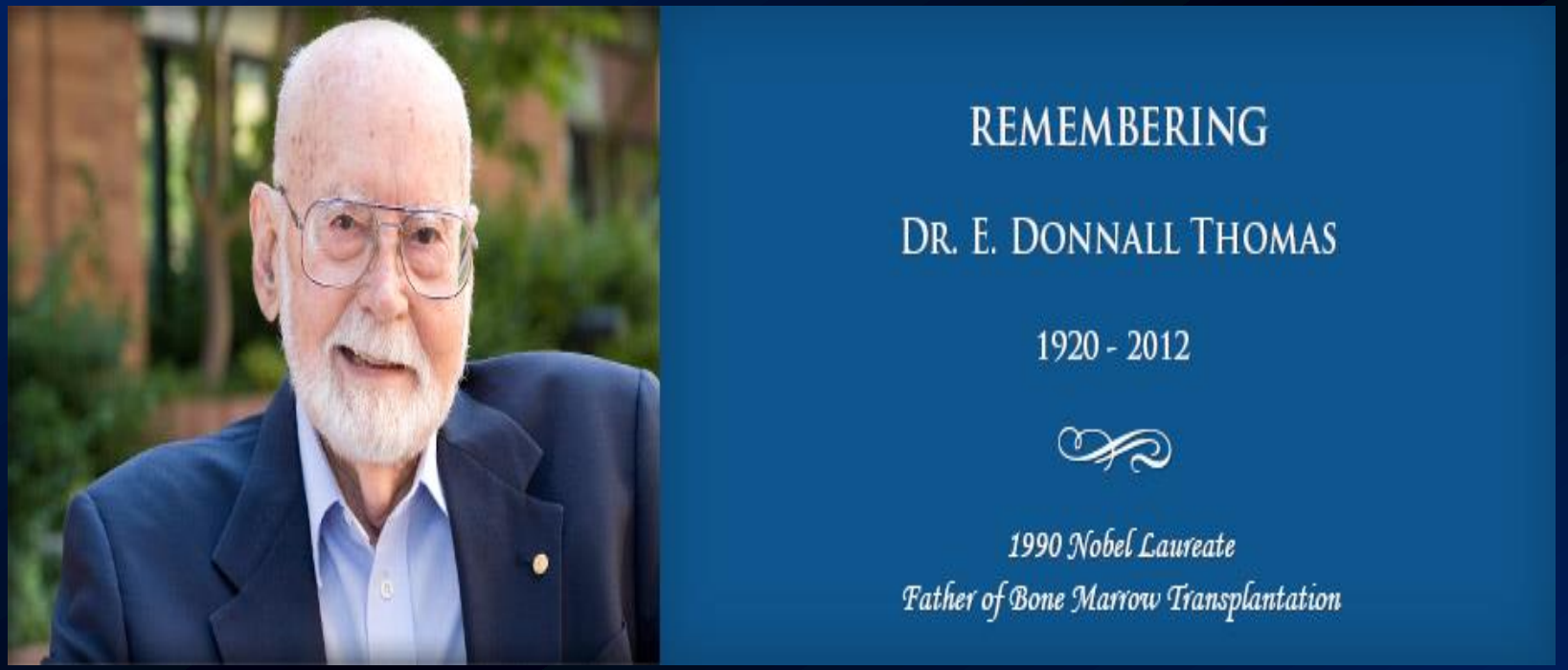
Future Trends in Blood and Marrow Transplantation



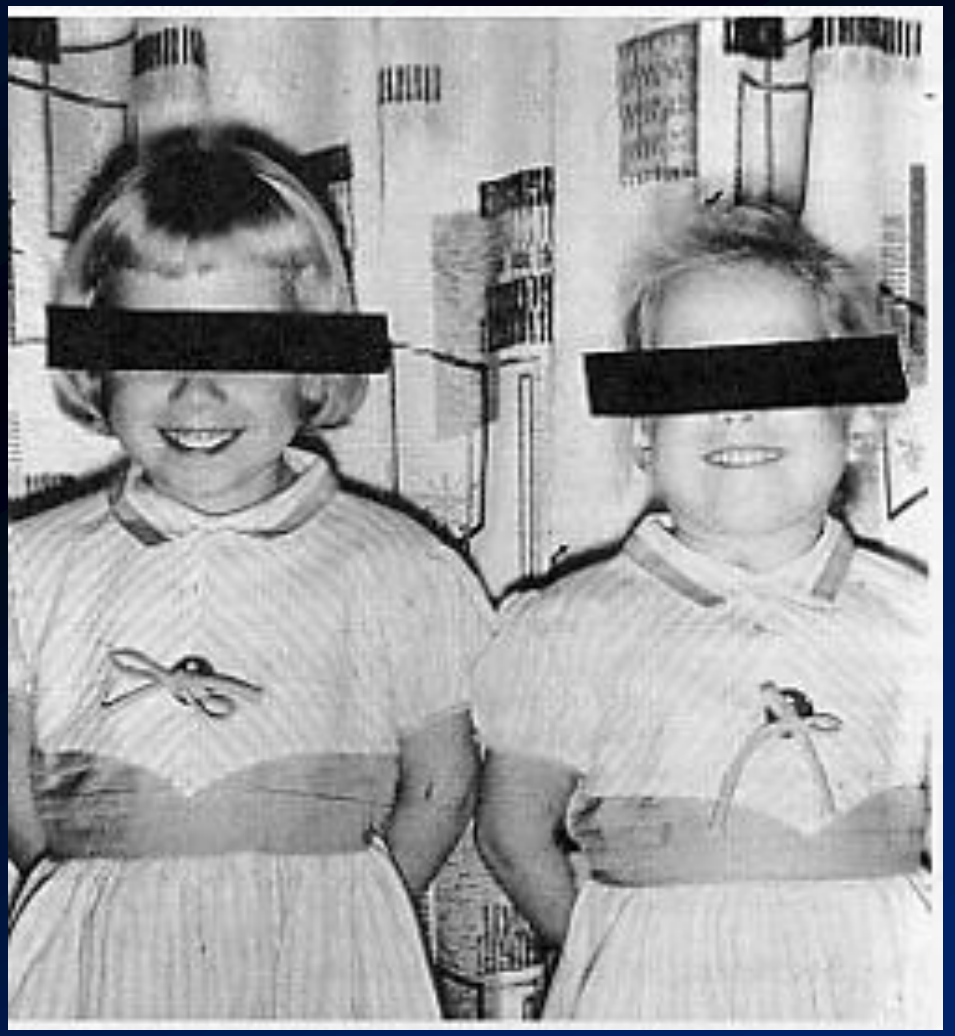
Evgenios Goussetis

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

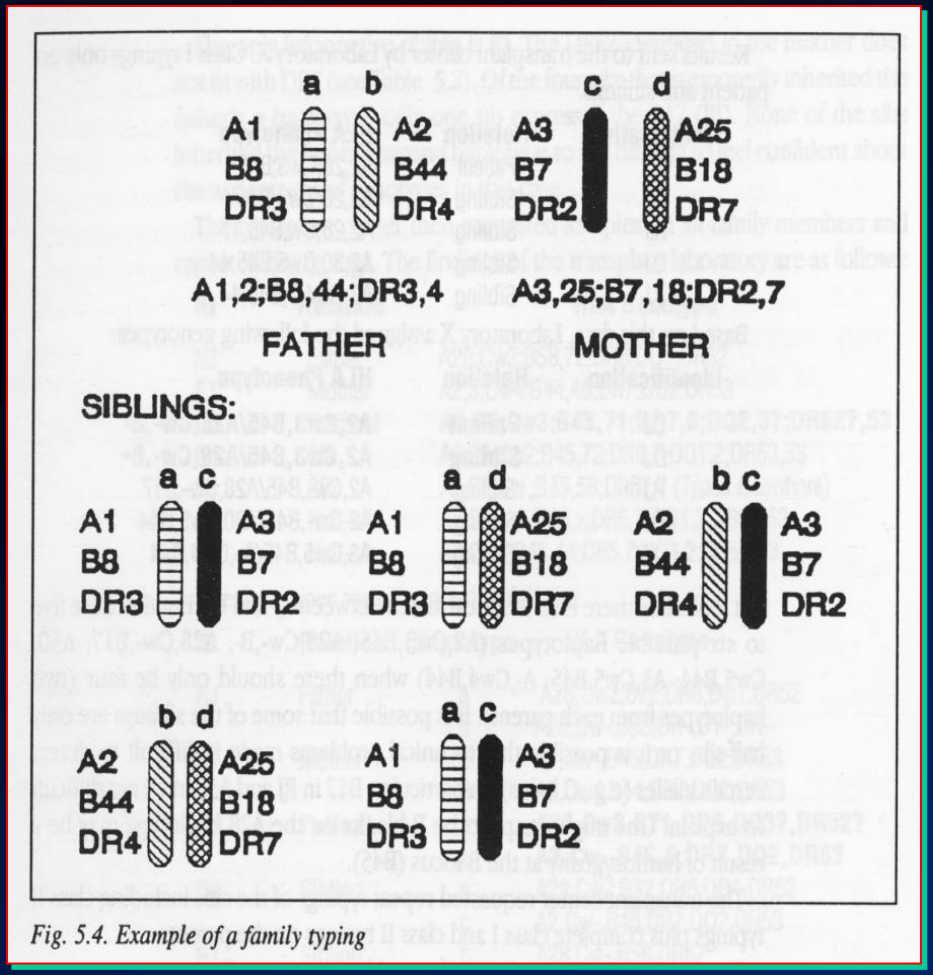
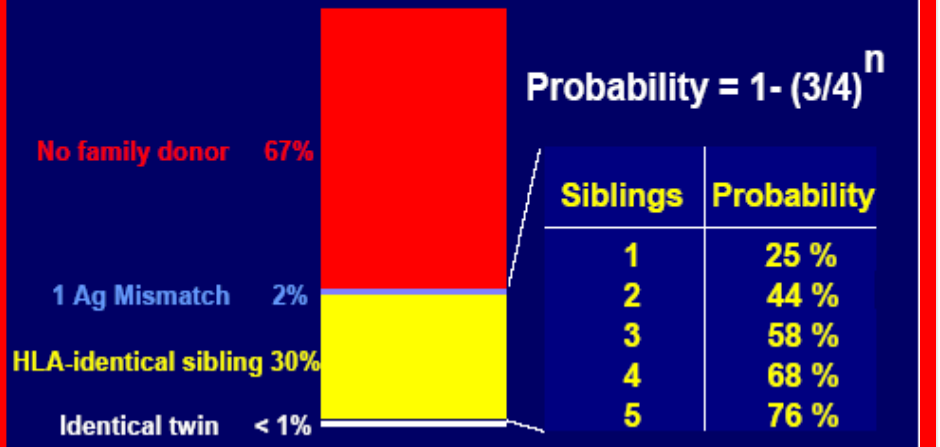


Fig. 5.4. Example of a family typing

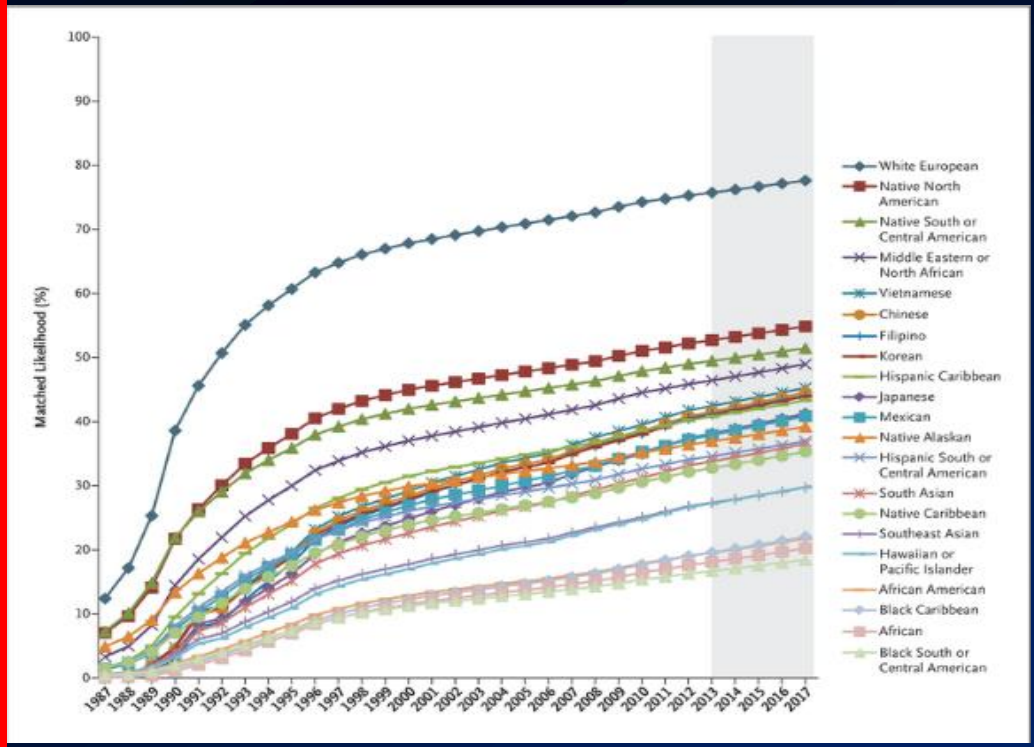
Probability of having compatible HSC donor

