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# Scientific News
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# Sven Bulterijs

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**PREPRINT**

# Against "Extending Healthspan but Not Lifespan" as a Goal for Biogerontology

David Gems *, Ekaterina R. Iijima, João Pedro de Magalhães

## Abstract

What is the ultimate goal of biogerontology (the study of the biology of aging)? Ask a biogerontologist and a common answer these days is that it is to extend healthspan rather than lifespan. But is this really a coherent aim for the field? Here we argue that it is not.

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# Living healthily to 120: Implications for entrepreneurship

Marco van Gelderen

Research on how to slow, halt, and reverse aging processes is making progress. Pharmaceutical, big-tech, and venture capital companies and their owners are making considerable investments in potential (*epi*)genetic, molecular, cellular, and organ-based interventions and therapies. This essay considers implications for entrepreneurship in the case that such interventions would eventually result in a doubling of the human healthspan. A vastly extended middle age would imply potential changes in opportunities, the future time perspective of entrepreneurs, the pace of time as experienced by entrepreneurs, and the relationship between age and entrepreneurial success. The essay also outlines a range of wider considerations. The final section ties the essay together by discussing how entrepreneurship scholarship can help move the longevity domain forward.

# Stealth BioTherapeutics' Forzinity

**FDA approval of Stealth's mitochondria-targeted therapy could pave the way for a host of longevity biotech companies.**

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# Ochre Bio

## Derisking clinical trials requires a 'human first' approach

Author: **Danny Sullivan** | Published on: **October 23, 2025** | Last updated: **October 23, 2025**

# Aging research articles

# Global, Regional, and National Burden of Cardiovascular Diseases and Risk Factors in 204 Countries and Territories, 1990-2023

CVDs were the leading cause of disability-adjusted life years (DALYs) and deaths estimated in the GBD. As of 2023, there were 437 million (95% UI: 401 to 465 million) CVD DALYs globally, a 1.4-fold increase from the number in 1990 of 320 million (292 to 344 million). Ischemic heart disease, intracerebral hemorrhage, ischemic stroke, and hypertensive heart disease were the leading cardiovascular causes of DALYs in 2023 globally. As of 2023, age-standardized CVD DALY rates were highest in low and low-middle Socio-demographic Index (SDI) settings and lowest in high SDI settings. The number of CVD deaths increased globally from 13.1 million (95% UI: 12.2 to 14.0 million) in 1990 to 19.2 million (95% UI: 17.4 to 20.4 million) in 2023. The number of prevalent cases of CVD more than doubled since 1990, with 311 million (95% UI: 294 to 333 million) prevalent cases of CVD in 1990 and 626 million (95% UI: 591 to 672 million) prevalent cases in 2023 globally. A total of 79.6% (95% UI: 75.7% to 82.5%) of CVD burden is attributable to modifiable risk factors 347 million [95% UI: 318 to 373 million] DALYs in 2023). Globally, high systolic blood pressure, dietary risks, high low-density lipoprotein cholesterol, and air pollution were the modifiable risks responsible for most attributable CVD burden in 2023. Since 1990, changes in exposure to modifiable risk factors have had mixed effects on CVD burden, with increases in high body mass index, high fasting plasma glucose, and low physical activity leading to higher burden, while reductions in tobacco usage have mitigated some of these increases. Population growth and population aging were the main drivers of the increasing burden since 1990, adding 128 million (95% UI: 115 to 139 million) and 139 million (95% UI: 126 to 151 million) CVD DALYs to the increase in CVD burden since 1990.

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# Genetic signatures of exceptional longevity: a comprehensive analysis of coding region single nucleotide polymorphisms (SNPs) in centenarians and supercentenarians

Aging, a complex biological process, entails sequential changes in organisms that elevate the risk of frailty, disease, and mortality, affecting individuals at the level of cellular, organ, and organism. This process is influenced by genetic diversity, socioeconomic status, healthcare infrastructure, lifestyle choices, and cultural practices. Gerontology delves into the factors shaping longevity, aging processes, and aging from both evolutionary and individual perspectives. Centenarians and supercentenarians serve as models for studying exceptional longevity, offering insights into the aging process and resistance to age-related diseases. This research investigates common genetic variations (SNPs) shared among 3 centenarians and 18 supercentenarians, individuals aged 110 years or older. 754,520 SNPs were found to be common among all the 21 samples. Utilizing SNPnexus, a genetic variant annotation tool, we annotated coding variants and assessed potential disease susceptibilities associated with these variants. Ensembl was used as an annotation system, we annotated 1,607,122 variants, and found 11,348 coding variants. Among them, 4980 had non-synonymous variants, and 110 variants were observed to have deleterious effects. These deleterious SNPs were linked with 79 genes among them 16 novel variants were identified in 9 genes. The population frequency comparison using the 1000 Genomes Project and gnomAD revealed that a subset of these common, non-synonymous SNPs and deleterious SNPs had minor allele frequencies (MAF) below 1% or were absent entirely, suggesting potential rare variants specific to this cohort. In addition, we also found statistically significant ($p < 0.05$) 148 enriched pathways, among them the top enriched pathways such as extracellular matrix (ECM) remodeling, signal transduction, disease-associated pathways, sensory processing and metabolism of proteins and RNA. These preliminary findings may help prioritize candidate variants and genes for future studies on larger cohorts with appropriate controls can help in understanding the genetic basis of exceptional longevity.

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## Protracted fate acquisition and epigenetic de-aging during induced neural stem cell conversion of human blood cells

Transcription factor-based direct conversion of somatic cells represents an interesting avenue for generating induced neural stem cells (iNSCs) from peripheral blood without transit through a pluripotent stage. While this paradigm has been shown to be associated with epigenetic de-aging, the dynamics of this process have remained unclear. Here, we used overexpression of the two reprogramming factors SOX2 and cMYC to generate iNSC from erythroid progenitors of donors ranging from neonatal to 101 years of age. Using an epigenetic clock algorithm, we corroborated our previous finding that iNSCs generated from aged donors show pronounced epigenetic de-aging, preserving around 13 % and 5 % of the original donor age at low and high passages, respectively. Studying the dynamic of epigenetic de-aging during iNSC conversion across time, we found that this process is largely protracted, continuing for several weeks and even beyond forced neuronal differentiation of iNSCs. Transcriptomic differences between young and old donor-derived iNSCs dissipate with extended time in conversion, too. Concordant with this observation, established iNSC lines lack age-associated cellular hallmarks, similar to induced pluripotent stem cells and their derivatives. Interestingly, time course analysis of DNA methylation and RNA sequencing data revealed that acquisition of a bona fide NSC signature extends greatly beyond the time point when proliferative PAX6-positive iNSCs emerge. The unexpected slow dynamics of these processes makes iNSC conversion an attractive model for dissecting the mechanisms underlying somatic transdifferentiation and de-aging.

