

Advanced methods to detect and eliminate aged cells

1837-2027
190
ΧΡΟΝΙΑ

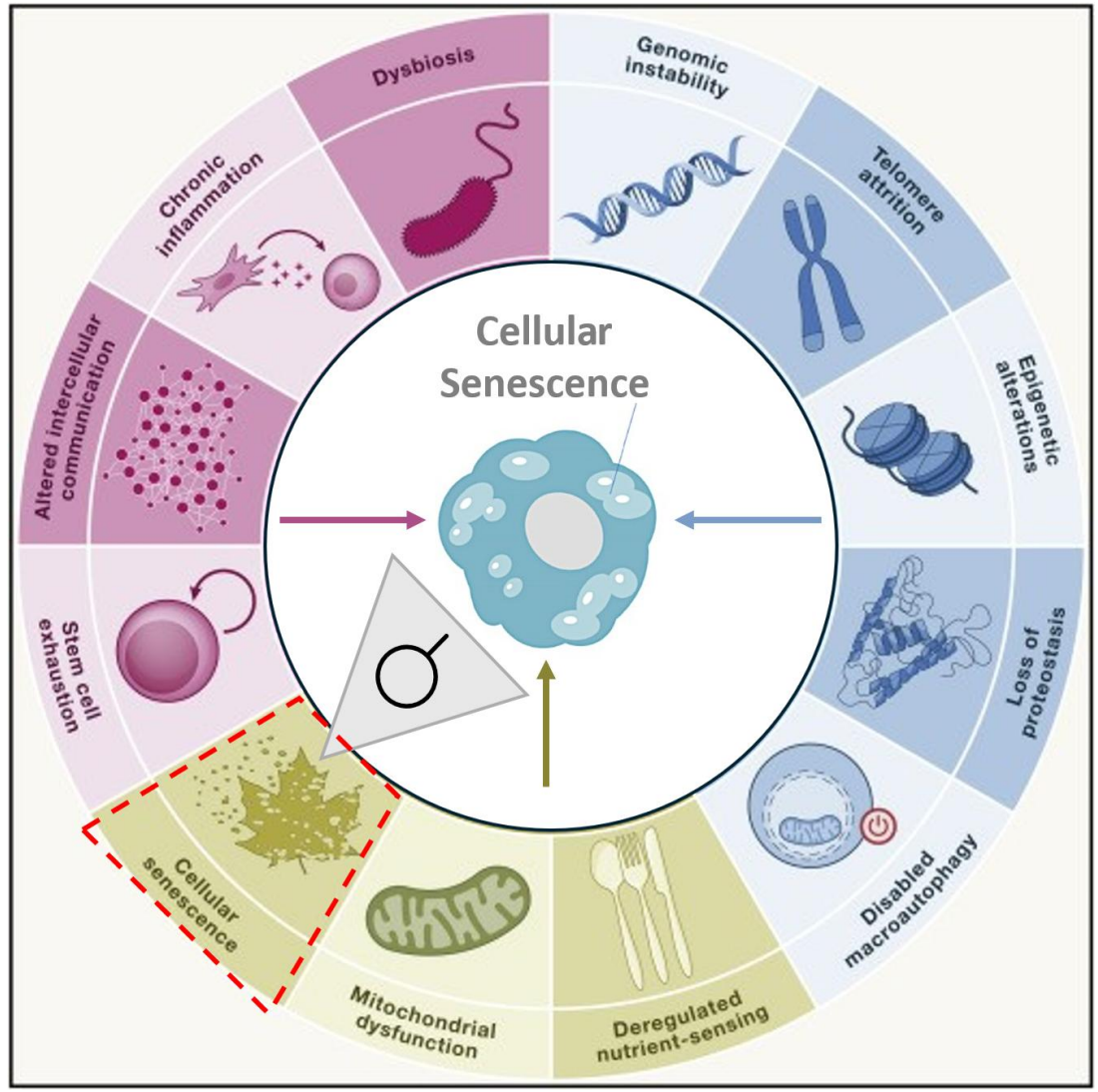
Εργαστήριο Ιστολογίας-Εμβρυολογίας, Ιατρική Σχολή,
Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών

Διευθυντής: Καθ. Βασίλης Γ. Γοργούλης (www.gorgoulis.gr)

Konstantinos Evangelou
Professor,
Molecular Biologist - Pathologist

Declaration of interest:
Senescence detection compound(s) described are under patents

To achieve Longevity, we need first to understand Aging and to eliminate Cellular Senescence, the reflection of aging at the molecular and cellular level!

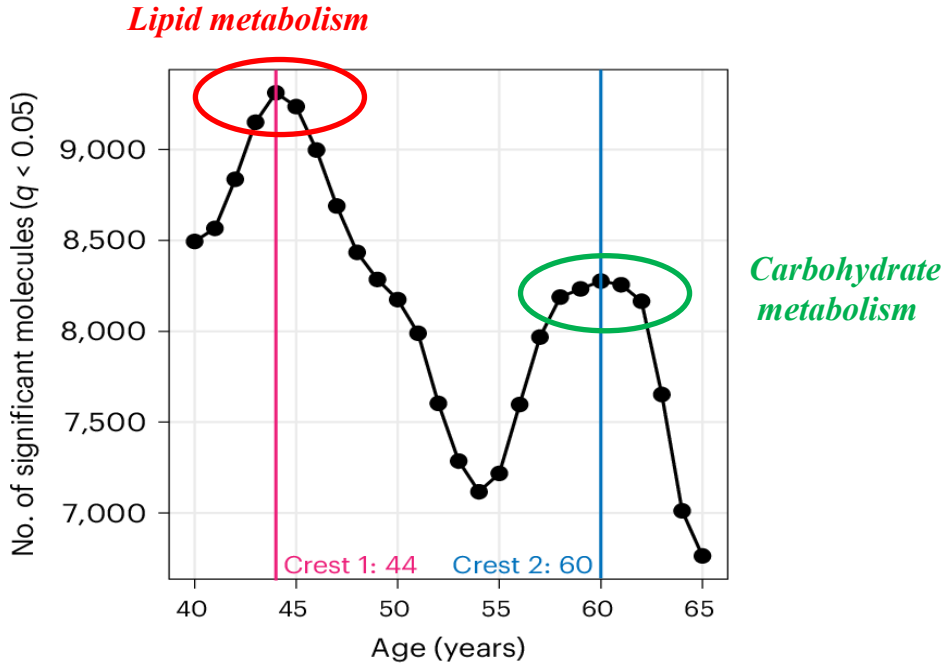


Carlos López-Otín, Cell 2023

What is Aging?

- Aging (WHO): the biological process of **accumulating molecular** and **cellular damage over time**, leading to a **gradual decrease in physical and mental capacity** and an **increased risk of disease**.

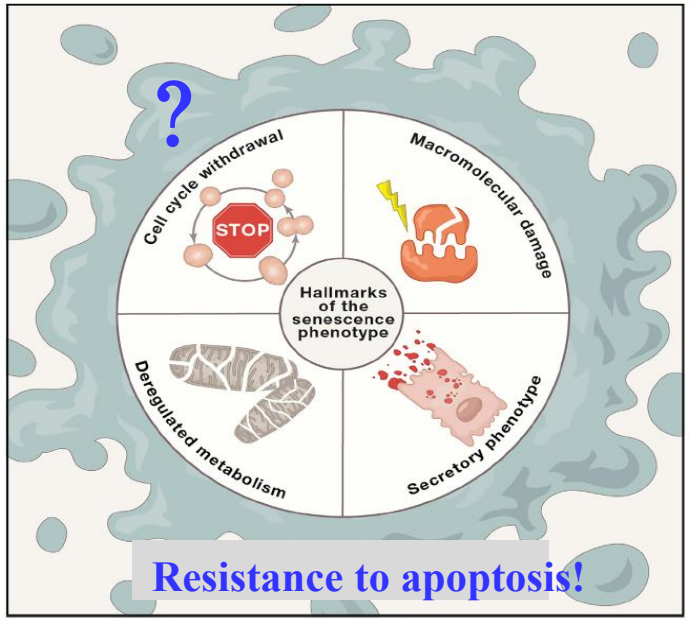
- Aging is not a linear process. It comes in **waves in our 40s and 60s**.

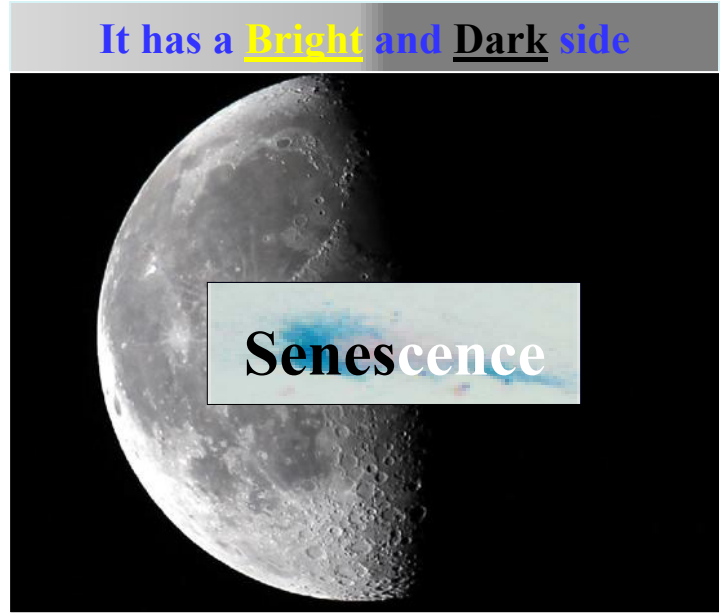


Shen, X., Wang, C., Zhou, X. et al., Nat Aging 2024

What is Cellular Senescence?

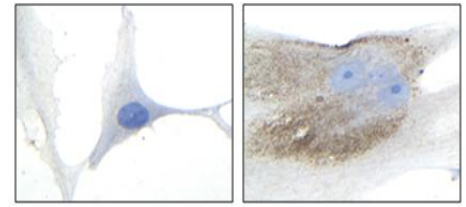
*A **cell state triggered by** **stressful cues** and **certain** **physiological processes**, characterized by:*







Normal Cell | Zombie Cell



2019

Leading Edge
Perspective

Cell

Cellular Senescence: Defining a Path Forward

Vassilis Gorgoulis,[1,20,25,26,*] Peter D. Adams,[2,27,28] Andrea Alimonti,[3,29,30,31] Dorothy C. Bennett,[4] Oliver Bischof,[5] Cleo Bishop,[6] Judith Campisi,[7] Manuel Collado,[8] Konstantinos Evangelou,[1] Gerardo Ferbeyre,[10] Jesús Gil,[11,32] Eiji Hara,[12] Valery Krizhanovsky,[13] Diana Jurk,[14] Andrea B. Maier,[15,33] Masashi Narita,[16] Laura Niedernhofer,[17] João F. Passos,[14]

(Author list continued on next page)

The “everyday battle” between stress and cells

Avoiding the hit!



Repair/ Recovery

Feeling dizzy!



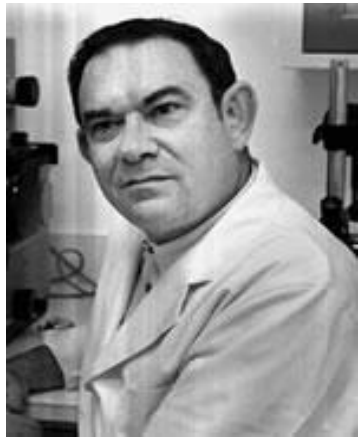
Cellular Senescence

Knockout hit!

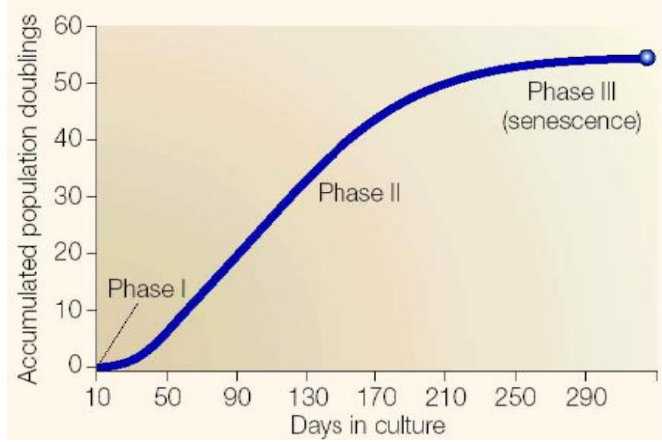


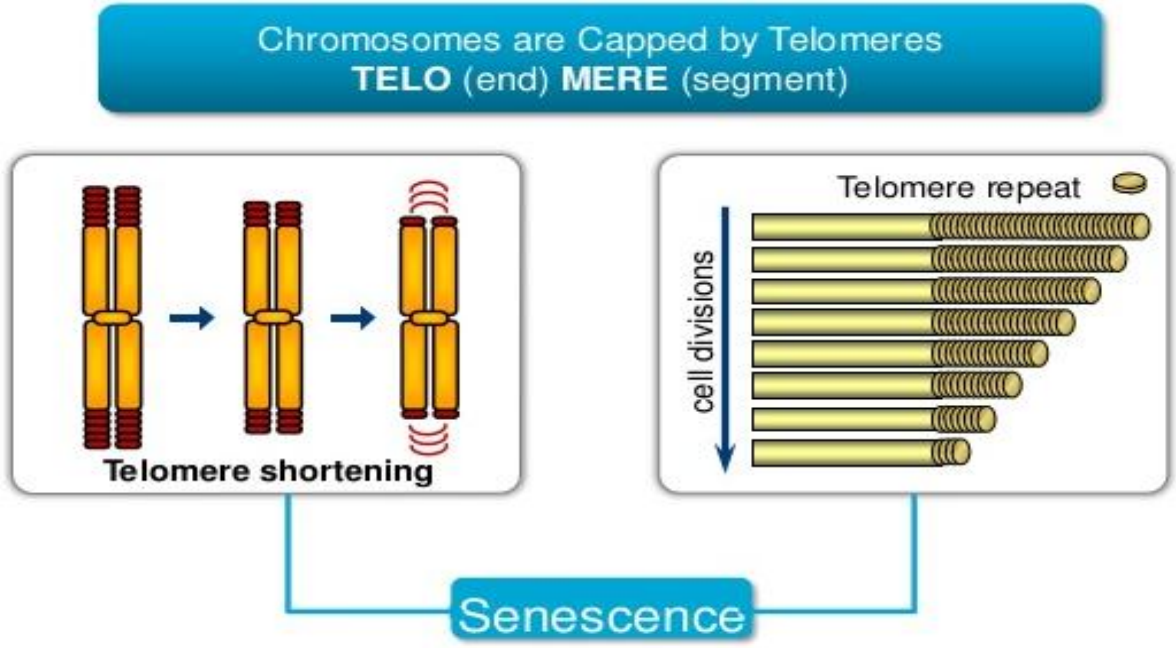
Cell death

First evidence: Replicative Senescence



Leonard Hayflick, 1961





Types of Cellular Senescence

Types

- **Replicative Senescence** (Hayflick, 1961) – *Telomere-dependent*
- **Stress induced premature senescence (SIPS)** – *Telomere-independent*

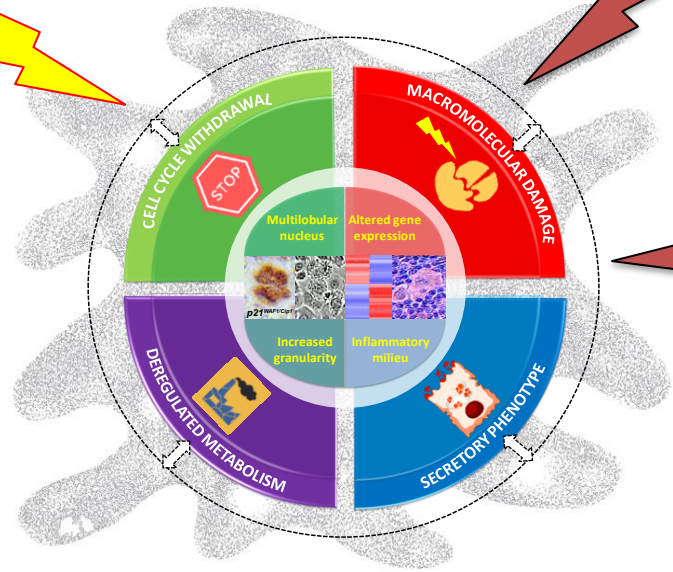
Stress induced premature senescence

Replicative senescence

Telomere dependent

DNA damage/genotoxic agents
Metabolic/oxidative stress
Growth factors, Cytokines, Viruses

