3RD SCIENTIFIC SYMPOSIUM OF THE WBMT

16th November 2014
Cape Town - South Africa

Hematopoietic Stem Cell Transplantation for Thalassemia Major

Alok Srivastava
Department of Haematology
Christian Medical College
Vellore, India

Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine

haematologica 2004; 89(10):October 2004

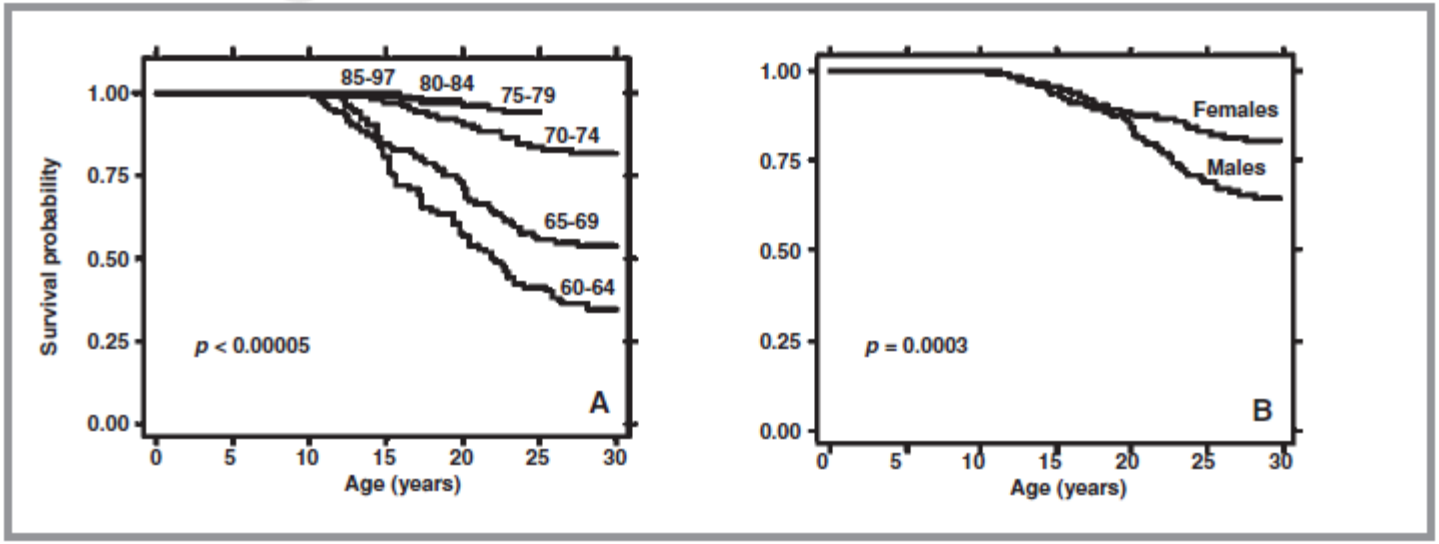


Figure 2. Kaplan-Meier survival curves, after the first decade of life, by birth cohort (A) and by sex (B).

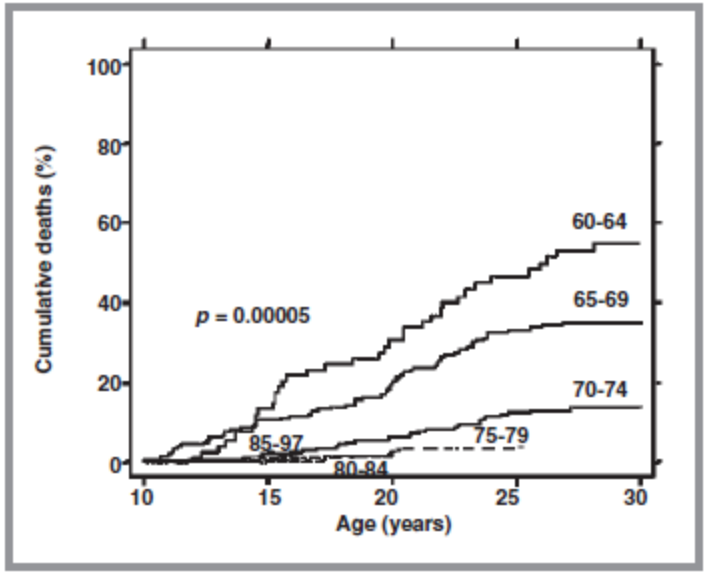


Table 2. Distribution of complications affecting 720 patients born after 1970.

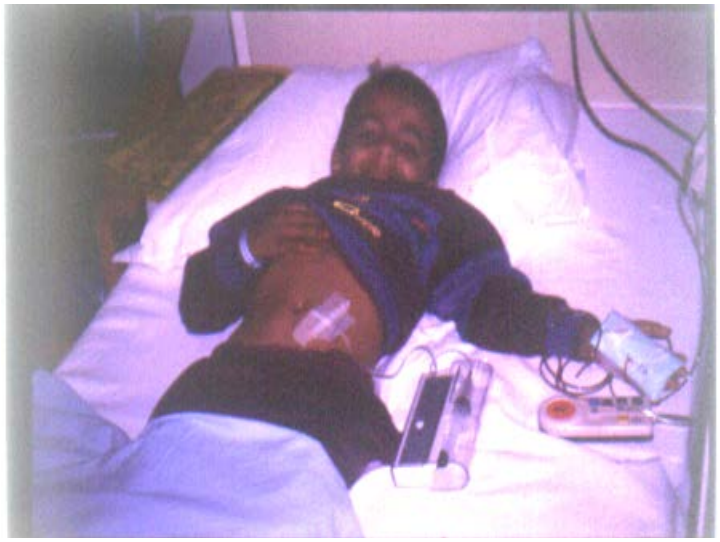
Complication	*N*	*%*
HEART FAILURE	49	6.8
ARRHYTHMIA	41	5.7
DIABETES	46	6.4
HIV INFECTION	12	1.7
THROMBOSIS	8	1.1
HYPOTHYROIDISM	78	10.8
HYPOGONADISM*	273	54.7

**Only 499 patients were old enough to be assessed for hypogonadism.*

Medical management of Thalassemia Major in Developing countries

- Transfusion
  - Packed red cells to maintain Hb>9gm%
  - Use leucodepletion filters
- Chelation
  - Initiate when ferritin is ~1000ng/ml
  - Desferrioxamine 50mg/Kg SC CI / Deferasirox 20-40mg/kg PO / Deferiprone 75-100mg/kg PO
  - Monitor ferritin

Challenges: Access / Compliance / Cost / Safety



Health | Updated Oct 03, 2011 at 12:29pm IST

Gujarat: 23 thalassemic kids turn HIV positive

CNN-IBN

Follow @ibnlive

Recommend Send

3 Tweet 0 Like Share

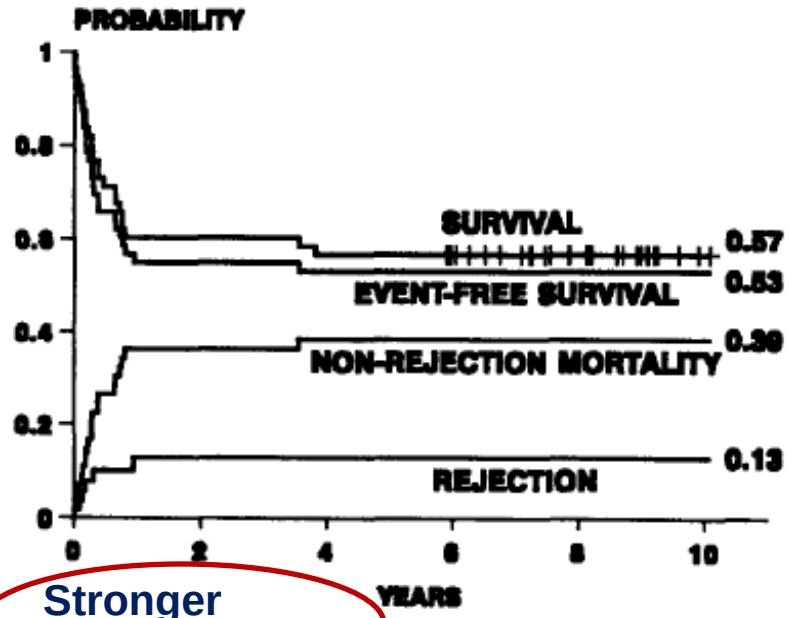
Click to play video

Junagadh: As many as 23 children, suffering from thalassemia in Junagadh in Gujarat, have been found to be HIV positive. Angry parents have blamed government hospital authorities for infecting the children during blood transfusions. But the medical staff have denied it.

