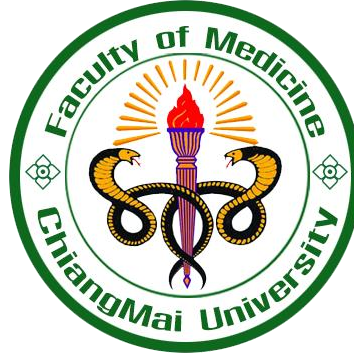
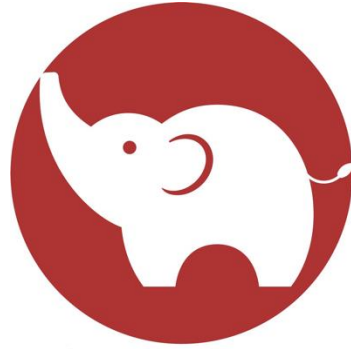




How to Define Graft Failure and Treatment

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Outlines

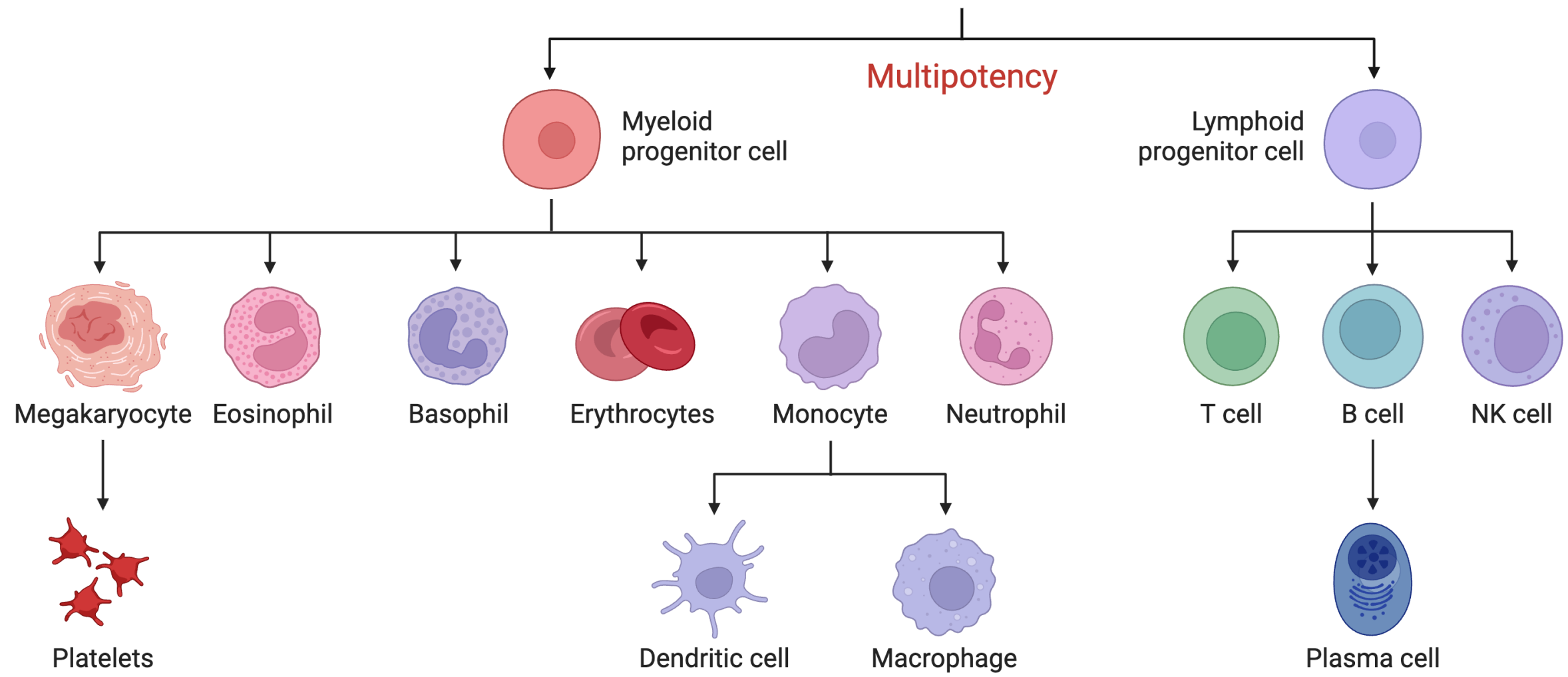
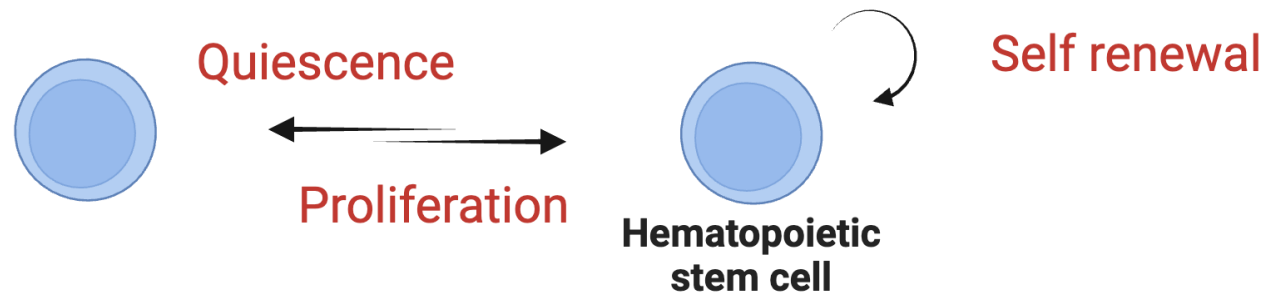
Case-based approach:

- Primary graft failure
- Secondary graft failure/graft rejection
- Poor graft function
- Mixed chimerism in non-malignant diseases
- Mixed chimerism in malignant diseases