HLA- Matched Family Donors Availability



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

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



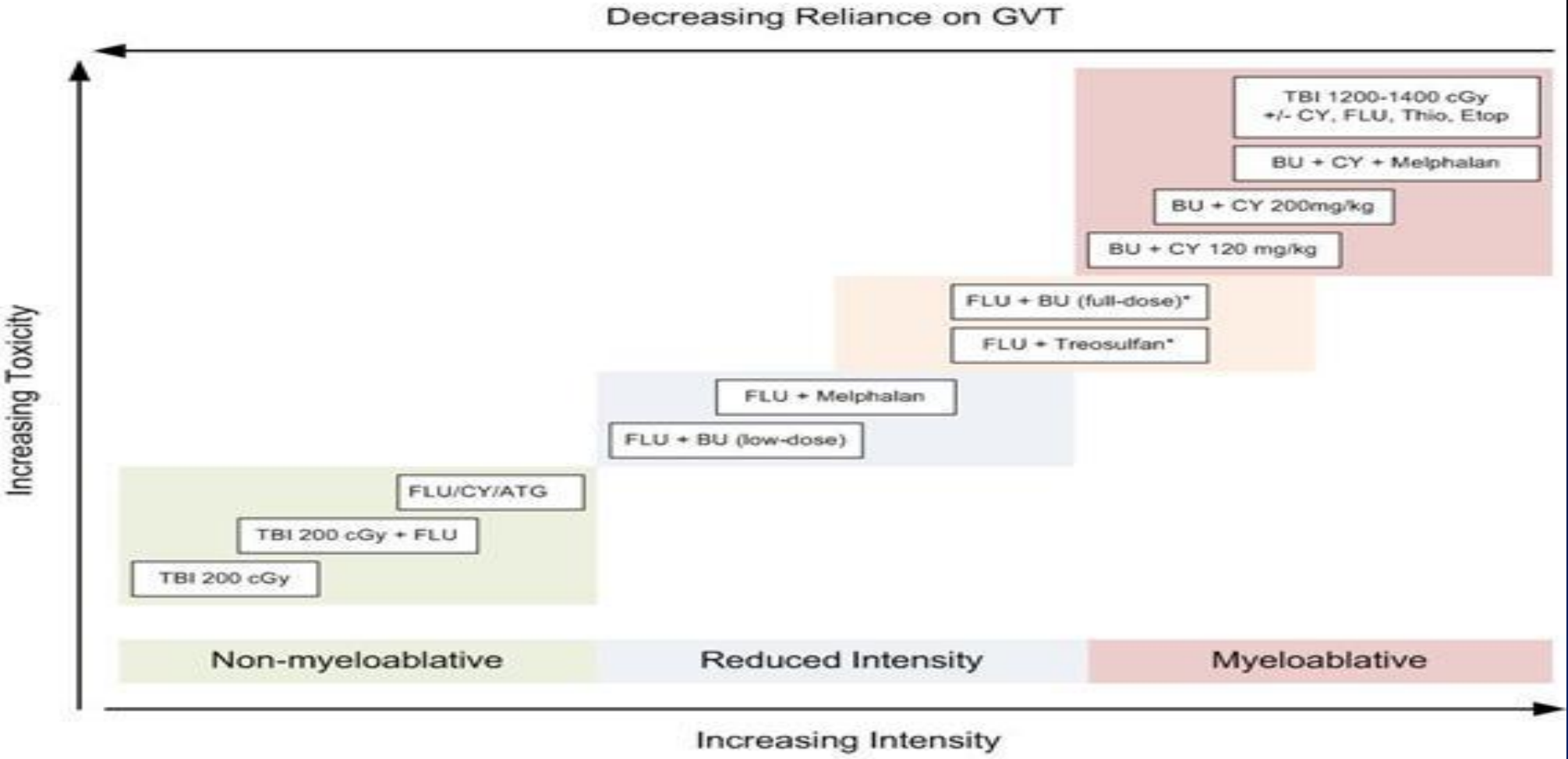
Increasing Donor Pool Essential

- 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



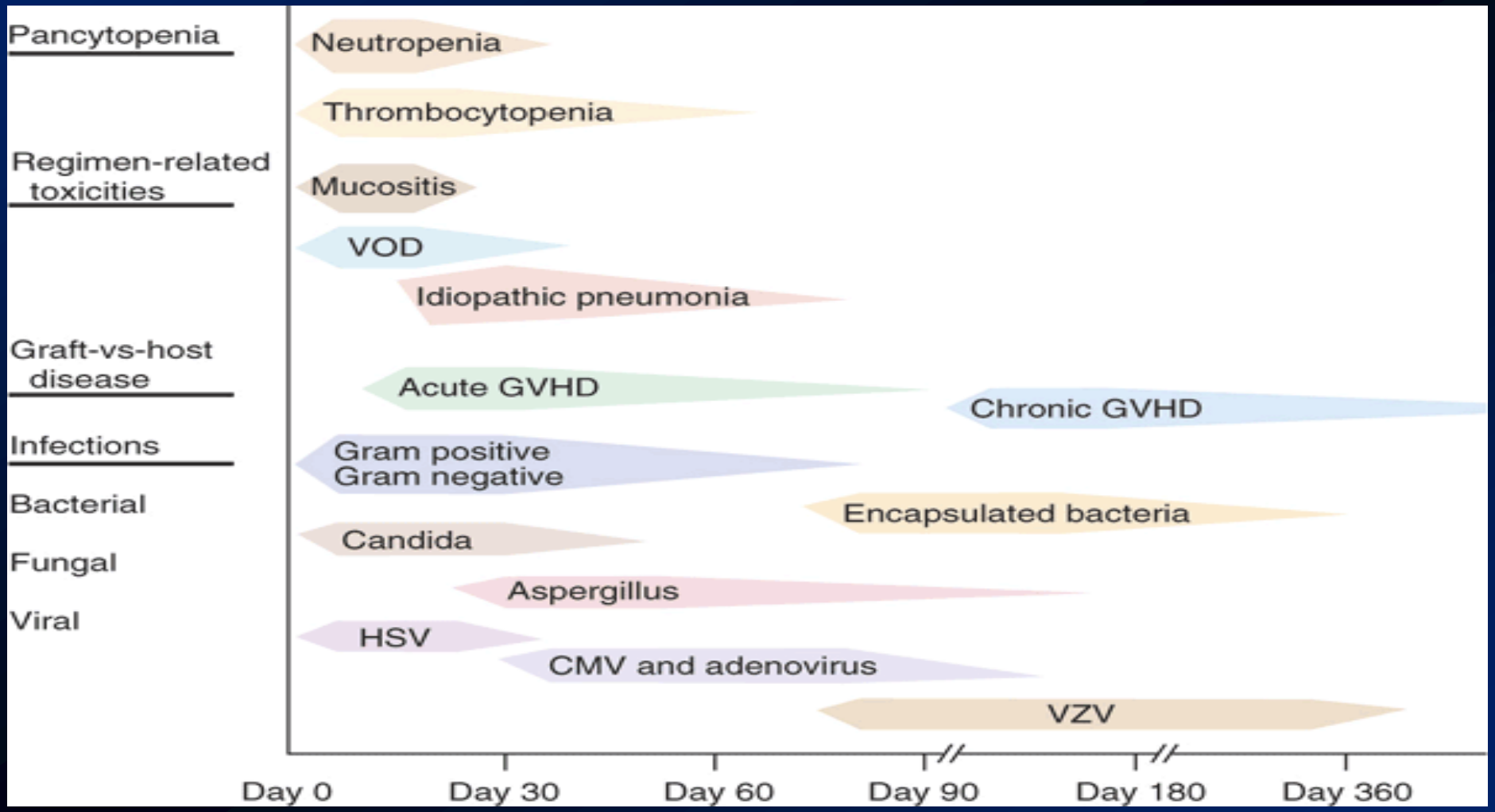
ATG = Antithymocyte globulin
BU = Busulfan
cGy = Centigray
CY = Cyclophosphamide
Etop = Etoposide
FLU = Fludarabine
GVT = Graft-versus-tumor effect
TBI = Total-body irradiation
Thio = Thiotepa

* 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.

