Haematology and blood transfusion

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Definition & function

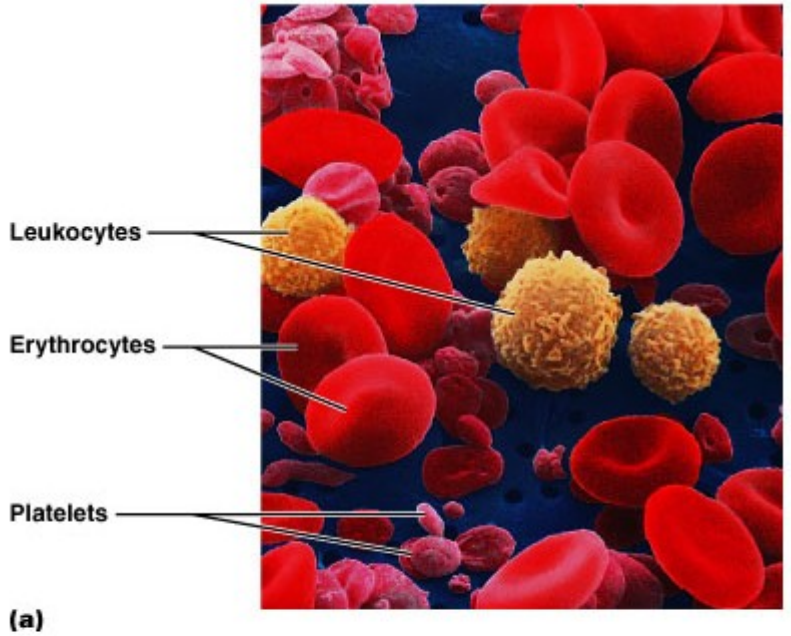
- the branch of medical science concerning blood and blood-forming tissues
- study of morphology, diagnosis, treatment, prognosis & prevention
- pathophysiology
  - Variations from normal blood element counts, function
  - Malignant disorders – leukaemia, lymphoma, myeloma
  - Haemoglobinopathies
- bone marrow and stem cell transplantation
- blood transfusion & blood banking

Organs & tissues

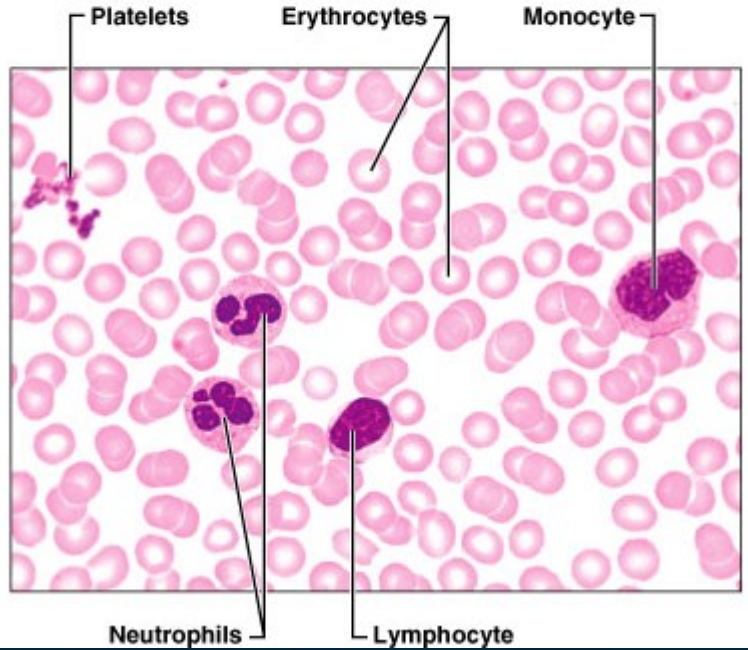
- peripheral blood
- bone marrow

- spleen
- lymph nodes
- liver

Scanning EM



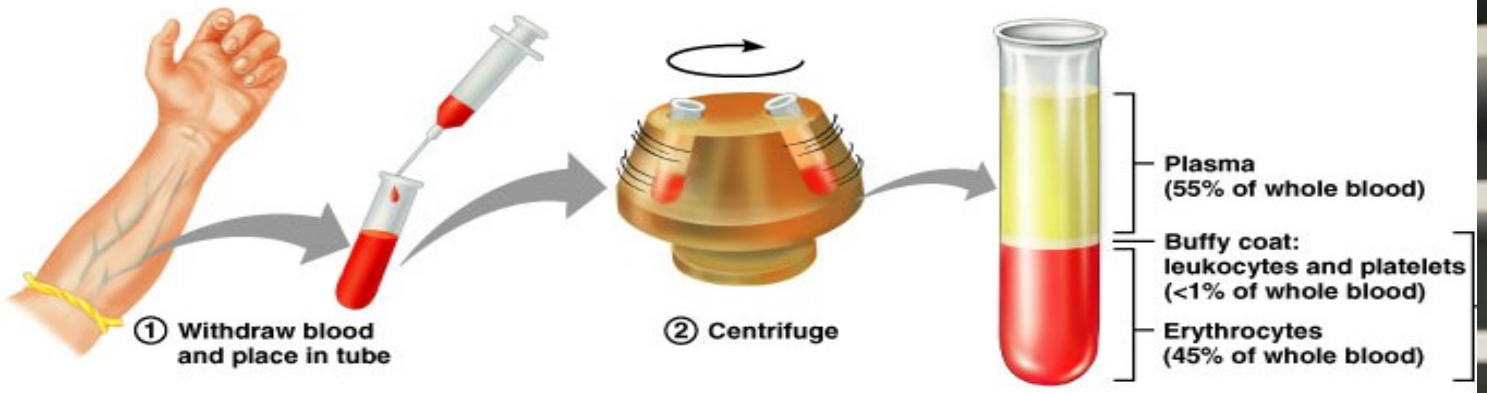
Light microscope



Composition of blood

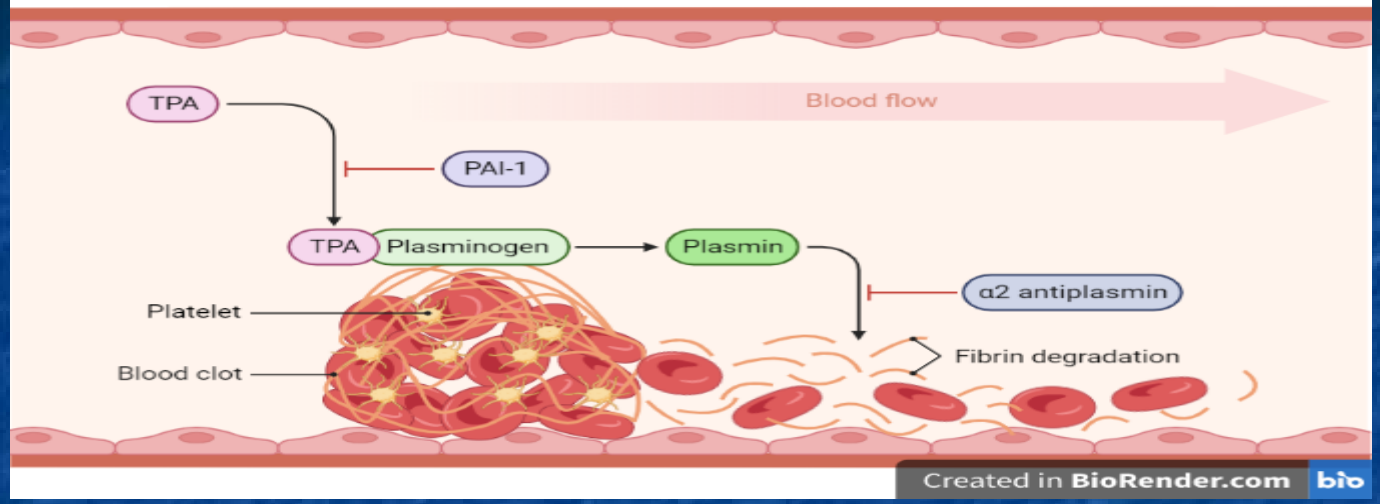
- Specialized connective tissue
- Blood cells (elements) suspended in plasma
- Blood volume: 5-6 litres in males and 4-5 litres in females
- Clinically important hematocrit
  - % of blood volume consisting of erythrocytes (red blood cells) to plasma volume
  - Male average 44-47; female average 39-42
  - Plasma contains water, ions, proteins: albumin, globulins, fibrinogen…

- Serum
  - Blood that is allowed to stand will clot
  - plasma without the clotting factors

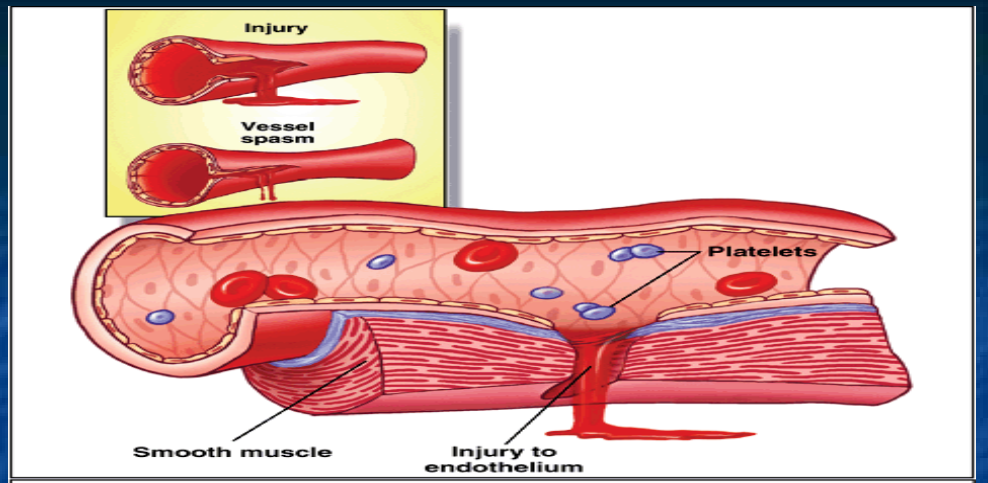


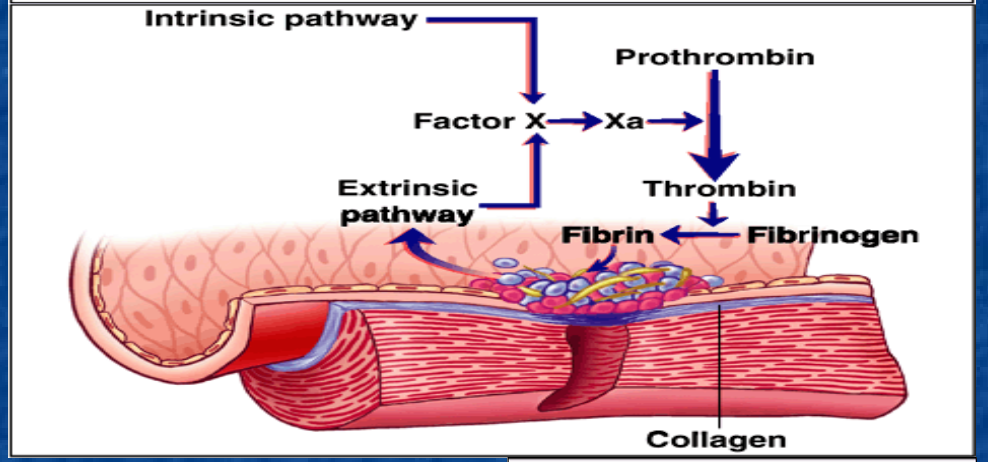
- Density gradient centrifugation
- Solution: Lymphoprep, Ficoll (1.077 g/ml)
  - layered over with whole blood or bone marrow as 1:1 volume
  - spin at 2,200 rpm for 20min, NO BRAKE
- Buffy coat