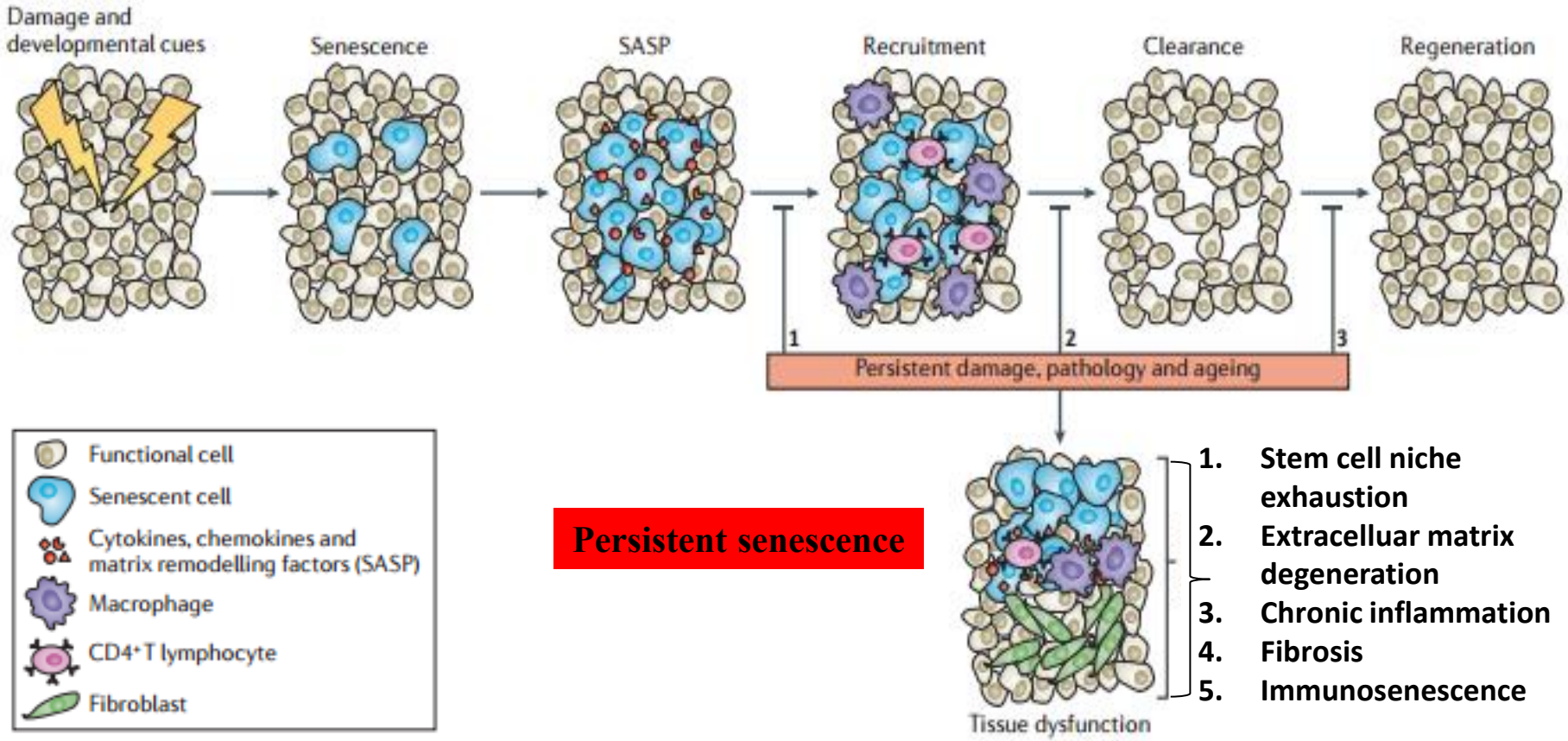
Oncogenes



Persistence of senescence promotes tissue dysfunction

Senescent cells are eliminated by immune cells

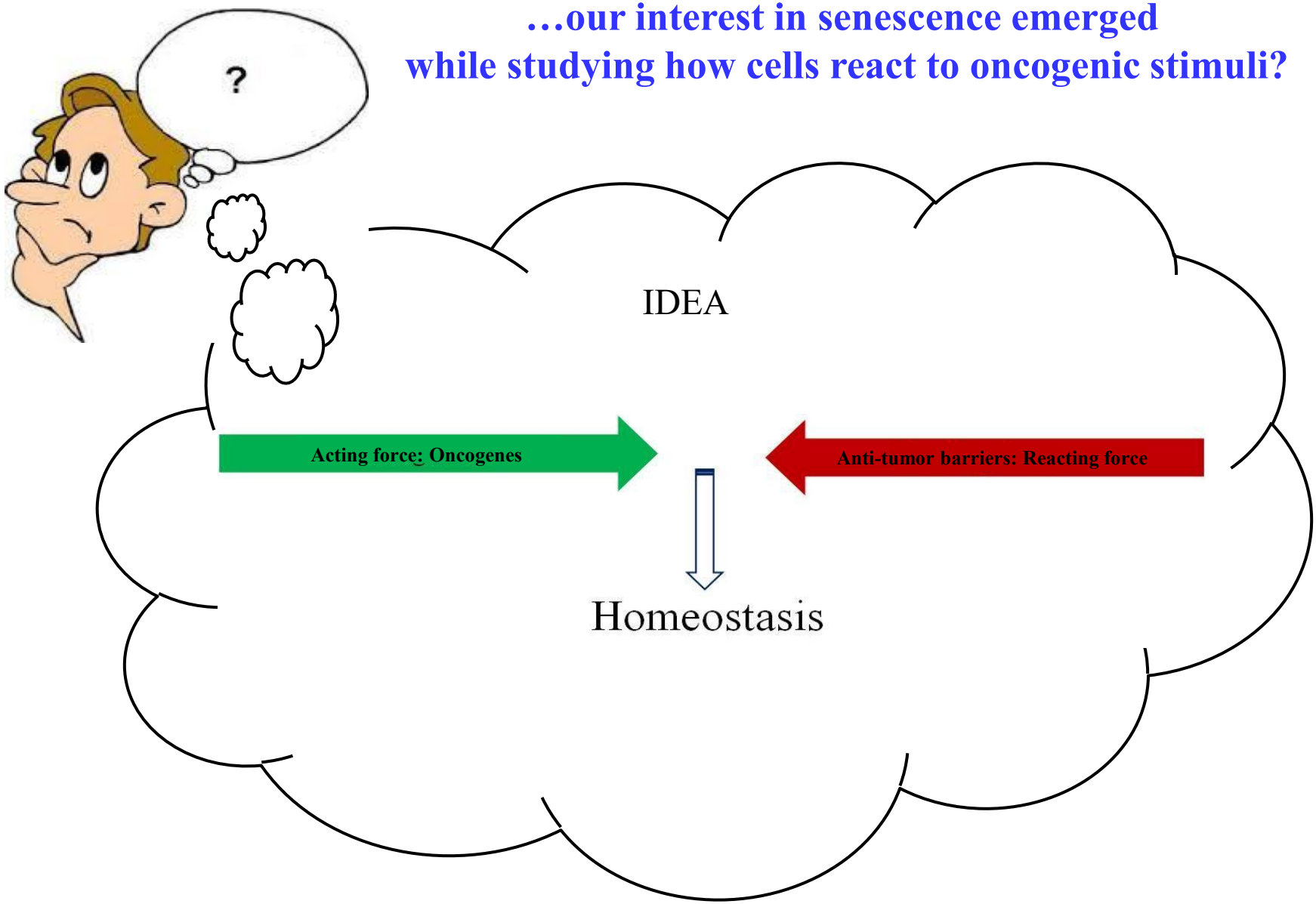
Acute senescence



1. **Stem cell niche exhaustion**
2. **Extracelluar matrix degeneration**
3. **Chronic inflammation**
4. **Fibrosis**
5. **Immunosenescence**

Nat Rev Mol Cell Biol 2014

…our interest in senescence emerged while studying how cells react to oncogenic stimuli?



Based on a series of studies, we conducted, we proposed the following model for cancer development …

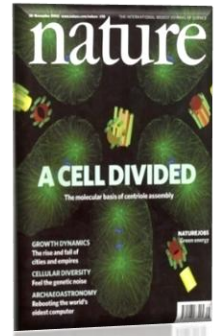
Oncogene (2008) 27, 3256–3264

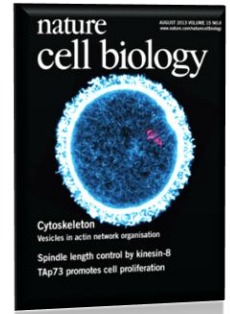
Oncogene-induced replication stress preferentially targets common fragile sites in preneoplastic lesions. A genome-wide study

Nature, Vol 444, Nov 2006

Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints







Cancer Research

Deregulated Expression of c-*mos* in Non-Small Cell Lung Carcinomas: Relationship with *p53* Status, Genomic Instability, and Tumor Kinetics Vol 61, Jan 2001

Vol 434, Apr 2005 letters to nature

Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions

Tumorigenesis and Neoplastic Progression AJP Oct 2004, Vol 165, No 4

Overexpression of the Replication Licensing Regulators hCdt1 and hCdc6 Characterizes a Subset of Non-Small-Cell Lung Carcinomas

Cancer Res 2007; 67: (22). November 15, 2007

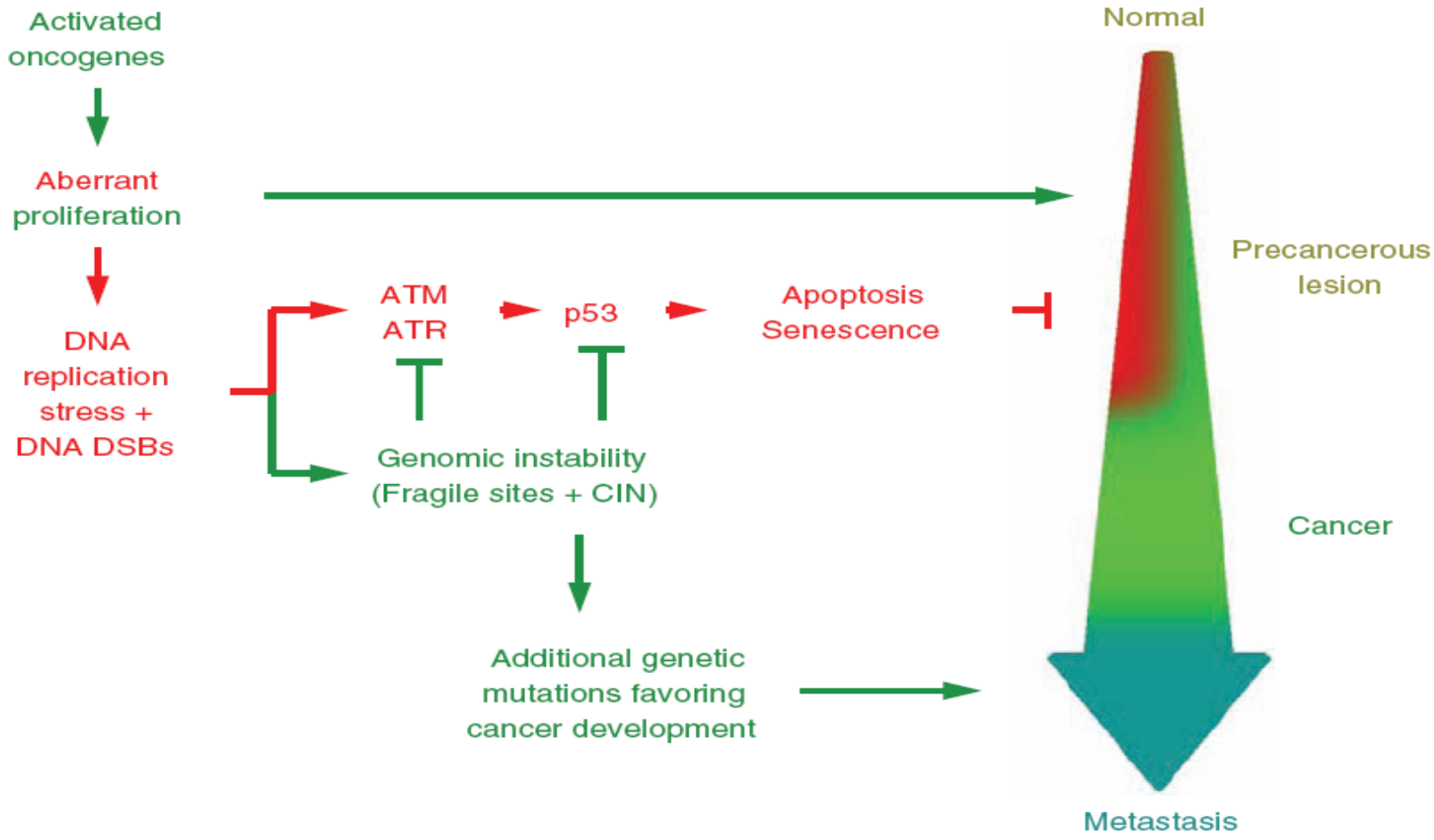
Research Article

Deregulated Overexpression of hCdt1 and hCdc6 Promotes Malignant Behavior

REVIEW 7 MARCH 2008 VOL 319 SCIENCE

An Oncogene-Induced DNA Damage Model for Cancer Development

Thanos D. Halazonetis,[1]* Vassilis G. Gorgoulis,[2] Jiri Bartek[3]



The model so far can explain:


REVIEW 7 MARCH 2008 VOL 319 SCIENCE

An Oncogene-Induced DNA Damage Model for Cancer Development

Thanos D. Halazonetis,[1]* Vassilis G. Gorgoulis,[2] Jiri Bartek[3]

1. The emergence of genomic instability in cancer

2. Evasion from apoptosis (e.g. by mutating p53)

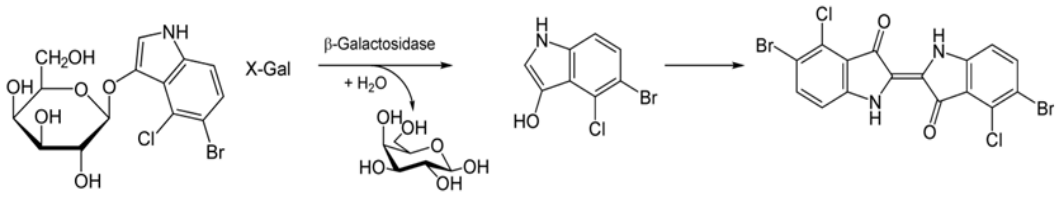
3. Early activation of senescence

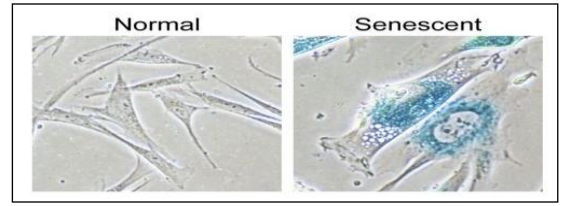
…but, could it explain *"escape"* from senescence?

The big challenge in the field is how to detect cellular Senescence accurately?

For many years...

The most widely used method to recognize senescent cells by detecting **Senescence Associated β-galactosidase (SA-β-gal) activity**, at pH=6



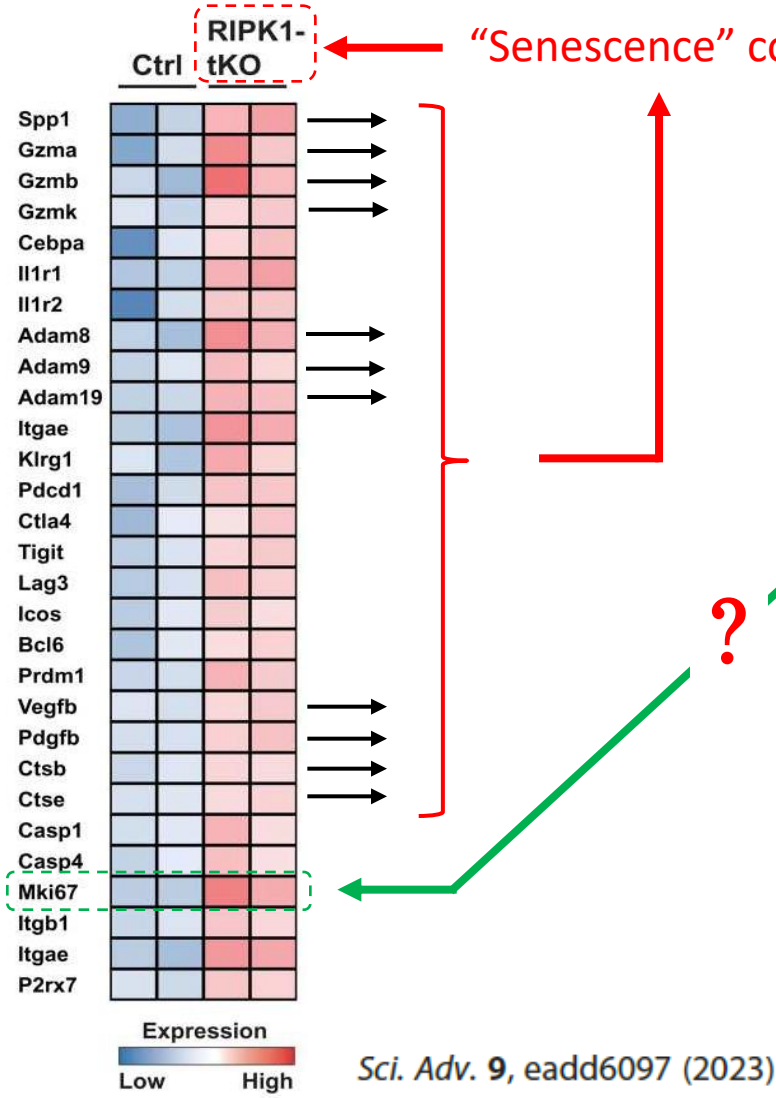


This assay is applicable only in **fresh samples/false positive or negative results**

Dimri et al, PNAS 1995

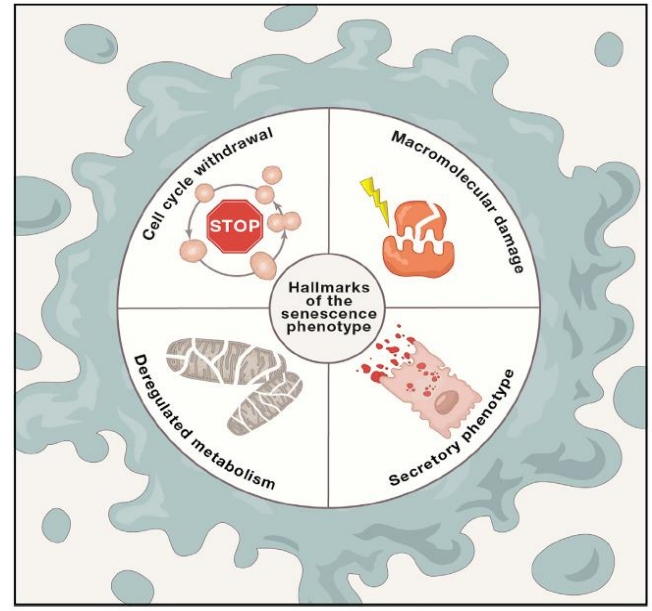
If cellular Senescence is not detected accurately, misleading conclusions emerge…

CD4 T-cells
(RNA-seq analysis)



"Senescence" condition

?

