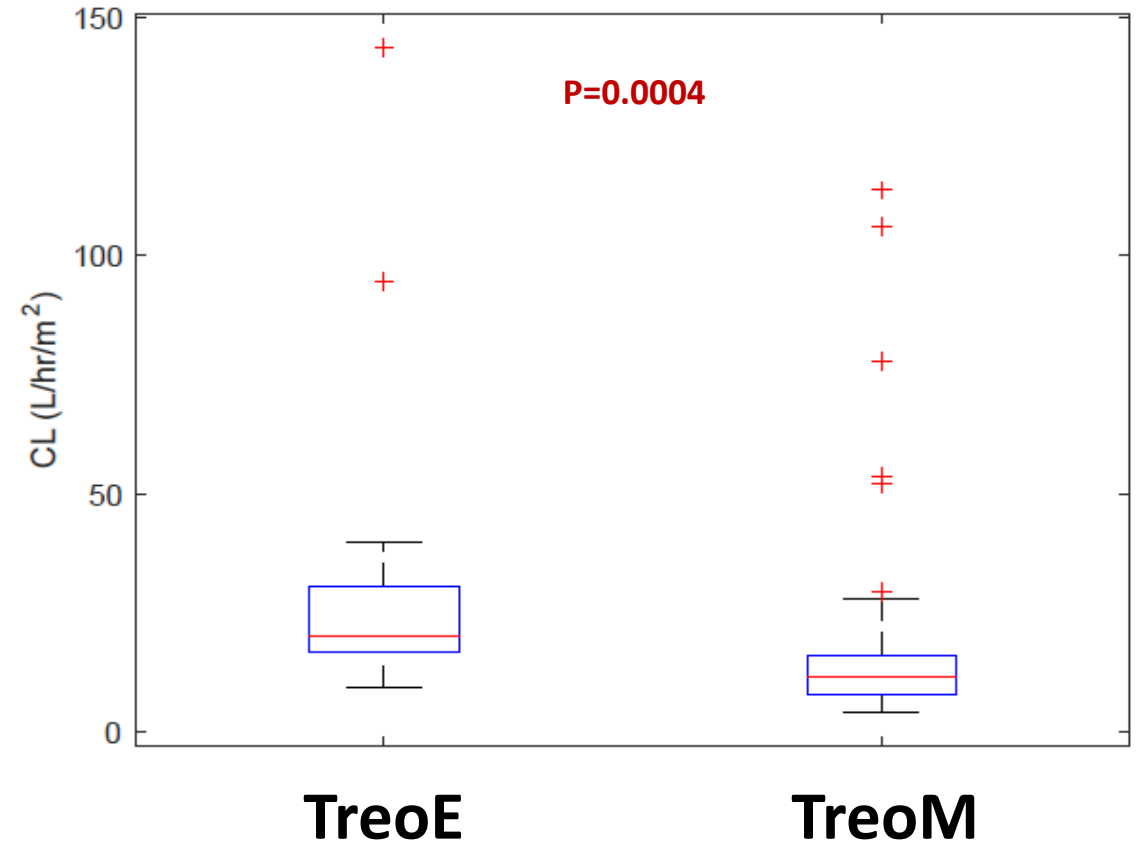
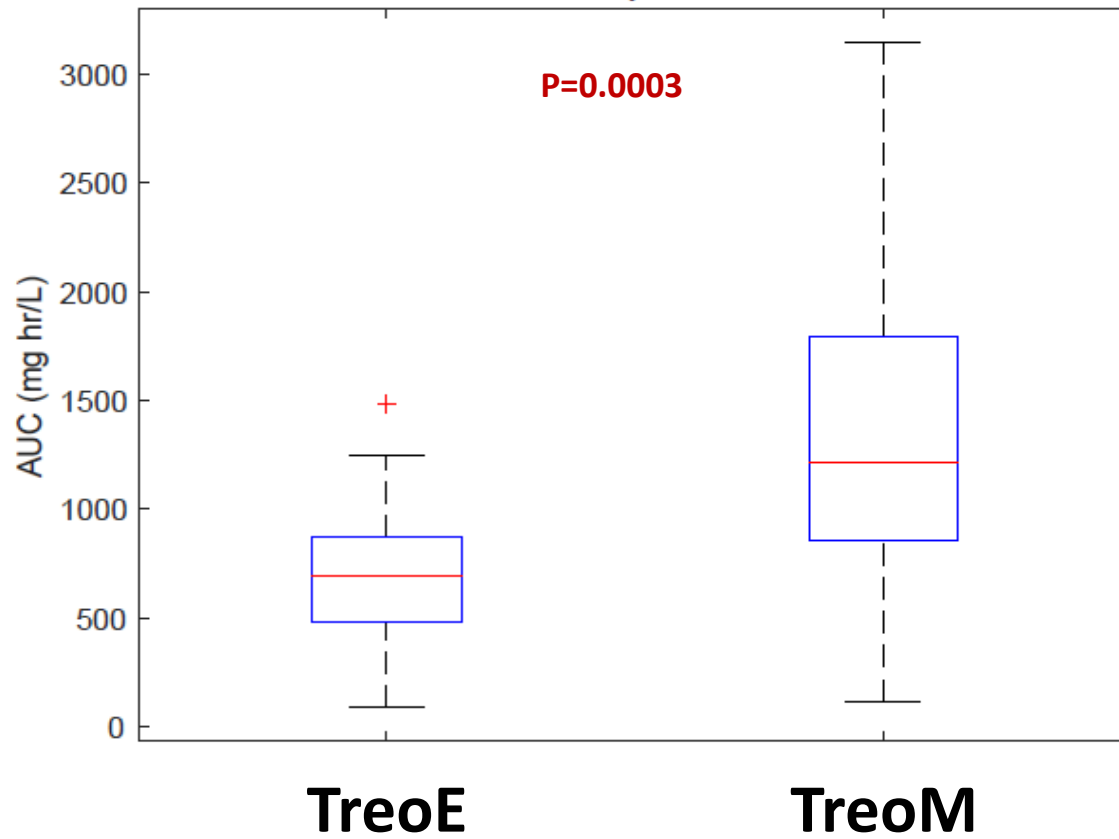
Variables	Bu/Cy (n=315) * N (%)	Treo/Thio/Flu (n=278) * N (%)	p value
Age			
≤ 15	309 (98.1)	248 (89.2)	0.000
>15	6 (1.9)	30 (10.8)	
Pesaro Risk group			0.000
Class	35 (11.1)	32 (11.5)	
1	164 (52.1)	54 (19.4)	
2	116 (36.8)	192 (69.1)	

