KU LEUVEN

Biology of aging and immunosenescence

Sigrid Hatse
Laboratory of Experimental Oncology, KU Leuven

BIUCGM 7 December 2022

3 different ways to look at ‘age’

An individual’s physical appearance, functioning and biological health may differ from other persons of the same chronological age!!!

CHRONOLOGICAL AGE

Number of years that have passed since birth

FUNCTIONAL AGE

Ability to function properly in daily physical and social environment

GERIATRIC ASSESSMENT
physical appearance mobility
strength
coördination
mental capacity

BIOLOGICAL AGE

Internal ‘footprint’ of passed lifespan, reflected by accumulated damage to cells/tissues/organs

AGE-RELATED MOLECULAR CHANGES

KU LEUVEN

Body aging starts at the molecular level

SUBCELLULAR LEVEL | CELLULAR LEVEL | TISSUE LEVEL | BODY LEVEL

Age-related molecular changes → **Cellular senescence** → **Tissue aging** → **Clinical aging (frailty)**

Immuno-senescence

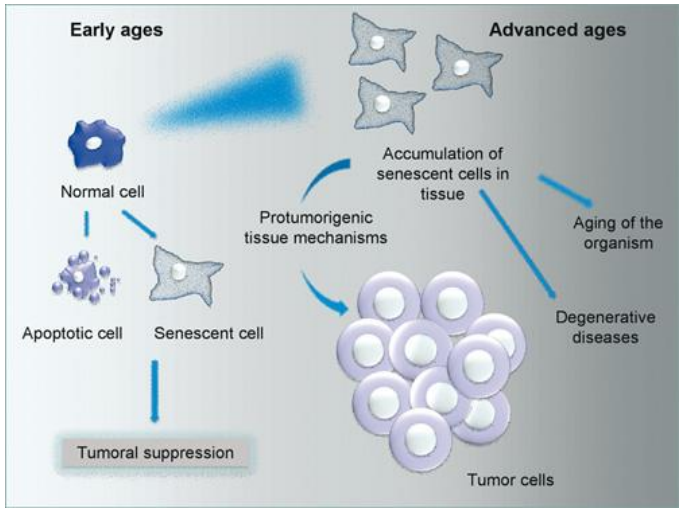
Neuro-senescence

Muscle aging

Decreased immune responses

Neurodegenerative diseases

Decreased strength & mobility



https://www.scielo.cl/pdf/ijmorphol/v28n1/art06.pdf

KU LEUVEN

Entangled molecular pathways drive the aging process

https://www.cell.com/fulltext/S0092-8674%2813%2900645-4

KU LEUVEN

DNA damage and genomic instability

Increased exposure with aging !!!

ENDOGENOUS FACTORS

- Replication errors (1 per 10^4-10^5 bp)
- Inflammation
- Cellular metabolism
- Mitochondrial dysfunction

EXOGENOUS INSULTS

- Smoking
- Pollution
- Ionizing ratiation
- UV light
- Toxins
- Drugs

ROS ROS ROS ROS ROS ROS ROS

DNA DAMAGE

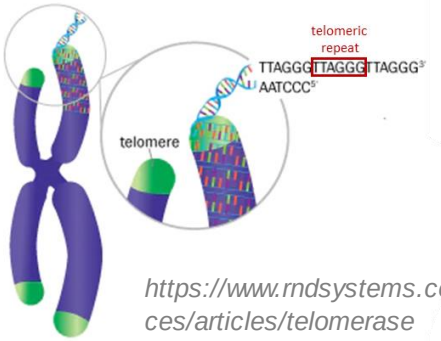
Base mismatch
Insertion/deletion
Deamination
Oxidation
Alkylation
Depurination
Dimer formation
ss or ds DNA breaks
Crosslinking
Hydrolysis

Decreased repair capacity with aging !!!

DNA REPAIR

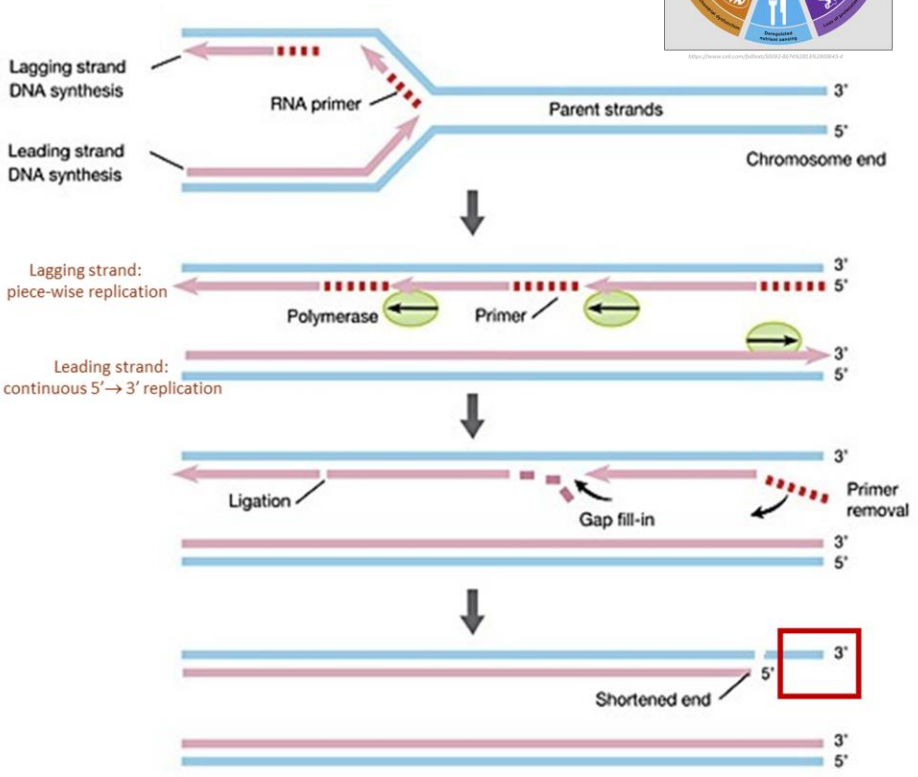
KU LEUVEN

Telomeres, the 'end replication problem' and the Hayflick limit

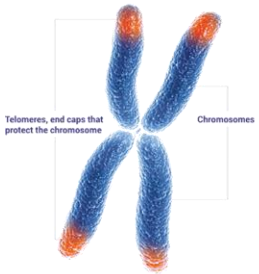


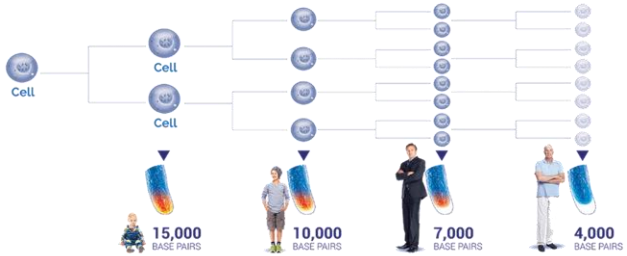
https://www.rndsystems.com/resources/articles/telomerase

- Hexanucleotide TTAGGG repeats capping the ends of eukaryotic (linear) chromosomes
- Maintain chromosome stability
- Ensure complete chromosome replication
- Shorten upon each cell division
- Cells stop dividing when chromosomes become critically short (Hayflick limit)
- Short telomeres resemble persistant dsDNA breaks

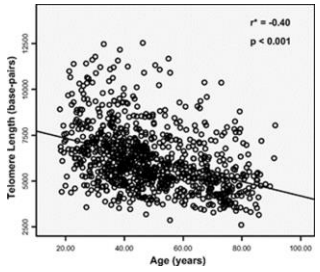


https://brainly.com/question/11002481



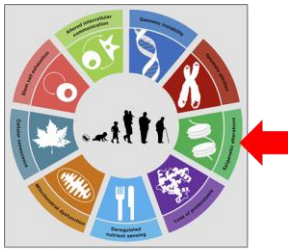


https://ta65-sciences.com/telomeres-cellular-aging/



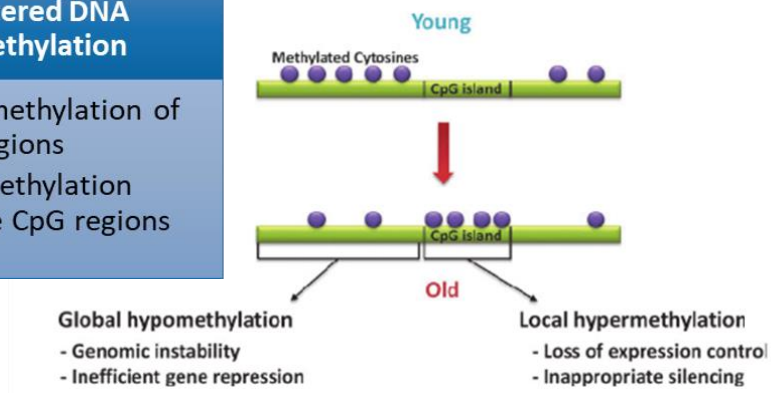
Njajou et al., PNAS 2007

Age-related epigenetic changes: DNA methylation and chromatin remodelling



Altered DNA methylation

- Hypermethylation of CpG regions
- Hypomethylation outside CpG regions



Johnson et al., Rejuvenation Res 2012, 15(5):483-94

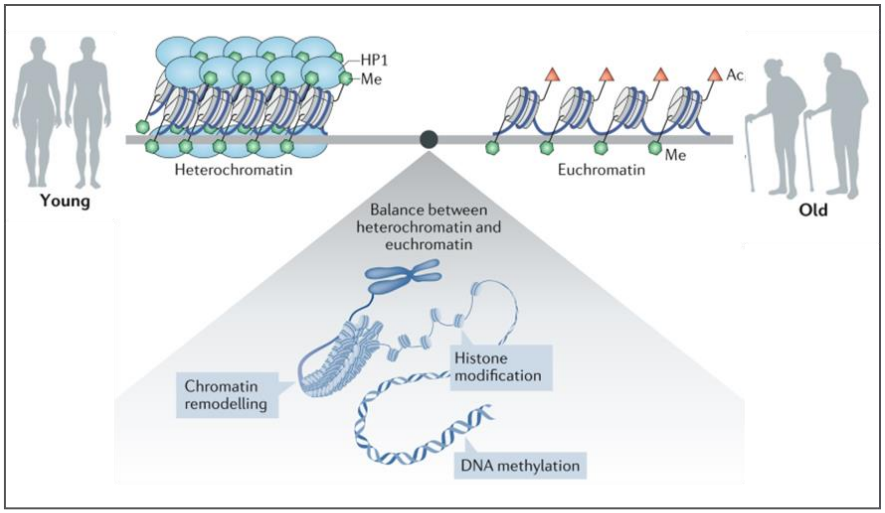
Histone variant changes

- H2A ↑ + redistribution
- H3.1 ↑
- H3.3 ↓

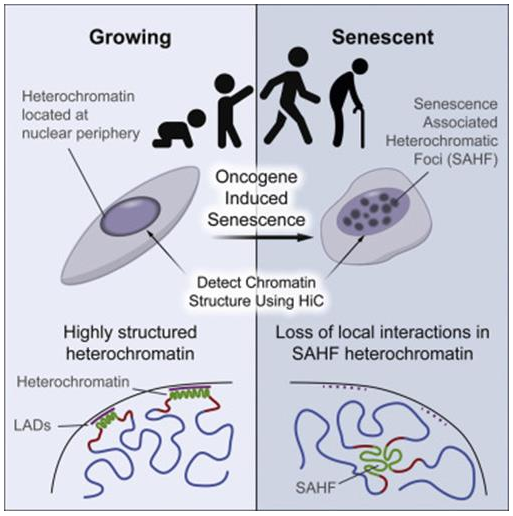
Altered pattern of histone modifications

- H3K4me3, H3K9me3, H3K27me3 ↓
- H4K20me3 ↑
- H4K16Ac ↓; H3K9Ac ↑

=> Loss and relocation of heterochromatin



Meiliana et al., Indones Biomed J. 2022; 14(1): 11-28



Chandra et al., Cell Rep 2015 Feb 3; 10(4): 471–483.

EPIGENETIC CLOCK: using DNA methylation to determine biological age

KU LEUVEN

Age-related mitochondrial dysfunction

- Increased leak => decreased membrane potential => **less ATP production**
- Altered stability of respiratory chain enzyme complexes => **more ROS generated**
- Decreased activity of anti-oxidant enzymes => ROS accumulation => **more DNA damage (incl mtDNA)**
- Less oxidation of NADH => **depletion of NAD+ pool**

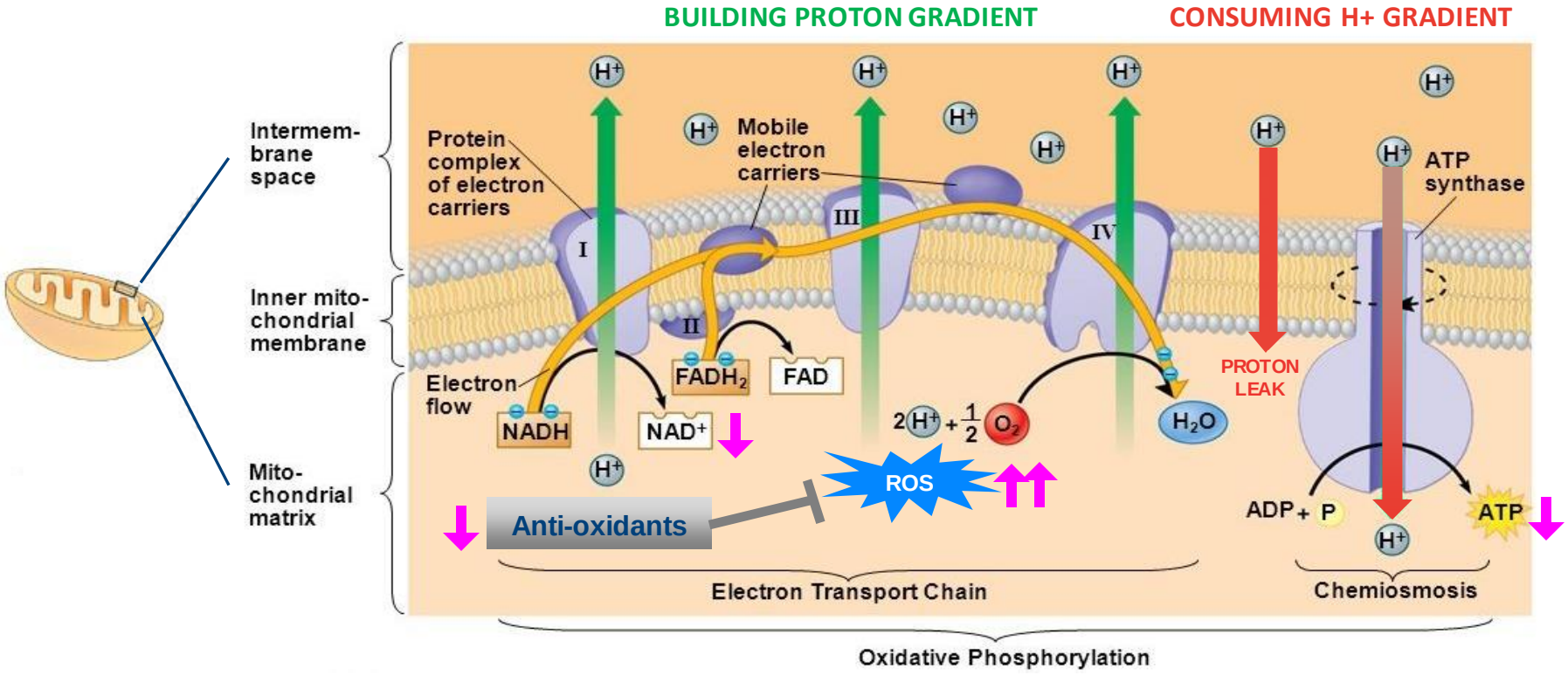


Figure adapted from https://www.premedhq.com/electron-transport-chain

KU LEUVEN

Age-related mitochondrial dysfunction (2)

