The MSKCC Experience

SBA^{-}E^{-} T Cell Depleted HLA Haploidentical Parental Marrow Transplants For Severe Combined Immunodeficiencies

T-CELL DEPLETION METHODS

METHOD OF T-CELL DEPLETION	CELLS DEPLETED	LOG T-CELL DEPLETION
MoAb CD2, CD3, CD5, or CD6	T	1-2.5
MoAb CD4 or CD8	T	1-2
T10B9 (TCR) + Cplt	T	1.0-1.5
Anti-CD5 Ricin A-chain Immunotoxin	T	1.5-2.0
Immunomagnetic separation (CD3-CD8)	T	2
Rat MoAb Campath-1 (CD52) + Human Cplt	T, B	1.7-2.5
Multiple E-rosetting depletion	T, NK, B	1.5-2.0
Counterflow Elutriation	T, +/-	Fixed: 1.5-3.0
Soybean Agglutinin + E-rosetting	T, NK, B	2.5-3.0
CellPro CD34+	T	1.5
Isolex CD34+	T	2
Milteny	T	3.0-4.0

Incidence of Graft versus Host Disease
Adult Good Risk Leukemia, Matched T-Depleted BMT

	n	Not Eval	ACUTE GvHD None	Grade I	Grade II	Grade III	Grade IV	Not Eval	Chronic GvHD None	Yes
	284	20	232	24	4	4	0	66	208	10
Percent			87.9	9.1	1.5	1.5	0		95.4	4.6
By Rejection Prophylaxis										
None	86	14	64 (88.9%)	6 (8.3%)	2 (2.8%)	0	0	25	60 (98.4%)	1 (1.6%)
Post ATG and Steroids	128	3	107 (85.6%)	13 (10.4%)	1 (0.8%)	4 (3.2%)	0	26	95 (93.1%)	7 (6.9%)
Pre ATG and Nothing Post	23	2	18 (85.7%)	3 (14.3%)	0	0	0	5	16 (88.9%)	2 (11.1%)
Pre ATG and Steroids Post	34	0	32 (94.1%)	2 (5.9%)	0	0	0	5	29 (100.0%)	0
Steroids Only	13	1	11 (91.7%)	0	1 (8.3%)	0	0	5	8 (100.0%)	0

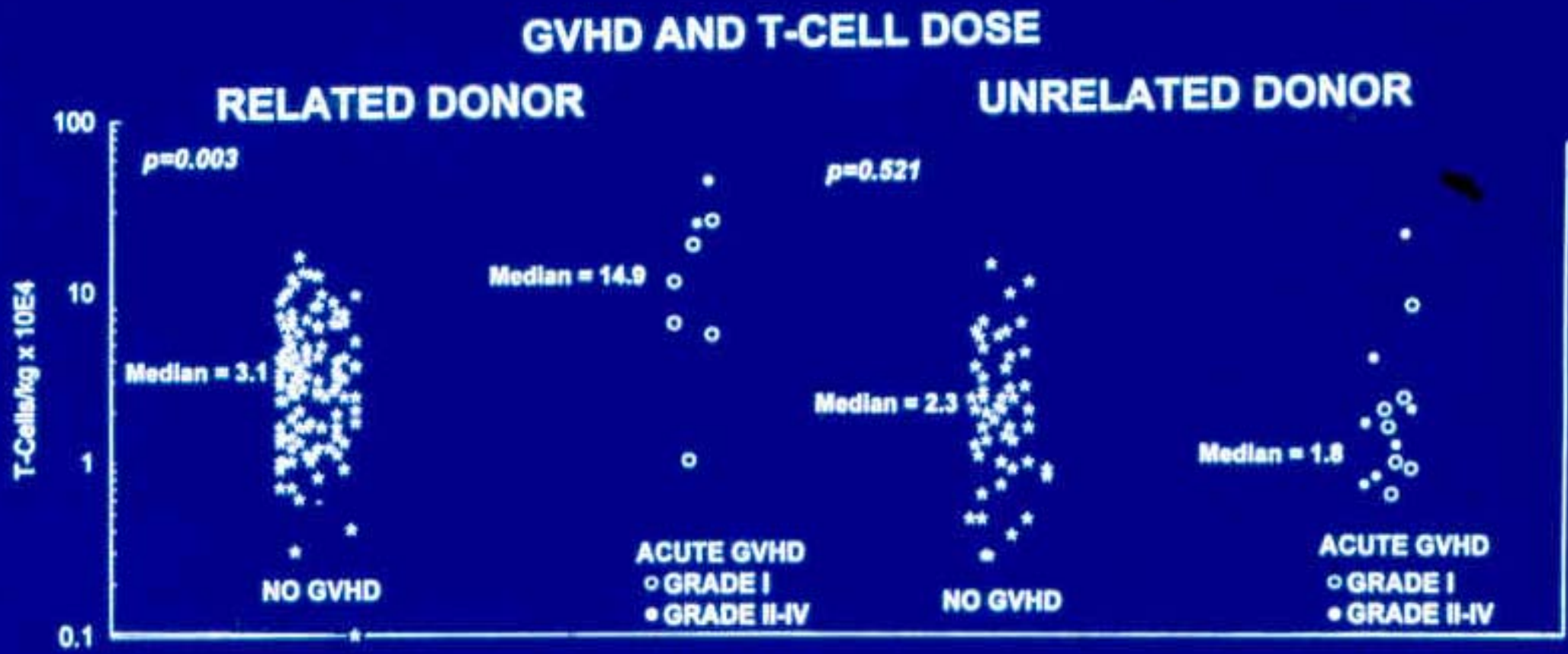
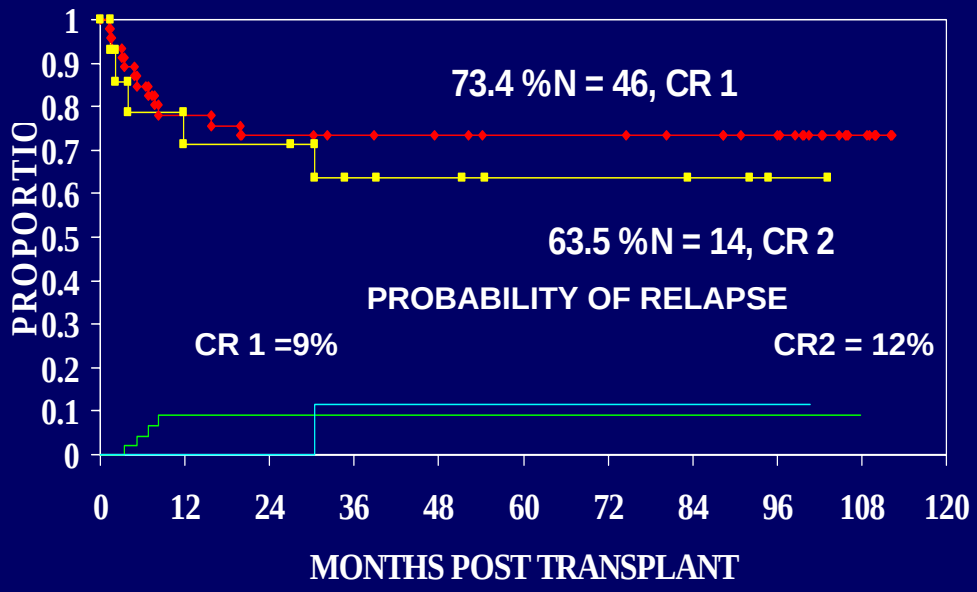
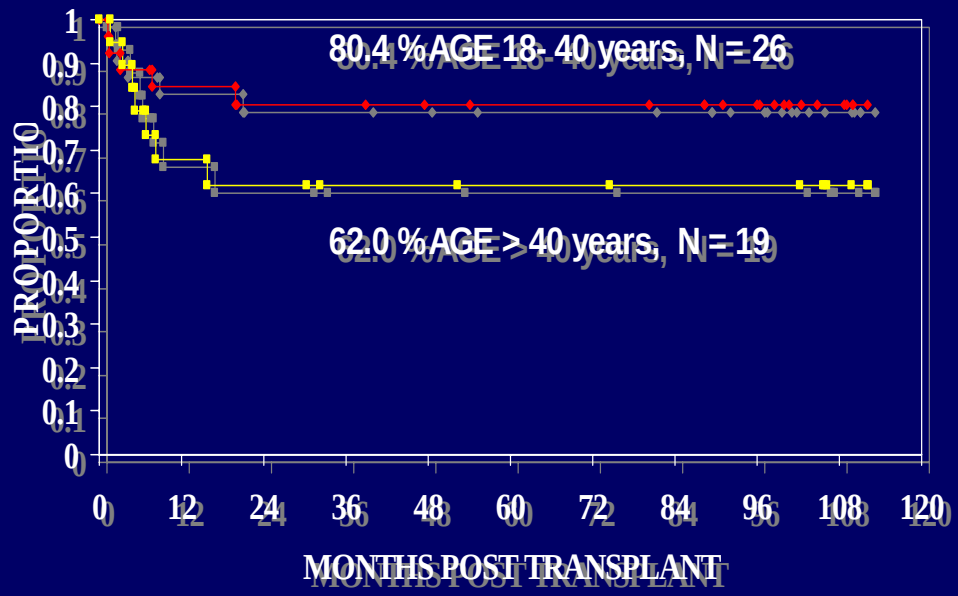


Figure 2: Limiting dilution analyses quantitating clonable T-cells in SBA^{-}E^{-} T-cell depleted grafts in patients who did and did not develop GvHD, by donor type.

