Aim of the practicals

- Basic of radiobiology
- Model of acute radiation syndrome - blood form
- Evaluation of the data obtained during practice on a model of acute radiation syndrome

MUNI
MED

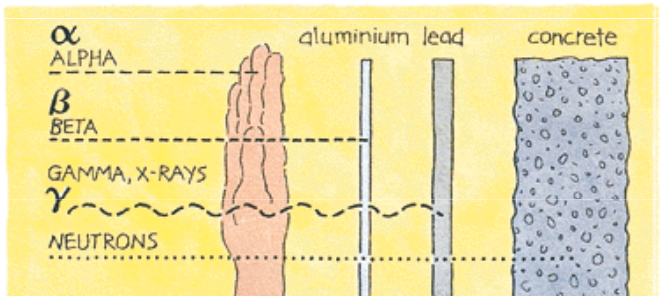
Ionizing radiation

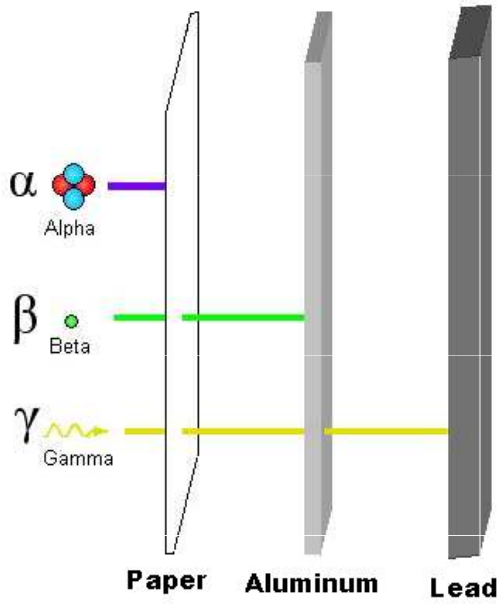
- The radiation emitted by radioactive nuclides
- Electromagnetic or corpuscular radiation, which causes the penetration of mass ionization (must have sufficiently high energy).
- Energy is in the range of keV-MeV

MUNI
MED

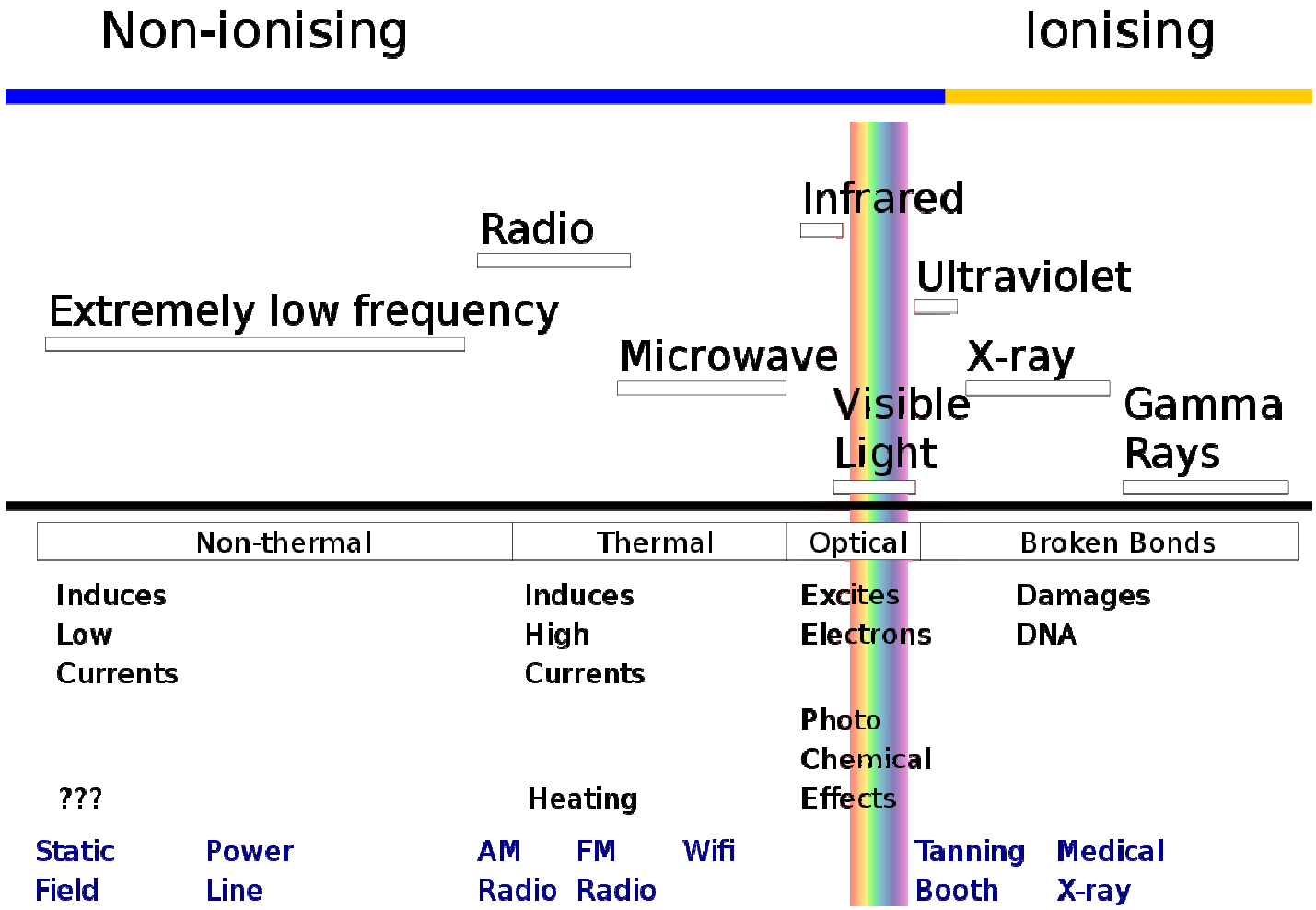
Types of ionizing radiation

Corpuscular α, β, neutrons
electromagnetic γ



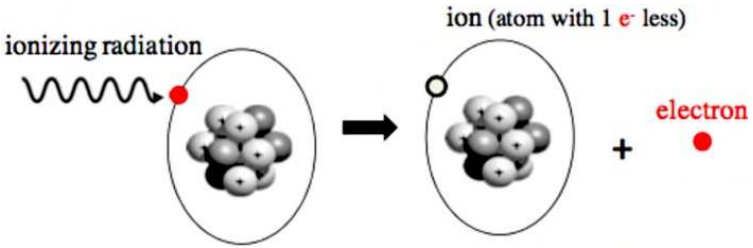


The energy spectrum of radiation



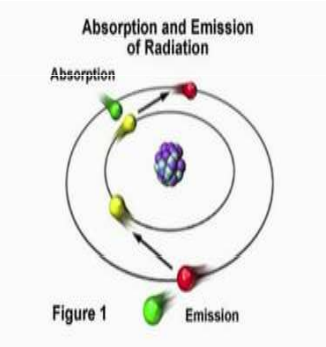
MUNI MED

Ionization vs. excitation



$$M \longrightarrow M^{+} + e^{-}$$

$$M \longrightarrow M^{excit}$$



- Ionization = process of creating charged atoms
- Excitation = atoms absorp energy without ionizazion. Orbital electrons are raised to next level.

- Both types of interactions are very fast

- Formed in a ratio of 1:2

MUNI MED

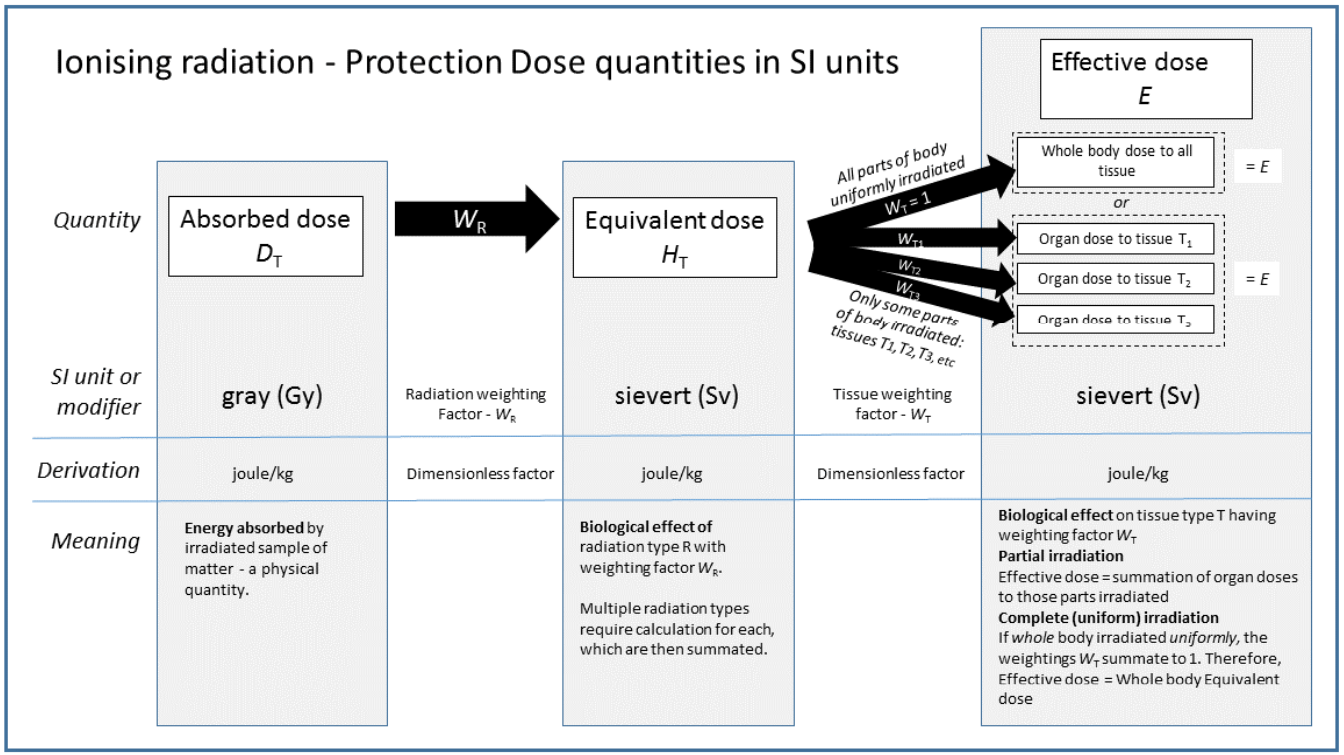
Units

- MeV, a unit of energy
- Roentgen, a unit of exposure [C / kg dry air]
- Becquerel, activity [s-1] ≈ Curie
- Gray, dose [J / kg] ≈ rad
- Sievert, dose equivalent [J / kg] ≈ rem

MUNI
MED

Units

- Dose equivalent
- = dose * constant WR
- γ, β, X = 1
- neutrons = 10
- α = 20



Radiation	Energy	W_R (formerly Q)
x-rays, gamma rays, beta particles, muons		1
neutrons	< 1 MeV	$2.5 + 18.2 \cdot e^{-[\ln(E)]^2/6}$
	1 MeV - 50 MeV	$5.0 + 17.0 \cdot e^{-[\ln(2 \cdot E)]^2/6}$
	> 50 MeV	$2.5 + 3.25 \cdot e^{-[\ln(0.04 \cdot E)]^2/6}$
protons, charged pions		2
alpha particles, Nuclear fission products, heavy nuclei		20

MUNI MED

Sources of ionizing radiation?