*3 patients received other conditioning regimen

**Cost of original Treosulfan
very high - Generic drug now
available in India**



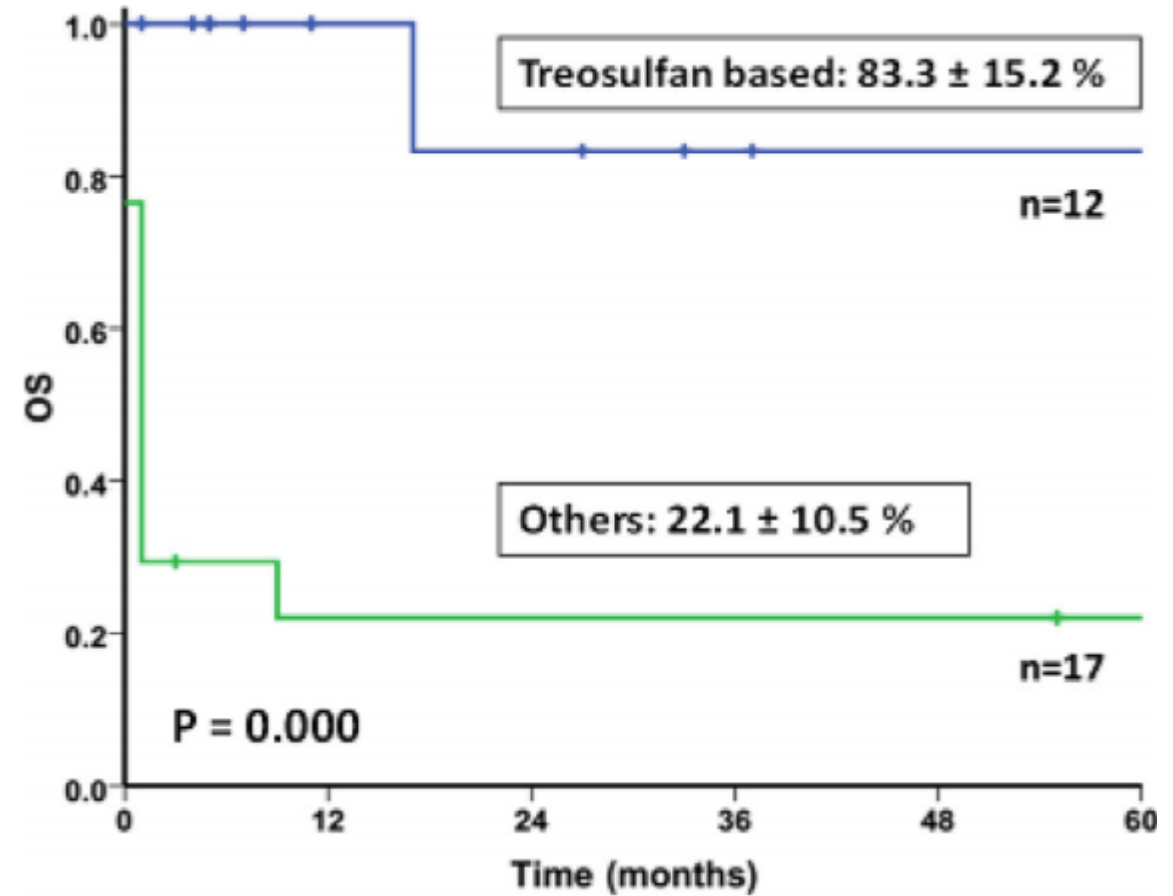
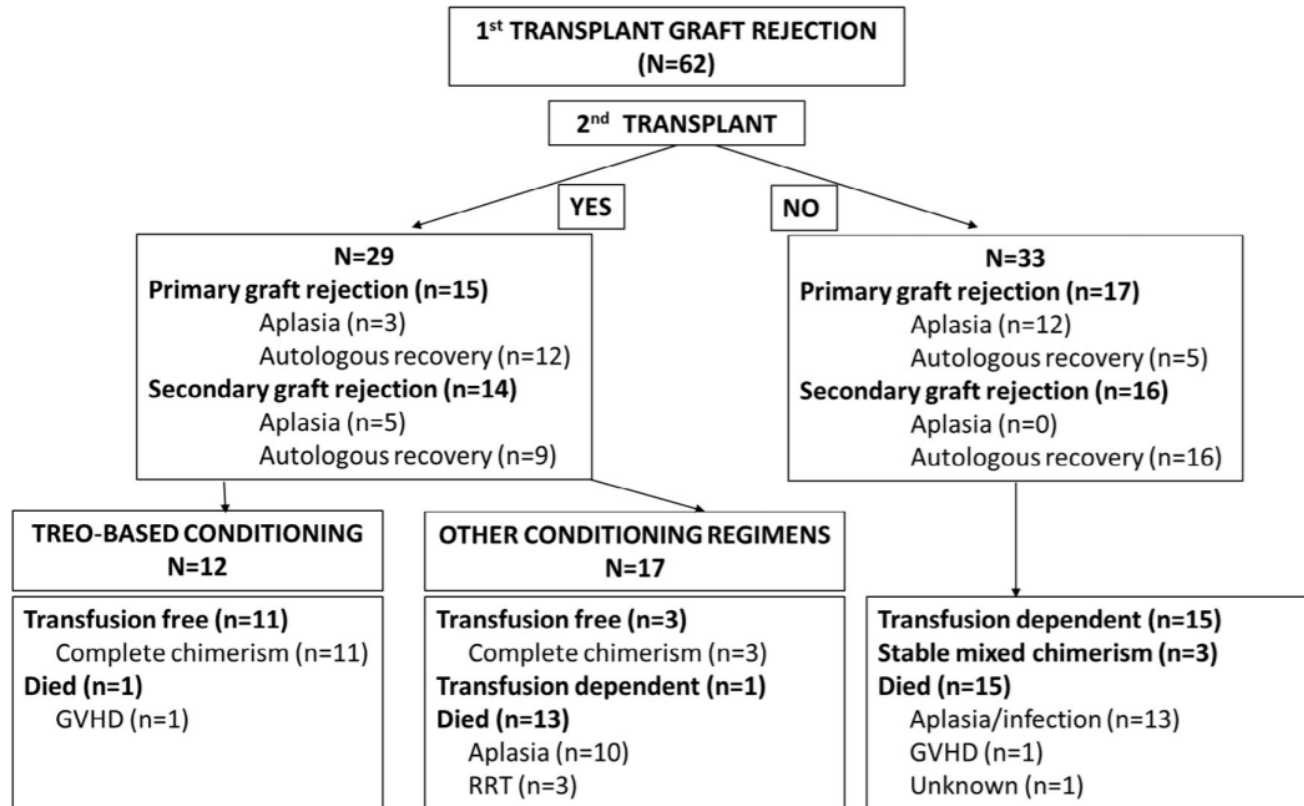
Evaluation of a generic Treosulfan in India



❖ Comparison of Treo PK parameters showed high **inter-individual variation (IIV)** in AUC and Cl. AUC values of 13/15 patients receiving TreoE were in the range of the lowest quartile of TreoM.

❖ The **IIV was >30 fold** with TreoM and **~10 fold** with TreoE for AUC.

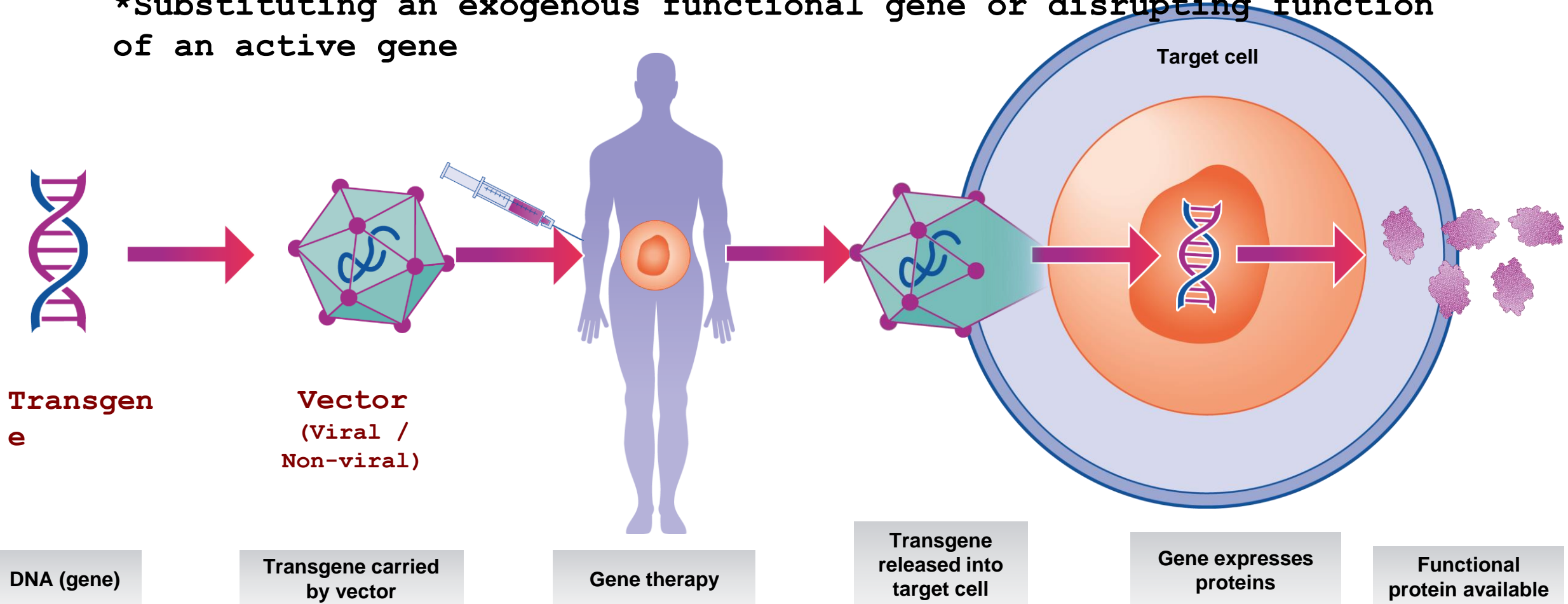
Second Hematopoietic Stem Cell Transplant for Thalassemia Major: Improved Clinical Outcomes with a Treosulfan Based Conditioning Regimen



