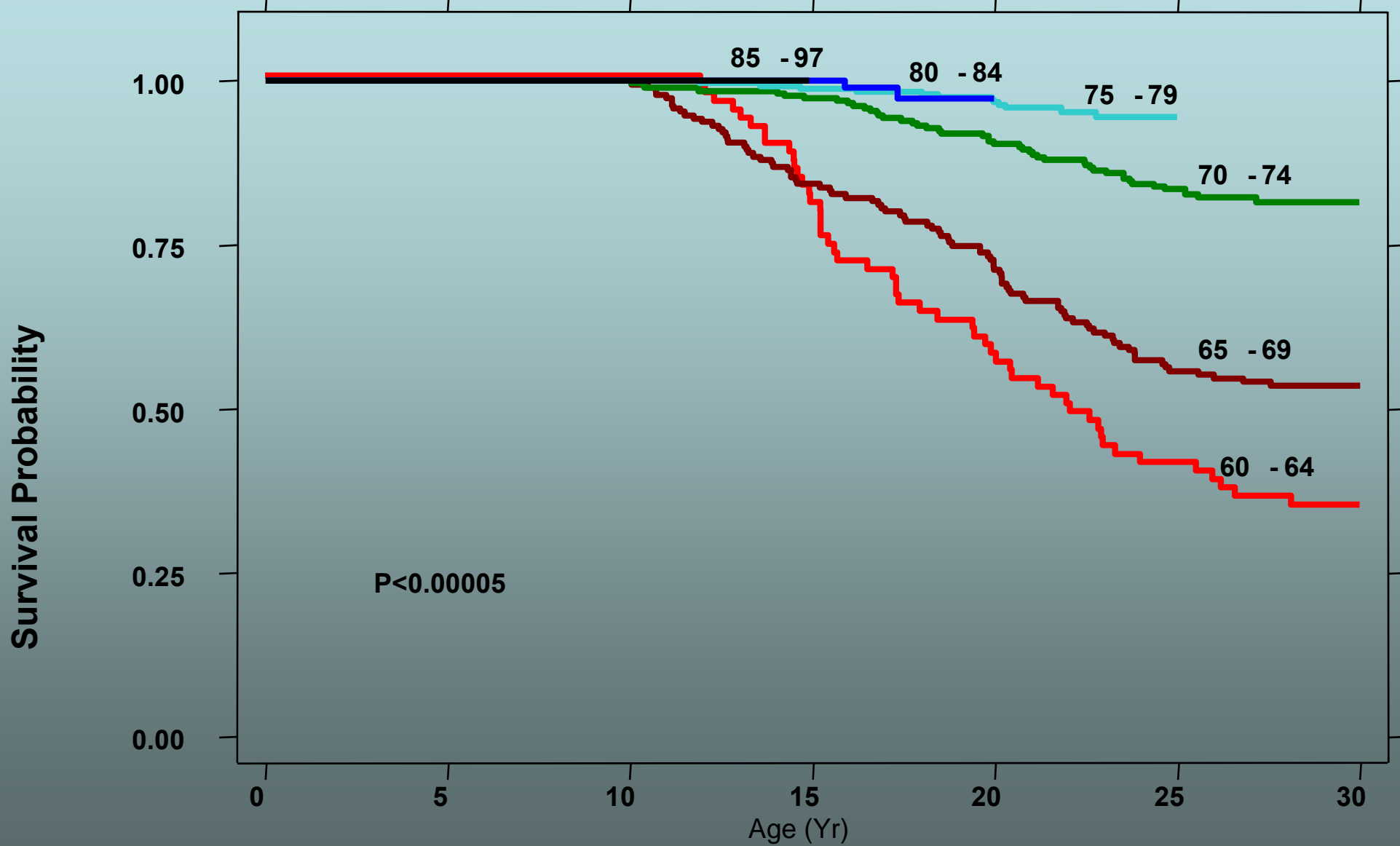


Stem cell transplantation for haemoglobinopathies

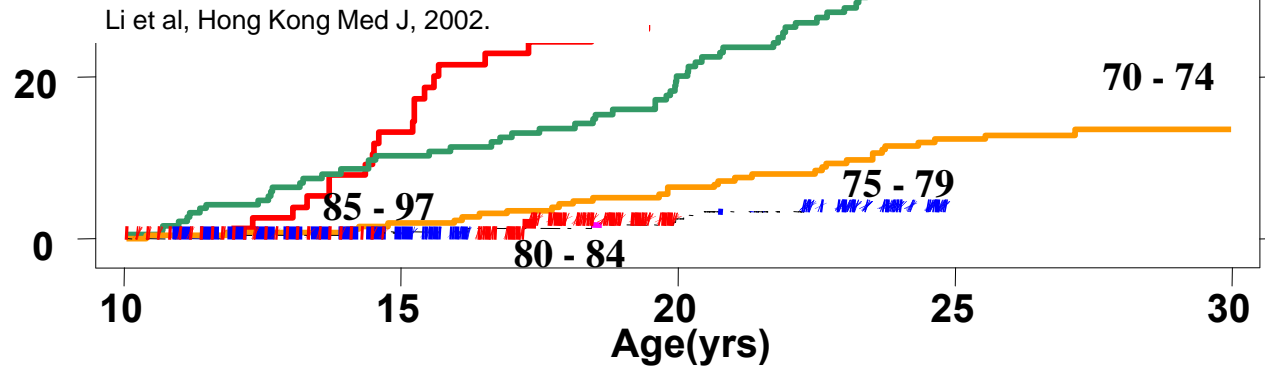
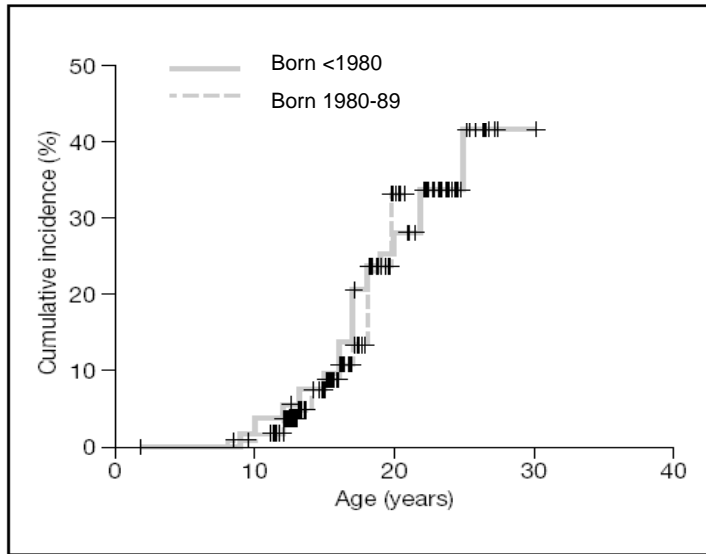
Dr P J Darbyshire

Birmingham Childrens Hospital

Survival by Cohort of Birth (N=977)



