AFAR: RCCN
Measuring Biologic Age
January 19, 2022

Are the things we can measure useful?

Senescence Burden

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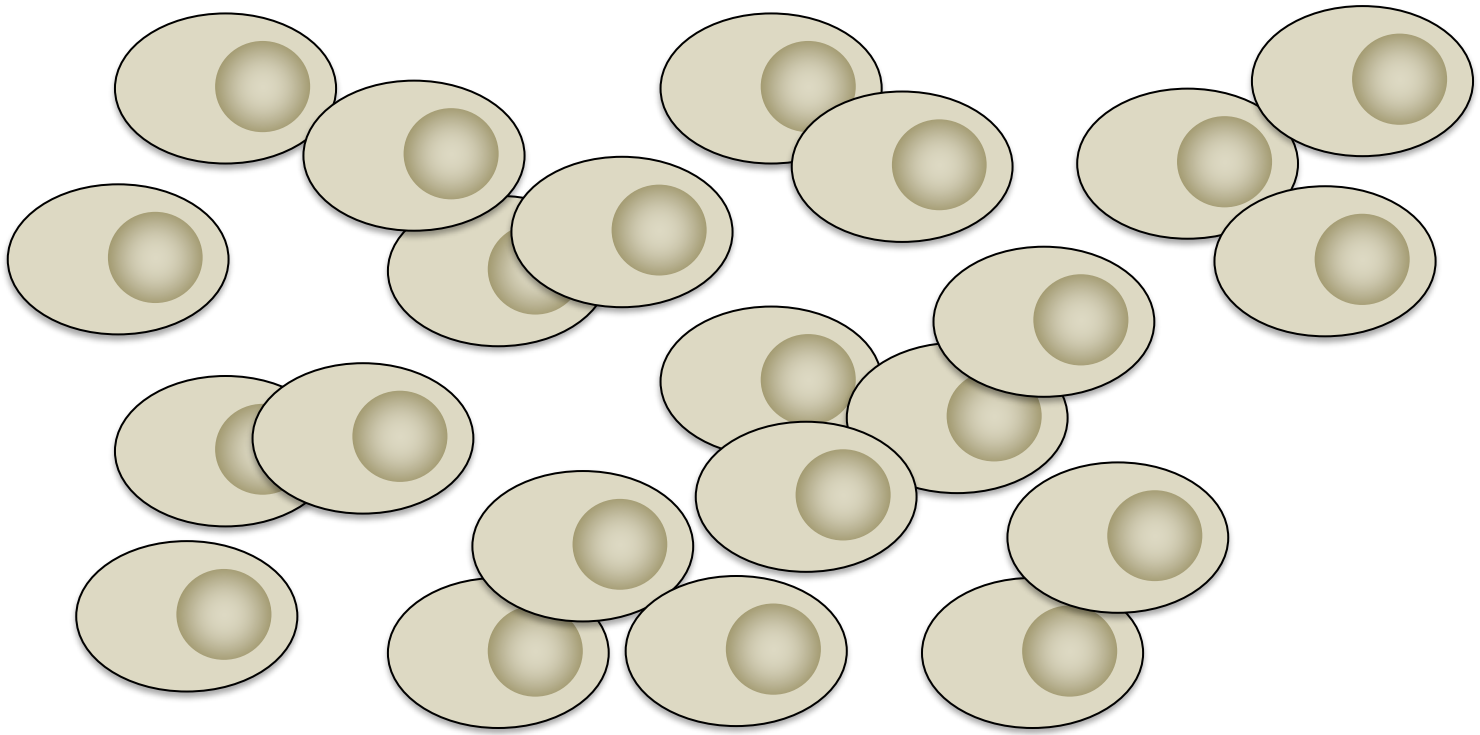
Cellular senescence: initially discovered as a limited replicative capacity *in vitro*.