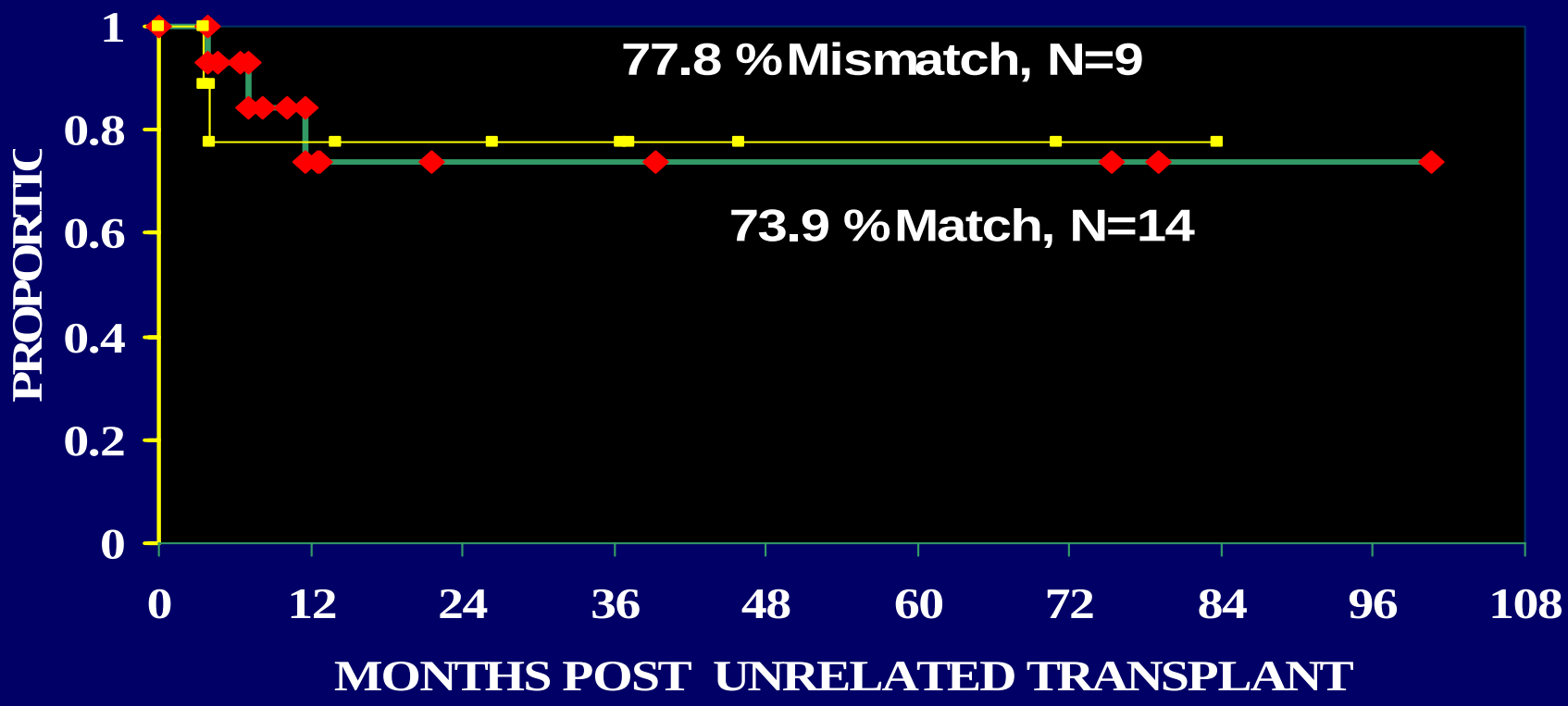
DFS OF AML CR1 & CR2; HLA IDENTICAL RELATED, TBI/THIO/CY/ATG



DFS OF AML CR1; HLA IDENTICAL RELATED TBI/THIO/CY/ATG; LSBMT; BY AGE



DFS OF Acute Leukemia CR1/CR2, UNRELATED PEDIACTRIC PATIENTS BY HLA TYPE, MSKCC



Mismatched T-Depleted BMT for SCID

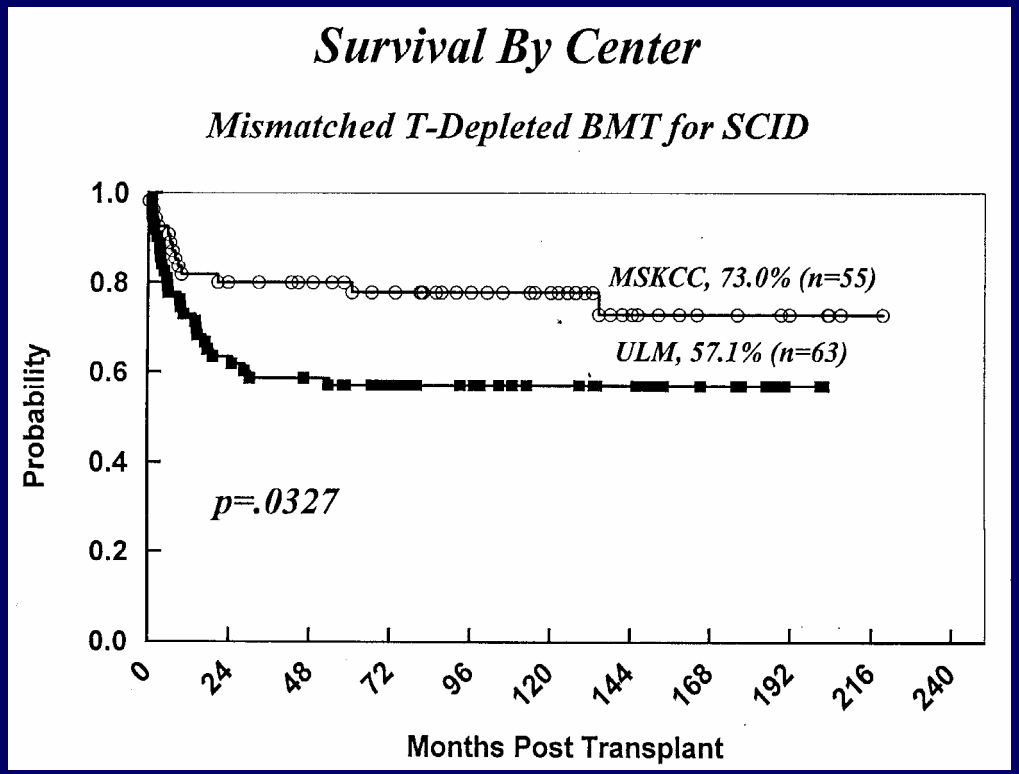
	MSKCC	ULM	OVERALL
N	**55**	**63**	**118**
Sex			
Male	**42**	**48**	**90**
Female	**13**	**15**	**28**
Median Age at BMT (Yrs.)	**0.56**	**0.48**	**0.52**
(Range)	**(.03-3.58)**	**(.03-2.89)**	**(.03-3.58)**
Diagnosis/Inheritance			
X-Linked	**14**	**18**	**32**
Recessive-Auto	**12**	**12**	**24**
SCID-Inheritance Unk	**14**	**19**	**33**
ADA Deficient	**8**	**4**	**12**
Reticular Dysgenesis	**1**	**6**	**7**
Ommens	**2**	**3**	**5**
Capping Defect-Auto	**3**	**0**	**3**
SL Dwarf/Ectoderm	**1**	**0**	**1**
Infectious History			
Yes	**27**	**36**	**63**
No	**28**	**27**	**55**
Maternal Engraftment			
Yes	**20**	**25**	**45**
No	**24**	**23**	**47**
Unknown (Not Tested)	**11**	**15**	**26**
NK Function Pre			
Yes	**28**	**26**	**54**
No	**20**	**20**	**40**
Unknown (Not Tested)	**7**	**17**	**24**

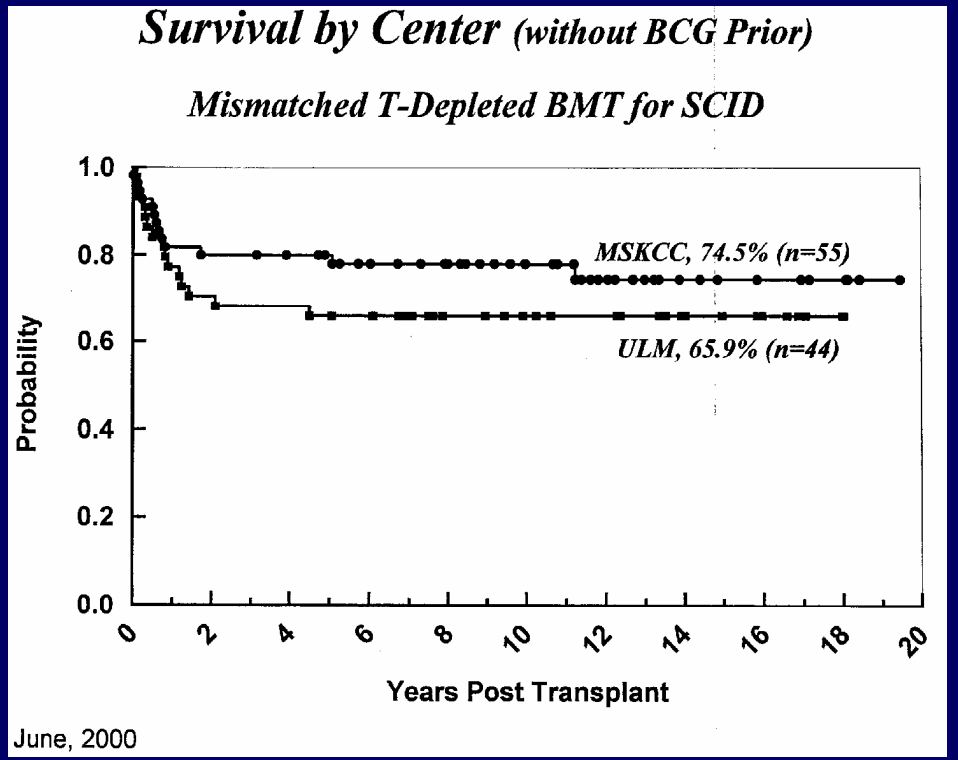
SBA-E- BMT FOR SCID
HLA DISPARITIES BETWEEN DONOR AND HOST

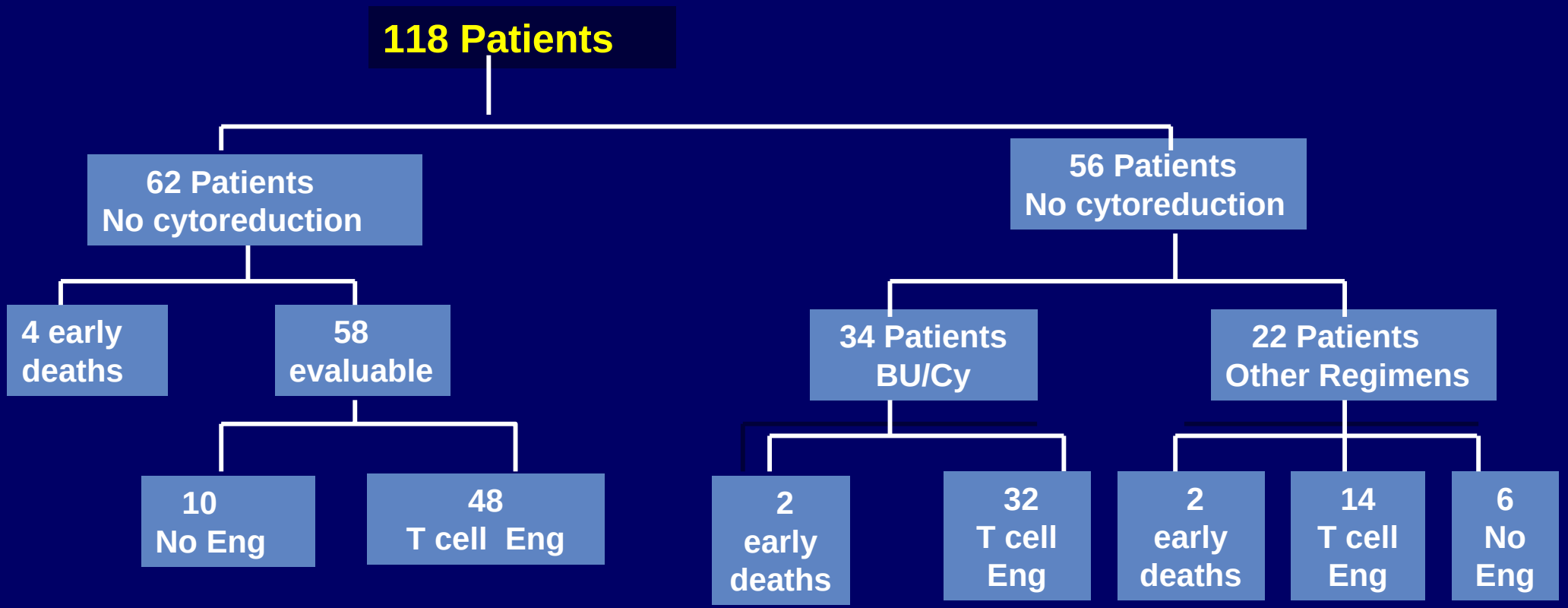
HLA ALLELES	HLA ALLELES UNIQUE TO DONOR (REJ.)	HLA ALLELES UNIQUE TO HOST (GvHD)
3 alleles	67	63
2 alleles	29	41
1 allele	12	10
no alleles	4	0

MSKCC AND ULM UNIVERSITY RESULTS
HLA-HAPLOTYPE NON-IDENTICAL
SBA-E- MARROW TRANSPLANTS FOR SCID

TOTAL PATIENTS	118	
EARLY DEATH: ENGRAFTMENT NOT EVALUABLE	5	
DURABLE ENGRAFTMENT	96	
ENGRAFTMENT NOT ACHIEVED	17	
T-CELL RECONSTITUTION		
Full	72	
Partial	20	
Early	10	
B-CELL RECONSTITUTION		
Donor B-Cells Present	35/42	
Donor B-Cells Absent	4/64	
GRAFT VS. HOST DISEASE	7/80 (1 Grade I; 6 II)	
DEATHS	40	
CAUSES OF DEATH		
Pneumonia and Infection	23	
Encephalitis	5	
EBV LPD	3	
GvHD	2	(Antecedant Materno Fetal GvHD)
Graft Failure	1	
Metabolic	3	
Other	3	
Survival (13-200 Months, MPO 112 Months)	78 (66%)	


Survival By Center
Mismatched T-Depleted BMT for SCID
Probability
1.0
0.8
0.6
0.4
0.2
0.0
MSKCC, 73.0% (n=55)
ULM, 57.1% (n=63)
p=.0327
0
24
48
72
96
120
144
168
192
216
240
Months Post Transplant


Survival by Center (without BCG Prior)
Mismatched T-Depleted BMT for SCID
Probability
1.0
0.8
0.6
0.4
0.2
0.0
MSKCC, 74.5% (n=55)
ULM, 65.9% (n=44)
0
2
4
6
8
10
12
14
16
18
20
Years Post Transplant
June, 2000

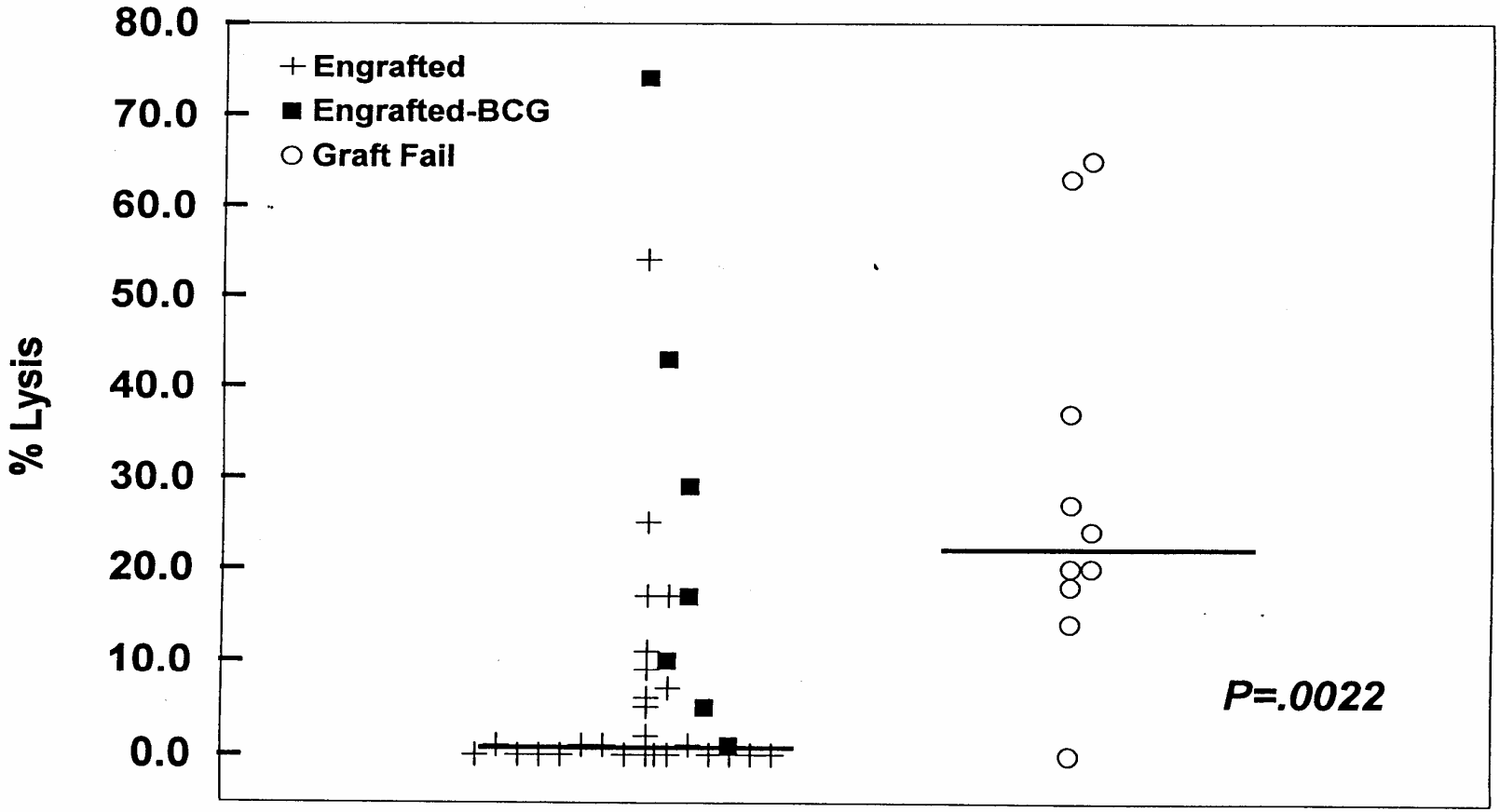

118 Patients
62 Patients
No cytoreduction
56 Patients
No cytoreduction
4 early
deaths
58
evaluable
34 Patients
BU/Cy
22 Patients
Other Regimens
10
No Eng
48
T cell Eng
2
early
deaths
32
T cell
Eng
2
early
deaths
14
T cell
Eng
6
No
Eng