# Chemical reprogramming ameliorates cellular hallmarks of aging and extends lifespan

Lucas Schoenfeldt, Patrick T Paine, Sara Picó, Nibrasul H Kamaludeen M, Grace B Phelps, Calida Mrabti, Gabriela Desdín-Micó, María del Carmen Maza, Kevin Perez, and Alejandro Ocampo | AUTHOR INFORMATION

The dedifferentiation of somatic cells into a pluripotent state by cellular reprogramming coincides with a reversal of age-associated molecular hallmarks. Although transcription factor induced cellular reprogramming has been shown to ameliorate these aging phenotypes in human cells and extend health and lifespan in mice, translational applications of this approach are still limited. More recently, chemical reprogramming via small molecule cocktails have demonstrated a similar ability to induce pluripotency in vitro, however, its potential impact on aging is unknown. Here, we demonstrated that chemical-induced partial reprogramming can improve key drivers of aging including genomic instability and epigenetic alterations in aged human cells. Moreover, we identified an optimized combination of two reprogramming molecules sufficient to induce the amelioration of additional aging phenotypes including cellular senescence and oxidative stress. Importantly, in vivo application of this two-chemical combination significantly extended *C. elegans* lifespan and healthspan. Together, these data demonstrate that improvement of key drivers of aging and lifespan extension is possible via chemical-induced partial reprogramming, opening a path towards future translational applications.

- A seven-compound (7c) reprogramming cocktail was shown to reverse multiple aging hallmarks in human dermal fibroblasts.
- A reduced two-compound (2c) cocktail was identified that retained rejuvenating effects in vitro and was sufficient to ameliorate additional aging phenotypes, including senescence, heterochromatin loss, genomic instability, and oxidative stress.
- 2c treatment in C. elegans improved stress resistance, thermotolerance, reproductive and healthspan markers, and extended median lifespan by over 42%.
- The results suggest that partial chemical reprogramming could modulate the underlying mechanisms of aging and reproductive aging, offering a potential strategy for extending both healthspan and overall lifespan in aging populations.

# Preservation of Autophagy May Be a Mechanism Behind Healthy Aging

Autophagy is intricately linked with protective cellular processes, including mitochondrial function, proteostasis, and cellular senescence. Animal studies have indicated that autophagy becomes dysfunctional with aging and may contribute to T cell immunosenescence. In humans, it remains unclear whether autophagy is impaired in $CD4^+$ T cells as people age. To answer this question, we examined basal and inducible autophagic activity in a series of experiments comparing $CD4^+$ T cells from younger (23-35 years old) and older (67-93 years old) healthy donors. We used immunofluorescence to detect LC3 (a marker of autophagosomes and autolysosomes) and LAMP2 (a marker of endolysosomes) in conjunction with bafilomycin $A_1$ (which inhibits the acidification of lysosomes) and CCCP (a mitochondrial uncoupler) to manipulate autophagic flux. We found a significantly higher autophagy flux in $CD4^+$ T cells from older compared to younger donors and a higher number of $LC3^+$ compartments among older donors. Since the overall amount of autophagosomes degraded was comparable between the two groups, we concluded that autophagosome biogenesis was reduced in the older group. Rather than a decline, our findings in healthy older donors point toward a compensatory enhancement of human $CD4^+$ T cell autophagy with age, which may be a mechanism behind healthy aging.

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## A multi-omics analysis of human fibroblasts overexpressing an *Alu* transposon reveals widespread disruptions in aging-associated pathways

During aging and cellular senescence, repetitive elements are frequently transcriptionally derepressed across species and cell types. Among these, the most abundant repeats by copy number in the human genome are *Alu* retrotransposons. Though *Alu* elements are often studied for their mutagenic potential, there is increasing appreciation for their contributions to other biological functions, including pro-inflammatory signaling and mitochondrial dysfunction. However, a comprehensive analysis of *Alu*-driven molecular changes remains to be conducted, and *Alu*'s potential contributions to aging features remain incompletely characterized. Here, we show that overexpression of an *AluJb* transposon in human primary IMR-90 fibroblasts leads to large-scale alterations across the transcriptome, cellular proteome, and secretome. Functional genomics analyses reveal alterations in aging pathways, broadly, and mitochondrial metabolism, proteostasis, cell cycle, and extracellular matrix pathways, more specifically. Our results demonstrate that *Alu* transcriptional upregulation is sufficient to drive widespread disruptions to cellular homeostasis that mirror aging-associated alterations.

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## FOXM1 enhances DNA repair in aged cells to maintain the peripheral heterochromatin barrier to senescence enhancers

DNA damage is a key driver of aging, contributing to epigenetic erosion, senescence, and chronic inflammation. However, genoprotective strategies to counteract aging remain intangible. Here we show that FOXM1 repression during aging accounts for a global transcriptional shutdown of DNA repair genes and the accrual of DNA damage. Restored FOXM1 activity in aged cells reduces DNA damage and epigenetic alterations driving senescence. Mechanistically, FOXM1 drives the transcription of DNA repair genes, which prevents the DNA damage-driven degradation of the G9a methyltransferase and subsequent loss of H3K9me2 at the nuclear periphery. Remarkably, we show that amendment of the H3K9me2 guidepost for peripheral heterochromatin by FOXM1 induction in aged cells inactivates enhancers of the AP-1-driven senescence and inflammation program. These findings establish FOXM1 as an age-reversal factor capable of restoring (epi)genetic integrity to inhibit the senescence enhancer landscape, offering a promising therapeutic avenue to address the fundamental causes of aging.

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# Mapping the Progression of Therapy-Induced Senescence to Therapy Tolerance: An Evolutionarily Conserved Mechanism for Optimizing Cancer Treatment with Senotherapeutics

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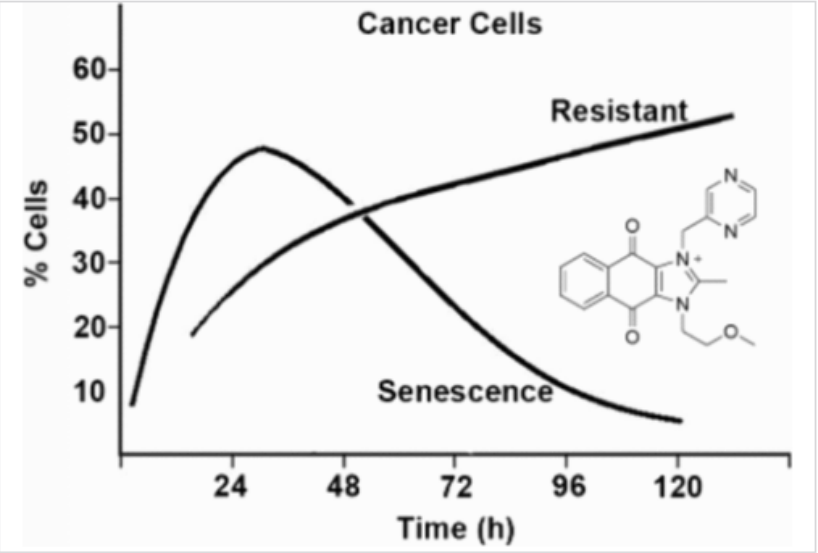


Therapy-induced senescence (TIS) is a reversible growth arrest induced by anticancer treatments, which may contribute to the development of long-term therapy resistance in tumor cells. Senotherapeutics, agents targeting senescent cells, are being tested in clinical trials to improve patient outcomes. Due to the transient nature of TIS, we hypothesized that senolytics would be most effective when administered at the appropriate time. We created a reliable TIS cell line model in triple-negative breast cancer (TNBC) using experimental drug YM155. We observed that a single dose of YM155 triggers a brief senescence, leading to a persistent drug-tolerant state that cannot be reversed by redosing. This reversibility is not limited to cancer cells. It extends to noncancerous human cells and live zebrafish larvae, suggesting a rapid adaptation mechanism against xenobiotics. We identified transforming growth factor-β (TGF-β), a cytokine linked to TNBC chemoresistance, as being expressed alongside the emergence of drug tolerance. We inhibited TGF-β signaling to eliminate the tolerant phenotype and promote the clearance of cancer cells by immune cells. However, this was most effective within a specific time window after TIS induction. We suggest that the timely use of senotherapeutics could improve the effectiveness of anticancer drugs in clinical settings.

