



ISBMT

Indian Society for Blood & Marrow Transplantation

BMT MASTER CLASS

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HSCT for BMFS

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The burden of SAA

- SAA – 2 per million North America and Europe, Asia 8 per million
- Median age at diagnosis is 8 years
- Mortality over 50%
- *Mellinkeri 2015, Heimpele 2000*

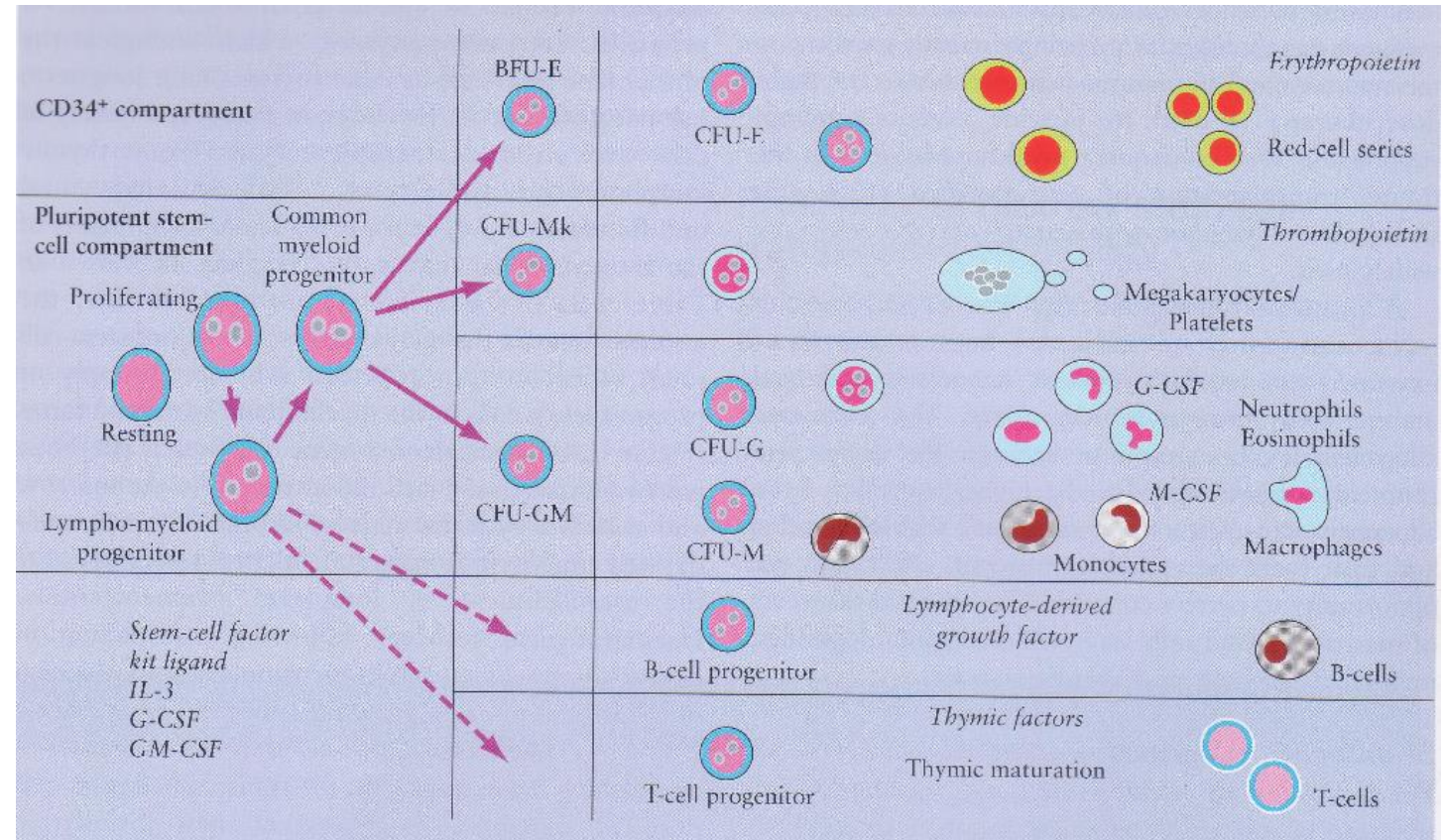
Table 1. Incidence of aplastic anemia according to age and sex.

	Age at diagnosis (years)					N. of cases	Total incidence ^a
	2-14	15-24	25-44	45-64	≥65		
Male							
N. of cases	17	25	22	28	31	123	
Incidence	1.92	2.83	1.52	2.56	5.89		2.54
Female							
N. of cases	12	11	15	31	43	112	
Incidence	1.43	1.41	1.00	2.58	4.89		2.16
Total							
N. of cases	29	36	37	59	74	235	
Incidence	1.68	2.16	1.26	2.57	5.33		2.34

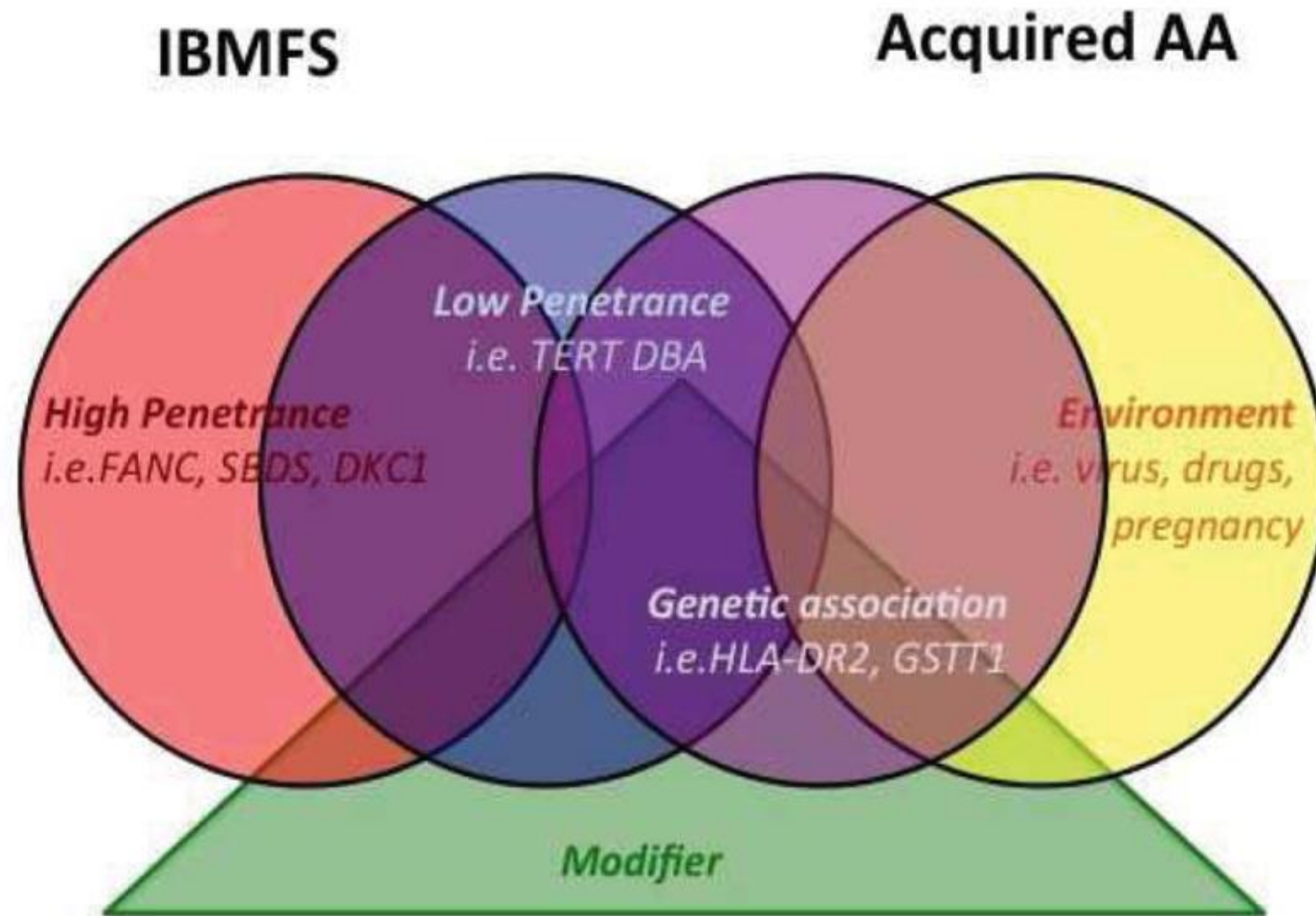
^aNumber of cases per one million people per year.

AA – Three distinct groups

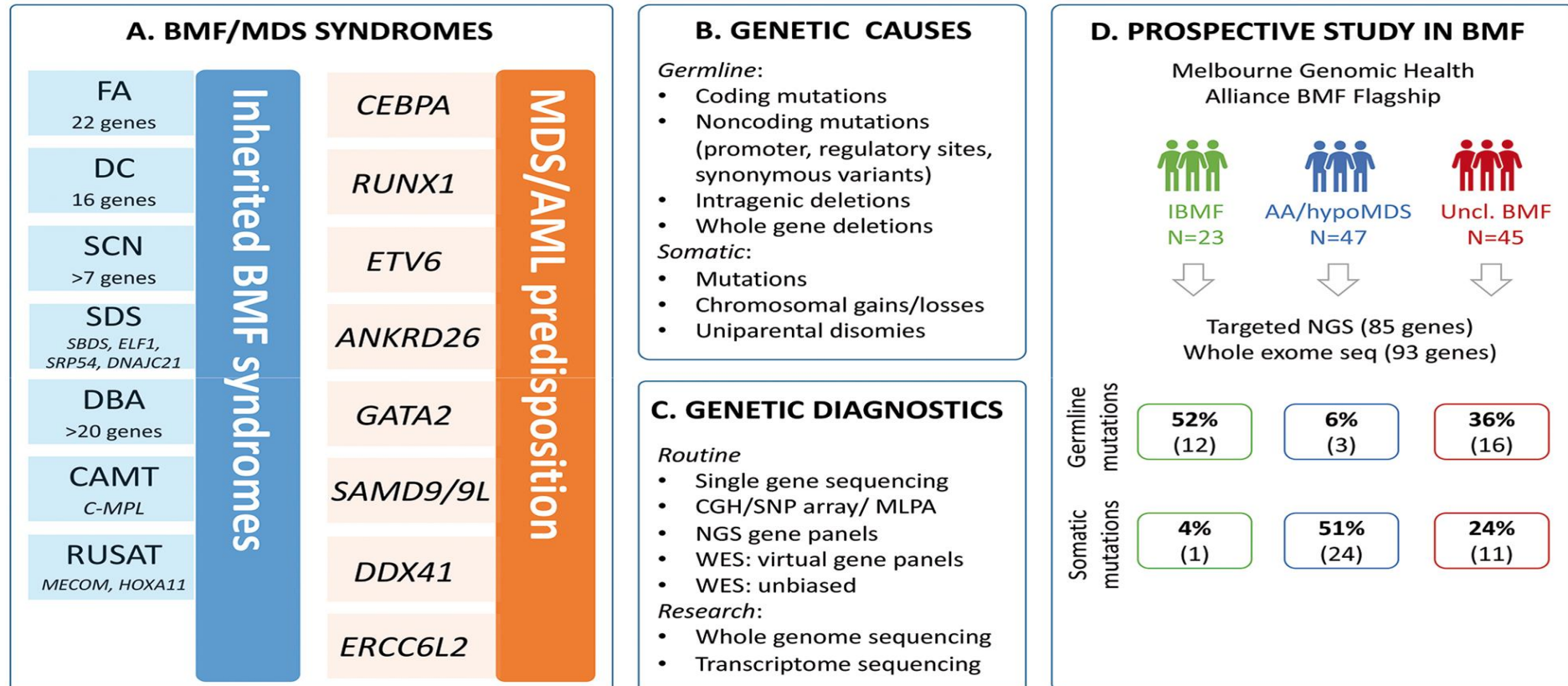
- Marrow injury
- Inherited
- Immune mediated



Have we got the right diagnosis?