Patients Transplanted Without Cytoreduction

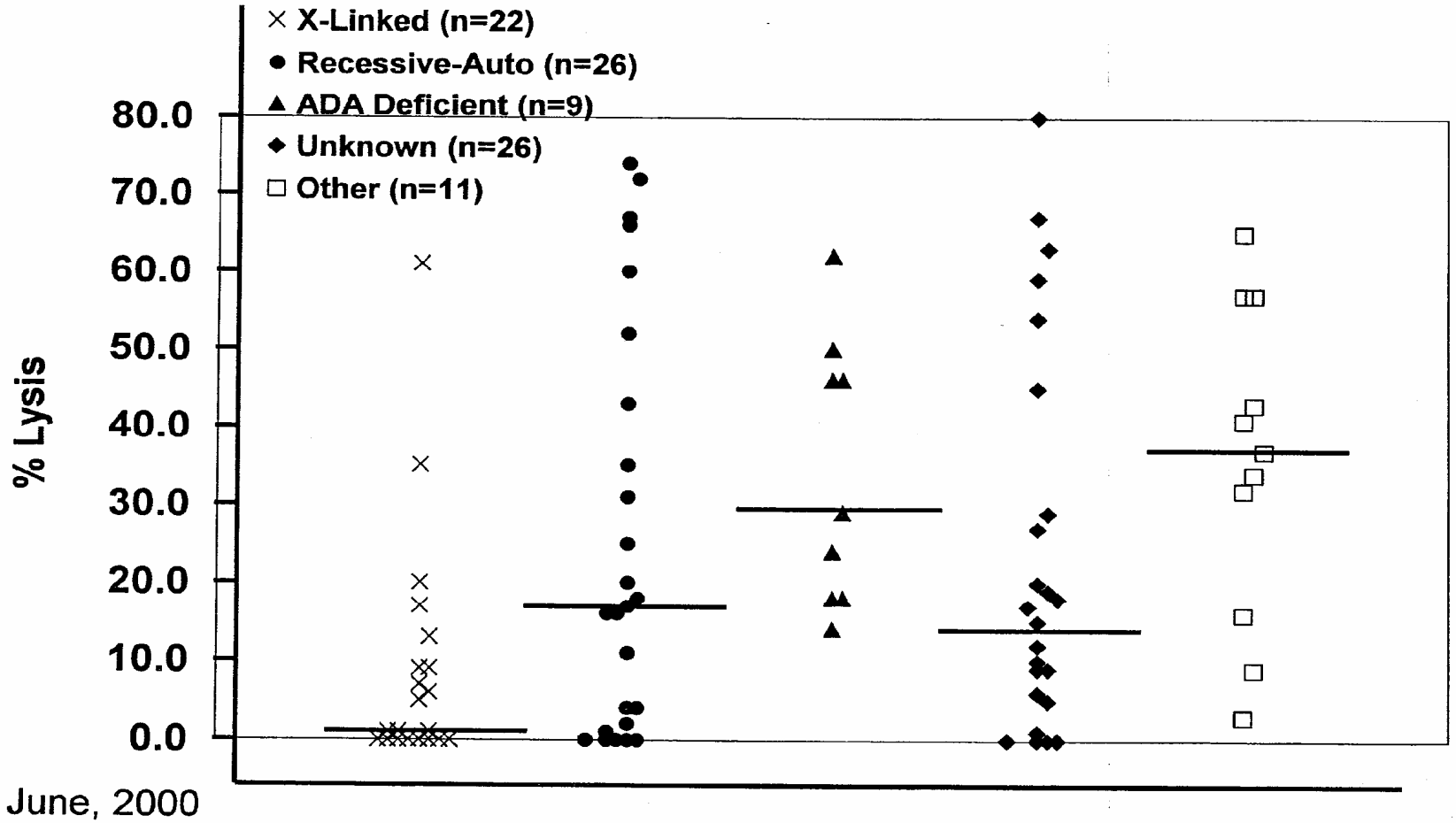
SCID VARIANT	N	ENG.	NO ENG.	NE
ADA Deficiency	3	1	2	
Autosomal Recessive	13	11	1	1
X-linked Recessive	22	22	0	
Omenn's Syndrome	1	0	1	
Ectodermal Dysplasia	1	0	1	
Reticular Dysgenesis	4	3	0	1
Unknown	18	11	5	2
	62	48	10	4

Engraftment by NK Function Pre

No Cytoreduction, Mismatched T-Depleted BMT for SCID



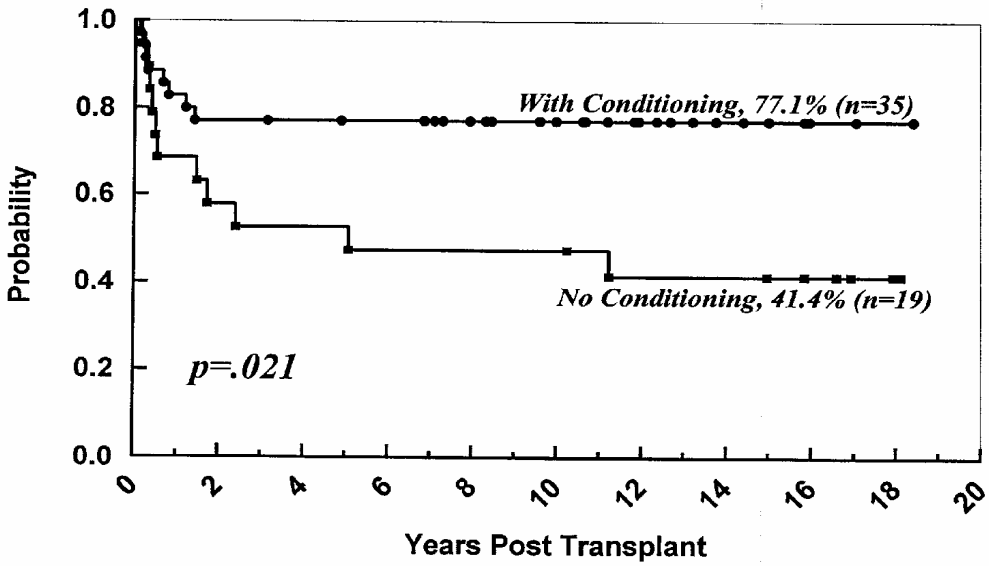
NK Function Pre by Variant of SCID



Preoperative Regimen			T Cell Engraftment		T Cell Function				B Cell Engraftment			B Cell Function			
	N	Eval	+	-	Full	Partial	None	NE	+	-	NE	Full	Partial	None	NE
BU/CY	33	31	31	0	24	7	0	3	25	3	3	22	2	3	4
THIO/CY	9	9	8	1	8	0	0	0	8	1	0	8	0	1	0
BU Alone	5	5	3	2	2	1	2	0	3	2	0	2	0	2	1
CY/ARA-C	3	2	2	0	2	0	0	0	1	1	0	1	0	1	0
ATG Alone	1	1	0	1	0	0	1	0	0	1	0	0	0	1	0
ATG/THIO	1	1	0	1	0	0	1	0	0	1	0	0	0	1	0
ATG/CY	1	1	0	1	0	0	0	0	0	1	0	0	0	1	0
CY Alone	2	2	1	1	1	0	0	0	1	1	0	1	0	1	0
Totals	*55*	*52*	*45*	*7*	*37*	*8*	*4*	*3*	*38*	*11*	*3*	*34*	*2*	*11*	*5*

Survival for Patients with NK Function Pre

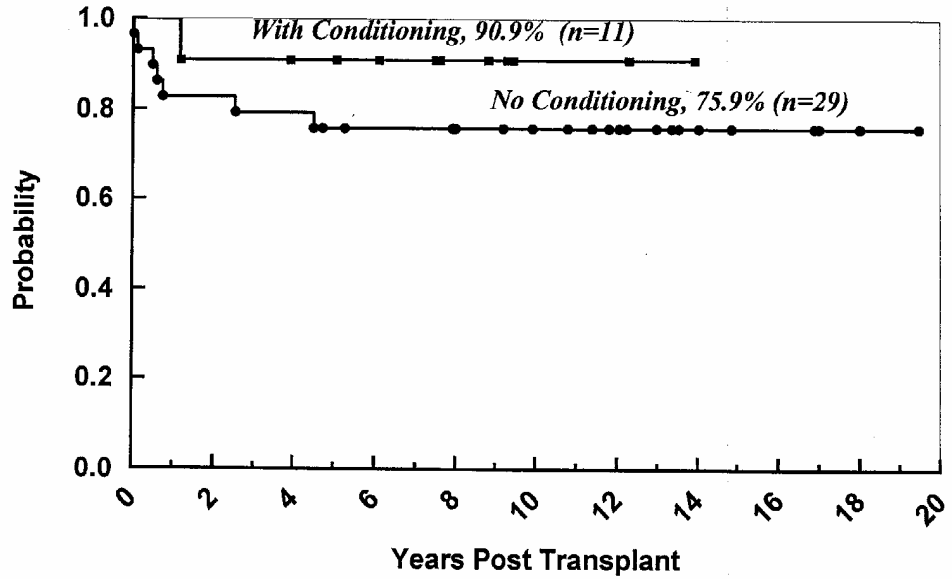
Mismatched T-Depleted BMT for SCID



June, 2000

Survival for Patients Without NK Function Pre

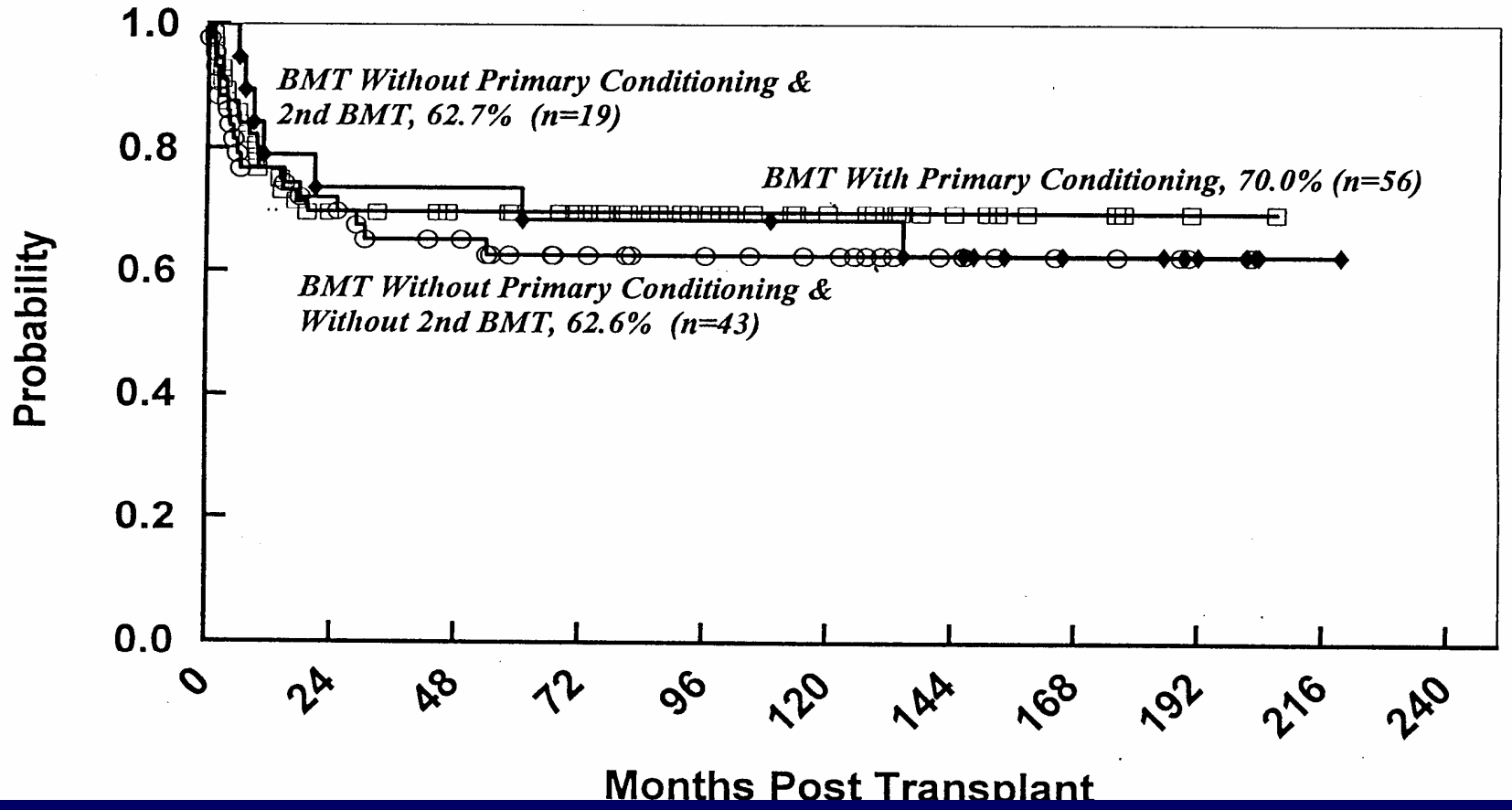
Mismatched T-Depleted BMT for SCID



June, 2000

Survival by Primary Conditioning and 2nd BMT

Mismatched T-Depleted BMT for SCID

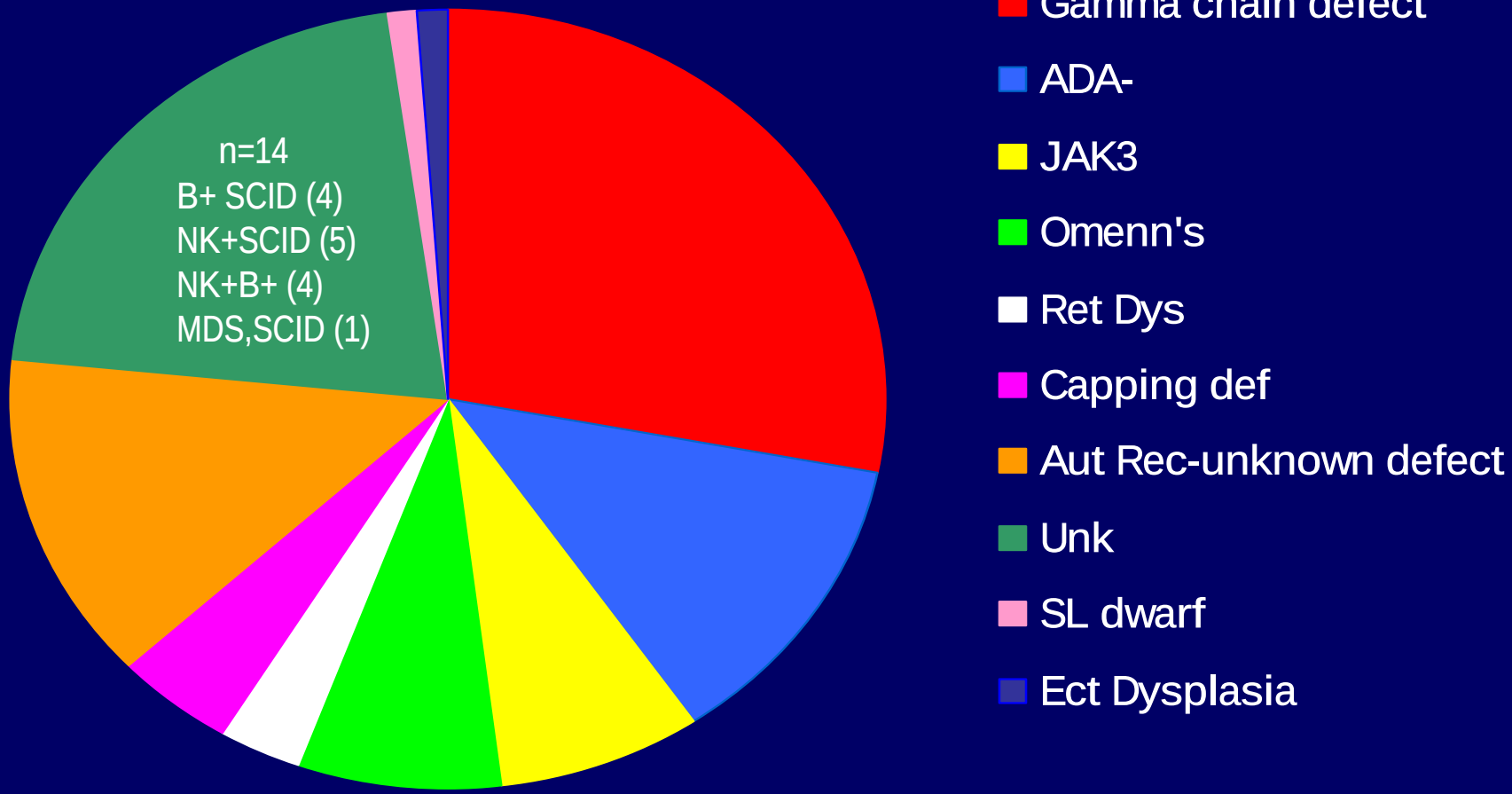


Composite of Engraftment and Function by Cytoreduction

Response to Primary Transplant

		ENGRAFTMENT					FUNCTION					
	N	EVAL	T +	T -	Full	Part	No	B +	NE -		B Full	Part	No	NE
no cytoreduction	62	58	48	10	34	12	12	5	51	2	5	0	46	7
+ cytoreduction	55	52	45	7	37	8	7	38	11	3	34	2	11	5