- **Perturbed mitochondria homeostasis:**
  - Biogenesis ↓
  - Imbalanced dynamics (fusion-fission)
  - Clearance ↓ (mitophagy)

} Accumulation of dysfunctional mitochondria

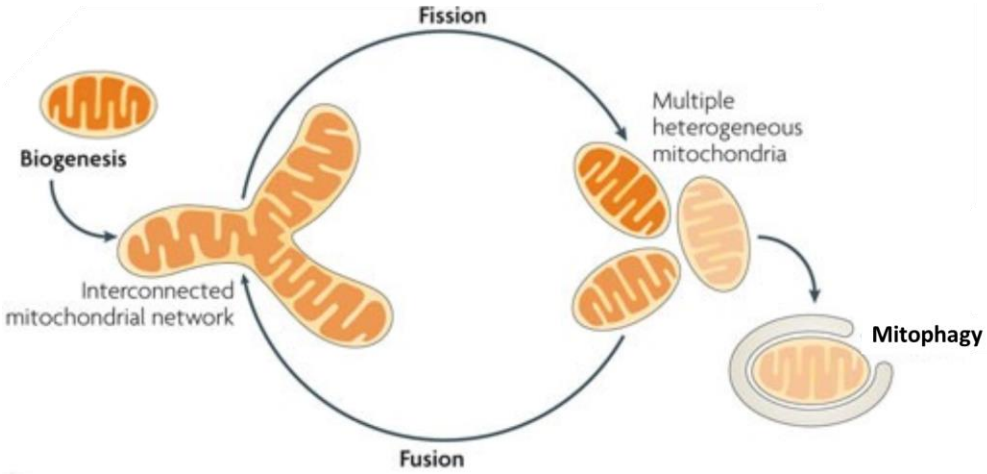


Figure adatpted from https://www.nature.com/articles/nrm3013

The aging cell is facing a self-reinforcing and self-accellerating problem that can be considered as a 'biological battery breakdown'

KU LEUVEN

NAD+ depletion, DNA repair and nutrient sensing

NAM

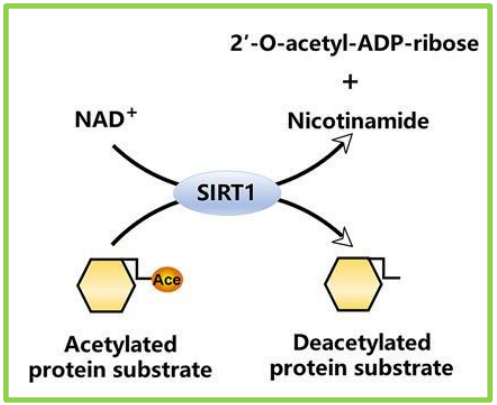
SALVAGE pathway

NAD+ consuming enzymes

CD38 — Ca2+ signalling

PARP — DNA repair

SIRT — Protein deacetylation



Trp

DE NOVO synthesis

Nicotinic acid

NAD+

Glycolysis/β-oxidation/TCA

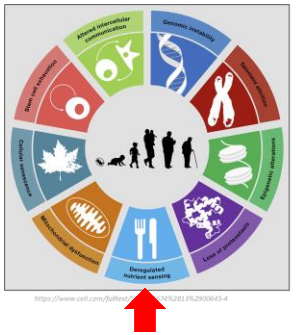
Electron transport chain (complex I)

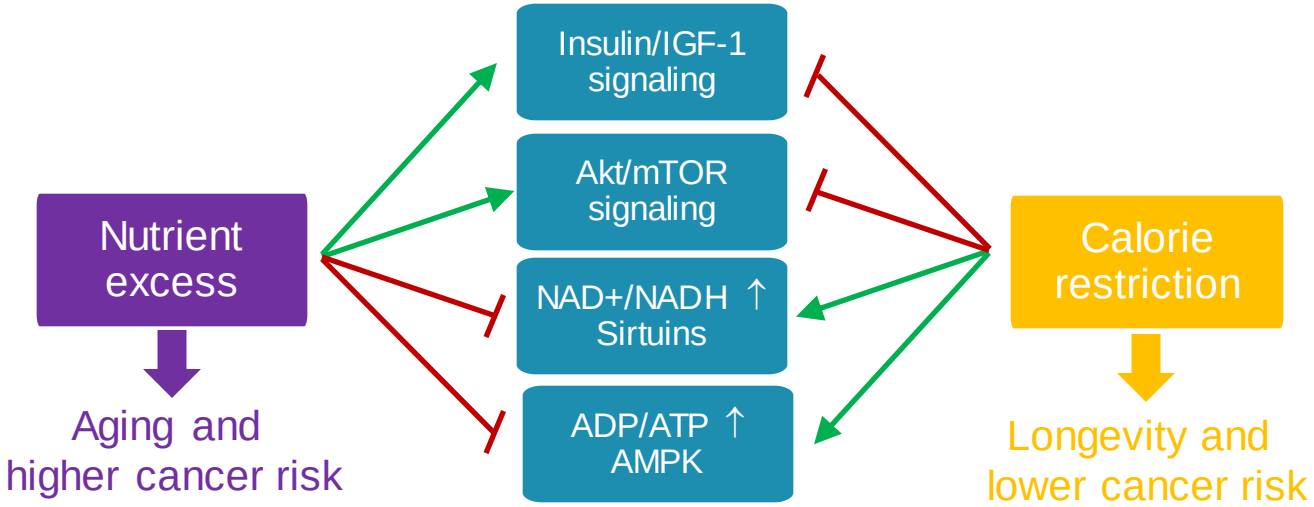
NADH

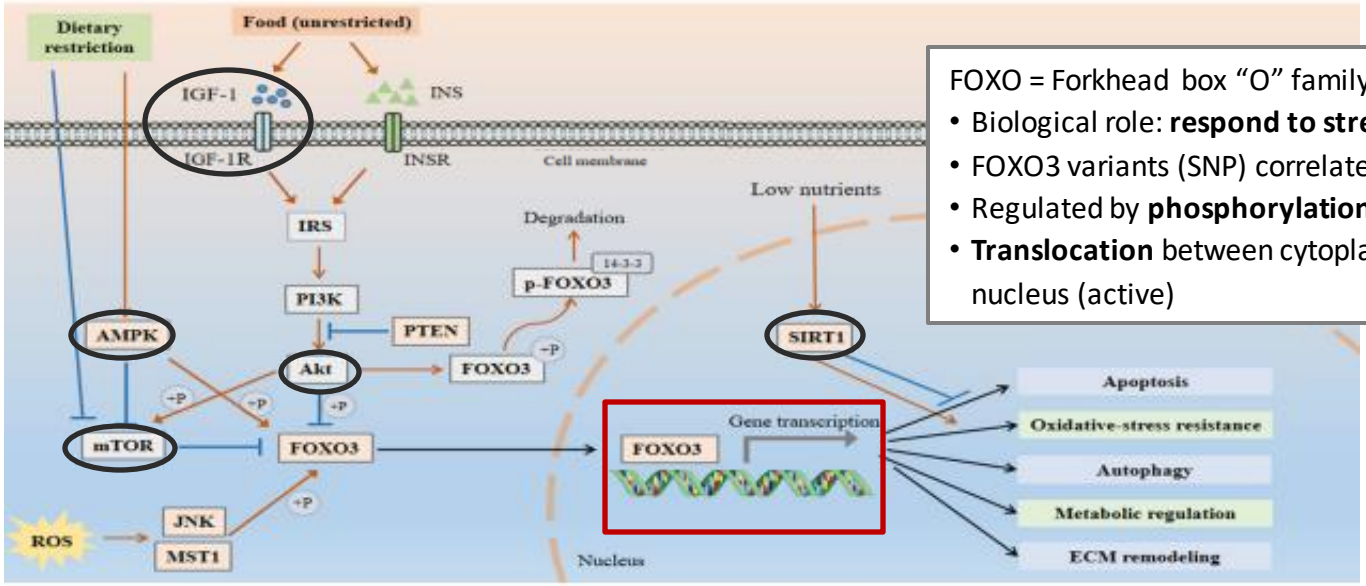
AGE-related DEPLETION

KU LEUVEN

Deregulated nutrient sensing







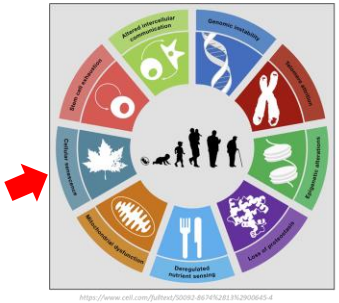
FOXO = Forkhead box "O" family of **transcription factors**

- Biological role: **respond to stress conditions**
- FOXO3 variants (SNP) correlate with human **longevity**
- Regulated by **phosphorylation** and **acetylation (SIRT)**
- **Translocation** between cytoplasm (inactive) and nucleus (active)

https://www.frontiersin.org/articles/10.3389/fcvm.2021.778674/full

KU LEUVEN

Cellular senescence: a defensive mechanism



Cellular senescence = stable state of growth arrest in which cells remain metabolically active but are unable to proliferate (G1/S arrest) despite optimal growth conditions and mitogenic stimuli.

Quiescence = transient G0 arrest caused by lack of nutrients or proliferation signals

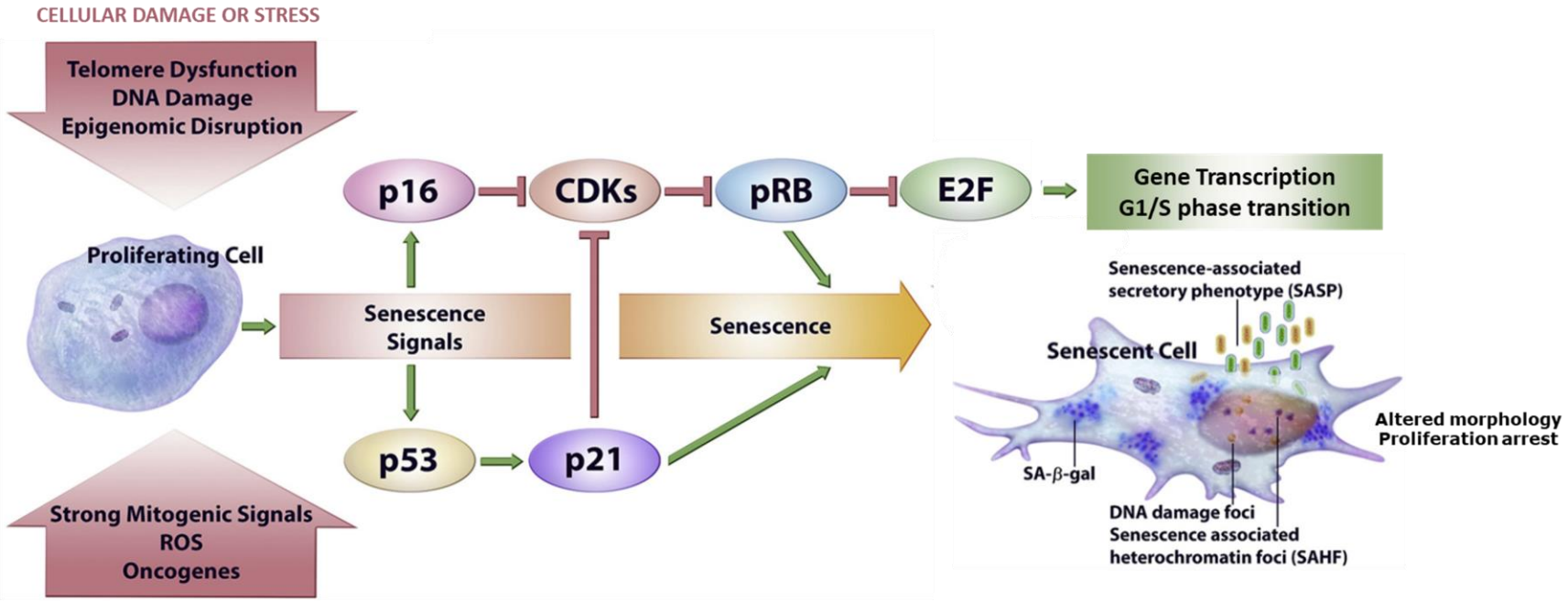
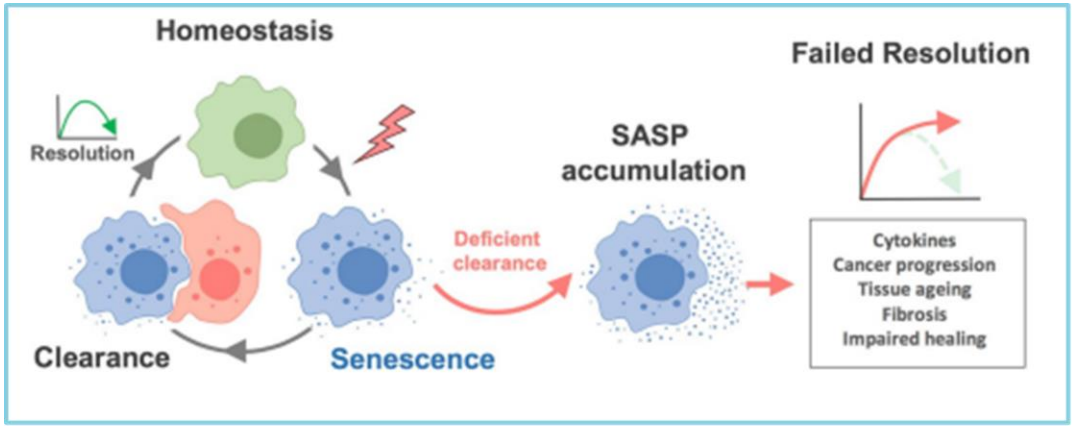


