Παθολογική
Φυσιολογία

The pathophysiology of inflammatory responses in Large Vessel Vasculitis

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Εθνικό &
Καποδιστριακό
Πανεπιστήμιο
Αθηνών

Rhodes 2022

Disclosures

Received Research Grants from Novartis, Pfizer, ABBVIE, Genesis, Eli-Lilly. NONE related to the current presentation

Coordinator of HarmonicSS, an EU sponsored Research Grant

Chairman of eSSential, the Study Group of EULAR, devoted to Sjögren's syndrome

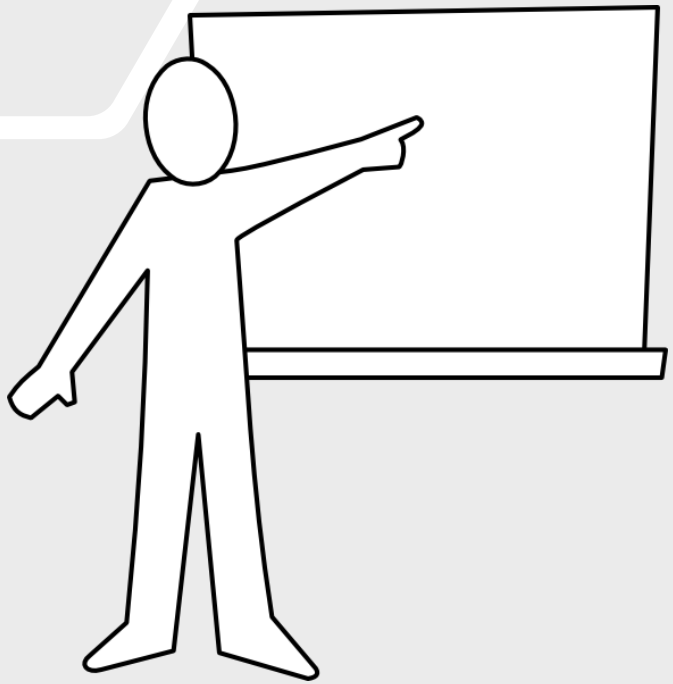
Lecture outline

Introduction

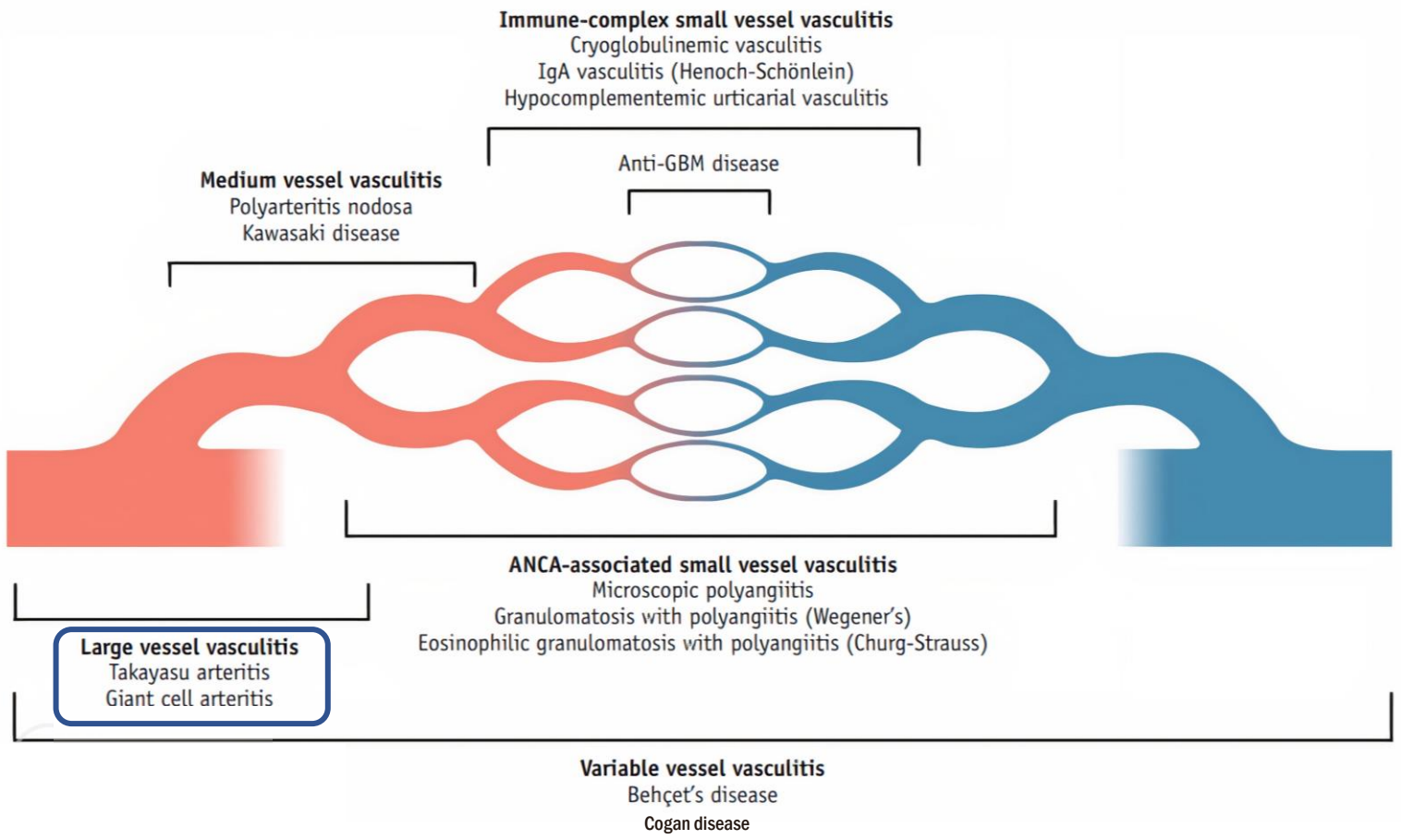
Cells participating/ Stages of inflammatory lesion

Histologic heterogeneity

Work in progress-Future perspectives



Nomenclature of Systemic Vasculitides

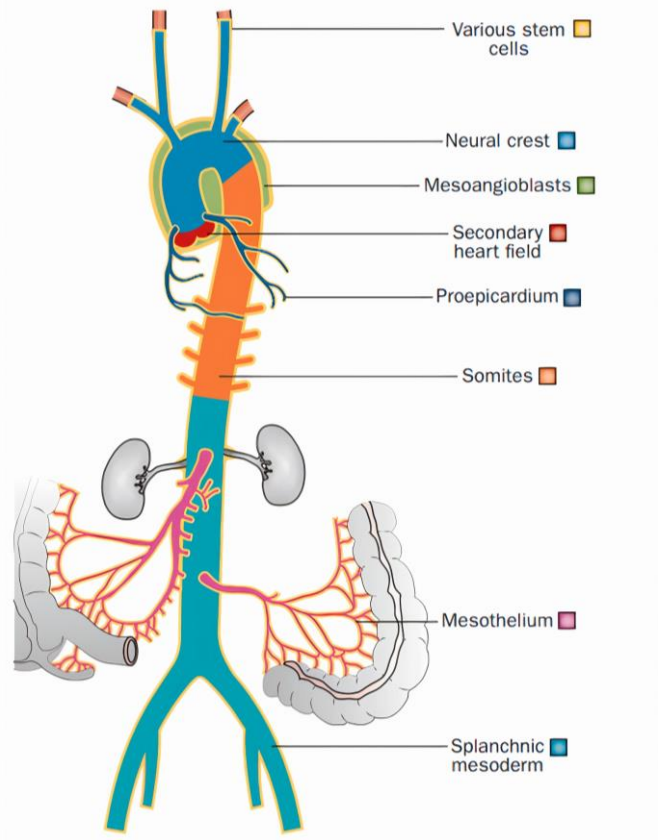


Modified from Jennette et al. Arthritis Rheum 2013

Jennette JC Nomenclature of systemic vasculitides: The proposal of an international consensus conference. Arthritis Rheum. 1994

Heterogeneity of Vascular inflammation (1)

- Commitment and specification from the earliest stages of embryonic development.
- Vessels of a specific caliber can be affected by <u>different</u> diseases and <u>different</u> pathogenetic mechanisms.
- Vessels of a specific caliber are <u>not</u> equally prone to injury



Different colours represent differences in embryonic origins of vascular smooth muscle cells

Gary S Hoffman et al Vasculitis: determinants of disease patterns. Nat. Rev. Rheumatology 2014

Heterogeneity of Vascular Inflammation-LVV (2)

GCA

GIANT CELL ARTERITIS

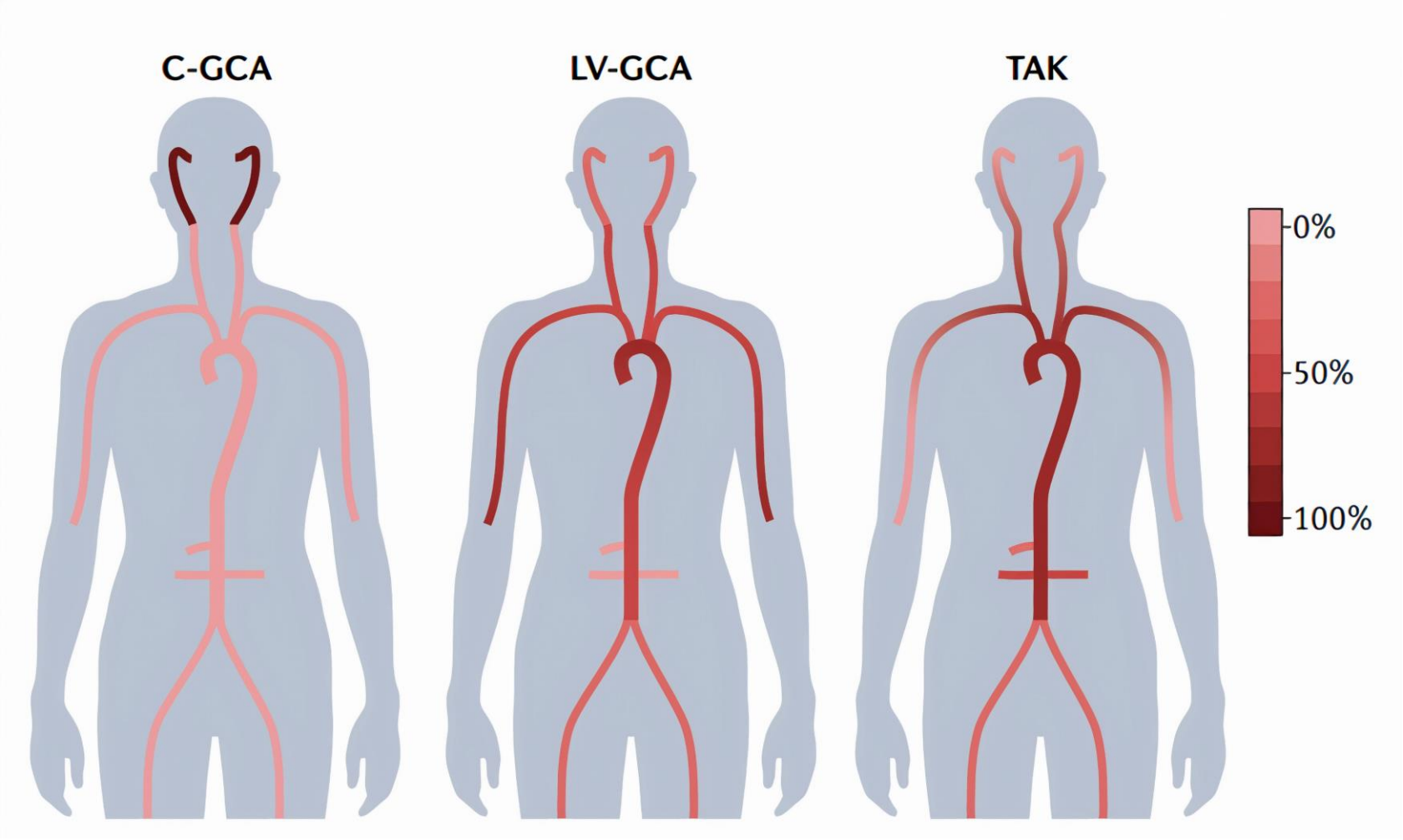
- Individuals over 50
- More common in Caucasian
- Association with genes in the HLA II region
- Jaw claudication/ Headache/ Visual loss
- Association with PMR
- Different vessels involved

TA

TAKAYASU ARTERITIS

- Young women below 40
- More common in Asian ancestry
- No clear genetic loci associations
- Arm claudication/diminished pulses/ pulseless disease
- Incidence rate of TAK is significantly lower in comparison with GCA
- Different vessels involved

Arterial involvement in LVV



Cranial GCA

- Temporal artery

Large vessel GCA

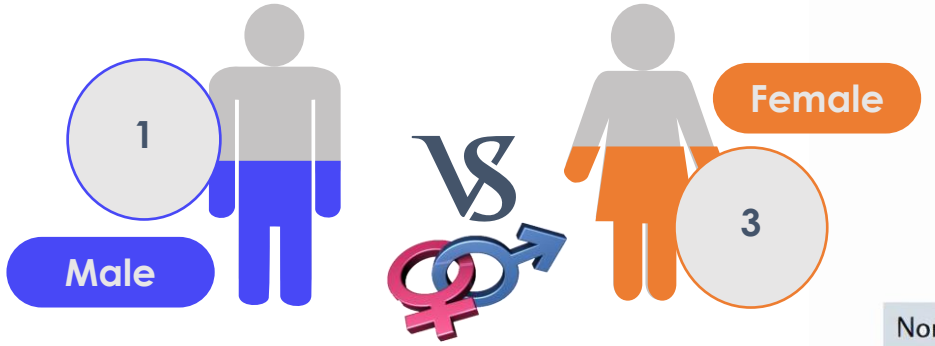
- Axillary arteries

Takayasu arteritis

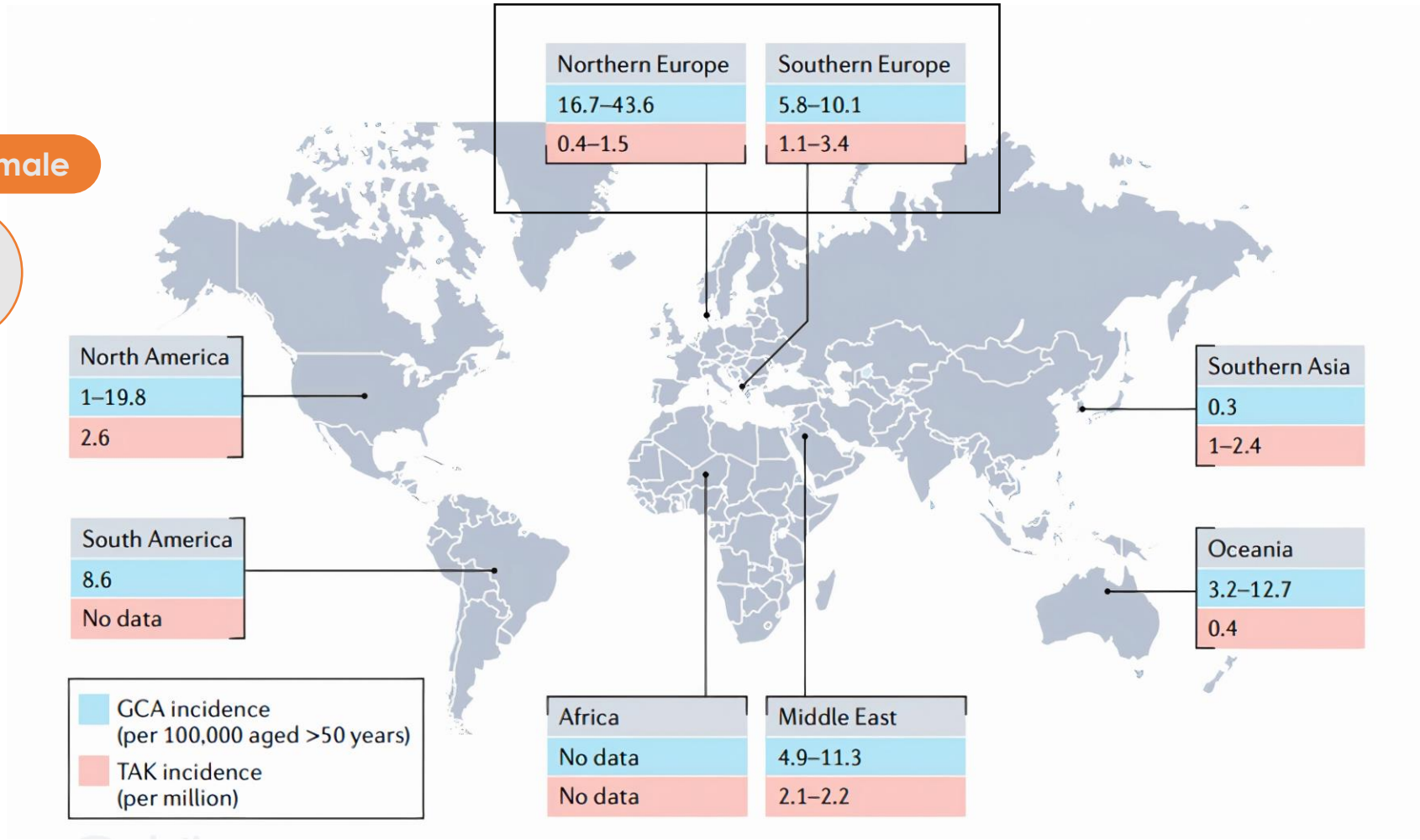
- Renal and mesenteric vessels
- Symmetrical involvement of with the possible exception of subclavian involvement
- Left subclavian is more commonly implicated