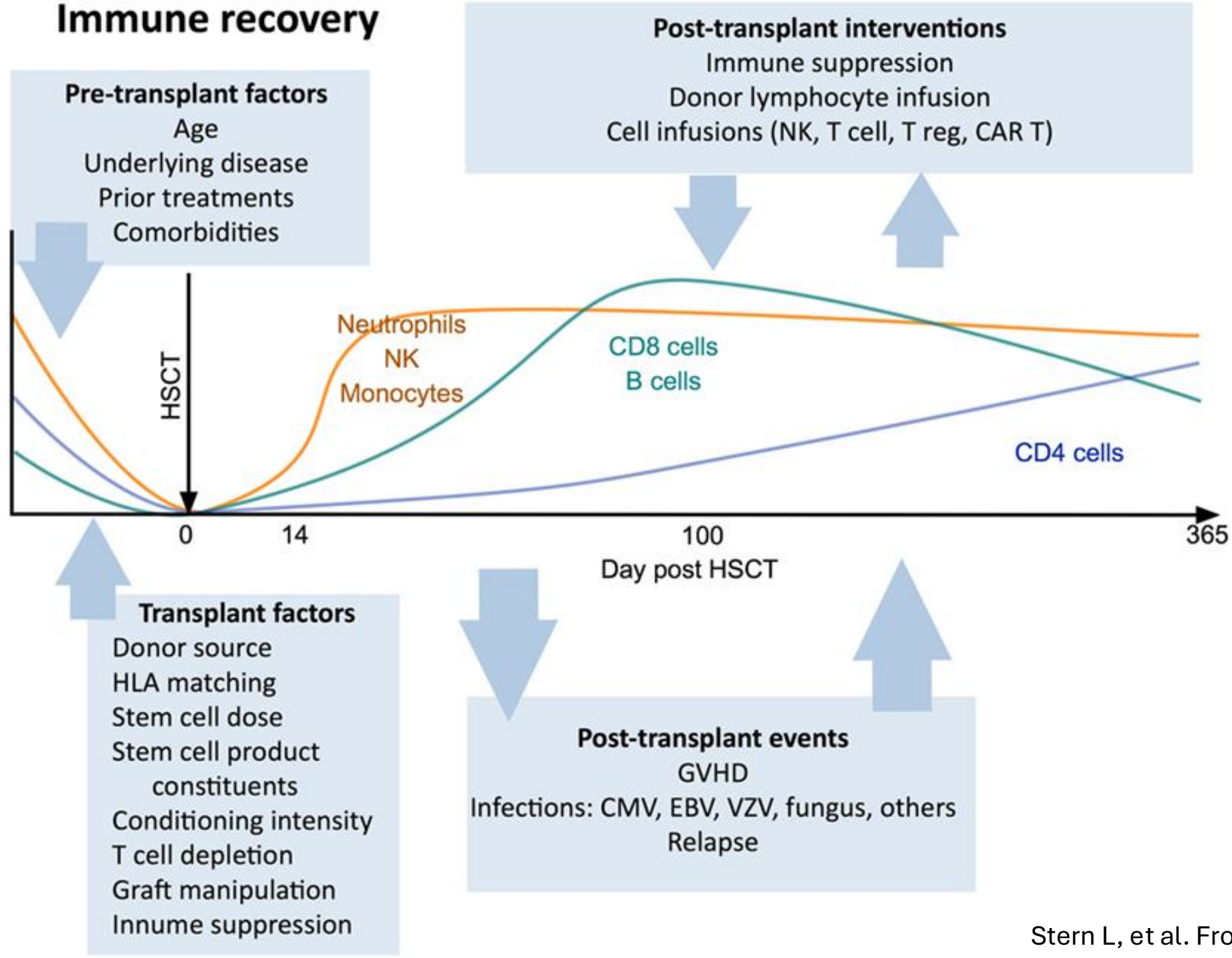
Harmonizing definitions for hematopoietic recovery, graft rejection, graft failure, poor graft function, and donor chimerism in allogeneic hematopoietic cell transplantation: a report on behalf of the EBMT, ASTCT, CIBMTR, and APBMT



Anna Sureda ¹✉, Paul A. Carpenter², Andrea Bacigalupo³, Vijaya Raj Bhatt ⁴, Josu de la Fuente ^{5,6}, Aloysius Ho⁷, Leslie Kean ^{8,9}, Jong Wook Lee ¹⁰, Isabel Sánchez-Ortega ¹¹, Bipin N. Savani ¹², Johannes Schetelig ¹³, Edward A. Stadtmauer¹⁴, Yoshiyuki Takahashi¹⁵, Yoshiko Atsuta ^{16,17}, John Koreth¹⁸, Nicolaus Kröger ¹⁹, Per Ljungman ²⁰, Shinichiro Okamoto²¹, Uday Popat ²², Robert Soiffer ¹⁸, Heather E. Stefanski²³ and Mohamed A. Kharfan-Dabaja ²⁴



Immune recovery



Definitions

- $\text{ANC} \geq 500 \text{ cells}/\mu\text{L}$ = hematopoietic engraftment?
 - NOT TRUE in the case of primary graft failure with autologous neutrophil recovery
- **Neutrophil recovery:** The first of 3 successive days with an ANC of $\geq 500/\mu\text{L}$ after post-transplantation nadir.
- **Platelet recovery:** The first of 3 consecutive days with a platelet count of $20,000/\mu\text{L}$ or higher in the absence of platelet transfusion for 7 consecutive days.

Case 1: 6 y.o. girl with Dyskeratosis congenita

- Progressive cytopenias with confirmed *TINF2* mutation
- Underwent 6/8 HLA-matched UCB HSCT, TNC dose: 5×10^7 cells/kg
- CMV (D/R): neg/neg, blood group (D/R): B Rh+/B Rh+
- Conditioning regimen: Alemtuzumab, fludarabine
- GVHD prophylaxis: Tacrolimus, MMF
- CBC on day +30:
Hb 7 g/dL, WBC 0.5×10^9 /L, ANC 0.1×10^9 /L, PLT 10×10^9 /L
- **Primary graft failure?**

Primary graft failure

Cell sources	Definitions
PBSCs	Lack of achievement of an ANC $\geq 500/\mu\text{L}$ by <u>day +30</u> with associated pancytopenia.
Unstimulated BM	Lack of achievement of an ANC $\geq 500/\mu\text{L}$ by <u>day +30</u> with associated pancytopenia.
UCB	Lack of achievement of an ANC $\geq 500/\mu\text{L}$ by <u>day +42</u> with associated pancytopenia.

Case 1: 6 y.o. girl with Dyskeratosis congenita

- History of febrile neutropenia; blood culture negative
- Became afebrile after starting piperacillin-tazobactam
- Virology screening (CMV, EBV, ADV, HHV6): All negative
- Persistent pancytopenia with $ANC < 0.5 \times 10^9/L$ beyond day +42
- Chimerism <5% donor cells
- **Diagnosed with primary graft failure**

Risk factors for graft failure

Pretransplant		Peri- and post-transplant
(difficult to modify)	(easy to modify)	
HLA mismatches	Graft source (UCB>BM>PBSC)	CD34+ cell count
Non-malignant disease	Conditioning (RIC>MAC)	Viral infections
Advanced disease	T-cell depletion	GVHD
Extensive marrow fibrosis		Drug toxicity
Donor age		
Splenomegaly		
Iron overload		
HLA antibodies		
Transfusion history		

Minimum cell content recommended

Progenitors	Type of transplant	Amount of cells
BM progenitors	Autologous	TNC: $2 \times 10^8/\text{kg}$
	Allogeneic	TNC: $3 \times 10^8/\text{kg}$
PB progenitors	Autologous	Minimum: $\text{CD34} > 1 \times 10^6/\text{kg}$
		Optimum: $\text{CD34} > 2 \times 10^6/\text{kg}$
	Allogeneic MAC	Minimum: $\text{CD34} > 2 \times 10^6/\text{kg}$
		Optimum: $\text{CD34} > 4 \times 10^6/\text{kg}$
Cord blood	Allogeneic RIC	Minimum: $\text{CD34} > 2 \times 10^6/\text{kg}$
		Optimum: $\text{CD34} 4\text{--}8 \times 10^6/\text{kg}$
	HLA 4–6/6	TNC $> 2.5\text{--}3 \times 10^7/\text{kg}$
		CD 34 $> 1 \times 10^5/\text{kg}$

Case 1: 6 y.o. girl with Dyskeratosis congenita

- Underwent second HSCT (3 months after first HSCT)
- 9/10 matched unrelated donor
- Cell source: PBSC, CD34+ dose 8×10^6 cells/kg
- Conditioning regimen: Alemtuzumab, fludarabine, cyclophosphamide
- GVHD prophylaxis: Tacrolimus, MMF, abatacept
- Clinical course: Full donor chimerism at day +30

Second HSCT for graft failure

Factor	Recommendation
Type of donor	Similar results using the same/different donor Always avoid donors with positive DSA
Conditioning regimen	Required (Better RIC)
Posttransplant IS	Required (CNI-based)
Stem cell source	PB or BM > CB
T-cell depletion	Avoid ex vivo T-cell depletion, especially if with immune graft rejection Good option in cases of poor graft function ATG or alemtuzumab have been used to foster IS and reduce the GVHD risk.