SCID defect of 68 consecutive recipients of a Parental HLA Haploidentical TCD BMT MSKCC 1980-2004, n=68



Transplants For SCID

Incidence of GVHD Post $SBA^{-}E^{-}$ BMT From Parental Donors

Host Unique HLA Disparities	N	Eng^{+} Eval.	Acute GVHD I	Acute GVHD II-III	Chronic GVHD
1	3	3	0	0	0
2	14	14	0	0	0
3	51	46	3	2	0
TOTAL	68	63	3	2	0

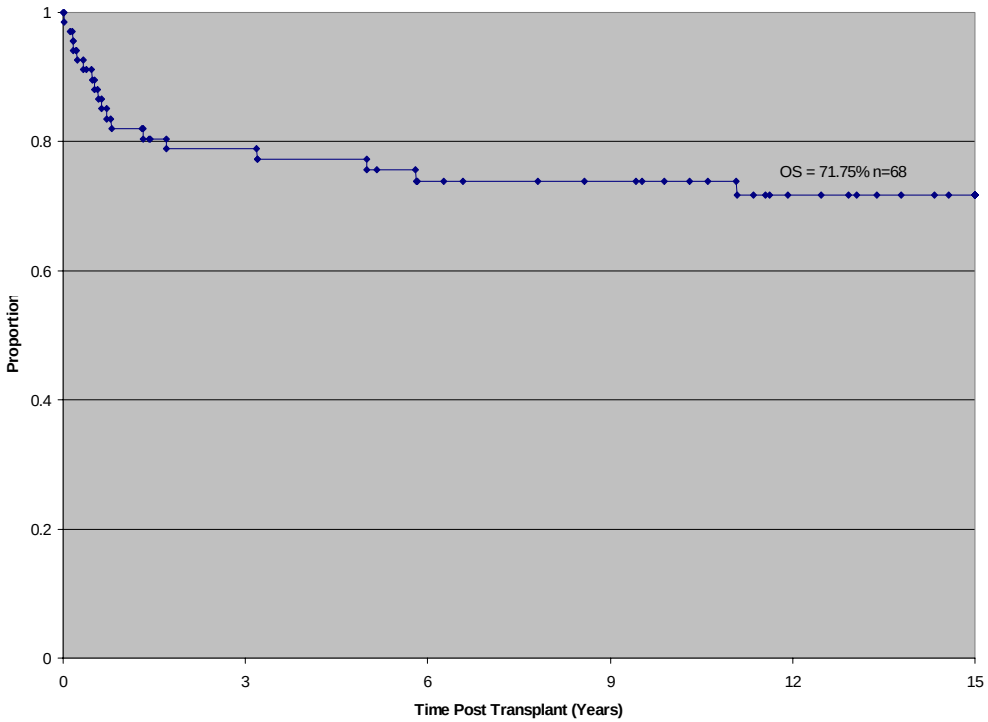
SCID

Results of HLA Haploidentical SBA^{-}E^{-} TCD Parental Marrow Grafts

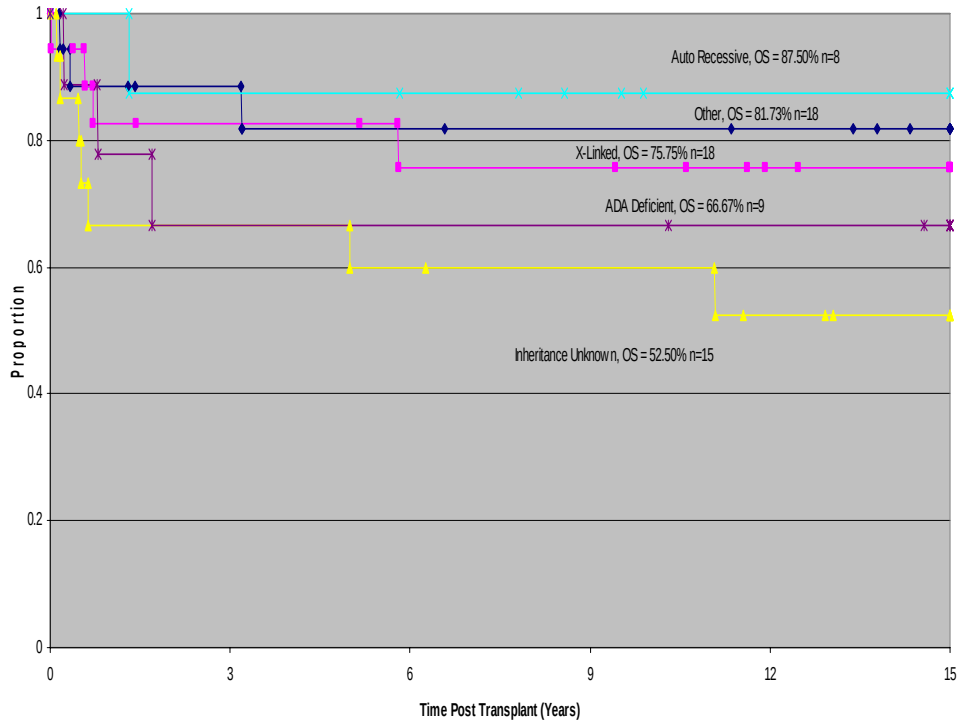
Total Consecutive Cases:	**68**
Surviving with Reconstitution	**48**
Dead	**20**

	Preparation For Primary Graft	
	No Myeloablation	**Ablation**
Total Patients:	**40**	**28**
Early Deaths:		
Pre Engraftment	**7 (17%)**	**2 (7%)**
Post Engraftment	**2 (5%)**	**3 (11%)**
Late Deaths:	**6 (15%)**	**0 (0%)**
TOTAL:	**15 (37.5%)**	**5 (18%)**

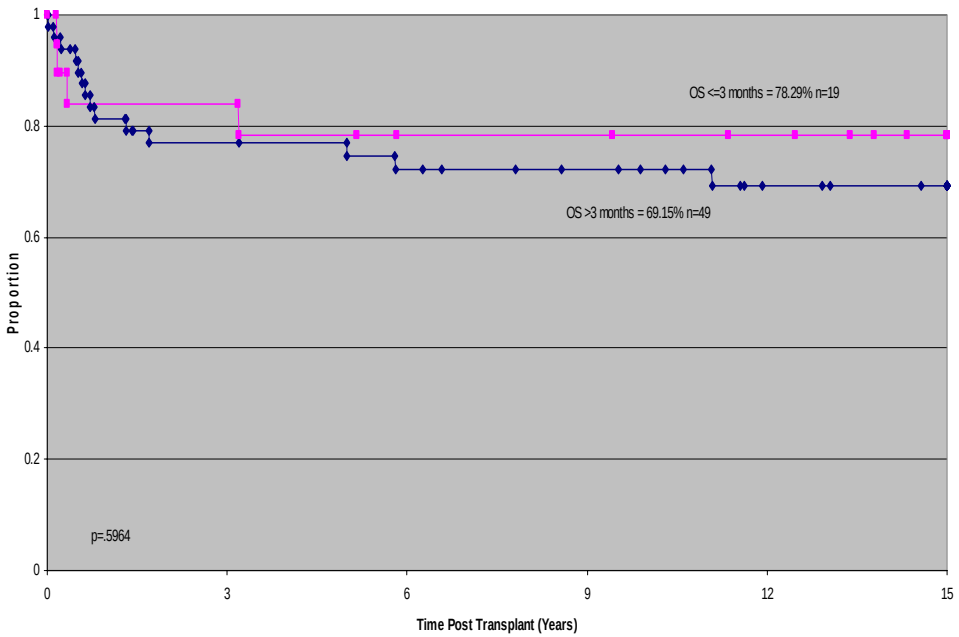
Survival at 15 Years for SCID Patients who Received a Mismatched T-Depleted BMT



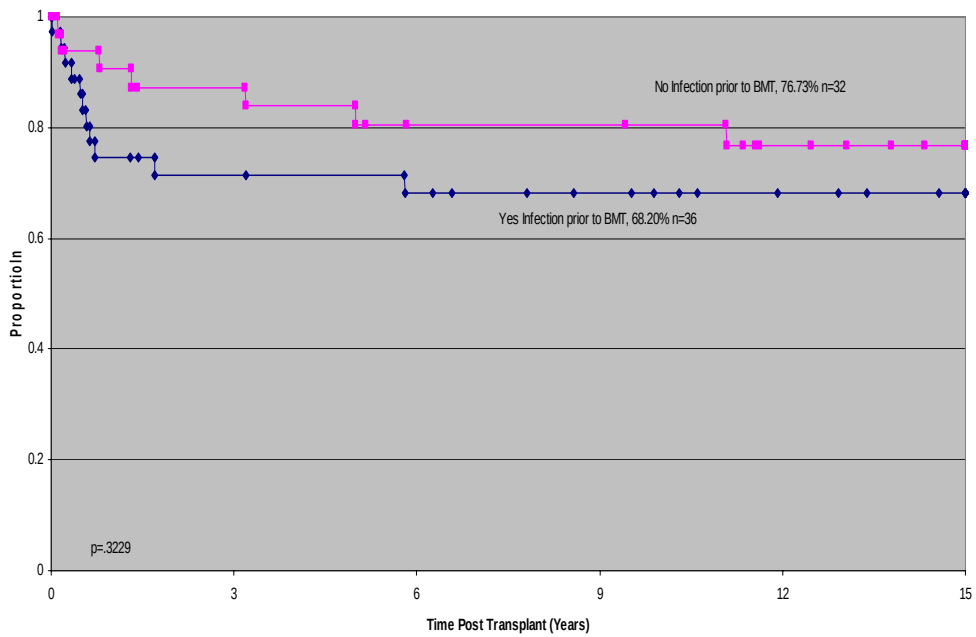
Survival at 15 Years by SCID Variant Mismatched T-Depleted BMT



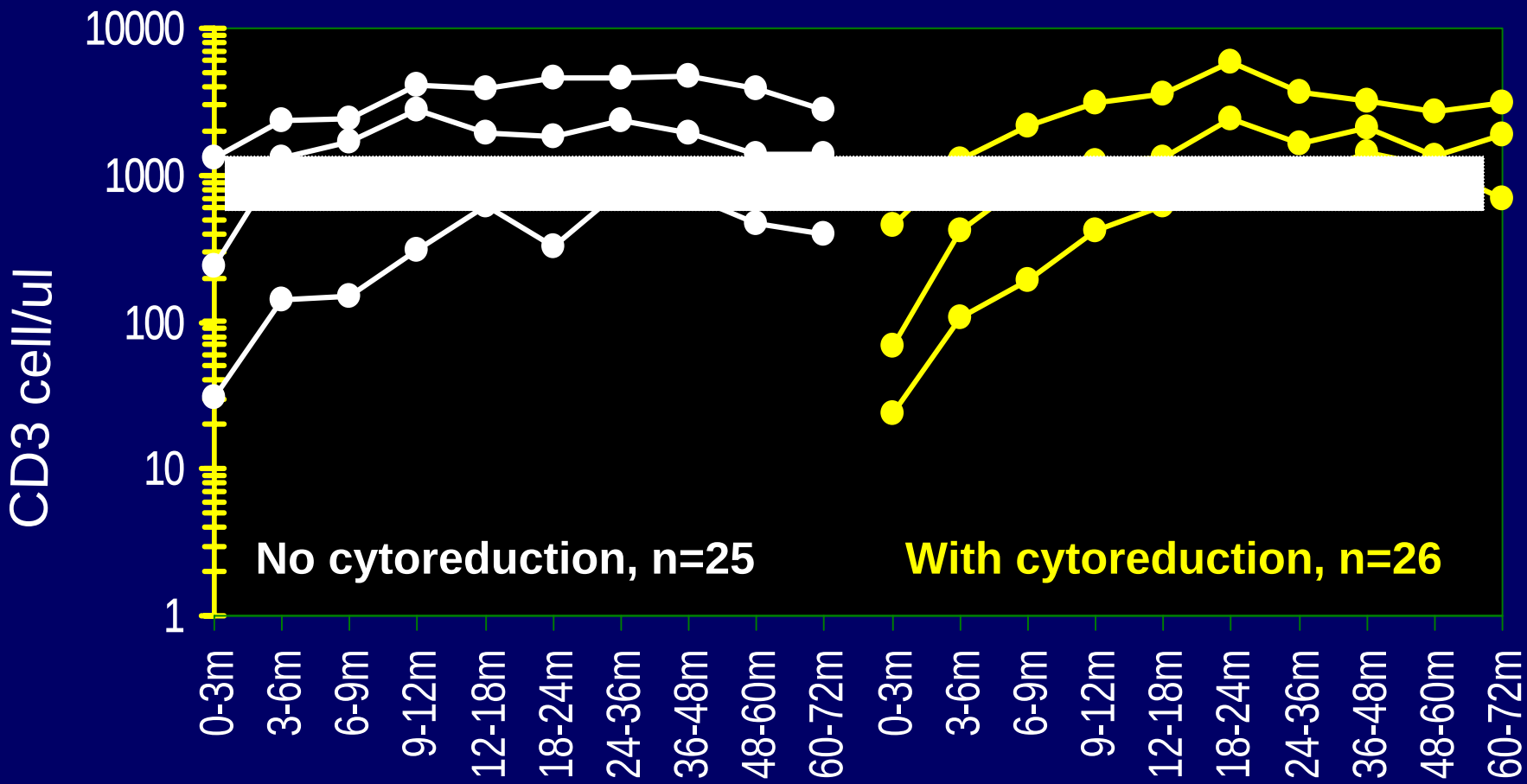
Survival at 15 Years of SCID Patients Transplanted at 3 months of Age Mismatched T-Depleted BMT



