National and
Kapodistrian University
of Athens, Greece

Ο ΧΑΡΑΚΤΗΡΙΣΜΟΣ ΤΩΝ ΓΗΡΑΣΜΕΝΩΝ ΚΥΤΤΑΡΩΝ ΣΕ ΚΡΟΤΑΦΙΚΕΣ ΑΡΤΗΡΙΕΣ ΑΣΘΕΝΩΝ ΜΕ ΓΙΓΑΝΤΟΚΥΤΤΑΡΙΚΗ ΑΡΤΗΡΙΤΙΔΑ ΑΠΟΚΑΛΥΠΤΕΙ ΦΛΕΓΜΟΝΩΔΗ ΦΑΙΝΟΤΥΠΟ ΚΑΙ ΙΣΧΥΡΗ ΣΥΣΧΕΤΙΣΗ ΜΕ ΤΗΝ ΙΝΤΕΡΛΕΥΚΙΝΗ-6

Dept. of
Pathophysiology
School of Medicine

Ουρανία Αργυροπούλου

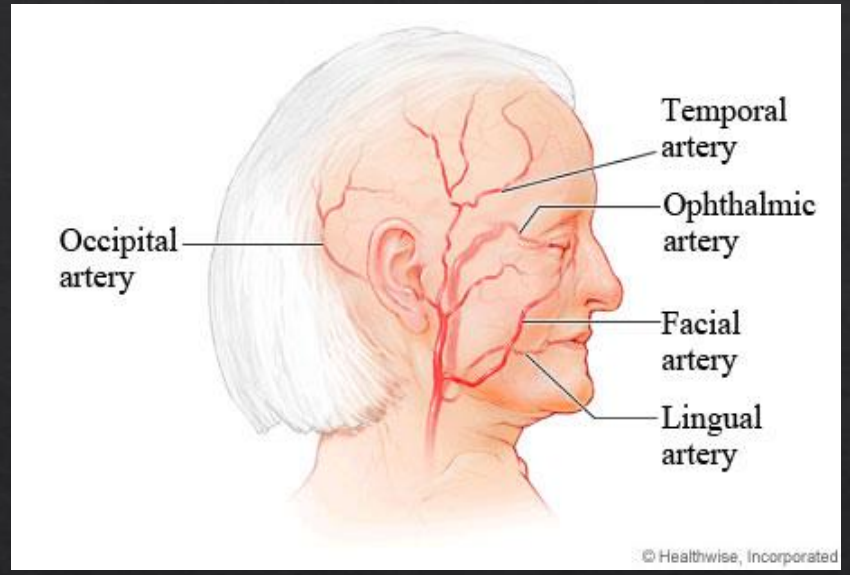
Ρευματολόγος

Ακαδημαϊκός υπότροφος ΕΚΠΑ, Παθολογική Φυσιολογία, Γ.Ν.Α ΛΑΙΚΟ

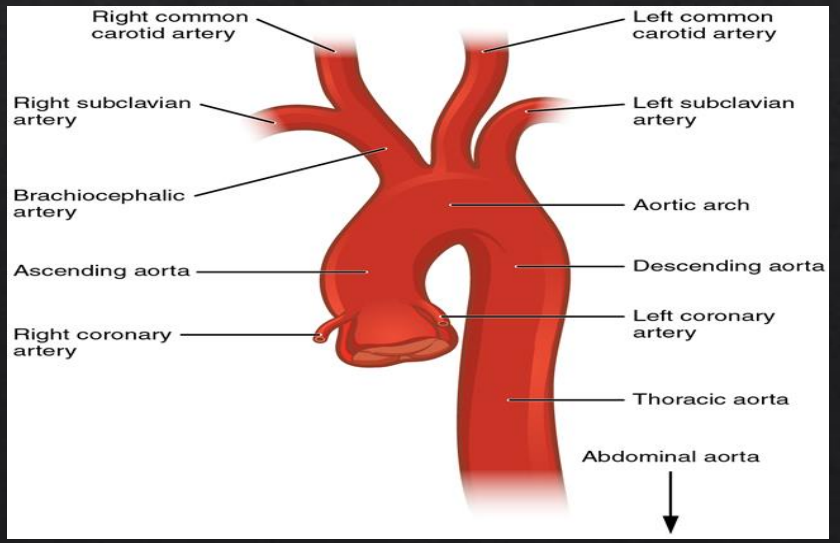
Sharma A et al. Seminars in Arthritis & Rheumatism 2020
Sammel AM et al. Arthritis Rheumatol 2019

Giant cell arteritis (GCA)

- Most prevalent form of systemic vasculitis >50 years
- White race
- 3-4 ♀ : 1 ♂
- The incidence increases with age (7^{th}-8^{th} decade >> 5^{th}-6^{th} decade)
- Annual incidence in Scandinavia & UK 14.6-43.6/100000, USA 19.8/100000, South Europe 1.1-11.1/100000



- Granulomatous inflammation of the inner elastic lamina of medium and large vessels:
  - Extra-cranial branches of carotid arteries
  - Aorta and its major branches (15-20% clinical & 50-80% PET/CT).



KSM van der Geest et al. Arthritis & Rheumatology 2018
Dejaco C et al . Rheumatology (Oxford) 2016
Salvarani C et al. Lancet 2008

✓ Multi-level HETEROGENEITY

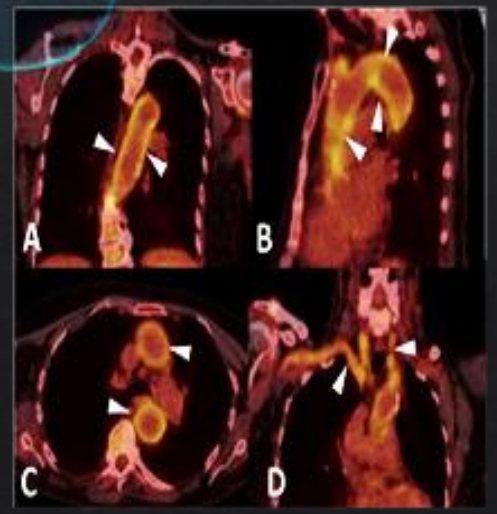
15%

Systemic inflammation:

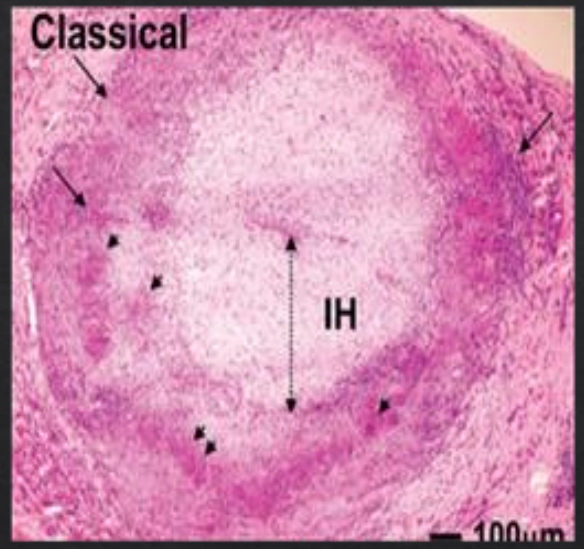
- Malaise
- Weight loss
- Night sweats
- Fever

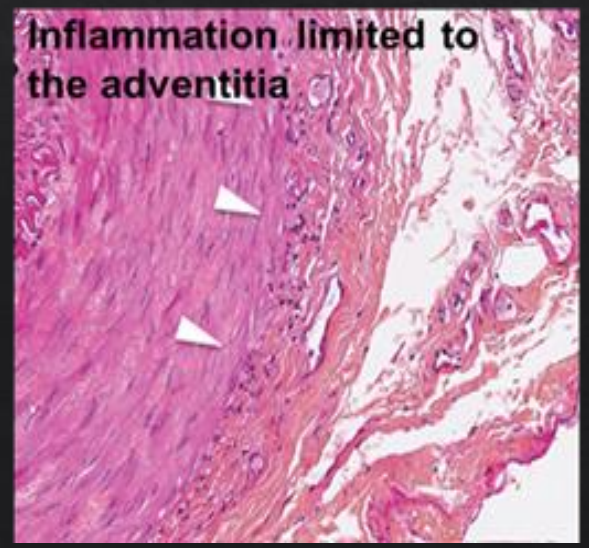
40%

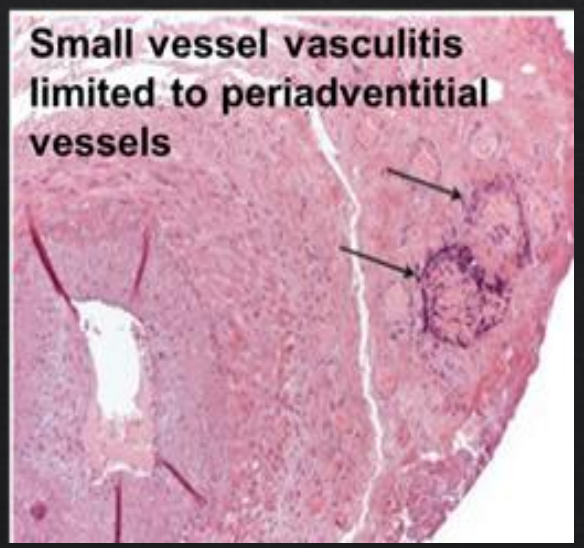
15-20%



20%







GCA: Pathogenesis

Álvarez-Lafuente R et al. Ann Rheum Dis 2005
Carmona FD et al. Expert Rev. Clin. Immunol. 2015
Gonzalez-Gay MA et al. Medicine 2000
Gonzalez-Gay MA et al. Ann Rheum Dis 2000
Carmona FD et al. Rheumatology (Oxford) 2014
Carmona FD et al. Am J Hum Genet 2015
Carmona FD et al. Am J Hum Genet 2017
Prieto-Pena D et al.Seminars in Arhritis & Rheumatism 2020

Elderly

* Impaired immune response
* Changes in the vascular microenvironment

HLA-DRB1*04:

* Resistance to Corticosteroids
* Severe disease with ischemic complications

Non-HLA genes:

* Cytokines and their receptors (e.g.IL-18, IL-10, IL-6, IL17A, IFGN, IL12RB2
* Endothelial function (e.g.ICAM-1, VEGF, MMP9, NOS2A)
* Innate immunity (e.g.TLR4, FCGR2A/3A, NLRP1)
* PTPN22, LRRC32, REL (Immunochip platform)
* PLG, P4HA2 (GWAS platform)

- ❑ The functional TYK2 variant rs34536443 (P1104A): a new potential protective factor against GCA (L. Ortiz-Fernández et al. Clin Exp Rheumatol 2020).