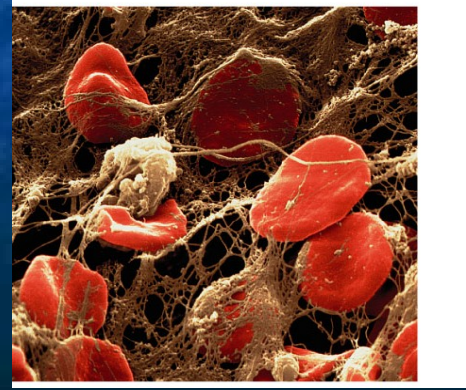
Process of Blood Clot Formation

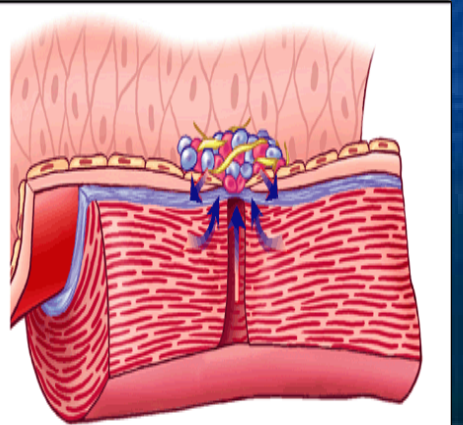


- Process in which blood changes from liquid to an insoluble clott
- conversion of fibrinogen into fibrin by the serine protease enzyme thrombin.
- Coagulation factors are generally indicated by Roman numerals
- Cofactors: calcium, phospholipids
- Fibrinolysis - blood clots are broken down & resorbed
  - TPA tissue plasminogen activator (serine protease) - conversion of plasminogen to plasmin
  - Hyperfibrinolysis (excessive bleeding, increased vascular permeability)
  - Hypofibrinolysis (thrombosis, embolism)
  - DVT, stroke, heart attack
  - Decreased platelet numbers (thrombocytopenia)

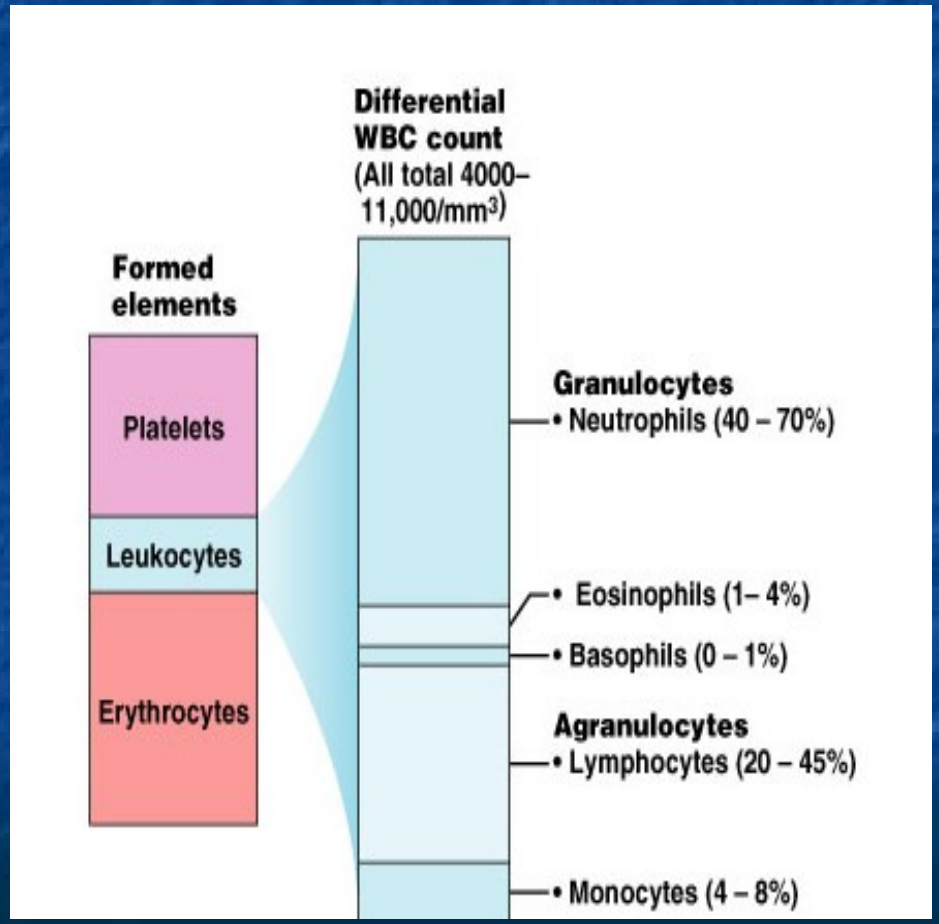








CBC test = complete blood count



Parameter	Male	Female
Haemoglobin g/dL	13.5 - 18.0	11.5 - 16.0
WBC x10^9/L	4.00 - 11.00	4.00 - 11.00
Platelets x10^9/L	150 - 400	150 - 400
MCV fL	78 - 100	78 - 100
PCV, packed cell vol hematokrit	0.40 - 0.52	0.37 - 0.47
RBC x10^{12}/L	4.5 - 6.5	3.8 - 5.8
MCH, mean cell hemoglobin pg	27.0 - 32.0	27.0 - 32.0
MCHC g/dL	31.0 - 37.0	31.0 - 37.0
RDW	11.5 - 15.0	11.5 - 15.0
Neutrophils	2.0 - 7.5	2.0 - 7.5
Lymphocytes	1.0 - 4.5	1.0 - 4.5
Monocytes	0.2 - 0.8	0.2 - 0.8
Eosinophils	0.04 - 0.40	0.04 - 0.40
Basophils	< 0.1	< 0.1

Cell count variations from normal

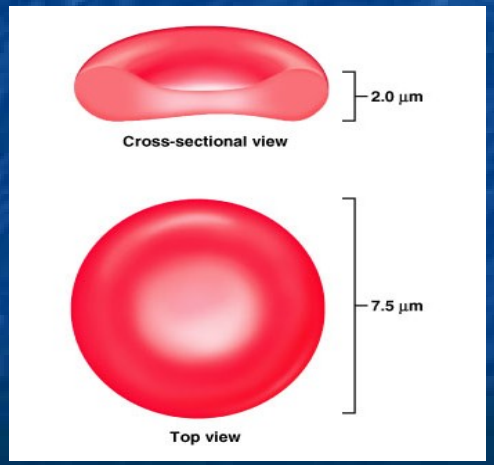
- Lymphopenia : too few lymphocytes
- Neutropenia: too few neutrophils
- Thrombocytopenia : too few platelets

- Neutrophilia: too many neutrophils
- Thrombocytosis: too many platelets
- Leucocytosis : too many WBC
- Erythrocytosis, Polycythemia: too many RBC