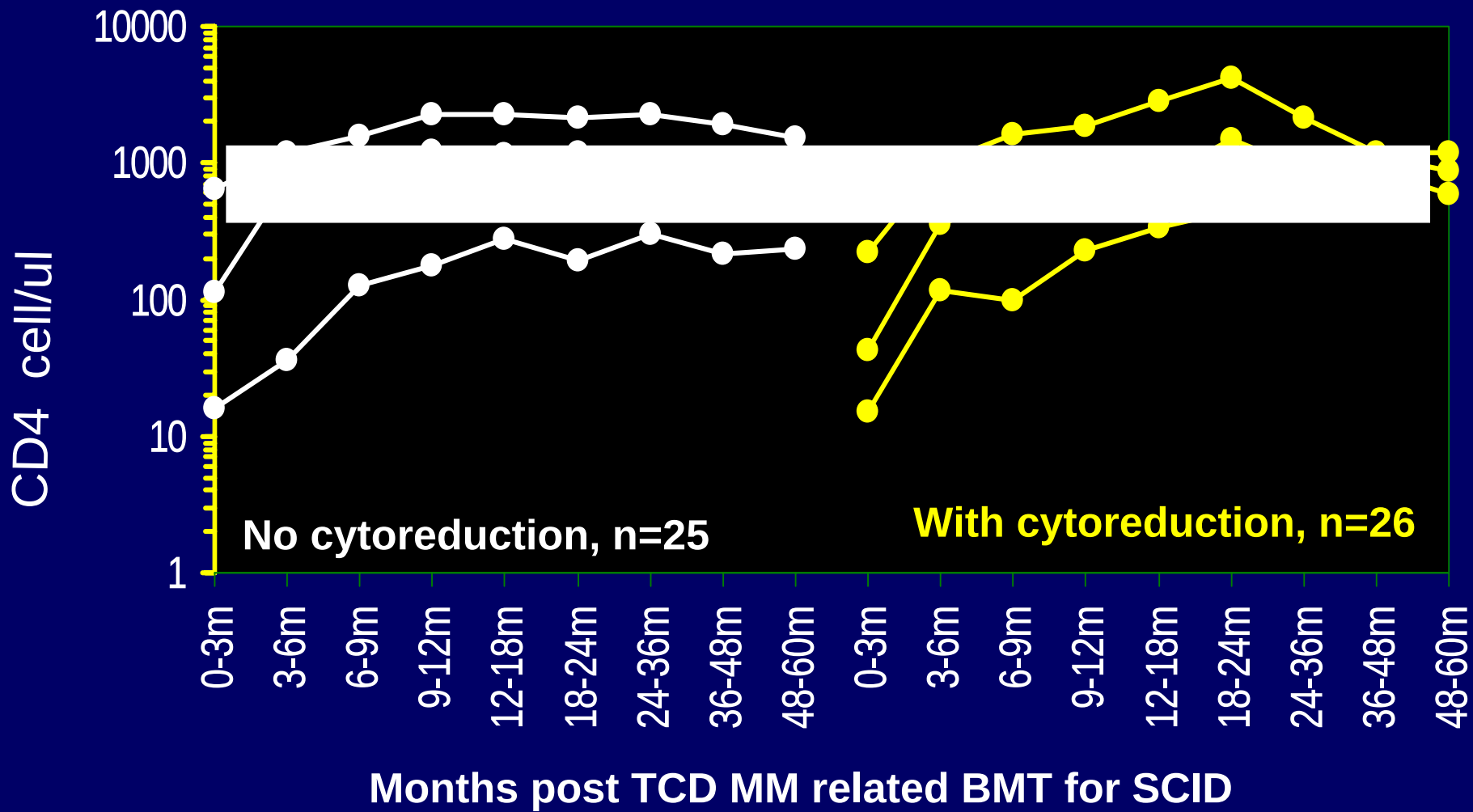
Survival at 15 Years of SCID Patients by Infection Mismatched T-Depleted BMT



