

Nov 17th - 19th, 2025

Venue: Spanish National Cancer Research Centre  
CNIO Auditorium — Madrid • Spain

# Molecular and Cellular Hallmarks of Aging: 3rd edition

## Organising committee

**Maria Blasco**  
Spanish National Cancer  
Research Centre,  
Madrid, Spain

**Alejo Efeyan**  
Spanish National Cancer  
Research Centre,  
Madrid, Spain

**Thomas Rando**  
University of California  
Los Angeles, US

## Speakers

**Adam Antebi**  
MPI for the Biology of Aging,  
Cologne, Germany

**Mary Armanios**  
Johns Hopkins, Baltimore,  
US

**Steve Artandi**  
Stanford Cancer Institute,  
California, US

**Salvador Aznar-  
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IRB Barcelona,  
Barcelona, Spain

**Ana Maria Cuervo**  
Albert Einstein  
College of Medicine,  
NY, US

**Jesús Gil**  
Medical Research Council  
(MRC), London, UK

**Vera Gorbunova**  
University of Rochester,  
NY, US

**Pekka Katajisto**  
Helsinki University,  
Helsinki, Finland

**María Mittelbrunn**  
Centre for Molecular Biology  
Severo Ochoa (CBM),  
Madrid, Spain

**Daniel Muñoz-  
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**Laura  
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University of Minnesota,  
US

**Lenhard Rudolph**  
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**Manuel Serrano**  
Altos Labs, Cambridge  
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**Sheila Stewart**  
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**Ashley Webb**  
Buck Institute for Research  
on Aging, Novato, US

**Eileen White**  
Rutgers Cancer Institute,  
Ludwig Institute for Cancer  
Research, New Jersey, US

Madrid 17<sup>th</sup> – 19<sup>th</sup> Nov 2025

**Molecular and Cellular  
Hallmarks of Aging:  
3rd edition**

Spanish National Cancer Research Centre (CNIO)  
Madrid, Spain

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Madrid 17<sup>th</sup> - 19<sup>th</sup> Nov 2025

# Molecular and Cellular Hallmarks of Aging: 3rd edition

# #CFM\_ MolCellAging

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# Molecular and Cellular Hallmarks of Aging: 3rd edition

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Madrid 17<sup>th</sup> – 19<sup>th</sup> Nov 2025

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Madrid 17<sup>th</sup> - 19<sup>th</sup> Nov 2025

# Molecular and Cellular Hallmarks of Aging: 3rd edition

## Organisers & Speakers

Madrid 17<sup>th</sup> – 19<sup>th</sup> Nov 2025

# Molecular and Cellular Hallmarks of Aging: 3rd edition

*Venue:*

Spanish National Cancer Research Centre – CNIO Auditorium, Madrid.

*Organizing committee:*

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Spanish National Cancer Research Centre,  
Madrid, Spain

**Alejo Efeyan**

Spanish National Cancer Research Centre,  
Madrid, Spain

**Thomas Rando**

University of California Los Angeles, US

**CNIO - CaixaResearch Frontiers Meeting**

*Speakers*

**Adam Antebi**  
MPI for the Biology of  
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**Vera Gorbunova**  
University of Rochester, NY,  
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**Lenhard Rudolph**  
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**Mary Armanios**  
Johns Hopkins, Baltimore,  
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**Pekka Katajisto**  
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**Sheila Stewart**  
Washington University in  
St. Louis, St. Louis, US

**Ana Maria Cuervo**  
Albert Einstein  
College of Medicine,  
NY, US

**Laura  
Niedernhofer**  
Institute on the Biology  
of Aging & Metabolism  
(iBAM) -

**Ashley Webb**  
Buck Institute for Research  
on Aging, Novato, US

**Jesús Gil**  
Medical Research Council  
(MRC), London, UK

University of Minnesota,  
US

**Eileen White**  
Rutgers Cancer Institute,  
Ludwig Institute for Cancer  
Research, New Jersey, US



Madrid 17<sup>th</sup> - 19<sup>th</sup> Nov 2025

# Molecular and Cellular Hallmarks of Aging: 3rd edition

## Programme

**Monday November 17th, 2025****08:30-09:15** *Registration - main hall***09:15-09:30** *Welcome address***09:30-12:30** **Session 1. Aging Pathways***Chair: **Maria Blasco***

**09:30-10:00** *NAD/NADH levels limit dietary restriction induced stress responses and health benefits in old mice*  
**Lenhard Rudolph**  
 Leibniz Institute Aging, Jena, Germany

**10:00-10:30** *Epigenetic reprogramming cellular age*  
**Thomas Rando**  
 University of California Los Angeles, US

**10:30-11:00** *Poster session - coffee break - social room*

**11:00-11:15** *short talk Mechanisms of Calorie Restriction: Lowering of Body Temperature.*  
**Bruno Conti**  
 San Diego Biomedical Research Institute, San Diego, US

**11:15-11:30** *short talk Orphan ribosomal proteins as drivers of aging and neurodegeneration.*  
**Óscar Fernández Capetillo**  
 Spanish National Cancer Research Centre (CNIO), Spain

**11:30-12:00** *Immunosenescence as the Central Mediator of Age-Related Vulnerability*  
**Maria Mittelbrunn**  
 Centre for Molecular Biology Severo Ochoa (CBM), Madrid, Spain

**12:00-12:30** *Multi-omics analysis of longevity mechanisms across species*  
**Vera Gorbunova**  
 University of Rochester, NY, US

**12:30-13:45** *Lunch break - Canteen*

Monday November 17th, 2025

13:45-15:45 Session 2. Cancer and Aging I

Chair: **Manuel Serrano**

- 13:45-14:15 *Aging triggers a pro-tumorigenic senescent microenvironment in the lung*  
**Daniel Muñoz-Espín**  
 CRUK Cambridge Centre,  
 Cambridge, UK
- 14:15-14:30 *short talk Fasting enhances chemotherapy: molecular, immune and metabolic insights*  
**Pablo Fernández**  
 Altos Labs UK Limited,  
 Little Abington, UK
- 14:30-15:00 *Dissecting the biology of time-restricted feeding*  
**Alejo Efeyan**  
 Spanish National Cancer Research Centre,  
 Madrid, Spain
- 15:00-15:15 *short talk "Resolvin" inflammation in senescence and ageing*  
**Ana O'Loghlen**  
 Center for Biological Research (CIB),  
 Madrid, Spain
- 15:15-15:45 *Cancer as a Systemic Disease*  
**Eileen White**  
 Rutgers Cancer Institute,  
 Ludwig Institute for Cancer Research,  
 New Jersey, US
- 15:45-16:15 *Poster session - coffee break - social room*

Monday November 17th, 2025

**16:15-17:30 Session 3. Regulation of Aging**

Chair: **María Mittelbrunn**

16:15-16:45 *Metabolic regulation of cell fate through age-selective segregation of organelles*

**Pekka Katajisto**

Helsinki University,

Helsinki, Finland

16:45-17:00 *short talk Using CRISPR barcoding as a molecular clock to capture dynamic processes at single-cell resolution*

**Irene Herráez**

Institute of Molecular Biology of Barcelona (IBMB),

Barcelona, Spain

17:00-17:30 *On Line Circadian rhythms during health and aging*

**Salvador Aznar-Benitah**

IRB Barcelona,

Barcelona, Spain

**17:30-19:00 Poster session - refreshments for all participants - social room**

*Tuesday November 18th, 2025*

**09:30-12:15 Session 4. Cancer and Aging II**

*Chair: **Guadalupe Sabio***

- 09:30-10:00 *Selective Autophagy in the fight against aging and age-related diseases*  
**Ana Maria Cuervo**  
 Albert Einstein College of Medicine, NY, US
- 10:00-10:15 *short talk Discovery of a new class of senolytics derived from animal venoms*  
**Maria Ikonopoulou**  
 IMDEA Nutrition, Madrid, Spain
- 10:15-10:45 *The cellular and pathophysiological effects of endogenous DNA damage, a primary hallmark of aging*  
**Laura Niedernhofer**  
 Institute on the Biology of Aging & Metabolism (iBAM);  
 University of Minnesota, US
- 10:45-11:30 Group picture CNIO main entrance / poster session & coffee break - social room**
- 11:30-12:00 *Transcriptional and epigenetic mechanisms of brain aging and neurodegeneration*  
**Ashley Webb**  
 Buck Institute for Research on Aging, Novato, CA, US
- 12:00-12:15 *short talk Modulating senescence in pancreatic adenocarcinoma with targeted nanotherapies.*  
**Magdalani Panagiotakopoulos**  
 Memorial Sloan Kettering Cancer Center,  
 New York, US
- 12:15-13:45 Lunch break - Canteen**

*Tuesday November 18th, 2025*

**13:45-17:00 Session 5. Telomeres and Aging Diseases**

*Chair: **Ana María Cuervo***

- 13:45-14:15 Regulation of telomere protection*  
**Maria Blasco**  
 Spanish National Cancer Research Centre,  
 Madrid, Spain
- 14:15-14:30 short talk Remodeling of Intracellular Ca<sup>2+</sup> Homeostasis in rat hippocampal neurons during In vitro aging*  
**Carlos Villalobos Jorge**  
 Institute of Biomedicine and Molecular Genetics of  
 Valladolid (IBGM), University of Valladolid & CSIC. Spain.
- 14:30-15:00 Lessons from Disorders of Telomere Length*  
**Mary Armanios**  
 Johns Hopkins, Baltimore, US
- 15:00-15:15 short talk Neuronal CG and non-CG DNA methylation coupling is a conserved hallmark of aging*  
**José Vicente Sánchez Mut**  
 Neuroscience Institute (UMH-CSIC),  
 Alicante, Spain
- 15:15-16:00 **Poster session - coffee break - social room***
- 16:00-16:30 Molecular Regulation of Telomerase in Aging and Cancer*  
**Steven Artandi**  
 Stanford Cancer Institute,  
 California, US
- 16:30-17:00 Stress signaling in T Cells: Balancing Metabolic Health, Immune Exhaustion, and Aging*  
**Guadalupe Sabio**  
 Spanish National Cancer Research Centre - CNIO, Spain
- 17:00-18:00 **Poster session - refreshments for all participants - social room***

Wednesday, November 19th, 2025

09:30-12:15 **Session 6. Senescence, Reprogramming and Aging**

Chair: **Thomas Rando**

09:30-10:00 *Nutrient regulation of age-reversal and senescence*

**Adam Antebi**

*MPI for the Biology of Aging,  
Cologne, Germany*

10:00-10:15 *short talk Senomorphic Effect of Genetic and Chemical Partial Reprogramming*

**Manuel Collado**

*Center for Research in Molecular Medicine  
and Chronic Diseases (CiMUS),  
Santiago de Compostela, Spain*

10:15-10:45 *Stromal senescence in tumor progression and therapy-induced comorbidities*

**Sheila Stewart**

*Washington University in St Louis,  
St. Louis, US*

10:45-11:15 **Poster session - coffee break - social room**

11:15-11:45 *Cellular senescence as a therapeutic target*

**Jesus Gil**

*Medical Research Council (MRC),  
London, UK*

11:45-12:15 *Decoding the Role of Mitochondria in Cellular Senescence*

**Manuel Serrano**

*Altos Labs,  
Cambridge, UK*

12:15 **Poster/talk prizes**

**Wrap up and closing**



Madrid 17<sup>th</sup> - 19<sup>th</sup> Nov 2025

# Molecular and Cellular Hallmarks of Aging: 3rd edition

Monday Nov 17<sup>th</sup> 2025

## Session #1 Aging Pathways

Chairperson: **Maria Blasco**

## NAD/NADH levels limit dietary restriction induced stress responses and health benefits in old mice

**K. Lenhard Rudolph, M.D.**

Research Group on Stem Cell and Metabolism Aging  
Leibniz Institute on Aging - Fritz Lipmann Institute (FLI)  
Jena, Germany

Dietary restriction (DR) extends health span and lifespan across various species, but for unknown reason its efficacy diminishes, when initiated at old age. Our study demonstrates that food-supplementation of nicotinamide adenine dinucleotide (NAD<sup>+</sup>) via nicotinamide riboside (NR) enhances DR-mediated nutrient stress response specifically in old mice. The study shows that late life NRDR has synergistic effects on the elevation of NAD<sup>+</sup>/NADH levels resulting in improvements in proteostasis and molecular hallmarks of aging in various tissues. Only the combined treatment leads to improvements in hematopoietic stem cell function and a robust elongation of lifespan in both sexes when the treatment is initiated in already old mice. Together, these results establish a new, limiting role of NAD/NADH for the induction of nutrient stress responses at old age and identify synergism between DR and NR supplementation in alleviating hallmarks of aging and promoting longevity when initiated at old age.







## Epigenetic reprogramming cellular age

### Thomas A. Rando, M.D., Ph.D.

Director, UCLA Broad Stem Cell Research Center  
Professor, Neurology  
Professor, Molecular, Cell and Developmental Biology  
UCLA Broad Stem Cell Research Center  
University of California, Los Angeles, US

The expression of Yamanaka factors induces the dedifferentiation and rejuvenation of various cell types into induced pluripotent stem cells. However, the effects of the expression of these factors in terminally differentiated cells has not been studied in detail. We have found that expression of Yamanaka factors in muscle myofibers does not lead to defects in muscle structure or function. Consistent with previous findings, we found that myofiber reprogramming positively impacts the function of associated muscle stem cells. In addition, we find that the expression of expression of Yamanaka factors in muscle myofibers leads to enhancement of distant tissues, presumably via the secretion of myokines from the muscle. We are currently examining the changes in the serum proteome as a result of myofiber reprogramming to identify putative myokines that mediate the beneficial effects on distant tissues.

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## Mechanisms of Calorie Restriction: Lowering of Body Temperature

**Bruno Conti**

San Diego Biomedical Research Institute

Calorie restriction (CR), defined as a reduction in caloric intake without malnutrition, has been shown to enhance both healthspan and lifespan through mechanisms that remain only partially understood. In this presentation, we will explore evidence suggesting that one key pathway through which CR confers its anti-aging and protective effects in rodents is by lowering core body temperature (T<sub>b</sub>). Furthermore, we will examine how reduced T<sub>b</sub> influences aging biology at the molecular and cellular levels.

Promoting health and survival through lowered body temperature.

Conti B, de Cabo R.

Nat Aging. 2025 May;5(5):740-749. doi: 10.1038/s43587-025-00850-0. Epub 2025 Apr 9.

PMID: 40205073







## Orphan ribosomal proteins as drivers of aging and neurodegeneration

Anabel Sáez-Mas, Guillermo de la Vega-Barranco, Oleksandra Sirozh, Amin El- Manchoud, Vanesa Lafarga and **Óscar Fernández-Capetillo**

Spanish National Cancer Research Centre, CNIO, Madrid, Spain

Aging is the strongest risk factor for most neurodegenerative diseases, yet the molecular mechanisms that explain how aging contributes to neuronal loss remain poorly understood. Recent work in our lab demonstrated that the most common genetic alteration in ALS—a hexanucleotide expansion in C9ORF72—is associated to the accumulation of orphan ribosomal proteins, which is a hallmark of other diseases known as “ribosomopathies”. Such an accumulation is known to be detrimental for animal cells, since due to the very high abundance of ribosomal proteins, this leads to the saturation of the machineries that clear protein aggregates such as the proteasome or autophagy pathways. Furthermore, our previous work shows that, in mice, this accumulation drives accelerated aging, further reinforcing the links between aging and neurodegeneration.

We are currently expanding our model and exploring whether the presence of a ribosomopathy is a general hallmark of aging and ALS. To do so, we are evaluating if the accumulation of orphan ribosomal proteins occurs in response to a wide range of mutations or environmental agents known to increase the incidence of ALS, as well as in experimental models of aging (such as killifish or mice). In addition, we are using transcriptional signatures from ALS patients, to determine if their transcriptome is also indicative of an underlying ribosomopathy. In summary, our objective is to determine if the accumulation of orphan ribosomal proteins might be one of the mechanisms by which aging impacts on the onset of neurodegenerative diseases.