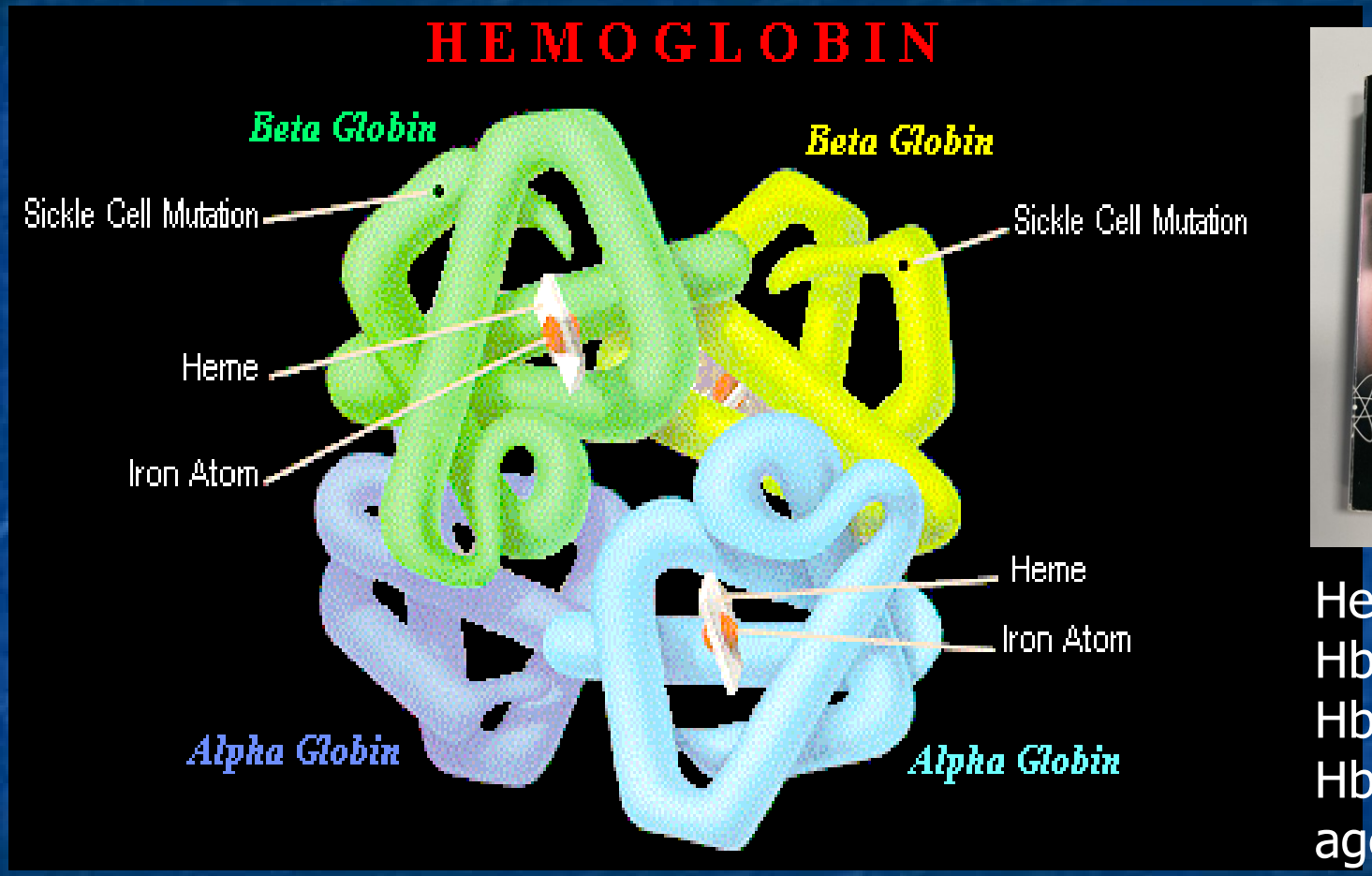
Erythrocytes

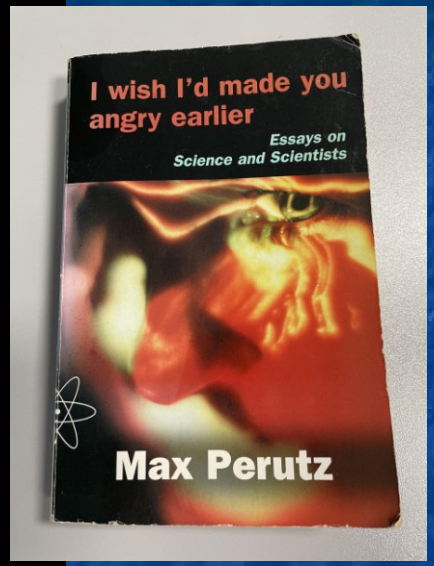
- Also called red blood cells (RBC)
- Biconcave discs and flexible
- Plasma membrane but no nuclei or organelles
- Packed with hemoglobin molecules
  - Oxygen carrying protein
  - 4 chains of amino acids, each with iron which is binding site for oxygen/CO2
  - young RBC still containing ribosomes are called ***reticulocytes***
- Lifespan 100-120 days

Parameter	Male	Female
Haemoglobin g/L	135 - 180	115 - 160
RBC x10^{12}/L	4.5 - 6.5	3.8 - 5.8



1962 Nobel Prize for Chemistry for his X-ray diffraction analysis of the structure of hemoglobin (share with John Kendrew)

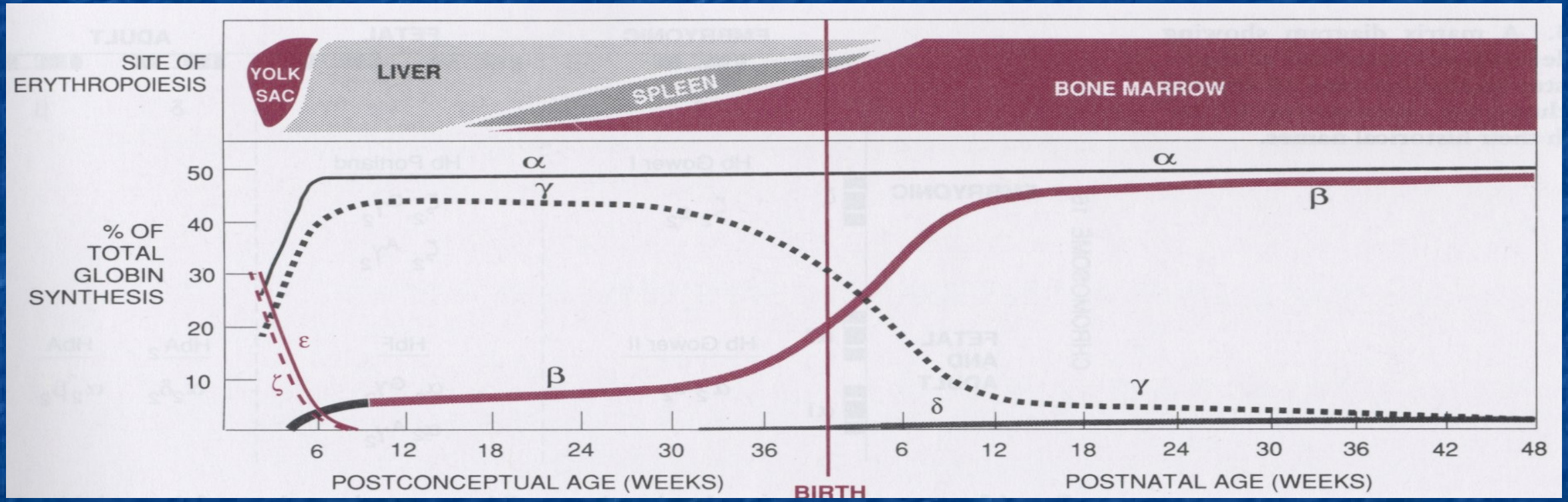




Heterotetramer
HbA_1 $\alpha_2\beta_2$ 96-98%
HbA_2 $\alpha_2\delta_2$ 2%
HbF $\alpha_2\gamma_2$ this dominates until 6 weeks of age

postnatally, Hb A dominates through life

Erythropoesis

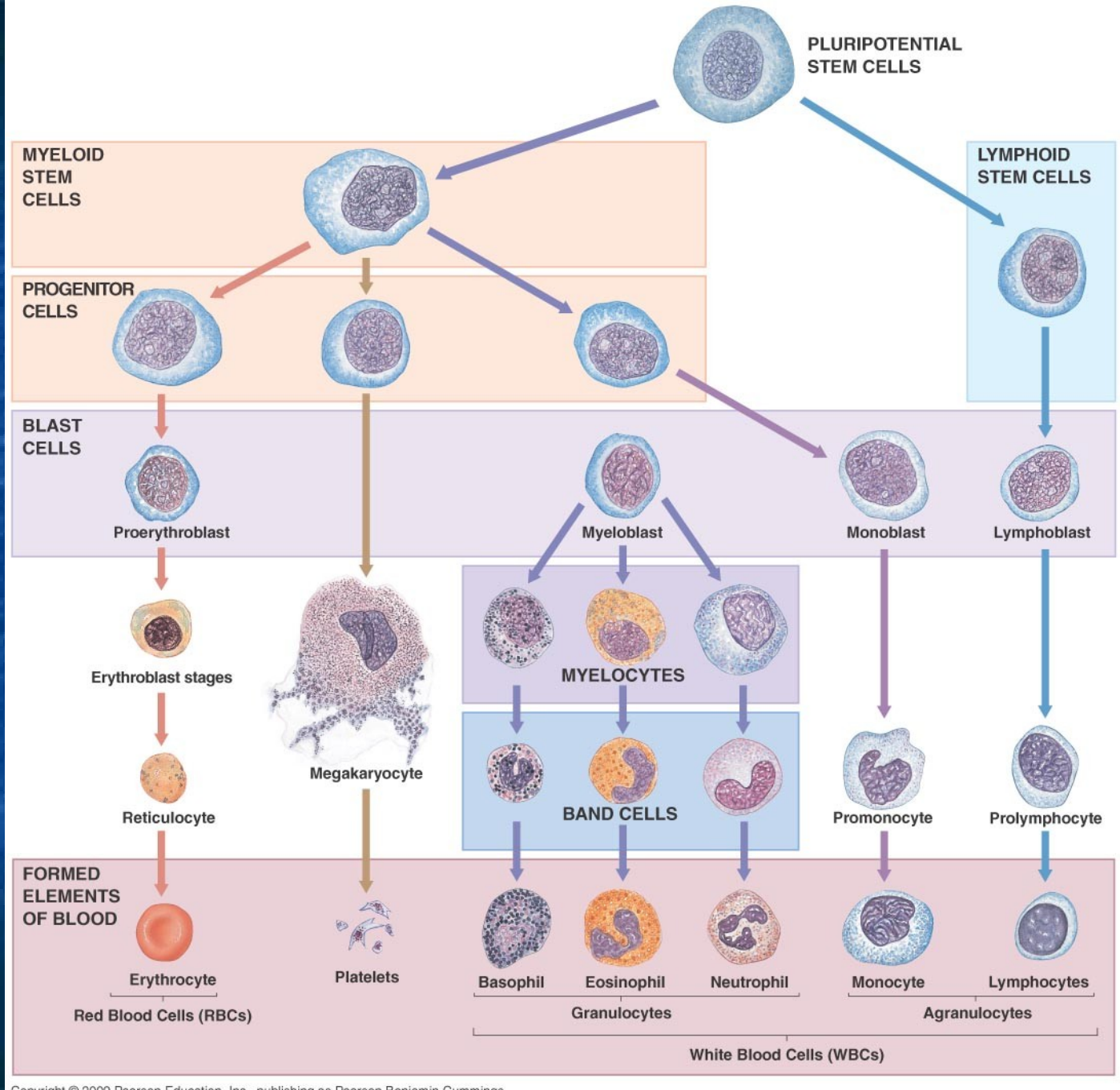


Methemoglobin

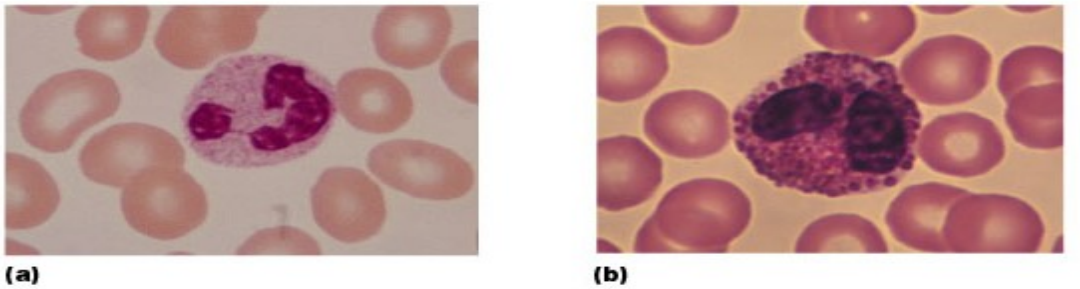
- MetHb is the derivative of Hb, in which the iron of the heme group is oxidized from Fe2+ to Fe3+
- MetHb is no longer completely capable of reversibly binding O2 (brown)
- MetHb forms continuously (present in RBC 1-2% c HB)
  - must be reduced actively by normal red cell metabolism or by ascorbic acid
  - cyanosis & fatigue 10%, coma & fatal 50-70%
  - nitrates in food and water, medication-local anesthetics, G6PD deficiency