# Lymphoma accelerates T cell and tissue aging

The combined effects of aging and cancer on immune cells were investigated in young versus aged mice harboring B cell lymphoma, and in T cells from young and aged B cell lymphoma patients. These analyses revealed that lymphoma alone is sufficient to trigger transcriptional, epigenetic, and phenotypic alterations in young T cells that manifest in aged T cells. In contrast, aged T cells are largely resistant to lymphoma-induced changes. Pathway analyses revealed open chromatin regions and genes controlling iron homeostasis are induced by both lymphoma and aging, and lymphoma-experienced and aged T cells have increased iron pools and are resistant to ferroptosis. Furthermore, both aged and lymphoma-experienced T cells have defects in proteostasis. B cell lymphoma also accelerates aging of other tissues, as evidenced by elevated expression of *Cdkn2a* and *Tnfa*. Finally, some lymphoma-induced aging phenotypes are reversible whereas others are fixed, indicating opportunities for improving some cancer-associated aging comorbidities.

# Contributions of inherited mtDNA to longevity: evidence from extended pedigrees with 176 million kinship pairs

## Background

Mitochondria are bacteria-like organelles with their own DNA (mtDNA) that exist in the cellular cytoplasm of almost every cell in the human body. Because mitochondria are critical for sustaining life, it follows that inherited mtDNA could be a key aetiologic element underlying longevity. Unfortunately, biometric approaches able to quantify heritable contributions of mtDNA have not been available.

## Methods

We directly leveraged the unique matrilineal inheritance pattern of mtDNA to estimate its effects on longevity (defined as the top 10% oldest survivors within their birth cohort). We employed the Utah Population Database (UPDB) to identify 176,348,110 unique kinship links amongst 1,018,929 individuals born between 1700 and 1925 with information on matrilineal versus patrilineal relatedness.

## Findings

Across 1st, 2nd, 3rd, 4th, and 5th degree kin, matrilineal relatives were more similar in their longevity outcomes than were non-maternal relatives. Variance component analyses indicated nuclear DNA heritability of 23–26% and mtDNA heritability of at least 5% – despite mtDNA constituting only ~16.6 k base pairs (versus 2,875,002 k base pairs for nuclear DNA). Moreover, sharing the maternal line of a longevous relative translated to an average of 11.3 months extra years of life.

# Association of Epigenetic Age Acceleration and Mitochondrial DNA-Based Aging Metrics Provides Insights Into Mechanisms of Aging-Related Diseases

Investigating the interplay between mitochondrial DNA (mtDNA) variations and epigenetic aging metrics may elucidate biological mechanisms associated with age-related diseases. We estimated epigenetic age acceleration (EAA) metrics from DNA methylation data and derived mtDNA metrics, including heteroplasmic variants and mtDNA copy number (mtDNA CN) from whole genome sequencing. Linear regressions and meta-analyses were conducted to assess associations between EAA and mtDNA metrics, adjusting for chronological age, self-identified sex, and other covariates in 6,316 participants (58% female, 41% non-White Americans). Mediation analysis was conducted to examine whether EAA mediated the relationship between mtDNA CN and metabolic traits. A higher burden of rare heteroplasmic variants was associated with accelerations of first-generation EAA metrics, while a lower level mtDNA CN was associated with accelerations of second- and third-generation EAA metrics. For example, one standard deviation (SD) higher MSS, a score based on the predicted functions of rare heteroplasmic variants, was associated with a 0.22-year higher EAA by the Hannum method ($p$ = 1.3E-6) among all participants, while one SD lower mtDNA CN was associated with higher DunedinPACE ($\beta$ = −0.005, $p$ = 6.0E-4). No significant association was observed between the heteroplasmy burden of common variants and EAAs. Furthermore, we observed DunedinPACE mediated 11.1% and 10.8% of the associations of mtDNA CN with obesity and T2DM in older FHS participants, respectively. Our analysis indicated that higher levels of heteroplasmy burden of rare variants and lower mtDNA CN were associated with accelerated epigenetic aging, and these associations showed stronger magnitudes among older participants.

# Lipid raft proteomics identify endothelial myosin-9 (MYH9) as a regulator of low-density lipoprotein transcytosis and atherosclerosis

Background: In early atherosclerosis, circulating Low-Density Lipoprotein (LDL) crosses the endothelium by transcytosis. This involves caveolar uptake of LDL by scavenger receptor BI (SR-BI) and activin-like kinase 1 (ALK1) and requires the protein caveolin-1 (Cav-1). We identified mediators of LDL transcytosis by isolating membrane microdomains enriched in caveolin-1 from human coronary endothelial cells (HCAECs) treated with LDL and performing mass spectrometry. One of the proteins identified was myosin-9 (MYH9). Methods: Total internal reflection fluorescence microscopy was conducted to measure LDL transcytosis by HCAECs. We measured LDL transcytosis in vivo in mice lacking endothelial MYH9 (EC-*Myh9*$^{-/-}$). Atherosclerosis studies were also performed in EC-*Myh9*$^{-/-}$ deleted of hepatic LDLR via (adeno-associated virus, AAV)-CRISPR. Additionally, we performed analysis of human transcriptomic data. Results: Gene ontology analysis in human aortic endothelial cells suggested a role for MYH9 in exocytosis. Both knockdown and pharmacologic inhibition of MYH9 inhibited LDL transcytosis. MYH9 depletion caused an accumulation of LDL-containing vesicles at the base of the cell; overexpression caused an increase in LDL exocytosis. EC-*Myh9*$^{-/-}$ mice accumulated less LDL in the aortic arch after acute injection with LDL. To investigate the role of MYH9 in atherosclerosis, we deleted hepatic LDL in EC-*Myh9*$^{-/-}$ mice using AAV-CRISPR and fed them a high-fat diet. The aortic arch and root of AAV-CRISPR; EC-*Myh9*$^{-/-}$ mice exhibited smaller plaques. Human transcriptomic data showed greater messenger RNA (mRNA) levels of aortic *MYH9* in atherosclerotic aortas compared to healthy controls. Conclusions: Lipid raft proteomics identified MYH9 as a regulator of LDL transcytosis. MYH9 is required for endothelial LDL exocytosis and contributes to early atherosclerosis.