Natural

space

exposure increases with altitude

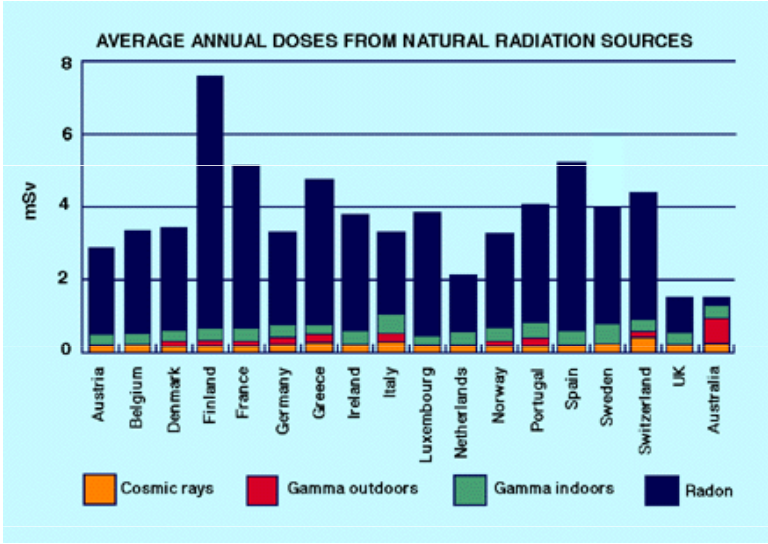
solar

terrestrial sources

natural radioactive decay of radioisotopes (soil and rock)

Radon

gas, there is a decay of radium-226 (Uranium)



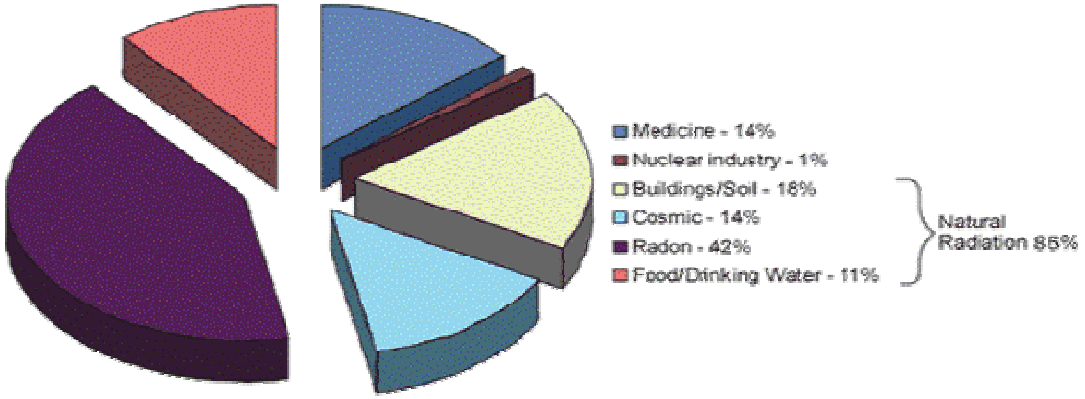
Artificial

medicine

diagnosis, therapy, sterilization

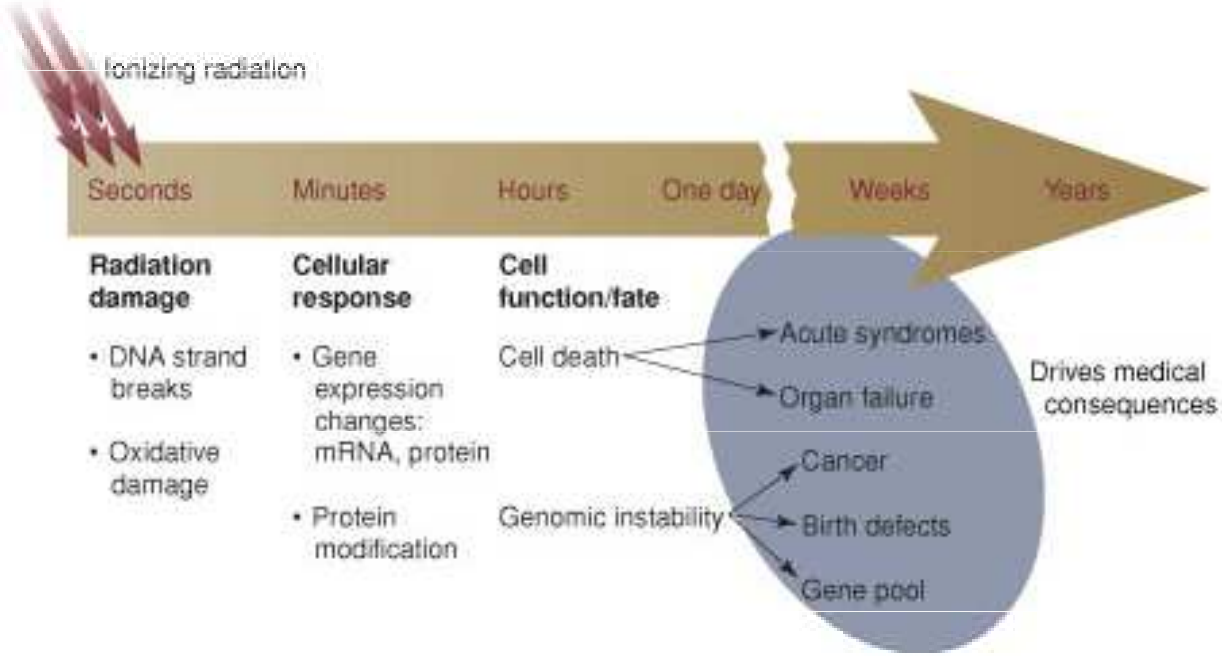
industrial

nuclear energy



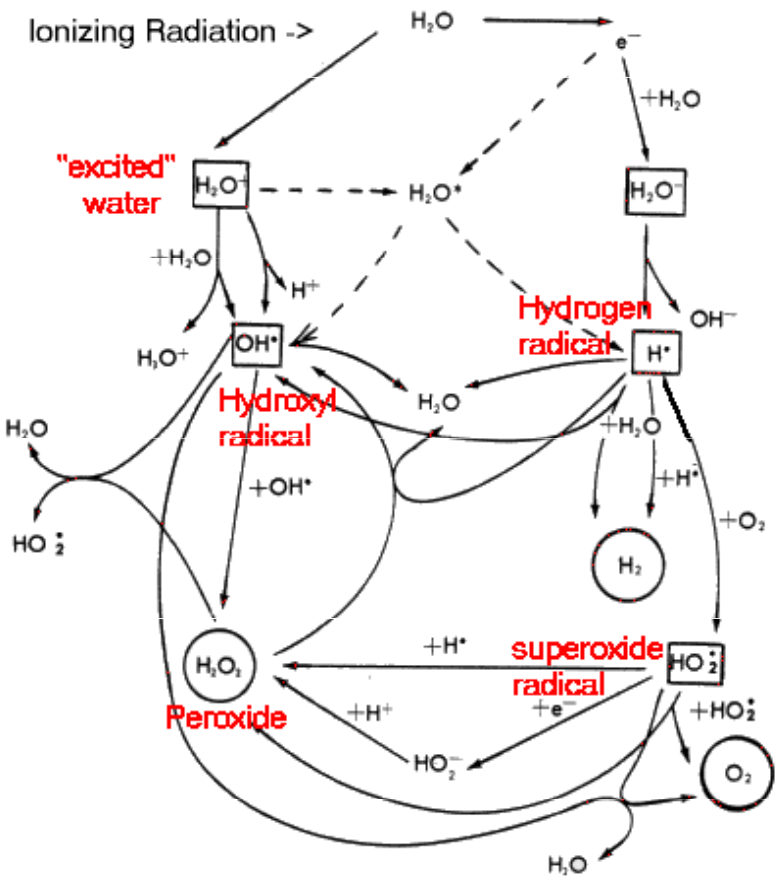
Biological effects of ionizing radiation

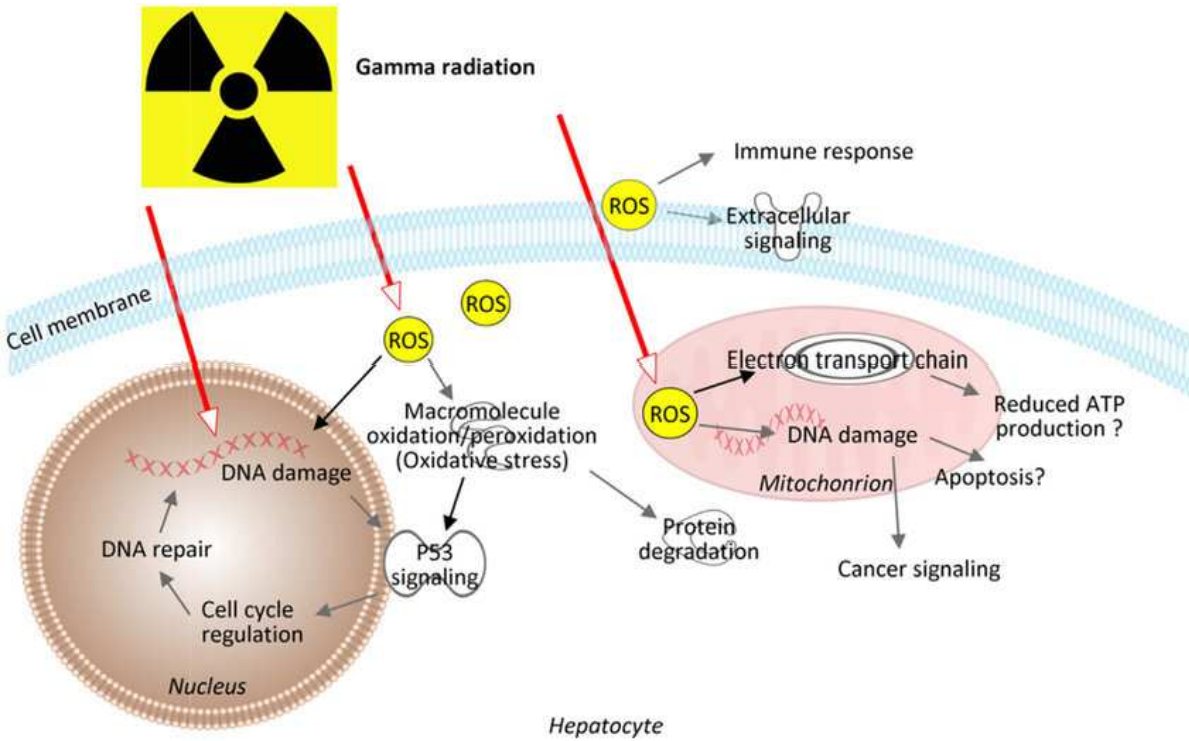
- Direct ionizing = direct destruction of biomacromolecules
- Indirectly (nondirect) effects = production of free radicals (radiolysis of water)



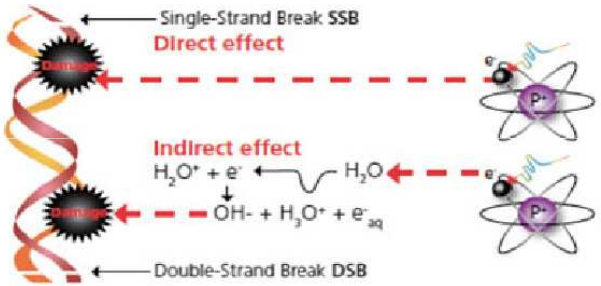
MUNI MED

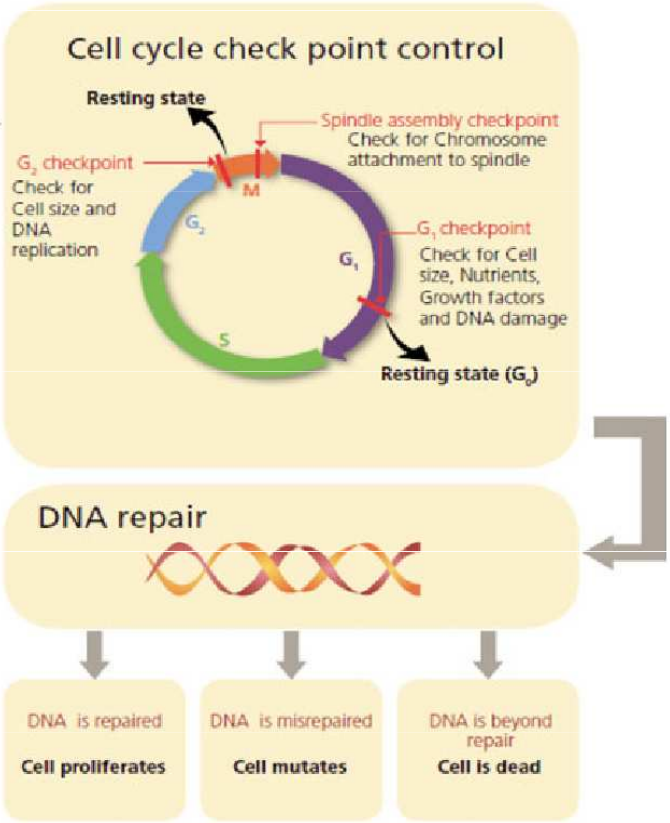
Chemical effect of ionizing radiation

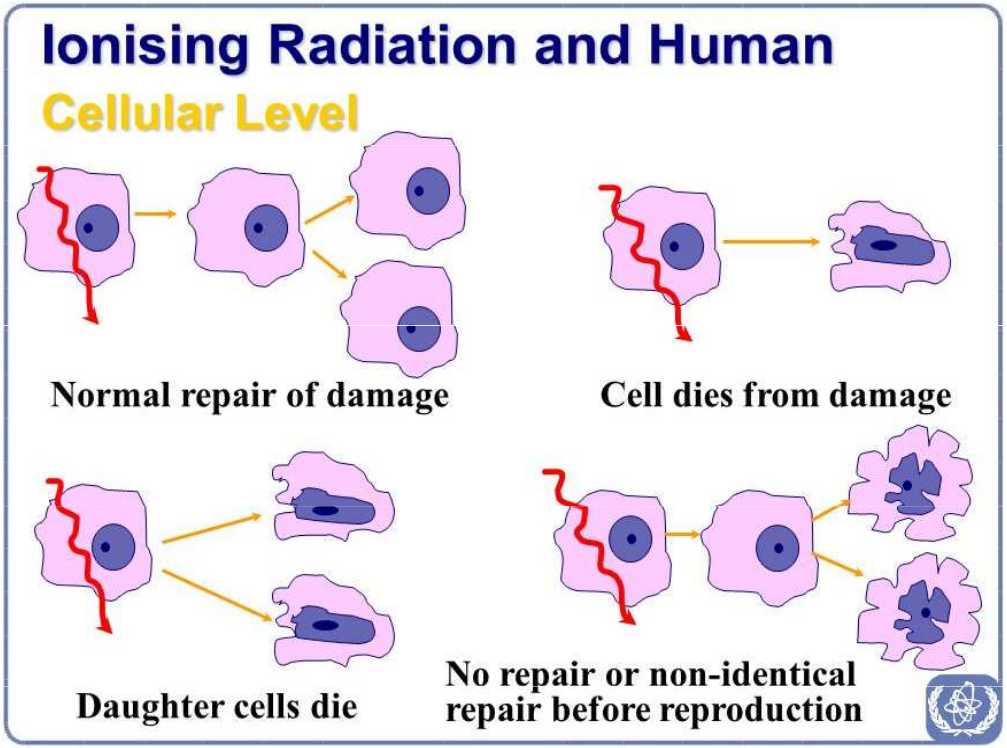


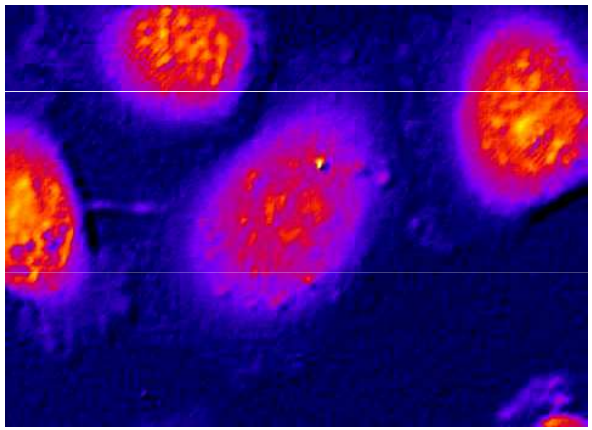


Oxygen effect!!