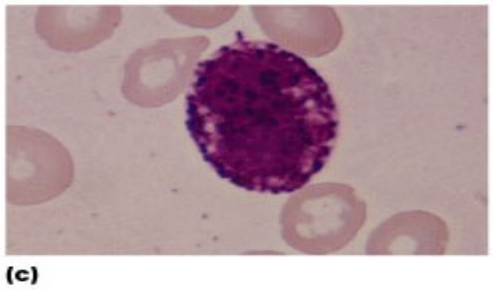
Hematopoiesis

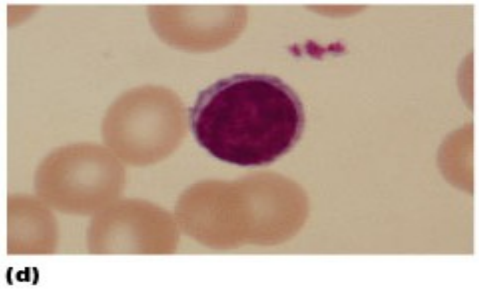
- Formation of blood cells
- Occurs mostly in red bone marrow
- All cells arise from the same pluripotent hematopoietic stem cells
- MSCs form fat cells, osteoblasts, chondrocytes, fibroblasts and muscle cells…

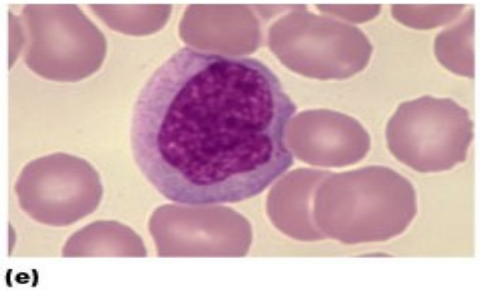


Leukocytes









- A) Granulocytes
  - Granules, lobed nuclei
  - All phagocytic
  - Neutrophil: Nuclei of 2-6 lobes
  - Eosinophil: Nuclei bi lobed
  - Basophil: Dark purple granules
- D) lymphocyte
  - Large nucleus
  - T, B lineage
  - NK cells
- E) monocyte diff. into MØ

CD numbers (clusters of differentiation)

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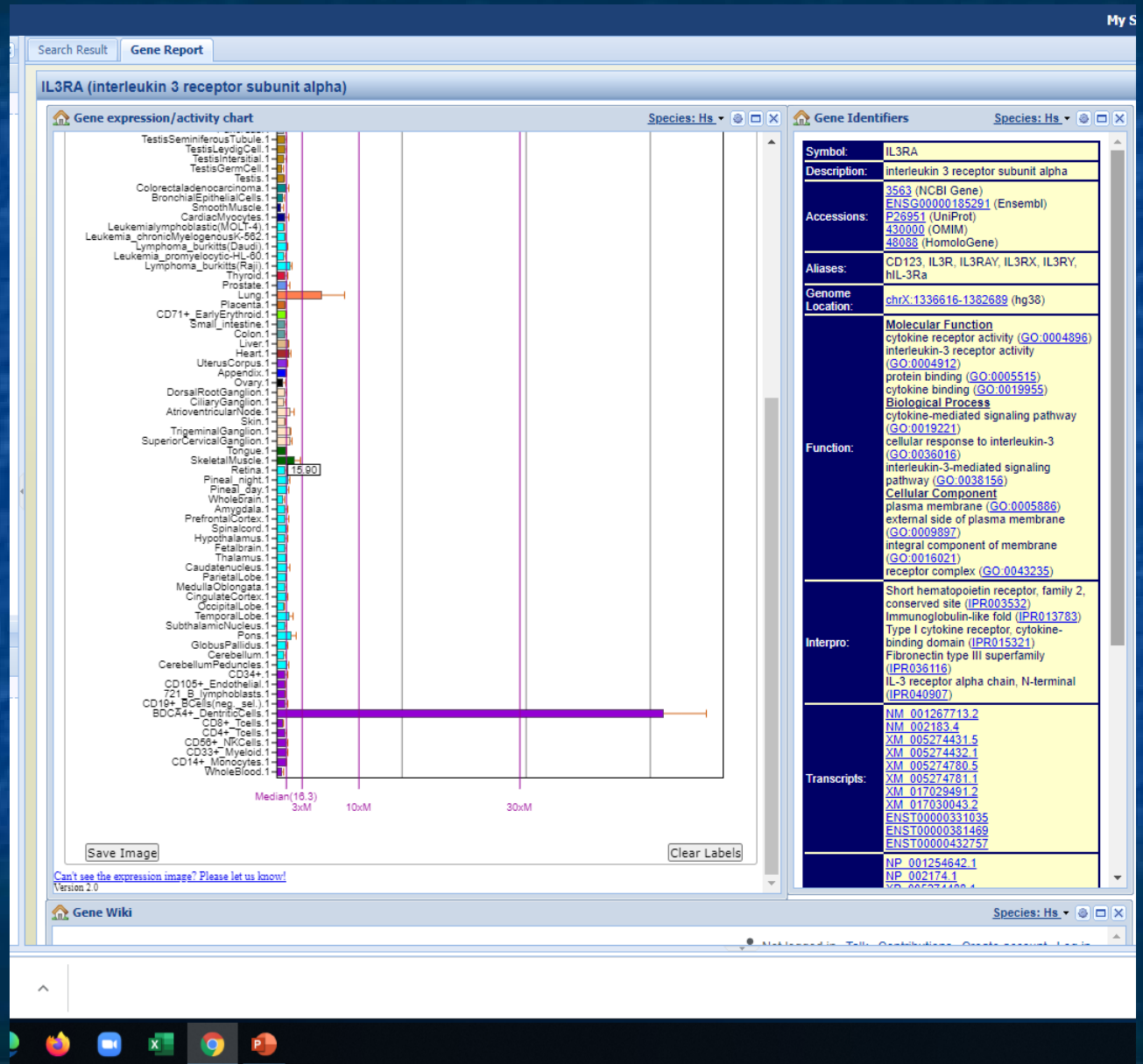
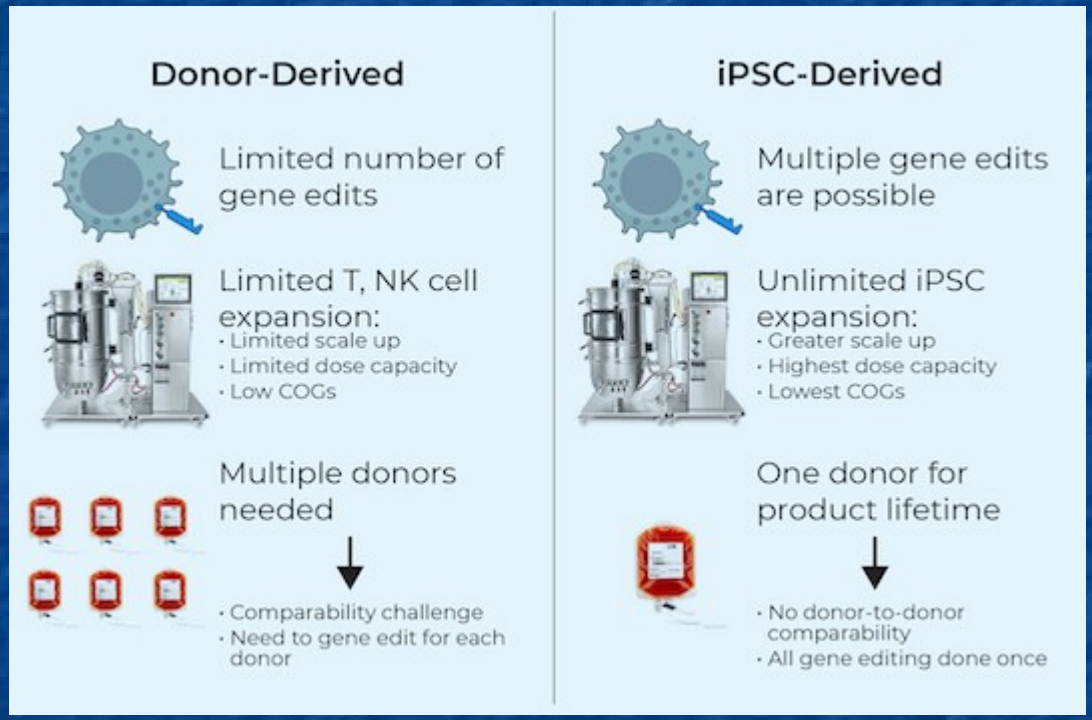


