

A microscopic view of numerous red blood cells, appearing as bright orange-red, biconcave discs against a dark background. The cells are scattered across the frame, with some in sharp focus and others blurred in the foreground and background.

# Transfusion support in haematopoietic transplantation

Dr Chris Hogan  
ARCBS Transfusion Update  
6th May 2009  
Powerhouse - Sydney

# 1865

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- Joseph Lister develops antiseptic system
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# 1866

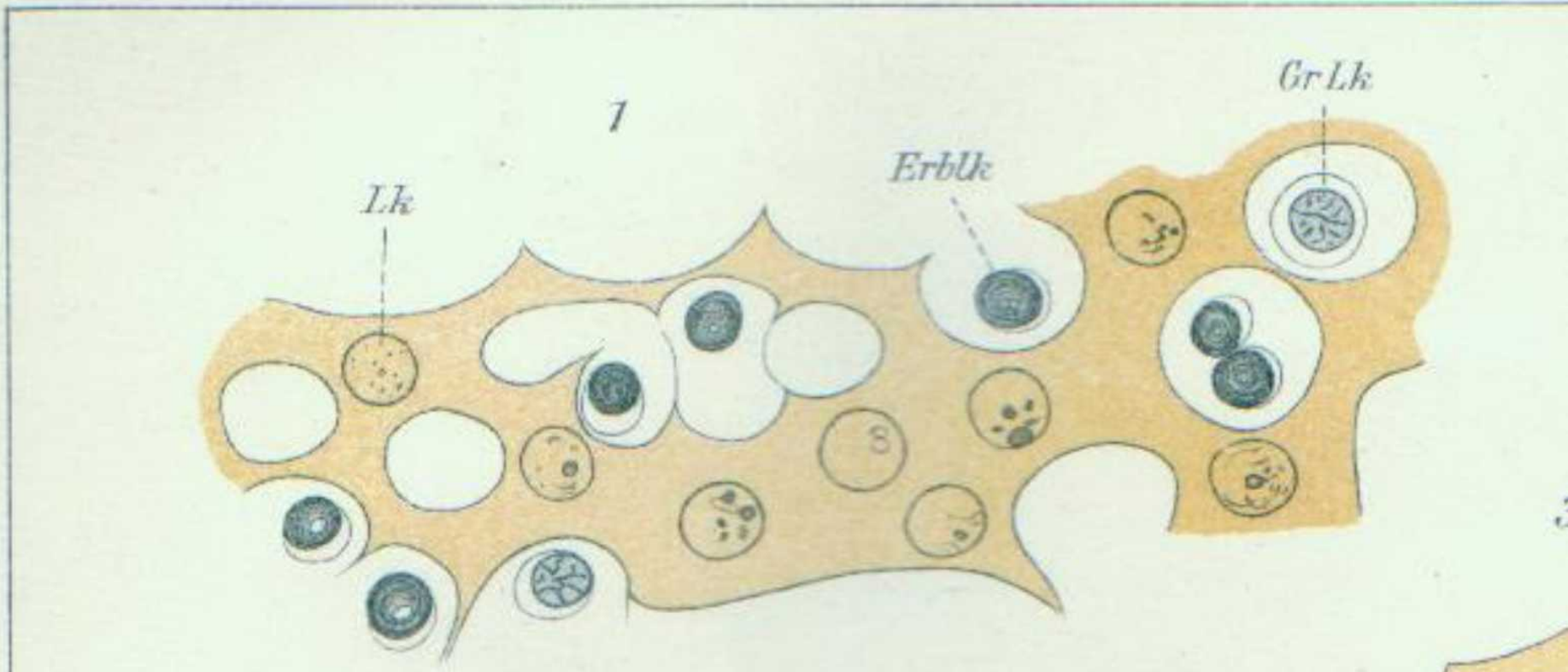
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- Gregor Johann Mendel discovers genes



*Neumann, Blut und Pigmente*



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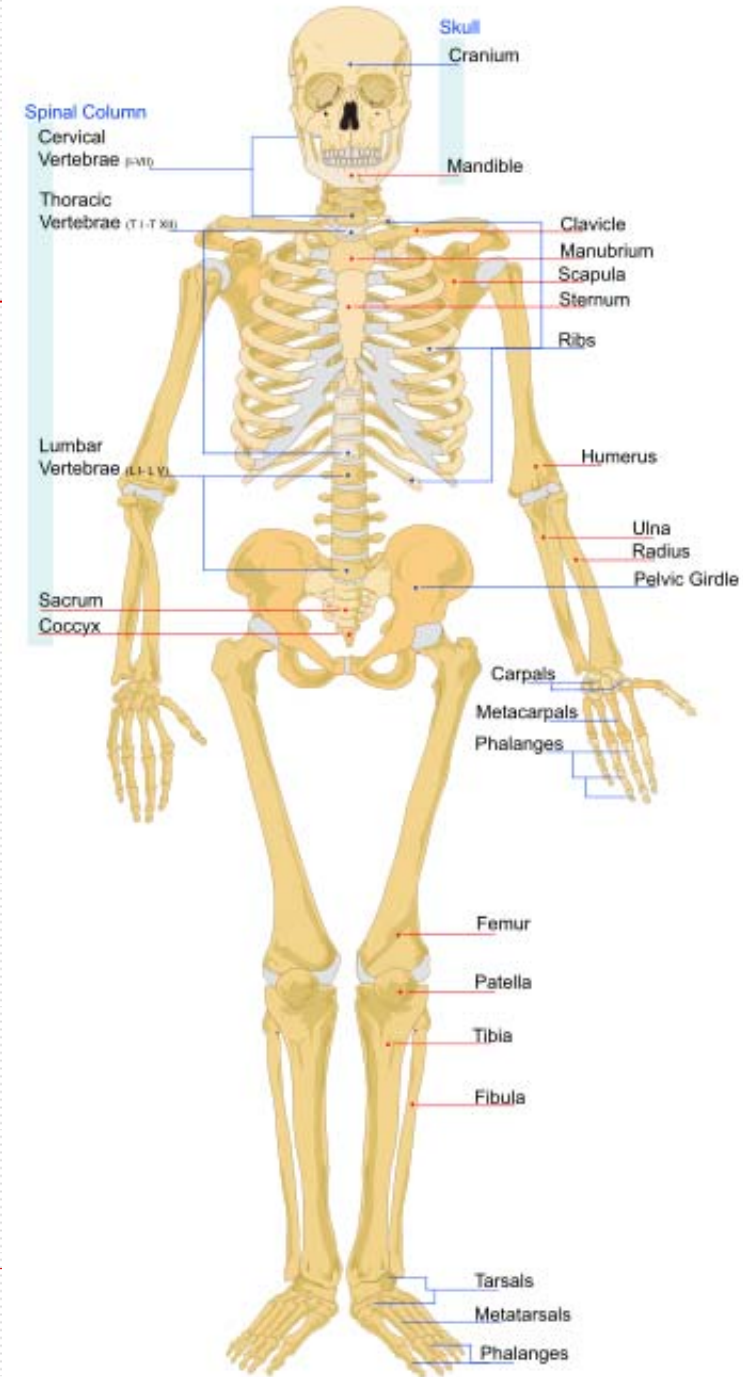


