What the Transfusion Scientist should know about Stem Cells

Dr Claire Wiggins – BBTS September 2017

What is a stem cell?

NHS Blood and Transplant

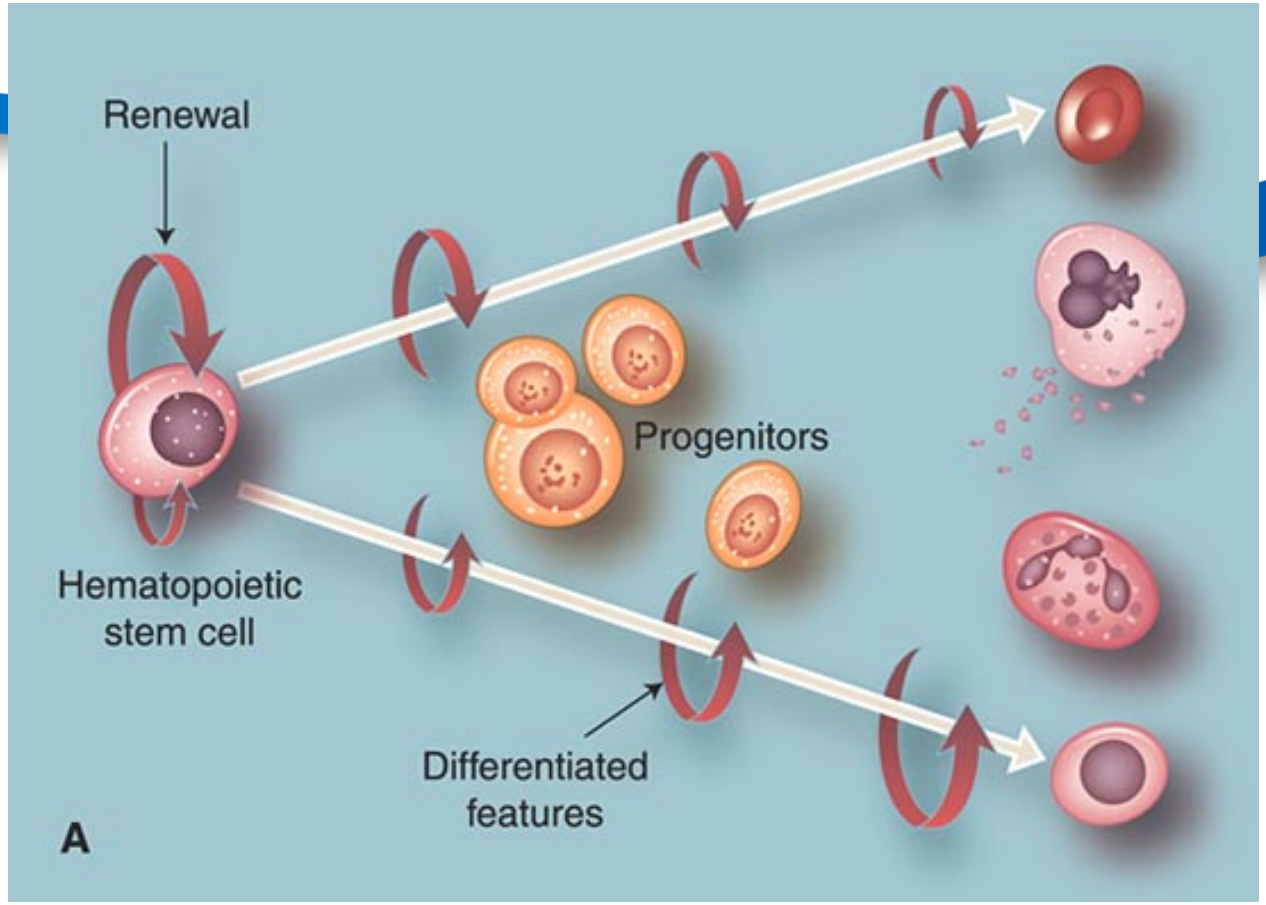
- Undifferentiated cell
- Can divide and self renew for long periods
- Differentiate into tissue or organ specific cells – specialised cells

What is a Haemopoietic Stem Cell?