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# Vascular Niches Are the Primary Hotspots in Cardiac Aging

**Background:** Aging is a major, yet unmodifiable, cardiovascular risk factor and is associated with vascular alterations, increased cardiac fibrosis, and inflammation, all of which contribute to impaired cardiac function. However, the microenvironment inciting age-related alterations within the multicellular architecture of the cardiac tissue is unknown.

**Methods:** We investigated local microenvironments in aged mice hearts by applying an integrative approach combining single-nucleus RNA sequencing and spatial transcriptomics of 3- and 18-month-old mice. We defined distinct cardiac niches and studied changes in their cellular composition and functional characteristics. We treated mice with broad-spectrum senolytics dasatinib and quercetin, and endothelial-specific senolytic fisetin and studied their effects on senescence and macrophage populations.

**Results:** Integration of spatial transcriptomics data across 3- and 18-month-old hearts allowed the identification of 11 cardiac niches, which were characterized by distinct cellular composition and functional signatures. Aging did not alter the overall proportions of cardiac niches but led to distinct regional changes, particularly in the left ventricle. While cardiomyocyte-enriched niches showed disrupted circadian clock gene expression, vascular niches showed major changes in proinflammatory and profibrotic signatures and altered cellular composition. We particularly identified larger vessel-associated cellular niches as key hotspots for activated fibroblasts and bone marrow-derived $Lyve1^-$ and resident $Lyve1^+$ macrophages in aged hearts, with interactions of both cell types through the C3:C3ar1 axis. These niches were also enriched in senescent cells exhibiting high expression of immune evasion mechanisms that may impair senescent cell clearance. Removal of senescent cells by senolytics reduced the presence of $Lyve1^-$ macrophages.

**Conclusions:** Our findings indicate that the perivascular microenvironment is particularly susceptible to age-related changes and serves as a primary site for inflammation-driven aging, so-called inflammaging. This study provides new insights into how aging reshapes cardiac cellular architecture, highlighting vessel-associated niches as potential therapeutic targets for age-related cardiac dysfunction.

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# Niche-specific dermal macrophage loss promotes skin capillary ageing

All mammalian organs depend on resident macrophage populations to coordinate repair and facilitate tissue-specific functions[1,2,3]. Functionally distinct macrophage populations reside in discrete tissue niches and are replenished through a combination of local proliferation and monocyte recruitment[4,5]. Declines in macrophage abundance and function have been linked to age-associated pathologies, including atherosclerosis, cancer and neurodegeneration[6,7,8]. However, the mechanisms that coordinate macrophage organization and replenishment within ageing tissues remain largely unclear. Here we show that capillary-associated macrophages (CAMs) are selectively lost over time, contributing to impaired vascular repair and reduced tissue perfusion in older mice. To investigate resident macrophage behaviour in vivo, we used intravital two-photon microscopy in live mice to non-invasively image the skin capillary plexus, a spatially well-defined vascular niche that undergoes rarefication and functional decline with age. We find that CAMs are lost at a rate exceeding capillary loss, resulting in macrophage-deficient vascular niches in both mice and humans. CAM phagocytic activity was locally required to repair obstructed capillary blood flow, leaving macrophage-deficient niches selectively vulnerable under homeostatic and injury conditions. Our study demonstrates that homeostatic renewal of resident macrophages is less precisely regulated than previously suggested[9,10,11]. Specifically, neighbouring macrophages do not proliferate or reorganize to compensate for macrophage loss without injury or increased growth factors, such as colony-stimulating factor 1 (CSF1). These limitations in macrophage renewal may represent early and targetable contributors to tissue ageing.

# Alzheimer Disease, Vascular Disease, and Blood-Brain Barrier Permeability Biomarkers in Middle-Aged Adults


## Methods

The study included middle-aged participants from the Offspring Study of Racial and Ethnic Disparities in AD who had MRI and plasma biomarker data available. Biomarker concentrations of vascular endothelial growth factor (VEGF) family members (VEGF-D, placental growth factor [PlGF], and basic fibroblast growth factor [bFGF]) were measured using the Meso Scale Discovery platform. β-Amyloid (Aβ42, Aβ40), phosphorylated tau 181 (p-tau181), astrocytosis (glial fibrillary acidic protein [GFAP]), and neurodegeneration (neurofilament light chain [NfL]) biomarkers were measured with Simoa immunoassays. White matter hyperintensity (WMH) volumes were derived from T2-weighted MRI scans. Bivariate relationships of WMH, Aβ42/Aβ40 ratio, p-tau181, GFAP, and NfL with VEGF biomarkers were tested, and path analyses examined potential causal pathways linking each VEGF biomarker concentration to WMH and GFAP, as well as their downstream associations with tau pathology and neurodegeneration.

## Results

We analyzed data from 488 participants (mean [SD] age = 54.3 [10.5]; 66.8% women). Higher PlGF levels were associated with older age (R [CI] = 0.25 [0.17–0.33]); greater WMH volume (R [CI] = 0.2 [0.11–0.29]); and higher levels of GFAP (R [CI] = 0.11 [0.02–0.2]), p-tau181 (R [CI] = 0.12 [0.03–0.21]), and NfL (R [CI] = 0.19 [0.1–0.27]). Higher VEGF-D was associated with increased GFAP (R [CI] = 0.11 [0.02–0.19]) and NfL (R [CI] = 0.16 [0.07–0.25]) levels. bFGF concentration was associated with a lower Aβ42/40 ratio (R [CI] = −0.1 [−0.19 to −0.02]) and higher p-tau181 levels (R [CI] = 0.13 [0.04–0.21]). The best fitting path model showed that PlGF had an indirect effect on GFAP levels mediated by WMH. GFAP subsequently had a direct positive effect on p-tau181, which in turn had a positive effect on NfL levels. VEGF-D and bFGF levels also had a positive direct effect on NfL.

# Single nucleus RNA sequencing unveils relationship between microglia and endothelial cells in mixed Alzheimer's disease and vascular pathology

Single-nucleus RNA sequencing (snRNAseq) allows for the dissection of cell type-specific transcriptional profiles. We evaluated differential gene expression using snRNAseq data generated from hippocampal region tissue donated by 11 Boston University Alzheimer's Disease Research Center (BU-ADRC) participants with neuropathologically confirmed Alzheimer's disease (AD) with or without co-existing pathology (AD only = 3, AD+vascular disease (Vas) = 6, AD+Lewy body disease (LBD) = 2). Expression of 19,893 genes was compared between AD+Vas and other AD groups for each cell type. Co-expression modules were identified in a set of 174 bulk RNAseq hippocampal samples from BU-ADRC. Modules enriched in differentially expressed genes (DEGs) were identified using Fisher's exact tests. The overlap between DEGs and co-expression modules was incorporated into quantitative gene set analysis. AD+Vas subjects showed decreased expression of genes related to immune activation in microglia ($t = -2.67$, $p = 2.72 \times 10^{-2}$). Expression of these genes was negatively associated with expression of receptors P2RY12 and CX3CR1 ($r = -0.87$, $p = 1.70 \times 10^{-2}$), which have been linked to microglial migration and activation, respectively. Expression of genes that negatively regulate angiogenesis in endothelial cells was decreased ($t = -4.84$, $p = 1.49 \times 10^{-3}$) and associated with expression of the microglial activation genes in the BU-ADRC dataset ($r = 0.68$, $p = 1.63 \times 10^{-2}$). This association and the finding of upregulation of P2RY12 in AD+Vas samples were replicated in 393 ROSMAP Study dorsolateral prefrontal cortex snRNAseq samples ($r = 0.34$, $p = 8.37 \times 10^{-12}$ and $z = 5.82$, $p_{FDR} = 8.73 \times 10^{-6}$, respectively). In summary, we found an expression profile in brain tissue from individuals with AD+Vas pathology that is associated with reduced activation and increased migration in microglia and angiogenesis in endothelial cells.