Figure adapted from Sultana et al., Am J Obstet Gynecol 2018;218(2S):S762-S773

KU LEUVEN

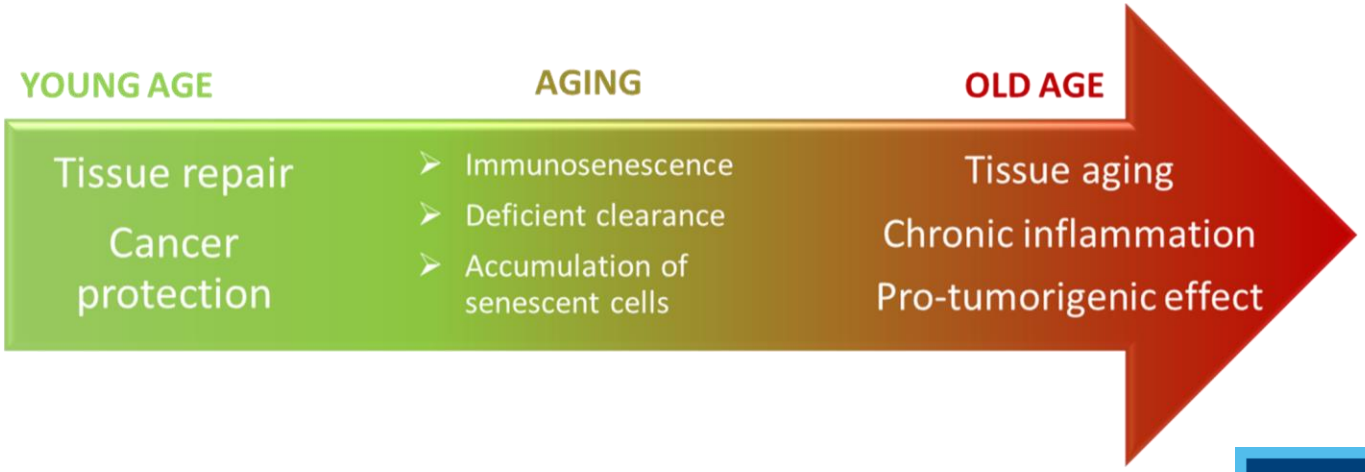
Cellular senescence : the dark side

Senescence-associated secretory profile (SASP)



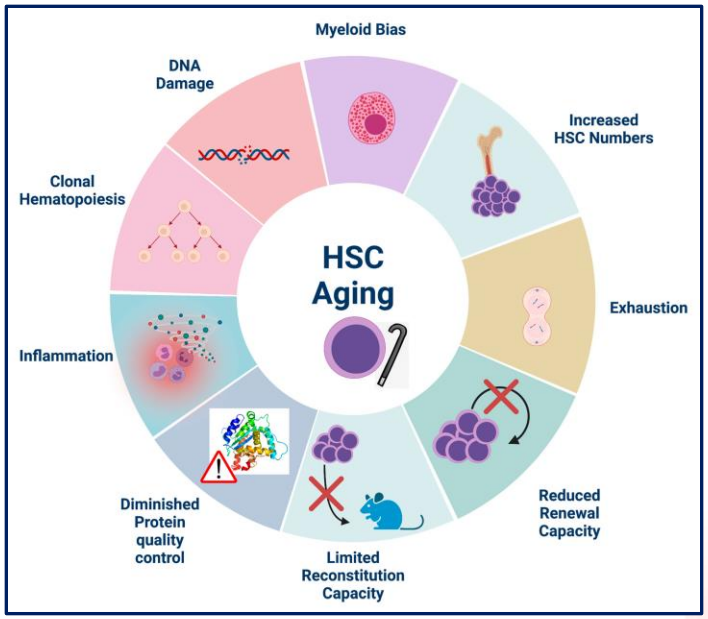
Davan-Wetton et al., Cell Mol Life Sci 2021, 78:3333-3354

- SASP signals the presence of senescent cells to the immune system and promotes their elimination
- Immune clearance becomes deficient with age
- Accumulation of senescent cells
- SASP becomes detrimental

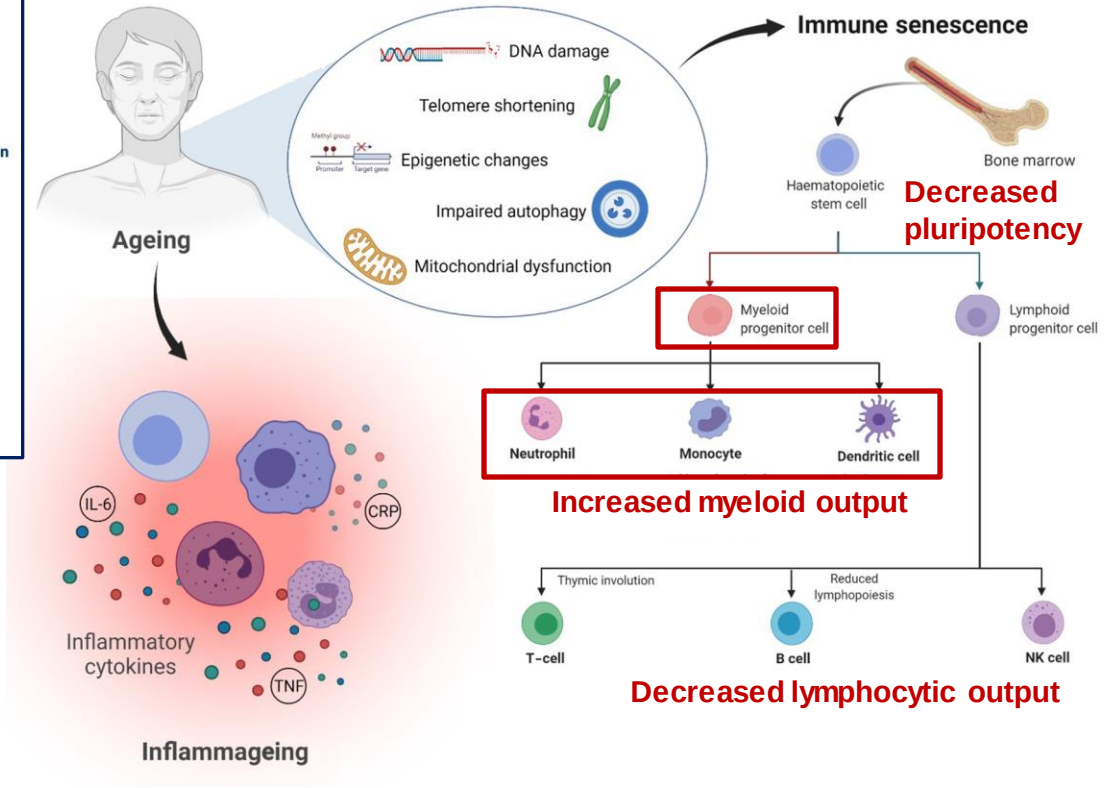


KU LEUVEN

Immunosenescence: aging of hematopoietic stem cells



*Bousounis et al., Cells **2021**, 10(6), 1386*



Conway et al., Br J Pharmacol.2022;179:1808–1824.

KU LEUVEN

Immunosenescence: aging within the 2 branches of the immune compartment

INNATE IMMUNITY

- First-line defense against pathogens. It is the most conserved protection system in the animal kingdom!
- Recognizes and reacts to conserved pathogen-associated molecular patterns (PAMPS) via pattern-recognition receptors (PRRs)
- Exhibits some capacity for memory-like responses: recognition of certain pathogenic features

ADAPTIVE IMMUNITY

- Attacking particular pathogens by specific antigen-recognition
- Building long-term memory

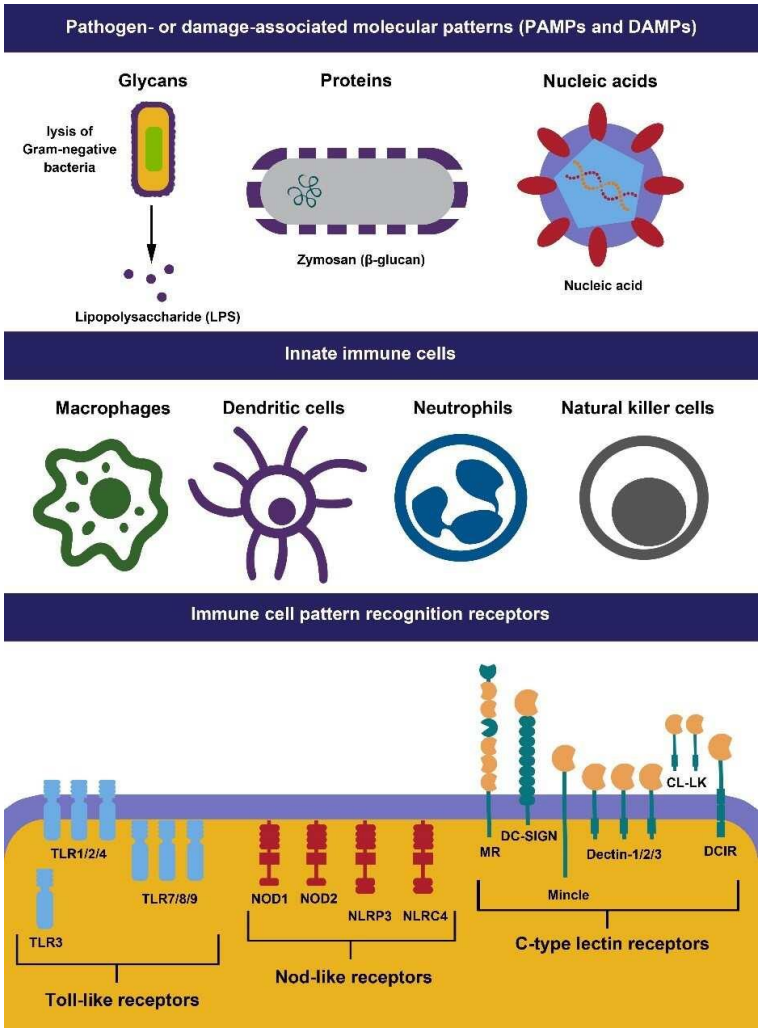
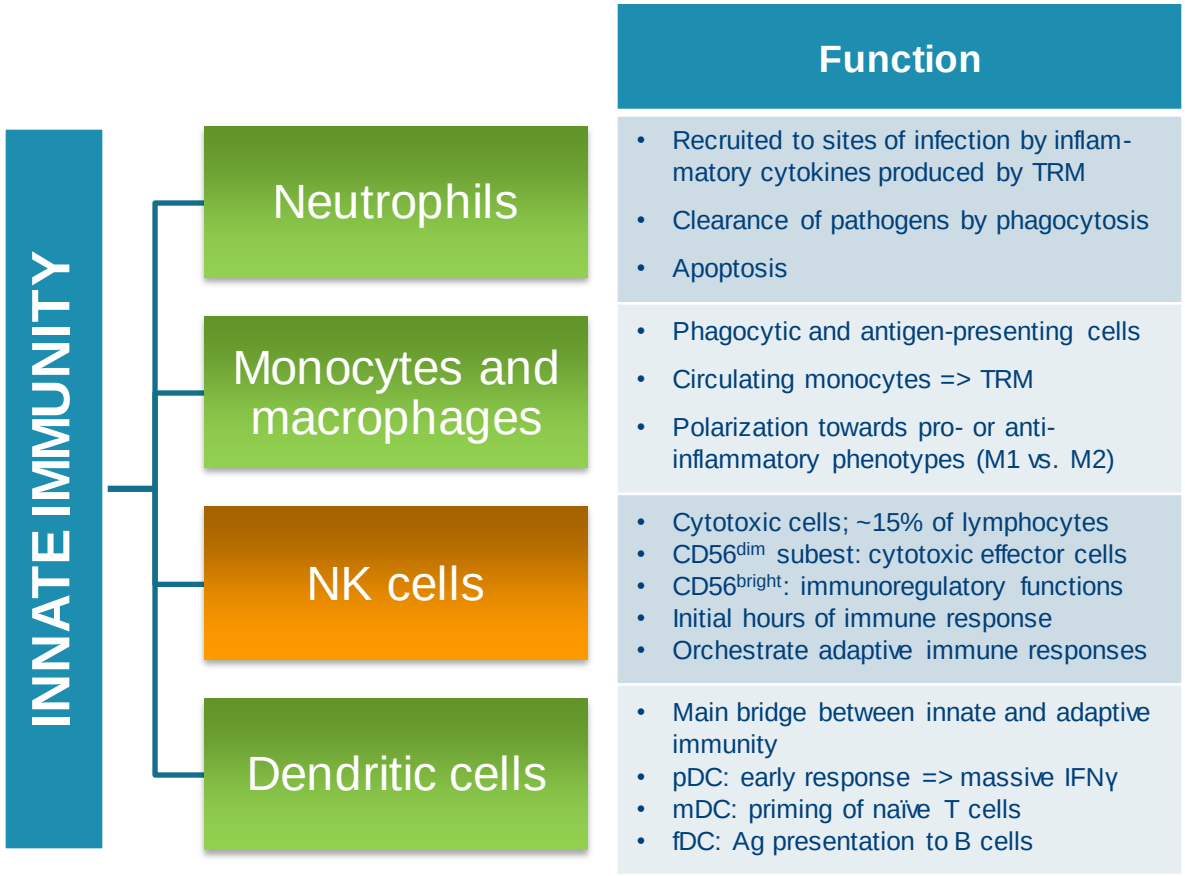


FIGURE: https://www.thermofisher.com/be/en/home/life-science/cell-analysis/cell-analysis-learning-center/immunology-at-work/pattern-recognition-receptors-overview.html

KU LEUVEN

Innate immune cell subsets and their functions

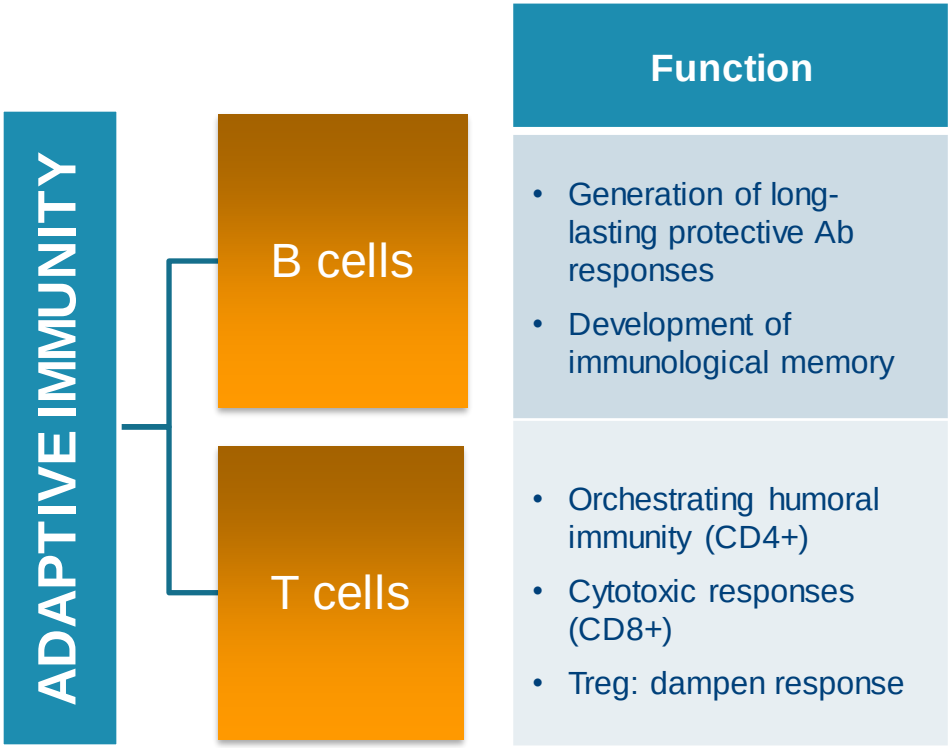


Crooke et al., Exp Gerontol 2019, 124:110632

KU LEUVEN

Abbreviations - TRM: tissue-resident macrophages; pDC: plasmacytoid dendritic cells; mDC: myeloid dendritic cells; fDC:follicular dendritic cells