NHS Blood and Transplant

- Haemopoietic progenitor cell, capable of reconstituting long term multi-lineage haemopoiesis
- 1-3% cells in normal bone marrow, extremely rare in PB
- Small lymphoid looking cells
- Express CD34

NHS
Blood and Transplant



Bone Marrow Transplantation

NHS Blood and Transplant

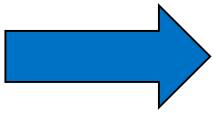
- 1956 – BMT transplants in leukaemic mice
- 1957 – First human allogeneic BMT transplants – 6 patients
- 1959 – 1st European BMT on 5 nuclear workers whose own bone marrow damaged
- 1968 – 1st BMT between siblings
- 1970s – Renewed interest in autologous BMT
- 1979 – 1st successful unrelated BMT
- 1980s – Formation of donor panels

Purpose of Stem Cell Transplantation

NHS Blood and Transplant

Treat a variety of blood related and metabolic disorders e.g.myeloma, lymphoma, leukaemia, SCID

Patient bone marrow cells eradicated through conditioning regimes



New population of HSC transplanted to repopulate the patients bone marrow. Also GvL effect

Types of Stem Cell Transplant

NHS Blood and Transplant

Autologous

- Stem cells collected from patient
- Cells ‘rescue’ patient after chemotherapy

Allogeneic

- Stem cells collected from healthy donor
- HLA matched sibling
- Haploidentical family relative (partially matched)
- Unrelated volunteer
- Cryopreserved cord blood unit

Sources of Haemopoietic Stem Cells

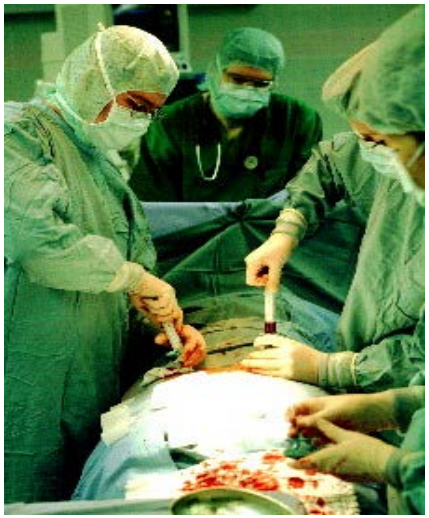
NHS Blood and Transplant

- Bone marrow
- Mobilised peripheral blood
- Cord blood

Sources of Haemopoietic Stem Cells - Bone Marrow

NHS Blood and Transplant

- GA
- Marrow aspirated using needles/syringes
- Iliac crest
- 20ml/kg
- Approximately 3 weeks to be replaced