Dan Pugh et al. Large-vessel vasculitis Nat Rev Dis Primers 2022

Epidemiology of LVV

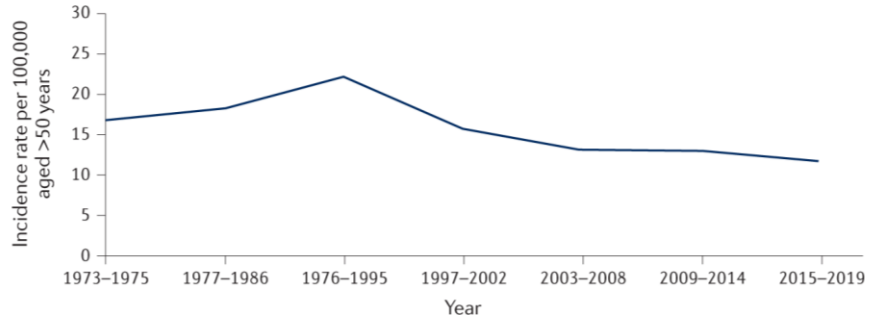


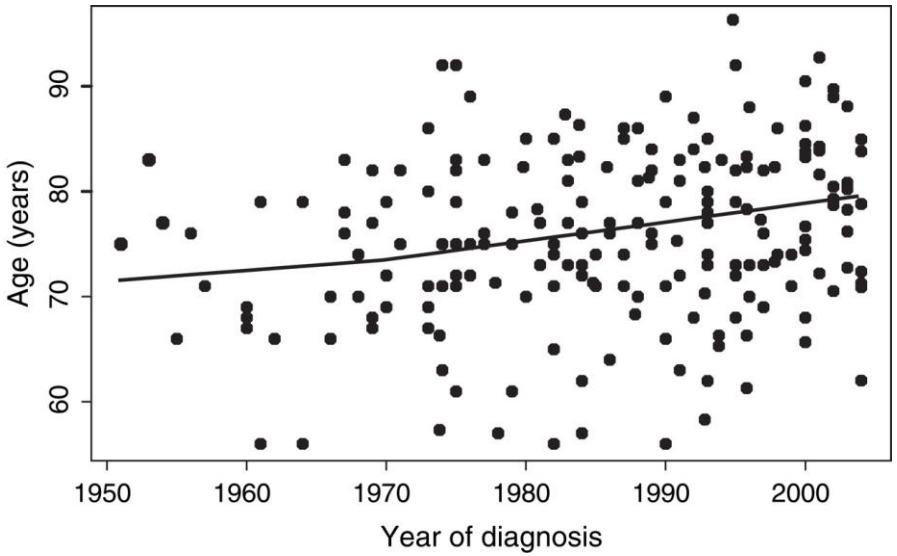
The incidence within Europe shows a marked north–south gradient and is reported to be <10 cases per 100,000 persons over the age of 50 years in Mediterranean population



Epidemiology of LVV

- GCA is observed in patients >50 years. The incidence increases with age to peak in the eighth decade. A 40-fold increase in disease risk over those aged 50–59 years
- Giant cell arteritis is gradually becoming less common, having peaked in incidence around 1990
- Patients with LV-GCA are younger at presentation, are more commonly females and more often present with bilateral arterial involvement than those with C-GCA



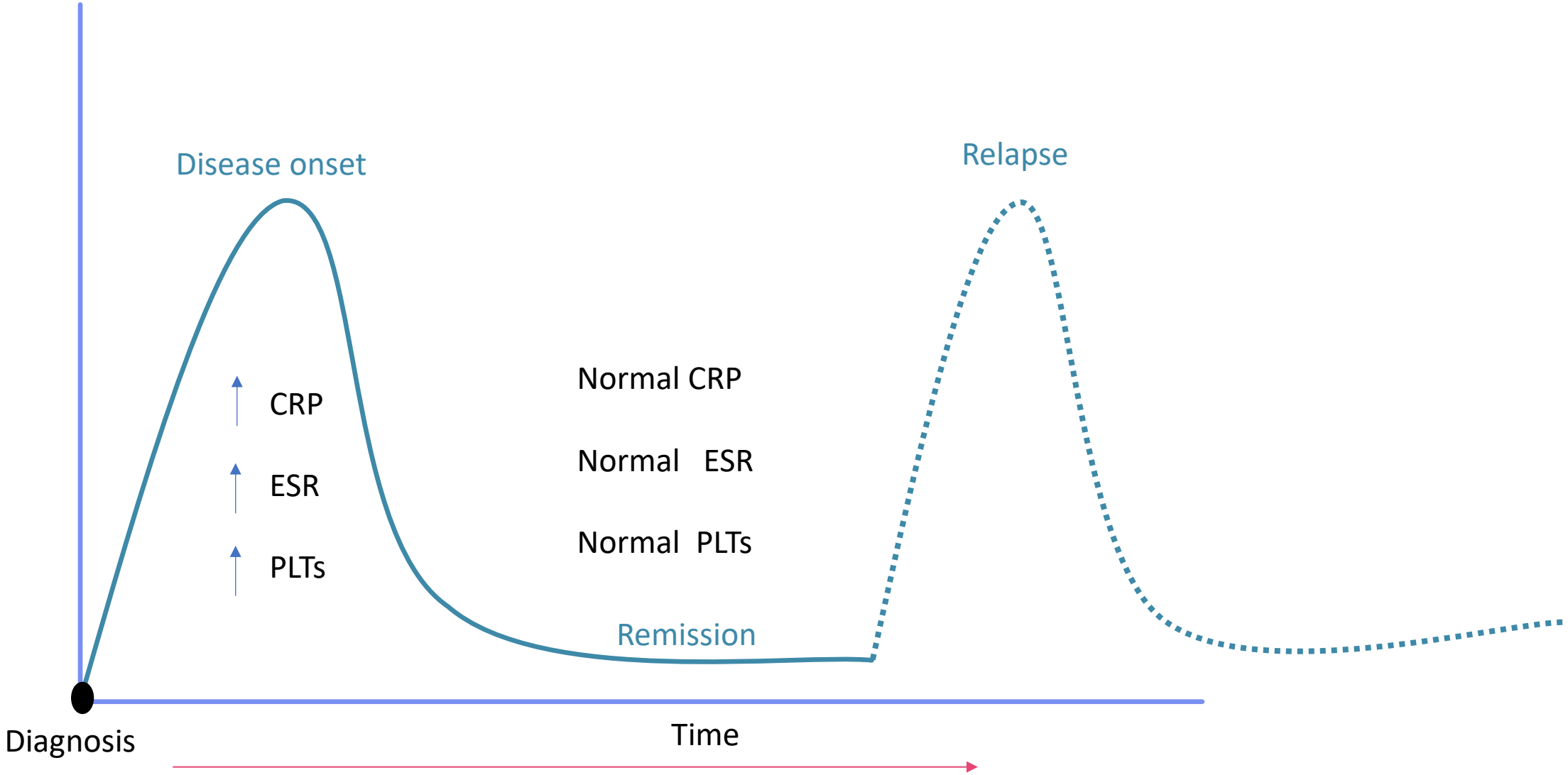


Richard A. Watts et al. Global epidemiology of vasculitis Nat. Rev. Rheumatology 2021

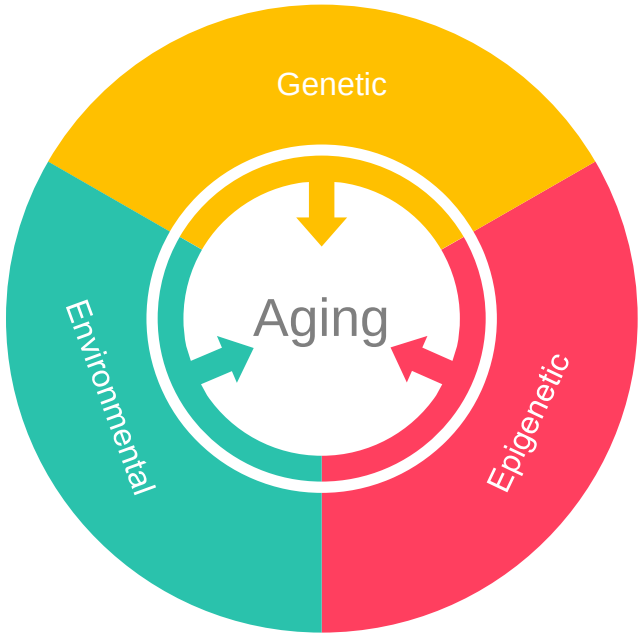
Tanaz A Kermani et al. Increase in age at onset of giant cell arteritis: a population-based study Ann Rhheum Dis 2010

Carlo Salvarani et al. Reappraisal of the epidemiology of giant cell arteritis in Olmsted County, Minnesota, over a fifty-year period Arthritis Rheum 2004

Clinical course of GCA



Disease determinants and risk factors



Genetic

Class II HLA [HLA-DRB1*04 alleles (OR 1.79)]
Other polymorphisms within IL17A, IL33, PLG, P4HA2

Epigenetic

Hypomethylation of genes implicated in the T-cell receptor-CD28 signaling pathways
Overexpression of miRNAs involved in the response of T cells, macrophages and dendritic cells

Environmental

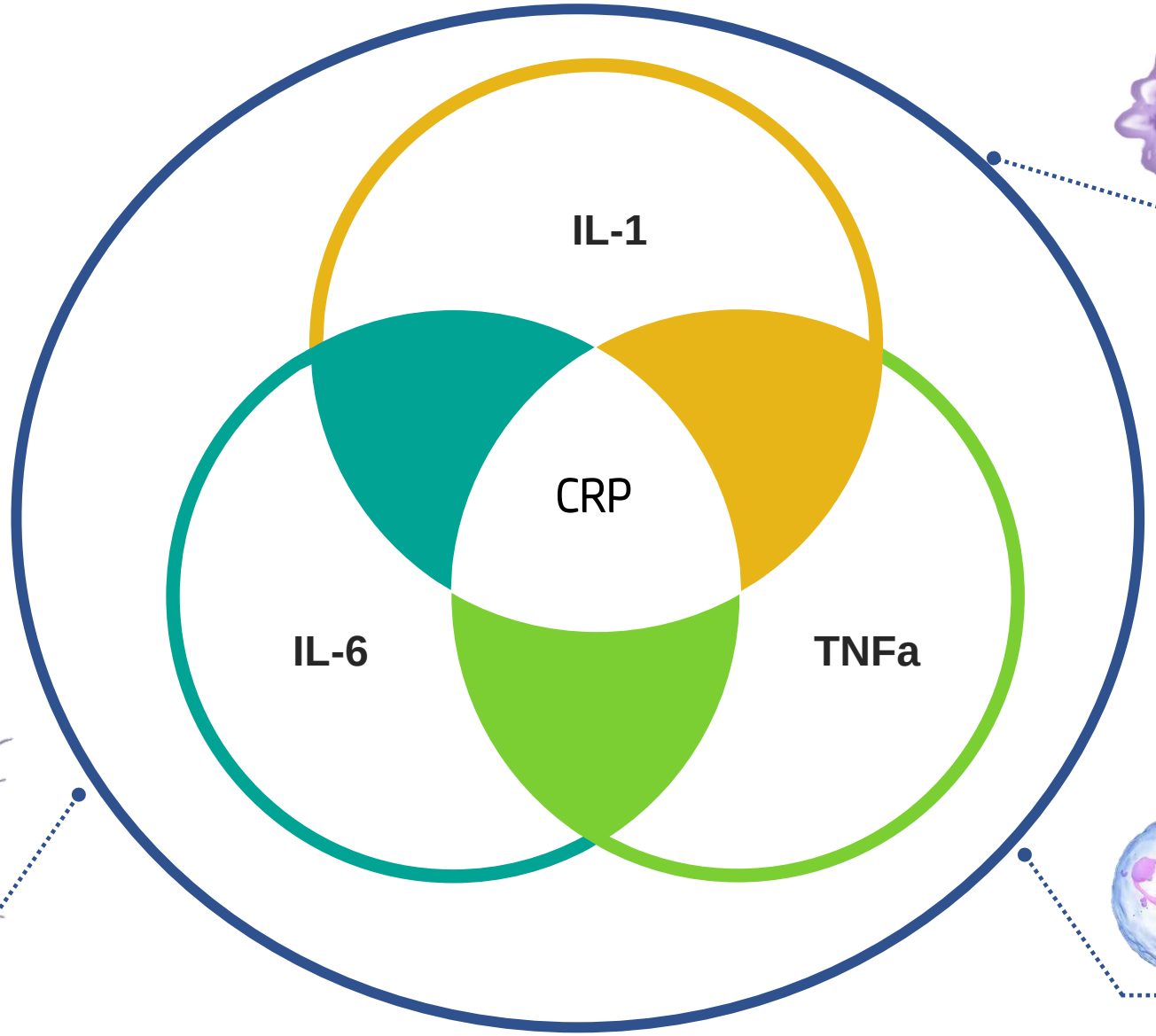
Varicella-zoster virus, Chlamydia pneumoniae, Mycoplasma spp. and parvovirus B19

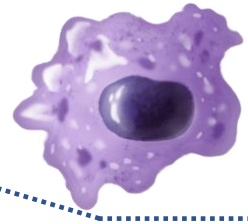
F. David Carmona et al. Genetic component of giant cell arteritis 2014

Carmona, F. D. et al. A genome-wide association study identifies risk alleles in plasminogen and P4HA2 associated with giant cell arteritis. Am. J. Hum. Gene, 2017

Patrick Coit et al. An update on the role of epigenetics in systemic vasculitis Curr Opin Rheumatol. 2017

