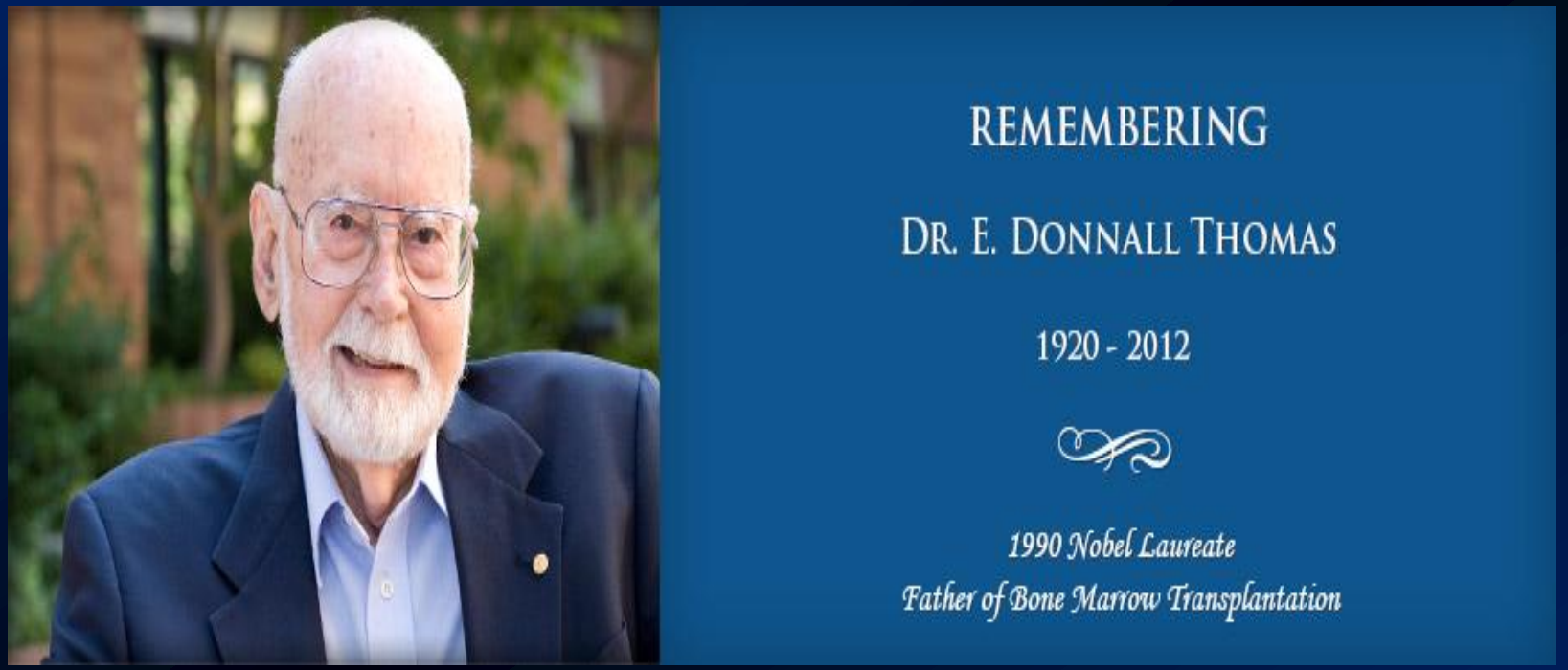
# Future Trends in Blood and Marrow Transplantation



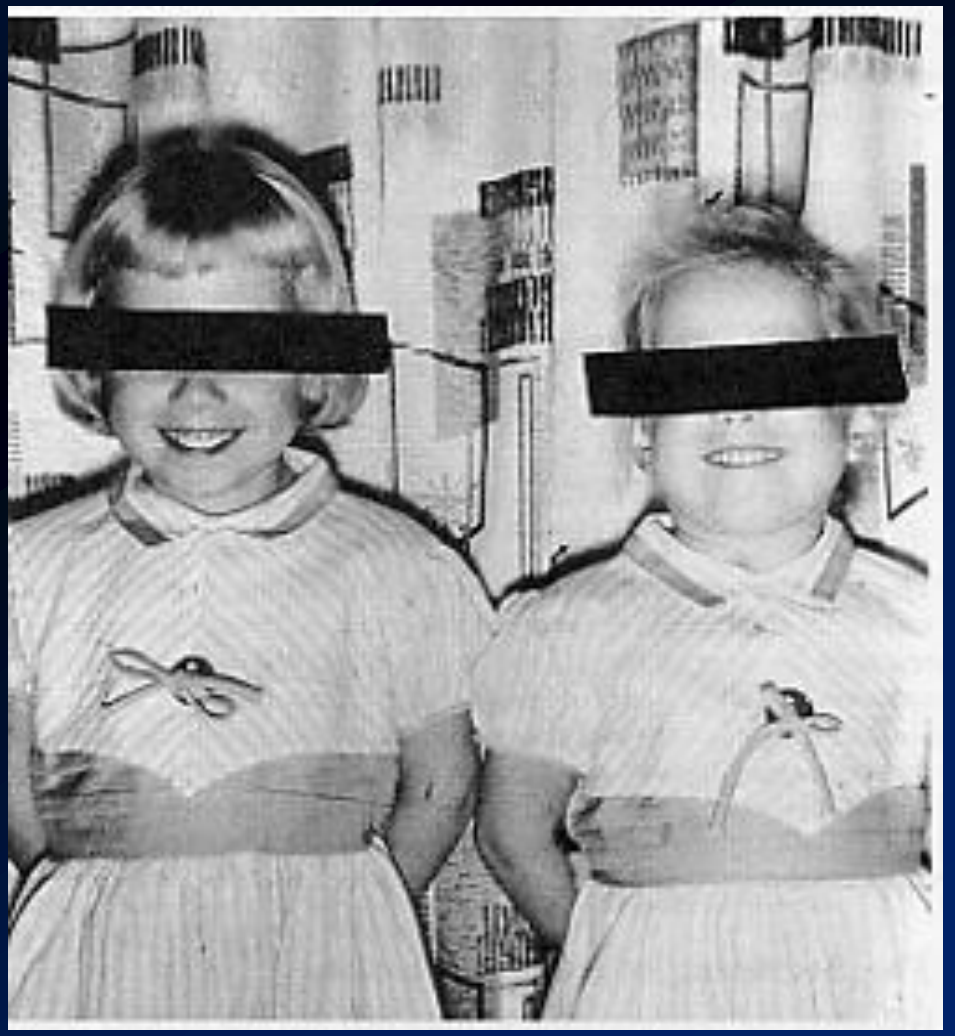

Evgenios Goussetis

# Agenda

- History of hematopoietic stem cell transplantation (HSCT)
  - From first human studies to current developments
- Overcoming HLA-Barriers
- Adoptive T-cell therapy
- Synthetic Immunology
- Stem Cells
- Gene Therapy
- Future directions of SCT

# Highlights in HSCT

- 1957: marrow infused intravenously
- 1958: reports of successful identical twin transplants
- 1958: HLA
- 1979: First unrelated donor
- 1989: peripheral blood stem cells
- 1990: first cord blood transplant
- 1996: first non-ablative transplant
- 2005: Haploidentical SCT
- 2007: iPSCs
- 2017: CAR-T cell therapy
- 2018: Gene therapy for β-thalassemia



Thomas et al J Clin Invest 1959

# HSCT

## Type of HSCT

- Allogeneic HSCT
  1. Identical twin (Syngeneic)
  2. HLA identical sibling donor
  3. HLA identical related (other than sibling) donor
  4. HLA matched unrelated donor
  5. HLA- Haploidentical related donor
- Autologous HSCT

## Source of Graft

- Bone Marrow
- Peripheral Blood Stem Cells
- Cord Blood Stem Cells

# Indications for HSCT

- Cancer:
  - Leukemia
  - Myelodysplasia
  - Lymphoma
  - Breast cancer
  - Testicular cancer
  - Ovarian cancer
  - Brain tumors
  - Pediatric tumors
  - Multiple myelomas
  - Sarcomas
  - Kidney cancers
- Non Cancers:
  - Aplastic Anemias
  - Metabolic disorders
  - Autoimmune diseases
    - Rheumatoid arthritis
      - Juvenile and adult
    - Multiple Sclerosis
    - Scleroderma
    - Systemic Lupus
  - Immune deficiency
  - Sickle cell anemia
  - Thalassemia

# Elements of HSCT

- Selection of donor
  - Based on tissue typing of 6-10 HLA antigens in allogeneic transplantation
  - Tissue typing unnecessary in autologous transplantation
- Harvest of stem cells from donor
  - Bone marrow harvest or apheresis of peripheral blood
- Preparative regimen
  - Chemo-radiation for ablation and immune suppression
- Stem cell infusion
- Post-transplant supportive care
  - Autologous 100 days
  - Allogeneic 180 days or longer for tolerance to develop

# HLA and HSCT

- Histocompatibility Locus Antigens (HLA) are determinants of immunologic "self" and "not-self"
  - Immunologic "password"
  - Allows for effective immune response against infections, cancer
- T cell reaction to foreign HLA molecules (donor) is a major problem of transplantation (alloreactivity)
  - Need good donor and recipient match for HLA sites
  - Cause of acute rejection in organ transplant, and of GVHD in BMT.

# HLA Typing in HSCT

- Family members typed with patient for HLA A, B and DR
  - Likelihood of 6/6 or 5/6 match depends on frequency of recipient HLA haplotype
- Likelihood of unrelated donor match related to haplotype frequency in general population
  - Some HLA combinations more frequently found among ethnic groups
    - Ethnic sequestration phenomenon

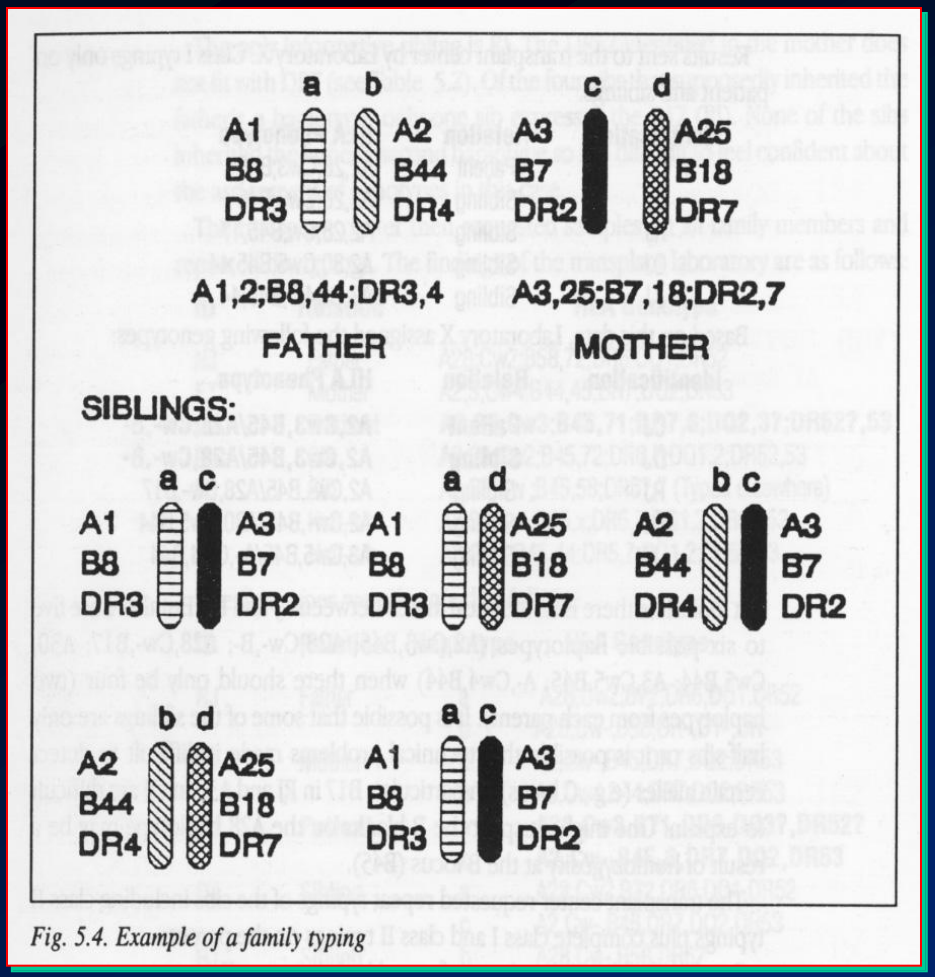
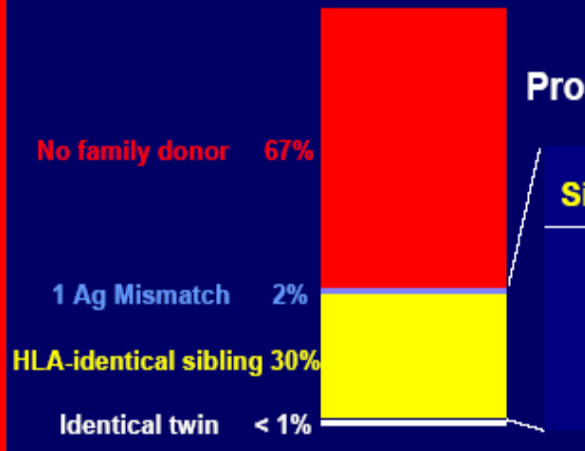