Dr. Leonard Hayflick, UCSF
Past President: GSA
Founding Member of the Council of the NIA

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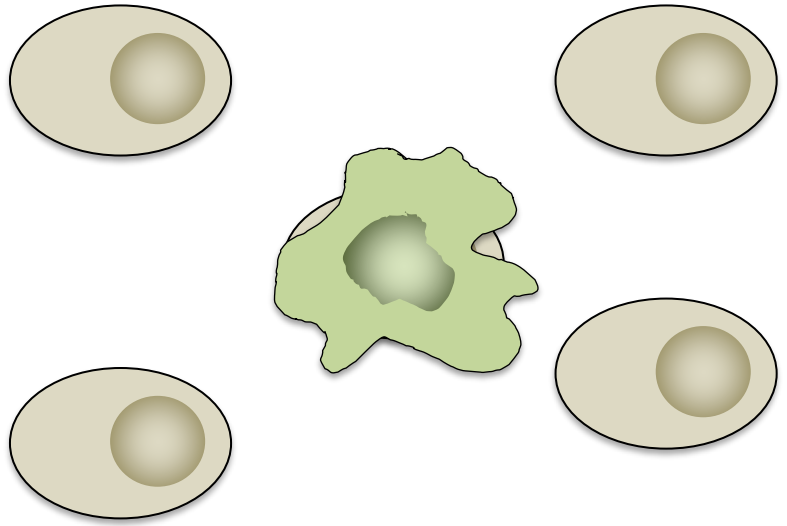
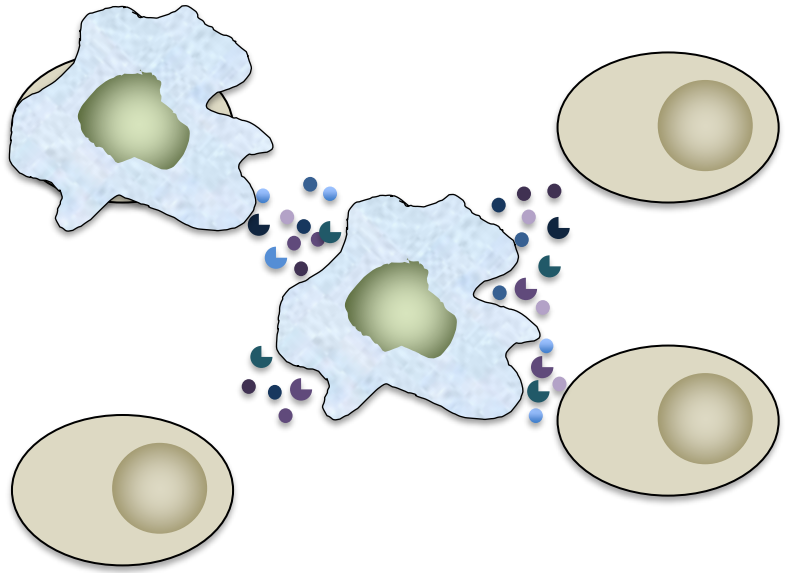


1961. Limited replicative capacity of human cells in culture: aging at the cellular level, “Cellular Senescence”. PMID: 13905658

Cellular senescence biomarker discovery *in vitro* has lead to their identification *in vivo*.



Biomarkers *In Vitro*.

1995. SA β-gal[1].

1996. *CDKN2A*/p16^{INK4a} regulates cell cycle arrest[2].

2008. Senescence associated secretory phenotype "SASP"[3].

2013. Senescence spread[4].

Senescence *In Vivo*

2002. Detected in human atherogenic plaques[5].

2006. Detected in aged primate skin[6].

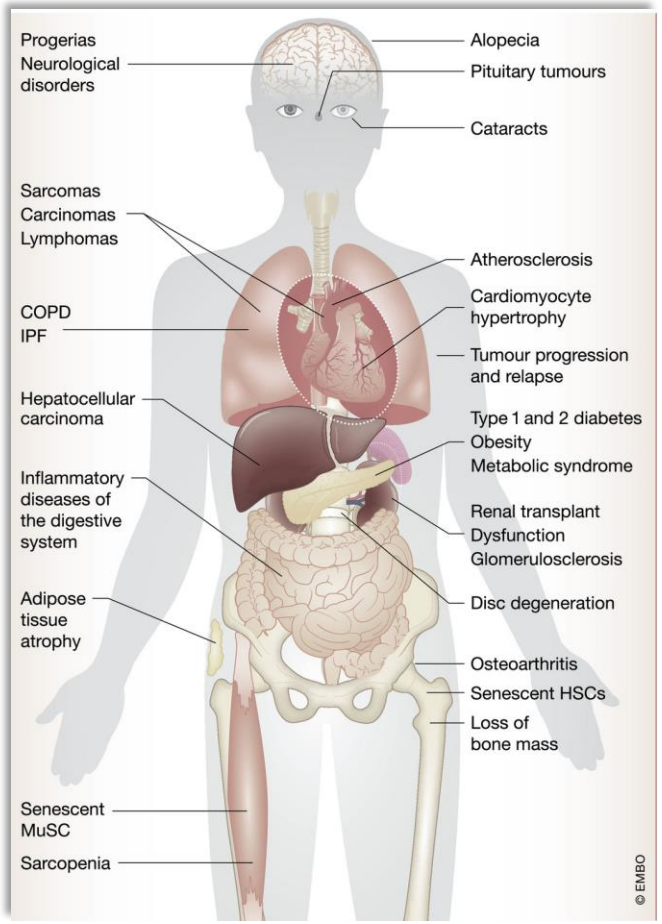
2011. Clearing senescent cells ameliorates progeroid phenotypes[7].