Next generation sequencing in IBMFS



Choice of therapy in SAA

- Immunosuppressive therapy
- IST - Horse ATG, cyclosporine and eltrombopag
- Haematopoietic Stem Cell transplantation
- MFD – Matched Family Donor
- MUD – Matched Unrelated Donor
- Haploidentical HSCT

Immunosuppressive therapy - IST

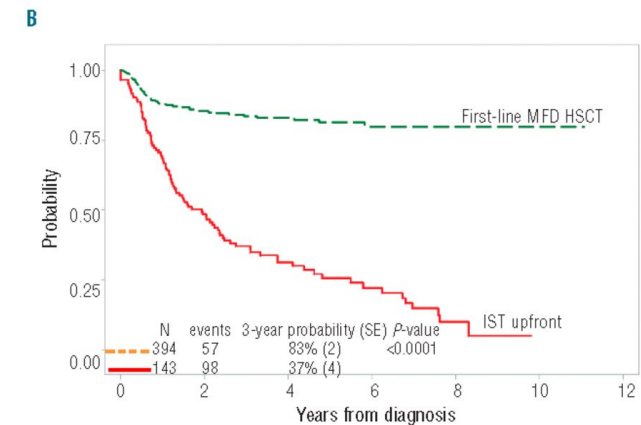
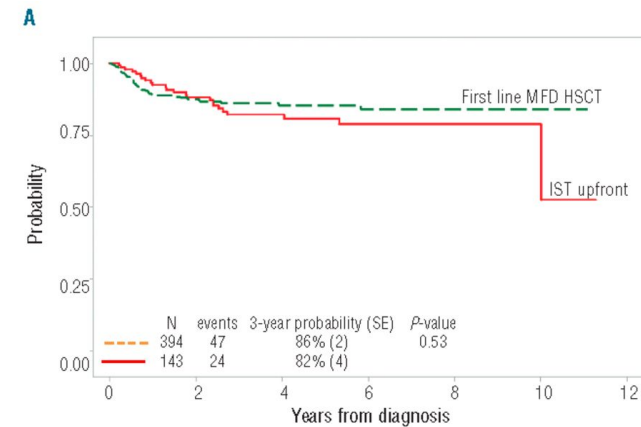
- IST – Timing – less than 10 weeks from diagnosis
- Child should have no active infections
- Addition of Eltrombopag improves efficacy

Matched Family HSCT versus IST

- OS
- MFD 91%
- IST 87%

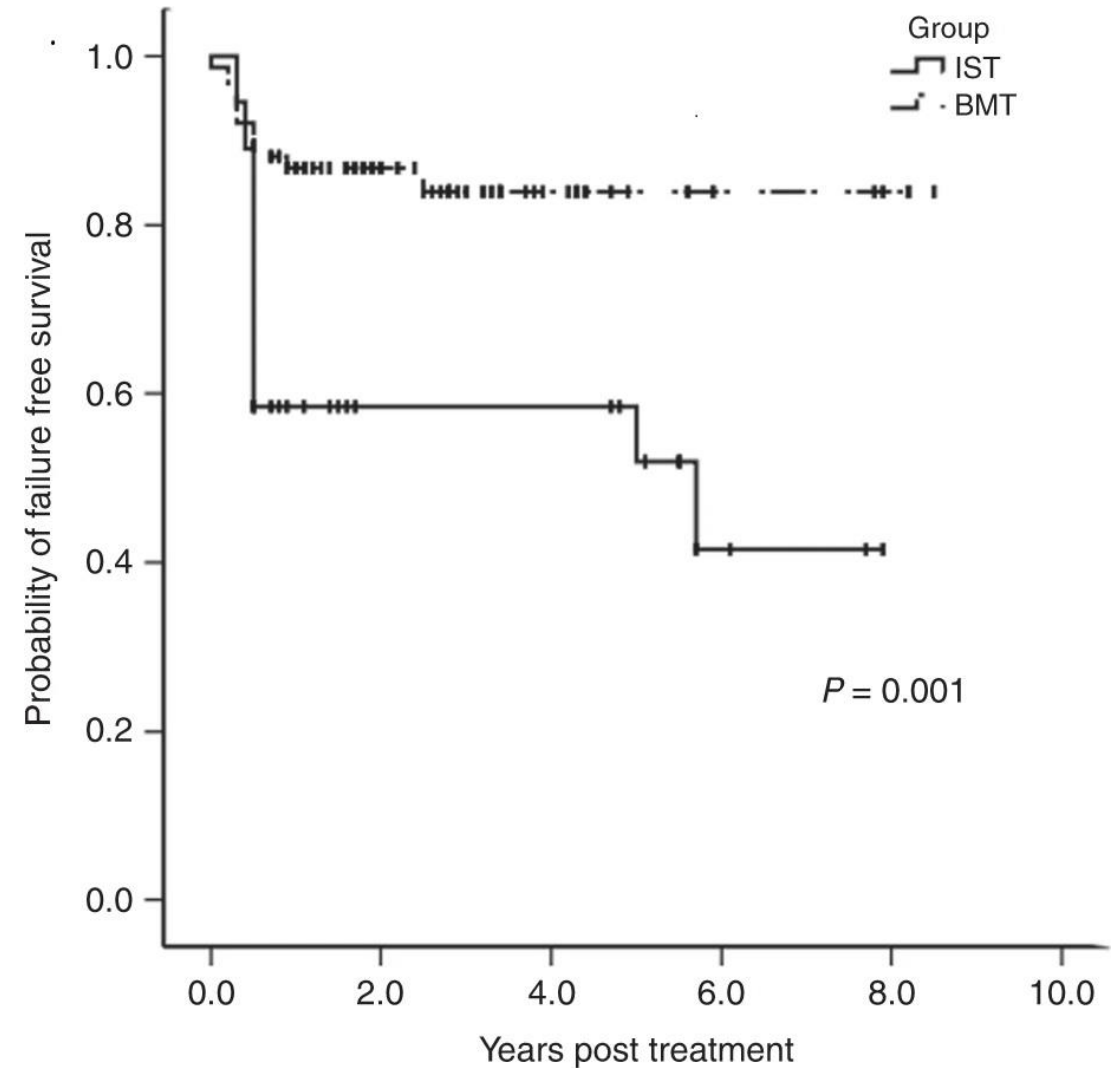
- EFS
- MFD 87%
- IST 33%

*Dufour et al- EBMT data
BJH 2015*



Matched Unrelated Donor versus IST

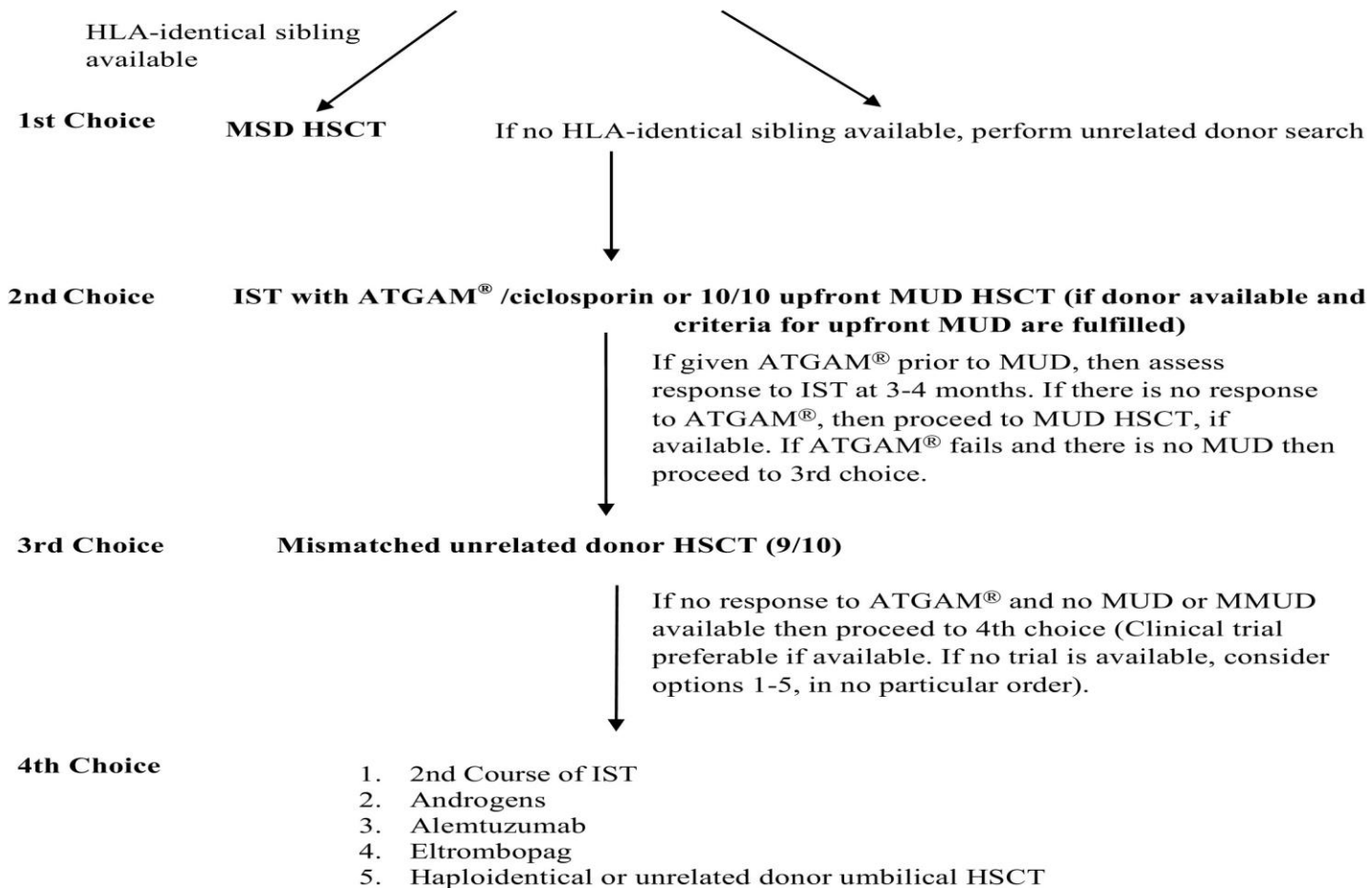
- HSCT conditioning was a fludarabine, cyclophosphamide and alemtuzumab (FCC) regimen
- Upfront MUD
- *Excellent outcome of matched unrelated donor transplantation in paediatric aplastic anaemia following failure with immunosuppressive therapy: A United Kingdom multicentre retrospective experience, BJH 2012, Samarasinghe et al*



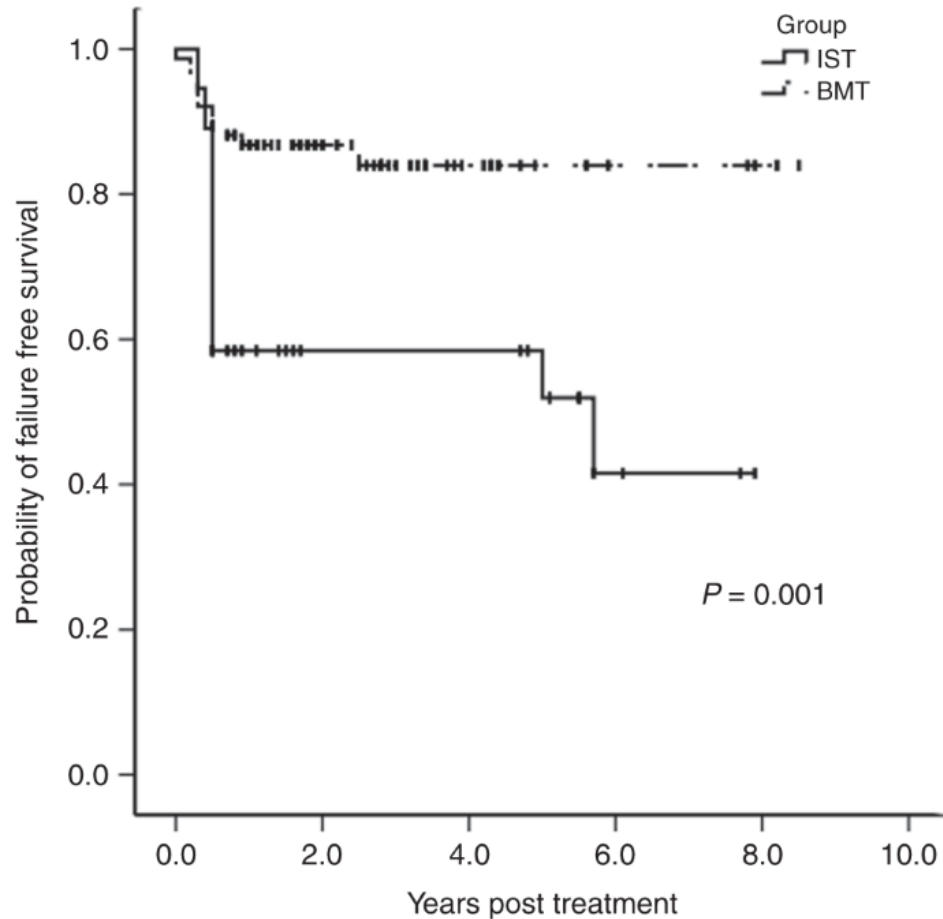
Recent algorithm - BSH

Algorithm for the management of Idiopathic Paediatric SAA

- Establish diagnosis of SAA/VSAA
- Exclude Inherited Bone Marrow Failure Syndrome
- Tissue Type Family and Patient