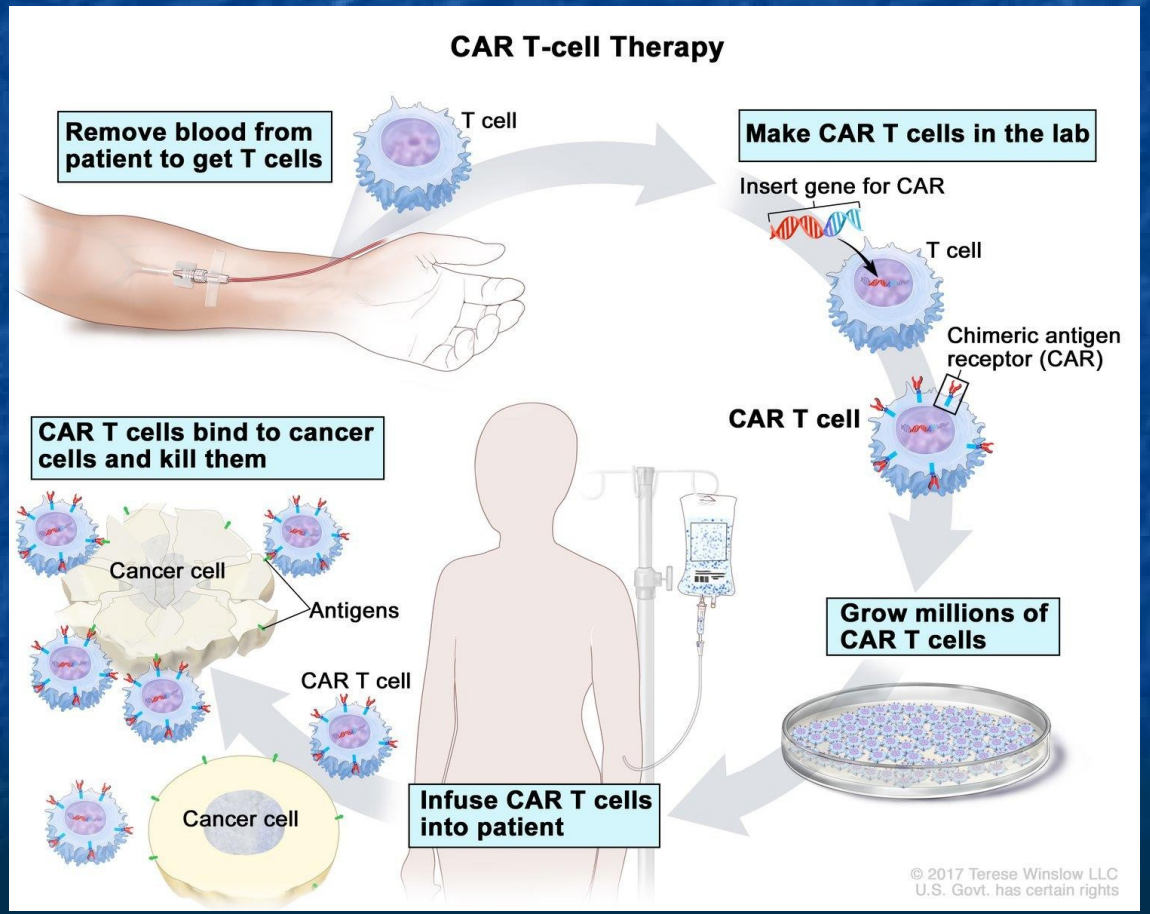
Bone marrow

- Red marrow (medulla ossium rubra)
  - Consists mainly of haematopoietic tissue
  - Site of haematopoiesis (red and white blood cells, platelets)
- Yellow marrow (medulla ossium flava)
  - Made up of fat cells
- With age more red BM is converted to yellow BM
- BM stroma
  - Creates a microenvironment
    - Fibroblasts, MØ, adipocytes, Osteoblasts, osteoclasts, Endothelial cells
- Mesenchymal Stem cells (MSC)
  - Pluripotent stem cells that can differentiate *in vitro* and *in vivo* into a number of cell types incl. osteoblasts, chondrocytes, myocytes, adipocytes
- Induced pluripotent stem cells (iPSC)
  - type of pluripotent stem cells that can be generated directly from a adult somatic cell
  - by Oct3/4, Sox2, Klf4, and c-Myc transcription factors (Yamanaka factors, Nobel prize 2012)
  - The Advanced Therapies 2023 congress

T cell therapy

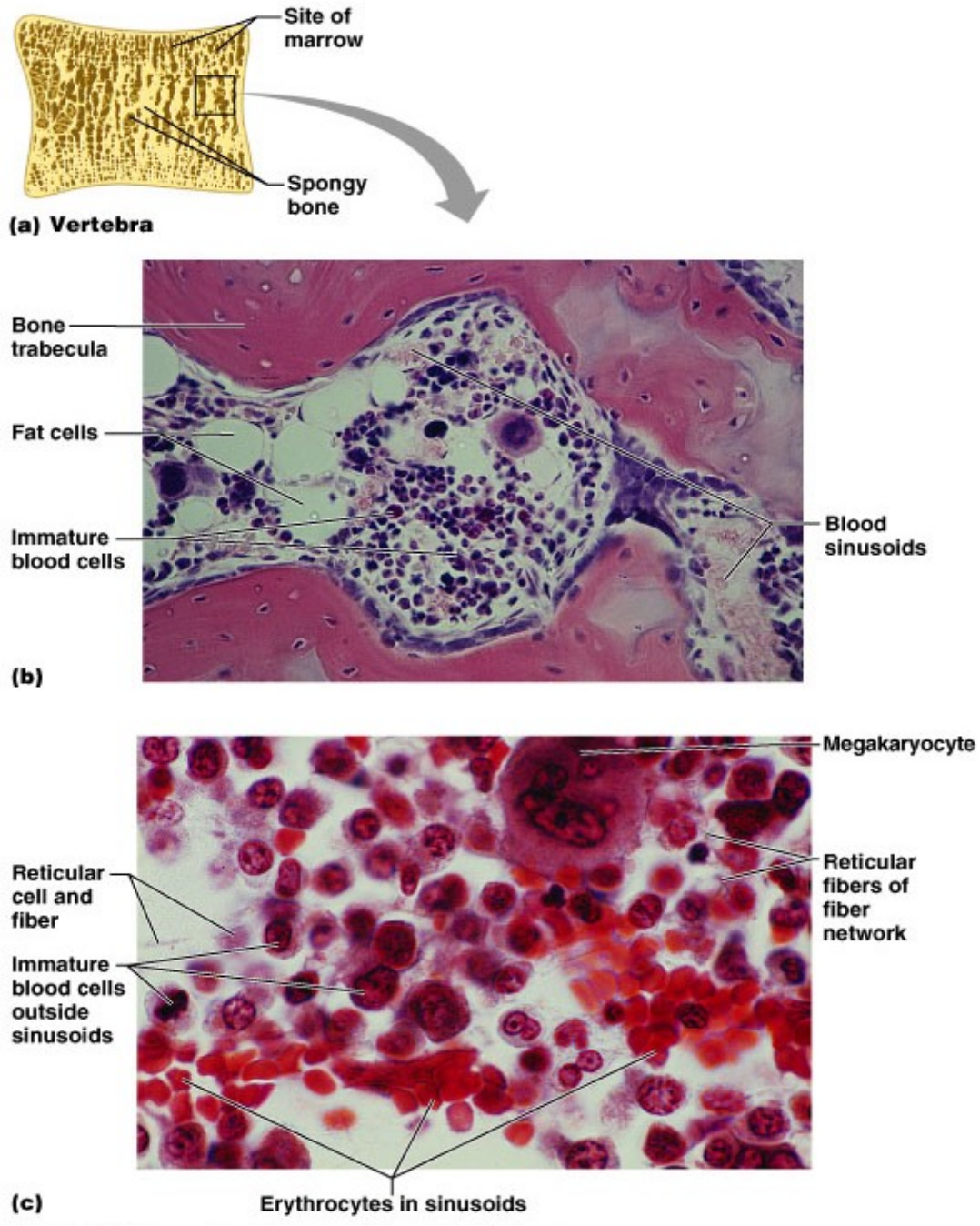


Century Therapeutics, Inc.



Examination of bone marrow

- Invasive procedure
- BM sample obtained via biopsy or aspiration (sternum, pelvis)
- Used to newly diagnose & confirm suspected pathology
- To examine haematopoiesis
- Parallel to analysis of venous PB drawn



Bone marrow harvest for transplantation

- BM is collected (pelvis under general anesthesia) and infused back:
  - Autologous Tx - same patient
  - Allogeneic Tx
    - Matched sibling
    - Matched Unrelated Donor (MUD)
  - Donor – recipient compatibility (MHC/HLA alleles)
  - Donor registers around the world

The Anthony Nolan Trust

- http://www.anthonynolan.org
  - story of Anthony Nolan (1971-1979)
  - born with a rare Wiskott-Aldrich syndrome
  - only cure was Tx but no donor was available
  - Shirley Nolan (1942-2002) and her legacy to start a donor register
  - Currently over 750 000+ potential donors fully typed
  - Important charity – please log-in & donate
  - Research Institute & project Allostem
    - major EU grant involved 13 countries including CZ
    - (Prof. Bartunkova, Prague)
  - Essential clinical and research contribution to EBMT

MHC proteins

- Major Histocompatibility Complex, locus on chr. 6
  - Highly polymorphic
  - Enormous MHC allelic diversity
- HLA, human leukocyte antigens
  - Transplant antigens to prevent graft rejection
- HLA I. class (HLA-A, B, C)
  - Expressed on all nucleated cells
- HLA II. class (HLA-DP, DQ, DR)
  - Expressed on cells of IS
- MHC III. class
  - Complement

- Prof. S Marsh at ANRI, President of the European Federation for Immunogenetics
- Allele frequencies vary in different populations and ethnic groups

Haematopoietic stem cell transplantation

- Stem cell transplantation derived from:
  - BM
  - peripheral blood
  - cord blood
- Autologous Tx
  - Requires extraction/apheresis of stem cells (HSC)
  - Stored in the liq. nitrogen
  - Patient undergoes high-dose chemo ± radiotherapy
  - Established as the second-line treatment for lymphoma
- Allogeneic Tx
  - HLA matching
  - Recipient's immunosuppression
    - Calcineurin inhibitors (cyclosporin, tacrolimus)
    - Corticosteroids (methylprednisolone, dexamethasone, prednisolone)
    - Cytotoxic immunosuppressants (azathioprine, chlorambucil, cyclophosphamide, methotrexate)
  - Full ablative vs Reduced intensity conditioning (RIC)
  - RIC pioneered by Prof Stephen Mackinnon at University College London
  - Numerous clinical trials ongoing

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Find a study (all fields optional)

Status

- ○ Recruiting and not yet recruiting studies
- ● All studies

Condition or disease (For example: breast cancer)

Other terms (For example: NCT number, drug name, investigator name)

Country

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Perutz.jpg

Zobrazit vše

Cell storage for transplantation

- Cells frozen in 5-10% DMSO/human serum
  - DMSO, Dimethyl sulfoxid
  - Prevents the formation of ice crystals during the freezing process
- Stored at liquid nitrogen (-196° C) for months/years
- Decreasing the temperature as 1°C per minute over night at -80°C in the Mr Frostie containing isopropyl alcohol