*Fig. 5.4. Example of a family typing*

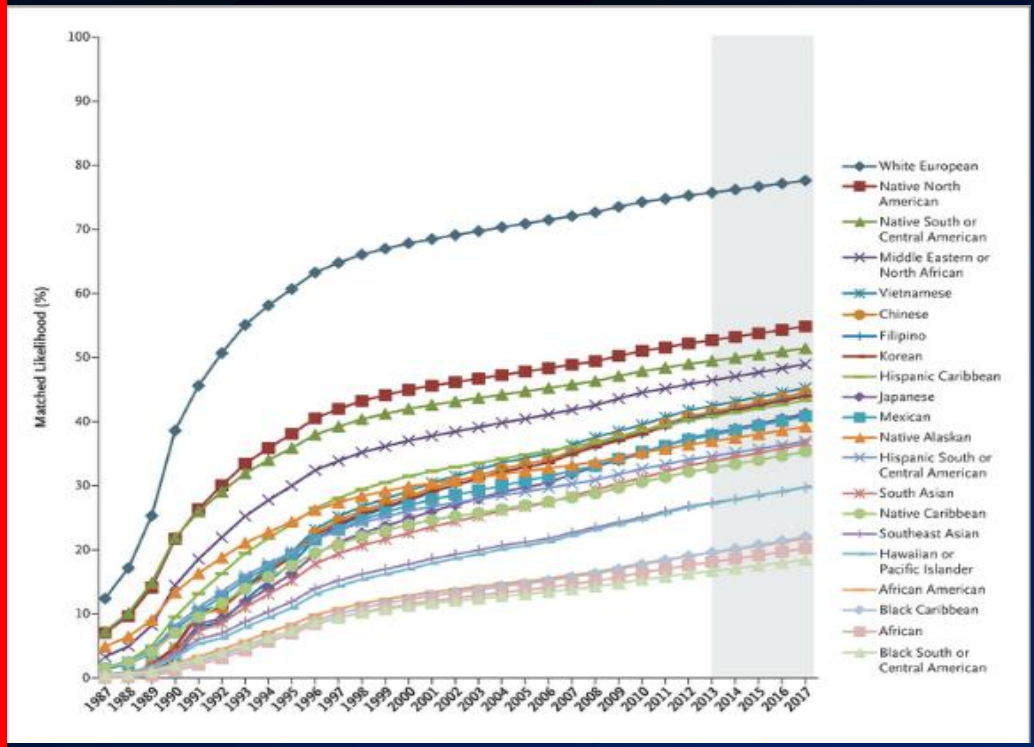
# Probability of having compatible HSC donor

## HLA- Matched Family Donors Availability




Probability = $1- (3/4)^n$

| Siblings | Probability |
|---|---|
| 1 | 25 % |
| 2 | 44 % |
| 3 | 58 % |
| 4 | 68 % |
| 5 | 76 % |



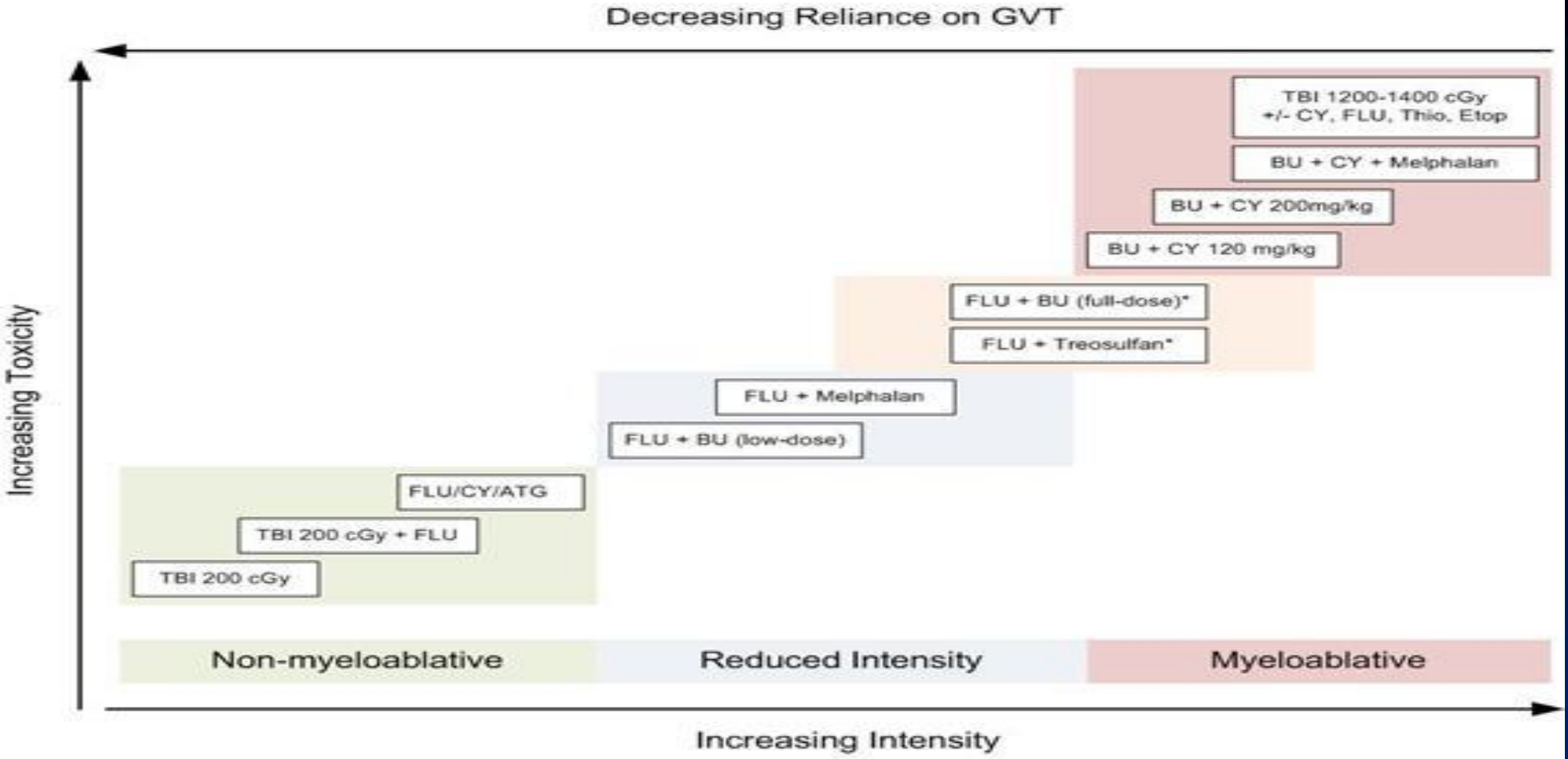
# Increasing Donor Pool Essential

- Time from search to unrelated donor: 4 months
  - Often relapse prevents coming to transplant
- Greater efforts are needed to increase participation and minority representation in the volunteer donor pool (NMDP)
  - Education regarding safety and need
- Increasing cord blood donation may help some
  - Everyone has umbilical cord blood they won't use
  - No risk to donate
  - Better reflects the local population demographics

# Preparation for SCT

- Immune suppression *and* myeloablation required
  - Bone marrow  failure states require more immunosuppression
  - Immune deficiency without empty marrow  leads to rejection.
    - Chemotherapy induces aplasia to allow engraftment
- Additional merits of marrow ablation
  - Provides marrow “space”
  - Eradicates malignant cells
  - Reset of the recipient immune system
- Preparative regimens before transplant provide aplasia *and* immune suppression

# Classification of Pediatric Conditioning Regimens




ATG = Antithymocyte globulin
BU = Busulfan
cGy = Centigray

CY = Cyclophosphamide
Etop = Etoposide
FLU = Fludarabine

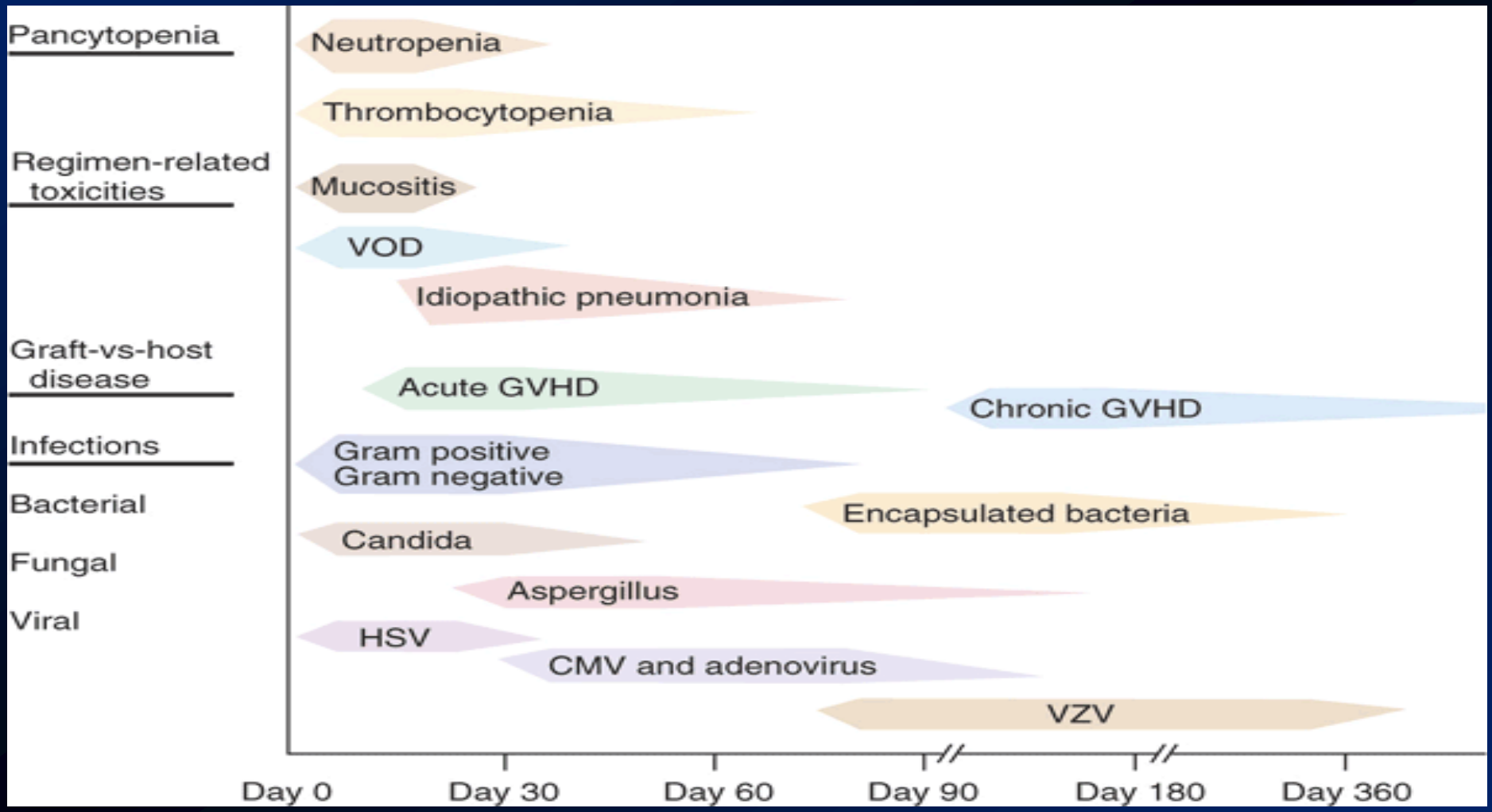
GVT = Graft-versus-tumor effect
TBI = Total-body irradiation
Thio = Thiotepa

* These two regimens have been associated with lower rates of transplant-related mortality compared with standard myeloablative approaches and are often referred to as reduced toxicity myeloablative regimens.

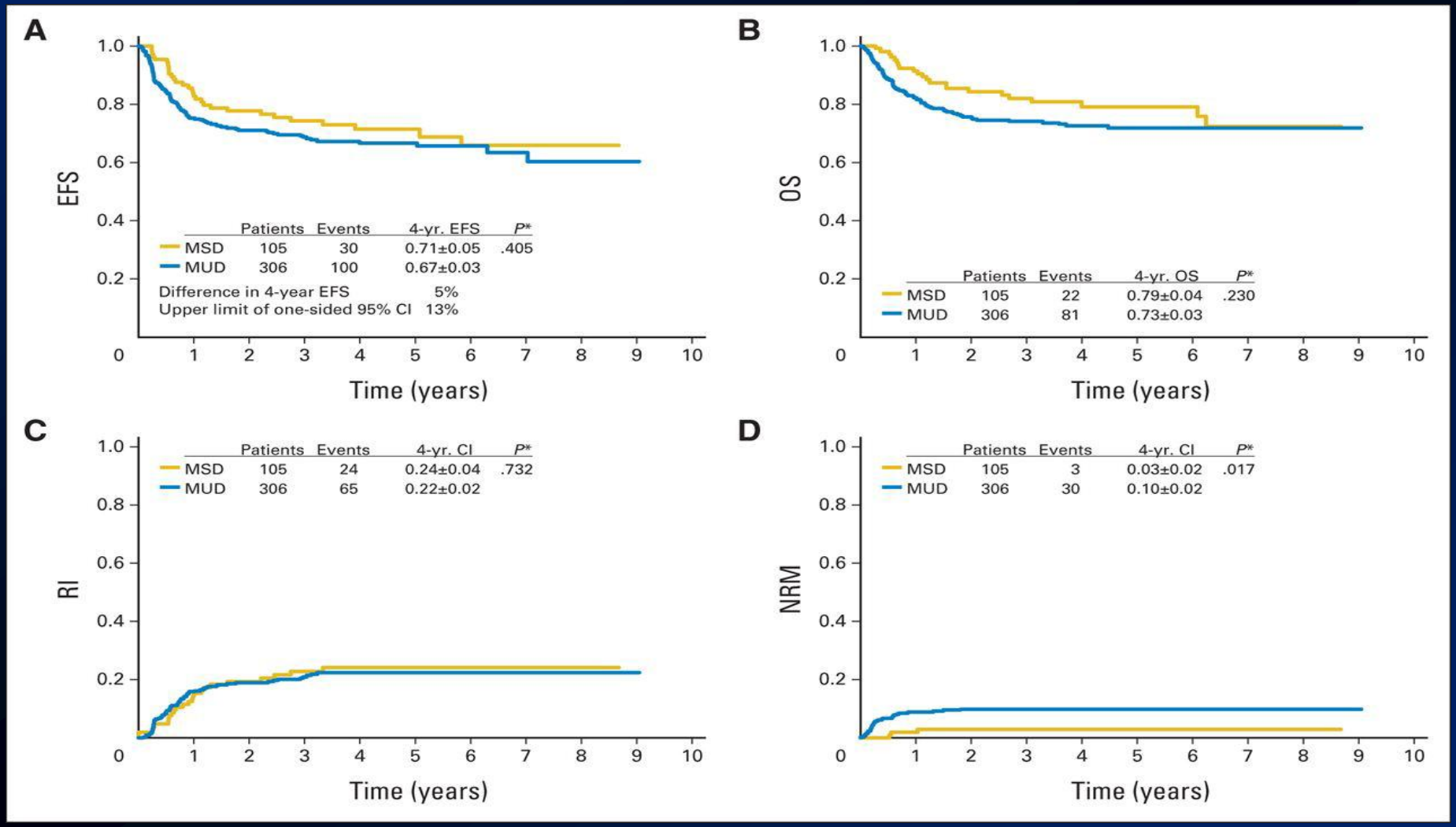
# Hematopoietic Reconstitution

- Bone marrow cellularity decreased months post transplant
- Immunologic reconstruction over 100 days post transplant
  - Graft-vs.-host disease (GVHD) delays immune reconstitution
- Immune deficits expected:
  - T cell and B cell dysfunction.
  - Low Ig levels for three months, normal IgG and IgM by one year, IgA by two year
    - Predisposes to fungal, viral and bacterial infection