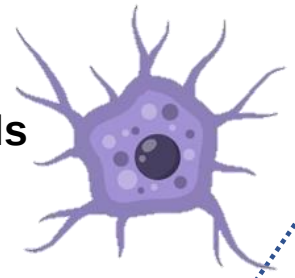
Best GCA marker for active disease

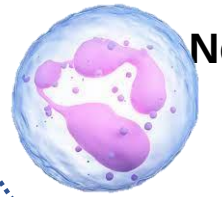




Macrophages

Dendritic cells





Neutrophils

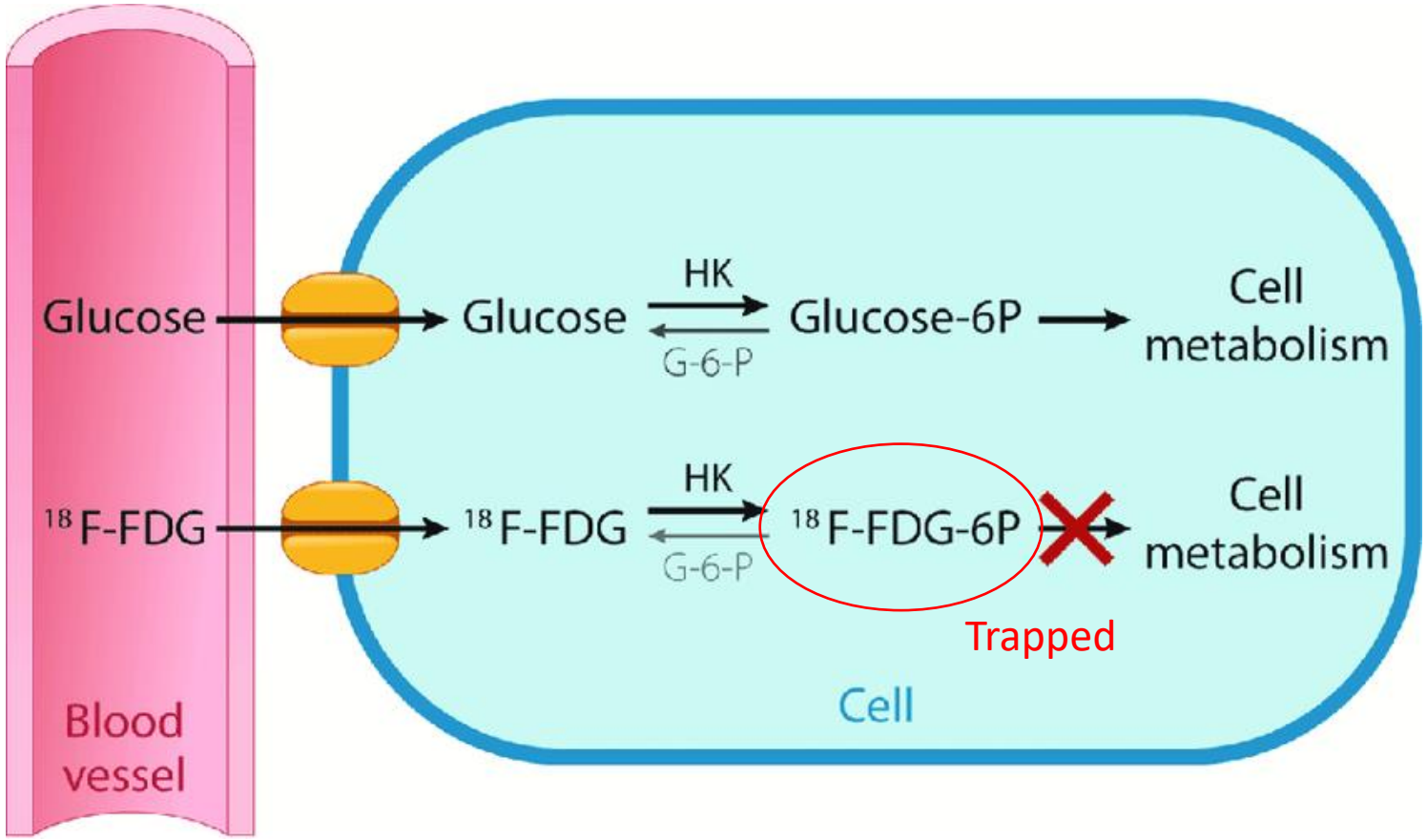
Metabolism

Metabolically active

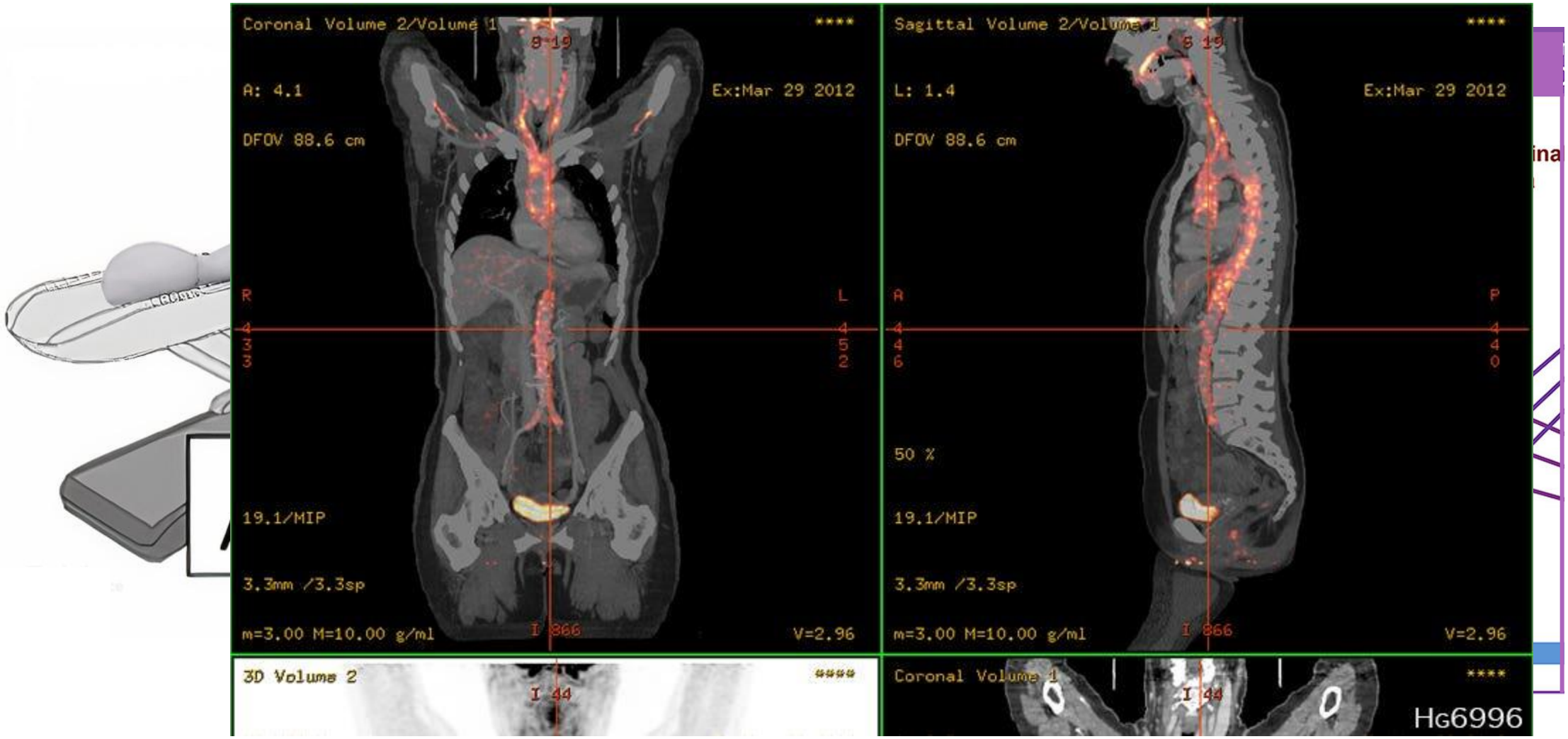
Neutrophils

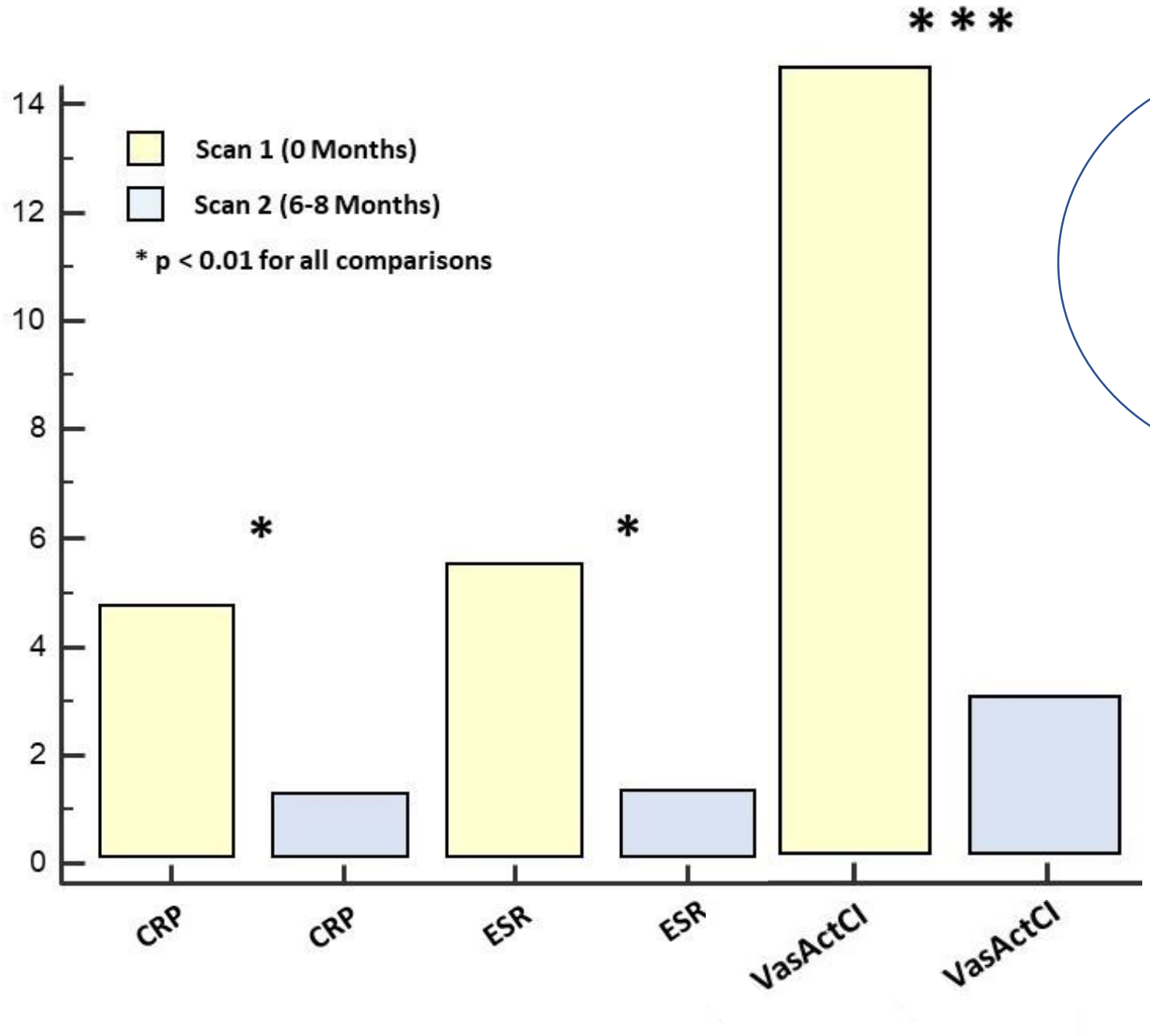
Macrophages

Dendritic cells



FDG-PET CT


Coronal Volume 2/Volume 1
A: 4.1
Ex:Mar 29 2012
DFOV 88.6 cm
R
L
19.1/MIP
3.3mm /3.3sp
m=3.00 M=10.00 g/ml
V=2.96
Sagittal Volume 2/Volume 1
L: 1.4
Ex:Mar 29 2012
DFOV 88.6 cm
A
P
50 %
19.1/MIP
3.3mm /3.3sp
m=3.00 M=10.00 g/ml
V=2.96
3D Volume 2
Coronal Volume 1
Hg6996



Composite index:
CRP + ESR + SUV
(PET)

Unpublished data

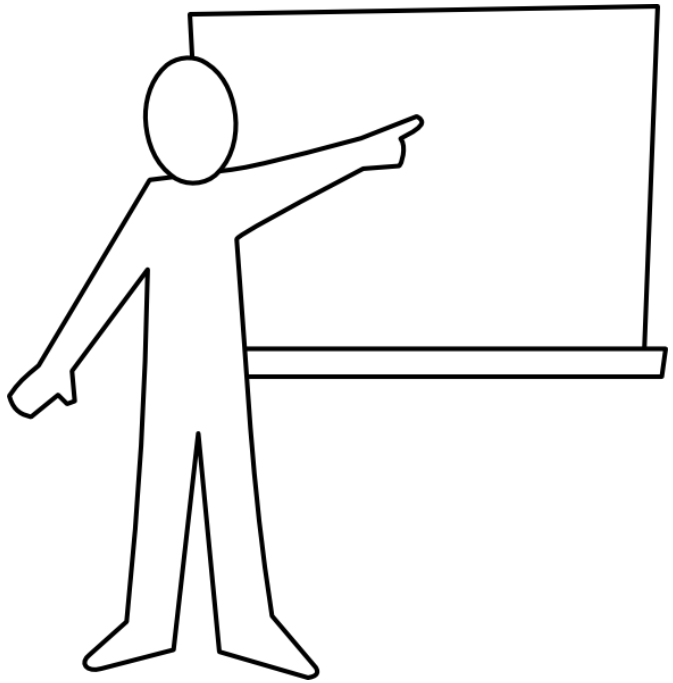
Lecture outline

Introduction

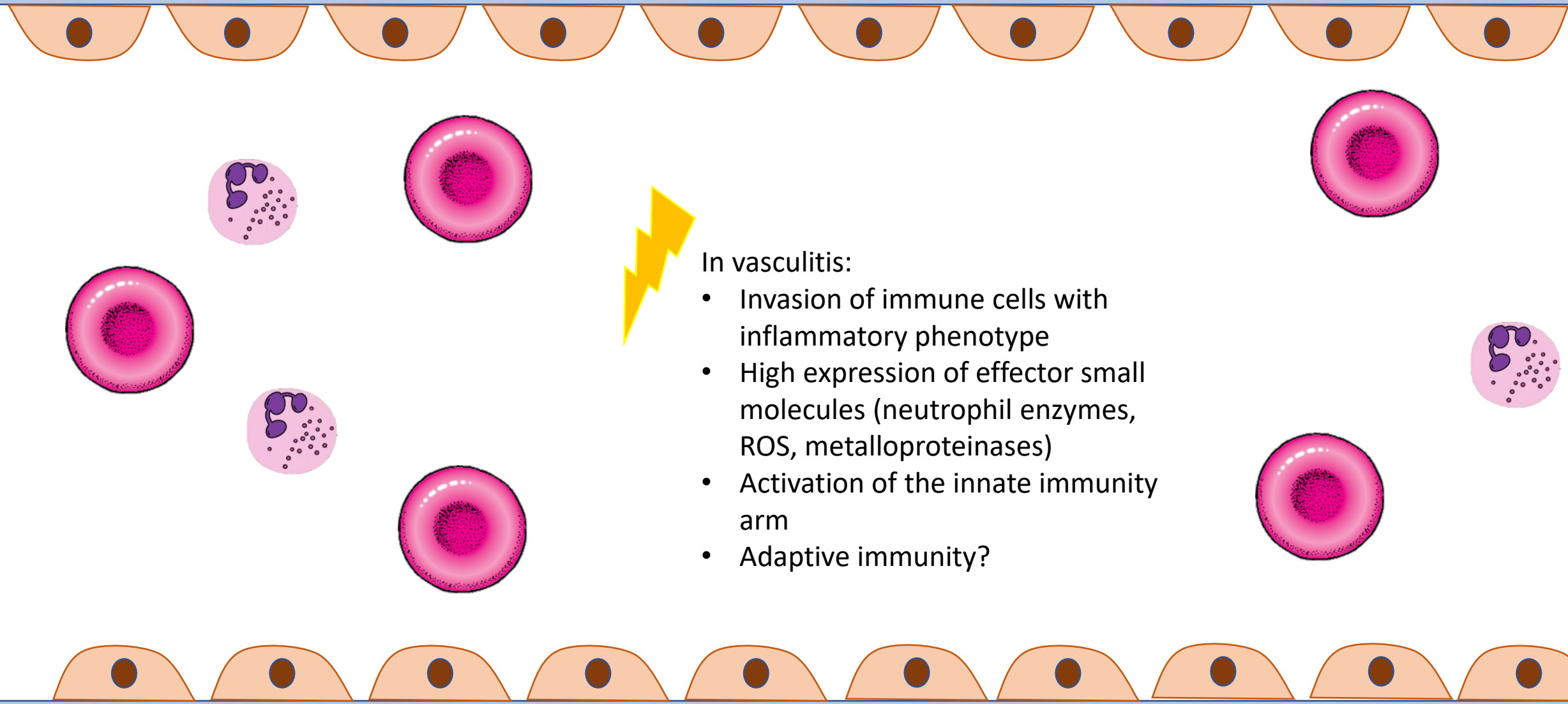
Cells participating/ Stages of inflammatory lesion

Histologic heterogeneity

Work in progress-Future perspectives



The arterial wall is an immune-privileged tissue site

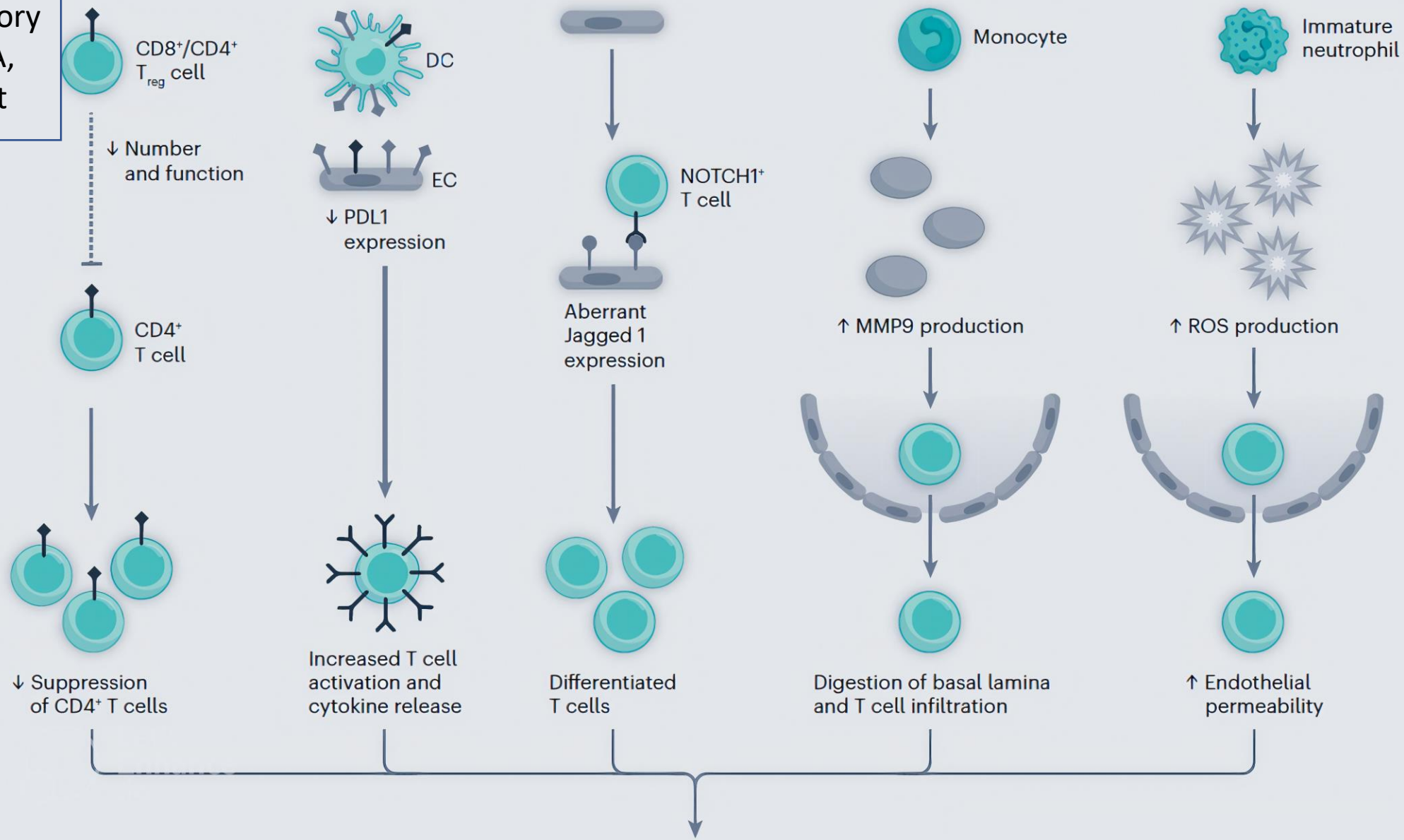


In vasculitis:

- Invasion of immune cells with inflammatory phenotype
- High expression of effector small molecules (neutrophil enzymes, ROS, metalloproteinases)
- Activation of the innate immunity arm
- Adaptive immunity?