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## A proteomic signature of vascular dysfunction linked to tauopathy and degeneration in the aging brain

Small vessel disease (SVD) impacts healthy aging of organs across the body, yet its contributions to adverse brain aging remain poorly defined. Here we show thromboinflammation, a core feature of SVD, as a driver of adverse brain aging. We identify cerebrospinal fluid fibrinogen as a marker of brain thromboinflammation and screen neurovascular biosignatures mediating its impact on synaptic vulnerability along the full spectrum of brain aging from cognitively typical, amyloid-negative to cognitively impaired, amyloid-positive older adults. We identified 53 proteins mediating the effect of fibrinogen on synaptic markers in 1,655 donors from three independent cohorts. Single-cell transcriptomic mapping revealed mediator enrichment in neurovascular unit cells. Pathway analysis demonstrated dysregulation of angiogenesis, fibrosis, and immune signaling. Vascular and microglial-enriched biosignatures associated with compromised white matter integrity. These findings implicate thromboinflammation as an early, amyloid-independent pathway to neurodegeneration and tauopathy, establishing vascular health as fundamental to preserving brain healthspan.

# Multiscale proteomic modeling reveals protein networks driving Alzheimer's disease pathogenesis

The molecular mechanisms underlying the pathogenesis of Alzheimer's disease (AD), the most common form of dementia, remain poorly understood. Proteomics offers a crucial approach to elucidating AD pathogenesis, as alterations in protein expression are more directly linked to phenotypic outcomes than changes at the genetic or transcriptomic level. In this study, we develop multiscale proteomic network models for AD by integrating large-scale matched proteomic and genetic data from brain regions vulnerable to the disease. These models reveal detailed protein interaction structures and identify putative key driver proteins (KDPs) involved in AD progression. Notably, the network analysis uncovers an AD-associated subnetwork that captures glia-neuron interactions. AHNAK, a top KDP in this glia-neuron network, is experimentally validated in human induced pluripotent stem cell (iPSC)-based models of AD. This systematic identification of dysregulated protein regulatory networks and KDPs lays down a foundation for developing innovative therapeutic strategies for AD.

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## Neuronal glycolytic reprogramming drives lethality via accelerated aging in a Drosophila model of tauopathy

Neurometabolic dysfunction is a hallmark of Alzheimer's disease (AD) and tauopathies. Whether these changes drive pathology or represent compensatory, protective responses remains unresolved. Here, we demonstrate that human tau induces Warburg-like metabolism in Drosophila neurons, characterized by coordinated upregulation of glycolytic enzymes and lactate dehydrogenase that mirrors metabolic signatures in human AD. Despite intact mitochondrial oxidative phosphorylation, tau-expressing fly neurons preferentially utilize glycolysis for ATP production and operate with diminished metabolic reserve. Crucially, this metabolic reprogramming drives rather than protects against pathology as genetic suppression of glycolysis or lactate dehydrogenase completely rescued tau-induced lethality. Further, Gompertz mortality analysis revealed that hyperactive glycolysis in tau neurons drives premature lethality by accelerating biological aging rate without affecting baseline mortality. Collectively, these findings establish aberrant neuronal glycolysis as a cause rather than a consequence of tau pathology, and demonstrate that sustained glycolytic metabolism in mature neurons exacts a specific cost in the form of accelerated aging.

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## Amyloid-beta precursor protein contributes to brain aging and learning decline

Brain aging is a key risk factor for many neurodegenerative diseases, yet its molecular and cellular mechanisms remain elusive. Amyloid-beta precursor protein (APP) is among the most studied proteins linked to brain pathology; however, its role in non-pathological brain aging remains poorly characterized. Here, we investigate the natural impact of APP on normal brain aging using the short-lived turquoise killifish (*Nothobranchius furzeri*), which exhibits rapid and spontaneous age-related decline. We found that a pyroglutamated APP derivative ($APP_{pE11}$) accumulates intra-neuronally in an age-dependent manner, co-localizing with a marker of cell death. We found that intraneuronal $APP_{pE11}$ is also present in brains from healthy elderly humans, suggesting deep evolutionary conservation. To determine APP's role in spontaneous brain aging, we knock-out "amyloid precursor protein a" (*appa*) in killifish via CRISPR/Cas9. The lack of *appa* mitigated brain aging from a proteome-wide perspective, reduced age-related cell death and inflammation, and improved neuronal activity and learning capacity in aged individuals. Our findings show an ancestral and previously unrecognized role of amyloid-beta precursor protein in non-pathological brain aging, making it an ideal target for anti-aging interventions.

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## Secondary nucleation of α-Synuclein drives Mitochondria dysfunctions and Lewy body formation in Parkinson's Disease

The seeding of α-Synuclein (αSyn) is a key driver of Lewy pathology propagation in Parkinson's disease (PD) and forms the basis for recent diagnostic advances. However, it remains unclear how the structural and biochemical features of αSyn seeds dictate their propagation efficiency, capacity to induce Lewy body formation, and resulting cellular toxicity. Using genetic and idiopathic PD cell models, we map the pathogenic cascade beginning with the seed-driven conversion of endogenous αSyn, followed by impaired degradation, mitochondrial dysfunction, and ultimately Lewy body formation. By coupling kinetic modelling of aggregation with functional readouts, we identify secondary nucleation as the predominant mechanism generating toxic αSyn aggregation intermediates, identifying the critical process that links seeding to pathology. Extending this framework to PD brain, we quantitatively correlate seeding capacity with the spatiotemporal spread and severity of Lewy pathology, revealing a mechanistic connection between αSyn aggregation dynamics and disease progression at molecular, cellular, and anatomical levels. By unifying molecular mechanism with clinicopathological progression, our work identifies catalytic αSyn fibrillar seeds as tractable targets for both disease-modifying therapy and biomarker development in PD.

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# Self-Reported Hearing Aid Use and Risk of Incident Dementia

Age-related hearing loss (HL) is a known risk factor for developing dementia.[1] We examined prospectively whether self-reported hearing aid use by people with HL was associated with lower risk of incident dementia in the Framingham Heart Study (FHS) original and offspring cohorts and explored interactions with age.