Case 2: 2 y.o. girl with R/R sJIA and MAS

- Underlying disease: Down syndrome
- Diagnosed with systemic JIA and macrophage activation syndrome
- Previous treatments: pulse methylprednisolone, anakinra, ruxolitinib
- Experienced R/R sJIA with MAS and interstitial lung disease
- Disease partially controlled with combination therapy:
Prednisolone, anakinra, emapalumab

Case 2: 2 y.o. girl with R/R sJIA and MAS

- Underwent 10/10 MSD HSCT
- Cell source: PBSC
- CD34+ dose 10×10^6 cells/kg
- Blood group (D/R): B Rh+/B Rh+
- Conditioning regimen:
Treosulfan, fludarabine, ATG
- GVHD prophylaxis:
Tacrolimus, prednisolone
- Neutrophil recovery on day +12
- Day +27: developed high-grade fever with progressive pancytopenia
Hb 7.7 g/dL, WBC 1.0×10^9 /L, ANC 0.4×10^9 /L, PLT 20×10^9 /L
- **Secondary graft failure?**

Secondary graft failure

- A decline in hematopoietic function (may involve hemoglobin and/or platelets and/or neutrophils) necessitating blood products or growth factor support, after having met the standard definition of hematopoietic (neutrophils and platelets) recovery.

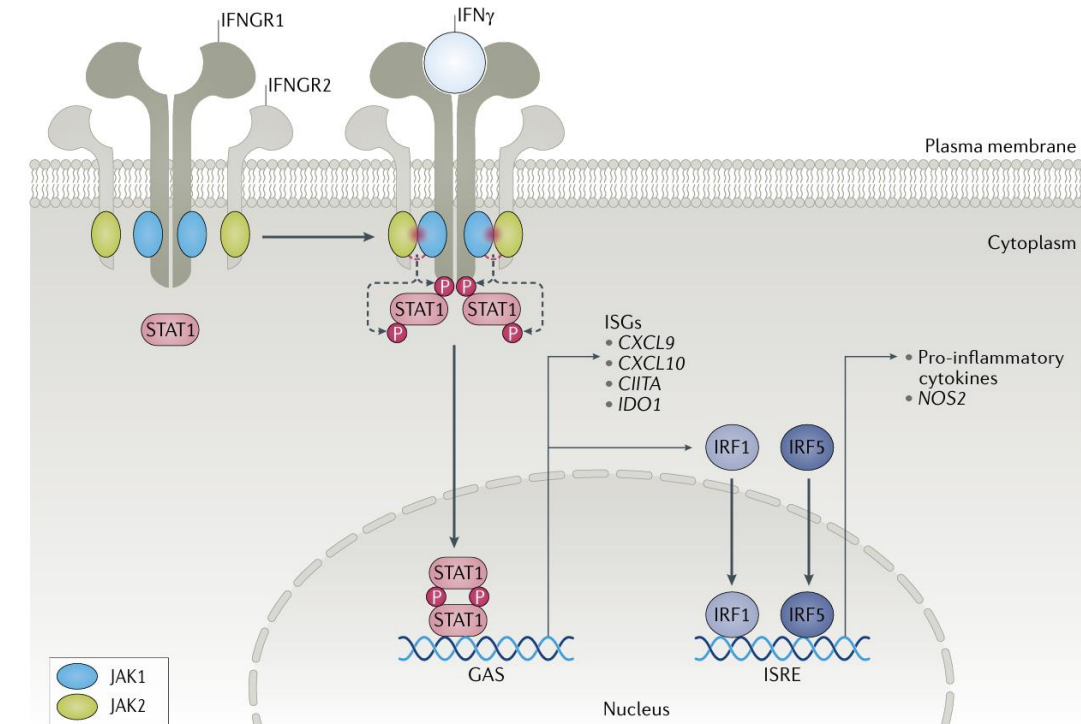
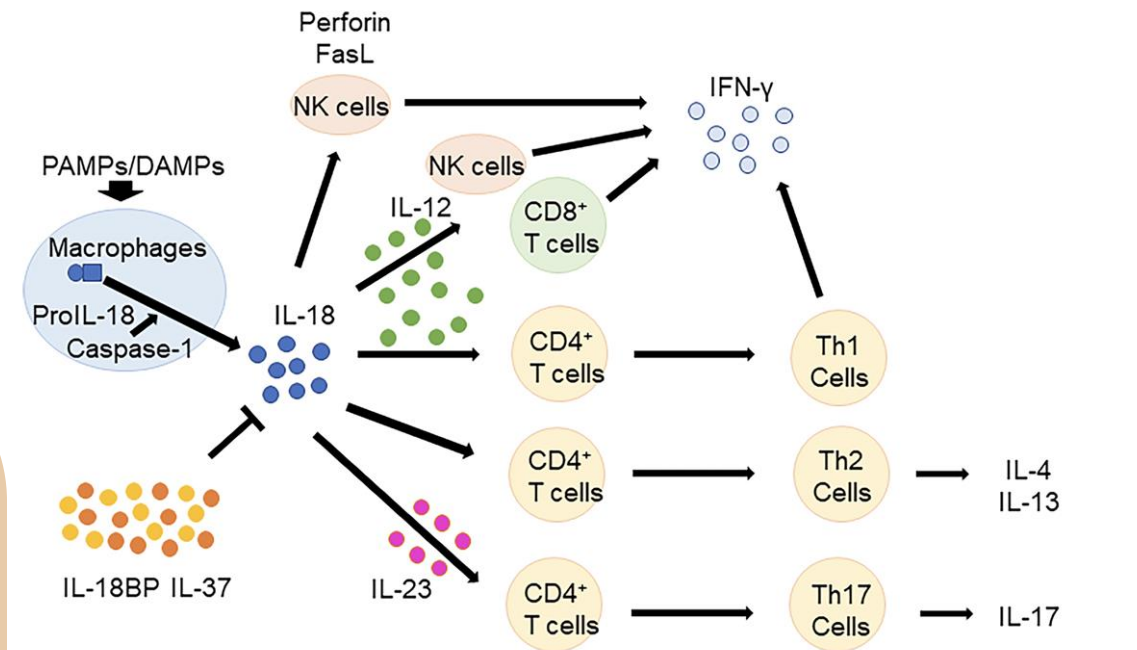
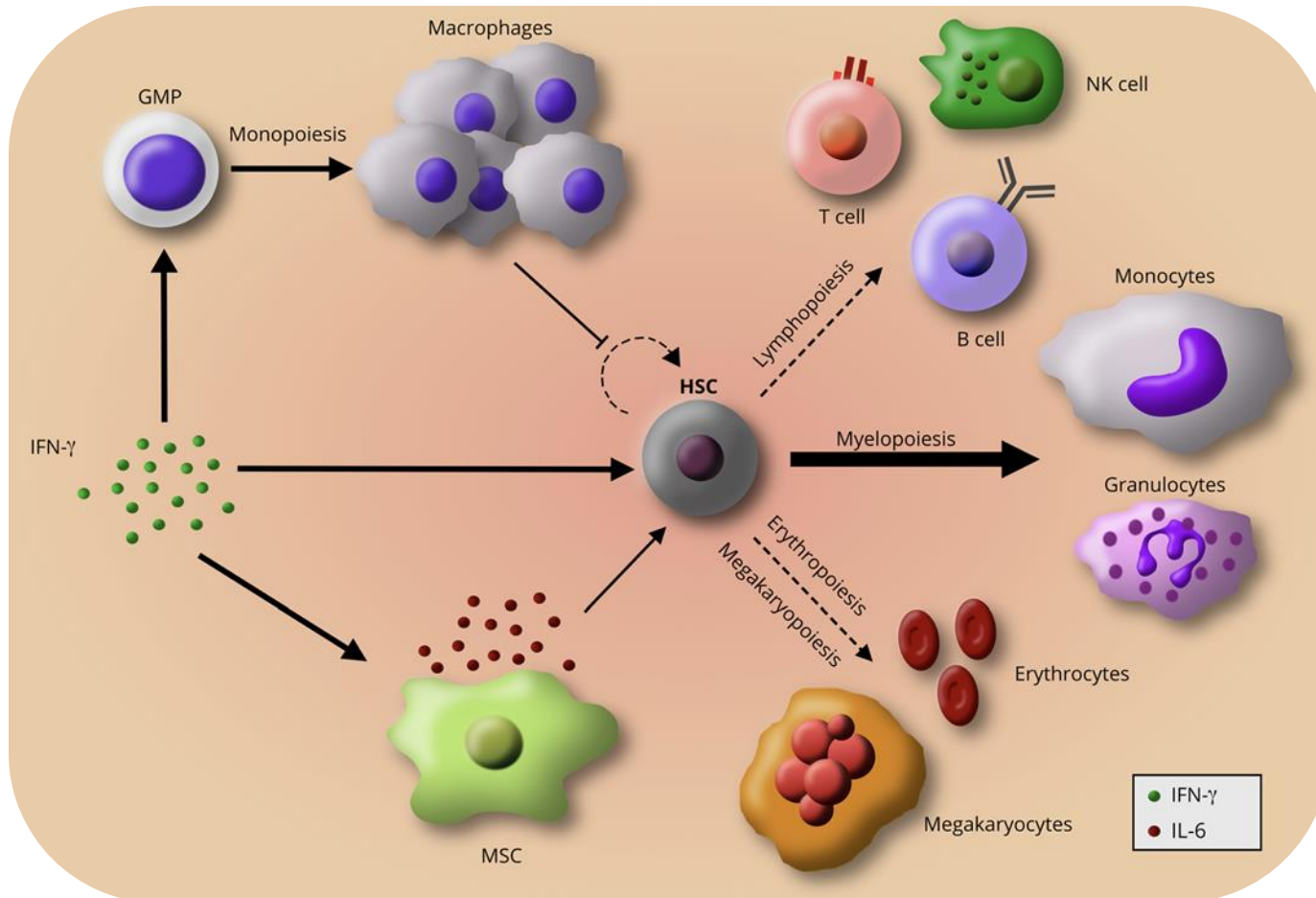
Case 2: 2 y.o. girl with R/R sJIA and MAS