**Ernst Neumann – 1866**  
**Königsberg, Prussia**

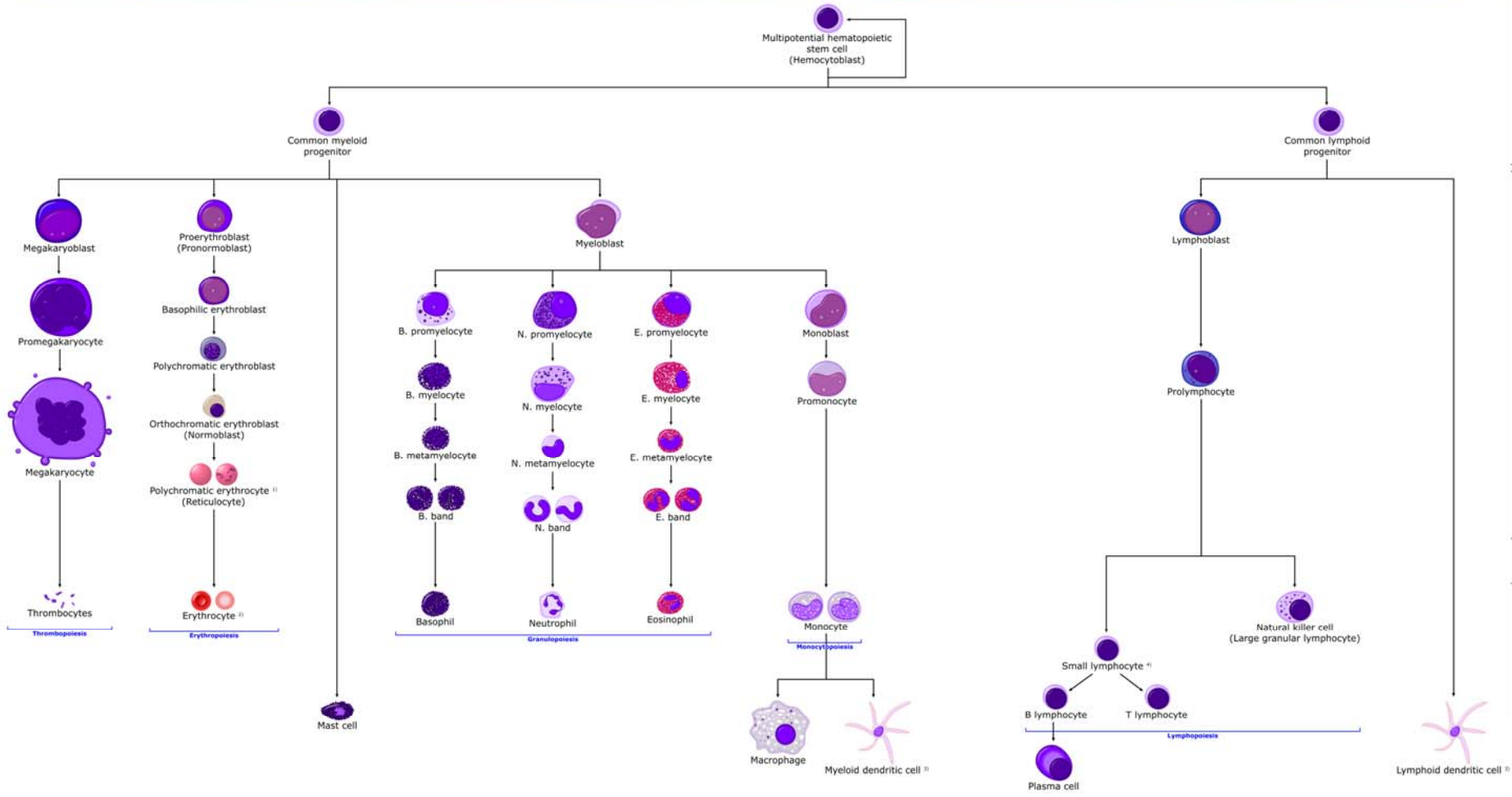


# Bone Marrow

- Red marrow
  - Functional haematopoiesis
- Yellow marrow
  - Predominantly fat
- Distribution is age defined
  - Contracts with increasing age
    - Adult – skull, pelvis, ribs, vertebrae and upper long bones
  - Increases with demand
- Varies with
  - Therapy
  - Disease