Lined area for notes



## Immunosenescence as the Central Mediator of Age-Related Vulnerability

### Maria Mittelbrunn

Group Leader of the Immunometabolism & Inflammation Laboratory  
Molecular Biology Center, CSIC-UAM  
Madrid, Spain

Aging is the strongest risk factor for cancer, infectious diseases, cardiovascular decline, and neurodegeneration. This increased vulnerability is largely driven by immunosenescence, the progressive deterioration of the immune system with age. Immunosenescence reshapes immune homeostasis, weakens immune surveillance, and contribute to inflammaging.

To investigate the consequences of the aging of the immune system, we have induced age-associated mitochondrial dysfunction prematurely in T cell. Targeting mitochondrial function in T cells recapitulates metabolic, phenotypic and functional features of aged T cells, including susceptibility to infections and premature inflammaging. We found that inducing age-associated mitochondrial decline in T lymphocytes, does not only cause an immunometabolic dysfunction that drives T cell senescence, but actually causes a general, body-wide deterioration of health with multiple aging-related features, including metabolic, musculoskeletal, cardiovascular and cognitive alterations, altogether resulting in premature death. Thus, premature aging of T lymphocytes may be 'contagious', driving a generalized acceleration of aging throughout multiple organ systems. Our results place the metabolism of T cells at the crossroad between inflammation, senescence and aging, highlighting that immunometabolism can be a therapeutic target to delay aging.

In this presentation, we will unravel the molecular mechanisms by which aged immune cells fuel inflammaging and age-related pathologies, and we will explore emerging therapeutic strategies aimed at promoting healthy aging. Notably, we are developing approaches to rejuvenate aged T cells and reverse immunosenescence. By integrating genetic, cellular, pharmacological, and nutritional interventions, we seek to restore T cell functionality, with the ultimate goal of enhancing immune competence to support healthy aging.

**FUNDING:** This study was supported by the European Regional Development Fund (ERDF) and the European Commission through H2020-EU.1.1, European Research Council grant ERC-2021-CoG 101044248-LetTBe



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## Multi-omics analysis of longevity mechanisms across species

### Vera Gorbunova, Ph.D.

Doris Johns Cherry Professor of Biology and Medicine  
Co-director Rochester Aging Research Center  
Director Nathan Shock Center of Excellence in Basic Biology of Aging  
University of Rochester - Department of Biology  
Rochester, NY, USA

The molecular basis of mammalian longevity remains poorly understood, particularly which pathways—and their constituent proteins—are differentially regulated across tissues and plasma in species spanning vastly different lifespans. We performed deep proteomic profiling across six tissues from over forty mammalian species, including the short-lived shrew and the long-lived bowhead whale, spanning more than a 100-fold range in maximum lifespan. Long-lived species exhibited lower expression of mitochondrial and ribosomal proteins but higher levels of stress-responsive chaperones, endoplasmic reticulum, lysosomal, membrane-organizing, nuclear structural, and extracellular matrix proteins, reflecting enhanced proteostasis and tissue maintenance. Comparative plasma proteomics implicated immune, proteasomal, and nutrient-sensing pathways in lifespan regulation. Notably, extracellular vesicle (EV) abundance correlated positively with lifespan, and EV proteomics and small RNA sequencing revealed enrichment of proteins and miRNAs targeting inflammatory and nutrient-sensing pathways. These findings reveal conserved molecular hallmarks of longevity, highlight protein candidates as potential lifespan interventions, and identify extracellular vesicles (EVs) as potential modulators of longevity.





Lined area for notes with horizontal ruling lines.



Madrid 17<sup>th</sup> - 19<sup>th</sup> Nov 2025

# Molecular and Cellular Hallmarks of Aging: 3rd edition

Monday Nov 17<sup>th</sup> 2025

## Session #2 Cancer and Aging I

*Chairperson: Manuel Serrano*

## Aging triggers a pro-tumorigenic senescent microenvironment in the lung

Diana Campos-Iglesias<sup>1,2,#</sup>, Joaquín Araos Henríquez<sup>1,2,#</sup>, Jianfeng Ge<sup>1,2</sup>, Robert Rintoul<sup>2</sup>, Scott Haston<sup>3</sup>, Juan Pedro Martínez-Barbera<sup>3</sup>,

**Daniel Muñoz-Espín<sup>1,2,\*</sup>**

1. Early Cancer Institute, Department of Oncology, University of Cambridge, UK

2. CRUK Cambridge Centre Thoracic Cancer Programme, Cambridge, UK

3. UCL Great Ormond Street Institute of Child Health, London, UK

\* Leading author

# These authors contributed equally

Lung adenocarcinoma (LUAD) is a disease of unmet need due to its high lethality and limited therapeutic options. In a previous study, we found a population of senescence macrophages playing a fundamental role in lung carcinogenesis and, notably, accumulating in naturally aged lungs in the absence of oncogenic stress. While the incidence of LUAD increases with age, the composition of an aged microenvironment and how it relates to lung cancer initiation and progression remains poorly understood.

Here, we investigated how ageing modulates the landscape of senescent cells (SnCs) in normal lungs prior tumour initiation and during early stages of carcinogenesis. To address this, we employed young (~3-months-old) and aged (~18-months-old) p16-FDR mice. In this model, mCherry and the diphtheria toxin receptor (DTR) are expressed under the control of the *Cdkn2a* promoter (encoding for p16<sup>INK4A</sup>) to selectively trace and eliminate SnCs in vivo. By leveraging three mouse models of LUAD based on the p16-FDR allele, including genetically-engineered mice (*Kras*<sup>G12D</sup>), orthotopic transplantation of KP cells (*Kras*<sup>G12D</sup>; *Trp53*<sup>fl/fl</sup>) and carcinogen-induced LUAD using N-methyl-N-nitrosourea, we show that the selective ablation of SnCs in aged lungs ameliorates tumorigenesis. Our results highlight the role of ageing on shaping a microenvironment featured by immune senescent cells, including specific populations of macrophages and dendritic cells.

This study has important implications for the identification of early carcinogenesis biomarkers and novel strategies for lung cancer prevention in high-risk individuals.

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## Fasting enhances chemotherapy: molecular, immune and metabolic insights

Andrés Pastor-Fernández<sup>1</sup>, Marta Barradas<sup>1</sup>, Adrian Plaza<sup>1</sup>, Manuel Montero Gómez de las Heras<sup>2</sup>, Jose Ignacio Escrig-Larena<sup>2</sup>, Cristina Pantoja<sup>1</sup>, Iolanda Lázaro<sup>3</sup>, Jose Luis Lopez-Aceituno<sup>1</sup>, Gonzalo Colmenarejo<sup>4</sup>, Lidia Daimiel<sup>5</sup>, Manuel Serrano<sup>6</sup>, Ana Ramirez de Molina<sup>7, 8</sup>, Alejo Efeyan<sup>9</sup>, Viviana Loria-Kohen<sup>7</sup>, Oscar J Pozo<sup>10</sup>, Aleix Sala-Vila<sup>3,11</sup>, Maria Mittelbrunn<sup>2</sup>, Lola Martinez<sup>12</sup>, **Pablo Jose Fernandez-Marcos<sup>1</sup>**

1. Metabolic Syndrome Group (BIOPROMET), Madrid Institute for Advanced Studies (IMDEA) Food, E28049, Madrid, Spain
2. Molecular Biology Center Severo Ochoa (CBMSO), Spanish National Research Council (CSIC), Madrid Autonoma University (UAM), Madrid, Spain
3. Cardiovascular risk and nutrition, Hospital del Mar Medical Research Institute—IMIM, Barcelona, Spain
4. Biostatistics and Bioinformatics Unit, CEI UAM+CSIC, Madrid Institute for Advanced Studies—IMDEA Food, Madrid, Spain
5. Nutritional Genomics of Cardiovascular Disease and Obesity, Madrid Institute for Advanced Studies—IMDEA Food, Madrid, Spain
6. Institute for Research in Biomedicine (IRB Barcelona), Barcelona Institute of Science and Technology (BIST), Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain
7. Nutrition and Clinical Trials Unit, Platform GENYAL, CEI UAM+CSIC, Madrid Institute for Advanced Studies—IMDEA Food, Madrid, Spain
8. Molecular Oncology and Nutritional Genomics of Cancer Group, CEI UAM+CSIC, Madrid Institute for Advanced Studies—IMDEA Food, Madrid, Spain
9. Metabolism and Cell Signaling Laboratory, Spanish National Cancer Research Centre (CNIO), Madrid, Spain
10. Applied Metabolomics Research Group, Hospital del Mar Medical Research Institute—(IMIM), Barcelona, Spain
11. Fatty Acid Research Institute, Sioux Falls, SD USA
12. Flow Cytometry Unit, Biotechnology Programme, Spanish National Cancer Research Centre (CNIO), Madrid, Spain

Fasting during chemotherapy treatment enhances anti-tumor efficacy and reduces secondary effects in mice and humans. In turn, fasting triggers a plethora of responses that are not yet fully understood: it shows a marked sexual dimorphism; it induces the expression of the *Cdnl1a/p21Cip1* gene and protein; and it changes the cell membrane lipid composition in different tissues. Using in vivo genetic and allograft models, human interventions and lipidomics, we have observed that: (I) Fasting-mediated changes in membrane lipid composition in erythrocytes is tightly associated to the ability of fasting to prevent chemotherapy toxicity in mice; (II) Induction of p21 is necessary for the fasting-mediated protection from chemotherapy toxicity and for the enhanced anti-tumor efficacy of the combination of chemotherapy and fasting; (III) Fasting shows a dramatic sexual dimorphism for certain tumour types and chemotherapy responses, affecting differently the antitumour immune responses in male and female mice. Our results constitute a consistent support to the beneficial potential of fasting during chemotherapy, illuminate novel mechanisms by which fasting exerts these beneficial effects, and open novel paths to personalized optimization of this nutritional intervention in the clinic.





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## Dissecting the biology of time-restricted feeding

### Dr. Alejo Efeyan

Group Leader, Metabolism and Cell Signaling Lab, Tumour Biology Programme  
Spanish National Cancer Research Centre - CNIO  
Madrid, Spain

Different forms of dietary restriction (DR) have been empirically used in model organisms as an extremely efficacious intervention to extend healthspan and lifespan. Caloric restriction, fasting-mimicking diet, ketogenic diet, intermittent fasting, and time-restricted feeding (TRF), among other interventions, all result in a spectrum of health benefits. In particular, TRF without a reduction in total daily caloric intake has shown safety and is relatively easy to comply with, as compared to other interventions. Our understanding of the molecular players in the effects of TRF is rudimentary. Yet, a discrete number of signaling pathways have been proposed to mediate its benefit, including those evolutionarily fixed to control cellular and physiological responses to nutrient availability. Among these, the mechanistic target of rapamycin complex 1 (mTORC1) pathway stands out by its ability to control anabolic metabolism across tissues in response to raises in intracellular levels of nutrients and insulin after feeding. We and others have shown that metabolic responses to fasting require inhibition of mTOR via the nutrient Rag GTPase signaling and the insulin-AKT-TSC axis. Moreover, we have observed that a daily regime of TRF results in a long period of mTORC1 inhibition in the liver, as compared to ad libitum fed mice. We hypothesize that nutrient and hormone sensing and signaling pathways in hepatocytes mediate at least part of the beneficial effects of TRF. I will present data, using the liver as a critical metabolic organ that responds to dietary regimes, on the genetic dissection of nutrient and hormone signaling cues to mTORC1 as critical mediators of the effects of long-term TRF. The dissection of specific players and components within these signaling cascades can pave the future way for the therapeutic adoption of DR-mimicking drugs.







**“Resolvin” inflammation in senescence and ageing**

Mario Mira-Carnicer<sup>1</sup>, Marta Menéndez-García<sup>1</sup>, Celia Palomino-Lozano<sup>1</sup>  
and **Ana O’Loghlen<sup>1</sup>**

1. Epigenetics & Cellular Senescence Group, Biological Research Centre (CIB), Spanish National Research Council (CSIC), Madrid, Spain

Ageing is considered as a process where molecular, cellular and tissular function is impaired. One classic cellular phenotype that increases during ageing is cellular senescence. Upon senescence, the cells stop proliferating and release a variety of cytokines, chemokines and extracellular vesicles. However, the release of biomolecules derived from lipids (lipid mediators, LM) are not well characterised in senescence and ageing. Here, we found that senescent cells release different resolvins (E1, D1, and D2) during senescence. In accordance, the resolvin biosynthesis pathway is activated as observed by an increase in their corresponding receptors and enzymes implicated in their biogenesis. This pathway is conserved not only during senescence but also in fibroblasts derived from aged human individuals, aged mice and during other inflammatory responses. Interestingly, resolvins are enriched in small extracellular vesicles (sEV) in comparison with the soluble fraction during senescence. In addition, sEV isolated from young human donors ameliorate inflammation and the biogenesis of resolvins both in vitro and in aged mice. In summary, here we will present data showing that the resolvins biogenesis pathway is induced in ageing and cellular senescence.







## Autophagy and Metabolic Dysregulation in Cachexia

### Eileen White

Rutgers Cancer Institute, Rutgers University  
Ludwig Princeton Branch, Ludwig Institute for Cancer Research, Princeton University

Cancer is a metabolic disease. Oncogenic events alter tumor cell metabolism to produce building blocks and mitigate redox stress while suppressing the high energy consuming functions of normal professional cells. Tumor cells also engage nutrient scavenging pathways (e.g. micropinocytosis for extracellular nutrients and autophagy for recycling of intracellular nutrients) to sustain metabolism. In advanced cancer, factors produced by tumors drive systemic inflammation and the wasting of host tissues, particularly the dedicated nutrient stores of muscle and fat, in a process known as cachexia. Cancer cachexia is responsible for most cancer deaths, but the underlying mechanisms are unclear. Determining who cancer metabolism is altered and how tumors alter the metabolism and function of host tissues can identify new targets for cancer therapy.

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Madrid 17<sup>th</sup> - 19<sup>th</sup> Nov 2025

# Molecular and Cellular Hallmarks of Aging: 3rd edition

Tuesday Nov 18<sup>th</sup> 2025

## Session #3 Regulation of Aging

*Chairperson: **María Mittelbrunn***

### Metabolic regulation of cell fate through age-selective segregation of organelles

**Pekka Katajisto**<sup>1,2</sup>

1. Faculty of Biological and Environmental Sciences, University of Helsinki, Finland

2. Department of Cell and Molecular Biology, Karolinska Institutet, Sweden

Heterogeneity within subcellular components, such as organelles, has emerged as a potent regulator of cellular functions and differentiation. We found that the chronological age of organelles within a cell provides a proxy for identifying functional organelle sub-classes. Moreover, cells can asymmetrically apportion such functionally distinct organelle age-classes in cell divisions, and thereby divert the functions of the two daughter cells. In asymmetric stem cell divisions, the age-selective segregation of mitochondria and peroxisomes drives a metabolic divergence that guides one daughter cell to remain a self-renewing stem cell whereas the other daughter cell differentiates. I will discuss how “old” mitochondria and peroxisomes differ from their “young” counterparts, the ongoing work on metabolic cell fate determination, and how organellar metabolism can be used to target tissue renewal and aging.