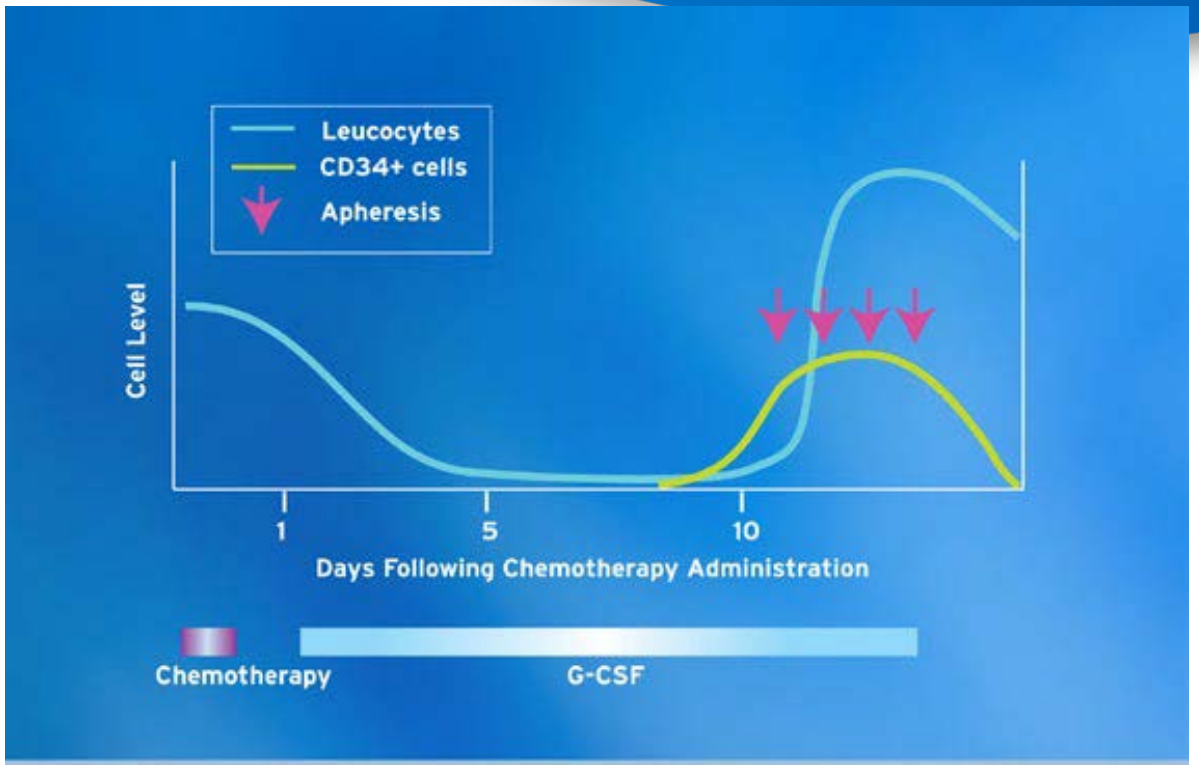
Sources of Haemopoietic Stem Cells - Mobilised Peripheral Blood

NHS Blood and Transplant

- Recovery after chemotherapy
- Donors stimulated - Haematopoietic cytokines e.g. G-CSF
- Combined use of chemotherapy + G-CSF
- Plerixafor

Mobilisation

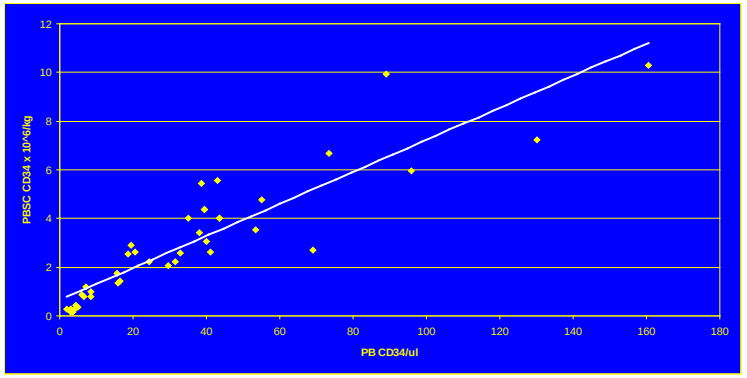
NHS
Blood and Transplant



CD34

NHS Blood and Transplant

- Stem Cell marker is CD34
- Correlation between blood and harvest
- PB CD34 expressed as cells/µl
- Harvest CD34 expressed as $x10^6$/kg