2016. Clearing senescent cells extends healthspan and lifespan[8].

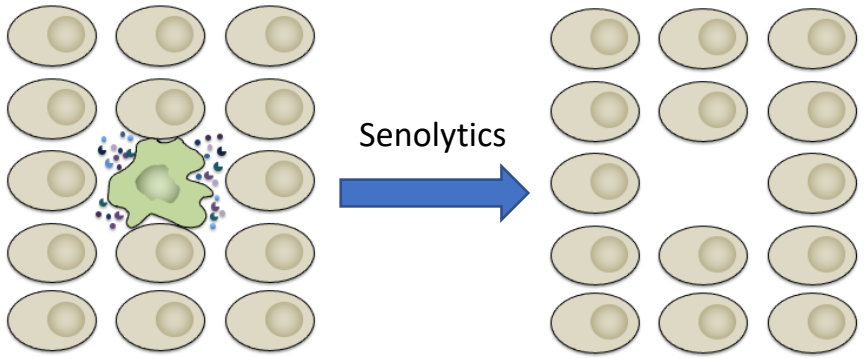
2016-Present: Senescent cell clearance head-to-tail…

[1]Dimri *et al*; (7568133); [2]Alcorta *et al* (8943005); [3]Coppe *et al* (19053174); [4]Acosta *et al* (23770676); [5]Minamino *et al* (11927518); [6]Herbig *et al* (16456035); [7]Baker *et al* (22048312); [8]Baker *et al* (26840489).

Senescent cells have been detected in tissues throughout the body, drug discovery and trials are underway.



Paez-Ribes *et. al*. (2019) *EMBO Mol Med.* PMID: 31746100



Zhu Y., *et al.,* (2015) *Aging Cell.* PMID: 25754370

Justice J., *et al.,* (2019) *J Gerontol A Biol Sci Med Sci.* PMID: 30616998

Table 1 | Selected biotech companies focused on senolytics

Company (year founded)	Business focus/technology
1E Therapeutics (2020)	Antisense oligonucleotide-based senolytics
Atropos Therapeutics (2018)	Targeting transition between quiescence and senescence (senescence after growth arrest, or SAGA)
Cleara Biotech (2018)	Targeting FOXO4 to release proapoptotic p53
Deciduous Therapeutics (2018)	Activating immune cells to clear senescent cells
Dialectic Therapeutics (2018)	Systemic delivery of senolytic agents using proteolysis-targeting chimeras (PROTACs)
Dorian Therapeutics (2018)	Targeting USP16, a deubiquitination enzyme, to reverse senescence
Eternans (2017)	FOXO4-binding peptide
FoxBio (2018)	Targeting p53/FOXO4 prosurvival pathways in senescent cells
Genome Protection (2018)	Stimulating innate immunity to eradicate genome-compromised cells
Geras Bio (2020)	SASP inhibitors
Insilico Medicine/ Taisho (2020)	AI target identification and generation/validation
NRTK Biosciences (2020)	Synthetic optimization of approved drugs and supplements
Numeric Biotech (2017)	Selective targeting of FOXO4-p53
Oisín Biotechnologies (2014)	Gene therapy with caspase-9 activated in p16-positive cells
Oncosence (2019)	Monoclonal antibodies targeting tumor cells after inducing them to senescence
OneSkin (2016)	Peptide that modulates senescence-related signaling pathways and enhances DNA repair
Recursion Pharma (2013)	AI drug discovery platform
Rejuversen (2020)	Antibody against PD-L2 that promotes immune-mediated clearance of senescent cancer cells
Rubedo Life Sciences (2018)	Small-molecule senolytics
Senisca (2020)	Antisense oligonucleotides against splicing factors
Senolytic Therapeutics (2017)	Senolytic and senomorphic drugs to treat fibrosis
SIWA Therapeutics (2006)	Antibody against glycation surface molecule
Unity Biotechnology (2011)	Targeting various senescence-related proteins (Bcl-xL)