- CMV reactivation – treated with Foscarnet
- Most recent CMV viral load: <1,000 IU/mL
- Blood cultures: negative
- Other virology screening (EBV, ADV, HHV6): all negative
- Ferritin: 5,400 µg/L
- CXCL9: 23,823 pg/mL (normal ≤898 pg/mL)
- IL-18: 6,952 pg/mL (normal ≤367 pg/mL)

Graft rejection VS graft failure

- **Graft rejection:** An immune-mediated process
- **Graft failure:** A wider array of possibilities, including cell dosing, disease, infection, drugs, and an immune-mediated event

IL18/interferon- γ /CXCL9 axis and the graft

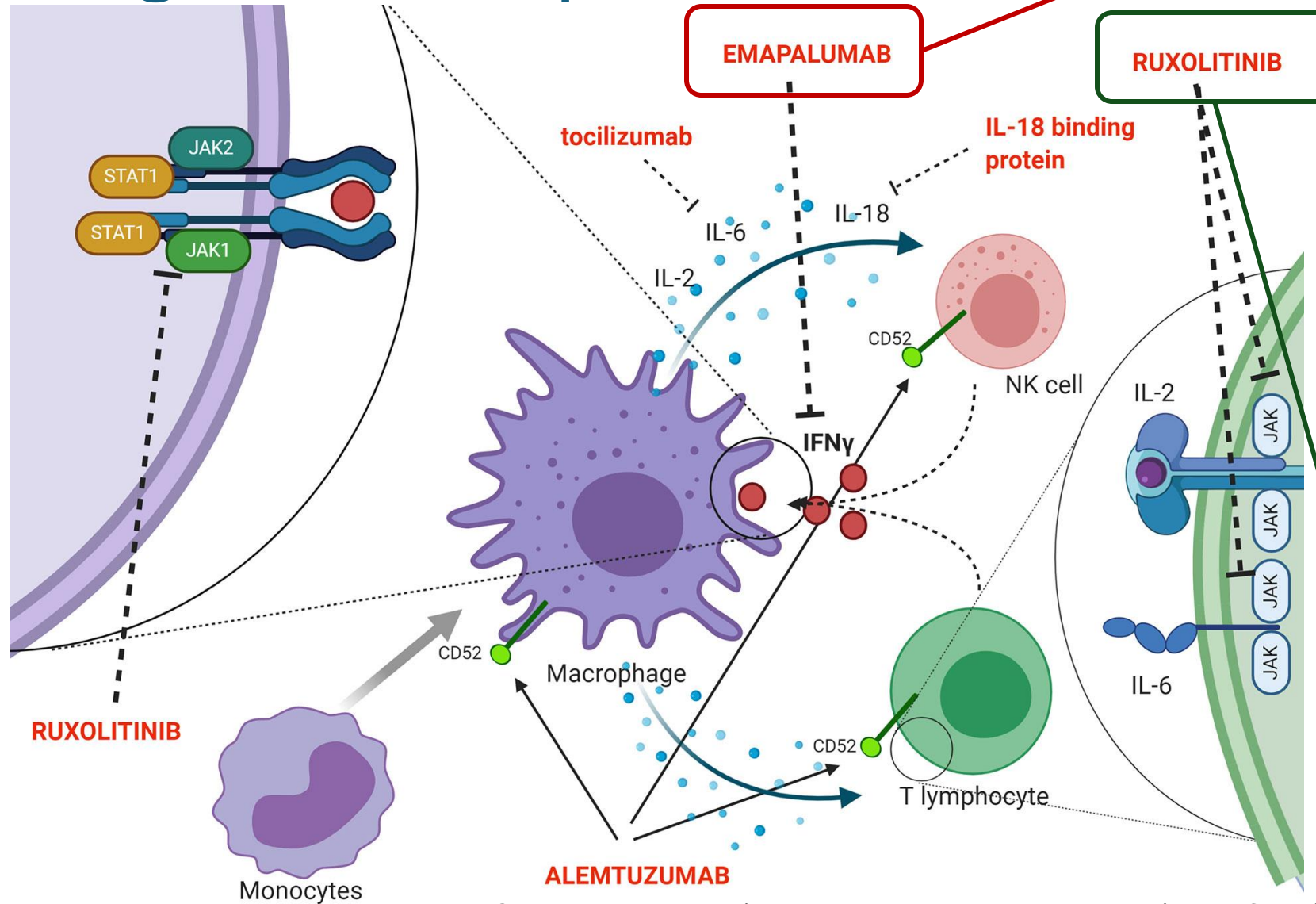


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Shimizu M, et al. Front Immunol. 2022.

Targeted therapies



Pros

- Salvage therapy for GF
- Highly specific (IFN- γ)
- Long half-life (~23 days)
- Target mediated clearance