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## Using CRISPR barcoding as a molecular clock to capture dynamic processes at single-cell resolution

Yolanda Andres-Lopez<sup>1</sup>, Alice Santambrogio<sup>2,3</sup>, Ioannis Kafetzopoulos<sup>2,3</sup>, Christopher Todd<sup>2,3</sup>, Celia Alda-Catalinas<sup>2,4</sup>, Stephen J. Clark<sup>2,3</sup>, Wolf Reik<sup>2,3</sup> & **Irene Hernando- Herrera**<sup>2,1</sup>

1. Department of Cells and Tissues, Instituto de Biología Molecular de Barcelona, Consejo Superior de Investigaciones Científicas (IBMB-CSIC), Barcelona, Spain
2. Epigenetics Programme, Babraham Institute, Cambridge, United Kingdom
3. Altos Labs, Granta Park, Cambridge, United Kingdom
4. Genomic Sciences, GSK, Stevenage, United Kingdom

Biological processes are fundamentally dynamic, yet existing methods for capturing these temporal changes are limited. We present scDynaBar, a novel approach that integrates CRISPR-Cas9 dynamic barcoding with single-cell sequencing to enable the recording of temporal cellular events. In this system, genetic barcodes accumulate mutations over a 4-week timeframe and then are sequenced together with the transcriptome of each single cell. We propose that this gradual accumulation of genetic diversity can be exploited to create a time-ordered record of cellular events. To demonstrate this, we apply the system to track the transition from a pluripotent state to a two-cell (2C)-like state in mouse embryonic stem cells (mESCs). The results provide compelling evidence for the transient nature of the 2C-like state. Additionally, our system shows consistent mutation rates across diverse cell types in a mouse gastruloid model, underscoring its robustness and versatility across various biological contexts. This technique not only improves our ability to study cellular dynamics but also creates exciting new opportunities for future applications based on recording temporal signals at the single-cell level—in other words, using dynamic barcoding as a molecular clock.



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## Circadian rhythms during health and aging

### Salvador Aznar

IRB Barcelona, Barcelona, Spain

Our body's circadian clock allows cells to "know" the time of the day and to function according to it. This incredible mechanism ensures that all tissues function in a synchronized manner, which is essential for remaining healthy. Importantly, our clock progressively fails as we age, significantly contributing to organ degeneration, obesity, arthritis, loss of vision, infections, and cancer. Within the brain, the central clock located in the hypothalamus senses changes in light and communicates this information to all tissues in our body, which themselves communicate with each other to perform their daily functions in a concerted manner. How does this communication network happen? Why is synchronicity lost during aging? How does the misalignment of peripheral clocks contribute to age-related pathologies?

In the past decade we have progressively defined tissue-intrinsic clock functions, and mapped systemic nodes that govern clock communication between the central clock in the brain and peripheral tissues. Our current efforts involve identifying systemic signals (proteins, metabolites and lipids) that synchronize tissue function to ensure daily physiology, which are mis-regulated during aging, and that might offer potential anti-aging intervention points. I will present data obtained from different complex in vivo models that are allowing us to pursue such objectives, to ultimately obtain an atlas of the connections that ensure a coherent daily physiology, and of the critical clock nodes that "fail" during aging and that can be targeted to promote a healthier aging.

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Madrid 17<sup>th</sup> - 19<sup>th</sup> Nov 2025

# Molecular and Cellular Hallmarks of Aging: 3rd edition

Tuesday Nov 18<sup>th</sup> 2025

## Session #4 Cancer and Aging II

*Chairperson: **Guadalupe Sabio***

## Selective Autophagy in the fight against aging and age-related diseases

**Dr. Ana Maria Cuervo MD PhD<sup>1</sup>**

1. Institute for Aging Studies, Albert Einstein College of Medicine, New York, NY 10461 USA

Malfunctioning of autophagy has recently been recognized as one of the primary drivers of aging. We have found that a selective form of autophagy, known as chaperone-mediated autophagy (CMA), is markedly reduced with age in most organs and tissues. Although the decline occurs in both males and females, the kinetics and mechanisms underlying CMA loss of function are sex-specific in many organs.

Using genetic approaches to mimic age-related changes in CMA, we have shown that CMA blockage in young animals phenocopies features of aging, often in an organ-specific manner. The selectivity of CMA normally allows for fine-tuned, timely adjustments in the proteome, but when this pathway fails with age, cells lose this capacity, leading to impaired homeostasis, energetics, adaptability, and responses to a constantly changing environment.

In this talk, I will discuss our chemical and genetic efforts to restore CMA activity in old organisms and the impact of these interventions on age-related diseases.







## Discovery of a new class of senolytics derived from animal venoms

Javier Moral-Sanz<sup>1\*</sup>, Isabel Fernández-Carrasco<sup>1\*</sup>, Valentina Ramponi<sup>2</sup>, Amanda Garrido<sup>3</sup>, Izhar Karbat<sup>4</sup>, Pablo Cabezas-Sainz<sup>5</sup>, Esperanza Rivera-de-Torre<sup>6,7</sup>, Osama Elsallabi<sup>8</sup>, Roberto Martín-Hernández<sup>9</sup>, José L. López-Aceituno<sup>1</sup>, Nathan L. Price<sup>3</sup>, Laura E. Sanchez<sup>5</sup>, Gonzalo Colmenarejo<sup>9</sup>, Álvaro Martínez-del-Pozo<sup>6</sup>, Irina Vetter<sup>10,11</sup>, Angel Cogolludo<sup>12,13</sup>, Francisco Perez-Vizcaino<sup>12,13</sup>, Jorge Del-Pozo<sup>14</sup>, Eitan Reuveny<sup>4</sup>, Manuel A. Fernández-Rojo<sup>15,16</sup>, Paul D. Robbins<sup>8</sup>, Rafael de Cabo<sup>3</sup>, Manuel Serrano<sup>2,17</sup>, **Maria P. Ikonopoulou**<sup>1,10</sup>  
\*Equally contributing authors

1. Translational Venomics Group, Madrid Institute for Advanced Studies in Food, Madrid, Spain
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3. Experimental Gerontology Section, Translational Gerontology Branch, National Institute on Aging, NIH, Baltimore, USA
4. Weizmann Institute of Science, Rehovot, Israel
5. Department of Zoology, Genetics and Physical Anthropology, University of Santiago de Compostela, Lugo, Spain
6. Department of Biochemistry and Molecular Biology, Complutense University, Spain

Senescence acts as a barrier to tumour progression, yet its persistent accumulation promotes inflammation and relapse. Therefore, the therapeutic success of chemotherapy may be compromised when senescence arises within the tumour microenvironment. We identified senolytic properties in a pore-forming toxin, TVS1, and its engineered, enhanced variant TVS1G. These compounds selectively impair the viability of chemotherapy-induced senescent cancer cells. This selectivity is driven by specific membrane lipid compositions and disrupted bilayer asymmetry characteristic of senescent cells.

Mechanistically, TVS1G induces sodium and calcium influx alongside sustained potassium efflux in senescent cells. Calcium influx activates calcium-dependent potassium channels, culminating in cell death via apoptosis and pyroptosis. In vivo, TVS1G synergizes with senescence-inducing chemotherapy to promote remission of solid tumours.

Our findings demonstrate that animal venoms contain compounds with senolytic activity and identify TVS1 and TVS1G as senotoxins: a novel class of senolytics with promising potential in cancer therapy.





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## The cellular and pathophysiological effects of endogenous DNA damage, a primary hallmark of aging

### Laura Niedernhofer

Director, Masonic Institute on the Biology of Aging and Metabolism  
Professor, Department of Biochemistry, Molecular Biology and Biophysics  
Laurence O. Pilgeram Endowed Research Chair in the Molecular Biology of Aging  
University of Minnesota Medical School

Genome instability, which encompasses nuclear DNA damage, is a type of cellular damage that drives aging biology, and a primary hallmark of aging. Endogenous DNA damage occurs through normal metabolism and the inherent instability of DNA under oxidative and hydrolytic conditions. Forty thousand DNA lesions is estimated to occur per day per cell in humans. Endogenous sources of genotoxic stress include anabolic, mitochondrial, and lipid metabolism, demethylation, DNA replication, and water, thus are largely unavoidable. We provide evidence that endogenous DNA damage is a potent driver of cellular senescence *in vivo*, which in turn is the most proximal cause of aging. Depletion of expression of the DNA repair endonuclease ERCC1-XPF in mice, increases the abundance of spontaneous oxidative DNA damage, and models a human progeroid syndrome driven by mutations in either gene. We are currently depleting *Ercc1* expression cell type-specifically to address the questions of which cell types are most vulnerable to endogenous DNA damage and which lineages of senescent cells are most deleterious. This causes early onset of chronic diseases in all seven organs tested to date, providing evidence that endogenous DNA damage contributes to age-related diseases. Further, we find that increased endogenous DNA damage as a primary insult exacerbates all other hallmarks of aging, illustrating the interconnectedness of the aging hallmarks as well as the hypothesis that therapeutically targeting any hallmark of aging is sufficient to alleviate the negative consequences of genome instability.







## Transcriptional and epigenetic mechanisms of brain aging and neurodegeneration

### Ashley Webb, PhD

Associate Professor  
The Buck Institute for Research on Aging  
Novato, CA, USA

Aging is the greatest risk factor for many diseases, including neurodegenerative conditions such as Alzheimer's disease. Our lab investigates the mechanisms responsible for brain aging, with the goal of identifying targets to improve healthy aging and treat neurodegeneration. Our work is focused on two different areas of the brain: the hippocampus and the hypothalamus. In this presentation, I will highlight our work investigating the mechanisms of hypothalamic aging and the changes that this area of the brain undergoes in neurodegeneration. The hypothalamus is a well-conserved brain region that controls homeostatic and survival-related behaviors such as sleep, circadian rhythms, metabolic homeostasis, reproduction, and hormone status. We have identified cell-type specific transcriptional and epigenetic changes with age in the mouse that correlate with loss of hypothalamic function. We have also discovered sex-specific features of the aging hypothalamus suggesting cell-intrinsic epigenetic changes that underlie differences in male and female aging. Finally, we have performed the first large-scale analysis of single cell transcriptomic changes in the human hypothalamus in aging and Alzheimer's disease, and I will present our findings suggesting major changes in specific hypothalamic subregions and cell types. In the long term, this work may lead to new strategies to enhance healthy brain function in the context of aging and neurodegeneration and improve quality of life in the elderly.







## Modulating senescence in pancreatic adenocarcinoma with targeted nanotherapies

**Magdalini Panagiotakopoulou**<sup>1</sup>, Ashley Sullivan<sup>1</sup>,  
Riccardo Mezzadra<sup>2</sup>, Ho Yujui<sup>2</sup>, Scott Lowe<sup>2</sup>, Daniel Heller<sup>1</sup>

(1) Memorial Sloan Kettering Cancer Center, Molecular Pharmacology, New York, NY,

(2) Memorial Sloan Kettering Cancer Center, Cancer Biology and Genetics, New York, NY

Pancreatic ductal adenocarcinoma (PDAC) remains one of the deadliest cancers, due to its resistance to chemo- and immunotherapies. Inducing senescence can expose therapeutic vulnerabilities, but lingering senescent cells pose risks both for relapse and aging-related toxicities. We developed a Galectin-3-targeted lipid nanoparticle system to selectively deliver a senolytic BRD4 degrader after senescence induction. This targeted approach significantly reduced tumor growth and improved survival in preclinical PDAC models. Our analysis revealed a major reconfiguration of the tumor microenvironment. scRNA-seq identified a pronounced reduction of the pro-inflammatory Senescence-Associated Secretory Phenotype (SASP) across ductal, fibroblast, and macrophage populations. In parallel, myeloid cells displayed striking transcriptional plasticity, positioning macrophage subpopulations as central mediators of the therapeutic response. These findings point to a promising strategy for overcoming PDAC resistance and targeting pathological senescence in cancer and potentially other age-related diseases.









Madrid 17<sup>th</sup> - 19<sup>th</sup> Nov 2025

# Molecular and Cellular Hallmarks of Aging: 3rd edition

Tuesday Nov 18<sup>th</sup> 2025

**Session #5**

**Telomeres and Aging Diseases**

*Chairperson: Ana María Cuervo*

## Regulation of telomere protection

### Maria A. Blasco

Telomeres and Telomerase group, Spanish National Cancer Research Center (CNIO), Madrid, Spain

The telomere-bound shelterin complex is essential for chromosome-end protection and genomic stability. We previously showed that TRF1 is phosphorylated by AKT, ERK, B-Raf and mTOR and identified residue T330 as common phosphorylation site for these kinases (Méndez-Pertuz et al., *Nature Comm.*, 2017; Bejarano et al., *EMBO Mol Med*, 2019; Sánchez-Vázquez et al., *PLoS Gen*, 2021). Therefore, we hypothesize that mouse TRF1 phosphorylation at T330 is important for telomere biology in vivo. To address this, we have generated homozygous knock-in (ki) mice with non-phosphorylatable T330A or phosphomimetic T330D Trf1 mutant alleles. In this meeting, we will report the effects of these mutations on telomere maintenance as well as in cancer and aging.

Pulmonary fibrosis is a lethal disease associated with damaging insults to the lung and organismal aging. Presence of short and dysfunctional telomeres have been placed at the origin of the disease in a percentage of both familial and sporadic cases. Recently, a mutation in the telomere-binding protein Protection Of Telomeres 1 in humans (hPOT1), the hPOT1 L259S mutation, was found in families with idiopathic pulmonary fibrosis. Here, we generate a Pot1a<sup>L261S</sup> knock-in mouse harboring the murine homologous hPOT1 L259S mutation. We find that the homozygous Pot1a<sup>L261S</sup> mice show shorter telomeres, a phenotype that is aggravated with increasing mouse generations, in striking analogy to the telomerase-deficient mouse models. Furthermore, we find that the POT1a-L261S mutant protein exerts a higher telomerase inhibitory effect at telomeres than wild-type POT1a, providing a mechanism by which Pot1a<sup>L261S</sup> knock-in mice phenocopy the short telomere phenotype of the telomerase knock-out model.







## Remodeling of Intracellular Ca<sup>2+</sup> Homeostasis in rat hippocampal neurons during In vitro aging

Enrique Pérez-Riesgo<sup>1</sup>, Elena Hernando-Pérez<sup>1,2,3</sup>, Lucía Núñez<sup>1,4</sup>,  
**Carlos Villalobos<sup>1</sup>**

1. Institute of Biomedicine and Molecular Genetics of Valladolid (IBGM), University of Valladolid & CSIC. Spain.

2. Dept. of Cell Biology, Histology and Pharmacology, School of Medicine, University of Valladolid. Spain

3. Health Sciences School, Miguel de Cervantes European University (UEMC), Valladolid, Spain

4. Dept. of Biochemistry & Molecular Biology & Physiology, School of Medicine, University of Valladolid. Spain

Neuronal aging is associated to changes in Ca<sup>2+</sup> signaling, a key integrator of excitability, plasticity, and cellular resilience. Here, we combined single-cell Ca<sup>2+</sup> imaging with bulk transcriptomic profiling and linear modelling to elucidate the basis of Ca<sup>2+</sup> remodeling in rat hippocampal neurons during in vitro aging. Accordingly, primary hippocampal cultures (neurons and glia) from neonatal Wistar rats were cultured for 6–8 days in vitro (DIV) or 18–21 DIV, representing young and aged neurons. Intracellular Ca<sup>2+</sup> responses to different stimuli and storeoperated Ca<sup>2+</sup> entry (SOCE) was monitored. IP3R-mediated Ca<sup>2+</sup> release was assessed using caged-IP3 photolysis and confocal microscopy. In parallel, Clariom D (rat) microarrays of the same samples were analyzed using limma (FDR<0.05), controlling for the random effect of culture origin (paired young/aged samples from the same primary preparation) and applying a Bayesian framework to infer neuronspecific expression changes from paired mixed and glia-enriched cultures within a curated Ca<sup>2+</sup> gene panel. We found that Ca<sup>2+</sup> responses mediated by voltagegated Ca<sup>2+</sup> channels, glutamate and acetylcholine receptors were enhanced in aged neurons, while SOCE nearly disappeared with age. IP3R-dependent Ca<sup>2+</sup> release also declined in aging neurons. Transcriptomic analysis revealed overexpression of a number of plasma membrane receptors including GluN2C, GluA1/2, GluK4/5, CHRM1/2, P2X5/6, P2Y4, and Cav3.1, the infraexpression of P2Y1 and CHRM3, along with altered expression of pumps/exchangers, SOCE components, ER channels, and the loss of mitochondrial Ca<sup>2+</sup> transporters. In summary, aging shifts Ca<sup>2+</sup> signaling in hippocampal neurons towards enhanced Ca<sup>2+</sup> influx but impaired SOCE, ER Ca<sup>2+</sup> release and mitochondrial Ca<sup>2+</sup> handling leading to enhanced susceptibility to neuron cell death and loss of dendritic spine stability required for memory formation. Thus, posing the question on whether Ca<sup>2+</sup> remodeling may be a novel aging hallmark.

