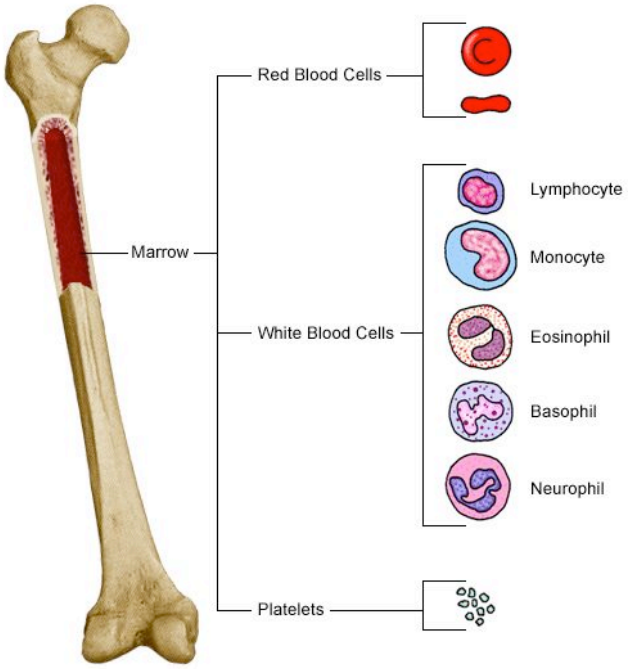
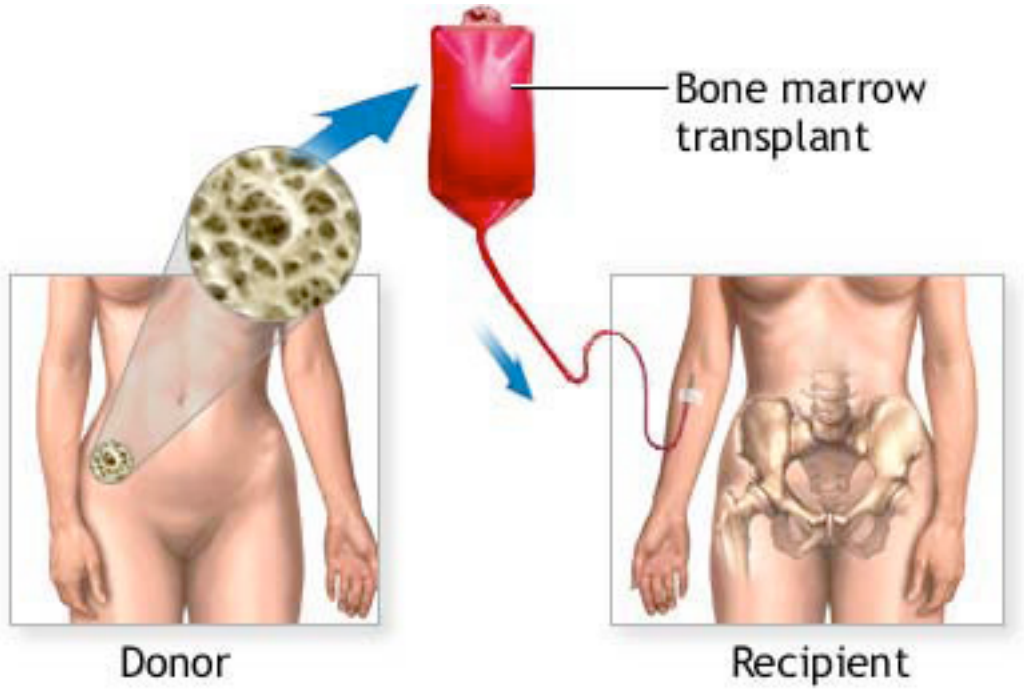

Lymphoma Tumor Board
Friday, December 2, 2016
Bone Marrow Transplantation:
The Nuts and Bolts

Hematopoietic cell transplantation for bone marrow failure – a simple concept



Bone marrow – the blood cell "factory" in postnatal life



Bone marrow is readily transplantable

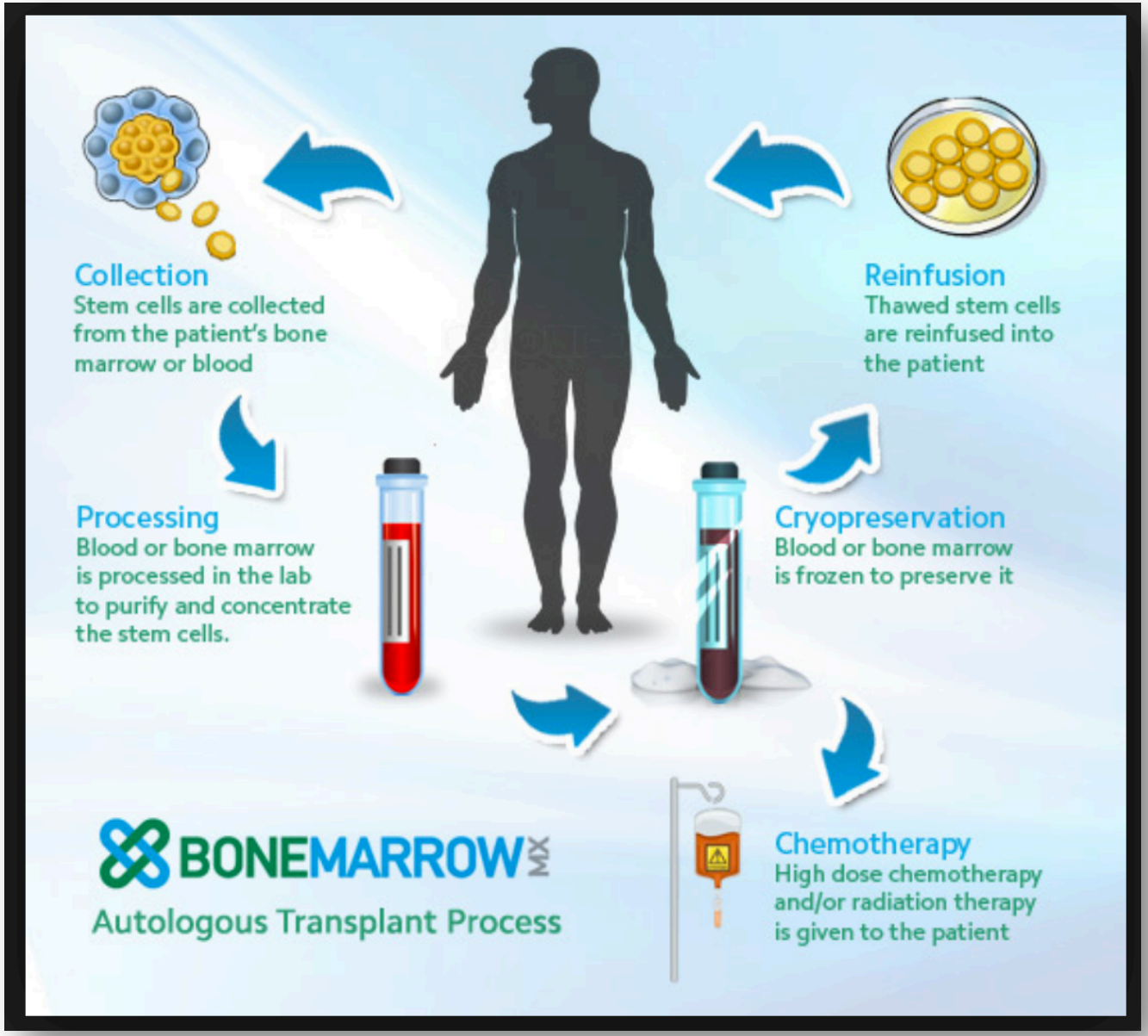
Two Types of Bone Marrow Transplants: Allogeneic and Autologous