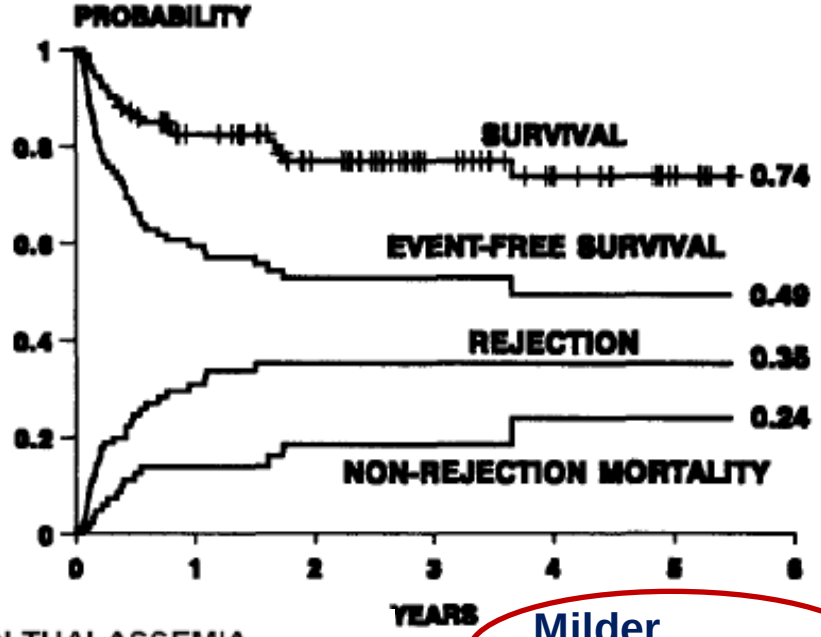
For the little kids, blood transfusions are a reality they have to live with, to stay alive. Since January, over 100 thalassemic children below the age of ten years have been getting transfusions at the Government hospital in Junagadh. But now parents allege the children have been infected with HIV by the hospital authorities.

Marrow Transplantation for Patients With Thalassemia: Results in Class 3 Patients

Blood, Vol 87, No 5 (March 1), 1996: pp 2082-2088

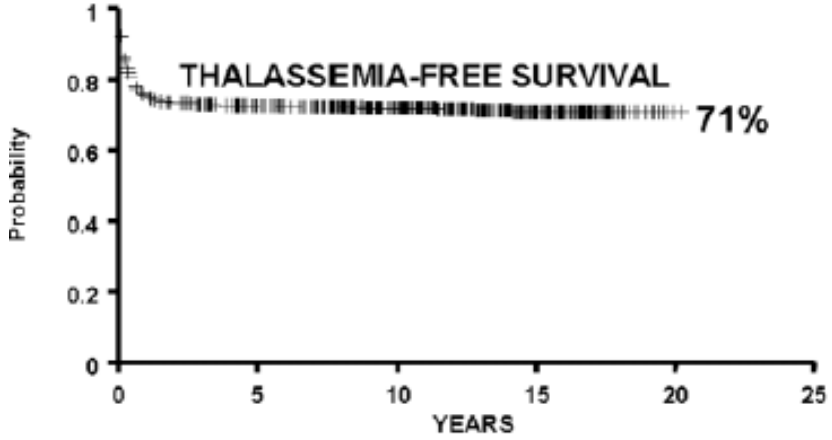


Stronger conditioning



Milder conditioning

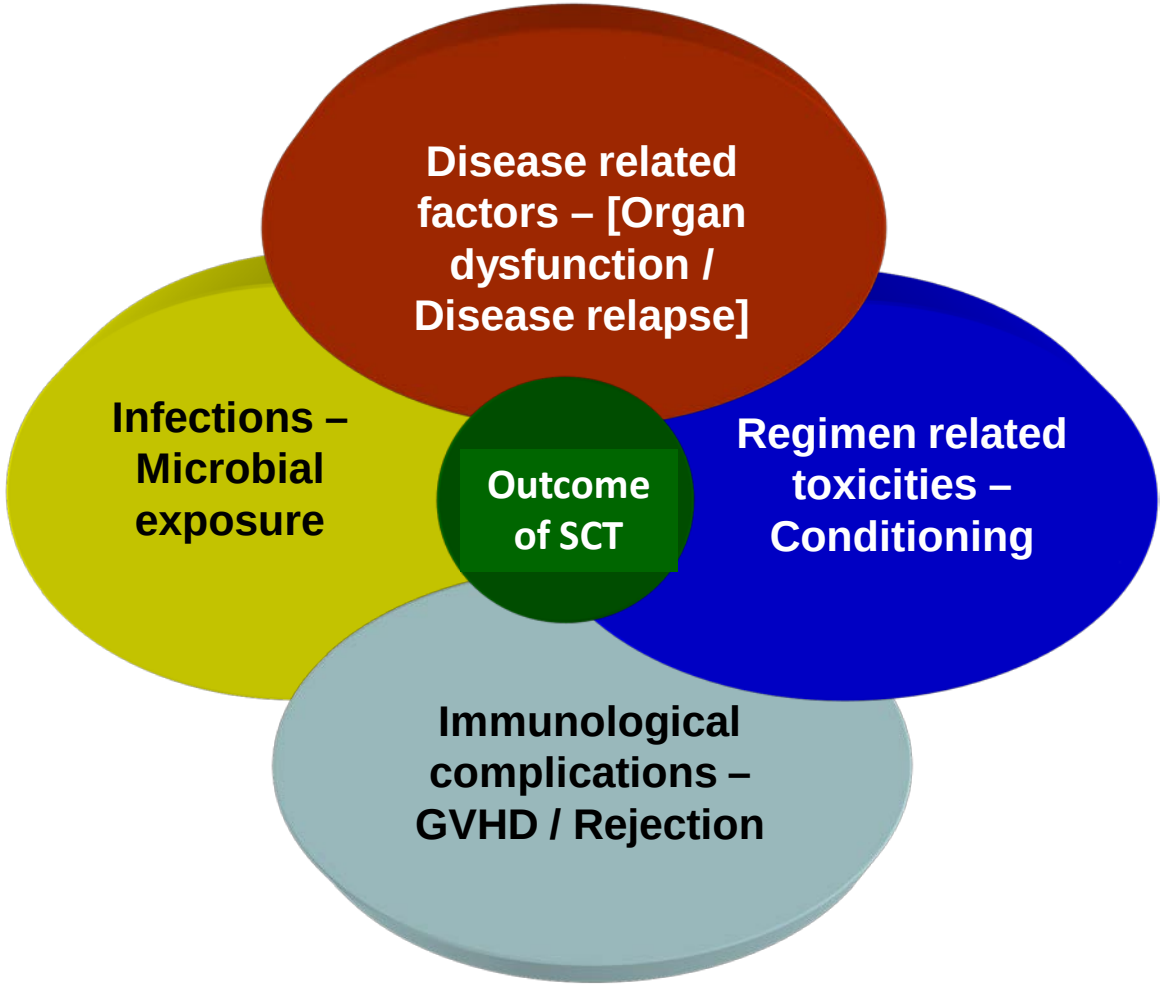
951 TRANSPLANTS IN THALASSEMIA
AGE 1 THROUGH 35 YEARS



Hematology 2003

~First BMT for thalassemia major done in 1981

Stem Cell Transplantation for Thalassemia –
Causes of failure



***HIGH INCIDENCE OF GRAFT REJECTION IN CLASS III PATIENTS (~35%)**

(42% rejection in the first 17 patients from India transplanted in Pesaro)

***HIGH INCIDENCE OF RRT - VOD / SOS (~30-40%)**

BMT for Thalassemia Major - CMC Vellore

- Between October 1991 to December 2011
- Total No of Patients: 355
  - Median age — 7 years (range: 2 – 24)
  - Males — 221 (62.3%)
  - BM graft — 317 (89.3%)

Risk stratification:	n	%
Class I	16	4.5
Class II	144	40.6
Class III	195	54.9

Liver size > 2cms	236 (66.5%)
Inadequate chelation	319 (89.9%)
Liver fibrosis	247 (69.6%)

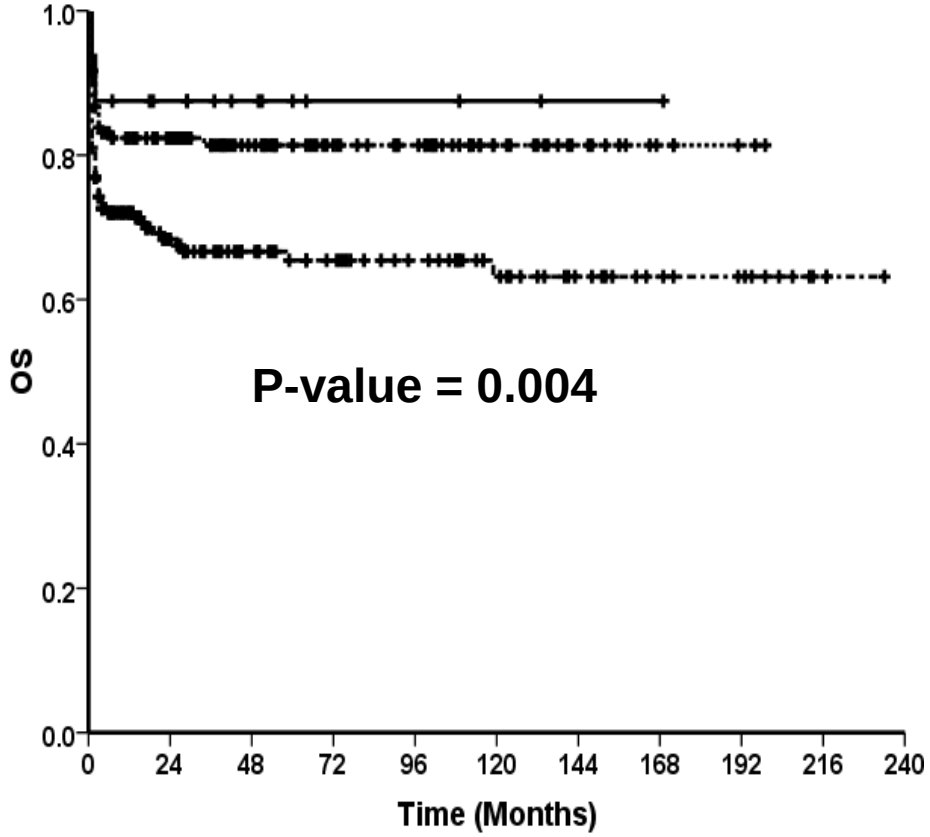
BMT for Thalassemia Major - CMC Vellore

Survival:

Mean follow up 171 months

5 year KM estimate of overall survival 73.2 ± 2.5%

5 year KM estimate of event free survival 66.8±2.6%



Class I : n=14 : 87.5±8.3%

Class II : n=144 : 81.4±3.3%

Class III : n=195 : 65.4±3.7%

Bone Marrow Transplantation (2005) 36, 839–845
© 2005 Nature Publishing Group All rights reserved 0268-3369/05 $30.00
www.nature.com/bmt

Conditioning regimens

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

M Chandy[1], P Balasubramanian[1], SV Ramachandran[1], V Mathews[1], B George[1], D Dennison[2], R Krishnamoorthy[3] and A Srivastava[1]

[1]*Department of Hematology, Christian Medical College, Vellore, India;* [2]*Sultan Qaboos University Hospital, Muscat, Oman; and* [3]*INSERM, U458, Hospital Robert Debre, Paris, France*

1. REGIMEN A [Bu600] – busulfan 600 mg/m^2 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.

***EVALUATE Bu KINETICS – CORRELATE WITH OUTCOME**
***REGULATORS OF Bu METABOLISM – GST/GSH LEVELS (HEPATIC / PLASMA) & GST POLYMORPHISM**

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

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*
Outcome by class					
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

95% CI: *0.08, 0.16; **–0.17, 0.07.

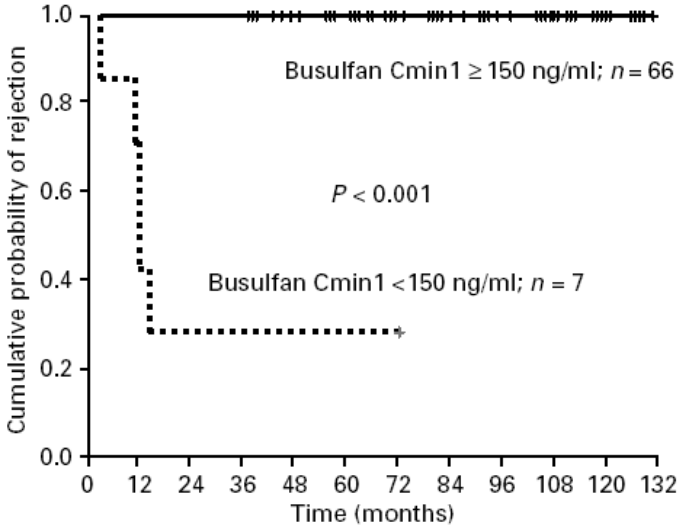


Figure 1 Probability of graft rejection depending on busulfan C_{min}-1.

Bone Marrow Transplantation (2005) 36, 839–845

blood
2004 104: 1574-1577
doi:10.1182/blood-2003-11-3778 originally published online May 13, 2004

Glutathione *S*-transferase M1 polymorphism: a risk factor for hepatic venoocclusive disease in bone marrow transplantation

Alok Srivastava, Balasubramanian Poonkuzhali, Ramachandran V. Shaji, Biju George, Vikram Mathews, Mammen Chandy and Rajagopal Krishnamoorthy

Table 2. Frequency of GSTM1 and GSTT1 genotypes in patients with and without HVOD

	GSTM1 null, n (%)	GSTM1 positive, n (%)	GSTT1 null, n (%)	GSTT1 positive, n (%)
$HVOD^-$	23 (53.5)	58 (81.7)	28 (82)	53 (66.5)
$HVOD^+$	20 (46.5)*	13 (18.3)*	6 (18)†	27 (33.5)†

Table 4. Busulfan pharmacokinetic parameters with reference to GSTM1 genotype

	Css-1, ng/mL	Cl/F-1, L/h/kg
GSTM1 null	544 ± 184	0.40 ± 0.064
GSTM1 positive	667 ± 256	0.333 ± 0.071
*P**	.001	.00001

Css-1 indicates steady-state concentration after the first dose of busulfan; and Cl/F-1, clearance after the first dose of busulfan.

**P* value calculated by 2-tailed *t* test.

Unpredictability of Intravenous Busulfan Pharmacokinetics in Children Undergoing Hematopoietic Stem Cell Transplantation for Advanced Beta Thalassemia: Limited Toxicity with a Dose-Adjustment Policy

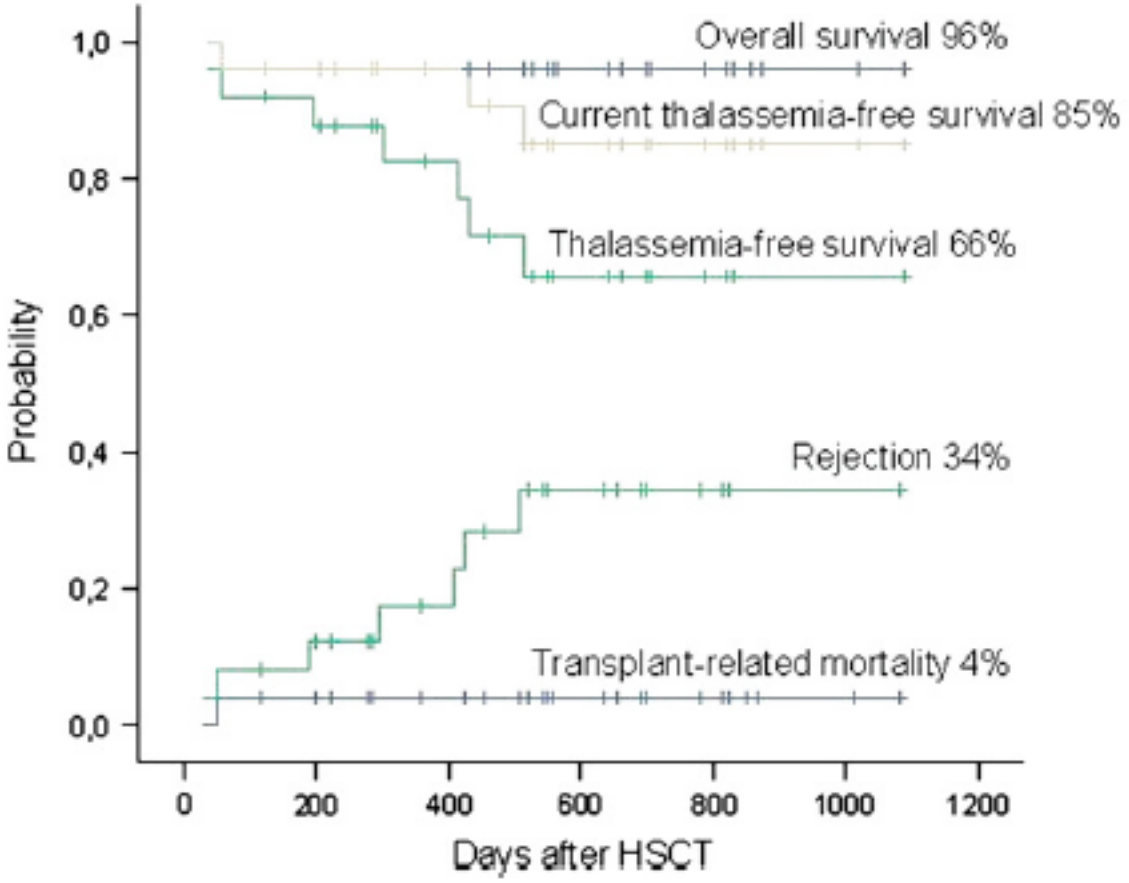


Figure 2. Kaplan-Meier probabilities of OS, DFS, current thalassemia-free survival, rejection, and TRM in 25 patients with class III thalassemia.