Probability of death due to heart disease after age 10 yrs



Survival in thalassaemia major in Torino: 257 patients

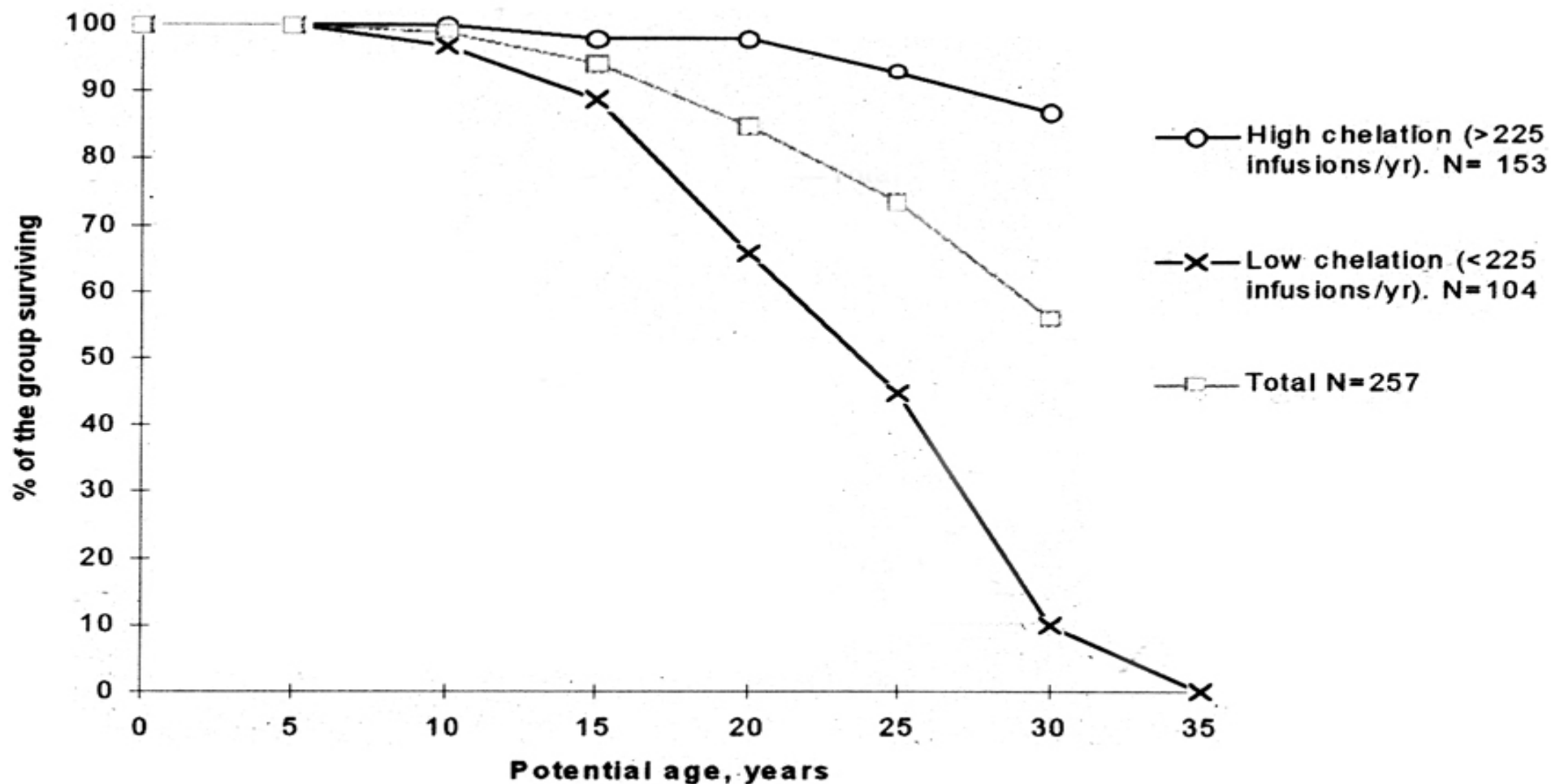
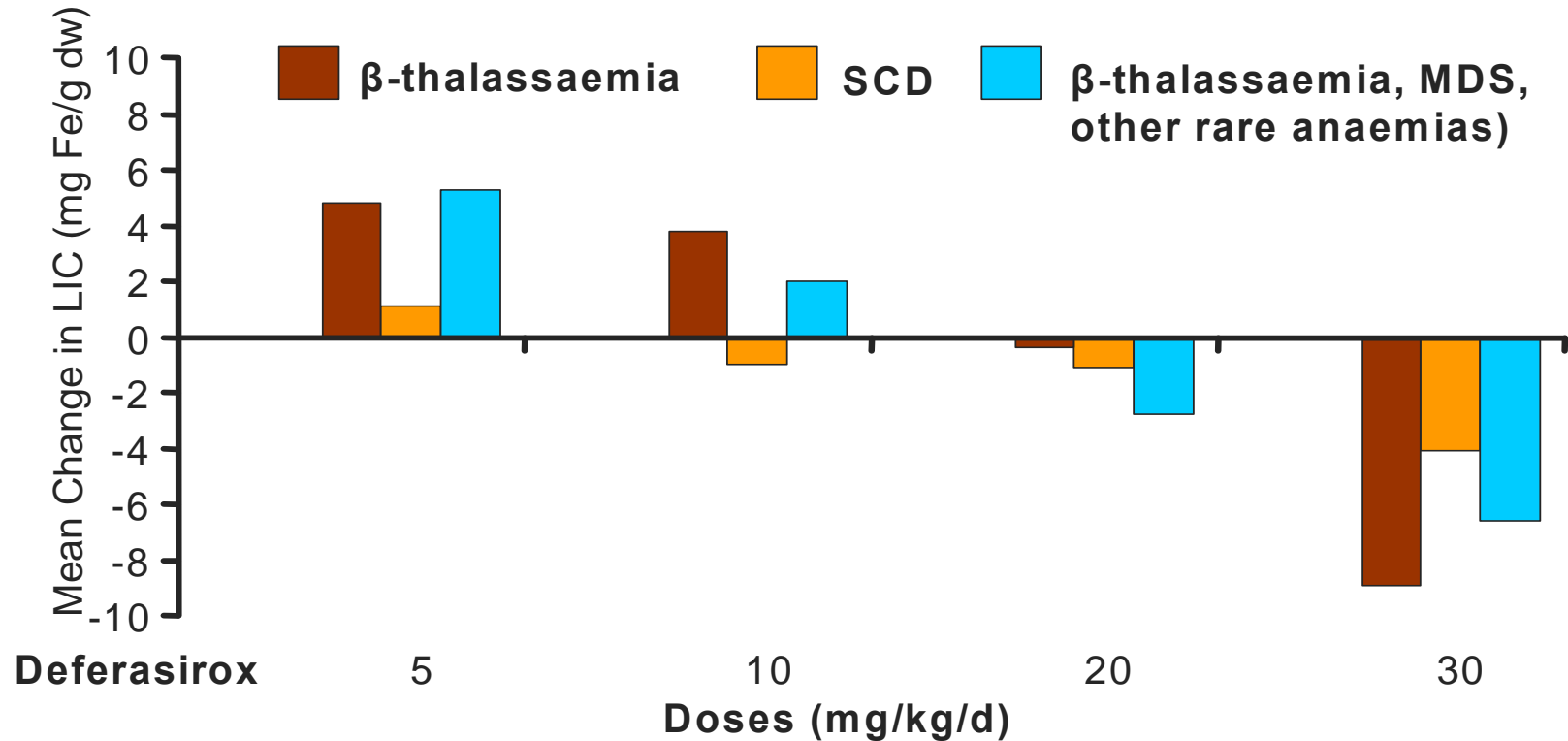


Figure 2. Results of a recent survival study in Torino, Northern Italy, showing the effect of strict compliance with desferrioxamine therapy on survival.

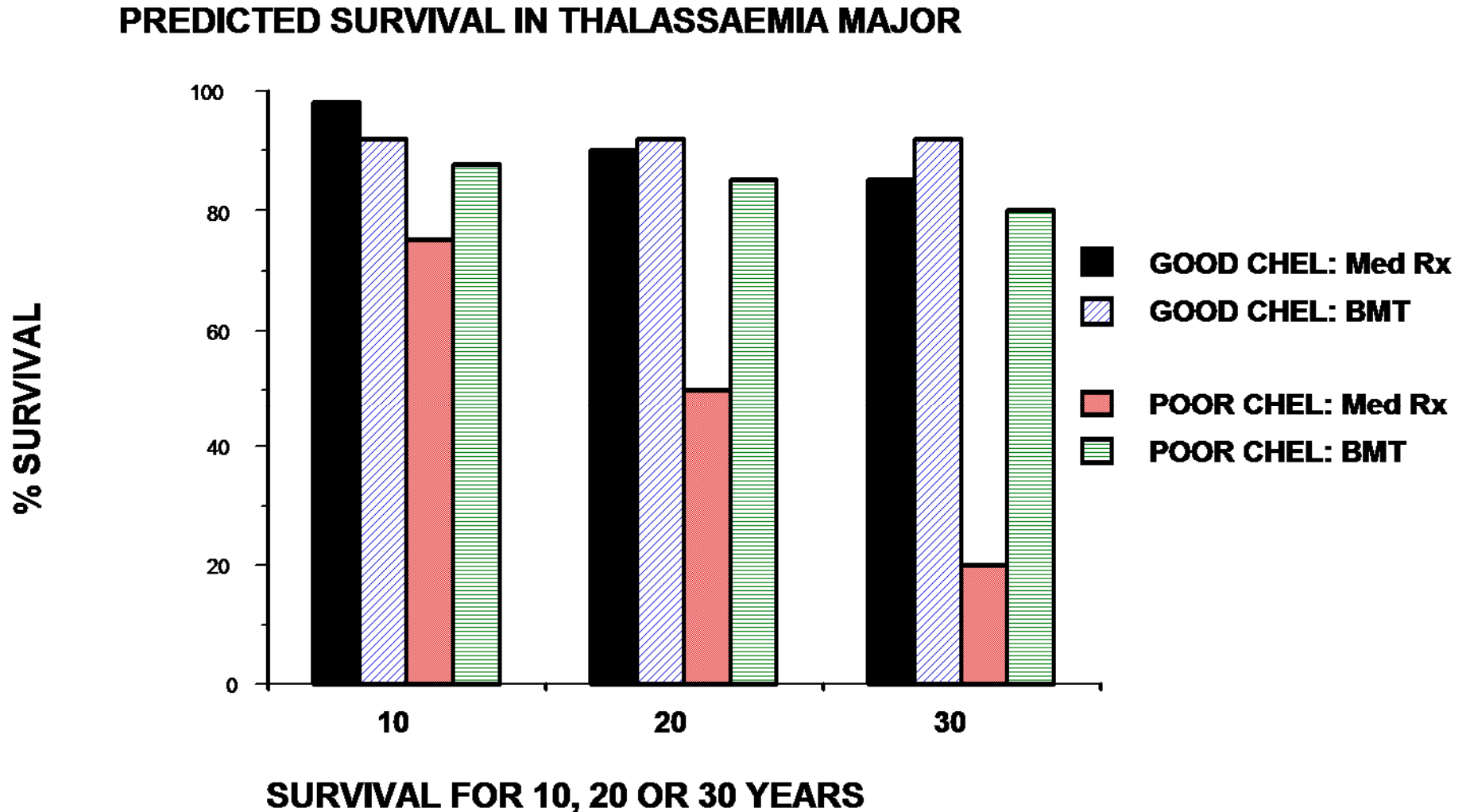
Effects of Iron Chelators on Liver Iron Concentration (LIC)

LIC: Good control with desferrioxamine or deferasirox; inconsistent effects with deferiprone

Deferasirox shown to maintain and reduce LIC in phase 2/3 clinical trials in adult and paediatric patients (12-month efficacy—LIC)