Post HSCT

- Cytokine storm
- Graft-versus-host disease (GvHD) as a major complication post SCT
  - T cells present in the transplant recognize the host's (recipient's) cells as foreign
  - Minor histocompatibility antigens
  - Acute within 100 days as major challenge to transplant mortality and morbidity (grade 1-4)
  - Chronic as moderate to severe
  - Skin, liver, gut and GI tract, lung
  - Donor T cells mediate graft -versus-tumour effect (versus leukaemia, lymphoma or myeloma)

Graft – versus - tumour effect

- GvL (versus leukaemia)
  - Most prominent in CML patients, (also in ALL)
- GvM (myeloma)

Cytotherapy, 2012; 14: 1110–1118

informa
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Human Vdelta1 gamma-delta T cells exert potent specific cytotoxicity against primary multiple myeloma cells

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Abstract

Background aims. Human gamma-delta (γδ) T cells are potent effector lymphocytes of innate immunity involved in anti-tumor immune surveillance. However, the Vδ1 γδ T-cell subset targeting multiple myeloma (MM) has not previously been investigated. *Methods.* Vδ1 T cells were purified from peripheral blood mononuclear cells of healthy donors and patients with MM by immunomagnetic sorting and expanded with phytohemagglutinin (PHA) together with interleukin (IL)-2 in the presence of allogeneic feeders. Vδ1 T cells were phenotyped by flow cytometry and used in a 4-h flow cytometric cytotoxicity assay. Cytokine release and blocking studies were performed. Primary myeloma cells were purified from MM patients' bone marrow aspirates. *Results.* Vδ1 T cells expanded from healthy donors displayed prominent cytotoxicity by specific lysis against patients' $CD38^+$ $CD138^+$ bone marrow-derived plasma cells. Vδ1 T cells isolated from MM patients showed equally significant killing of myeloma cells as Vδ1 T cells from normal donors. Vδ1 T cells showed similarly potent cytotoxicity against myeloma cell lines U266 and RPMI8226 and plasma cell leukemia ARH77 in a dose-dependent manner. The interferon (IFN)-γ secretion and Vδ1 T-cell cytotoxicity against myeloma cells was mediated in part through the T-cell receptor (TCR) in addition to involvement of Natural killer-G2D molecule (NKG2D), DNAX accessory molecule-1 (DNAM-1), intracellular cell adhesion molecule (ICAM)-1, CD3 and CD2 receptors. In addition, Vδ1 T cells were shown to exert anti-myeloma activity equal to that of Vδ2 T cells. *Conclusions.* We have shown for the first time that Vδ1 T cells are highly myeloma-reactive and have therefore established Vδ1 γδ T cells as a potential candidate for a novel tumor immunotherapy.

SCT and CMV

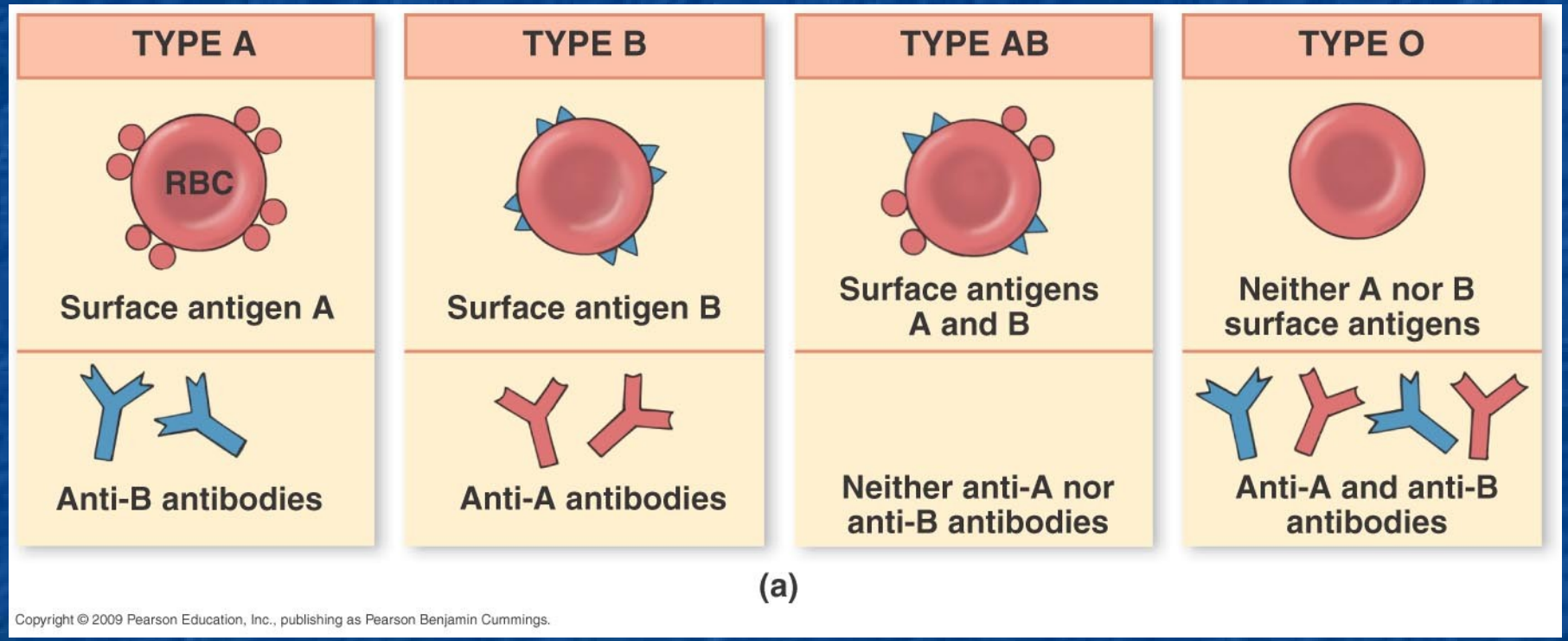
- HCMV cytomegalovirus
- Common beta-herpes virus (HHV5)
- Primary infection followed by a latent infection
- Vigorous immune response, persistent suppression of viral replication
- CMV seropositivity associated with immune senescence of virus-specific CD4+ and CD8+ T cells (Prof. Paul Moss, Graham Pawelec, Mark Wills)
- Multiple strategies to evade the host immune system
- Immunocompetent vs immunocompromised host

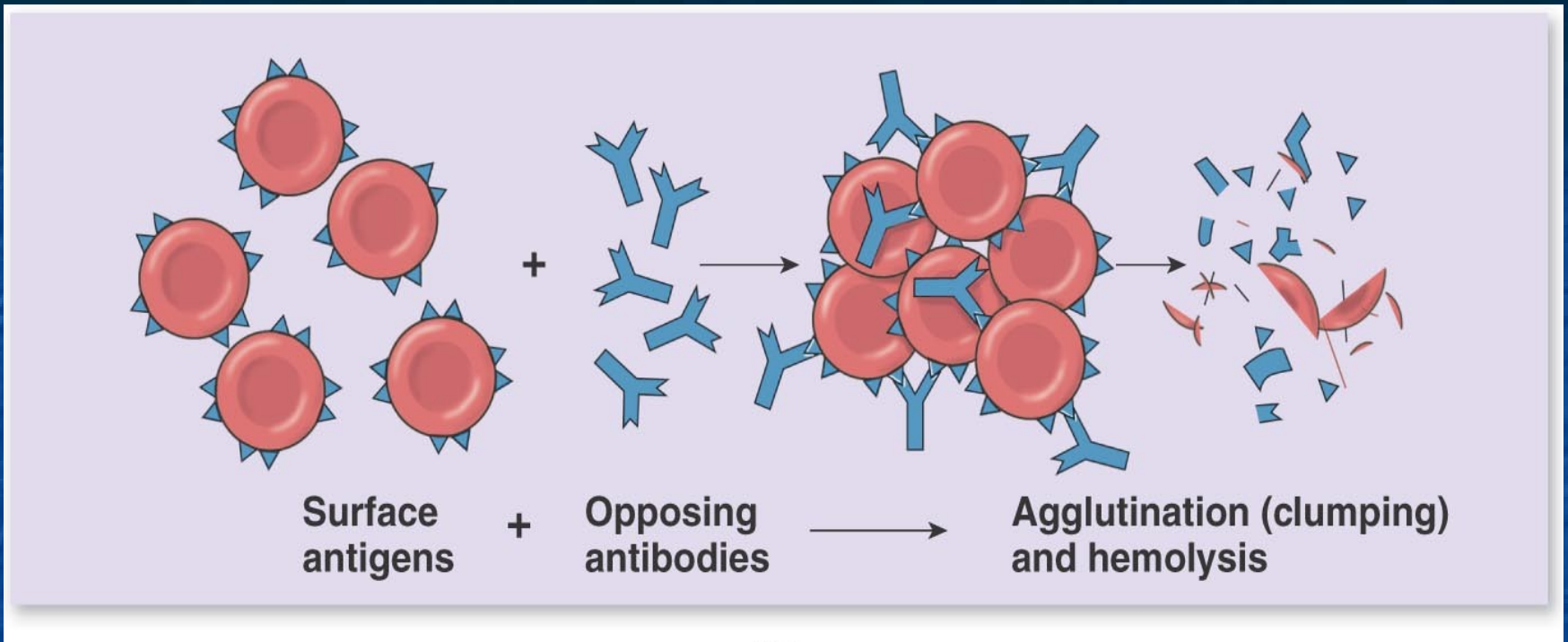
  - Donor+ Recipient+
  - D+ R-
  - D- R+
  - D- R-

Blood transfusion

- process of receiving blood intravenously
- to replace a lost blood component (red blood cells, plasma, platelets or clotting factors)
- donated blood processed/separated by centrifugation
- tested for infections (HIV 1, 2, HTLV 1, 2, Hep B, C, syphilis, CMV)
- stored in Blood Bank
- compatibility testing between D and R
- typing of recipient's blood determines the ABO blood groups and Rh status
- sample tested for any alloantibodies that may react with donor blood

ABO blood groups





- If a blood transfusion is given to a person who has antibodies to that type of blood, then the transfused blood will be attacked and destroyed (transfusion reaction)

ABO blood group types

- Europe:
  - A 45%
  - B 16%
  - AB 6%
  - O 33%

TABLE 20.4 Differences in Blood Group Distribution

Population	Percentage with Each Blood Type				
	O	A	B	AB	Rh^+
U. S. (average)	46	40	10	4	85
Caucasian	45	40	11	4	85
African-American	49	27	20	4	95
Chinese	42	27	25	6	100
Japanese	31	39	21	10	100
Korean	32	28	30	10	100
Filipino	44	22	29	6	100
Hawaiian	46	46	5	3	100
Native North American	79	16	4	<1	100
Native South American	100	0	0	0	100
Australian Aborigines	44	56	0	0	100