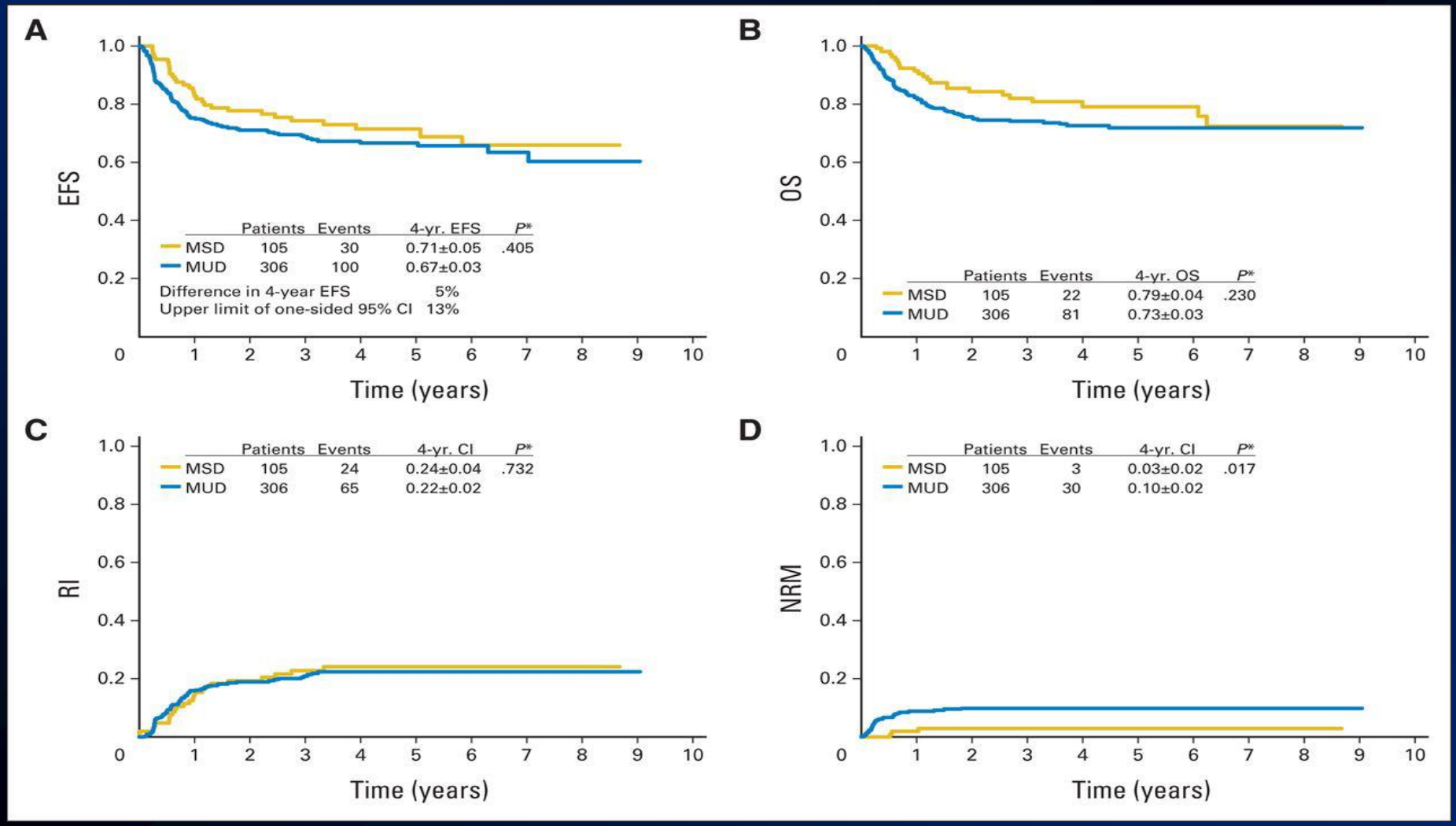
Hematopoietic Reconstitution

- 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

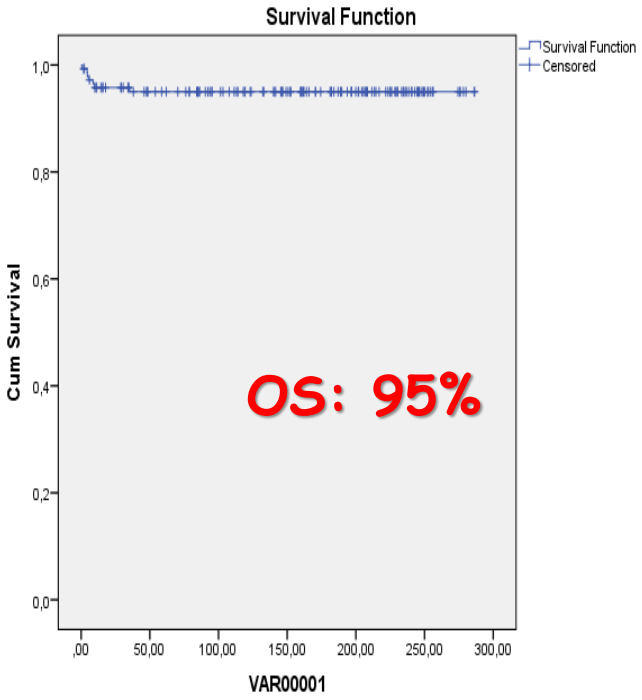
Complications

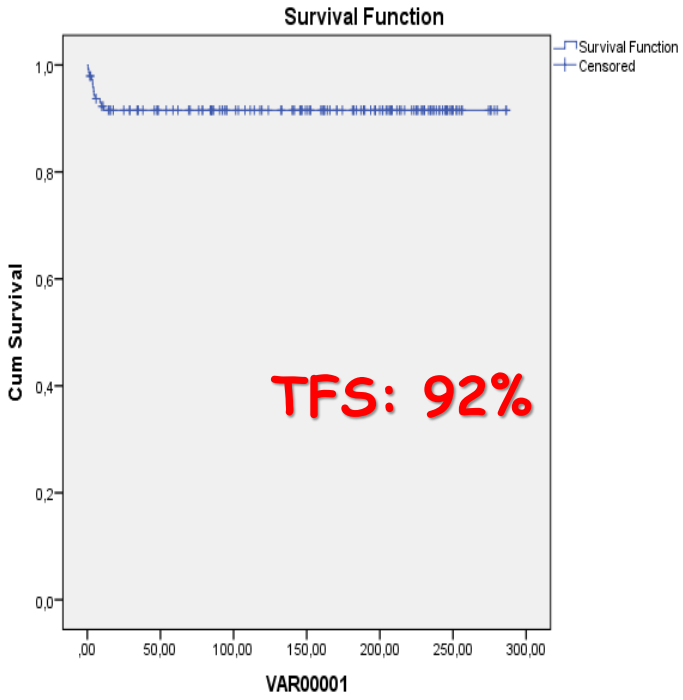


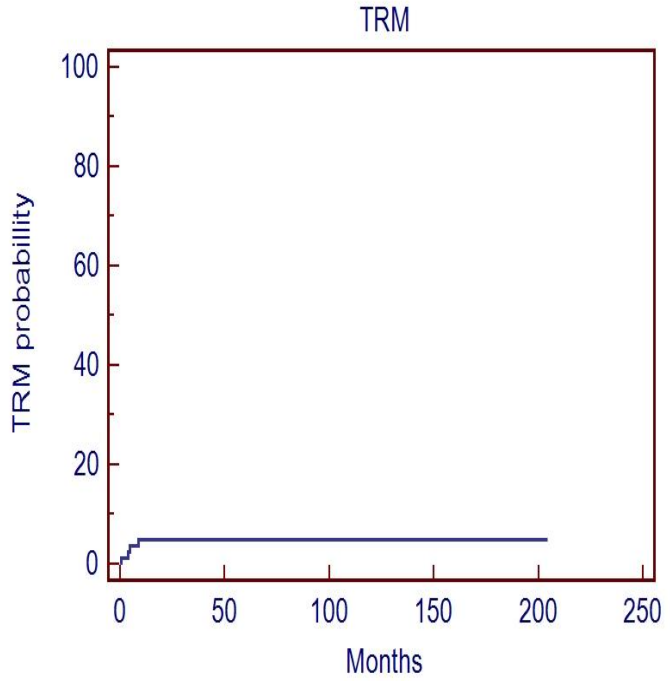
Outcome in pediatric ALL



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

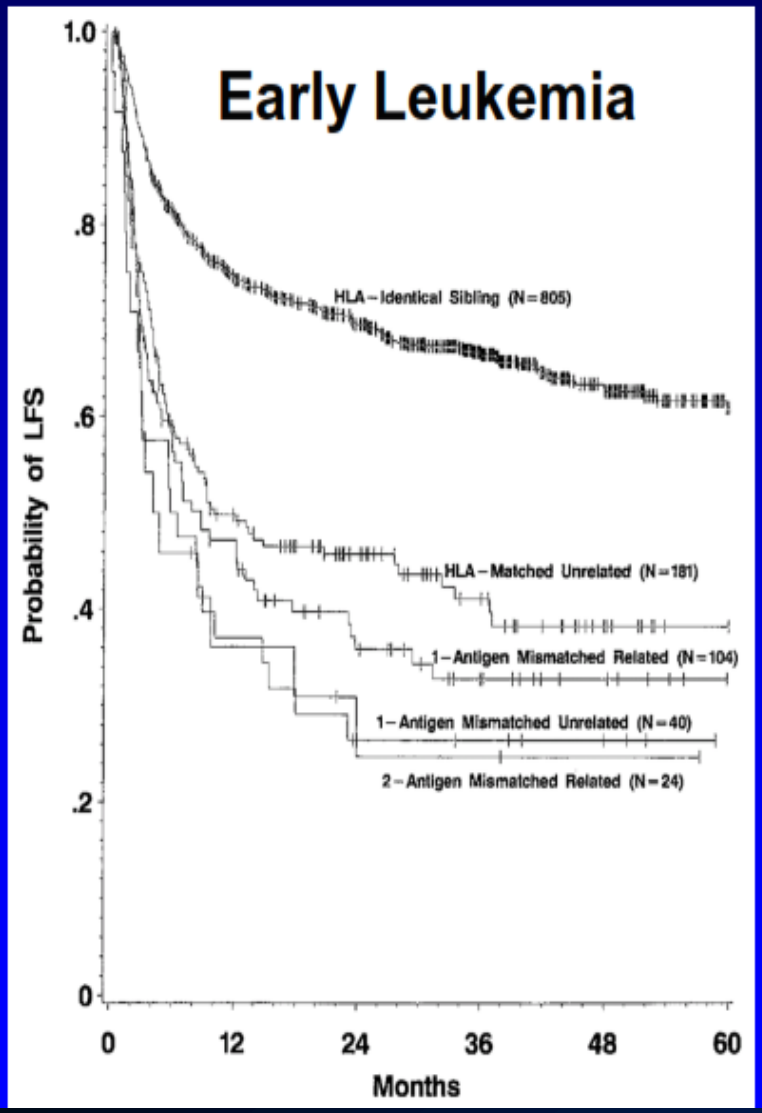
E Goussetis[1], I Peristeri[1], V Kitra, G Vessalas, A Paisiou, M Theodosaki, E Petrakou, MN Dimopoulou and S Graphakos

The limits of success today

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

What We Learned Over the Decades

HLA mismatches are prohibitively toxic

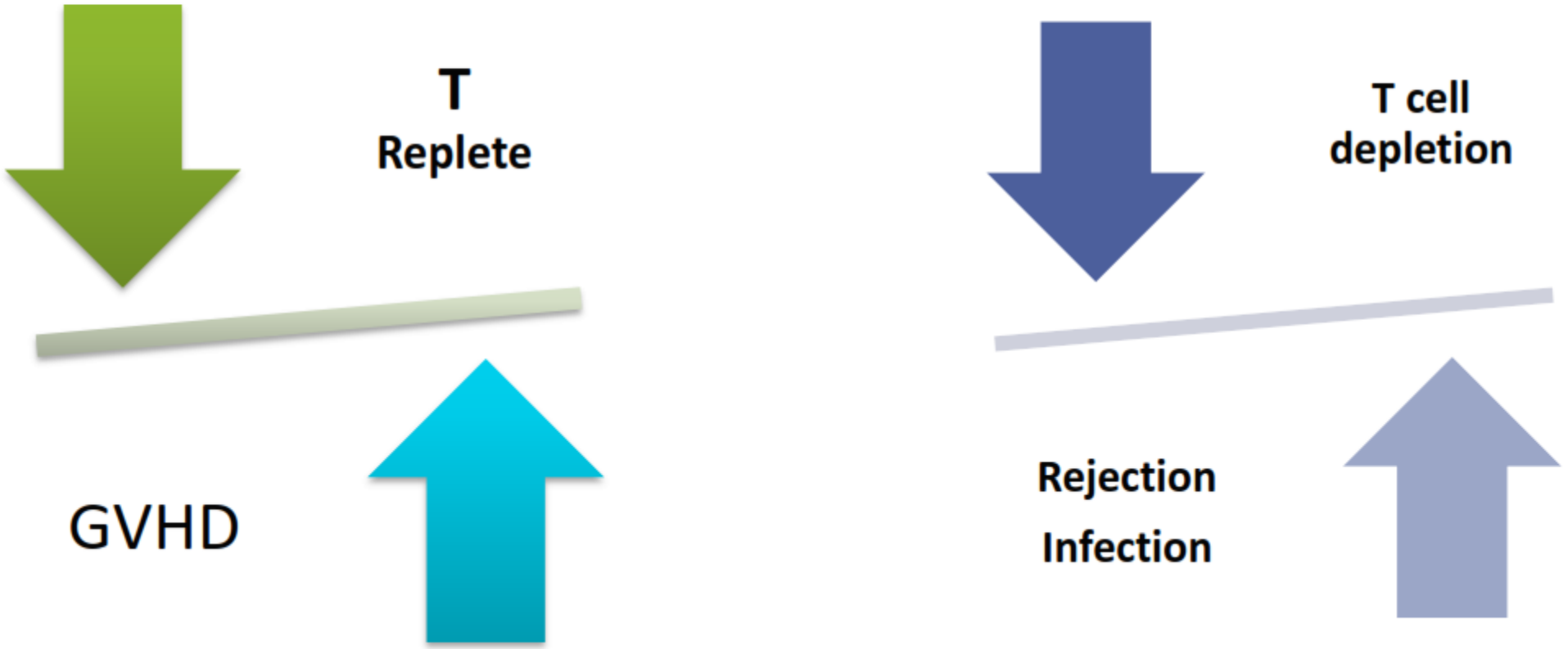


Disease State/Type of Donor†	No.	TRM (%)	P*
Early			
HLA-identical sibling	805	21 ± 2	—
1-Antigen mismatched related	104	53 ± 5	< .001
2-Antigen mismatched related	24	55 ± 11	< .001
Matched unrelated	181	53 ± 4	< .001
1-Antigen mismatched unrelated	40	69 ± 8	< .001