Haploidentical HSCT versus IST



- Xu, ZL., Zhou, M., Jia, JS. et al. Immunosuppressive therapy versus haploidentical transplantation in adults with acquired severe aplastic anemia. *Bone Marrow Transplant* **54**, 1319–1326 (2019)

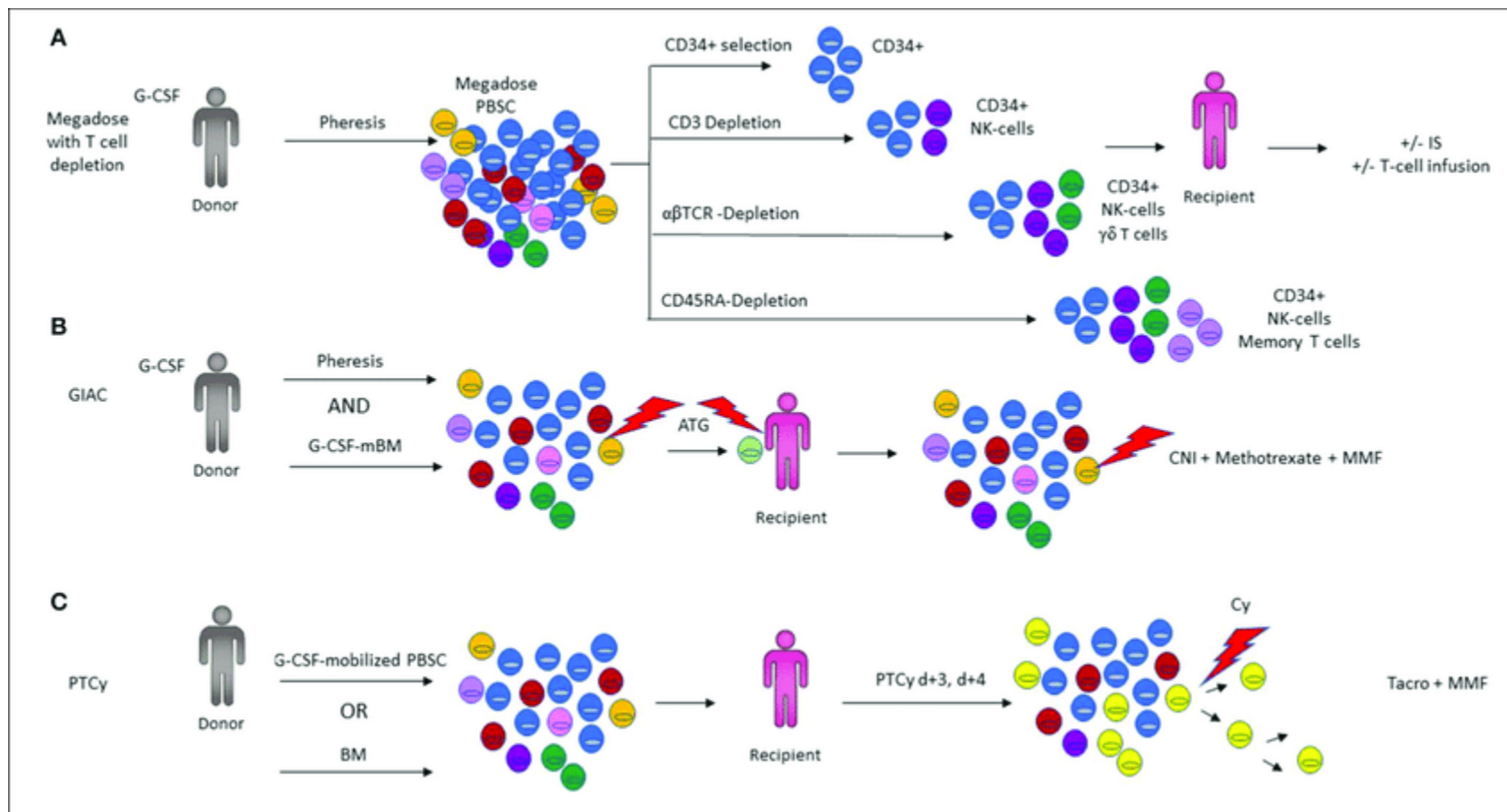
Challenges in India

- 10/10 donor choice limited
- SAA – heavily transfused
- Alloimmunisation rates are high – no leucodepletion or irradiation
- Infections – drug resistant bacteria and fungal infections

How do we proceed...

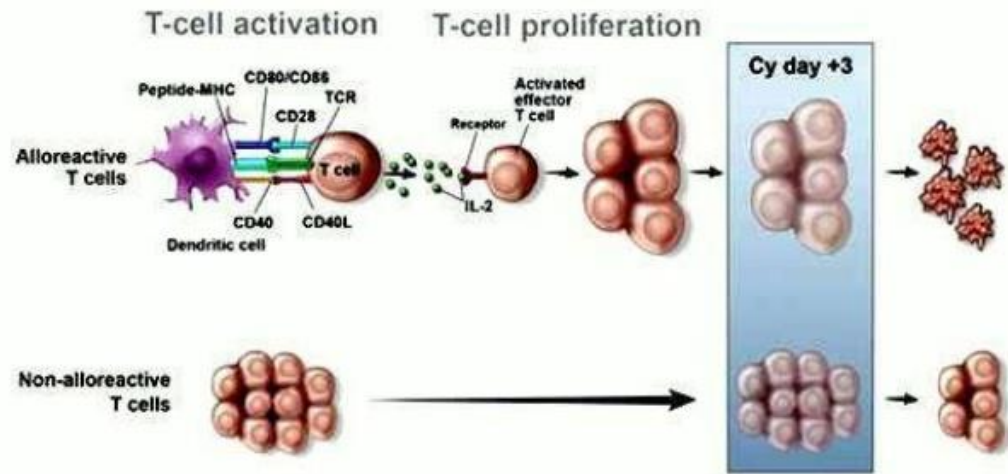
- HLA typing of child and family at diagnosis
- NGS and MMC at diagnosis
- IBMFS – HSCT – Matched family or haploidentical
- Acquired - Look for a matched donor – family donor MUD
- If ill proceed with haploidentical donor transplant

Haploidentical HSCT – which method?



PTCY versus TCR alpha beta depletion

Mechanism Post-transplant Cyclophosphamide

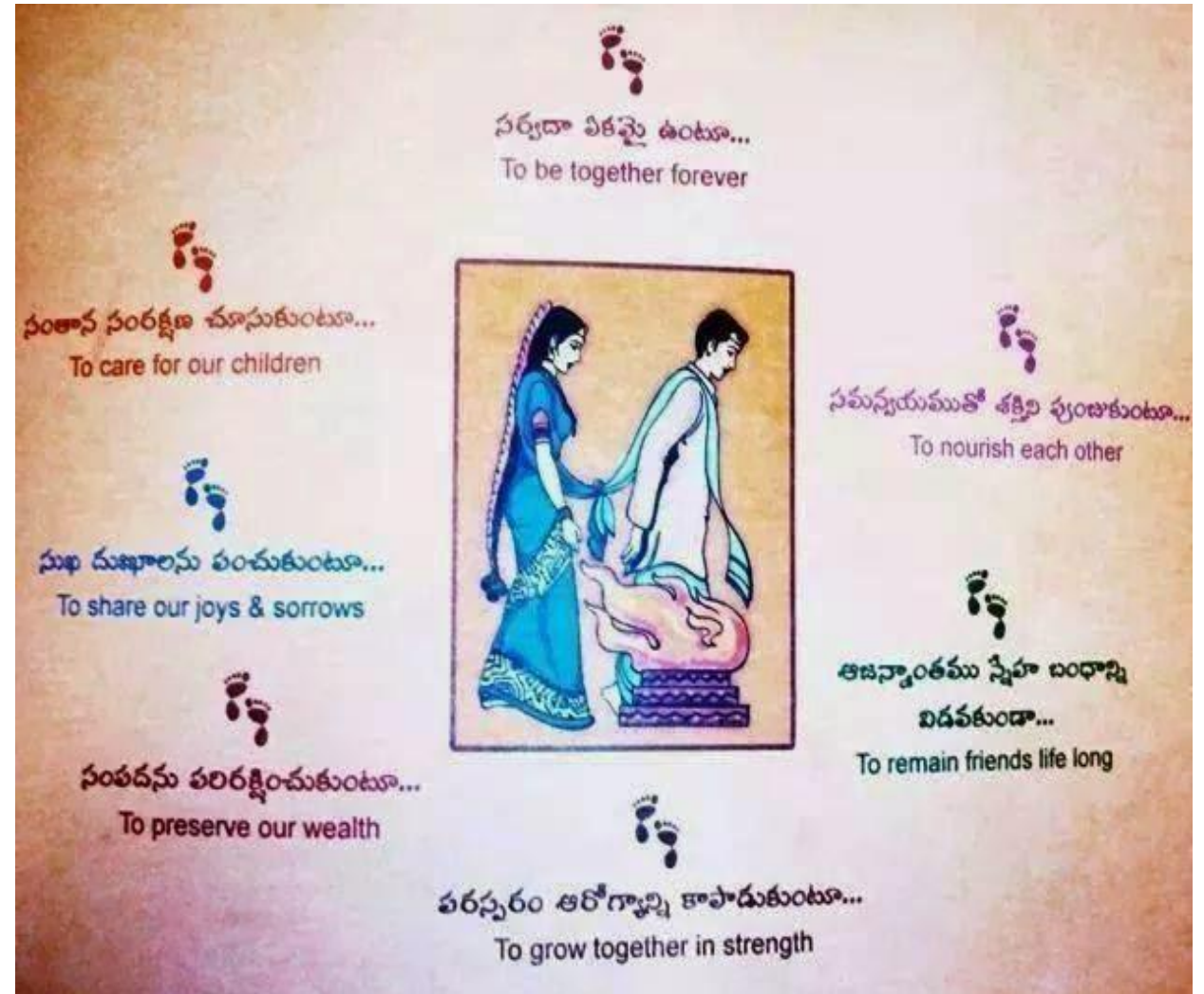


Luznik L, et al. Blood. 2001;98:3456-3464.



The seven steps of Hindu marriage and HSCT

- Patient selection
- Donor selection
- Conditioning
- Infusion of stem cells
- Supportive care
- Prevent rejection and GVHD
- Long term follow up



Step 1

The right patient



Story

- 16 year old girl from Bangladesh
- Pancytopenia for 3 months
- Leg ulcer – operated – wound unhealthy with drug resistant Klebsiella and enterococcus
- Consolidation right upper lobe

How did we manage this child?