Role of BMT for Thalassaemia major



Outcomes of 1189 beta thalassaemia conceptions in the UK

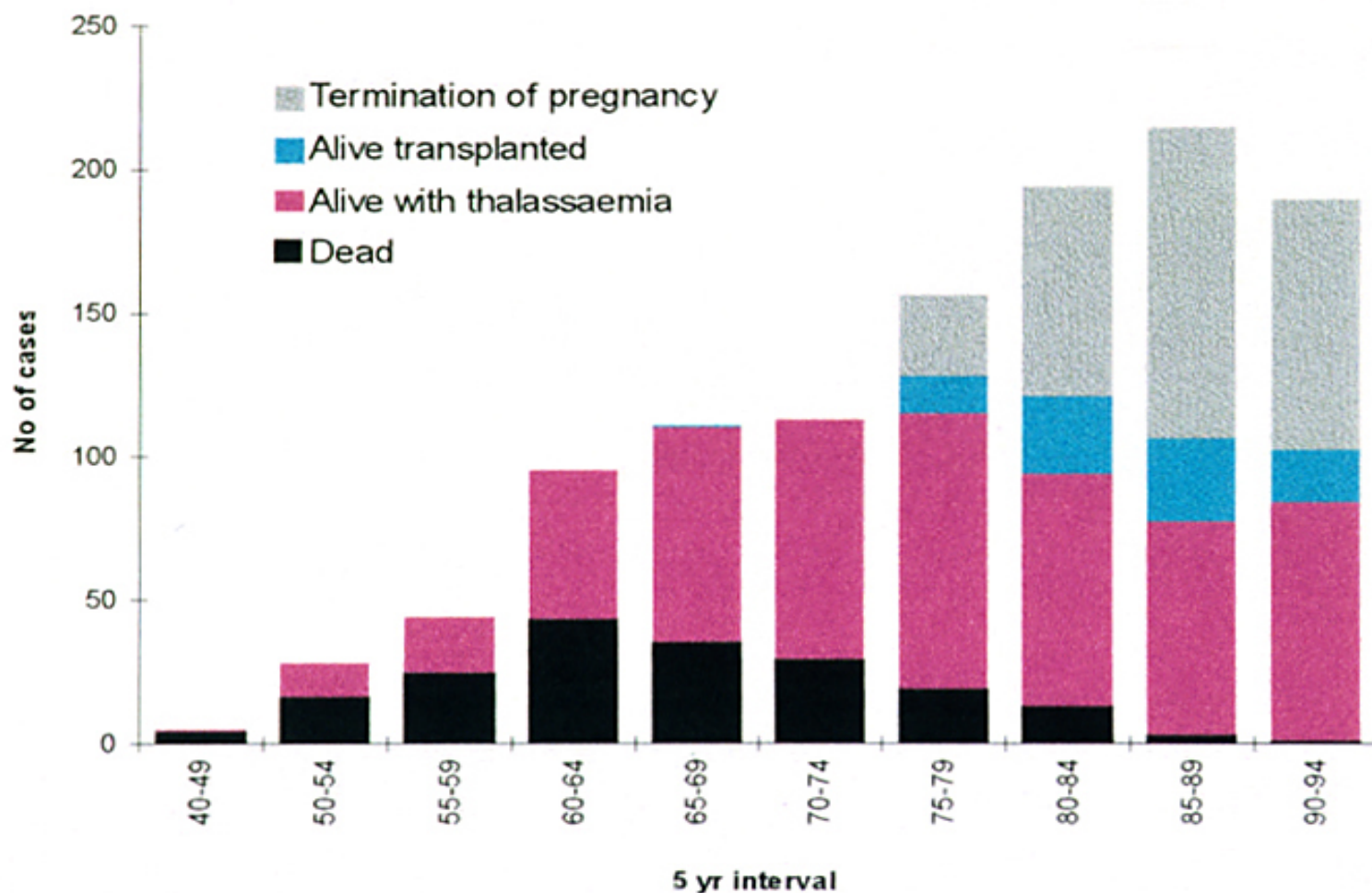


Figure 6. Total affected thalassaemia conceptions and outcomes since thalassaemia appeared in the UK in the late 1950s, by 5-year intervals.

Management of β -thalassaemia: the role of BMT

Patient choice:

quality of life

Physician choice:

poor compliance

failure of medical Rx

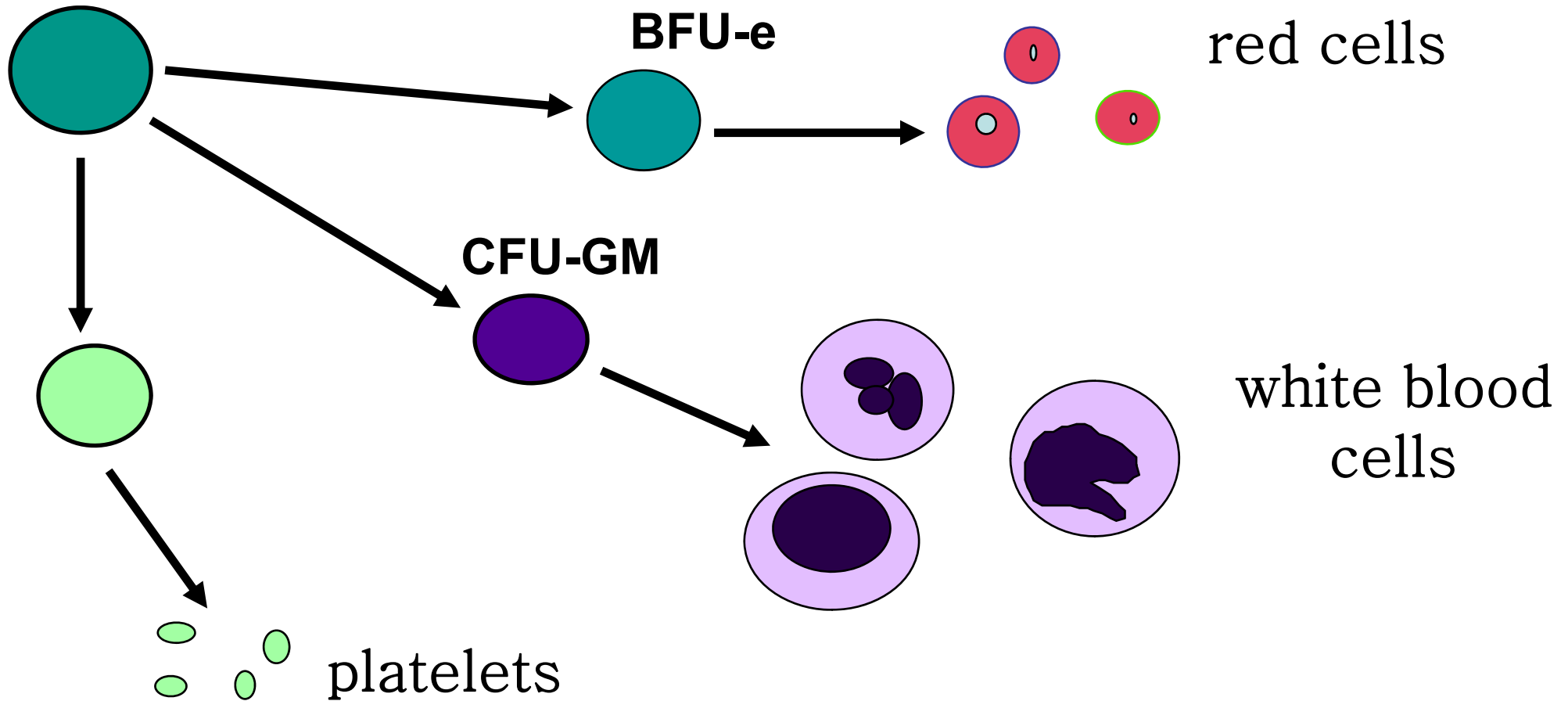
Political choice:

unavailability of good

quality medical care

Principles of stem cell transplantation for haemoglobinopathies

Multipotent stem cell



SCT in Haemoglobinopathies

CCLG

β Thalassaemia Major

- Offered to all children ≤ 16 years with transfusion-dependent thalassaemia

PLUS

- HLA-identical family donor
- Consider carefully:
 - patients >16 years
 - previous failed SCT

Sickle Cell Disease

- Stroke
- Recurrent Chest Syndrome*
- Recurrent VOC*

* if hydroxycarbamide fails

Emerging indication:

- CNS disease
- Risk of CNS disease

SCT for haemoglobinopathies: conditioning

Drug

Dose

Schedule

Busulphan

14 mg/kg

day -9 to -6

**Cyclophosphamide
to -2**

200 mg/kg

day -5

Campath 1H

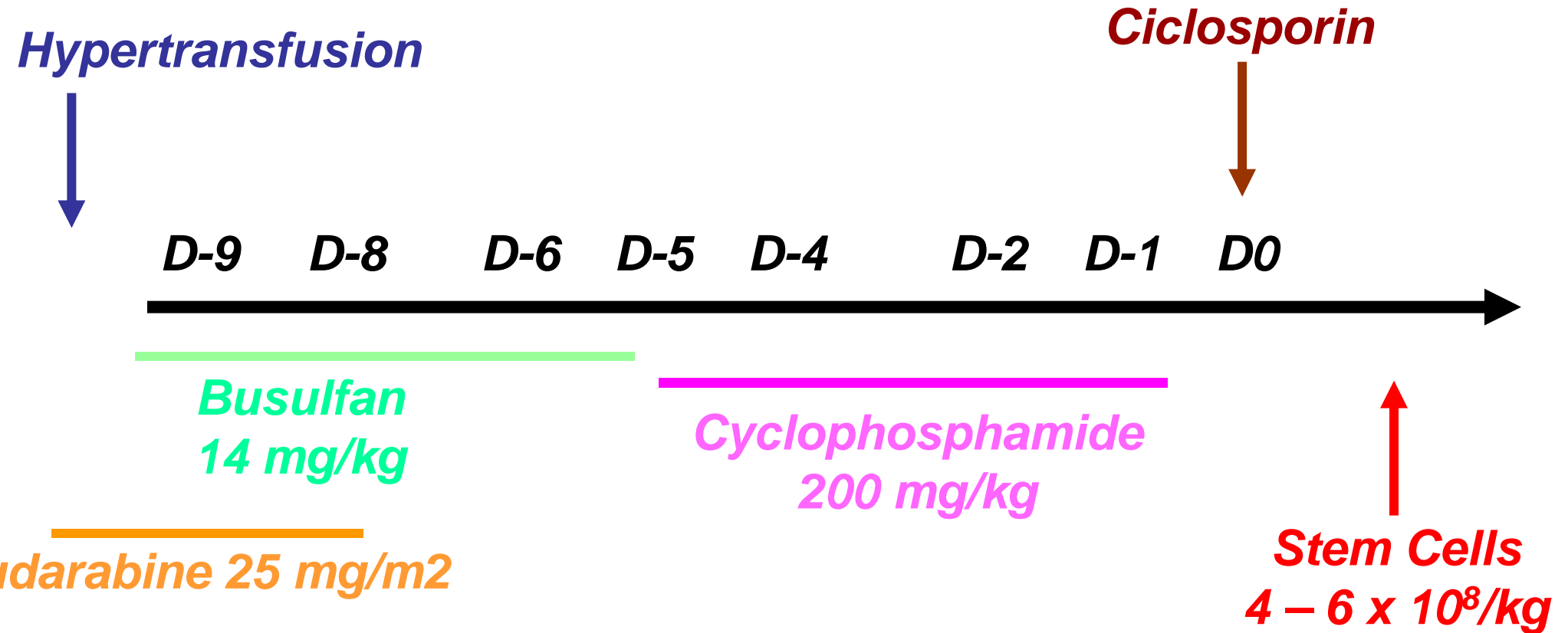
0.1 mg/kg

day -9 to -7

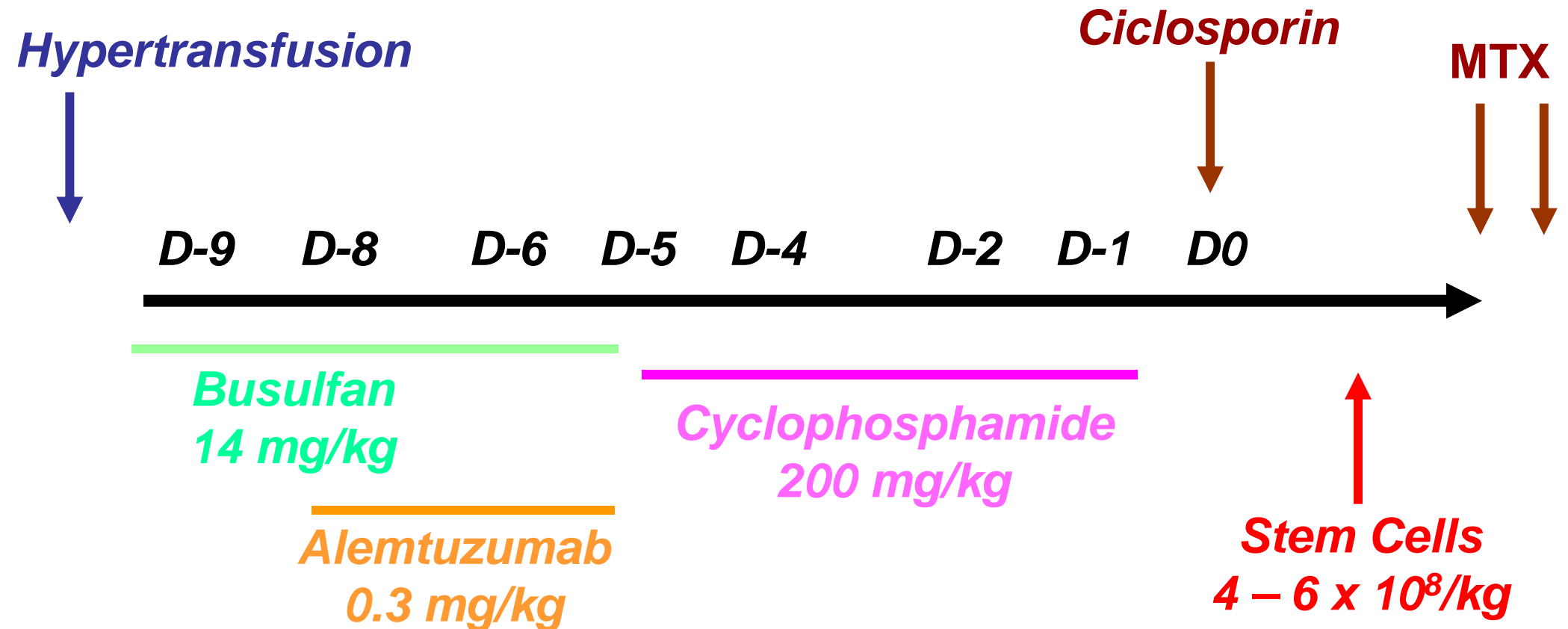
SCT for haemoglobinopathies: conditioning

Drug	Dose	
Schedule		
Fludarabine	125 mg m²	day -12 to -7
Busulphan	14 mg/kg	day -9 to -6
Cyclophosphamide to -2	200 mg/kg	day -5

BCH Conditioning Protocol



Imperial College Healthcare Conditioning Protocol



BMT for Children with Thalassaemia at BCH/HH

DONOR CHARACTERISTICS

No of Children

HLA-IDENTICAL:

Brother 26

Sister 25

Parent 3

AGE (years): 5.8 (1.4 to 32)

THALASSAEMIA TRAIT: 34

Lawson et al, 2003

BMT for thalassaemia: the Birmingham and Hammersmith experience

No of Children	54
Age (years)	6.6 (2 - 16)
Ethnic origin:	
Pakistani / Indian	39
Mediterranean	10
Arabic	5

Lawson et al, Br J Haematol, 120: 289,

BMT for Children with Thalassaemia at BCH/HH

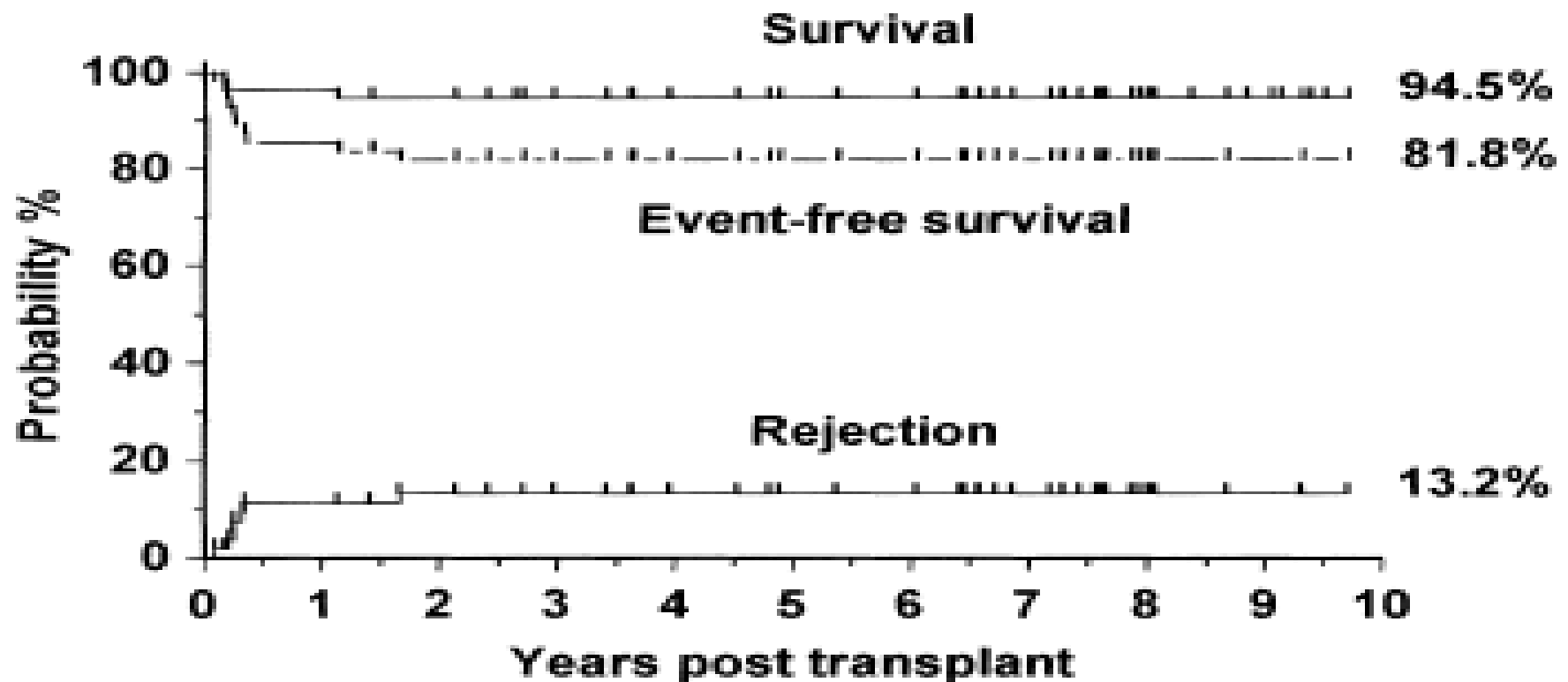


Fig 1. The probability of survival, EFS and rejection post-HLA-matched related BMT in the UK.