Giant cell arteritis

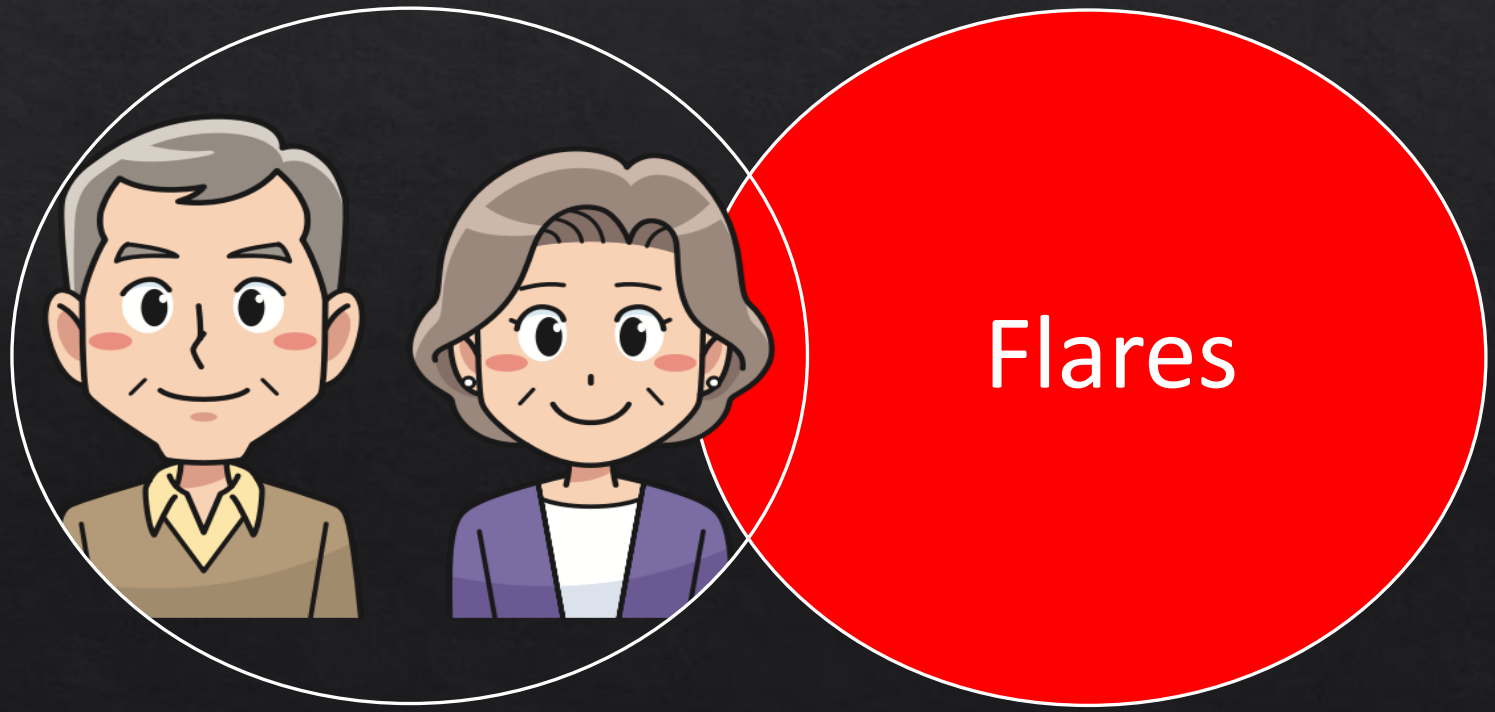
Sammel AM et al. Arthritis Rheumatol 2019
Mohan SV et al. Arthritis Res Ther 2011
Gonzalez –Gay MA et al. Arthritis Rheum 2009
Larsson K et al. Ann Rheum Dis 2006

VASCULAR AGEING:

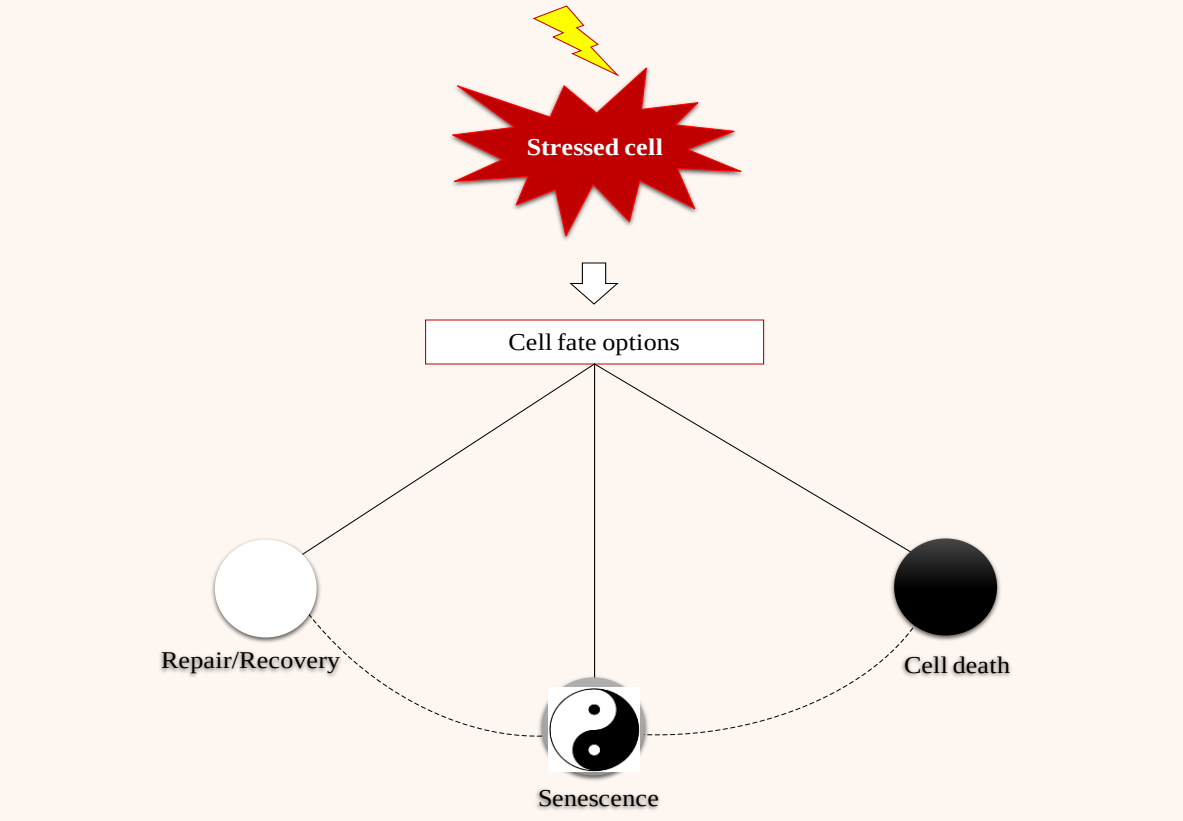
- reactive oxygen species (ROS) mitochondrial dysfunction
- cellular senescence,
- genomic instability,
- increased apoptosis,
- epigenetic alterations
- clonal hematopoiesis of indeterminate potential (CHIP)

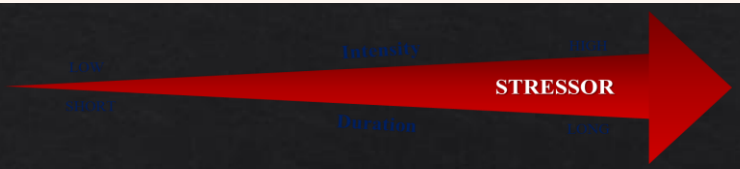
Flares:

- Glucocorticosteroids (34-75%)
- DMARDs (methotrexate: 50%)
- Anti-IL6R (Tocilizumab) (25%)

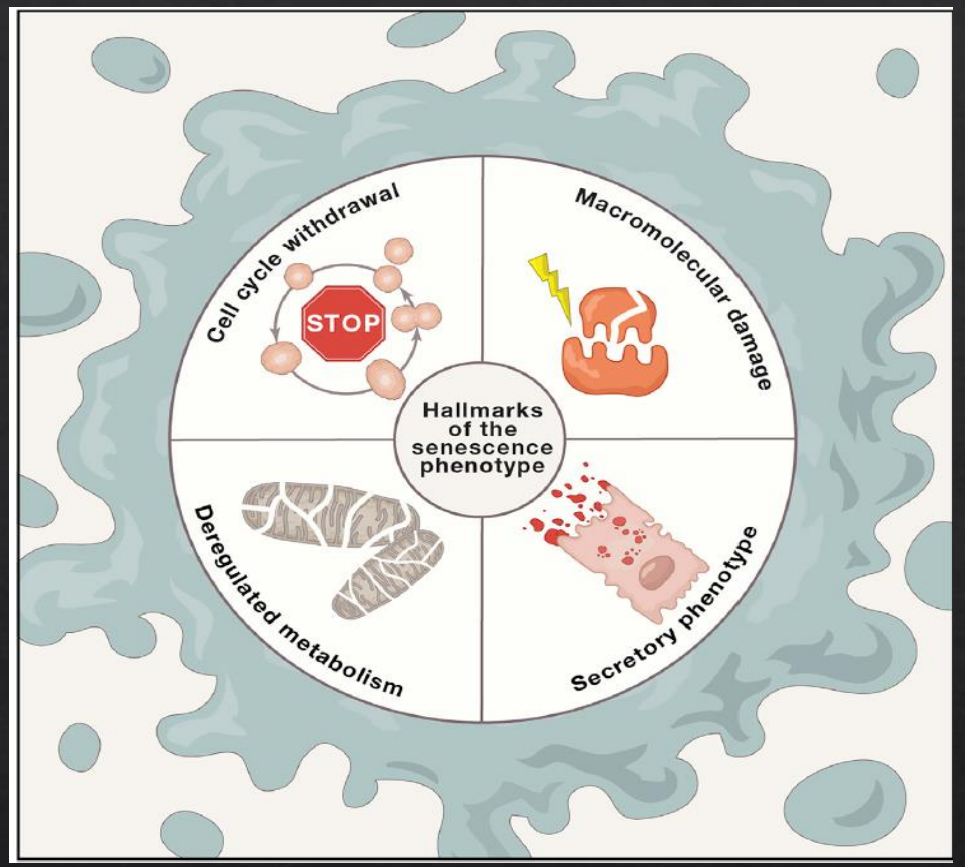


Cellular Senescence: a stress response mechanism that preserves cellular homeostasis