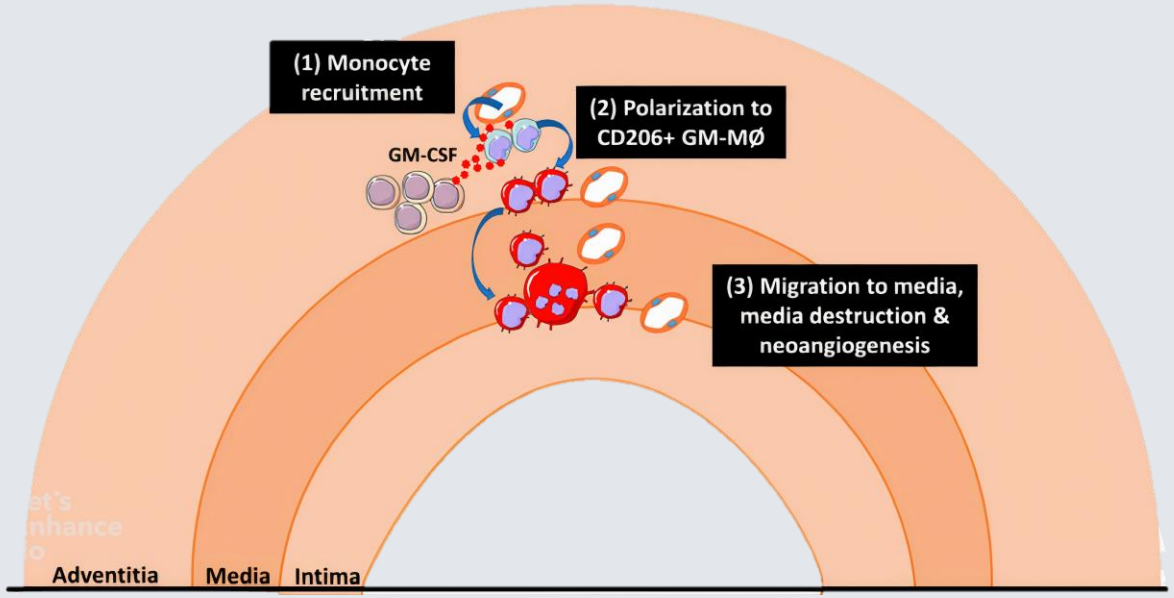
Absence of resident leukocytes, except for the vascular dendritic cells strategically placed at the adventitia interface

1. Inflammatory cells in GCA, early event



Initiation and propagation of inflammation in LVV via pro-inflammatory mediators, stimulation of vascular dendritic cells (DCs) recruitment of immune cells and differentiation of monocytes

2. Main role of Myeloid cells



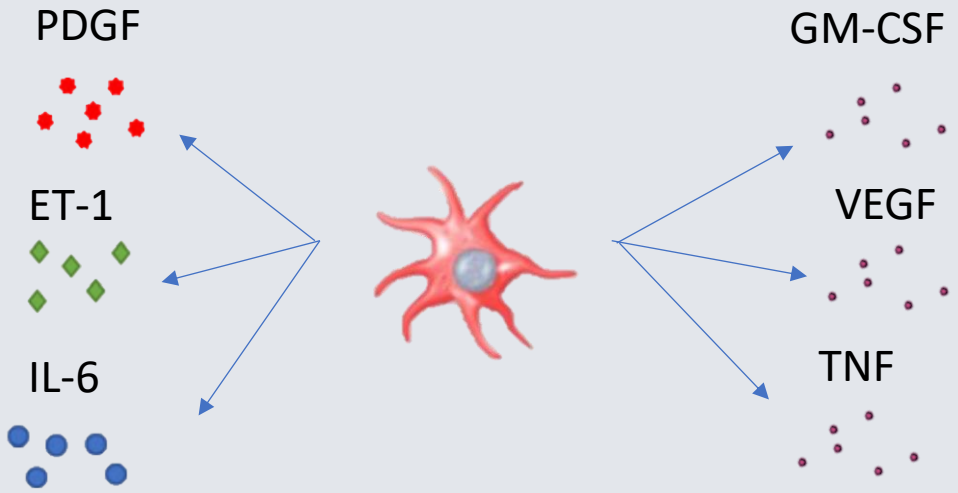
At least three macrophage population phenotypes were identified:

i) pro-inflammatory macrophages,

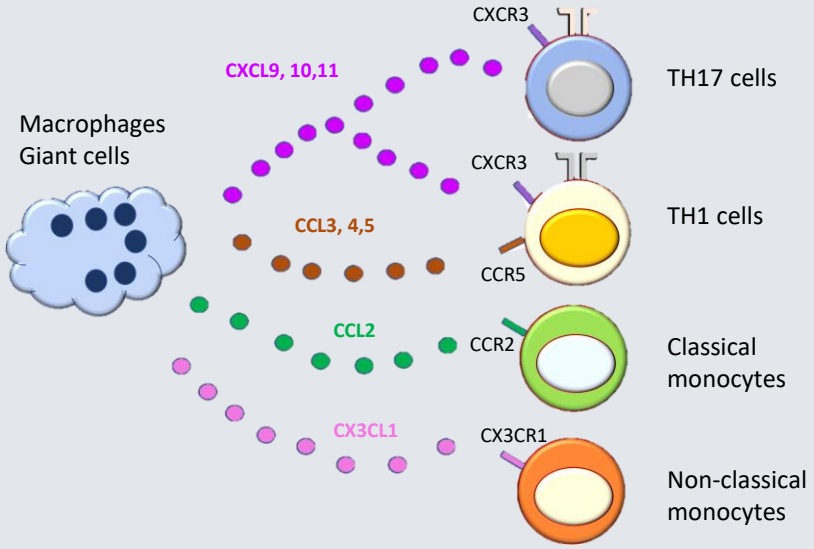
ii) $CD206^{+}$ MMP-9-producing macrophages

iii) folate receptor β-expressing macrophages

Macrophages use the metalloproteinase MMP-9 to gain access to the arterial wall by degrading the vasa vasorum barrier as a preliminary step for invasion by innate and adaptive immune cells.



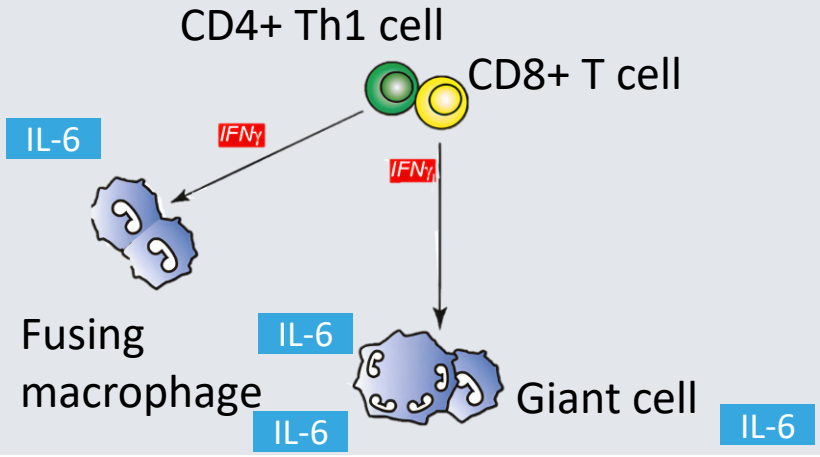
Cytokines

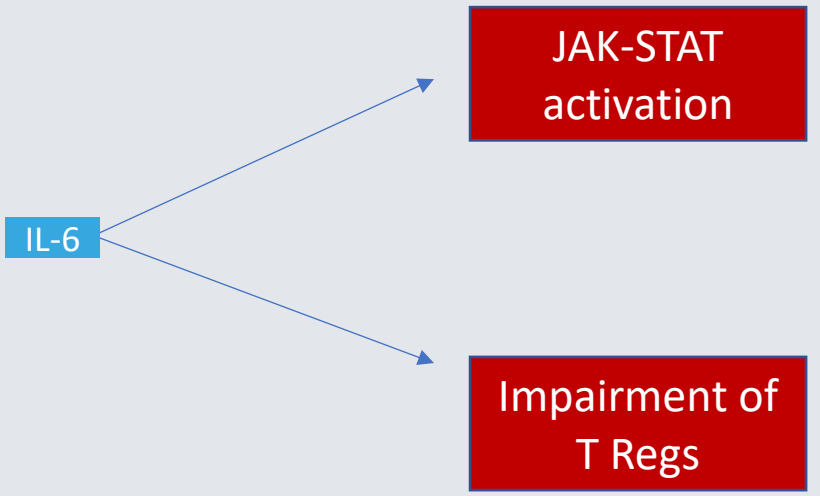


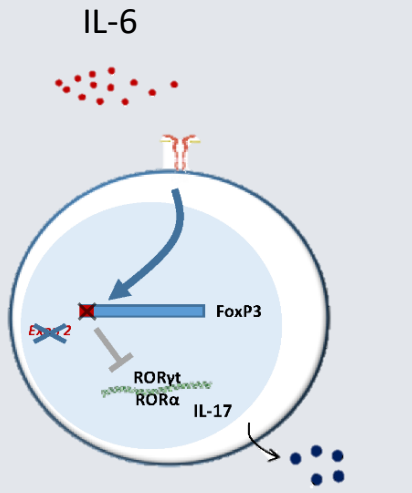
Chemokines

3. Studies on Th1 and Th17 cells

- $CD4^+$ T cells are activated by DCs and polarize into Th1 and Th17 cells through the effect of IL-12, IL-23, IL-6 and IL-1β, which are produced by activated DC.
- Th1 and Th17 lymphocytes release IFN-γ and IL-17, respectively.
- A substantial increase in a multitude of cytokines produced by T cells was shown in giant cell arteritis lesions, including IL-6, IFNγ, IL-2, IL-17, IL-9, IL-21, IL-22, IL-23p19, and GM-CSF.
- IFN-γ induces the activation of vascular smooth muscle cells (VSMC) in the media and enables them to produce chemokines (CCL2, CXCL9, CXCL10, CXCL11), which trigger the recruitment of additional T cells ($CD4^+$ and $CD8^+$) and monocytes.
- Monocytes differentiate into macrophages and merge into multinucleated giant cells, the hallmark of GCA

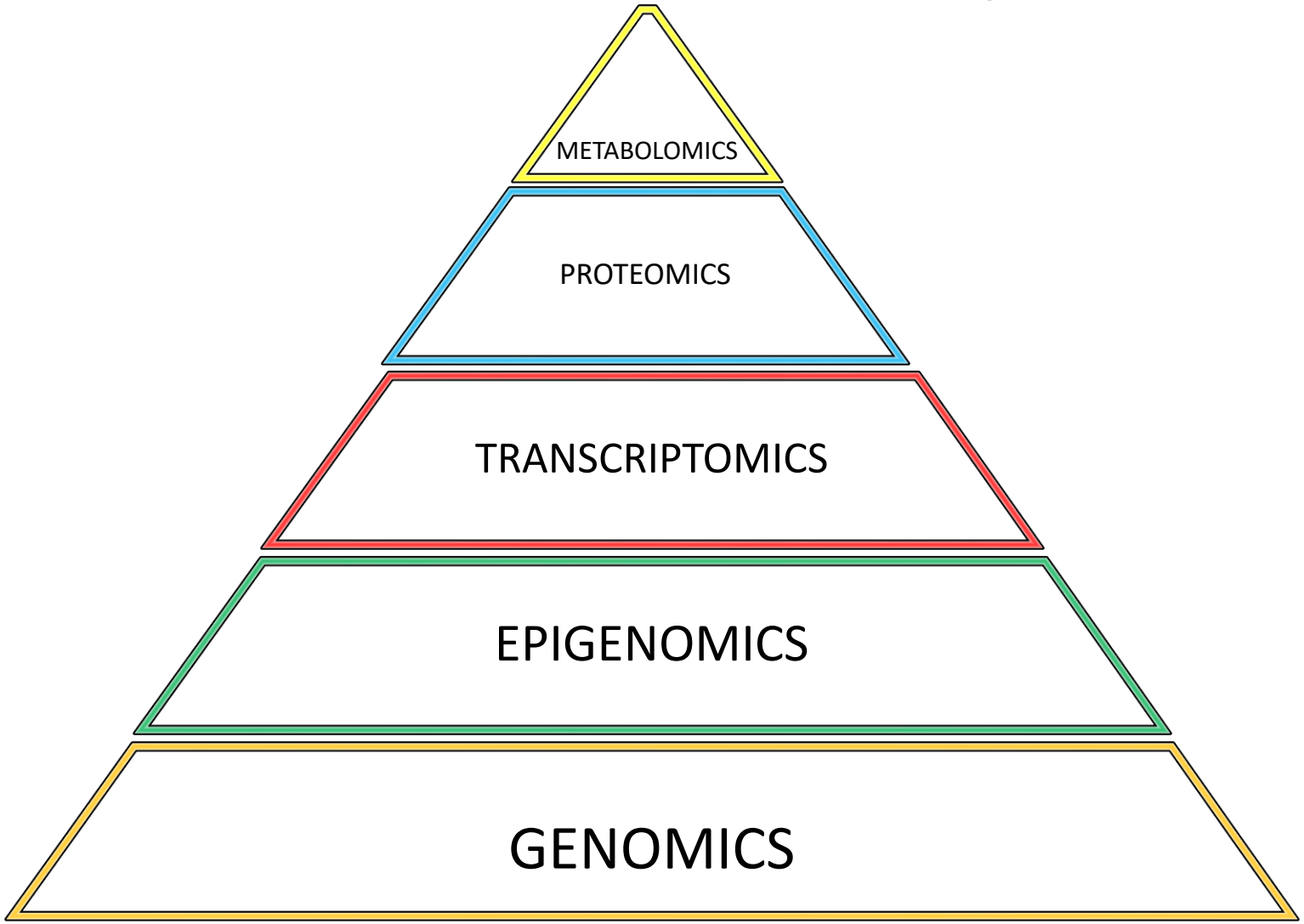


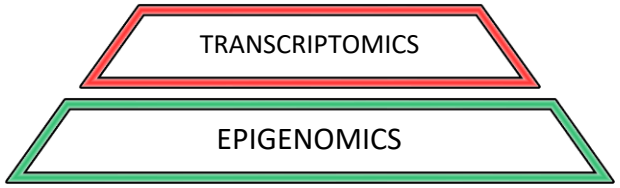




Systems biology

- Detection of all molecules of interest in a given tissue





TRANSLATIONAL SCIENCE

Methylome and transcriptome profiling of giant cell arteritis monocytes reveals novel pathways involved in disease pathogenesis and molecular response to glucocorticoids

Elkyn Estupiñán-Moreno [1], Lourdes Ortiz-Fernández [1], Tianlu Li [2], Jose Hernández-Rodríguez [3], Laura Ciudad [2], Eduardo Andrés-León [1], Laura Carmen Terron-Camero [1], Sergio Prieto-González [3], Georgina Espígol-Frigolé [3], Maria Cinta Cid [3], Ana Márquez [1,4], Esteban Ballestar [2], Javier Martín [1]

Study Design

31 age and sex-matched healthy controls

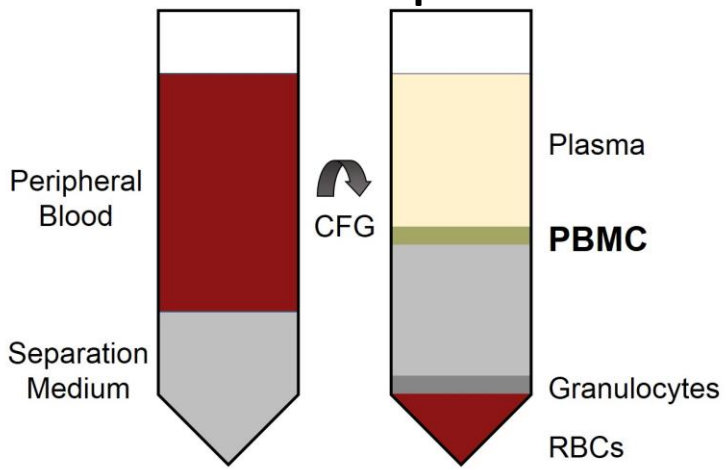
82 biopsy-proven GCA patients

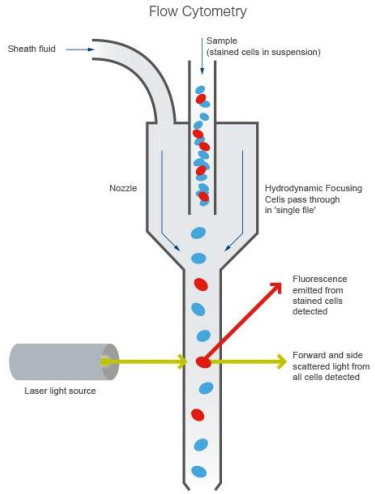
a) active disease (n=20): newly diagnosed (n=16) or 2 days of GC start (n=2), or disease relapse (n=2);

b) in remission with treatment (n=33): prednisone (< 10mg/day)

c) in remission without treatment (n=29): without any treatment for at least 1 month.