Rh blood group system

- consists of 50 defined blood-group antigents
- The commonly used terms *Rh factor*
  - *Rh positive* (85%)
  - *Rh negative* (15%) refer only to the *D antigen*
- We either have or don't have it on the surface of red cells
- Condition of hemolytic disease of the newborn
  - Incompatibility between mother and the fetus

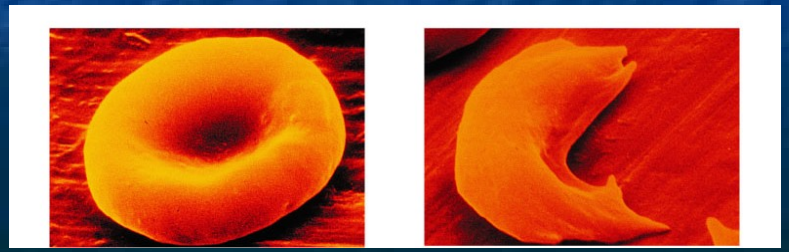
Haematological disorders

Disorders of Erythrocytes

- Polycythemia: high RBC, increased Hb and hematokrit
- Anaemia: low RBC
- over 400 types of anaemia
- develops when:
  - Decrease in the total number of red blood cells (RBC)
    - **Blood loss –** pregnancy, Acute: trauma and surgery, Chronic: many types of cancers (colon, bladder carcinomas), IBD patients
    - **Decreased production of RBC - result of BM failure & differentiation of stem cells**
    - **Increased destruction of RBC**
  - Decrease of the amount of haemoglobin and/or its reduced ability to carry oxygen

Disorders of Erythrocytes - Hemoglobinopathies

- are inherited single-gene disorders
- characterized by decreased and/or unstable haemoglobin
  - Thalassemia
    - usually results in underproduction of normal globin proteins often through mutations in regulatory genes
    - Beta; subtypes major (both beta globin genes missing) and intermedia
    - Alpha; subtypes Hb H and hydropsis fetalis
    - Minor; either alpha or beta globin gene missing
  - Sickle cell disease
    - Estimated that 7% of world's population (~420 million) are carriers
    - Inheritance of two abnormal B-globin gene (chr 11)
    - The gene defect is a SNP (single nucleotide polymorphism) where GAG changes to GTG and results in glutamic acid being substituted by valine (E6V)



G6PD Deficiency

- Glucose-6-phosphate dehydrogenase deficiency
  - enzyme involved in the pentose phosphate pathway
  - important in red blood cell metabolism
- Perhaps most common, world-wide congenital abnormality
  - > 300 variants identified
  - X-linked inheritance
- Common G6PD deficient variants are associated with an acute intermittent hemolysis and anaemia
- vast majority never symptomatic!

Disorders of Platelets

- Thrombocytopenia
  - normal platelet count ranges from 150,000 - 450,000 per µL
  - platelet count below 50,000 per µL
  - occasional bruising, nosebleeds, bleeding gums
  - !! internal bleeding
  - many causes: decreased production or increased destruction (SLE, HIV)
    - Vitamin B12 or folic acid deficiency
    - Leukaemia, MDS
    - Decreased production of trombopoietin by the liver in liver failure
    - Bacterial, viral infections, sepsis
    - Hereditary: Fanconi anemia
  - Treatment depending on the cause
    - Corticosteroids
    - Platelet transfusion

Disease of the bone marrow

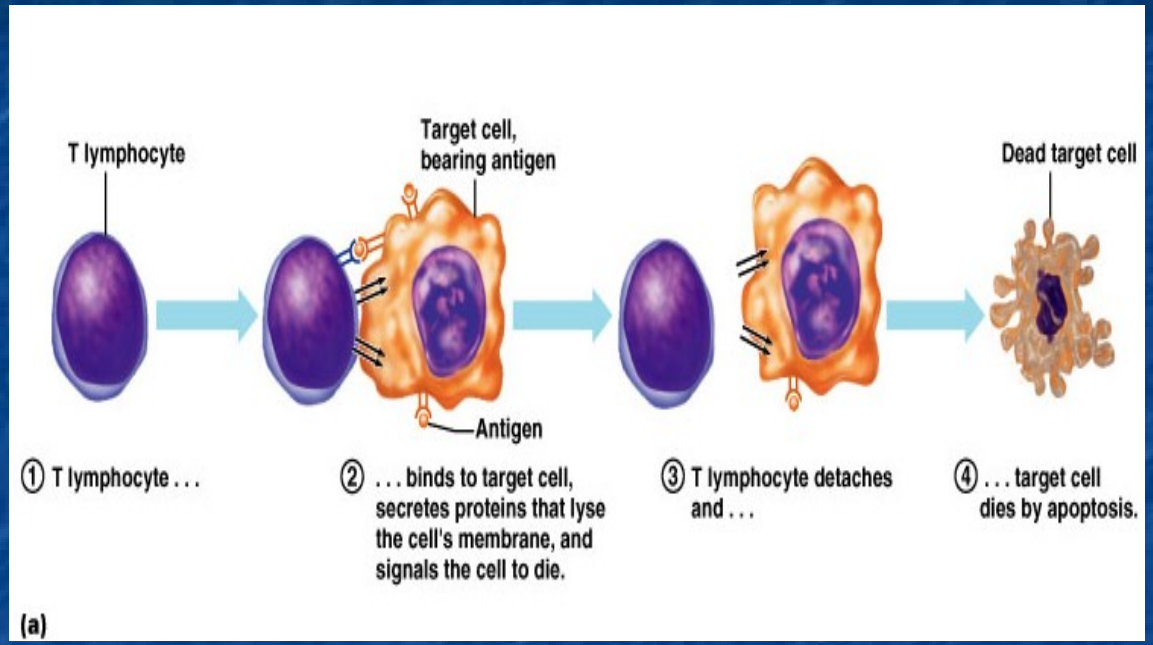
- Congenital defects
- Aplastic anemia
- Malignancies
  - Leukaemia
  - Lymphoma
  - Multiple myeloma

Congenital defects

- Dyskeratosis congenita (DKC)
  - is a rare progressive congenital disorder resembling premature aging
  - Essen. bone marrow failure syndrome
  - DKC typically develop between ages 5-15 years
  - is a result of one or more mutations in the long arm of the chr X in the gene DKC1
  - Heiss NS, Knight SW, Vulliamy TJ, et al." May 1998, *Nat. Genet.* **19** (1): 32–38

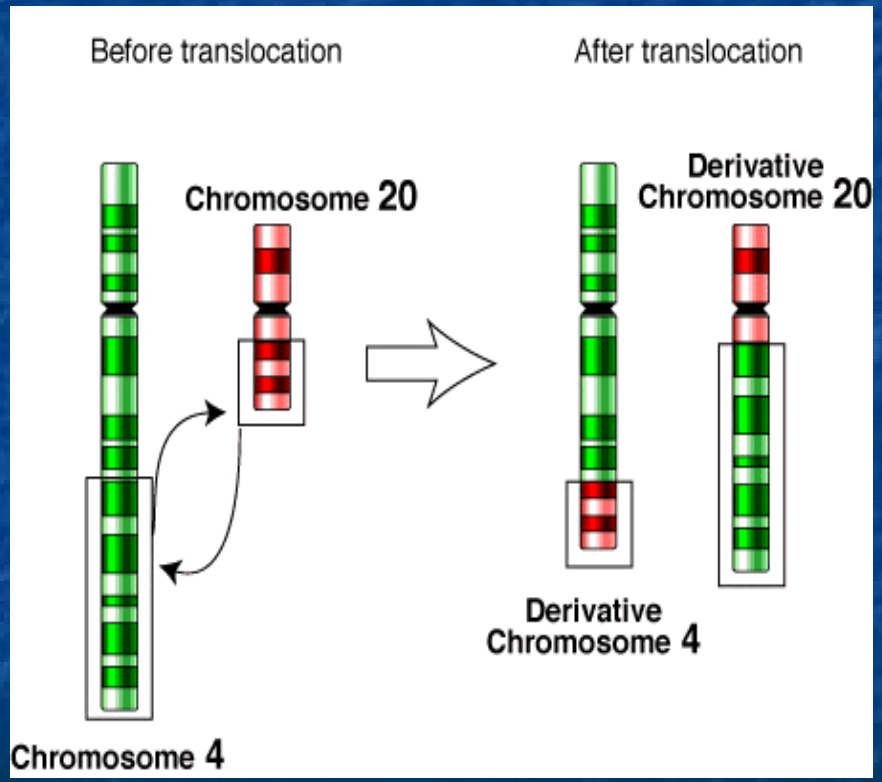
Haematological Malignancies

- Understand the pathogenesis
  - Genetic alterations including translocations, mutations, SNPs…
  - Leukaemogenesis
    - Hereditary factors (Fanconi A, Down sy)
    - Radiation, chemicals, drugs
    - Virus related (EBV, CMV)
    - Retrovirus mediated (HTLV-1)
    - Age related
    - Oncogenes, tumour suppressor genes
- Understand the pathophysiology
- Able to list down the laboratory investigations required for diagnosis
- Therapy & clinical trials
- Research !



Stress ligands
Immune surveillance
Tumour evasion
Shedding
Trogocytosis

Leukaemia and chromosomal translocations



- Ionising radiation can caused breakage of the phosphodiester backbone of both strands of DNA
- Double-strand breaks are very efficiently repaired
  - Potential loss of genetic material
  - Double-strand ends recognised as "foreign" DNA and destroyed
- If double-strand breaks occur in two different chromosomes then possibility for incorrect repair taking place

Frequent translocations

B-ALL	t(1;19)	5%
B-ALL (in children)	t(12;21)	22%
T-ALL	t(5;14)	20%
T-ALL	1p32 deletion	25%
AML	t(15;17)	13%
AML	t(8;21)	7%
CML	t(9;22)	99%

Leukaemia I.

- heterogenous group of malignant disorders which is characterised by uncontrolled clonal and accumulation of blasts in the bone marrow and body tissues
- Excessive production of WBC
- Often non fully differentiated cells called “blasts”
- WBC have abnormal function
  - Resistant to apoptosis
  - Excessive proliferation
  - Tumour microenvironment in the bone marrow
- Disruption of normal haematopoesis in bone marrow

Leukaemia II.