Single-Strand Break SSB
Direct effect
Indirect effect
$H_2O^+ + e^-$
H_2O
$OH^- + H_3O^+ + e^-_{aq}$
Double-Strand Break DSB


Cell cycle check point control
Resting state
Spindle assembly checkpoint
Check for Chromosome attachment to spindle
G_2 checkpoint
Check for Cell size and DNA replication
G_1 checkpoint
Check for Cell size, Nutrients, Growth factors and DNA damage
Resting state (G_0)
M
G_2
G_1
S
DNA repair
DNA is repaired
Cell proliferates
DNA is misrepaired
Cell mutates
DNA is beyond repair
Cell is dead

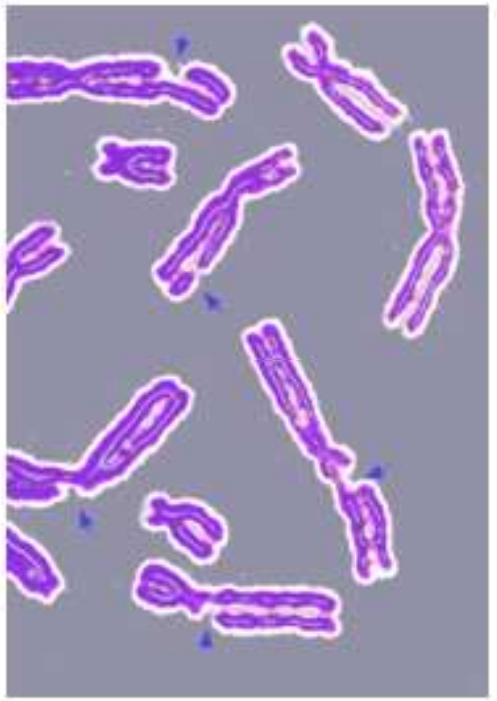

Ionising Radiation and Human
Cellular Level
Normal repair of damage
Cell dies from damage
Daughter cells die
No repair or non-identical repair before reproduction
Module 26
6



MUNI MED

DNA damage

Very serious state
DNA damage is reflected in the synthesis of damaged proteins
DNA repair mechanisms
Reproductive ability of cells



Eukaryotic Chromosomes

Eukaryotic chromosomes:

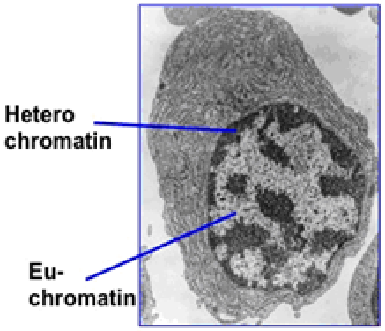
-linear chromsomes;

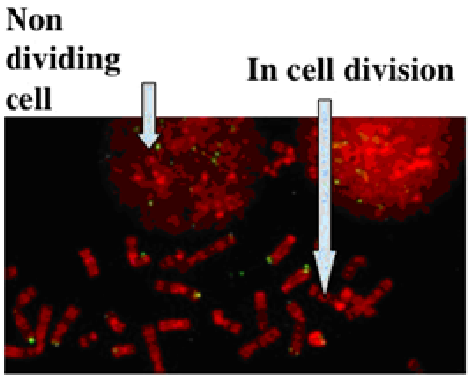
-every species has a different number of chromosomes;

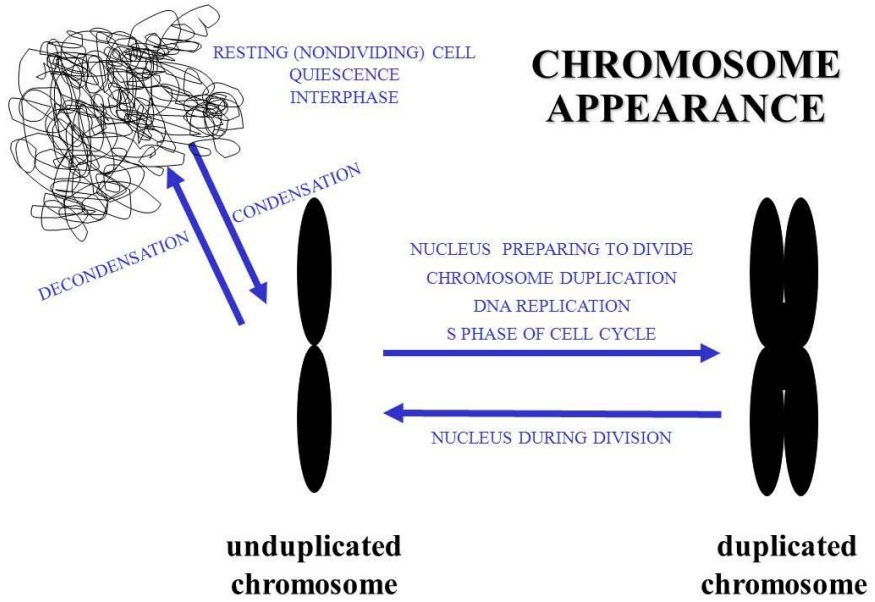
-composed of **chromatin** – a complex of DNA and proteins

