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www.bhs.be

BHS training course

Transfusion and Cell therapy

2nd March 2024

Blood-derived cell therapy products: processing and regulations

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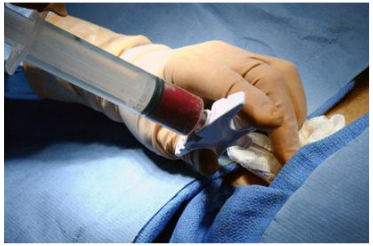
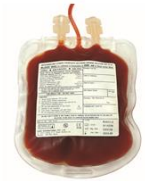
Blood-derived Cell Products

Bone marrow



Cord blood

Peripheral blood (apheresis)



Stem cells
HPC (M)

Stem cells
HPC (CB)

Stem cells
HPC (A)

STEM CELL TRANSPLANTATION

T-lymphocytes

Dendritic cells

IMMUNE EFFECTOR CELL THERAPY

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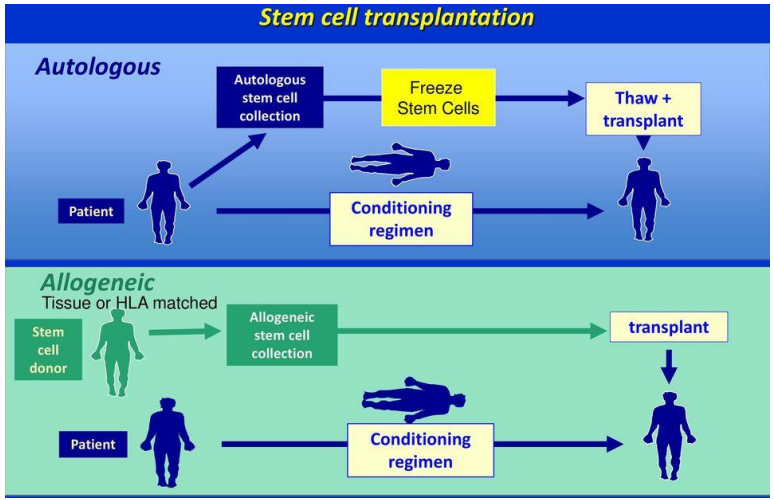
Stem cell transplantation and donor types



Autologous

Allogeneic

Related

- ❑ Sibling (HLA-matched brother or sister) > first choice (for 1/3 patients available)
- ❑ Haplo-identical (half-matched family member) > increasing incidence
- ❑ Syngeneic (identical twin pair) > exceptional

Unrelated

- ❑ Donor registries worldwide (volunteer donors) > 39 milj. donors available (70-10% availability)
- ❑ Cord blood donors > 850.000 units available worldwide – slightly decreasing incidence