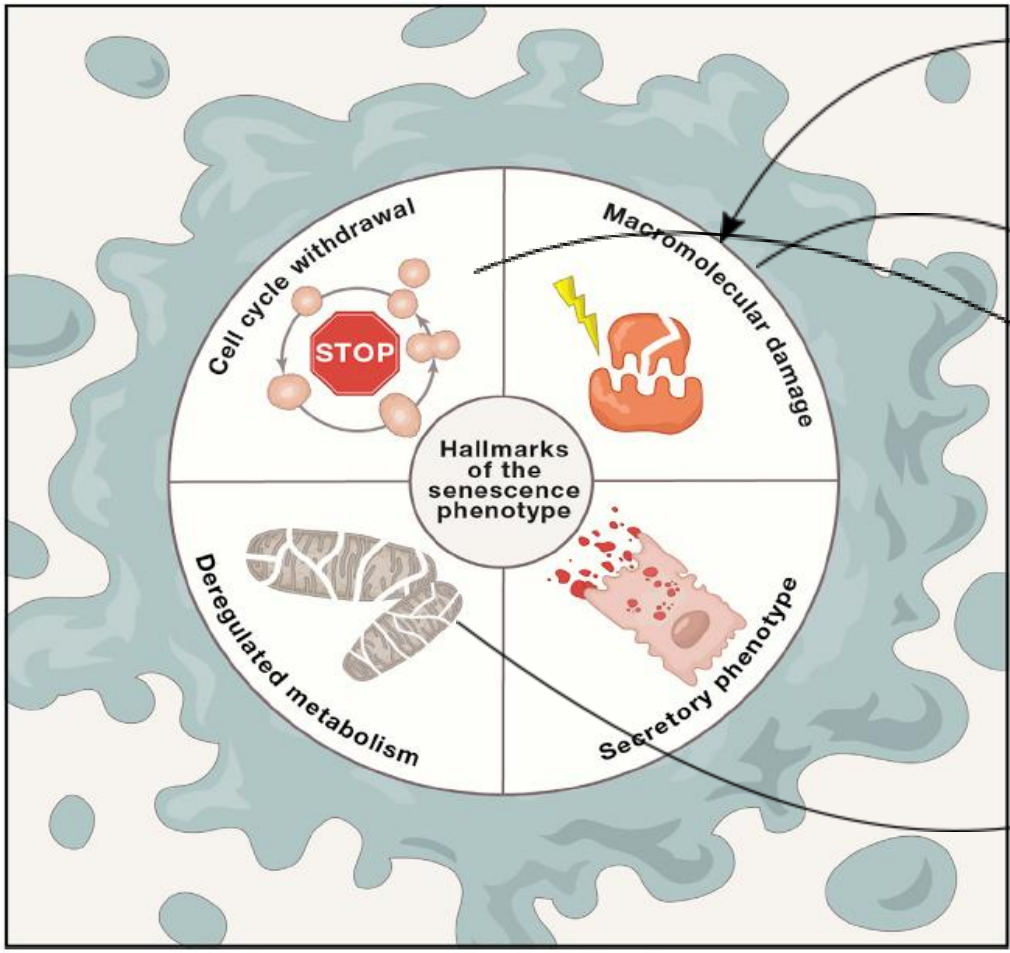



Cell *179*, October 31, 2019
Leading Edge
Perspective
Cell
Cellular Senescence: Defining a Path Forward

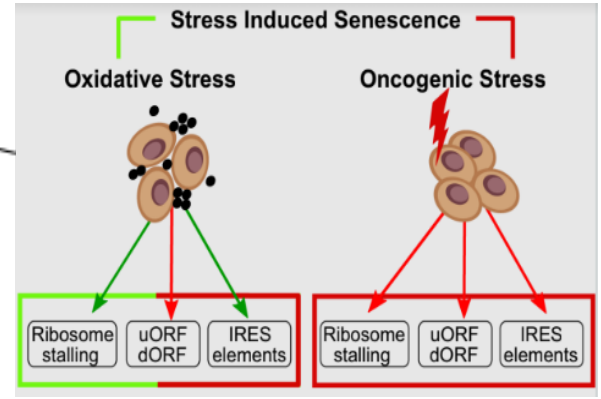
Sci. Adv. **9**, eadd6097 (2023)

2019

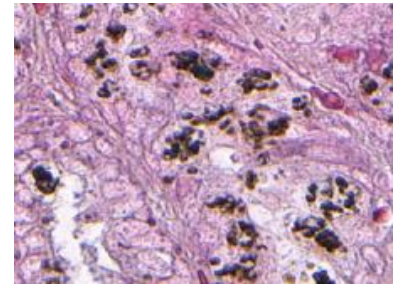
Lipofuscin (dark matter) is a non-degradable byproduct of cellular senescence



Translation deficiency



Lipofuscin accumulation (auto-fluorescence)



2019 2024 2023

Cell *179*, October 31, 2019
Leading Edge
Perspective
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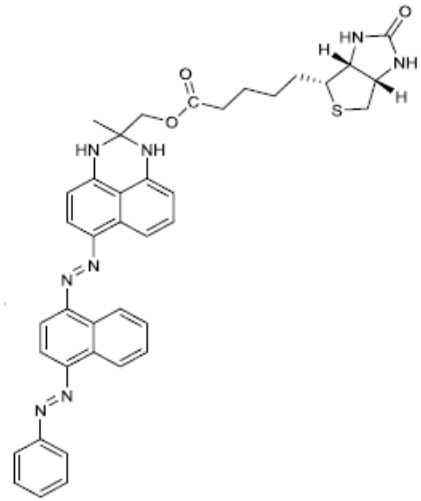
Cell *187*, August 8, 2024 4151
Cell
Leading Edge
Primer
Guidelines for minimal information on cellular senescence experimentation *in vivo*

Mikolaj Ogrodnik,[1,2,3,*] Juan Carlos Acosta,[4,5] Peter D. Adams,[6] Fabrizio d'Adda di Fagagna,[7,8] Darren J. Baker,[9] Cleo L. Bishop,[10] Tamir Chandra,[11] Manuel Collado,[12,13] Jesus Gil,[14,15] Vassilis Gorgoulis,[16,17,18,19,20] Florian Gruber,[21,22] Eiji Hara,[23,24] Pidder Jansen-Dürr,[25] Diana Jurk,[26] Sundeep Khosla,[27] James L. Kirkland,[28,29] Valery Krizhanovsky,[30]
(Author list continued on next page)

Aging Cell Open Access
ANATOMICAL SOCIETY
RESEARCH ARTICLE | Open Access
Decoding of translation-regulating entities reveals heterogeneous translation deficiency patterns in cellular senescence
Aging Cell. 2023;22:e13893.
https://doi.org/10.1111/acel.13893

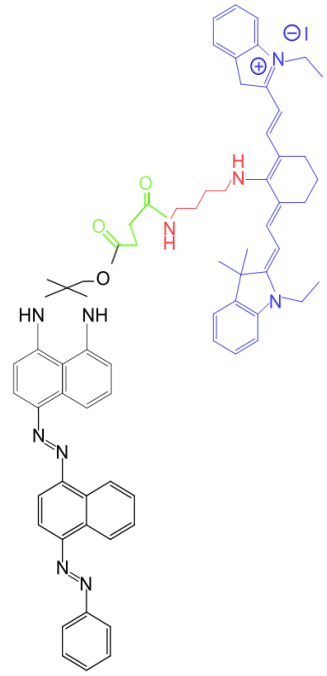
GL13 (SenTraGor™), GLF16 and mGLF16 probes to detect senescence

GL13 (SenTraGor™)



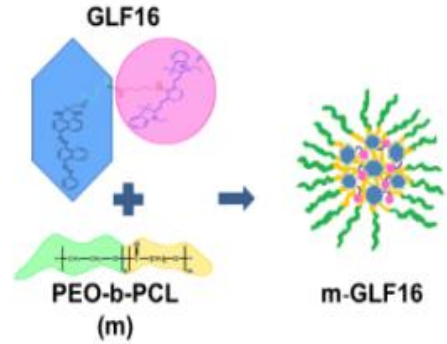


GLF16



mGLF16

QR LABS



In vitro
and archival material (morphology)

***In vitro*, archival (IF), FACS**
and downstream analyses

In vivo

2017

Aging Cell (2016) pp1–6 Doi: 10.1111/acel.12545

SHORT TAKE

Robust, universal biomarker assay to detect senescent cells in biological specimens

2023

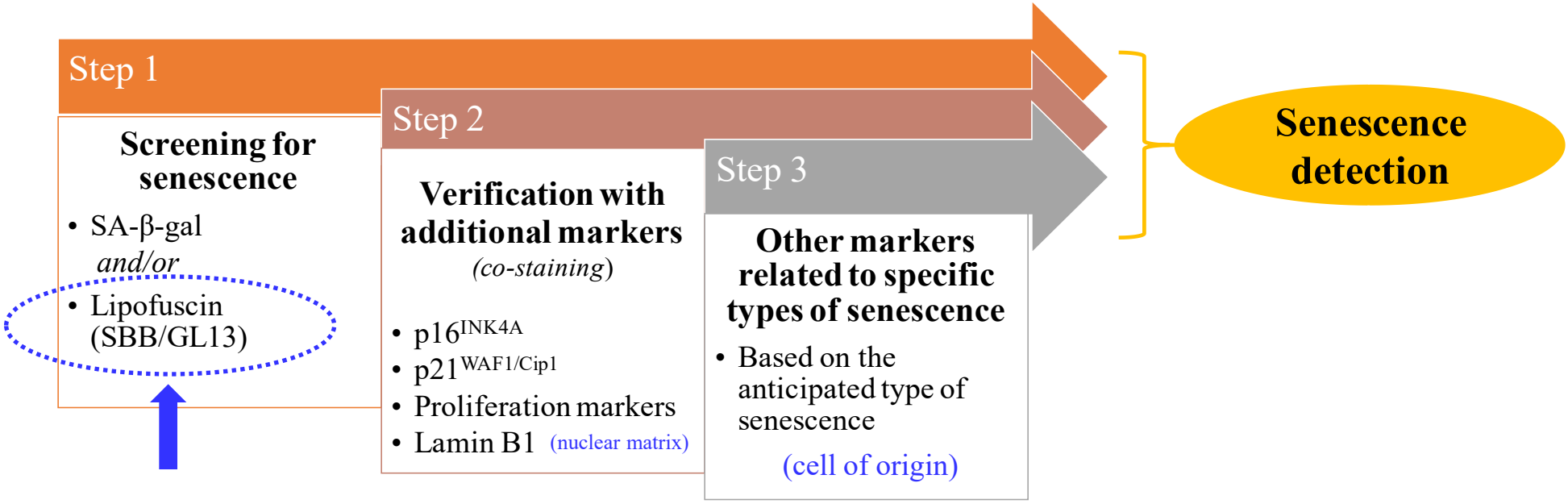
Molecular Cell Technology

A fluorophore-conjugated reagent enabling rapid detection, isolation and live tracking of senescent cells

Magkouta et al., 2023, Molecular Cell *83*, 3558–3573
October 5, 2023 © 2023 Elsevier Inc.

To accurately detect senescence the senescence community proposed…

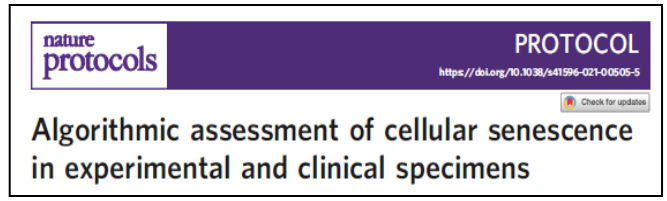
A multi-marker, three-step workflow for the detection of senescence



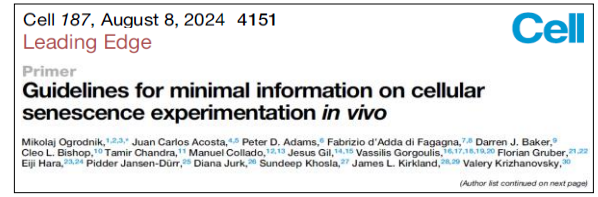
2019


Cell 179, October 31, 2019
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Cell
Cellular Senescence: Defining a Path Forward

2021


nature protocols
PROTOCOL
https://doi.org/10.1038/s41596-021-00505-5
Algorithmic assessment of cellular senescence in experimental and clinical specimens

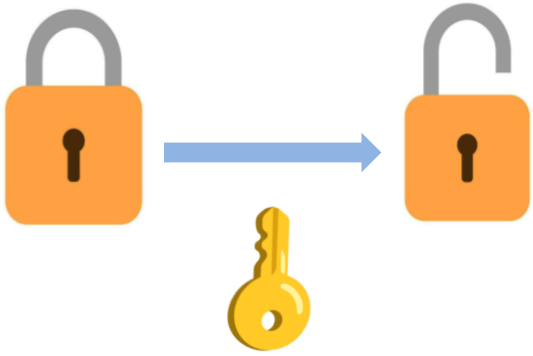
2024


Cell 187, August 8, 2024 4151
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Primer
Guidelines for minimal information on cellular senescence experimentation *in vivo*

GL13 and GLF16 Senoprobes: unlocked the field allowing for elucidation of the role of senescence in human aging and age-related diseases



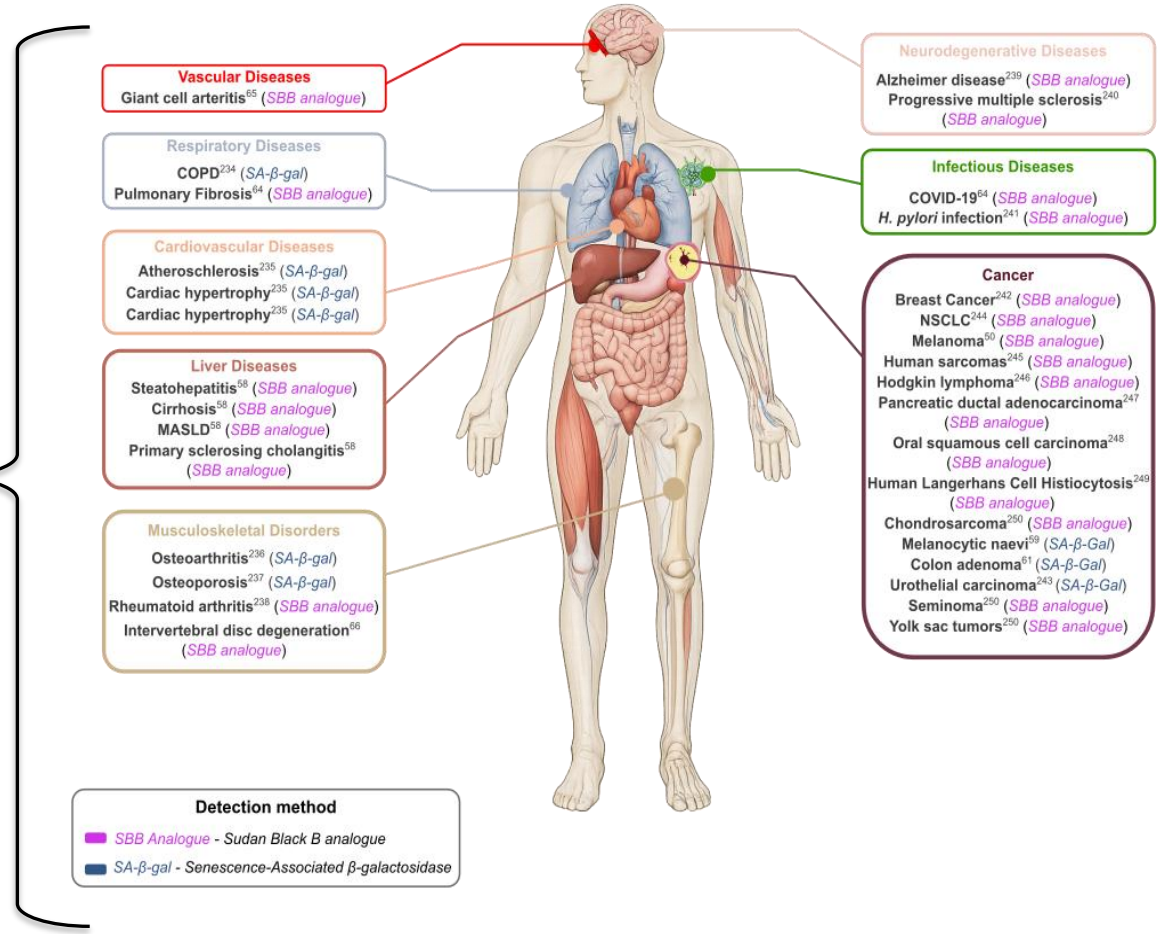
Senescence in Human Diseases and Aging?