- Hematopoietic stem cell transplantation (HSCT) is derived from either bone marrow, peripheral blood or umbilical cord blood

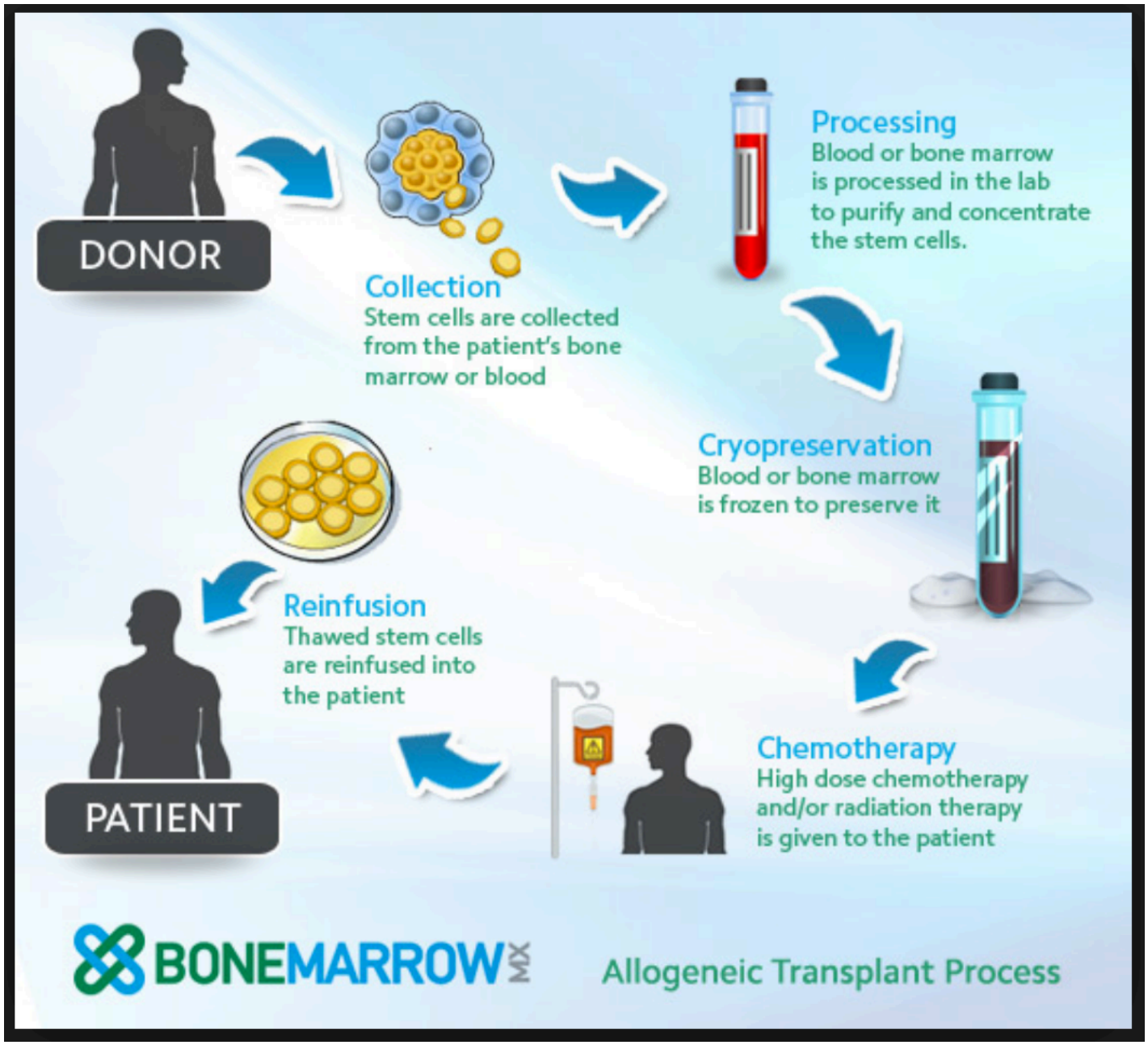
- **Autologous**: Patient's own stems cells are used
  - Requires apheresis of hematopoietic stem cells from patient which are cryopreserved
  - Patient receives high-dose chemotherapy with or without radiation to eradicate malignant cell population
  - Patient's own stem cells are then transfused into their bloodstream to replace destroyed tissue
  - Rejection incidence is typically low
- **Allogeneic**: Stem cells come from a donor or identical twin
  - Involves two people where donor must be HLA tissue typed to match recipient
  - Recipient requires immunosuppressive medications to alleviate Graft-versus-host-disease
  - Donors can be siblings or family members or unrelated donors that match

- Immune system is depleted with radiation and/or chemotherapy before transplantation in either setting