# Hematopoiesis in humans



Bone marrow

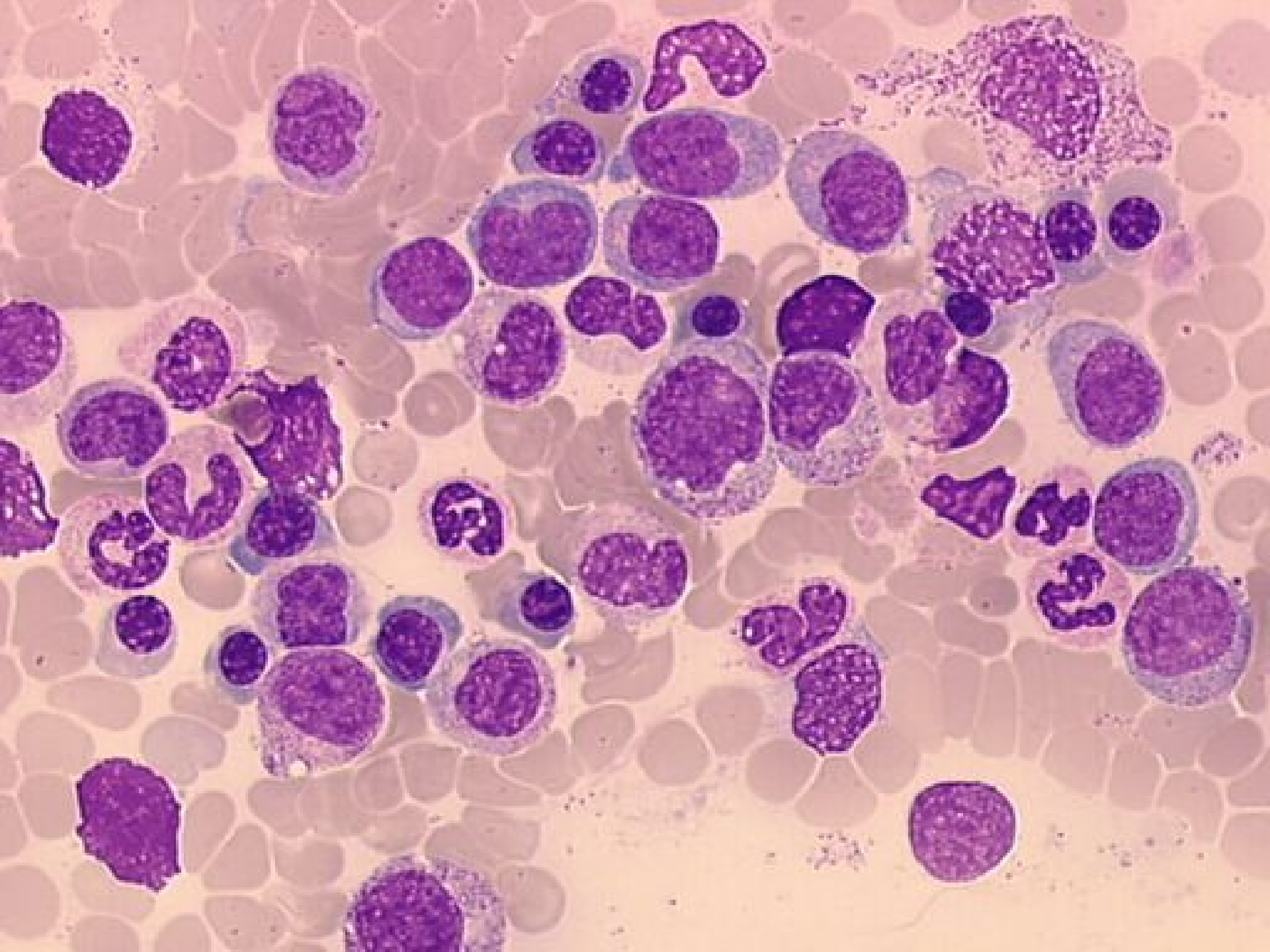
Blood

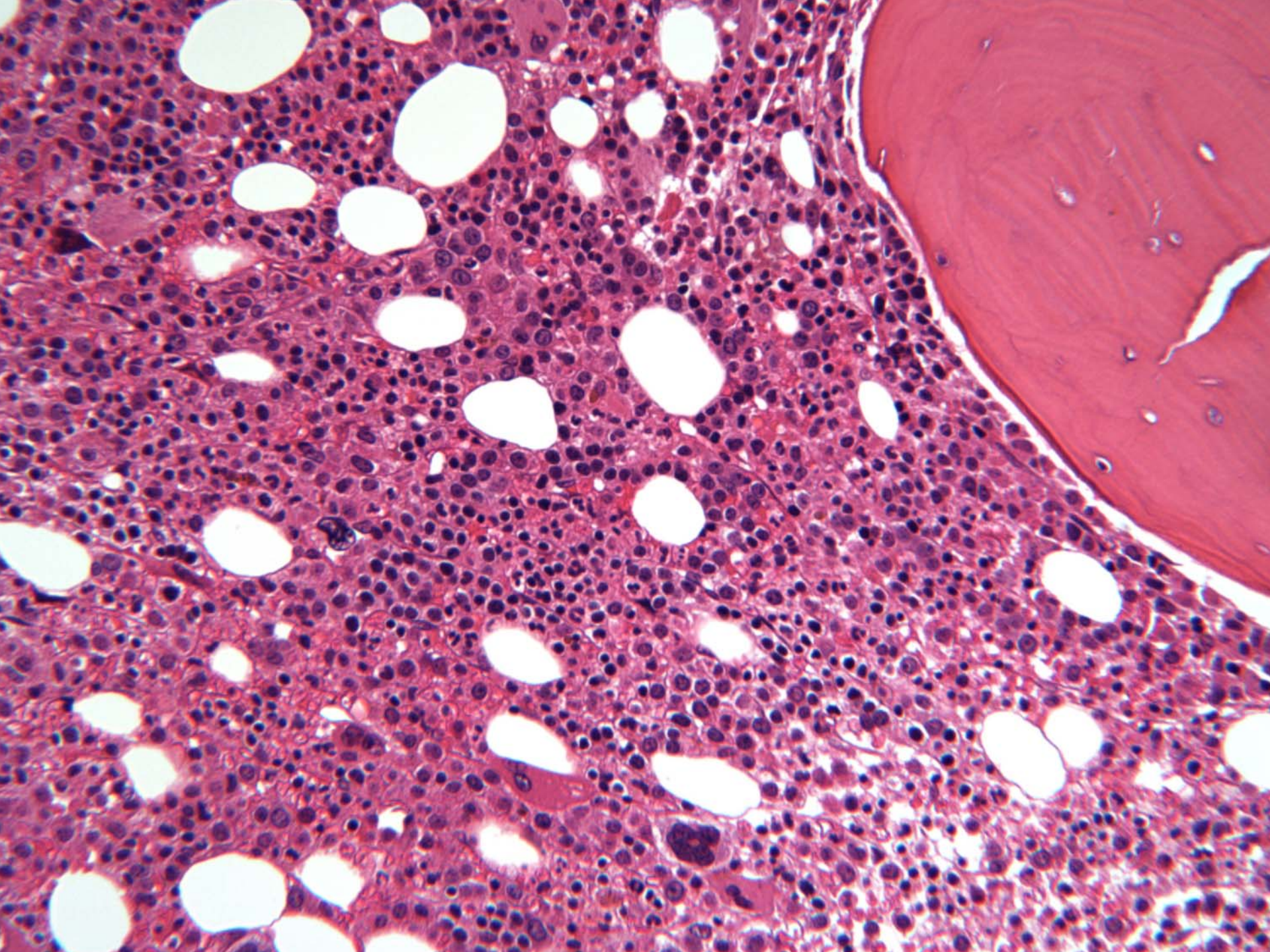
Tissue

Stem cell

Committed progenitor

Mature cell





# Haematopoietic stem cell transplantation

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- Allogeneic
    - Bone marrow
    - Peripheral blood progenitor cells
    - Umbilical cord progenitor cells
  
  - Autologous
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# Haematopoietic stem cell transplantation

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- Allogeneic
  - Myeloablative
  - Non-myeloablative (“mini-allo”)
    - Reduced intensity of immunosuppression
    - More reliance on graft-vs-tumour effect
    - Persistent mixed-chimerism
    - Diminished transfusion requirements
  - PBSCT c/w BMT – diminished transfusion requirements – especially for platelet support
  
- Autologous
  - Reduced intensity of pre-treatment
  - Reduced risks of infection during the immunocompromised period
  - Rapid haematopoietic reconstitution
  - Diminished transfusion requirements



# Some Indications

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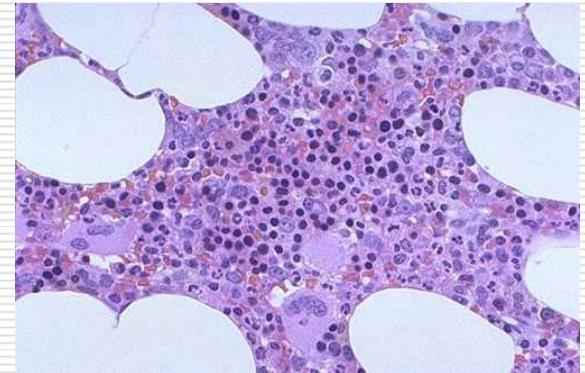
- Acute leukaemia
- Lymphomas
- Primary immunodeficiency disorders
- Auto-immune disorders
- Myeloma
- Chronic myeloid leukaemia
- Aplastic anaemia
- Sickle cell disease
- Beta thalassaemia major
- Some solid tumours – Ewings, Neuroblastoma, Choriocarcinoma
- Myelodysplastic disorders
- Myeloproliferative disorders
- Amyloidosis
- Storage disorders



# Haematopoietic reconstitution

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- Transfusion dependency affected by:
  - Intensity of myeloablation
  - PBSC vs BM
  - Dose of CD34+ cells



# Transfusion in HSCT

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- Permissive / Supportive
- However, inappropriate transfusion support might compromise transplantation outcome
  - TA-GVHD
  - HLA alloimmunisation
  - Red cell alloimmunisation
  - Immunomodulation and infection
  - TRALI
  - Morbidity related to consequences of ABO and other RBC antigenic mismatch
  - Venocclusive disease



# History of TA-GVHD

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- 1916: syndrome in chicks after injection of cells from adult chicken spleens & bone marrow into chick embryos - development of enlarged spleen & disseminated nodules
- 1950s: Simonsen similar experiments in chickens & mice & interpreted as cw GVHD
- 1955: Shimoda described condition called POE - post-operative erythroderma - 1st report of Ta-GVHD
  - 12 pts skin rash & high fever 6-13d after surgery. Half died.
- 1965: similar syndrome in 2 children with congenital immunodeficiency



# Blood products associated with Ta-GVHD

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- All cellular blood products:
  - red cells
  - platelets
  - granulocytes
  
- ?? issue of FFP: contains viable immunocompetent T lymphocytes & has been implicated. Not irradiated routinely



# Risk factors for the development of Ta-GVHD

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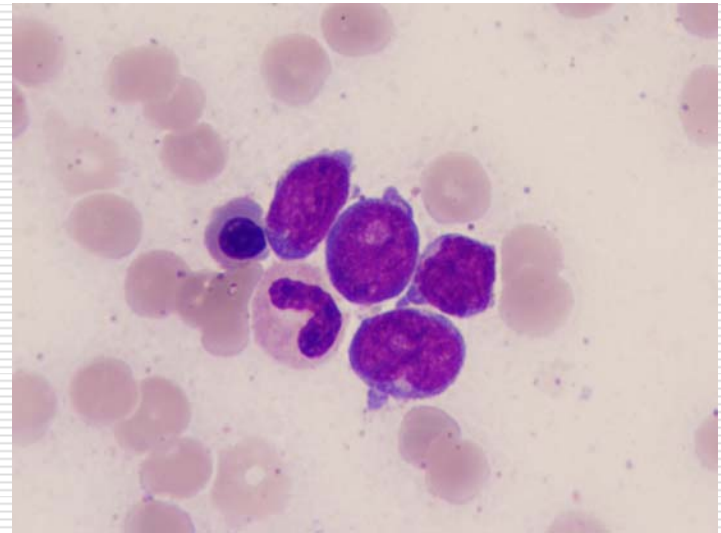


- Significantly increased risk
    - Congenital immunodeficiency syndromes
    - Bone marrow transplantation (Allogeneic and autologous)
    - Transfusions from blood relatives
    - Intrauterine transfusions
    - HLA-matched platelet transfusions
    - Hodgkin Lymphoma
    - Patients treated with purine analogue drugs
-