Cellular Senescence

Senoprobes

GL13 **GLF16**



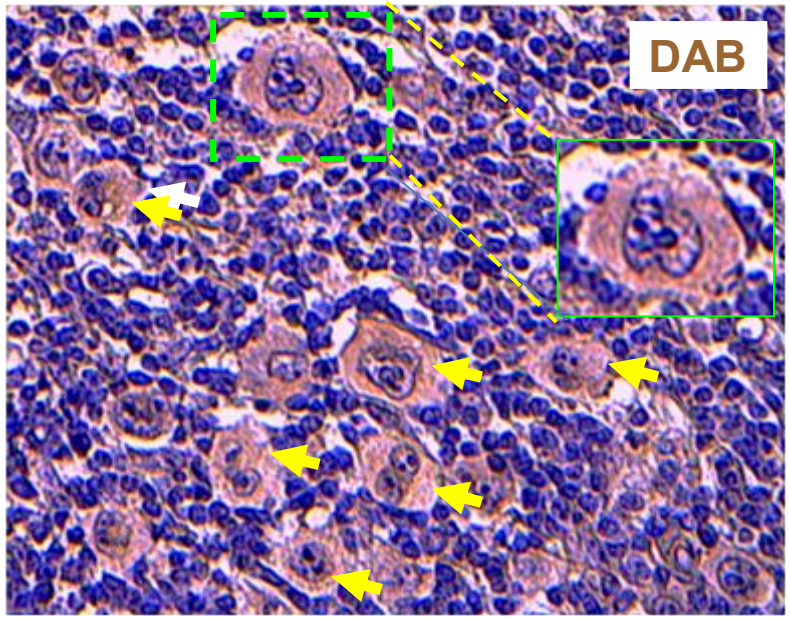
2026

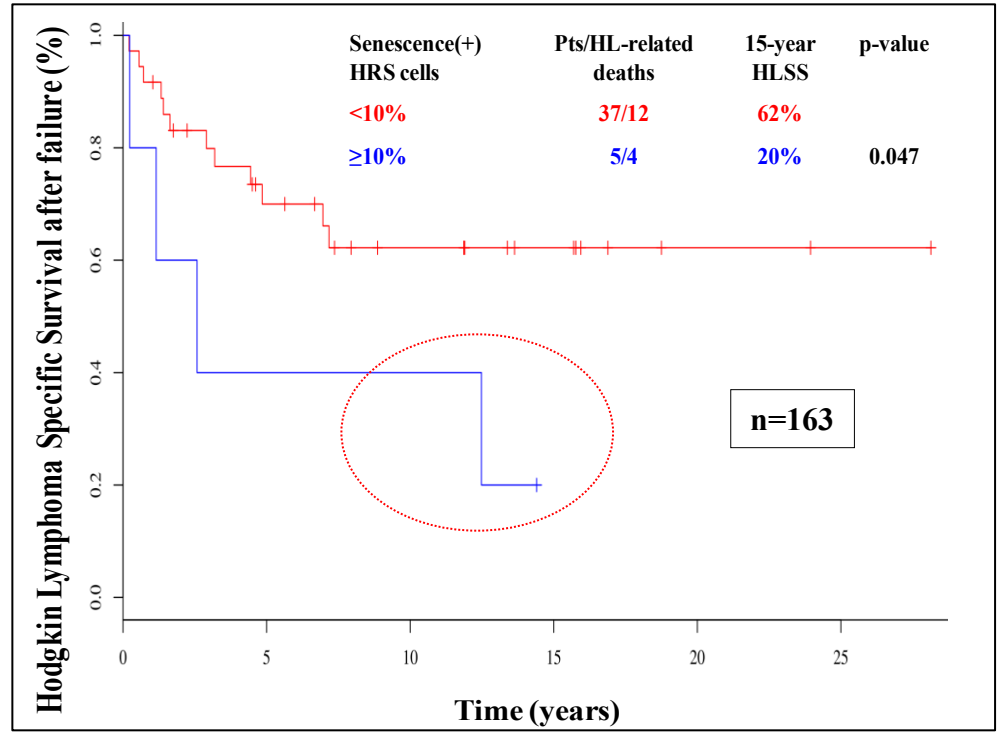
Nature Rev Bioengineering

By applying GL13, cellular senescence was retrospectively identified in a wide range of clinical settings (I)

Hodgkin lymphoma: *the first clinical entity*

SenTraGor™ Staining (+ HRS Cells)





2023

Journal of Pathology
J Pathol August 2023; **260:** 649–665
Published online 7 August 2023 in Wiley Online Library
(wileyonlinelibrary.com) DOI: 10.1002/path.6164

INVITED REVIEW

Escape from senescence: molecular basis and therapeutic ramifications

2019

Contents lists available at ScienceDirect

Pharmacology and Therapeutics

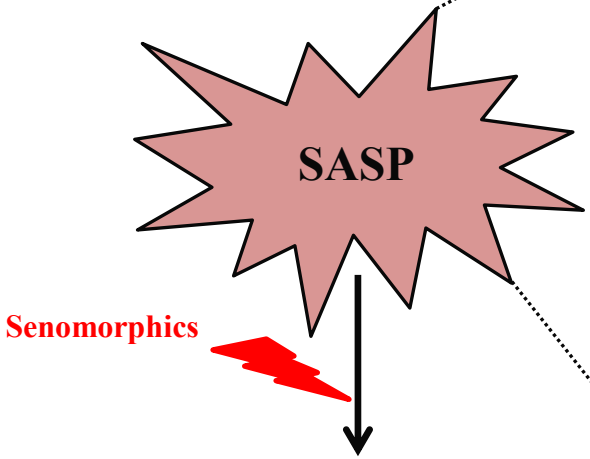
journal homepage: www.elsevier.com

ELSEVIER

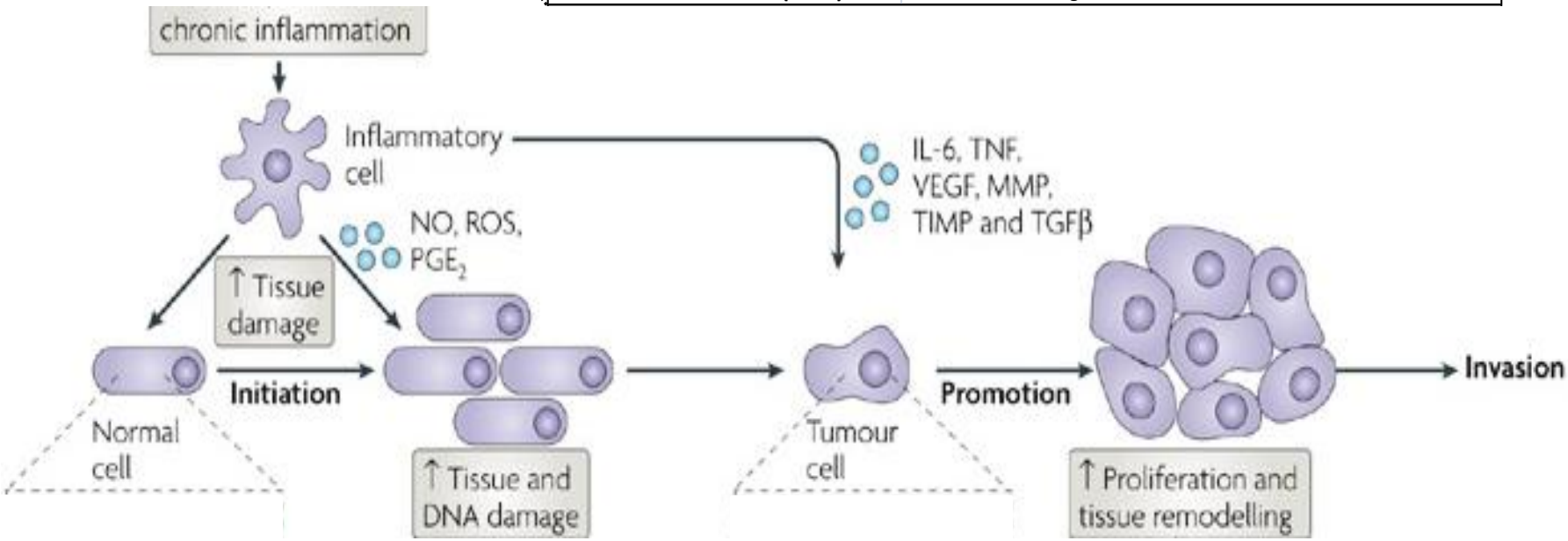
Senescence and senotherapeutics: a new field in cancer therapy

SASP exerts pro-tumorigenic properties

The "dark" Side of Cellular Senescence



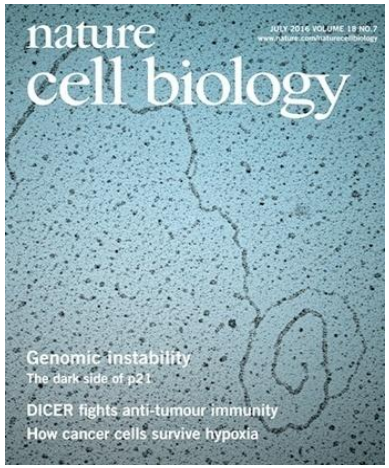
Soluble factors	
Interleukins (IL)	IL-6, IL-7, IL-1a, -1b, IL-13, IL-15
Chemokines (CXCL, CCL)	IL-8, GRO-a,-b,-gc, GRO-a,-b,-gc, MCP-2,
	MCP-4, MIP-1a, MIP-3a, HCC-4, Eotaxin, Eotaxin-3, MIP-1a,
	MIP-3a, HCC-4, Eotaxin, Eotaxin-3, TECK, ENA-78, I-309, I-TAC
Other inflammatory factors	GM-CSE, G-CSE, IFN-?, BLC, MIF
Growth factors and 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, ICAM-1, -3
Soluble or shed receptors or ligands	OPG, sTNFRI, TRAIL-R3, Fas, sTNFRII, Fas, uPAR, SGP130, EGF-R
Nonprotein soluble factors	PGE2, Nitric oxide, Reactive oxygen species
Insoluble factors (ECM)	Fibronectin, Collagens, Laminin



Nature Reviews Cancer 2014

In three consecutive studies we showed that "escape" from senescence occurs and genomic instability is the driving force

2016

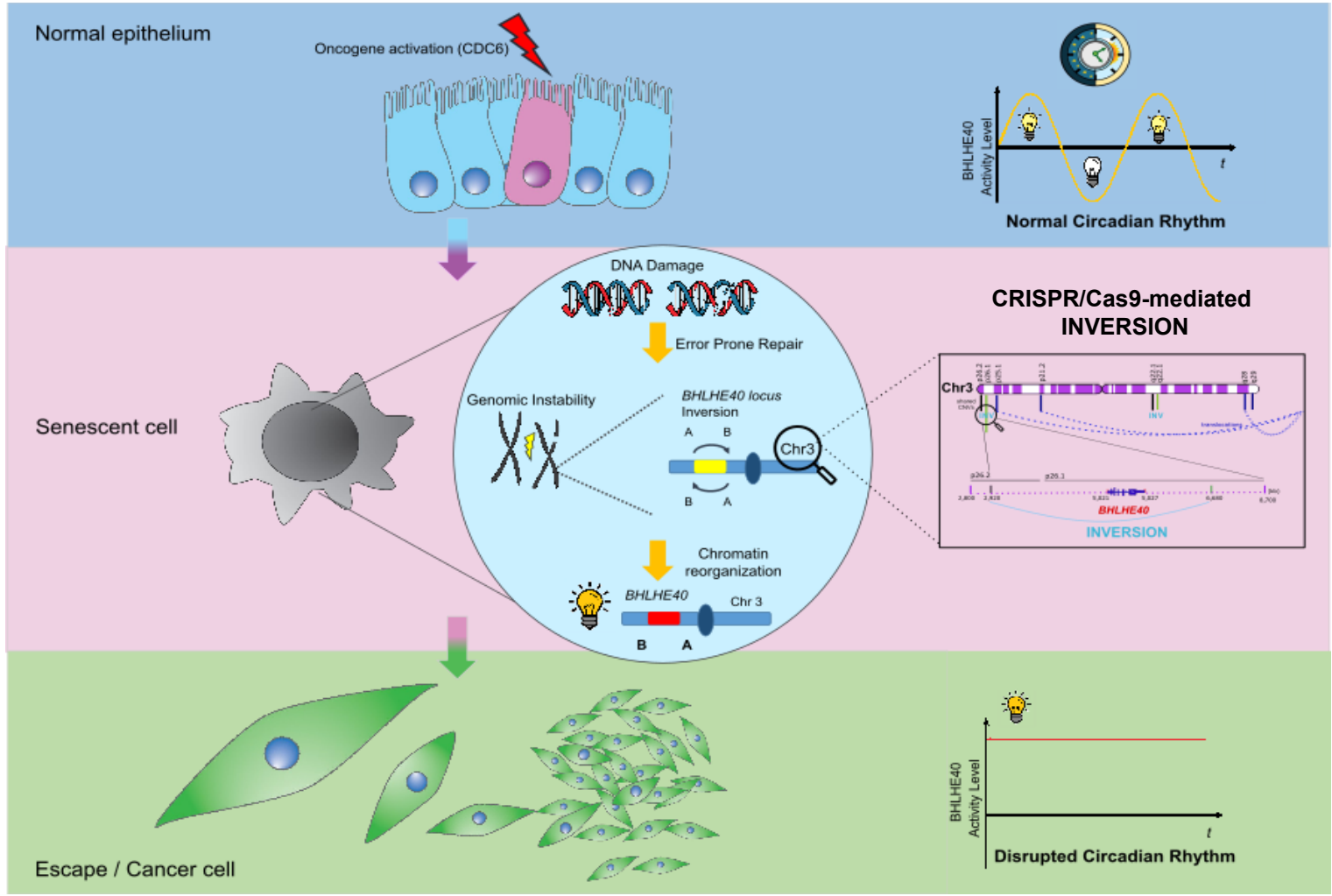


2018

Genome Biology (2018) 19:37

RESEARCH

Mutational signatures reveal the role of RAD52 in p53-independent p21-driven genomic instability



2021

Molecular Cell

Article

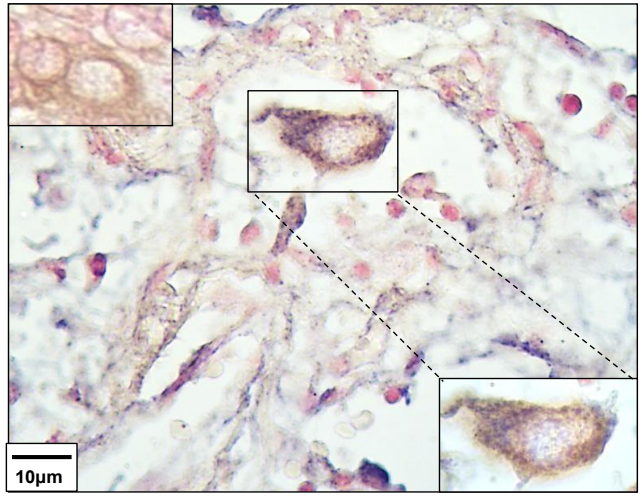
A recurrent chromosomal inversion suffices for driving escape from oncogene-induced senescence via subTAD reorganization

Zampetidis et al., 2021, Molecular Cell 81, 4907–4923
December 2, 2021 © 2021 Elsevier Inc.
https://doi.org/10.1016/j.molcel.2021.10.017

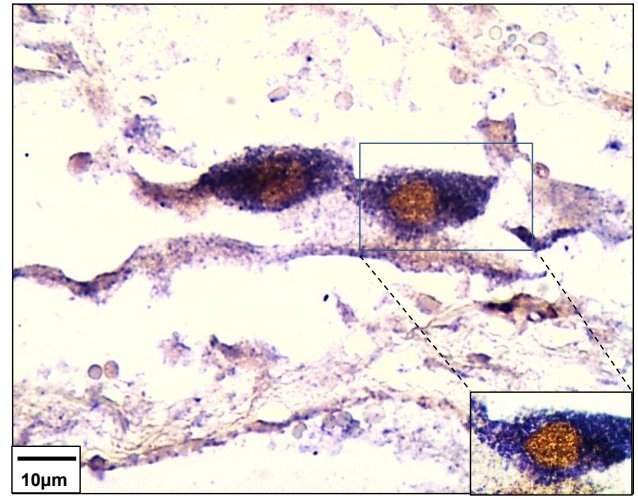
Identification of cellular senescence in COVID-19

Lung from a COVID-19 patient

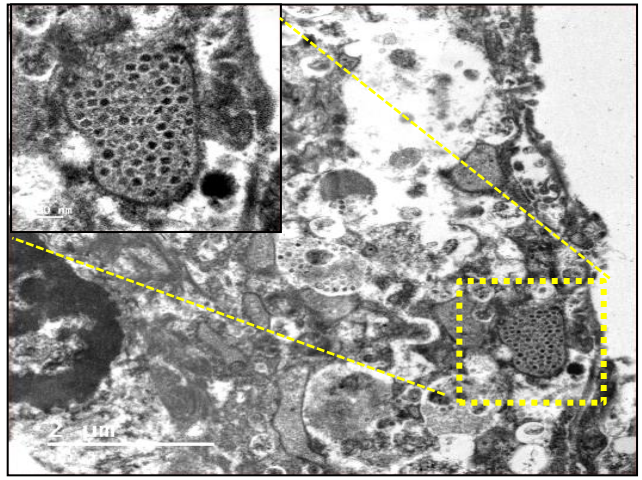
Anti-SARS-CoV-2 ab(+) / SenTraGor(+)