Dolgin E. (2020) *Nat Biotechnol.* PMID: 33184478

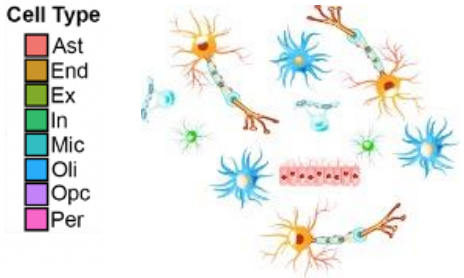
Single senescence biomarkers may have cell type biases and have highlighted the need for multi-analyte approaches.

PMID: 33799628
PMID: 33671362

- **Neurons**
  - p21 — PMID: 22882466 — Jurk D *et al.,* (2012) *Aging Cell*
  - NFT-transcriptome — PMID: 30126037 — Musi…OrrME, (2018) *Aging Cell*
  - SA β-gal — PMID: 31636448 — Chow HM *et al*., (2019) *Nat. Neurosci.*
  - p21 — PMID: 31543366 — Riessland M *et al.,* (2019) *Cell Stem Cell*
  - Transcriptome (p19 + NFTs) — *In Press* — Dehkordi…OrrME (2021) *Nature Aging*
- **Astrocytes**
  - p16 — PMID: 22984612 — Bhat R *et al.,* (2012) *PLoS One*
  - Lamin B1 — PMID: 29386135 — Chinta SJ *et al.,* (2018) *Cell Reports*
- **Microglia**
  - p16 — PMID: 30232451 — Bussian TJ *et al.,* (2018) *Nature*
  - p16 — PMID: 33470505 — Ogrodnik, M. *et al.,* (2021) *Aging Cell*
  - SA β-gal, SASP, MMP3 — PMID: 34669475 — Brelstaff *et al.,* (2021) *Sci. Adv.*
- **Oligodendrocyte precursor cells**
  - p16 — PMID: 30936558 — Zhang P *et al.,* (2019) *Nat. Neurosci.*
  - p16 — PMID: 33470505 — Ogrodnik, M. *et al.,* (2021) *Aging Cell*
- **Endothelial cells**
  - SASP — PMID: 33041998 — Bryant AG *et al.,* (2020) *Front Neurol*

We observed cell-type heterogeneity using single marker genes as surrogates for “senescence”.

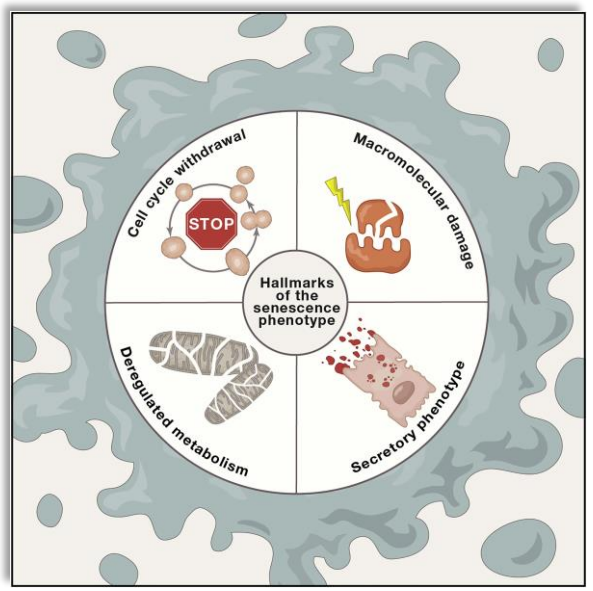
snRNASeq



~140,000 Cells
76 brains

Mathys *et. al.,* 2019. PMID: 31042697
Zhou *et. al.,* 2020. PMID: 31932797

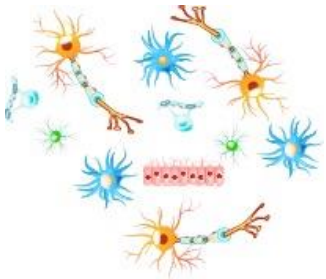
Dr. Habil Zare &
Shiva K. Dehkordi, UTHSA