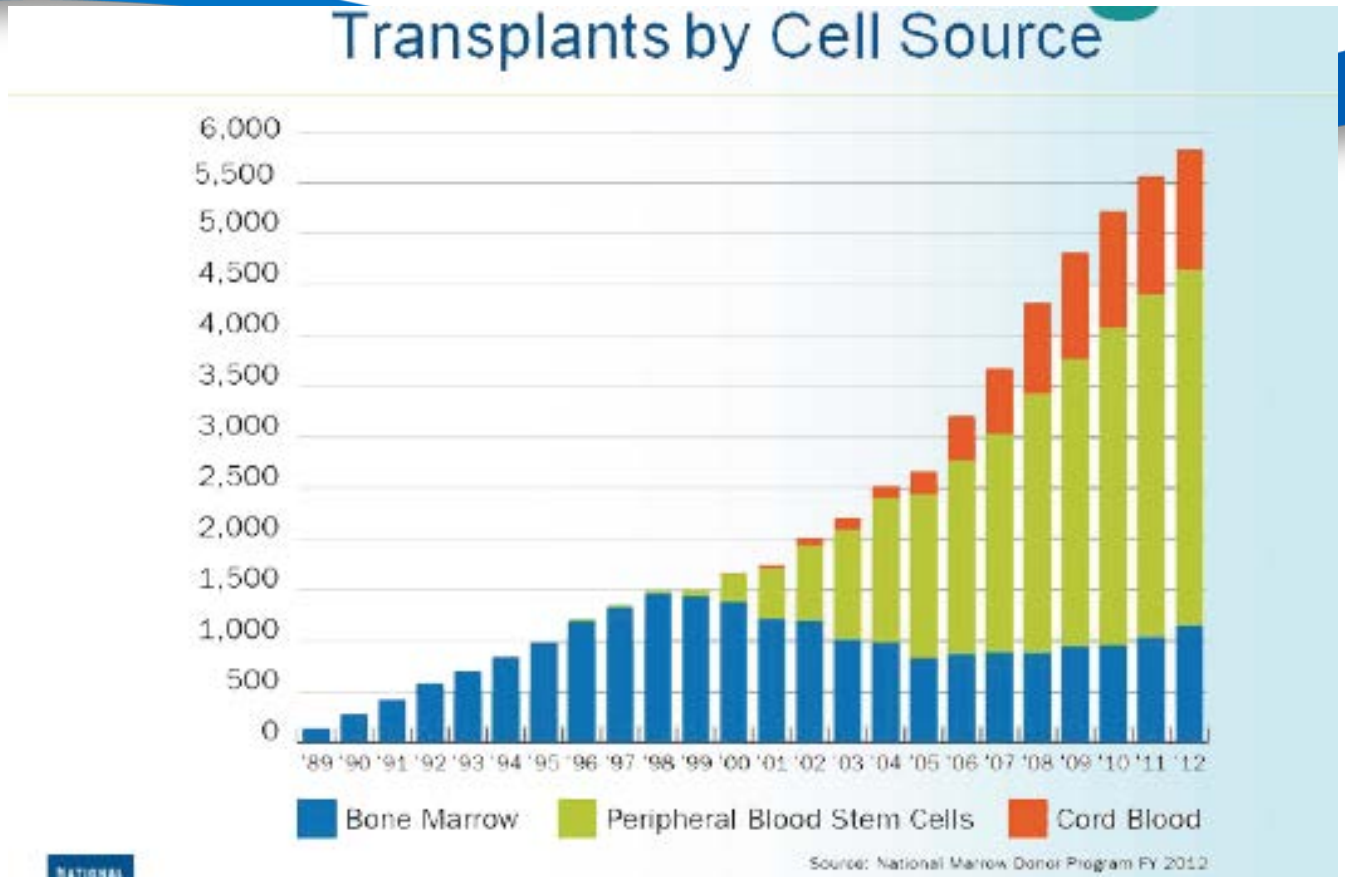


Cord

NHS Blood and Transplant

- Blood left on placenta and umbilical cord from delivery of baby
- Rich in stem cells
- Collected at registered hospitals

NHS
Blood and Transplant



What we require prior to collection/processing

NHS Blood and Transplant

- Notice of intended dates for harvest
- Request form
- Consent form
- Virology results/samples within 30 days of collection
- Notice of time cells arriving on the day
- Long line left on the collection bag

Virology Screening – Minimum Requirement

NHS Blood and Transplant

- 30 days prior to donation
- Anti-HIV 1 & 2
- Hepatitis B - HBsAg, Anti-HBc
- Anti-Hepatitis C Ab
- Anti HTLV 1 & 2 (if applicable)
- Syphilis
- Hep E from Sept 2017
- Additional tests (e.g. CMV, EBV, toxoplasma)
- Separate VAT for positive products
- Quarantine VAT for unscreened