This work has been funded by the spanish Agencia Estatal de Investigación (AEI) grants PID2024-159238OB-I00 and PID2021-125909OB-I00, the IBGM Unit of Excellence program from Junta de Castilla y León CLU-2025-2-01 and the CSIC Deep-MaX excellence program. EPR has been supported by the Momentum program from CSIC MMT24-IBGM-01.







## Lessons from Disorders of Telomere Length

### Mary Armanios, MD

Johns Hopkins School of Medicine

Telomere shortening is acquired with aging and at certain thresholds, short telomere length predisposes to a premature aging phenotype in the Mendelian short telomere syndromes. These disorders have distinct manifestations in children and adults with adult-onset pulmonary fibrosis being their most prevalent manifestation. Because of the prevalence of germline mutations in pulmonary fibrosis, this makes them the most common premature aging syndromes. While these observations may imply longer telomere length has an advantage in vitro, there is emerging evidence that Mendelian disorders associated with excessively long telomere length are also marked by premature aging marked by a high incidence of cancer. Here I will discuss emerging data that link long telomere length to propensity to clonal evolution across multiple tissues, highlighting the premature aging phenotype associated long telomere syndromes.







## Neuronal CG and non-CG DNA methylation coupling is a conserved hallmark of aging

Alejandro González-Ramón<sup>1</sup>, Matthew Kocher<sup>1</sup>, Raúl F Pérez<sup>2</sup>, Agustín F Fernández<sup>2</sup>, Mario F Fraga<sup>2</sup>, **Jose V Sanchez-Mut<sup>1</sup>**

1. Laboratory of Functional Epi-Genomics of Aging and Alzheimer's disease, Instituto de Neurociencias, Universidad Miguel Hernández-Consejo Superior de Investigaciones Científicas (UMH-CSIC), 03550 Alicante, Spain.
2. Cancer Epigenetics and Nanomedicine Laboratory, Centro de Investigación en Nanomateriales y Nanotecnología-Consejo Superior de Investigaciones Científicas (CINN-CSIC), Universidad de Oviedo, 33011, Oviedo, Spain.©

Aging is associated with widespread DNA methylation (DNAm) changes, particularly at CG dinucleotides (mCG), yet the role of non-CG methylation (mCA) and its coordination with mCG over time remains poorly understood. This study identifies a conserved, age-associated increase in co-methylation (CoM) between mCG and mCA in postmitotic neurons, defined by the correlation of DNAm levels within 100 bp genomic windows. Using whole-genome bisulfite sequencing (WGBS) and enzymatic methylation sequencing (EM-seq) across two independent human cohorts aged 5 to 103 years, we observe a consistent increase in CoM slope with age. CoM gain (CoM<sup>+</sup>) predominantly occurs in Polycomb-repressed developmental genes, while CoM loss (CoM<sup>-</sup>) localizes to actively transcribed and repetitive regions. These patterns are enriched for DNMT3A and TET3 sequence motifs, respectively, suggesting distinct regulatory mechanisms.

Cross-species analysis in chimpanzees and mice confirms the increase in CoM slope with age, and suggestive results in other vertebrate species. These findings reveal CoM as a previously unrecognized epigenetic feature of neuronal aging, and suggest distinct CoM<sup>+</sup> and CoM<sup>-</sup> dynamics respectively linked to programmed and stochastic aging processes, offering a potential epigenetic bridge between major theories of aging.

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## Molecular Regulation of Telomerase in Aging and Cancer

**Steven Artandi**

Stanford Cancer Institute, California, US







## Stress signaling in T Cells: Balancing Metabolic Health, Immune Exhaustion, and Aging

### Guadalupe Sabio, DVM, PhD

Organ Crosstalk in Metabolic Diseases Group Leader  
Tumour Biology Programme  
Spanish National Cancer Research Centre (CNIO)  
Madrid, Spain

Aging profoundly impacts both immune and metabolic homeostasis, and recent evidence suggests that immune cell signaling pathways can act as critical regulators of systemic metabolism and lifespan. Among these, the stress-activated kinase p38 emerges as a central molecular regulator linking inflammation, metabolism, and aging.

Our recent work reveals that p38 signaling in T cells plays a dual and context-dependent role. On one hand, p38 activation promotes obesity development by influencing T-cell-mediated inflammation and metabolic dysfunction. Mice lacking p38 in T cells are partially protected from diet-induced obesity, displaying improved metabolic profiles by increasing adipose tissue thermogenesis. However, this protection comes at a cost. The absence of p38 accelerates T-cell exhaustion, limiting their long-term functionality and reducing their ability to sustain effective immune surveillance. This premature exhaustion not only impairs immune responses during aging but also compromises the capacity of these cells to mount efficient anti-tumor immunity.

These findings highlight p38 as a key molecular switch coordinating the delicate balance between metabolic health, immune fitness, and longevity. Understanding how p38 integrates stress and nutrient signals in T cells provides new insights into the crosstalk between the immune system and systemic metabolism, and may open therapeutic opportunities to modulate aging and age-related diseases.









Madrid 17<sup>th</sup> - 19<sup>th</sup> Nov 2025

# Molecular and Cellular Hallmarks of Aging: 3rd edition

Wednesday Nov 19<sup>th</sup> 2025

**Session #6**

**Senescence, Reprogramming and Aging**

*Chairperson: **Thomas Rando***

## Nutrient regulation of age-reversal and senescence

### Prof., Dr. Adam Antebi, Ph.D.

Max Planck Institute for Biology of Ageing  
Cologne, Germany

Diapause is a long-lived state of resilience that allows organisms to outlast adversity, whose study has led to seminal insights into the biology of aging. *C. elegans* can endure months in a fasting-induced adult reproductive diapause (ARD), and upon refeeding, regenerate and reproduce. Interestingly, we observe that during their time fasting in ARD, animals gradually age, as measured by transcriptomic clocks, but remarkably rejuvenate their transcriptional age upon refeeding, suggesting that nutrient provision promotes restoration. Thus, while fasting is usually thought to promote anti-aging effects, our studies suggest that refeeding may also play an equally important role. Through genetic screens, we discover that *hlh-30*/TFEB transcription factor is a master regulator of ARD whose mutation results in loss of survivorship and reproductive capacity. Closer examination reveals that such mutants arrest in a novel senescent-like state during ARD and refeeding, resembling mammalian cellular senescence--a phenotype not previously noted before in worms. Germline stem cells are characterized by DNA damage, nucleolar expansion, cell cycle arrest, and mitochondrial dysfunction. On the organism level, we observe dysregulated immune and growth metabolic signatures, elevated SA $\beta$ -gal and accelerated aging. This senescent-like state is likely induced by a misalignment of nutrient availability with metabolism and growth signaling. Suppressor screens reveal that inhibition of systemic TGF $\beta$  signaling bypasses *hlh-30* senescence, and restores survivorship and stem cell longevity, implicating this axis as an evolutionarily ancient regulator of metazoan senescence. Importantly, we demonstrate that TFEB's vital role is conserved in mouse embryonic and human cancer diapause. Further genetic dissection of ARD also identifies a fasting-induced epigenetic factor whose mutation impacts biological age, resilience and restoration, and whose mammalian counterparts show a strikingly similar regulation and physiological role. Thus, ARD offers a powerful model to study resilience, restoration and senescence in vivo, directly relevant to mechanisms of mammalian longevity.







## Senomorphic effect of genetic and chemical partial Reprogramming

**Manuel Collado**

Laboratory of Cell Senescence, Cancer and Aging, CNB-CSIC, CIMUS-USC, Santiago de Compostela, Spain

Over the past few years, partial reprogramming via short, repetitive expression of Oct4, Sox2, Klf4, and c-Myc (OSKM) has emerged as a promising approach for rejuvenation. The accumulation of cells undergoing senescence, an irreversible state of growth arrest, with time is considered one of the major contributors to aging.

However, the impact of partial reprogramming on cellular senescence and its potential to reverse senescence phenotypes remains unclear.

Our study aimed to investigate the effects of OSKM expression on senescent cells.

Our findings demonstrate that OSKM expression in senescent cells leads to a significant reduction in certain senescence markers, restores mitochondrial function, and reduces the senescence-associated secretory phenotype (SASP) without altering growth arrest. This effect of OSKM on the SASP is observed at the functional level, as shown by experiments with conditioned media from senescent cells with or without OSKM expression, as well as through in vivo analyses, both of which reveal a reduction in pro-inflammatory secretions and an enhancement of cellular health.

Furthermore, we have tested partial chemical reprogramming and observed a similar capacity to alter the senescent phenotype, reducing SASP, and restoring mitochondrial function, thus mimicking the rejuvenating effects of OSKM and opening possibilities for clinical translation. These partial chemical reprogramming also showed a senomorphic effect in vivo leading to an amelioration of the aging hallmarks of very old mice (2.5 year-old).

In conclusion, our study reveals that OSKM expression and partial chemical reprogramming exert a senomorphic effect, modulating the senescence phenotype without inducing cell proliferation. These findings shed light on the complex interplay between partial reprogramming and senescence, providing valuable insights into the rejuvenation potential of partial reprogramming strategies.







## Stromal senescence in tumor progression and therapy-induced comorbidities

### Dr. Sheila A Stewart, Ph.D.

Gerty T. Cori Professor  
Professor of Cell Biology & Physiology  
Professor of Medicine  
Vice Chair of Cell Biology and Physiology  
Associate Director for Basic Sciences, Siteman Cancer Center  
St. Louis, MO, US

Age is the single largest risk factor for the development of cancer, but how age impacts the molecular mechanisms that drive cancer remain poorly understood. While it is clear that age-related accumulation of cell autonomous mutations contributes to tumorigenesis, the central role age-related changes in the tumor microenvironment play in the transformation process is becoming more fully appreciated. Underscoring the importance of an aged microenvironment in cancer development are findings that senescent fibroblasts, which accumulate with age, directly stimulate preneoplastic and neoplastic cell growth and tumor progression. Investigations into how senescent fibroblasts promote tumorigenesis revealed that they express a plethora of growth factors, extracellular matrix remodeling enzymes, chemokines, and cytokines collectively referred to as the senescence associated secretory phenotype (SASP). We find that a subset of senescent cancer associated fibroblasts (senCAFs) limit NK cell killing, increasing tumorigenesis. Chemotherapy induces similar changes that can negatively impact a patients quality of life. We will discuss how these changes impact tumor progression and therapy-induced bone loss.







## Cellular senescence as a therapeutic target

### Jesus Gil<sup>1,2</sup>

1. MRC Laboratory of Medical Sciences (LMS), Du Cane Road, London, W12 0NN, UK
2. Institute of Clinical Sciences (ICS), Faculty of Medicine, Imperial College London, Du Cane Road, London W12 0NN, UK.

Senescent cells are present in cancerous and fibrotic tissues and are associated with multiple age-related diseases. Recently, drugs that selectively kill senescent cells, termed senolytics, have proven beneficial in improving the outcomes of many of these pathologies. While the potential of senotherapies is great, there are several limitations to translating them to the clinic. In particular, we need to better understand the complex biology of senescence, identify effective senolytics, and develop ways to detect senescent cells. I will describe functional approaches that my lab has employed to identify senotherapies and how we can take advantage of machine learning to detect senescence.







## Decoding the Role of Mitochondria in Cellular Senescence

### Dr. Manuel Serrano

Principal Investigator  
Cambridge Institute of Science  
Altos Labs  
Cambridge, UK

The importance of mitochondria in cellular senescence is well-established. However, the molecular details are poorly understood. I will present our most recent work on mitochondrial biology in cellular senescence. In particular on the role of cyclophilin D and the transition permeability pore (mPTP) to allow the exit of calcium and compensate for its elevated influx from the endoplasmic reticulum. Also, mitochondria play a key role in the SASP by releasing dsDNA and dsRNA into the cytosol. These, in turn, activate the cGAS/STING and the RIGI/MDA5/MAVS pathways, both converging on the activation of the interferon and NFkB pathways. This work has led to the identification of new senolytic and senomorphic treatments.









Madrid 17<sup>th</sup> - 19<sup>th</sup> Nov 2025

# Molecular and Cellular Hallmarks of Aging: 3rd edition

## Organisers & Speakers' Biographies



## Maria A. Blasco PhD

Telomeres and Telomerase Group - Humanism and Science Foundation  
Molecular Oncology Programme  
Spanish National Cancer Research Centre

Maria A. Blasco obtained her PhD in 1993 for her research on a viral DNA polymerase at the Centro de Biología Molecular “Severo Ochoa” (CSIC-UAM, Madrid, Spain) under the supervision of Prof Margarita Salas. Following a postdoctoral stay with Carol C. Greider at her Cold Spring Harbor Laboratory (New York, USA) where she isolated the first mammalian telomerase gene and generated the first knockout mouse for telomerase, Blasco returned to Spain in 1997 her own laboratory at the Centro Nacional de Biotecnología-CSIC (Madrid, Spain). She joined the CNIO in 2003 as Head of the Telomere and Telomerase Group and Director of the Molecular Oncology Programme (2003-2012). She served as CNIO Vice Director (2005-2011), and CNIO Director (2011-2015).

Blasco's Group has made an array of seminal contributions to the understanding the role of telomere biology in ageing, tumorigenesis, DNA repair and epigenetics, and the biology of both adult and induced pluripotent stem cells (ESC and iPSC), as well as on the translation of that knowledge into therapeutic strategies and applications. Blasco has generated two spin-off companies: Life Length SL and Telomere Therapeutics. Blasco was a runner-up for the EU Women's Innovator Award in 2014.

She has authored to date more than 280 publications, and her h-index >100.

She was elected EMBO Member in 2000 and sat at the EMBO Council during 2008-2011. She was the recipient the EMBO Gold Medal in 2004. She has also been distinguished with the European Association of Cancer Research “Young Investigator Award” (2002), the Joseph Steiner Award (2003), the Rey Jaime I Prize for Basic Research (2007), the Körber European Science Award (2008), the Spanish National Research Award in Biology Santiago Ramón y Cajal (2010), the Lilly Foundation Preclinical Research Award (2010), the Miguel Catalan Career Achievement Award awarded by the Regional Government of Madrid (2016). Maria became a Fellow of the Royal Society of Pharmacy in 2013. In 2021 she was named Chair of SOMMA (‘Severo Ochoa’ Centres and ‘María de Maeztu’ Units of Excellence Alliance) and also became a member of the Board of Trustees of Museo del Prado (Madrid, Spain). She has been conferred Doctorate Honoris Causa titles from Universidad Carlos III de Madrid (Spain) in 2014 and from Universidad de Murcia (Spain) in 2018.



## Alejo Efeyan

Group Leader, Metabolism and Cell Signaling Lab,  
Tumour Biology Programme  
Spanish National Cancer Research Centre - CNIO  
Madrid, Spain

After obtaining his BSc degree in Buenos Aires, Alejo Efeyan received his PhD from the Autonomous University of Madrid, for his studies on mechanisms of tumor suppression. His postdoctoral training was in the David M Sabatini Lab, studying the biology of mTOR and nutrient signaling. Alejo Efeyan settled his laboratory at the CNIO in 2016, and his team studies the links between nutrients and metabolic homeostasis and the impact of deregulated nutrient and growth factor signaling in cancer and aging.



## Thomas A. Rando, MD, PhD

Director

Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research  
University of California Los Angeles  
Los Angeles, CA, US

Dr. Rando is the Director of the Broad Stem Cell Research Center at UCLA where he is a professor of Neurology and Molecular, Cell, and Developmental Biology. Research in the Rando laboratory lies at the intersection of basic stem cell biology and the biology of aging. Dr. Rando's research has revealed how stem cells respond to cues from their environment to modulate their ability to maintain tissues or engage in tissue repair. His laboratory is credited with pioneering work in stem cell aging that revealed how factors in blood can promote stem cell activity in young individuals and suppress it in older individuals. This work has led to novel clinical trials for age-related disorders.