The science behind gene therapy

*Use of nucleic acids (DNA / RNA) for treatment, cure or prevention of human disorders¹

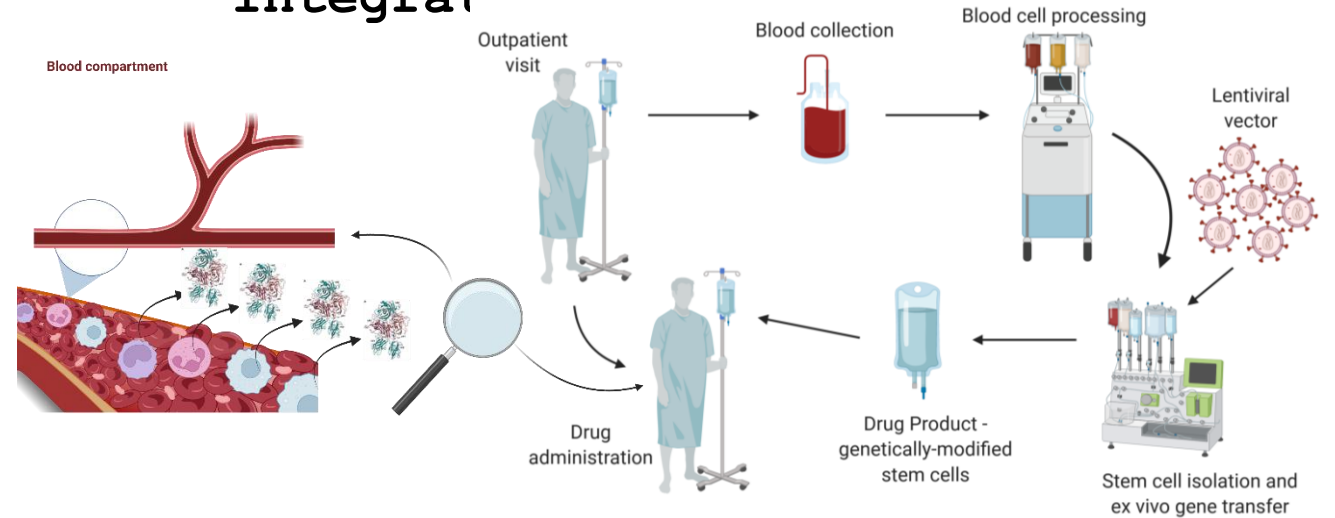
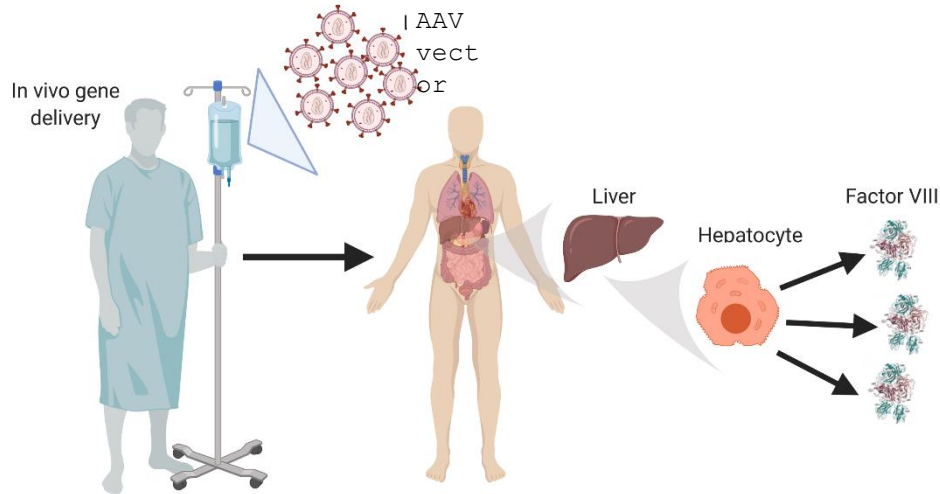
*Substituting an exogenous functional gene or disrupting function of an active gene



Modalities of Gene Therapy – *In vivo* and

In vivo tissue-targeted
AAV (or LV) gene therapy

Ex vivo hematopoietic stem cell
(HSC) or Immune cell –
Integrating Vector (D17/T17)



In-Vivo Pros

- *Bulk central manufacturing
- *Easy peripheral vein administration
- *High safety profile

In-vivo Cons

- *Variable initial expression
- *Decline of expression after 6-12 months (FVIII)
- *High ineligibility due to anti-AAV Ab
- *Only for >12

Ex-vivo Pros

- *No age barrier
- *Anti-AAV Ab not a barrier
- *Sustained expression

Ex-vivo Cons

- *Personalized product manufacturing
- *Stem cell transplant related toxicities
- *Offsite integration issues

Allogeneic transplantation

Healthy donor's HSCs

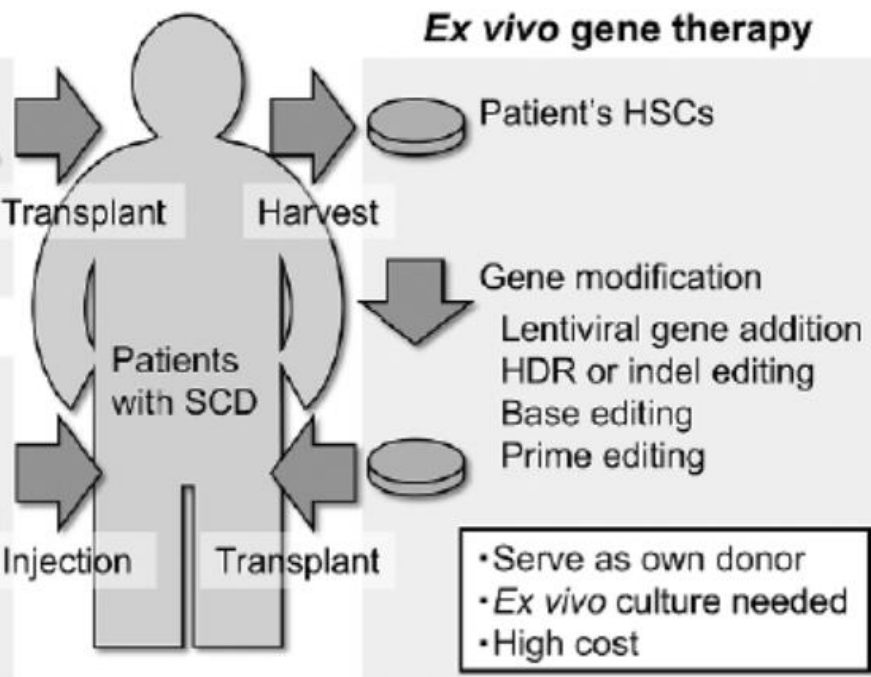
- Cure most patients
- Limited donors found
- Risk of rejection and GVHD