- -**heterochromatin** – not expressed
- -**euchromatin** – expressed regions

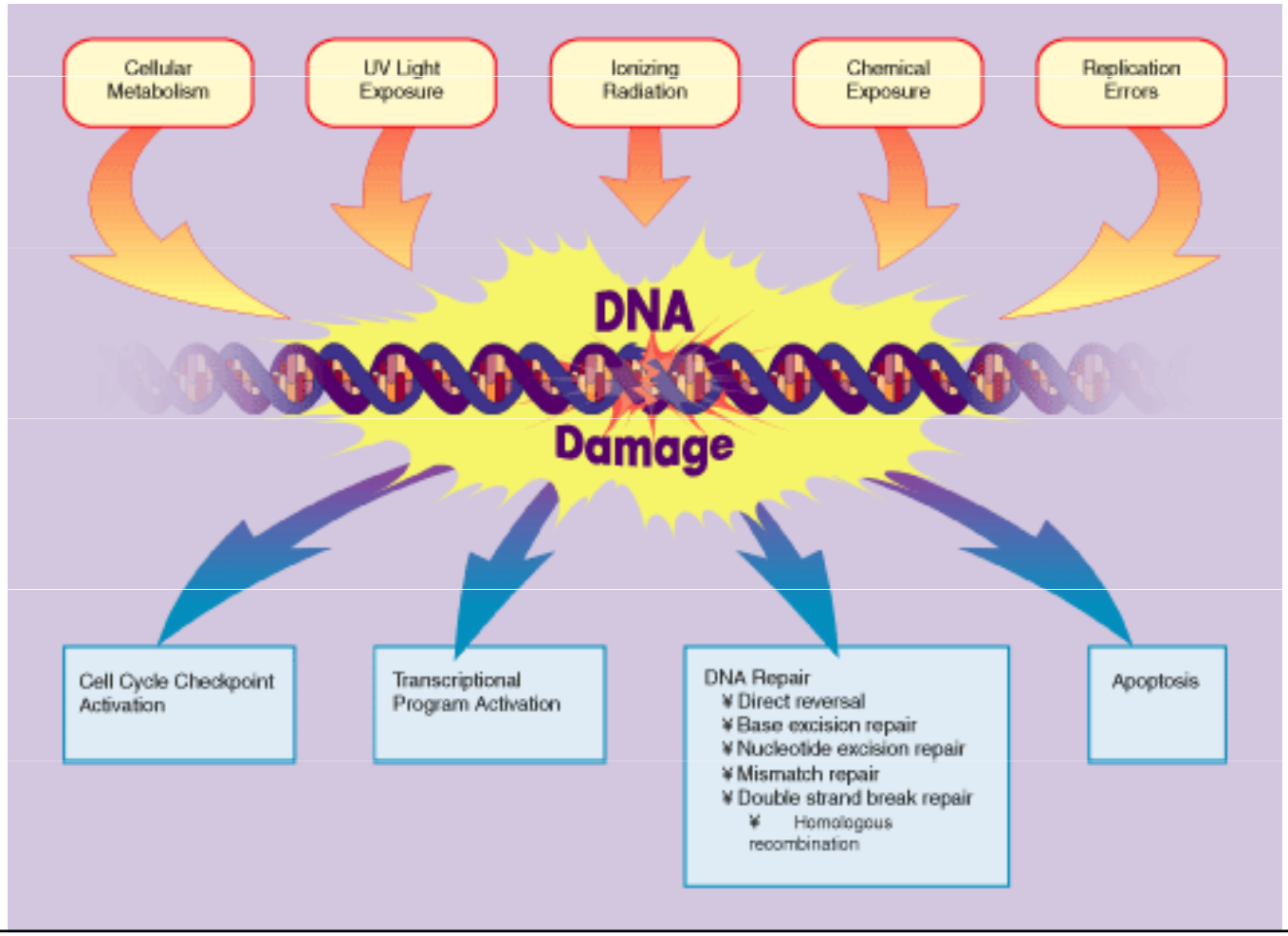
Cell and Chromatin





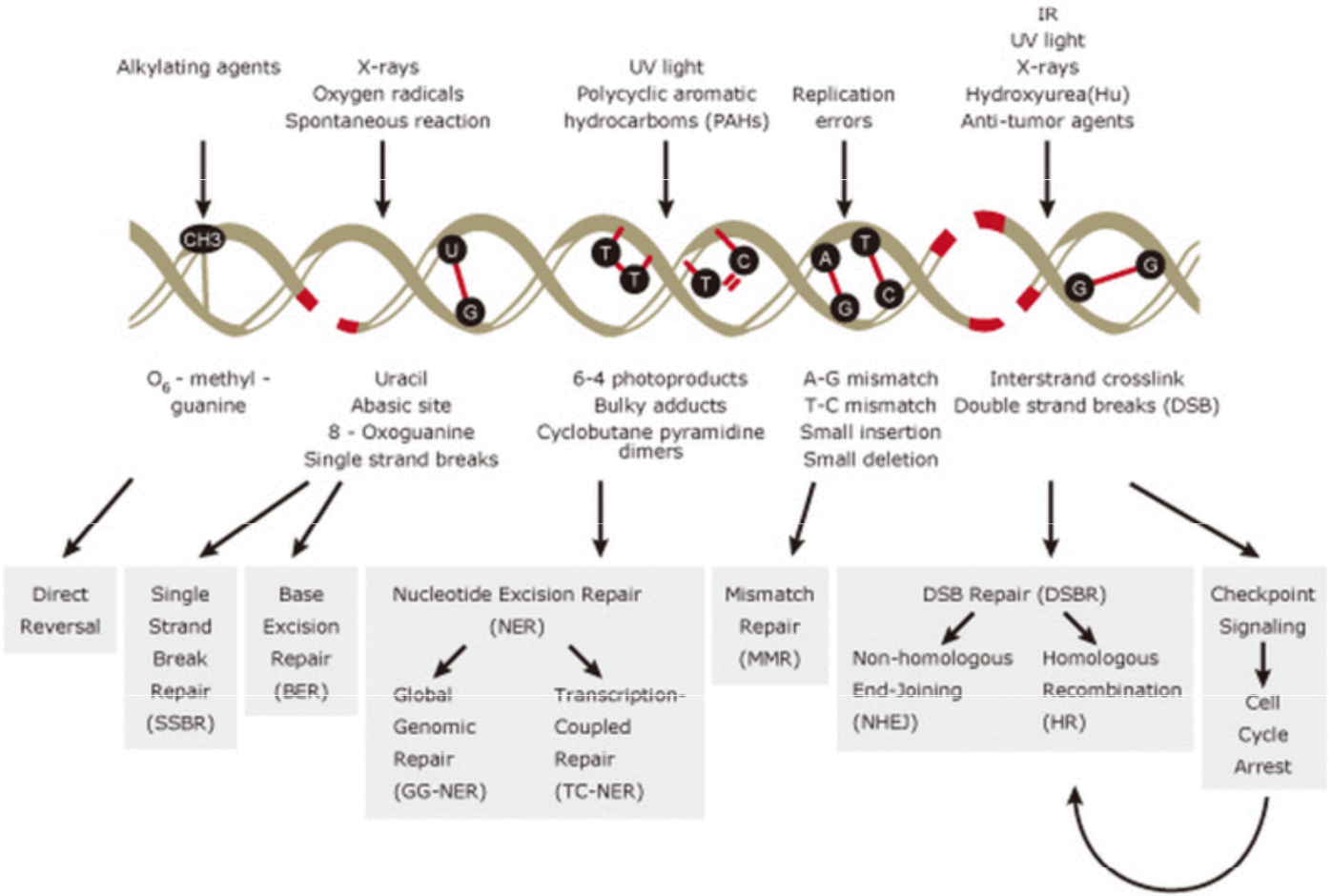


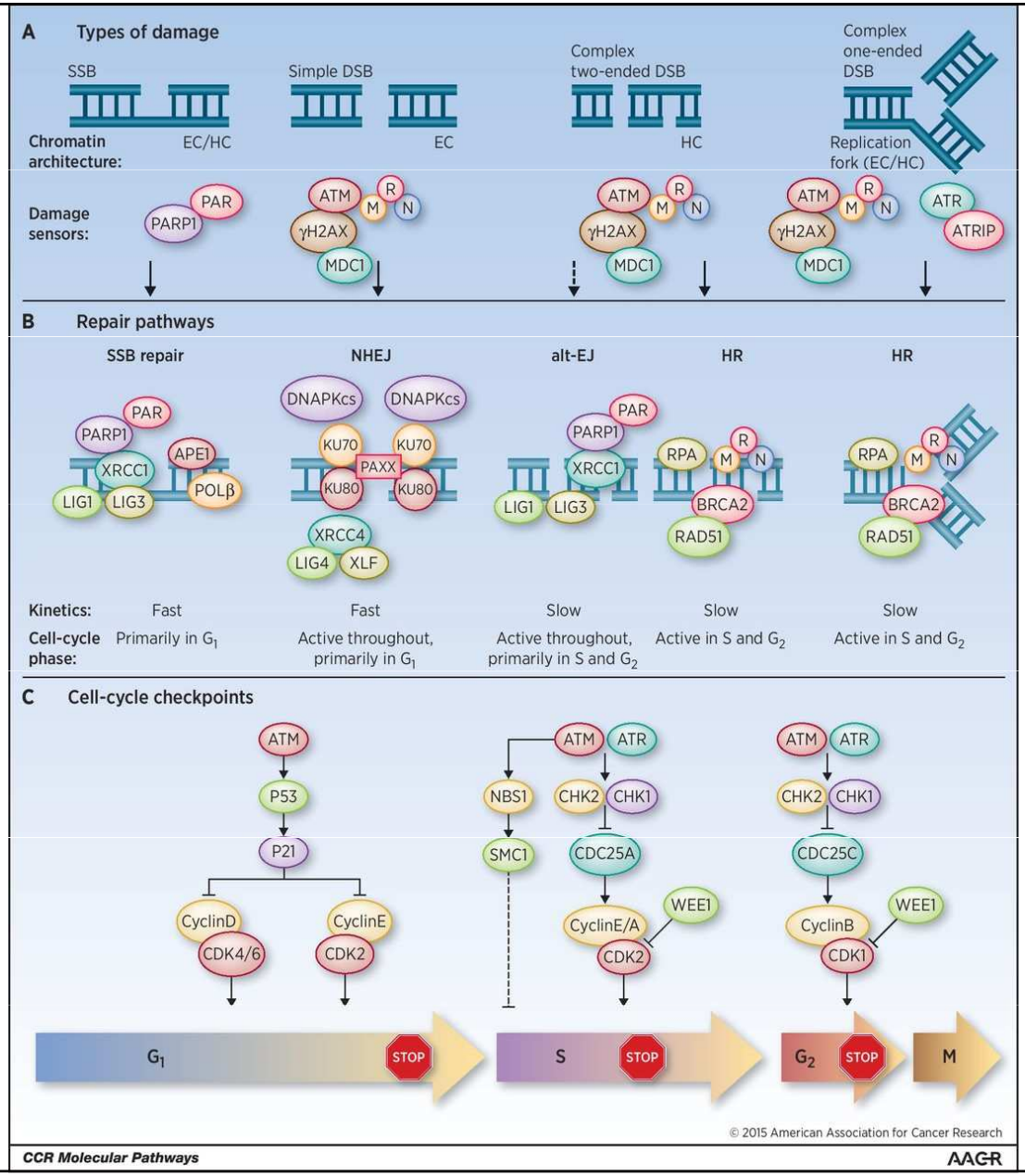
Mechanisms of DNA damage



MUNI MED

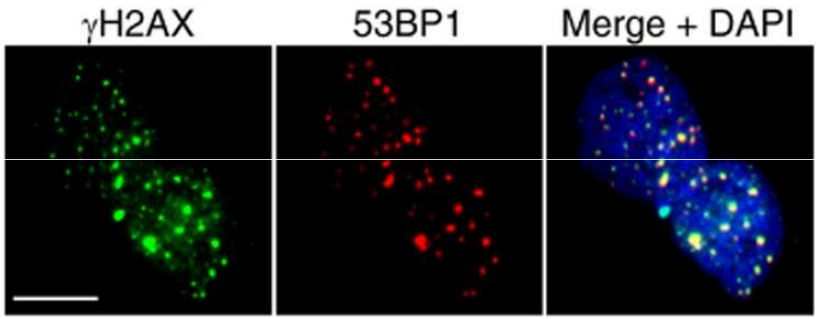
damage response enables life on earth as we know it

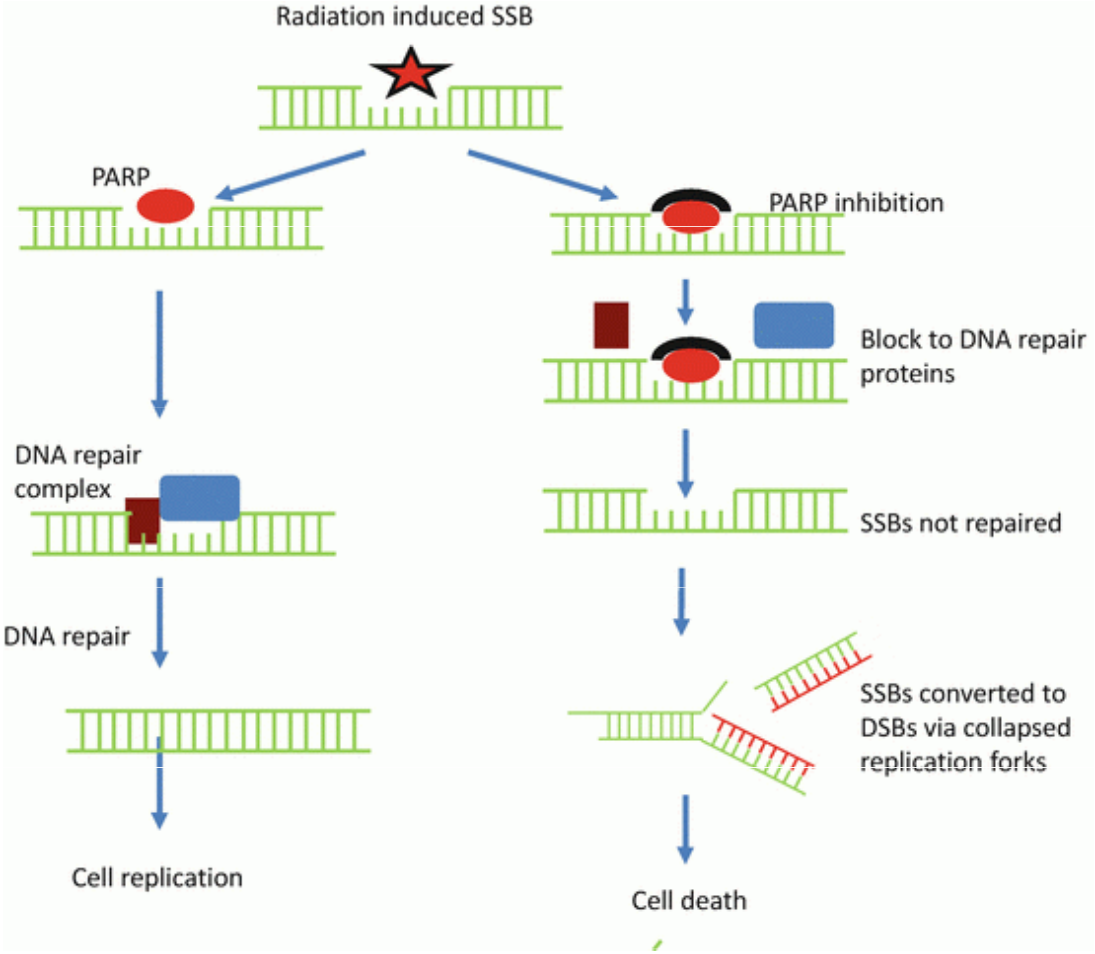


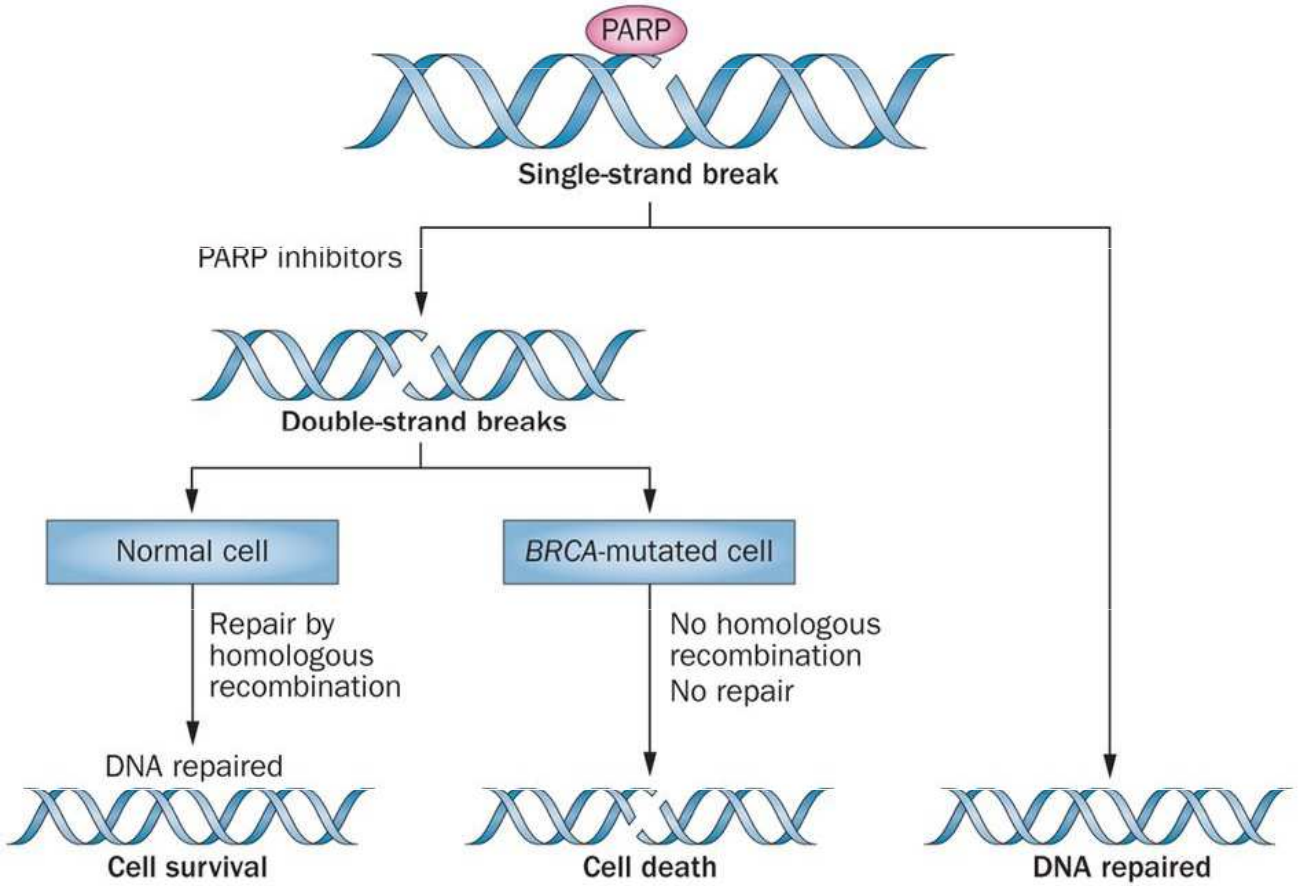

A Types of damage
SSB
Simple DSB
Complex two-ended DSB
Complex one-ended DSB
Chromatin architecture:
EC/HC
EC
HC
Replication fork (EC/HC)
Damage sensors:
PAR
PARP1
ATM
R
M
N
γH2AX
MDC1
ATR
ATRIP
B Repair pathways
SSB repair
NHEJ
alt-EJ
HR
HR
XRCC1
APE1
LIG1
LIG3
POLβ
DNAPKcs
KU70
PAXX
KU80
XRCC4
LIG4
XLF
RPA
BRCA2
RAD51
Kinetics:
Fast
Fast
Slow
Slow
Slow
Cell-cycle phase:
Primarily in G1
Active throughout, primarily in G1
Active throughout, primarily in S and G2
Active in S and G2
Active in S and G2
C Cell-cycle checkpoints
P53
P21
CyclinD
CDK4/6
CyclinE
CDK2
NBS1
SMC1
CHK2
CHK1
CDC25A
CyclinE/A
WEE1
CDC25C
CyclinB
CDK1
G1
STOP
S
G2
M
© 2015 American Association for Cancer Research
CCR Molecular Pathways
AACR