# Haematology patients and TA-GVHD

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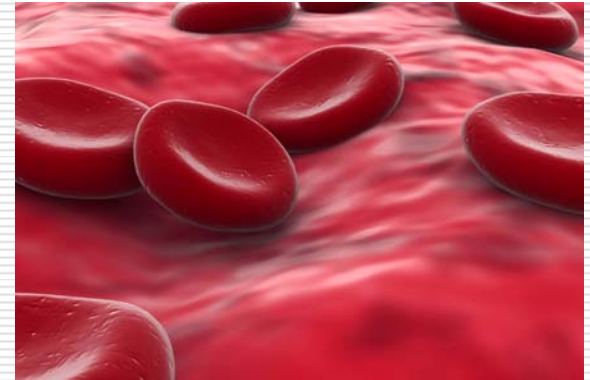
- Haematological malignancies
  - Hodgkin lymphoma
  - Acute leukaemia Rx with intensive chemo have been reported
  - NHL lesser risk - but has been reported
  - CLL Rx with purine analogues eg fludarabine
  
- Bone marrow transplant patients
  - Allo BMT: routine irradiation
  - Auto BMT: NOT routine irradiation in/for many centres



# Age of blood and TA-GVHD Risk

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- Different transfusion practices
  - Japan: use of warm, fresh blood <24hrs old
- Majority of reported cases Ta-GVHD occur with blood <4 days old
  - Ohto & Anderson (1996) - review of Japanese cases - 62% occurred with blood <72 hrs old
  - Petz et al (1993) reported in USA - about 90% of cases of Ta-GVHD transfused blood ws <4d old



# Clinical picture Ta-GVHD

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- ❑ Similar to GVHD after BMT
  - ❑ Several differences - time of onset, presence of marrow hypoplasia & course of disease
  - ❑ Both - fever, rash, liver dysfunction & diarrhoea
  - ❑ Ta-GVHD onset earlier
  - ❑ Fever >38 presenting sx - early as 4d after tx, median onset 10d
  - ❑ Rash - trunk, extremities
  - ❑ Variable liver dysfn
  - ❑ Leucopenia & pancytopenia later (median 16d)
  - ❑ MR: >90% Death: infection
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# Diagnosis of TA-GVHD

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- Clinical & relevant lab findings:
  - leucopenia & pancytopenia
  - abnormal LFTs
- Biopsy findings:
  - skin: eg epidermal basal cell vacuolization (grade I), mononuclear cell infiltration epidermal basal layer(grade II), bulla formation (grade III)
  - liver: small interlobular & marginal bile ducts
  - +/- bone marrow aspirate/biopsy: hypocellular or aplastic with a lymphocytic or histiocytic infiltration
- HLA typing :donor cells or DNA in patient's circulation or in cellular infiltrates in association with clinical picture.

ID of additional or different HLA antigens to those in patient confirms engraftment of transfused cells.



# Diagnosis of TA-GVHD

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- ❑ Presence of donor lymphocytes ALONE without the clinical picture is NOT indicative of Ta-GVHD
  - ❑ Mixed microchimerism ie presence of both host & donor cells has been shown to exist in some cases years after a blood transfusion. State of tolerance developed & NO evidence GVHD
  - ❑ **Diagnosis: Clinical features AND evidence of blood donor lymphocyte engraftment**
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# Irradiation - dosing

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- ❑ Dose: a decrease in T cells of  $>2$  log is needed to prevent GVHD
- ❑ studies to determine optimum radiation dose to inhibit lymphocyte proliferation with NO significant adverse effect on red cell, platelet or granulocyte function
- ❑ Doses 2 500 - 3000 cGy shown to completely inhibit T-cell proliferation (Pelszynski et al 1994)
- ❑ Dose 2500 cGy recommended



# Gamma irradiation

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- ❑ Induces cross-linking of the DNA in the “contaminating” lymphocytes of the red cell or platelet product
- ❑ Psoralens may produce similar cross-linking. Evidence not sufficient re the equivalence to gamma irradiation for TA-GVHD avoidance
- ❑ Thus prevents lymphocyte proliferative response
- ❑ Necessary for pre/peri and post HSCT care
- ❑ No data re how long post is long enough
- ❑ Also need to avoid transfusion of non-irradiated components into planned donors



# Effect of irradiation on blood components

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- Red cells
  - decreased red cell survival in irradiated blood ONLY after prolonged storage
  - Davey et al, 1992: red cells irradiated on d 0 with 3000 cGy stored in an additive preservative solution for 42d. Mean 24h recovery of red cells 68% versus 78% in non-irrad
  - increases level of extracellular potassium
  
- Platelets
  - no significant changes in platelet function
  
- Granulocytes
  - evidence for irradiation damage to granulocyte function conflicting



# Inventory disadvantages of irradiation

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- Reduced shelf life red cells - 14 days from irradiation. (can be irradiated up to 14 days after collection)



**Table II.** Comparison of radiation guidelines, including methods and indications.

	<b>Britain<sup>1</sup></b>	<b>US<sup>2</sup></b>	<b>Japan<sup>3</sup></b>
<b>Techniques</b>	<b>Irradiation</b>	<b>Irradiation</b>	<b>Irradiation</b>
<b>Dose</b>	<b>Minimum –2500 cGy</b> No part > 5000 cGy	<b>2500 cGy at centre of product</b> Minimum 1500 cGy at any point	<b>Between 1500 cGy and 5000 cGy</b>
<b>Type of product</b>	<b>All cellular products:</b> Whole blood RBCs Platelets Granulocytes	<b>All cellular products:</b> Whole blood RBCs Platelets Granulocytes	<b>All cellular products:</b> Whole blood RBCs Platelets Granulocytes Fresh plasma
<b>Age of product</b>	RBCs < 14 d after collection Platelets – any time during 5 d storage For exchange or intrauterine transfusion: < 24 h	RBCs – any time Platelets Granulocytes	RBCs: ≤ 3 d – regardless of recipient ≤ 14 d – if clinically indicated at any time – if patient immunocompromised
<b>Expiration</b>	RBCs stored 14 d after irradiation	RBCs stored up to 28 d after irradiation or original outdate, whichever is sooner	Irradiated RBCs – up to 3 weeks after collection
<b>General</b>	All blood from relatives All HLA selected products All granulocytes	All blood from relatives All HLA selected products	All blood from relatives All HLA selected products
<b>Neonates</b>	Intrauterine transfusions (IUT) exchange transfusions in IUT babies	Intrauterine transfusions	Intrauterine and exchange transfusions
<b>Top-up transfusion</b>	IUT babies	*	Pre-term
<b>SCID</b>	All	All	All
<b>BMT – Allogeneic</b>	All – at least 6 months post BMT; longer in selected patients	All	All
<b>Autologous</b>	All – at least 3 months post BMT; 6 months if TBI used		
<b>Leukaemia</b>	No	*	To be considered
<b>Hodgkin's disease</b>	All stages	*	To be considered
<b>Purine analogues</b>	All	*	Not discussed
<b>Non-Hodgkin's lymphoma</b>	Not necessary – under review	*	To be considered
<b>Solid tumours</b>	No	*	To be considered
<b>Solid organ transplants</b>	No	*	To be considered
<b>Aged &gt; 65 years</b>	Not discussed	No	Yes
<b>Massive blood loss</b>	Not discussed	No	Yes
<b>CV surgery</b>	No	No	Yes
<b>AIDS</b>	No	No	No

# Royal Melbourne Hospital Irradiation Guidelines

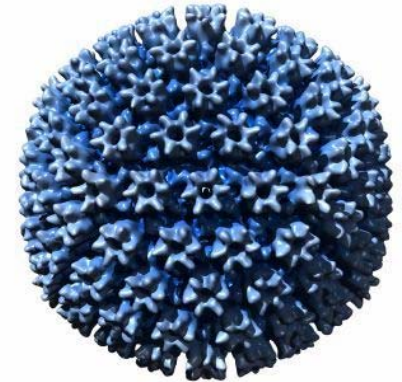
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- Patients receiving directed blood donations from family members
  - Recipients of HLA-matched single donor platelets
  - Recipients of granulocyte transfusions
  - Bone marrow or stem cell transplant recipients
  - Patients planned for bone marrow/stem cell transplantation.
  - Patients undergoing chemotherapy for acute leukaemia
  - Aplastic anaemia
  - Hodgkin lymphoma
  - Patients receiving nucleoside analogue therapy e.g. cladribine, fludarabine
-