Transport of Products to Lab

NHS Blood and Transplant

- Product in sealed secondary bag in sealed thermally insulated validated transport box
- Traceability
- Labelled with contact details (to and from), inside and outside container
- Labelled with handling instructions
- Temperature – transport times <1 hour temperature range 2 – 37^{o}C. Transport times 1 – 12 hours temperature range 2 – 24^{o}C. Transport times >12 hours temperature range 2-8^{o}C. Loggers sometimes used

Receipt of Product in Lab

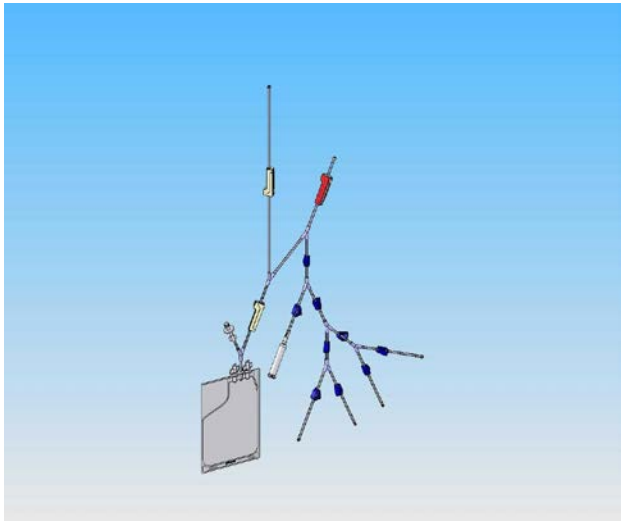
NHS Blood and Transplant

- Inspect product for integrity, condition, labelling
- Temperature of product on arrival
- Sample taken from product for wcc, mnc, CD34 (stem cells), viability, bacteriology, CD3 (allogeneic)
- Product may be stored overnight at 4^{o}C in monitored temperature controlled fridge
- Product may require diluting for o/n storage

Processing/Preparation for Cryopreservation

NHS Blood and Transplant

- Some stem cell labs use cryodoc on bench using sterile dockers and heat sealer

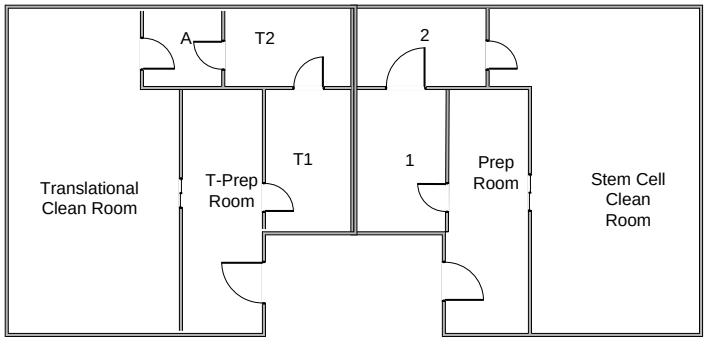


- Some stem cell labs perform processing in cleanrooms

Clean room Suite

NHS Blood and Transplant

- HEPA filtered, positive pressure
- Clean room – grade B
- Cabinets – grade A
- Over garments and undergarments
  - Over garments sterile
  - Prevent contamination
  - Non-shedding synthetic fibres
  - Private changing areas



Cryopreservation

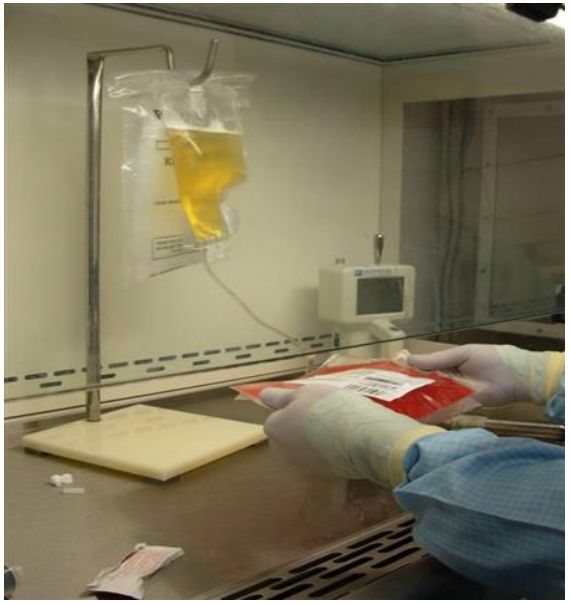
NHS Blood and Transplant

- Dehydration and ice formation damage cells and tissues
- Cryoprotectant required to protect the cells from freezing damage – DMSO most commonly used
- Penetrating cryoprotectant
- DMSO is cytotoxic, temperature dependant
- Final concentration of 10% DMSO commonly used