Lawson et al, 2003

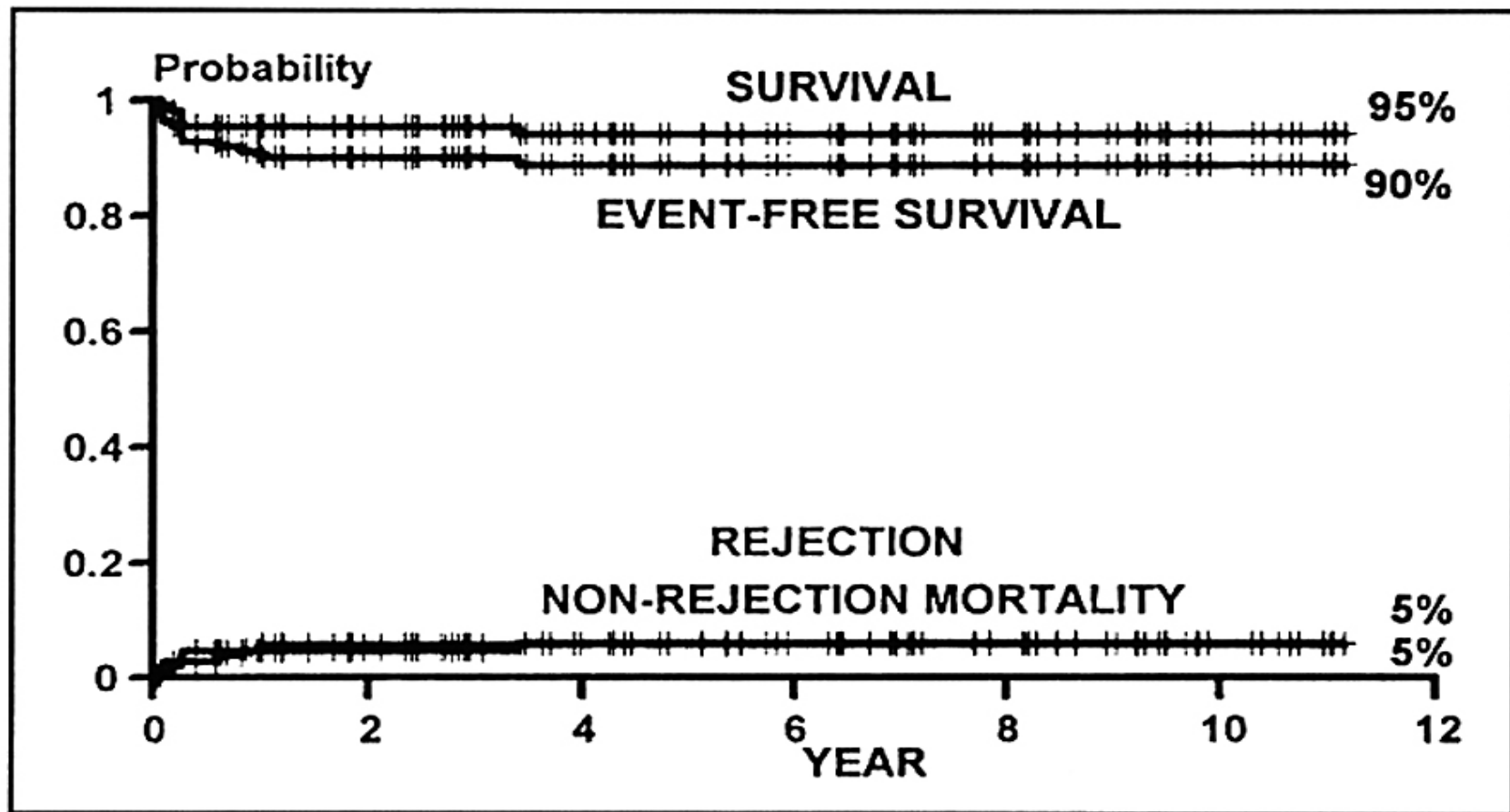


FIGURE 2. Kaplan-Meier probabilities of survival, event-free survival, rejection, and non-rejection mortality for 121 thalassaemic patients aged less than 17 years, transplanted from HLA-identical donors after preparation with busulfan (14mg/kg), cyclophosphamide (200 mg/kg), and cyclosporine alone from January 2, 1986 through April 10, 1997 and calculated on May 15, 1997.

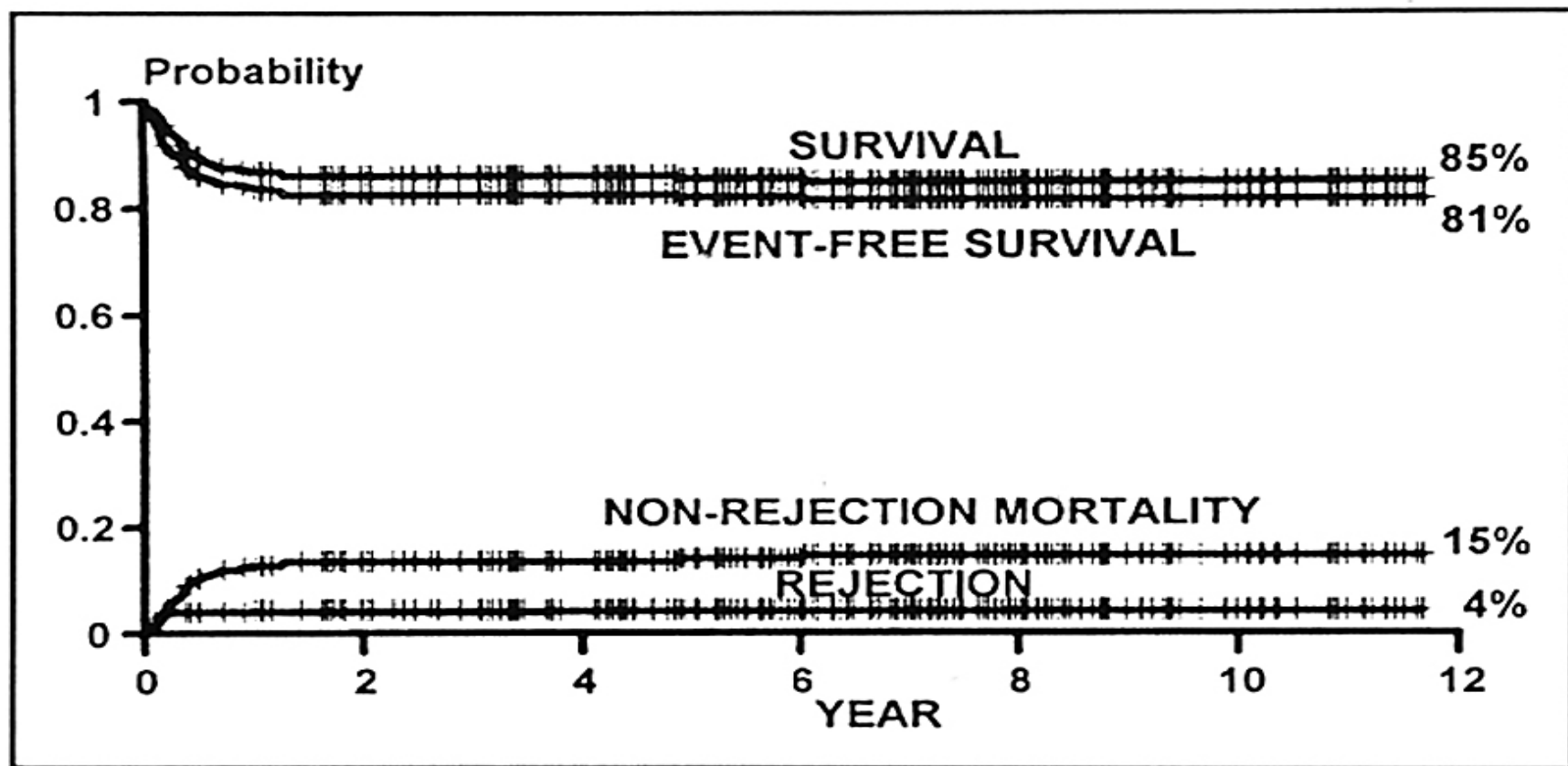


FIGURE 3. Kaplan-Meier probabilities of survival, event-free survival, rejection, and non-rejection mortality for 272 Class 2 thalassaemic patients aged less than 17 years, transplanted from HLA-identical donors after preparation with busulfan (14mg/kg), cyclophosphamide (200 mg/kg), and cyclosporine alone from June 6, 1985 through April 10, 1997 and calculated on May 15, 1997.