# Infections which may be sometimes be leukocyte-borne

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- ❑ **CMV**, HIV-1/2, HTLV-1/2, EBV
- ❑ Yersinia
- ❑ Trypanosomiasis
- ❑ Toxoplasmosis
- ❑ ? Prions - ? in passengers  
B lymphocytes



# Cytomegalovirus infection in immunocompromised settings

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- Serious morbidity
    - Pneumonitis
    - Hepatitis
    - Myelosuppression
    - Retinitis
    - Cardiac and renal toxicity
-

# Difficulties with CMV serology & transfusion risk assessment

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- ❑ Very high seroprevalence of IgG positivity
  - ❑ IgG, IgM & total Ig as a predictor of risk in donor
  - ❑ Only < 1% of leucocytes from seropositive donors may be infected
  - ❑ Urinary versus serum sampling
  - ❑ PCR methodologies
  - ❑ Re-activation & “second strain” patterns
-

# Reducing transfusion related CMV

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- CMV negative cellular blood products
    - difficulties with testing interpretation
    - effects on blood supply & inventory
  - Leukodepletion
    - Leucofiltration
      - Bedside
      - "In house" (in Blood Centre)
    - Buffy coat poor products
  - Patient treatments
    - Gancyclovir
    - Anti-CMV immunoglobulins
-

## Leucodepletion filters in CMV prophylaxis

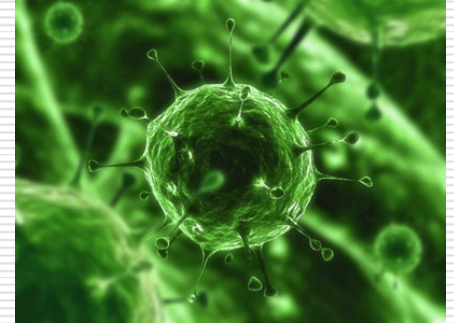
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- ❑ Many major trials demonstrate equal efficacy c/w use of CMV negative products
  - ❑ Possibly extra benefit with CMV negative products in neonates & some CMV negative transplant recipients
  - ❑ Need at least 3 log reduction in WBC's to achieve prophylaxis (aim for  $< 5 \times 10^6$  WBC's)
-

# Optimisation for CMV seropositive HSCT patients

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- ❑ Role for preemptive antivirals (gancyclovir / foscarnet)
- ❑ Quantitation of CMV antigenaemia and semi-quantitative CMV PCR
- ❑ Highest risk if seropositive recipients of T-lymphocyte depleted (or cord) grafts, worse with HLA mismatched
- ❑ These patient outcomes may be additional compromised by the anti-CMV Rx



# CMV seronegative HSCT patients

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- If given CMV negative products
    - Avoidance of the myelosuppressive and nephrotoxicity of anti-CMV Rx
    - Leukodepleted products not completely equivalent (controversy)
    - No clarity in literature on the length of time that the CMV negative support should continue post transplantation
-

# Survival of donor cells 25 years after intrauterine transfusion

Vietor HE, Hallensleben E, van Bree SP, van der Meer EM, Kaal SE, Bennebroek-Gravenhorst J, Kanhai HH, Brand A, Claas FH

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□ *Blood* 2000 Apr 15; 95(8):2709-14

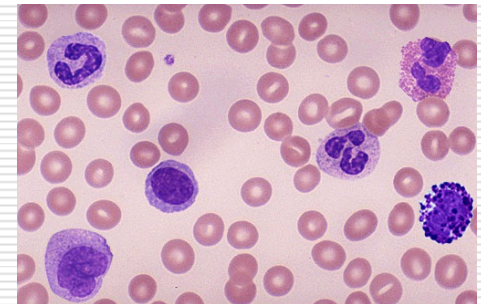
- Twenty-four surviving IUT recipients between 1966 and 1976 were tested for autoimmune disease and autoantibodies at follow-up
  - Seven female recipients could be tested for chimerism using a Y- chromosome-specific polymerase chain reaction (PCR) because they received at least 1 IUT from a male donor
  - Y-chromosome-specific sequences were detected by PCR in 6 of the 7 women.
  - The current study provides evidence that IUT can result in the persistence of donor cells in the recipient for a period longer than 20
-

# Leukodepletion

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## Potential Adverse Consequences of Leukocytes in Blood Components

- Alloimmunisation
- Immunomodulation
- Graft-Versus Host Disease
- TRALI
- Leukocyte borne infections
- Impaired Quality of Stored Blood



# Transfusion-Related Alloimmunisation

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- ❑ Against HLA and leukocyte specific antigens
  - ❑ Febrile non-haemolytic transfusion reactions - especially with platelets
  - ❑ Refractoriness to platelet transfusions
  - ❑ Potentially reduces success of transplantation procedures
  - ❑ Non-ABO haemolytic transfusion reactions
  - ❑ Shortening of transfused cell survival
-

# Platelets for transfusion

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Preparations available:

- ❑ separated from whole blood:
  - made from platelet-rich plasma or buffy coat pools
- ❑ collected by apheresis technology

Stored at 20-24C for up to 5 days:

- ❑ viability
  - ❑ function
  - ❑ risks:
    - WBC metabolism → cytokine production
    - bacterial proliferation
-

# Apheresis platelets

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- Donor's WB separated by machine, platelets/plasma collected, and other components returned to donor
- Platelets suspended in plasma:
- Special features
  - can split one unit very low WBC can HLA-match if required
  - expensive to collect: staff, equipment, time, donors
  - donor issues: recruitment, CMV status, risks of procedure



# Why is bacterial contamination of platelet transfusions such a problem?

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- ❑ Platelets stored at 20-24C for up to 5 days to maintain viability and function
  - ❑ This can permit:
    - Cytokine accumulation
    - (IL-1 $\beta$ , IL-6, IL-8, TNF- $\alpha$  etc)
    - Bacterial proliferation
  - ❑ Refrigerated storage not (yet) available
-

# Reticulated platelets

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- *Bone Marrow Transplantation* (2007) **39**, 501–507, 2007
  - **Immature platelet fraction for prediction of platelet engraftment after allogeneic stem cell transplantation**
  - A Takami et al
  - Reticulated platelets (IPF) by flow
  - Rise of the IPF relates to early trilineage engraftment
-

# Thrombopoietin (TPO)

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- ❑ 1990 viral oncogene v-Mpl described in murine myeloproliferative leukaemia
  - ❑ c - Mpl mRNA - present in platelets, MK's and CD34+ cells
  - ❑ c - Mpl ligand cloned - had properties of TPO
  - ❑ TPO synthesized in the liver - 353 a.a. then cut to 332 and glycosylated
  - ❑ First 123 a.a. have homology with human EPO
-

# Effects of Mpl Ligands

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- ❑ Act in very early haematopoiesis on pluripotent cells
  - ❑ Late maturational effect only on MK's
  - ❑ Increase size, number and ploidy of MK's
  - ❑ Increase number of Meg-CFC's and GEMM-CFC's
-

# Clinical Studies of Mpl-Ligands

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- Initial randomized dose escalation PEG-rHuMGDF studies in cancer patients
  - ↑ MK number, size, nuclear lobulation, platelet counts
  - rHuTPO after chemotherapy for NSCLC
    - ↑ platelet nadir
    - platelet recovery foreshortened from 21 to 14 days
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# Clinical Studies (continued)

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- rHuTPO following high-dose chemotherapy for gynaecological cancer → 23 vs 50% platelet transfusion requirements
  - In patients having PBPC transplants after high-dose therapy for breast cancer - no reduction in platelet transfusions
  - II-11
    - to patients following high-dose therapy for breast cancer
      - 1 CVA
      - 30% vs 96% requirement for platelet transfusion
-