Cons

- High cost
- Limited availability

Pros

- Use in PIRDS and GVHD
- JAK inhibitor

Cons

- Cytopenias
- Short half-life

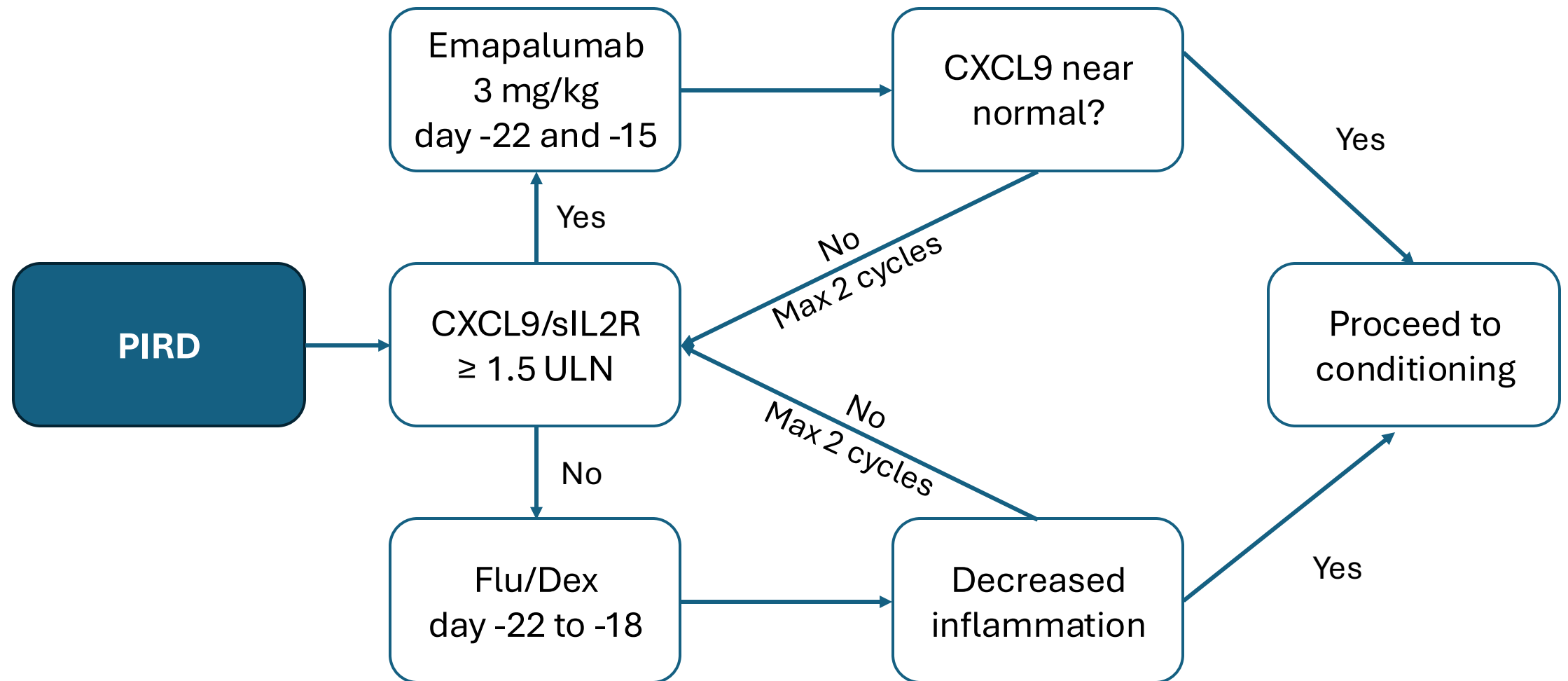
Case 2: 2 y.o. girl with R/R sJIA and MAS

- Received one dose of Emapalumab (3 mg/kg IV)
- Fever subsided with good hematologic recovery
- Clinical course complicated by Grade IV GVHD (Skin Stage 4), completely resolved with Prednisolone, Etanercept, Basiliximab, and topical Betamethasone
- 1-year follow-up: Full donor chimerism, normal blood counts, no recurrence of sJIA or MAS, no chronic GVHD, improvement in ILD, with no further oxygen requirement

BRIDGE trial: Ongoing phase 2 study

- Bridging pre-transplant inflammatory dampening for primary immune regulatory disorders
- Objective: To assess the effects of a targeted inflammatory cool-down prophylaxis prior to myeloablative allogeneic HSCT on achieving and maintaining trilineage engraftment through d+100
- Inclusion:
 - PIRD with or without identified genetic lesion
 - Autoinflammatory diseases with active autoinflammation
 - Able to tolerate cytoreduction

BRIDGE trial: Treatment schema

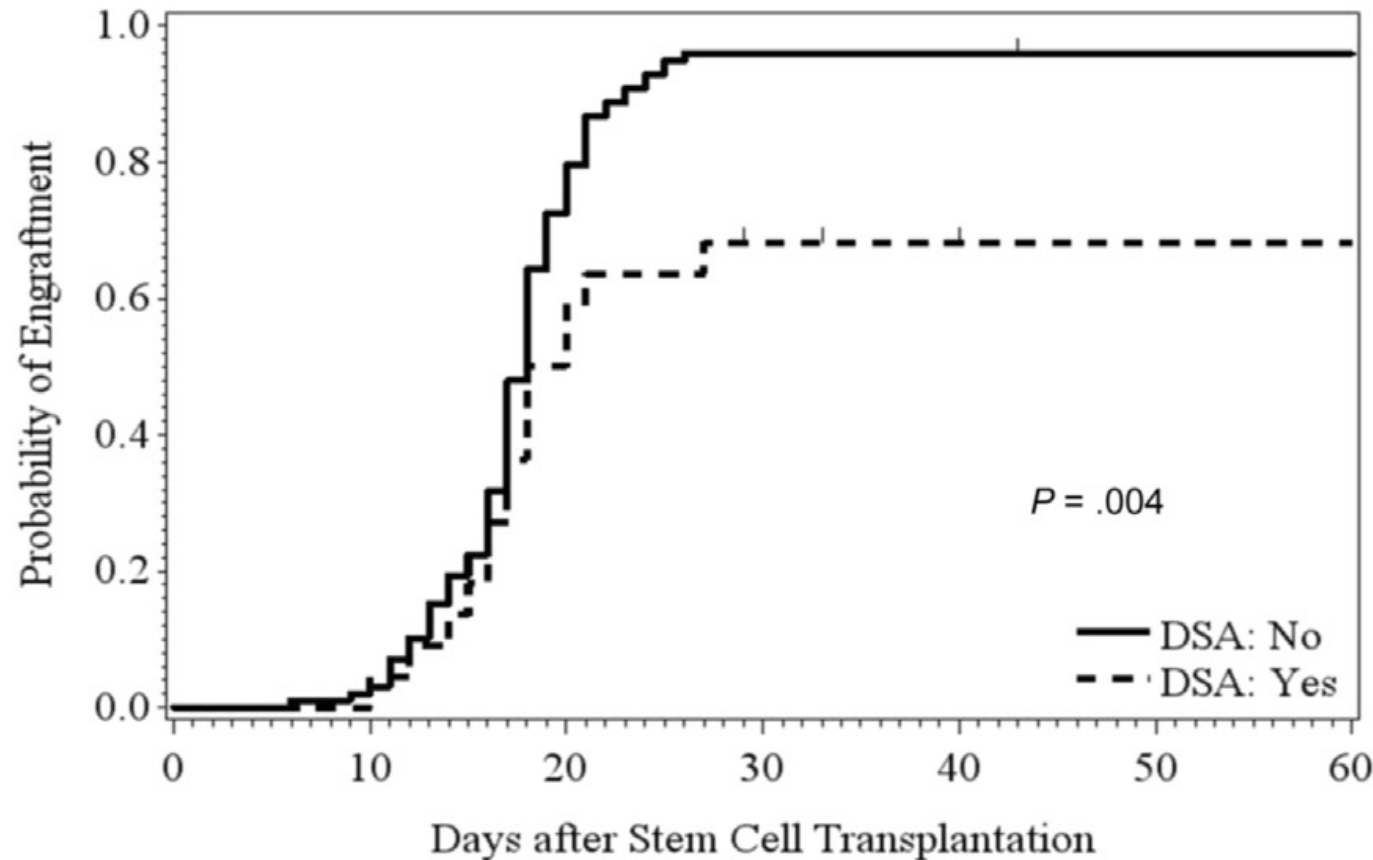


BRIDGE trial: Treatment schema

- Rabbit ATG: Days -12 to -10
- Emapalumab arm: 3 mg/kg/dose on Days -8 and -1 only
- Busulfan (AUC 90 mg·h/L) + Fludarabine: Days -5 to -2
- Donor types: All eligible except UCB
- Pre-HSCT: Weekly CXCL9 and cytokine panel
- Post-HSCT: Twice-weekly CXCL9/inflammatory markers
- Post-HSCT: Weekly cytokine panel