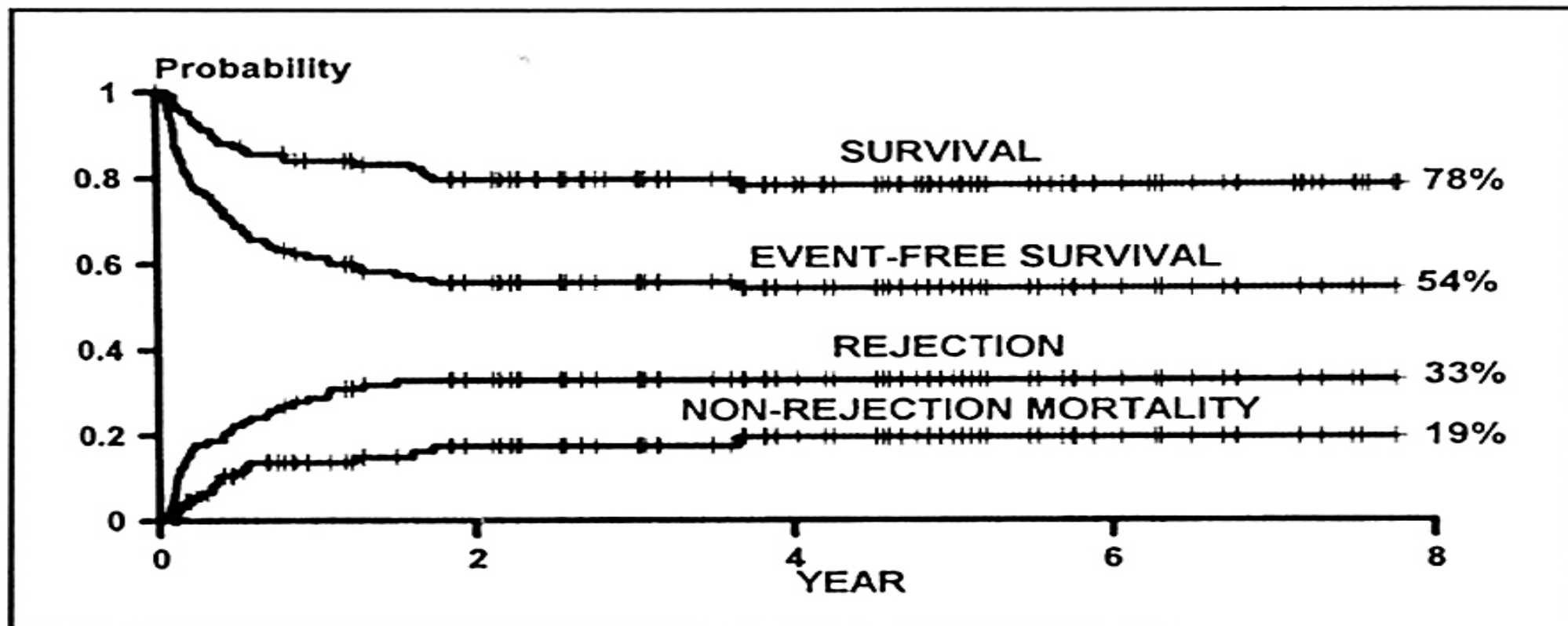


FIGURE 4. Kaplan-Meier probabilities of survival, event-free survival, rejection, and non-rejection mortality for 125 Class 3 thalassaemic patients aged less than 17 years, transplanted from HLA-identical siblings after preparation with busulfan (14mg/kg), cyclophosphamide (120-160 mg/kg), and cyclosporine plus “short” methotrexate from March 1989 through April 10, 1997 and calculated on May 15, 1997.

SCT for thalassaemia major: Class 3

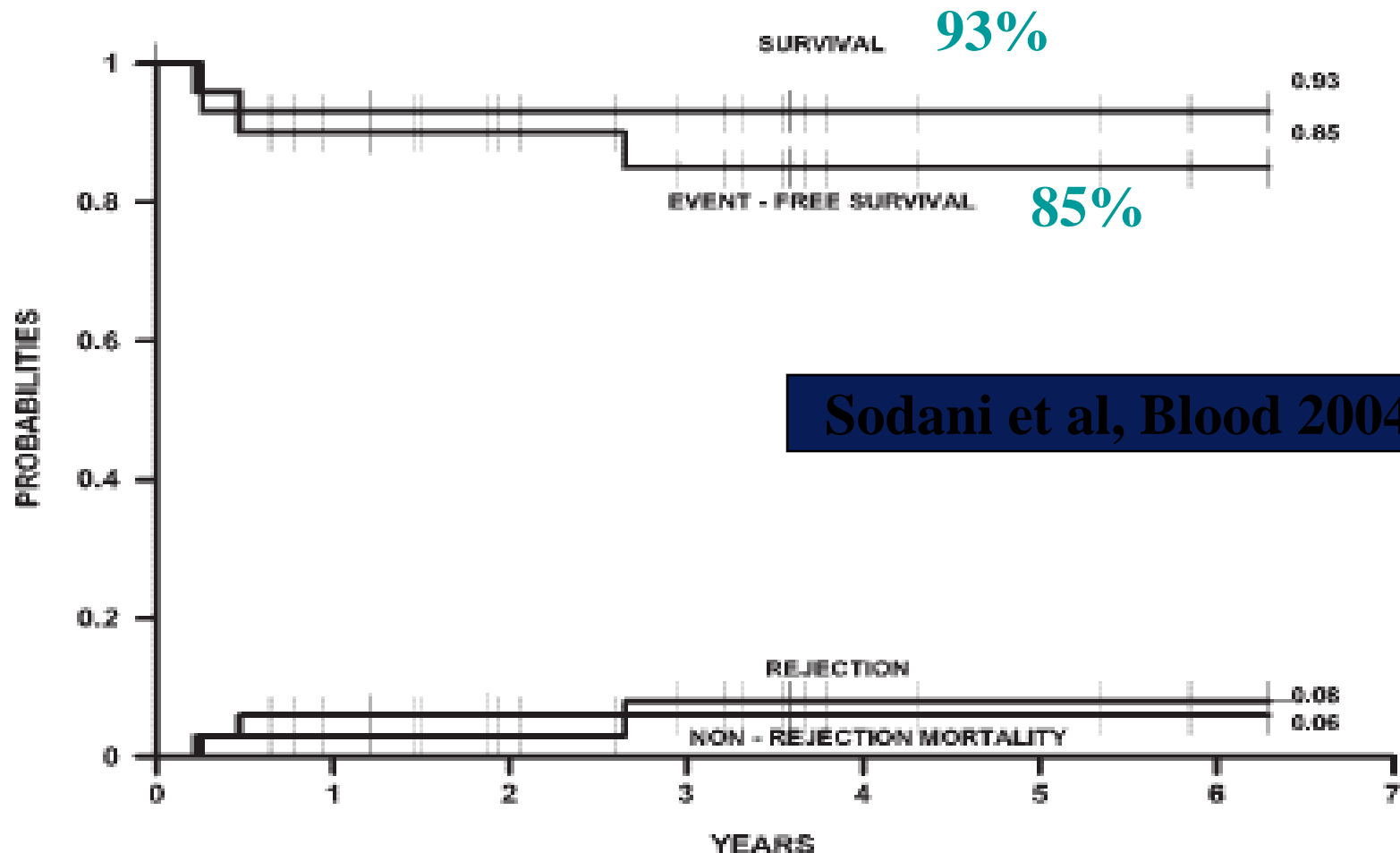


Figure 1. Kaplan-Meier probabilities of survival, thalassaemia-free survival, and cumulative incidences of rejection and nonrejection mortality in 33 thalassaemic patients aged younger than 17 years, prepared for transplantation with protocol 26.

BMT for Thalassaemia major:

Long-term effects

GROWTH

**Usually normal
or improved**

SEXUAL DEVELOPMENT

Delayed in 50%

FERTILITY

**Gonadal failure
common, esp girls**

OTHER

**Iron overload
Malignancy (0.9%)**

Follow up

- Must include venesection
- 34% women 67 % of men went through puberty normally
- Spontaneous pregnancy very rare after Bu/Cy conditioning
- New in vitro techniques may benefit some men

Follow up

- Second malignancy rare mostly EBV and GVH related less than 1%
- One case of mouth SCC in Birmingham
- No real studies of quality of life but reasonable assumption that life without DFO better than life with infusions
- No one asked the patients !

Limitations of SCT

- Lack of donors
 - Length of Treatment:
 - 2 months as an inpatient
 - 4 months as outpatient
 - Transplant Related Mortality
 - Long Term Effects:
 - Infertility
 - Pubertal failure
 - Chronic GvHD
 - Organ toxicity
 - Secondary malignancy

SCT for thalassaemia: mismatched related donors

No of patients

Transplanted	29
m/m sibling donors	13
m/m parental donors	8
other relatives	8
Event-free survival	21%
Overall survival	65%

Gaziev et al, 2000

Unrelated donor BMT for thalassaemia

No of patients

Transplanted	32	(10 aged >16 years)
Class 1 or 2:	15	
Class 3:	17	
Survived	26	(81%)
Cured	22	(69%)

Unrelated donor BMT for thalassaemia

No of patients

Transplanted	32	
Extended haplo match	22	
Survived	19 / 22	(86%)
Cured	17 / 22	(77%)

Unrelated cord blood transplants for thalassaemia major

- 5 children aged 2-11 years
 - Conditioning: Bu 14/Cy 200 + ATG
 - 1-2 antigen mismatched CB mononuclear cells
 - Engraftment: 5/5
 - Acute GVHD > grade II: 1
 - Survival with 100% donor cells: 5/5 (follow up 6-15m)
-

Jaing et al, Biol Blood Marrow Transplant 11: 349-53, 2005

BMT for Adults with Thalassaemia Major: Outcome 1988-1996

	No of patients	%
Transplanted	107	-
Survival	69	64
Event-free survival	66	62
Recurrence	4	4