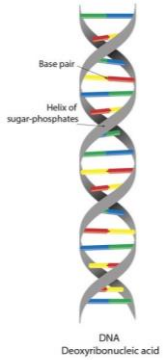
Ficoll-Paque



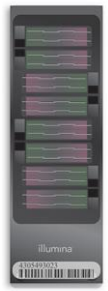


Monocyte isolation

CD14+CD15-



Bisulfite converted



Infinium Methylation **Bead Chip array**

Methylome

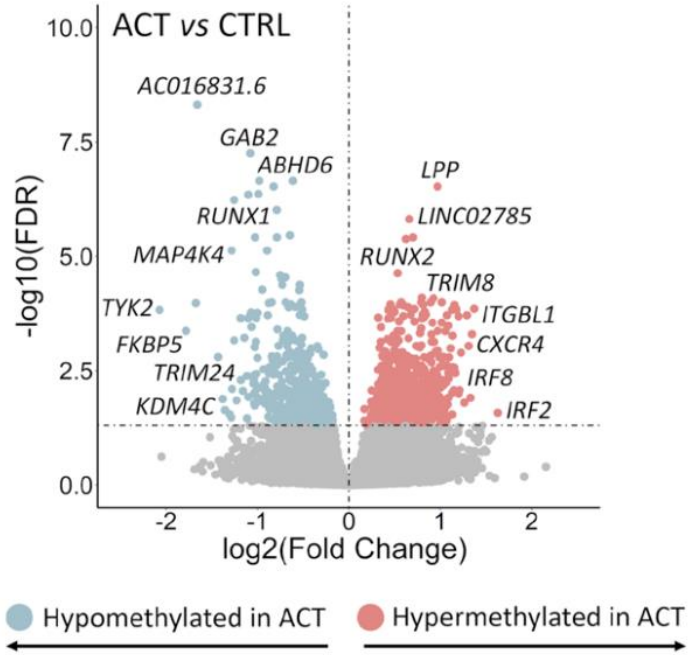
TruSeq Stranded mRNA Library Prep

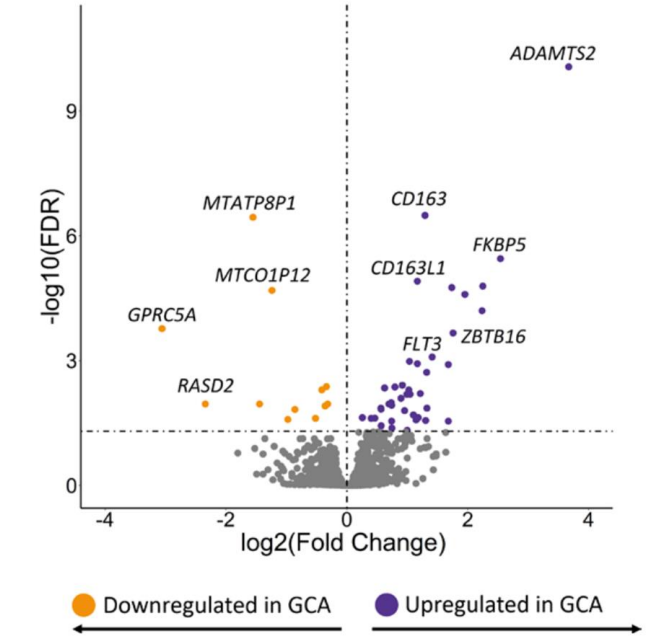
mRNA sequencing

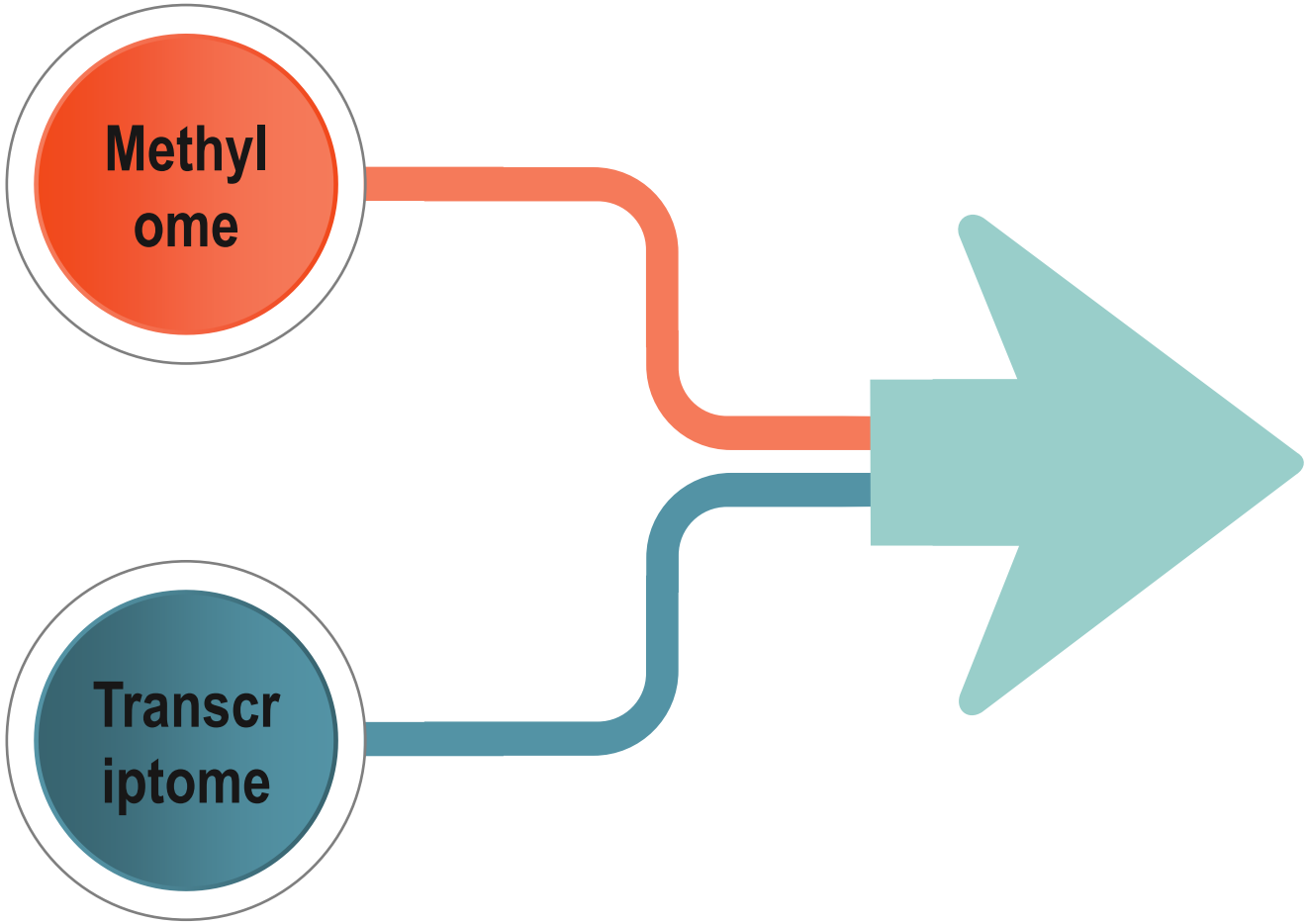
Transcriptome

Enrichment analysis

Integrative analysis


ACT vs CTRL
AC016831.6
GAB2
ABHD6
RUNX1
MAP4K4
TYK2
FKBP5
TRIM24
KDM4C
LPP
LINC02785
RUNX2
TRIM8
ITGBL1
CXCR4
IRF8
IRF2
-log10(FDR)
log2(Fold Change)
Hypomethylated in ACT
Hypermethylated in ACT


ADAMTS2
MTATP8P1
MTCO1P12
GPRC5A
RASD2
CD163
FKBP5
CD163L1
FLT3
ZBTB16
-log10(FDR)
log2(Fold Change)
Downregulated in GCA
Upregulated in GCA


Methyl ome
Transcr iptome

Conclusions

01

Monocytes from active patients possess more proinflammatory phenotype

Dysregulation of pathways involving cytokines and growth factors already known to have a key role in GCA, such as IL-6, TNF, IL-1, IL-4, IL-2, PDGF and VEGF

02

IL-11 pathway

Enriched among the DMPs hypomethylated in active patients with respect to controls and patients in remission with and without treatment

03

Chemokines

CCL2 and CCL7, involved in the recruitment of monocytes, were overexpressed in active patients

Genes encoding several integrins were also overexpressed

04

Remission

The proinflammatory methylation and expression profiles observed in the active disease are lost during remission

CD163 and CD163L1, were the most significant overexpressed genes in patients in remission with treatment compared with nontreated patients

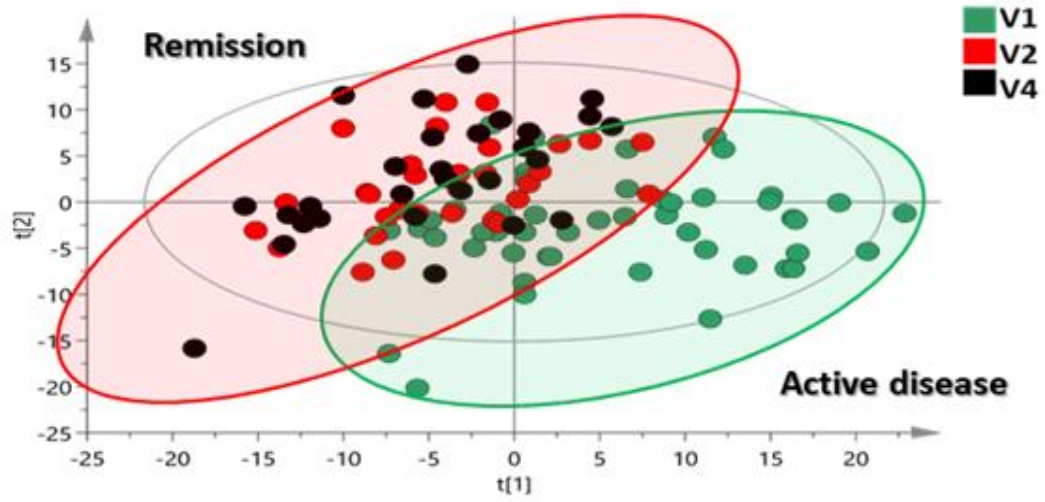
05

Corticosteroids

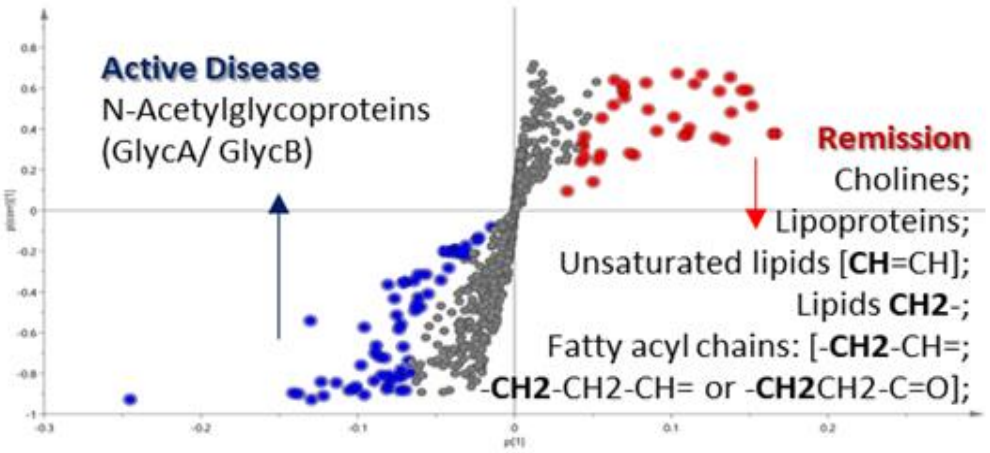
GCs modify gene expression levels through DNA demethylation of target genes

Study of the metabolome in GCA and PMR Patients in sequential serum samples

A



B



Study Design

GCA

V1	V2	V4
Naive, n=33 25 Female; 8 Male	1st month after treatment, n=22	6th month after treatment, n=21

PMR

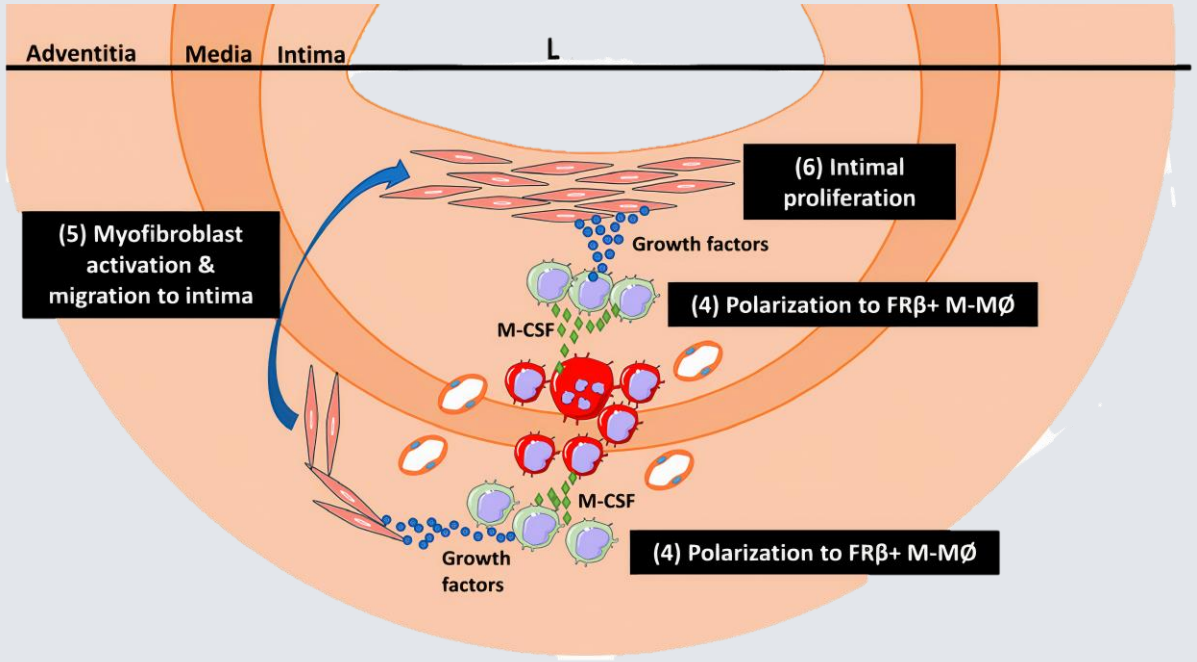
V1	V2	V4
Naive, n=15 9 Female; 8 Male	1st month after treatment, n=10	6th month after treatment, n=8

Results

- ✓ Metabolic differences in active and inactive disease.
- ✓ N-acetyl glycoproteins, increased in inflammation, while cholines, lipoproteins, and lipids were decreased in these patients.

Unpublished data

4. Vascular remodeling



- **Tissue remodeling: a maladaptive repair process**
- In vessels it is characterized by the destruction of the internal elastic lamina and the proliferation and migration of VSMC into the intima.

The process is governed by direct and indirect action of specific subpopulations of activated macrophages

Direct actions

- Polarization towards a M-CSF-differentiated (FRβ) type in the late stages of inflammation

- Production and release of several factors such as Platelet-Derived Growth Factor (PDGF), VEGF, FGF, Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)

Indirect actions.

- VSMCs and endothelial cells release ET-1

- Activation and proliferation of VSMCs and consequent invasion into the intima where they deposit extracellular matrix proteins.