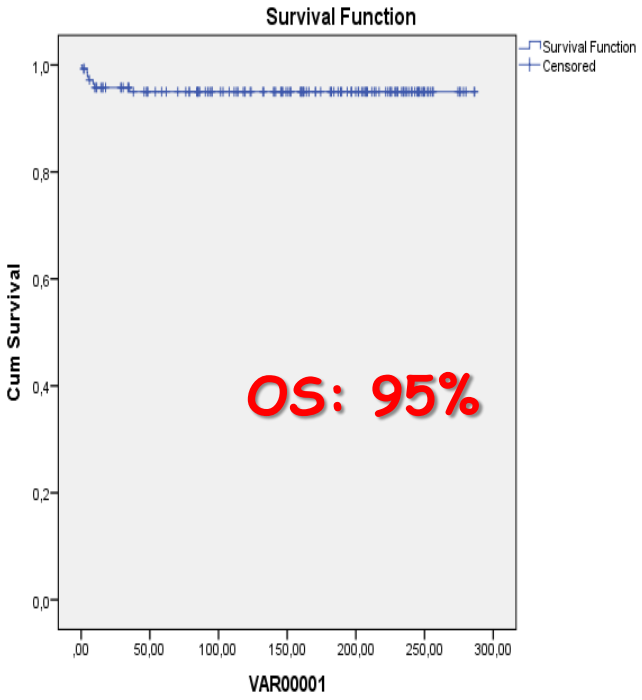
# Complications

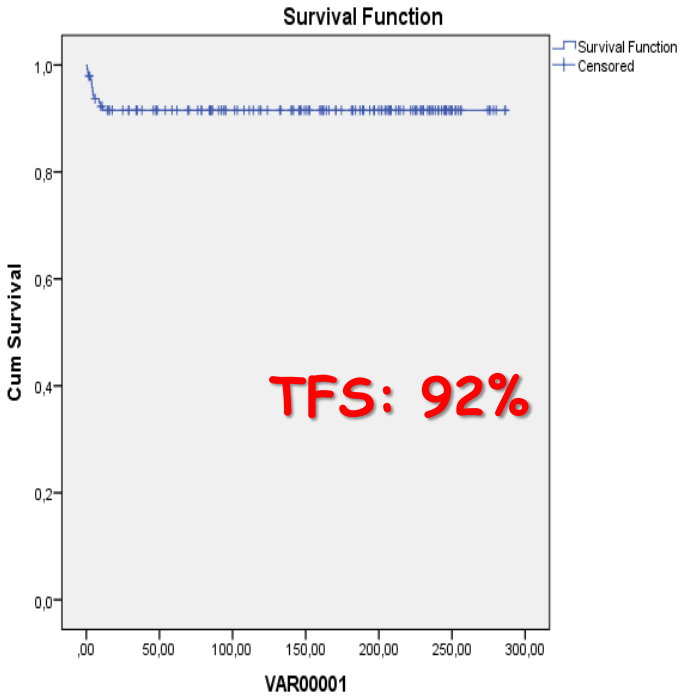


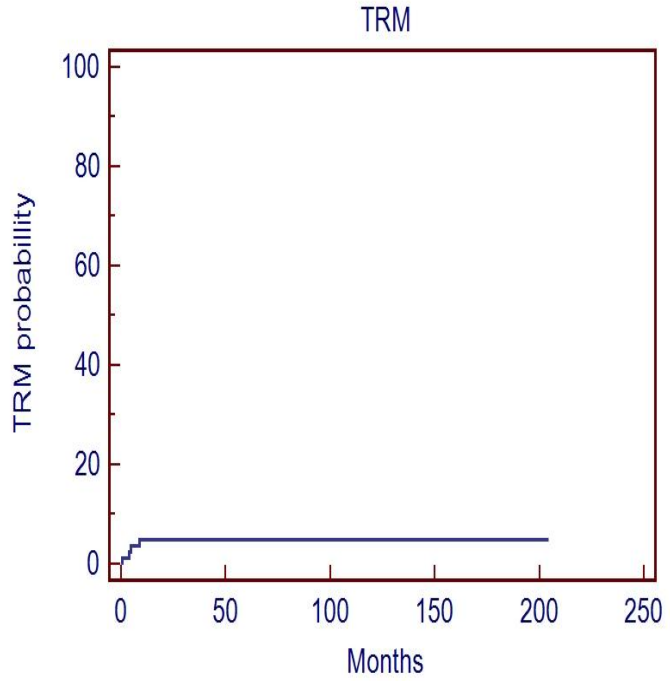
# Outcome in pediatric ALL



# Outcome in β-Thalassemia




Bone Marrow Transplantation (2012) **47**, 1061 - 1066

**www.nature.com/bmt**

**ORIGINAL ARTICLE**

## HLA-matched sibling stem cell transplantation in children with β-thalassemia with anti-thymocyte globulin as part of the preparative regimen: the Greek experience

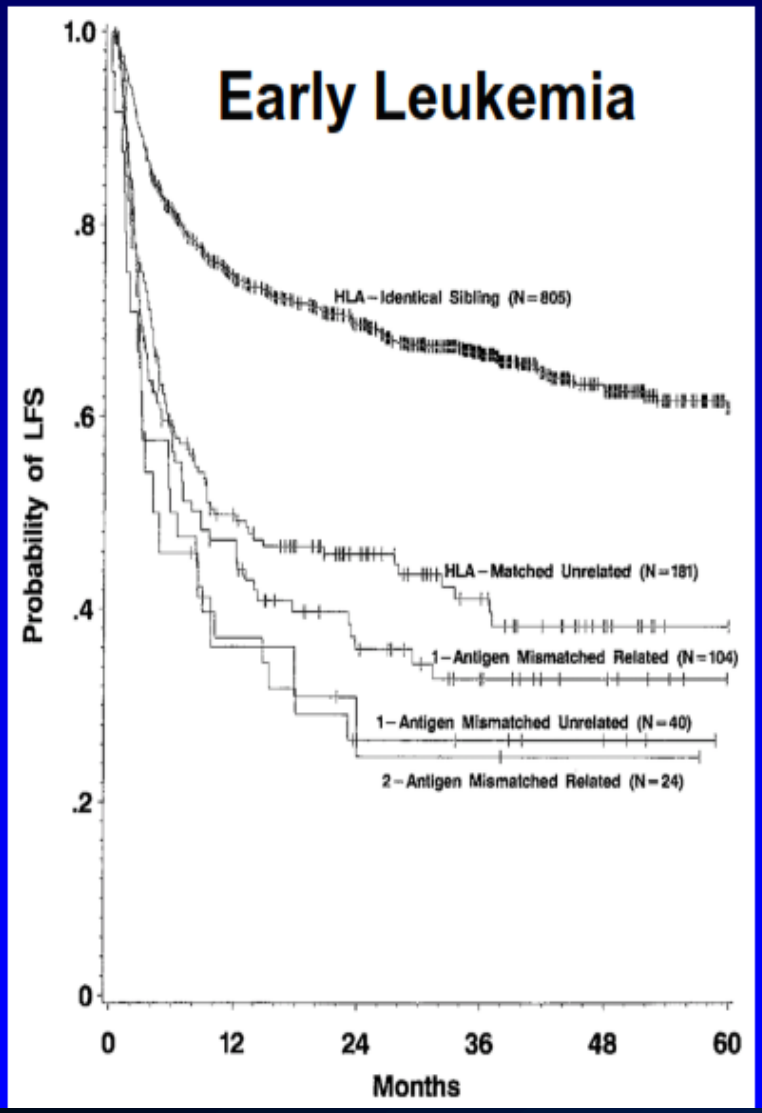
E Goussetis[1], I Peristeri[1], V Kitra, G Vessalas, A Paisiou, M Theodosaki, E Petrakou, MN Dimopoulou and S Graphakos

# The limits of success today

- HLA-Barrier
- Relapse
- GVHD
- Infections
- Regimen related mortality

# What We Learned Over the Decades

## HLA mismatches are prohibitively toxic

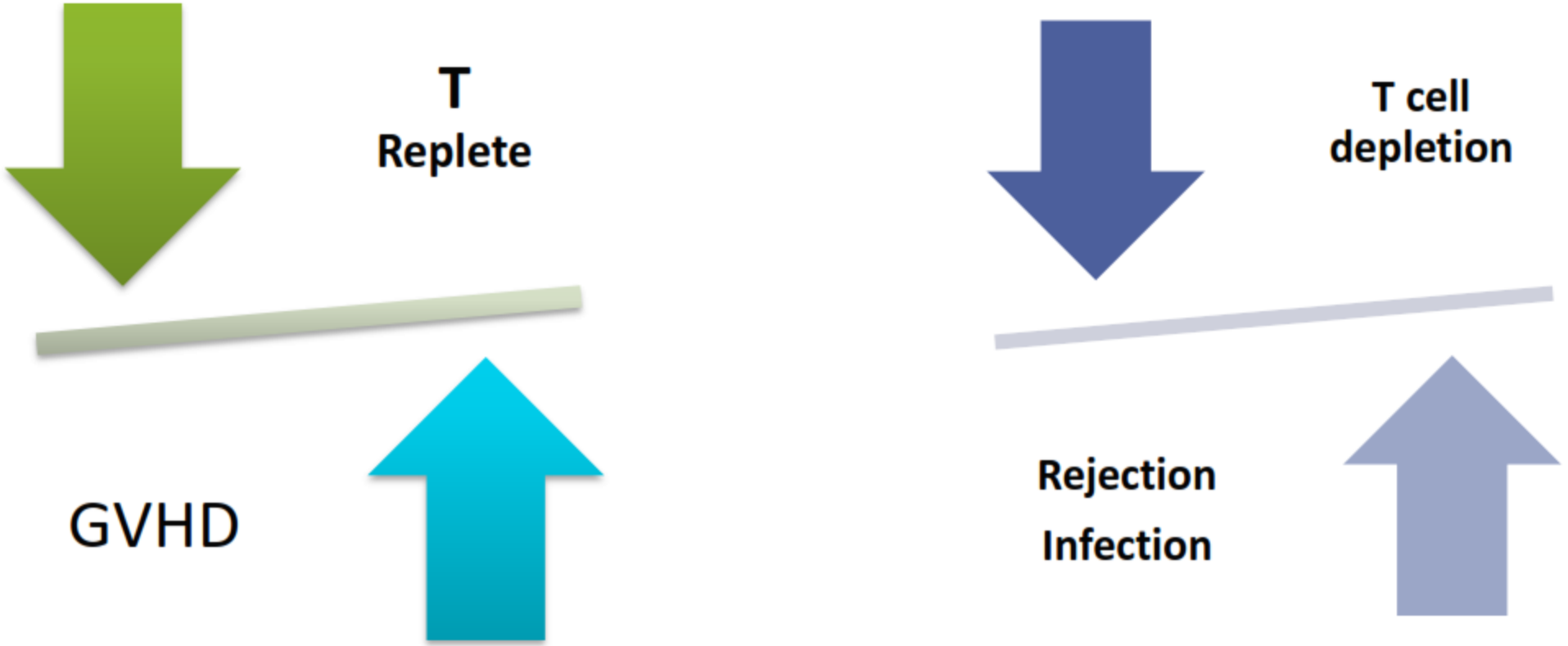



| Disease State/Type of Donor† | No. | TRM (%) | P* |
|---|---|---|---|
| Early | | | |
| HLA-identical sibling | 805 | 21 ± 2 | — |
| 1-Antigen mismatched related | 104 | 53 ± 5 | < .001 |
| 2-Antigen mismatched related | 24 | 55 ± 11 | < .001 |
| Matched unrelated | 181 | 53 ± 4 | < .001 |
| 1-Antigen mismatched unrelated | 40 | 69 ± 8 | < .001 |