Lucarelli et al, Blood 93:1164, 1999

Eurocord Study: thalassaemia

- Largest series, multicentre study: 33 patients with β -thalassaemia major and 11 patients with SCD.
- The median age for the whole group was 5 years (range 1-20 years).
- Pesaro staging (hepatomegaly, portal fibrosis and a history of irregular iron chelation therapy):
 - Class I: 20/33
 - Class II: 13/33
 - Class III: 0/33
- Donor cells:
 - All were transplanted with family donors: 32/33 were fully matched, one being A locus mismatch.
 - median number of TNC infused: $4.0 \times 10^7/\text{kg}$ (range 1.2-13)
- Conditioning regimens:
 - Bu/Cy: 26 patients (10 with added ATG)
 - Added thiotepa: 16 patients
- GvHD prophylaxis: 12 methotrexate

- **Engraftment:**

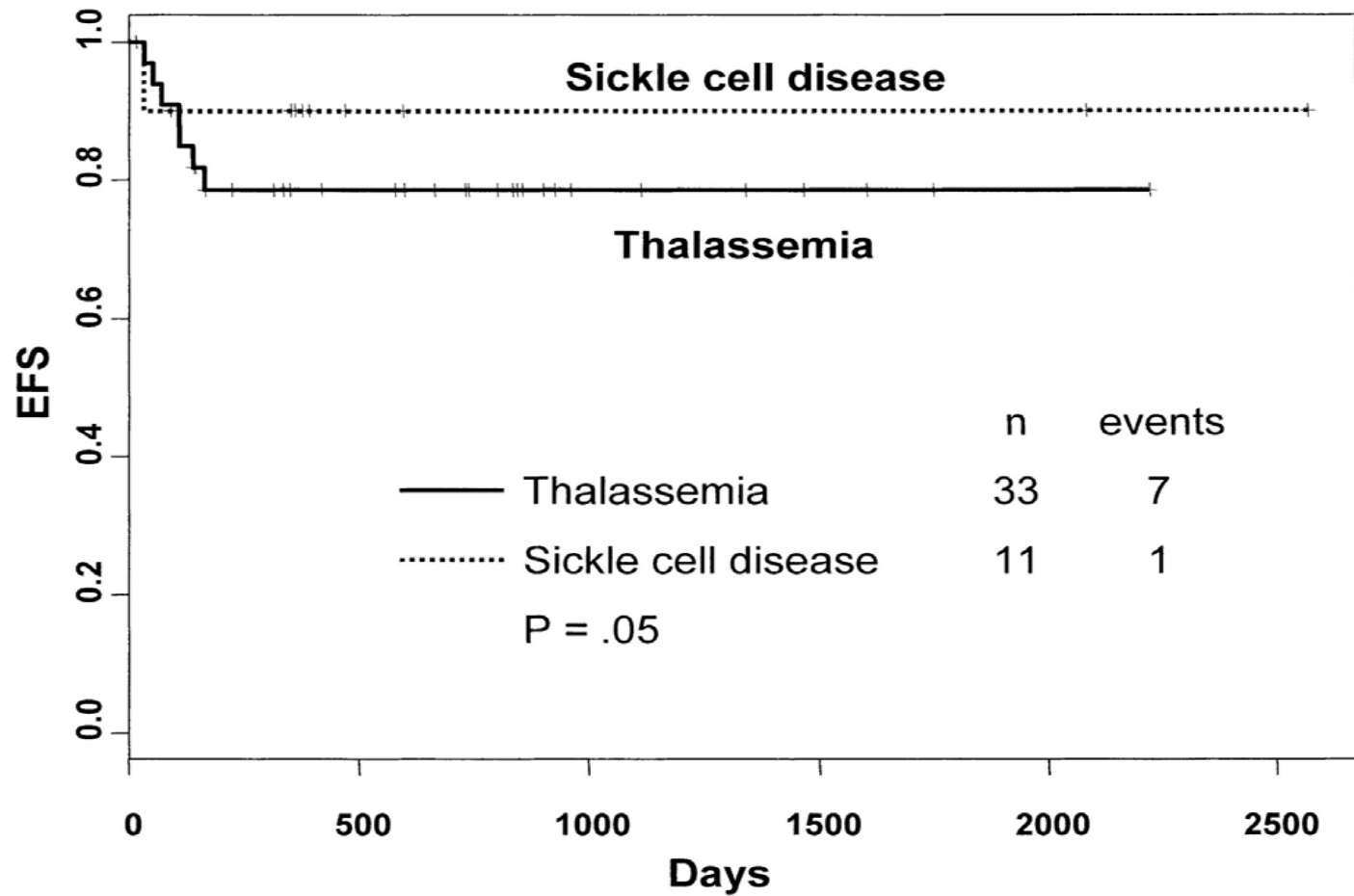
- 7/33 patients experienced graft failure including the patient who received the class I mismatched CBT.
- TNC doses given to the patients who experienced graft failure varied from 1.2 to $10 \times 10^7/\text{kg}$ (median 5.0).
- They were subsequently rescued with either re-injection of autologous back-up marrow or BMT at a later date when the matched sibling donors could donate marrow cells.
- Neutrophil and platelet recovery kinetics occurred as standard.
- Persistent mixed chimerism: 3/33, but transfusion-independent.