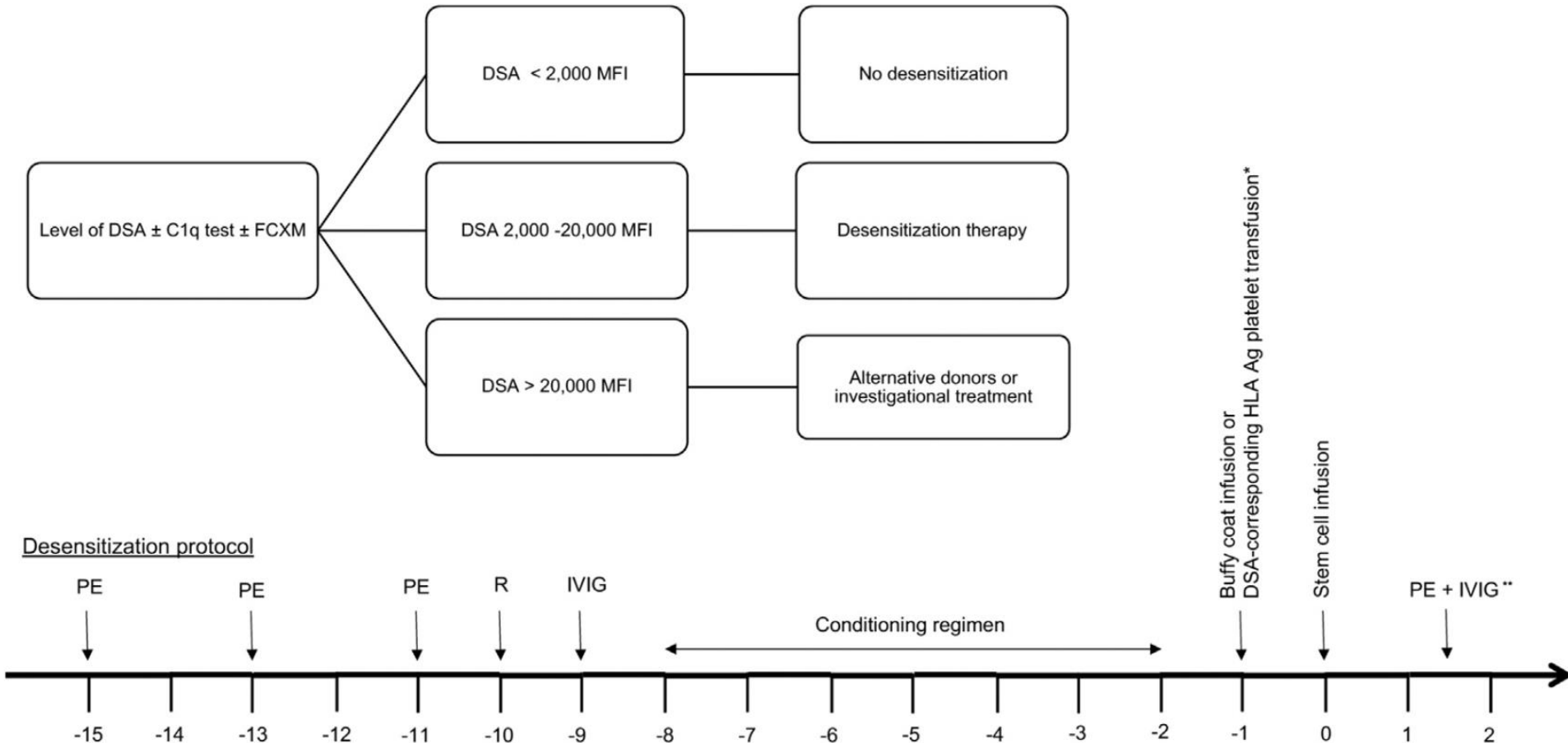
Other important cause of graft rejection: DSA

A



DSA: No	98	96	27	4	4	3	3
DSA: Yes	22	22	11	6	5	4	4

ASTCT consensus recommendations: DSA



Monitoring DSA weekly after initiation therapy & D-1 until engraftment
PE: Plasma exchange 1-1.5 total plasma volume
R: Rituximab 375 mg/m²
IVIg dose 1 g/kg
* For DSA class I only
** Consider in case of trending up of DSA after starting desensitization

Case 3: 7 y.o. boy with XL-CGD

- Diagnosed with X-linked chronic granulomatous disease
- History of recurrent infections and inflammatory bowel disease
- Underwent haploidentical HSCT from father (DSA-negative)
- Conditioning regimen: Treosulfan, fludarabine, cyclophosphamide, ATG
- GVHD prophylaxis: Post-transplant cyclophosphamide, Tacrolimus, MMF

Case 3: 7 y.o. boy with XL-CGD

- Neutrophil recovery on day +17
- Day +30 chimerism: Full donor
- Developed symptomatic adenoviremia:
treated initially with cidofovir,
then brincidofovir
- Hb 7.5 g/dL, WBC $1.2 \times 10^9/\text{L}$,
ANC $0.5 \times 10^9/\text{L}$, PLT $9 \times 10^9/\text{L}$
- Required platelet transfusions
and filgrastim
- **Secondary graft failure VS
poor graft function**

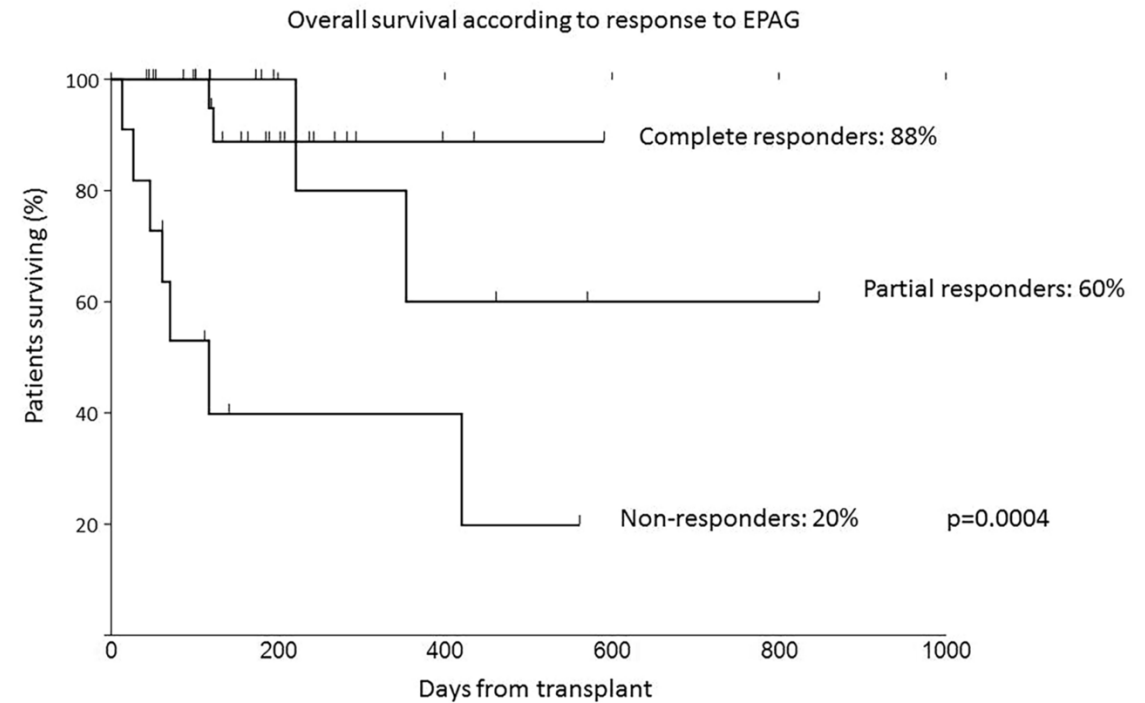
Poor graft function

- Frequent dependence on blood and/or platelet transfusions and/or growth factor support in the absence of other explanations, such as disease relapse, drugs, or infections