In vivo gene therapy

HSC-targeted delivery system

Viral vectors
Nanoparticles

- Under development
- Walk-in basis
- Low cost



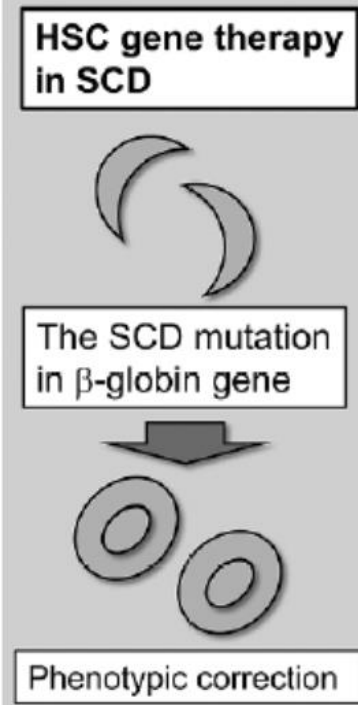
Ex vivo gene therapy

Patient's HSCs

Gene modification

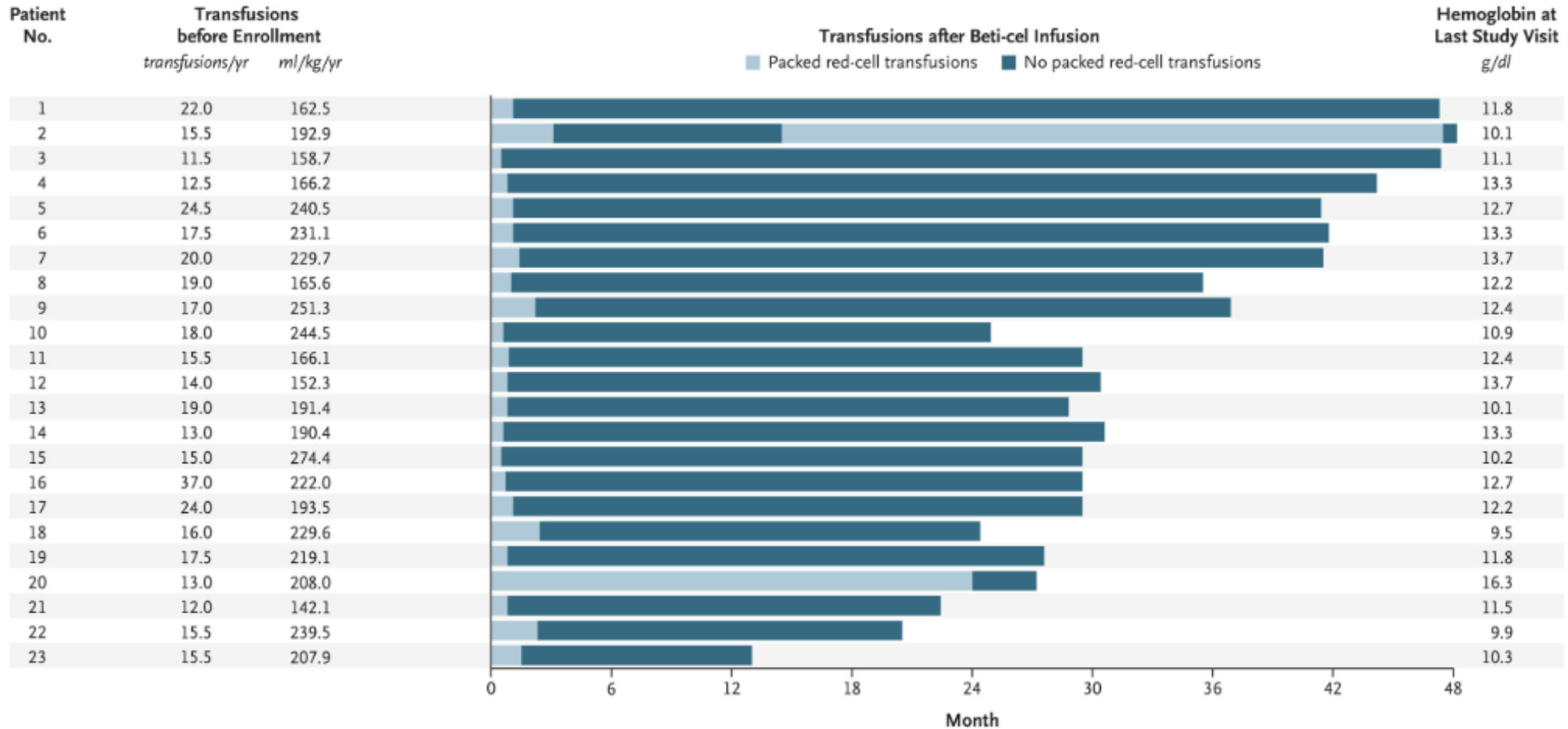
- Lentiviral gene addition
- HDR or indel editing
- Base editing
- Prime editing

- Serve as own donor
- Ex vivo culture needed
- High cost



<p>β-globin \uparrow</p>	<p>Gene addition</p> <p>Lentiviral transduction</p>	<ul style="list-style-type: none"> • β-globin gene • γ-globin gene
<p><i>BCL11A</i> \downarrow γ-globin \uparrow</p>	<p>Gene silencing</p> <p>Lentiviral transduction</p>	<ul style="list-style-type: none"> • RNA interference with <i>BCL11A</i> gene
<p>Cas9 <i>BCL11A</i> \downarrow γ-globin \uparrow</p>	<p>Gene disruption</p> <p>Indel editing Base editing</p>	<ul style="list-style-type: none"> • <i>BCL11A</i> enhancer • Repressor binding site of γ-globin promoter
<p>Cas9 GAT \rightarrow GTG</p>	<p>Gene correction</p> <p>HDR editing Base editing Prime editing</p>	<ul style="list-style-type: none"> • β-globin gene

Betibeglogene Autotemcel Gene Therapy for Non- β^0/β^0 Genotype β -Thalassemia



Engraftment:

Neutrophil: 23 days (Range: 13-32)
 Platelet: 46 days (Range: 20-94)
 Duration of severe cytopenia??

Conditioning:

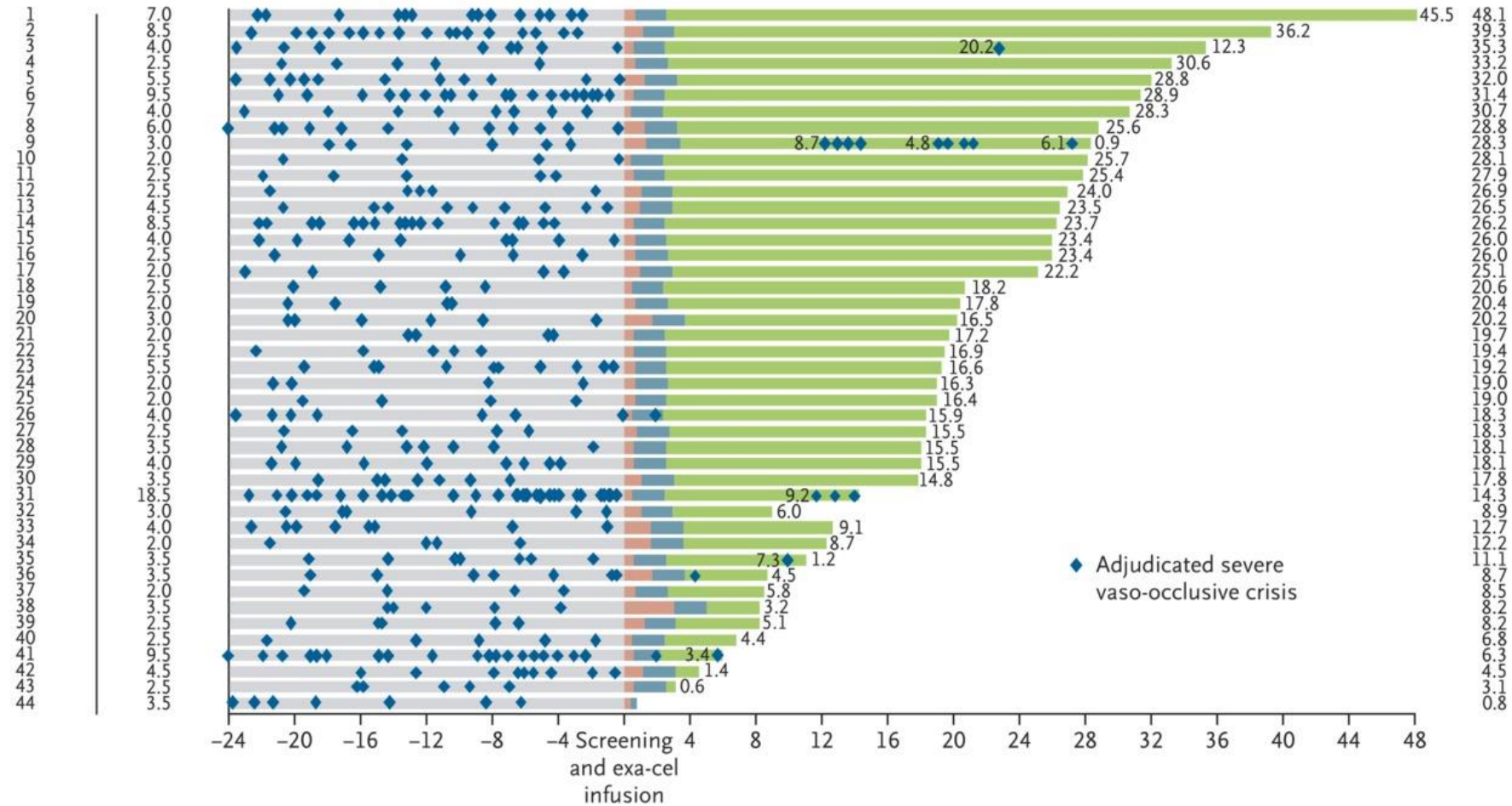
Busulfan (IV) - 3.2mg/kg/day x 4 days
 (Dose targeted with PK)