- A cellular transformation from VSMC to a myofibroblast phenotype is observed

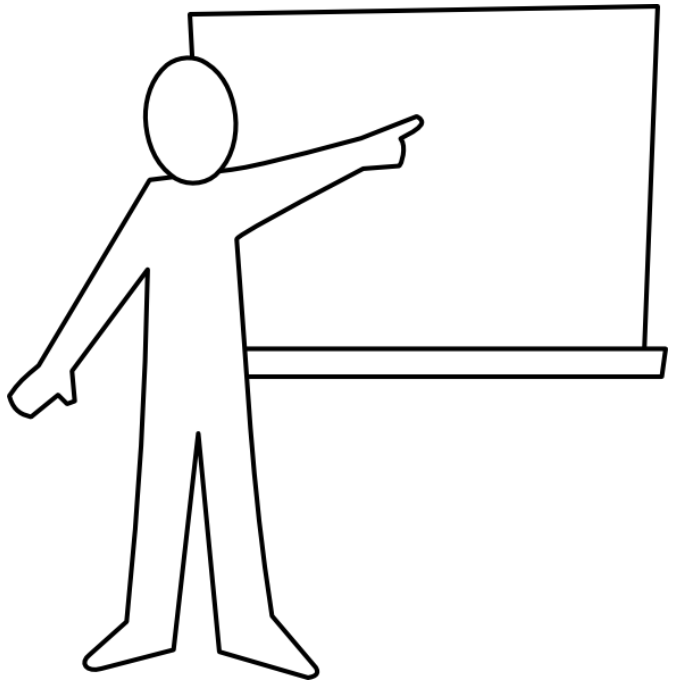
Lecture outline

Introduction

Cells participating/ Stages of inflammatory lesion

Histologic Heterogeneity

Work in progress-Future perspectives



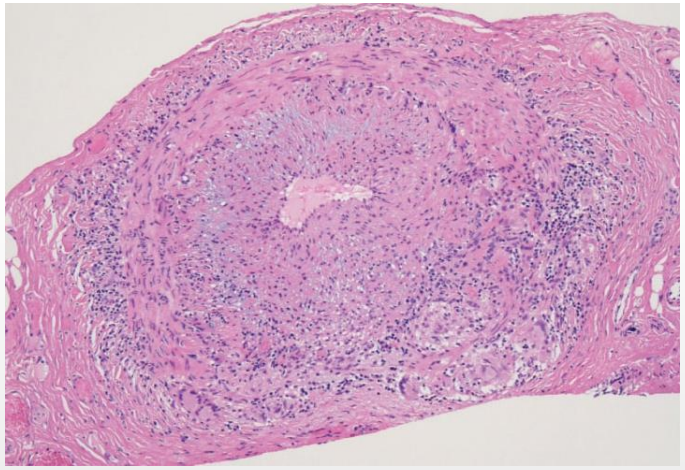
Histologic heterogeneity in untreated systemic autoimmune diseases depends on:

- Stage of the disease
- Clinical phenotype of a given disease

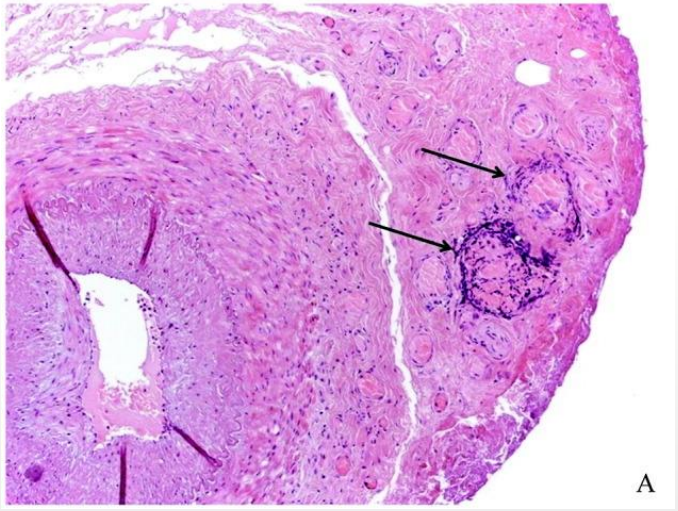
UNMET NEEDS:

- Does a specific histologic type follow a specific clinical phenotype?
- Does a specific histologic type underlie a specific endotype?
- Response to treatment

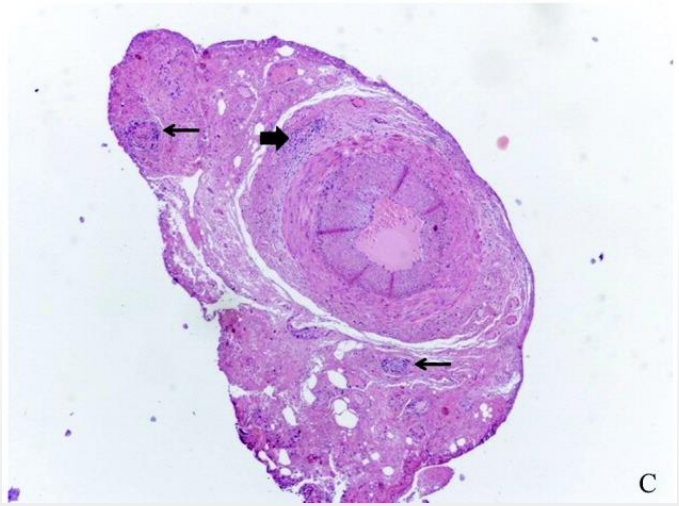
Histologic heterogeneity in GCA



Panarteritis



Small Vessel Vasculitis



Coexistence of SVV (**thin arrows**) and vasa vasorum vasculitis (**thick arrow**)

Giovanna Restuccia et al. Small-vessel vasculitis surrounding an uninflamed temporal artery and isolated vasa vasorum vasculitis of the temporal artery: Two subsets of giant cell arteritis Athr. Rheum. 2011

G. Restuccia et al. Pathogenic role of monocytes/macrophages in large vessel vasculitis Front. Immun.. 2022

Clinical significance

- The extent to which these patterns are reported and their role in GCA diagnosis remains to be elucidated.
- The question whether limited disease and vasa vasoritis is a different phenotype or the early stage of full blown transmural involvement is answered
- Other forms of vasculitis, infection, and certain hematologic malignancies can present with Restricted Inflammation and particularly with SVV.

Elena Galli et al. Significance of inflammation restricted to adventitial/periadventitial tissue on temporal artery biopsy Semin. Arhtirits Rheum. 2020

Giovanna Restuccia et al. Small-vessel vasculitis surrounding an uninflamed temporal artery and isolated vasa vasorum vasculitis of the temporal artery: Two subsets of giant cell arteritis Athr. Rheum. 2011

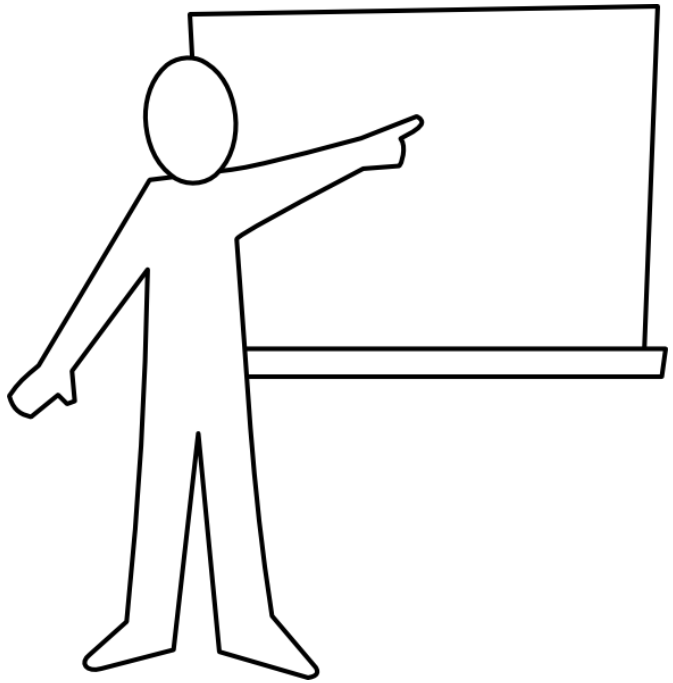
Lecture outline

Introduction

Cells participating/ Stages of inflammatory lesion

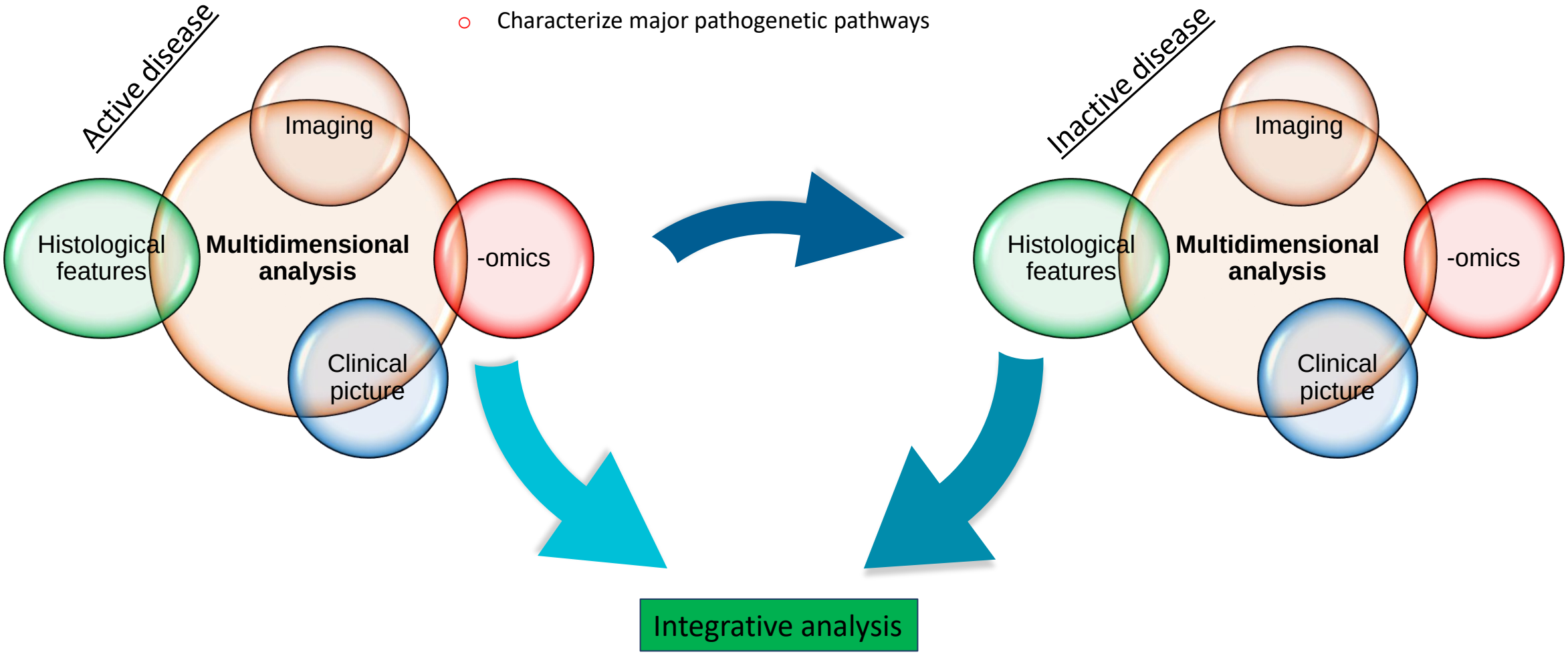
Histologic heterogeneity

Work in progress-Future perspectives



❑ Combination and integration of different types of data

- Define phenotypes
- Characterize subgroups by tissue stratification
- Develop non-invasive biomarkers
- Characterize major pathogenetic pathways



Ageing is the strongest independent risk factor

Aging
- Age > 50

Malignant neoplasms
Infectious diseases (Covid-19)
Neurogenerative diseases

Vascular Aging
Mechanical and structural damage

reactive oxygen species (ROS)
mitochondrial dysfunction
cellular senescence,
genomic instability,
increased apoptosis,
epigenetic alterations
clonal hematopoiesis of indeterminate potential (CHIP)

Low grade inflammation

Atherosclerosis
- ??

High grade inflammation

Giant cell arteritis
- ??

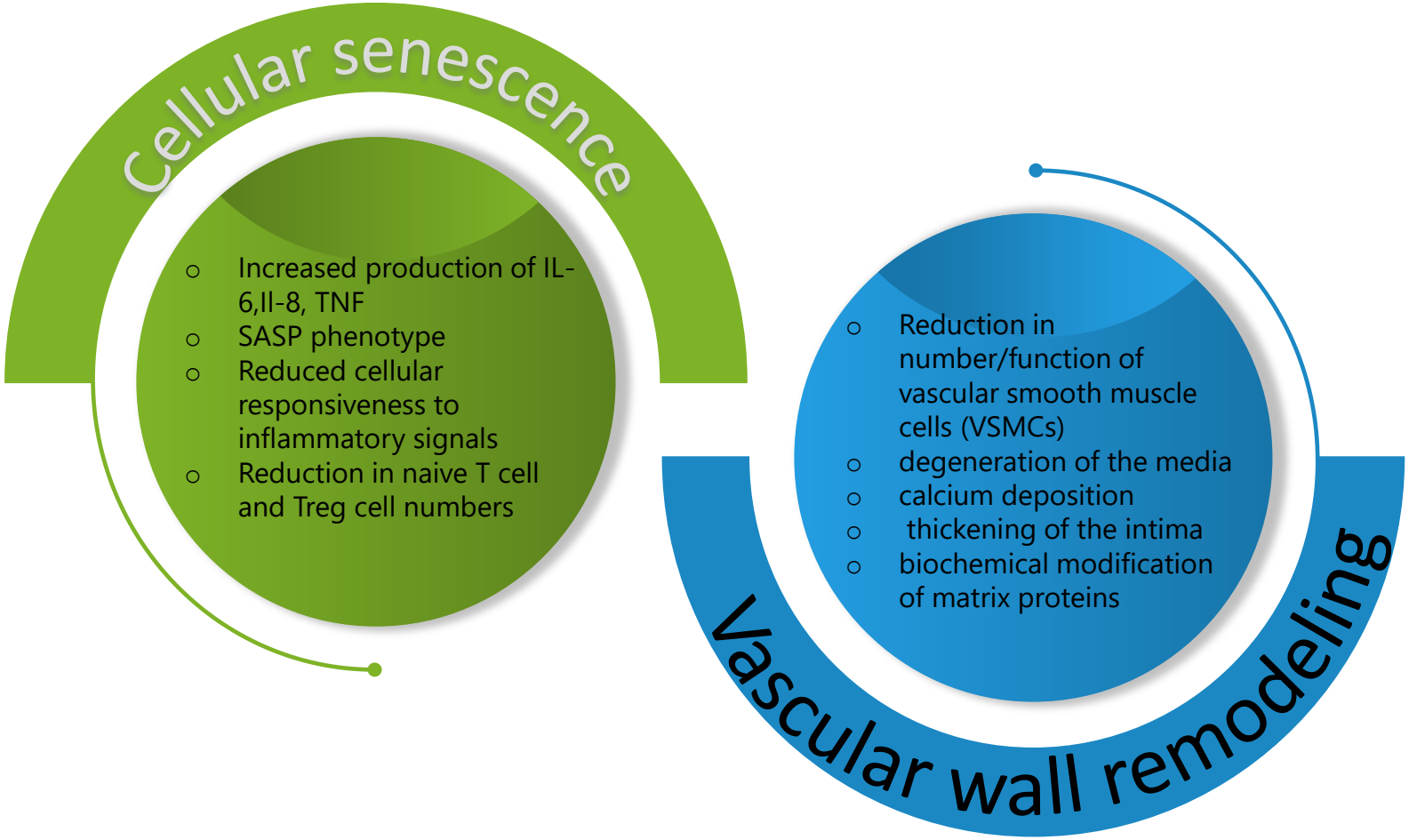
Siddhartha Jaiswal et al. Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease NEJM 2017

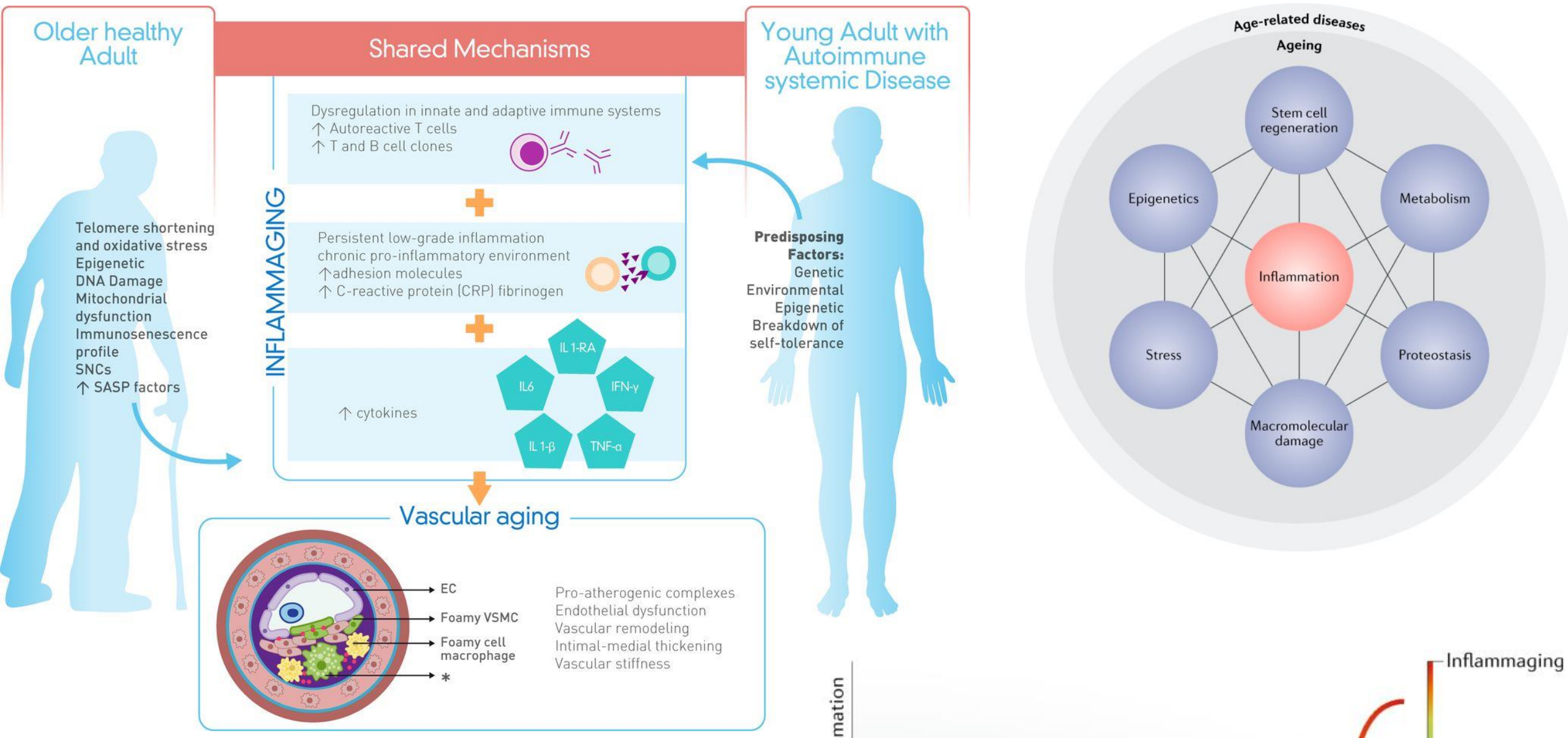
Ryu Watanabe et al. Aging-Related Vascular Inflammation: Giant Cell Arteritis and Neurological Disorders Front. Aging Neurosci 2022*