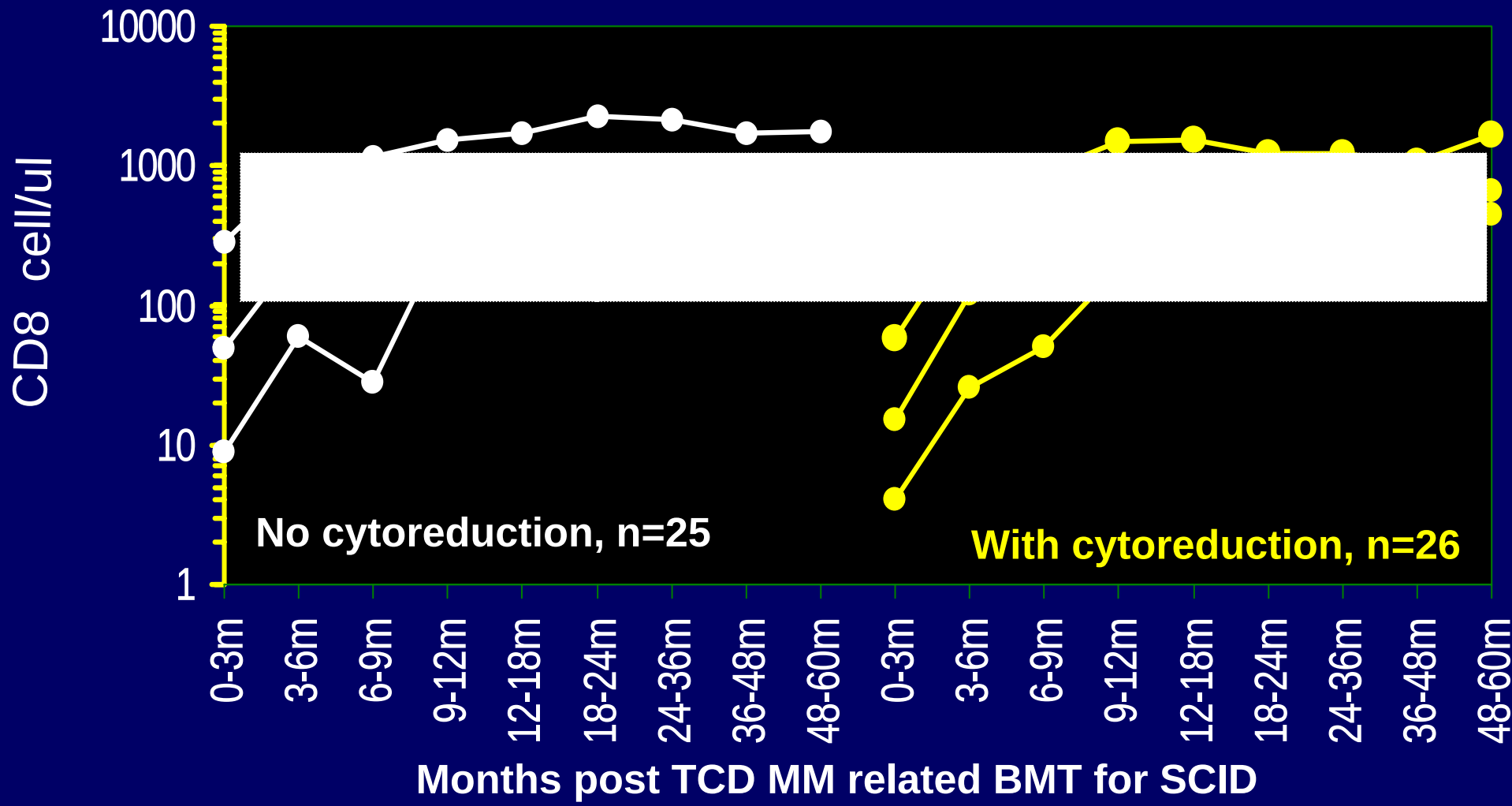
10th-50th-90th CD3 cell counts/ul



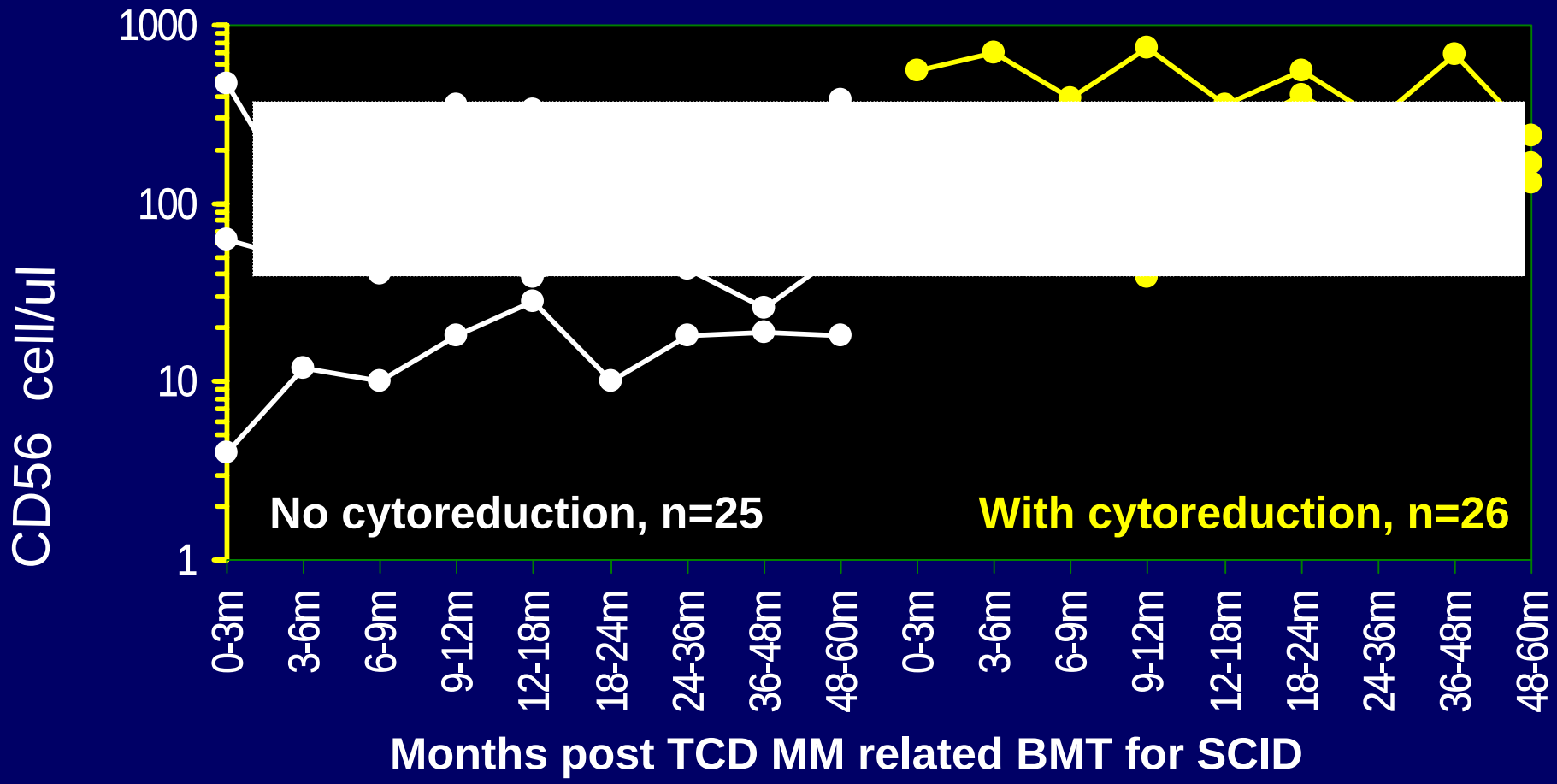
10th-50th-90th CD4 cell counts/ul



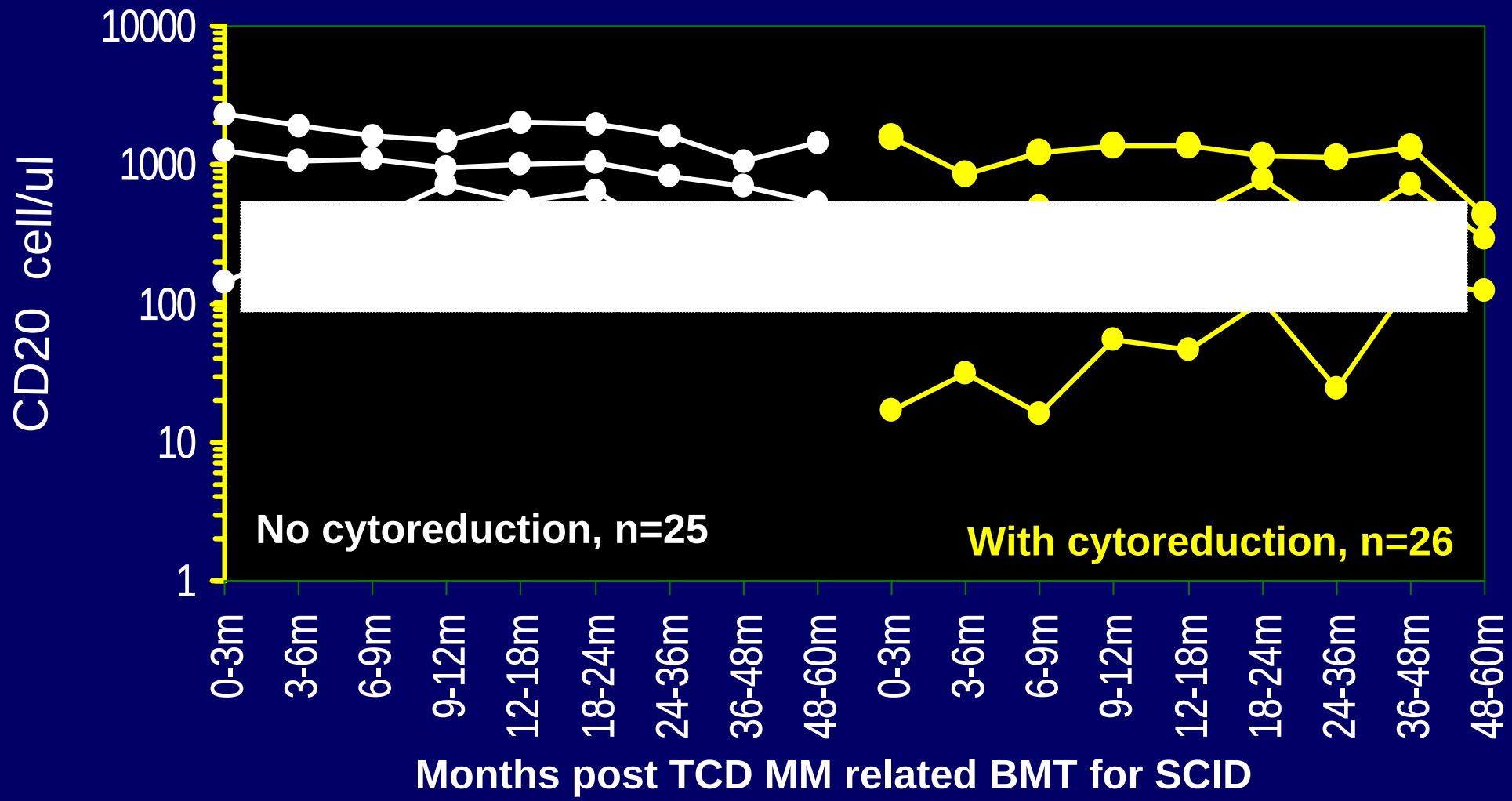
10th-50th-90th CD8 cell counts/ul



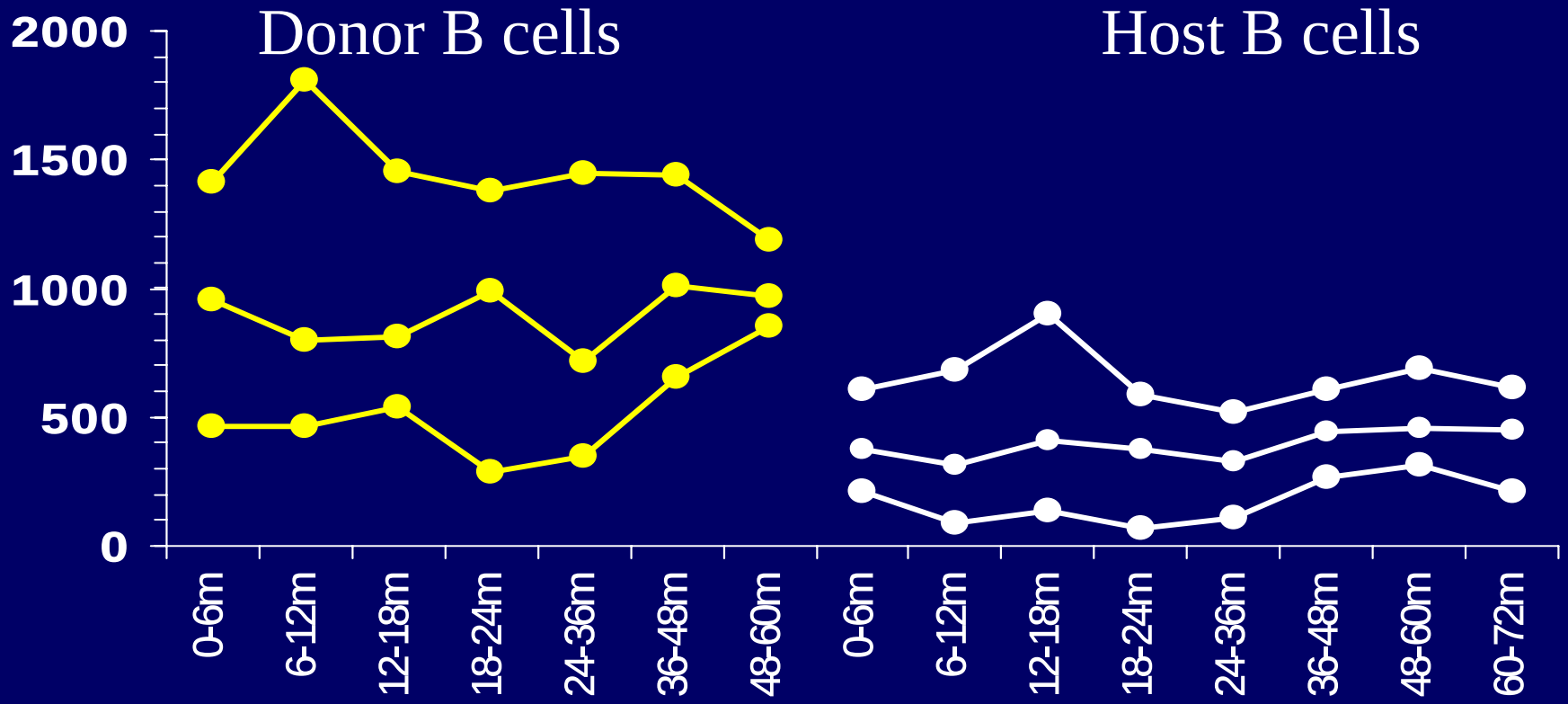
10th-50th-90th CD56 cell counts/ul



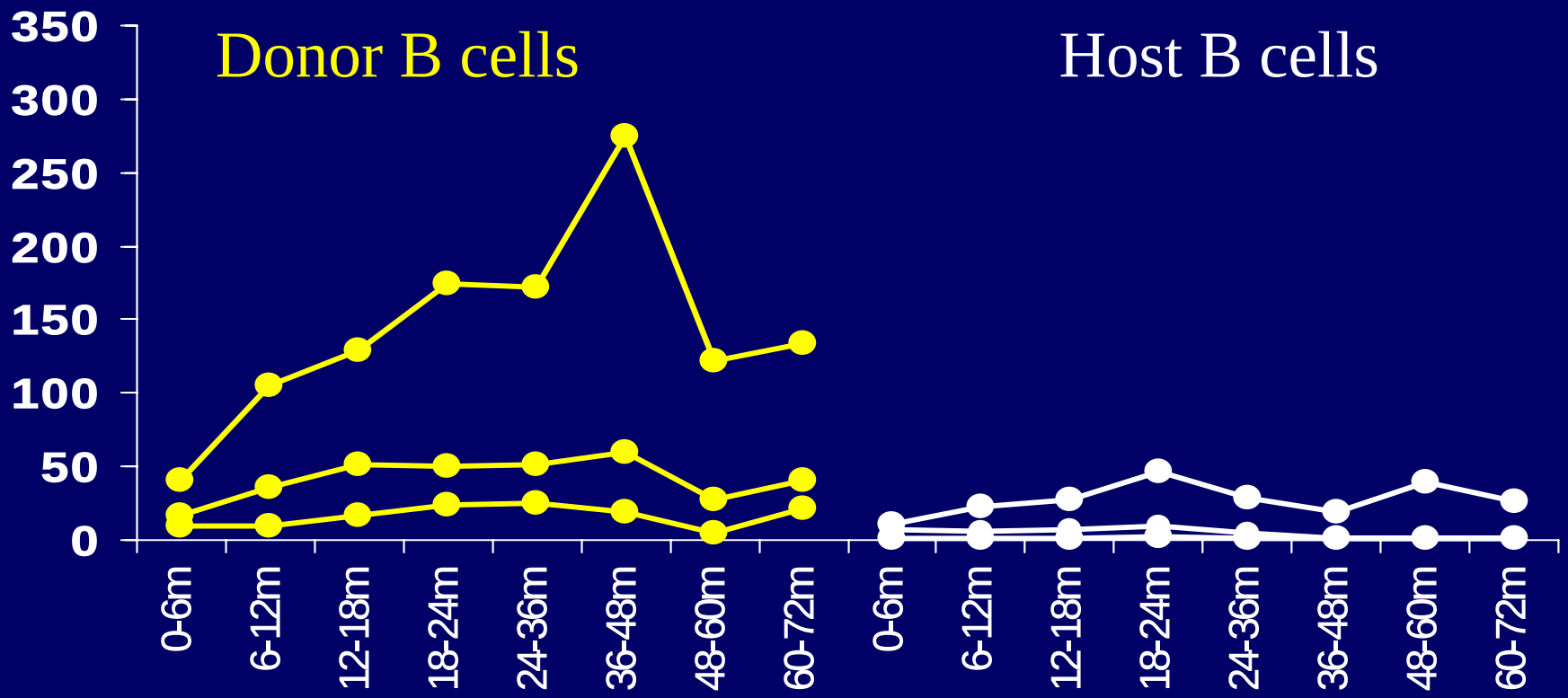
10th-50th-90th CD20 cell counts/ul



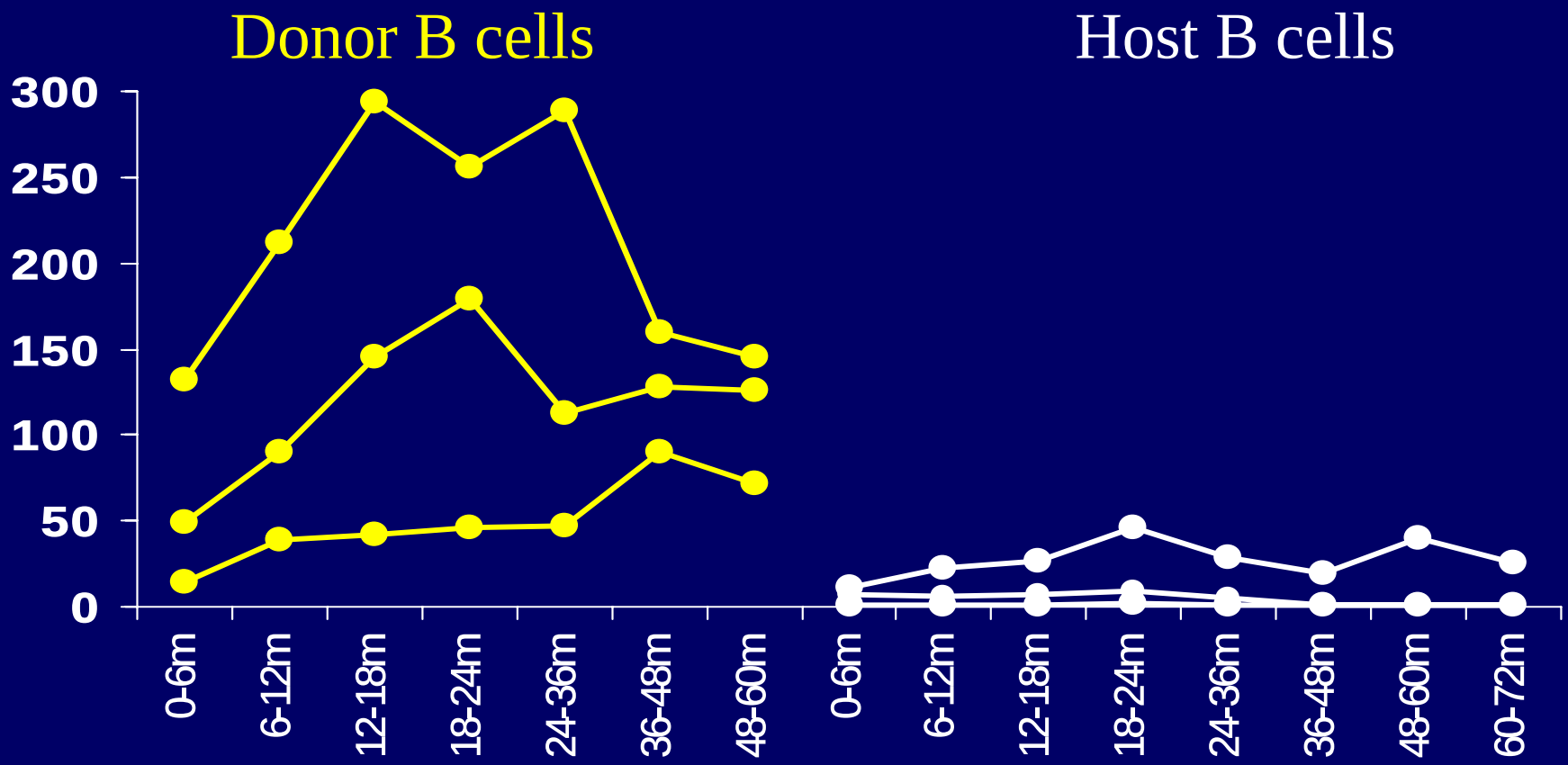
10^{th}-50^{th}-90^{th} percentile of serum IgG post Mis-matched TCD BMT for SCID: Donor B versus Host B cells



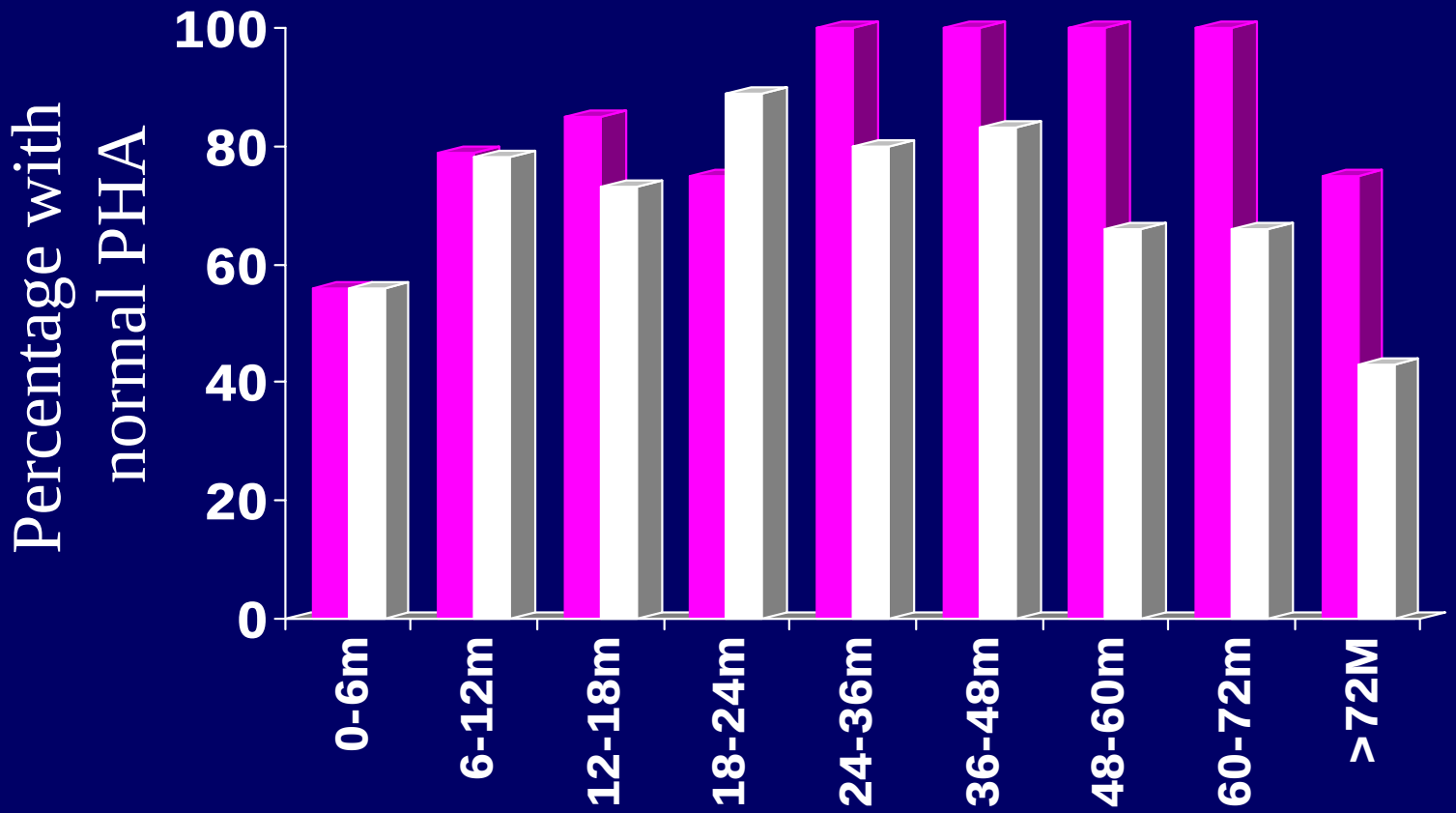
10^{th}-50^{th}-90^{th} percentile of serum IgA post Mis-matched TCD BMT for SCID: Donor B versus Host B cells