FHS participants without dementia aged 60 years or older who underwent pure-tone audiometry at the 15th biennial examination (1977-1979; original cohort) and the 6th quadrennial examination (1995-1998; offspring cohort) were subsequently followed up for incident dementia for up to 20 years. The Boston University Medical Center Institutional Review Board approved the study protocols. All participants provided written informed consent. We followed the STROBE reporting guideline.

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# PREPRINT

## The impact of sex, age, and genetic ancestry on DNA methylation across tissues

Understanding the consequences of individual DNA methylation variation is crucial for advancing our knowledge of human biology and disease. Yet, the collective impact of individual traits on DNA methylation and their downstream effects on gene expression across human tissues remain poorly understood. Here, we quantify the contributions of sex, age, genetic ancestry, and BMI on autosomal DNA methylation variation across 9 human tissues and 424 individuals from the Genotype-Tissue Expression project. We show that genetic ancestry and age have a greater impact on DNA methylation than sex, with aging effects being more widespread but less pronounced. On average, less than 10% of the gene expression variation in sex, age, and ancestry are mediated by DNA methylation differences, with ancestry showing the largest proportion of mediation. We further show that ancestry-associated DNA methylation differences accumulate at CpG sites with extreme methylation states and are largely under genetic control. The female autosomal genome exhibits consistent hypermethylation across tissues at Polycomb-repressed regions. Ultimately, we show that age-related Polycomb target hypermethylation is observed across multiple tissues, but not in the gonads. Our multi-individual, multi-tissue approach defines the key drivers of human DNA methylation variation in healthy conditions, establishing a baseline for the interpretation of DNA methylation changes in disease contexts.

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# PREPRINT

## TranslAGE:A Comprehensive Platform for Systematic Validation of Epigenetic Aging Biomarkers

Epigenetic clocks are powerful biomarkers of biological aging, however, their performance varies across studies and contexts. Current limitations include siloed datasets, inconsistent validation methods, and the absence of a standardized framework for systematic comparison. Here, we introduce TranslAGE: a publicly available online resource that addresses this gap by harmonizing 179 human blood DNA methylation datasets and precalculating a suite of 41 epigenetic biomarker scores for each of the >42,000 total samples. Users can explore these data through interactive dashboards that evaluate four fundamental performance domains: Stability, Treatment response, Associations, and Risk, collectively forming the STAR framework. Stability quantifies robustness to multiple types of technical and biological noise. Treatment response measures biomarker sensitivity to aging interventions and environmental exposures. Associations capture cross-sectional relationships with age, demographics, disease, and other phenotypes, and Risk assesses predictive power for future functional decline, morbidity and mortality. The STAR framework unifies these test metrics into a single composite scoring system that enables researchers to identify, benchmark, and validate biomarkers best suited to their scientific or clinical applications. TranslAGE will be continually updated, with rapid scaling by adding datasets, biomarkers, or analyses. By providing harmonized datasets, precomputed biomarker scores, and interactive data tools, TranslAGE establishes the first standardized, reproducible framework for benchmarking epigenetic aging biomarkers across populations, and accelerates the translation toward clinical use.

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# CD4 T cells acquire Eomesodermin to modulate cellular senescence and aging

Aging is characterized by the progressive deterioration of tissue structure and function, leading to increased vulnerability to diseases. Senescent cells (SCs) accumulate with age, but how the immune system regulates their burden is unclear. Here we show that CD4 T cells differentiate into Eomesodermin (Eomes)$^{+}$CCL5$^{+}$ T lymphocytes (CD4-Eomes) in a SC-rich environment and that a reduction in the SC load, achieved using senolytic drugs, was sufficient to halt this differentiation. We further demonstrate that eliminating CD4-Eomes cells at advanced age by selectively deleting the Eomes transcription factor in CD4 T cells results in increased accumulation of SCs, profound physical deterioration and a decreased lifespan. In liver cirrhosis, a model of localized chronic inflammation, CD4-Eomes cell elimination increased fibrosis, SC load and worsened the disease. Collectively, our findings demonstrate the fundamental role of CD4-Eomes cells in modulating tissue senescence, with implications for age-related diseases and longevity.

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# Aging as a Loss of Goal-Directedness: An Evolutionary Simulation and Analysis Unifying Regeneration with Anatomical Rejuvenation

Léo Pio-Lopez, Benedikt Hartl, Michael Levin

Although substantial advancements are made in manipulating lifespan in model organisms, the fundamental mechanisms driving aging remain elusive. No comprehensive computational platform is capable of making predictions on aging in multicellular systems. Focus is placed on the processes that build and maintain complex target morphologies, and develop an insilico model of multiscale homeostatic morphogenesis using Neural Cellular Automata (NCAs) trained by neuroevolution. In the context of this model: 1) Aging emerges after developmental goals are completed, even without noise or programmed degeneration; 2) Cellular misdifferentiation, reduced competency, communication failures, and genetic damage all accelerate aging but are not its primary cause; 3) Aging correlates with increased active information storage and transfer entropy, while spatial entropy distinguishes two dynamics, structural loss and morphological noise accumulation; 4) Despite organ loss, spatial information persists in tissue, implementing a memory of lost structures, which can be reactivated for organ restoration through targeted regenerative information; and 5) rejuvenation is found to be most efficient when regenerative information includes differential patterns of affected cells and their neighboring tissue, highlighting strategies for rejuvenation. This model suggests a novel perspective on aging as loss of goal-directedness, with potentially significant implications for longevity research and regenerative medicine.

# Timing and duration of calorie restriction determine its impact on ovarian aging in female mice

This study investigated how the timing and duration of 30% caloric restriction (CR) affect ovarian aging in mice. Mice were assigned to one of four groups: ad libitum (AL) control, long-term CR (3–11 months; CR/CR), early short-term CR (3–7 months; CR/AL), and late short-term CR (7–11 months; AL/CR). Long-term CR reduced body mass, improved insulin sensitivity, preserved the ovarian primordial follicle reserve, and attenuated ovarian macrophage infiltration compared to AL-fed mice. Metabolic benefits from CR were quickly reversed upon returning to AL feeding. Short-term CR, whether initiated early or late, did not preserve the ovarian reserve. Some benefits were observed with an early start CR, including reduced ovarian collagen deposition at 7 months and reduced macrophage infiltration at 11 months. Our findings indicate that only long-term CR preserves the ovarian reserve. Short-term CR positive effects on other ovarian aging hallmarks depended on an early age of onset.

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# Interplay of glycated hemoglobin and traditional risk factors for the risk of atherosclerotic cardiovascular disease and all-cause mortality in people without diabetes

**Background:** To assess the impact of glycated hemoglobin A1c (HbA1c) in individuals without diabetes and at the extremes of cardiovascular risk factor (CVRF) burden on the incidence of atherosclerotic cardiovascular disease (ASCVD) and all-cause mortality.