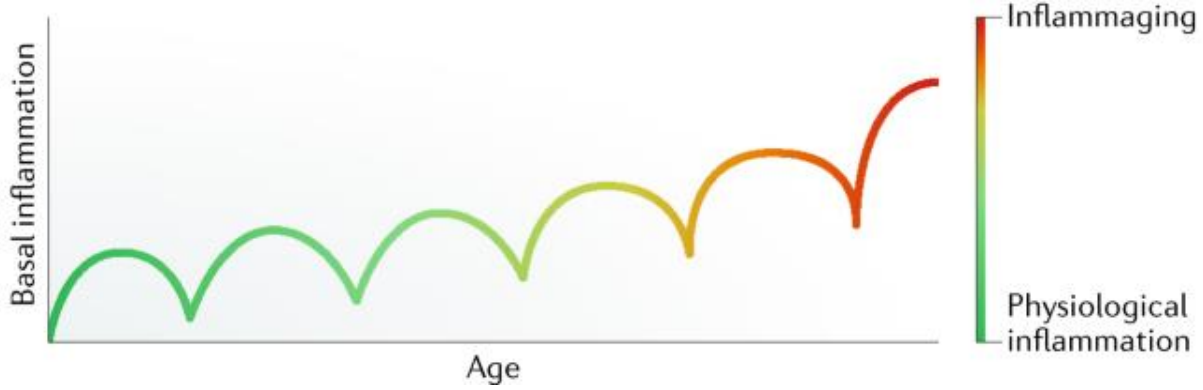
Shalini V Mohan Giant cell arteritis: immune and vascular aging as disease risk factors Arthritis Res Ther 2011

Giant cell arteritis

- ??



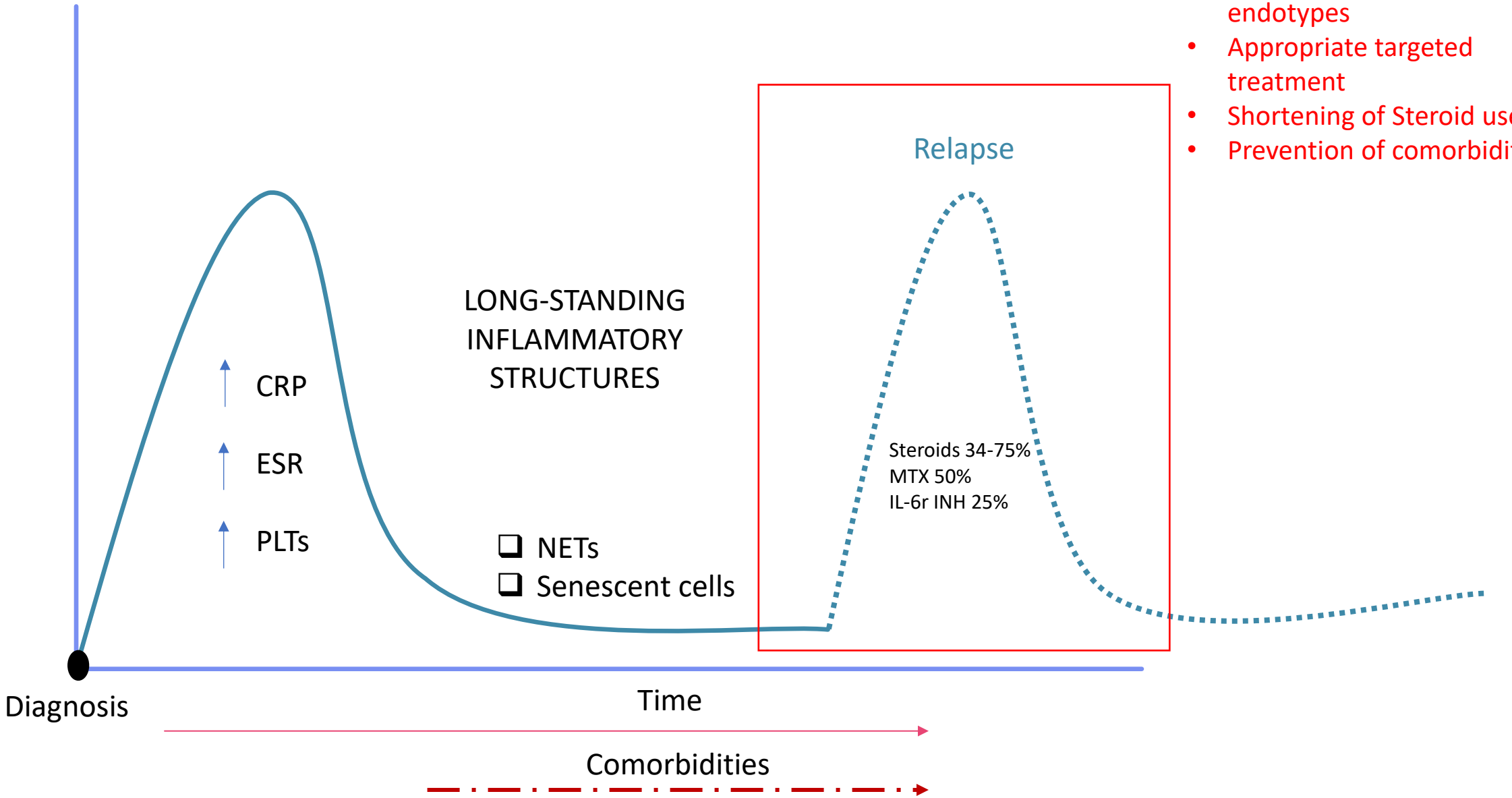




Claudio Franceschi et al. Inflammaging: a new immune–metabolic viewpoint for age-related diseases Nat Rev 2018

Pedro Santos-Moreno et al Inflammaging as a link between autoimmunity and cardiovascular disease: the case of rheumatoid arthritis RMD Open 2021

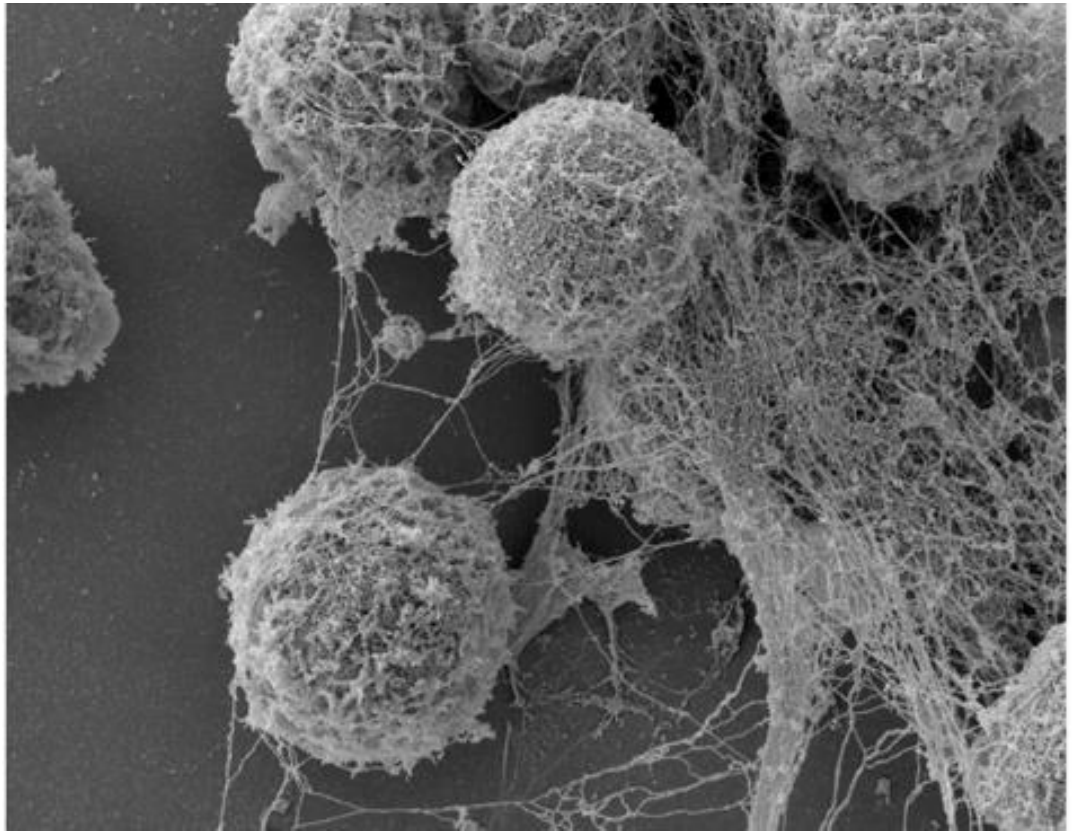
Clinal course of GCA



Unmet Needs

- Clinical phenotyping and definition of the relevant endotypes
- Appropriate targeted treatment
- Shortening of Steroid use
- Prevention of comorbidities

Cellular Extracellular Traps (Most common NETS)

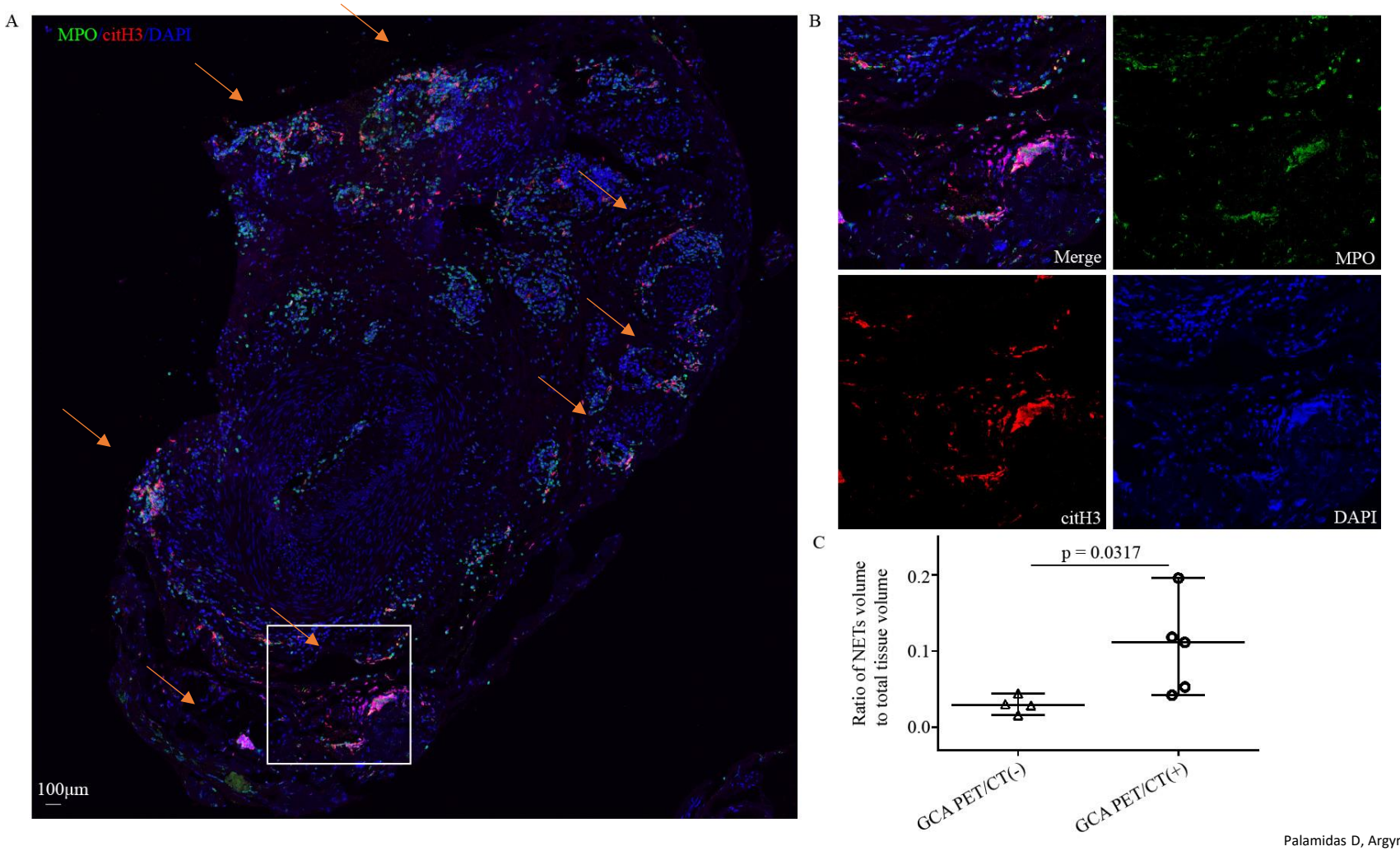


Scanning electron microscopy image of polymorphonuclear neutrophils undergoing NETosis, after in vitro treatment with PMA for 3 h.

"NETs are large extracellular structures of neutrophils composed of cytoplasmic and nuclear proteins located in a deconcentrated chromatin/histone scaffold"

- Extracellular DNA -> genomic and mitochondrial
- Present in chronic inflammation (SLE, RA, ANCA –Vasculitis ect).
- Permanent in tissue injury (poor clearance).
- Decorated with inflammatory cytokines and/or autoantigens

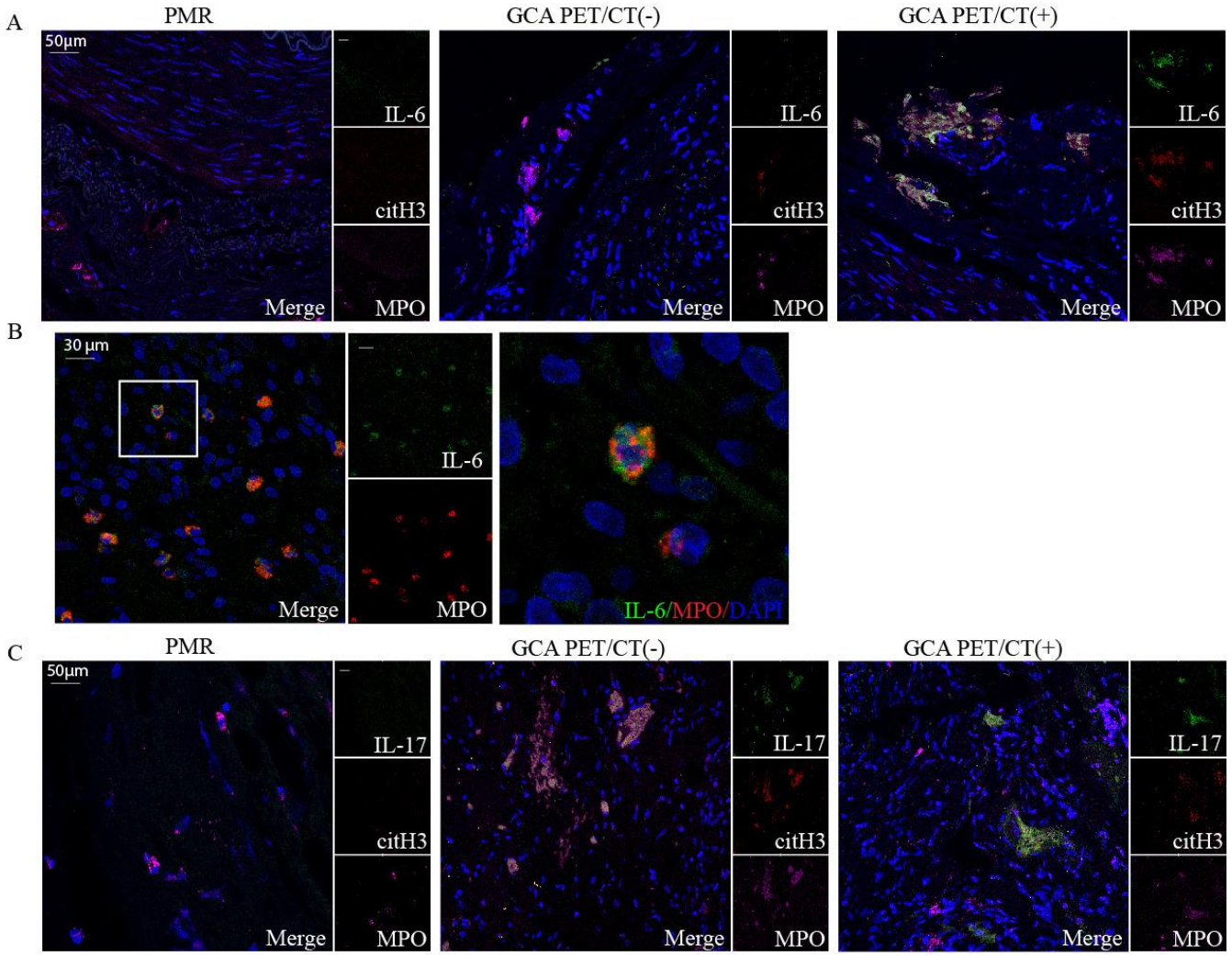
Neutrophil extracellular traps in GCA biopsies: presentation, localization



- Neutrophil Extracellular Traps in GCA TABs are mainly located in the adventitia close to the vasa vasorum.