- Urgent HLA typing of child and family at diagnosis
- NGS and MMC at diagnosis – not genetic
- Acquired - Look for a matched donor – family donor MUD
- As critically ill proceed with haploidentical donor transplant

Clinical course

- Two weeks to stabilise the child
- Antibiotics, antifungal, granulocyte transfusions
- Stabilise
- Fludarabine, cyclophosphamide, 2 Gy TBI / PTCY – Engrafted and well

Step 2

The right donor



Story

- 7 year old girl with SAA
- Haploidentical parents and haploidentical twin sister
- Father 38 year old male, major blood group mismatch, 8/10 match
- Twin sister 5/10 match, donor specific antibodies negative

Our haploidentical donor selection algorithm

- The European Society for Blood and Marrow Transplantation (EBMT) consensus recommendations for donor selection in haploidentical hematopoietic cell transplantation – Ciurea et al
- Age less than 35 years
- Male sex
- DSA negative

Clinical course

- Flu/Cy/TBI 2Gy/Bone marrow/PTCY
- Clinically well
- Did not engraft till day 37 and died due to gram negative sepsis

Step 3

Conditioning

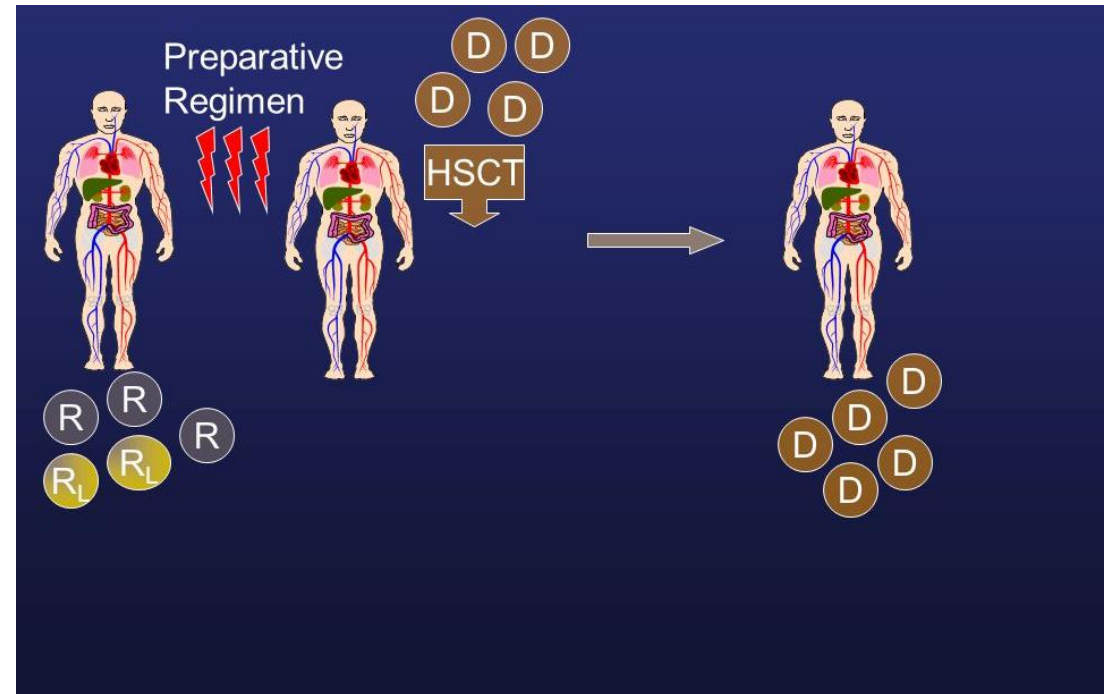


Story

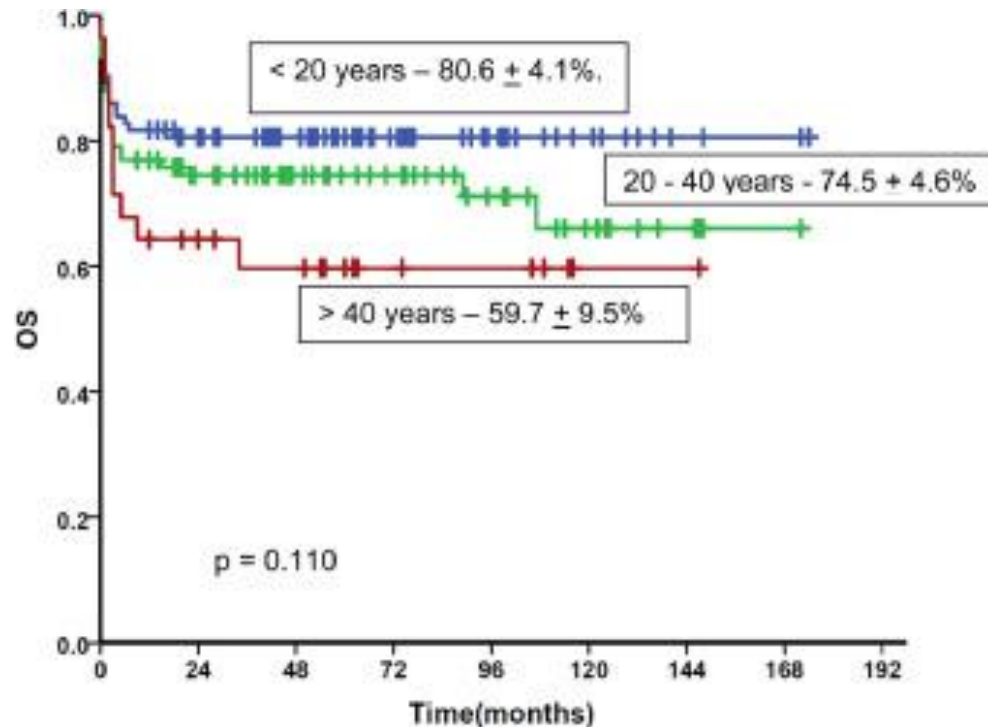
- 3 year old girl
- IST in November 2021
- Transfusion dependent – coming in for HSCT in April 2022
- Over 50 units transfusion

Conditioning - Host lymphopoiesis

- Fludarabine
- Cyclophosphamide
- Antithymocyte globulin
- 2 Gy TBI



Data from India – ATG free regimen

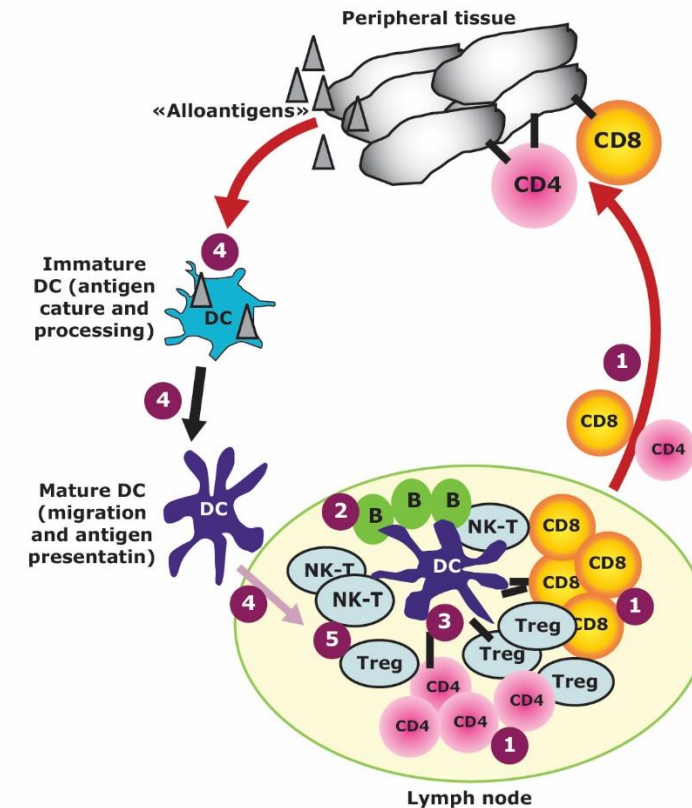


- George B
- An Antithymocyte Globulin-Free Conditioning Regimen Using Fludarabine and Cyclophosphamide Is Associated with Good Outcomes in Patients Undergoing Matched Related Family Donor Transplantation for Aplastic Anemia.
- Transplant Cell Ther. 2021

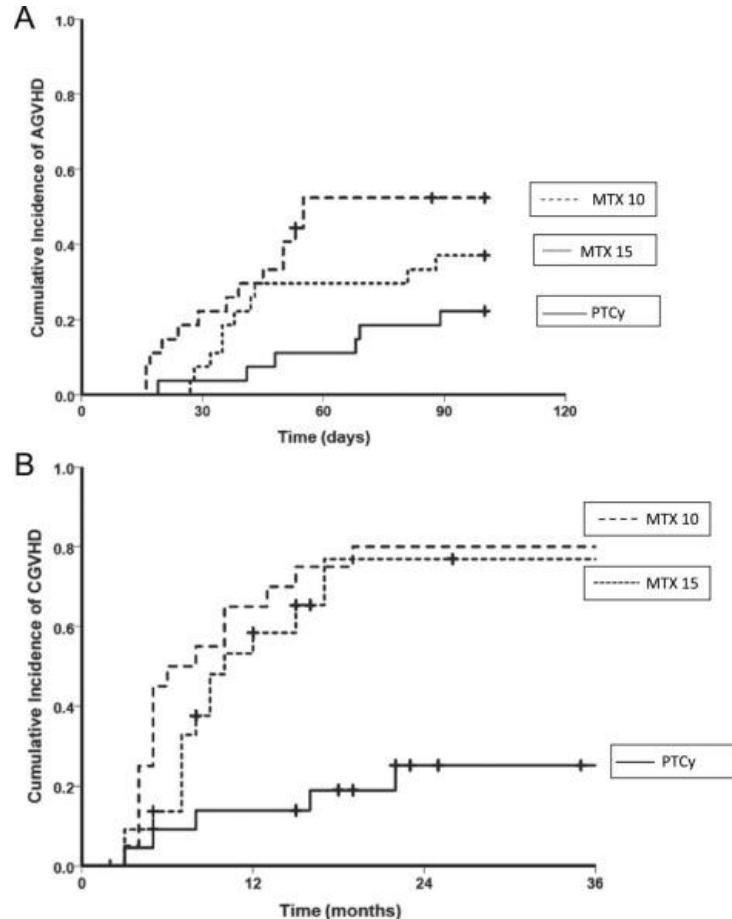
Polyclonal nature of ATG and the immune system

- 1 T-cell depletion in blood and peripheral lymphoid tissues through complement-dependent lysis and T-cell activation and apoptosis
- 2 Induction of B-cell apoptosis
- 3 Modulation of key cell-surface molecules that mediate leukocyte/endothelium interactions
- 4 Interference with DC functional properties
- 5 Induction of Treg and NK-T cells

Proposed mechanisms through which ATG can interfere with the immune response



PTCY based GVHD prophylaxis



- George B
- Post-Transplant Cyclophosphamide as Sole Graft-versus-Host Disease Prophylaxis Is Feasible in Patients Undergoing Peripheral Blood Stem Cell Transplantation for Severe Aplastic Anemia Using Matched Sibling Donors.
- Biol Blood Marrow Transplant. 2018 Mar

How did we manage this child?

- Checked donor specific antibodies – negative
- Flu/Cy/TBI 2Gy/PTCY
- Addition of Rabbit ATG 1.5 X 3
- Engrafted an well over 15 months with no GVHD

What can we do for patients with antibodies?

- Fludarabine
 - Cyclophosphamide
 - Rabbit ATG
 - 2 Gy TBI
-
- Donor specific antibodies if positive
 - Rituximab
 - Plasma exchange before HSCT

Step 4

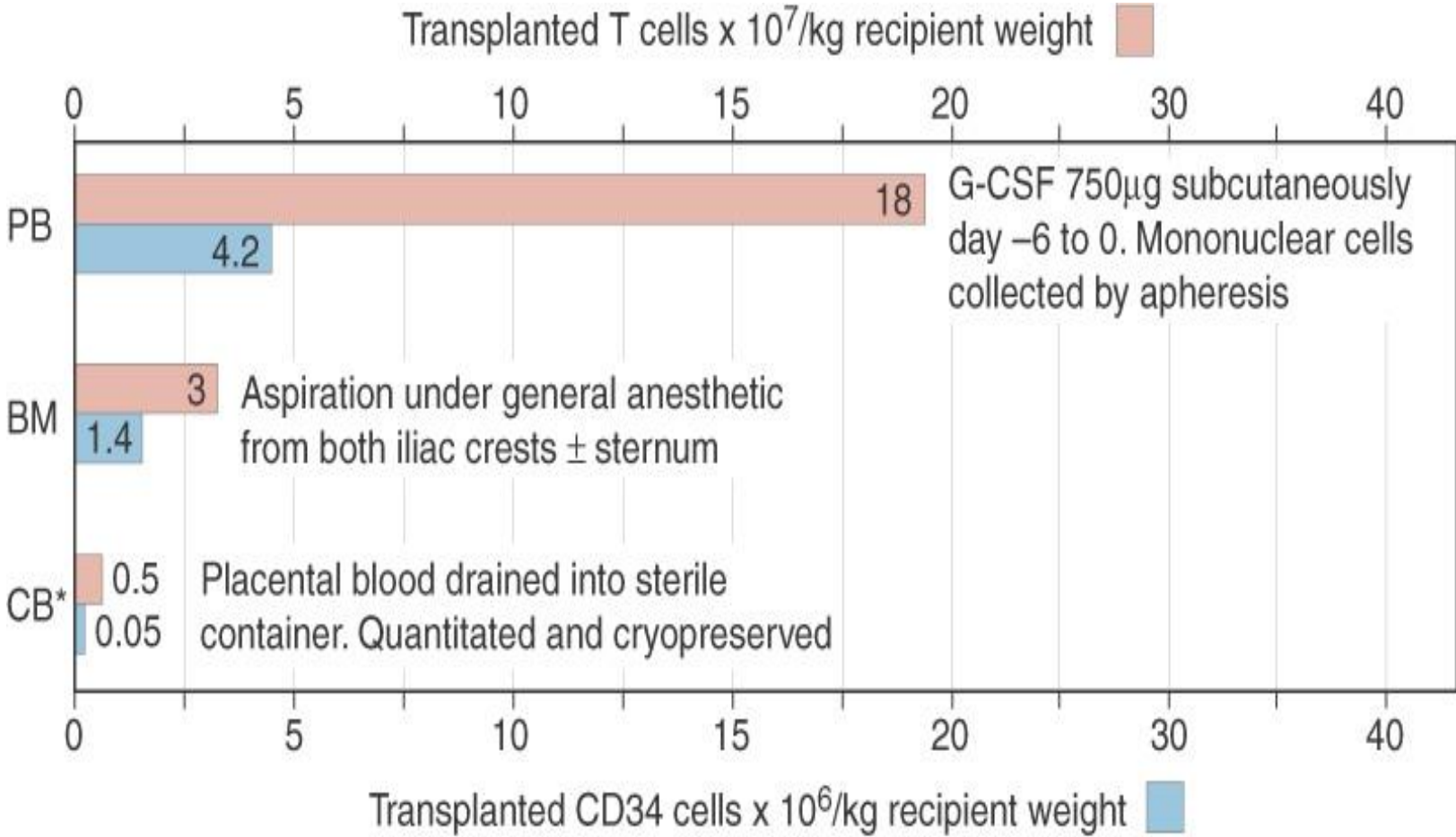
Infusion of stem cells



Story

- 16 year old boy weighing 57 kg with SAA
- Matched sibling – younger sister 13 years old weighing 30 kg
- Over 40 units transfusions