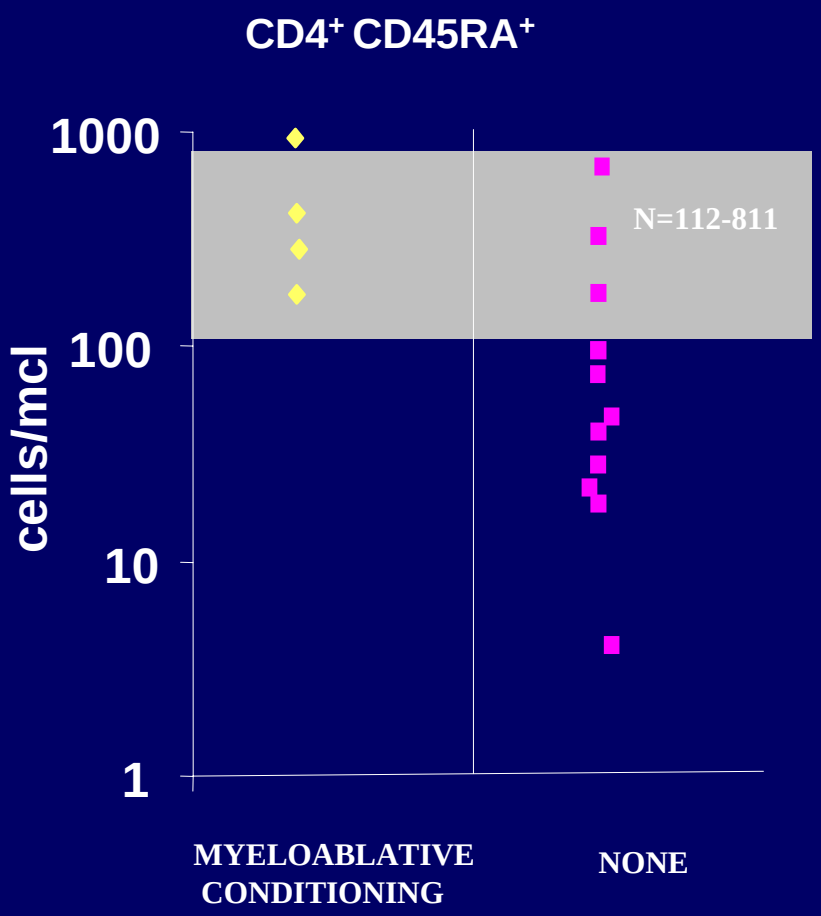
10th-50th-90th percentile of serum IgM post Mis-matched TCD BMT for SCID: Donor B versus Host B cells

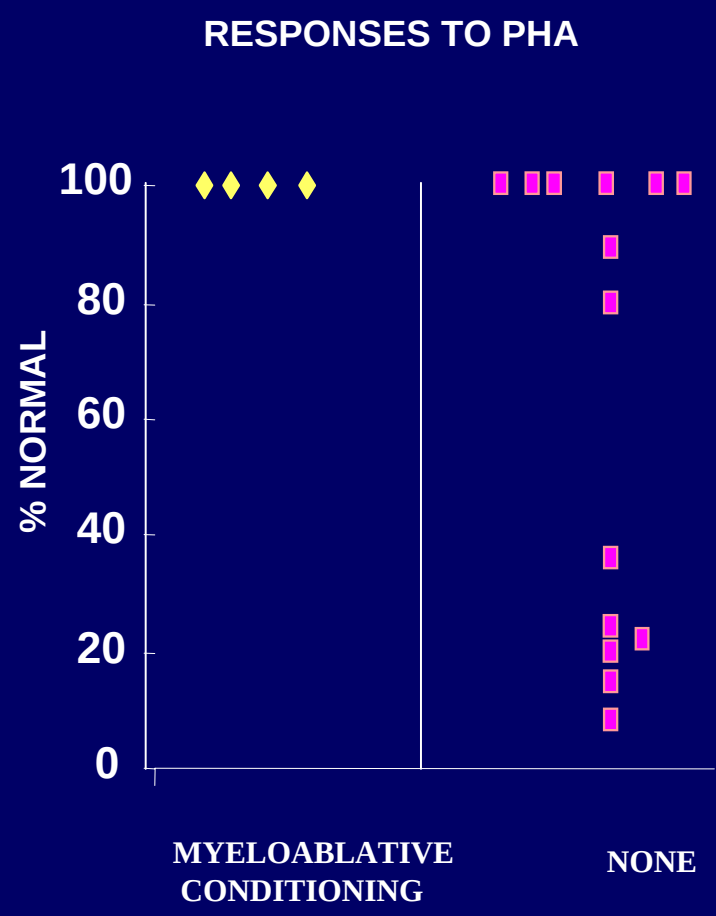


Comparison of the percentage of children with PHA greater or equal to 80% of the lower limit of normal post SBA- E- BMT for SCID: With OR Without pre-transplant cytoreduction



ANALYSIS OF T-CELL RECONSTITUTION 10+ YRS POST TRANSPLANT





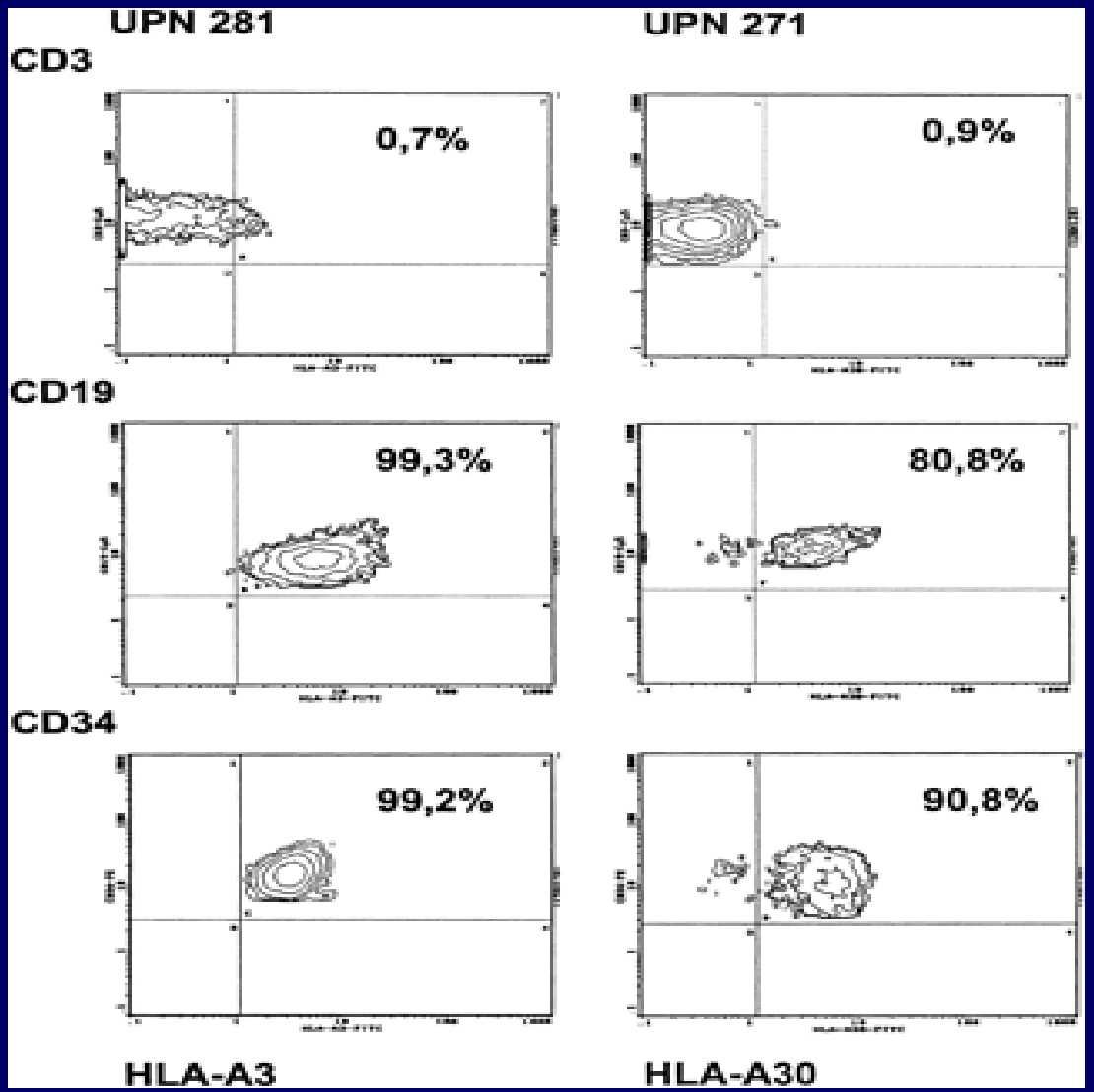
CAUSES OF DEATH

EARLY				LATE	
Pre or Without Engraftment		Post Engraftment		Post T cell Reconstitution	
N	9		5		6
CMV hepatitis or CMV Encelphalitis	3	Viral Pneumonia**	1	COPD	4
Viral Pneumonia*	4	Sepsis	1	Chronic Hepatitis	1
Toxicity (2^0 BMT)	1	Toxicity	2	Metabolic Seizures	1
EBV LPD		GVHD	1		

LATE COMPLICATIONS IN T-CELL RECONSTITUTED HOSTS

	Cytoreduced	Non-cytoreduced
TOTAL PATIENTS	25	28
Affected Patients	2 (8%)	11 (37%)
→ COPD + Hepatitis + Nephritis	0	1
→ Chronic Obstructive Lung Disease	0	7
→ Chronic Non-Viral Hepatitis	0	1
→ Interstitial Nephritis	0	2
→ Polymyopathy	2	0
Persistent Infections	0	6
Warts		5
Molluscum		1

CONDITIONING



Outcome of patients with X-linked SCID, n=18

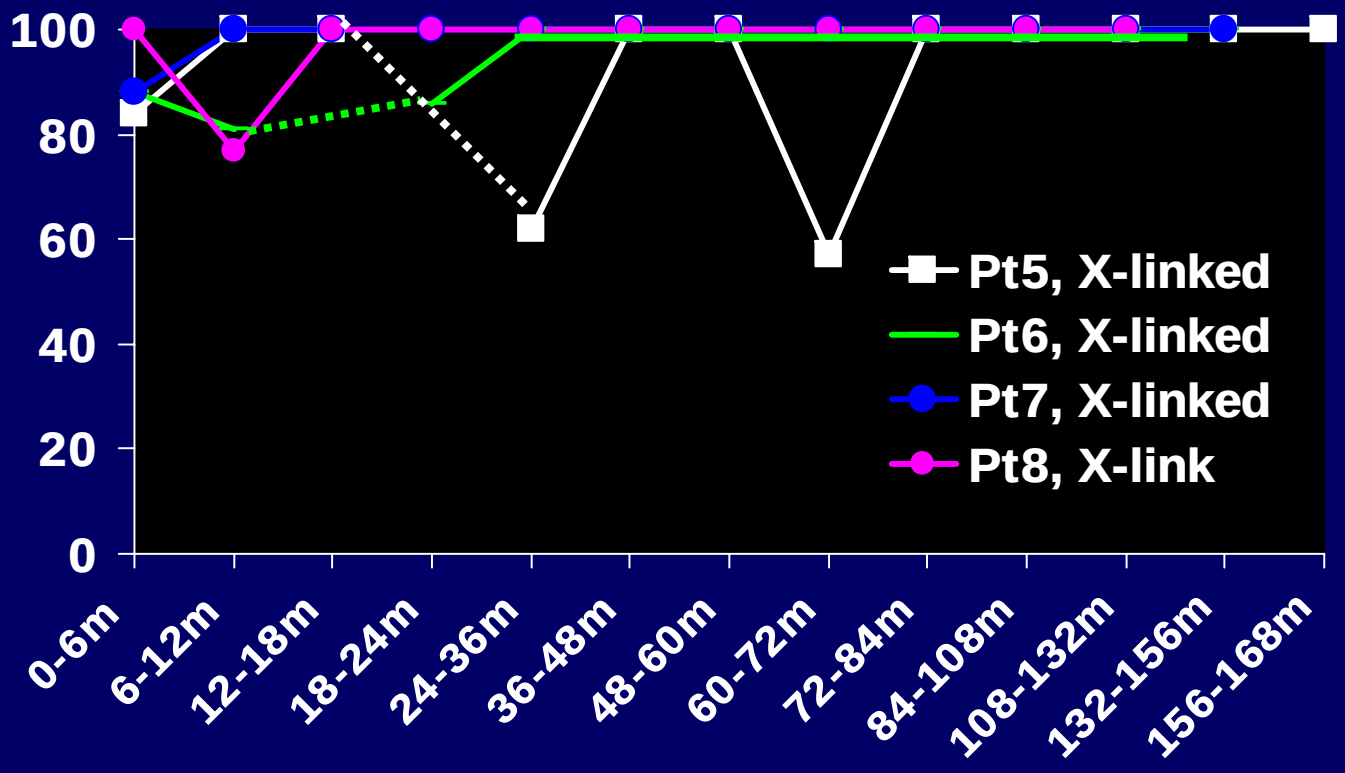
	Cause of Infection	Alive	COD
(-) Cytoreduction, n=10 Without infection	N	**10/10** 152 (63-263m+)	**NA**
(-)Cytoreduction, n=6 With antecedent infection	RSV IP, n=1 PCP, n=2 Measles, n=1 Paraflu 3, n=1 IP, unk, n= 1	**2/6** 4.7m+ 129.6m+	Measles Paraflu Graft failure Late Liver failure @70 months
(+) Cytoreduction, n=2 With antecedent infection	RSV, n=1 Cryptosporidium , n=1	**2/2** 19.7m+ 144.5m+	**NA**

