# CELL PROCESSING LABORATORY

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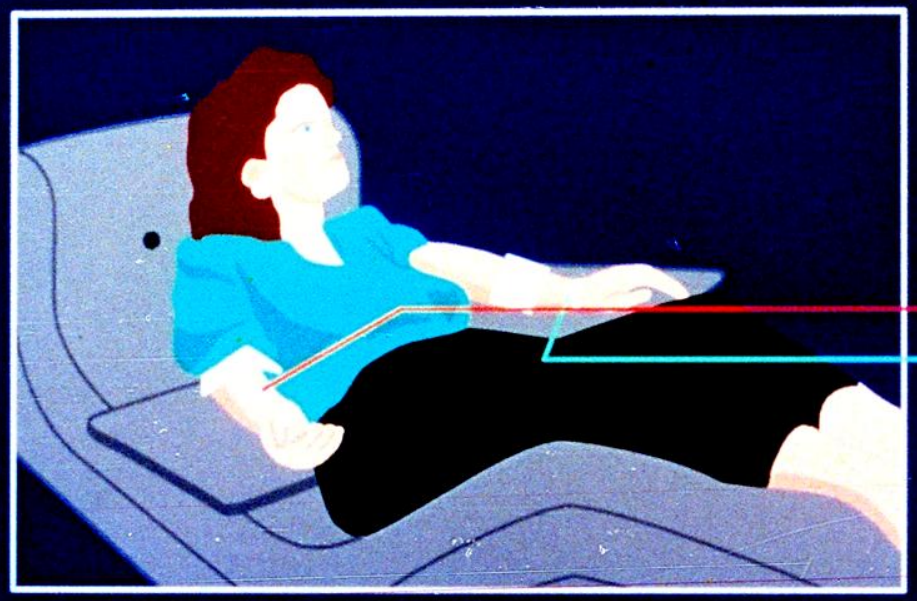
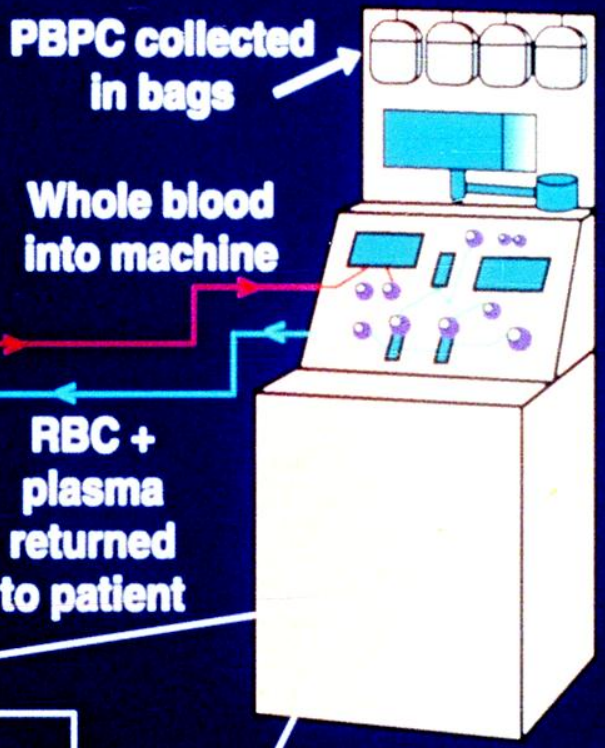
# MINIMAL MANIPULATION OF THE GRAFT: which methods can be used ?