Bone marrow versus PBSC

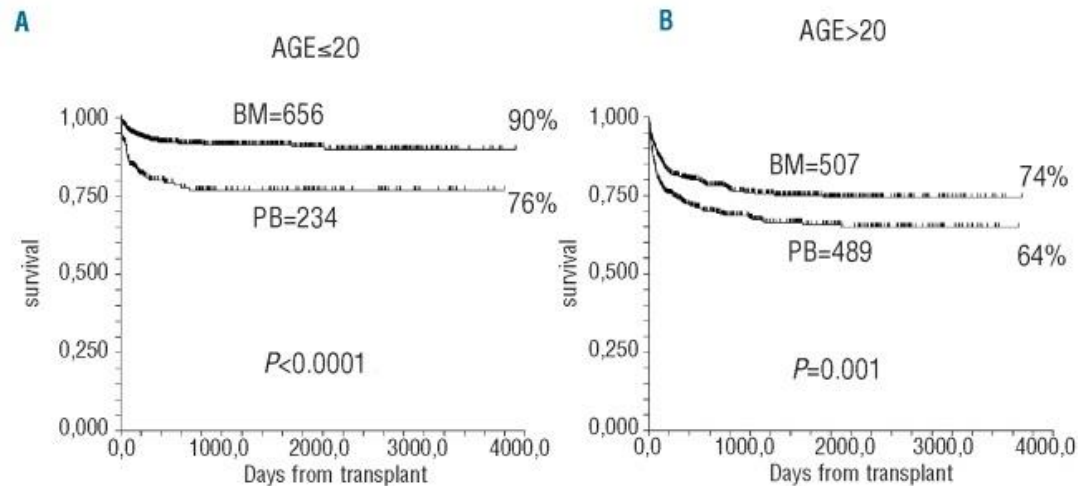


Chronic GVHD with PBSC



- High incidence of graft versus host disease with peripheral blood stem cells in severe aplastic anaemia
- Graft rejection with bone marrow

Bone marrow is superior



- Bacigalupo
- Aplastic Anemia Working Party of the European Group for Blood and Marrow Transplantation (WPSAA-EBMT). Bone marrow versus peripheral blood as the stem cell source for sibling transplants in acquired aplastic anemia: survival advantage for bone marrow in all age groups. *Haematologica*. 2012 Aug;97(8):1142-8

How did we manage this child?

- Flu/Cy/TBI 2Gy / PTCY
- PBSC harvested with mozobil to target 7 million cells per kg of recipient body weight

Step 5

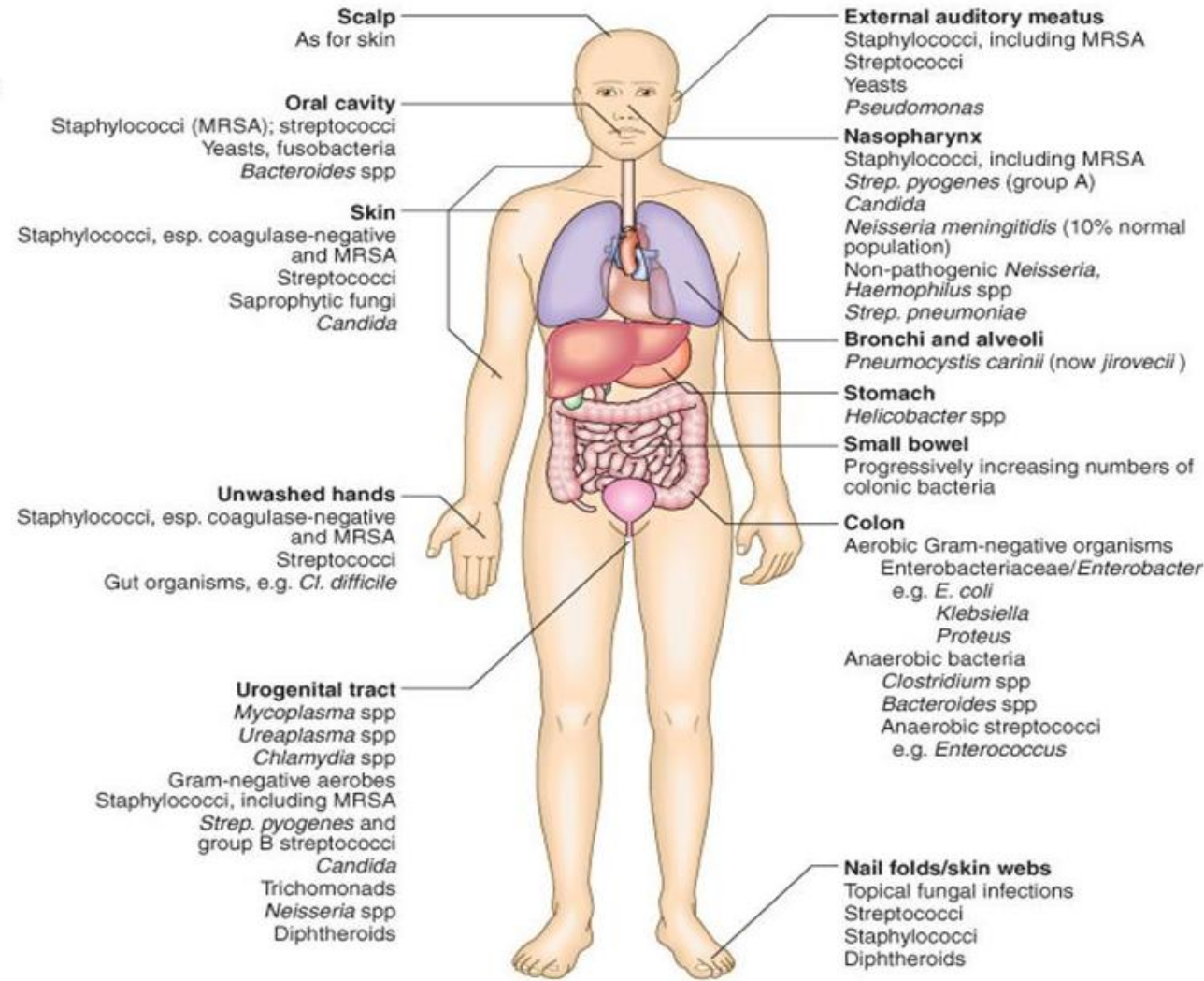
Supportive care



Story

- 6 year old boy with SAA
- Start of HSCT
- Recent Klebsiella sepsis – Carbapenam resistant

The two week challenge

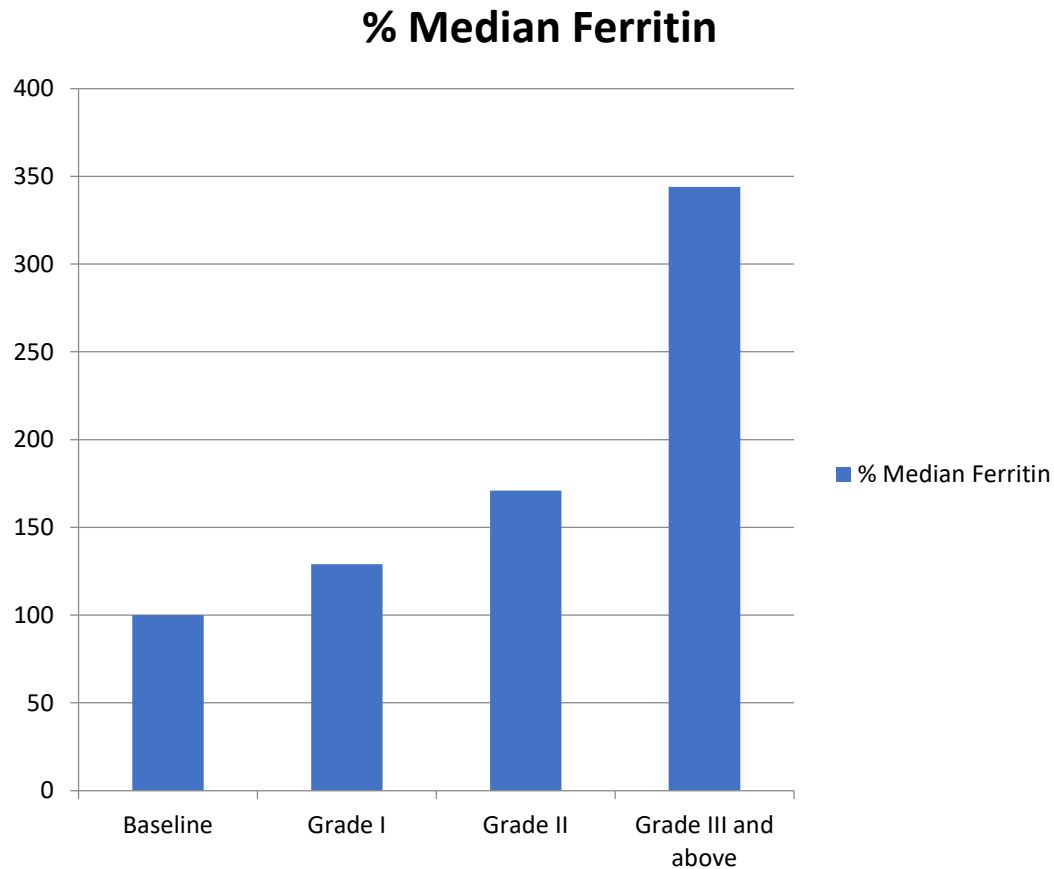


Measures to prevent XDRO

- Perianal care – 60% source for KPC
- Trophic feeds only to avoid gut bacterial translocation
- Surface cleaning of the unit
- Infection control team



Engraftment syndrome



- Cytokine release can cause multiorgan dysfunction
- Early recognition is the key
- Treatment with steroids prevents progression
- IL 6 blocker Tocilizumab must be used in select cases

What did we manage this child?