https://wmda.info

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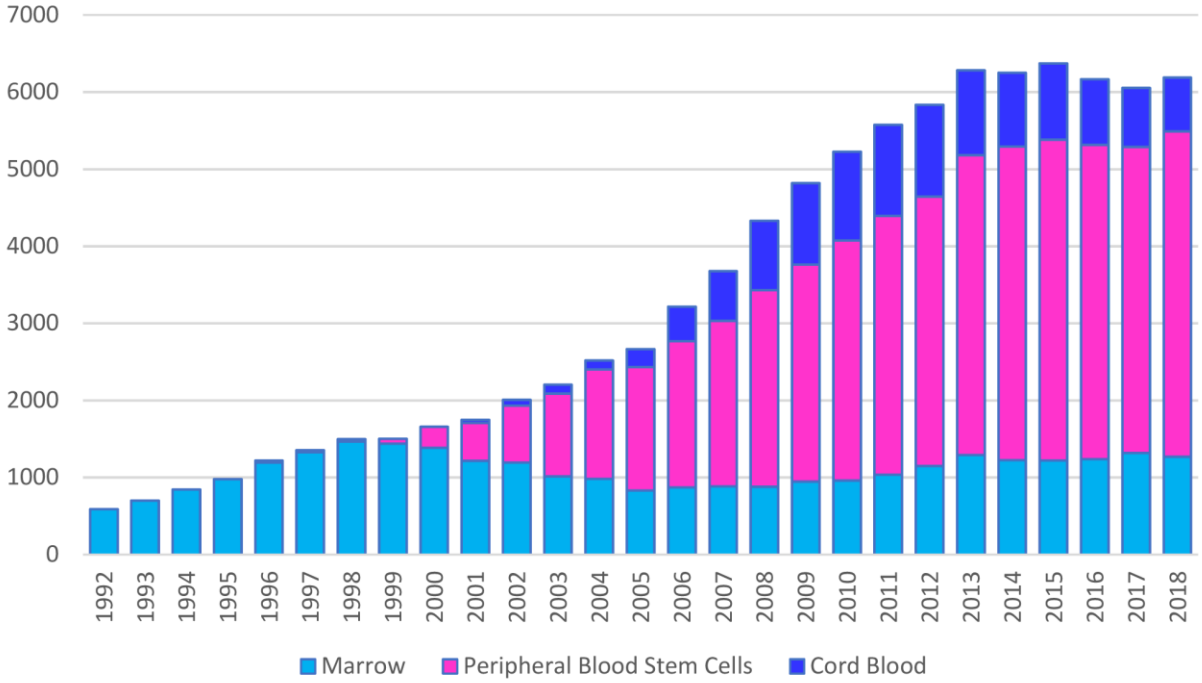
Unrelated donor transplants by cell source in the last decades



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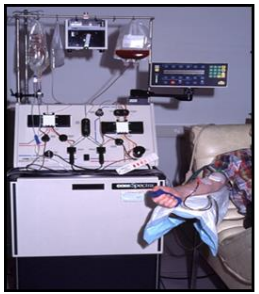
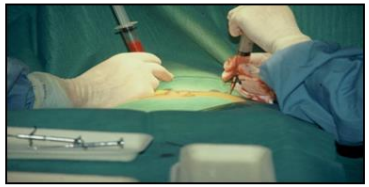
Hematopoietic (stem) cell therapy: collaboration and teamwork





Cell bank (collection and processing facilities)

Clinical facility

HLA-typing laboratory

National donor registry (MDPB)

Laboratory Hematology
Laboratory Immunology
Laboratory Microbiology

Hospital Pharmacy
Laboratory Pathology
Dept. Radiotherapy
ICU
+ other supportive depts

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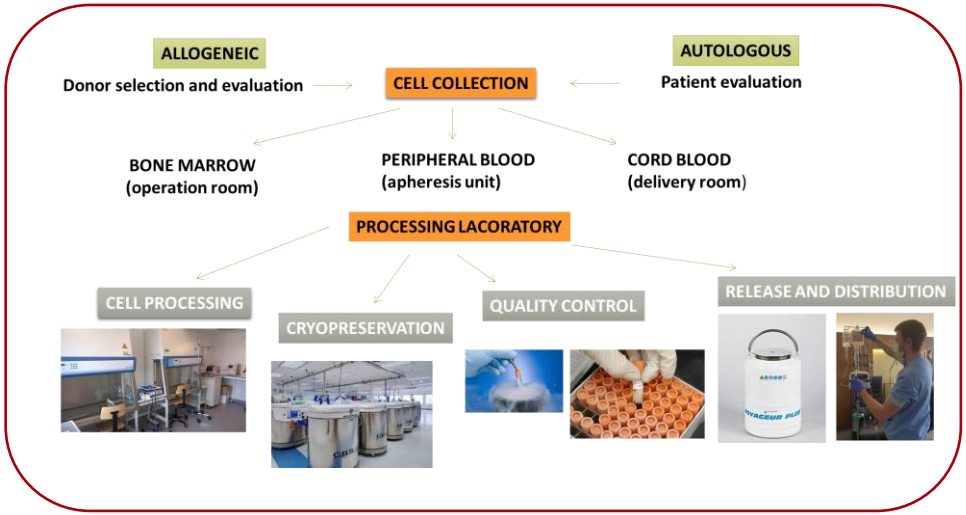
Hematopoietic (stem) cell bank