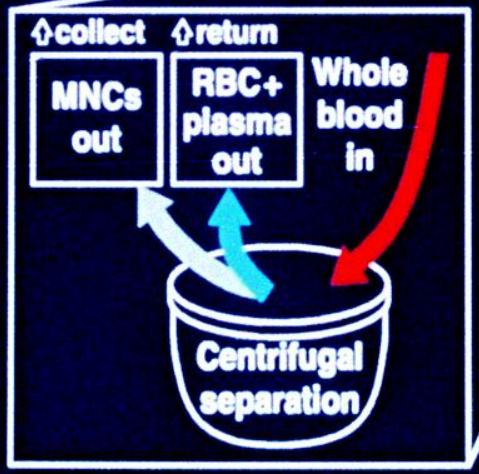
**METHODS USED SHOULD NOT MODIFY CELL FUNCTION AND STRUCTURE**

Standards for Haemopoietic Progenitor Cell Collection, Processing &Transplantation- JOINT ACCREDITATION OF EBMT AND ISCT : JACIE 3° edition

# COLLECTION OF PBPC BY LEUKAPHERESIS



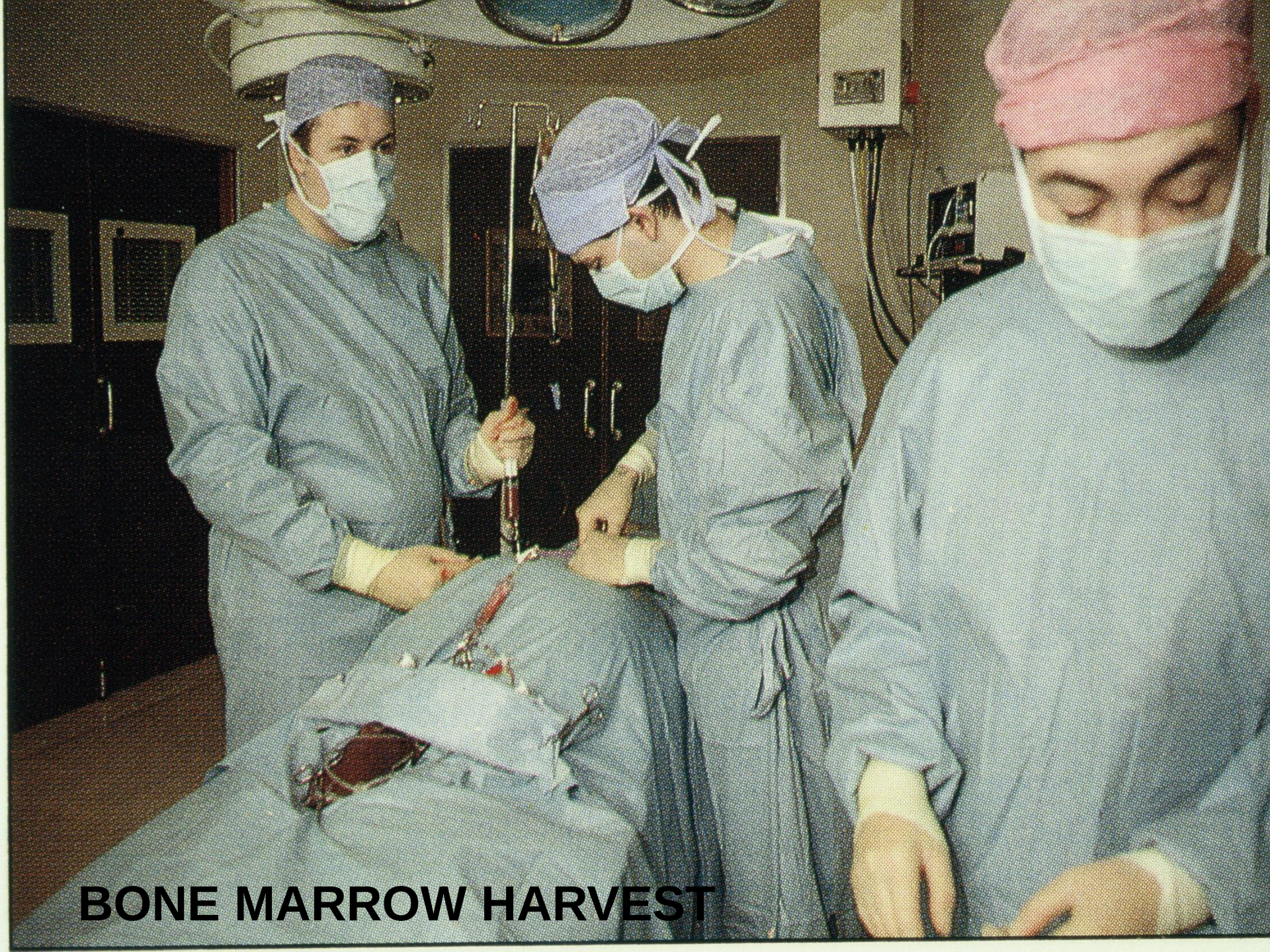



**LOOK AT :**

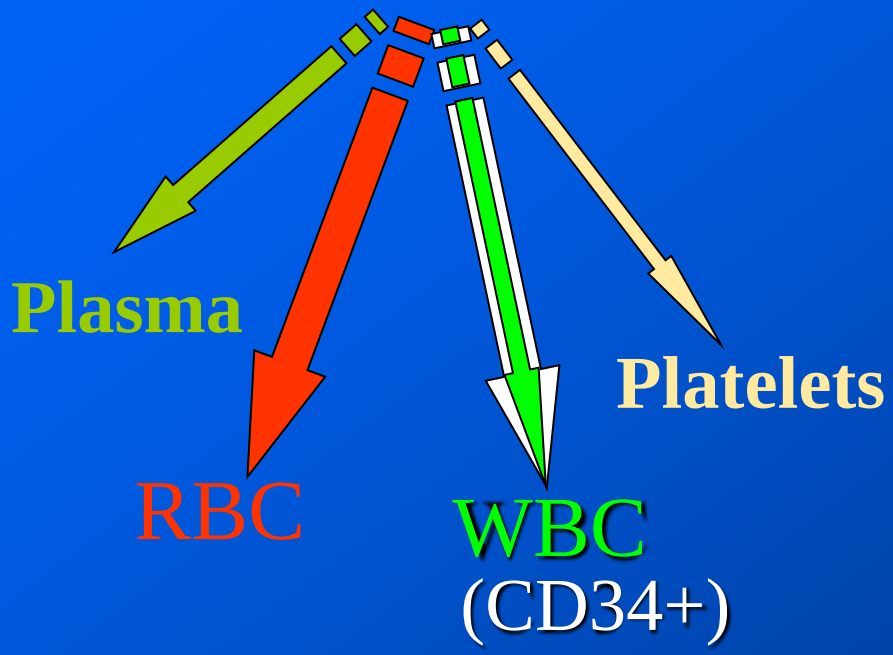