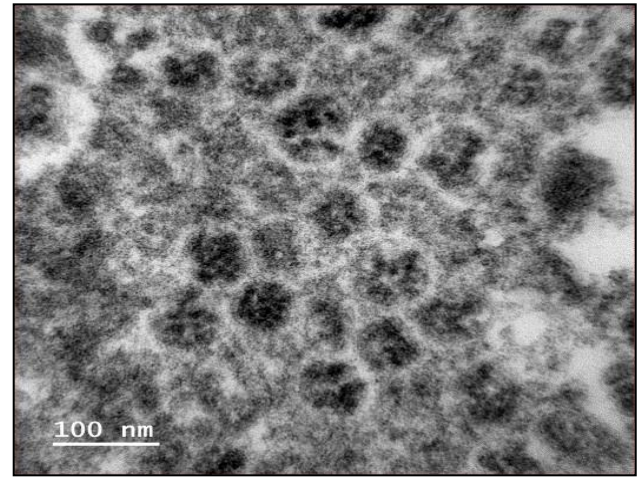


p16^{INK4A}(+) / SenTraGor(+)



2023





2022

Journal of Pathology
J Pathol August 2023; **260:** 649–665
Published online 7 August 2023 in Wiley Online Library
(wileyonlinelibrary.com) DOI: 10.1002/path.6164

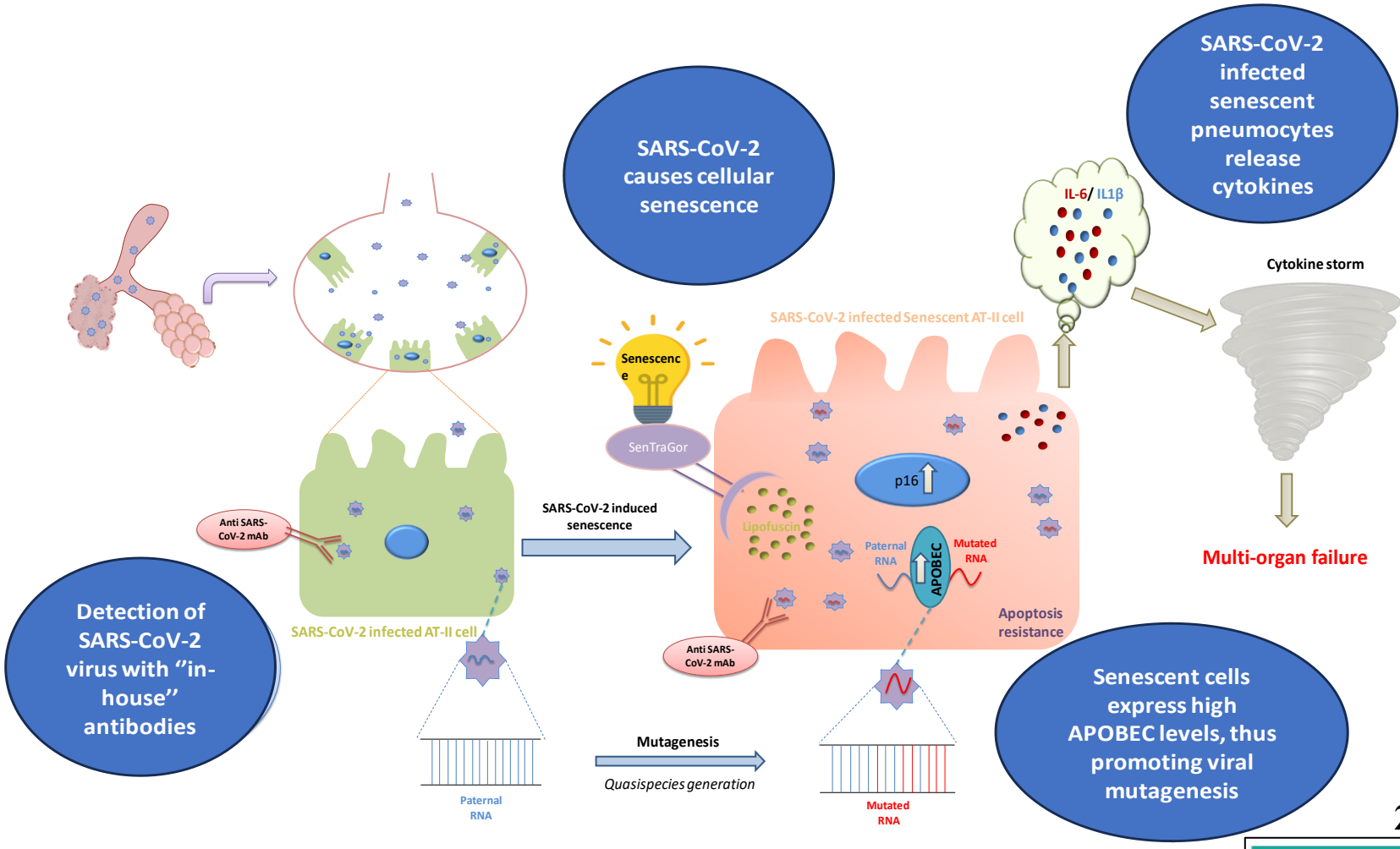
INVITED REVIEW

Escape from senescence: molecular basis and therapeutic ramifications

EUROPEAN RESPIRATORY *journal*
FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Pulmonary infection by SARS-CoV-2 induces senescence accompanied by an inflammatory phenotype in severe COVID-19: possible implications for viral mutagenesis

Proposed model of the role of cellular senescence in COVID-19



2022

EUROPEAN RESPIRATORY *journal*
FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Pulmonary infection by SARS-CoV-2 induces senescence accompanied by an inflammatory phenotype in severe COVID-19: possible implications for viral mutagenesis

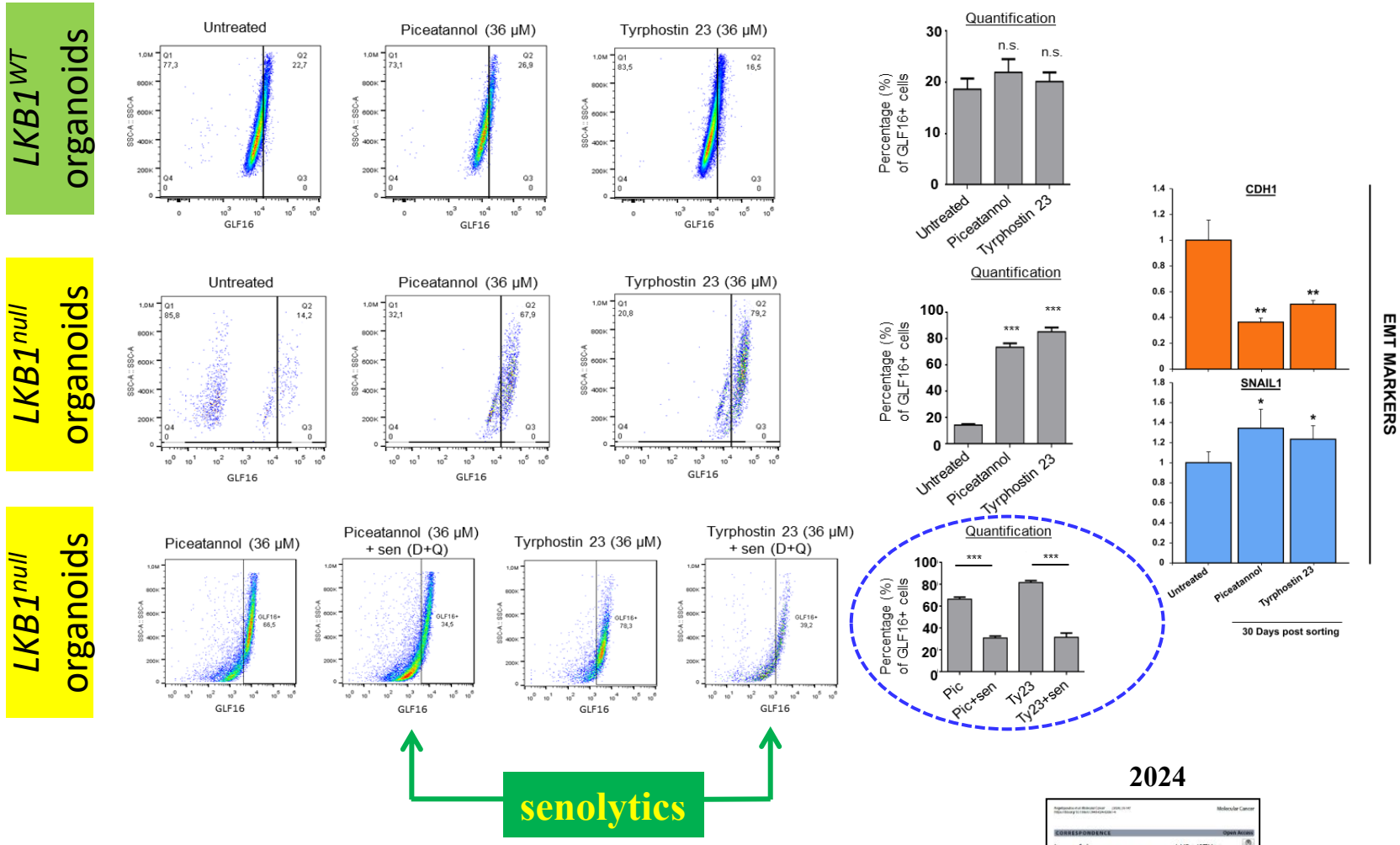
2021

The FEBS Journal

Review | Open Access

Cellular senescence as a source of SARS-CoV-2 quasispecies

Therapy induced senescence (TIS) as an outcome of anticancer interventions



2024

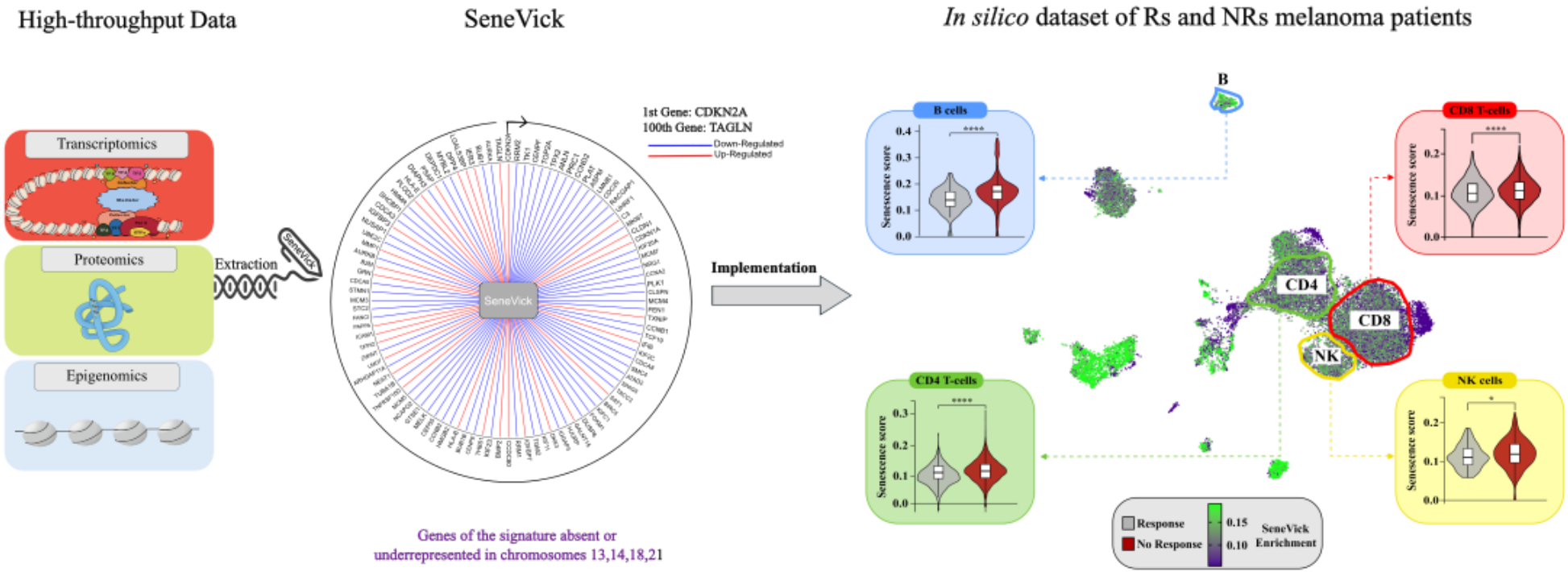


We're entering a transformative era from **“big data analytics”** to **“smart data”.** New tools (e.g GenAI, RAG) and practices have taken over making data practices less about collecting great volumes of information and more about delivering **real value**…

Within this context…

… and exploiting our senoprobes we generated SeneVick, a molecular signature that allows detection of cellular senescence, retrospectively

Using this tool, we identified increased immune cell senescence in the TME of Non-responders (NRs to immunotherapy melanoma patients