Collected and/or processed cell products

- Hematopoietic stem cells
- Donor lymphocytes
- Leukapheresis products as starting material for:

Advanced Therapy Medicinal products
(CAR-T cells and other immune effector cells)

Pharmaceutical companies

Academic centers

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Classification of blood-derived cell products according to complexity of in vitro processing

MINOR MANIPULATIONS

- Cell selection and/or separation
- Volume reduction
- Cell washing
- Cryopreservation

Stem cell grafts (auto and allo), donorlymphocytes

Basic Licence of tissue/cell bank

MORE COMPLEX MANIPULATIONS
(**A**dvanced **T**herapy **M**edicinal **P**roducts)

Genetic modification

In vitro expansion and differentiation

CAR-T cells, mesenchymal stem cells,..

Basic Licence of tissue/cell bank
+
GMP certificate
+
Manufacuring Licence
+
Clinical trial approval

McGrath E et al. EBMT Handbook: Hematopoietic Stem Cell Transplantation and Cellular Therapies [Internet]. 7th edition. Cham (CH): Springer; 2019.

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How can graft processing influence clinical outcome?

Transplantation type	Advantages	Disadvantages
Allogeneic	No tumor contamination of the graft Graft versus tumor reaction Direct infusion after collection	Donorselection required Graft versus host disease ABO-incompatibility (donor vs patient) Low stem cell numbers in cord blood (adult recipient)
Autologous	No donorselection required No Graft versus host disease No ABO-incompatibility (donor vs patient)	Tumor contamination of the graft (possible) No Graft versus tumor reaction Stem cell storage limited < 72 hr (non-frozen)

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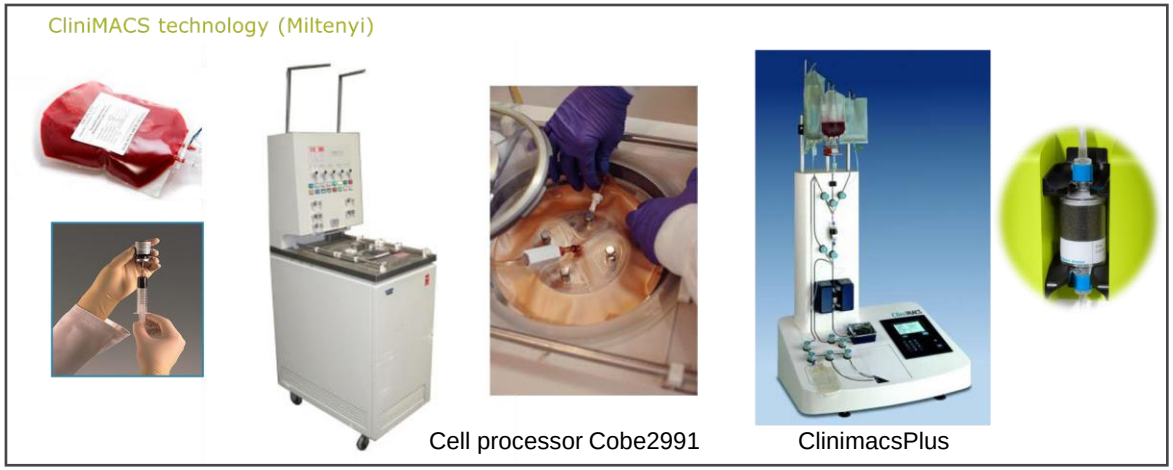
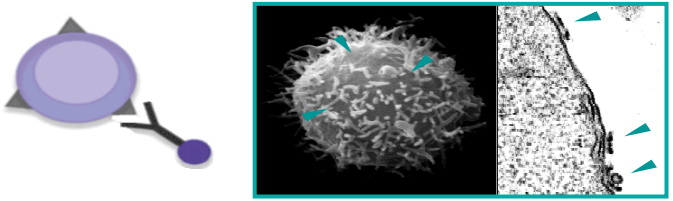
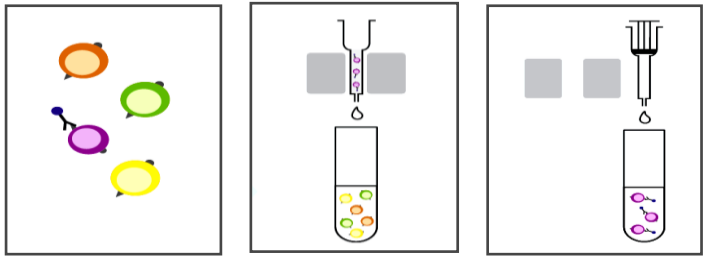
In vitro (immunomagnetic) cell selection: a tool for T cell or tumor cell depletion from the graft