# Adverse Effects of Mpl-Ligands

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- ❑ Rare injection site reactions
  - ❑ Fatigue, arthralgias, headache, nausea (mild to moderate only)
  - ❑ 1 CVA (platelet count not elevated)
  - ❑ 10% of volunteers developed antibodies to PEG-rHuMGDF
  - ❑ ? Potential of malignant stimulation with some CD34 + cells expressing Mpl receptors
  - ❑ Mild anaemia with IL-11 due to  $\uparrow V_D$
-

# Platelets and bleeding risk

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- ❑ Persistent counts  $<10$  associated with significant bleeding risk
  - ❑ Soft data re the effect of concurrent degree of anaemia and the bleeding risk
  - ❑ Bleeding in HSCT is often multi-factorial – thrombocytopenia, mucositis, line related, infection related, haemorrhagic cystitis, DAD
-

# Veno-occlusive disease with HSCT

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- ❑ More likely with ABO mismatch
  - ❑ Case reports post minor-side platelet transfusion mismatch in transplanted children
  - ❑ VOD itself is associated with an increased platelet transfusion requirement
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# Granulocytes transfusion in HSCT settings

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- ❑ Variable practice in fungaemia
  - ❑ Significance of potential for CMV transmission
  - ❑ Often use stimulated apheresis donors
  - ❑ Increase risk of HLA alloimmunisation
  - ❑ Need for gamma irradiation
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# Historical Background

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- First described in 1950s
    - Non-cardiogenic lung oedema as result of blood transfusion (Barnard 1951)
  - Early 1970s – Leukoagglutinins to HLA and non-HLA antigens postulated as aetiological agents
    - (Ward 1970, Thompson *et al* 1971)
  - 1983 – Popovsky *et al* recognised this transfusion reaction as a distinct clinical entity → “TRALI”
  - 1985 – Popovsky & Moore analysed 36 patients and proposed minimum criteria
    - Acute respiratory distress, new bilateral lung infiltrations in CXR within 6h of blood transfusion; absence of volume overload or cardiac malfunction
-

# TRALI risk

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- ❑ Higher in HSCT (soft data)
  - ❑ Especially at time of commencement of engraftment
  - ❑ Cryoprotectants may also cause infusional pulmonary toxicities
-

# Historical Background

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- 1997 – Silliman *et al* reported association of biologically active lipids with development of TRALI
  - 2003-05 – consensus guidelines
    - TRALI Consensus Conference Committee 2004 and European Haemovigilance Network
    - 2003 – National Heart Lung and Blood Institute / NIH
-

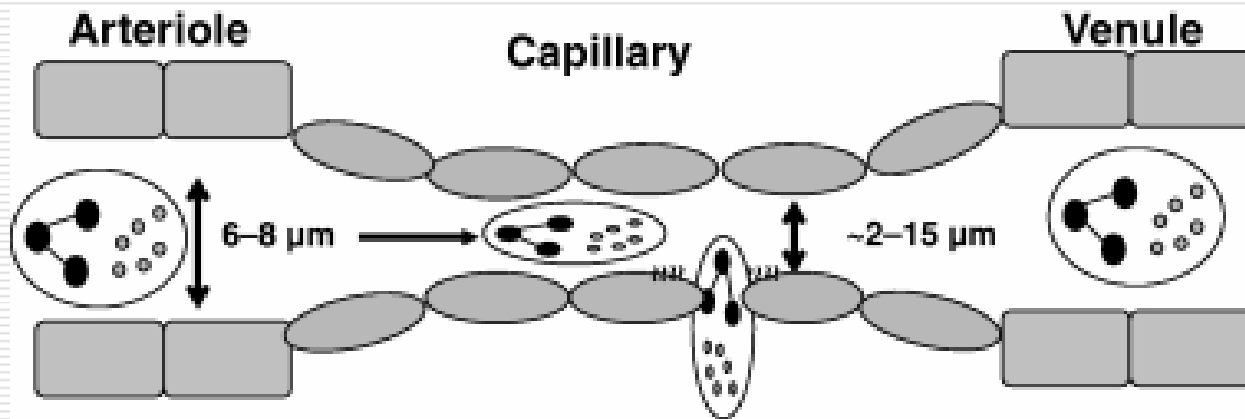
# Criteria for TRALI

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- TRALI Consensus Conference Committee 2004 and European Haemovigilance Network
    - Acute respiratory distress
    - Bilateral lung infiltrations in CXR
    - Occurrence during / within 6h after completion of transfusion
    - No evidence of TACO/cardiogenic lung oedema
    - Hypoxaemia ( $\text{PaO}_2/\text{FiO}_2 < 300\text{mmHg}$  or  $\text{O}_2$  saturation  $< 90\%$  or other clinical evidence
    - New acute lung injury and no other ALI risk factors
    - If one or more ALI risk factors present, possible TRALI should be diagnosed
-

# Neutrophil Passage in TRALI

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Bux *et al*, 2007 *Br J Haematol* 136;788-799

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# Priming and activation of neutrophils

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## Priming due to underlying co-morbidity

- Recent surgery, active infection, cardiovascular disease, leukaemic infiltrate identified as additional risk factors
  - Variety of neutrophil priming agents released by dying / necrotic cells or stimulated endothelial cells, monocytes or lymphocytes:
    - PAF, TNF- $\alpha$ , IL-8, G/M-CSF, IFN- $\gamma$ , infectious agents (influenza A virus, bacteria-derived LPS)
-

# Priming & activating substances in blood components

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- Substances in blood components induce TRALI by priming / activating neutrophil or endothelial cells of pulmonary vasculature
    - Leucocyte antibodies to human neutrophil antigens or human leucocyte antigens
    - Bioactive lipids
    - Other factors – CD40-ligand (CD40L), immune complexes
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# Blood components

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## Antibodies to HNA

- HNA-1a, -1b, -2a, -3a (?HNA-4a)
  - Sachs *et al*, Blood, 2006
    - HNA-2a expression heterogeneous in humans with large subpopulation (70%) or small subpopulation (30%)
    - Used neutrophils from healthy donors in rat lung model
    - Application of corresponding Ab induced TRALI if HNA-2a present on majority of neutrophils (70%)
    - If HNA-2a present on minority of cells (<30%), no TRALI reaction induced
    - fMLP added (mimics activity of bacterially derived peptides; approximates active infection) → TRALI induced in presence of both types of neutrophils
  - Therefore induction of TRALI depends on appropriate density of Ag, but additional stimuli can overcome inability of initial stimulus to activate neutrophils
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# Blood components

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## Antibodies to HLA

### □ HLA-Class II Ab

- Biological mechanism of TRALI induction uncertain
  - ?binding of Ab to monocytes with subsequent release of cytokines and neutrophil activation
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# Blood components

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## Bioactive Lipids

- Breakdown products of membrane lipids
    - E.g. lysophosphatidylcholine species
  - Act on neutrophils through PAF receptors to prime respiratory burst reaction
  - Do not develop in stored acellular plasma
    - Generation is dependent on presence of blood cells
-

# Blood components

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## Other Factors

- CD40-ligand (CD40L)
    - Neutrophil-priming breakdown product
    - Platelet-derived proinflammatory mediator
    - Accumulates in platelet concentrates during storage
    - Binds CD40 (present on surface of monocytes, macrophages and neutrophils)
    - CD40L primes neutrophils through CD40, which are then able to be activated
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# IVIg in BMT

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- Through the 1980's and early 90's several trials showed IVIg to reduce GVHD, interstitial pneumonia and infections following allogeneic BMT
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# IVIg and CMV

BMT 1993;12:273-282

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- Revealed significant reductions in
    - Fatal CMV infections,
    - CMV pneumonia,
    - Non-CMV interstitial pneumonia
    - Transplant related mortality
    - Acute GVHD (RR=0.68)
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# IVIG

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'High-Dose weekly Intravenous Immunoglobulin to prevent infections in patients undergoing Autologous Bone Marrow Transplantation or severe myelosuppressive therapy: a study of the American Bone Marrow Transplant Group'