**Methods:** We studied 20,360 U.S. adults, initially free of diabetes and ASCVD from the CARDIA, MESA, ARIC, and FOS cohorts, all with available HbA1c data. The mean (standard deviation) age was 57.1 (9.1) years [56.2% women and 21.9% Black]. Using multivariable Cox proportional hazard regression, ASCVD and all-cause mortality were analyzed over a median 16.7-year follow-up across categories of CVRF burden (0, 1, 2, or 3 of dyslipidemia, smoking, and hypertension) and HbA1c levels (< 5.0%, 5.0-5.4% [reference], 5.5-5.9%, and 6.0-6.4%).

**Results:** During follow-up, 3592 ASCVD events (17.6%) and 6627 deaths (32.6%) occurred. The hazard ratios (HRs) and 95% confidence intervals (CIs) for HbA1c levels of 5.5-5.9% and 6.0-6.4% for ASCVD were 1.17 (1.09-1.26) and 1.59 (1.42-1.78), respectively. The corresponding HRs for mortality were 1.14 (1.07-1.20) and 1.35 (1.24-1.47). HbA1c < 5.0% was also associated with an elevated mortality risk (HR, 95% CI 1.17, 1.07-1.28). Among individuals with 0 CVRFs, the HRs for ASCVD risks ranged from 1.26 (1.02-1.55) for HbA1c 5.5-5.9% to 1.68 (1.12-2.54) for HbA1c 6.0-6.4%. For those with 3 CVRFs, the corresponding HRs were 1.22 (0.88-1.69) and 2.00 (1.35-2.97). Similar findings were observed for all-cause mortality. Subgroup analysis revealed that HbA1c ≥ 5.5% was associated with an increased mortality risk in non-Black individuals but did not reach statistical significance in Black individuals (P interaction < 0.001).

**Conclusions:** HbA1c testing in individuals without diabetes may help identify those at higher risk for ASCVD and mortality, even at the extremes of CVRF burden.

# Metabolic regulation of behavior by the intestinal enzyme FMO-2

Many elements of an organism's behavior are intertwined with the organism's health. Over a long period of time, health status is also indicative of life span, with improved health correlating with a longer life. However, the relationship between longevity and behavior remains relatively unexplored. Here, we report that modification of a single longevity gene downstream of dietary restriction and hypoxia markedly alters behavior in *Caenorhabditis elegans*. We found that modified expression of flavin-containing monooxygenase (*fmo-2*) leads to altered sensory perception and decision-making in a variety of behavioral paradigms. This cell nonautonomous signaling pathway is linked to changes in tryptophan metabolism, where loss of *fmo-2* requires the tryptophan metabolite serotonin and overexpressed *fmo-2* requires the tryptophan metabolite quinolinic acid to change behavior. These results suggest a unique mechanism for gut metabolism to communicate positive satiety signals and negative depressive signals to the organism by modifying an essential amino acid. They also demonstrate the importance of examining pleiotropic effects in promising longevity interventions.

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**PREPRINT**

# Single-cell exploration of ovarian aging across vertebrate models

Mammalian female reproductive span is thought to be limited by a fixed "ovarian reserve" determined at birth. With age, a dwindling ovarian reserve leads to infertility, culminating in menopause in humans. In addition to infertility, accumulating evidence has shown that age-related ovarian functional decline contributes to multisystem aging and frailty, making post-menopausal women most susceptible to an array of chronic diseases. However, due to limited tissue accessibility and lack of reliable research models, molecular drivers of ovarian aging remain poorly understood. A key barrier in the field has been the limited establishment and benchmarking of preclinical models faithfully recapitulating human ovarian biology. To address this, we curated publicly available single-cell/nucleus ovarian RNA-seq datasets from human, macaque, mouse, and goat, and processed them using a consistent and stringent pipeline. Datasets were then annotated in a harmonized fashion across studies in order to conduct a robust, integrative, cross-species analysis of ovarian aging with single cell resolution. We systematically evaluated cell-type composition, global transcriptional perturbations, gene-level changes, pathway and network features, and drug-response alignments. Across analyses, granulosa and theca cells emerged as the cell-types most affected by aging. We observed limited but promising consistencies across species, including granulosa-specific signature genes (*FSHR* and *OSCIN2*) and cell type–linked pathways, with extracellular matrix/adhesion programs in granulosa and ribosomal/mitochondrial programs in theca cells. These convergences suggest that cross-species modeling likely capture core aspects of ovarian aging. Together, our meta-analysis approach may help refine model selection, generate testable hypotheses, and cautiously inform preclinical and translational work in ovarian aging.

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# PREPRINT

## Evidence for an energetic trade-off model linking inflammaging and immunosenescence in the US Health and Retirement Study and UK Biobank

Later life is characterized by the development of chronic inflammation, termed inflammaging, alongside changes in immune cell profiles, or immunosenescence. While these features contribute to health risk, they have also been interpreted as adaptive remodeling of the immune system in response to accumulating somatic damage. Here we consider a recently developed theoretical framework to understand these processes as interrelated: the Brain-Body Energy Conservation model of aging. This model views functional declines, such as immunosenescence, as part of an energy conserving response to the rising energy expenditure of inflammaging. This response promotes short term survival against somatic damage at the expense of future health risk. For example, naïve T cells, which enhance defense against future infections, decline with age. We find evidence consistent with this model in the US Health and Retirement Study (HRS) and UK Biobank (UKB). TNFR1, a key marker of inflammaging, mediated 10% and 5% of the age-related declines in naïve CD4T and CD8T cells respectively in the HRS (n = 8,261). Consistent with an impaired immune response to future infections, TNFR1 also mediated 16% of the age-related increased risk of hospitalization or death from COVID-19 in the UKB (n = 522 hospitalized or died, full sample n = 40,638). GDF15, which is produced in response to metabolic stress and has been found to induce immune tolerance in response to chronic inflammation, mediated 28% of the TNFR1-related COVID-19 health risk, as well as 38% of the age-related increased risk independent of TNFR1.

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# Multi-omic profiling reveals age-related immune dynamics in healthy adults

The generation and maintenance of immunity is a dynamic process that is dependent on age[1,2,3]. Here, to better understand its progression, we profiled peripheral immunity in more than 300 healthy adults (25 to 90 years of age) using single-cell RNA sequencing, proteomics and flow cytometry, following 96 adults longitudinally across 2 years with seasonal influenza vaccination. The resulting resource generated a single-cell RNA-sequencing dataset of more than 16 million peripheral blood mononuclear cells with 71 immune cell subsets from our Human Immune Health Atlas and enabled us to interrogate how immune cell composition and states shift with age, chronic viral infection and vaccination. From these data, we demonstrate robust, non-linear transcriptional reprogramming in T cell subsets with age that is not driven by systemic inflammation or chronic cytomegalovirus infection. This age-related reprogramming led to a functional T helper 2 ($T_H2$) cell bias in memory T cells that is linked to dysregulated B cell responses against highly boosted antigens in influenza vaccines. Collectively, this study reveals unique features of the immune ageing process that occur prior to advanced age and provides novel targets for age-related immune modulation. We provide interactive tools for exploring this extensive human immune health resource at https://apps.allenimmunology.org/aifi/insights/dynamics-imm-health-age/.