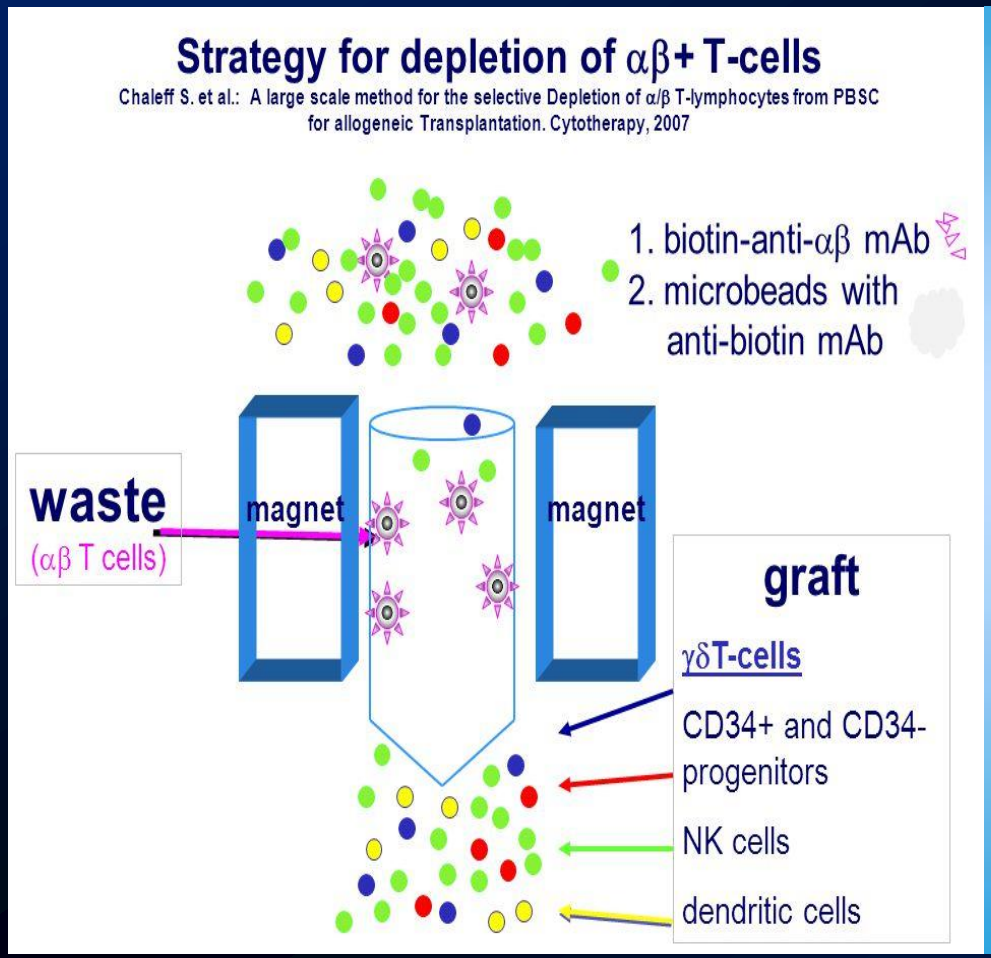
IBMTR

Szydlo et al *JCO* 1997

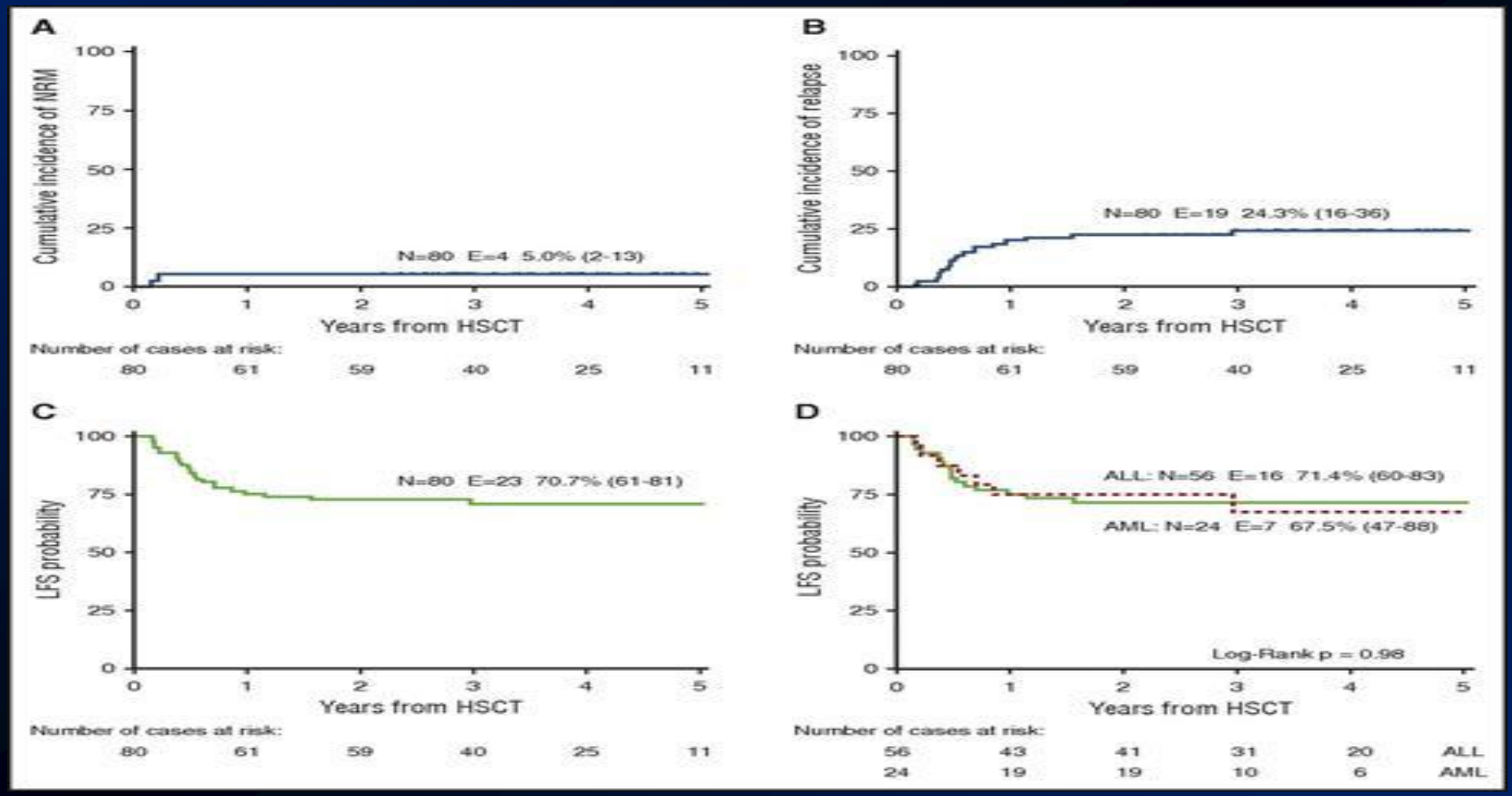
# Overcoming the mismatch



# *Ex vivo* T-cell depletion




# T-cell depleted haploidentical Transplantation in children with acute leukemia

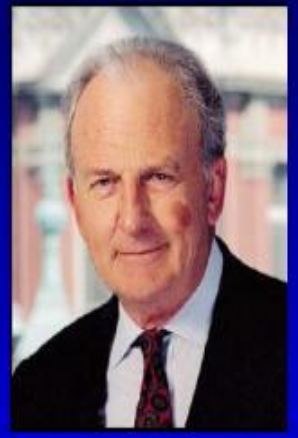



Blood 2017 Aug 3;130(5):677-685

# Development of Post-Transplant Cy

## Back to the future (Santos & Owens, 1960s-70s)

- Cy post alloBMT prevented GVHD in mice (Santos/Owens - 1960s)
  - Only high doses (150-300 mg/kg) effective
  - Lower doses - limited activity



- Standard Hopkins prophylaxis (1975-1984)
  - Low dose - 7.5 mg/kg/d x 4 because of hematologic toxicity fears

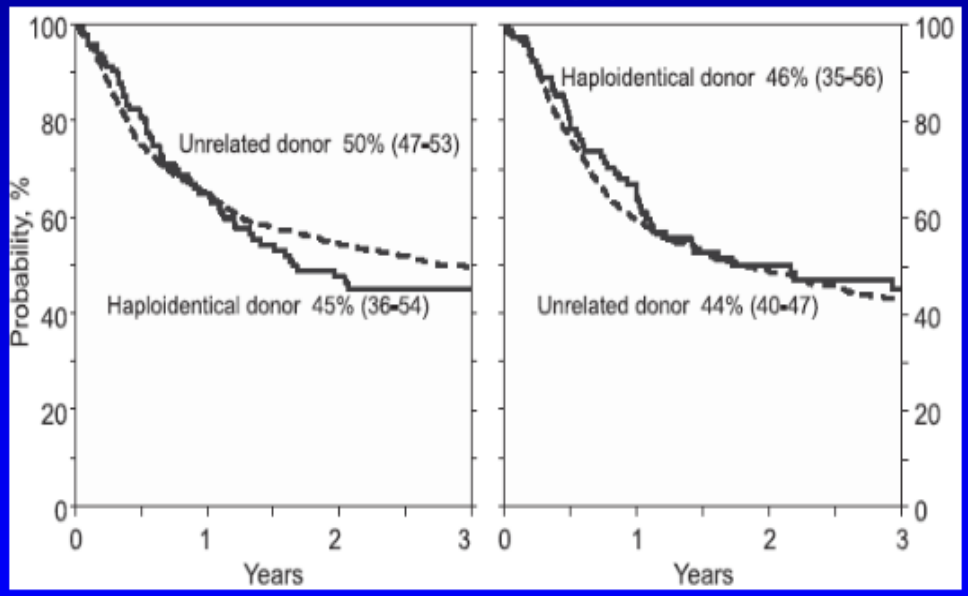
- Randomized trial - less effective than CsA (Santos *et al Clin Transplant* 1986)

# Haploidentical transplant with posttransplant cyclophosphamide vs matched unrelated donor transplant for acute myeloid leukemia

Stefan O. Ciurea,[1] Mei-Jie Zhang,[2,3] Andrea A. Bacigalupo,[4] Asad Bashey,[5] Frederick R. Appelbaum,[6] Omar S. Aljitawi,[7] Philippe Armand,[8] Joseph H. Antin,[8] Junfang Chen,[2] Steven M. Devine,[9] Daniel H. Fowler,[10] Leo Luznik,[11] Ryotaro Nakamura,[12] Paul V. O'Donnell,[6] Miguel-Angel Perales,[13] Sai Ravi Pingali,[1] David L. Porter,[14] Marcie R. Riches,[15] Olle T. H. Ringdén,[16] Vanderson Rocha.[17] Ravi Vii.[18] Daniel J. Weisdorf.[19] Richard E. Champlin,[1] Mary M. Horowitz,[2] Ephraim J. Fuchs,[11] and Mary Eapen[2] *Blood.* 2015;126(8):1033-1040

## However, it is now time to unlearn

### No survival difference



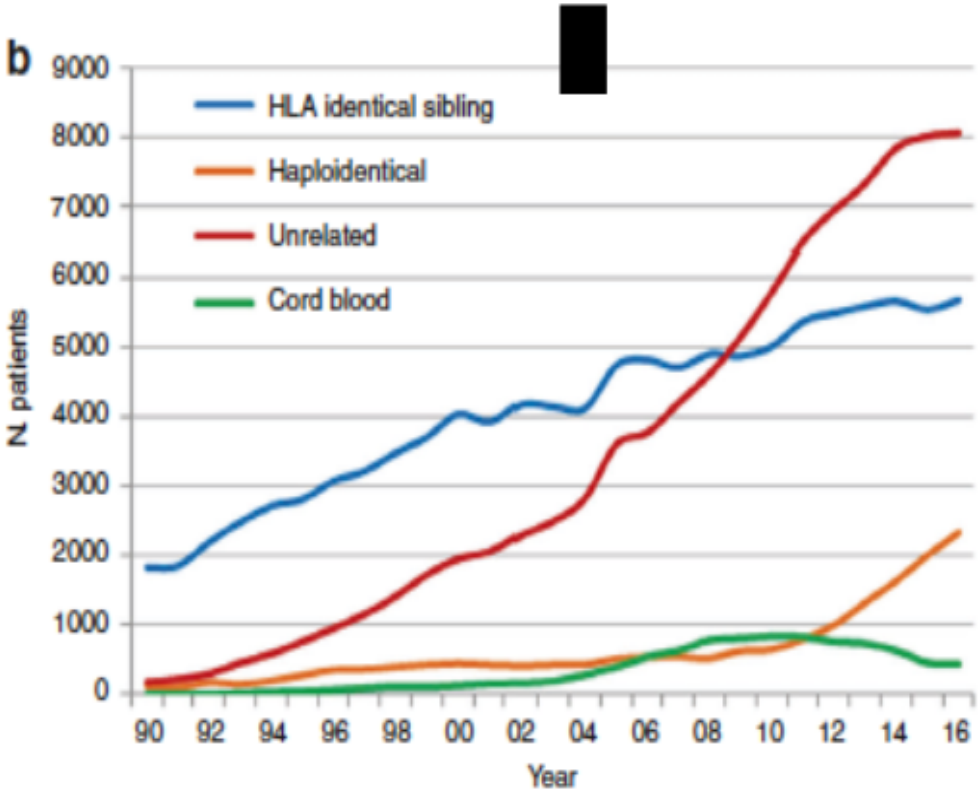

Myeloablative RIC

### Less GVHD with Haplo/PTCy

**Table 5. Multivariate analysis (subset): risks of acute and chronic GVHD, nonrelapse mortality, relapse, and OS by donor type**

| Outcome | Transplant conditioning regimen intensity: Myeloablative* Hazard ratio (95% CI) | Reduced intensity† Hazard ratio (95% CI) |
|---|---|---|
| **Grade 2-4 acute GVHD** | | |
| Matched unrelated donor | 1.00 | 1.00 |
| Haploidentical donor | 0.37 (0.23-0.61) *P* = .0001 | 0.71 (0.44-1.15) *P* = .16 |
| **Grade 3-4 acute GVHD** | | |
| Matched unrelated donor | 1.00 | 1.00 |
| Haploidentical donor | 0.33 (0.14-0.81) *P* = .02 | 0.21 (0.05-0.86) *P* = .03 |
| **Chronic GVHD** | | |
| Matched unrelated donor | 1.00 | 1.00 |
| Haploidentical donor | 0.44 (0.29-0.66) *P* = .0001 | 0.45 (0.28-0.71) *P* = .0006 |