Cryopreservation of Stem Cells

NHS Blood and Transplant

- Equal volume of chilled 20% DMSO in plasma or HAS added slowly
- Dispensed into 2 or more Cryo-storage bags
- Also cryovials



Cryopreservation of Stem Cells

NHS Blood and Transplant

- Controlled-rate freezer or passive freezing
- Cooling rate is important
- Controlled rate freezing programmes not standardised
- End point range -100 to -160^{o}C
- Cells frozen at even rate throughout bag

Storage

NHS Blood and Transplant

- Bags stored below -135°C (glass transition point)
- Cross contamination is minimised
  - Vapour-phase nitrogen
  - Double-wrapped
- Temperature is continuously monitored

Quality control measures on products

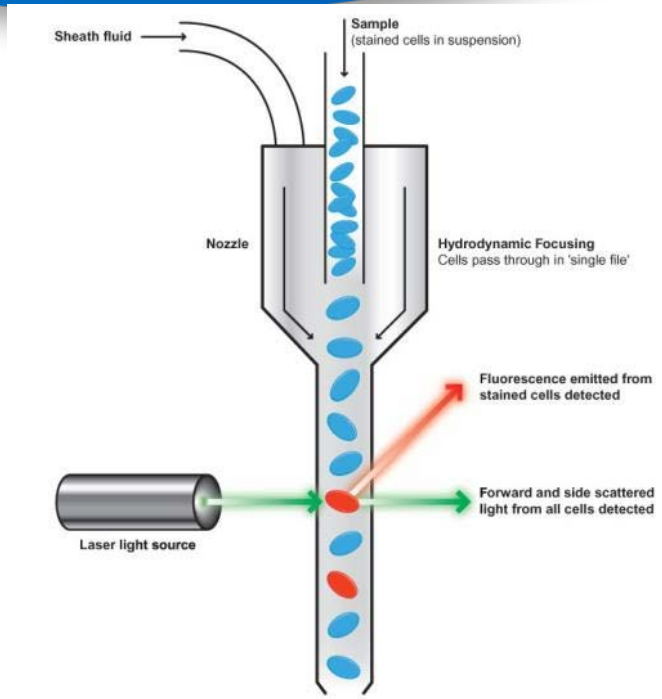
NHS Blood and Transplant

- Total nucleated cell count (TNC)
- Mononuclear cell count (MNC)
- CD34 count (stem cells)
- CD3 count (T cells for allogeneic products)
- Viability
- CFU assays
- Bacteriology

Flow Cytometry

NHS Blood and Transplant

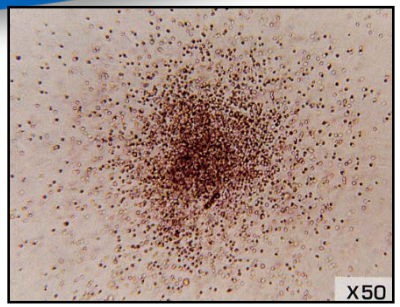
- Viable CD34 positive cells enumerated, ISHAGE gating
- Viability measured using 7-aminoactinomycin D (7-AAD)
- Absolute number/µl
- Calculated CD34 x 10^6/kg
- Also viable CD3 positive cells measured for allogeneic products



CFU Assay

NHS Blood and Transplant

- Cells plated in methyl cellulose
- Good indication of cell viability, functional assay
- Requires 14 day culture period
- Can be time consuming to read
- Number of colonies counted, expressed as CFU x 10^4/kg



Bacteriology

NHS Blood and Transplant

- Minimum requirements, post processing
- Positive results, sensitivities performed
- Reported to transplant clinician
- Can still be transplanted, issued under concession
- Antibiotic cover