Autologous Transplant

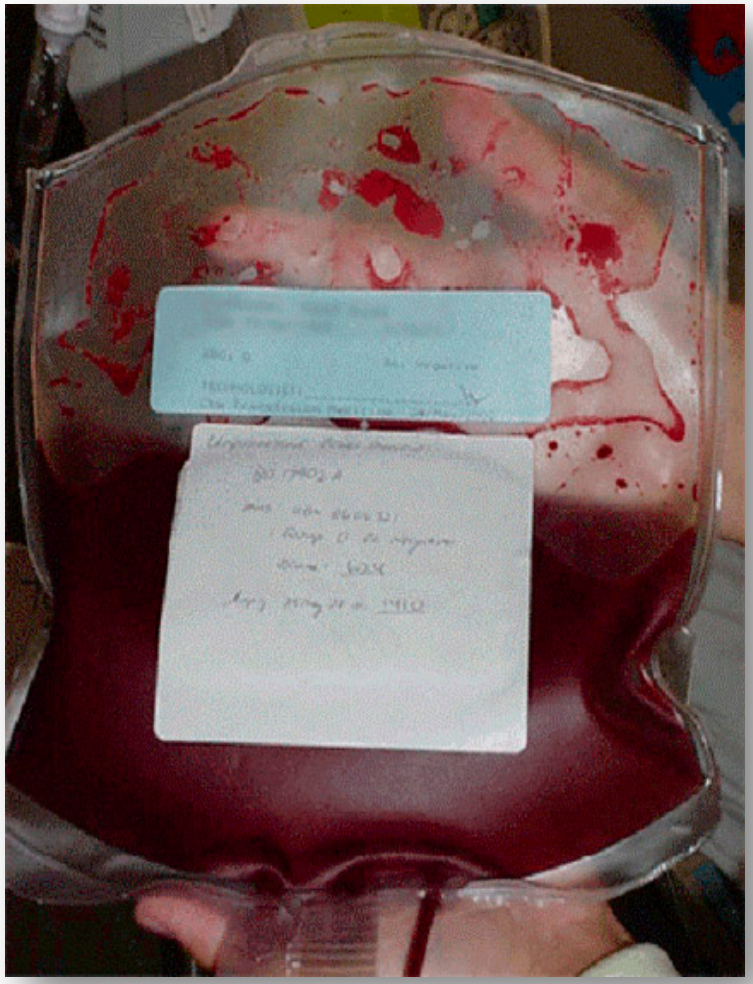


Allogeneic Transplant



Transplant Products

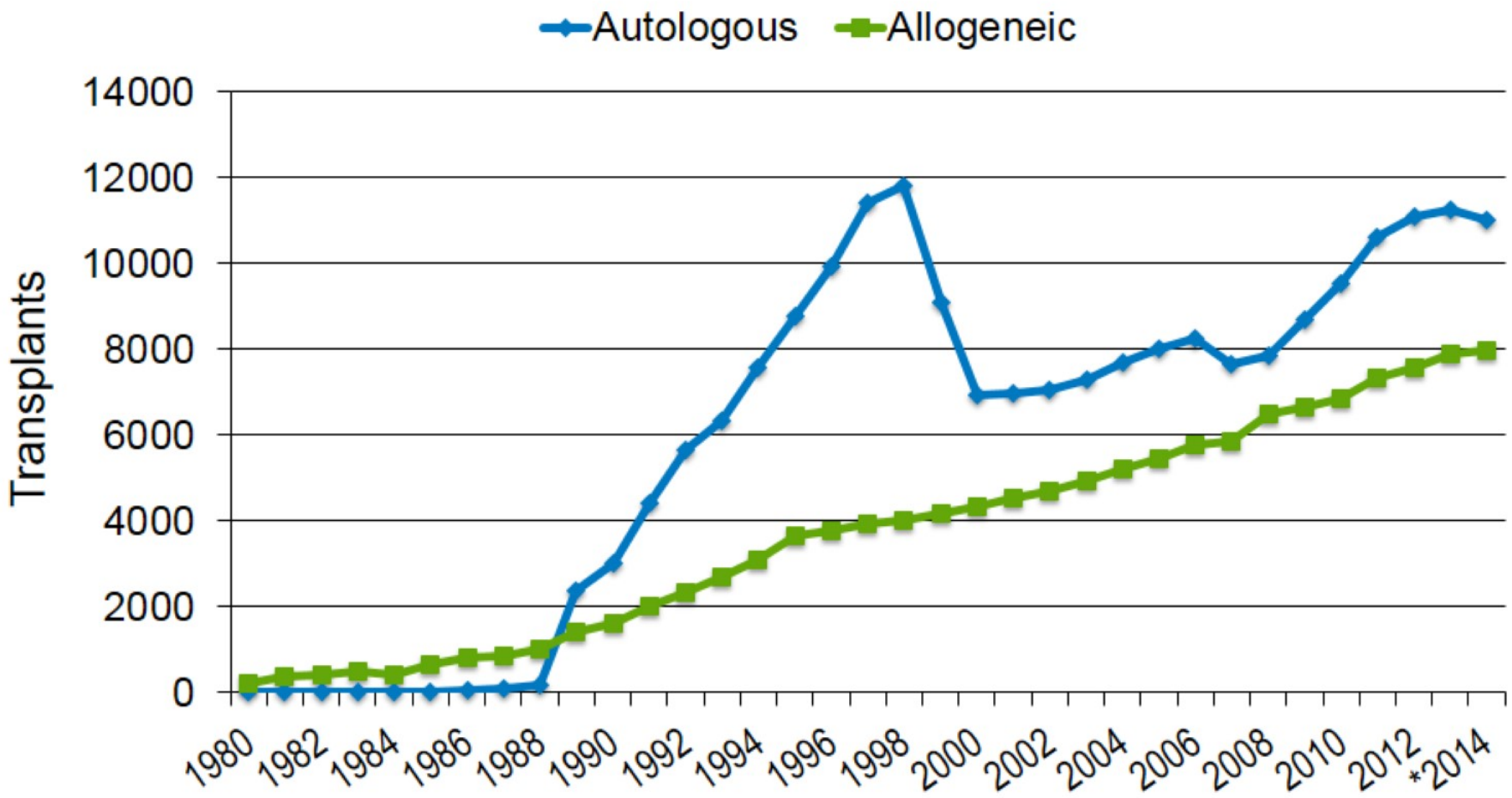
Bone Marrow Harvest



PBSC Collection

Cord Blood Unit

Annual Number of Transplant Recipients in the US by Transplant Type



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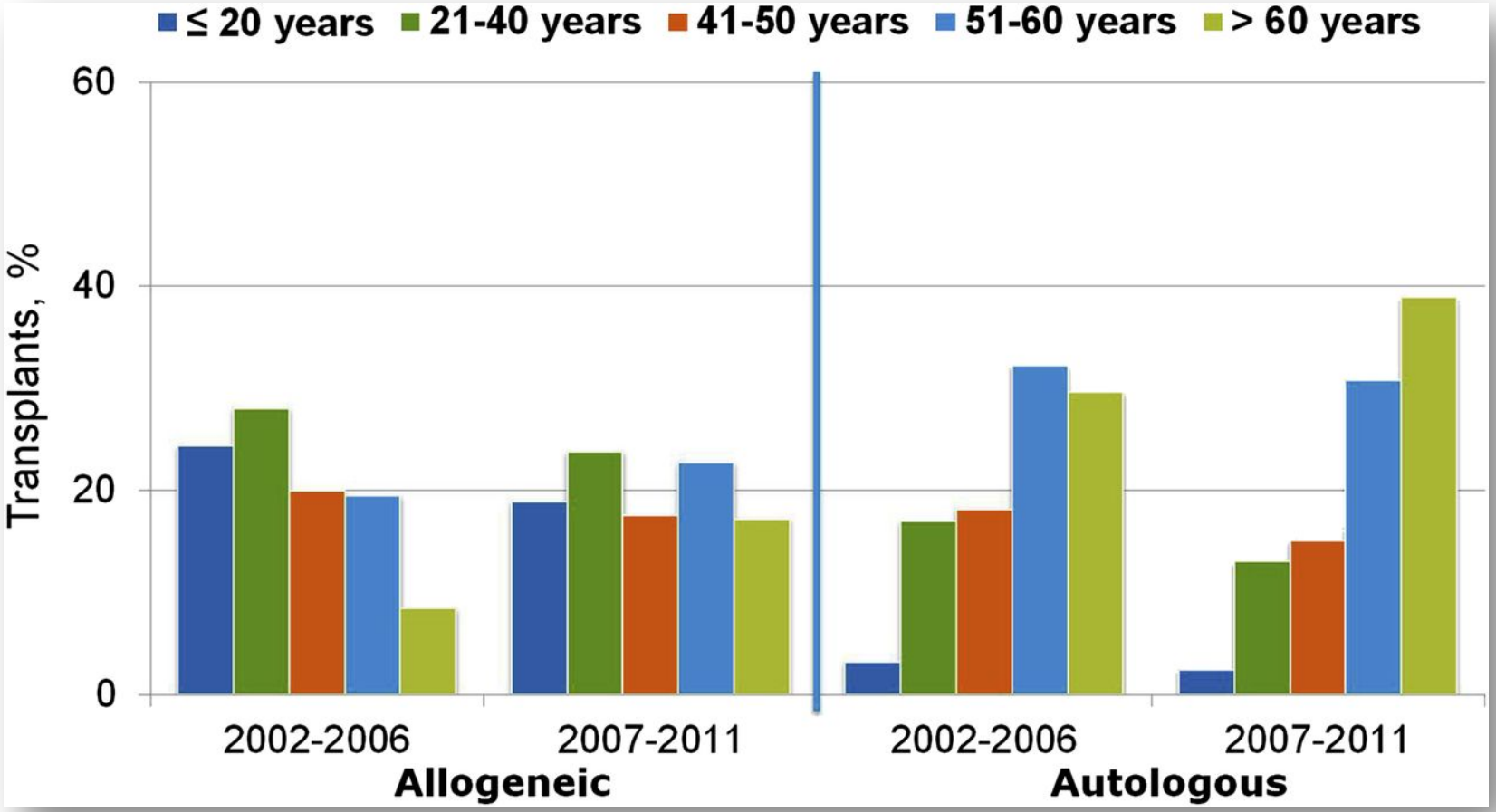
*2014 Data incomplete