# Are VUD transplants levelling off



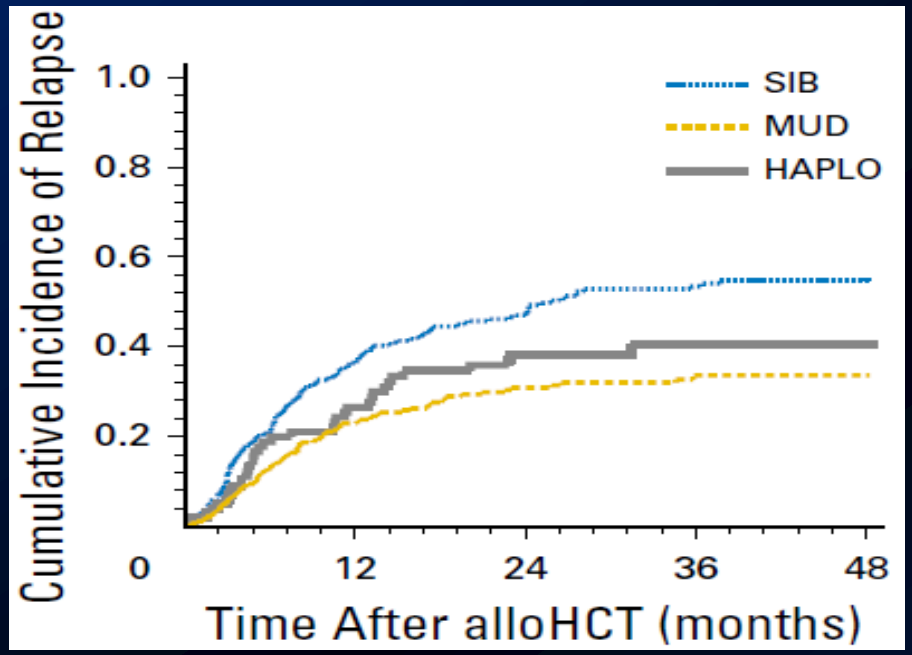

Transplant rates per 10 million inhabitants

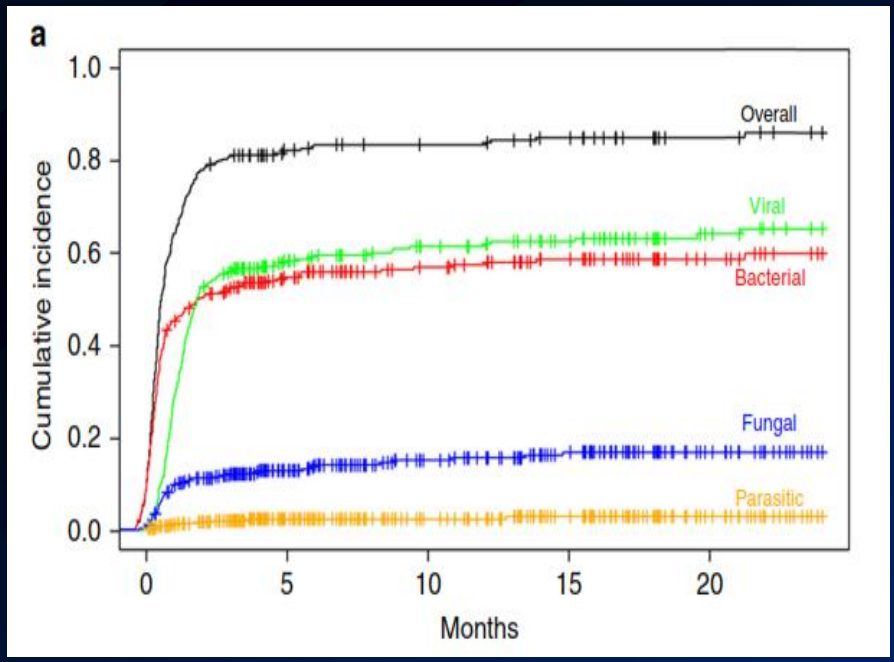
| | Donor type | | |
|---|---|---|---|
| | Identical Sibling | Haploidentical family | Unrelated |
| Income group | | | |
| Very high | 390 | 77 | 978 |
| High | 283 | 106 | 321 |
| Upper middle | 102 | 16 | 16 |

Transplant rates per 10 million inhabitants (TR) over the years 2012–2016 by donor choice and income group

*Passweg et al, BMT 2018*

# Relapse and infections still remain…



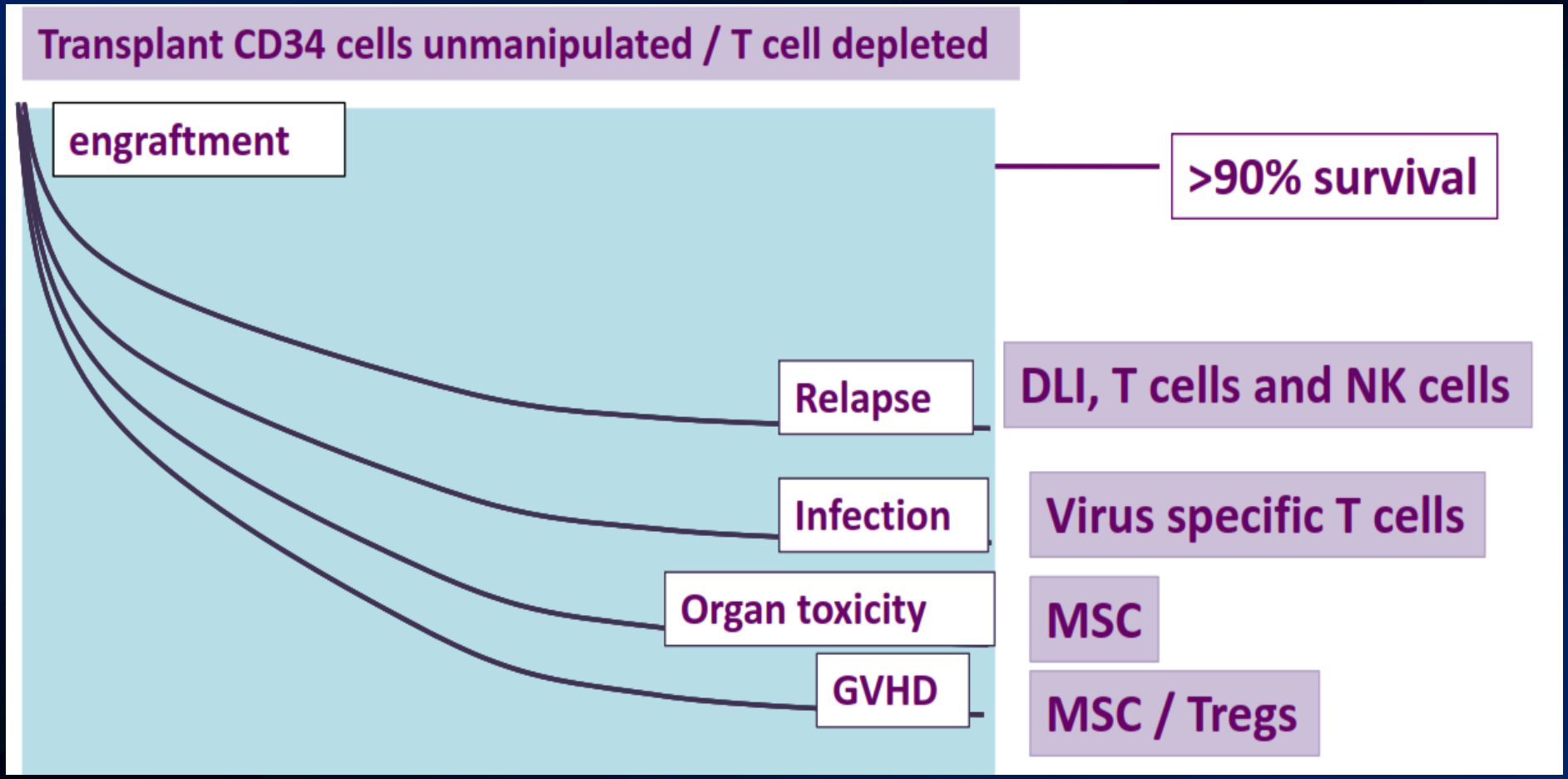
**Cellular therapies:**

**Adoptive transfer of viral-specific T-cells**

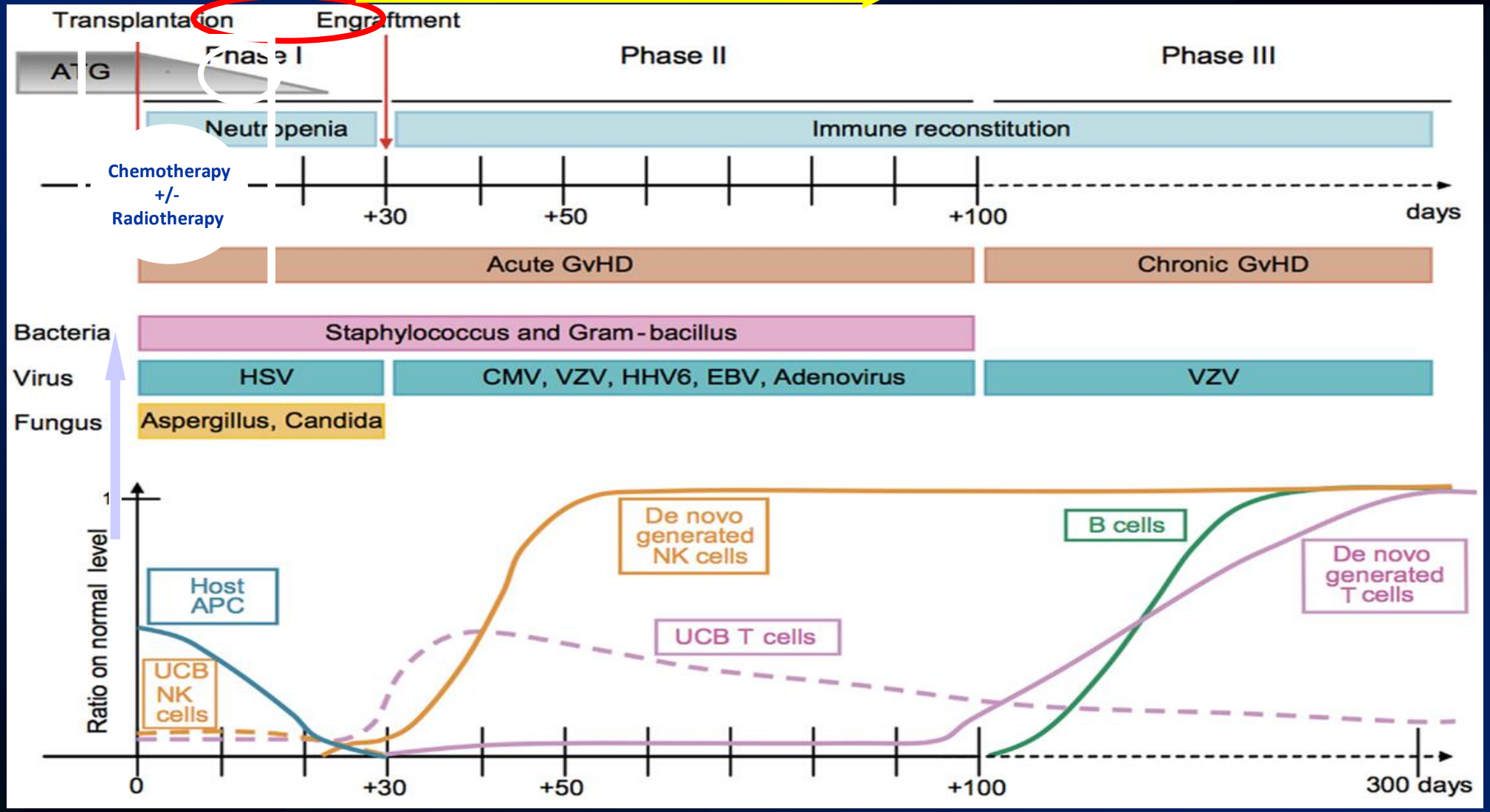
**Donor Cell (T-cells or NK cells) Infusions**