- Super-paramagnetic , biodegradable particles conjugated to monoclonal antibody
- Permanent magnet and separation column with ferromagnetic matrix
- computerized device
- Targets: CD34+ stem cells, T cells, NK cells, B cells, myDC's,…





https://www.miltenyibiotec.com/

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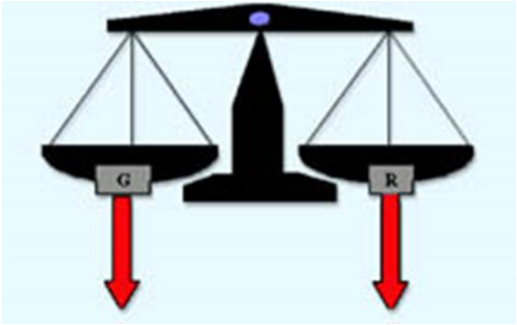
In vitro T cell depletion in allo grafts

Positive selection : CD34+ cell targetting

	Median	Range
Before selection:		
Number NC (x10^8/kg)	11,4	4-31,8
Purity CD34+ cells	0,3	0,1-1,6
Number CD34+ cells (x10^6/kg)	8,6	3,1-35,9
Number CD3+ cells (x10^6/kg)	286,5	105-1425
After selection:		
Purity CD34+ cells	**98,O %**	41,1-99,5
Number CD34+ cells (x10^6/kg)	6,0	1,9-17,4
Yield CD34+ cells	**71,5 %**	50,0-100
Number CD3+ cells (x10^3/kg)	7,6	3-50,7
T cell depletion	**4,5 log**	3,4-5,1

40 procedures – Clinimacs Plus device - allo grafts - UZ Brussel
Saad A. et al, Bone Marrow Transplantation (2017) 52, 1241–1248



GVHD Relapse

Delayed engraftment ?
Delayed immune reconstitution

Optimal dose: T cell add back?

Depletion of T cell subsets (Tαβ, CD45 R0,…) by direct cell targetting (negative selection) ?

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In vitro tumor cell depletion in autografts

Presence of **(minimal) residual amounts** of tumor cells in autografts can be demonstrated for several indications (Multiple Myeloma,…) using flowcytometry, PCR or NGS

Tumor cell purging *in vitro* **technically feasable** (CD34+ cell selection or targeted tumor cell removal)

No significant clinical benefit demonstrated in (most) randomized clinical trials (Multiple Myeloma, neuroblastoma,…)

Tumor contamination in graft can reflect also higher residual disease in vivo

Considering *in vivo purging* (monoclonal antibodies) prior to autograft collection or after autograft infusion ???

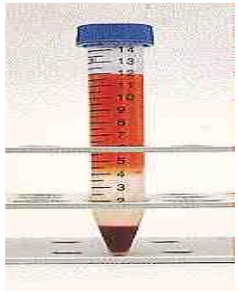
No reimbursement by RIZIV/INAMI (in contrast to T cell depletion for allografts)

Mellilo L et al , Acta Haematol. 2005;114(4):206-13
Alvarnas J., Oncology 2004 Jun;18(7):867-76

ABO incompatibility between donor and recipient: how to process the graft?