Trends in transplant by type and recipient age, 2002 to 2006 and 2007 to 2011, CIBMTR data



Boglarka Gyurkocza, and Brenda M. Sandmaier Blood 2014;124:344-353

Diseases commonly treated with allogeneic hematopoietic [stem] cell transplantation

Cancers

- Acute myeloid leukemia
- Acute lymphoblastic leukemia
- Chronic myeloid leukemia
- Myelodysplastic syndromes
- Myeloproliferative disorders
- Non-Hodgkin lymphoma
- Hodgkin lymphoma
- Chronic lymphocytic leukemia
- Multiple myeloma
- Juvenile chronic myeloid leukemia

Non-malignant diseases

- Aplastic anemia
- Paroxysmal nocturnal hemoglobinuria
- Fanconi's anemia
- Blackfan-Diamond anemia
- Thalassemia major
- Sickle cell anemia
- Severe combined immunodeficiency
- Wiskott-Aldrich syndrome
- Inborn errors of metabolism

Conditioning Regimens

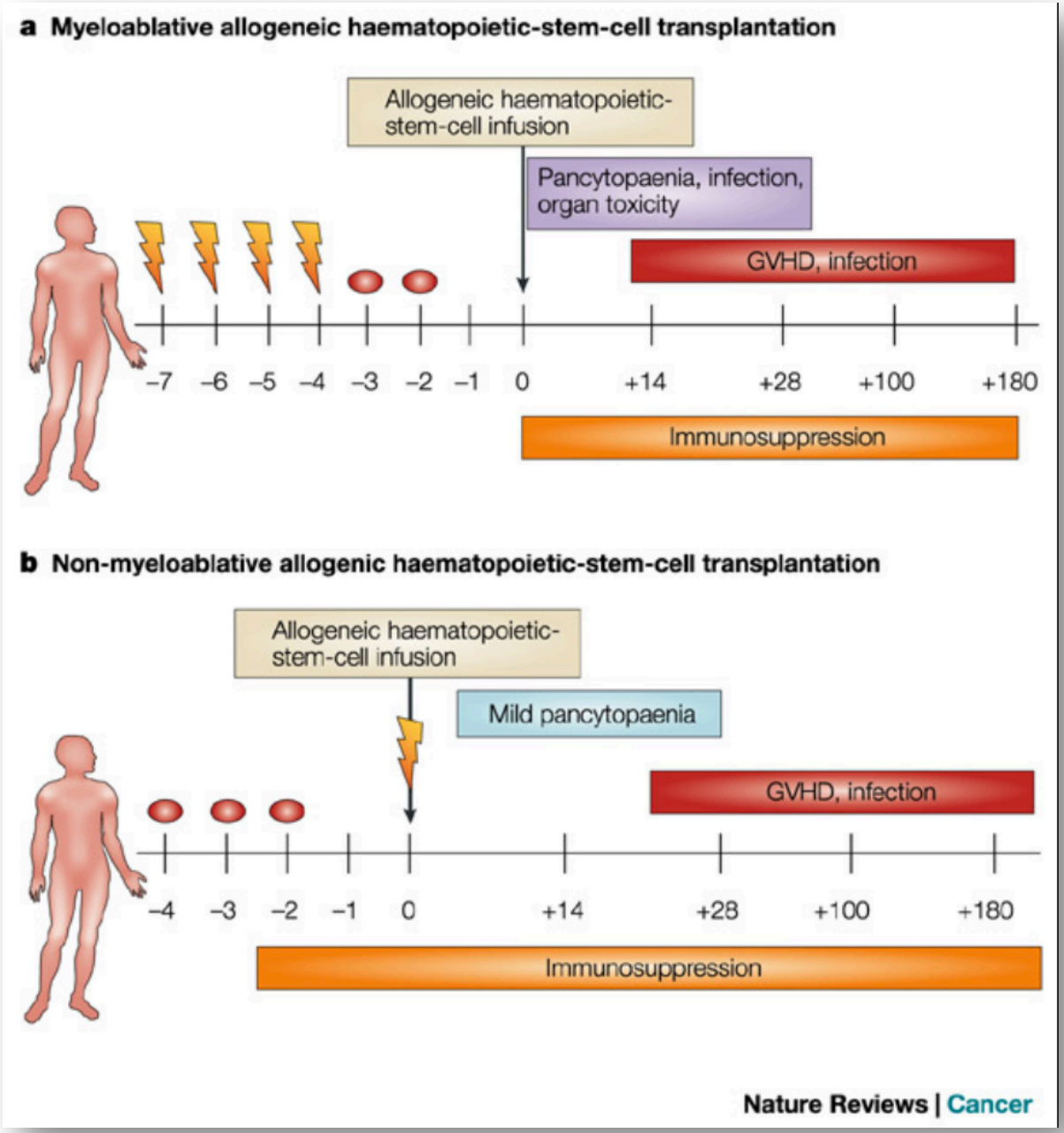
Myeloablative

- The purpose of the conditioning regimen, which is the chemotherapy or irradiation given prior to transplant, is to eradicate the patient's disease prior to the infusion of HSC.
- Bone marrow is ablated with dose-levels that ensure minimal injury to other tissues
- Allogeneic transplants use cyclophosphamide and total body irradiation
- This allows for an immunosuppressive effect that prevents rejection of the bone marrow graft.