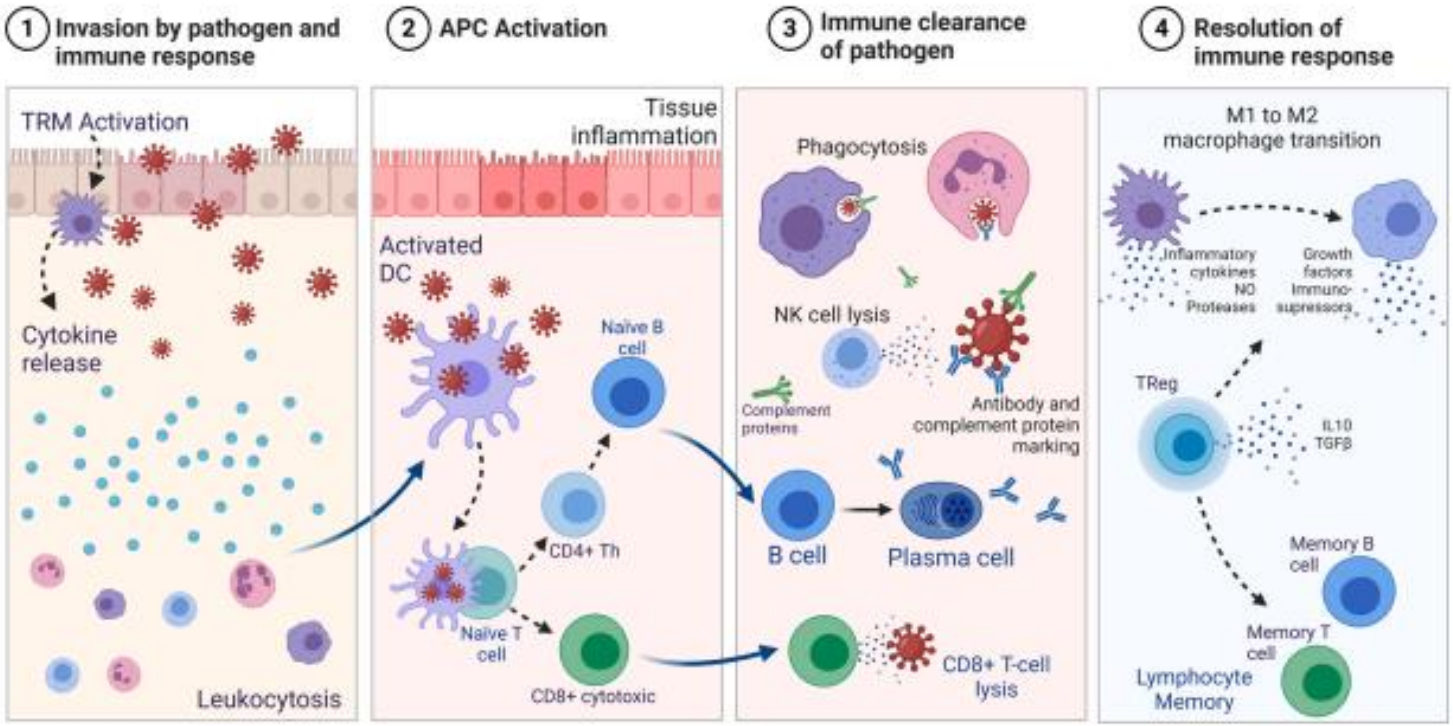
Adaptive immune cell subsets and their functions



Crooke et al., Exp Gerontol 2019, 124:110632

Abbreviations – Ab: antibody; Treg: regulatory T cell

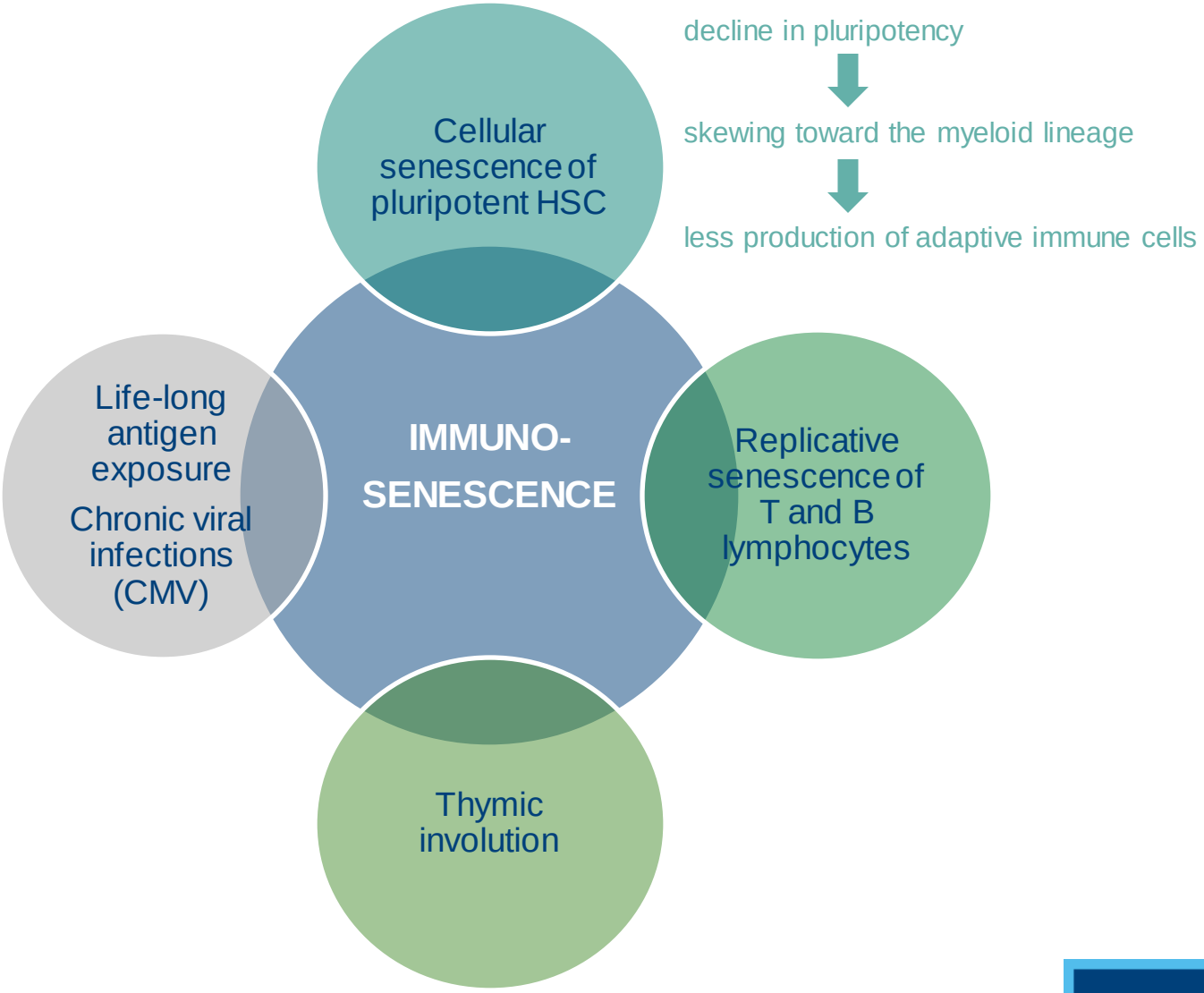
Phases of the immune response



https://www.maturitas.org/article/S0378-5122(21)00023-2/pdf

KU LEUVEN

Immunosenescence: the causes



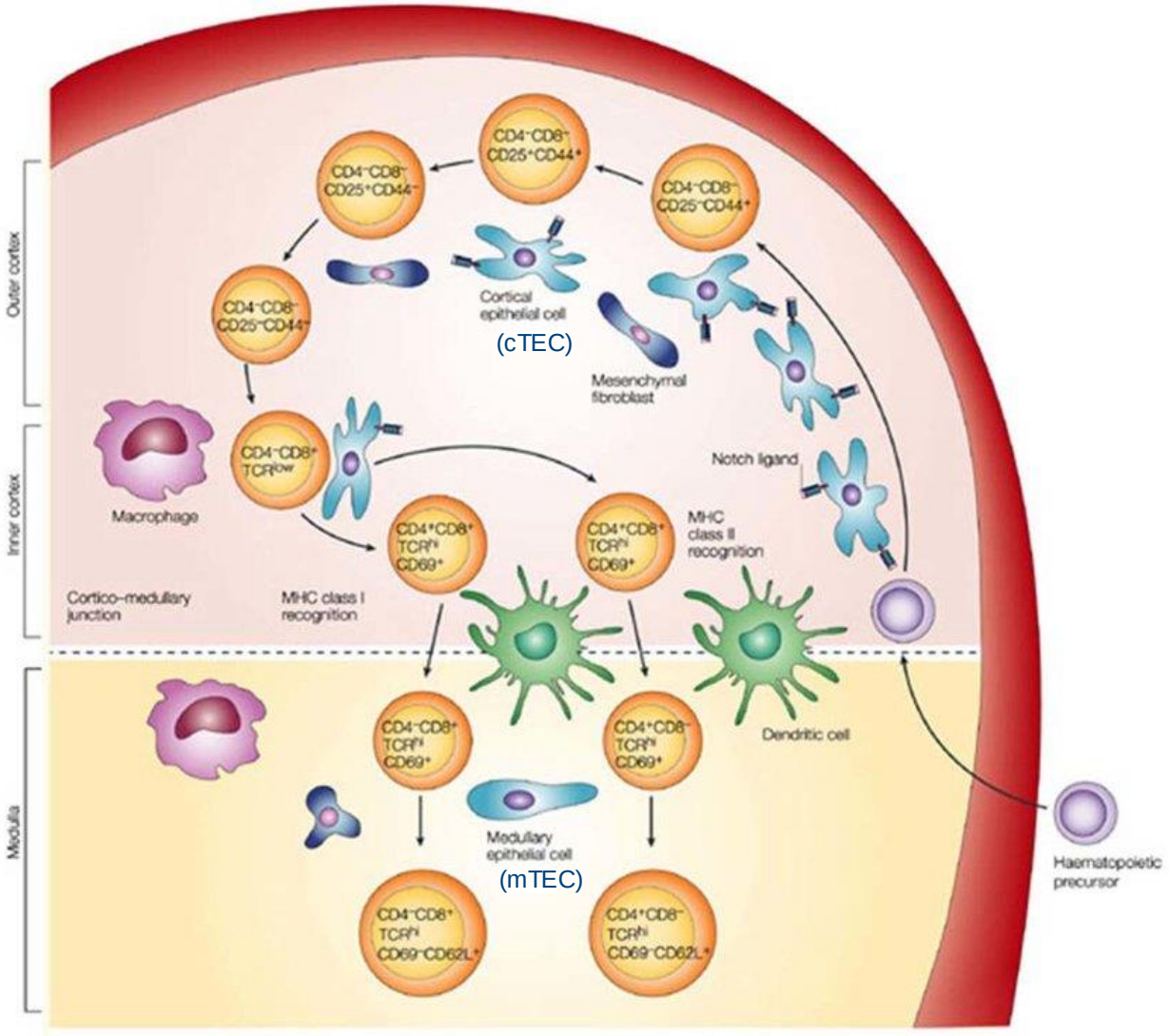
KU LEUVEN

T cell development in the thymus

TCR gene rearrangements
↓
TCR repertoire

Positive selection for proper interaction of TCR with MHCI or MHCII
↓
CD8+ and CD4+ SP thymocytes

Depletion of self-reactive cells



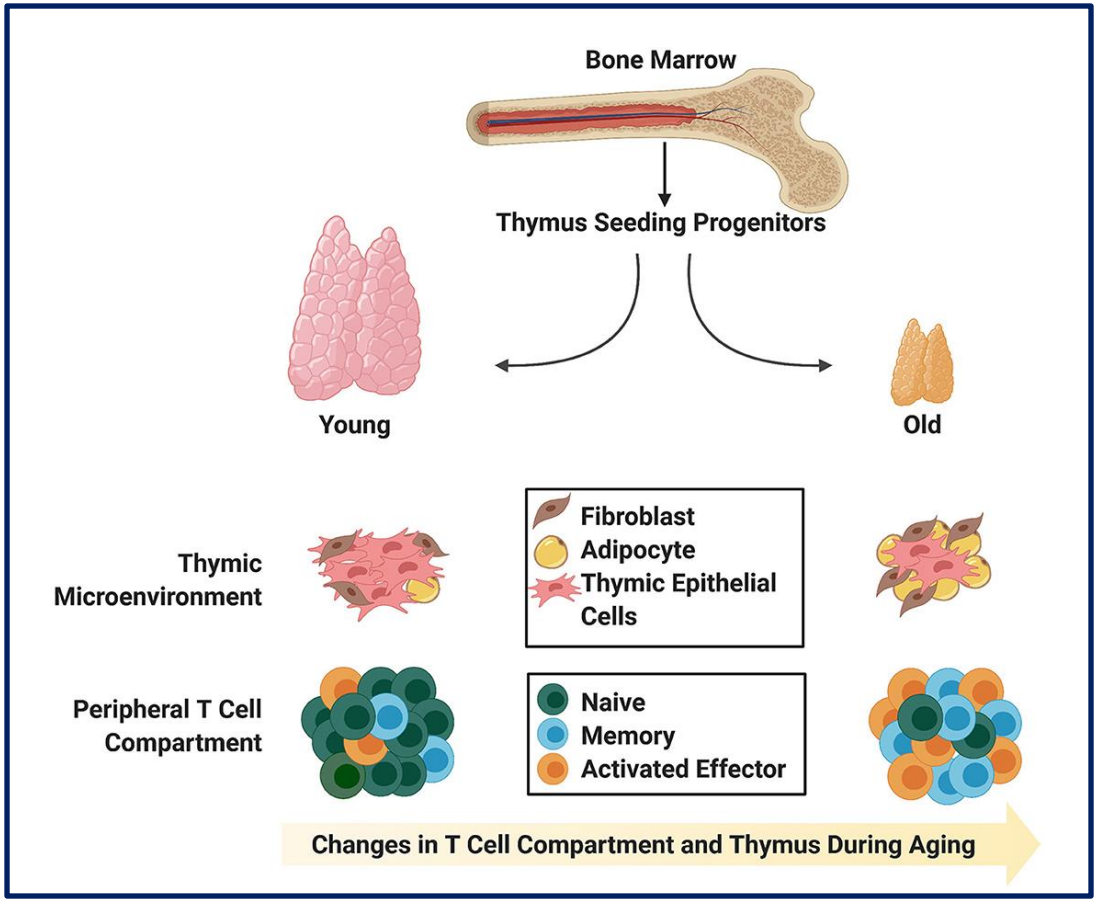
Zúñiga-Pflücker, Nat Rev Immunol 4: 67–72 (2004)