Gorgoulis V *et. al.,* 2019 PMID: 31675495

We used a bioinformatic approach to included multiple genes, each set representing distinct aspects of cellular senescence.

snRNASeq



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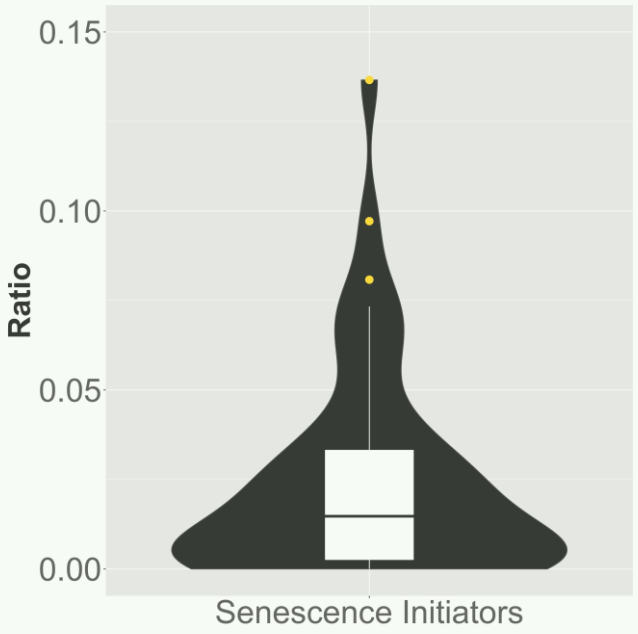
2.0%



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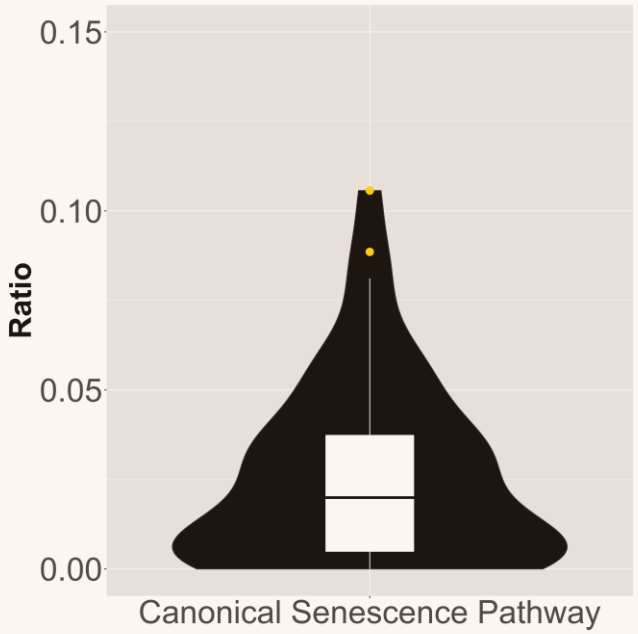