**1) COLLECTION EFFICIENCY !**

**2) BLOOD VOLUME PROCESSED**

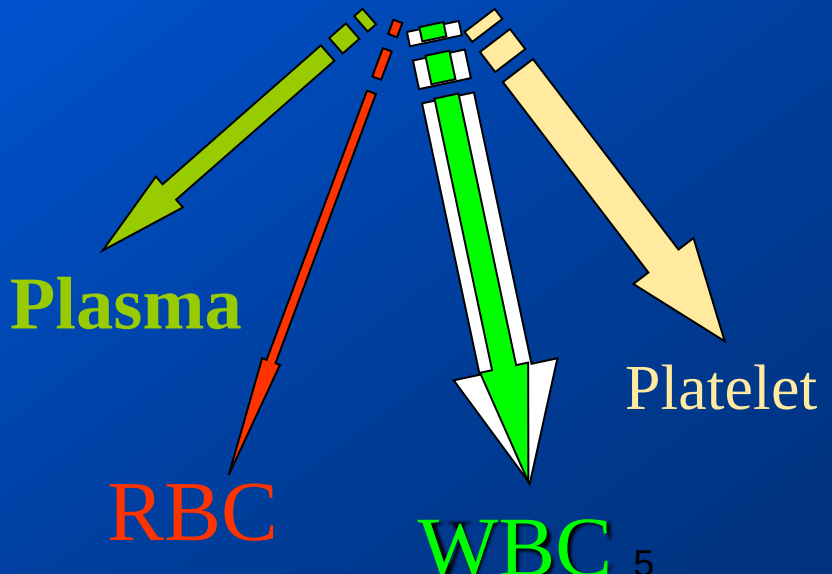

BONE MARROW HARVEST

# GRAFT COMPOSITION

## BONE MARROW




## PBSC




**TOTAL NUCLEATED CELLS (TNC) and MNC**

**(CD34+)**

# *PERIPHERAL BLOOD STEM CELL TRANSPLANTATION*

REFERENCE PARAMETERS

- **CD34+ CELLS**
  - > 20 uL (mobilised blood)
  - > 2 x $10^6$/kg (minimum)
  - > 5 x $10^6$/kg (optimal)