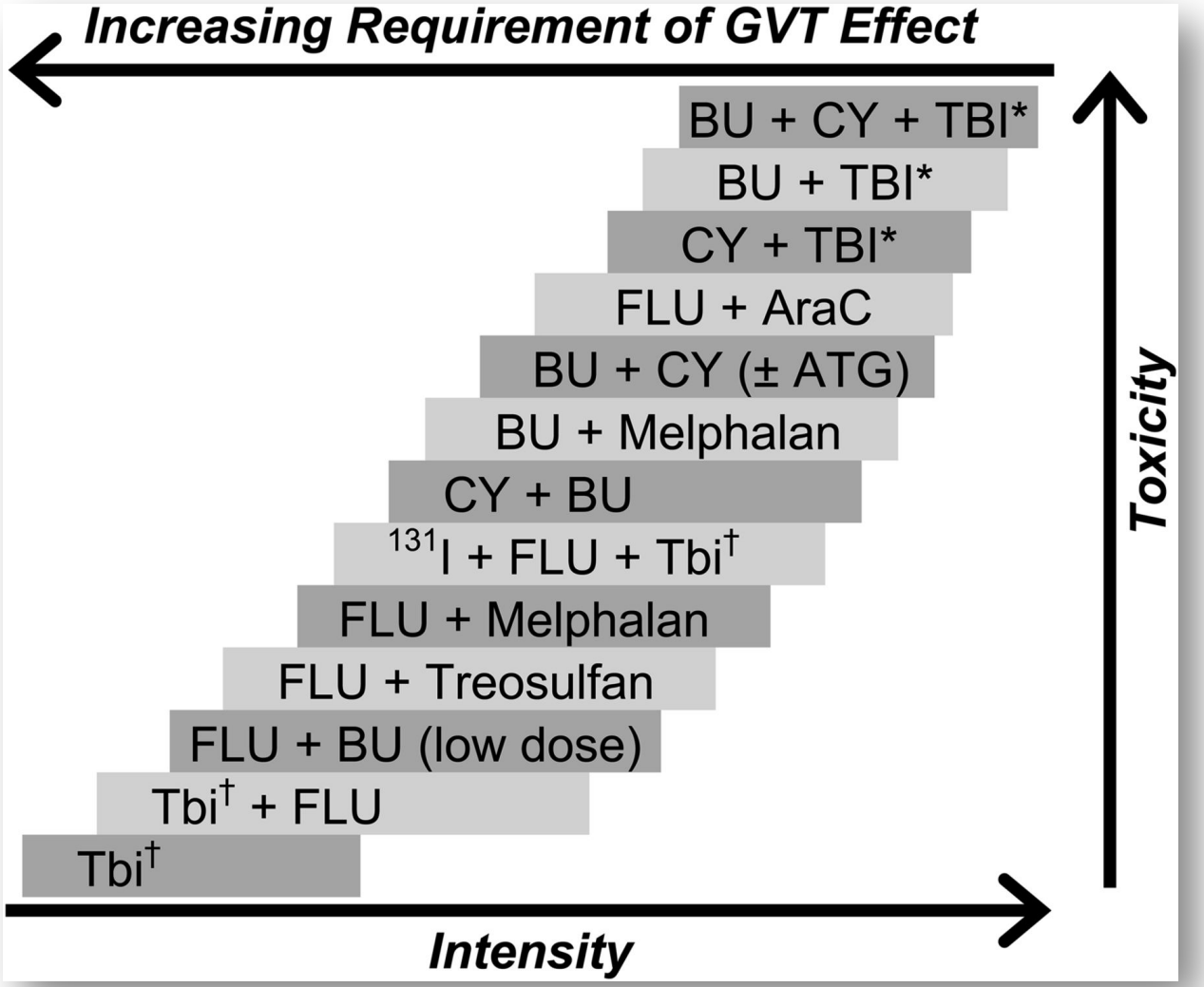
Non-myeloablative also known as reduced-intensity conditioning (RIC)

- "Uses low doses of chemotherapy and radiation too low to eradicate all the bone marrow cells of the recipient"
- Run lower risks of serious infections and transplant-related mortality
- "Requires high doses of immunosuppressive agents in the early stages of treatment, less than for conventional transplants"
- Often associated with lower risk of transplant-related mortality

Conditioning Regimens



Selected conditioning regimens of different dose intensities



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blood

Bone Marrow Transplantation (2014) **49,** 1098–1105; doi:10.1038/bmt.2014.104; published online 16 June 2014

Essential requirements for setting up a stem cell processing laboratory

T Leemhuis[1], D Padley[2], C Keever-Taylor[3], D Niederwieser[4], T Teshima[5], F Lanza[6], C Chabannon[7], P Szabolcs[8], A Bazarbachi[9] and M B C Koh[10,11] on behalf of the Graft Processing Subcommittee of the Worldwide Network for Blood and Bone Marrow Transplantation (WBMT)

Abstract

▲ Top

The Graft Processing subcommittee of the Worldwide Network for Blood and Marrow Transplantation wrote this guideline to assist physicians and laboratory technologists with the setting up of a cell processing laboratory (CPL) to support a hematopoietic stem cell transplant program, thereby facilitating the start-up of a transplant program in a new location and improving patient access to transplantation worldwide. This guideline describes the minimal essential features of designing such a laboratory and provides a list of equipment and supply needs and staffing recommendations. It describes the typical scope of services that a CPL is expected to perform, including product testing services, and discusses the basic principles behind the most frequent procedures. Quality management (QM) principles specific to a CPL are also discussed. References to additional guidance documents that are available worldwide to assist with QM and regulatory compliance are also provided.

Table 1. Equipment needed to start a cell processing lab

Required equipment:		
Biosafety cabinet (or equivalent)	Refrigerator	Balance (Scale)
Water bath	Centrifuge (with carriers to hold 600 mL blood bags)	Freezer (≤ −70 °C)
Plasma extractor	Tubing sealer	Tubing stripper
Cryo-transporter (−80 °C) or liquid nitrogen dry shipper	Micropipettes (100 µL and 1000 µL)	Reference thermometer
Pipette aid	Hemostats	
Desired equipment:		
Sterile connecting device	Controlled rate freezer	LN_2 storage freezer
Label printer	CO_2 incubator	Hemocytometer
Microscope	Personal computer	
Shared equipment:		
Flow cytometer	Automated instrument for cell processing	Microbiology lab for bacterial and fungal culture
Hematology analyzer		

Abbreviation: LN_2=liquid nitrogen.

Table 2. Minimal supplies needed to start a cell processing lab

Miscellaneous laboratory supplies		
Cryobags (for example: 50; 250; 500 mL)	Transfer packs (300; 600 mL)	Syringes (1, 3, 10, 30, 60 mL)
Safety needles; couplers	Spike to needle, spike to spike adapters; stopcocks	Alcohol swabs, iodine swabs, syringe caps, sterile swabs
Labels, laminating tags; zip ties	15, 50, 175 mL conical tubes	Pipettes (1–50 mL)
Biohazard sample bags	Tube racks	Pipette tips
Cryovials, microtubes	Biohazard bags; sharp containers; garbage bags; trash can	Dry ice
Sterile overwrap bags		
Sample reagent list (will vary depending on products and services offered)		
DMSO	Plasmalyte (or equivalent)	ACD-A
Human serum albumin	Hetastarch	Heparin
70% IPA; bleach; bactericidal and fungicidal detergent	Flow cytometry reagents	Trypan blue

Abbreviations: ACD-A=acid citrate dextrose solution A; DMSO=dimethyl sulfoxide; IPA=isopropyl alcohol.