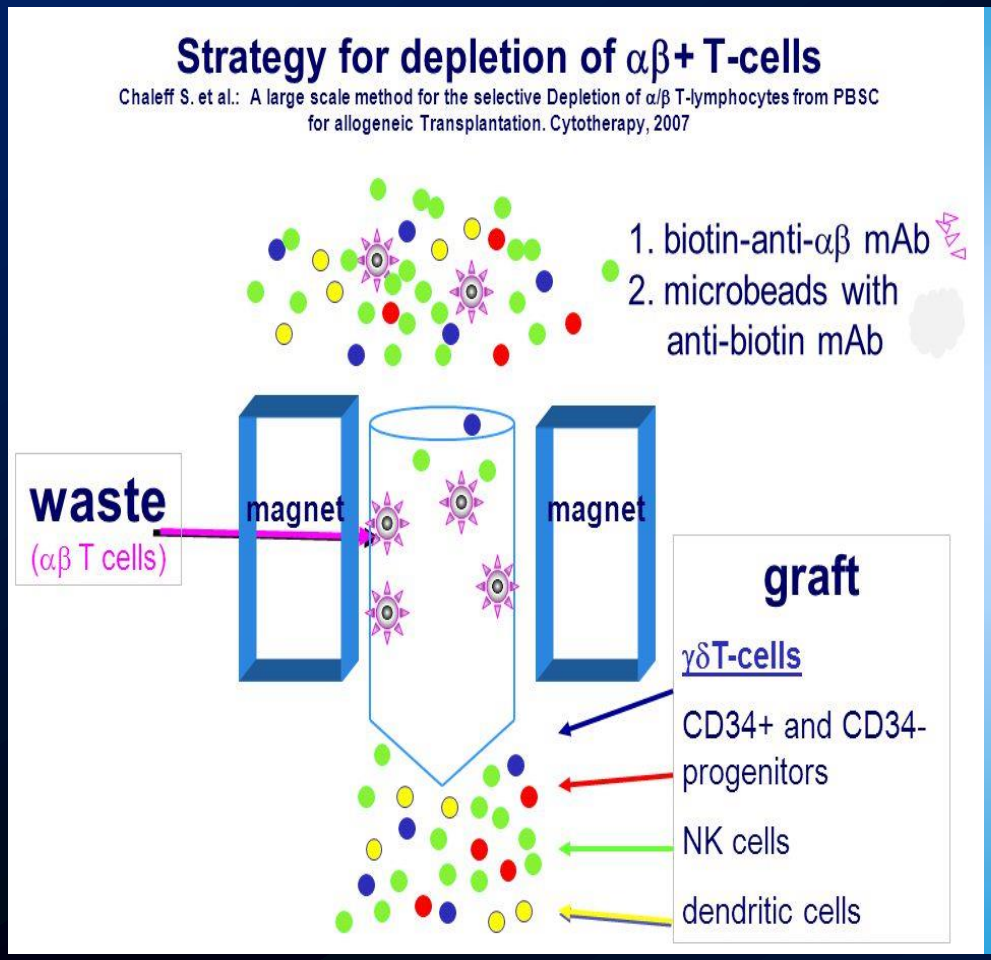
IBMTR

Szydlo et al *JCO* 1997

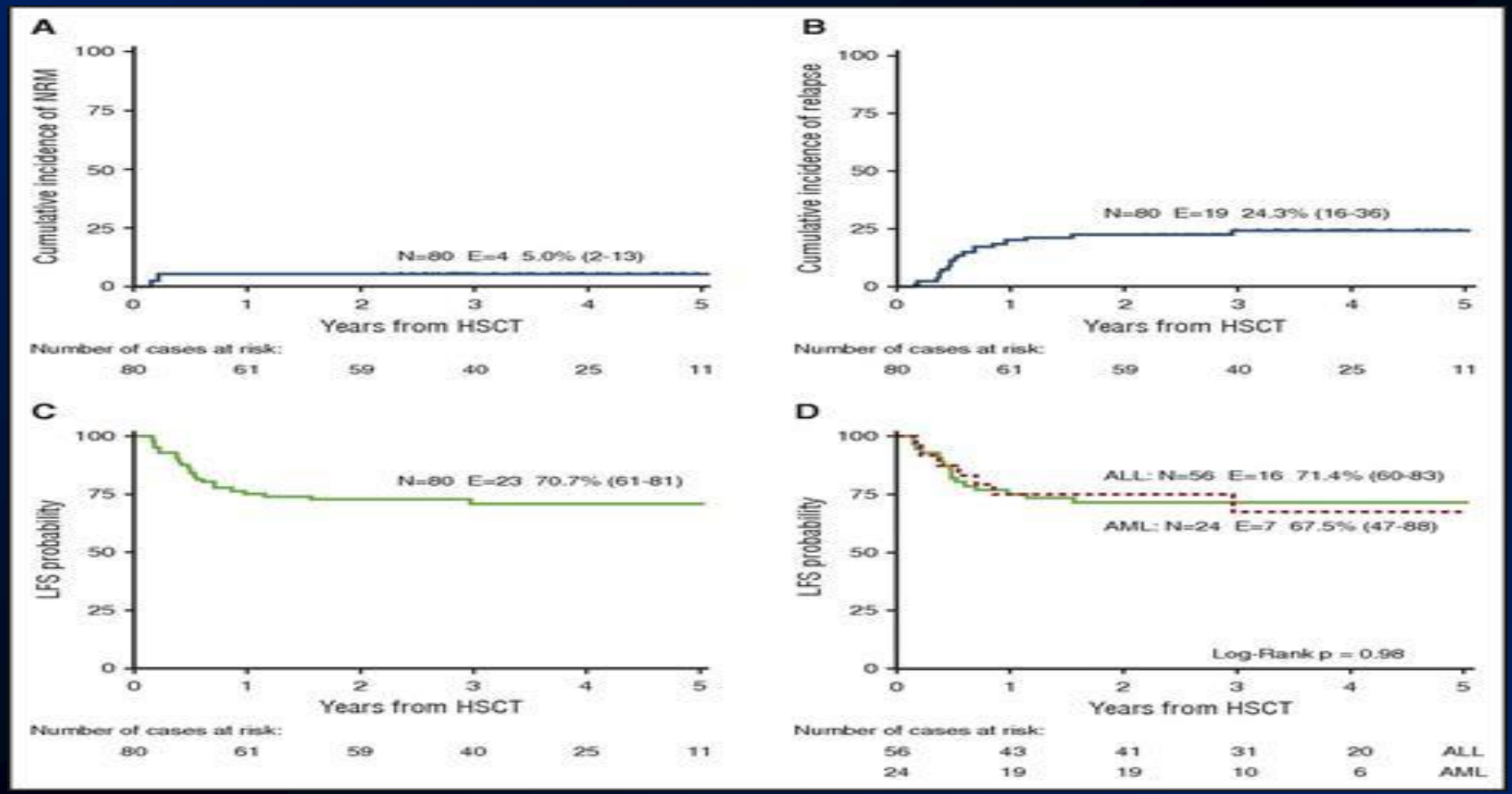
Overcoming the mismatch



Ex vivo T-cell depletion



T-cell depleted haploidentical Transplantation in children with acute leukemia

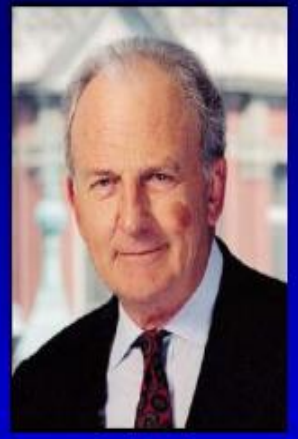


Blood 2017 Aug 3;130(5):677-685

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

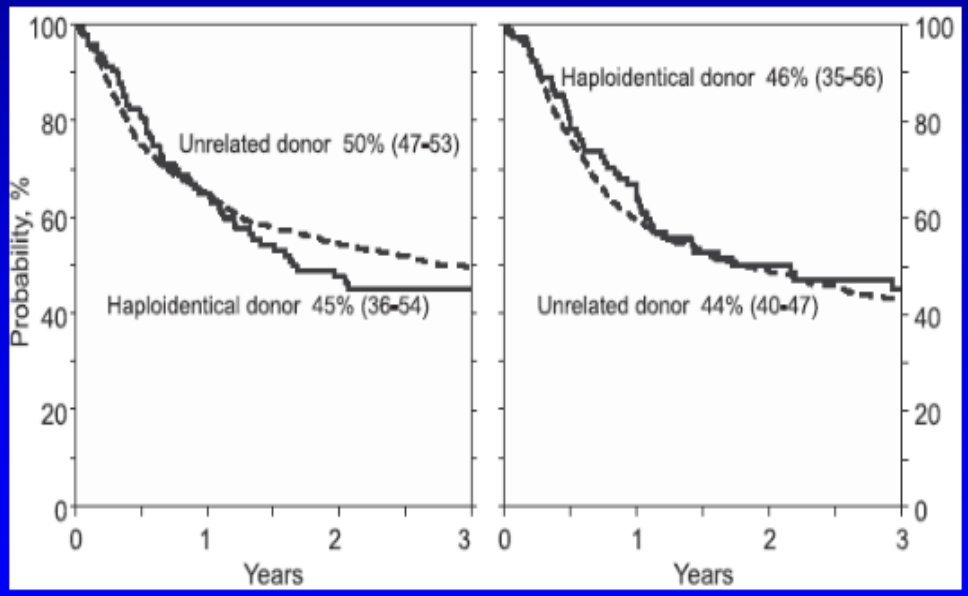
- Randomized trial - less effective than CsA (Santos *et al Clin Transplant* 1986)