Dr. Rando has published over 200 peer-reviewed articles and has trained over 100 students, fellows, and visiting scholars, most of whom have continued in biomedical research careers. He has received numerous honors and awards for his work, including an NIH Director's Pioneer Award, an Ellison Medical Foundation Senior Scholar Award in Aging, the "Breakthroughs in Gerontology" Award from the American Federation for Aging Research, and a Transformative Research Award from the NIH. He is a Fellow of the American Association for the Advancement of Science, a member of the American Institute for Medical and Biological Engineering, the National Academy of Medicine, and the American Academy of Arts and Sciences.

**K. Lenhard Rudolph, M.D.**

Research Group on Stem Cell and Metabolism Aging  
Leibniz Institute on Aging - Fritz Lipmann Institute (FLI)  
Jena, Germany

My main contributions include the description of the role of telomere dysfunction and DNA damage signaling in stem cells and organism aging. Work of my group showed that alterations in epigenetic stress responses contribute to stem cell aging by activation of developmental pathways. Our current work focuses on the influence of early life growth signals on the pace of aging, particularly the development of aging-associated metabolic alterations that limit the function of stem cells and organ function. We aim to delineate the potential of new interventions to reverse aging-associated impairments in metabolism and nutrient stress responses to improve healthy aging.



## Maria Mittelbrunn

Group Leader of the Immunometabolism & Inflammation Laboratory  
Molecular Biology Center, CSIC-UAM  
Madrid, Spain

Maria Mittelbrunn is Head of the Immunometabolism & Inflammation Laboratory at the Molecular Biology Center (Madrid). Among her original contributions as PI are the demonstration that the deterioration of immune system function with aging not only compromises the response to infection, cancer, vaccination, or predisposes to autoimmunity but also increases the risk for cardiovascular, metabolic, and cognitive decline, thereby placing the immune system as a controller of healthy aging. Dr. Mittelbrunn has contributed to decoding the molecular mechanisms by which aged T cells contribute to inflammation and age-related diseases. Additionally, she has proposed new therapeutic targets to delay age-related multimorbidity and to reverse aortic aneurysms, thus preventing sudden death due to aortic dissections.

Since 2024, she is Visiting Professor at Columbia Center for Human Longevity and elected member of the Royal Academy of Science in Spain.

**Vera Gorbunova, Ph.D.**

Doris Johns Cherry Professor of Biology and Medicine  
Co-director Rochester Aging Research Center  
Director Nathan Shock Center of Excellence in Basic Biology of Aging  
University of Rochester - Department of Biology  
Rochester, NY, USA

Vera Gorbunova is an endowed Professor of Biology at the University of Rochester and a director of the Rochester Aging Research Center. Her research is focused on understanding the mechanisms of longevity and genome stability and on the studies of exceptionally long-lived mammals. Dr. Gorbunova pioneered comparative biology approach to study aging. She demonstrated that LINE1 elements trigger age-related inflammation. Her work received awards from Ellison Medical Foundation, Glenn Foundation, AFAR and the NIH. Her work was awarded Cozzarelli Prize from PNAS, prize for research on aging from ADPS/Aliaz, France, Prince Hitachi Prize in Comparative Oncology, Japan, and others.



## Daniel Muñoz-Espín, PhD

Group Leader ECI | Co-lead of Thoracic Cancer Programme  
 CRUK Cambridge Centre | Early Cancer Institute (ECI)  
 Department of Oncology - University of Cambridge, UK

Prof Daniel Muñoz-Espín is Principal Investigator at the Early Cancer Institute, Department of Oncology, University of Cambridge, and Co-Director of the CRUK Cambridge Centre Thoracic Cancer Programme. His research focuses on the fundamental processes and mechanisms triggering aging-related diseases, including cancer. His group applies this knowledge toward the validation of novel tools to promote rejuvenation, extend lifespan, and convert lethal diseases like fibrosis and aggressive cancer types into curable diseases.

Prof Muñoz-Espín completed his PhD (2002–2006) in the laboratory of Prof. Margarita Salas at the Autonomous University of Madrid, with collaborative training in the group of Prof. Jeff Errington at the University of Oxford. He then undertook postdoctoral research at the Centre of Molecular Biology Severo Ochoa in Madrid (2007–2010), followed by a second postdoctoral period (2011–2015) in the laboratory of Prof. Manuel Serrano at the Spanish National Cancer Research Centre (CNIO). There, he specialized in cellular senescence and in vivo models of cancer and ageing. His seminal work, published in *Cell* (2013) and highlighted in *Nature Reviews Molecular Cell Biology* (2014), was the first to describe the phenomenon of developmental senescence. This work was recognized with two major research awards: the Ramón y Cajal Programme Senior Grant and a National Programme Grant for Research Aimed at H2020 Societal Challenges.

In 2016, Prof Muñoz-Espín established his independent research group at the University of Cambridge. He has since secured multiple competitive funding awards, including an MRC New Investigator Research Grant (2017–2021), a CRUK Early Detection Project Grant (2018–2021), and a CRUK Programme Foundation Award (2020–2026). As principal investigator and corresponding author, he has published recent research in high-impact journals including *EMBO Molecular Medicine* (2018, 2025 Accepted), *Aging Cell* (2020), *European Respiratory Journal* (2022), *Cancer Cell* (2023), *Angewandte Chemie* (2024), *Cell Death & Disease* (2024), and *Nature Aging* (2025, Accepted).

Prof Muñoz-Espín's laboratory has developed a suite of novel tools targeting senescence and promoting transient reprogramming for diagnostic and therapeutic purposes, resulting in several filed patents. Prof Ljiljana Fruk (Department of Biotechnology, University of Cambridge) and Prof Muñoz-Espín have co-founded Senesys Bio start-up to develop therapeutic and detection tools to target ageing and cancer.



## **Eileen White, PhD**

Rutgers Cancer Institute, Rutgers University  
Ludwig Princeton Branch, Ludwig Institute for Cancer Research, Princeton University  
New Jersey, US

Eileen White, PhD is a cancer biologist known for her work establishing that a DNA tumor virus oncogene functions by inhibiting programmed cell death by apoptosis and is a homologue of the human BCL-2 oncogene. She is also known for establishing that tumor cells induce intracellular nutrient scavenging by autophagy, which promotes their metabolism, growth, survival, and malignancy. These findings informed the means to target the apoptosis and autophagy pathways for cancer therapy. Eileen is Deputy Director and Chief Scientific Officer at the Rutgers Cancer Institute at Rutgers University, and Associate Director of the Ludwig Princeton Branch of the Ludwig Institute for Cancer Research at Princeton University. She is also the Lead PI for the Cancer Research United Kingdom/United States National Cancer Institute Cancer Grand Challenge grant to address the mechanisms causing cancer cachexia through the CANcer Cachexia Action Network (Team CANCAN). Amongst Eileen's honors are election to the US National Academy of Sciences and the American Academy of Arts and Sciences, and she is an elected fellow of the American Association for the Advancement of Science, the American Academy of Microbiology, and the American Association for Cancer Research Academy.



## Pekka Katajisto

Professor  
Principal Researcher Department of Cell and Molecular Biology  
University of Helsinki and Karolinska Institutet  
Stockholm

After his PhD from University of Helsinki, Finland in 2009, Dr. Katajisto conducted postdoctoral training in the Whitehead Institute and MIT, where he continued research on cell-cell interactions in the context of stem cells and their surrounding niche. In 2013 Katajisto started his own laboratory in the University of Helsinki, and in 2015 he started in joint position with the Karolinska Institutet, Sweden. His laboratory focuses on aging induced alterations in tissue renewal and has discovered multiple niche mediated mechanisms that reduce stem cell capacity in the old tissue. Their discoveries have also illuminated how tumor cells bias clonal competition by employing mechanisms that reduce stem cell capacity during normal aging. In addition to the work on cell extrinsic mechanisms, his lab has made key discoveries highlighting the role of cellular metabolism in stem cells. They discovered that asymmetrically dividing stem cells segregate metabolic organelles selectively, and that instead of being a consequence of other fate determining programs, cellular metabolism can function as a pioneering fate determinant after cell division.

### Selected publications

Andersson et al.; Old mitochondria regulate niche renewal via stem cell metabolism

Nature metabolism. 2025 in press

Pentimikko et al.; Cellular shape reinforces niche to stem cell signaling in the small intestine

Science Advances. 2022 Oct 14;8(41)

Döhla et al.; Metabolic determination of cell fate through selective inheritance of mitochondria.

Nature Cell Biology, 2022 Feb;24(2):148-154,

Flanagan et al.; Notum from Apc-mutant cells biases clonal competition to initiate cancer.

Nature. 2021 Jun;594(7863):430-435.

Pentimikko et al.; Notum produced by Paneth cells attenuates regeneration of aged intestinal epithelium.

Nature. 2019 Jul;571(7765):398-402.

Katajisto et al.; Asymmetric apportioning of aged mitochondria between daughter cells is required for stemness. Science. 2015 Apr 17;348(6232):340-3.



## Salvador Aznar

IRB Barcelona,  
Barcelona, Spain

Salvador Aznar Benitah obtained his Honours BSc in Biochemistry and Molecular Biology at McGill University in 1998. In 2007, after a postdoctoral work in the laboratory of Fiona Watt at the London Research Institute (Cancer Research UK), he established his own lab at the Center for Genomic Regulation (CRG) as a Junior ICREA researcher. In 2014 Salvador was promoted to ICREA Research Professor, one of the most prestigious awards in research in Spain, and moved to the Institute for Biomedical Research (IRB) in Barcelona as a senior researcher. In 2015, he was also appointed as a Foundation Botin Researcher. His lab aims at understanding the molecular mechanisms underlying adult stem cell function during homeostasis, ageing and cancer, with special interests in epigenetics, spatiotemporal regulation of stem cells (circadian rhythms), and the link between metastatic-initiating-cells and their epigenetic and metabolic mechanisms. Based on his work on metastasis, Salvador funded ONA Therapeutics in 2019 as a spin-off of IRB Barcelona and ICREA to develop new therapies targeted against metastasis. In 2020 ONA Therapeutics closed a 30M Series A round of investment. In 2025, Salvador also created the company HelixAI to develop AI-based deep learning computational methods applied to biomedicine. Salvador is the recipient of the Banc Sabadell Award in Biomedicine (2015), Doctor Diz Pintado Award in Biomedicine (2016), Beug Foundation Metastasis Award (2015), City of Barcelona Award in life sciences (2017), National Award on life sciences (2017), the Foundation Serra Award in Life Sciences (2019), the Severo Ochoa Award (2019), the Lilliane Bettencourt Award (2020), and The Atresmedia Award in life sciences (2024). In 2018, he was appointed as an EMBO member and has received a Consolidator and an Advanced ERC grant. In 2024, Cell journal named him among the 50 most innovative and disruptive current biomedical scientists worldwide. Salvador also has a long-lasting interest in the evolution of writing and language, theater, guitar playing, and graphic novels. He is the happy father of Mateo (15 years old) and Lucía (13 years old).



## Ana Maria Cuervo MD PhD

The Robert and Renee Belfer Chair for the Study of Neurodegenerative Diseases  
Distinguished Professor Department of Developmental and Molecular Biology  
Co-director Institute for Aging Research  
Albert Einstein College of Medicine  
New York, US

Dr. Ana Maria Cuervo MD PhD is distinguished professor and co-director of the Institute for Aging Research at Albert Einstein College of Medicine. She is a recognized leader in the field of biology of aging for her studies on the role of protein degradation in age-related disorders, with emphasis in metabolic conditions and neurodegenerative disorders. She has been the recipient of prestigious awards in cell biology and biology of aging and is elected member of the Valencian Royal Academy of Medicine, the Spanish Royal Academy of Sciences, The Spanish Royal Academy of Pharmacy and the American Academy of Arts and Sciences. In 2019, she was elected to the National Academy of Sciences.



## Laura Niederhofer

Director Institute on the Biology of Aging and Metabolism  
Professor Department of Biochemistry, Molecular Biology and Biophysics  
University of Minnesota Medical School, US

Dr. Niedernhofer, M.D./Ph.D. directs the Masonic Institute on the Biology of Aging and Metabolism at University of Minnesota. Previously, at the Scripps Research Institute she helped discover and name a new class of gerotherapeutics: senolytics. She trained at Duke University, M.I.T., Vanderbilt University, and Erasmus Medical Center in the Netherlands. Her expertise is in DNA damage and repair, with an emphasis on endogenous DNA damage. Laura's current research program is focused on studying fundamental mechanisms of aging with a primary focus on cellular senescence. Laura is currently serving as the Chair of the Steering Committee of the SenNet Consortium.



## Ashley Webb, PhD

Associate Professor  
The Buck Institute for Research on Aging  
Novato, CA, USA

Dr. Ashley Webb received her PhD from the University of Washington and did her postdoctoral work at Stanford University investigating mechanisms of neural stem cell aging, with a focus on transcriptional networks in the aging neurogenic niche. She is currently an Associate Professor at The Buck Institute for Research on Aging and continues to investigate the molecular mechanisms of brain aging and neurodegeneration. Her work has advanced our understanding of the cellular mechanisms that accelerate brain aging, and has the potential to reveal new approaches to promote healthy aging and treat age-associated disease.



## Mary Armanios, MD

Johns Hopkins School of Medicine  
Baltimore, US

Mary Armanios is Professor of Oncology and Genetic Medicine at the Johns Hopkins University School of Medicine. Her research program has focused on understanding the role of telomeres and telomerase in human disease. She has defined the clinical spectrum, genetics and underlying mechanisms that mediate the short and long telomere syndromes. Her discoveries have led to advances in treatment paradigms for patients with idiopathic pulmonary fibrosis and cancer predisposition. Dr. Armanios is cofounder and Director of the Telomere Center at Johns Hopkins, an internationally recognized hub for translational research related to the role of telomeres in human disease. As part of this role, she oversees a long-standing multi-disciplinary Telomere Clinic well as a telomere length testing laboratory. The latter serves as a reference lab in the United States and has to date provided diagnostic information to more than 15,000 patients.



## Steven Artandi, MD, PhD

Laurie Kraus Lacob Director of the Stanford Cancer Institute  
Jerome and Daisy Low Gilbert Professor of Medicine  
Biochemistry at Stanford University, US

Steven Artandi, MD, PhD is the Laurie Kraus Lacob Director of the Stanford Cancer Institute and the Jerome and Daisy Low Gilbert Professor of Medicine and Biochemistry at Stanford University. He also serves as the inaugural Senior Associate Dean for Cancer Programs for Stanford School of Medicine and the Chief Cancer Officer for Stanford Health Care. He received his undergraduate degree from Princeton University, and MD and PhD degrees from Columbia University. He trained in Internal Medicine at Massachusetts General Hospital and in Oncology at Dana-Farber Cancer Institute before joining the Stanford faculty in 2000. Dr. Artandi is an oncologist and cancer biologist whose research work has focused on the role played by the enzyme telomerase in cancer, aging and stem cell function. His work has produced new insights into the origins of cancer, revealing how telomerase endows cells with immortal growth properties and how aspiring cancers circumvent critical bottlenecks encountered during carcinogenesis. He has received a number of awards including an Outstanding Investigator Award from the National Cancer Institute and is an elected member of the American Association for the Advancement of Science, the American Society for Clinical Investigation and the Association of American Physicians. He serves on the Editorial Boards of the journals *Molecular Cancer Research* and *Stem Cells*.



## Guadalupe Sabio, DVM, PhD

Organ Crosstalk in Metabolic Diseases Group Leader  
 Tumour Biology Programme  
 Spanish National Cancer Research Centre (CNIO)  
 Madrid, Spain

Guadalupe Sabio leads the Organ Crosstalk in Metabolic Diseases Group at the CNIO. She obtained her PhD from the University of Extremadura and the MRC Protein Phosphorylation Unit (University of Dundee), where she identified key substrates of the stress kinases p38 $\gamma$  and p38 $\delta$ . After a postdoctoral stay at the University of Massachusetts with Roger Davis, she elucidated the tissue-specific role of JNK1 in obesity and diabetes.