Major incompatibility

- If CD34+ cell selection peformed: all RBC are removed from the allo graft
- if no CD34+ cell selection:
  - ❑ determine titer anti-A/anti-B antibodies (recipient)
  - ▪ If > 1/16:
  - ❑ determine Hct and volume of graft:
  - if volume RBC **< 15 ml**: slow graft infusion (<25ml/hour)
  - If volume RBC **> 15 ml:** RBC reduction (apheresis device) or plasma-exchange (recipient)



Booth G. et al, Biol Blood Marrow Transplant. 2013;19(8): 1152-8.

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Cryopreservation and storage of hematopoietic stem cells

- Viability of stem cells starts to decrease after 72 hours of storage between 2-8°C
- Longer storage necessitates prior freezing and cryopreservation
- Freezing bags : fixed cell concentration 100-200x10e6 nucleated cells/ml > 2-8 bags/graft
- Freezing medium with cryoprotectant (7,5 to 10% DMSO)
- Controlled rate freezing (computerized device)
- Storage in vapor phase liquid nitrogen (up to > 20 years possible)

Jahan S, Transfusion Medicine Reviews 35 (2021) 95–102

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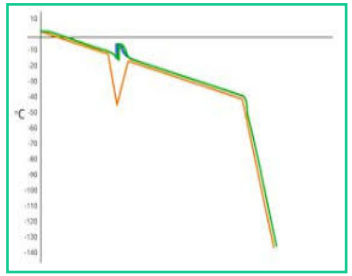
Cryopreservation and storage of hematopoietic stem cells



Jahan S, Transfusion Medicine Reviews 35 (2021) 95–102

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Cryopreservation and storage of hematopoietic stem cells

- Entrance cryopreservation unit restricted to authorized staff members
- Continuous temperature monitoring and registration (24/7)
- Quarantine zone
- Alarm System
- Inventarisation system
- Informed consent between donor, patient and stem cell bank (storage duration)

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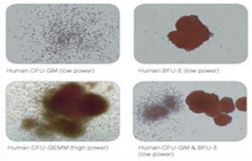
Quality control and release of hematopoietic stem cells

Cytometry



Cell cultures

Microbiological analyses

- Donor eligibility testing (IDM markers)
- Control of (frozen) test samples (viability, cell yield, microbiology)
- Release report (specified criteria)
- Exceptional release by medical director if normal release not possible (medical need)

Stem cell yield after thawing:
- 80% (50-95) (CD34+/7-AAD-)

Stem cell yield after isolation:
- 70% (50-90)

Purity after stem cell isolation:
- 98% (93-99)

Positive microbiology testing:
- Lower than 1%C

Processed stem cell grafts UZ Brussel

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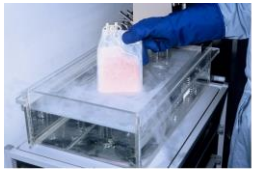
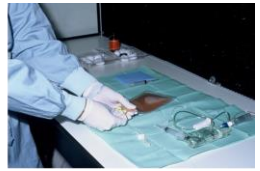
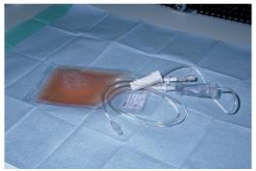
Distribution and infusion of stem cell grafts

- Transport of graft to cell therapy unit
- Shipper / courier
- Thawing by laboratory staff or nursing staff
- Waterbath or automatic thawing device (Plasmatherm)
- Mostly no washing and/or volume reduction
- Infusion by nursing staff / supervision by transplant physician
- DMSO amount < 1ml/kg/day







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Regulations, licences and accreditations (1)

National

- Collection and/or processing of human body material with the intention of clinical use can in Belgium only be performed by a licenced tissue/cell bank (hospital or academic organisation with faculty of medicine)
- All tissue/cell banks must have a specific licence for each type of tissue- or cell type
- Licence(s) can be obtained after inspection by **FAGG/AFMPS** (profesional inspectors)
- Inspections are performed according to regulations in national laws and royal decrees (based on EU directives)
- Human body material for manufacturing of **advanced therapy medicinal products (ATMPs)** (also by third parties) must be collected in centers that have the appropriate tissue/cell bank licence
- ATMP manufacturing in academic/hospital (non-commercial) setting requires additional certificates from FAGG/AFMPS (GMP certificate, manufacturing licence, clinical trial approval)

www.fagg-afmps.be

https://webgate.ec.europa.eu/eucoding/reports/te/index.xhtml(externe link)
https://webgate.ec.europa.eu/eucoding/reports/te/activities.xhtml(externe link)