MUNI MED

A


γH2AX
53BP1
Merge + DAPI

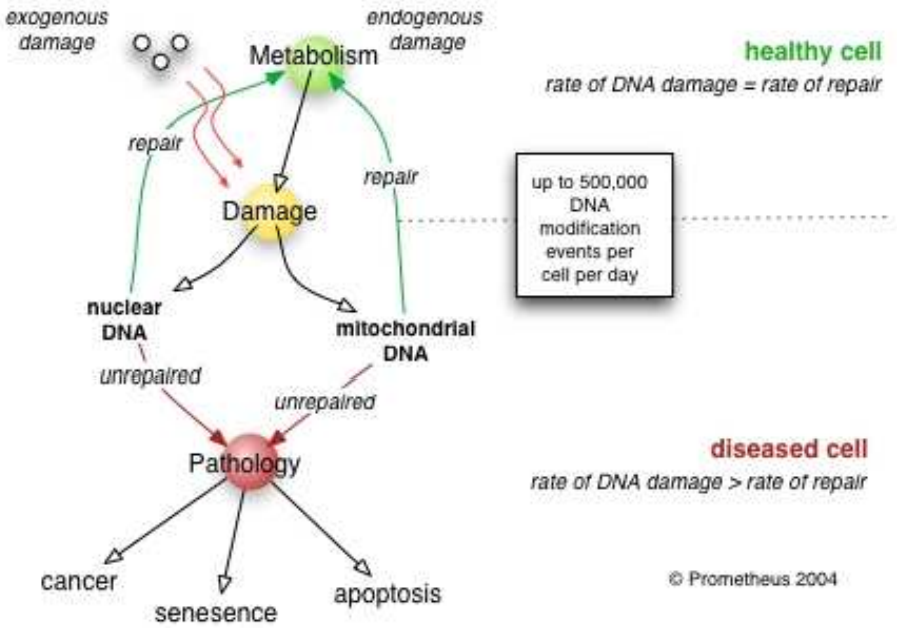

Radiation induced SSB
PARP
PARP inhibition
Block to DNA repair proteins
DNA repair complex
SSBs not repaired
DNA repair
SSBs converted to DSBs via collapsed replication forks
Cell replication
Cell death


PARP
Single-strand break
PARP inhibitors
Double-strand breaks
Normal cell
BRCA-mutated cell
Repair by homologous recombination
No homologous recombination
No repair
DNA repaired
Cell survival
Cell death
DNA repaired

MUNI MED

Repair mechanisms

Direct repair
Excision repair
Mismatch repair
SSB repair
(DSB repair)



Effects of ionizing radiation on the human body

- **Deterministic effects**
- **Stochastic effects**

MUNI
MED

Deterministic effects

- There are those that will take effect after total body irradiation, or a tissue once
- The dependence of the probability of occurrence of damage to an equivalent dose have the sigmoid character

MUNI
MED

Deterministic effects

- Acute radiation sickness (radiation syndrome)
- Local acute skin damage
- Damage to fetus
- Infertility
- Lenticular opacities

MUNI
MED

Stochastic effects

- They result from damage to a small number of cells
- They can occur after a single exposure of sub-threshold dose or chronic radiation tissue or whole body

MUNI
MED

Character of biological effects

Deterministic
– **severity dependent** ("determined") **on the dose**
– manifestation is **specific**
– effect only when exposure **exceeded threshold**
– damage of **large amount of cells**
– onset rather close to the exposure (**short latency**)
– types:
  acute radiation syndrome
  chronic post-radiation syndrome
  cataract, radiation dermatitis,
  damage of the foetus *in utero*
  sterility

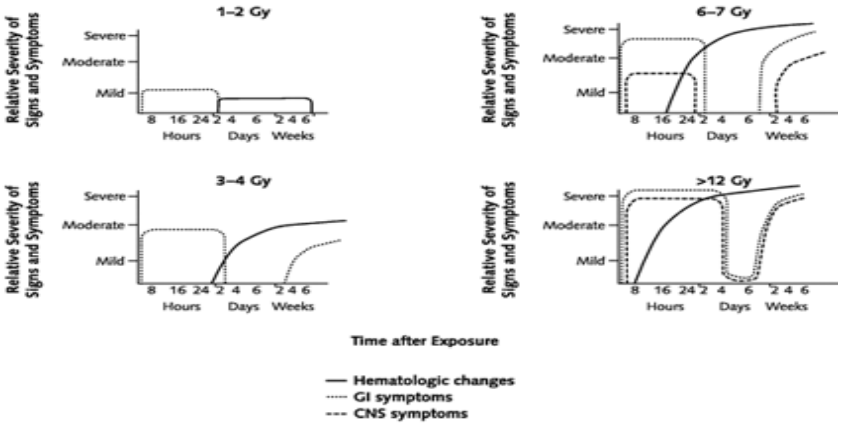
Stochastic
– **probability increases with the dose** (not the severity!)
– manifestation **non-specific**
– gradual increase of the risk **without "safe" threshold**
– damage of the **single cell** enough to cause effect
– **manifestation delayed** (typically years)
– types:
  somatic mutation - cancer
  leukemias, thyroid, lung, breast, bones
  germinative mutation (oocyte, sperm cell)

Acute radiation syndrome

affecting the hematopoietic, gastrointestinal system and cerebrovascular

timing, extent and severity graded according to the dose - deterministic effect!!

from several hours to several months after exposure



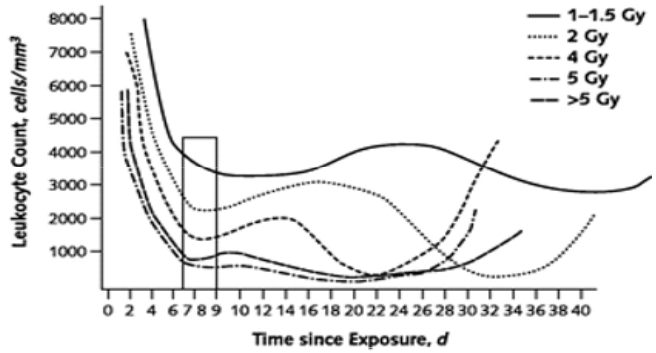
Acute radiation syndrome

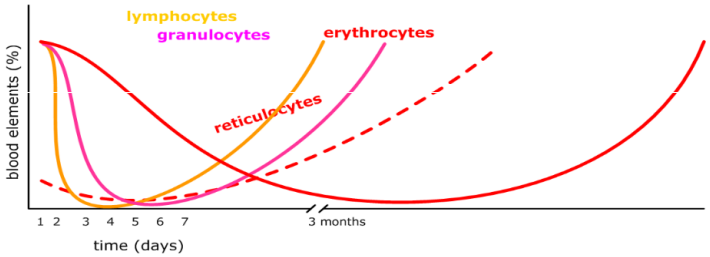
- Haematopoetic syndrome (> 1GY)
- GI syndrome (> 10Gy)
  - early (hours) - nausea, vomiting, diarrhea
  - late (days) - loss of intestinal integrity
    malabsorption, dehydration, toxemia / sepsis, ileus, bleeding
- Cerebrovascular syndrome (tens of Gy)
  - headache, cognitive impairment, disorientation, ataxia, convulsions, fatigue and hypotension
- Cutaneous
  - erythema, burns, edema, impaired wound healing

MUNI
MED

Hematopoetic syndrome

bone marrow irradiation (> 1GY) leads to an exponential cell death
- haematological crisis
marrow hypoplasia to aplasia + peripheral pancytopenia (infection, bleeding)
subpopulation of stem bb is selectively more radioresistant, (probably due to predominance bb. stage in Go)
necessary for regeneration
anemia is the result of late (erythrocytes ~ 120 days)!
massive stress response (glucocorticoids) contribute to lymphopenia (cytolytic effect) and paradoxically delay the onset of granulocytopenia (release stocks. granulocytes from the spleen)