Autologous Transplant

NHS Blood and Transplant

- Request from transplant unit to issue cells for transplant
- Patient receives high dose chemotherapy
- Transported in cryoshipper, temperature monitored and alarmed
- Temperature <-150 degC
- Accompanying paperwork for traceability

Infusion of Cryopreserved Stem Cells

NHS Blood and Transplant

- Pre-medication (Piriton, Hydrocortisone) given to reduce risk of reaction to DMSO

- Bags thawed on the ward one at a time
  - Each bag infused within 20 minutes

- Infusion guidelines are <1mL DMSO/Kg/day
  - Split infusion over 2 days

Infusion Reactions

NHS Blood and Transplant

- Mild – Nausea, Vomiting, Abdominal cramps, Headache, Tachycardia, Shortness of breath, Shivering, Rigors, Aftertaste
- Moderate/Severe – Reported to HTA on line and investigated, untoward occurrence, unintended response, fatal, life threatening, disabling, incapacitating or which results in, or prolongs, hospitalisation or morbidity

Engraftment

NHS Blood and Transplant

- Stem cells migrate to patients bone marrow
- Requirement to monitor engraftment
- Daily FBC
- Neutrophil count >0.5, mean usually 11-13 days
- Platelet count >20, mean usually 13-20 days
- >28 days, delayed engraftment

Allogeneic Transplants
HLA Typing and Matching

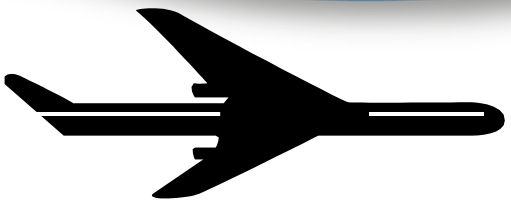
NHS Blood and Transplant

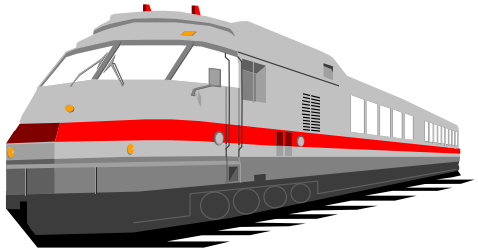
- HLA (A, B, Cw, DR and DQ)
- Ideal is 10 out of 10 match.
- 30% of patients have a suitable fully matched related donor
- Unrelated donor, high resolution testing recommended
- ABO groups can be different
- CMV matching important - Transplant of a CMV positive graft into a negative recipient can cause a CMV infection - CMV negative graft into a positive recipient can cause CMV reactivation
- Age and gender of donor also taken into account

Allogeneic Transplants

NHS Blood and Transplant

- Patient has chemotherapy/radiotherapy
- Collection on hospital ward/clinic/operating theatre, via registry if unrelated
- Transported to the Stem Cell Lab
- Checks on receipt of product

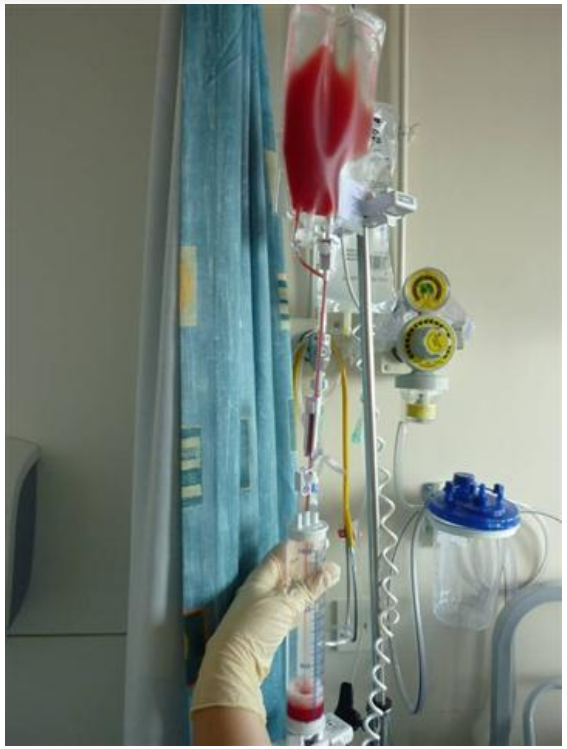




Allogeneic Transplants

NHS Blood and Transplant

- Processing of product may be required
- Analysis of product, CD34, CD3, viability, bacteriology
- Issue of cells to hospital ward in cool box
- Traceability
- Infusion to patient
- Monitor engraftment, neutrophils >0.5, platelets >20