Palamidas D, Argyropoulou OD et al. Neutrophil extracellular traps in giant cell arteritis biopsies Rheumatology (Oxford) 2022

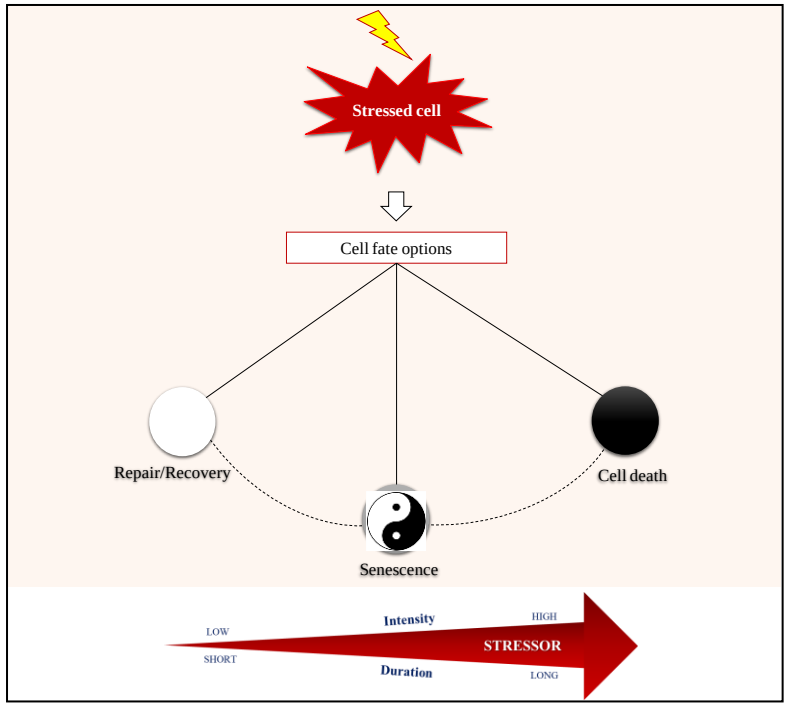
Neutrophil extracellular traps in GCA biopsies: co-expression with inflammatory cytokines



- Decoration of neutrophil extracellular traps with IL-6 and IL-17A may suggest a pro-inflammatory and/or immunoregulatory role
- IL-6 may participate actively through neutrophils in the tissue inflammatory loop

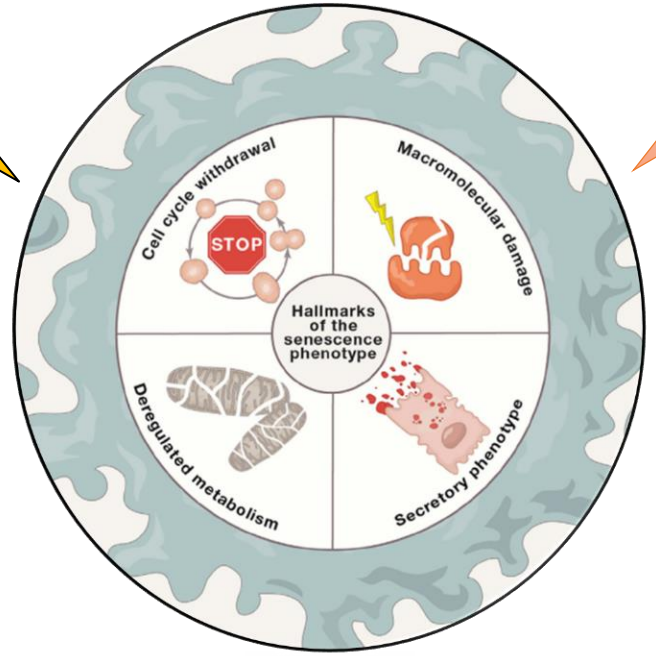
Palamidas D, Argyropoulou OD et al. Neutrophil extracellular traps in giant cell arteritis biopsies Rheumatology (Oxford) 2022

Cellular Senescence: a stress response mechanism that preserves cellular homeostasis



Replicative senescence

Stress induced senescence



Cellular senescence is a cell state triggered by stressful insults and certain physiological processes

Senescent cells secrete a plethora of factors (SASP) including pro-inflammatory cytokines and chemokines, growth modulators, angiogenic factors, and matrix metalloproteinases (MMPs) (MAJOR PLAYER IN INFLAMMAGING)

Table 2. Senescence-Associated Secretory Phenotype (SASP) Components

Class	Component
Interleukins	IL-6; IL-7; IL-1; IL-1b; IL-13; IL-15
Chemokines	IL-8; GRO-a, -b, -g; MCP-2; MCP-4; MIP-1a; MIP-3a; HCC-4; eotaxin; eotaxin-3; TECK; ENA-78; I-309; I-TAC
Other inflammatory molecules	TGFβ; GM-CSE; G-CSE; IFN-γ; BLC; MIF
Growth factors; regulators	Amphiregulin; epiregulin; heregulin; EGF; bFGF; HGF; KGF (FGF7); VEGF; angiogenin; SCF; SDF-1; PIGF; NGF; IGFBP-2, -3, -4, -6, -7
Proteases and regulators	MMP-1, -3, -10, -12, -13, -14; TIMP-1; TIMP-2; PAI-1, -2; tPA; uPA; cathepsin B
Receptors; ligands	ICAM-1, -3; OPG; sTNFRI; sTNFRII; TRAIL-R3; Fas; uPAR; SGP130; EGF-R
Non-protein molecules	PGE2; nitric oxide; ROS
Insoluble factors	Fibronectin; collagens; laminin

Based on SenTraGorTM methodologies a guideline algorithmic approach for the detection of senescence is recommended (Cell 2019, Nat Protoc 2021)

Step 1

Screening for senescence

- SA-β-gal *and/or*
- Lipofuscin (SBB/GL13)

Step 2

Verification with additional markers *(co-staining)*

- $p16^{INK4A}$
- $p21^{WAF1/Cip1}$
- Proliferation markers
- Lamin B1

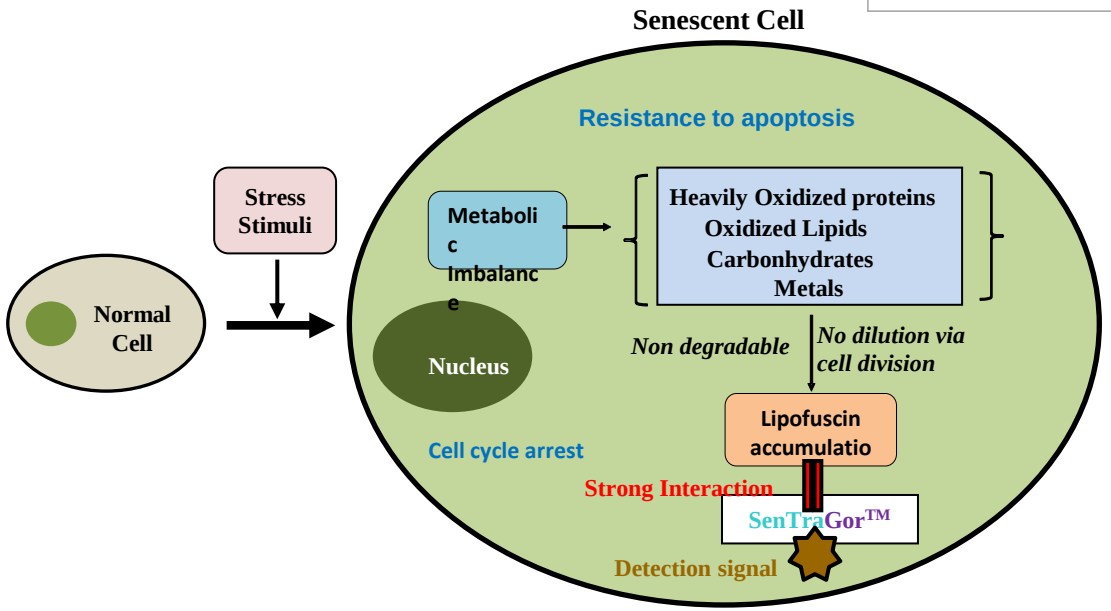
Step 3

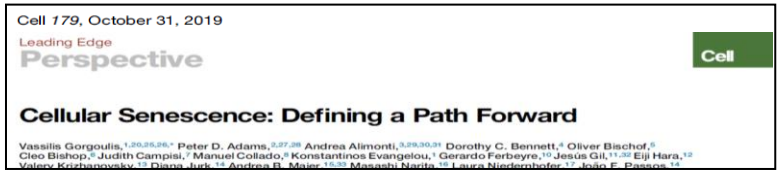
Other markers related to specific types of senescence

- Based on the anticipated type of senescence

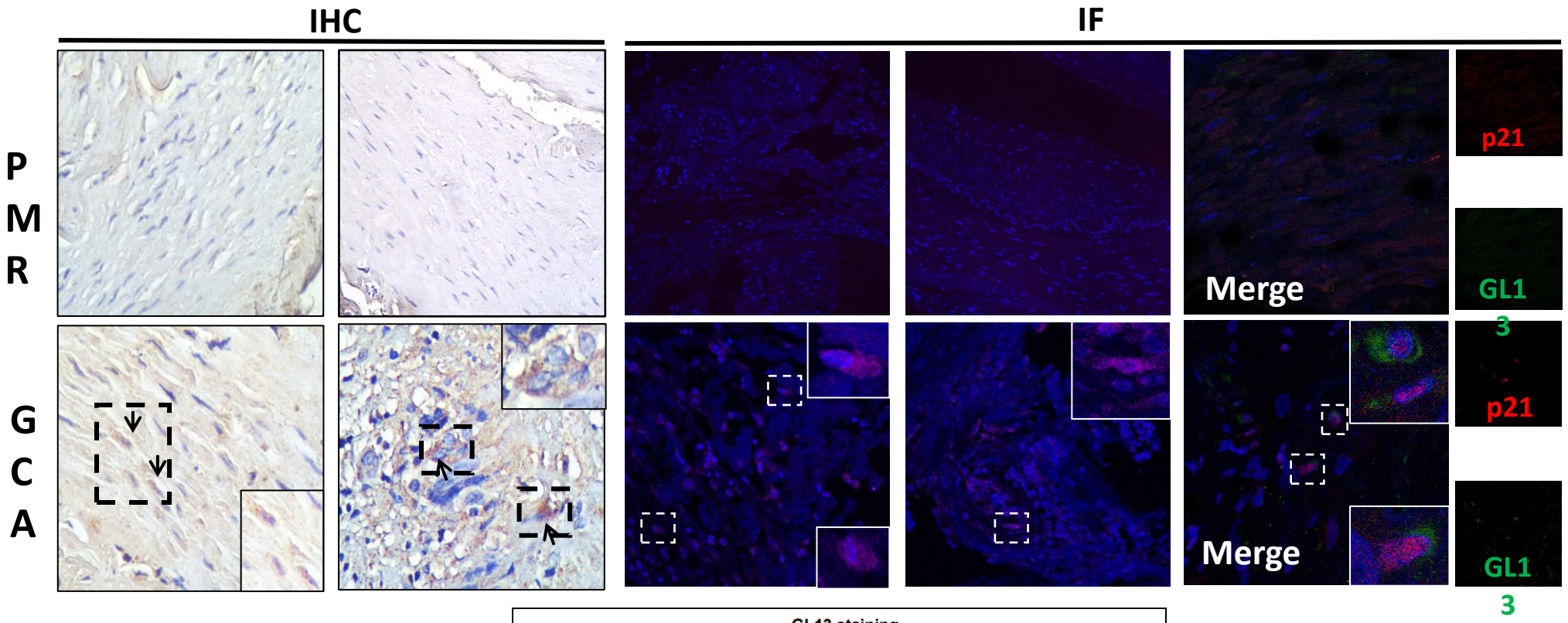
Senescence detection

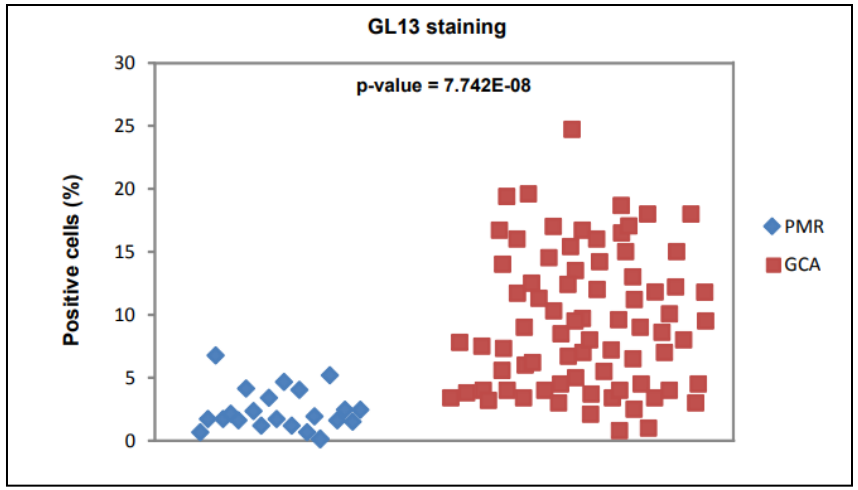
Lipofuscin accumulation is the most reliable marker of cellular senescence detection





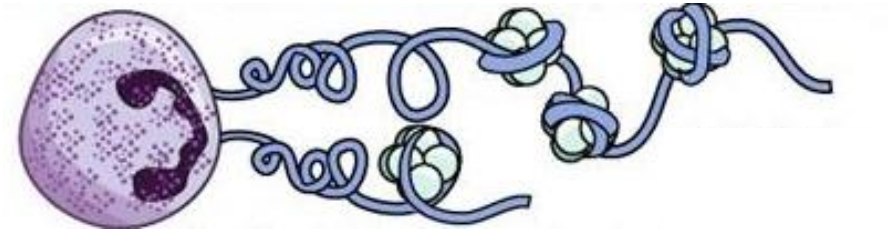
Cellular senescence detection on PMR and GCA tissues



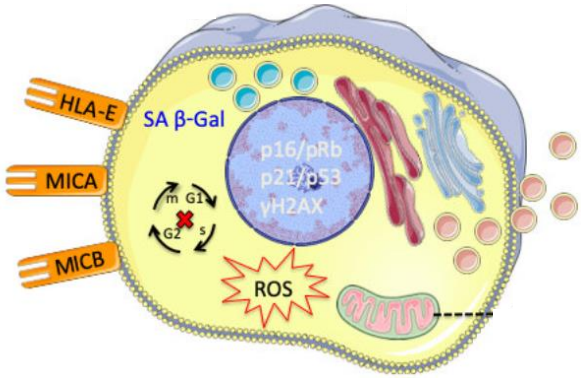


Veroutis et al. Manuscript in preparation

NETs



Senescent cells



Turnover time/Clearance from tissue is unknown. They have been found in fibrotic lesions in NSIP several months after inflammatory phase.

They remain in tissues for prolonged periods past inflammatory phase.

They carry various inflammatory cytokines in a disease specific manner, such as IL-1β, IL-6, IL-17.

They acquire SASP producing IL-1β, IL-6, matrix metalloproteinases etc.

They are involved in tissue remodeling

They are involved in tissue remodeling

Senescent cells can attract neutrophils and produce NETs through SASP, resulting in tissue remodeling

Summary

- GCA is a prototype disease to study acute and chronic autoinflammatory responses
- The predominant cells appear to be the activated macrophage-monocytes
- Predominant pathogenetic mechanisms involved those of the innate immunity arm
- The inflammaging through long permanent inflammatory structures seems to play a role in both relapses of the disease and remodeling

Future directions

- Multidimensional approaches with a variety of data in both active and inactive forms of the disease are anticipated to provide:

  A) new disease relevant biomarkers

  B) stratify patients more precisely according to the endotype of the disease

  C) disclose new targets for treatment

Acknowledgments

Vascular inflammation group (Pathophysiology – Histology)

Principal investigator: Athanasios G Tzioufas
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- Anagnostopoulos K.

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