A Duration of Periods Free from Severe Vaso-Occlusive Crises after Exa-cel Infusion in All Patients

Patient Annualized Rate

	No. before Screening	24 Months before Screening	After Exa-cel Infusion	Total Follow-up
	<i>no. of crises/yr</i>			<i>mo</i>



◆ Adjudicated severe vaso-occlusive crisis

Months before and after Exa-cel Infusion

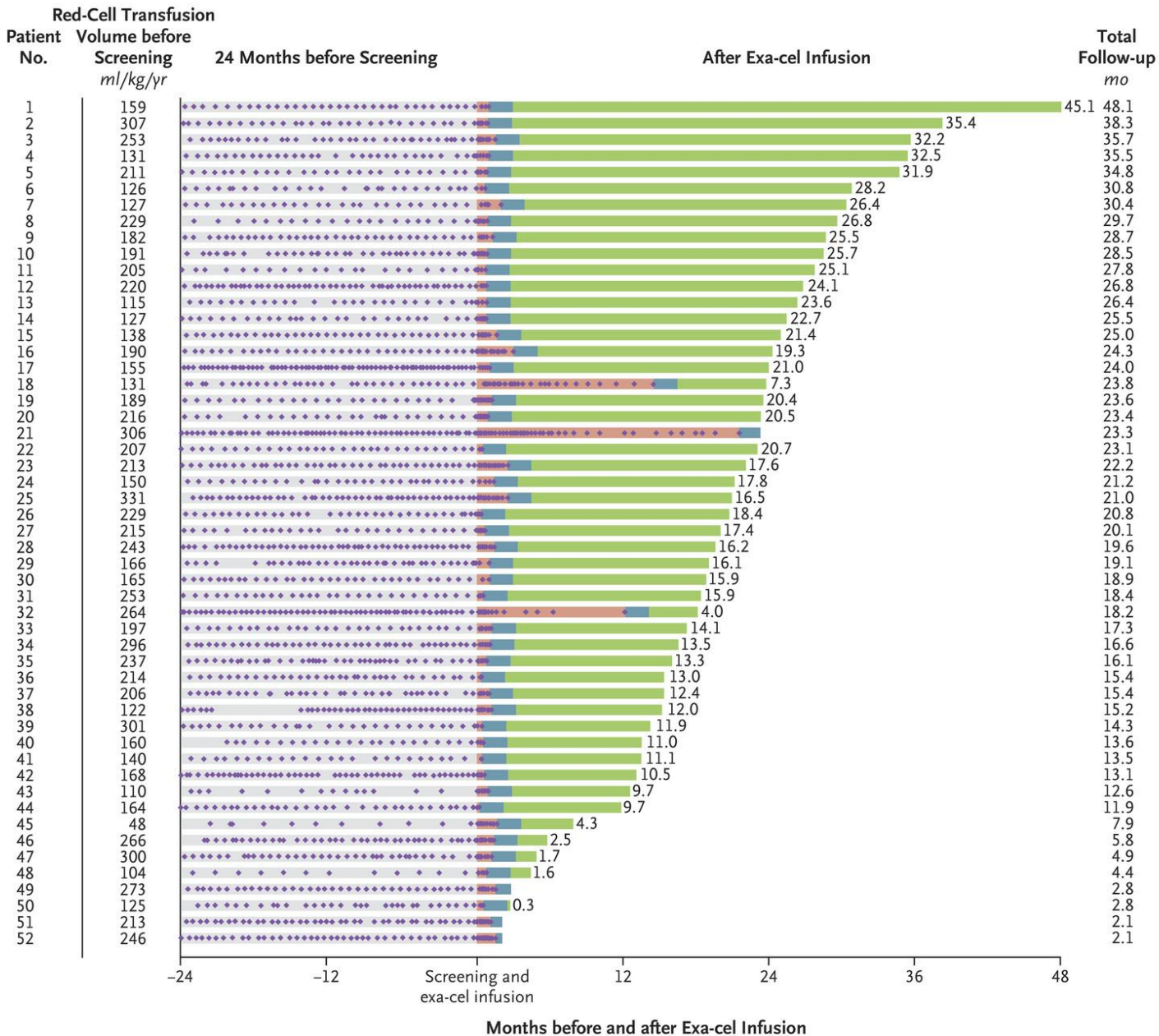
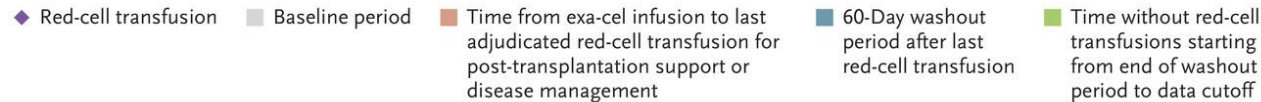


Exagamglogene Autotemcel for Transfusion-Dependent β -Thalassemia

NEJM 2024 May 9;390(18):1663-76

Table 2. Primary and Key Secondary Efficacy Results.

End Point	Primary Efficacy Population (N = 35)
Primary end point: weighted average hemoglobin level of ≥ 9 g/dl without red-cell transfusion for ≥ 12 consecutive months	
No. of patients	32
Percentage of patients (95% CI)*	91 (77–98)
P value	<0.001
Key secondary end point: weighted average hemoglobin level of ≥ 9 g/dl without red-cell transfusion for ≥ 6 consecutive months	
No. of patients	32
Percentage of patients (95% CI)*	91 (77–98)
P value	<0.001



Betibeglogene Autotemcel Gene Therapy for Non- β^0/β^0 Genotype β -Thalassemia

Table 2. Summary of Adverse Events and Serious Adverse Events after Beti-cel Infusion.*

Event	All Patients (N = 23)
	no. (%)
Grade ≥ 3 adverse events occurring in ≥ 2 patients through 2 yr of follow-up	
Thrombocytopenia	22 (96)
Neutropenia	18 (78)
Anemia	14 (61)
Stomatitis	14 (61)
Leukopenia	13 (57)
Febrile neutropenia	8 (35)
Epistaxis	5 (22)
Pyrexia	4 (17)
Decreased appetite	3 (13)
Hepatic veno-occlusive disease	3 (13)
Increased alanine aminotransferase level	2 (9)
Increased blood bilirubin level	2 (9)
Hypoxia	2 (9)
Lymphopenia	2 (9)
Neutropenic sepsis	2 (9)
Pharyngeal inflammation	2 (9)
Serious adverse events reported in ≥ 2 patients through last follow-up	
Hepatic veno-occlusive disease	3 (13)
Thrombocytopenia	2 (9)
Pyrexia	2 (9)

Busulfan based
MAC
conditioning:
Targeted Bu
levels

Grade 3-4 mucositis:
40-60%
SOS/VOD: 10-13%

Engraftment:
Neutrophil: 29 days (range, 12 to 56)

Platelet: 44 days (range, 20 to 200)

Engraftment:
Neutrophil: 23 days (range, 13 to 32)

Platelet: 46 days (range, 20 to 94)

Exagamglogene Autotemcel for Transfusion-Dependent β -Thalassemia

Table 3. Grade 3 or 4 Adverse Events after Exa-Cel Infusion.