- Granulocyte transfusions during neutropenic period alternate days
- BCID panel – early and rapid ESBL identification
- Antifungal prophylaxis
- Engrafted and doing well – CMV reactivation needed therapy

Step 6

Balance graft rejection and graft
versus host disease



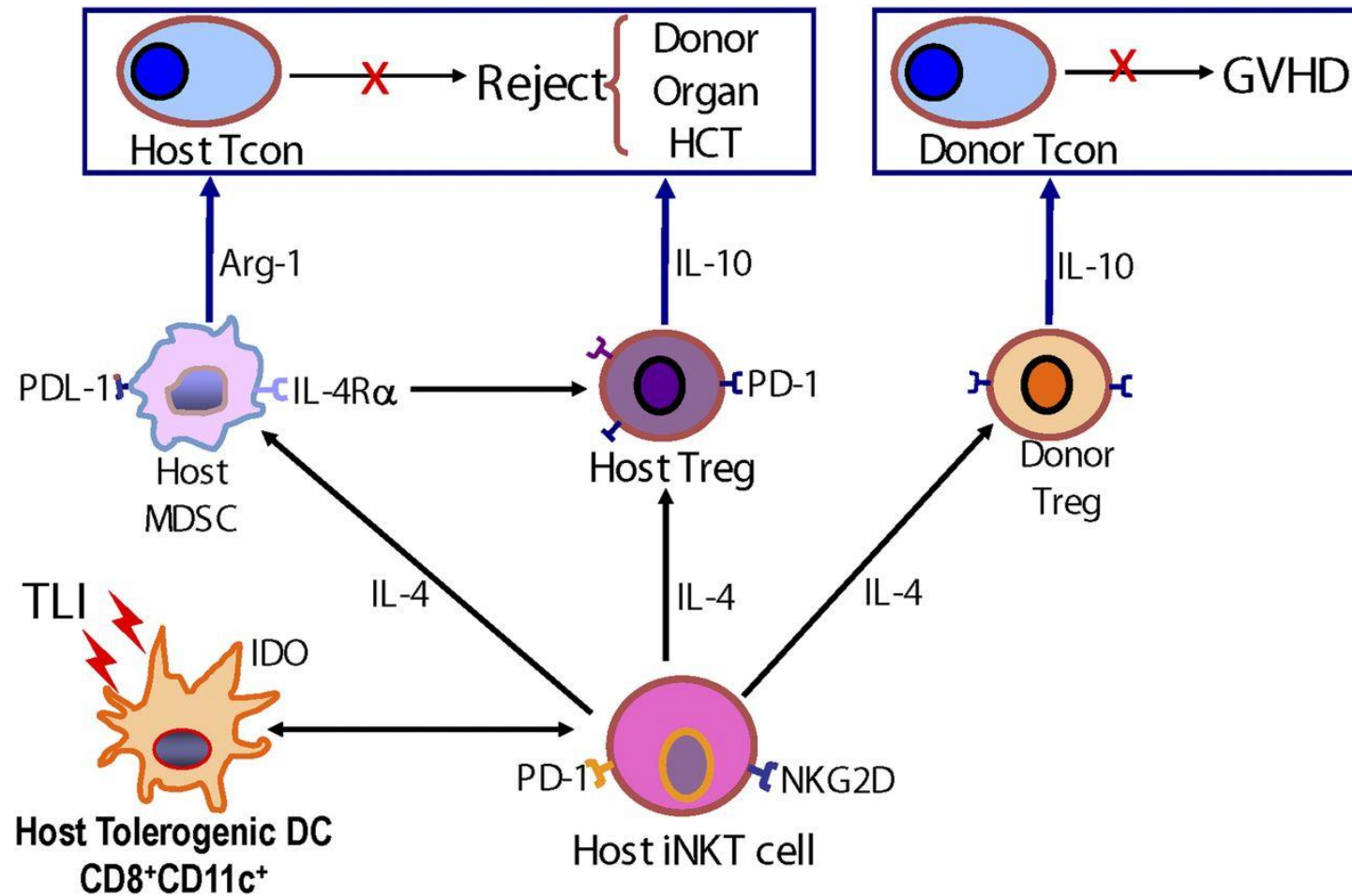
Story

- 13 year old post MSD HSCT for SAA
- Mixed chimerism at 4 months – T cell chimerism 93%
- CBC normal
- Well child

How did we manage this child?

- Added MMF to CNI
- Recheck chimerism in 2 to 4 weeks
- Further drop in chimerism to 81%
- DLI $1 \times 10^5/\text{KG}$ – Complete chimerism

Fine balance between rejection and GVHD



Step 7

Late effects



Story

- 15 year old girl post HSCT
- Chronic skin GVHD – vitiligo on face, dry eyes
- Mild effort intolerance

What did we do for this child?

- Cyclosporin plus steroids plus Jakavi
- Offered ECP – Extracorporeal photopheresis
- Improved after 4 sessions

Late effects follow up

- Vaccination
- Growth
- Puberty
- Fertility
- Endocrinopathy
- Second malignancy

Improving survival

- NGS based patient selection
- Reducing GVHD – low dose prophylaxis – ATG and PTCY
- Optimal supportive care
- Prevent GVHD - Abatacept

IBMFS

Step 1

The right patient

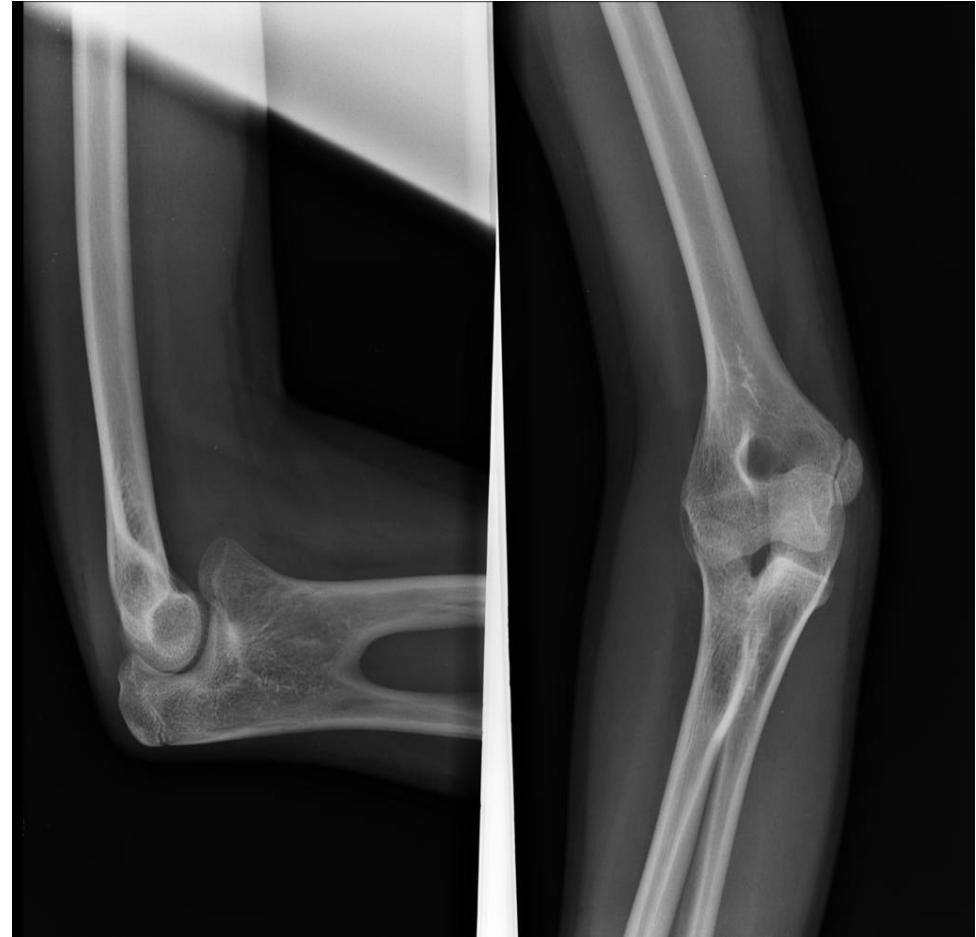


Story

- Eight year old boy
- Pancytopenia from 6 years of age
- Hb 6.7, TC 1900, N10%, Platelets 15000
- Required PRBC, platelets twice
- BMA – absent megakaryocytes
- Stress cytogenetics - negative

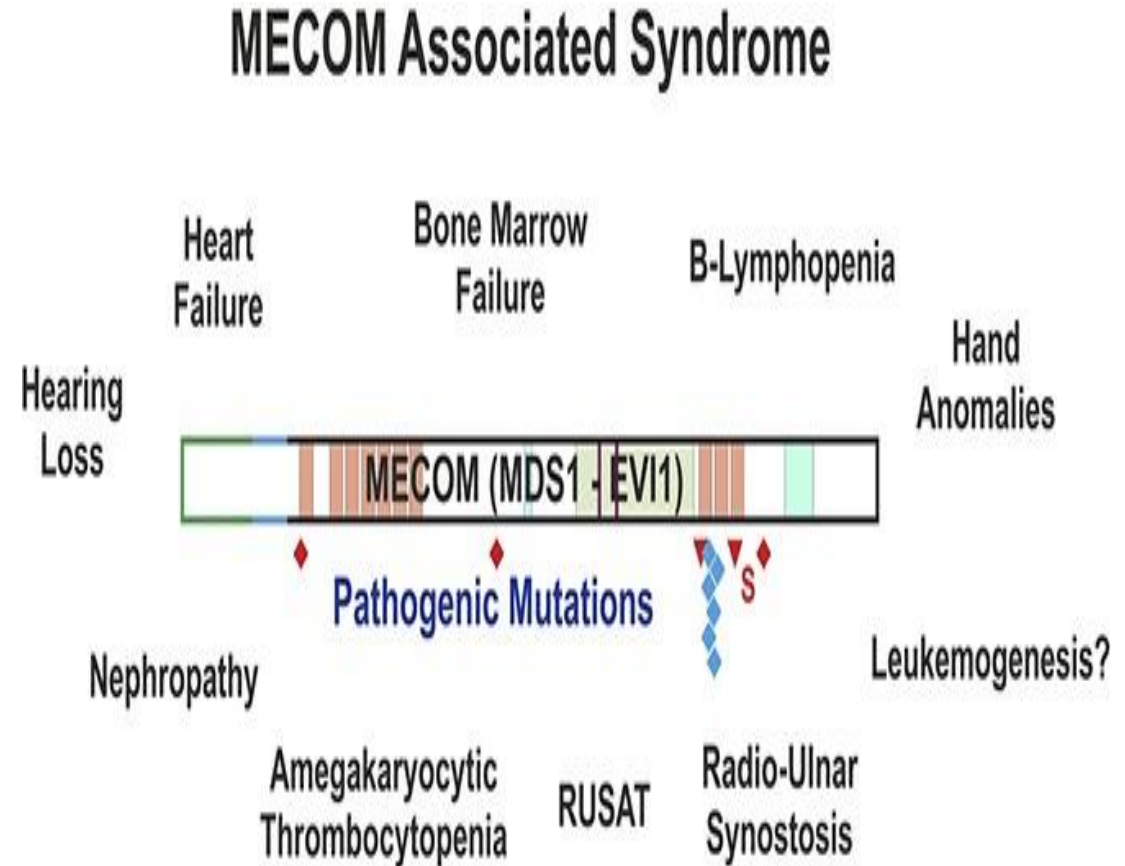
Examination

- Radioulnar synostosis
- Dysplastic right thumb
- Dysmorphic ears
- Retrognathia



Whole exome sequencing

- MECOM mutation



Step 2

The right donor

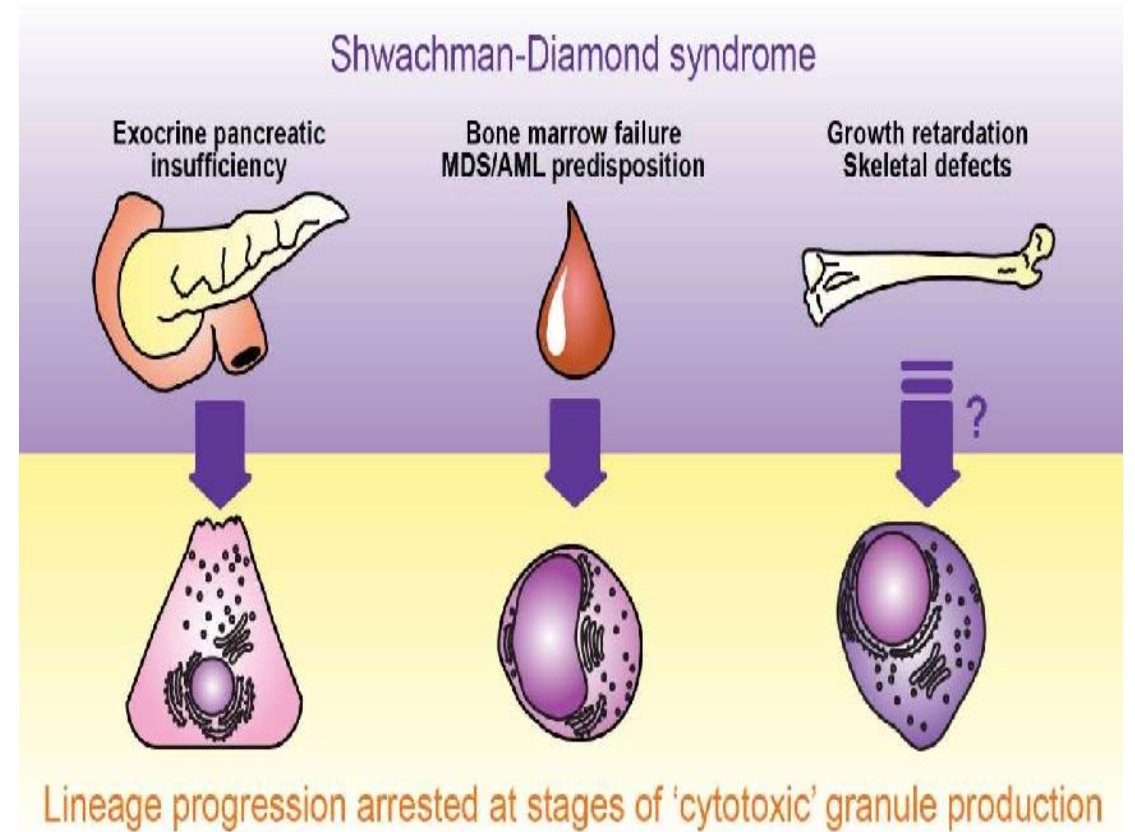


Story

- Five year old boy, one of twins
- Evaluated for recurrent febrile illnesses from infancy
- PRBC transfusion at 3 months of age for Hb 4g%
- Oily stools at 18 months of age
- Pneumonia at 2 years of age
- Culture positive E.coli UTI at 3 years of age
- Pancreatic insufficiency - on enzyme replacement