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



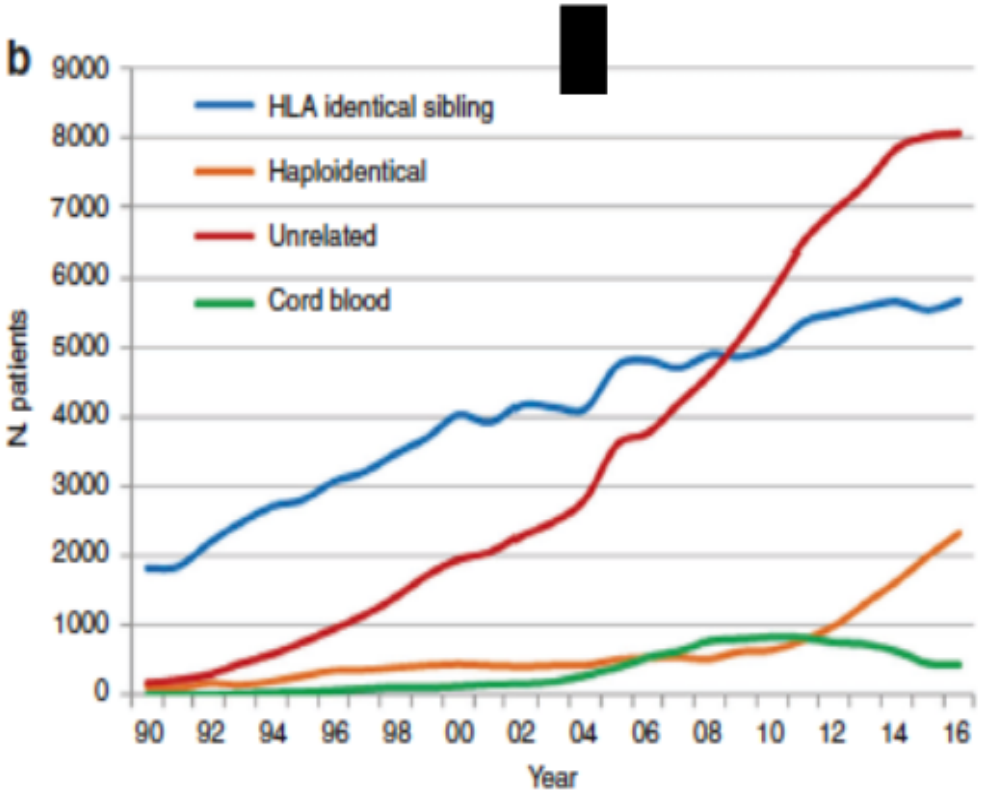
Myeloablative RIC

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

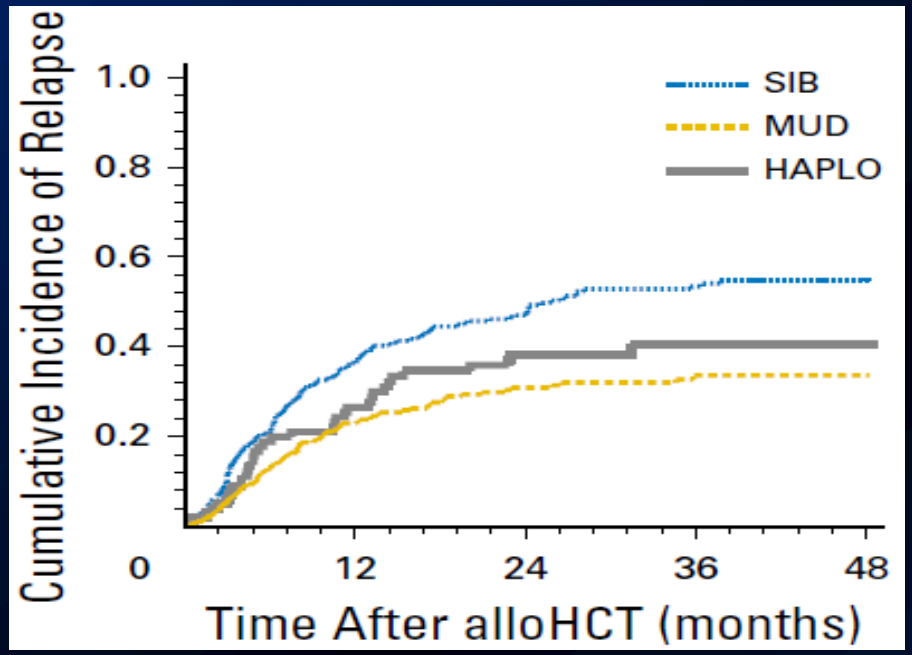


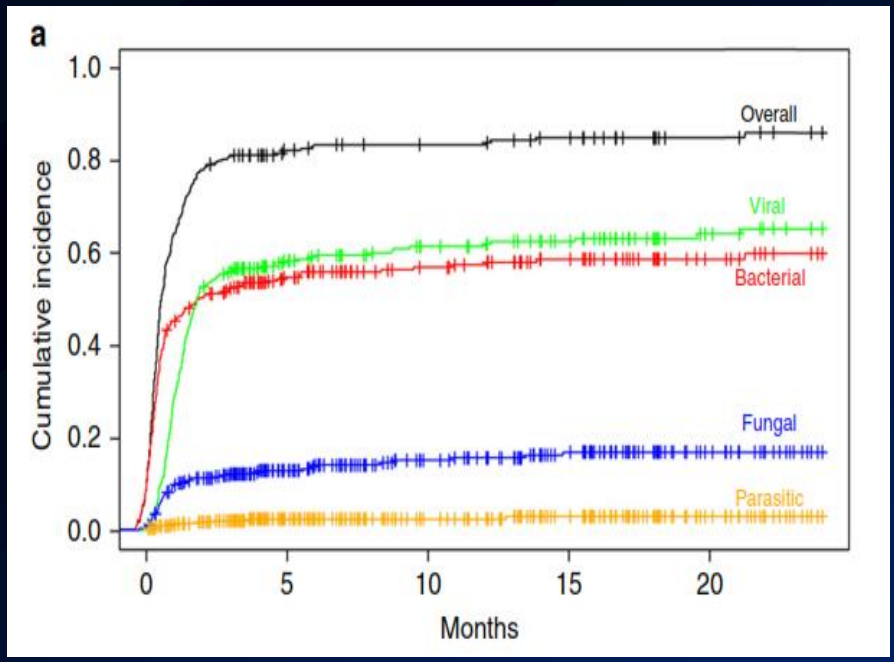
Transplant rates per 10 million inhabitants			
	Donor type		
	Identical Sibling	Haploidentical family	Unrelated
Income group			
Very high	390	77	978
High	283	106	321
Upper middle	102	16	16

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…





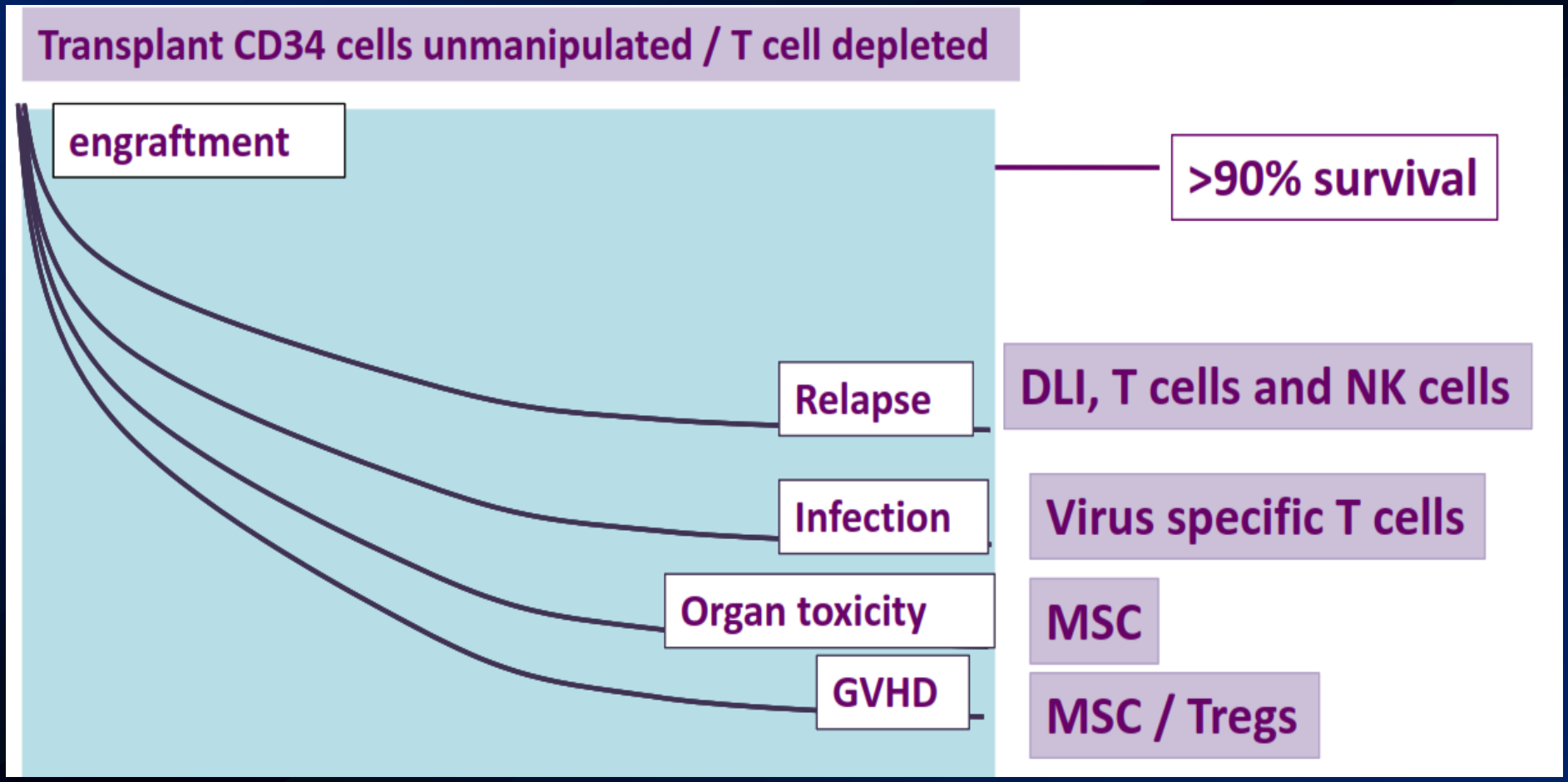
Cellular therapies:

Adoptive transfer of viral-specific T-cells

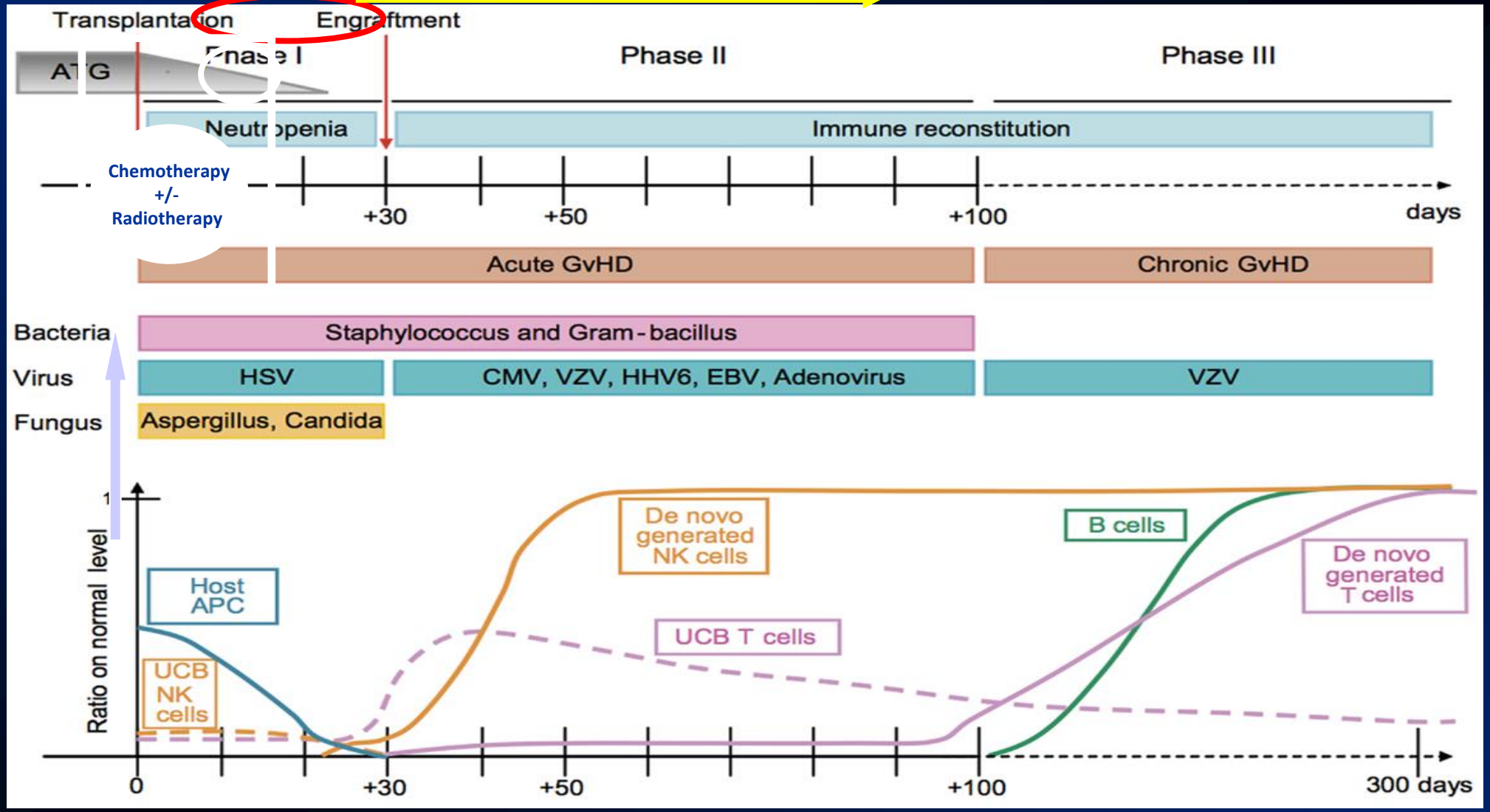
Donor Cell (T-cells or NK cells) Infusions