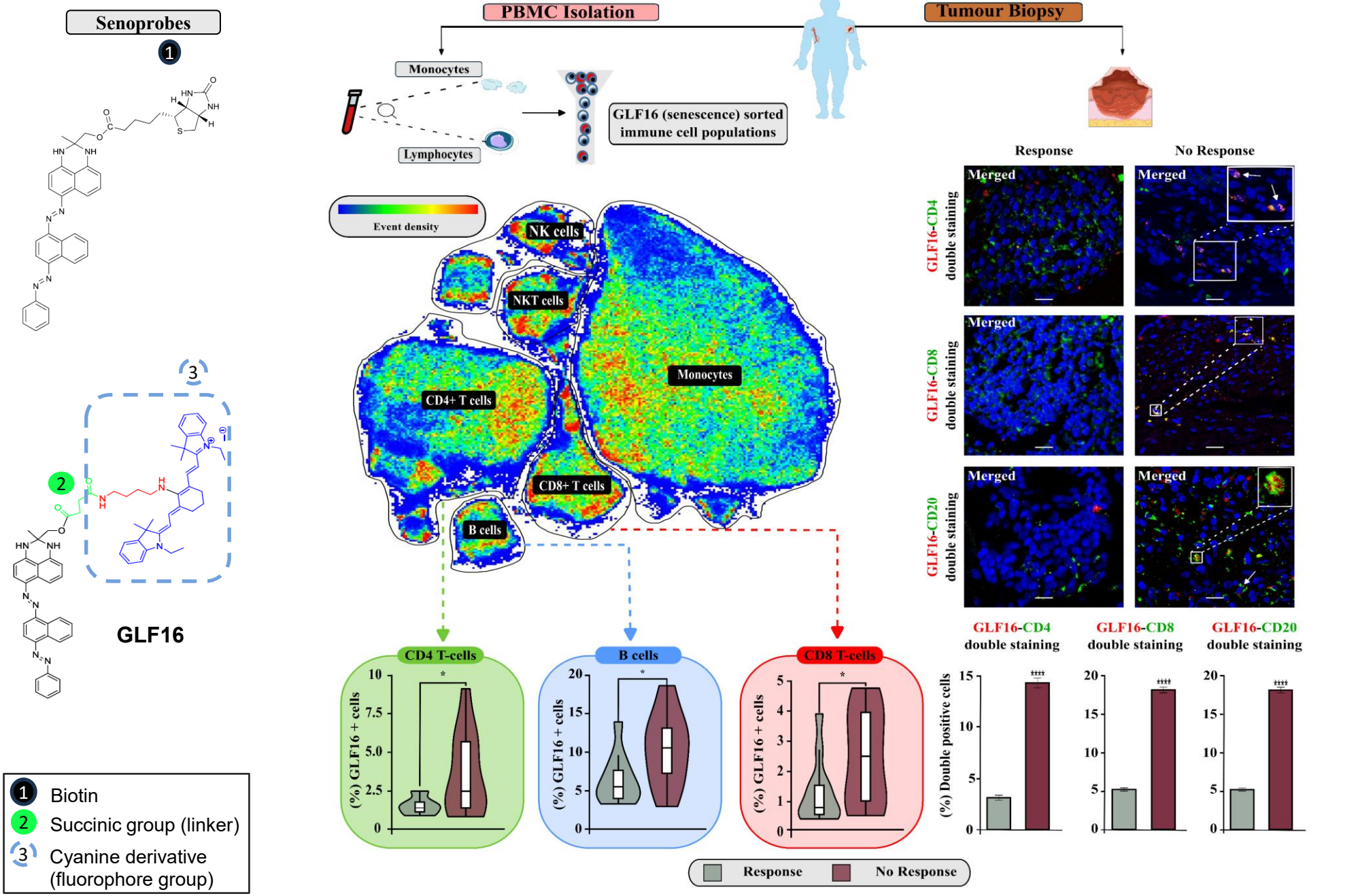
2025


Molecular Cancer

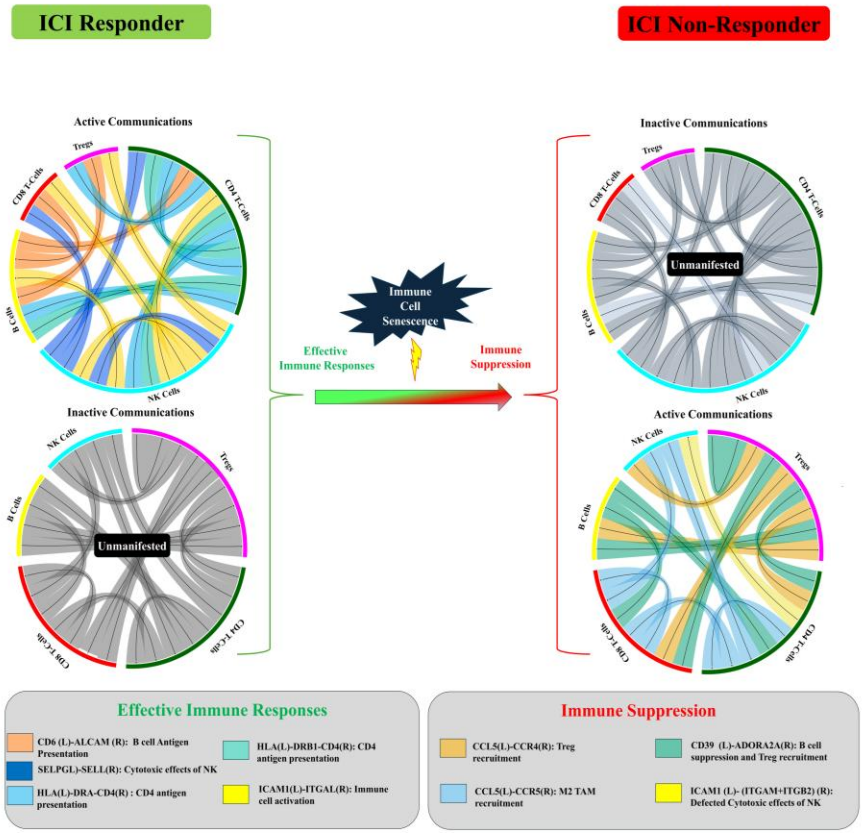
RESEARCH Open Access

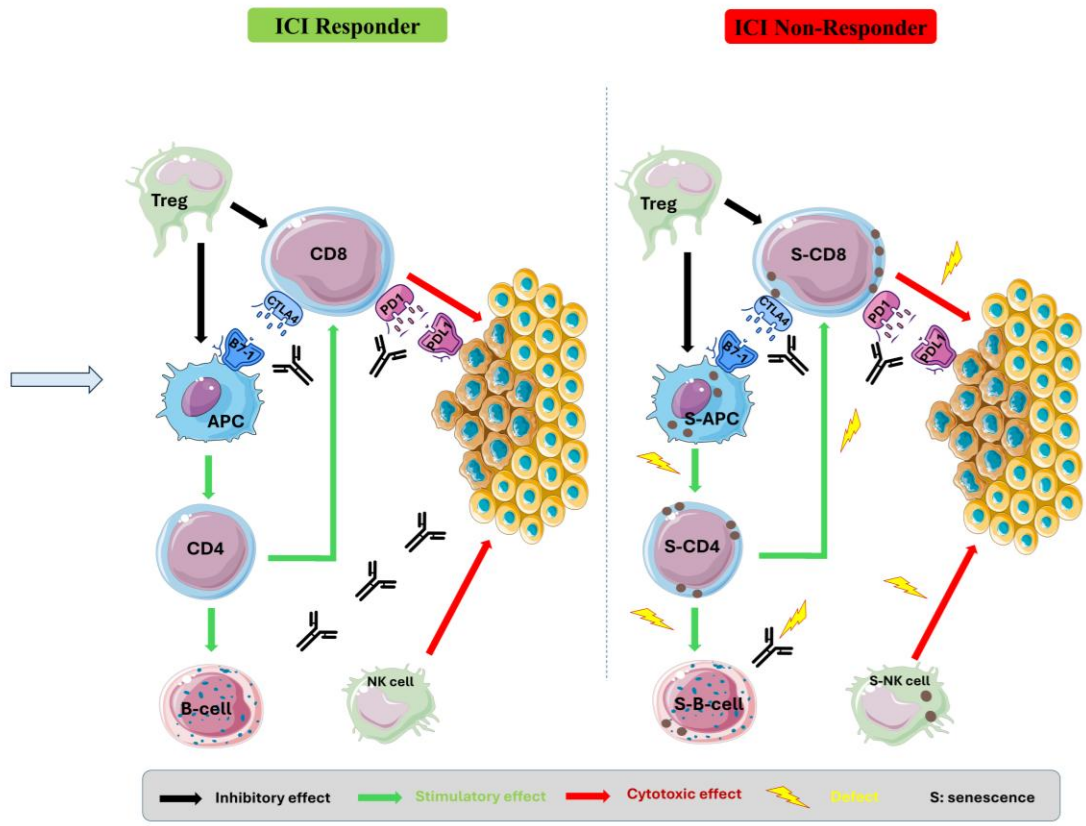
Immune cell senescence drives responsiveness to immunotherapy in melanoma

Our senoprobes unveiled increased immune senescence in an *ex vivo* cohort of NRs melanoma patients



Increased immune cell senescence in the TME of NRs leads to altered immune cell interactions promoting an immunosuppressive environment

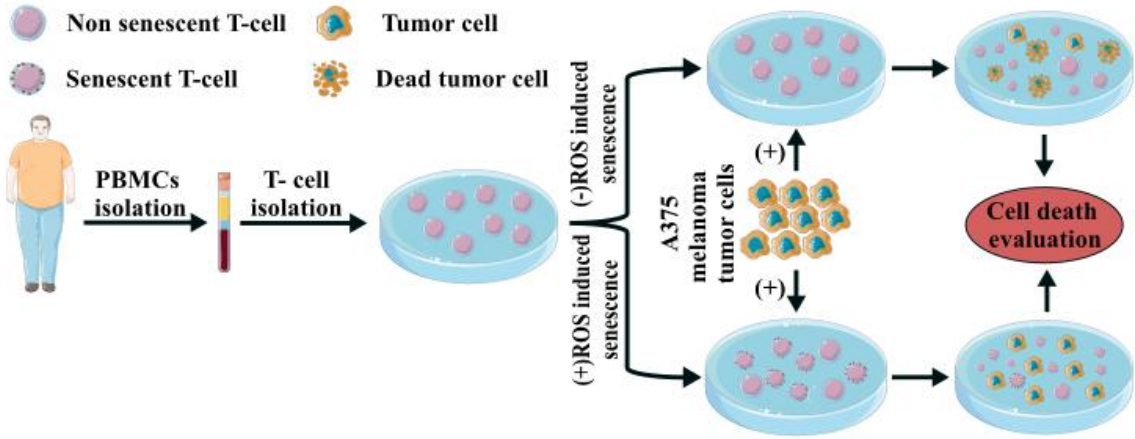


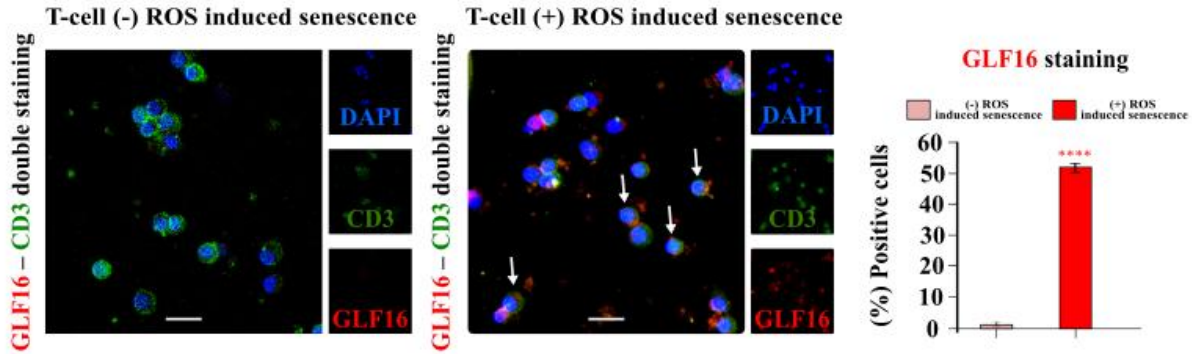


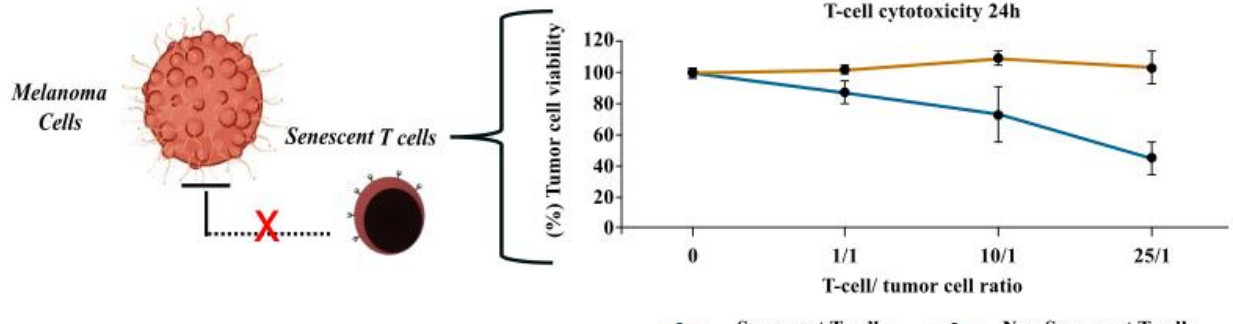
2025



At the functional level, senescent T-cells demonstrate ineffective immune responses (impaired cytotoxicity)



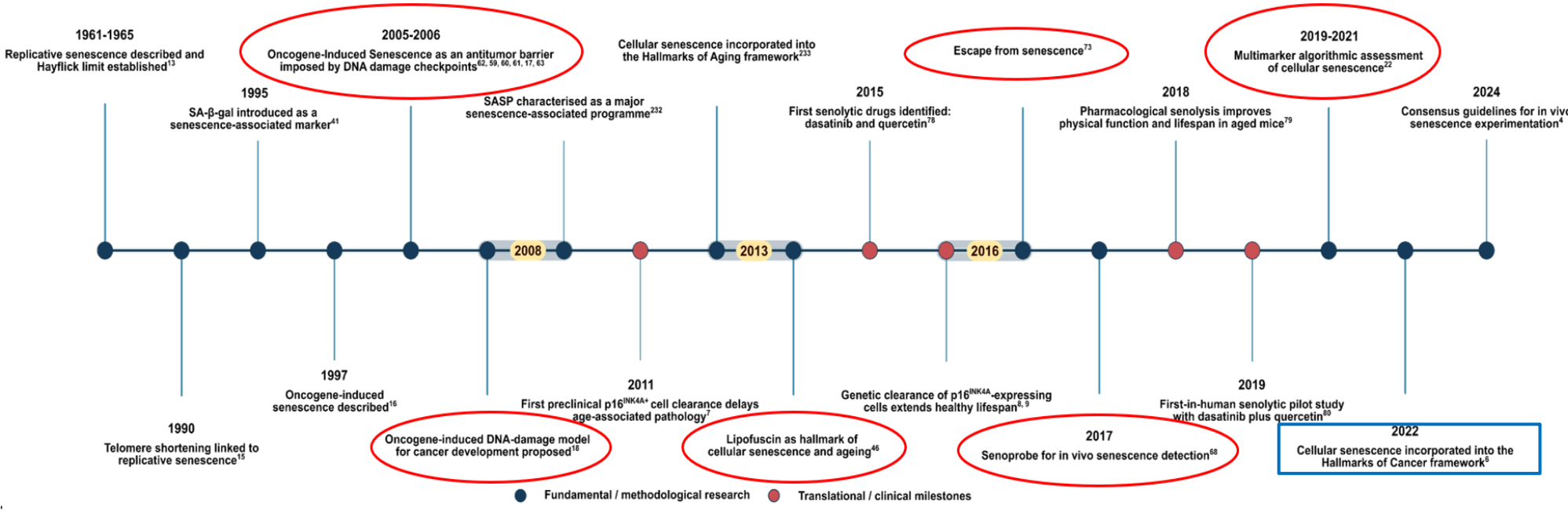




2025



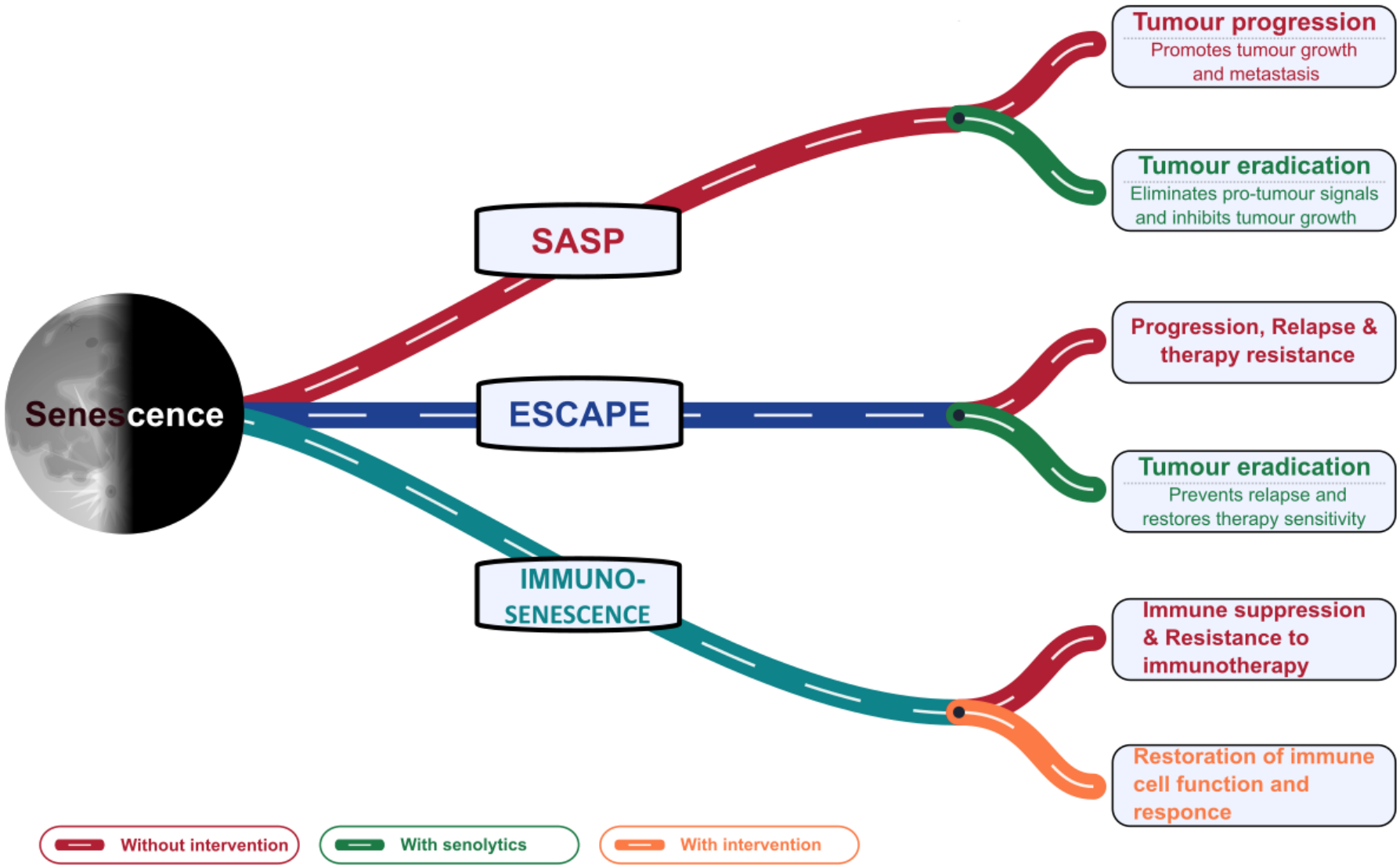
The aforementioned discoveries rank among the milestones in research on cellular senescence



2026

Nature Rev Bioengineering

Targeting cellular senescence as a holistic anticancer strategy



2026

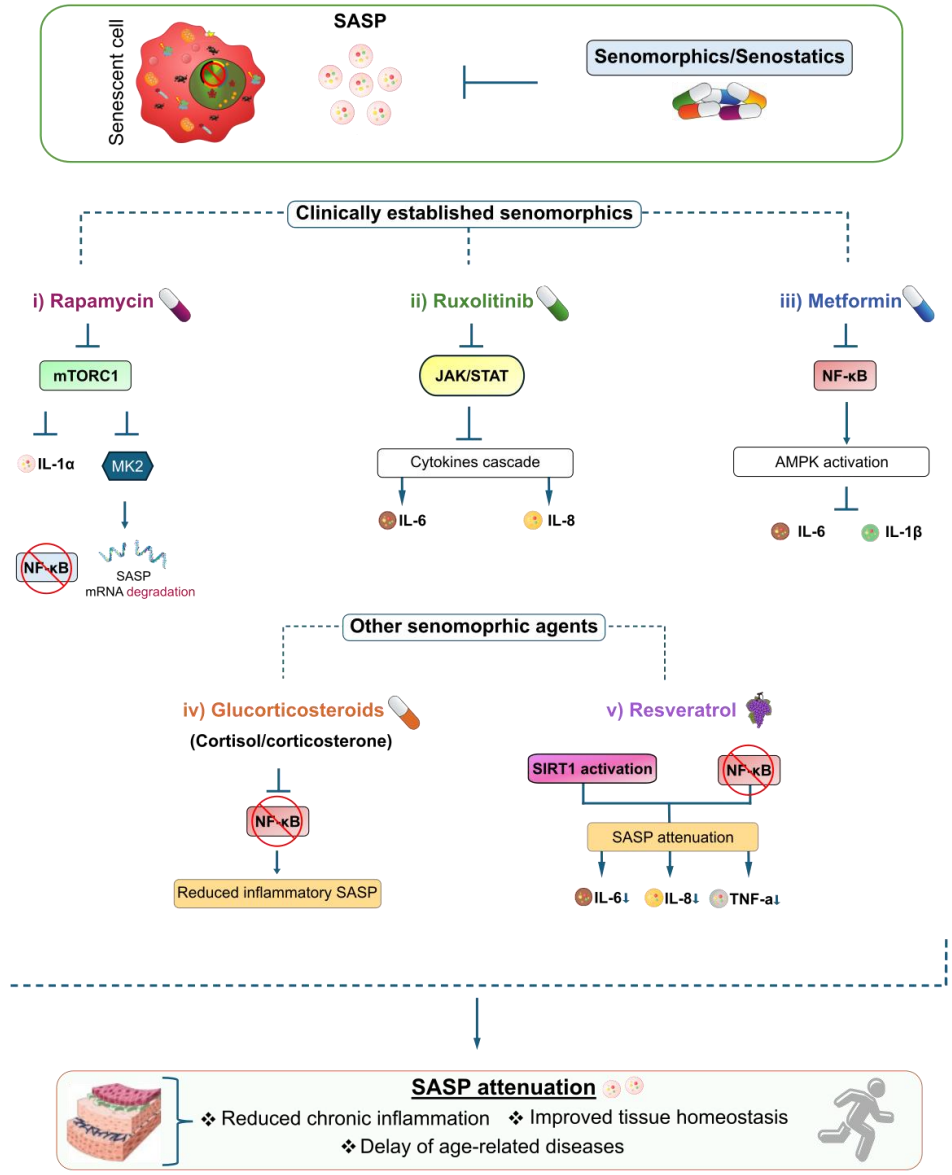
Nature Rev Bioengineering

… given that on a long-term basis senescence displays harmful effects the question that emerges is …



…how to neutralize their adverse effects or eliminate senescent cells (senolysis)?