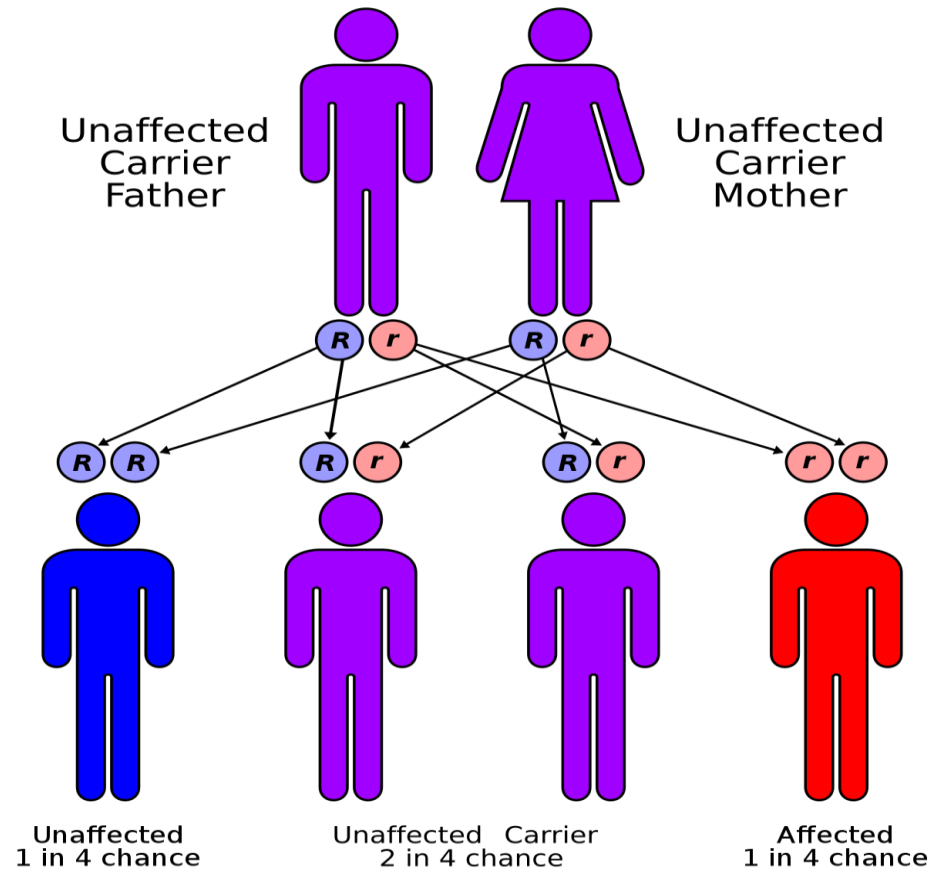
Whole exome sequencing

- Shwachmann Diamond syndrome
- Twin unaffected
- Twin fully matched



Zambetti N. 2016.

Screening of donors



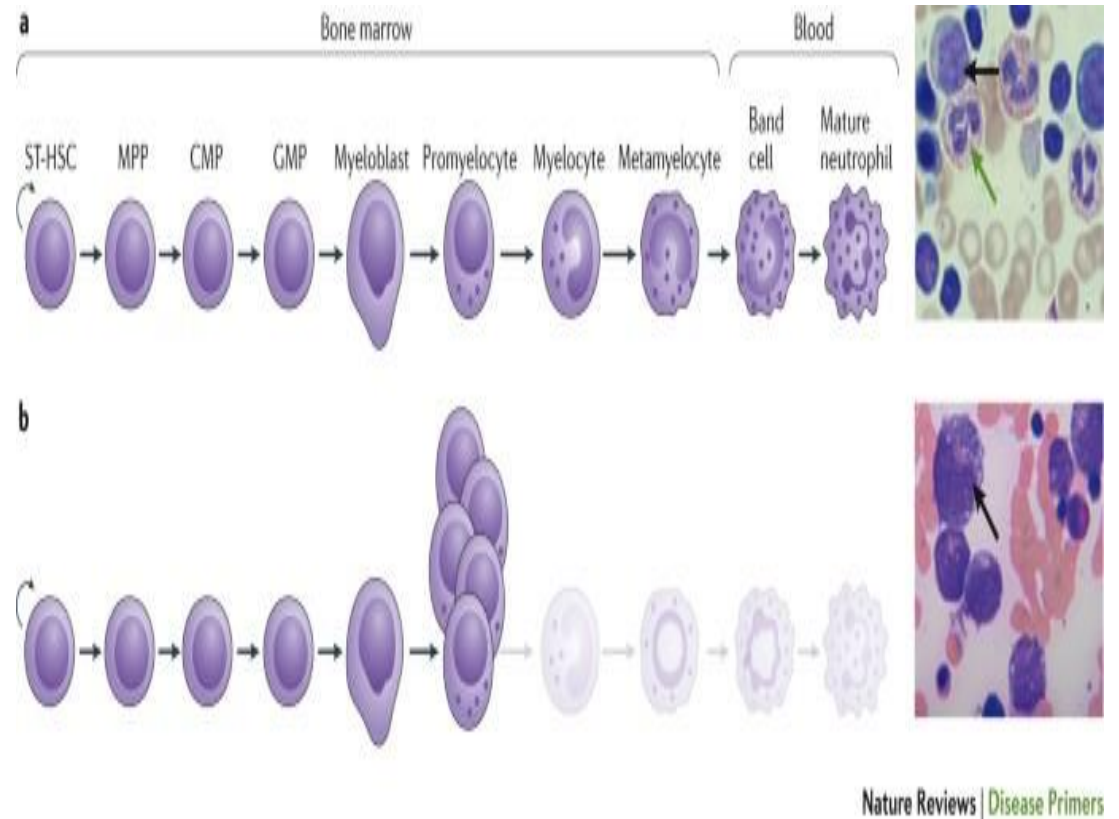
Step 3

Conditioning



Story

- Ten year old boy
- Recurrent gingivitis since infancy
- Recurrent febrile illness since infancy
- Persistent neutropenia
- Bone marrow aspiration – myeloid maturation arrest
- Gene mutation – ELANE mutation



Myeloablative conditioning

- Fully matched father

D-7

Thiotepa 8mg/kg

D-6 to
D-4

Treosulphan 14gram/m²

D-6 to
D-3

Fludarabine 40mg/m²

- GVHD prophylaxis
- Tacrolimus
- Short course methotrexate

Step 4

Infusion of stem cells



Story

- Thirteen year old girl
- Evaluated by several endocrinologists for short stature
- Incidentally found pancytopenia
- Fanconi anaemia
- Fully matched sibling
- RIC and bone marrow

Step 5

Supportive care




Story


- Five year old boy
- Fanconi anaemia diagnosed at 3 years of age
- Multiply transfused with PRBC and platelets
- High ferritin – 6700
- Elevated liver enzymes – SGPT 250
- Hepatitis C positive

Measures for supportive care

- N- acetyl cysteine infusion along with cyclophosphamide
- 10 gram/kg/day as continuous infusion
- Extend MESNA infusion on D+5

Biol Blood Marrow Transplant 26 (2020) 2292–2298

 **Biology of Blood and Marrow Transplantation**
journal homepage: www.bbmt.org



Haploidentical Stem Cell Transplantation with Post-Transplant Cyclophosphamide in Fanconi Anemia: Improving Outcomes with Improved Supportive Care in India

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Haploidentical stem cell transplantation
Post-transplant cyclophosphamide
Ruxolitinib
N-acetyl cysteine
Ferritin

ABSTRACT
Fanconi anemia is the most common inherited bone marrow failure syndrome, and hematopoietic stem cell transplantation (HSCT) is the only curative option. Post-transplant cyclophosphamide (PTCY) is challenging in this group of children, given their increased sensitivity to chemotherapy. We performed a retrospective analysis of the data on children diagnosed with Fanconi anemia who underwent a haploidentical HSCT with PTCY from January 2014 to December 2019. Nineteen children (male/female, 0.75:1) underwent 21 haplo-HSCTs with PTCY. Fludarabine, low-dose cyclophosphamide, and 200 centi-gray total body irradiation were included in the conditioning regimen with 25 mg/kg PTCY on days +3 and +4. Haplo-graft was from a sibling in 38% and father in 57% of transplants. The source of stem cells was peripheral blood stem cells in 81% and bone marrow in 19% of transplants, with a median CD34 dose of $5.0 \times 10^6/\text{kg}$. We documented engraftment in 84% and primary graft failure in 10% of transplants. N-acetylcysteine (NAC) was infused concomitantly during cyclophosphamide in 13 children. Grade 2 and 3 mucositis was lower among those who received NAC as compared to those who did not (30% and 15% versus 33% and 50%), while transaminitis was higher among those who did not receive the infusion. The incidence of acute graft-versus-host disease (GVHD) was 68%, and 81% of these were steroid responsive (grade III). We documented chronic GVHD in 25% children, predominantly involving the skin and mouth, which responded to low-dose steroids and ruxolitinib. Serum ferritin was monitored twice weekly as a surrogate marker for cytokine release syndrome due to nonavailability of IL-6 levels. A 1- or 2-log increase in the titers of ferritin associated with clinical features guided the early addition of steroids in the preengraftment period. The mean survival was found to be less among those with high serum ferritin ($>10,000$ ng/dL) in the periengraftment period as compared to those with ferritin $\leq 10,000$ ng/dL (mean survival of 25 ± 10 months versus 50 ± 6 months, respectively). The overall survival in our cohort was 68.4%, with a mean survival time of 41.5 months (95% confidence interval, 29.3 to 53.8 months), with a statistically significant correlation between inferior outcome and having received over 15 transfusions before HSCT ($P = .01$). PTCY can be considered a viable option in children with Fanconi anemia, particularly in resource-limited settings given the high costs of HSCTs. Focused interventions in this subset of children help improve survival outcomes. Early identification of cytokine release syndrome and risk-adapted steroid therapy during engraftment helps prevent mortality. The concomitant use of NAC during cyclophosphamide infusion helps reduce oxygen free radical related tissue damage and regimen-related toxicity.
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Fanconi anemia (FA) is the most common inherited bone marrow failure syndrome. Presentations can be varied, ranging from asymptomatic, phenotypically normal to features of dysmorphism and severe pancytopenia [1,2]. Twenty genes have been linked to FA, with FANCA documented most often [3,4]. The diagnosis is confirmed by performing stress cytogenetics with mitomycin C or diepoxybutane that help identify excessive breaks per cell in the metaphases. Whole-exome sequencing and mutation analysis further confirm the diagnosis [1]. FA is associated with a high propensity for hematologic malignancies, marrow failure, aplasia, and head and neck malignancies [5]. Hematopoietic stem cell transplantation

Financial disclosure: See Acknowledgments on page 2297.
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E-mail address: ramya.december@gmail.com (R. Uppuluri).