**Recipient or allogeneic CAR-T or CAR-NK cell infusions**

# Cell therapy for HSCT - The vision



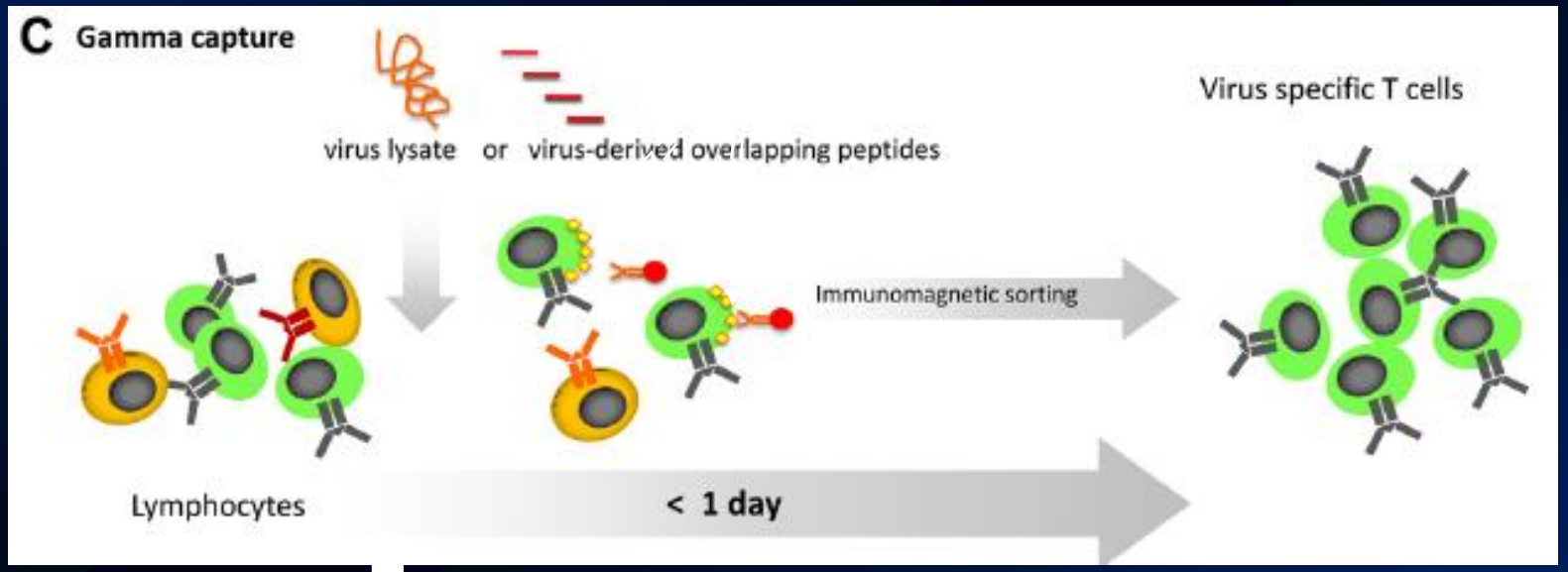
# Immunosuppression



# *Viral specific T-cytotoxic cells*

## IFNγ capture method

IFNγ



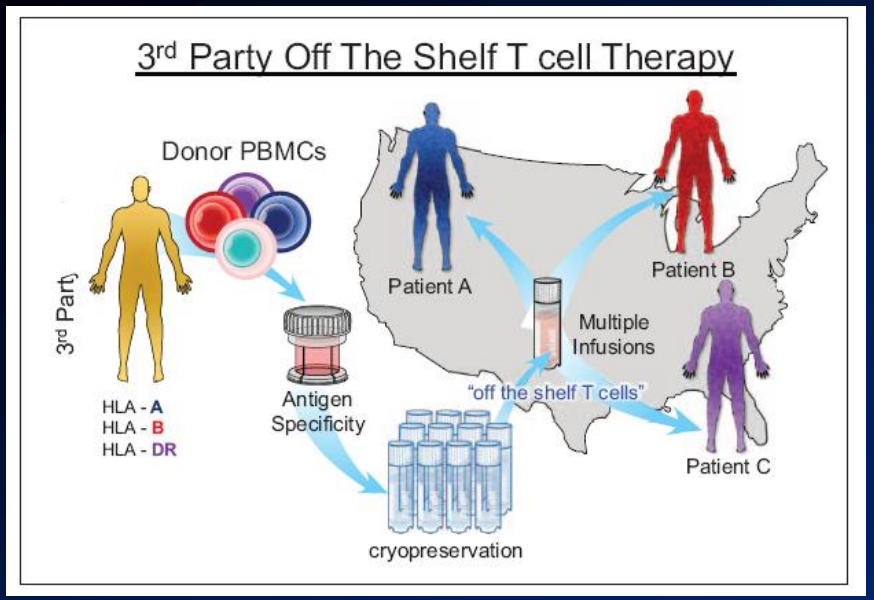

12-16 hours

Antibody-conjugated magnetic beads

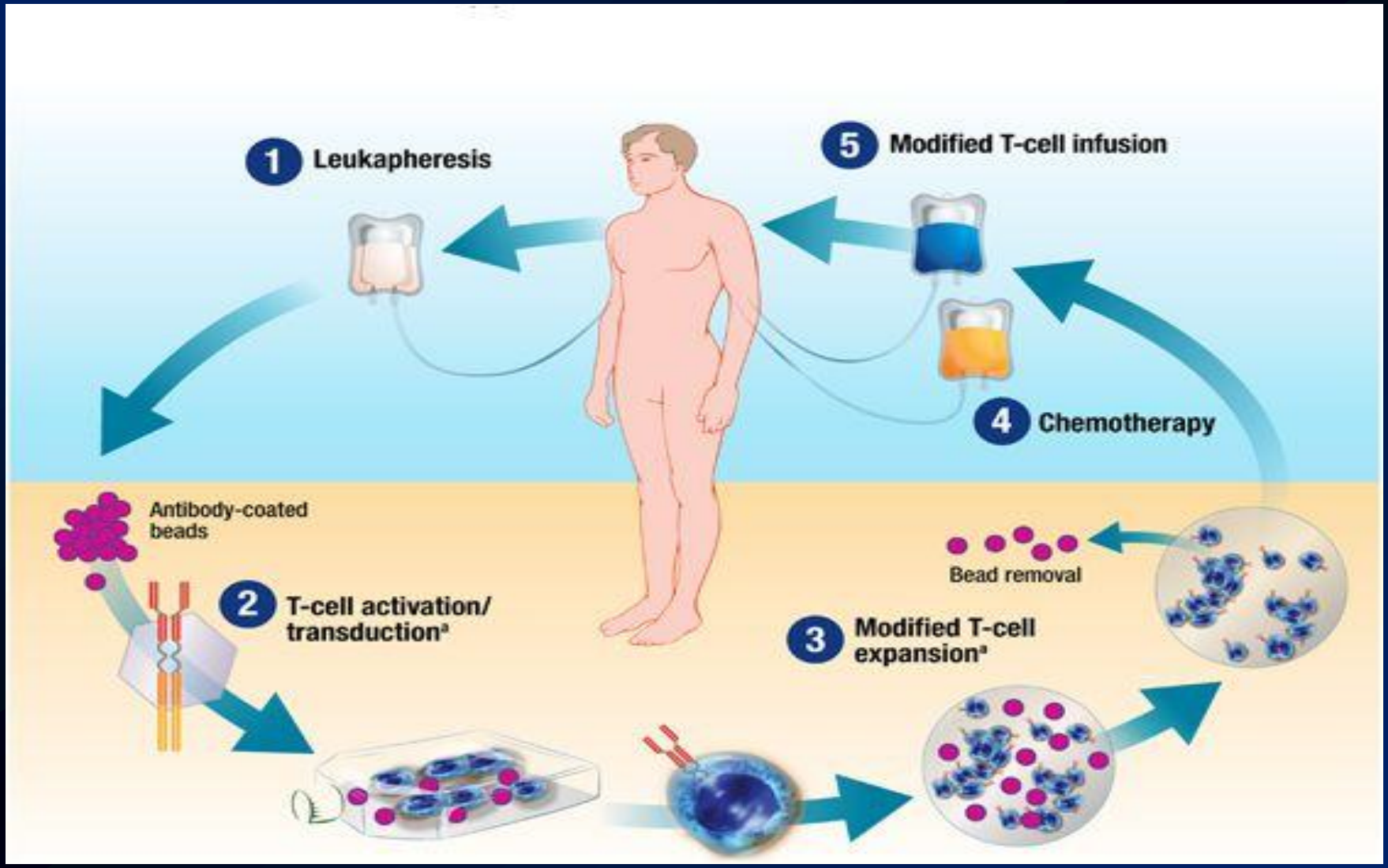
*Saglio et al, 2014*

# CliniMACS Prodigy Cytokine Capture System

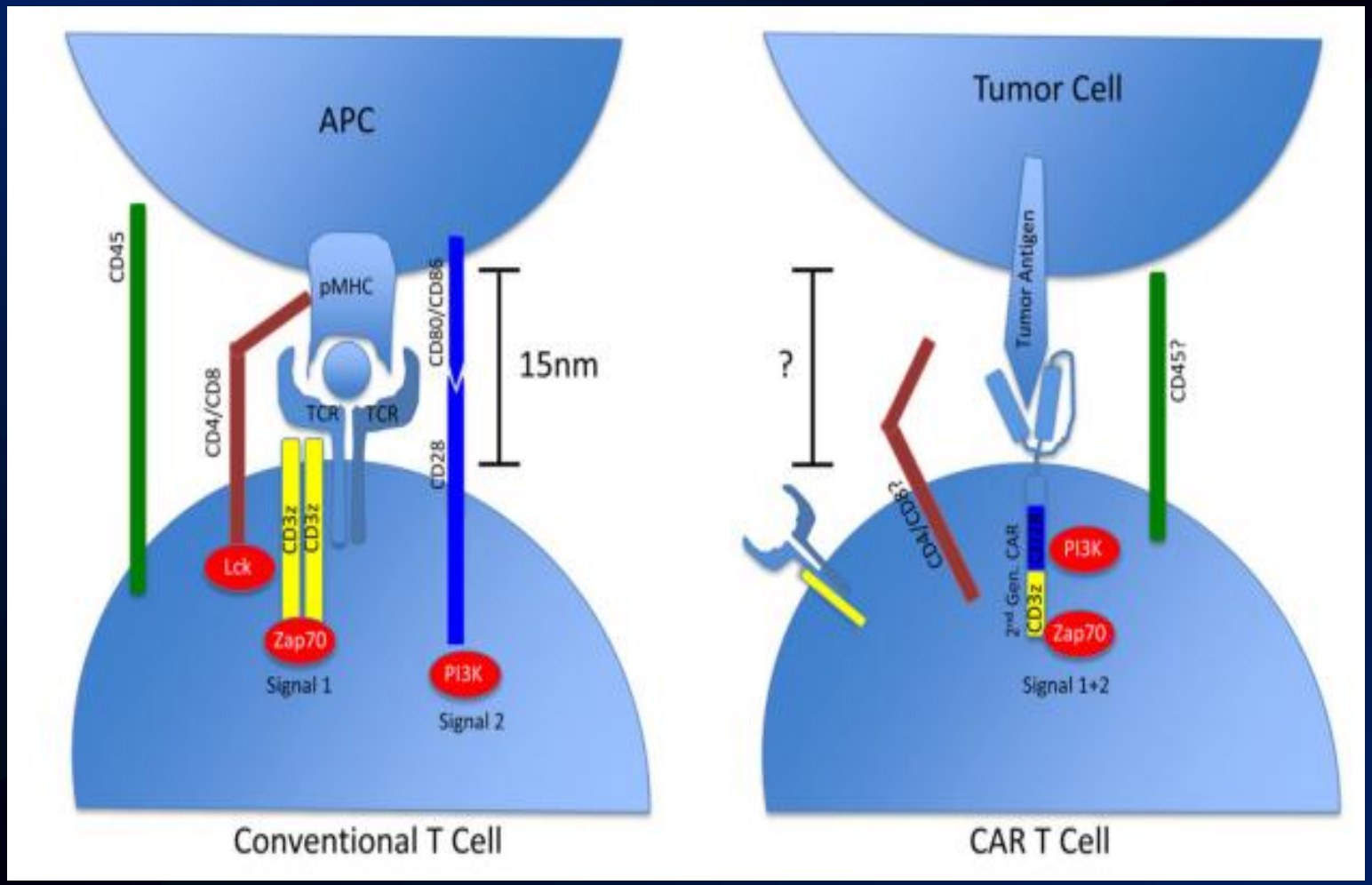

# Third party recipients



# CAR-T Cells

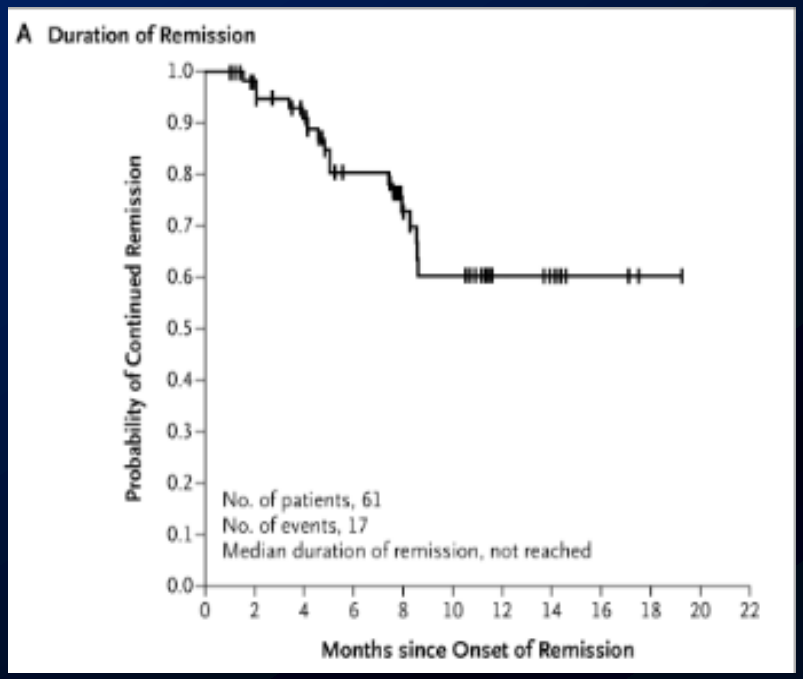

1 Leukapheresis
5 Modified T-cell infusion
4 Chemotherapy
Antibody-coated beads
2 T-cell activation/ transduction[a]
Bead removal
3 Modified T-cell expansion[a]

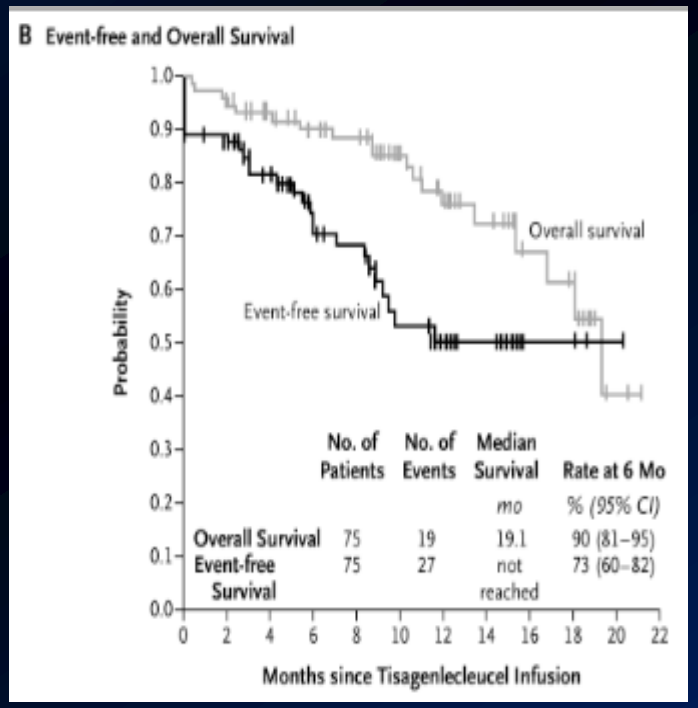
# Signaling of conventional and CAR-T cells



# Eliana clinical trial: Results

N Engl J Med. 2018 February 01; 378(5): 439-448.