KU LEUVEN

Age-related thymic involution

AGING:

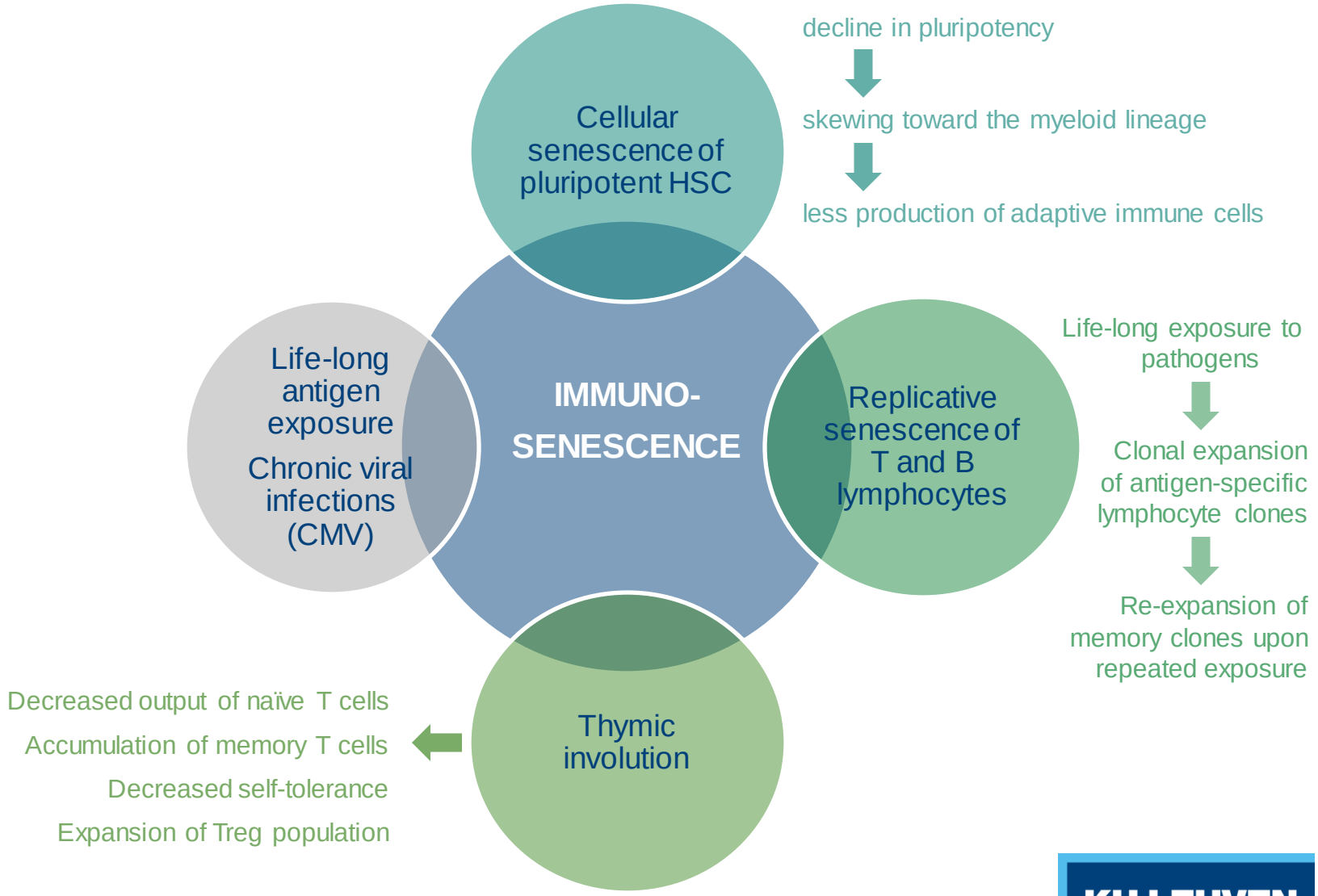
- Less BM progenitors reach the tymus
- Thymus atrophy and replacement by adipose tissue



Singh et al, Front Immunol, 2020 (https://doi.org/10.3389/fimmu.2020.01850)

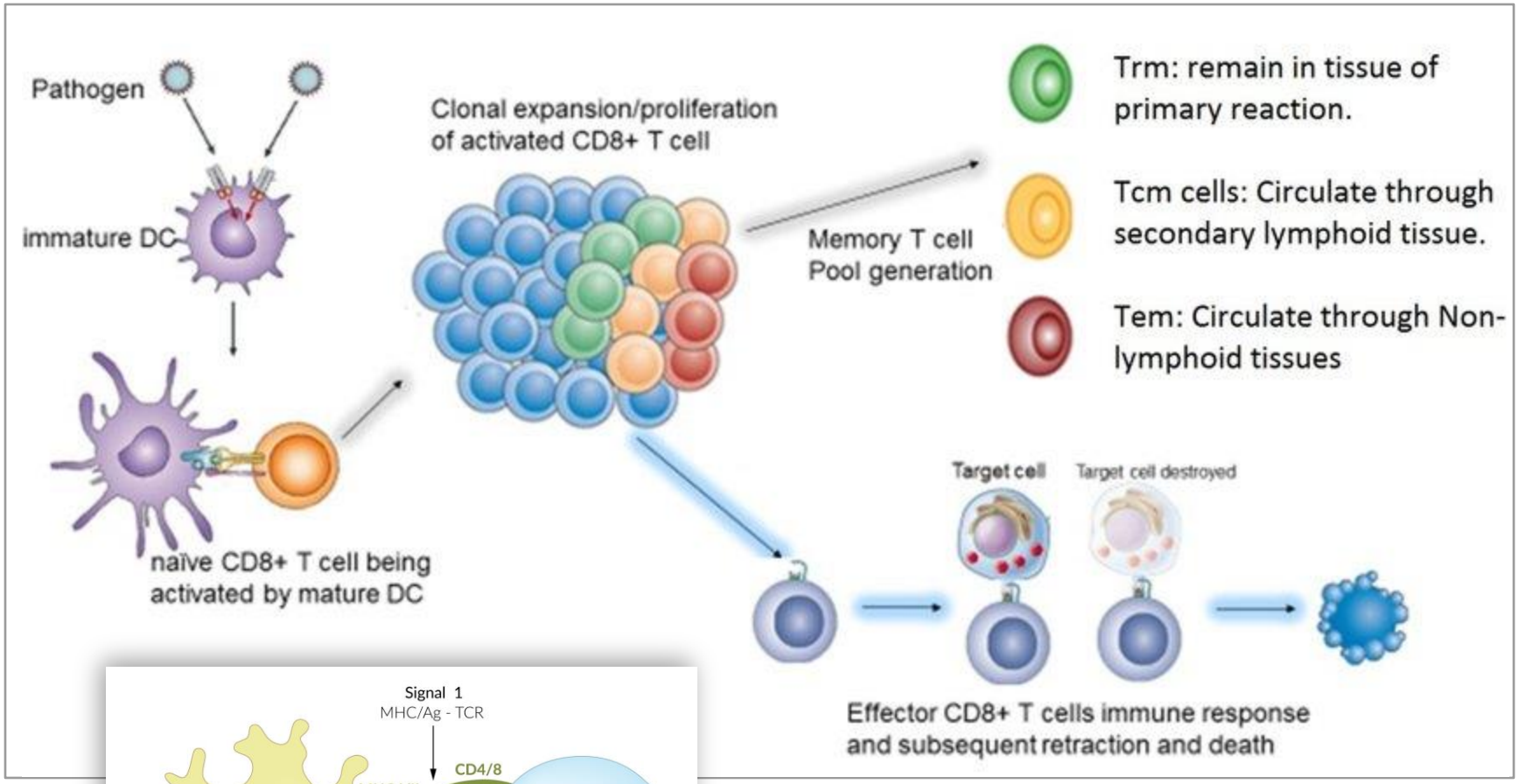
KU LEUVEN

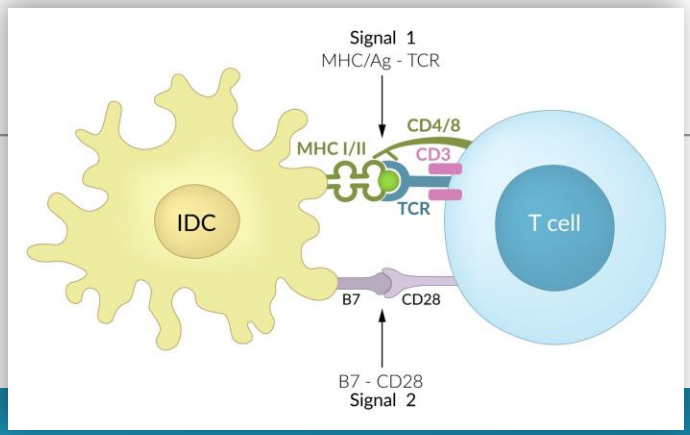
Immunosenescence: the causes



KU LEUVEN

T cell differentiation upon antigen stimulation



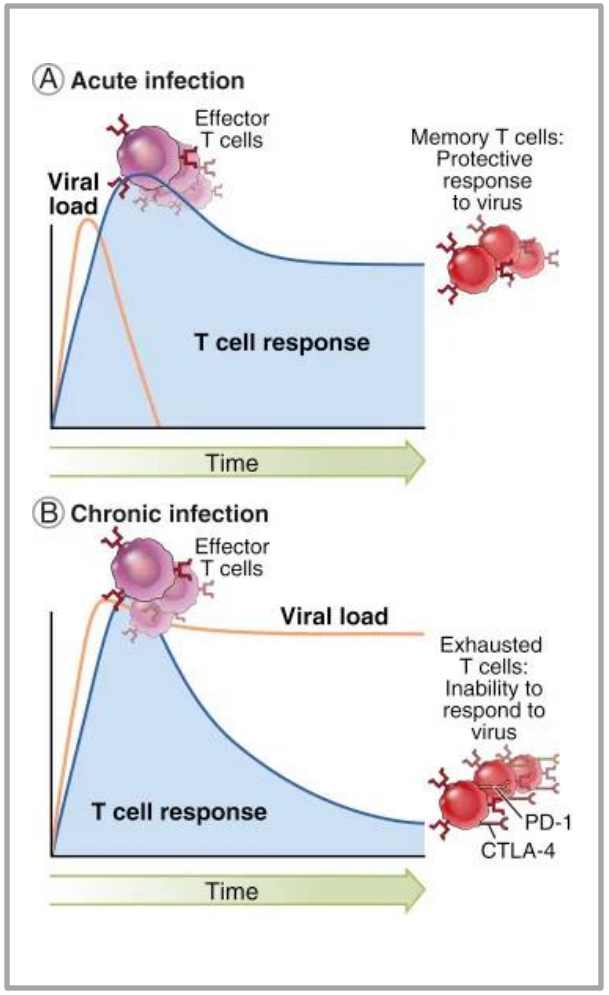


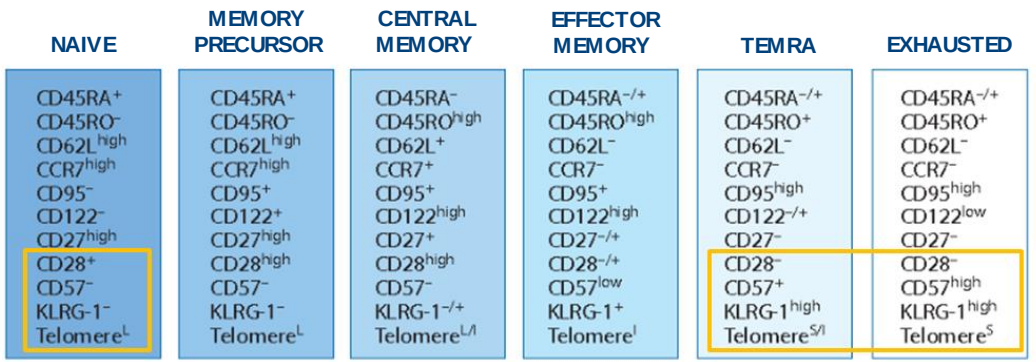
Kaech SM, Cui W. Nat Rev Immunol. 2012;12(11):749-61.