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Table 3. Quality control testing for HPC products

Attribute	*Test method*	*Specification*
Donor screening	Summary of records; donor eligibility form	Donor eligible
Infectious disease testing	Certified laboratory	Negative (exclusive of CMV)[a]
Infusion volume	Measurement	⩽20 mL/kg/infusion
DMSO volume	Calculation	⩽1 mL/kg/day
Total nucleated cell (TNC) count	Automated cell counter; or hemacytometer	As measured
RBC content (if ABO incompatible)	Automated cell counter	⩽20–30 mL/adult infusion
CD34+ cell count	Flow cytometry	⩾2×10^6/kg
CD3+ cell count (if allogeneic)	Flow cytometry	As measured
Viability (pre-freeze)	Flow cytometry	⩾80%
Sterility	Bacterial culture	No growth
Sterility	Fungal Culture	No growth
Final product Labeling	Observation	Labeled correctly

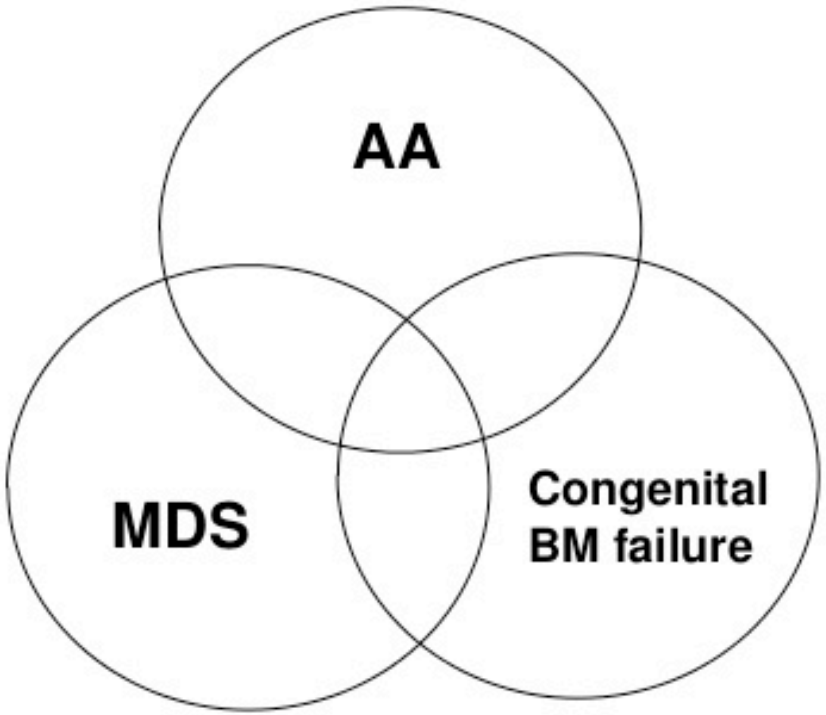
Abbreviation: HPC=hematopoietic progenitor cell.

[a] Infectious disease testing of autologous products is not universally required worldwide. Consult national regulations.

Table 4. Cell processing laboratory quality management plans

Quality system element	***Description***
Organization	Organizational charts; reporting structures, inter-institutional relationships
Personnel	Human resources policies; job descriptions; personnel qualifications, training and competency
Communications	Processing prescriptions; result reporting
Facilities, work environment and safety	Floor plans; cleaning schedules; mechanical systems; environmental monitoring; disaster plans
Suppliers and materials management	Materials management; supplier qualification
Equipment	Qualification; calibration; maintenance; cleaning schedules
Process controls	Change control; methods to prevent mix-ups and cross-contamination; process validation; product release; quarantine storage; product tracking; label control
Documents and records	Standard operating procedures; document and version controls; record review; record retention
Management of non-conforming events	Deviation and adverse event reporting; documentation of urgent medical need; regulatory agency reporting
Monitoring and assessment	Donor eligibility; product testing; outcome analysis; audits

Differential diagnosis of AA / MDS in children



Autoimmune
Immunodeficiency
Metabolic disease
Mitochondrial deficiency
Vit B12 deficiency
Folate deficiency
Infection
Drug

Fanconi anemia
Shwachman-Diamonod syndrome
Dyskeratosis congenita
Congenital amegakaryocytic thrombocytopenia

Standard conditioning regimens for children with acquired BMF

AA/RCC

Matched related donor:

CY (200 mg/kg) + ATG ± low dose TBI

Alternative donor:

FLU + CY (100 mg/kg) + ATG ± low dose TBI

→ **Is everything all right?**

Conditioning regimen for acquired BMF

… needs to be reconsidered

AA/RCC → Risk for donor-type aplasia

Matched related donor:

CY (200 mg/kg) + ATG ± low dose TBI

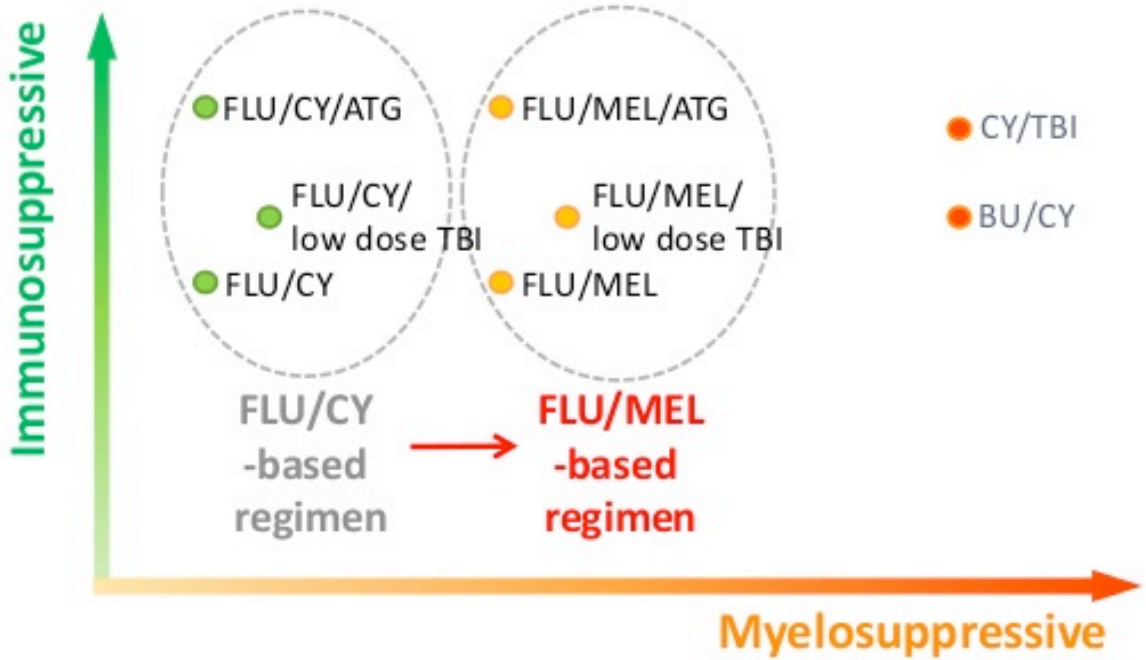
Alternative donor:

FLU + CY (100 mg/kg) + ATG ± low dose TBI

CY 100 mg/m² → Risk for donor-type aplasia
CY 200 mg/m² → Risk for heart failure

Risk factors for "Donor-type aplasia"

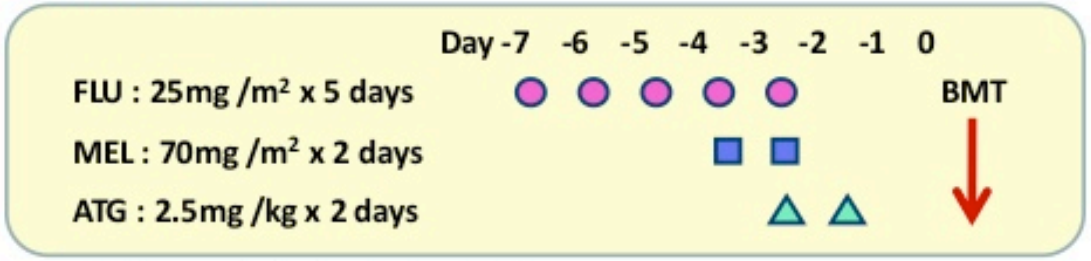
Optimal conditioning regimen for acquired BMF children with high risk of donor-type aplasia?



Risk factors for "Donor-type aplasia"

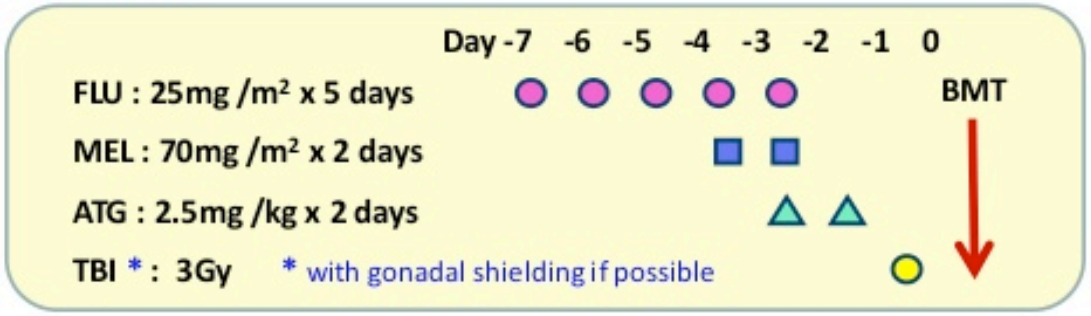
Conditioning regimen and GVHD prophylaxis :

1) Matched related donor:



GVHD prophylaxis : CyA + sMTX

2) Alternative donor:



GVHD prophylaxis : FK + sMTX

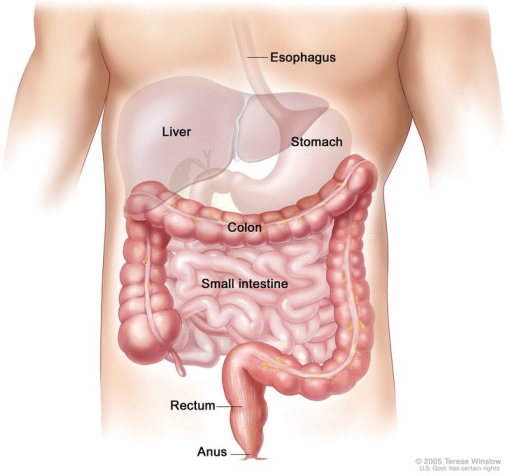
Proposal of prospective study

What is GVHD?

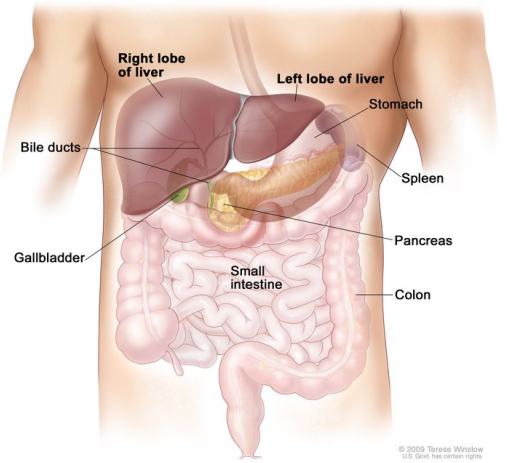
- Graft vs. Host Disease (GVHD)
- Occurs after bone marrow transplantation or any tissue transplantation
- Transplanted immune cells attack host's body cells
- Symptoms include:
  - Rash
  - Immune-mediated pneumonitis
  - Damage to connective tissue and exocrine glands
  - Sloughing of mucosal membrane
  - Diarrhea
  - Abdominal pain
  - Nausea
  - Vomiting
  - Eye irritation
- Can be fatal
- Treatment includes glucocorticoids such as prednisone