Biol Blood Marrow Transplant 16:622-628, 2010

Busulfan PK in Thalassemia Major – Oral vs IV

Age	2-7 yrs	2-7 yrs	2-7 yrs
	n=100	n=41	n=16
Bu Dose	16 mg/kg oral	600mg/m2 oral	0.8 mg/kg IV
AUC1 (mg*h/L)	5.02 ±1.38	5.88 ±1.42	2.96 ± 1.34
Cl (ml/min/kg)	3.58 ±1.08	4.6 ±1.16	6.62 ±5.01

IV Busulfan data using 'generic' Indian product

The oral Busulfan PK data is calculated using NONMEM software by population PK analysis (Carl Panetta, St Jude)
IV Bu PK data is calculated using trapezoidal rule

B Poonkuzhali, CMC Vellore (2010, unpublished)

Population pharmacokinetics of cyclophosphamide in patients with thalassemia major undergoing HSCT

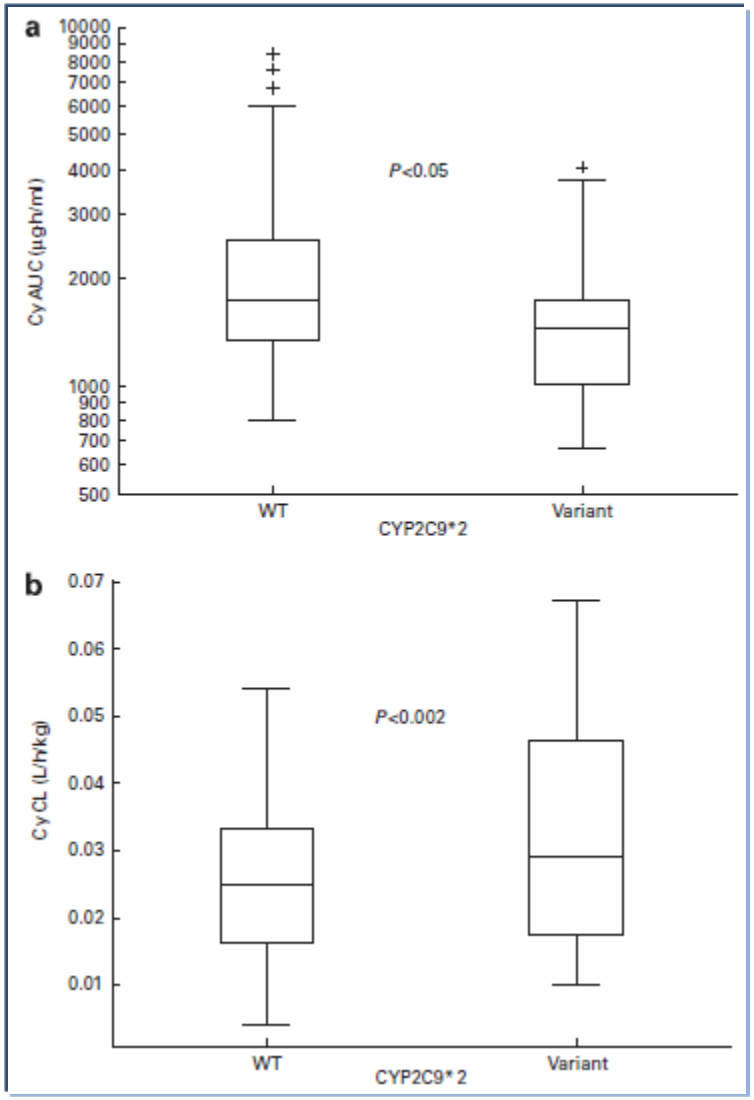
P Balasubramanian[1,4], S Desire[1,4,5], JC Panetta[2,4], KM Lakshmi[1], V Mathews[1], B George[1], A Viswabandya[1], M Chandy[1,6], R Krishnamoorthy[3] and A Srivastava[1]

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

	Population mean (range)	*IIV (CV%)*	*IOV (CV%)*
CY AUC (μg h/mL)	1858.8 (866–9445)	51	62
CY C_{max} (μg/mL)	637.6 (292–2664)	41	48
CY C_{min} (μg/mL)	6.7 (0.06–121)	92	103
CY $t_{1/2}$ (h)	1.59 (0.54–4.2)	24	35
HCY AUC (μg h/mL)	5200 (953–22573)	45	24
HCY C_{max} (μg/mL)	1700 (250–6542)	49	33
HCY C_{min} (μg/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**



Bone Marrow Transplantation (2012) **47**, 1178–1185

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 Lakshmi[1], V Mathews[1], B George[1], A Viswabandya[1], M Chandy[1,6], R Krishnamoorthy[3] and A Srivastava[1]

Table 3. Comparison of CL (clearance) (L/h/m^2) in children receiving myeloablative doses of CY

Group	No. of cases	Disease	Age (years) Median (range)	CY CL (L/h/m^2)
Qiu *et al.*[30a]	8	Leukemia	11.2 (2.8–15.9)	2.56 (1.69–3.95)
Chinnaswamy *et al.*[11]	11	Rhabdomyosarcoma	12.8 (5.4–21)	3.02 (2.3–4.0)
Yule *et al.*[43]	38	Cancer		2.9 (1.2–10.6)
Present study	55	Thalassemia	7.3 (2–14)	0.55 (0.12–1.43)

[a]CY PK data from pediatric patients included in the study were requested from investigators and obtained.

Table 4. Comparison of CY and HCY pharmacokinetic parameters between this study and previous studies using BU/CY-conditioning regimen for HSCT

Group	No. of cases	Disease	Age (years) median (range)	BU dose (mg/kg)	Total CY dose (mg/kg)	CY C_{max} (μg/mL)	CY $t_{1/2}$ (h)	CY AUC (μg h/mL)	HCY C_{max} (μg/mL)	HCY $t_{1/2}$ (h)	HCY AUC (μg h/mL)	Ratio HCY/CY
Hassan *et al.*[20]	12	CML/AML	37 (5-51)	16	100-120	390 ± 223	10.9 ± 2.9	1913 ± 1019	1.14 ± 0.54	8.14 ± 0.25	8.34 ± 2.13	0.0053 ± 0.002
Slattery *et al.*[19]	7	BrCa/AML	42 (12-52)	Variable	120-200	227 ± 56	2.36 ± 0.37	861 ± 172	–	2.19 ± 0.74	–	0.123 ± 0.036
McCune *et al.*[17]	75	MDS/AML/CML	47 (20-66)	Variable[a]	120	87.1 ± 48	–	715 ± 332	10.2 ± 5.3	–	8.5 ± 2.9	0.011
Present study	55	Thalassaemia major	7.3 (2-14)	16	200	723 ± 375	1.73 ± 0.5	2276 ± 1585	1.98 ± 1.1	0.13 ± 0.024	6.04 ± 3.3	0.0037 ± 0.0032
P-value 1 vs 4			0.0001			0.004	0.000	0.452	0.013	0.0001	0.024	0.102
P-value 2 vs 4			0.0001			0.001	0.002	0.022	–	0.0001	–	0.0001
P-value 3 vs 4			0.0001			0.0001	–	0.0001	0.0001	–	0.0001	0.0001

Abbreviations: AUC = area under the concentration curve; BrCa = breast cancer; HCY = hydroxy CY. *P*-values generated using two-tailed *t*-test. [a]These patients received targeted BU doses.

HSCT for Thalassemia Major

***Extensive PK and PG evaluation of Busulfan and Cyclophosphamide.**

***Elaborate assessment of immunological aspects of graft composition and post-engraftment recovery**

***Not a major impact on outcome of high risk patients**

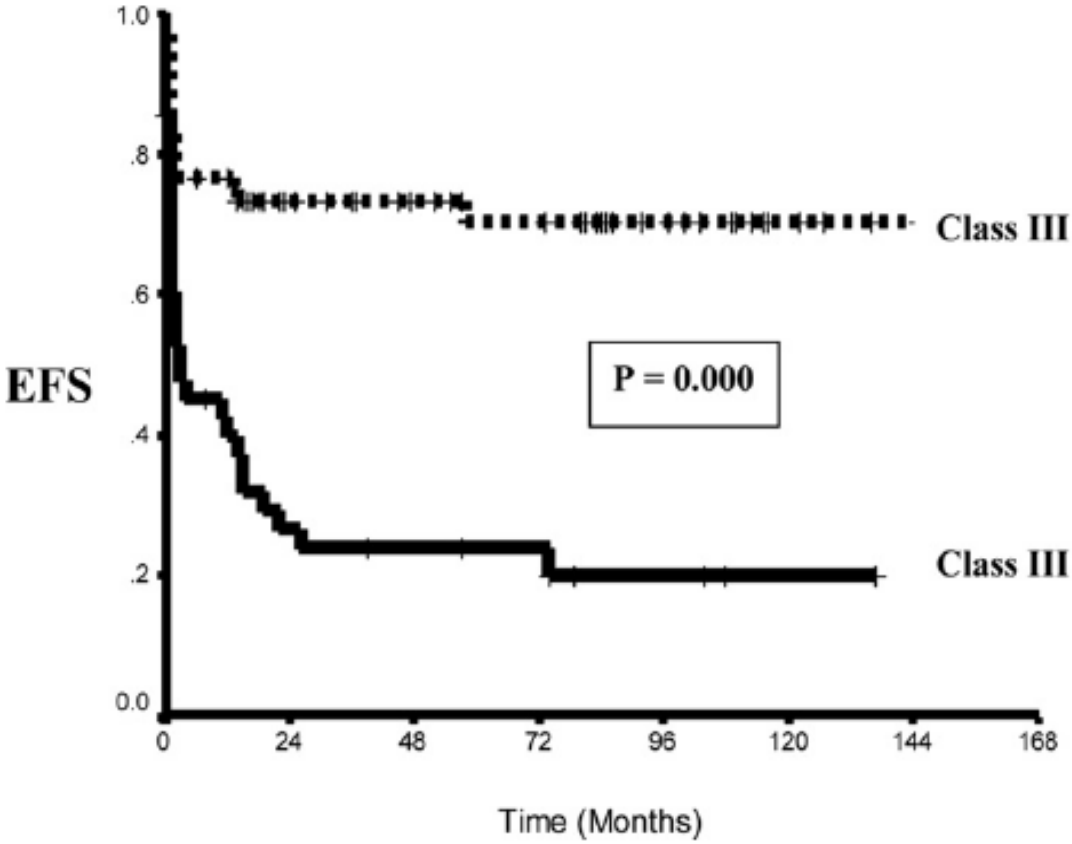
BMT for Thal Major - CMC Vellore
Risk Stratification - Limitations