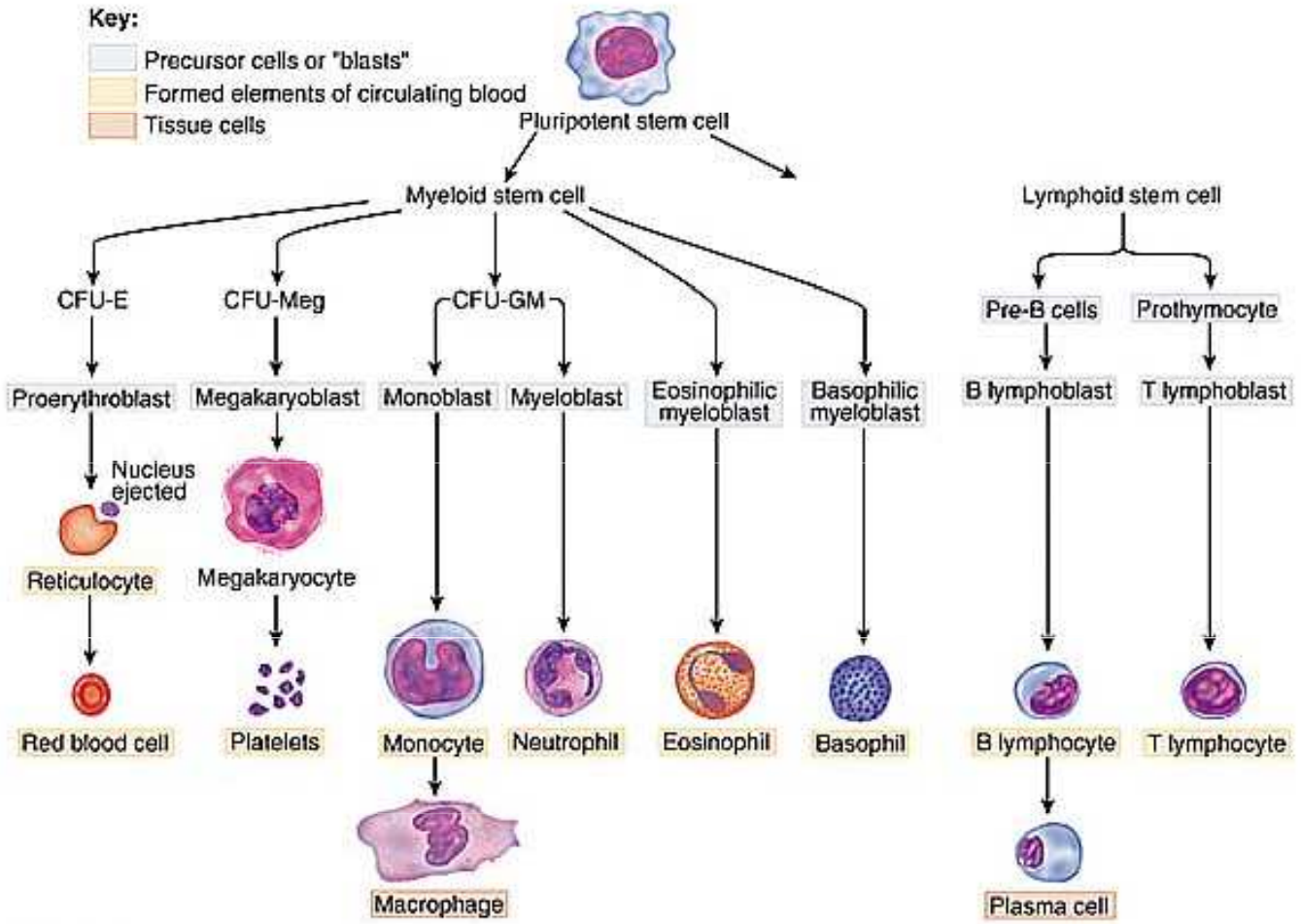


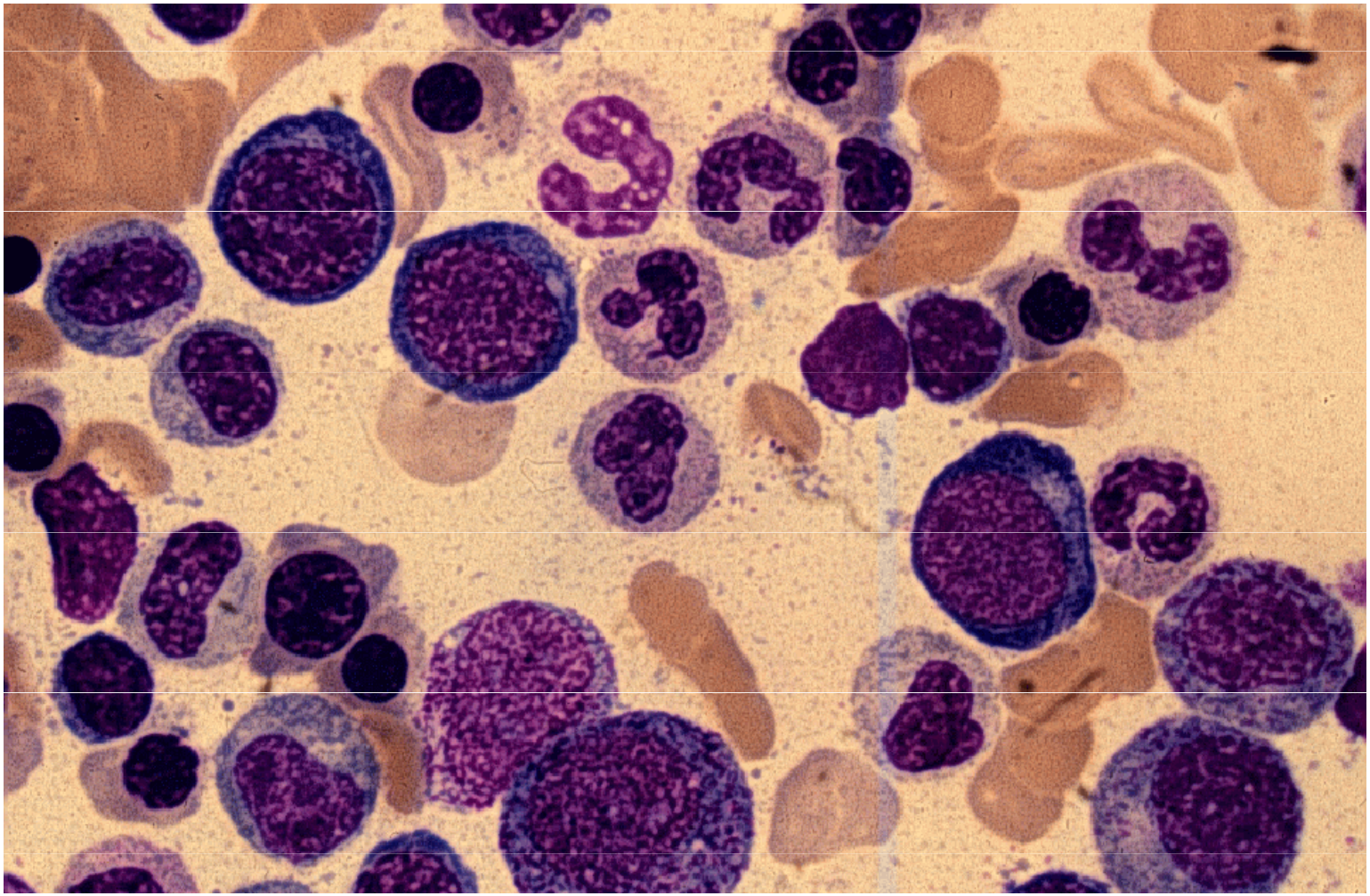
Therapeutic effects of ionizing radiation

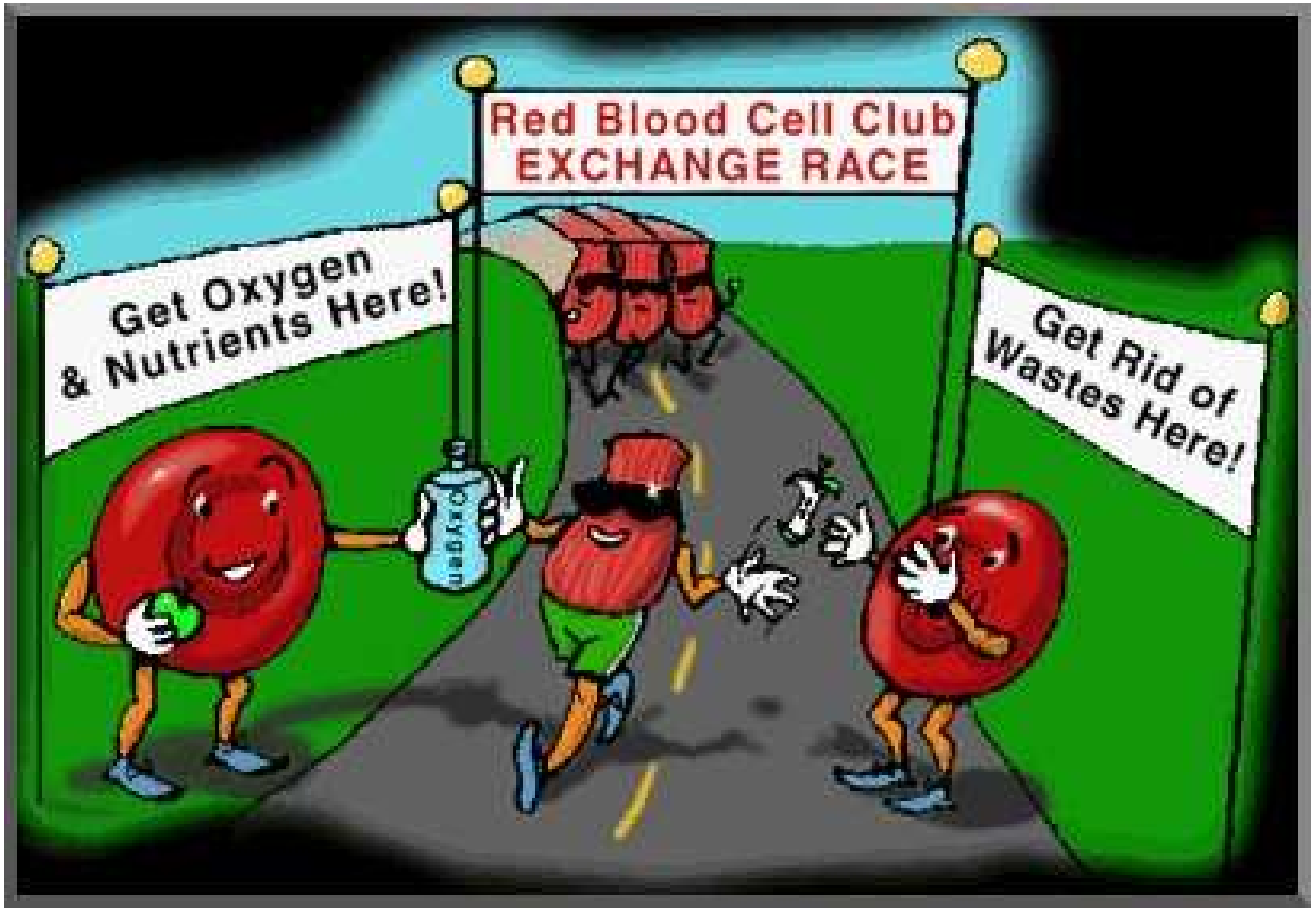
- Teleterapy (^{60}Co)
- Contact therapy (^{32}P, ^{90}Sr)
- Brachytherapy (^{60}Co, ^{137}Cs)
- Endotherapy ($Na^{131}I$)
- Radioimunotherapy

MUNI
MED

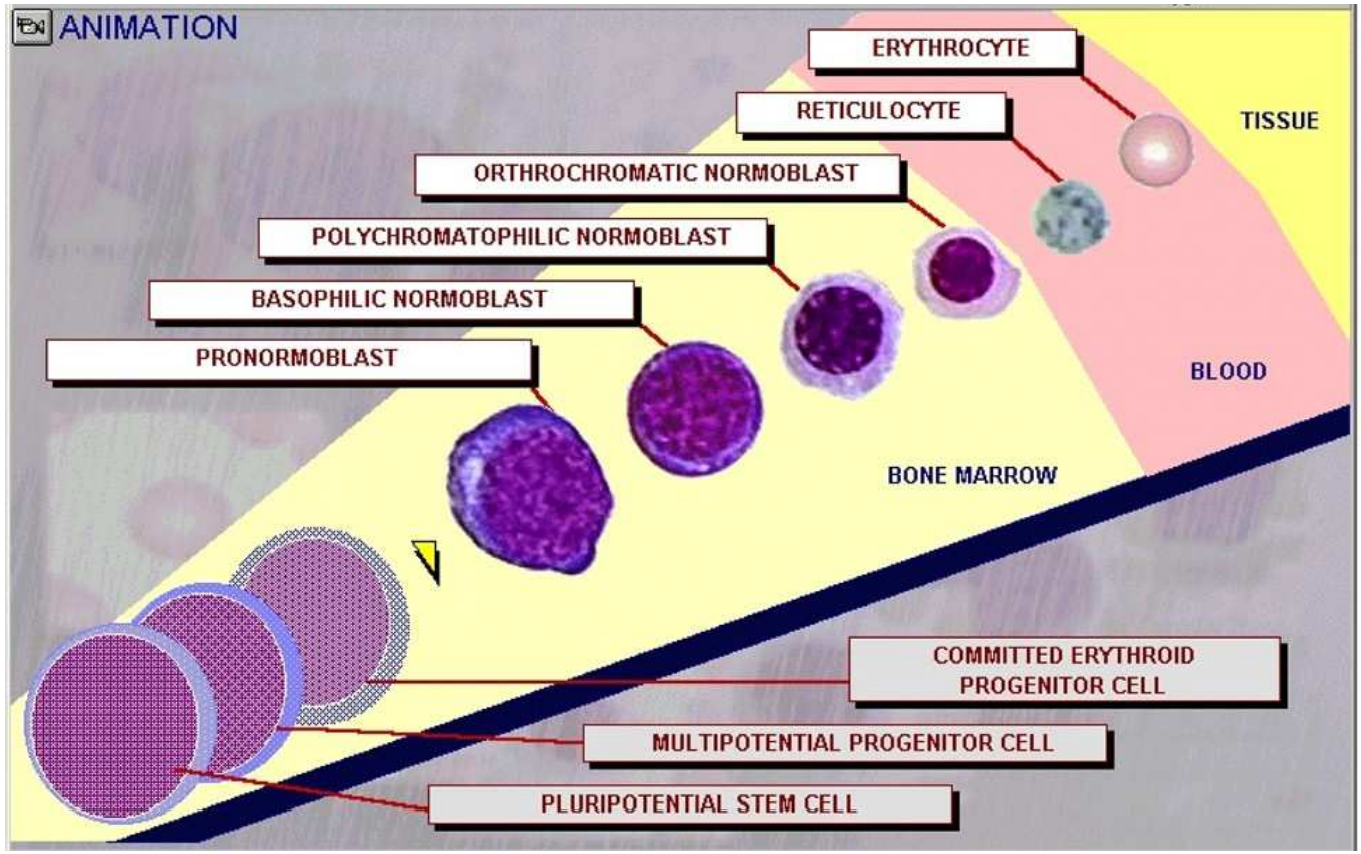
Hemopoesis


Key:
Precursor cells or "blasts"
Formed elements of circulating blood
Tissue cells
Pluripotent stem cell
Myeloid stem cell
Lymphoid stem cell
CFU-E
CFU-Meg
CFU-GM
Pre-B cells
Prothymocyte
Proerythroblast
Megakaryoblast
Monoblast
Myeloblast
Eosinophilic myeloblast
Basophilic myeloblast
B lymphoblast
T lymphoblast
Nucleus ejected
Reticulocyte
Megakaryocyte
Red blood cell
Platelets
Monocyte
Neutrophil
Eosinophil
Basophil
B lymphocyte
T lymphocyte
Macrophage
Plasma cell




Red Blood Cell Club
EXCHANGE RACE
Get Oxygen
& Nutrients Here!
Get Rid of
Wastes Here!
Oxygen