Major sites of graft-versus-host disease

Skin

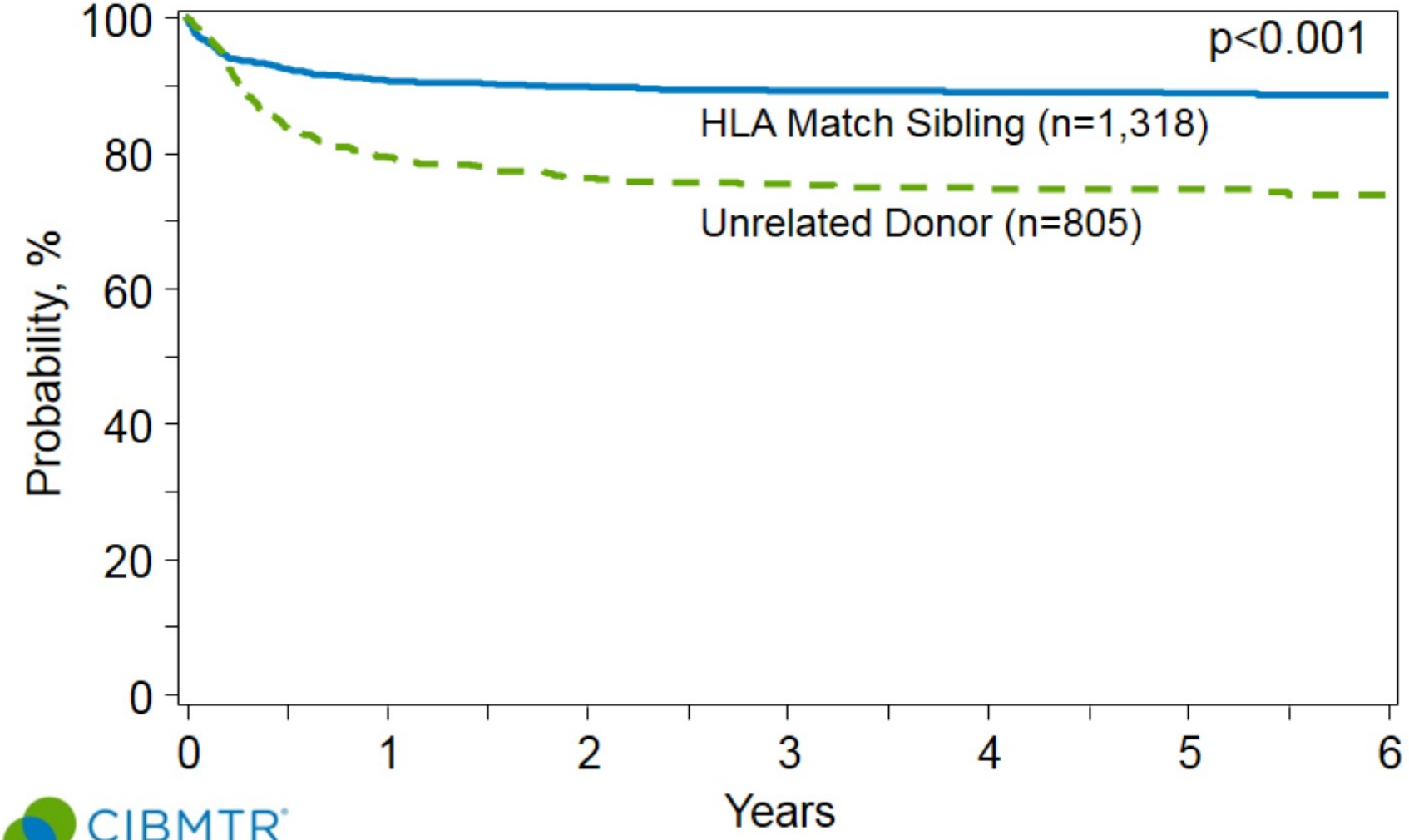


GI Tract



Liver

Survival after Allogeneic Transplants for Severe Aplastic Anemia, <20 Years, 2003-2013



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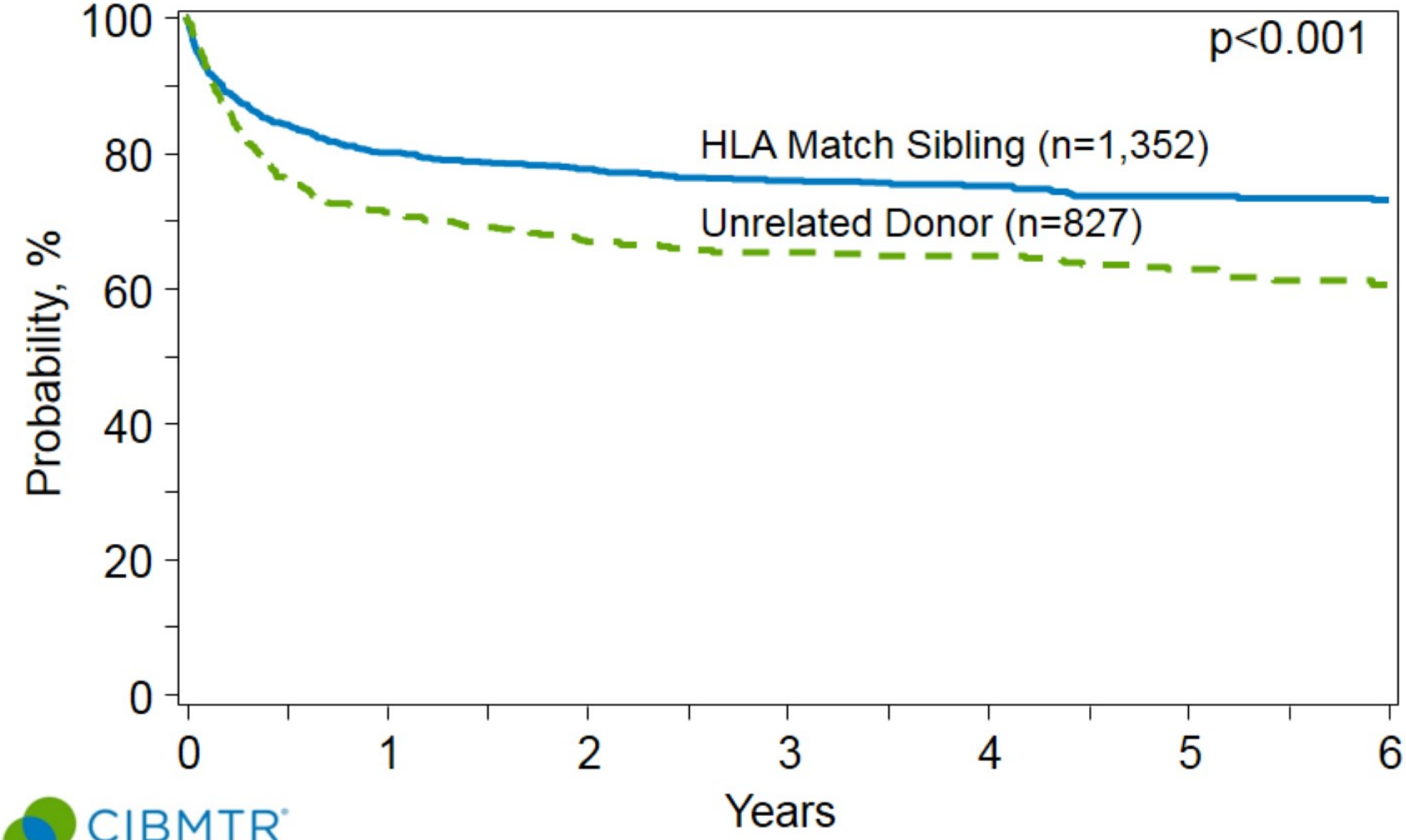
By Donor Type

Survival after Allogeneic Transplants for Severe Aplastic Anemia, ≥20 Years, 2003-2013



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By Donor Type

Summary

- Infusion of autologous and allogeneic hematopoietic stem cells is a standard and quite common procedure in contemporary hematology and oncology
- Eradication of malignant cells in recipients of allogeneic HCT is mediated by the donor's immune system – providing the clearest example of effective cancer immunotherapy
- Proper infrastructure, patient care, and management is essential to ensure transplant success

References

- https://www.cibmtr.org
- Gyurkocza, B., & Sandmaier, B. M. (2014). Conditioning regimens for hematopoietic cell transplantation: one size does not fit all. Blood, 124(3), 344-353. Accessed November 23, 2016. http://dx.doi.org/10.1182/blood-2014-02-514778.
- Bleakley, M. and S. R. Riddell (2004). "Molecules and mechanisms of the graft-versus-leukaemia effect." Nat Rev Cancer **4**(5): 371-380.
- Leemhuis, T., et al. (2014). "Essential requirements for setting up a stem cell processing laboratory." Bone Marrow Transplant **49**(8): 1098-1105.
- https://en.wikipedia.org/wiki/Hematopoietic_stem_cell_transplantation#Conditioning_regimens
- New England Journal of Medicine 257:491-496 (1957)
- http://www.slideshare.net/spa718/2-seiji-kojima
- https://crohntransplant.wordpress.com/