Adverse Event	Full Analysis Population (N= 52)
	no. of patients (%)
Any grade 3 or 4 event	46 (88)
Grade 3 or 4 events occurring in $\geq 5\%$ of patients*	
Febrile neutropenia	28 (54)
Stomatitis	21 (40)
Anemia	20 (38)
Platelet count decrease	18 (35)
Thrombocytopenia	18 (35)
Mucosal inflammation	17 (33)
Neutrophil count decrease	14 (27)
Decrease in appetite	12 (23)
Epistaxis	7 (13)
Neutropenia	7 (13)
White-cell count decrease	7 (13)
Veno-occlusive liver disease	5 (10)
Blood bilirubin increase	4 (8)
Hypokalemia	4 (8)
Hypophosphatemia	4 (8)
Iron overload	4 (8)
Nausea	4 (8)
Vomiting	4 (8)
CD4 lymphocyte count decrease	3 (6)
Hematuria	3 (6)
Headache	3 (6)
Hypoxia	3 (6)

The role of the conditioning regimen for autologous and ex vivo genetically modified hematopoietic stem cell-based therapies: recommendations from the ISCT stem cell engineering committee

Landmark studies of allogeneic ex vivo genetically modified hematopoietic stem cell therapy.

Indication	Pathophysiology	Landmark studies	Recommendation
Hemoglobinopathies	Intrinsic erythropoiesis defect	<p>Hgb 206 – Lentiviral gene addition for sickle cell disease. Busulfan targeting AUC 82mg*hr/L in groups B & C (Kanter J NEJM 2021)[40]</p> <p>Hgb 207 & 212 – Lentiviral gene addition for thalassemia. Busulfan targeting AUC 70mg*hr/L (Locatelli F NEJM 2021)[45]</p> <p>CLIMB THAL-111 & CLIMB SCD-121 – CRSPR/CAS9 gene editing for thalassemia and sickle cell disease. Busulfan targeting AUC 74-90mg*hr/L (Frangoul H NEJM 2020, NEJM 2024 & Blood 200)[17,34,46]</p>	Myeloablative regimen. Therapeutic drug monitoring using pop-PK models for busulfan targeting an AUC of 80-100mg*hr/L
Lysosomal storage and Metabolic Disorders	Disorders of enzymatic pathways leading to accumulation of toxic substrates	<p>ALD102 – Lentiviral gene addition for X-linked CALD. Busulfan targeting AUC 70–85 mg*h/L & Cyclophosphamide 200 mg/kg (Eichler F NEJM 2017)[62]</p> <p>MLD – Lentiviral gene addition. Busulfan targeting AUC 67 – 85 mg*h/L (Fumagalli F Lancet 2022)[63]</p> <p>MPS-1 – Lentiviral gene addition. Busulfan targeting AUC 85mg*h/L. Fludarabine 40mg/m² x 4 and Rituximab 375mg/m² x1 (Genter B NEJM 2021)[61]</p>	Myeloablative regimen. Therapeutic drug monitoring using pop-PK models for busulfan targeting an AUC of 80–100 mg*h/L. Disease-specific immune ablation.
Inherited Bone Marrow Failure	Intrinsic defects of one or more lineages of hematopoiesis	RP-L102 – Lentiviral gene addition. No conditioning regimen due to competitive advantage of transduced HSCs (Rio P Nat Med 2019)[28]	Need to balance risk of underlying disease with need to engraft. Conditioning will be disease specific.
Inborn Errors of Immunity	Genetic lesion that affects one or multiple arms of the immune system	<p>SCID – Lentiviral gene addition. ARTEMIS SCID Busulfan targeting 20 mg*h/L (Cowan M NEJM 2022). ADA SCID single dose Busulfan 4mg/kg (Kohn DB NEJM 2021). X-SCID Busulfan targeting 22 mg*h/L (Mamcarz E NEJM 2019) [67]</p> <p>X-CGD – Lentiviral gene addition. Busulfan targeting 70–75 mg*h/L (Kohn DB Nat Med 2020)[68]</p> <p>WAS – Lentiviral gene addition. Busulfan 4 mg/kg/day with Fludarabine 40 mg/m²/day. Anti-CD20 and/or Anti-CD52 for significant autoimmunity (Magnani A Nat Med 2022) [70].</p>	Need to balance risk of underlying disease with need to engraft. For SCID, genotype dependent. For other non-radiosensitive PIDs likely higher Busulfan exposure needed.



ORIGINAL ARTICLE

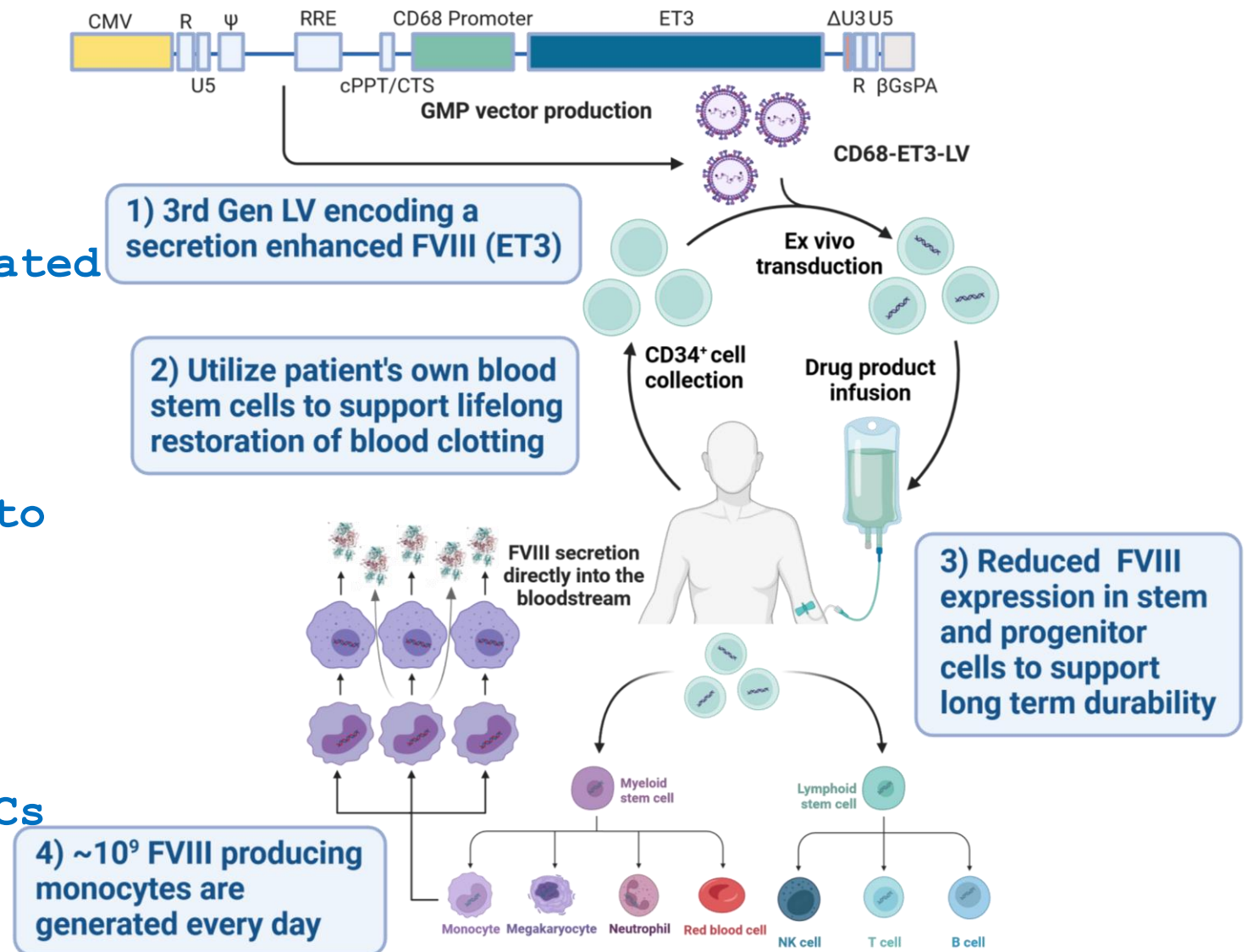
Lentiviral Gene Therapy with CD34+ Hematopoietic Cells for Hemophilia A

A. Srivastava, A. Abraham, F. Aboobacker, G. Singh, T. Geevar, U. Kulkarni, S. Selvarajan, A. Korula, R.G. Dave, M. Shankar, A.S. Singh, A. Jeba, N. Kumar, C. Benjamin, K.M. Lakshmi, V.M. Srivastava, R.V. Shaji, S.C. Nair, H.C. Brown, G. Denning, P. Lollar, C.B. Doering, and T. Spencer

Department of Hematology, Christian Medical College Vellore, Ranipet Campus, Vellore, India (A.S., A.A., F.A., U.K., S.S., A.K., A.S.S., A.J., N.K., C.B., K.M.L., R.V.S.); Center for Stem Cell Research Unit of inStem, Bengaluru, Christian Medical College Vellore, Vellore, India (A.S., G.S., M.S., R.V.S.); the Department of Immunohematology and Transfusion Medicine, Christian Medical College Vellore, Town Campus, Vellore, India (T.G., R.G.D., S.C.N.); the Department of Cytogenetics, Christian Medical College Vellore, Vellore, India (V.M.S.); Expression Therapeutics, Tucker, GA (H.C.B., G.D., P.L., C.B.D., T.S.); and Emory University School of Medicine, Children's Healthcare of Atlanta, Atlanta (P.L., C.B.D., T.S.).