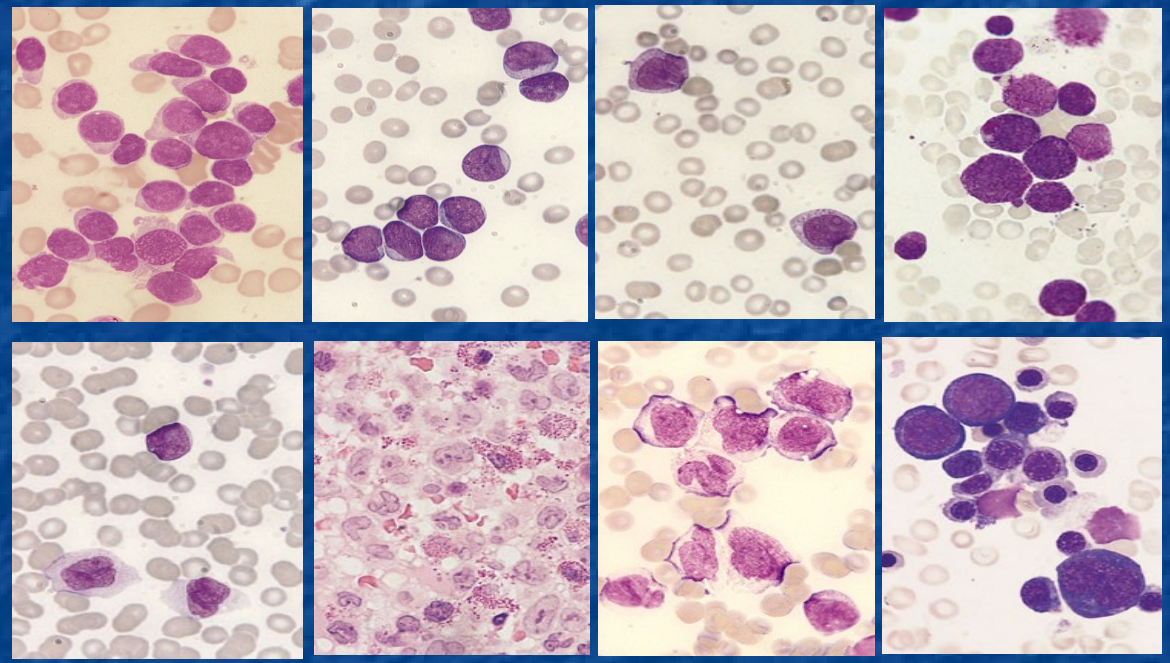
- Classification
  - Acute
    - Acute lymphoblastic leukemia (T-ALL & B-ALL)
    - Acute myeloid leukemia
  - Chronic
    - Chronic myeloid leukemia
    - Chronic lymphocytic leukemia

Acute Lymphoblastic Leukaemia

- Cancer of the blood affecting the white blood cell *LYMPHOCYTES*
- Commonest in the age 2-10 years
- Peak at 3-4 years.
- Incidence decreases with age, and a secondary rise after 40 years.
- In children - most common malignant disease
- 85% of childhood leukaemia

Acute Myeloid Leukaemia

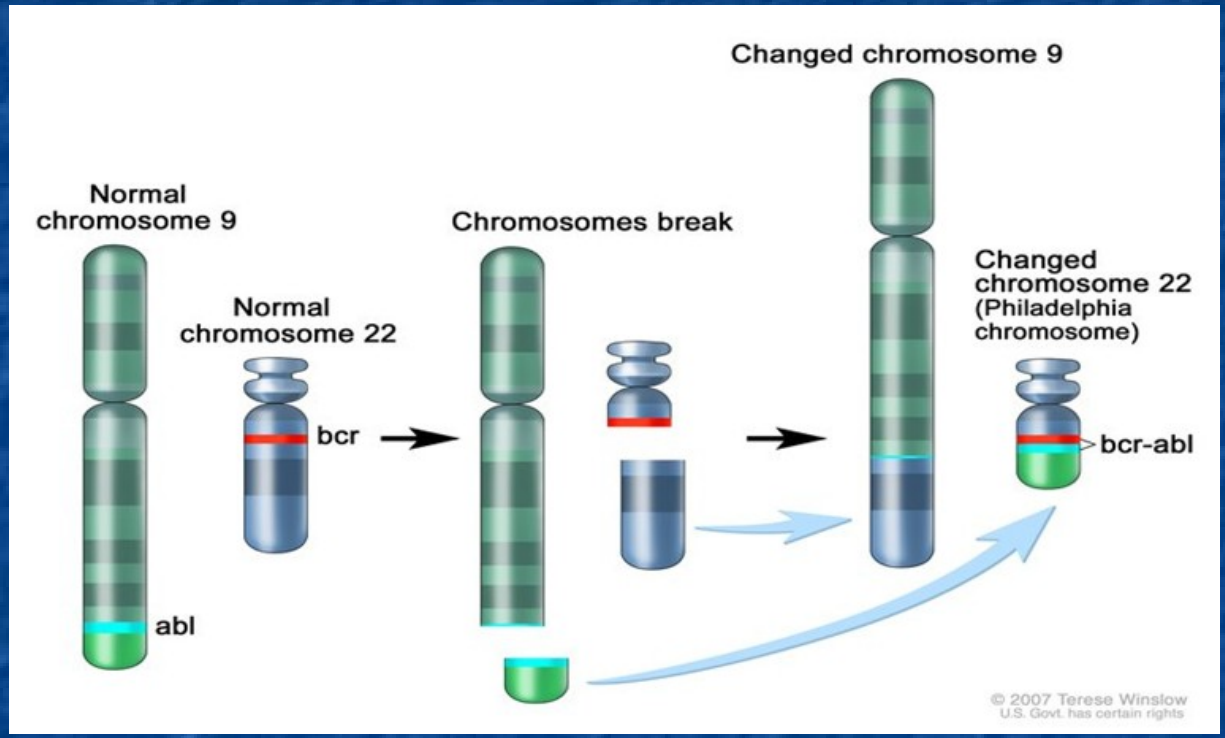
- Arise from the malignant transformation of a myeloid precursor
- Rare in childhood (10%-15%)
- The incidence increases with age
- 80% in adults
- FAB classification
  - M0 Undifferentiated blasts
  - M1 AML without maturation
  - M2 AML with maturation
  - M3 Acute promyelocytic leukemia
  - M4 Acute myelomonocytic leukemia
  - M5 Acute monocytic leukemia
  - M6 Acute erythroblastic leukemia
  - M7 Acute megakaryoblastic leukemia



From lecture by Dr NJ Dodd BS967-7-SP: Session 6
*courses.essex.ac.uk/bs/**bs967**/restricted/NJD%20Leukaemia.ppt*

Molecular biology of CML

- Philadelphia Chromosome (Ph)
- t(9;22) balanced translocation
- disruption of the ABL (Chr 9) and BCR (Chr 22) genes
- formation of two hybrid genes
  - 5'BCR/3'ABL
  - 5'ABL/3'BCR
- BCR/ABL mRNA,
- p210 'fusion' oncoprotein as constitutively active tyrosine kinase resulting in the permanent activation of the RAS pathway



visualsonline.cancer.gov/addlb.cfm?imageid=7153

New CML Treatment

- Design compounds that specifically target the p210 protein
- p210 is CML specific
- imatinib (Gleevec, Glivec, STI571)
- specifically inhibits the ABL kinase
  - imatinib inhibits the growth of CML cells in culture
  - progression-free survival at 24 months is 87%
- Prof John Goldman Hammersmith Hospital London
- Prof John Barret NIH Washington
- Prof Francois Mahon Bordeaux
- Prof Mayer FN Brno
- New generations of the TKI – dasatinib, nilotinib

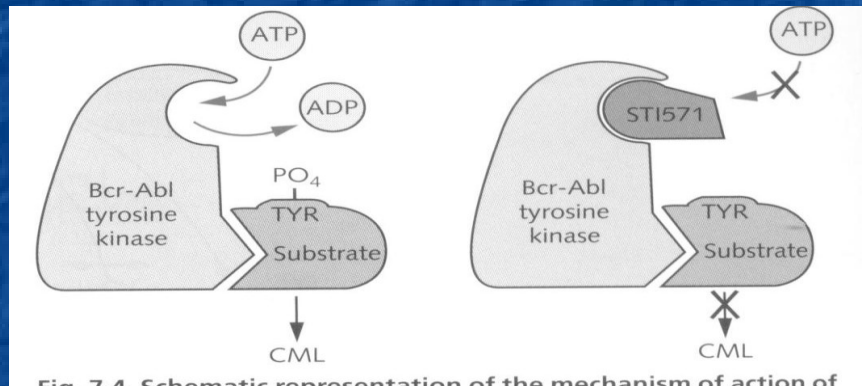


Fig. 7.4 Schematic representation of the mechanism of action of the BCR-ABL tyrosine kinase and its inhibition by imatinib

Molecular Haematology Provan & Gribben

Cancer Immunology, Immunotherapy
https://doi.org/10.1007/s00262-022-03312-3

RESEARCH

Check for updates

Expansions of tumor-reactive Vdelta1 gamma-delta T cells in newly diagnosed patients with chronic myeloid leukemia

Andrea Knight[1] · Martin Piskacek[1] · Michal Jurajda[1] · Jirina Prochazkova[2] · Zdenek Racil[3] · Daniela Zackova[2] · Jiri Mayer[2]

Received: 6 July 2022 / Accepted: 12 October 2022

Chronic lymphocytic leukaemia

- Most common leukaemia in the Western countries
  - lymphocytosis of > 5000 cells/μl for
  - > 3 months
- Flow cytometry of peripheral blood (phenotype CD19, CD5, CD23)
- Bone marrow biopsy
- Staging according to Rai (I-IV)
- Mutated IgVH
- Del11q (ATM)
- Del17p
- Del13q (RB1)
- +12
- TP53
- Prof Michael Doubek, IHOK, FN Brno

Multiple Myeloma

- B cell maligancy of plasma cells CD38+CD138+ in the bone marrow
- Pre-malignant stage:
  - MGUS – monoclonal gammopathy of undetermined significance
  - Progression of 1% per annum
- Bone marrow biopsy
- Therapy (IMIDS)
- Prof. Roman Hájek FN Ostrava

Questions