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



Senescent cells are characterized by:

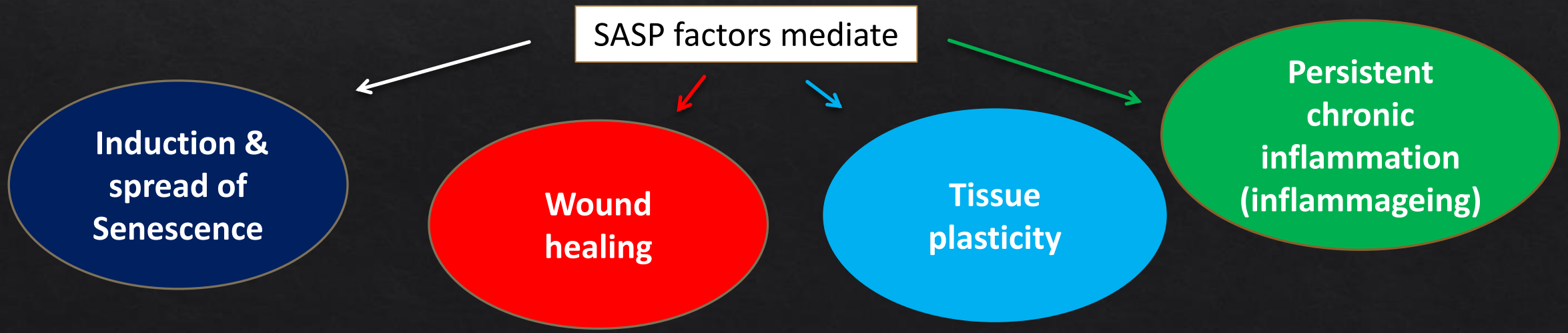
1. **Prolonged and generally irreversible cell-cycle arrest**
2. **Macromolecular damage**
3. **Altered metabolism**
4. **Secretory features (SASP)**

Senescence Associated Secretory Phenotype (SASP)

- Senescent cells secrete a plethora of factors, including pro-inflammatory cytokines and chemokines, growth modulators, angiogenic factors, and matrix metalloproteinases (MMPs), collectively termed the Senescent Associated Secretory Phenotype (SASP)

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

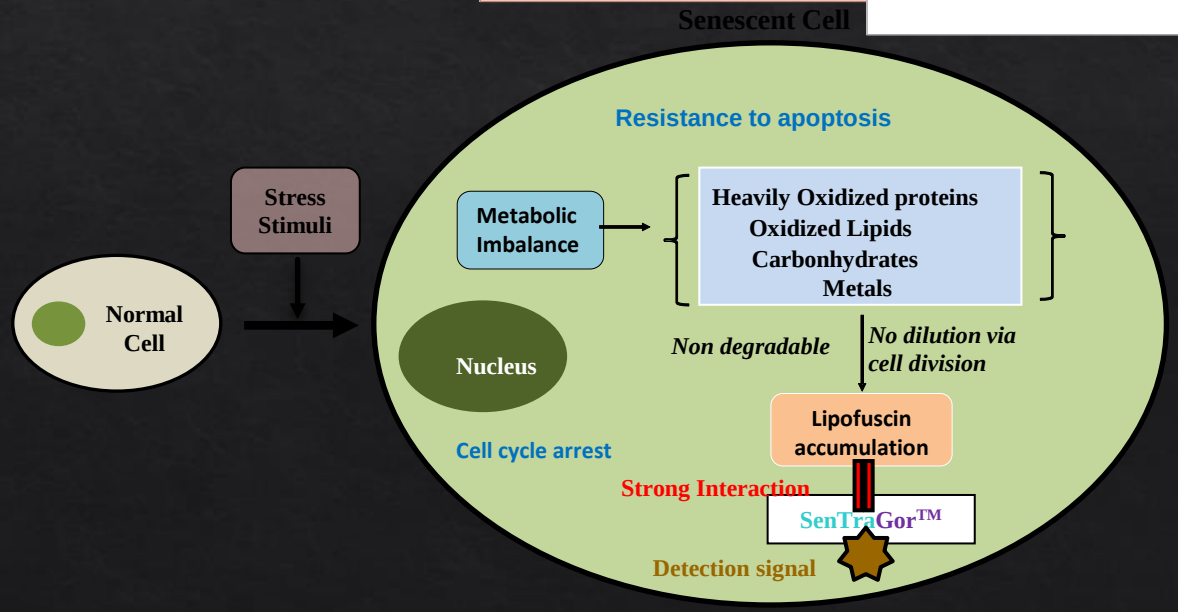
Step 3

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



Ο ΧΑΡΑΚΤΗΡΙΣΜΟΣ ΤΩΝ ΓΗΡΑΣΜΕΝΩΝ ΚΥΤΤΑΡΩΝ ΣΕ ΚΡΟΤΑΦΙΚΕΣ ΑΡΤΗΡΙΕΣ ΑΣΘΕΝΩΝ ΜΕ ΓΙΓΑΝΤΟΚΥΤΤΑΡΙΚΗ ΑΡΤΗΡΙΤΙΔΑ ΑΠΟΚΑΛΥΠΤΕΙ ΦΛΕΓΜΟΝΩΔΗ ΦΑΙΝΟΤΥΠΟ ΚΑΙ ΙΣΧΥΡΗ ΣΥΣΧΕΤΙΣΗ ΜΕ ΤΗΝ ΙΝΤΕΡΛΕΥΚΙΝΗ-6.

Βερούτης Δ.1, Αργυροπούλου Ο.2, Γουλές Α.2, Καμπάς Κ.3, Παλαμηδάς ΔΑ.2, Ευαγγέλου Κ.1, Χαβάκη Σ.1, Πολύζου Α.1, Χίντζη Ε.4, Καρατζά Ε.5, Μποκή Κ.6, Cavazza A.7, Κίττας Χ.1,8, Θάνος Δ.9, Ricordi C.10, Marvisi C.10, Muratore F.10, Galli E.10, Croci S.11, Salvarani C.10, Γοργούλης Β.1,13,14,15, Τζιούφας Α.2,12

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AIMS OF THE STUDY

1. Detection of cellular senescence level in GCA TAB compared to PMR using GL13 (SenTraGor) reagent and senescence detecting algorithm.

2. Analysis of senescent cell subpopulations in GCA biopsies compared to PMR by applying specific markers for its cell population.

3. Analysis of SASP components (IL-6, MMP-9) in GCA biopsies compared to PMR per different senescent subpopulations.

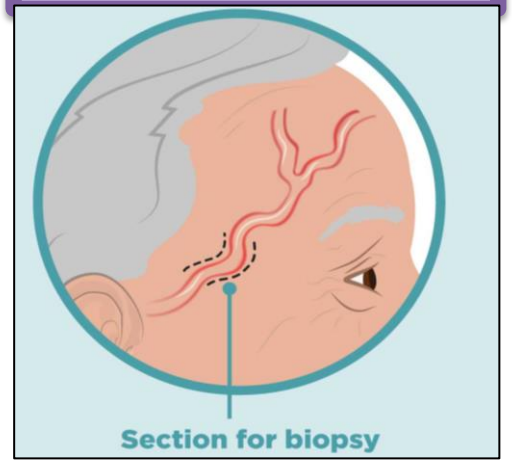
Biomarker	Populations
Vimentin	Fibroblasts
CD34	Endothelial cells
CD68	Macrophages
aSMA	Smooth Muscle cells

Patients and Methods

- 75 patients with GCA and 22 with PMR who were diagnosed and followed up in 3 Rheumatology centers from Greece and Italy (University of Athens, Sismanoglio General Hospital, Reggio Emilia Hospital) between 2009 and 2022, were retrospectively included in the study.
- All participants underwent temporal artery biopsy as part of standard of care and fulfilled the international classification criteria for each disease.
- The extents of vascular involvement in GCA patients as well as the absence of underlying subclinical vasculitis in PMR patients were confirmed by 18FDG PET/CT.
- The study was approved by the local ethical committees of all the involved Institutions (1718016656), after obtaining patients' informed consent and in compliance with the general data protection regulations (GDPR) of the European Union and Declaration of Helsinki.