- **Class III a heterogeneous group (in the setting of sub optimal medical therapy prior to transplant)**

	Patient I	Patient II
Liver size	3 cms	7cms
Inadequate chelation	+	+
Fibrosis	+	++
Age	6 years	14 years
Spleen size	NP	5 cms

A New Stratification Strategy That Identifies a Subset of Class III Patients with an Adverse Prognosis among Children with β Thalassemia Major Undergoing a Matched Related Allogeneic Stem Cell Transplantation

Biology of Blood and Marrow Transplantation 13:889-894 (2007)

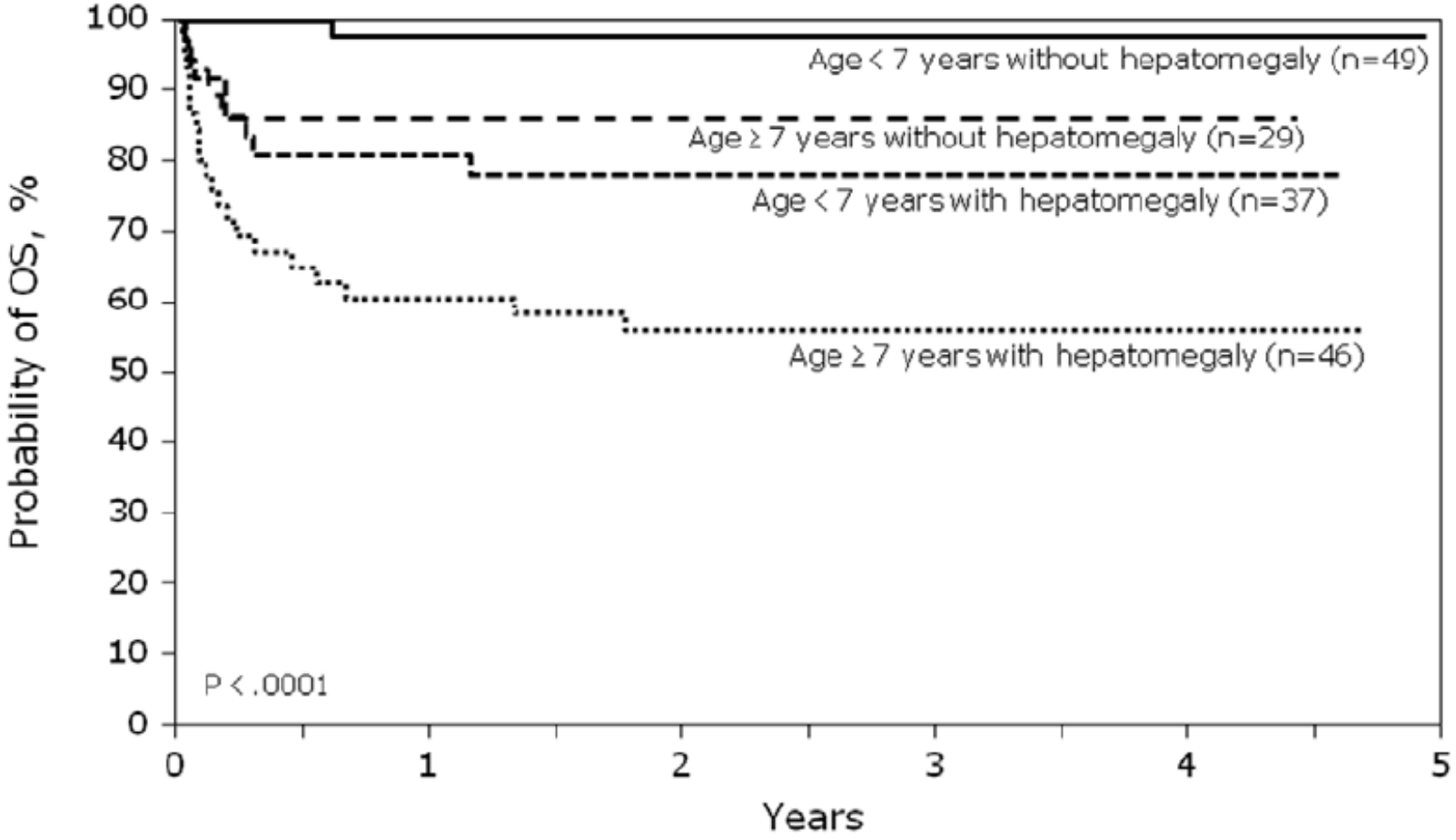


Age	≥ 7years
Liver size	≥5cms
Class III HR	40%

!. Comparison of 5-year EFS of Class III transplants in the high-risk group (n = 41) and rest of transplants in Class III (n = 64).

blood
2011 117: 1745-1750
doi:10.1182/blood-2010-09-306829 originally published online November 30, 2010