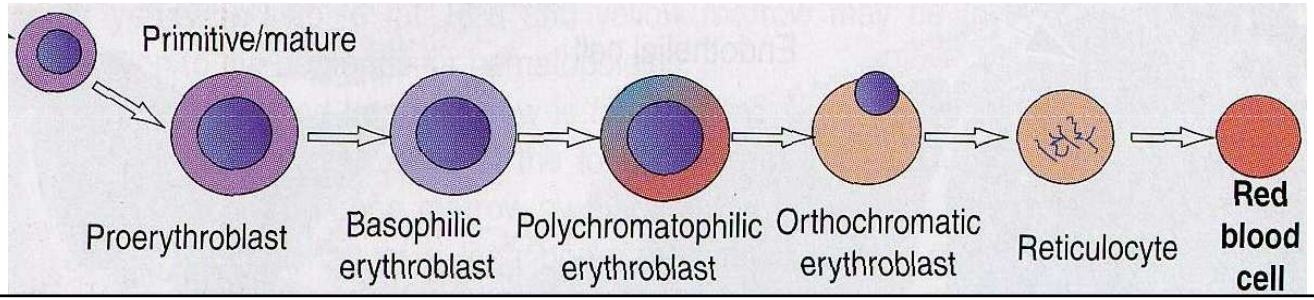
Erythropoiesis



http://www.noblesmedart.com/morph.mov

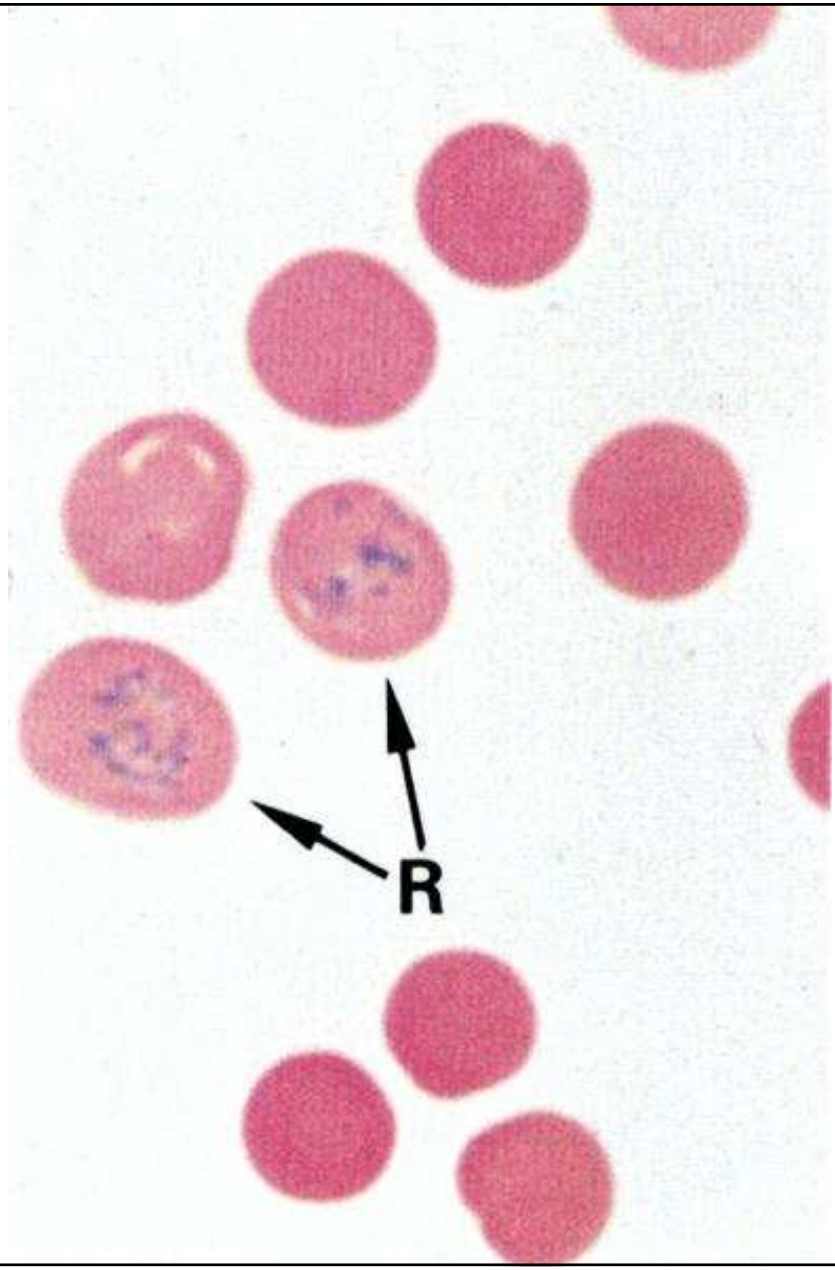
Erythropoiesis

- Erythropoiesis is the development of mature red blood cells (erythrocytes).
- Like all blood cells, erythroid cells begin as **pluripotential stem cells**.
- The first cell that is recognizable as specifically leading down the red cell pathway is the **proerythroblast** .
- As development progresses, the nucleus becomes somewhat smaller and the cytoplasm becomes more basophilic, due to the presence of ribosomes. In this stage the cell is called a **basophilic erythroblast** .
- The cell will continue to become smaller throughout development. As the cell begins to produce hemoglobin, the cytoplasm attracts both basic and eosin stains, and is called a **polychromatophilic erythroblast** .
- The cytoplasm eventually becomes more eosinophilic, and the cell is called an **orthochromatic erythroblast** .
- This orthochromatic erythroblast will then extrude its nucleus and enter the circulation as a b . Reticulocytes are so named because these cells contain reticular networks of polyribosomes. As reticulocytes loose their polyribosomes they become **mature red blood cells**.



MUNI MED

Reticulocyte

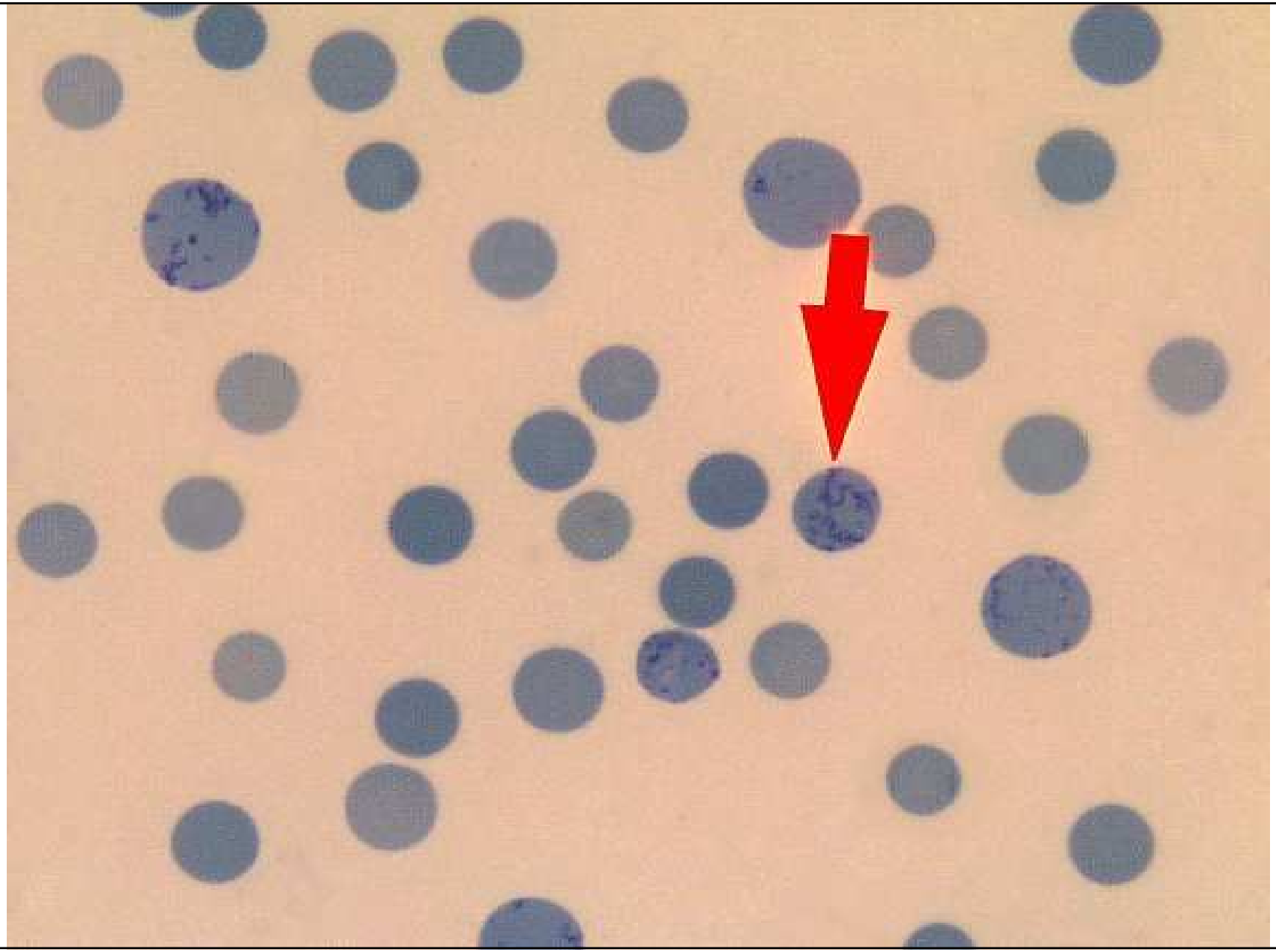


MUNI
MED

Reticulocytes

- **Reticulocytes** are immature red blood cells, typically composing about 1% of the red cells in the human body.
- Reticulocytes develop and mature in the red bone marrow and then circulate for about a day in the blood stream before developing into mature red blood cells.
- Like mature red blood cells, reticulocytes do not have a cell nucleus.
- They are called reticulocytes because of a reticular (mesh-like) network of ribosomal RNA that becomes visible under a microscope with certain stains such as new methylene blue.

MUNI
MED



Classification of reticulocyte counts

- Counting with imersion, magnification 100
- **Out of 1000 RBC in the moving viewing field, the number of RET will be counted**
- Normal counts RAT: app. 20 ‰ RET
- Normal counts MAN: app 0.5-1.5% (5-15‰) RET

↑ number: increasing bloodforming (regeneration)
↓ number: inhibition of erythropoiesis

MUNI
MED

Differential leucocyte counts

- Changes in WBC number (%)
  - Indication of infection, poisoning, leukemia, chemotherapy, alergic reaction

- Normal WBC counts in man:
  - neutrophils 60-70% (incr. in bacterial infection)
  - lymphocytes 20-25% (incr. in viral infection)
  - monocytes 3-8 % (incr. in fungal/viral infection)
  - eozinophils 2-4 % (incr. in alergic reaction and parasital infect.)
  - bazophils <1% (incr. in alergic reaction)

MUNI
MED

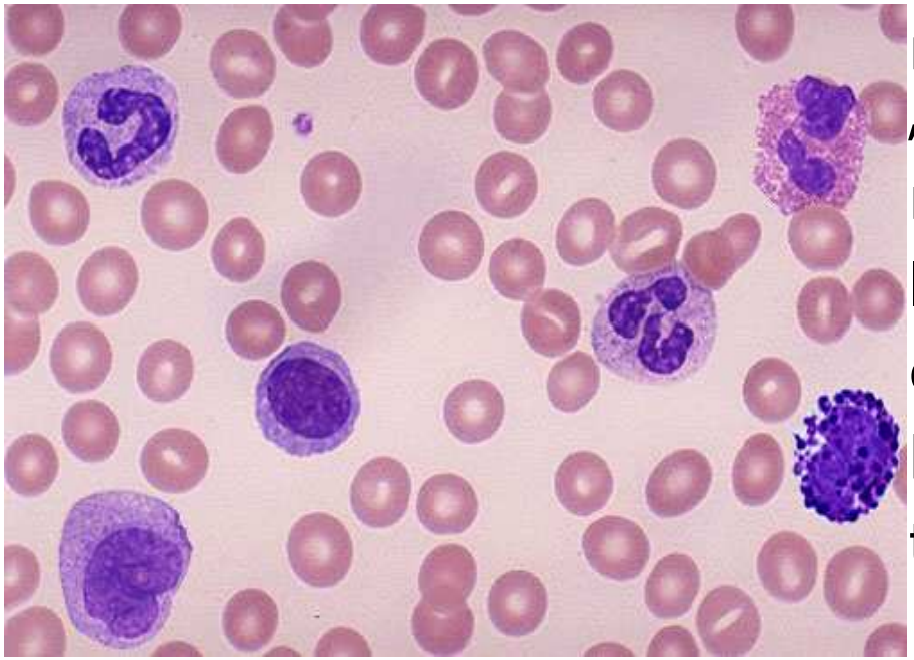
Normal counts of WBC in rat

– Number of WBC.....cca 12.5 tis.mm-3

- neutrophil granulocytes 18 - 36%
- eozinophil granulocytes 1 - 4%
- bazophil granulocytes 0 - 1%
- lymphocytes 62 - 75%
- monocytes 1 - 6%