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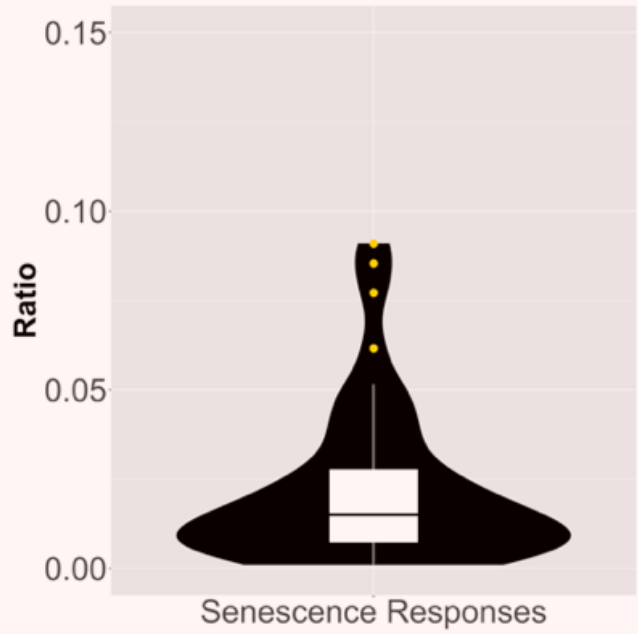
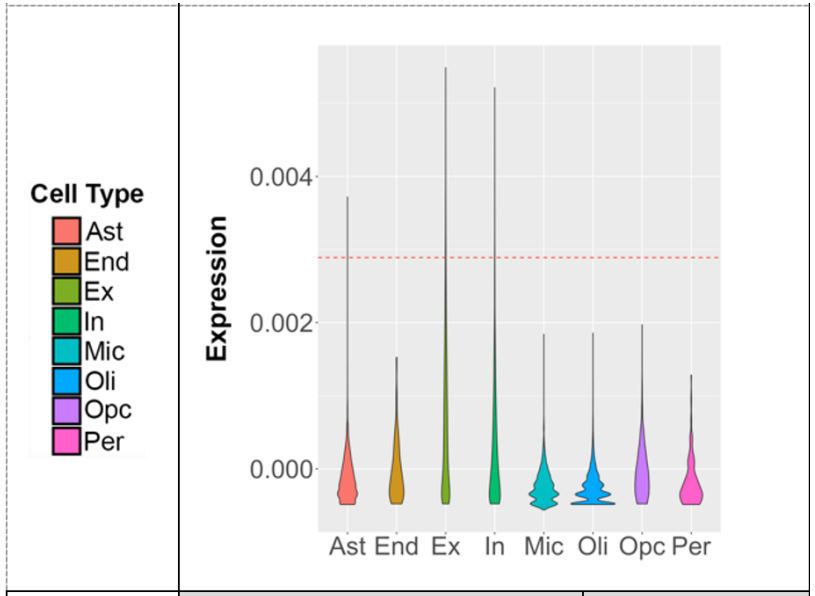


What are these cell types?

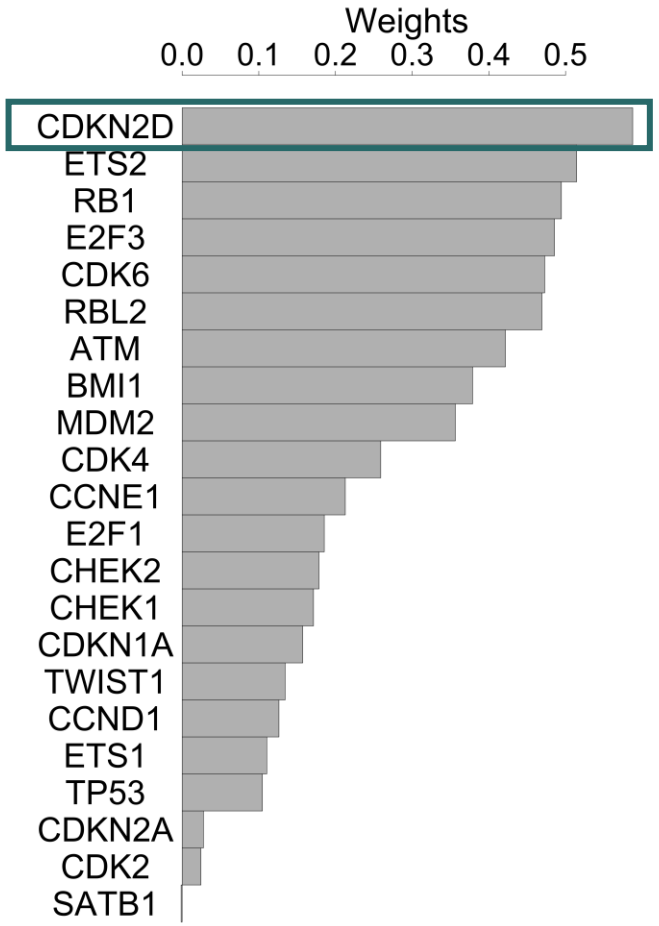
Excitatory neurons are the only cell type that are represented in all three eigengenes.