Senomorphics/Senostatics



2026

Nature Rev Bioengineering

Genetic ablation of senescent cells increases lifespan and health span



Increased burden of senescent cells/aging



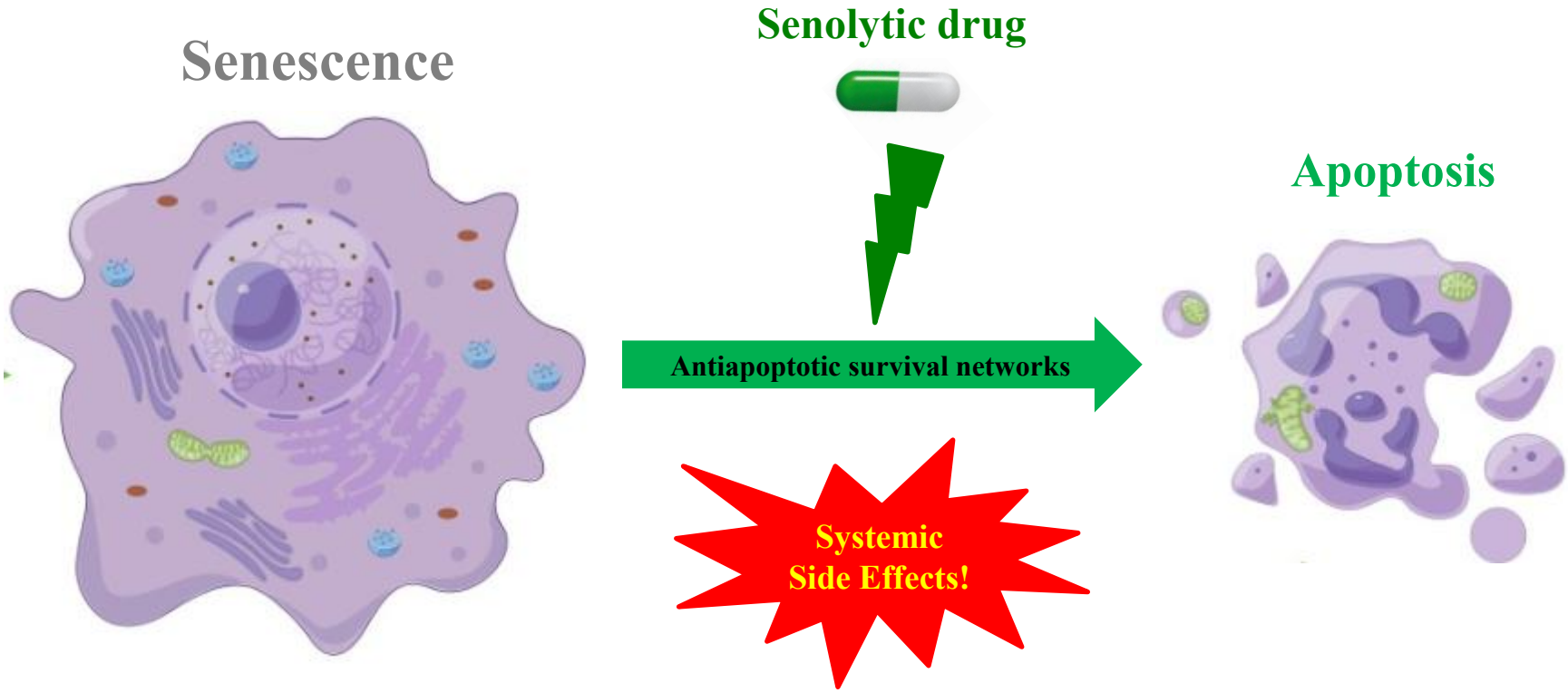
Two littermates, almost 2 years old; the mouse on the right had its senescent cells cleared by a drug from 1 year of age onwards. Credit: Jan Van Deursen



Elimination of senescent cells/longevity

Baker et al, Nature 2016
Callaway, Nature 2016

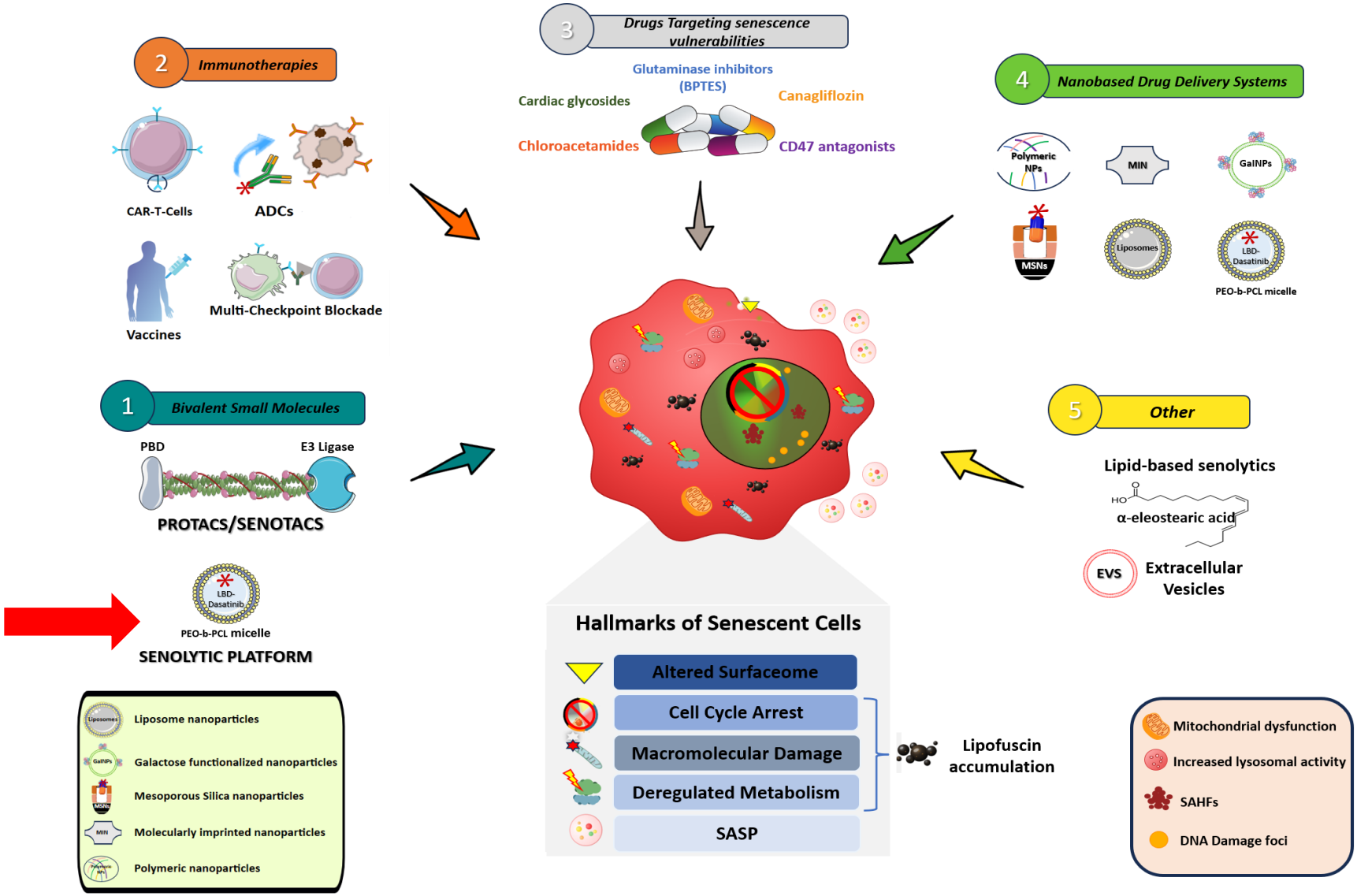
Senolytics (…result from drug repurposing strategies)





…how to achieve selective senolysis?

Existing next generation approaches for targeted senolysis



2026

Nature Rev Bioengineering


TARGETED ELIMINATION
of senescent cells

1837-2027
190
ΧΡΟΝΙΑ

Εργαστήριο Ιστολογίας-Εμβρυολογίας,
Ιατρική Σχολή ΕΚΠΑ

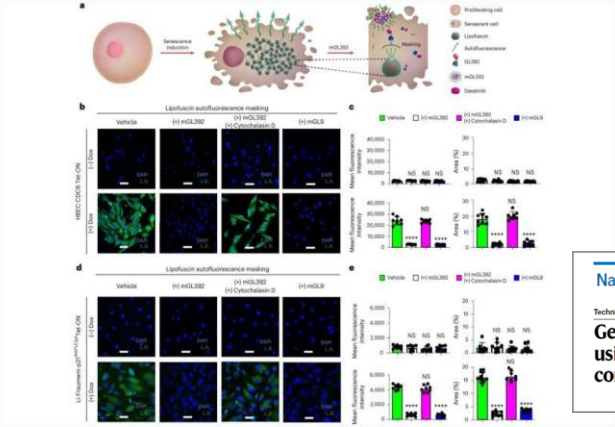
Διευθυντής: Καθ. Βασίλης Γ. Γοργούλης

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HMN 2025: How Platform paves the way in which for brand spanking new therapies towards age-related ailments

HEALTHMEDICINET / MARCH 25, 2025 / HEALTH



Nature Aging | Volume 5 | January 2025 | 162–175

Technical Report https://doi.org/10.1038/s43587-024-00747-4

Generation of a selective senolytic platform using a micelle-encapsulated Sudan Black B conjugated analog

Proper internalization of mGL392 and particular binding of its cargo to lipofuscin was validated primarily based on lipofuscin auto-fluorescence. Credit: *Nature Aging* (2024). DOI: 10.1038/s43587-024-00747-4

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FIGHT AGING!

Do you want to live a longer life in good health? Simple practices can make some difference, such as exercise or calorie restriction. But over the long haul all that really matters is progress in medicine: building new classes of therapy to repair and reverse the known root causes of aging. The sooner these treatments arrive, the more lives will be saved. Find out how to help »

January 7th, 2025

Senolytics Targeting Lipofuscin as a Signature of Cellular Senescence

Permalink (With Comments) Permalink (No Comments) Posted by Reason

No Comments Yet Add a Comment

Researchers here propose a way to make existing senolytic small molecules more efficient by attaching them to a compound that binds to lipofusin, a form of molecular waste originating in lysosomes that is hard to break down. The resulting compound senolytic molecule is encapsulated in some form of delivery system for cell uptake, here a micelle, but other forms of nanoparticle would probably also do the job. Lipofuscin accumulation is a feature of senescent cells. It is also found in very long-lived non-dividing cells in old tissues, such as neurons, so some thought should probably be given to limiting their exposure to such a drug.

The emerging field of senolytics is centered on eliminating senescent cells to block their contribution to the progression of age-related diseases, including cancer, and to facilitate healthy aging. Enhancing the selectivity of senolytic treatments toward senescent cells stands to reduce the adverse effects associated with existing senolytic interventions. Taking advantage of lipofuscin accumulation in senescent cells, we describe here the development of a highly efficient senolytic platform consisting of a lipofuscin-binding domain scaffold, which can be conjugated with a senolytic drug via an ester bond.

As a proof of concept, we present the generation of GL392, a senolytic compound that carries a dasatinib senolytic moiety. Encapsulation of the GL392 compound in a micelle nanocarrier (termed mGL392) allows for both in vitro and in vivo (in mice) selective elimination of senescent cells via targeted release of the senolytic agent with minimal systemic toxicity. Our findings suggest that this platform could be used to enhance targeting of senotherapeutics toward senescent cells.

Link: https://doi.org/10.1038/s43587-024-00747-4

Search

First Steps
- Read an Introduction to Living Longer
- Read the Fight Aging! FAQ
- Sign up for the Fight Aging! Newsletter
- Fund Meaningful Aging Research

Services
- Longevity Industry Consulting
- How to Start a Longevity Biotech Company

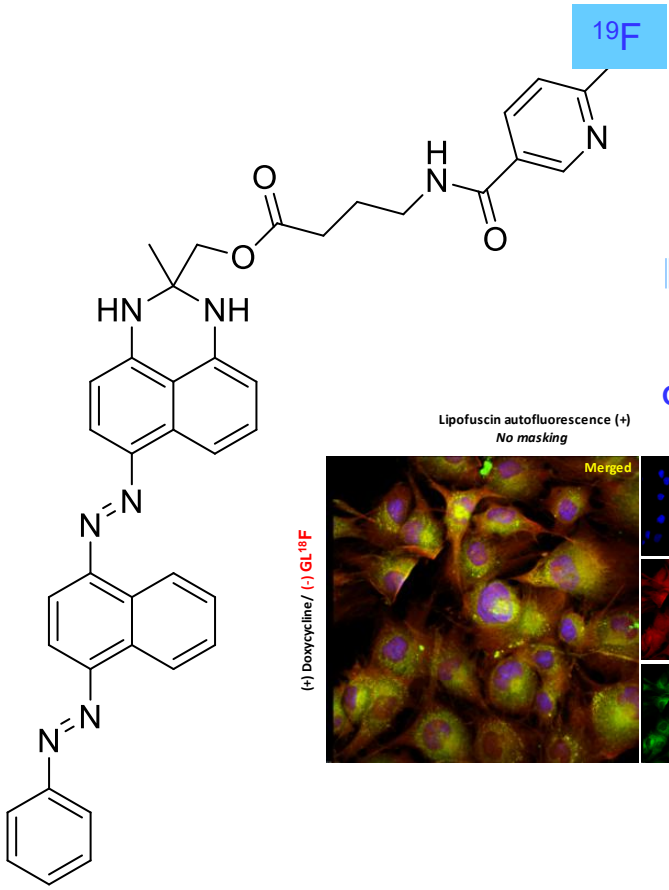
The Root Causes of Aging
- Aging is Caused by Damage
- Accumulating Cross-Links
- Buildup of Amyloid Between Cells
- The Failing Adaptive Immune System
- The Failing Innate Immune System
- Declining Lysosomal Function
- Mitochondrial DNA Damage
- Nuclear DNA Damage
- Buildup of Senescent Cells

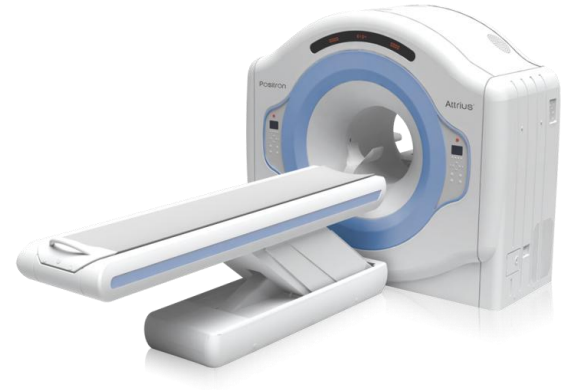
Archives and Feeds
- Monthly News and Blog Archives
- Newsletter Archive
- RSS Posts Feed
- RSS Comments Feed
- Using the Fight Aging! Content Feeds

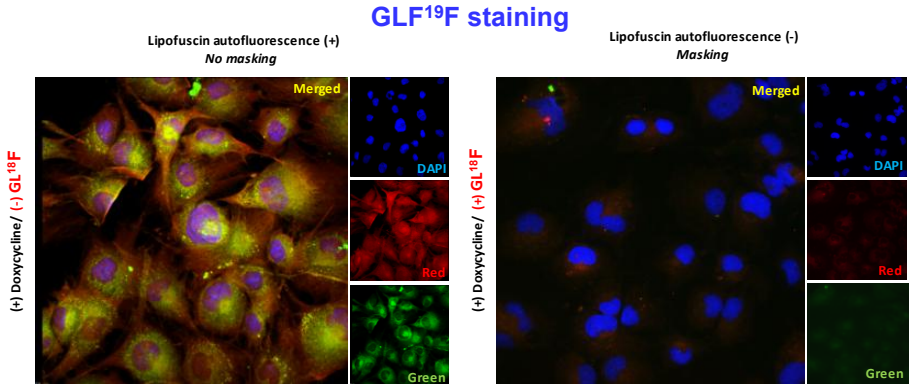
Required Reading
- A Proposal to Accelerate Progress
- Calorie Restriction and Longevity
- Cryonics, the Necessary Backup Plan