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

Cell therapy for HSCT - The vision



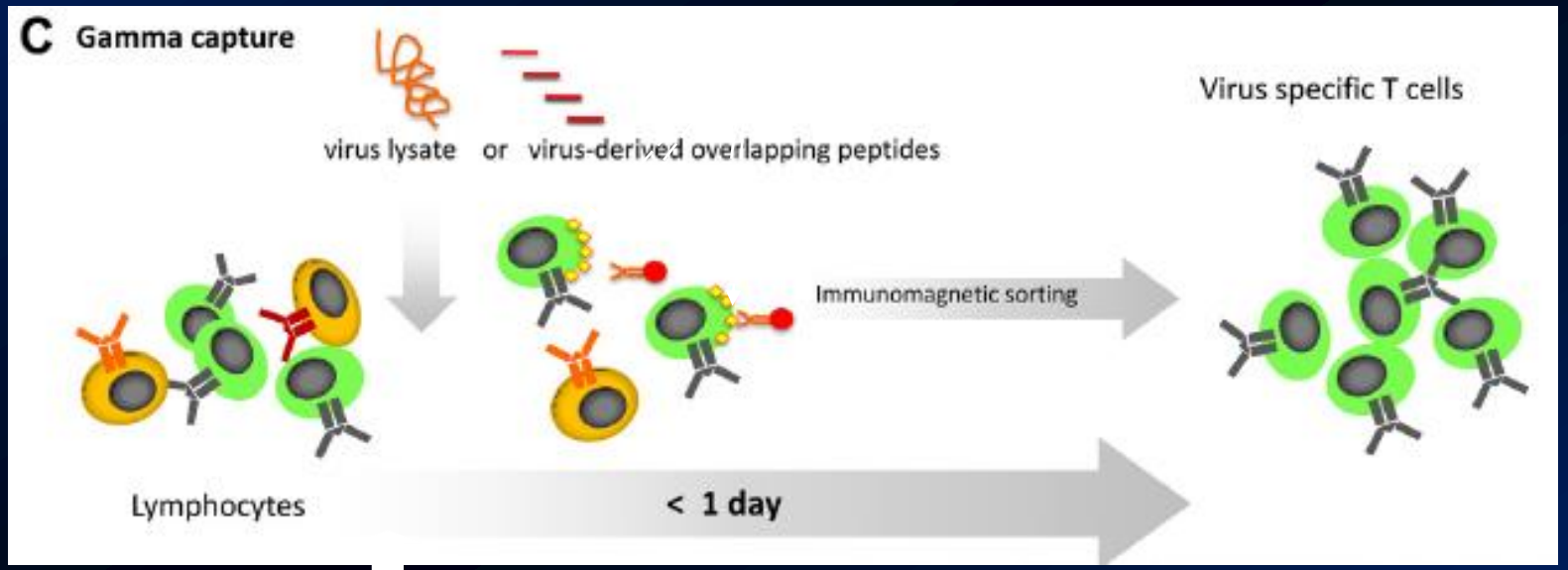
Immunosuppression



Viral specific T-cytotoxic cells

IFNγ capture method

IFNγ



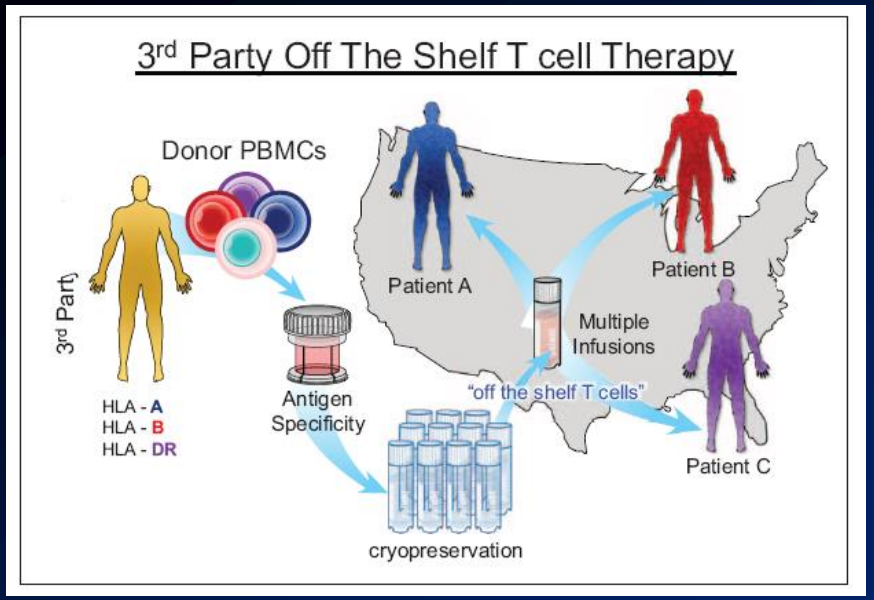
12-16 hours

Antibody-conjugated magnetic beads

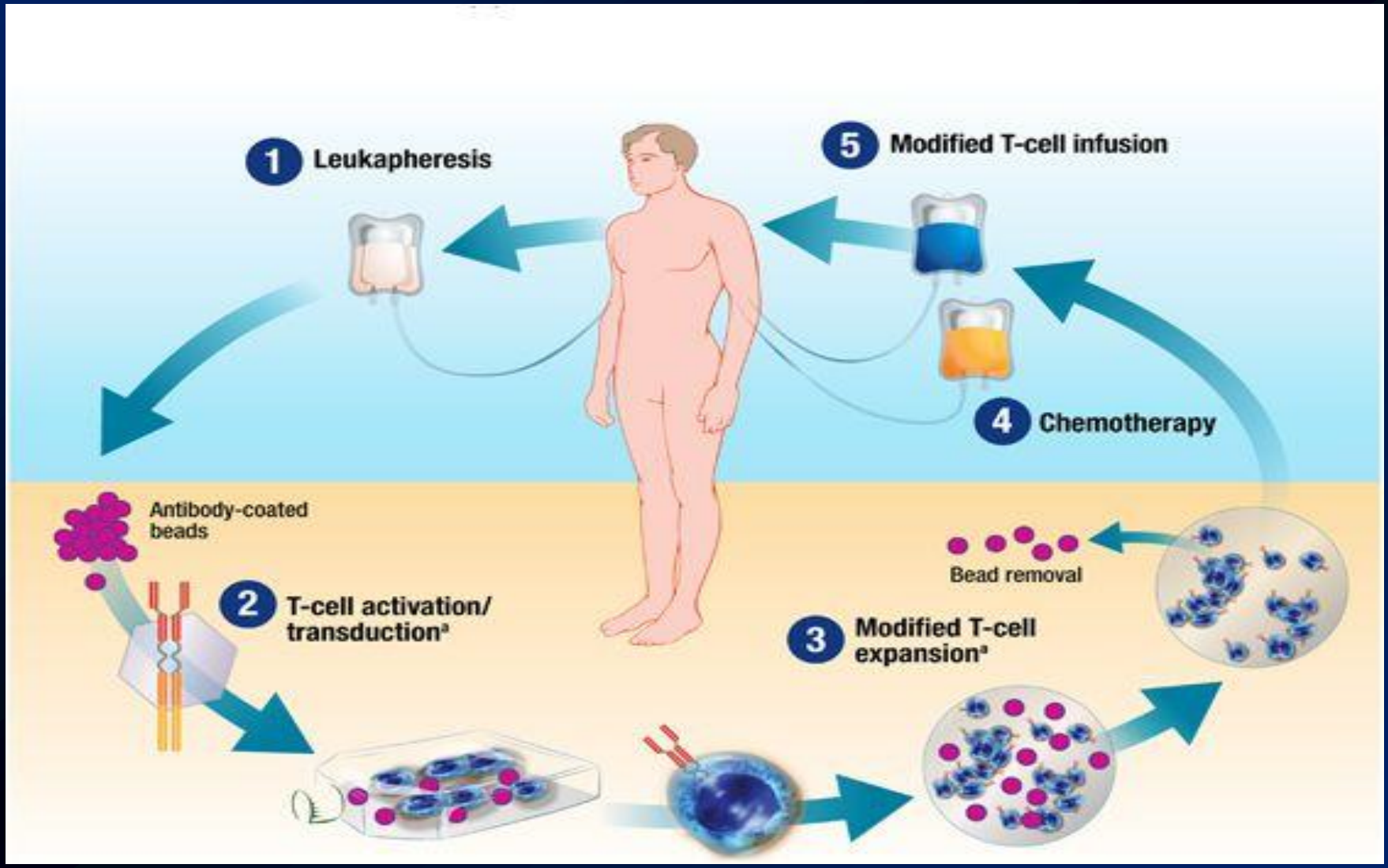
Saglio et al, 2014

CliniMACS Prodigy Cytokine Capture System

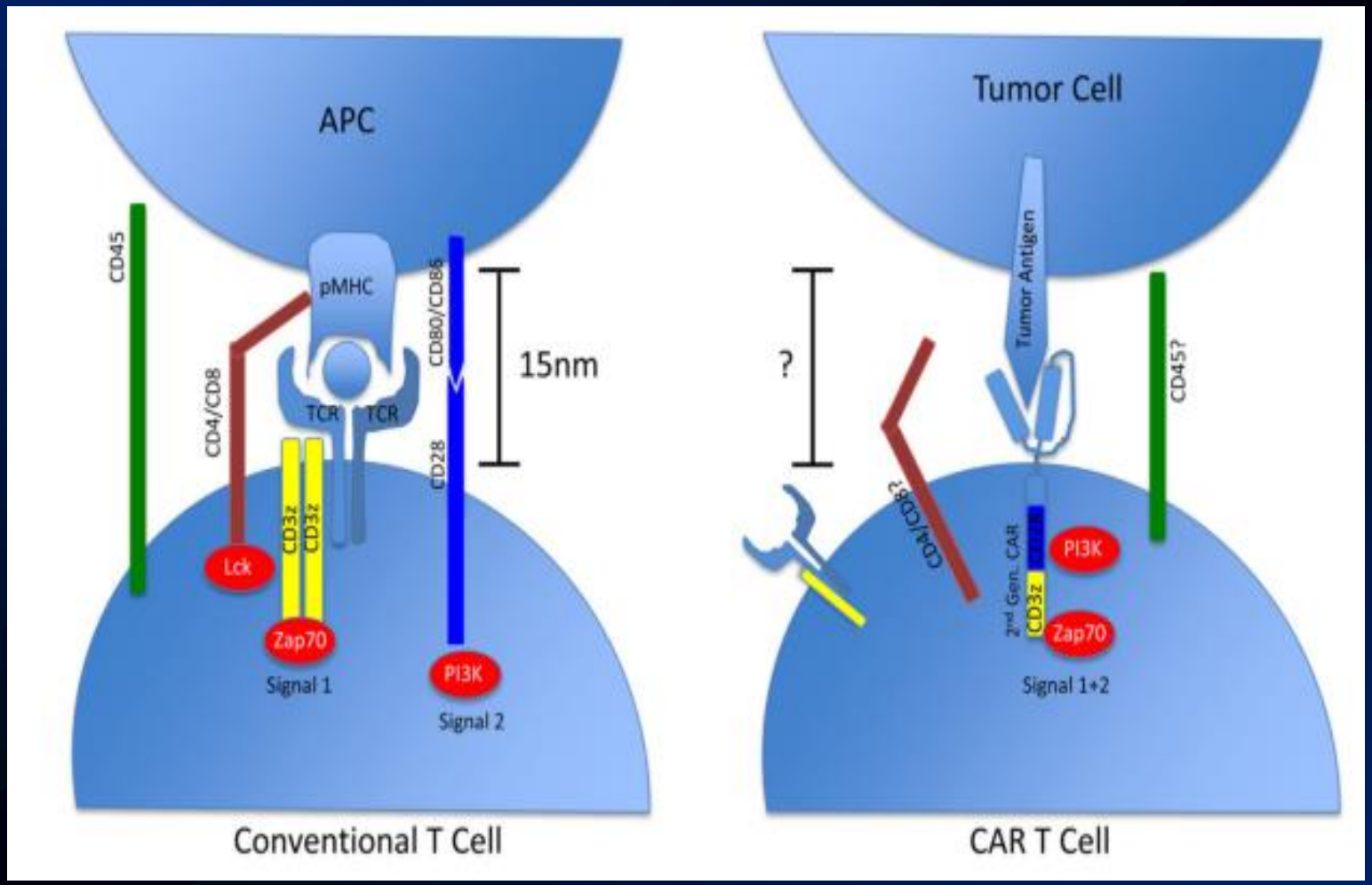
Third party recipients



CAR-T Cells

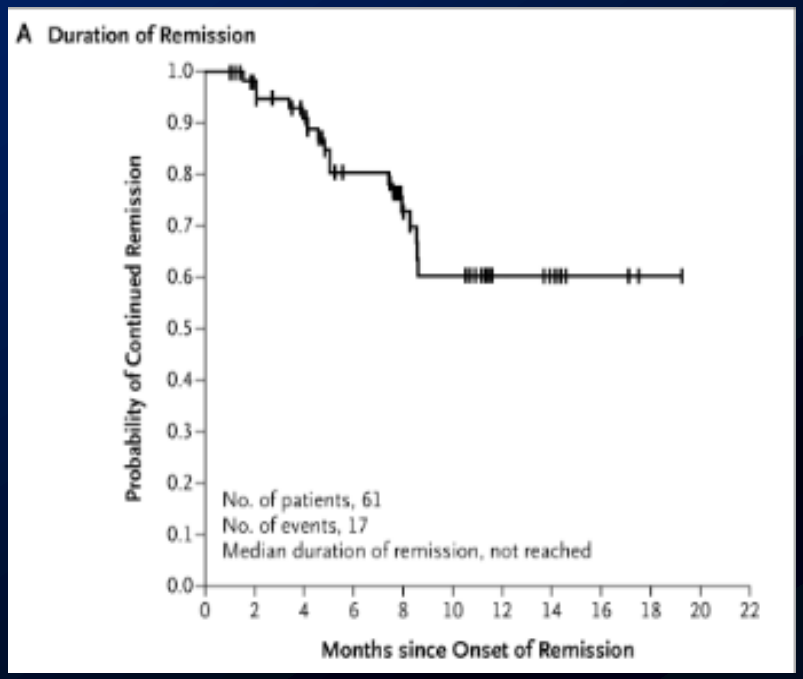

1 Leukapheresis
5 Modified T-cell infusion
4 Chemotherapy
Antibody-coated beads
2 T-cell activation/ transduction[a]
Bead removal
3 Modified T-cell expansion[a]