Her group investigates how metabolic alterations and obesity promote cancer and other diseases by evaluating inter-organ communication. Current research focuses on four main areas: (1) dysfunction of adipose tissue and its circadian and mitochondrial regulation, (2) how exercise protects against tumor development through myokine secretion, (3) chronic inflammation driven by stress kinases, and (4) metabolic rewiring as a disease driver and therapeutic target.

Dr. Sabio has received numerous awards, including the L'Oréal-UNESCO for Women in Science Award, the Princess of Girona Foundation Prize for Scientific Research, and the Banco Sabadell Award for Biomedical Research.

**Adam Antebi, Ph.D.**

Max Planck Institute for Biology of Ageing, Director  
Cologne, Germany

Prof. Antebi received his PhD in Biology from Massachusetts Institute of Technology, and performed his post-doctoral studies at the Johns Hopkins University. He is currently one of the founding Directors of the Max Planck Institute for Biology of Ageing, Cologne, Germany. Dr Antebi's work has focused on dietary, metabolic and endocrine regulation of longevity from worms to vertebrates. His research seeks to decipher convergent mechanisms of longevity across pathways and evolution, using molecular and systems approaches.



## Sheila A Stewart, Ph.D.

Gerty T. Cori Professor  
 Professor of Cell Biology & Physiology  
 Professor of Medicine  
 Vice Chair of Cell Biology and Physiology  
 Associate Director for Basic Sciences, Siteman Cancer Center - St. Louis, MO, US

Dr. Stewart is a Professor in the Department of Cell Biology and Physiology and Medicine at Washington University in St. Louis and is the Associate Director for Basic Science at the Siteman Cancer Center. She received her Ph.D. in Microbiology and Immunology from UCLA in 1997 and completed her postdoctoral fellowship in Cancer Biology at the Whitehead Institute at MIT in Robert Weinberg's laboratory. Dr. Stewart is an American Cancer Society Scholar and her research is focused on understanding how age-related changes in the tumor microenvironment impact tumorigenesis. Her laboratory has shown that senescent stromal cells, similar to cancer associated fibroblasts express a plethora of pro-tumorigenic factors, many of which are subject to post-transcriptional stabilization. This work led to the identification to the p38MAPK-MK2 pathway where Dr. Stewart and colleagues are targeting MK2 in a metastatic breast cancer trial. Dr. Stewart's team is also using this knowledge to combine MK2 inhibition with immune therapy approaches to reduce metastatic breast cancer progression. Her group is also engaged in exploring the role senescent cells play in chemotherapy-induced comorbidities. Finally, the laboratory is also examining how age-related changes in the premetastatic niche facilitate tumor cell seeding and outgrowth and how these changes alter the local immune response to facilitate tumor cell proliferation and impact tumor cell dormancy.



## Jesus Gil

Senescence Group  
MRC Laboratory of Medical Sciences (LMS)  
Imperial College  
Hammersmith Hospital Campus  
Du Cane Road - London, UK

Prof. Jesús Gil was born in Zaragoza, Spain, and earned his PhD in 2000 at Universidad Autónoma in Madrid. During his postdoc, he worked at UCL, CRUK, and CSHL on p16INK4a regulation and senescence. Since 2005, he has led the Senescence Group at MRC LMS, Imperial College, investigating the molecular mechanisms regulating cellular senescence and how we can exploit that information therapeutically. Jesus Gil is a Professor at Imperial College, where he heads the Department of Molecular Sciences. He is a named inventor on several patents on senolytics and has collaborated with different companies on the development of senotherapies.



## Manuel Serrano

Principal Investigator  
Cambridge Institute of Science  
Altos Labs  
Cambridge, UK

Dr. Manuel Serrano obtained his PhD in 1991 in Madrid, Spain. During his postdoctoral period with David Beach, at Cold Spring Harbor Lab, NY, USA, he discovered p16, a key anti-cancer gene and inducer of cellular senescence. From 1997 to 2022, Serrano developed his career at the CNIO, Madrid, and at the IRB, Barcelona. His laboratory has made important contributions to the fields of senescence and reprogramming. In 2023, Serrano moved to Altos Labs, Cambridge, UK, where he continues investigating senescence and reprogramming with the aim of understanding and treating aging and its associated diseases.



Madrid 17<sup>th</sup> - 19<sup>th</sup> Nov 2025

# Molecular and Cellular Hallmarks of Aging: 3rd edition

Monday Nov 17<sup>th</sup> 2025

## Poster Session

01

## Functional interplay between HSF1 and HSF2 isoforms defines tumor cell response to proteotoxic stress

Kristína Bednářová<sup>1,2</sup>, Oliver Simončík<sup>1</sup>, Veronika Koci<sup>1</sup>, Zuzanna Aleksandra Trybala<sup>1,2</sup>, Petr Müller<sup>1</sup>

1. Research Centre for Applied Molecular Oncology (RECAMO), Masaryk Memorial Cancer Institute, Zluty kopec 7, Brno, 65653, Czech Republic
2. Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic

The ability of tumor cells to withstand proteotoxic stress is a feature of malignancy. Central to this resilience is the heat shock response (HSR), orchestrated by HSF1 and fine-tuned by its paralog HSF2. While HSF1 directly activates transcription of chaperones such as Hsp70, HSF2 provides a modulatory layer through heterooligomerization and isoform-specific interactions. HSF2 exists in two isoforms: transcriptionally competent HSF2 $\alpha$  and truncated HSF2 $\beta$ , which lacks a transactivation domain and may act as a dominant-negative regulator.

Our analysis across multiple tumor cell lines revealed variability in both expression levels and relative ratios of HSF2 isoforms, suggesting that isoform balances may underlie lineage-specific stress adaptation. Using H1299 lung carcinoma cells and their HSF1- and HSF2-deficient counterparts, we dissected the functional contribution of individual isoforms. Expression of HSF2 $\beta$  resulted in slower proliferation and reduced viability under HSP90 inhibition (62.5 nM NVP-AUY922), whereas HSF2 $\alpha$  promoted stress tolerance. Mechanistically, HSF2 $\beta$  attenuated HSF1-mediated transcription of HSPA1A (Hsp70), a key chaperone involved in proteostasis recovery.

Interestingly, under stress conditions, both HSF2 isoforms colocalized with nuclear stress bodies even without HSF1, indicating that HSF2 recruitment to nSBs does not depend on HSF1. However, without HSF1, these complexes failed to activate HSPA1A transcription, showing that HSF2 can associate with stress-induced chromatin independently but remains transcriptionally inactive on its own.

In summary, our results show that HSF2 $\beta$  negatively regulates HSF1 activity and stress adaptation, and that the ratio of HSF2 isoforms could serve as a potential marker of tumor stress sensitivity and therapeutic response.

### Funding:

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## A non-canonical role for BRAF in telomere biology and cancer

**Giuseppe Bosso**<sup>1</sup>, Jörg Müller<sup>2</sup>, Nicolás González-Calero Totoki<sup>1</sup>, Oscar Laguna<sup>1</sup>,

María Dolores Moreno<sup>3</sup>, Jorge Martínez-Torrecuadrada<sup>4</sup>, Rafael Fernández-Leiro<sup>3</sup>, Rosa Serrano<sup>1</sup>,  
Eduardo Zarzuela<sup>5</sup>, Marta Isasa<sup>5</sup> and Maria A. Blasco<sup>1</sup>

1. Telomeres and Telomerase Group, Molecular Oncology Program, Spanish National Cancer Centre (CNIO), Melchor Fernández Almagro 3, Madrid, E-28029, Spain.

2. University of Bayreuth, Department of Computer Science, Universitätsstraße 30, Bayreuth, 95447, Germany.

3. Genome Integrity and Structural Biology Group, Structural Biology Programme, Spanish National Cancer Center

4. Protein Production Unit, Structural Biology Program, Spanish National Cancer Center (CNIO), Madrid, E-28029, Spain.

5. Proteomics Unit, Structural Biology Program, Spanish National Cancer Center (CNIO), Madrid, E-28029, Spain.

Telomeres are ribonucleoproteic structures at the end of eukaryotic chromosomes that consist in tandemly repeated DNA sequences bound by the six-protein complex shelterin. Shelterin protects eukaryotic telomeres from degradation, unwanted DNA repair activities at chromosome ends and plays an active role in the maintenance of genome stability. Growing evidence indicates that the shelterin factor TRF1 is a stemness marker, is essential for pluripotency induction and is upregulated in several human cancers. BRAF, is a master kinase of the ERK-signaling cascade, a well-known mitogenic pathway that promotes cell proliferation in the presence of external growth factors. Oncogenic lesions affecting BRAF encoding gene frequently occur in a plethora of human malignancies and result in a continuous proliferative cue. Here, we describe an unanticipated function for BRAF in telomere biology that antagonizes its oncogenic role at chromosome ends mediated via the canonical ERK-pathway. We make the unprecedented finding that BRAF depletion *in vitro* as well as in murine models results in a post-translational overexpression of TRF1, which occurs independently of ERK1/2 phosphorylation and relies on BRAF catalytic activity. Such dysfunctional BRAF-dependent TRF1 upregulation induces telomere shortening, telomeric fragility and DNA damage response activation at chromosome ends in a tissue-specific fashion. Mechanistically, we demonstrate that BRAF displays telomere-binding properties and co-localizes with TRF1. We also find that BRAF physically interacts with and directly phosphorylates TRF1 *in vitro* and *in vivo*. This non-canonical phosphorylation is required for the regulation of TRF1 binding to telomeres. Importantly, the human orthologs of such BRAF-dependent TRF1 phosphosites, are mutated in human cancer, thus potentially suggesting that such post-translational modifications may be dysregulated in human malignancies. In line with this, we uncover the BRAF-mediated TRF1 upregulation occurs in human cancer and is associated with a worse survival of breast cancer patients. Our findings challenge current perspectives and reveal a novel function of BRAF which antagonizes its oncogenic canonical activity through the regulation of telomere integrity.

03

## Metabolic reprogramming of microglia as a determinant factor in the development and progression of brain metastases

**Pablo Castillo Serrulla**, Myriam Jaraiz Rodríguez, Raquel Losada de Paz, Natalia del Pozo Ramos, Eduardo Balsa Martínez

Centro de Biología Molecular Severo Ochoa, Universidad Autónoma de Madrid.

Brain metastasis occurs when cancer cells spread to the brain, where the immunosuppressive environment and unique metabolic demands complicate treatment. Aging exacerbates these challenges by altering the tumor microenvironment, affecting nutrient availability, inflammation, and immune function. These age-related changes can influence metastatic progression, highlighting aging as a critical but often overlooked factor in brain metastasis. A fundamental question to address is how aging impacts the metabolic fitness of immune cells and how these changes negatively affect the progression of brain metastasis. We hypothesize that the decline in immune activity during aging is caused by dysregulated metabolism. Therefore, restoring the metabolic fitness of immune cells would be highly beneficial in enhancing their ability to suppress brain metastasis. Our preliminary data, obtained from RNA-seq analysis of tumor-associated and resting microglia in young and old mice, revealed significant differences in microglial activation between the two age groups. Notably, pathways related to mitochondrial oxidative metabolism were particularly impaired with age. These findings suggest that mitochondrial metabolism is crucial for microglial activation and anti-tumor function, while aging hinders these adaptive mechanisms.

To further investigate this, we developed a model featuring specific ablation of mitochondrial complex III in microglia by crossing *Uqcrcq* floxed and *Tmem119-cre/ERT2* mice (referred to as QPC KO). This model demonstrated that mice with ETC-deficient microglia developed larger brain metastases. We propose to utilize this newly generated mouse model, which mimics the effects of aging through deficient mitochondrial metabolism in microglia, to examine the role of mitochondrial metabolism in the anti-tumor function of microglia. Our aim is to elucidate how and why these processes are impaired with age.

## Dilucidating the nutritional dynamics in the aged tumor microenvironment

**Daniel Curbelo Piñero**<sup>1</sup>, Pablo Castillo Serrulla<sup>1</sup>, Víctor M. Cruz-Vilchez<sup>1</sup>, Lucía del Prado Montero<sup>1</sup>, Sara Laine Menéndez<sup>1</sup>, Marcos Javier Zamora Dorta<sup>1</sup>, Eduardo Balsa Martínez<sup>1</sup>.

1. Centro de Biología Molecular Severo Ochoa (CBM-UAM) / Universidad Autónoma de Madrid (UAM).

Metastasis remains the primary cause of cancer-related deaths, yet its study has largely ignored the metabolic and cellular alterations associated with aging. This project aims to elucidate how aging remodels the tumor microenvironment (TME), reprogramming tumor and immune cells toward pro-metastatic phenotypes through changes in nutrient availability and metabolite composition. The central hypothesis is that age-dependent metabolic shifts in the TME modulate metastatic potential and therapeutic response, revealing new intervention strategies adapted to elderly populations.

To address this, the project integrates multi-omic and single-cell approaches with murine models of melanoma and breast cancer in young (<3 months) and aged (>18 months) mice. Untargeted metabolomic profiling of tumor interstitial fluid and clonal tracking will map nutrient-driven metastatic memory in cancer cells. Genomic, transcriptomic, and epigenetic analyses will define metabolic and regulatory signatures linked to metastasis. Functional validation involves CRISPRbased screens, overexpression assays, and metabolic interventions both in vitro and in vivo. Immune heterogeneity in the aged TME will be analyzed using single-cell RNA sequencing of CD45<sup>+</sup> immune cells from metastases across multiple organs, comparing both tumor types and age groups. Adoptive transfer of metabolically conditioned T cells will assess their functional adaptation to aged environments. A novel “synchronous metastasis” mouse model will be developed to induce concurrent lesions in multiple tissues, minimizing inter-animal variability while meeting ethical standards. Finally, metabolic pathways and biomarkers identified in mice will be validated in human melanoma and breast cancer samples and through public datasets.

Overall, this project aims to uncover how aging reshapes the metabolic landscape of the TME to promote metastasis, providing mechanistic insight and preclinical evidence for age-adapted metabolic therapies.

Other Information: Clinical samples are given in a collaboration with Hospital 12 de Octubre. The aim of presenting this project as a poster is to have a valuable insight of relevant experts in the field of aging and cancer, despite not having any results yet. Giving the ambitious nature of the project, this congress is an excellent opportunity to discuss the different aspects of the project, especially important for a young PhD student starting in research.

## Apoptotic Secreted Factors as Drivers of Apoptosis-Induced Senescence (AIS)

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Senescence and apoptosis are two essential cellular responses that maintain tissue homeostasis by eliminating damaged unwanted cells. Although both share evolutionary significance, they have traditionally been considered alternative, if not antagonistic, cell fates. Cellular senescence implies that damaged cells remain in the tissue, allowing intercellular communication to promote tissue regeneration. In contrast, apoptosis has long been considered a silent process in which damaged cells are removed without affecting the surrounding environment. However, nowadays there is robust scientific evidence showing that apoptotic cells release specific factors as they progress towards cell death.

The cascade of events linking tissue damage to cell replacement following stem cell mobilization has only been partially characterized. In addition, the chemical cues that lead to the appearance of senescent cells in tissues, as well as the physiological triggers that mediate their time-dependent accumulation, are yet to be deciphered. Here, we put forward the hypothesis that factors emitted by apoptotic cells prior to their removal by phagocytic cells promote the induction of senescence in neighboring cells. We suggest that Apoptosis-Induced Senescence (AIS) is an essential step for the replacement of damaged cells in response to injury and account for proficient tissue regeneration.

Our results elucidate the link between these two cell fates, revealing a paracrine induction of senescence by apoptotic conditioned media derived from different inducers of cell death. To uncover the mechanisms behind this effect, we examined the role of mitochondrial integrity and inflammatory signaling. Inhibition of BAX impaired mitochondrial apoptosis and reduced the paracrine induction of senescence. Likewise, blocking STING and NF- $\kappa$ B pathways in apoptotic cells significantly decreased senescence in recipient cells, highlighting the key contribution of inflammatory signaling to AIS.