Experimental procedure: 1ST STEP

PMR / GCA tissue biopsy



Fresh material

FFPE material

Transmission electron microscopy (TEM)

Immunohistochemistry (IHC)
Immunofluorescence (IF)

Detection of senescence

Detection of senescent cell types

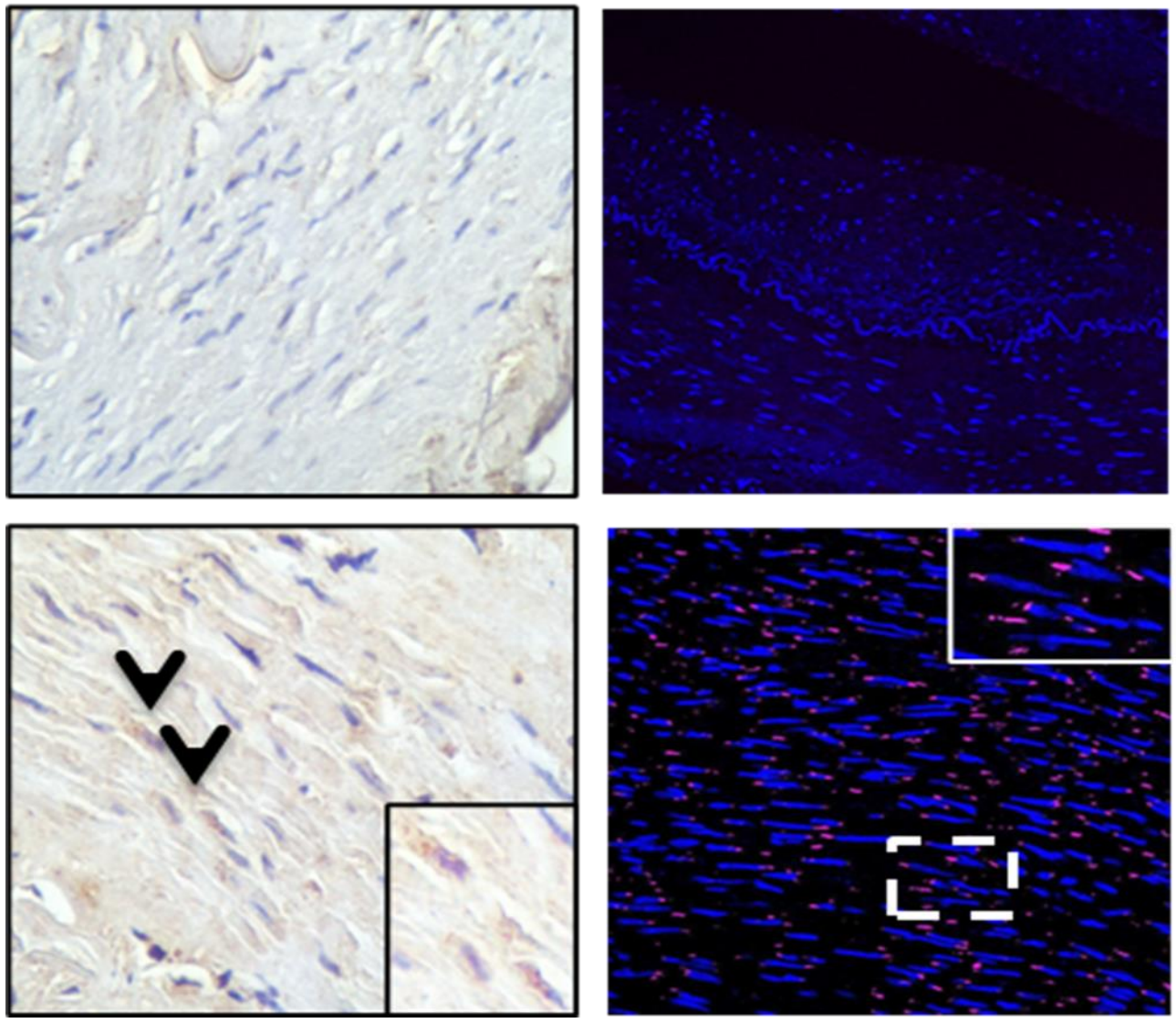
Detection of SASP components

Detection of cellular senescence on GCA and PMR artery biopsies (I)

GL13 STAINING

P
M
R

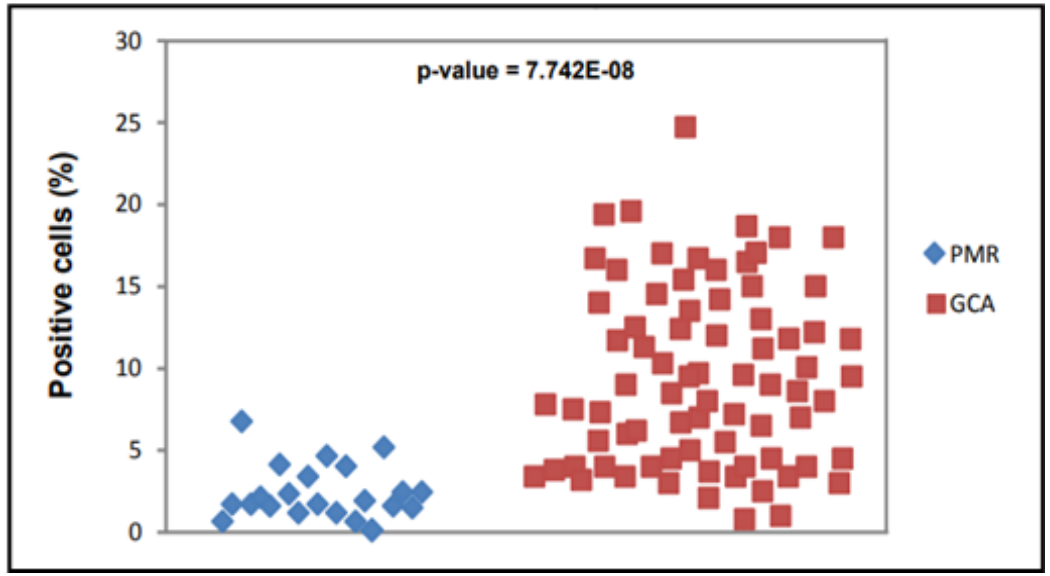
G
C
A



- GCA vs PMR: 0.8-28 % vs 0-6.8%

Mean proportion 9.5% vs 2.66%

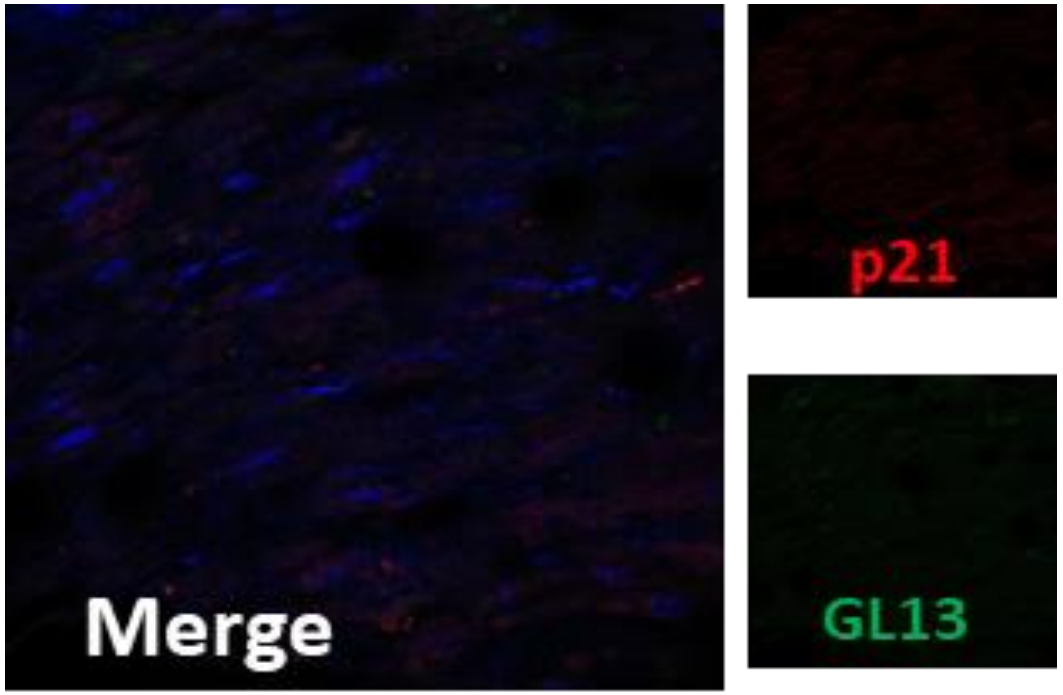
GL13 staining on GCA and PMR artery tissues



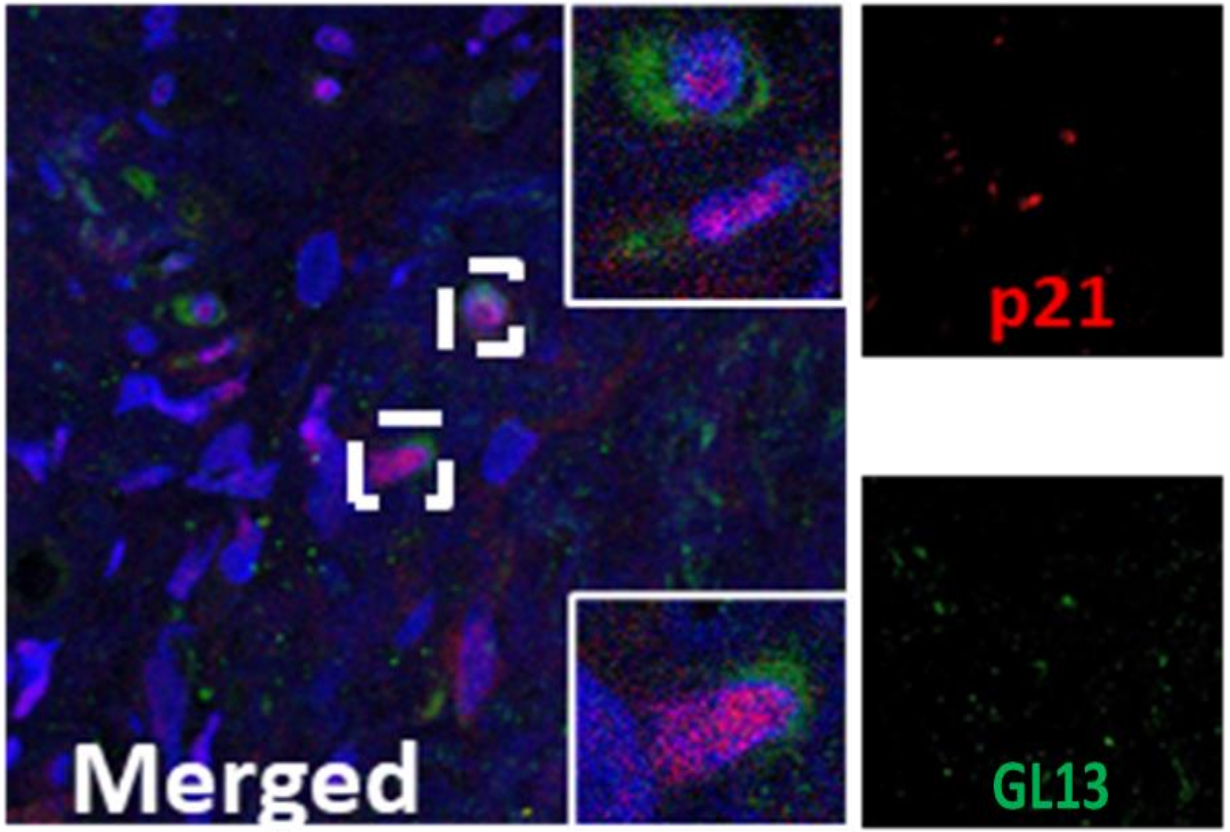
Detection of cellular senescence on GCA and PMR artery biopsies (I)