SIP: Initiating

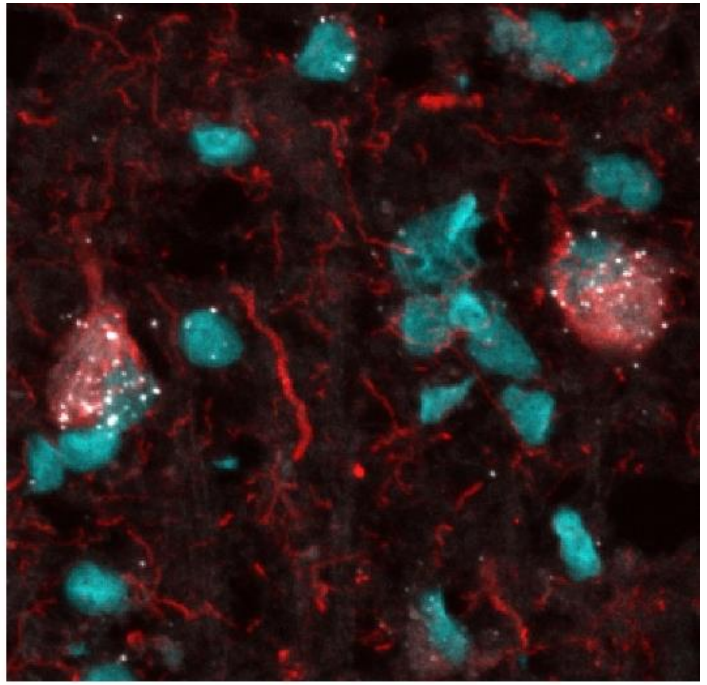


Cell Types	Cell Numbers		log10(p-value)
	Normal	Senescent	
Ast	3,391	1	~0
End	121	0	0
Ex	33,734	1,242	-2019
In	9,088	108	~0
Mic	1,920	0	0
Oli	18,235	0	0
Opc	2,627	0	0
Per	167	0	0
Total	69283	1351	

Target validation using RNAscope, immunohistochemistry and immunofluorescence indicate p19-expressing neurons have tau neuropathology.