  } **Graft**

- **CFU-GM** (> 6-8 x $10^4$/kg)

  **Functional assay**

  (Obsolete parameter)

# *CLINICAL IMPACT OF CD34+ CELLS QUANTIFICATION*

- **REAL TIME ANALYSIS (less than 1 hour)**
- **SELECTION OF PATIENTS WHO MOBILIZE A SUFFICIENT NUMBER OF PROGENITORS AFTER A MOBILIZATION REGIMEN (> 20 μl) (SUITABLE FOR COLLECTION BY LEUKOAPHERESIS)**
- **TECHNIQUE OF CHOICE FOR THE CLINICAL MANAGENMENT OF PBSC COLLECTIONS (enables the optimal timing of the apheresis sessions and the number of procedures ensuring the harvest of at least 2-5 X $10^6$/Kg CD34+ cells)**

**Hematology Section, Cremona, Italy**

# *CLINICAL IMPACT OF CD34+ CELLS QUANTIFICATION*

- **ACCURACY AND RELIABILITY IN PREDICTING THREE-LINEAGE SHORT AND LONG-TERM ENGRAFTMENT FOLLOWING HEMOPOIETIC STEM CELL TRANSPLANTATION (GOOD CORRELATION WITH THE CLONOGENIC ATTITUDE OF THE GRAFT)**

- **FIRST STEP IN QUALITY ASSESSMENT OF HEMATOPOIETIC STEM CELL GRAFTS**

**Cremona- Italy**

***THE DEVELOPMENT…***

# Flow Cytometric Methods for CD34 Enumeration

- **Milan** → **ISHAGE**
- **Single parameter** → **Multiparameter**
  **CD34,CD45, 7-AAD**
  **CD34 SUBSETS**
- **Dual platform** → **Single Platform**
  **(Counting beads)**
- **Automated** → **Abs counts**
- **methods**

# Sequential gating For True viable CD34+ Cells (ISHAGE GUIDELINES)

- **R1** = selection of leukocytes ($CD45^{+}$ from dim to bright
- **R2** = selection of $CD34^{+}$ cells among the leukocytes
- **R3** = selection of $CD45^{dim}$, $SSC^{low}$ HPC
- **R4** = selection of $FSC^{low\ to\ intermediate}$ HPC
- **R8** = selection of apoptotic/dead cells: gate out applied to all dot-plots