GL13-p21 co-staining

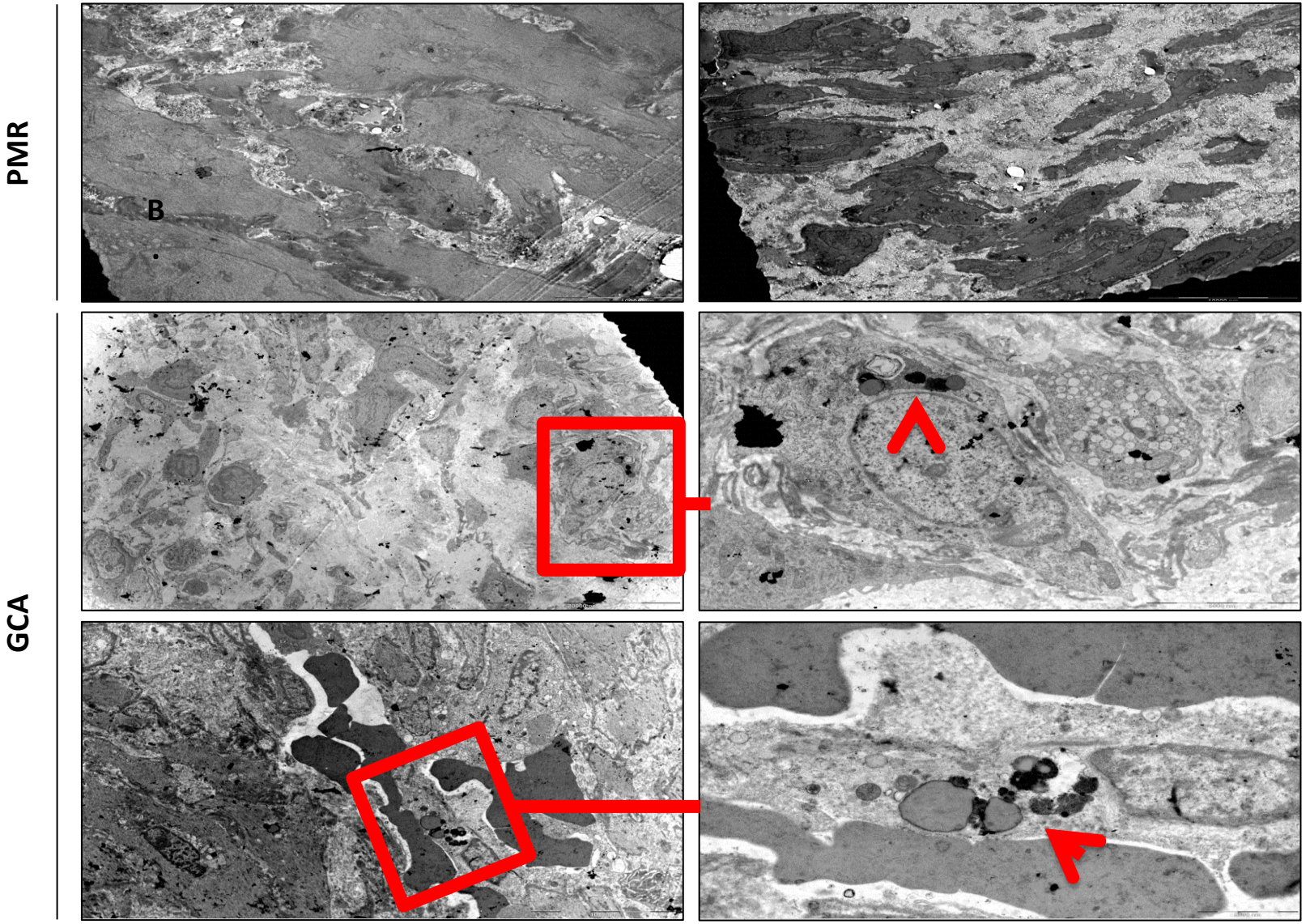
PMR



GCA

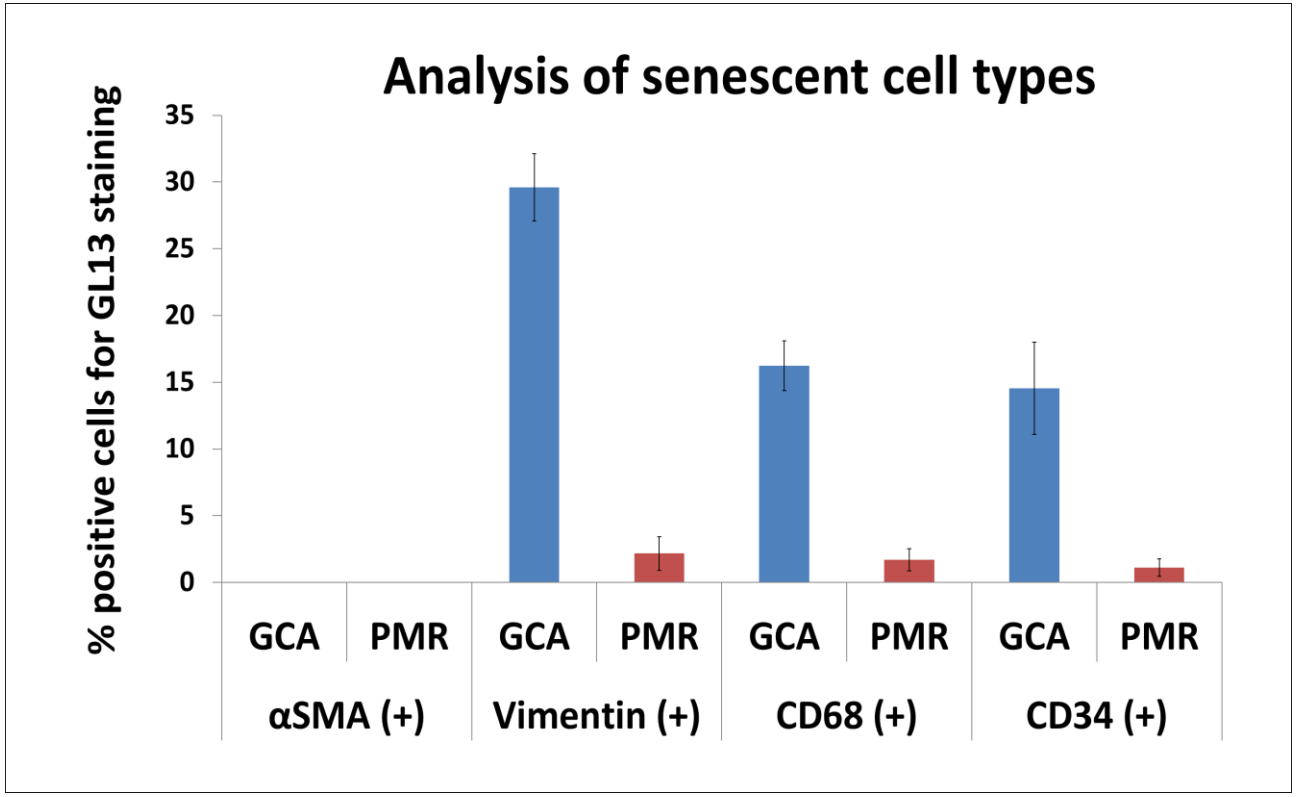


Detection of cellular senescence on GCA and PMR artery biopsies (I)



Detection of Cellular Senescence on PMR (A) and GCA (B) artery tissues by Transmission electron microscopy (TEM). Red arrows indicate the accumulation of Lipofuscin in Fibroblasts.

Analysis of senescent cell types (II)

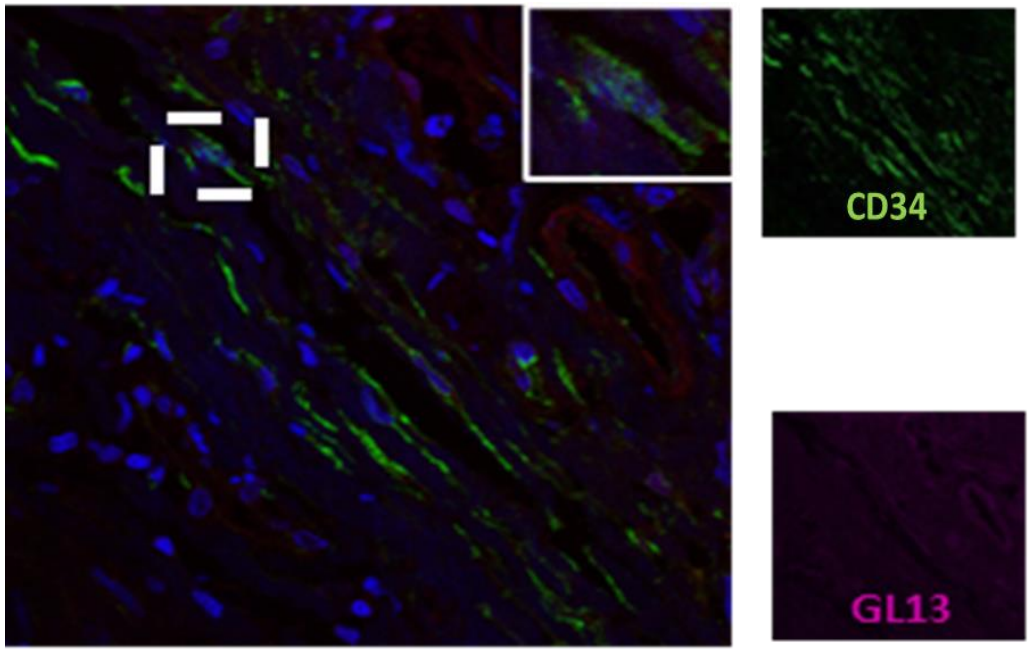


- **GCA vs PMR:**
  - Vimentin (+) (29.6% vs 2.2%),
  - CD68 (+) (16.23% vs 1.7%) and
  - CD34 (+) (14.3% vs 1.1%)