Donor Lymphocytes

NHS Blood and Transplant

- Cryopreservation of specific doses of T cells (CD3/kg) from donor
- Treatment of relapse
- Treatment of mixed chimerism
- Risk of GvHD

Blood Requirements

NHS Blood and Transplant

- Irradiated blood products – any third party leucocytes could engraft and initiate an alloreactive response, TA-GvHD
- CMV Negative – all CMV seronegative recipients, and seronegative donors, patients likely to proceed to transplant. CMV transmitted via leucocytes

Blood Group Changes
Major ABO incompatibility

NHS Blood and Transplant

- Presence in recipient's plasma of anti-A, anti-B or anti-A,B antibodies incompatible with donor red cells

  e.g. group A donor group O recipient

- *Risks:*
  - Acute haemolysis at time of HPC infusion
  - Delayed haemolysis due to production of antibodies by residual host lymphocytes

    Both above depend on vol. rbcs infused and titre of Ab (1:32 or greater)
  - Delayed red cell engraftment and pure red cell aplasia may occur

Blood Group Changes
Minor ABO incompatibility

NHS Blood and Transplant

- Presence of anti-A, anti-B, or anti-A,B antibodies in donor's plasma reactive with recipient's red cells

  e.g donor group O and recipient group A

- *Risks:*
  - Acute haemolysis at time of infusion caused by anti-A or anti-B in plasma of donor product
  - Delayed haemolysis of recipient cells due to passenger lymphocytes syndrome

    Relates to volume of plasma and isoagglutinin titres of donor, and also body size of recipient

Management of Group Changes

NHS Blood and Transplant

- **Pre-transplant:** samples from both donor and recipient for:
  - ABO and Rh D grouping and antibody screen
  - Anti-A and anti-B titres by indirect antiglobulin test (IAT) where indicated
  - Direct antiglobulin test (DAT)
- **Post transplant**:
- Observe for group changes
- Transient or permanent chimera

NHS Blood and Transplant

- For major ABO mismatch use group O red cells products, irrespective of ABO group until recipient ABO antibodies are undetectable and the antiglobulin test is negative and platelets and plasma from donors of the recipients ABO type until recipient red cells no longer detected.

- For minor ABO mismatch use red cells of the donor type i.e. group O throughout. Give platelets and plasma of recipient type until recipient-type red cells are no longer detected.
- For major and minor ABO mismatch use group O red cells until recipient ABO antibodies are undetectable and the antiglobulin test is negative and then switch to donor type. For platelets and plasma use group AB until recipient red cells are undetectable.

NHS Blood and Transplant

Incompat-ibility	Donor Group	Recipient Group	Before Gp change RBs	Before Gp change Platelets	Before Gp change Plasma	After Gp change RBC ,Plt & Plasma Groups
Major	A	O	O	A	A	Rbc : A Platelets: A Plasma: A or AB
Minor	O	A	O	A	A	Rbc: O Plt: O FFP: O,A,B,AB

Regulation - HTA

NHS Blood and Transplant

- April 2004 every tissue bank licensed
- Mandatory inspection started 2004-2005
- EU Cells and Tissue Directive
- HTA given authority over consent, procurement, testing, storage and distribution of cells

HTA
Human Tissue Authority

Regulation - JACIE

NHS Blood and Transplant

- JACIE accreditation voluntary
- Clinical, collection and processing
- Standardisation of practice to international standards

JACIE
joint accreditation committee
isct ebmt

NHS
Blood and Transplant

Any Questions?