*Keeney-Lanza, JBRJA, 2005

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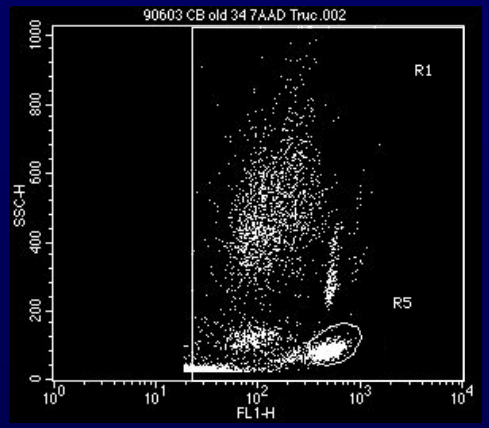
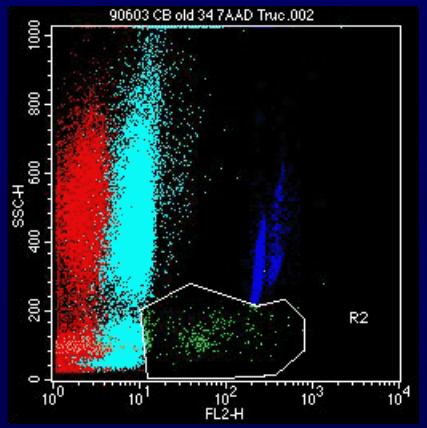
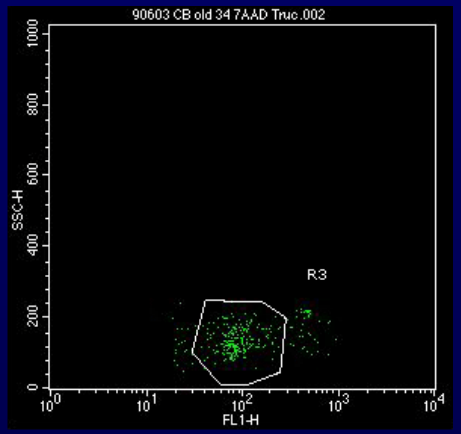
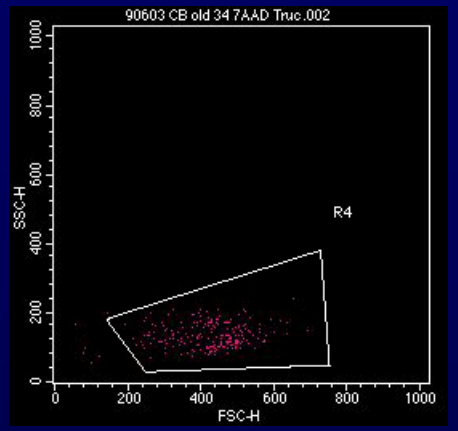
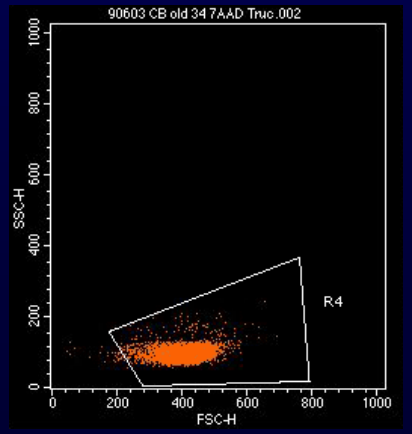
# ISHAGE/ISCT protocol for CD34+ cells enumeration and apoptotic/dead cells exclusion: single platform platform Boolean gating strategy-lyse no wash

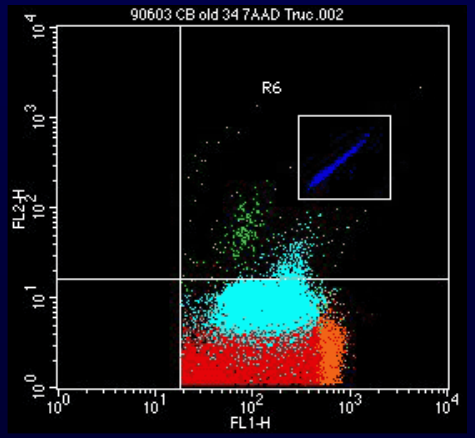


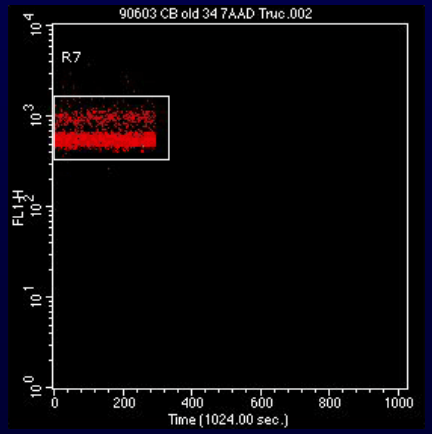



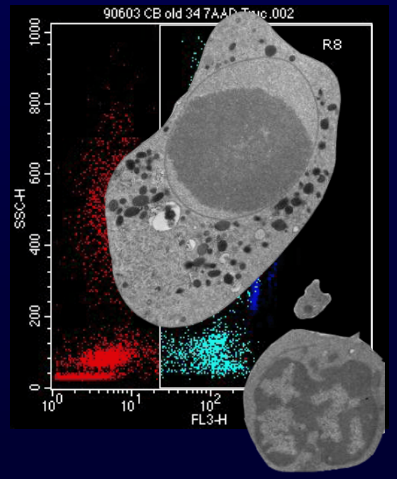




Keeney, Sutherlande, Lanza et al, JBRHA, J Biol Regul Homeost Agents. 2003: 17(3):247-53