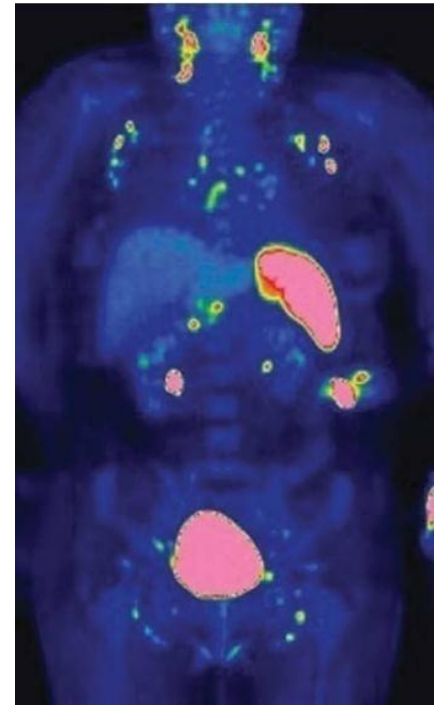
Current Research: Develop a radiotracer analogous to fluorodeoxyglucose (FDG) applied in ONCOLOGY to detect senescence in HUMANS applying PET-CT (Positron emission tomography–computed tomography)

Towards this direction we generated a "cold" analogue…

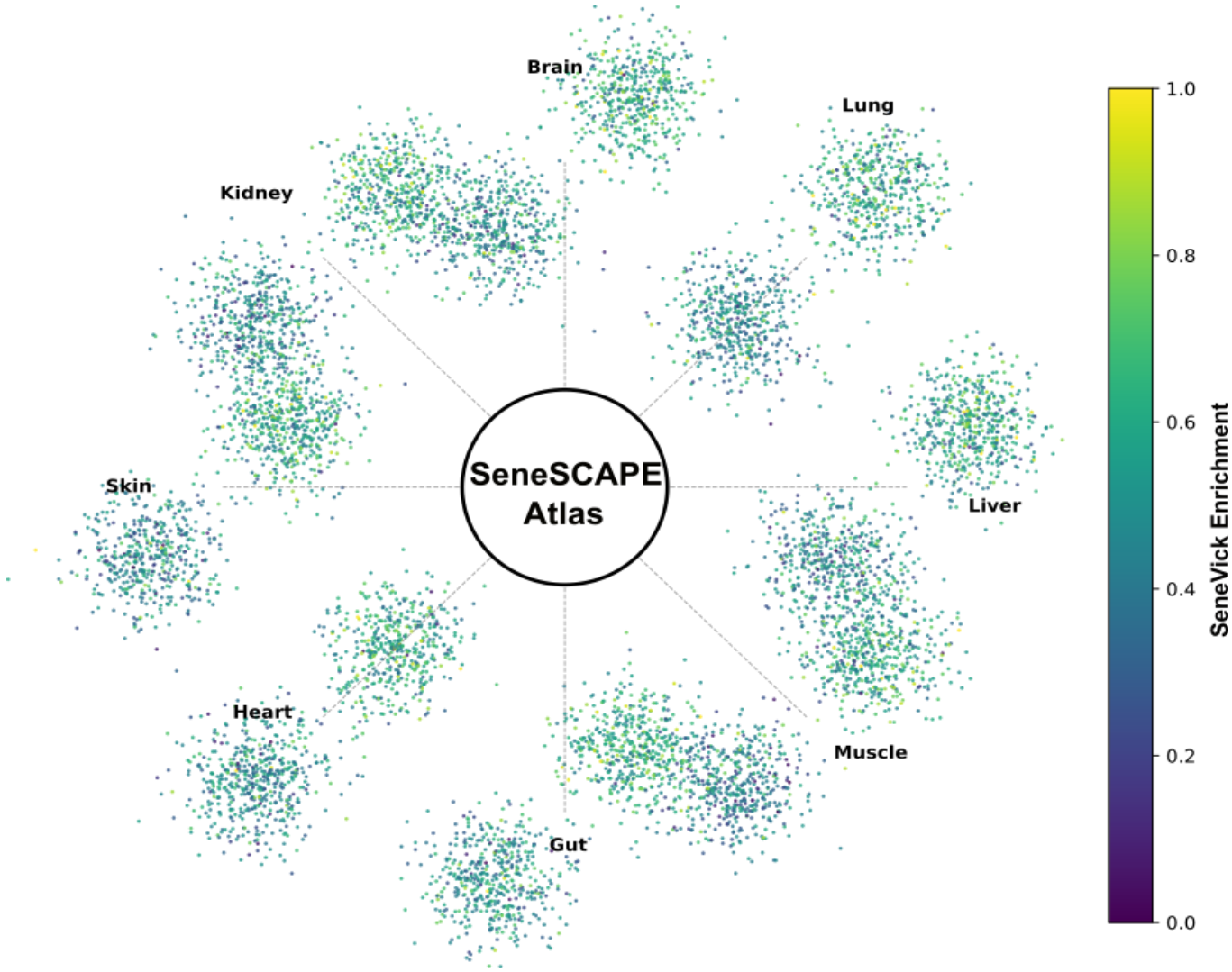




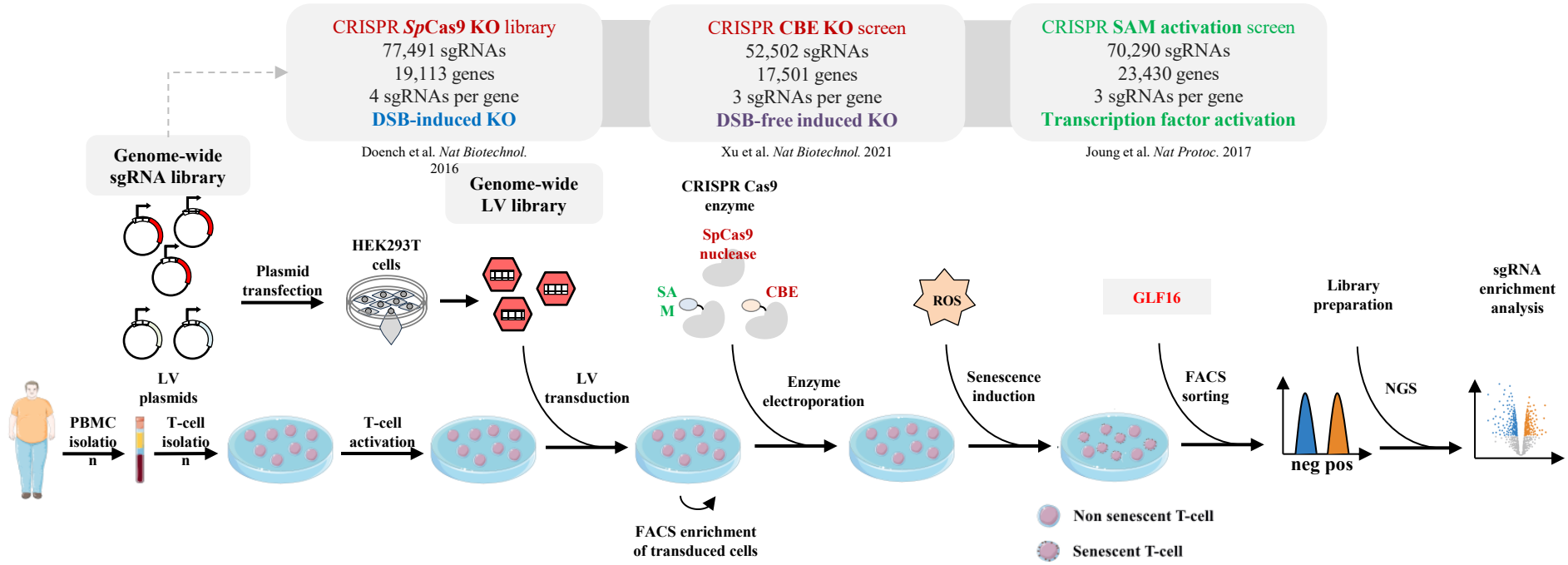




Development of a SENESCENCE ATLAS employing SeneVick



Identification of senescence drivers and pathways that could render T cells tolerable to senescent signals



Genome-wide LV library is produced by HEK293T transfection with:
- LV packaging and envelope plasmids
- Genome-wide sgRNA library

Activated T-cells are transduced by LV library at a MOI = 0.3
> Each cell incorporates only 1 sgRNA

Transduced T-cells are electroporated to receive the CRISPR Cas enzyme:
- 1 gene KO / cell
- 1 gene activation / cell

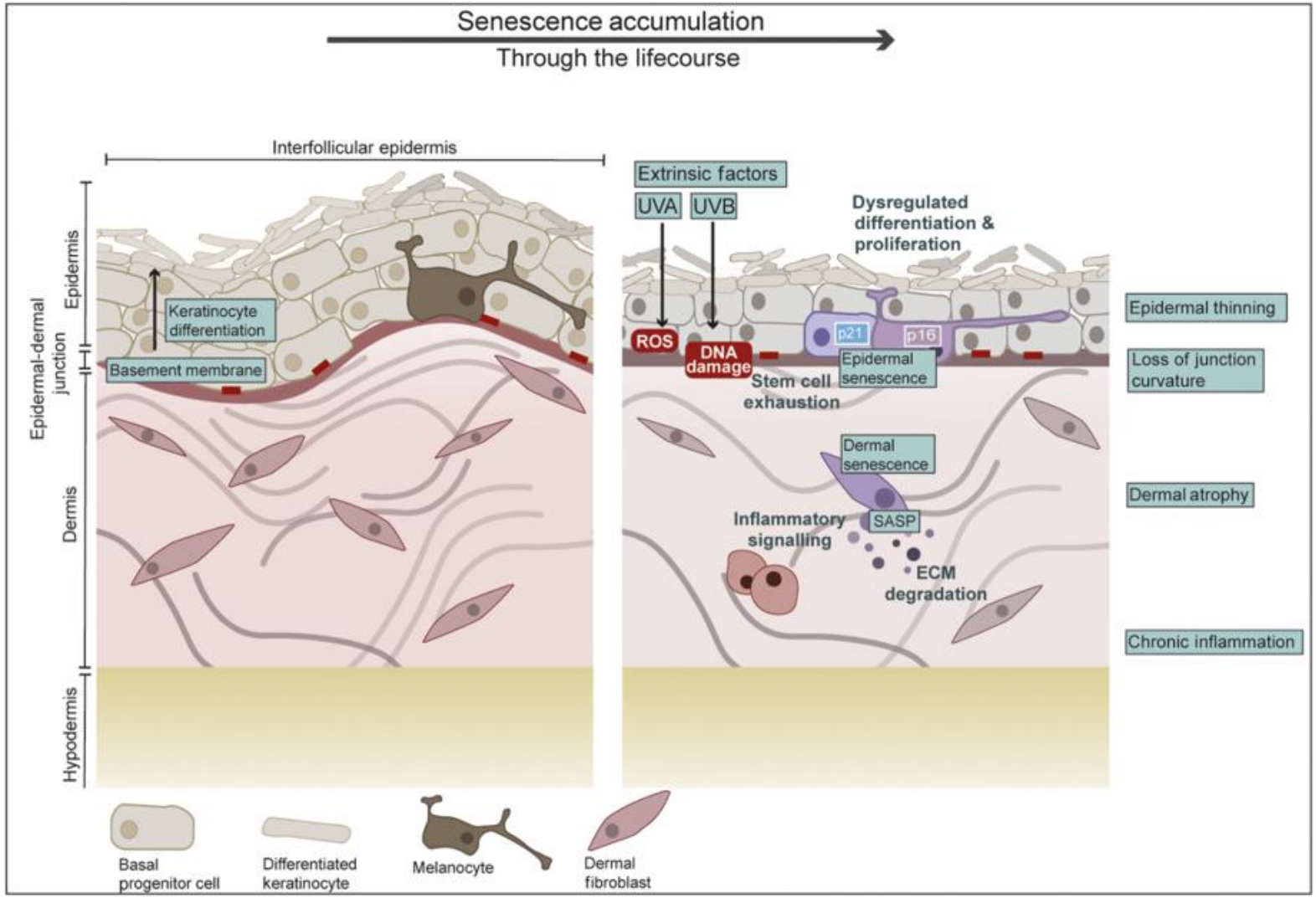
Senescence is induced

T-cells are sorted to
- senescent population
- non-senescent population

NGS analysis of sgRNA enrichment reveals target-genes that:
- Block T-cell senescence
- promote T-cell senescence

Clustered regularly interspaced short palindromic repeats, CRISPR; Knock-out, KO; Single-guide RNA, sgRNA; Double-strand break, DSB; Cytosine base editor, CBE; Synergistic activation mediator, SAM; Lentiviral vector, LV; Peripheral blood mononuclear cell, PBMC; Multiplicity of infection, MOI; Flow activated cell sorting, FACS; Reactive oxygen species, ROS; Next generation sequencing, NGS.

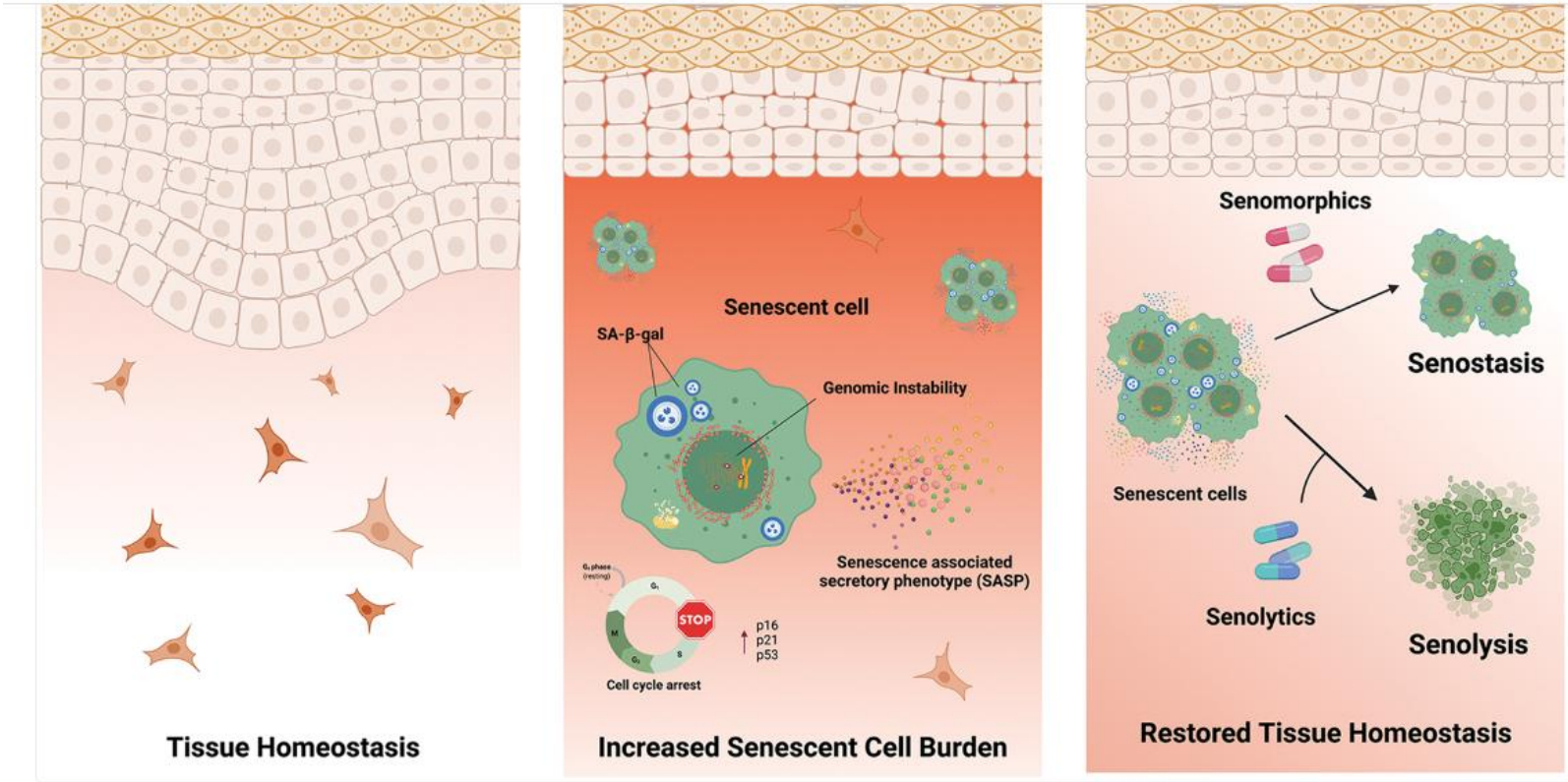
Cellular senescence and skin aging



Smith and Carroll, FEBS 2024

Senotherapeutics and Cosmetics

While laboratory models and small pilot trials demonstrate that topical senolytics/senomorphics can decrease cellular senescence burden, improve epidermal thickness, and support barrier function, most products occupy a gray zone between advanced cosmeceuticals and experimental science rather than FDA-approved or heavily regulated clinical drugs



Thompson et al, Plast Reconstr Surg 2022



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Partnership Agreement 2014 - 2020
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Εθνικόν και Καποδιστριακόν Πανεπιστήμιον Αθηνών



Thank you!