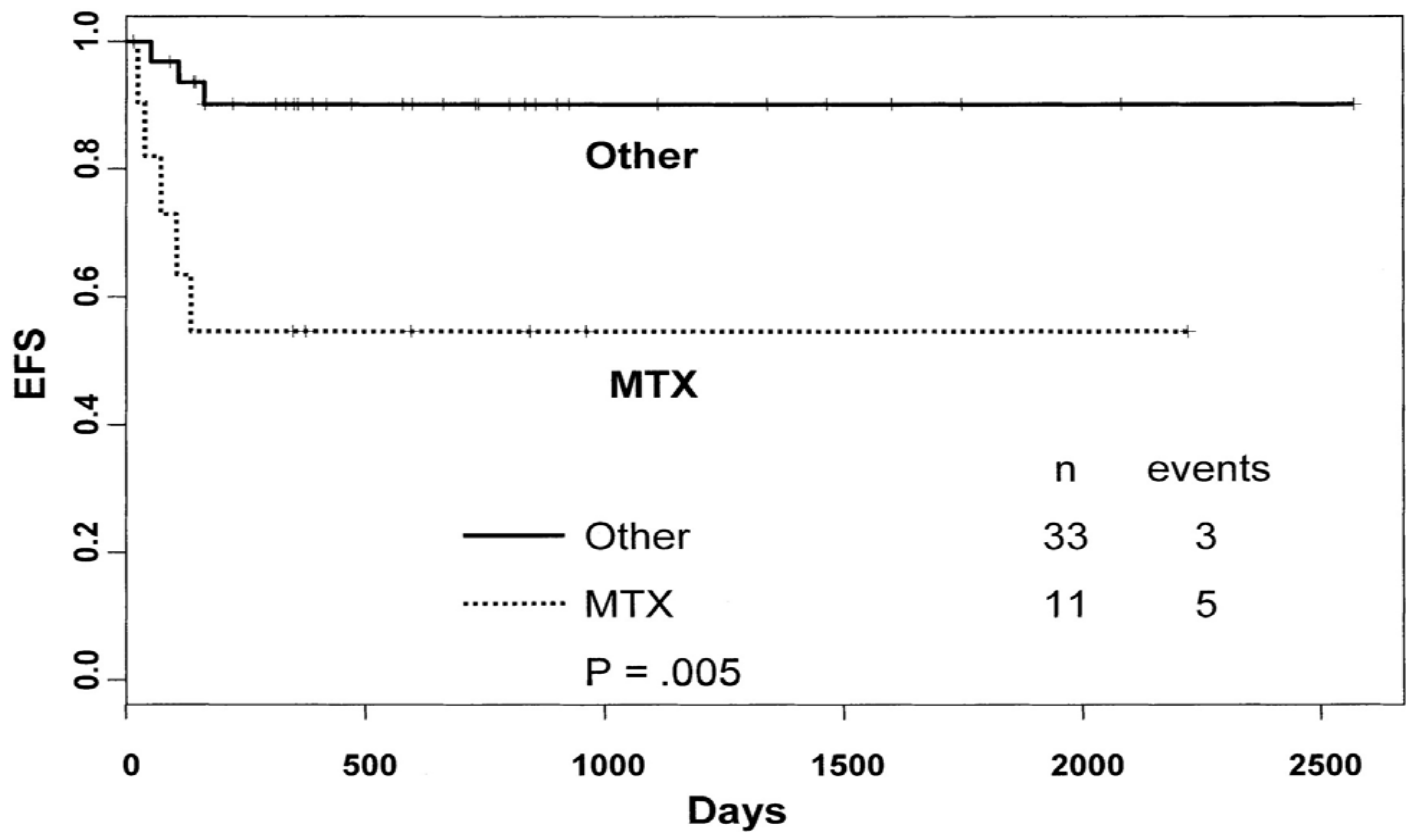
- **Transplant-related complications:**

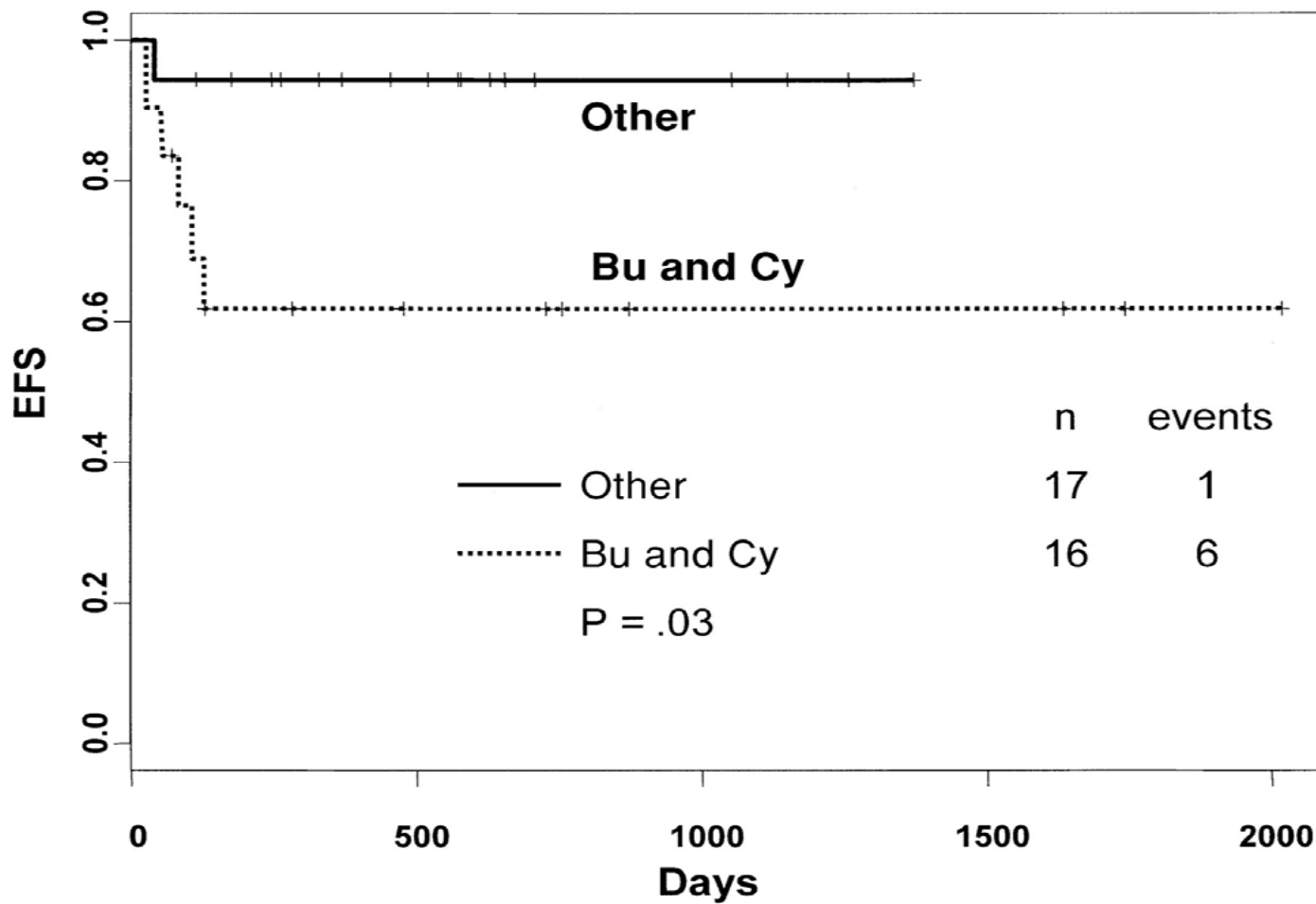
- OS: 100%
- EFS at 2 years:
 - Pesaro class I: 89%
 - Pesaro class II: 62%
- No cases of life-threatening infection
- GvHD:
 - Acute: 11%
 - Chronic: 6%

Outcome

- Early transplant related mortality (TRM) has been reported to be higher following CBT in some series (possibly because of slower engraftment rates and an increased risk of infection) (Rocha, *et al* 2001).
- Results vary according to the population studied and prognostic factors for these observations are not clear.
- By contrast, it is now widely established that overall survival (OS) and event-free survival (EFS) rates after CBT = unrelated donor marrow transplant.
- CB contains sufficient haemopoietic stem cells to transplant most patients lacking an HLA-matched sibling marrow donor, especially when double cord transplants are considered.







Engraftment

- Engraftment of neutrophils and platelets following CBT tends to be delayed compared to that following BMT or PBSCT
- Long-term results are similar, especially in terms of immune reconstitution (Inoue, *et al* 2003).
- Higher total nucleated cell (TNC) doses (> 2.5 to $3.7 \times 10^5/\text{Kg}$ recipient) yield shorter recovery times and better outcomes (Gluckman, *et al* 1997).
- Major concerns with CBT in adolescents and adults, where the cell doses given tend to be lower.

Directed Sibling CB in the NBS

- 10 year experience
- Based at NBS Stem Cell Services in Oxford
- 44 units collected from newborn siblings from families with major haemoglobinopathies:
 - Thalassaemia: 36
 - SCD: 8
- Usage:
 - Thalassaemia: 7 (20%).
 - SCD: 0/8

Sibling Donor Cord Blood Program

- National CB bank for medically indicated banking of sibling CB in the US
- More than 1600 CB collections over a period of 6 years since 1998:
 - Thalassaemia: 6%
 - SCD: 28%
- 32/96 (33%) donor-recipient pairs with β -thalassaemia were HLA identical and 14 of them (44%) received a CBT
 - Eleven survived free of disease with a median follow up of 12.4 months.
 - Fewer than 1 in 6 have so far been used, mainly because of HLA-incompatibility.
- Number of cords banked for SCD is much greater and the usage even lower:
 - 163 CB units collected and stored
 - 4 (2%) used. Median age at the time of transplant was 8.3 years (range 2 to 13.6 years) and median cell dose 4.4×10^7 TNC/kg (range 1.67 to 9.15).
 - All 4 patients engrafted and 3/4 survived, all disease-free, median follow-up of 22.3 months (range 5.2 to 25.4 months).
 - 2005 report: total number of CB banked from SCD families had risen to more than 450 but the number used for CBT (8) remained low (2%).

Conclusion

- The outcome of CBT from related donors is increasingly approaching the results for bone marrow transplants.
- Main complication is graft rejection, which may be reduced by increasing pre-transplant immune suppression and modifying existing GvHD prophylaxis.
- Combination CB and BM is extremely useful when there is a large weight and size difference between donor and patient.
- Unrelated donor CBT have resulted in successful outcomes in a very small number of patients, albeit with a higher mortality and morbidity than conventional transplantation.

Future developments

Reduced intensity grafts

Ability to utilise either VUDs or unrelated
Cords

Will HSCT still be a useful procedure in 10 years
time ?

Limitations of stem cell transplantation for thalassaemia major

- Transplant-related mortality**
 - Lack of donors for the majority of children**
 - Long-term effects (concerns about fertility)**
 - Role of SCT in treatment of adults**
-
-

CB Graft Characteristics

- Much smaller in volume (50-200mL) and cellularity.
- Higher numbers and proliferative capacity of the progenitor cells (CFU-GM, BFU-E, CFU-GEMM) (Broxmeyer, *et al* 1992, Mayani, *et al* 1998).
- Higher quantity and the quality of repopulating cells in SCID-NOD mice transplant models (Wang, *et al* 1997).
- Lower numbers of CB cells can effectively restore a full haematopoietic repertoire after transplant.
- Lower numbers of CD4+, CD8+ and CD3+ T-cells, with a higher CD4/CD8 ratio and a higher proportion of naïve CD45RA+ T-cells (producing lower amounts of Th1-type cytokines) and NK-cells with higher cytotoxic activity.
- Together these immunological differences are likely to be responsible for the lower rates of GVHD and preserved GVL responses (Gardiner, *et al* 1998, Harris, Nomura, *et al* 2001).

GvHD

- Incidence and severity of acute and chronic GVHD with both related and unrelated CBT is reduced when compared with unmanipulated marrow or PB, even where donor/recipient are not fully HLA-matched.
- HLA-matching:
 - Low resolution A, B and high resolution DR instead of high resolution A, B, C, DR, DQ
 - More disparity is tolerated: 1-2 mismatches (Gluckman, *et al* 2004)
- For non-malignant haematological diseases, where a GVL effect is not required, the criteria for finding the optimal unrelated donor should include both a higher cell dose and as few HLA disparities as possible.
- This is particularly the case in haemoglobinopathies for which unrelated CBT remains an experimental procedure.

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CB and BM

	A	B	C
Age (years)	4	15	13
Donor with β -thal trait	No	Yes	Yes
Major ABO incompatibility	Yes	Yes	No
CD34+/kg	0.6×10^5	1.7×10^5	0.5×10^5
Number of BM TNC/kg	0.7×10^8	1.6×10^8	1.7×10^8

Reality of obtaining CB

- Human Tissue Authority regulations:
 - Procurement
 - Collection and Processing
- Who should be collected from? – how to balance the economic cost and resources, the needs of the donor and cell dose requirements
 - Thalassaemia: all siblings of affected patients
 - SCD: if on transfusions or HU
- Pre-implantation diagnosis and HLA typing

Thalassaemia major: rationale for transplantation

- **morbidity**
 - **mortality**
 - **quality of life**
-

“Mini-allografting” in thalassaemia

Hongeng et al, BMT 30: 409-410, 2002

- **Girl aged 10 years received PBSC from 4 yr old sib**
 - **Conditioning:**
 - busulphan 8 mg/kg**
 - fludarabine 175 mg/m²**
 - ALG**
 - TLI (500cGy)**
 - **GVHD prophylaxis:** **CSA (d+100),MMF (d+35)**
 - **Outcome:Alive & well with full donor engraftment at 1yr**
-