# CRYOPRESERVATION

- **STORAGE AT 2-8°C is acceptable for 24-48 hrs**
- **Volume reduction for BM graft, WBC >250.000/μl or high neutrophil count requires plasma dilution to preserve cell viability**
- **The use of ACD is recommended**
- **PBSC AND BM MAY BE STORED YEARS (decades) before reinfusion**
- **Liquid nitrogen storage is optimal for long-term storage (controled rate freezing)**
- **-70-80°C mechanical freezer is suitable for PBSC storage up to 6 months**
- **Cryoprotectants: DMSO 10% or DMSO 5%+ HES )**

# CELLULAR PRODUCTS THAWING

- **The products (PBSC/BM) should be thawed rapidly, in a 37°C waterbath if possible, but without letting the product warm past ambient temperature prior to infusion.**
- **Exposure time to DMSO after thawing should be minimized to avoid cell death.**
- **Special attention should be paid to minimizing the chances of contaminating the product during the thaw process.**

# GRAFT INFUSION AFTER THAWING

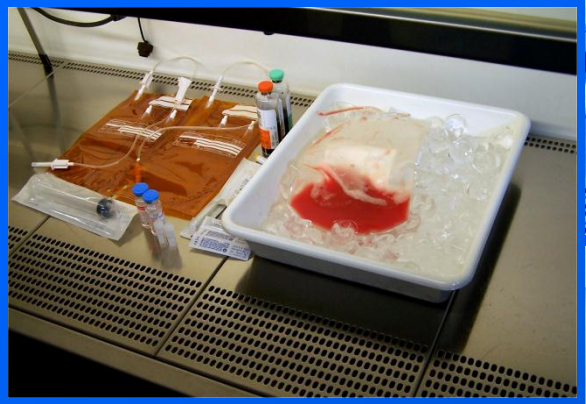
- **WHERE: BEDSIDE ?**

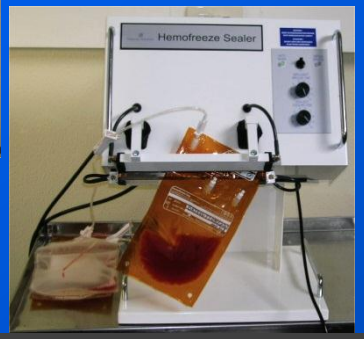
products that are directly thawed are done so in close proximity to the patient, requiring the transport of the product and equipment (waterbath) needed for thawing by laboratory personnel and the presence of laboratory staff throughout the infusion.

**CELL LAB ?**

Cell therapy facilities may deliver products that have been thawed, washed and controlled in the lab; this is partly the result of continuous pressure from competent authorities to create a situation that enables to monitor infused cell products.