Function of surviving patients with X-linked SCID, n=14*

	Function		ACHIEVED NORMAL	Late Decline in T
	B cell	NK	T cell function	Cell function
(-) Cytoreduction, n=13 Without infection	0	3/14	13/13	6/13
(+) Cytoreduction, n=2	2 /2	2/2	2/2	0/2

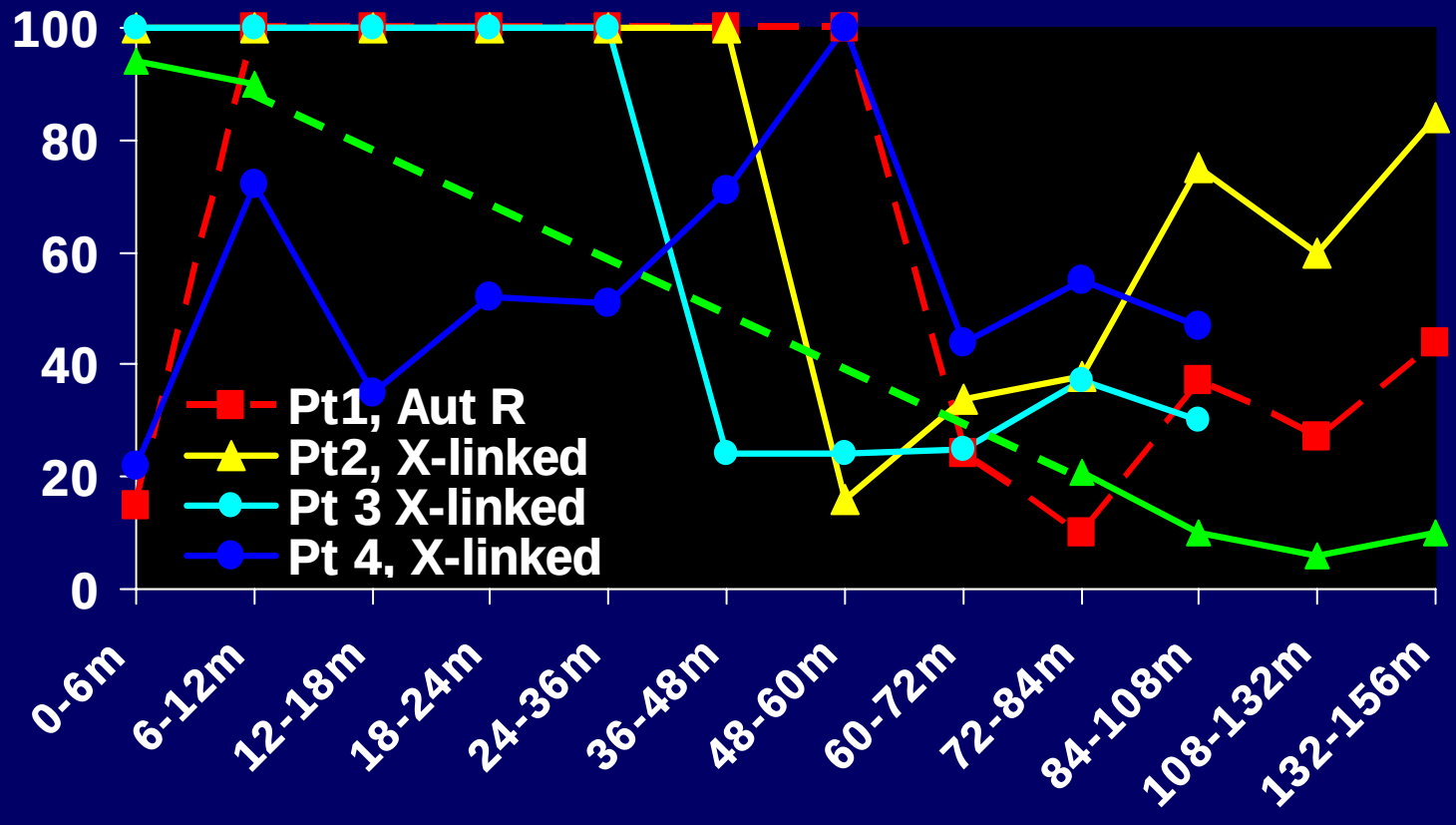
* 1 patient TETE (4 m+), 1 late death @ 70m

Kinetics of PHA response (% normal) in SCID patients who did not develop secondary T cell deficiency post TCD MM BMT



Months post SBA- E- MM-Related BMT: No cytoreduction

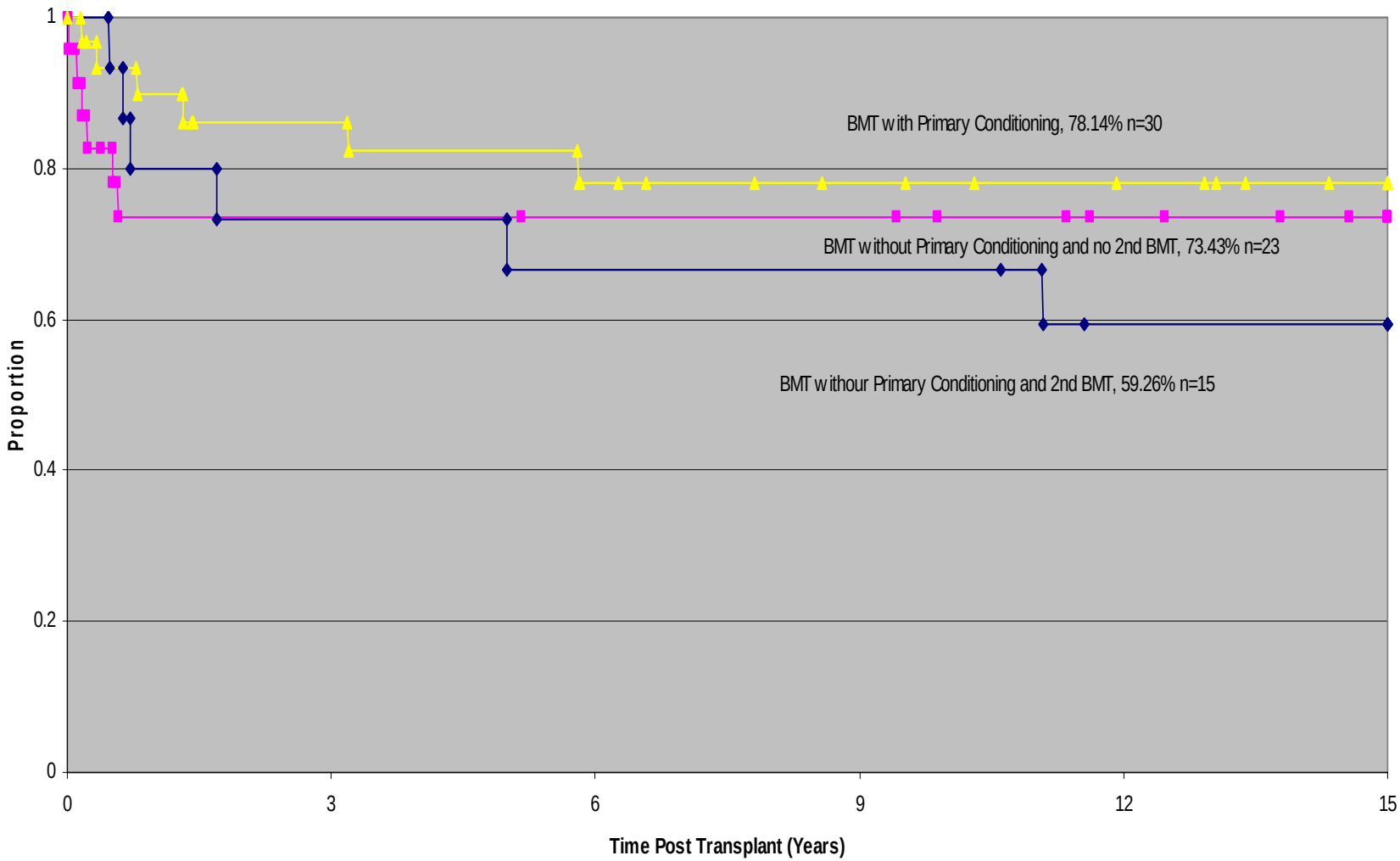
Kinetics of PHA response (% normal) in SCID patients who developed secondary T cell deficiency post TCD MM BMT



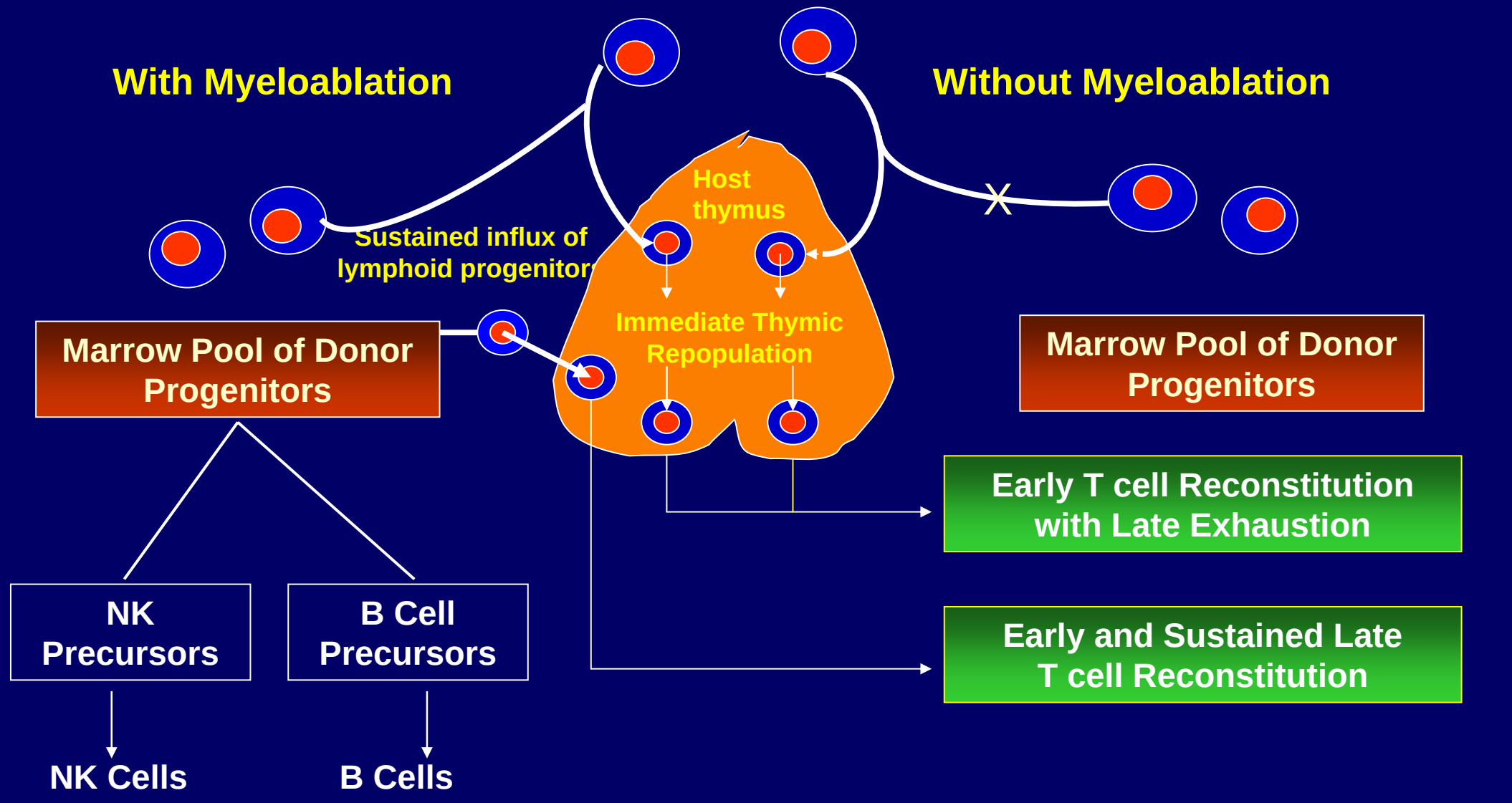
Months post SBA- E- MM-Related BMT: No cytoreduction

HLA Haplotype Disparate SBA^{-}E^{-} T cell Depleted Marrow Grafts

	No Cytoreduction	Myeloablative Cytoreduction
T cell chimerism	+	+
T cell function	+	+
B cell chimerism	Rare	Consistent mixed or full chimerism
Antibody production	Rare	Consistent
NK cell function	Persistent deficiency	Consistent normal function
Late reductions in thymic output and new T cell production	>80%	10%
Late development of “autoimmune” nephritis and hepatitis	5-10%	0%
HPV infections	Common	Rare
Viral hepatitis	Significant risk	Rare


Survival at 15 Year by Primary Conditioining and 2nd BMT
Mismatched T-Depleted BMT for SCID
BMT with Primary Conditioning, 78.14% n=30
BMT without Primary Conditioning and no 2nd BMT, 73.43% n=23
BMT withour Primary Conditioning and 2nd BMT, 59.26% n=15
Proportion
1
0.8
0.6
0.4
0.2
0
0
3
6
9
12
15
Time Post Transplant (Years)

Hematopoietic Cell Transplant



The Importance of Sustained Engraftment of a Pool of Donor-Derived Self-Renewing Hematopoietic Progenitor Cells

1. Engraftment of donor NK cells and reconstitution of NK cell function
2. Engraftment of donor B cells for reconstitution of humoral immunity
3. Maintenance of an adequate pool of thymic precursors to sustain T cell reconstitution
4. Maintenance of thymic constituents critical for positive selection of antigen-reactive cells and negative selection against auto-reactive T cells?

Cell Mediated Cytotoxic Responses of an Immunoreconstituted SCID Patient in Response to Allogeneic Stimulation

Priming Combination	Priming Antigens	E:T Ratio	FA A/B	MO C/D	SIS A/C	PT-PBL B/C + C/D	PT-B-LCL B/C Pre-transplant	DR-3 B-LCL (B)	K562
Mother versus Father$_x$	Aw32 B8,B15 DR3,DR2	100:1	62	4	53	28	69	69	38
		50:1	68	5	39	24	69	69	33
Patient versus Father$_x$	Aw32 B8,B15 DR3,DR2	100:1	22	4	20	10	3	3	37
		50:1	23	6	20	7	2	1	14
Mother (50%) + Patient (50%) versus Father$_x$	Aw32 B8,B15 DR3,DR2	100:1	60	5	51	18	59	64	32
		50:1	56	3	41	15	55	60	32