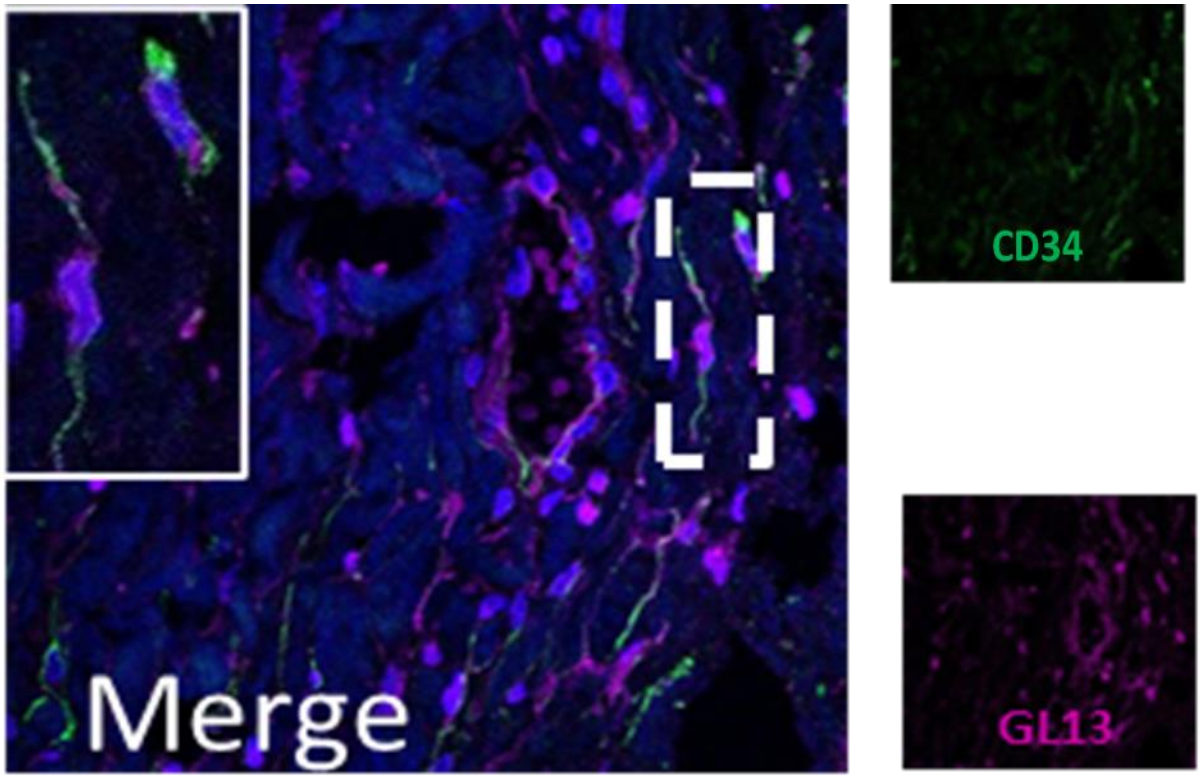
Biomarker	Populations
Vimentin	Fibroblasts
CD34	Endothelial cells
CD68	Macrophages
aSMA	Smooth Muscle cells

Analysis of senescent cell types (II)

PMR

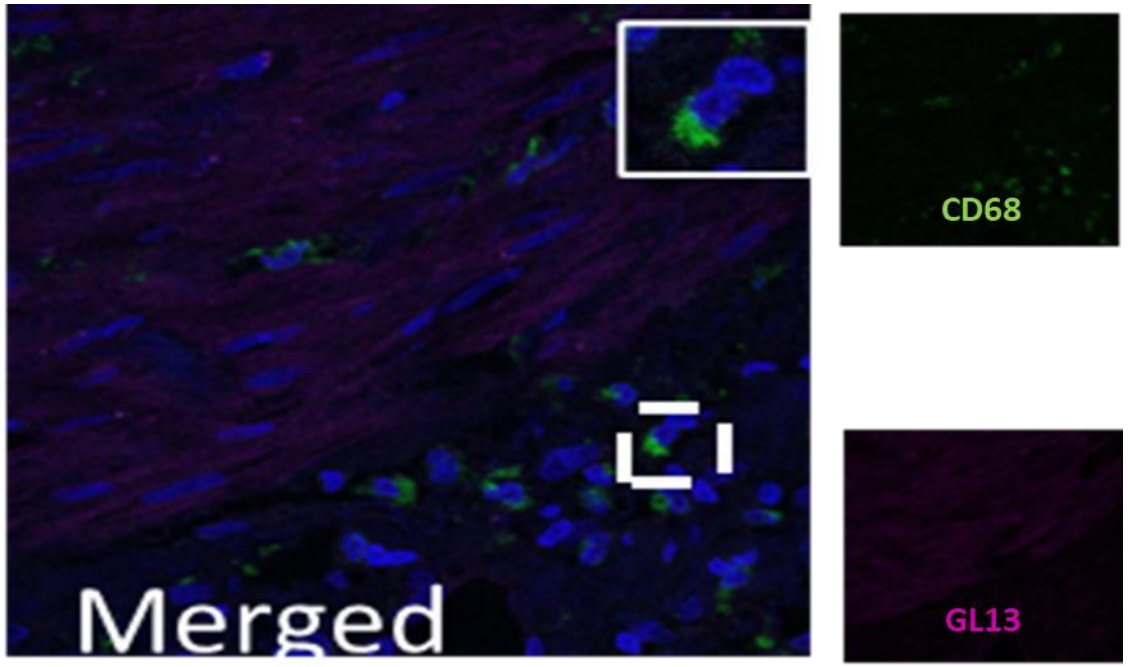


GCA

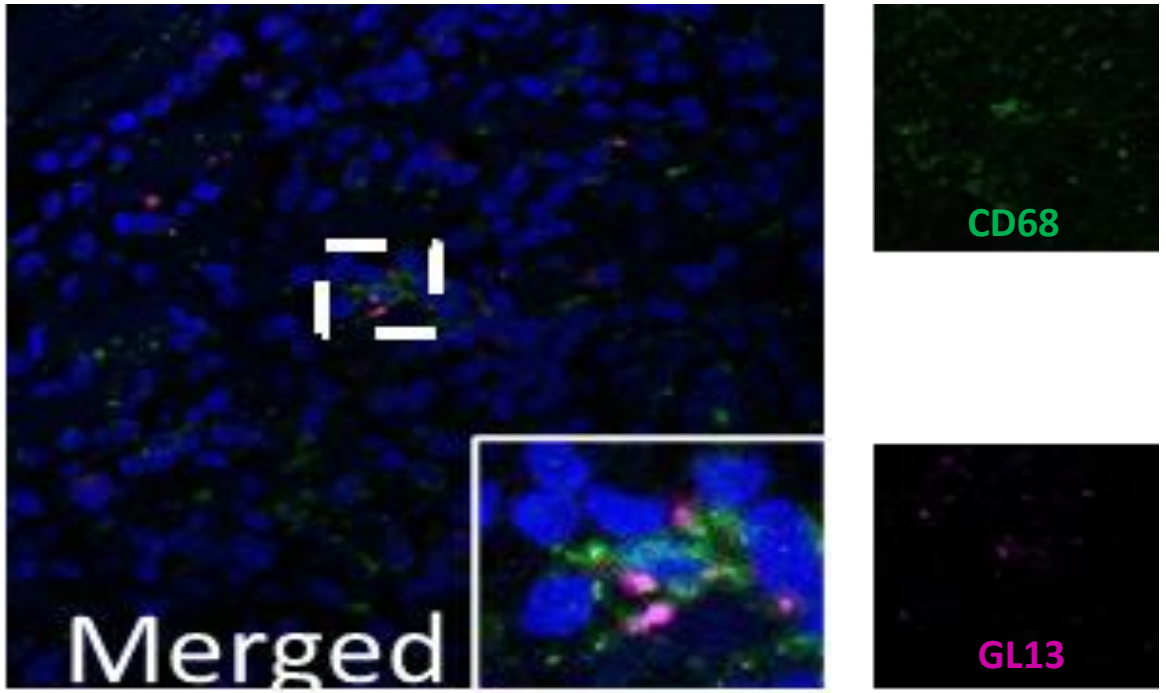


Analysis of senescent cell types (II)