# Cryopreservation : methods





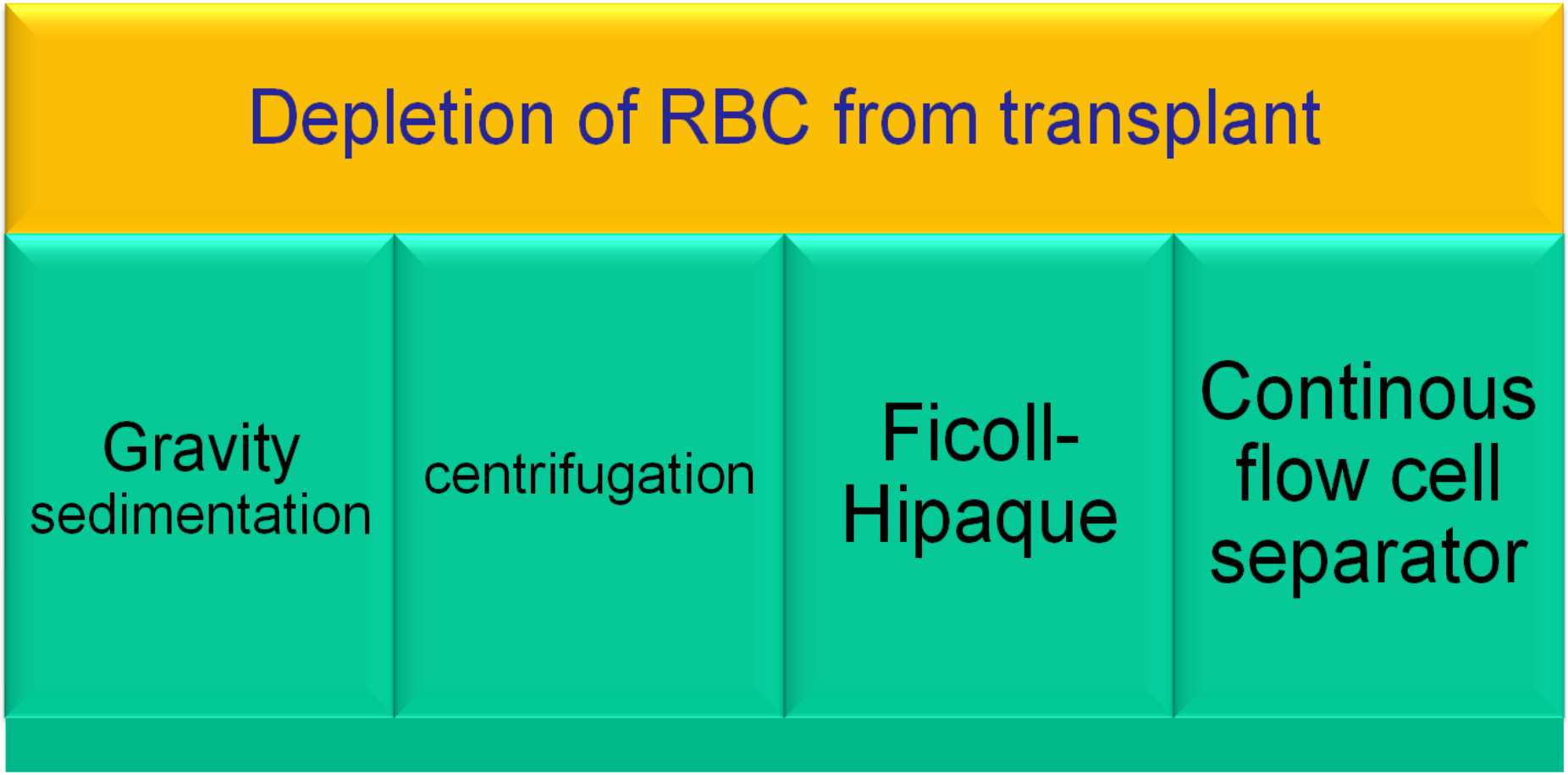

## Cryopreservation : devices

# ALLO-SCT: RBC DEPLETION for major AB0 mismatched transplant (BM)

- 23-30% of SCT
- Major mismatch is due to the presence of hemoagglutinin directed against donor RBC antigens **(> 1:64 IgM, > 1:256 IgG)**
- Minor Incompatibilitity →is due to the presence of hemoagglutinin directed against recipient RBC antigens

- **Mixed mismatch** : major and minor mismatch can be detected

# Techniques in major ABO-incompatible-HSCT



# ALLO-SCT: PLASMA DEPLETION for minor ABO mismatched transplant (BM)

- Plasma depletion is not required but highly recommended ;

- PD by Centrifugation (10 moin, 4°C, 3000g) is the preferred method (simple and effective )