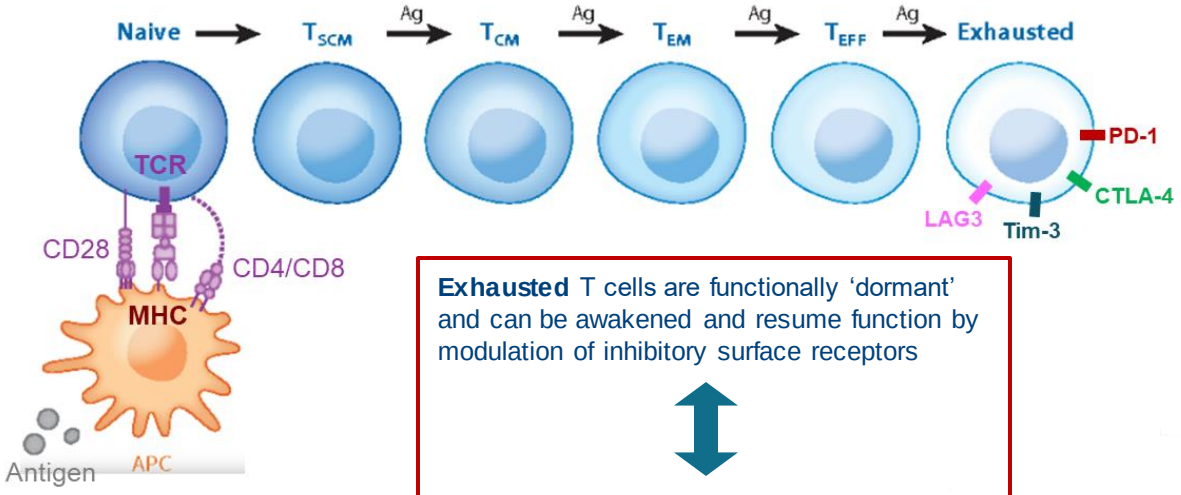
https://www.amboss.com/us/knowledge/Adaptive_immune_system

KU LEUVEN

T cell differentiation upon chronic antigen stimulation







Exhausted T cells are functionally 'dormant' and can be awakened and resume function by modulation of inhibitory surface receptors

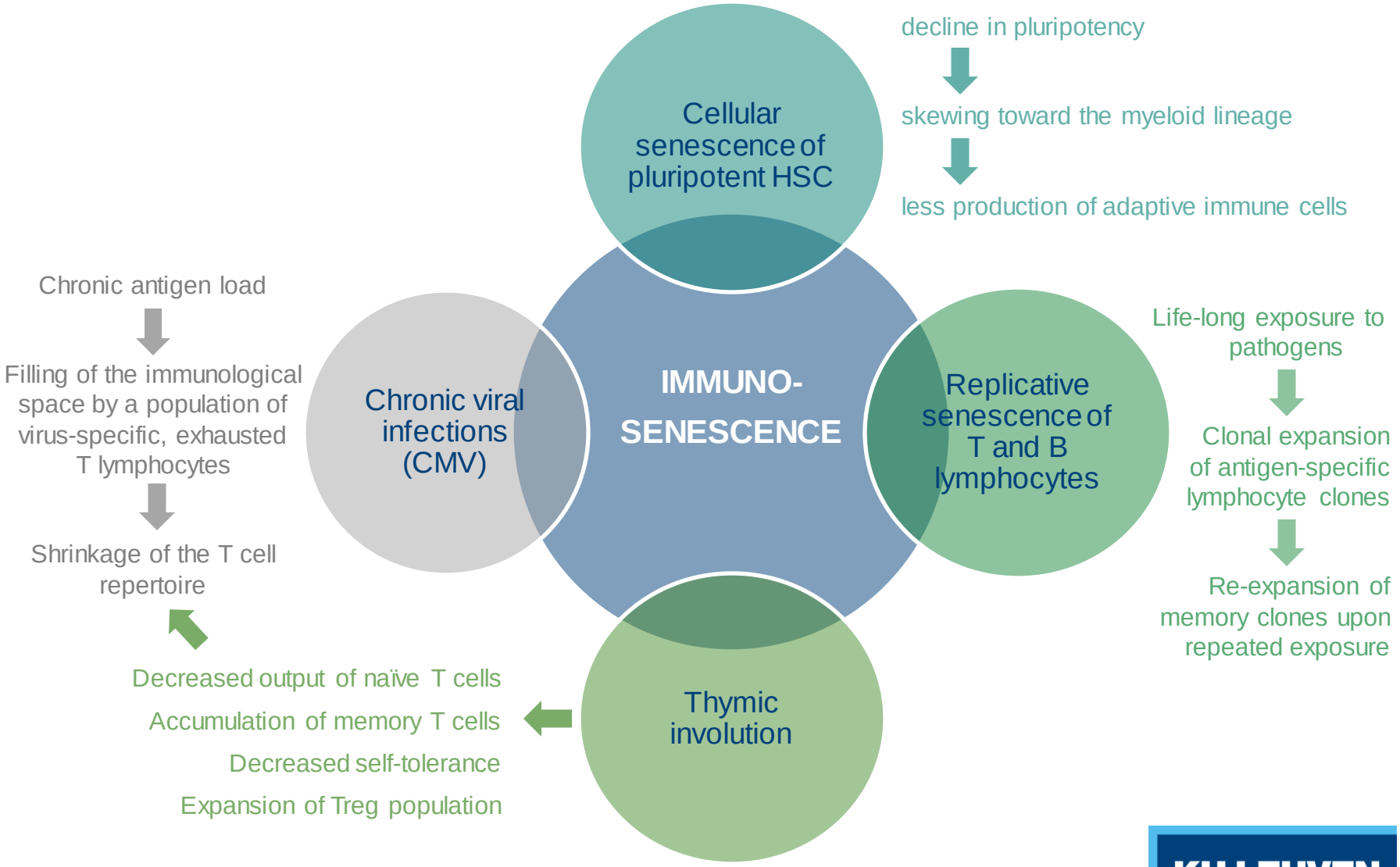
Senescent T cells are functionally 'inert'

Basic Immunology: Functions and Disorders of the Immune System, Chapter 6, 129-146

Figure adapted from Annu Rev Immunol. 2014 ; 32: 189–225. doi:10.1146/annurev-immunol-032713-120136.

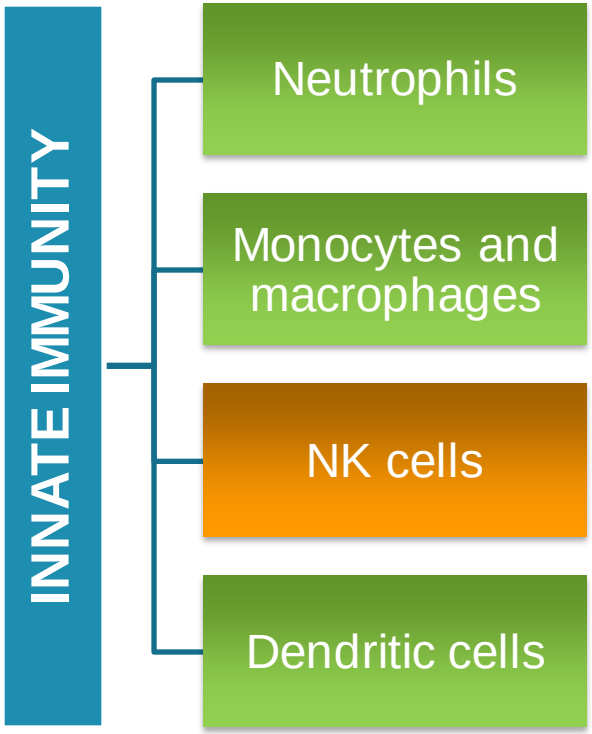
KU LEUVEN

Immunosenescence: the causes



KU LEUVEN

Age-related changes in innate immune cell subsets



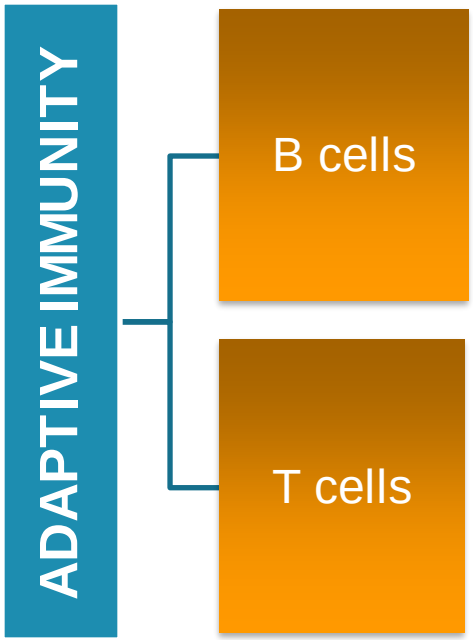
	Function	Age-related changes
Neutrophils	• Recruited to sites of infection by inflammatory cytokines produced by TRM • Clearance of pathogens by phagocytosis • Apoptosis	• Disrupted migration (PI3K ↑) • Impaired phagocytosis (CD16 ↓, ROS ↓) • Increased susceptibility to apoptosis
Monocytes and macrophages	• Phagocytic cells • Circulating monocytes => TRM • Polarization towards pro- or anti-inflammatory phenotypes (M1 vs. M2)	• Decreased IL-6 and TNF-α production • Decline in phagocytic function • Decreased response to IFNγ => MHCII ↓ • non-classical $CD14^{+}CD16^{+}$ monocytes ↑ • Altered M2 macrophage phenotype
NK cells	• Initial hours of immune response • Orchestrate adaptive immune responses • Cytotoxic cells; ~15% of lymphocytes • $CD56^{dim}$ subset: cytotoxic effector cells • $CD56^{bright}$: immunoregulatory functions	• Loss of $CD56^{bright}$ subset => decrease in regulatory cyto/chemokine production • Expansion of terminally differentiated $CD56^{dim}$ subset with superior cytotoxic capacity
Dendritic cells	• Main bridge between innate and adaptive immunity • pDC: early response => massive IFNγ • mDC: priming of naïve T cells • fDC: Ag presentation to B cells	• Decreased capacity of IFNγ production • Reduced phagocytosis and chemotaxis • Reduced Ag presentation • PI3K signaling ↓ • NFκB signaling ↑ => inflammation ↑

Crooke et al., Exp Gerontol 2019, 124:110632

KU LEUVEN

Abbreviations - TRM: tissue-resident macrophages; pDC: plasmacytoid dendritic cells; mDC: myeloid dendritic cells; fDC:follicular dendritic cells; ROS: reactive oxygen species; TNF: tumor necrosis factor; IFN: interferon; Ag: antigen

Age-related changes in adaptive immune cell subsets



Function	Age-related changes
• Generation of long-lasting protective Ab responses • Development of immunological memory	• Reduced output of B-cells from BM (decreased IL7 production by stroma) • Compensatory extension of B-cell life span • Loss of BCR diversity • Less responsive to BCR stimulation • Less effective antibodies • Production of dysfunctional (self)antibodies
• Orchestrating humoral immunity (CD4+) • Cytotoxic responses (CD8+) • suppress immune response and maintain self-tolerance (Treg)	• Dramatic shifts in composition : • less naïve T cells (thymic involution) • Expansion of memory compartment • Latent viral infections (CMV): oligoclonal expansion of CMV-specific memory CD8+ T cells • Loss of costimulatory receptor CD28 by increased TNFα context (inflammaging) • Increase in Treg

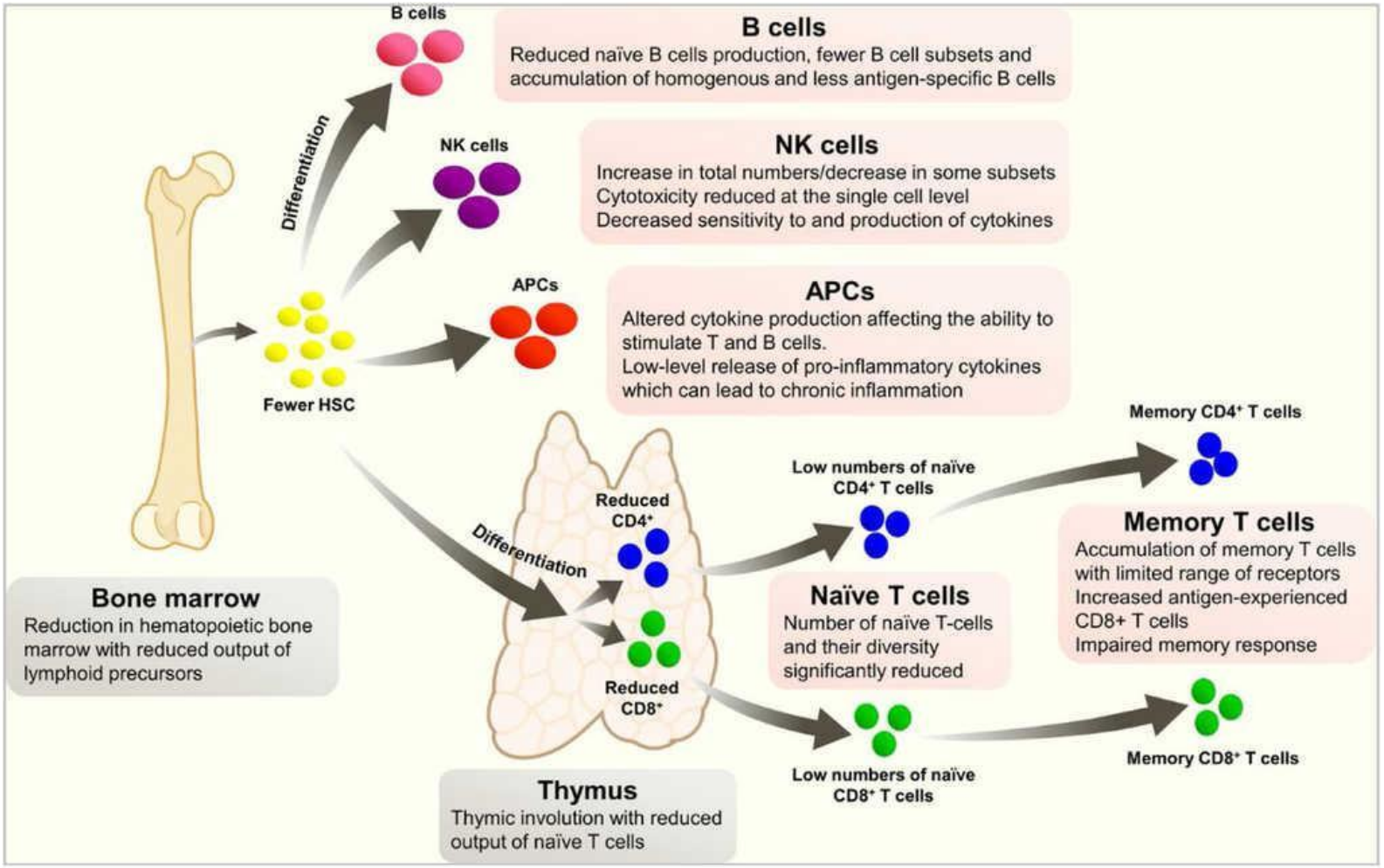
The age-related alterations occur at different levels across multiple cell types involved in both innate and adaptive immuntiy. Alterations in innate immunity may affect priming of adaptive immunity. This crosstalk leads to perturbations throughout the entire immune response.