Step 6

Balance graft and infections



Story

- 12 year old boy
 - Fanconi anaemia
 - Multiply transfused
 - PRBC, platelets
 - High ferritin
-
- Matched unaffected sibling donor HSCT

Stormy course

- Acute grade I/II skin and gut GVHD
- Responsive to steroids

- Hyperglycemia
- Requires insulin

- Hypertension with PRES
- Required antihypertensives

18 months post HSCT

- Well
- 100% donor chimerism
- GVHD settled
- Steroids stopped

- Hyperglycemia persistent
- On insulin

- Elevated ferritin
- On phlebotomy

Experience from our centre in Fanconi anaemia





Biology of Blood and Marrow
Transplantation

Volume 26, Issue 12, December 2020, Pages 2292-2298

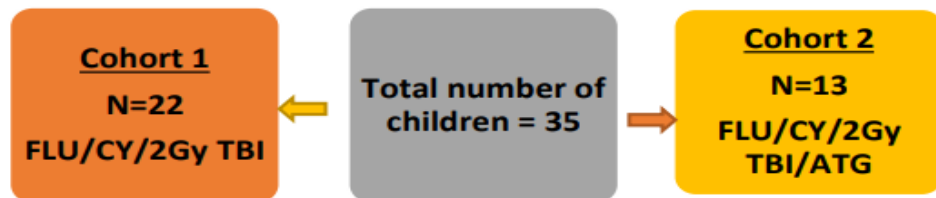


Haploidentical Stem Cell Transplantation with Post-Transplant Cyclophosphamide in Fanconi Anemia: Improving Outcomes with Improved Supportive Care in India

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[Balasubramaniam Ramakrishnan](#)¹, [Indira Jayakumar](#)², [Revathi Raj](#)¹

Day -7 to Day -2	INJ.FLUDARABINE 50 mg in 100ml <u>NS</u> over 1 hr FROM D-7, D-6 AND D-5 INJ.FLUDARABINE 25 mg in 100ml <u>NS</u> over 1 hr D-4 AND D-3 INJ.FLUDARABINE 10 mg in 100ml <u>NS</u> over 1 hr D-4 AND D-3	30mg/m ² /day X 6 days
Day -3 AND Day -2	Inj CYCLOPHOSPHAMIDE 170 mg in 100ml NS over 2 hrs Inj. <u>Mesna</u> 250 mg in 240 ml NS to be given over 23 hours after cyclophosphamide	5mg / kg / day x 2 days
Day -2	Tab. <u>Pangraf</u> 0.5 mg BD	
Day- 1	TBI 200cGy in single dose	
Day - 0	Peripheral blood stem cells to be infused	
Day +3 to Day +4	Inj CYCLOPHOSPHAMIDE 850 mg in 100ml NS over 1 hr Inj. <u>Mesna</u> 1200 mg in 250 ml NS to be given over 23 hours after cyclophosphamide Inj N- Acetyl Cysteine 8150 <u>mgs</u> IN 250 ML OF 5% Dextrose iv over 23 hours post -cyclophosphamide	25mg / kg / day x 2 days

Comparison of two cohorts – Impact of ATG



VARIABLES	Cohort 1	Cohort 2
No of patients	22	13
Conditioning regimen	FLU/CY/2GYTBI	FLU/CY/2GYTBI/ATG
Male:female	1.2:1	0.8:1
PBSC:Bone marrow	80:20	100% PBSC
Acute GVHD	73% (16/22)	7% (1/13)
Chronic GVHD	36% (8/22)	15% (2/13)
Viral Reactivation	45%(10/22)	30.7% (4/13)
Engraftment	77% (17/22)	77% (10/13)
Graft failure	23% (5/22)	23% (3/13)
Mortality	36.3% (8/22)	30.7% (4/13)

Serotherapy with ATG has a significant impact on reducing GVHD from 73% to 7% in children with Fanconi anaemia

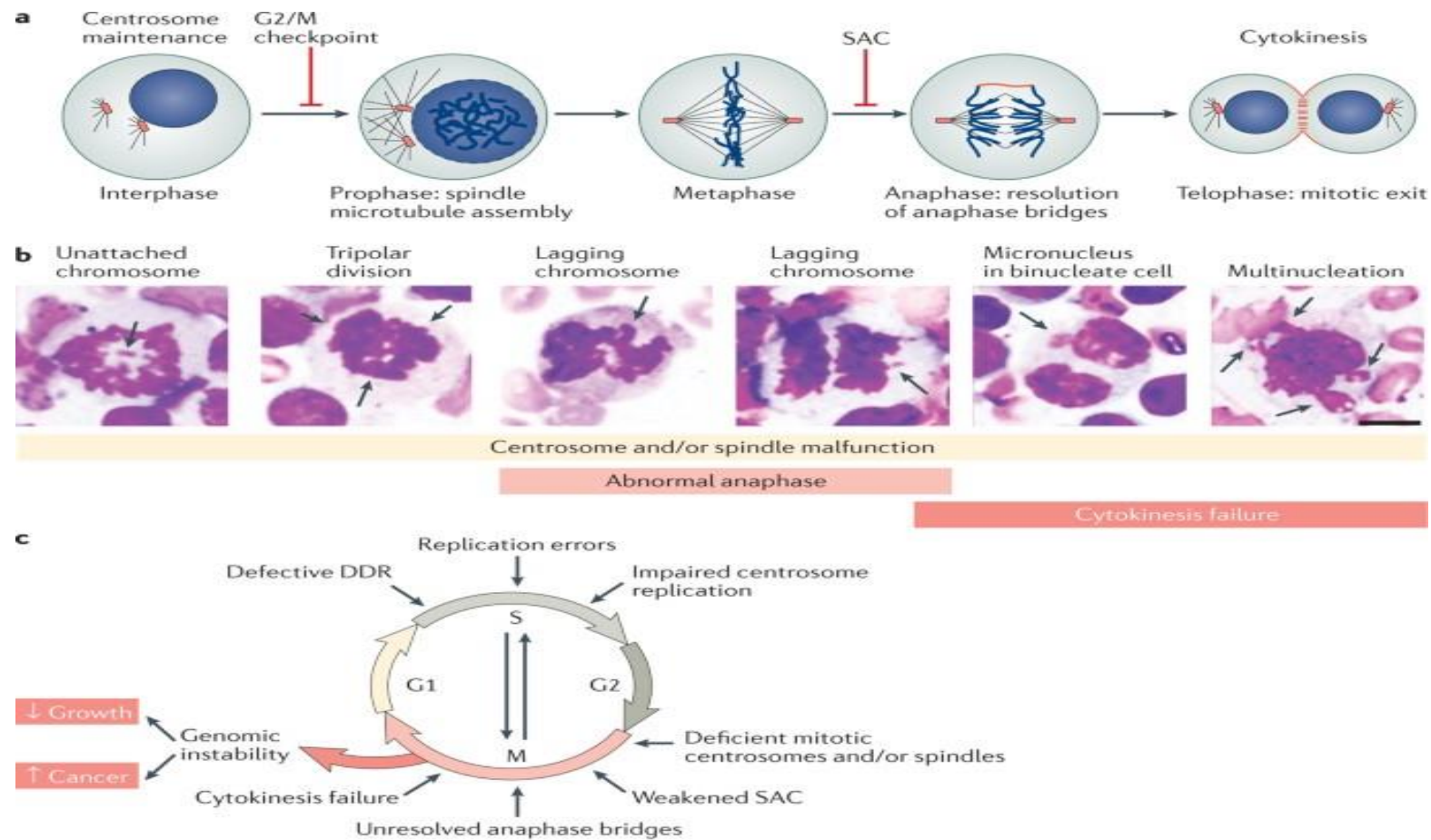
Increase in overall survival from 64% to 70% although it did not have an impact on graft failure

Step 7

Late effects



Late effects – mortality



Nature Reviews | Cancer

Story

- Diagnosed at 2.5 years of age
- Ectopic crossed fused right kidney
- Fanconi anaemia
- HSCT - At the age of 7 years (2010)
- From fully matched father

Detection of malignancy

- 12 years post HSCT – Painful lesion over the tongue for 6 weeks with trismus, swallowing difficulty, weight loss
- Biopsy showed moderately differentiated squamous cell carcinoma
Carcinoma tongue with nodal metastasis

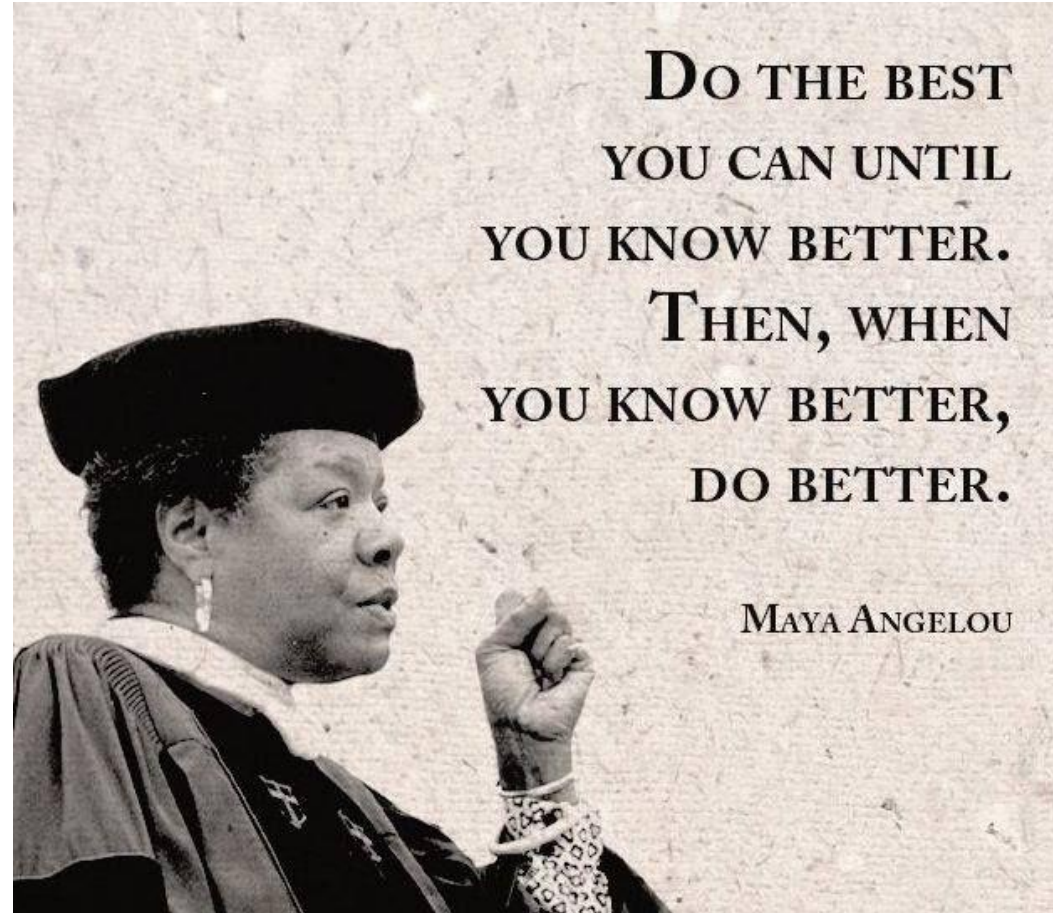
Clinical course

- Offered for surgical resection after counseling regarding morbidity and mortality
- Family chose palliative care
- Succumbed to illness 4 months later at home

IBMFS

- Alkylator and radiation free regimens and TDM based conditioning are ideal but expensive
- FA, DKC and Cerunnunos require RIC
- DBA, SDBS, CN and CAMT require MAC conditioning
- Treosulfan based conditioning is well tolerated for the MAC regimens
- Rabbit ATG is essential to improve survival
- NAC offers organ protection
- Watch for late effects


Thank you!



Quiz 1

- Child with pancytopenia and monosomy 7 with SAMD9
- Blasts 19%
- Option 1 - Upfront HSCT
- Option 2 – Chemotherapy then HSCT

ACUTE MYELOID LEUKEMIA (AML) PANEL BY FLUORESCENCE IN-SITU HYBRIDIZATION-REPORT

Aberration	Image	Probe	Cells scored	RESULT
del 7q (-7)		Vysis D7S486/CEP 7 FISH probe	400	POSITIVE for Monosomy 7 (49%)
nuc ish(CEP7,D7S486)x1[196/400]				

Percentages in parentheses indicate the percentage of nuclei scored showing the abnormality. Validated normal Cut-off for FISH probes: - Fusion signal 2%, Break apart 5%, deletion 5% and chromosome enumeration 5%.

INTERPRETATION: FISH analysis for 400 interphase nuclei shows -

- **Monosomy of chromosome 7 in 49%** of nuclei scored.
- **Negative for ETO::AML1, PML::RARA fusions, rearrangement of MLL, CBFβ genes and deletion 5q.**

Comments: Please correlate with clinical, hematological findings and conventional karyotyping.

Limitation: The probes in this FISH panel detect only specific aberrations. Chromosomal alterations present outside the regions targeted by the probes will not be detected.

References:

1. Ros J, Hastings Sarah Moore, Nicole Chia IScN 2024 An International System for Human Cytogenomic Nomenclature (2024). S.Karger AG ISBN print:978-3-318-07330-0 DOI:https://doi.org/10.1159/isbn.978-3-318-07331-7.
2. WHO Classification of Tumours Editorial Board, Haematolymphoid Tumours, International Agency for Research on Cancer, January 2024. ISBNs 978-9-28-324520-9.

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Quiz 2

- BMFS the combination of abatacept and PTCY reduces the chance of GVHD
- True or False?