# GRAFT QUALITY:
# ISSUES TO BE DEALING WITH

- **MOBILIZATION & PBSC COLLECTION**

  HSC MATURITY, **CD34 MEASUREMENT BEFORE AND AFTER CRYOPRESERVATION**,
- CLONOGENIC ASSAY, CELLULAR COMPOSITION, leukapheresis.
- **SCT**: PMNs and PLATELET ENGRAFTMENT; HOSPITALIZATION TIME, No. OF INFECTION DISORDERS, DAYS ON ANTIBIOTICS, number of transfusions of blood component (plts and/or RBC), IMMUNOLOGICAL RECONSTITUTION, ORGAN TOXICITY, SAE, EARLY DEATH , QOL ASSESSMENT, DISEASE RELAPSE.

# JACIE CELEBRATES THE 10TH ANNIVERSARY OF THE FIRST EUROPEAN INSPECTION VISIT WITH IMPROVED OUTCOME IN STEM CELL TRANSPLANTATION!

**Christian CHABANNON 1,2, Derwood PAMPHILON 2,3, Christiane VERMYLEN 2,4, Alois GRATWOHL 5, Dietger NIEDERWIESER 6, Eoin McGRATH 2, Cor LAMERS 7, Francesco LANZA 8, Ineke SLAPER-CORTENBACH 9, Alessandro MADRIGAL 10, Jane APPERLEY**

**Cytotherapy**. 2011 Jul;13

**Bone Marrow Transplant**. 2012 Jan;47(1):15-7. doi: 10.1038/bmt.2011.32. Epub 2011

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# THANK YOU

Leemhuis T,Padley D,Keever-Taylor C, Niederwieser D,Teshima T, Lanza F, Chabannon C,Szabolcs P,Bazarbachi A **Mickey BC Koh**; for the **Graft Processing Subcommittee of WBMT /WHO**

# AB0 incompatible transplant

J L Gajewski: A review of transfusion practice before, during, and after hematopoietic progenitor cell transplantation, Blood 2008 112: 3036-3047

- If a major ABO incompatibility exists, efforts should be made to minimize RBC content in the HPC graft, even at the expense of a slightly lower HPC content.
- Because the volume of RBC in the bone marrow HPC product may be significant (equal to or greater than that of a unit of blood), significant hemolysis is possible. To substantially mitigate this risk, RBC may be removed from the donor's BM by Hetastarch separation, mononuclear cell concentration by machine, or chemically (through density gradient separation). TARGET VALUE: incompatible RBC 0,3-0,5 ml/kg recipient body weight
- :

# EFFECTS OF ABO MISMATCH ON THE ENGRAFTMENT KINETICS OF BM CELLS

- **Sniecinski I**. Journal of Clinical Apheresis, 1985;2:231-234 → attecchimento e graft simili ai controlli, aumentata richiesta GR
- **Kimura F.** (Japan Marrow Donor Prog) , Haematologica, 2008; 93(11),1686-93 → OS significativamente inferiore (ABO-identical 63.0%; major mismatch, 56.9%; minor mismatch, 57.1% at 1 year); incompatibilità maggiore e minore significativi fattori di rischio per TRM e alta incidenza GVHD 3°-4° grado
- **Blin N**, Biol Blood Marrow Transplant, 2010;16(9):1315-23 → maggiore necessità trasfusionale nei BM ABO incomp; in PBSC lieve correlazione tra incompABO e incidenzaG**VHD**
- **Ozkurt ZN**, Transplant Proc 2009;41(9):3851-8 → TRM maggiore e OS minore in riceventi con incomp ABO minore; PRCA e ritardato attecchimento RBC in incomp ABO maggiore

# AB0 incompatible transplant

| Recipient RBC group | Donor's RBC group | Category of ABO mismatch† |
|---|---|---|
| A | O | Minor |
| B | O | Minor |
| AB | O | Minor |
| AB | A | Minor |
| AB | B | Minor |
| O | A | Major |
| O | B | Major |
| O | AB | Major |
| A | AB | Major |
| B | AB | Major |
| A | B | Minor and major |
| B | A | Minor and major |
| A | A | None |
| B | B | None |
| AB | AB | None |
| O | O | None |