## Senotoxins: A Novel Strategy to Target Chemotherapy-Induced Senescence

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Although senescence initially suppresses tumor growth, the accumulation of senescent cells in the tumour microenvironment compromises chemotherapy by promoting inflammation and disease relapse. We identified a pore-forming toxin derived from animal venom with potent senolytic activity, StnI. Its optimized form, StnIG, selectively targets chemotherapy-induced senescent cancer cells across diverse cancer models, senescence-induction methods and in vivo systems. This specificity arises from differences in lipid composition between senescent and proliferative cells. StnIG disrupts ion homeostasis by inducing sodium and calcium influx while sustaining potassium efflux. The calcium influx activates calcium-dependent potassium channels, ultimately triggering cell death. In vivo, StnIG enhanced the efficacy of senescence-inducing chemotherapy, leading to solid tumour remission. Our findings reveal a novel class of senolytics introduced as senotoxins that exploit vulnerabilities in ion homeostasis and lipid profiles.

## A breast tissue-specific epigenetic clock provides accurate chronological age predictions and reveals de-correlation of age and DNA methylation in tumoradjacent and tumor samples

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Epigenetic clocks have been widely used to estimate biological age across various tissues, but their accuracy in breast tissue remains suboptimal. Classical models such as Horvath's and Hannum's clocks, perform poorly in predicting chronological age in breast tissue, underscoring the need for a tissue-specific approach. In this study, we introduce a Breast Tissue-specific Epigenetic Clock (BTEC), developed using DNA methylation data from 553 healthy breast tissue samples across seven different studies. BTEC significantly outperformed existing clocks, demonstrating superior correlation with chronological age ( $r=0.88$ ) and lower prediction errors (MAE=3.27 years) without requiring for dataset-specific regressions adjustments. BTEC's chronological age predictions for tumor-adjacent samples showed distortions, with an average deviation of -1.76 years, which was even more pronounced in tumor samples, where the average difference between predicted and chronological age was -12.29 years. When analyzed by molecular subtype, the distortion was greater in the more aggressive HER2+ and TNBC tumors compared to HR+ tumors. Overall, our findings indicate that breast tumors do not generally exhibit accelerated epigenetic aging. The probes used by BTEC were associated with known oncogenes and genes involved in DNA binding and modification. Importantly, extreme deviations in epigenetic age, as measured by BTEC-derived epigenetic age acceleration (EAA), were associated with patient survival in two HR+ and TNBC cohorts, highlighting the prognostic value of tissue-specific epigenetic aging measures. These findings demonstrate that BTECs not only improve age prediction in breast tissue but also captures biologically meaningful alterations in tumor epigenetic aging with potential clinical implications.

## T Cell Senescence, Activation, and Exhaustion Define Prognosis in Elderly Patients with Metastatic Soft Tissue Sarcoma

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Soft tissue sarcomas (STS) are rare, heterogeneous malignancies whose incidence increases with advancing age. Older patients ( $\geq 70$  years) experience poorer clinical outcomes due to aggressive tumors, advanced stage at diagnosis, and limited therapeutic options, yet they remain underrepresented in clinical trials. This prospective, multicenter, randomized trial enrolled 39 elderly patients with unresectable, untreated metastatic STS to compare metronomic cyclophosphamide versus standard doxorubicin.

A total of 37 evaluable patients (median age 76.4 years; interquartile range 74.8–79.2), 21 receiving cyclophosphamide and 16 doxorubicin, underwent baseline immune profiling to assess CD4<sup>+</sup> and CD8<sup>+</sup> T-cell activation (CD38<sup>+</sup>HLA-DR<sup>+</sup>), exhaustion (PD1<sup>+</sup>, TIGIT<sup>+</sup>, CTLA-4<sup>+</sup>), and senescence (CD28<sup>+</sup>CD57<sup>+</sup>). Analyses were adjusted for age and performed in RStudio with variables dichotomized according to the best cut-off value.

Severe toxicities (grade 3–4) were more frequent with doxorubicin (50% vs 19%,  $p=0.046$ ), while tumor response rates were comparable between the two treatment arms.

No significant treatment–biomarker interaction emerged for overall survival (OS). In the entire cohort, high frequencies of senescent CD4<sup>+</sup> cells (HR=3.89,  $p=0.037$ ), activated CD4<sup>+</sup> and CD8<sup>+</sup> cells (HR=2.59,  $p=0.029$ ; HR=2.54,  $p=0.05$ ), and exhausted CD4<sup>+</sup>CTLA-4<sup>+</sup> cells (HR=2.95,  $p=0.024$ ) were associated with higher mortality risk. For progression-free survival (PFS), significant biomarker–treatment interactions occurred for CD4<sup>+</sup>TIGIT<sup>+</sup> ( $p=0.005$ ), CD4<sup>+</sup>CTLA-4<sup>+</sup> ( $p=0.022$ ), and CD8<sup>+</sup>PD1<sup>+</sup> ( $p=0.027$ ). In the doxorubicin arm, high levels of exhausted CD4<sup>+</sup>TIGIT<sup>+</sup> (HR=8.51,  $p=0.002$ ), CD4<sup>+</sup>CTLA-4<sup>+</sup> (HR=7.65,  $p=0.003$ ), and CD8<sup>+</sup>PD-1<sup>+</sup> (HR=9.21,  $p=0.004$ ) cells were strongly linked to poorer PFS.

In conclusion, T-cell senescence, activation, and exhaustion predict poor prognosis in elderly STS. With the limits of the small sample size, dictated by the rarity of the tumor and underserved population, the observed association between high levels of exhaustion markers in patients treated with doxorubicin and worse PFS suggests a possible role of immune markers in treatment outcomes.

## Colorectal location specific mechanisms of aging in healthy individuals: implication for colorectal cancer risk

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**Introduction:** Advanced age is a well-established risk factor for colorectal cancer (CRC). While studies in blood and other tissues has revealed that aging is associated with alterations in processes such as metabolism and genomic instability, few studies have investigated potential age-related mechanisms of CRC risk in normal colorectal tissues.

**Methods:** RNA was isolated from the right (ascending, n=169) and left (descending, n=191) colon, and rectum (n=196) biopsies of a cohort of healthy individuals using Qiagen AllPrep Mini kits. Paired-end RNA-seq reads were aligned to GENCODE v48 using STAR and RSEM. Location-specific regressions of aging were performed in DESeq2, while accounting for smoking, body mass index and sex.

Differentially expressed genes (DEGs) were significantly associated with aging if they survived a 5% false discovery rate (FDR) correction (age-DEGs). Pathway analysis was performed on DEGs using STRING. Repeated-measures regression analysis of RNA-seq data from matched right colon tumor and normal adjacent tissues from The Cancer Genome Atlas Colon Adenocarcinoma (TCGA-COAD) cohort (n=16 pairs) was also performed.

**Results and Discussion:** Drastically more age-associated DEGs (age-DEGs) were identified in right colon (n=982), than left colon (n=45) or rectum (n=59), with 369 genes being unique to right colon. Pathway analysis of age-DEGs in right colon revealed numerous enrichments highly relevant to CRC, such as cellular metabolic process (FDR=4.32E-09), DNA repair (FDR=1.50E-04) and epithelial cell development (FDR=8.60E-04). Fisher's exact test also revealed a significant enrichment for right colon age-DEGs and DEGs identified in TCGA-COAD (P=5.71E-10). We posited that if age-DEGs were relevant to CRC development, then, for example, genes overexpressed in CRC tumors would increase with aging. Remarkably, 79.20% (99 of 125) of overlapping significant genes followed this pattern, revealing a novel mechanistic link between aging and CRC risk.

The work was supported by research grants from the National Cancer Institute (NCI) Cancer Disparities SPORE Planning Grant (P20 CA233216), NCI (CA143237; R21 CA283132-01), as well as a pilot grant from the University of Virginia Cancer Center (P30 CA044579). All sample pre-processing steps were carried out in Rivanna, a high-performance cluster environment of the University of Virginia.

## Pharmacological Modulation of the Human Longevity Gene FOXO3

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Among all genes associated with longevity in model organisms, FOXO3 is one of the very few consistently linked to extreme human longevity. FOXO3 belongs to the FOXO family of transcription factors, which play pivotal roles in cellular adaptation to various stress conditions. FOXO proteins function as contextdependent tumor suppressors, and their dysregulation has been implicated in multiple age-related diseases. Consequently, FOXO proteins have emerged as promising targets for the development of therapeutic agents and geroprotectors.

We have established a FOXO3 drug discovery pipeline that integrates comprehensive virtual screening of small-molecule libraries with wet-lab testing of both compounds with known mechanisms of action and newly synthesized molecules. Primary screening is conducted using high-content FOXO3 nuclear translocation assays, followed by FOXO3-dependent gene reporter assays. Here, we present our workflow and highlight examples of compounds capable of modulating FOXO3 activity.

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## TERRA functions are driven by their loci of origin

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Telomeric repeat-containing RNAs (TERRAs) are long non-coding RNAs transcribed from subtelomeric regions toward telomeres. Despite extensive *in vitro* characterization, their physiological roles *in vivo* remain elusive. Here, we generate mouse models lacking the prominent TERRA loci on chromosomes 18 and 9 to dissect their locus-specific functions. We find that TERRA activity is governed by its genomic origin, as Chr18- and Chr9-TERRA knock-outs exhibit distinct and non-overlapping phenotypes. Chr18-TERRA deficiency leads to telomere elongation, progressive obesity, sex-specific disease susceptibility, and reduced lifespan, particularly in males. Proteomic and metabolic profiling reveal impaired fasting-induced fatty acid  $\beta$ -oxidation, uncovering TERRA as a novel player in hepatic energy homeostasis. In contrast, Chr9-TERRA knock-out mice display telomere shortening and a mild increase in DNA damage without affecting overall survival—possibly offset by the striking upregulation of the mitochondrial ADP/ATP translocator SLC25A31. These findings establish that TERRA molecules exert locus-specific roles in organismal physiology, unveiling a novel regulatory axis linking telomere biology to systemic homeostasis.

## The tyrosine kinase inhibitor GNF-7 targets senescent cells through allosteric activation of GCN2

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The one-two-punch approach refers to the sequential administration of two different chemotherapies, the second of which targets cancer cells that resisted the initial treatment. To find such a second punch, we performed a chemical screen to find drugs that are preferentially toxic for cells with an activated DNA damage response (DDR). This screen identified the tyrosine kinase inhibitor GNF-7 as a top hit. Subsequent work revealed that GNF-7 is a potent senolytic, even when senescence is triggered by therapies that do not activate the DDR. Consistently, GNF-7 is highly efficacious to kill cancer cells previously treated with CDK4/6 inhibitors, including in patient-derived organoids and mouse xenografts. Surprisingly, the senolytic effect of GNF-7 is not mediated by the inhibition of a tyrosine kinase (TK), but rather by the activation of GCN2, an effect previously reported for other TK inhibitors. Together, our study reports the discovery of a novel senolytic agent that strongly synergizes with CDK4/6 inhibitors when applied sequentially and expands our understanding of the mechanisms behind the anticancer effects of TK inhibitors.

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## The cGAS-STING pathway: Targeting inflammation and senescence to prevent ageing

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Our group identified the protein NSMCE2, as a suppressor of cancer and aging in mammals. NSMCE2, as part of the SMC5/6 complex, is thought to have a role in the resolution of DNA concatenates during replication. Lacking this protein, similar to the lack of Topoisomerase II, leads to chromosome segregation errors and an accumulation of micronuclei.

In this context, we speculated that part of the pathologies that are observed in NSMCE2-deficient mice, such as premature aging, could derive from the accumulation of cytoplasmic DNA. Interestingly, these mice present a rare inflammatory kidney disease, known as Karyomegalic Interstitial Nephritis (KIN). Studying these mice, we have now performed the first single/cell characterization of KIN, which further enhanced the importance of cytoplasmic DNA in the pathology. Cytoplasmic DNA is known to activate the viral sensing pathway cGAS/STING. Whether the origin of the DNA is exogenous or endogenous, it activates the cGAS/STING pathway leading to the transcription of a battery of inflammatory genes. This transcriptome induced by STING is tightly related to the Senescent Associated Secretory Phenotype. The single/cell analysis in NSMCE2-deficient mice kidneys is consistent with the activation of cGAS/STING.

Considering the cGAS/STING pathway's importance in inflammation and senescence, we hypothesize that the inhibition of this pathway in the NSMCE2-deficient mice would ameliorate their ageing phenotype. Because of this, we are exploring the use of H-151, a recently generated inhibitor of STING, as a potential new therapy.

Altogether, in the context of KIN and other aging-associated pathologies, this would make one of the first examples of possible treatment for a cGAS/STING-driven disease in response to genome instability.

## Multiplexed Quantitative Epigenomic Profiling for Aging Research

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Epigenetic changes are the hallmark of aging and hold important clues for drug discovery. Recent studies suggest that studying both, histone posttranslational modifications (hPTMs) and DNA methylation, can improve the predictive power of molecular aging clocks for disease risk management, diagnostics and precision medicine, yet current methods lack the throughput and quantitative rigor to deliver holistic epigenetic data at scale.

The EpiFinder™ Genome is a multiplexed ChIPseq platform that enables the simultaneous profiling of up to 8 hPTMs across 24 samples in a single workflow. Its pool-split strategy minimizes technical noise and supports spike in-free quantitative comparisons between conditions. EpiFinder™ Genome is compatible with compatible with native or formalin fixed cells and frozen tissues. In addition, the platform easily integrates DNA methylation profiling, allowing integrative analysis of multiple critical epigenetic layers within the same study.

EpiFinder™ cNUC is the first and one of a kind high throughput, multiplex, and quantitative epigenomics platform dedicated for liquid biopsies. EpiFinder™ cNUC allows studying hPTMs and DNA methylation simultaneously from nucleosome directly in plasma or serum, without extraction, across multiple liquid biopsies, facilitating the discovery of epigenetic biomarker signatures.

In Summary, the EpiFinder's high throughput, multiplexed, and quantitative approach can advance the aging clock models, supporting the discovery and evaluation of new epigenetic changes as well as drug-epigenome interactions, ultimately accelerating the development of interventions that target the aging process.

## Mice carrying the homologous human shelterin POT1-L259S mutation linked to pulmonary fibrosis show a telomerase deficiency-like phenotype with telomere shortening with increasing mouse generations

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The telomeric protein POT1 has been found mutated in several types of human cancer, both sporadic and familial. Several POT1 mutations found in cancer favour acquisition of the malignant features, such as longer telomeres and a higher chromosomal instability. A mutation in POT1, the L259S substitution, has also been found in patients with idiopathic pulmonary fibrosis (IPF). Pulmonary fibrosis is a lethal disease associated with damaging insults to the lung and with organismal aging. Presence of short and dysfunctional telomeres have been placed at the origin of this disease in a percentage of both familial and sporadic cases. IPF patients' cells harboring POT1-L259S mutation in heterozygosis show telomere loss, lagging strand defects, telomere damage and premature senescence (Kelich et al., JEM, 2022). To address the molecular mechanisms underlying the telomeric defects induced by POT1-L259S mutant protein in humans, we have generated a Pot1aL261S knock-in mouse harboring the murine homologous hPOT1 L259S mutation. We find that the homozygous Pot1aL261S mice show shorter telomeres and degenerative pathologies in intestine, testis and lungs at old ages, a phenotype that is aggravated with increasing mouse generations, in striking analogy to the telomerase-deficient mouse models. Furthermore, we find that the POT1a-L261S mutant protein binds more strongly to TPP1 and to telomerase and impedes telomerase-dependent telomere lengthening *in vivo*. We show that telomerase activity at telomeres is reduced in the presence of POT1a-L261S that behaves as a dominant negative mutant, thus providing a potential mechanism by which Pot1aL261S knock-in mice phenocopy the short telomere phenotype of the telomerase knock-out model. This model constitutes a useful tool to understand POT1-mediated telomeropathies including IPF and to study therapeutic strategies to treat these diseases.