Secondary graft failure		Poor graft function	
Initial engraftment	✓		✓
Initial hematologic recovery	✓		✓
Cytopenias	✓		✓
BM cellularity	↓		↓
Relapse	X		X
Chimerism	Recipient/mix		Donor

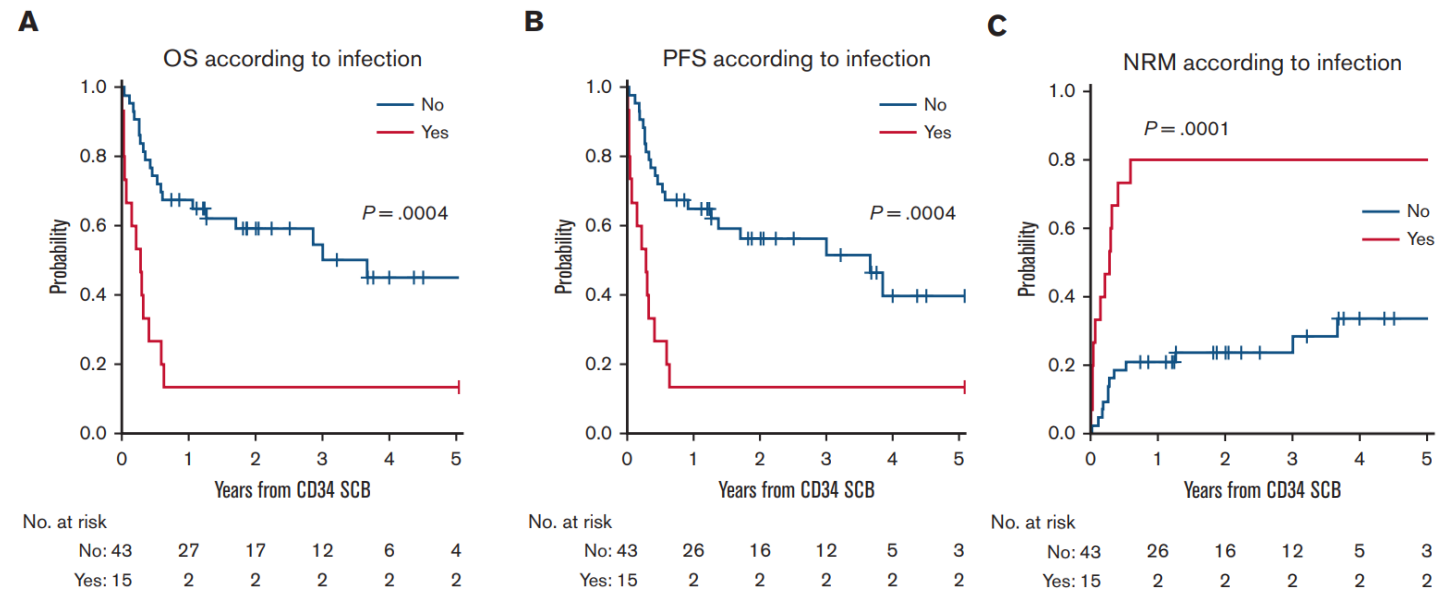
Eltrombopag (EPAG) for poor graft function

- Outcomes (N=48):
- Overall response rate: 75%
 - CR: 24 patients; PR: 12 patients
- Positive predictors of response:
 - HLA-matched donor
 - CD34+ cell dose $> 4 \times 10^6$ cells/kg
 - EPAG initiation ≥ 90 days post-HSCT



Stem cell boost (SCB) for poor graft function

- CD34+ cell selection using CliniMACS
- Median SCB dose: 3.88×10^6 CD34+ cells/kg
- Outcomes (N=58):
- CR + PR: 62%
- 2-year OS: 47%, 2-year NRM: 38%
- Grade 3–4 acute GVHD at 6 months: 3.4%
- Moderate-severe chronic GVHD at 2 years: 12%
- Multivariable analysis: Active infection at SCB infusion strongly predicts poor response and survival ($p=0.013$)
- Conclusion: SCB effectively restores hematopoiesis, especially in PGF without active infection at infusion.



Case 3: 7 y.o. boy with XL-CGD

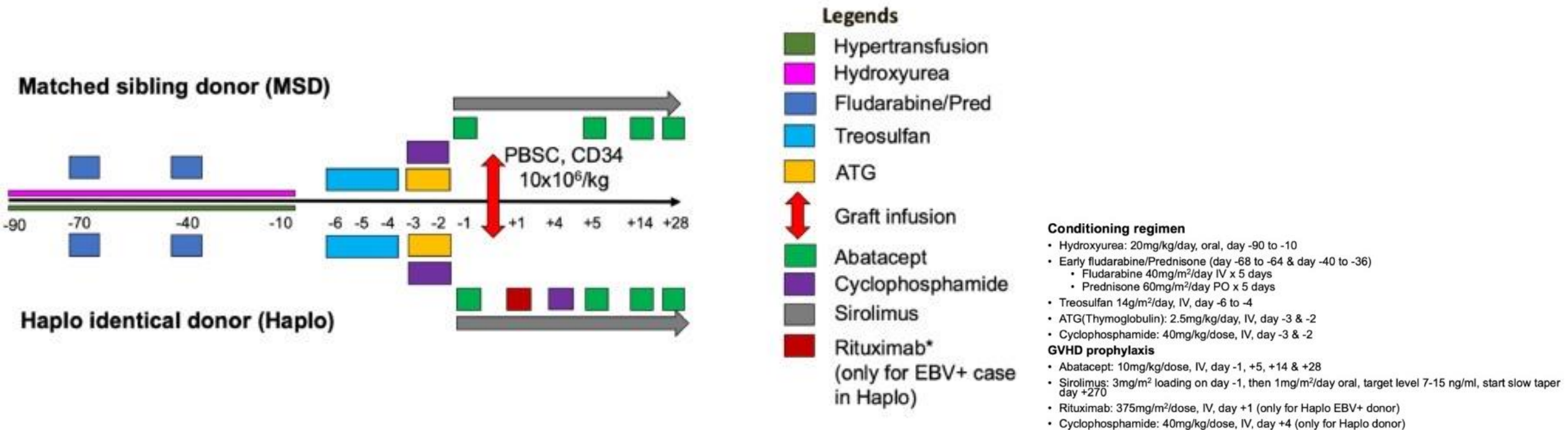
- Repeat chimerism: Full donor
- Bone marrow exam: hypocellular marrow, no MDS
- Initiated treatment with eltrombopag
- Transfusion interval extended, but did not achieve partial response
- Follow-up adenovirus viral load: negative
- Underwent CD34+-selected stem cell boost
- Full donor chimerism with complete response

Case 4: 8 y.o. boy with homo. alpha-thal-1

- Prenatal diagnosis: Homozygous alpha thalassemia 1
- Received intrauterine transfusions, continued with regular transfusion support
- Moderate hepatic iron overload (LIC 8.5 mg/g)
- Underwent a 10/10 MSD HSCT
- Cell source: PBSC
- CD34+ dose 10×10^6 cells/kg
- CMV (D/R): neg/neg, blood group (D/R): A Rh+/B Rh+

Case 4: 8 y.o. boy with homo. alpha-thal-1

Thal-FABs Protocol



Case 4: 8 y.o. boy with homo. alpha-thal-1

- Neutrophil recovery on day +18
- Chimerism at Day +30: CD33 98.0%, CD3 97.5%
- Chimerism at Day +100: CD33 81.0%, CD3 89.5% (Hb 11.5 g/dL)

Management: Reduce immunosuppression (sirolimus)