PMR

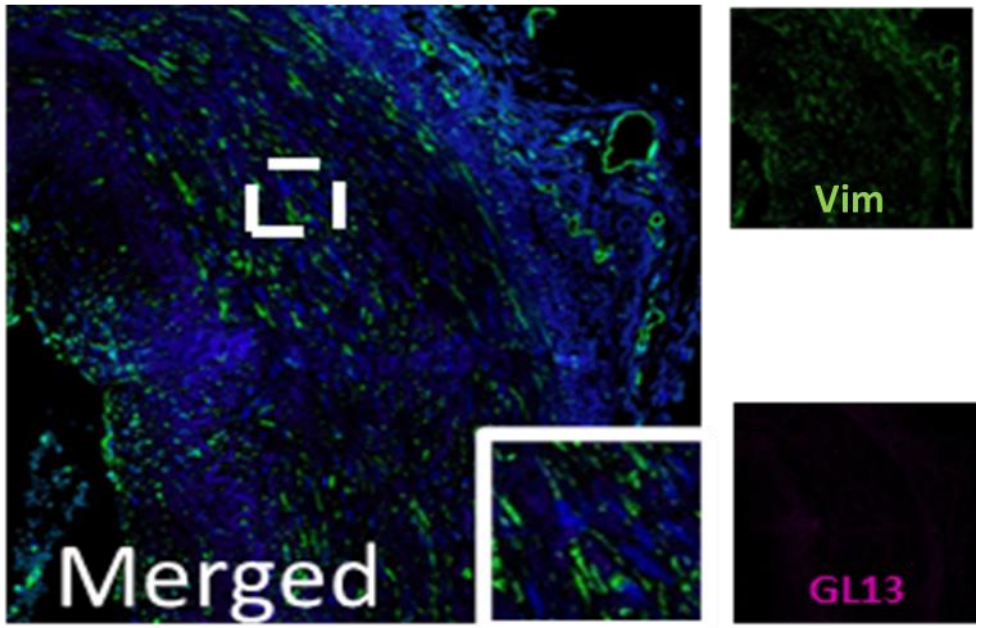


GCA

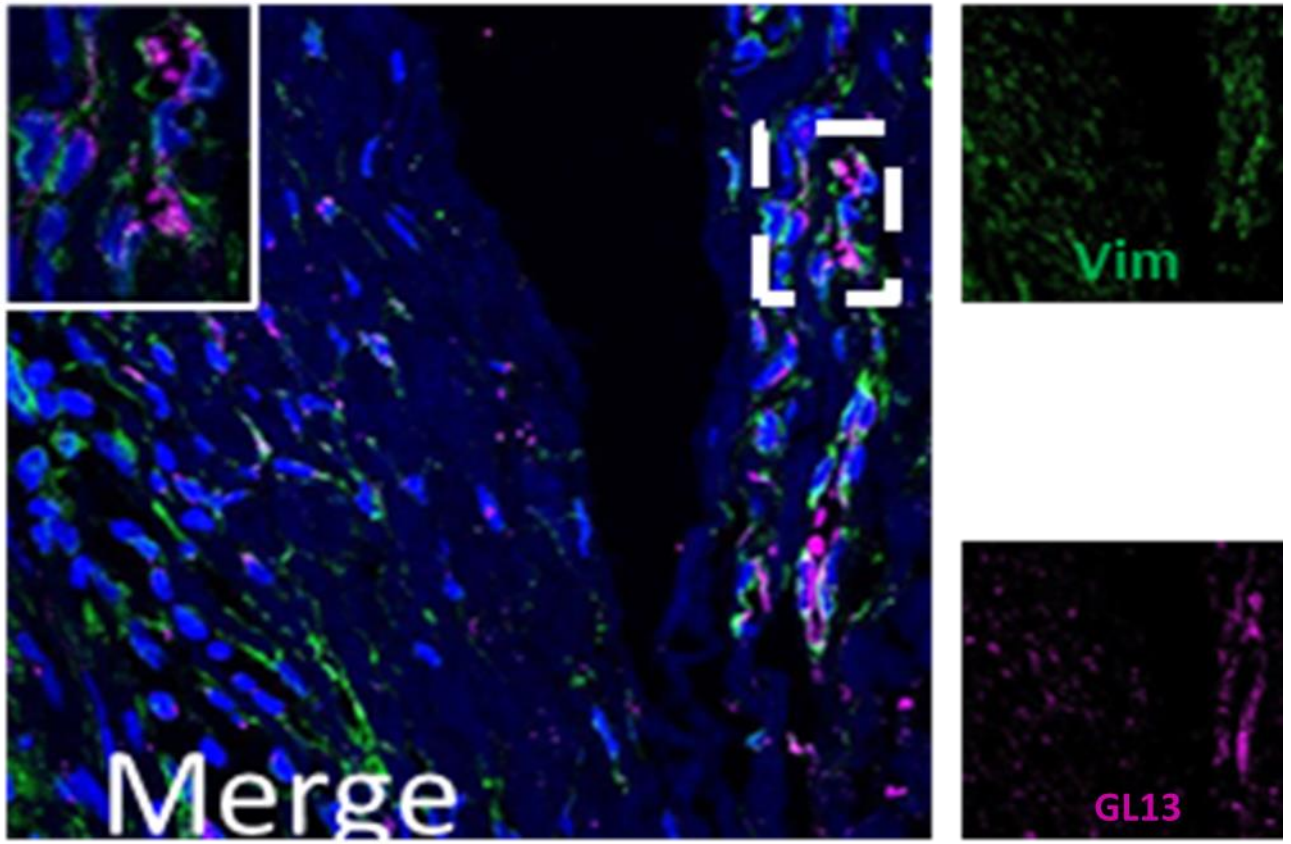


Analysis of senescent cell types (II)

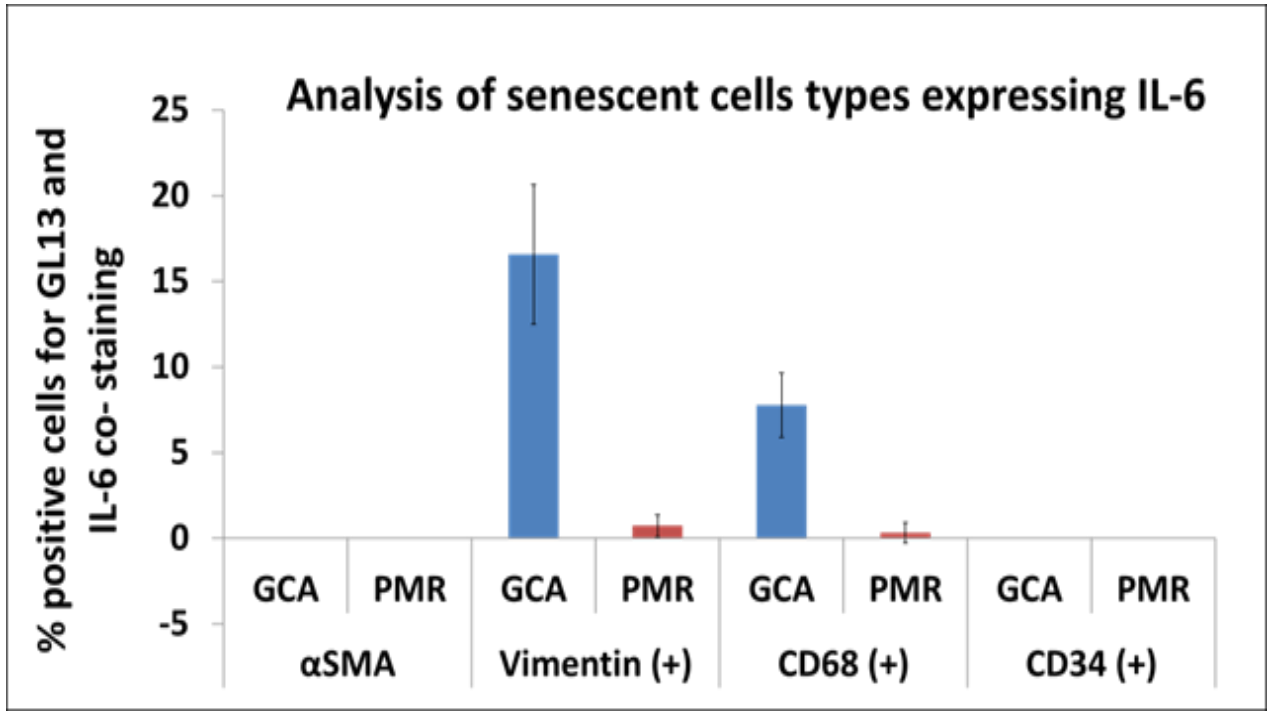
PMR



GCA



Analysis of senescent cell types expressing SASP components (III)



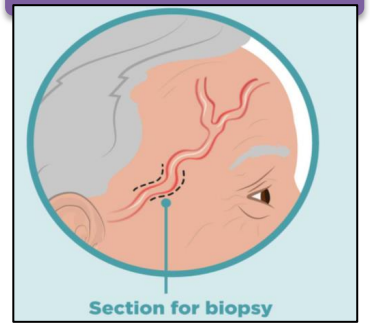
- **GCA vs PMR:**
  - GL13/Vimentin+/IL-6+ : 21.9 vs 0.7%
  - GL13/Vimentin+/MMP9+ : 13.43 vs 1.4%

- GL13/CD68+/IL-6+ : 7.76 vs 0.33

- **GCA Trasmural (n=68) vs limited (n=7):**
  - GL13/IL-6 double positive cells were more prominent in GCA patients with extended disease compared to those with limited disease (10.02% vs 4.37%, $p<0.0001$)

Experimental procedure: 2ST STEP

PMR / GCA tissue biopsy



Fresh material

FFPE material

Transmission electron microscopy (TEM)

Immunohistochemistry (IHC)
Immunofluorescence (IF)

Detection of senescence

Detection of senescent cell types

Detection of SASP components

Blocking of IL-6 receptor or IL-1β receptor
(2h pre-treatment)

Tissue culturing (24h)

Supernatant collection (24h)