MUNI MED

Blood smear



neutrophil „band“

neutrophil „segment“

monocyte

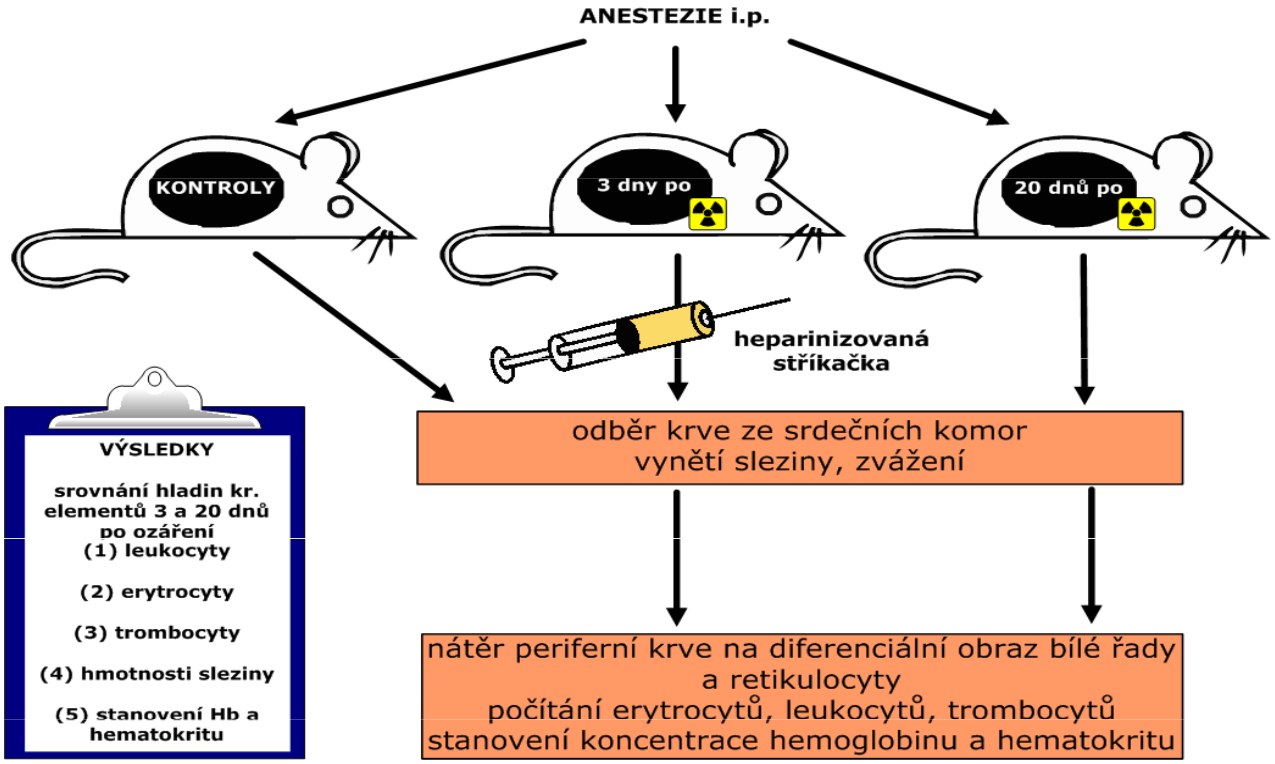
lymphocyte

eozinophil

bazophil

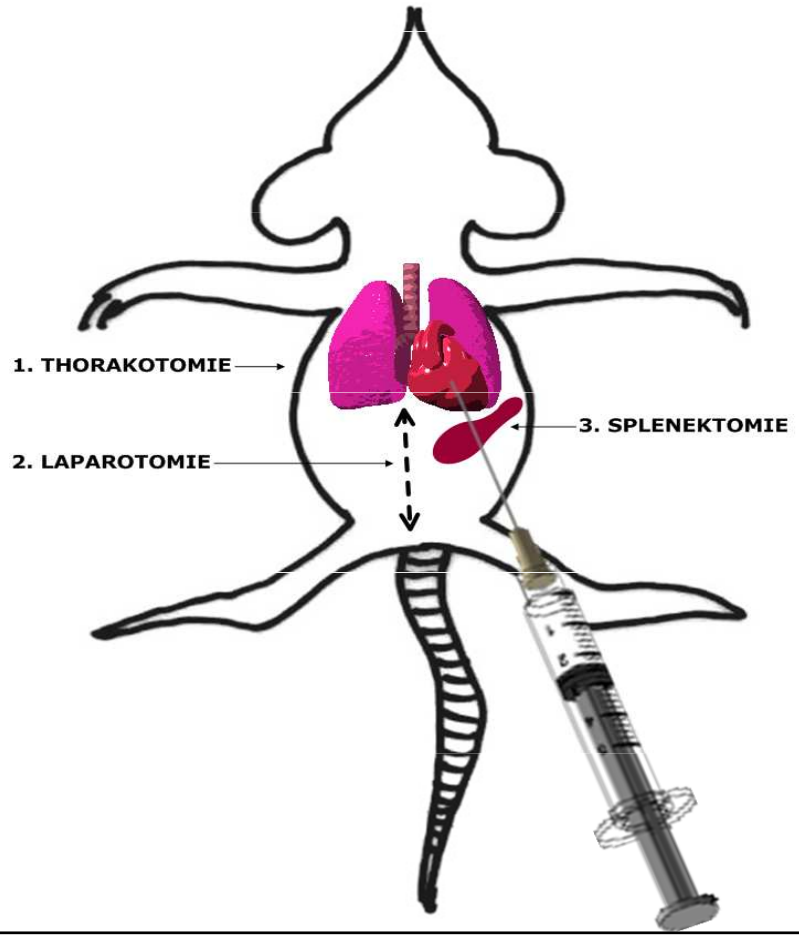
trombocyte

Praktikum I - design



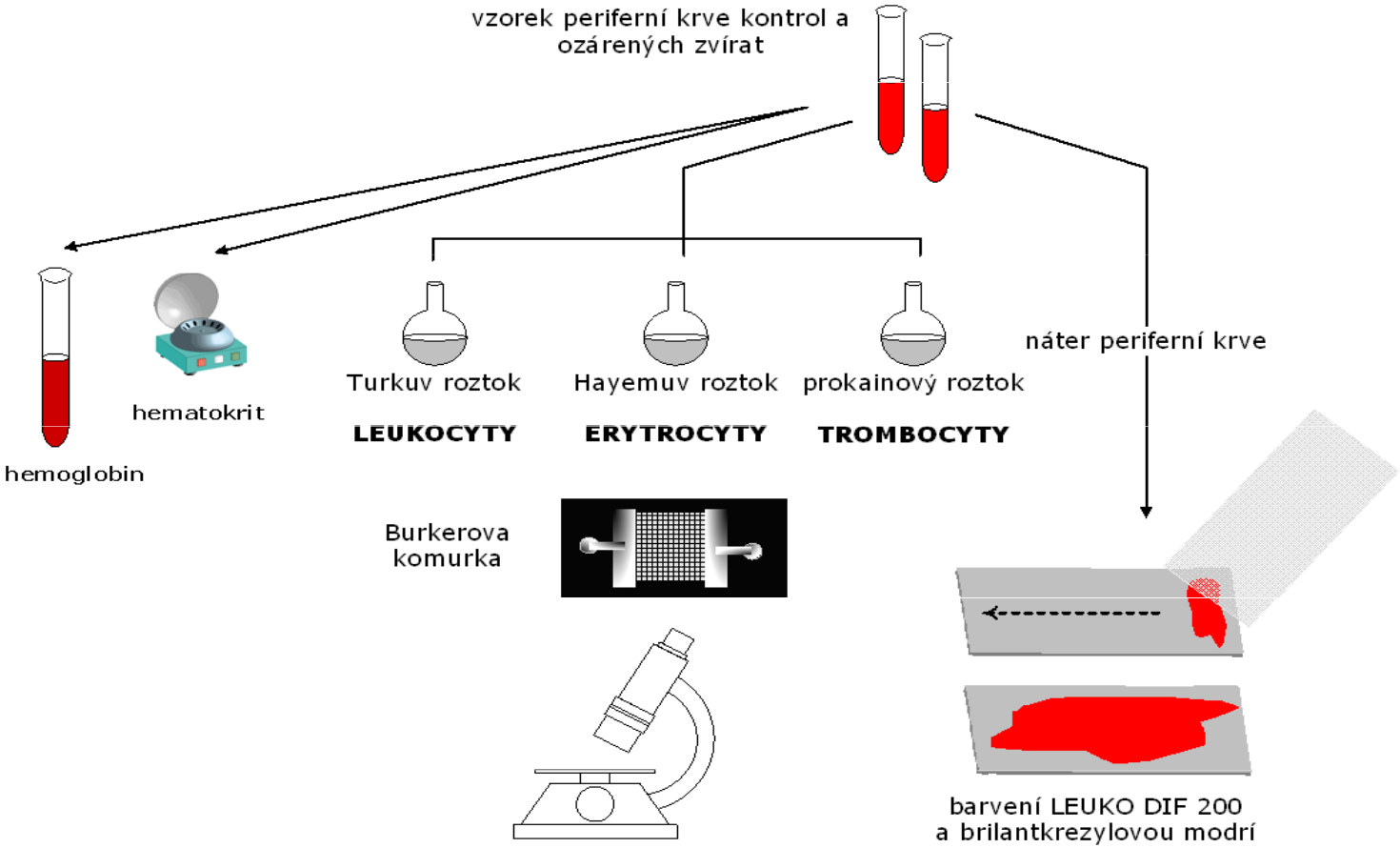
MUNI MED

Praktikum I – operační postup

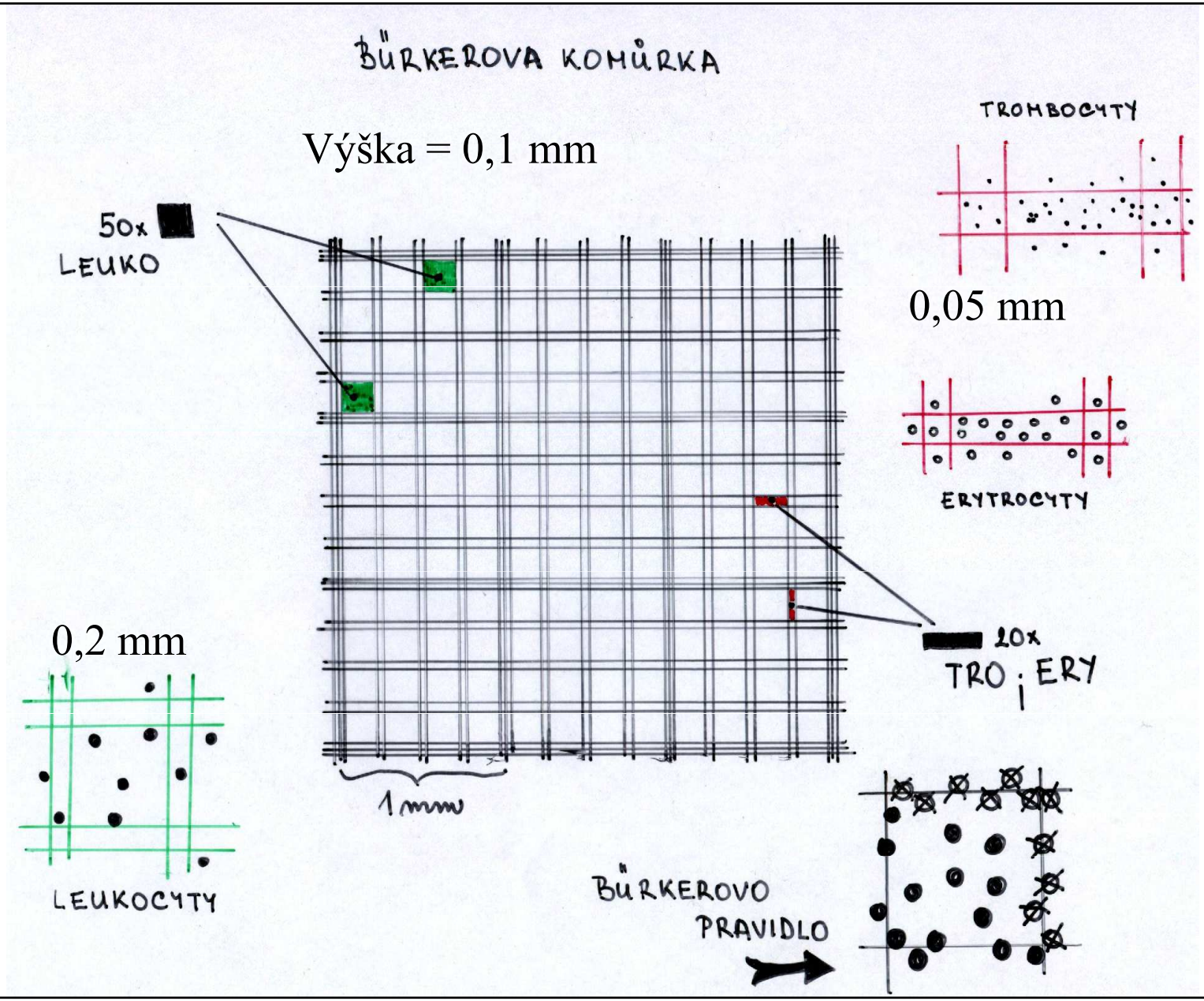


MUNI
MED

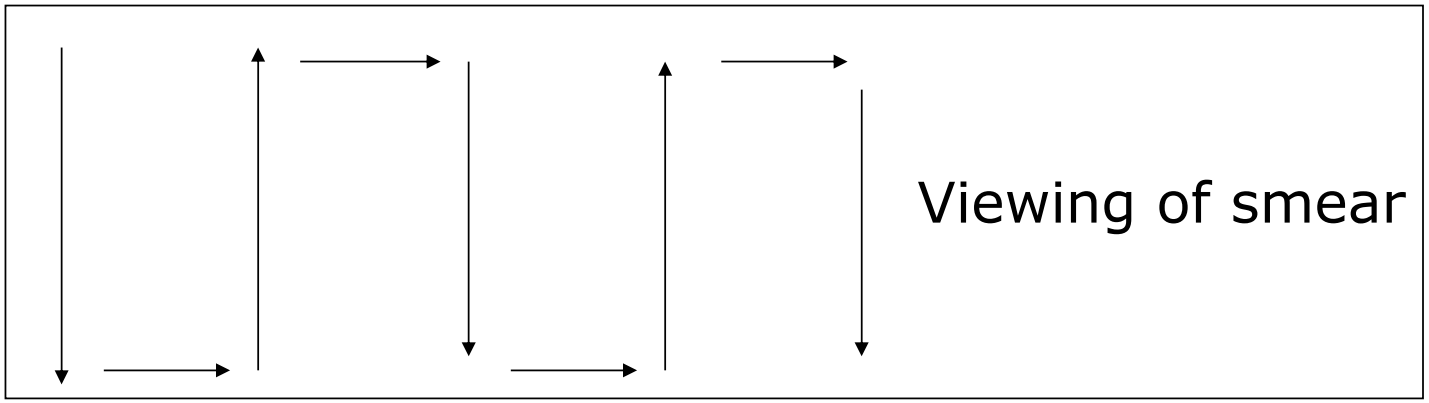
Praktikum I - hodnocení



MUNI MED


BŮRKEROVA KOMŮRKA
Výška = 0,1 mm
50x
LEUKO
TROMBOCYTY
0,05 mm
ERYTROCYTY
10x
TRO ; ERY
0,2 mm
1 mm
LEUKOCYTY
BŮRKEROVO
PRAVIDLO

MUNI
MED



Viewing of smear

- The stained smear will first be viewed at a low magnification and an area where white cells are not overlaid by red cells will be selected.
- Using an immersion objective, a total of 100 WBC will be registered and identified as to their individual type.
- The viewing field will be moved in order to count the prescribed number of leucocytes.

j.gumulec@med.muni.cz | @jarogumulec | www.med.muni.cz/masariklab