HLA-matched sibling bone marrow transplantation for β-thalassemia major



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

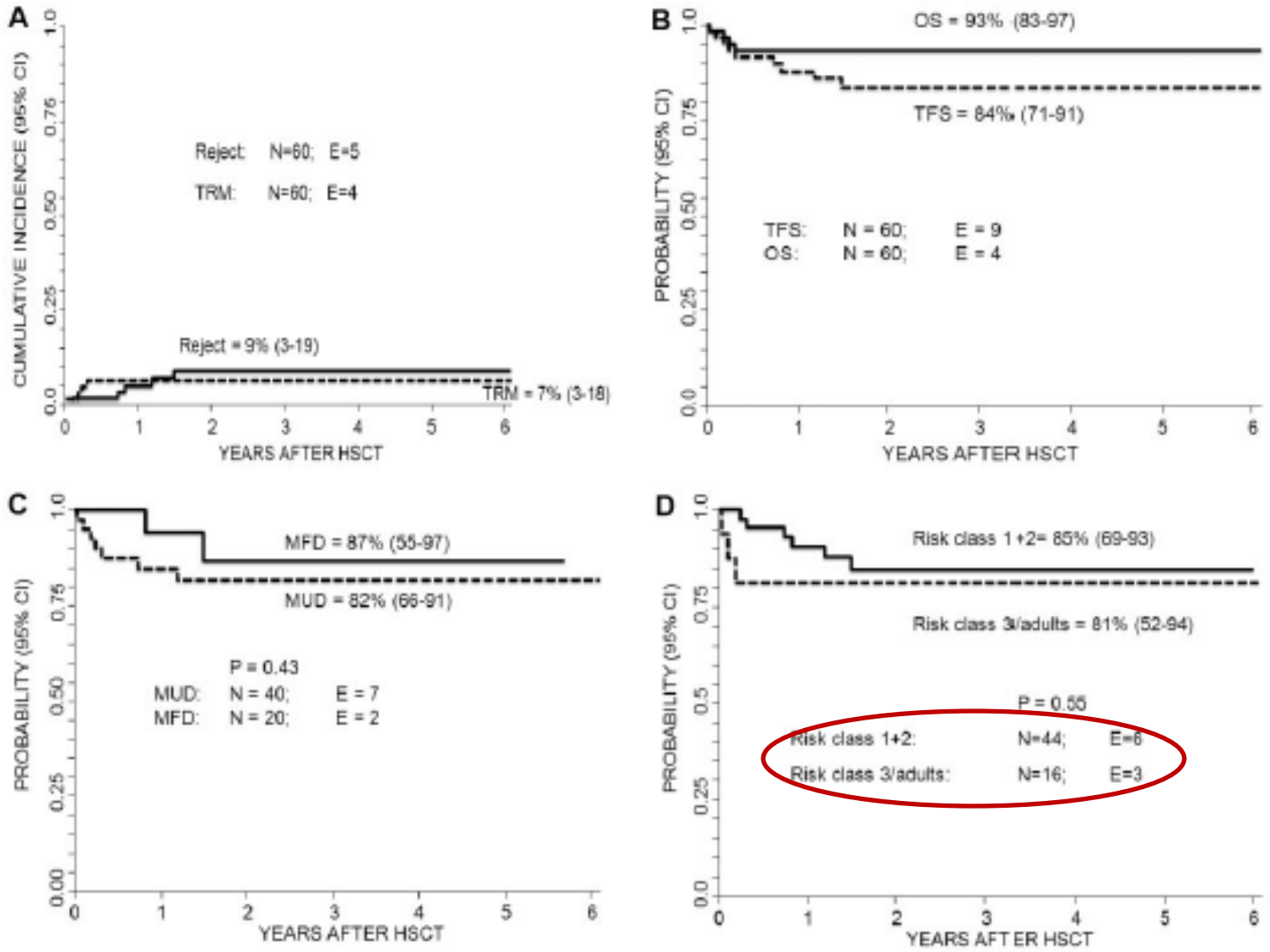


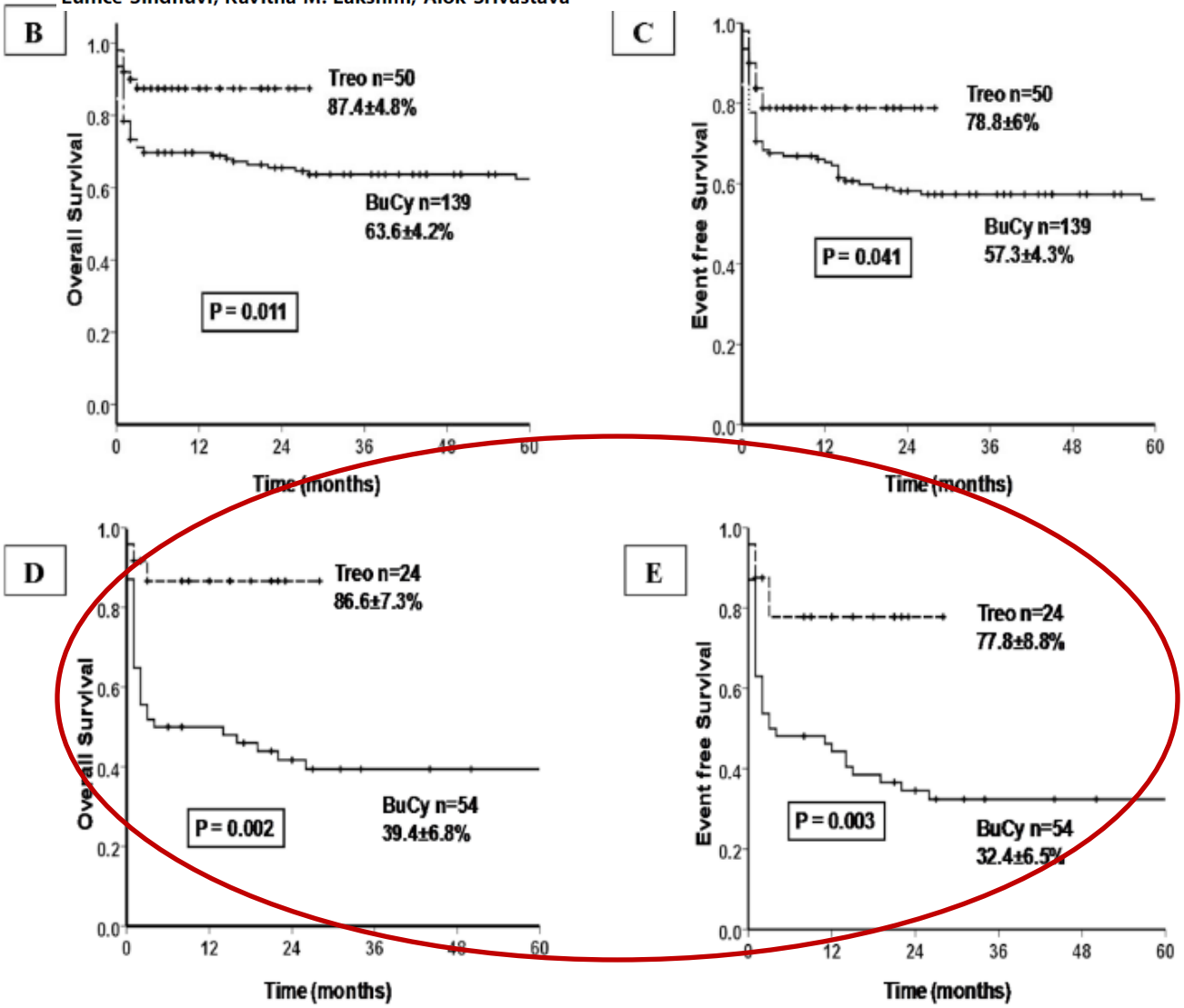
Figure 1. Outcomes of the study population. (A) Cumulative incidence of transplantation-related mortality (TRM) and graft rejection (Reject). (B) Five-year Kaplan-Meyer estimate of overall survival (OS) and TFS for the whole cohort of patients. (C) Five-year Kaplan-Meyer estimate of TFS according to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

Blood 2012; 120: 473-6

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

PLOS ONE | April 2013 | Volume 8 | Issue 4 | e61637

Vikram Mathews*, Biju George, Auro Viswabandya, Aby Abraham, Rayaz Ahmed, Abhijeet Ganapule, Eunice Sindhuvi, Kavitha M. Lakshmi, Alok Srivastava



Treosulfan based conditioning for Thalassemia Major

***Better toxicity profile – Larger therapeutic window**

***Higher incidence of mixed chimerism requiring manipulation of post HSCT immunosuppression / DLI**

***Major impact on outcome of high risk patients**

***Limited data on PK so far**

***Cost a major limitation**

****Generic Treosulfan – received conditional approval from DCGI***

****Subject to PK data being submitted – Study being initiated to be completed over the next 4-6 months***

blood
2012 120: 3875-3881
doi:10.1182/blood-2012-03-417998 originally published online September 11, 2012

A novel conditioning regimen improves outcomes in β-thalassemia major patients using unrelated donor peripheral blood stem cell transplantation

Chunfu Li, Xuedong Wu, Xiaoqing Feng, Yuelin He, Huaying Liu, Fuyu Pei, Jianyu Liao, Lan He, Lei Shi, Na Li, Qiujun Liu, Shiting Liu, Geyu Chen, Qingxia Su, Yuqiong Ren, Yanhua Wang and Wanxia Tan

Cyclophos: 55mg/kg/day days -10, -9
Busulfan: 2.8-4.4mg/kg/day days -8 to -6
Thiotepa: 10mg/kg/day day -5
Fludarabine: 40mg/m^2/day days -8 to -4

Table 1. NF-index of categorization of NF-08-TM

Group	Ferritin	Hepatomegaly	Age, y
I	< 3000 μg/L	< 2.5 cm under the costal margin	< 4
II	Neither group I nor group III		
III	> 5000 μg/L	> 4 cm	> 8

Table 4. Clinical outcome according to transplant groups

	MSD-HSCT	UD-PBSCT	Total	*P*
Engraftment, median day (range)*				
ANC > 500/mm^3	17.5 (12-30)	19 (11-26)	19 (11-30)	.230
PLT > 20 × 10^9/mm^3	17 (9-56)	15.5 (9-42)	16 (9-56)	.344
Hgb > 8.0 g/dL	13 (2-42)	13 (6-28)	13 (2-42)	.073
Duration of ANC < 500/mm^3	18 (1-33)	22 (13-32)	21 (1-33)	.010
Acute GVHD				
Grade III-IV (%)	1 (3.6)	5 (9.6)	6 (7.5)	.328
Transplantation-related complications				
HC (%)	3 (10.7)	9 (17.3)	12 (15.0)	.431
CMV reactivation (%)	9 (32.1)	22 (42.3)	31 (38.8)	.373
VOD (%)	3 (10.7)	2 (3.8)	5 (6.3)	.226
Mucositis (%)	10 (35.7)	33 (63.5)	43 (53.8)	.018
IFD (%)	1 (3.6)	5 (9.6)	6 (7.5)	.328
Results of transplant				
OS (at 3 y)	.900	.923	.915	.678
TFS (at 3 y)	.833	.904	.878	.309
TRM (at 3 y)	.100	.077	.085	.678
GR (at 3 y)	.069	.019	.037	.259

All patients received Azathioprine / Hydroxyurea from day -45

Stem cell transplant for thalassemia major:

1. Only curative treatment for thalassemia major that is standard of care

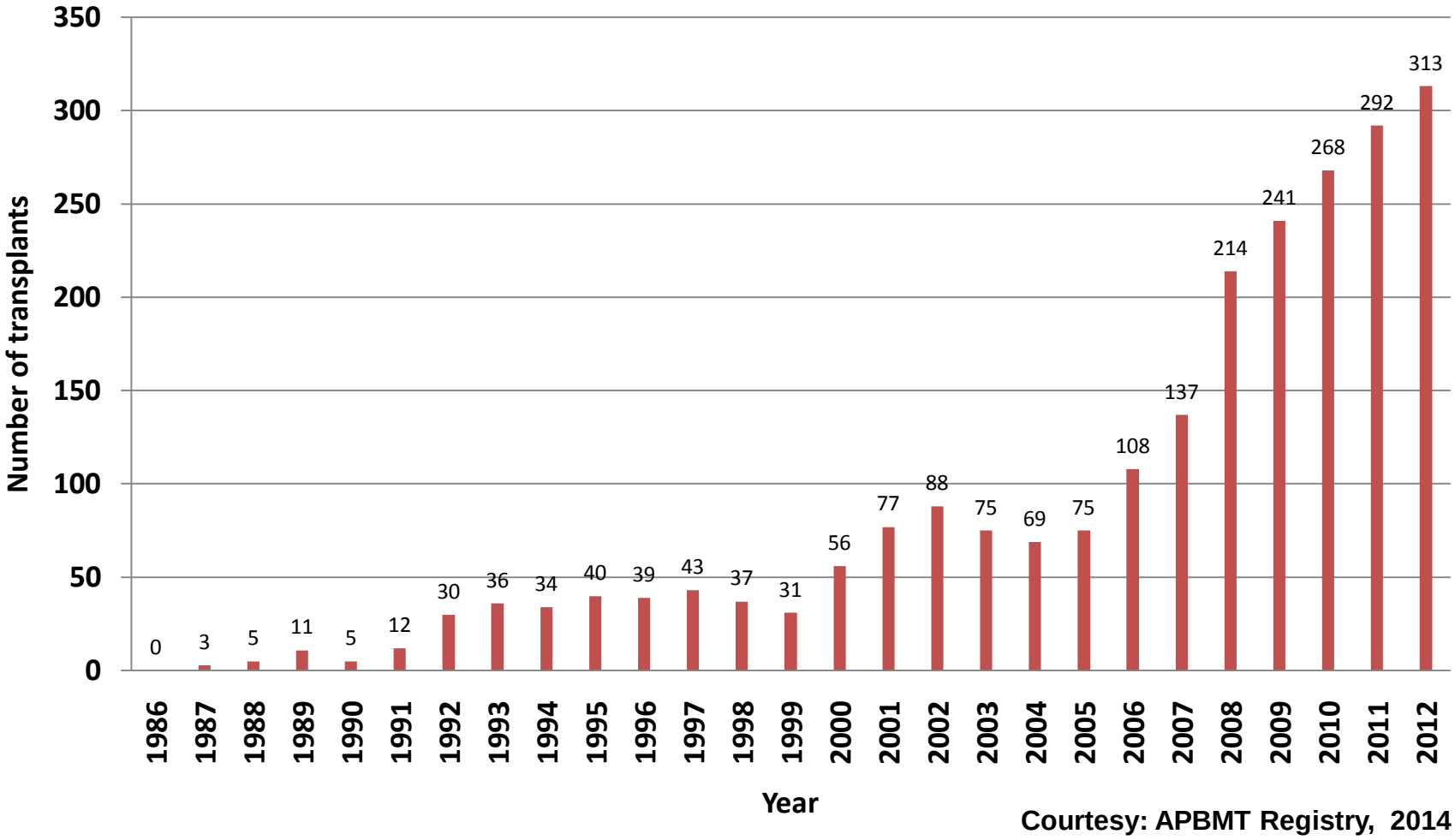
2. Results have improved over last 10-15 years
   - Low risk patients : 85-95% survival
   - High risk patients : 70-80% survival (?)

   → *These are the best results – not universal experience*

3. Many challenges
   - Risk stratification
   - Conditioning regimen and RRT
   - Suitable donor – Matched / mismatched
   - Immunological complications – GVHD / Rejection
   - Geographic variabilities

4. Impact of co-existing morbidities

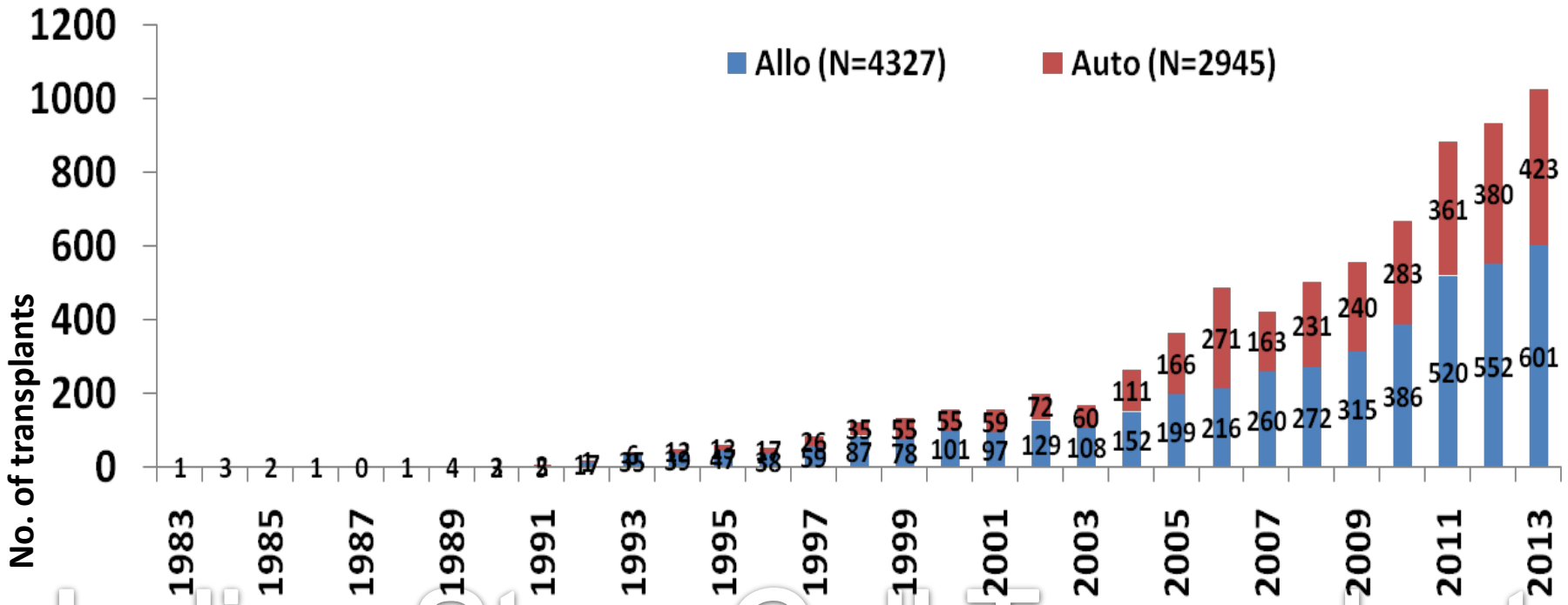
APBMT Registry - HSCT for Thalassemia Major



Courtesy: APBMT Registry, 2014

INDIAN STEM CELL TRANSPLANT REGISTRY

Number of Transplants – India (N=7242)



Indian Stem Cell Transplant Registry Data

~150 HSCT for Thalassemia Major in India / year

~10,000 children with Thalassemia Major /year

Total centers – 37 centers

Data submitted - 33 centers

What is gene therapy?

- **Gene Therapy** - technique that uses genes as a means of treatment and/or prevention of a particular disease caused by genetic defects

- **Includes**:

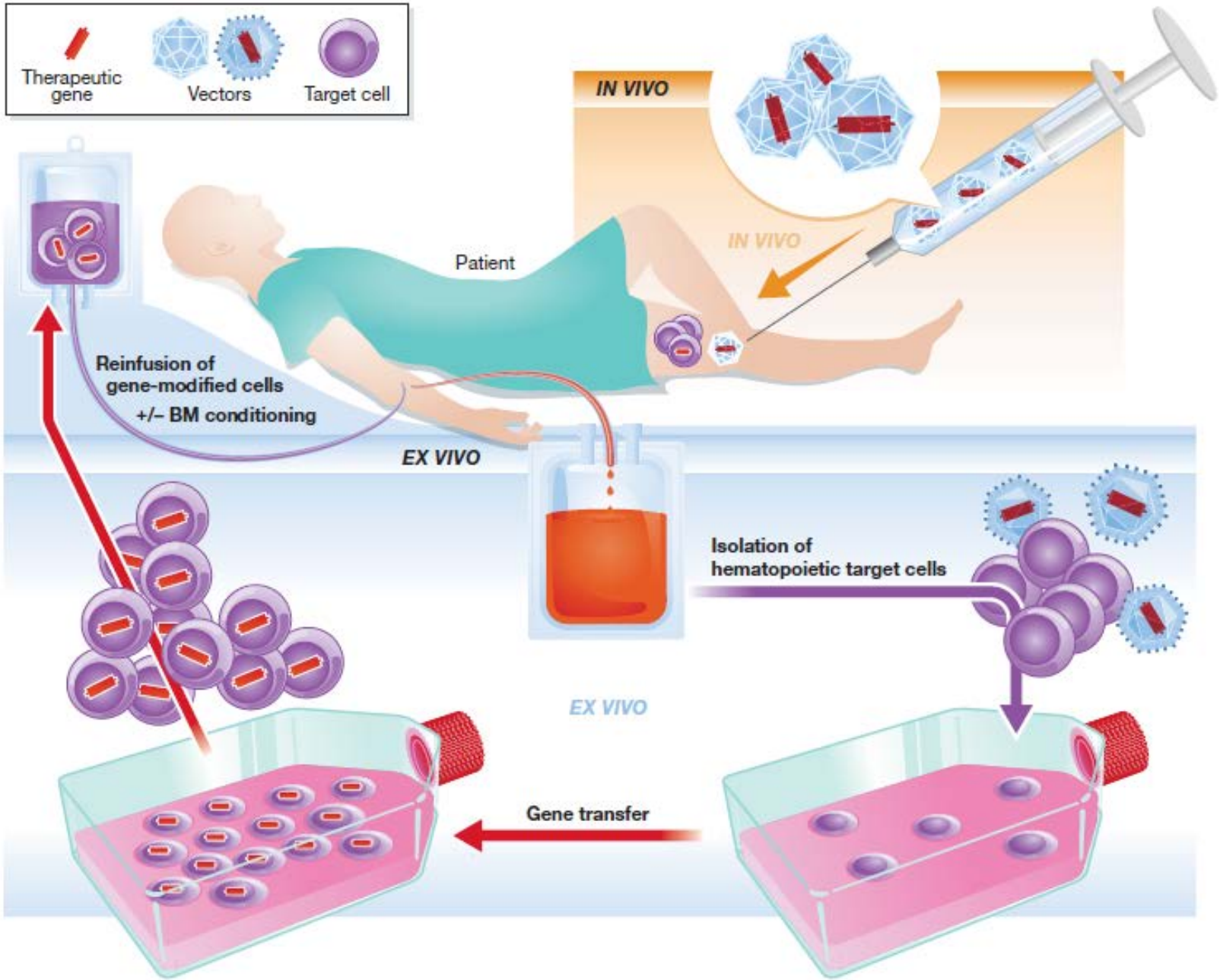
  -Replacing a mutated gene with a healthy copy,