CD68-ET3-LV CD34⁺: Mechanism of action

- 3rd generation SIN-LV
- Bioengineered ET3 transgene:
 - Enhanced secretion and reduced cell stress related toxicity
- Human CD68 internal promoter:
 - Directs ET3 expression to CD68 expressing cells (monocytic phagocytes, osteoclasts, and macrophages)
 - No ET3 expression in HSCs or HSPCs
- Designed for lifelong durability



Lentiviral gene therapy with CD34+ Hematopoietic Cells for Hemophilia A

A Phase 1 Clinical Trial -

Stem cell mobilisation for HSCT

- G-CSF 5µg/kg/day x bd x 5 days + Plerixafor 0.24mg/kg

Stem Cell Dose - $>2 \times 10^6$ CD34+/kg after transduction

→ An aliquot of unmodified CD34+ HSCs will be cryopreserved for rescue, if needed

HSCT - Conditioning Protocol

- Treosulfan - 14 g/m² x 3 days (Days -5 to -3)
- Fludarabine - 40mg/m² x 4 days (Days -5 to -2)
- Autologous transduced HSC infusion (Day 0)

Lentiviral gene therapy with CD34+ Hematopoietic Cells for Hemophilia A

Baseline Characteristics of Treated Participants and the Final Gene-Therapy Drug Product

Characteristic	Participant 1	Participant 3	Participant 6	Participant 7	Participant 8
Age — yr	33	31	34	22	41
Genotype	Duplication of exon 7 to 13	Exon 13, c.1967G→A, p.Trp656Stop	Intron 22 inversion	Exon 13, c.1911T→G, p.Asn637Lys	Exon 11, c.1733T→A, p.Val578Glu
Annualized bleeding rate†	30	20	36	120	52
HJHS total score‡	46	33	26	36	46
Factor VIII replacement (no. of exposures)	Episodic (>100)	Episodic (>100)	Episodic (>100)	Episodic (>100)	Episodic (>100)
Enriched CD34 cell dose — cells/kg of body weight	7.5×10 ⁶	7.0×10 ⁶	8.7×10 ⁶	9.2×10 ⁶	7.2×10 ⁶
Drug-product dose — cells/kg of body weight	5.6×10 ⁶	5.3×10 ⁶	5.9×10 ⁶	6.1×10 ⁶	5.0×10 ⁶
VCN in drug product — copies/cell	1.0	0.6	1.5	0.6	2.2
Duration of follow-up — mo	27	19	14	12	9

Group 1: P1 & 3; Group 2: P6, 7, 8

Hemophilia A

HSC Transplantation: Engraftment, Toxicities & FVIII

expression
*HSCT - Conditioning Protocol - Treosulfan

/ Fludarabine
*Engraftment:

-Neutrophil (median): 11 days (range: 10-12)

-Platelet (median): 15 days (range: 12-15)

*Duration of Cytopenia:

-Severe Neutropenia (median): 8 (range: 7-11)

-Severe Thrombocytopenia (median): 3 days (range:
1-7)

*Last day of CFC infusion (median): 16 days
(range: 11-20)

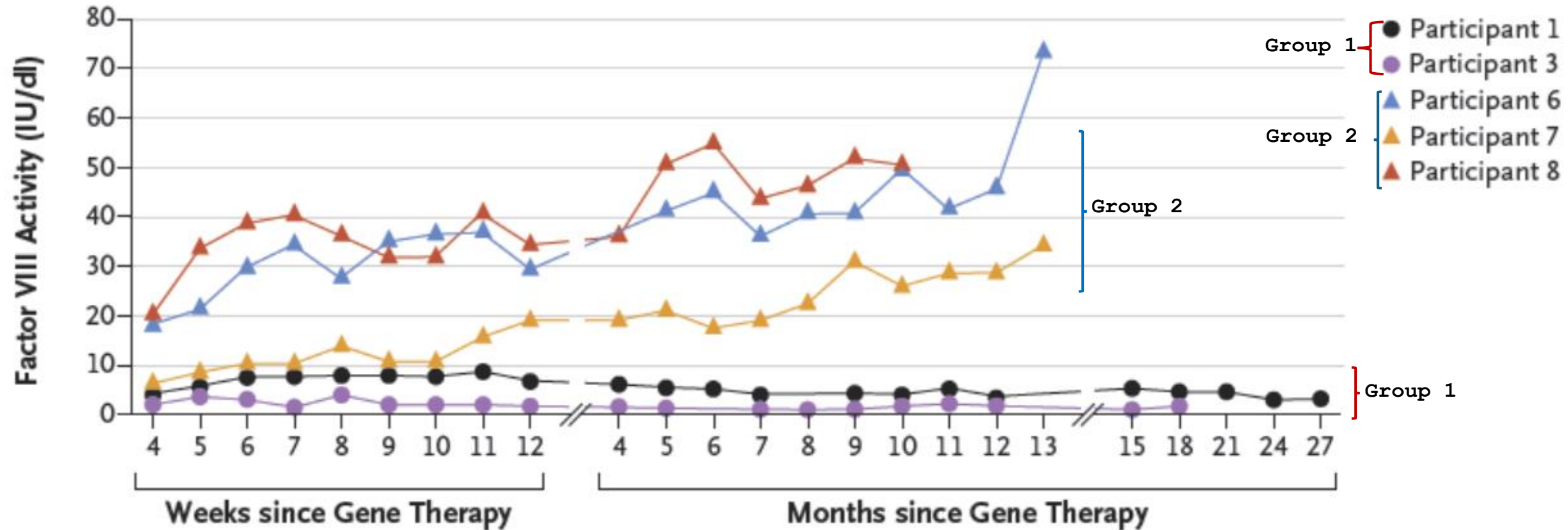
*Follow-up (median): 20 months (range: 15-33)

*Conditioning related mucositis: <Grade 2

-All participants maintained oral intake
→ Semen examination (first 4 participants at >6
through-out HSCT months post-GT) *Sperm count - Normal range: 4
*Clinically relevant hepatic, renal or other
/ 4 (1, with reduced motility)

Lentiviral gene therapy with CD34+ Hematopoietic Cells for Hemophilia A

Serial Factor VIII Activity (One-Stage Assay) after Gene Therapy



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All participants continue to maintain consistent expression of FVIII with no safety related signals

FERTILITY in HAEMATOLOGICAL MALIGNANCIES

TABLE 2 Risks of treatment-related gonadotoxicity in female patients with hematologic malignancies.