Crooke et al., Exp Gerontol 2019, 124:110632

KU LEUVEN

Abbreviations – Ab: antibody; Treg: regulatory T cell; BM: bone marrow; BCR: B cell receptor; CMV: cytomegalovirus

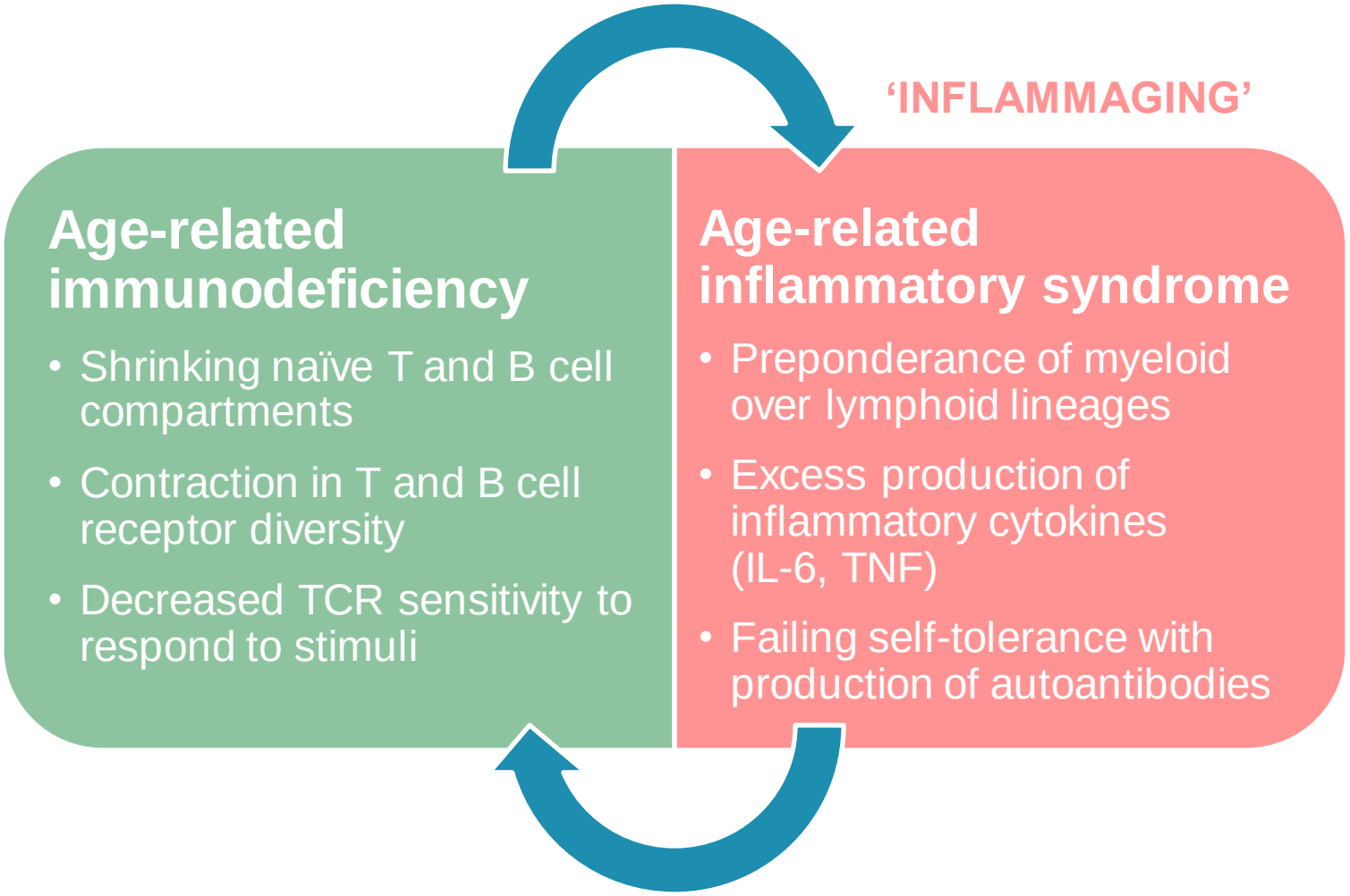
Hallmarks of immunosenescence



Villar-Alvarez et al., Open Respiratory Archives 4 (3) 2022, 100181

KU LEUVEN

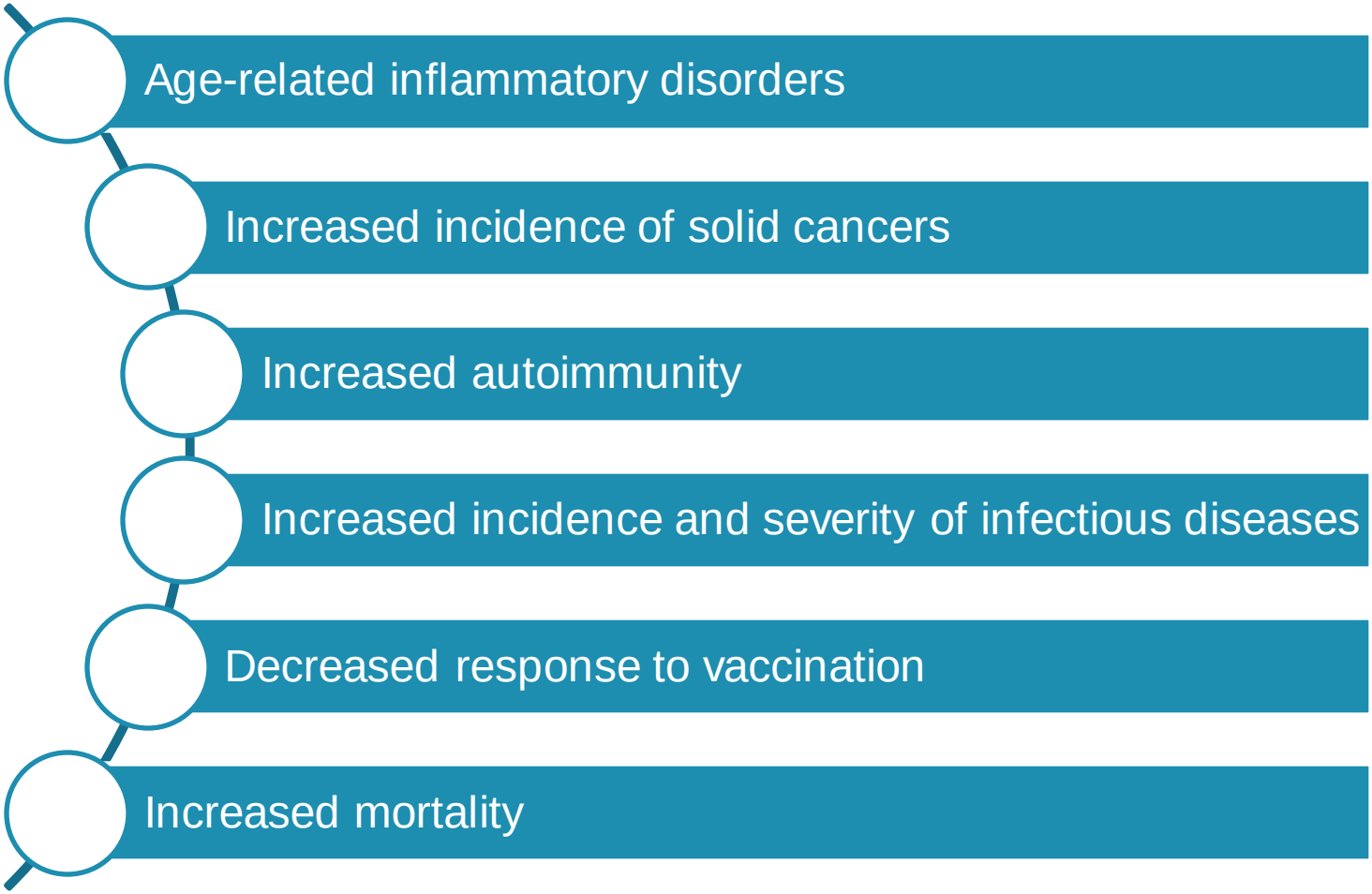
The duality of immune aging



Goronzy et al., Front Immunol. 2013; 4: 131

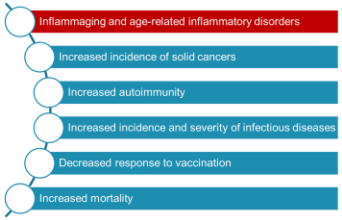
KU LEUVEN

Immune aging: clinical implications



KU LEUVEN

Age-related inflammatory disorders



Immunosenescence

Immune system

Other system

Immune cells
(e.g. T cell, monocyte, NK cell)

Senescent cells
(e.g. endothelia, smooth muscle cells)

Shifted subpopulations
Altered cytokine production

- Shifted subpopulation
- Increased inflammatory mediates expression
- Impaired imflammatory inhibition

Impaired clearance

Pro-inflammatory state

SASP

Inflammaging

Pro-inflammatory

Anti-inflammatory

IL-1
IL-6
TNF-α

IL-10

Sytemic inflammation and tissue damage

Diet, nutrition and gut microbiota can influence the magnitude and persistance of inflammation

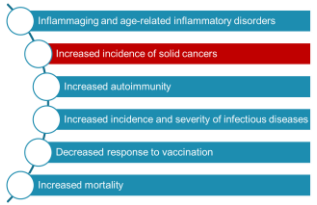
Atherosclerosis & CVD
Osteoporosis
Sarcopenia
Neurodegenerative diseases
Rheumatoid arthritis
…

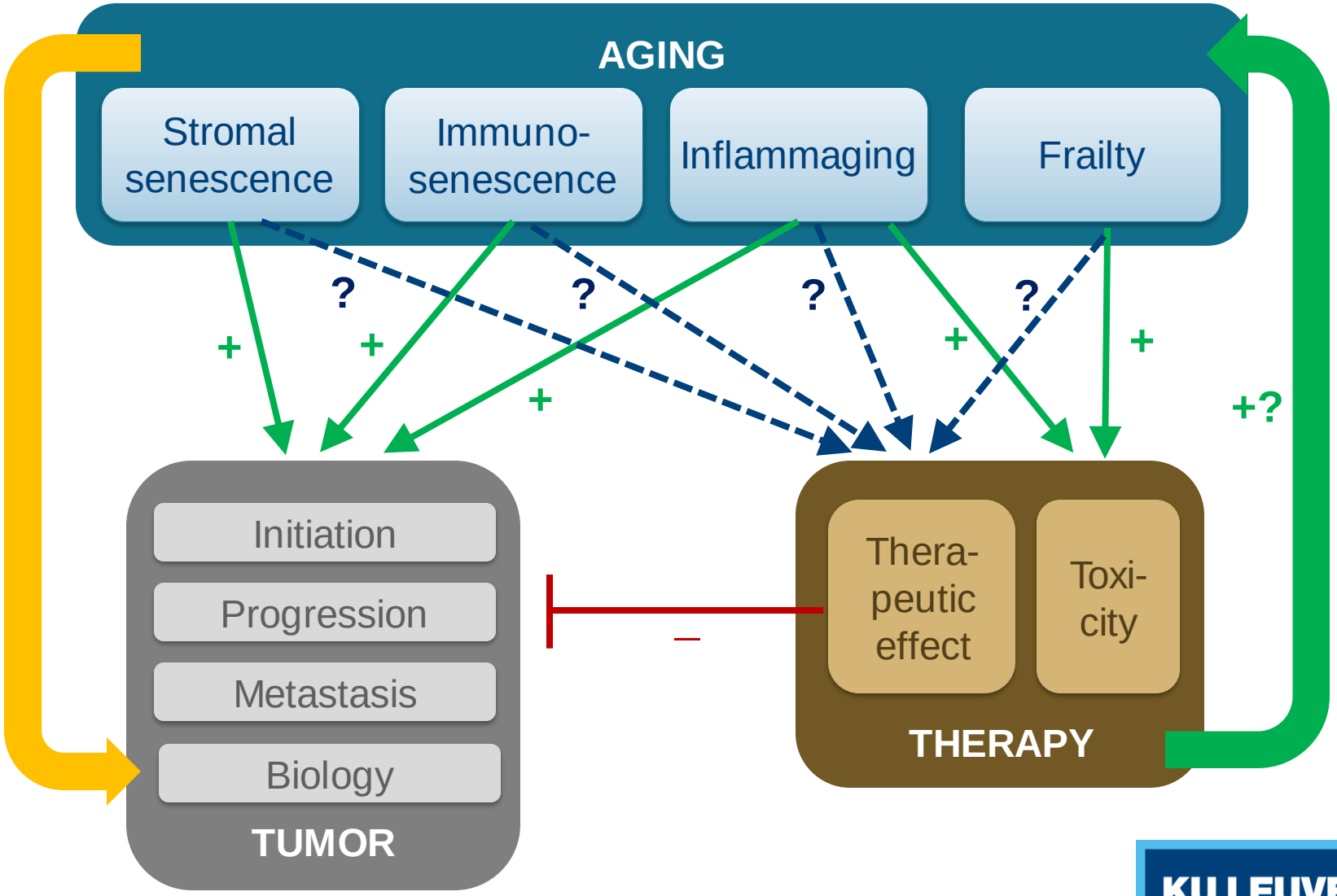
KU LEUVEN

Figure adapted from https://www.frontiersin.org/articles/10.3389/fimmu.2020.585655/full

Cancer: an age-related disease

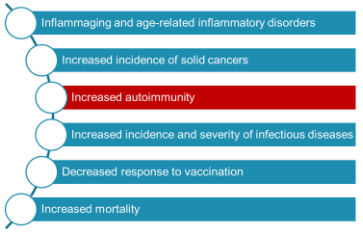
Complex interaction between aging and cancer

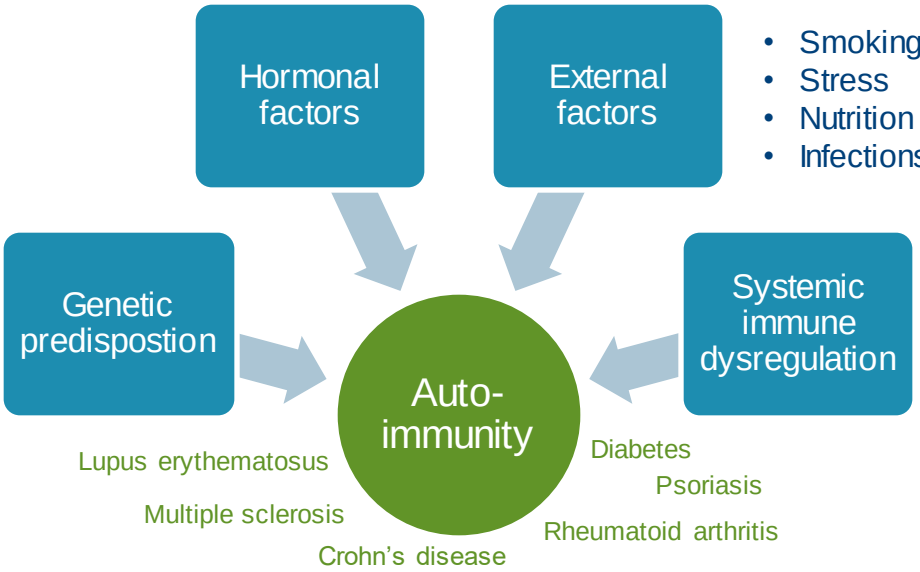




KU LEUVEN

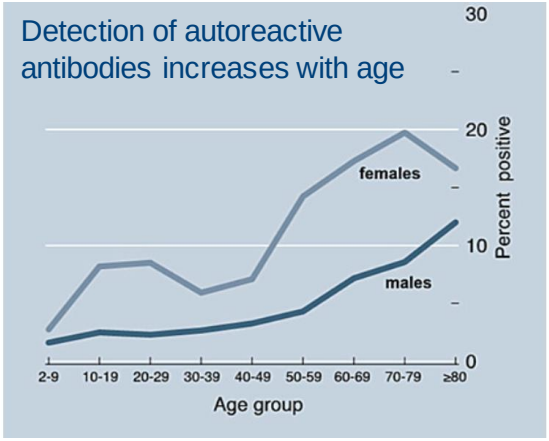
Auto-immunity and autoimmune disease



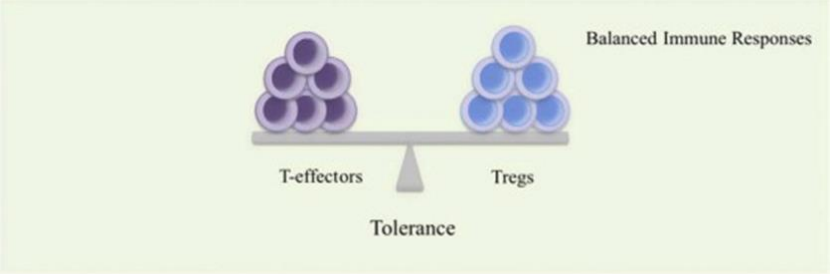


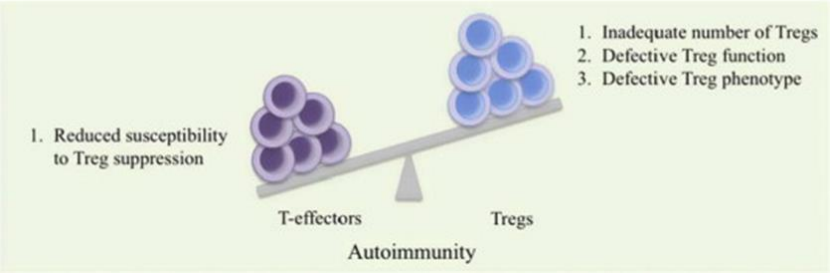
- Smoking, drugs
- Stress
- Nutrition (food antigens)
- Infections/pathogens