  -Inactivating a mutated gene

  -Introducing a new gene into the body

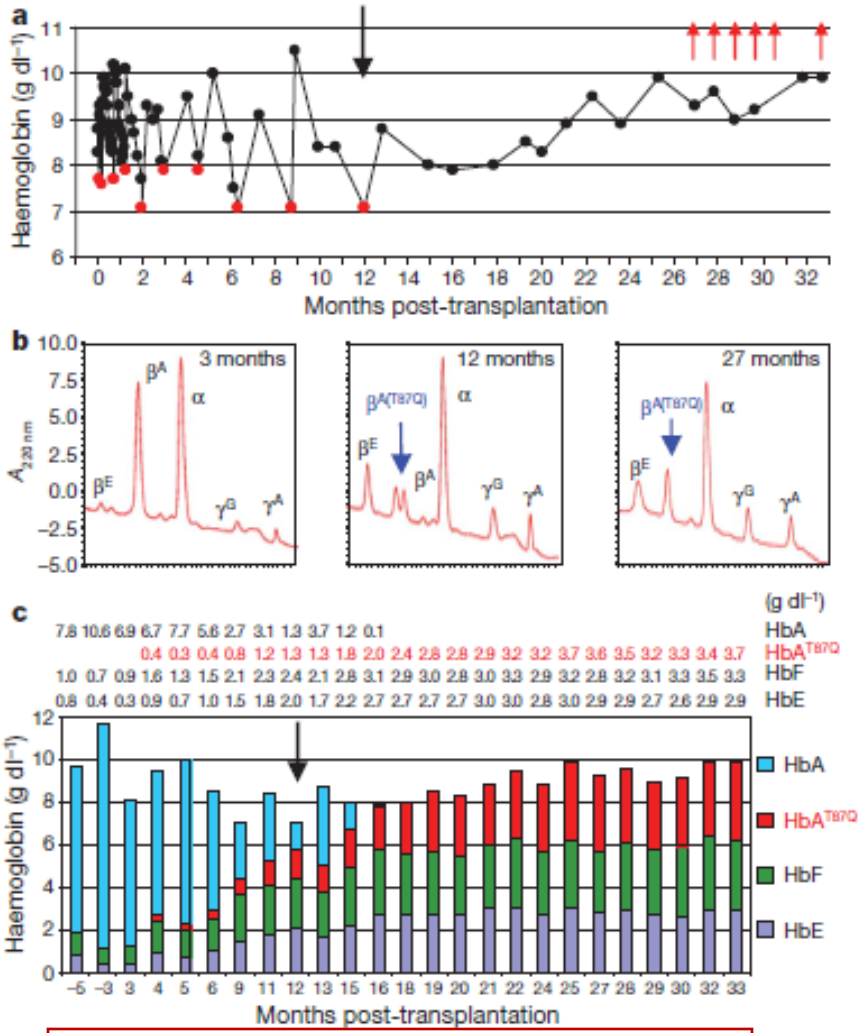
- **Technology of molecular genetics /cellular biology** made this possible from the 1980's - insert a gene into the cell

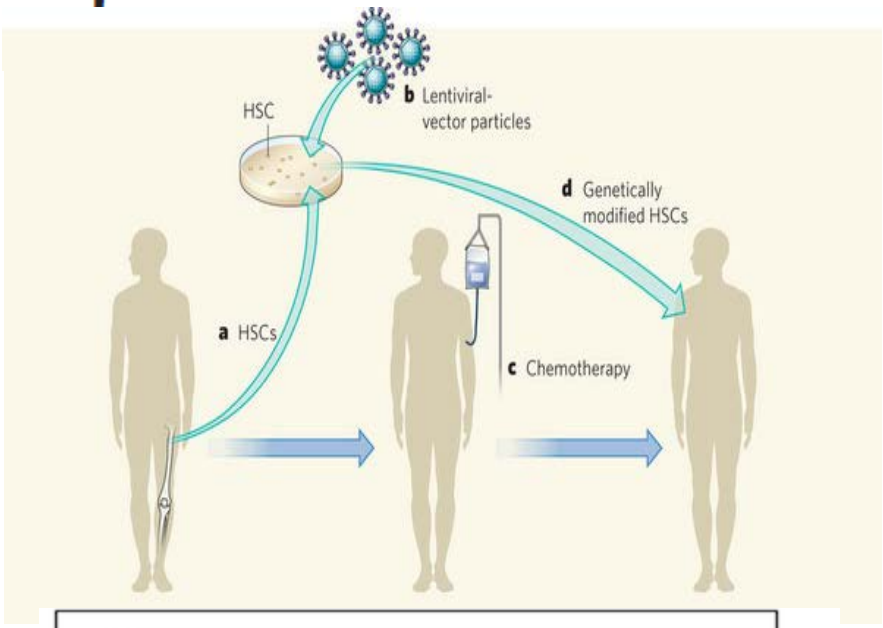
- **First gene therapy** done at NIH, USA in 1990 for SCID

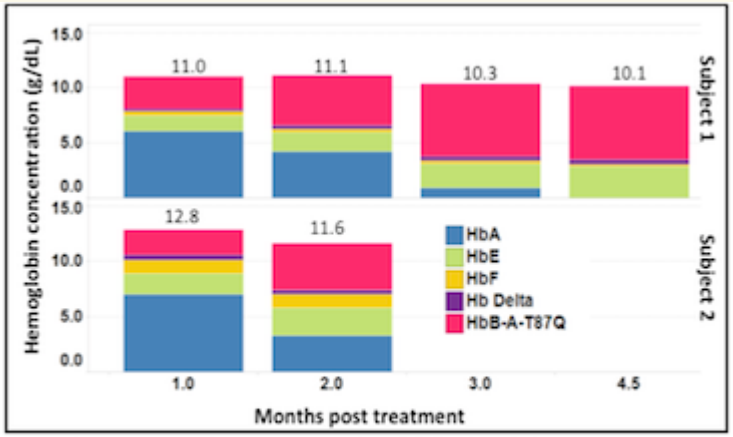


EMBO Mol Med (2013) 5, 1642–1661

Transfusion independence and *HMGA2* activation after gene therapy of human β-thalassaemia







- Subject 1: producing 6.6 g/dL of $\beta^{A\text{-}T87Q}$ -globin at 4.5 months
- Subject 2: producing 4.2 g/dL of $\beta^{A\text{-}T87Q}$ -globin at 2 months

2-4 gm% rise in Hb over 12-24 months

EHA 2014

nature Vol 467|16 September 2010|doi:10.1038/nature09328

Cure for Major Hemoglobin Disorders

***Increasing number of HSCT → With good results**
***Continued thrust on enhancing access**

***The focus should also be on reducing long term complications (LTC)**

***For best results several steps needed:**

-Good transfusion chelation before HSCT
-Early HSCT (2-5 years of age), before 7 years
-Reduce HSCT related LTC: Safer conditioning
-Early and planned attention to post HSCT
iron chelation / immunization / growth monitoring

***Initiate an effective control program**
***Work towards successful gene therapy!**

Acknowledgements

CMC, Vellore

Dr. Vikram Mathews
Dr. Biju George
Dr. Auro Viswabandya
Dr. Aby Abraham
Dr. Abhijeet G
Dr. Fouzia NA

Dr. Poonkuzhali B
Dr. Shaji R.V.

Ms. M.L. Kavitha

Dr. Mammen Chandy

Nurses / Registrars/ PhD students

Patients and families

Hospital Robert Debre, Hospital St. Louis, Paris

Dr. R. Krishnamoorthy
Dr. Evelyn J. Aigrain
Dr. D. Charron
Dr. R. Tamouza

Funding agencies

ICMR: 1994-1999
CEFIPRA: 2001-2004
DBT: 2007- 2010

CSCR (DBT): 2008 onwards