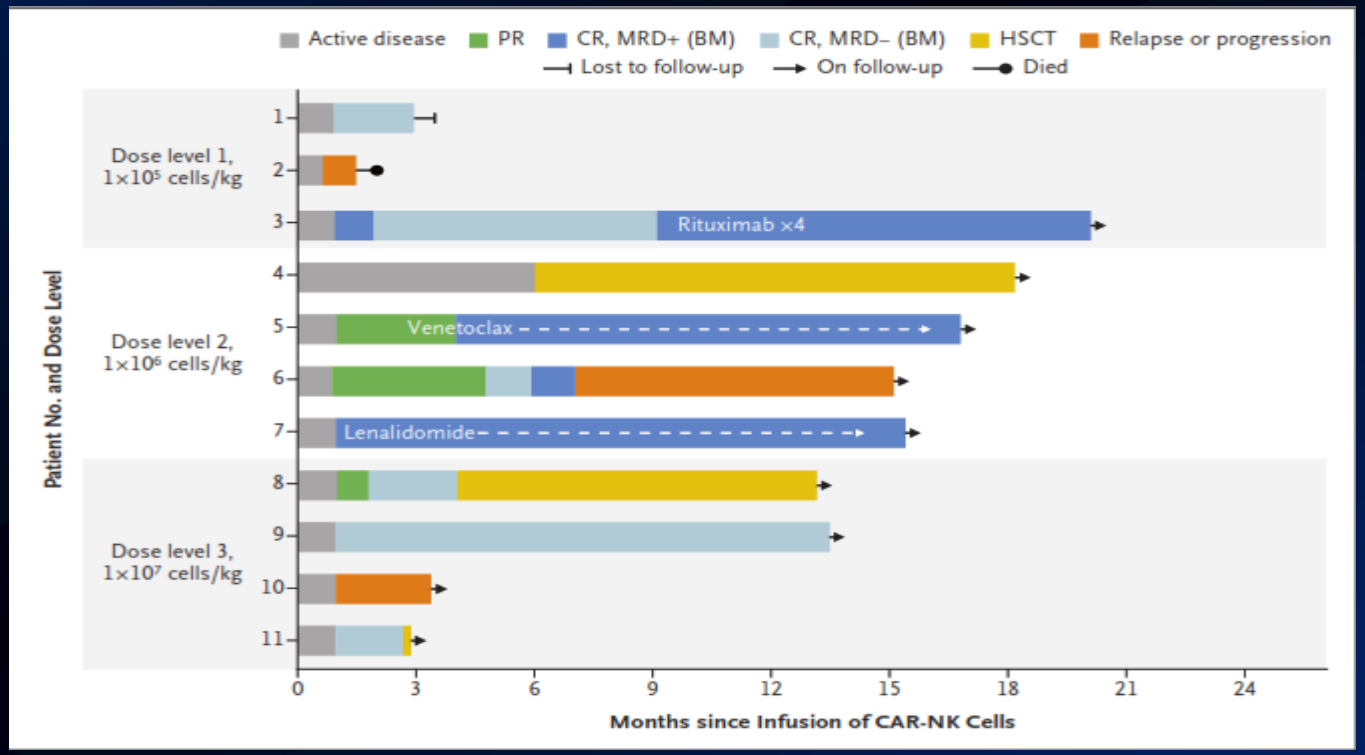

The rates of event-free survival and overall survival were 73% and 90%, at 6 months and 50% and 76% at 12 months

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

# Use of CAR-Transduced Natural Killer Cells in CD19-Positive Lymphoid Tumors

N ENGL J MED 382;6 NEJM.ORG FEBRUARY 6, 2020



# Types of Stem Cells on the basis of their Differentiation Potential

Totipotent:

- potential to become any cell type in the body including placenta.

Pluripotent:

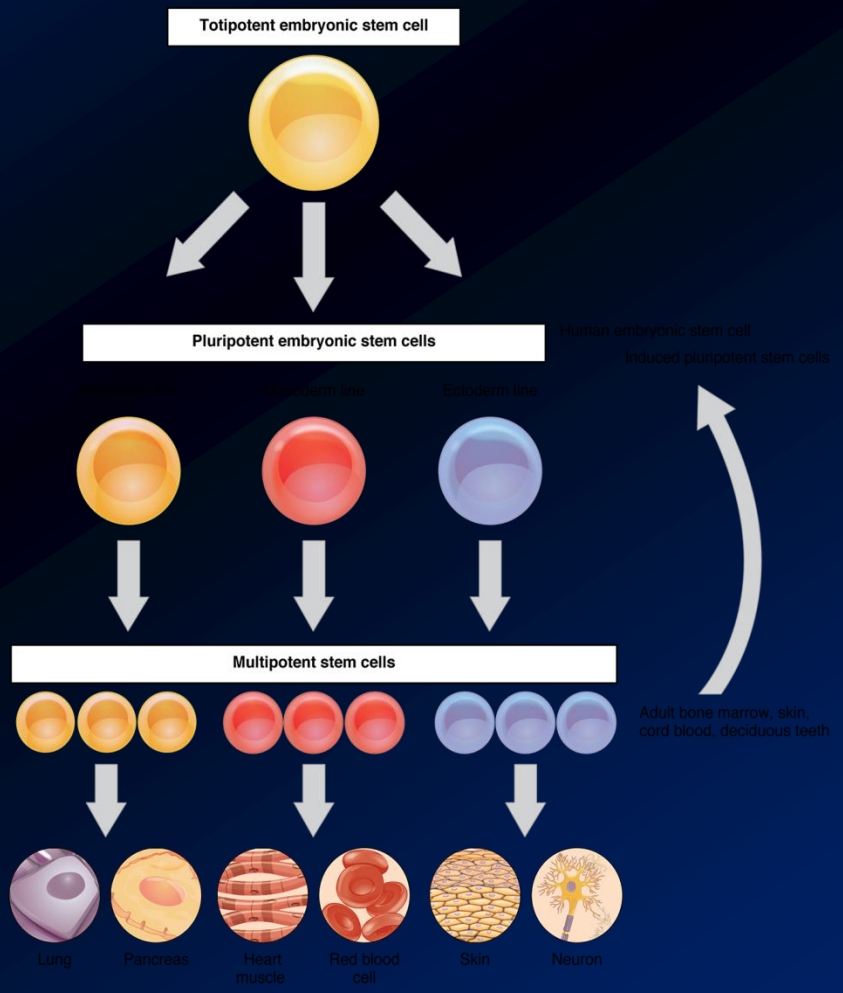
- potential to make any differentiated cell in the body

Multipotent:

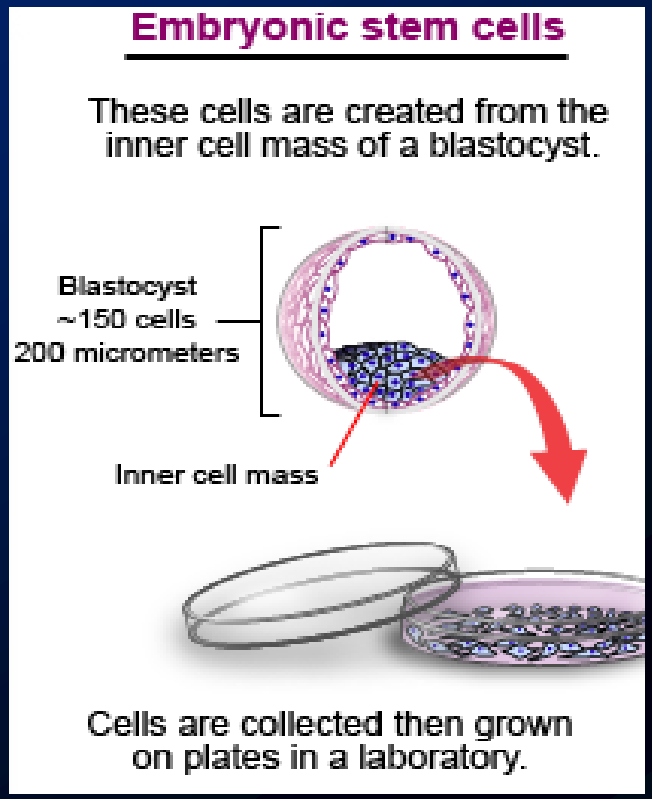
- produce only cells of a closely related family of cells e.g. hematopoietic stem cells.