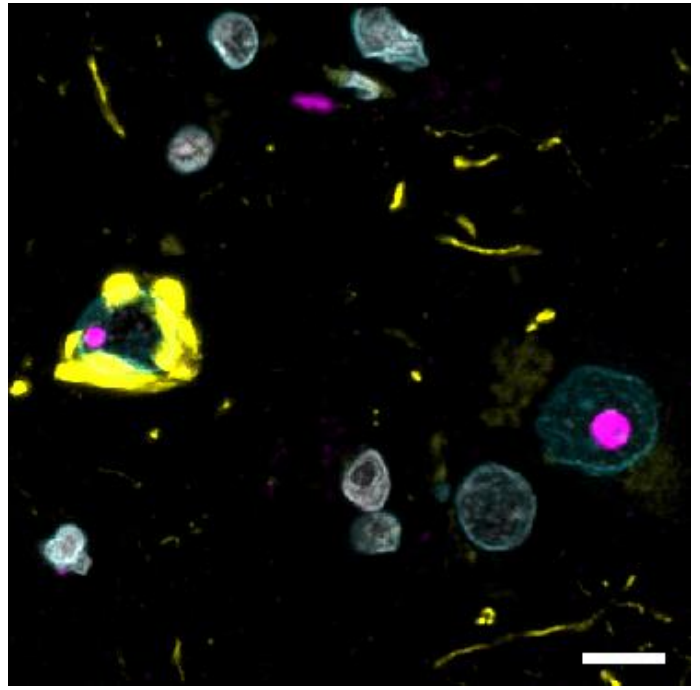


RNAscope



Dr. Rachel Bennett & Benjamin Woost, MGH/Harvard

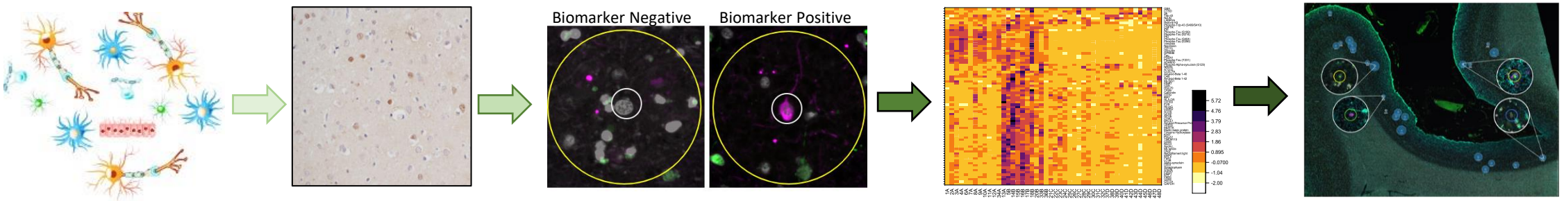
p19 Histology



Eric Jamie Walker, UTHSA

Dehkordi S…**Orr ME** (2021) *Nature Aging*

These data and approaches open new areas of research with opportunities for major advances.



1. Unbiased Screen
Transcriptomics

2. Low-plex Validation
IHC

3. Cell Selection Using Validated Biomarkers

4. High-Plex Analyses
GeoMx DSP

5. High Resolution Maps of Cells and Environments

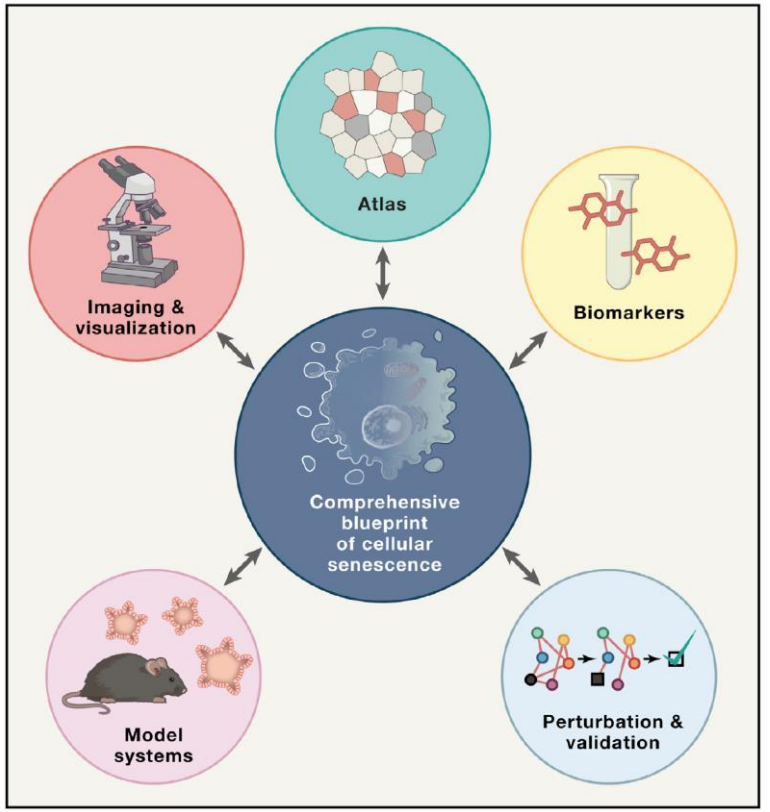
- Once senescent cells are identified, determine if they have functional consequences in people.
  - Cognitive resiliency vs dementia.
  - Clearance in clinical trials.

- Identify peripheral biomarkers unique and specific to distinct senescent cell type.
- Use biomarkers to develop *in vivo* imaging techniques.
- Understand mechanisms governing cellular senescence in post mitotic cells.

Major gaps in the field with opportunities for research.

The Common Fund's **Cellular Senescence Network (SenNet)** Program

Vision: To identify and functionally characterize the heterogeneity of senescent cells across multiple tissues in human health and lifespan at single cell resolution.



A Blueprint for Characterizing Senescence. Roy *et al. Cell.* 2020

Acknowledgements

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- Dr. Tamara Tchkonia

MGH 1811

- Dr. Rachel Bennett
- Benjamin Woost

Orr Lab is Recruiting!
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Department of Veterans Affairs

New Vision Research

Averill Family

Cure Alzheimer's Fund

Alzheimer's Drug Discovery Foundation

NIH NIA

Wake Forest School of Medicine

Clinical and Translational Science Institute

NCDRC North Carolina Diabetes Research Center

NIH National Institute of Neurological Disorders and Stroke

OAIC National Coordinating Center
Claude D Pepper Older Americans Independence Centers