Ann Intern Med 1993, 118(12):937-942

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# IVIG

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## **'Should Immunoglobulin Therapy be used in Allogeneic Stem-cell Transplantation'**

**A randomized, double-blind, dose effect, placebo  
controlled, multicenter trial**

**Ann Intern Med 2003;139:8-18**

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# Study design

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- 19 centers of the Société Française de Greffe de Moëlle in France enrolled Jan 98-Apr 00
  - 200 patients having **HLA-identical sibling allogeneic stem-cell transplants**
  - >2yrs, 1<sup>st</sup> transplant, Myeloablative conditioning, No T-cell depletion.
  - Excluded previous BuCy or TBI autografts, hypogammaglobulinaemia (<4g/l) at enrollment or HIV+
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# Conclusions from meta-analyses

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- No significant benefit from administration of immunoglobulin during the first 3 months after HLA-matched sibling allogeneic stem-cell transplant
  - Trend for more severe VOD with high dose IVIG may outweigh a possible benefit in preventing aGVHD
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# Red cell alloimmunisation

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- **Clinical predictors of alloimmunisation after red blood cell transfusion**
  
  - Transfusion. 47(11):2066-2071, November 2007.  
*Bauer, Martijn P.; Wiersum-Osselton, Jo; Schipperus, Martin; Vandenbroucke, Jan P.; Briet, Ernest*
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# ABO mismatched HSCT

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- ❑ Some studies report delayed rbc engraftment time
  - ❑ Some studies report delayed platelet engraftment time
  - ❑ Slightly increased risk of veno-occlusive disease complications
  - ❑ Possible reduced graft survival time in 2 studies
  - ❑ No negative impact on patient survival demonstrated in studies
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# Major and minor side ABO incompatibility

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- Red cell depletion of graft pre transplant
    - Centrifugation / +/- density gradient separations
    - Hetastarch separation
  - Role for recipient isohaemagglutinin titres
  - Plasma exchange may be offered in high-titre recipients
  - Reduce plasma volume for mini-side mismatch donation
  - Bi-directional ABO mismatch
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# A difficult month

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- ❑ For example:
  - ❑ Start out as A
  - ❑ Receive a B transplant
  - ❑ Middle of month – mixed field reactions in front group
  - ❑ End of month – engraftment of B red cells
  - ❑ Importance of back-ground monitoring
  - ❑ Red cell vs plasma product support protocols
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# Predictors of likely rbc alloimmunisation

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- females
  - diabetes mellitus
  - solid malignancy
  - previous allogeneic PBPC transplantation
  - lower incidence in patients with lymphoproliferative disorders
-

# A2 variants

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- ❑ Described by von Dungren & Hirszfeld – 1911
  - ❑ Essentially a quantitative variant
  - ❑ Different antigenic densities
  - ❑ Different transferase Km values
  - ❑ Group A people – 78% A1, 22% A2
  - ❑ Similar with A1B & A2B
  - ❑ 1 to 8% of A2s have anti-A1
  - ❑ 35% of A2Bs have anti-A1
  - ❑ A1 cells agglutinate with Dolichos biflorus lectin
-

## Number of A and B Antigen Sites per Red Cell of Adults and Newborn Infants of Different ABO Phenotypes (from Economidou et al (8))

Phenotype	Number
A <sub>1</sub> Adult	810,000 to 1,170,000
A <sub>1</sub> Cord	250,000 to 370,000
A <sub>2</sub> Adult	240,000 to 290,000
A <sub>2</sub> Cord	140,000
A <sub>1</sub> B Adult	460,000 to 850,000 A sites 310,000 to 560,000 B sites
A <sub>1</sub> B Cord	220,000 A sites
A <sub>2</sub> B Adult	120,000 A sites
B Adult	610,000 to 830,000

## Variation in clinical haemolysis in patients with the same titre – Why?

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- Variable binding of anti-A to recipient red cells
  - Variations in antigenic density
  - Variations in antigen structure
  - Variable degrees and rates of clearance of antibody coated red cells
    - Activity of the reticulo-endothelial system
    - Variable complement binding and lytic activity
    - Variations in splenic function
  - Possible unrecognised mild red cell membrane defects
  - Differences in titre methodology
  - Differences in definition of the cut-off which defines the titre
  - IgG vs. IgM
  - Secretor status of recipient
  - Antibodies vs. haemolysins
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# Antibody Titres - Methods

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**Method:**

**Mainly looks at:**

RT (room  
temperature)

Cold reacting IgM

37°

Warm reacting IgM

IAT

Warm reacting IgG

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**RMH Transfusion Laboratory  
Titration Worksheet**

Patient name:

UR no.:

SID:

Date: 28/11/15

Ward:

Unit:

ABO/Rh: O POS

Antibody screen: E

Performed by:

**Different titre methods & Parallels**

Note: Always use the strongest (homozygous) expression of the antigen for titres. Eg. Jk(a+b-) for an anti-Jka or R2R2 for an Anti-E

Antibody specificity	Cells Used	Phase	Reactions													4096	Neg ctrl	Titre
			Neat	2	4	8	16	32	64	128	256	512	1024	2048				
Antibody 1: A 28/11/15 serum	Cell#: Donor	Room temp. (30 min)	12	12	12	12	12	10	8	8	3	0	0	0	0	0	256	
	Batch: 28/11/15	37oC (30 min)	12 <sup>H++</sup>	12 <sup>H</sup>	12	12	10	10	8	3	0	0	0	0	0	0	128	
	Expiry:	IAT	12	12	12	12	12	10	10	8	5	3	3	0	0	0	1024	
		Validation																
	Is the antibody haemolytic? YES / NO																✓	✓
Antibody 2: parallel. A 22/11/15 serum	Cell#: Donor	Room temp. (30 min)	12	12	12	12	12	10	8	5	3	0	0	0	0	0	256	
	Batch: <del>28/11/15</del> 28/11/15	37oC (30 min)	12 <sup>H++</sup>	12 <sup>H</sup>	12	12	10	10	5	3	0	0	0	0	0	0	128	
	Expiry:	IAT	12	12	12	12	12	10	10	8	5	3	3 <sup>w</sup>	0	0	0	1024	
		Validation																
	Is the antibody haemolytic? YES / NO																✓	✓

\*. DONOR cells -

# Titre methods – other issues

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- ❑ Enhancers or not in the Coomb's method (e.g. RAM)
  - ❑ Valency of the Coomb's reagent (e.g. anti-human IgG only)
  - ❑ Serum vs plasma
  - ❑ DTT treatment of the serum
  - ❑ Definition of the titre
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# Different primary titre methodologies

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- Test-tubes
  - Micro-column technology
    - Diamed gel system
    - Biovue solid phase system
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# Different scoring systems for agglutination reactions

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Scoring out of 12 usually  
used with test-tube  
systems

e.g. 0, 3, 5, 8, 12

Scoring out of 4 usually  
used with micro-column  
technology

e.g. 0, 1, 2, 3, 4

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# Titres vs Haemolysins

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- Power of titres alone to predict in-vivo haemolysis / tissue damage
- Recording of presence of haemolysis in-vitro with titre tests

vs.