	ESMO [5]	ESHRE [6]	FertiPROTEKT [7]
High risk (>80% risk of amenorrhea)	Hematopoietic stem cell transplant (especially alkylating agent-based MAC with CY, BU, melphalan, or TBI) RT >6 Gy to a field including the ovaries 6 to 8 cycles of escalated BEACOPP of ≥30 years	Conditioning regimens for Hematopoietic stem cell transplant with CY and/or TBI RT, including the ovaries	Hematopoietic stem cell transplant with CY-TBI or BU-CY RT, including the ovaries Escalated BEACOPP ≥30 years Procarbazine, Chlorambucil
Intermediate risk	6 to 8 cycles of escalated BEACOPP <30 years 6 cycles of CHOP of ≥35 years 6 cycles of DA-EPOCH of <35 years	Alkylating agent-based regimens (such as MOPP, BEACOPP, CHOP, and CHOPE) in patients with lymphoma	Escalated BEACOPP <30 years
Low risk (<20% risk of amenorrhoea)	2 cycles of escalated BEACOPP ABVD 6 cycles of CHOP of <35 years 6 cycles of DA-EPOCH <35 years AML therapy (anthracycline/cytarabine) ALL therapy (multi-agent)	Non-alkylating agent-based regimens (such as ABVD or EBVP) in lymphoma patients aged ≥32 years	ABVD ≥32 years 4 to 6 cycles of CHOP CVP AML therapy (anthracycline/cytarabine) ALL therapy (multi-agent)
Very low or no risk	N/A (This risk is not set)	Non-alkylating agent-based regimens (such as ABVD or EBVP) in lymphoma patients aged <32 years Single-agent methotrexate	ABVD <32 years Methotrexate, Fluorouracil, and Vincristine

Conventional Conditioning - Allo-HSCT

Gonadal Recovery – Conventional Conditioning

	CY-TBI	BU-CY
Purpose	Eradicate tumor cells using radiation plus chemotherapy Achieve potent immunosuppression	Eradicate tumor cells using chemotherapy Achieve potent immunosuppression
Advantages	Possibly more effective in lymphoid malignancies Strong immunosuppression (beneficial in mismatched transplants) Effective against chemo-resistant tumors Reaches sanctuary sites (such as central nervous system)	Avoids radiation-induced toxicities (growth retardation) No need for radiation equipment
Disadvantages	Gonadal toxicity Risk of growth retardation in children Requires radiation facilities	Gonadal toxicity Possibly less effective in lymphoid malignancies Busulfan-specific toxicities (such as hepatic toxicity)

Type of SCT	Conditioning	Sex	n	Gonadal recovery
Allogeneic	CY	Female	43	74%
Allogeneic	CY	Female	103	54%
Allogeneic	CY	Male	109	61%
Allogeneic	BU-CY	Female	73	1%
Allogeneic	BU-CY	Male	146	17%
Allogeneic	TBI	Female	74	13.5% (100% < 18; 15% > 18)
Allogeneic	TBI	Female	532	10%
Allogeneic	TBI	Male	463	17.5%
Autologous	BEAM	Female	10	60%

Busulfan & TBI based conditioning associated with high incidence of gonadal toxicity with recovery of function in 10-18% of patients

Gonadal Function after Busulfan Compared with Treosulfan in Children and Adolescents Undergoing Allogeneic Hematopoietic Stem Cell Transplant

Biol Blood Marrow Transplant 25

(2019) 17(6):1701-1707

Characteristics of the 137 Eligible Patients

	Population (N = 137)	Busulfan (n = 118)	Treosulfan (n = 19)	P
Puberty at HSCT				
No	89 (64.96)	77 (65.25)	12 (63.16)	.1957
Yes	48 (35.04)	41 (34.75)	7 (36.84)	
Median age at HSCT, yr (range) [IQR]	11.04 (5.02-18.55) [7.61-15.01]	10.7 (5-18.6) [7.6-15.1]	12.2 (6.1-18.4) [8.4-14.3]	.6161
Median age at diagnosis, yr (range) [IQR]	8.11 (0-18.04) [4.05-13.14]	7.7 (0-17.5) [4-13.1]	10.8 (1.3-18) [5.4-13.1]	.3486
Median interval between diagnosis and HSCT, mo (range) [IQR]	10.85 (.3-207.15) [5.57-54.62]	11.8 (.3-207.1) [5.5-56.8]	8.9 (.3-129.3) [5.7-42.7]	.6074
<12 mo	71 (51.82)	59 (50)	12 (63.16)	.2867
≥12 mo	66 (48.18)	59 (50)	7 (36.84)	
Median years from HSCT to last follow-up (range) [IQR]	7.79 (2.07-22.69) [6.2-10.51]	7.8 (2.1-22.7) [6.3-11.2]	7 (3-9.1) [5.9-8.2]	.1286
Median age at last follow-up, yr (range) [IQR]	19.39 (8.23-33.86) [16.1-23.53]	19.2 (8.2-33.9) [16.2-23.6]	19.8 (12-26.1) [14.9-21.7]	.4057

Spontaneous puberty achievement in pre-pubertal children

No. of Patients (%)	Bu n (%)	Treo n (%)	P
85 prepubertal patients*	74	11	
64 Spontaneous puberty (75.3%)	53 (71.6%)	11 (100%)	.06
58 prepubertal boys	53	5	
50 Spontaneous puberty (86.2%)	45 (84.9%)	5 (100%)	1
27 pre pubertal girls	21	6	
14 Spontaneous puberty (51.8%)	8 (38.0%)	6 (100%)	.02

Bu= Busulfan; Treo=Treosulfan.

* In 4 of 89 prepubertal children, data were missing.

SM in Prepubertal and Postpubertal Girls [SM = Spontaneous

menarche]

No. of Girls	Bu *	Treo	P
25 prepubertal	20	5	
7 SM (28%)	2 (10)	5 (100)	.001
21 postpubertal	17	4	
14 SM (66.6%)	11 (64.7)	3 (75)	.70

Values are n or n (%).

* In 2 of 27 prepubertal girls, data were missing.

*Treo associated with a favourable post-HSCT hormonal profile

Gonadal Toxicity of Treosulfan vs Busulfan containing regimen

Publish (year)	Study type	n	Primary disease	Chemotherapy dose	Result (BU vs. Treo)	Reference number
2024	Multicenter, retrospective	521	Malignant	BU: 12.8-16 mg/kg Treo: 30-42 g/m ²	All groups: Gonadal toxicity was 38% vs. 10% ($p = 0.02$), RR was 0.51 (95% CI 0.34-0.76, $p < 0.001$) Postpubertal group ($n = 197$): Gonadal toxicity was 37% vs. 17% ($p = 0.03$)	8
2023	Single center, retrospective	88	Nonmalignant	BU: High ^a 66% vs. Low ^b 34% Treo: High ^a 26% vs. Low ^b 74%	Gonadal dysfunction: 63% vs. 28% Gonadal dysfunction (permanent): 55% vs. 13%	9
2022	Single center, retrospective	156 (children only)	Nonmalignant	Both regimens used a myeloablative conditioning regimen	Ovarian dysfunction: 94% vs. 33% Testicular dysfunction: 46% vs. 14%	10
2020	Retrospective	66	Malignant, nonmalignant	BU: 14 mg/kg ($n = 3$), 16 mg/kg ($n = 16$), 20 mg/kg ($n = 6$) Treo: 36 mg/kg ($n = 16$), 42 mg/kg ($n = 24$), and unknown ($n = 1$)	Female mean AMH: 0.11 μ g/L vs. 1.59 μ g/L ($p < 0.001$) Male Inhibin B SDS: -1.23 ± 1.41 vs. -0.506 ± 2.112 ($p < 0.05$)	11
2019	Multicenter, retrospective	137	Malignant, nonmalignant	BU: ≥ 8 mg/kg Treo: 36-42 g/m ²	Spontaneous puberty achievement in prepubertal children Female: 38% vs. 100% Male: 85% vs. 100% Spontaneous menarche in Prepubertal and Postpubertal Girls Prepubertal: 10% vs. 100% Postpubertal: 65% vs. 75% Hormone levels Female: median of FSH is 78 vs. 7 ($p = 0.003$) Male: median of LH is 5.6 vs. 5.5 ($p = 0.03$)	12

Much higher gonadal toxicity if Busulfan at >8 mg/kg compared to Treosulfan even at 36-42 g/m²

Conditioning Regimen in Haematopoietic Stem Cell Transplantation

- Choice depends on**
- 1. Disease & its status
 - 2. Age & comorbidities
 - 3. Type of donor
 - 4. Source of graft
 - 5. Prior therapy received
 - 6. Planned GVHD prophylaxis
 - 7. Options available
- MAC for younger / fit patients
 - -AML (<55 yrs) / ALL (<45 yrs)
 - TBI based conditioning - Mostly for ALL
 - Treosulfan good substitute for Busulfan in all MACs
 - FM140 - Close to any MAC
 - RIC for Lymphomas - FM100/2 Gy TBI or Flu/Cy/2 Gy TBI
 - RIC also for elderly (>55yrs) /

First Busulfan conditioning related death in thalassemia gene therapy

(Reported at ASH 2025)

After nearly 65 years of HSCT practice, science of conditioning therapy remains

imprecise