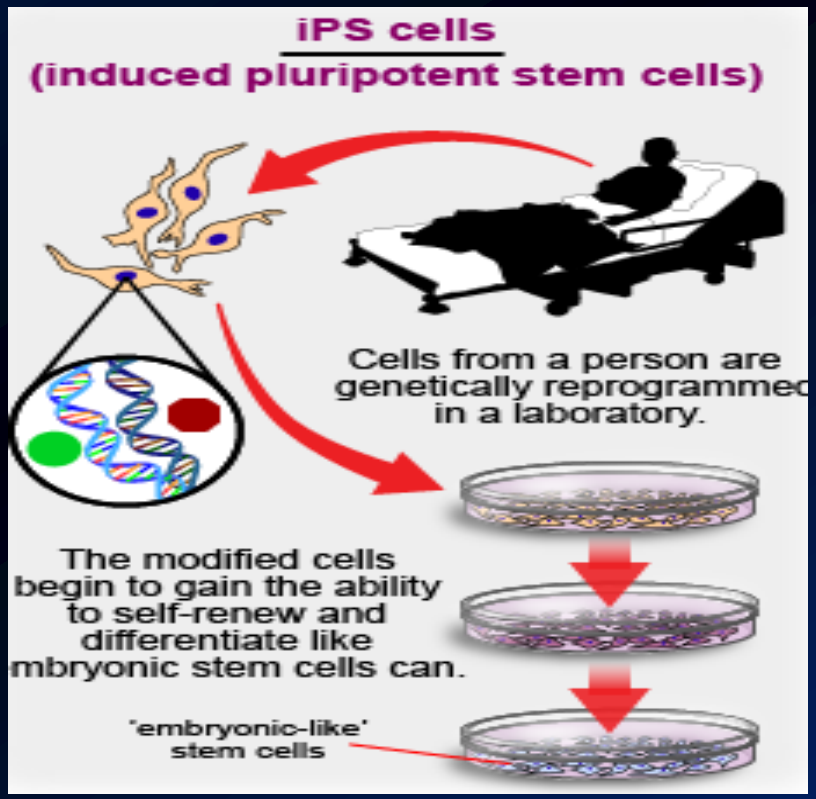
Unipotent

- produce only one cell type, but have the property of self-renewal

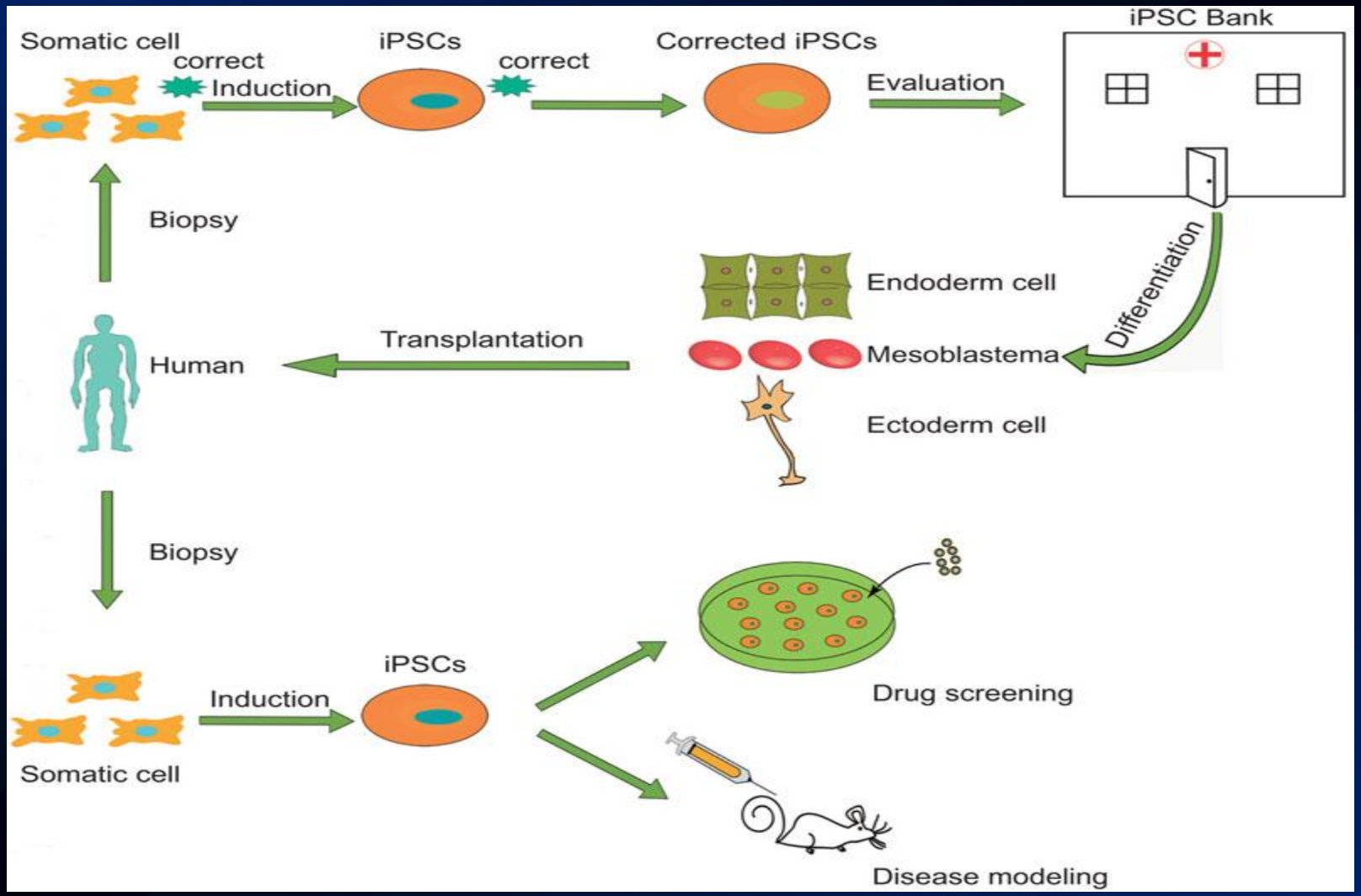


# Where do pluripotent stem cells come from?



# Disease specific iPSCs for the study and therapy



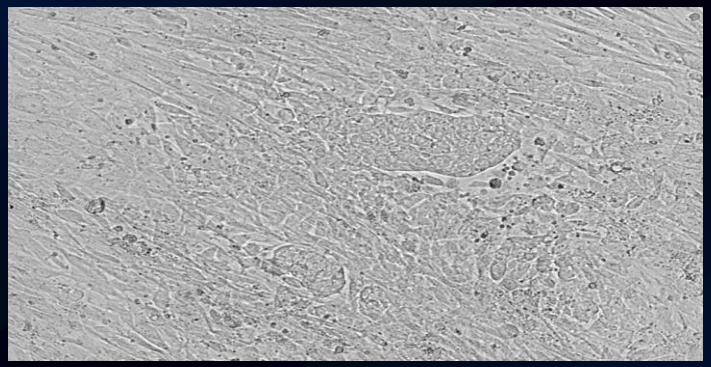
CELLULAR REPROGRAMMING
Volume 16, Number 6, 2014

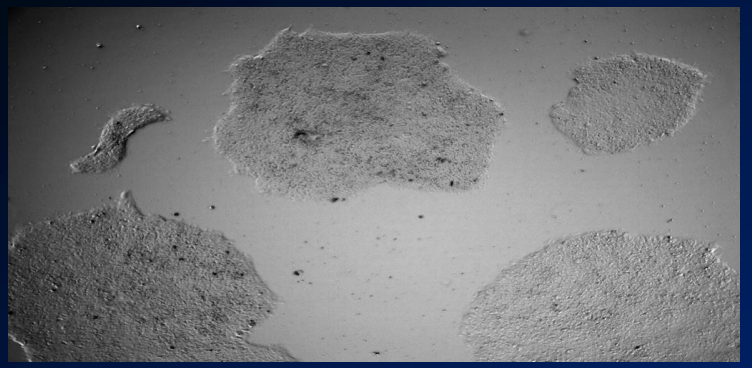
DOI: 10.1089/cell.2014.0050

***Original Article***

# Generation of Human β-Thalassemia Induced Pluripotent Cell Lines by Reprogramming of Bone Marrow–Derived Mesenchymal Stromal Cells Using Modified mRNA

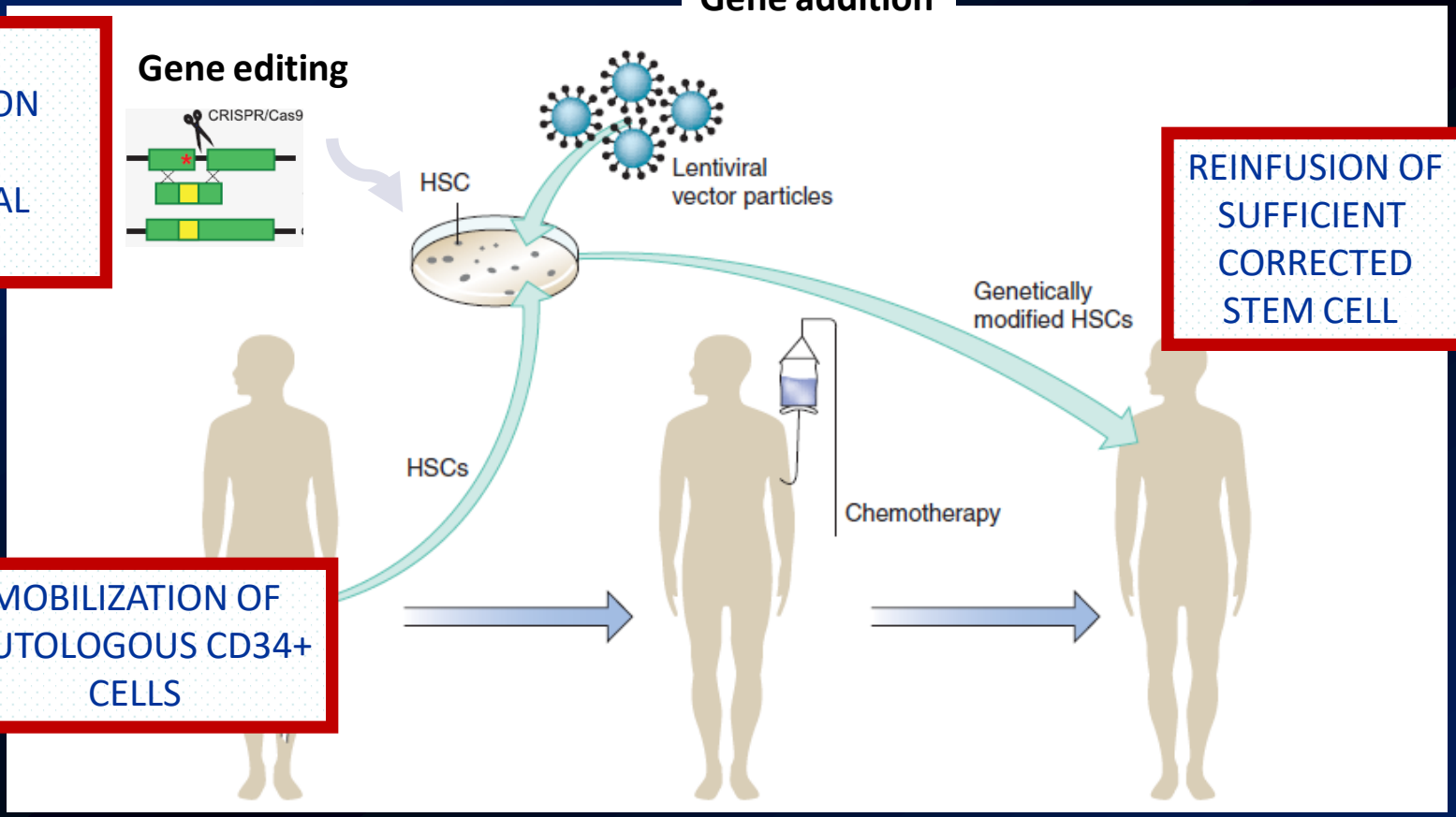
Ioanna Varela,[1,5] Angeliki Karagiannidou,[1] Vasilis Oikonomakis,[2] Maria Tzetis,[2] Marianna Tzanoudaki,[3] Elena-Konstantina Siapati,[4] George Vassilopoulos,[4] Stelios Graphakos,[1] Emmanuel Kanavakis,[2,5] and Evgenios Goussetis[1]





# Gene therapy in the clinic

EX VIVO CORRECTION OF THE ABNORMAL GENE




REINFUSION OF SUFFICIENT CORRECTED STEM CELL

MOBILIZATION OF AUTOLOGOUS CD34+ CELLS

MYELOABLATIVE CONDITIONING

*Nienhuis et al, Cold Spring Harb Perspect Med 2012;2:a011833*

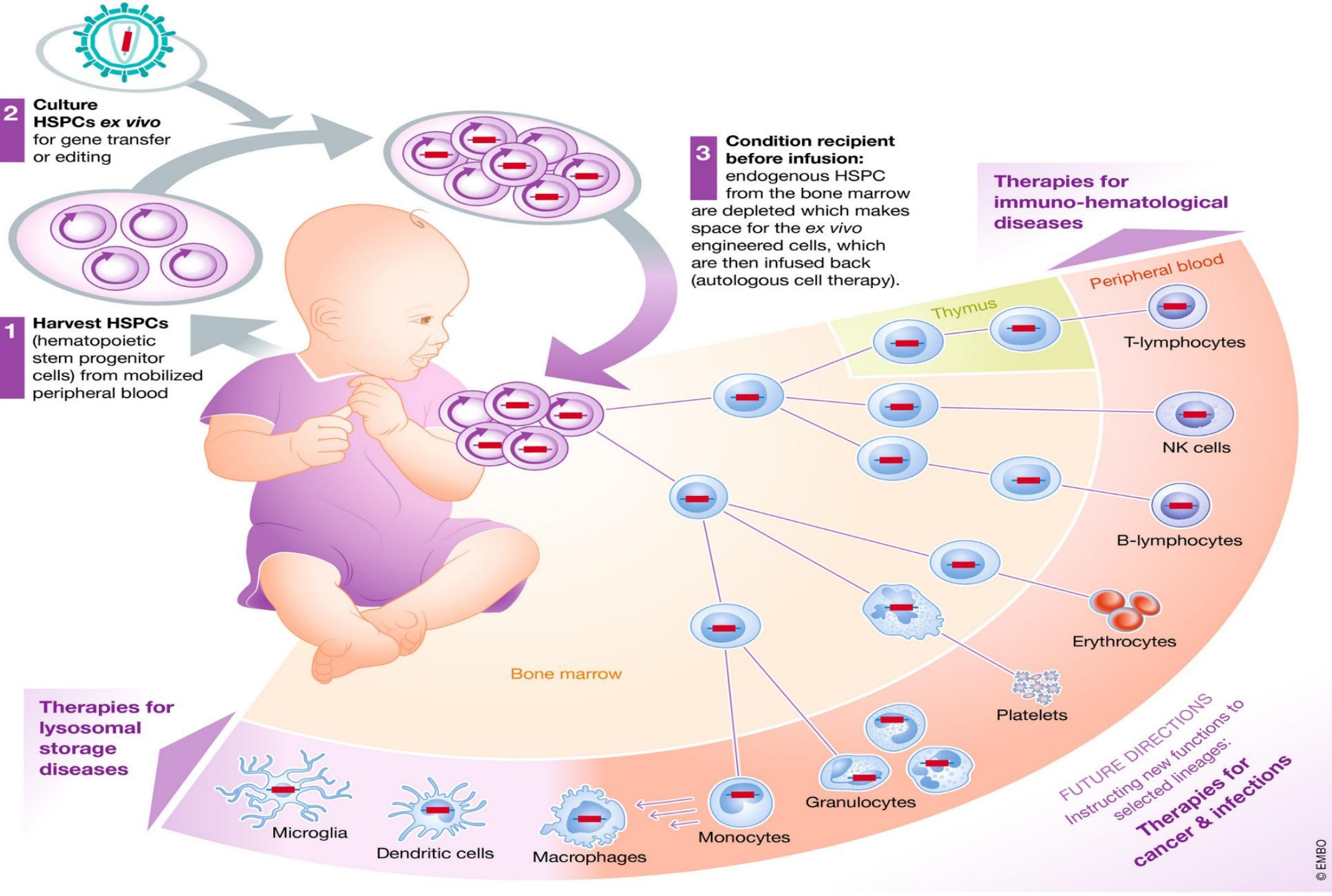
# The future


Basic Immunology/ Immunotherapy

Stem cell biology Lymphoid development

Genome engineering

## “Off-the-shelf” lymphocytes for the treatment of cancer


2 Culture HSPCs *ex vivo* for gene transfer or editing
3 Condition recipient before infusion: endogenous HSPC from the bone marrow are depleted which makes space for the *ex vivo* engineered cells, which are then infused back (autologous cell therapy).
1 Harvest HSPCs (hematopoietic stem progenitor cells) from mobilized peripheral blood
Therapies for immuno-hematological diseases
Peripheral blood
Thymus
T-lymphocytes
NK cells
B-lymphocytes
Erythrocytes
Platelets
Granulocytes
Monocytes
Macrophages
Dendritic cells
Microglia
Bone marrow
Therapies for lysosomal storage diseases
FUTURE DIRECTIONS
Instructing new functions to selected lineages:
Therapies for cancer & infections
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