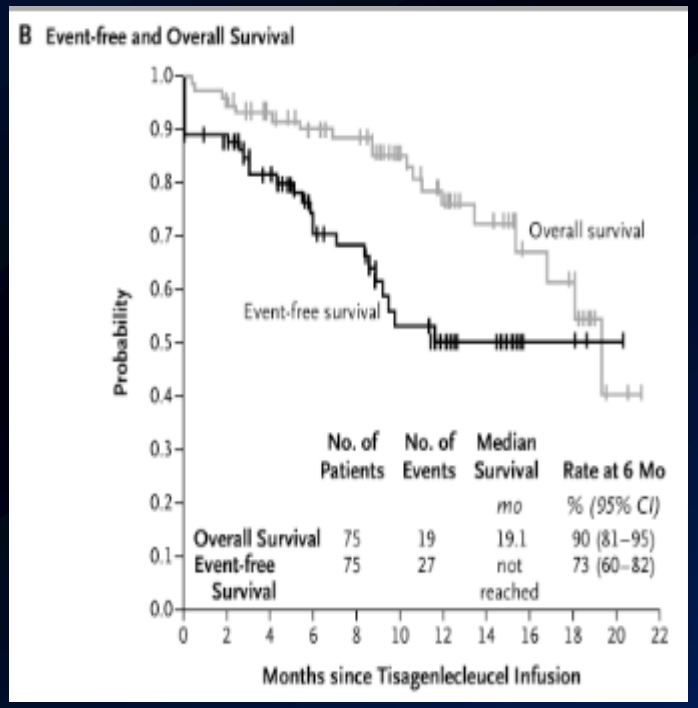
Signaling of conventional and CAR-T cells



Eliana clinical trial: Results

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





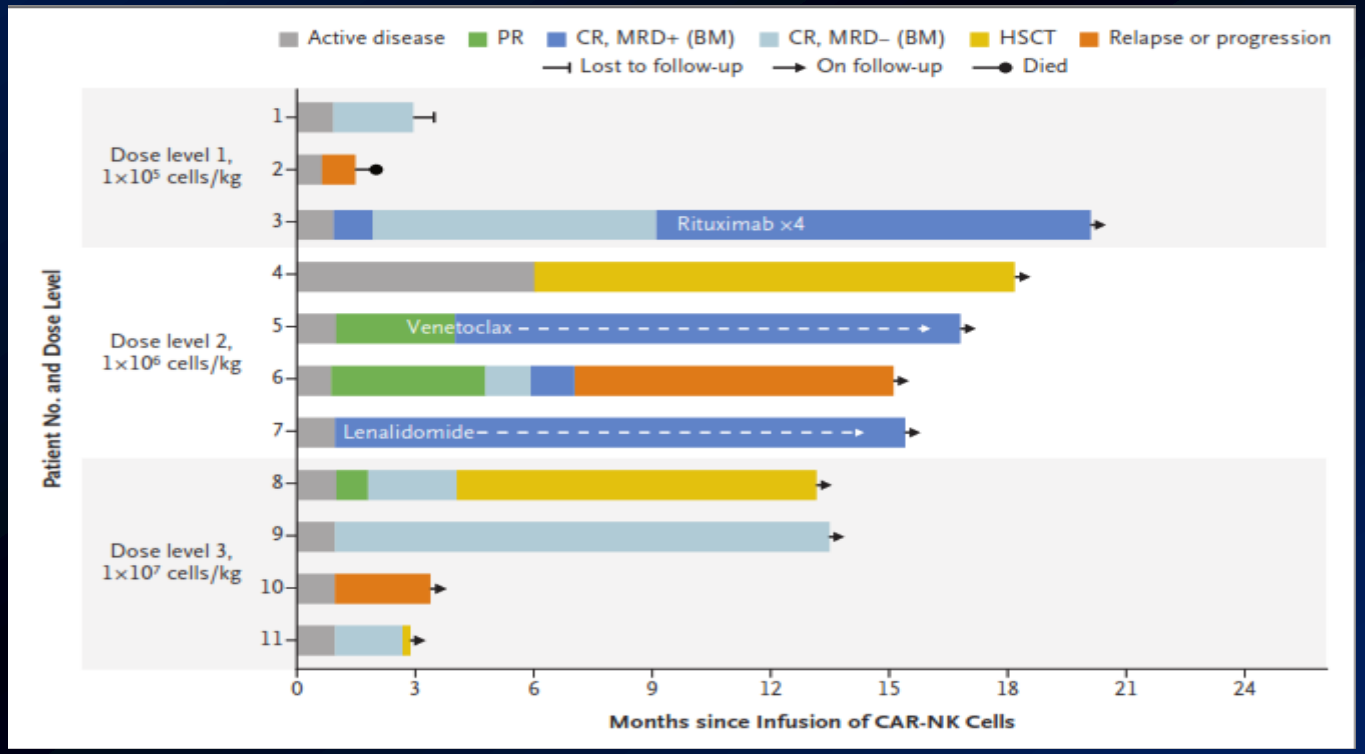
The rates of event-free survival and overall survival were 73% and 90%, at 6 months and 50% and 76% at 12 months

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:

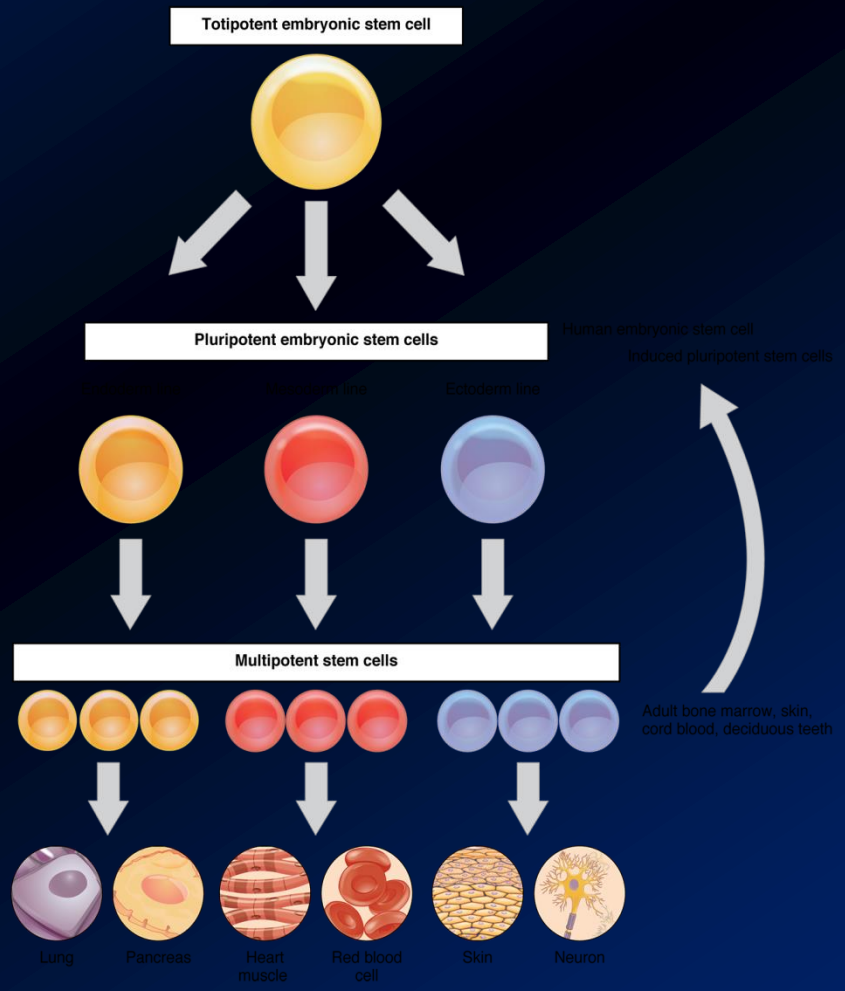
- potential to make any differentiated cell in the body

Multipotent:

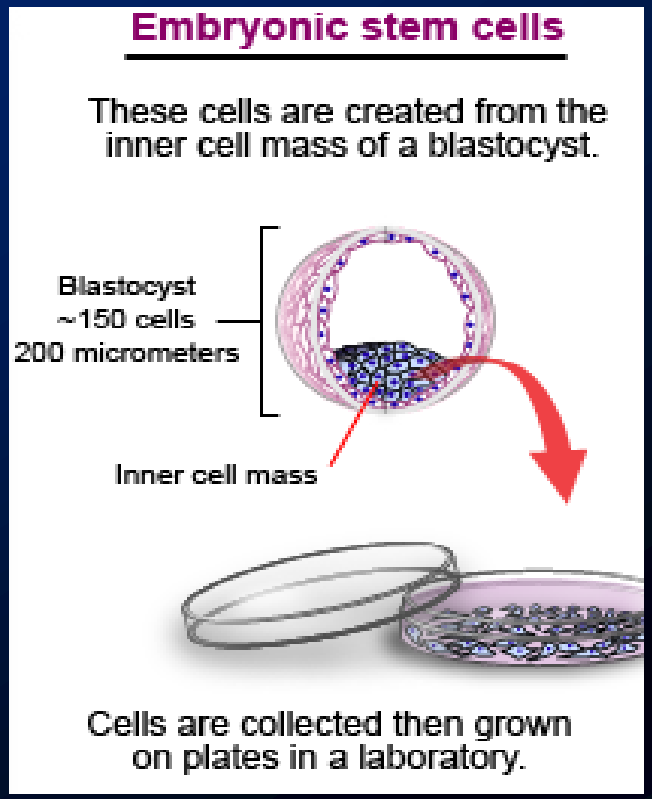
- produce only cells of a closely related family of cells e.g. hematopoietic stem cells.