- Formal haemolysin testing
    - Different incubation times
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# Definitions of titres vary

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- Reciprocal of highest doubling dilution to demonstrate a positive reaction (i.e.  $>0$ )

OR

- Reciprocal of highest doubling dilution to demonstrate a reaction strength  $\geq 5$  or 8 in test tubes (1 or 2 in microcolumns)
-

**RMH Transfusion Laboratory  
Titration Worksheet**

**Definition of highest titre**

Patient name:

UR no.:

SID:

Ward:

ABO/Rh: C

Performed by: *[Signature]*

Date: 28/11/15

Unit:

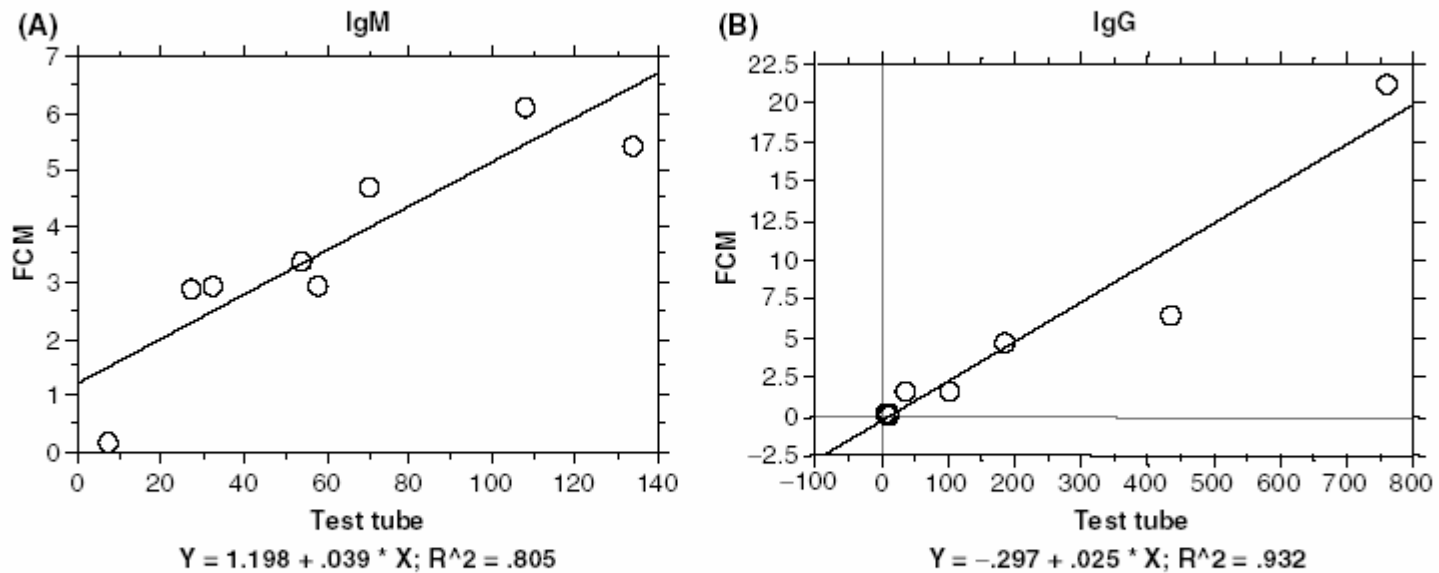
y screen: E

Note: Always use the strongest (homozygous) expression of the antigen for titres. Eg. Jk(a+b-) for an anti-Jka or R2R2 for an Anti-E

Antibody specificity	Cells Used	Phase	Reactions													Titre	
			Neat	2	4	8	16	32	64	128	256	512	1024	2048 ....	4096		Neg ctrl
Antibody 1:  A 28/11/15 serum	Cell#: Donor	Room temp. (30 min)	12	12	12	12	12	10	8	8	3	0	0	0	0	0	256
	Batch: 28/11/15	37oC (30 min)	12 <sup>H<sup>++</sup></sup>	12 <sup>H</sup>	12	12	10	10	8	3	0	0	0	0	0	0	128
	Expiry:	IAT	12	12	12	12	12	10	10	8	5	3	3	0	0	0	1024
		Validation													✓	✓	✓
Is the antibody haemolytic? <b>YES</b> / NO																	
Antibody 2: Parallel  A 22/11/15 serum	Cell#: Donor	Room temp. (30 min)	12	12	12	12	12	10	8	5	3	0	0	0	0	0	256
	Batch: <del>28/11/15</del> 28/11/15	37oC (30 min)	12 <sup>H<sup>++</sup></sup>	12 <sup>H</sup>	12	12	10	10	5	3	0	0	0	0	0	0	128
	Expiry:	IAT	12	12	12	12	12	10	10	8	5	3	3 <sup>2</sup>	0	0	0	1024
		Validation													✓	✓	✓
Is the antibody haemolytic? <b>YES</b> / NO																	

\*. DONOR cells -

**Kobayashi and Saito**



*Fig. 1.* Correlation between mean values measured by test tube and values measured by flow cytometry. Anti-A/B immunoglobulin M (IgM) and IgG antibody (Ab) titers were measured in serum samples from blood groups A, B and O (each  $n = 2$ ). Anti-A Ab titers ( $n = 4$ ) and anti-B Ab titers ( $n = 4$ ) are combined in each correlation chart of IgM (A) and IgG (B). Both IgM and IgG showed significant correlations between the values measured by test tube and by flow cytometry. The value of flow cytometry mean (FCM) on the vertical axis represents mean fluorescence intensity.

# Chain types

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- ❑ Many non-RBC ABO antigens on type IV chains
  - ❑ RBC ABO Ag on I, II, III, IV – especially II & III
  - ❑ Endothelia especially I & III, but not IV
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# Proteins carrying ABH

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- In endothelia ABH & alpha GAL Ags carried on:
    - vWF
    - Alpha 1, 3, 5 integrins
    - Beta 1, 3 integrins
  - Different density levels of group A determinants according to dominant chain subtype
-

# Patient identification

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- Identification of the proposed, or possible HSCT patient to the laboratory as soon as possible after diagnosis / clinical plan formed
  - Vitally important for other (e.g. rural or regional centres) offering transfusion support to these patients, away from the transplanting hospital
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# Pre-transplantation

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- For cellular products:
    - Leukodepleted
    - CMV negative
    - Irradiated
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# Transplant haemolysis

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- ❑ Immediate related to ABO mismatch
  - ❑ Emergence of transfusion or transplant induced red cell alloimmunisation (non-ABO directed)
  - ❑ DMSO – non-immune haemolysis
  - ❑ Sepsis related non-immune haemolysis
  - ❑ Important to sort out cause and distinguish from VOD, or GVHD, transplant related microangiopathy - which may share some features
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# Passenger lymphocytes

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- ❑ Donor derived lymphocytes – engrafting/persisting forming anti-red cell antibodies in the transplant recipient
  - ❑ Usually ABO type, but passenger lymphocyte haemolysis due to anti-Kidd, anti-Duffy, anti-Kell also described in post HSCT settings
  - ❑ Higher risk of passenger lymphocyte haemolysis syndromes in BMT, than in PBSCT
  - ❑ No solid data re expected higher rates in non-myeloablative regimens
  - ❑ Higher risk if the GVHD prophylaxis is without methotrexate
  - ❑ Classically occurring 1 to 2 weeks post transplantation
  - ❑ This haemolysis is often severe, persisting for 5 to 10 days
  - ❑ Innocent bystander haemolysis in this setting also to transfused red cell product
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# Pure red cell aplasia

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- ❑ Secondary to major ABO mismatch transplantation
  - ❑ Secondary to parvovirus infection
  - ❑ May occur early, or late (>100 days post BMT)
  - ❑ Isohaemagglutinin titre and reticulocyte counts inverse ratio
  - ❑ Although the original iso-haemagglutinins usually disappear by one month, can persist. Interpretation complicated by administration of IVIG Rx
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# Delayed AIHA post transplantation

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- ❑ Low incidence
  - ❑ Usually occurs 2 to 3 months post transplantation
  - ❑ More likely with T-cell depleted grafts
  - ❑ T cell dysregulation
  - ❑ Deleterious to graft survival
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# Potential Uses of Mpl-Ligands

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- ❑ To assist platelet recovery following chemotherapy
  - ❑ To improve platelet counts in ITP, HIV, MDS
  - ❑ Increased PBPC yields
  - ❑ Ex-vivo expansion protocols
  - ❑ Ex-vivo “bio-reactors”
  - ❑ To donors to increase platelet apheresis yields
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# Some Planning Implications

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- ❑ Need for supply & inventory planning as these procedures increase in number and with changing population demographics
  - ❑ Doesn't just affect hospitals that perform stem cell transplantation
  - ❑ Other hospitals may be providing part of the transfusion support/care of these patients
  - ❑ Need for highly specialised products in support of HSCT patients' care
  - ❑ Need for appropriately trained medical scientists and and transfusion specialist medical and nursing staff in support of these clinical activities
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Thank you