5 days treatment

Primary skin fibroblast

GCA and PMR patients

Serum collection

5 days treatment

Primary skin fibroblast

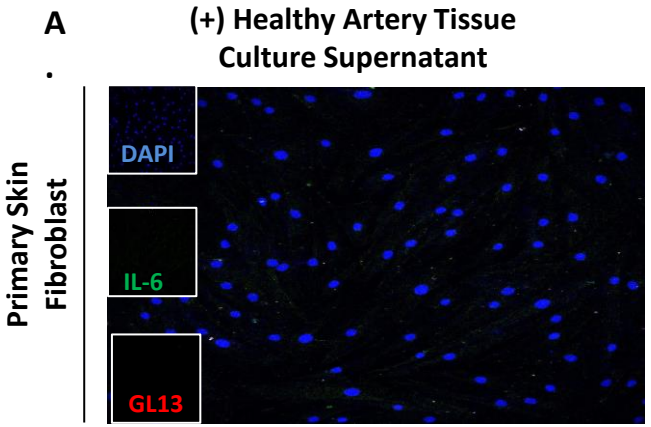
Immunofluorescence

Confocal Microscopy

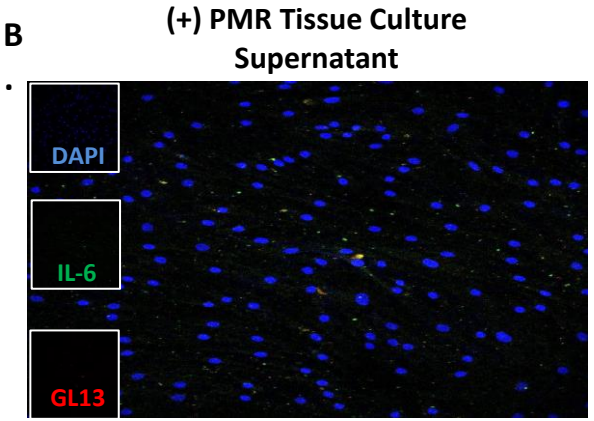
Detection of
- Senescence (GL13)
- SASP (IL-6)

Induction of cellular senescence in primary fibroblasts by GCA tissue culture supernatant (IV)

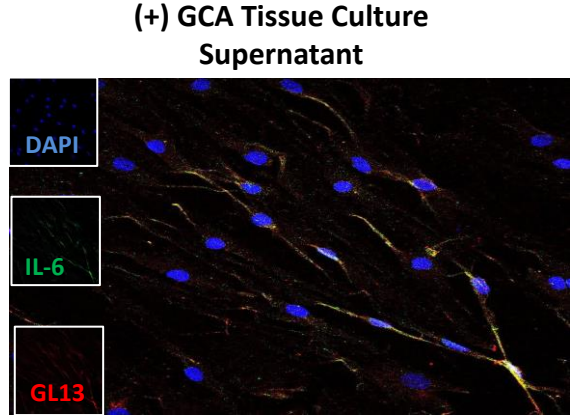
A.



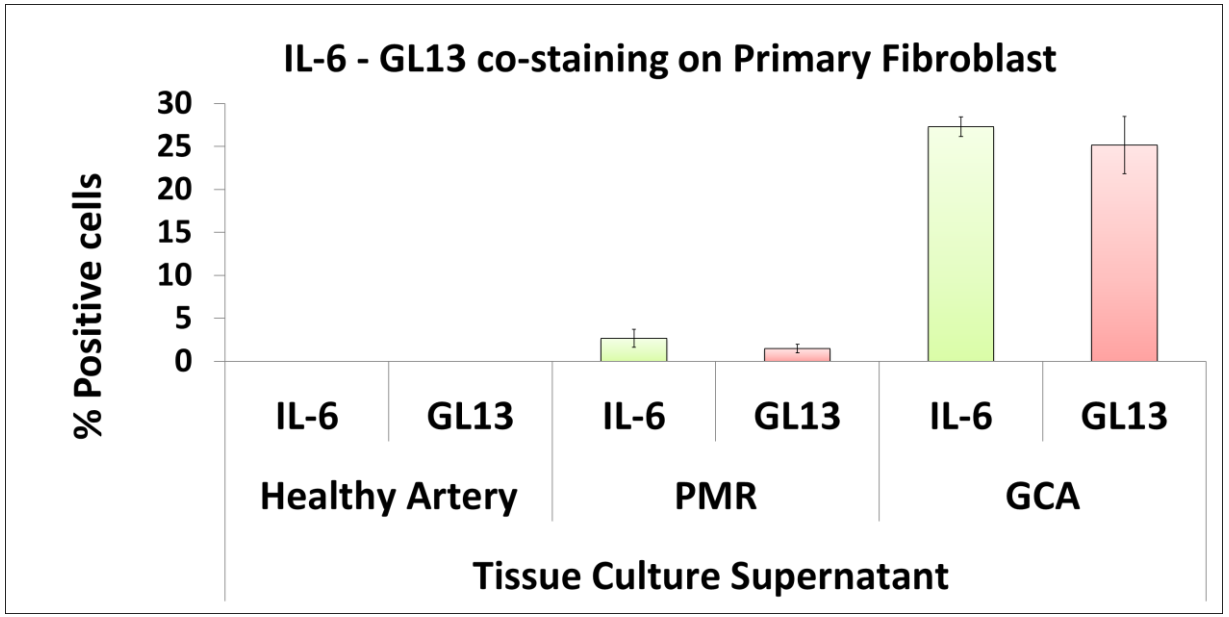
B.



C.

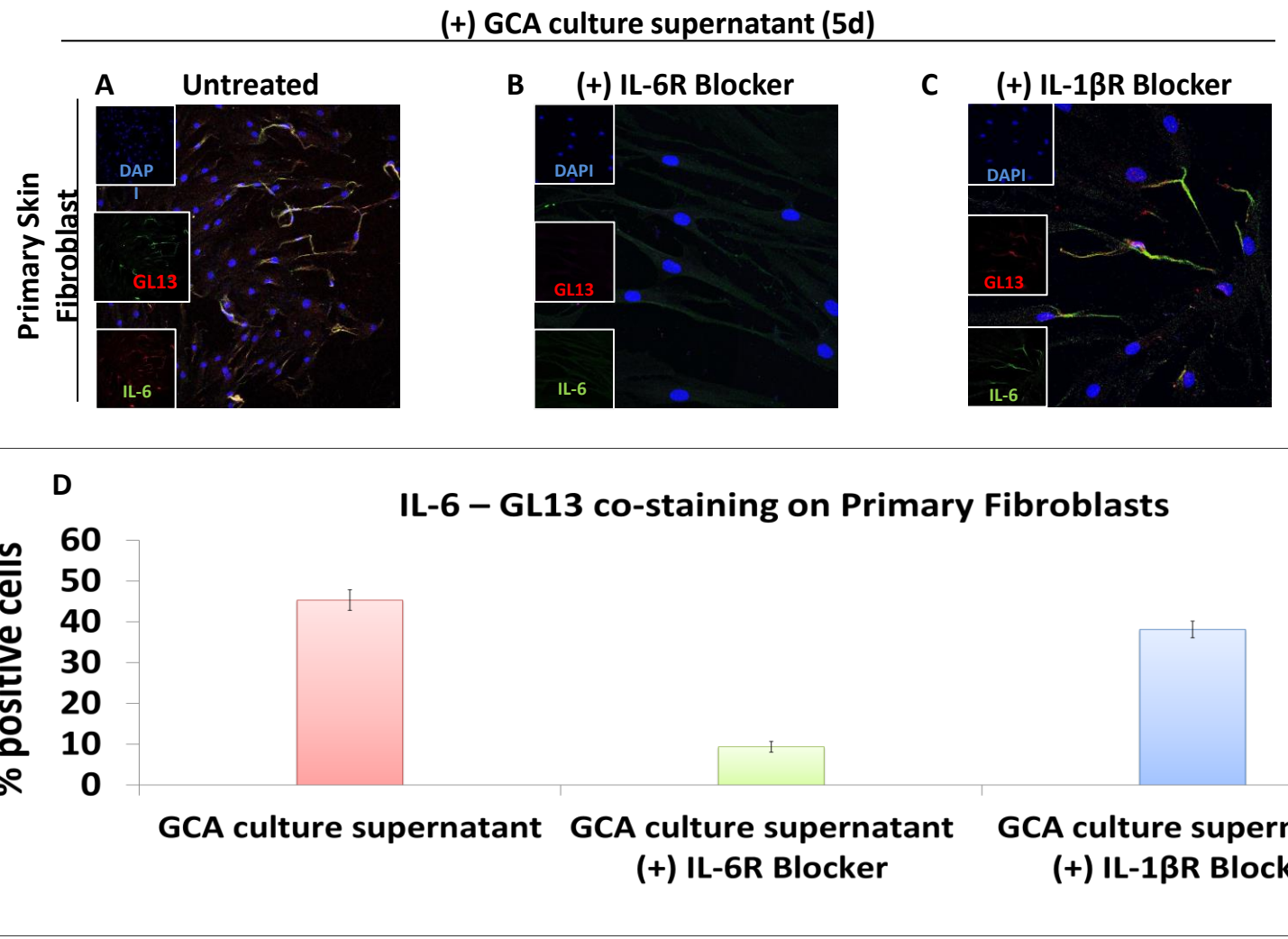


D.



- 24h temporal artery culture supernatant from GCA and PMR had comparable levels of IL-6.
- The proportion of double positive GL13/IL-6 fibroblasts after treatment with GCA supernatants was significantly higher vs PMR supernatants (25.17% vs 1.50%, $p<0.0001$).

Inhibition of cellular senescence in primary fibroblasts by (24h) GCA tissue culture supernatant via IL-6 Receptor blocking (V)



Cells were pre-treated with IL-6 receptor inhibitor or IL-1β receptor inhibitor for 2 hours and then tissue culture supernatant were applied for 5 days. Representative images from IL-6 – GL13 double immunofluorescence staining on Primary Fibroblasts treated only with GCA tissue culture supernatant (A) and in combination with IL-6R Blocker (B) or IL-1βR blocker (C). Quantification analysis of results showed colocalization and increased levels of IL-6 production and cellular senescence on GCA supernatant only treated cells compared to IL-6R Blocker or IL-1βR blocker treated cells (D).

Conclusions

- Senescent cells are present in GCA arteries and display an IL-6/MMP-9 SASP
- IL6+ senescent cells follow the extension and bulk of the inflammatory infiltrate and can be induced by soluble mediators of the microenvironment of GCA arteries in an IL-6 dependent way, through an IL-6 centered positive loop.
- Their detection in tissue biopsy may serve as a potential tissue biomarker for disease severity, response to treatment and prognosis of serious complications.
- In addition, the implication of cellular senescence in GCA pathogenesis and the IL-6 dependent vicious cycle of inflammation, sets reasonable questions on the efficacy of senolytic treatment as a complementary strategy in the management of GCA.
- Future studies focusing on these issues are anticipated to shed light at both clinical and molecular level regarding the pathogenesis, diagnosis and treatment of GCA.


To be
continued