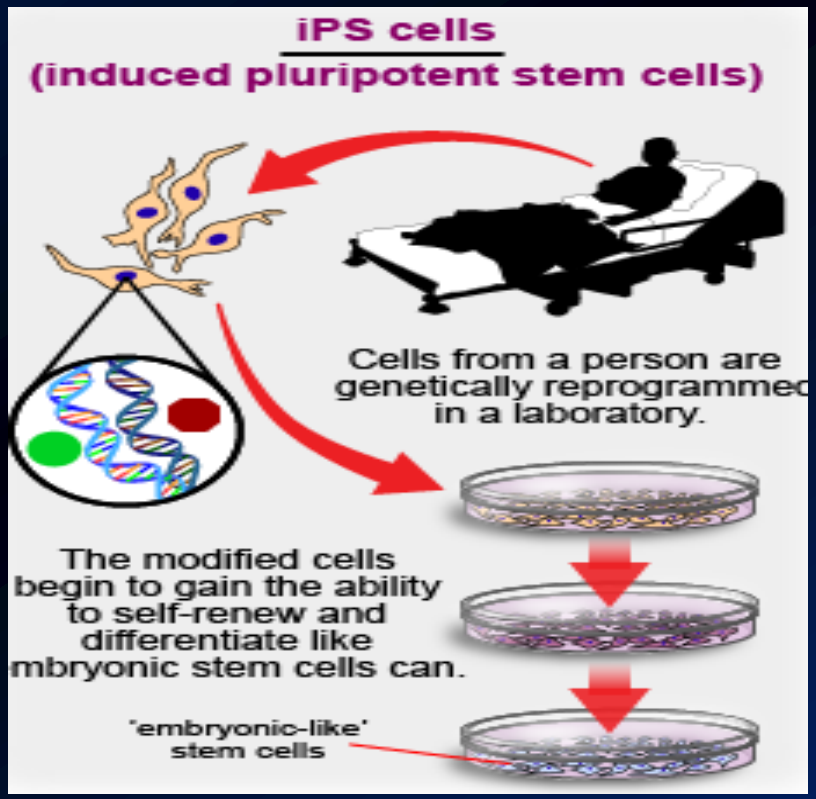
Unipotent

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

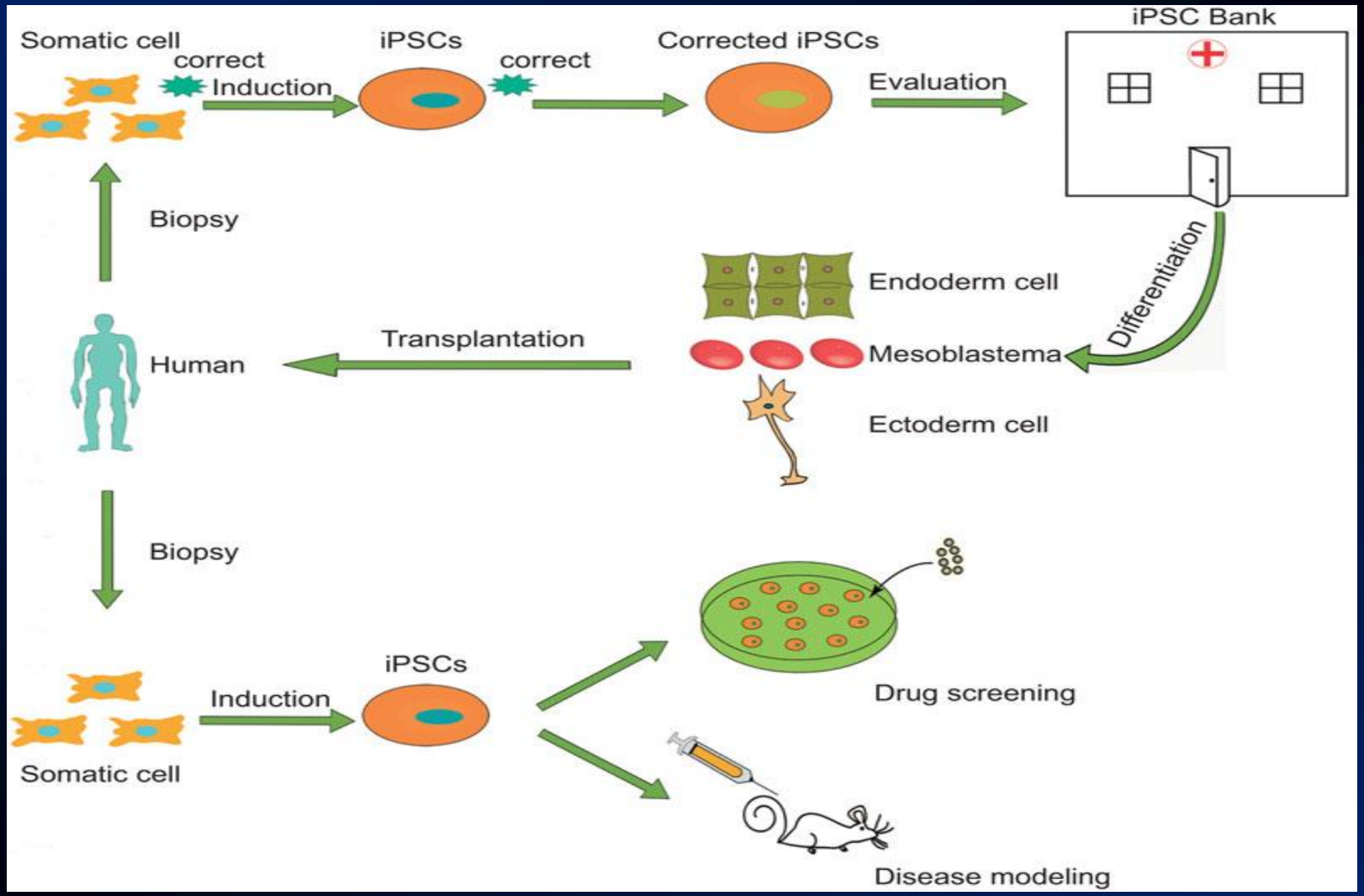


Where do pluripotent stem cells come from?





Disease specific iPSCs for the study and therapy



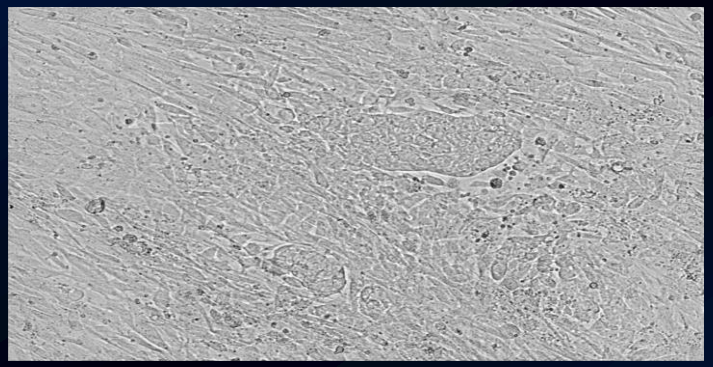
CELLULAR REPROGRAMMING
Volume 16, Number 6, 2014

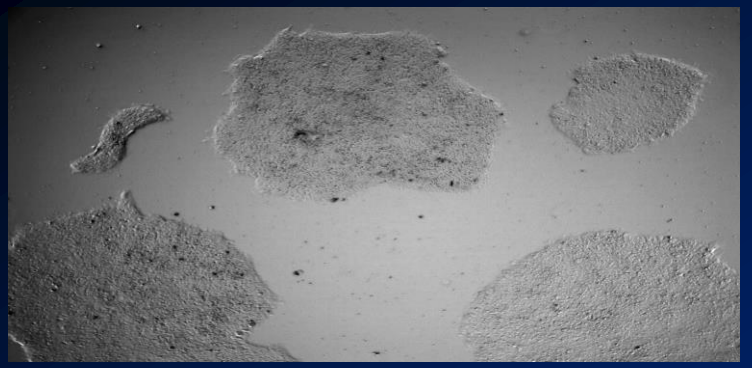
DOI: 10.1089/cell.2014.0050

Original Article

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

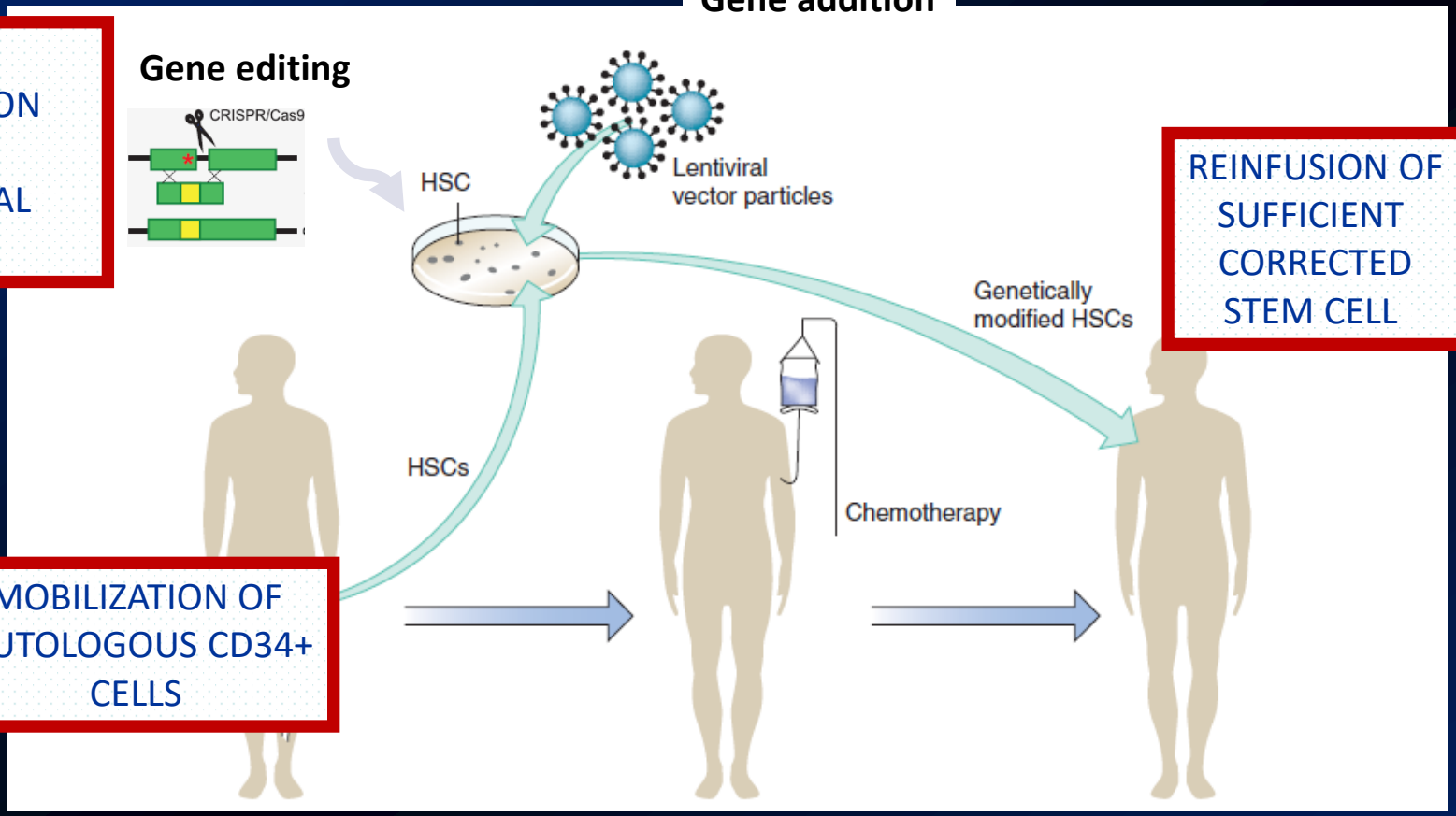
Ioanna Varela,[1,5] Angeliki Karagiannidou,[1] Vasilis Oikonomakis,[2] Maria Tzetis,[2] Marianna Tzanoudaki,[3] Elena-Konstantina Siapati,[4] George Vassilopoulos,[4] Stelios Graphakos,[1] Emmanuel Kanavakis,[2,5] and Evgenios Goussetis[1]





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

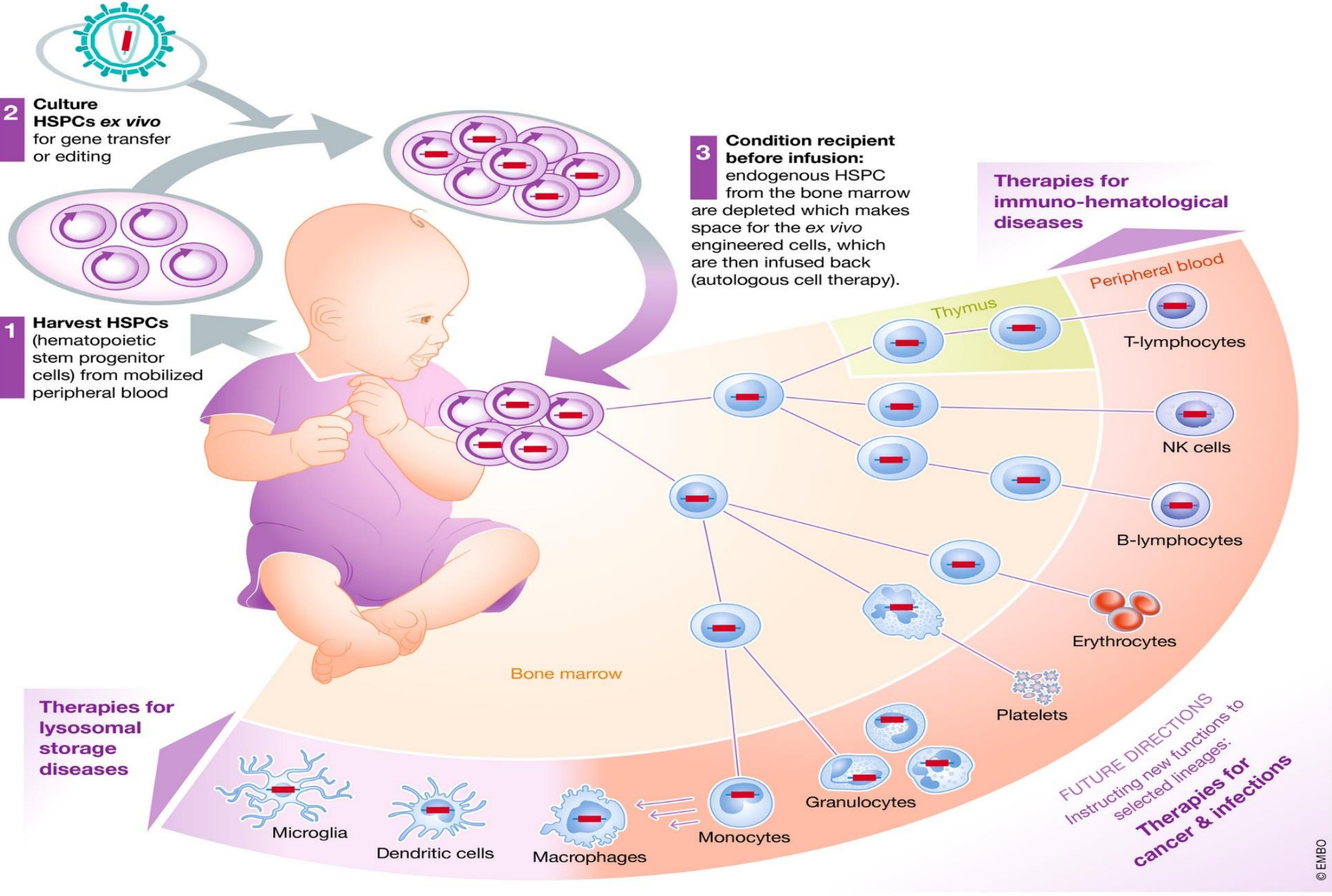
The future

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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