- Chimerism at Day +180: CD33 85.0%, CD3 86.5% (Hb 11.7 g/dL)

Donor chimerism

- **Full:** >95% for both myeloid and lymphoid lineages.
- **Mixed:** 5%–95% for either one or both myeloid and lymphoid lineages
- **Absent:** <5% for both myeloid and lymphoid lineages

Recommended donor chimerism levels

Non-malignant disorder	Lineage specificity	Minimum goal for donor Chimerism
Immunodeficiencies		
HLH	NK cell/Lymphoid	>30%
IPEX, ALPS	Lymphoid	>50%
Severe Combined Immunodeficiency	T, B, NK cell	100%
Chronic Granulomatous Disease	Myeloid	>50%
Wiskott-Aldrich Syndrome	Lymphoid/Myeloid	>50%
Hemoglobinopathies		
Sickle Cell Disease	Erythroid/myeloid	20–25%
Thalassemia	Erythroid/myeloid	20–25%
Metabolic disorders		
ALD, Hurlers, Krabbe's	Myeloid	70–100%
Osteopetrosis	Myeloid	>10%
Bone marrow failure syndromes		
SCN, SDS, DBA, FA	Myeloid	100%
	Lymphoid	>50%

Mixed chimerism does not predict graft failure in children with β -thalassemia

- **UCB recipients (N=27):**

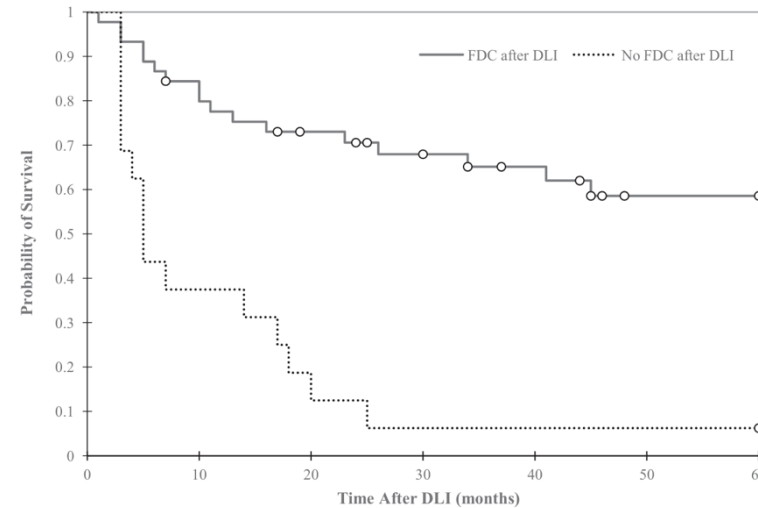
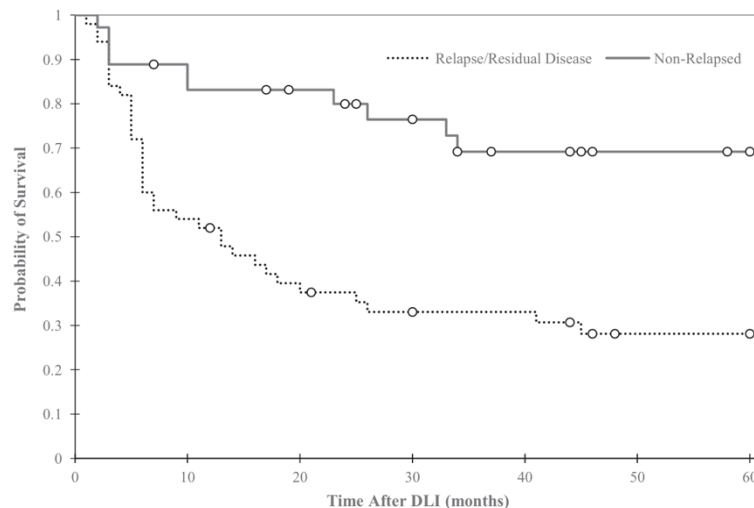
- 27/27 had initial mixed chimerism
- 13/27 converted to full donor
- 14/27 maintained stable mixed chimerism

- **BMT recipients (N=79):**

- 51/79 had initial mixed chimerism
- 40/51 converted to full donor
- 3/51 maintained stable mixed chimerism
- 8/51 eventually lost graft and became transfusion-dependent

Mixed chimerism in malignant diseases

- Mixed chimerism (MC) after HSCT predicts relapse risk.
- Donor lymphocyte infusions (DLI) can enhance graft-versus-malignancy effect.
- 86 patients receiving dose-escalated DLI:
50 with relapsed/residual (RR) disease, 29 with MC,
7 categorized as "other"
- 93% of MC patients converted to full donor chimerism (FDC) post-DLI.
- Nonrelapsed groups had significantly better OS vs. RR:
 - MC group: 83% (1-year), 70% (5-year)
 - Other group: 86% (1-year), 69% (5-year)
 - RR group: 28% (5-year OS) ($P = .00032$)
- Conversion to FDC correlated with higher survival:
 - 2-year OS: 71% (FDC) vs. 13% (no FDC); $P < .0001$
- DLI is significantly improving survival in nonrelapsed patients and supporting its pre-emptive use to reduce relapse risk in MC after HSCT.



Timing and dosing of prophylactic and preemptive DLI

Sureda A, et al, editors. The EBMT Handbook: Hematopoietic Cell Transplantation and Cellular Therapies . 8th ed. 2024.

	Timing of first dose	MRD	MUD	MMUD/Haplo	Number of DLI	References
Prophylactic	3 months (ex vivo TCD: any risk; no ex vivo TCD: high-risk disease)	$1 \times 10^5/\text{kg}$	$1 \times 10^5/\text{kg}$	$1 \times 10^5/\text{kg}$	1–3 ^b	Schmid et al. (2012)
	6 months ^a (high-risk disease)	$1 \times 10^6/\text{kg}$	$1 \times 10^6/\text{kg}$	$5 \times 10^5/\text{kg}$		Tsirigotis et al. (2016)
Pre-emptive	3 months	$1\text{--}5 \times 10^5/\text{kg}$	$1 \times 10^5/\text{kg}$	$1 \times 10^5/\text{kg}$	1–4 ^c	De Witte et al. (2021)
	6 months ^a	$1\text{--}3 \times 10^6/\text{kg}$	$1 \times 10^6/\text{kg}$	$5 \times 10^5/\text{kg}$		
Relapse	After chemotherapy	$1 \times 10^7/\text{kg}$	$1 \times 10^7/\text{kg}$	$1 \times 10^6/\text{kg}$	1–4 ^d	Santoro et al. (2023) van der Zouwen et al. (2023)

Interval between DLI: at least 4–6 weeks
^a Starting at 4 months, higher doses in the range employed at 6 months can be discussed
^b Second and third dose: increase each DLI dose by 0.5–1 log
^c Number of DLI: MRD/chimerism guided, consider continuation when MRDneg/full chimerism; in absence of MRD marker: GvHD guided
^d Second to fourth dose: increase each DLI dose by 1 log, to a maximum dose of $1 \times 10^8/\text{kg}$

Conclusions

Condition	Prevention	Treatment
Primary graft failure	Identify risk factors	Second HSCT
Secondary graft failure	Identify risk factors	Control the possible causes e.g., viral infection, drug toxicity
Graft rejection	Control inflammatory process Avoid using DSA+ donor Desensitization (DSA+ donor)	Emapalumab (IFN- γ driven)
Poor graft function	Identify risk factors	Eltrombopag, stem cell boost
Mixed chimerism in non-malignant diseases	Identify risk factors	Disease specific management, Reduce immunosuppression, +/- DLI
Mixed chimerism in malignant diseases	Identify risk factors	Reduce immunosuppression, Preemptive DLI

Acknowledgement



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- Pediatric CMU laboratory staffs

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