## Precision chromosome engineering, a tool for studying links between rDNA stability and senescence

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Nucleoli form around tandem arrays of ribosomal DNA (rDNA) repeats, termed nucleolar organizer regions (NORs), located on the short p-arms of the five human acrocentric chromosomes (13, 14, 15, 21, 22) [1]. NORs are transcribed by RNA polymerase I to produce the major ribosomal RNAs (rRNAs). Because rDNA accounts for >90% of cellular RNA synthesis, these arrays are highly vulnerable to DNA damage such as double-strand breaks (DSBs), arising from defects in rDNA metabolism or from external insults including radiation and chemotherapy. rDNA instability has been implicated in senescence and aging, as demonstrated in yeast [2]. Our laboratory has previously used site-specific nucleases (I-PpoI and CRISPR/ Cas9) to introduce DSBs into rDNA and revealed an elaborate nucleolar DNA damage response [3]. A limitation of this approach is that I-PpoI recognition site lies within the 28S rRNA coding region of every rDNA repeat. Thus, I-PpoI introduces widespread rDNA damage, leading to poor survival. Here, we show that while high levels of rDNA damage are lethal, intermediate levels can be tolerated but drive cells into senescence, accompanied by classic markers including increased SA- $\beta$ -gal activity, induction of p21, and senescence-associated heterochromatin. It remains unclear whether this outcome reflects saturation of repair capacity or a programmed senescence response to rDNA DSBs, analogous to yeast aging [4]. Defining a threshold of rDNA damage sufficient to trigger senescence may provide insights comparable to telomere-induced senescence [5]. To address this, we developed a precision chromosome engineering system (PCE) [6] to genetically tag a single NOR with recognition sites for the meganuclease IScel, absent from the human genome. This enables controlled induction of DSBs in a single NOR, producing biologically relevant, survivable levels of rDNA damage. Using this system, we aim to characterise both immediate and long-term consequences of localised rDNA instability.

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## Activation of the Integrated Stress Response to overcome multidrug resistance in cancer therapy

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Resistance to therapy is estimated to contribute to treatment failure in up to 90% of metastatic cancer patients and remains a fundamental challenge in cancer.

In this regard, we recently found that several kinase inhibitors were able to kill multidrugresistant cancer cells, through a mechanism independent of their proposed targets.

Instead, these compounds killed cells showing multidrug resistance (MDR) by activating the so-called Integrated Stress Response (ISR) (Sanchez-Burgos et al., EMBO Mol Med, 2022). Other reports also converge on similar observations in that: (a) activation of the ISR can overcome MDR in cancer and (b) several drugs activate the ISR by an unknown mechanism.

To systematically address the potential of drugs to activate the ISR, we conducted both chemical and CRISPR/Cas9 genome-wide genetic screens using a fluorescent reporter of CHOP, a pro-apoptotic factor that is expressed upon activation of the ISR.

Chemical screens have allowed us to obtain a panoramic view of how drugs activate the ISR and to identify some surprising inductors of the response: benzimidazole derivatives. We subsequently tested these compounds in both in vitro and in vivo models of drug resistance, yielding very promising results.

As mentioned above, in addition to chemical screens, we have also performed genome-wide CRISPR/Cas9 knockout screens to evaluate which targets, when depleted, lead to a more vigorous activation of the ISR. The idea here is to identify the best targets for which an inhibitor would be an efficacious ISR activator. The screen, also based on our CHOP reporter model, has led us to identify several promising hits, including some chaperones which we are currently investigating. In summary, our work aims to provide a general overview of the genes and drugs that modify the ISR, and to use this information to develop novel strategies to overcome multidrug resistance in cancer therapy.

## The tyrosine kinase inhibitor GNF-7 targets senescent cells through allosteric activation of GCN2

**Matilde Murga**<sup>1</sup>, Gema Lopez-Pernas<sup>1</sup>, Wareed Ahmed<sup>2</sup>, Maria Haggblad<sup>2</sup>, Elena Jiménez-Ortega<sup>3</sup>, Alicia G. Serrano<sup>1</sup>, Carlota Cardona<sup>1</sup>, Mario Lopez- Prieto<sup>1</sup>, Samuele Fiscaro<sup>2</sup>, Jorge Mota-Pino<sup>1</sup>, Belén Navarro-Gonzalez<sup>1</sup>, Eduardo Zarzuela<sup>4</sup>, Marta E. Anton<sup>1</sup>, Louise Lidemalm<sup>2</sup>, Sonia Martínez<sup>5</sup>, Marta Isasa<sup>4</sup>, Joaquín Pastor<sup>5</sup>, Rafael Fernandez-Leiro<sup>3</sup>, Daniela Huhn<sup>2</sup>, Oscar Fernández Capetillo<sup>1,2</sup>

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Most cancer therapies fail to kill all tumor cells, and the few that remain have often been cataloged as “dormant”, “persister” or “senescent”. In this context, one emerging concept for cancer therapy is the “one-two-punch”, whereby the initial chemotherapy (punch) is shortly followed by a second treatment that aims to eradicate cancer cells that resisted the initial one. To find such a second punch, we performed a chemical screen to find drugs that are preferentially toxic for cells with an activated DNA damage response (DDR). This screen identified the tyrosine kinase inhibitor GNF-7 as a top hit. Subsequent work revealed that GNF-7 is a potent senolytic, even when senescence is triggered by therapies that do not activate the DDR. Consistently, GNF-7 is highly efficacious to kill cancer cells previously treated with CDK4/6 inhibitors, including in patient-derived organoids and mouse xenografts.

Surprisingly, the senolytic effect of GNF-7 is not mediated by the inhibition of a tyrosine kinase (TK), but rather by the activation of GCN2, an effect previously reported for other TK inhibitors, and for which we now provide a molecular mechanism. Together, our study reports the discovery of a novel senolytic agent and opens the door for the rational design of stress kinase agonist molecules as anticancer agents.

## Targeting senescent cells with nucleoside analogues

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Senescence is a permanent cell cycle arrest associated to the activation of an inflammatory secretory program (SASP), and which can be triggered by several stimuli such as chemotherapy or aging. Since most chemotherapies target highly replicating cells, senescent cells are often resistant to these therapies, providing a mechanism for therapy-evasion and potentially contributing to future tumor relapses. One emergent concept to overcome this problem is the so-called, “onetwo punch” strategy. The idea behind this approach is use a sequential combination of two drugs: the first one to induce senescence and the second one to selectively kills senescent cells (senolytic). While searching for efficient “second punches” that can target senescent cells, our laboratory has identified a class of nucleoside analogues that have these properties. This is rather intriguing, as nucleoside analogues are commonly thought to target DNA replication and thus would preferentially target growing cells rather than arrested one. We are currently involved in a comprehensive *in vitro* and *in vivo* characterization of these new senolytic agents and trying several approaches to decipher their mechanism of action. Our progress and ideas for the future in this topic will be presented.

## Remodeling of Intracellular Ca<sup>2+</sup> Homeostasis in Hippocampal Neurons during In Vitro Aging

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Neuronal aging is associated to changes in Ca<sup>2+</sup> signaling, a key integrator of excitability, plasticity, and cellular resilience. Here, we combined single-cell Ca<sup>2+</sup> imaging with bulk transcriptomic profiling and linear modelling to elucidate the basis of Ca<sup>2+</sup> remodeling in rat hippocampal neurons during in vitro aging. Accordingly, primary hippocampal cultures (neurons and glia) from neonatal Wistar rats were cultured for 6–8 days in vitro (DIV) or 18–21 DIV, representing young and aged neurons. Intracellular Ca<sup>2+</sup> responses to different stimuli and storeoperated Ca<sup>2+</sup> entry (SOCE) were monitored. IP3R-mediated Ca<sup>2+</sup> release was assessed using caged-IP3 photolysis and confocal microscopy. In parallel, Clariom D (rat) microarrays of the same samples were analyzed using limma (FDR<0.05), controlling for the random effect of culture origin (paired young/aged samples from the same primary preparation) and applying a Bayesian framework to infer neuronspecific expression changes from paired mixed and glia-enriched cultures within a curated Ca<sup>2+</sup> gene panel. We found that Ca<sup>2+</sup> responses mediated by voltagegated Ca<sup>2+</sup> channels, glutamate and acetylcholine receptors were enhanced in aged neurons, while SOCE nearly disappeared with age. IP3R-dependent Ca<sup>2+</sup> release also declined in aging neurons. Transcriptomic analysis revealed overexpression of a number of plasma membrane receptors including GluN2C, GluA1/2, GluK4/5, CHRM1/2, P2X5/6, P2Y4, and Cav3.1, the infraexpression of P2Y1 and CHRM3, along with altered expression of pumps/exchangers, SOCE components, ER channels, and the loss of mitochondrial Ca<sup>2+</sup> transporters. In summary, aging shifts Ca<sup>2+</sup> signaling in hippocampal neurons towards enhanced Ca<sup>2+</sup> influx but impaired SOCE, ER Ca<sup>2+</sup> release and mitochondrial Ca<sup>2+</sup> handling leading to enhanced susceptibility to neuron cell death and loss of dendritic spine stability required for memory formation. Thus, posing the question on whether Ca<sup>2+</sup> remodeling may be a novel aging hallmark

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## Dual Targeting of Senescent Cells through Ferroptosis-Inducing Senolysis and Nuclear Remodeling Senomorphics

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Cellular senescence is crucial in embryogenesis, tissue remodeling and cancer protection, but the aberrant accumulation of senescent cells drives ageing and age-related pathologies. Senotherapy, either selectively eliminating senescent cells with senolytics or modulating their harmful secretory phenotype (SASP) with senomorphics, offers promising therapeutic approaches.

Using high-throughput screening of the EU-OPENSSCREEN library (>100,000 compounds) in both proliferative and senescent cell models, we identified, validated, and patented zhertin, a novel senolytic agent with broad activity across cancer cell lines, primary fibroblasts, and multiple models of senescence.

Mechanistic studies revealed that zhertin induces ferroptosis, a non-apoptotic, iron-dependent cell death, selectively eliminating senescent cells while sparing proliferating ones. Transcriptomic and functional assays confirmed suppression of senescence-associated programs. Zhertin reduced senescence markers in human fibrotic lung explants, and in a preliminary *in vivo* study in aged (24-month) mice, PLGA-encapsulated zhertin significantly decreased senescence burden in targeted tissues, underscoring its translational potential.

In parallel, using the screening data, we identified several senomorphic compounds that attenuate the senescent phenotype without killing cells, reducing nuclear size and SASP via autophagy- and inflammation-related pathways. Cytokine profiling and transcriptomic analyses confirmed this suppressive effect on the pro-inflammatory secretory program. Importantly, functional assays revealed that senescent cells treated with these compounds had a reduced capacity to induce paracrine senescence in neighboring cells, suggesting a shift toward a less deleterious cellular state.

Together, this dual senolytic/senomorphic strategy uncovers key vulnerabilities of senescent cells and provides complementary therapeutic approaches for aging- and fibrosis-related diseases.

## Immune activation and aging profile: risk factors for cancer onset in kidney transplant recipients

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Kidney transplant recipients (KTR) have a higher risk of malignancy and cancer-related mortality than the general population. This study aimed to investigate whether inflammation, immune activation, and aging profile may represent prognostic biomarkers of cancer onset in KTR.

Within a cohort of 850 KTR, this study included 21 patients who developed cancer (KTR-C) after a median time of 0.9 [interquartile range-IQR: 0.5-1.8] years from transplantation, and 66 age- and therapy-matched patients who remained cancer-free after transplantation (KTR-NC) with a median follow-up of 7.4 [6.7-8.4] years, as controls. Immune profiling (activation, exhaustion and senescence of CD8+ T cells) was assessed by flow cytometry. Relative telomere length (RTL) in PBMC, and plasma levels of PAMPs (16S rDNA) and DAMPs (mtDNA) were measured by realtime PCR. Circulating pro-inflammatory cytokines (IL-10, IL-6, TNF- $\alpha$ ) were quantified by ELISA. Statistical analyses were performed using RStudio software.

At transplantation, KTR-C showed significantly higher percentages of activated ( $p=0.011$ ) and senescent ( $p=0.051$ ) CD8+ T cells, higher levels of 16S rDNA ( $p=0.024$ ), pro-inflammatory TNF- $\alpha$  ( $p=0.002$ ) and IL-6 ( $p=0.031$ ), and shorter telomeres ( $p=0.012$ ) compared to KTR-NC. Notably, high percentages (up to the best cut-off value) of activated (HR=3.17,  $p=0.019$ ), exhausted (HR=3.39,  $p=0.021$ ) and senescent (HR=3.98,  $p=0.011$ ) CD8+ T cells, as well as high levels of proinflammatory markers (IL-10: HR=6.35,  $p=0.021$ ; TNF- $\alpha$ : HR=4.58,  $p=0.016$ ) and short telomere length (RTL: HR=5.67,  $p=0.023$ ) were associated with a higher risk of developing cancer.

In conclusion, immune activation and aging profiles are promising cancer prognostic biomarkers for minimally invasive monitoring in kidney transplant recipients.

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## Ribosomopathies at the core of aging and neurodegeneration

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Aging is the strongest risk factor for most neurodegenerative diseases, yet the molecular mechanisms linking aging to neuronal loss remain poorly understood. Recent work in our lab demonstrated that the most common genetic alteration in ALS—a hexanucleotide expansion in C9ORF72—leads to the accumulation of orphan ribosomal proteins (oRPs), a hallmark of diseases known as “ribosomopathies” [1]. This accumulation is deleterious for animal cells because the excess of unassembled ribosomal proteins overwhelms the proteostasis machinery, saturating pathways such as the proteasome and autophagy. Notably, our previous data showed that this phenotype is sufficient to drive accelerated aging in mice, pointing to a potential mechanistic bridge between aging and neurodegeneration. We are now extending these findings to assess whether ribosomal and nucleolar stress are generalizable features across multiple sources of ALS (mutational or toxin-related) and aging contexts. In addition, we are integrating transcriptomic data from ALS, ribosomopathy, and aging models to assess their convergence at the gene expression level. Preliminary analyses suggest that ribosome-related pathways are a unifying hallmark that links aging and neurodegeneration. Altogether, our work explores the possibility that ribosomopathy-like states may underlie the interplay between aging and neurodegeneration, potentially offering new targets for therapeutic intervention.

[1] Sirozh O, Saez-Mas A, Jung B, Sanchez-Burgos L, Zarzuela E, Rodrigo-Perez S, Ventoso I, Lafarga V, Fernandez-Capetillo O. Nucleolar stress caused by argininerich peptides triggers a ribosomopathy and accelerates aging in mice. *Mol Cell*. 2024 Apr 18;84(8):1527-1540.e7.

Currently working on the manuscript

## Diallyl Disulfide Promotes Dopaminergic Neuronal Resilience and Mitochondrial Integrity in Preclinical Models of Parkinson's Disease

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Parkinson's disease (PD) arises from a cascade of interrelated pathologies, including  $\alpha$ -synuclein ( $\alpha$ -syn) aggregation, synaptic and mitochondrial dysfunction, and axonopathy, ultimately leading to nigrostriatal dopaminergic (DA) degeneration. To target these converging mechanisms, we performed a high-throughput phenotypic screen and identified diallyl disulfide (DADS) as a lead compound capable of restoring neuronal structure and network connectivity in preclinical PD models. DADS is a bioactive organosulfur molecule with recognized cytoprotective, antioxidant, anti-inflammatory, and anti-apoptotic properties, although its neuroprotective potential in PD has not been previously characterized. In primary rat ventral midbrain cultures exposed to rotenone, a mitochondrial complex I inhibitor that mimics  $\alpha$ -syn aggregation and DA vulnerability, DADS significantly preserved DA neuron number and network complexity using our patented 3D neuronal profiling platform. In vivo, chronic oral DADS administration (2 mg/kg every 48 h for 3 months) in a unilateral AAV9-A53T  $\alpha$ -syn model improved exploratory behavior and postural control, reduced  $\alpha$ -syn accumulation, preserved nigrostriatal integrity, and normalized synaptic and mitochondrial protein expression. Collectively, these findings identify DADS as a novel driver of DA resilience, integrating mitochondrial protection, structural preservation, and network recovery, with strong translational promise as a safe and non-invasive disease-modifying therapy for PD.

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# Molecular and Cellular Hallmarks of Aging: 3rd edition

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Madrid 17<sup>th</sup> - 19<sup>th</sup> Nov 2025

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## Notes

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