- Chronic immune stimulation
- Chronic inflammation (inflammaging!)
- Defective Treg function



Guo YP et al Curr Ther Res, 2014

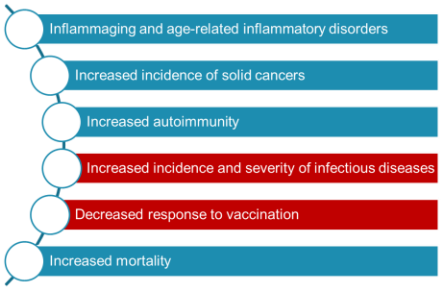


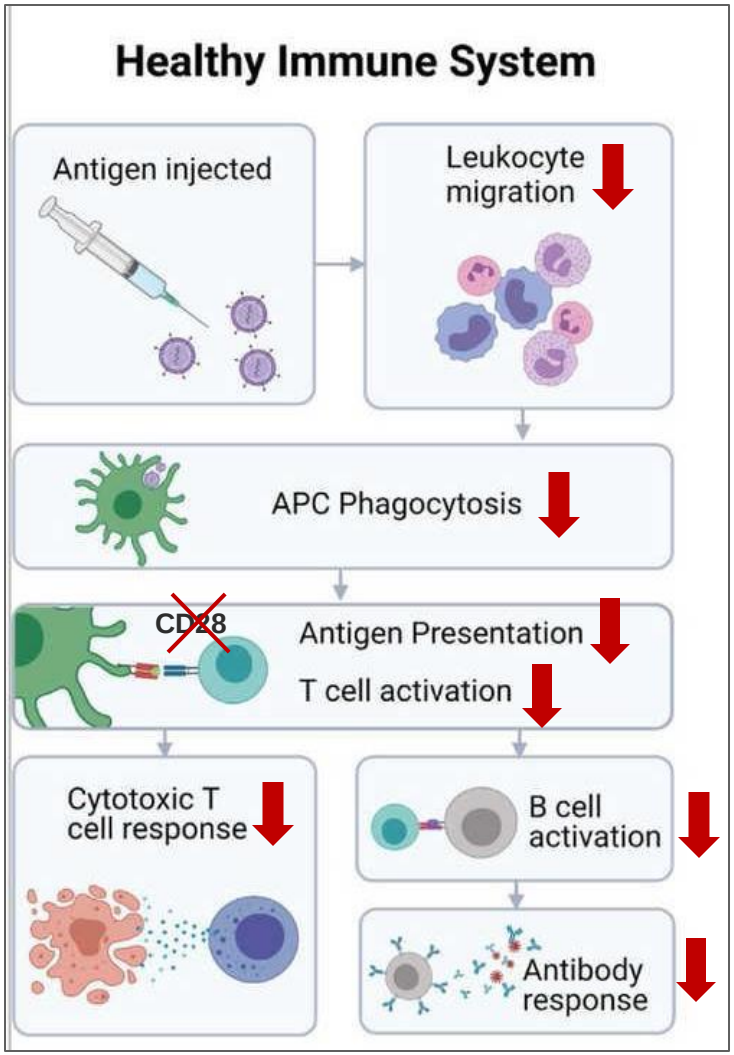


K.-M. Chavele, FEBS Letters 585 (2011) 3603–3610

KU LEUVEN

Increased susceptibility to infectious disease and decreased vaccination response





Immunosenescence

Decreased leukocyte migration

Diminished phagocytosis

Diminished antigen-presentation by APC
Loss of costimulatory receptors on T cells
Decreased T cell activation

Decreased B cell activation

Decreased cytotoxicity
Diminished cellular immunity

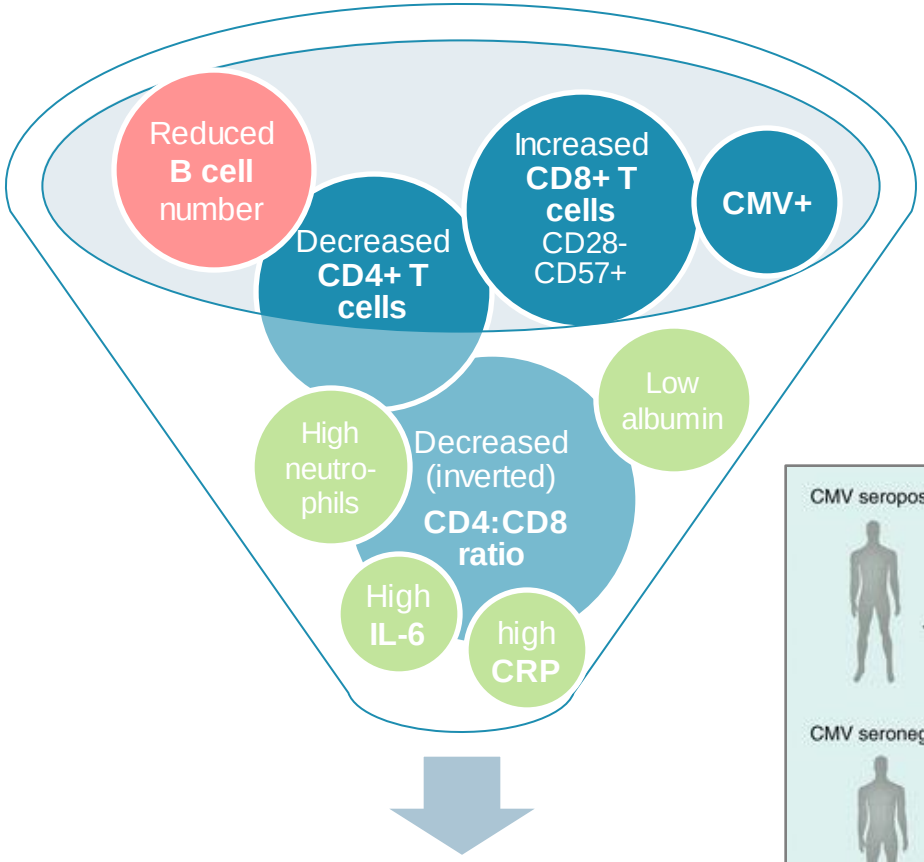
Dysfunctional antibodies
Diminished antibody response

Adapted from Villar-Alvarez et al., Open Respiratory Archives 4 (3) 2022, 100181

KU LEUVEN

Mortality : the 'immune risk profile' (IRP)

Pawelec, Ferguson and Wikby (2001)
The SENIEUR protocol after 16 years. Mech Ageing Dev 122:132-134

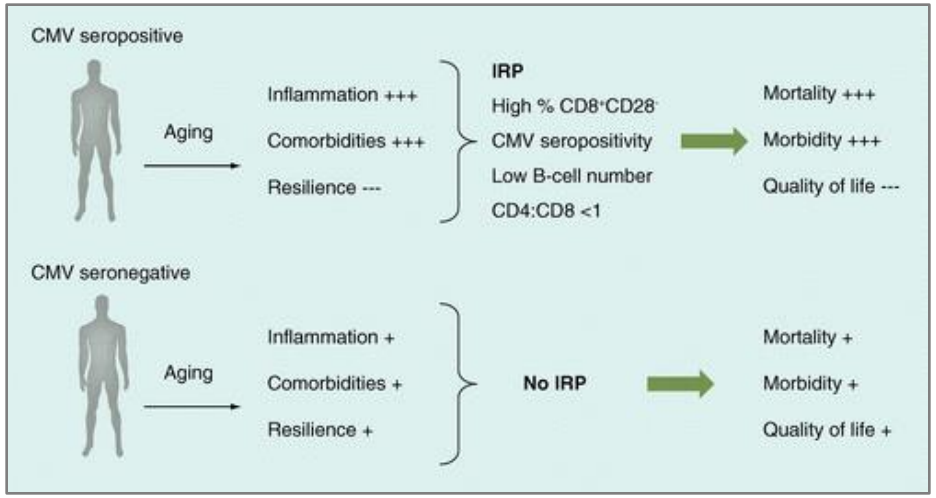


Risk of all-cause **mortality**

Immune parameters associated with survival may vary in distinct populations :

- Differences between **sexes**
- **Genetic** background (ethnicity)
- Differential **susceptibility to CMV**
- **Lifestyle:** nutrition, exercise

=> IRP must be matched to the context !



https://www.futuremedicine.com/doi/10.2217/ahe.12.78

KU LEUVEN

Can immunosenescence and inflammaging be controlled/reversed?

- **Diet**
  - Mediterranean, less animal proteins (red meat) => attenuation of inflammation and oxidative stress
  - Micronutrients:
    - Vit E, Vit C: counteracting age-related oxidative stress and inflammaging
    - Zinc: important for proper immune functioning
  - Pro-biotics: immunomodulators (gut microbiota influence the host's immune system)
  - Caloric restriction
  - Metformin (= caloric restriction mimetic): activator of AMPK
- **Senolytic drugs**
  - e.g.TK inhibitor dasatinib, flavonoid quercetin, mTOR inhibitors (rapamycin, everolimus)
  - Selective apoptosis induction in senescent cells
- **Growth factors: IL-7**
  - Produced by thymic epithelial cells and BM stroma => survival and proliferation of developing lymphocytes
  - High expression of IL-7R on naïve T cells => maintenance of the pool
  - Beneficial effect smaller than initially hoped (loss of thymic structure)

Can immunosenescence and inflammaging be controlled/reversed? (continued)

- **Immune checkpoint inhibitors**
  - PD-1/PD-L1: important role in inhibition and fine-tuning of T cell responses
  - PD-1 expression on T cells increases with age
  - PD-L1 is expressed by APC, tumor cells BUT ALSO senescent cells !
    THUS : PD-L1/PD-1 immune checkpoint is partially responsible for defective clearance of senescent cells !
  - PD-1 blockade partially restores the cells' functional competence
  - Cancer immunotherapy: significant benefit in older patients up to 75 years (selective proliferation of CD28+ cells)
  - >75 years: too much CD28 loss
  - Elimination of senescent cells in vivo??
    Improvement of senescent phenotype??
  - Balance of enhanced immune clearance, tolerance of acute inflammation
    and risk of auto-immune diseases.

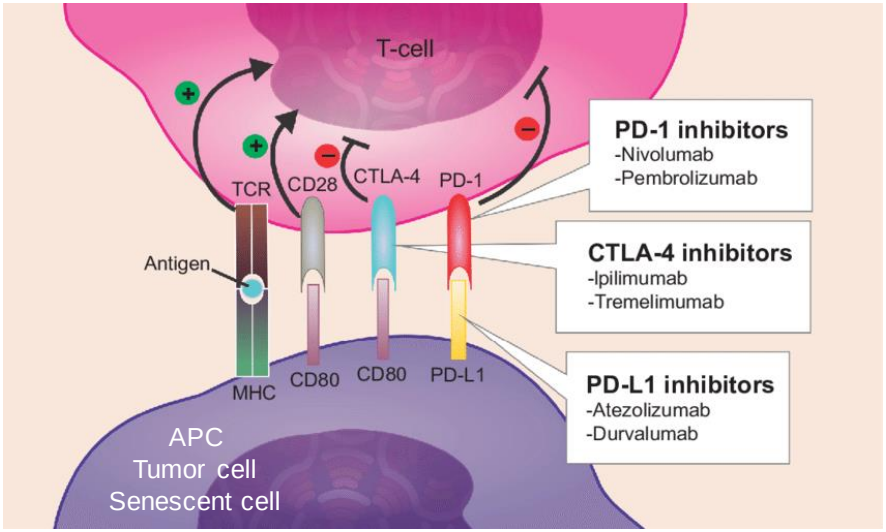


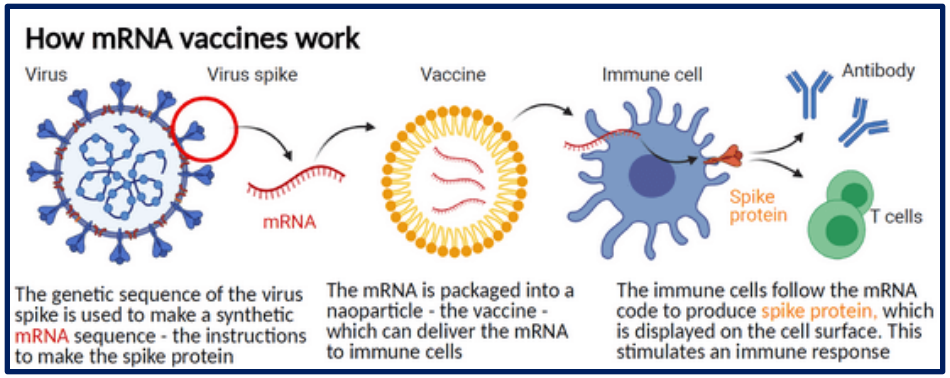
FIGURE: Ramon Andrade de Mello,Onco Targets Ther. 2017; 10: 21–30.

KU LEUVEN

Improving vaccination efficiency

- Increasing the **dose** of antigen (e.g. hemagglutinin in influenza vaccine)
- Addition of liposomal- and emulsion-based **adjuvants**
  - ⇒ Establishment of antigen-depot
  - ⇒ Slow relase of antigen
  - ⇒ APC recruitment to site of injection
  - ⇒ Enhanced antigen uptake and presentation
- Addition of TLR agonists: direct stimulation of early response pathways
- Addition of PAMPs, triggering pattern-recognition receptors
- Senolytic drugs
- Immunomodulatory drugs
- New vaccine technologies: mRNA

Weak vaccines conspire with weaker immunity in older adults to reduce vaccine efficiency, but improved vaccine formulations are able to overcome these deficits



https://www.benaroyaresearch.org/blog/post/11-things-know-about-mrna-vaccines-covid-19

KU LEUVEN


Thank you !!!