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Regulations, licences and accreditations (2)

International

- All European (stem) cell therapy centers can apply for an international accreditation for all activities that relate to collection, processing and clinical use of stem cells and immune effector cells
- Accreditation can be obtained after inspection by **JACIE** (accreditation organisation of the EBMT) (peer inspectors)
- Inspection performed according to international JACIE–FACT quality standards
- Accreditation is not a legal requirement for Belgian centers BUT obligatory for **reimbursement by RIZIV/INAMI** and **collaboration with the national donor registry (MDPB)**

https://www.ebmt.org

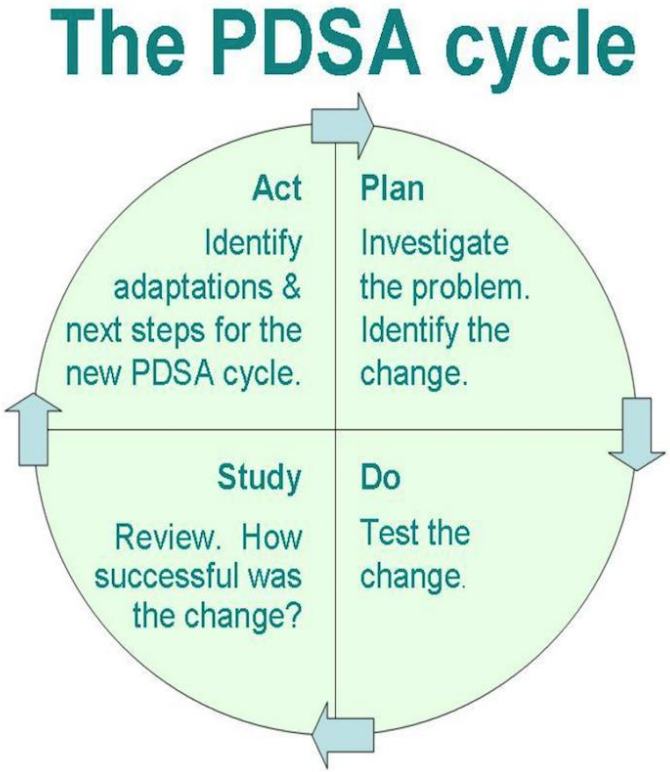
Quality management : the tools and the duties

To use

- Standards
- Quality manual (Standard operation procedures)
- Audit policy
- Regular QM meetings
- Registration policy (Biovigilance)
- Service Level Agreements
- Central role quality manager !

To do

- Read SOP's, propose changes, approve, apply them!
- Communicate / participate to QM meetings
- Follow (re-) training and education (e-learning)
- Registrate/report deviations (biovigilance)



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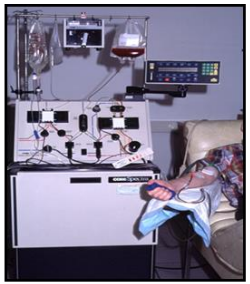
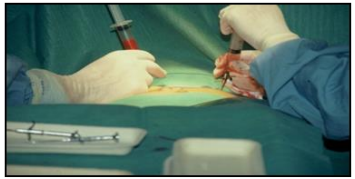
Analysis of quality indicators





Collection efficiency
Number of procedures to reach target

Cell yield / recovery
Cell viability / bioactivity
Sterility of cell product
Storage stability

Engraftment kinetics
Incidence of graft failure
Treatment-related mortality
Overall survival
Incidence of SAE/SAR

Regular trend analysis > changing policy and/or procedures
when QI's outside normal ranges (references/in house experience

International benchmarking EBMT

Saccardi et al, Bone Marrow Transplant. 2023 Jun;58(6):659-666

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THANK YOU!

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