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# Towards a reference cell atlas of liver diversity over the human lifespan

The goal of the Human Liver Cell Atlas (HLiCA) is to create a comprehensive map that defines the normal functions of diverse liver cell types and their spatial relationships over the human lifespan. This project fits within the goals of the Human Cell Atlas to create comprehensive reference maps of all human cells as a basis for both understanding human health and diagnosing, monitoring and treating disease. Through collection of samples from diverse individuals, data integration across technologies and overcoming liver-specific challenges for experimental methods, the HLiCA will map as many cell types and states as possible in healthy human livers from individuals across all ages and many ancestries. Establishing this HLiCA of healthy livers is a critical step to begin to understand perturbations in disease. The HLiCA will be available on an open-access platform to facilitate data sharing and dissemination. We expect that creation of the HLiCA will help to lay the foundation for new research initiatives to advance our understanding of liver disease, improve methods of tissue engineering, and identify novel prognostic biomarkers and therapies to improve patient outcomes. We describe key experimental and computational challenges to overcome in building the atlas and the potential impact of the atlas on disease research.

# *C. elegans* aging research

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# Age Deceleration and Reversal Gene Patterns in Dauer Diapause

Khrystyna Totska, João C. V. V. Barata, Walter Sandt, David H. Meyer, Björn Schumacher

The aging process is characterized by a general decrease in physical functionality and poses the biggest risk factor for a variety of diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders among others. Understanding the naturally evolved mechanisms that slow aging and rejuvenate an animal could reveal important concepts on how to prevent age-associated diseases and even revert aging. The *C. elegans* dauer stage is a robust and long-lived alternative developmental state that, after dauer exit, has a normal adult lifespan with fully retained fecundity. To understand how longevity during dauer and rejuvenation following dauer exit is mediated, we characterized the gene expression changes during dauer and upon exit. We assessed how biological age, as determined via BiT Age, a transcriptome aging clock, is affected during dauer and upon dauer exit. During the dauer stage, we measured a decelerated increase in age compared to the chronological age and an age reversal following dauer exit. Transcriptomic analyses revealed major metabolic shifts and enhanced biomolecular degradation that are reversed during exit. Moreover, we show that transcription-blocking lesions can induce lasting transcription stress in dauers that is rapidly resolved by transcription-coupled nucleotide excision repair during dauer exit. Our data provide new insights into the underlying mechanisms of naturally occurring age deceleration and rejuvenation.

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# Rewarding touch limits lifespan through neural to intestinal signaling

In multicellular organisms, sensory perception affects many aspects of behavior and physiology. Perception of environmental stressors like food scarcity often leads to physiological changes that promote survival and slow aging. However, recent work shows that perception of attractive food smells can block the health benefits of dietary restriction in multiple model organisms. While it is known that sensory perception and cell nonautonomous signaling can modulate health and longevity, our knowledge of the specific sensory cues and mechanistic pathways that define this signaling is still limited. Here we find that the sense of touch interacts with nutritional state to modulate lifespan in *Caenorhabditis elegans*. Worms subjected to dietary restriction are shorter-lived when they perceive tactile stimuli that mimic bacterial food and/or protective soil. Touch modulation of dietary restriction requires putative mechanoreceptor proteins, the neurotransmitters dopamine and tyramine/adrenaline, and the neuropeptides INS-11 and GnRH. Ultimately, the touch circuit regulates the longevity effectors DAF-2/IGF1R and FMO-2/FMO5. These results establish a physiological touch circuit and connect neural reward pathways to the growth and reproductive axes. Finding that texture mechanosensation can modulate longevity suggests a role for touch in lifespan.

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# Wide-band electrical-impedance-spectroscopy integrated microfluidic organism-on-a-chip device for simultaneous monitoring of multi-organ degradation along C. elegans aging

Monitoring the multi-dimensional tissue-specific aging signatures in Caenorhabditis elegans (C. elegans) provides unusual insight into senescent pathology across organ systems. However, the intricate imaging protocols and feature-extraction algorithms in conventional optical methods pose challenges to the synchronous tracking of multiple degraded organs in C. elegans. Herein, we report on a wide-band electrical impedance spectroscopy (EIS) integrated microfluidic organism-on-a-chip device that maintains C. elegans on-chip aging progression and facilitates simultaneous characterization of four organic phenotypes by EIS signals from 50 kHz to 50 MHz. This microfluidic biosensor enables precise manipulation of the C. elegans using a pneumatic valve and implements long-term EIS detection of different worm body regions via a strategically configured microelectrode sensing array. Coupled multi-organ degradation in aging worms, including morphology variation, intestinal necrosis, and cuticle rupture, are accurately identified by the customized linear regression model and opacity estimation from multi-frequency EIS signals. Optical inspection and finite element modeling results decisively validate the correlation between multi-organ degradation and EIS signal dynamics, illustrating a label-free monitoring approach of organ systems that potentially applicable to age-related mechanism research or drug screening.

# REVIEWS/COMMENTS/ METHODS/EDITORIALS

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# Meeting Report

## Meeting Report

**Bridging Physics and Aging Biology: Insights from the First Physics in Aging Biology Workshop**

## Authors

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## Abstract

Research in aging has often been descriptive, with few quantitative laws to explain how organisms change with age. However, physicists often build quantitative models to explain observed phenomena and how they change with time. We believe that merging physics with aging biology will help us to develop a deeper understanding of the biology of aging. The Physics in Aging Biology Workshop brought together a diverse group of aging biologists and physicists to exchange ideas on how theoretical physics and complexity science approaches could lead to a better understanding of the aging process.

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# Epigenetic Clocks: Beyond Biological Age, Using the Past to Predict the Present and Future

Predicting health trajectories and accurately measuring aging processes across the human lifespan remain profound scientific challenges. Assessing the effectiveness and impact of interventions targeting aging is even more elusive, largely due to the intricate, multidimensional nature of aging—a process that defies simple quantification. Traditional biomarkers offer only partial perspectives, capturing limited aspects of the aging landscape. Yet, over the past decade, groundbreaking advancements have emerged. Epigenetic clocks, derived from DNA methylation patterns, have established themselves as powerful aging biomarkers, capable of estimating biological age and assessing aging rates across diverse tissues with remarkable precision. These clocks provide predictive insights into mortality and age-related disease risks, effectively distinguishing biological age from chronological age and illuminating enduring questions in gerontology. Despite significant progress in epigenetic clock development, substantial challenges remain, underscoring the need for continued investigation to fully unlock their potential in the science of aging.

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# A receptor for glycation end products (RAGE) is a key transmitter between garb-aging and inflammaging

Zulfiya G. Guvatova [a b], Anna Vakhrusheva [c] ✉, Alexey Moskalev [a] ✉

A receptor for glycation end products (RAGE) plays a key role in the pathogenesis of many chronic diseases associated with aging. Acting as a multi-ligand sensor, RAGE is able to bind a wide range of stimuli, which fuels inflammation. This makes it a key link between garb-aging and inflammaging. We propose that RAGE functions as the missing molecular link between garb-aging, the progressive buildup of biological waste, and inflammaging, the chronic inflammatory state that drives degenerative disorders. This review summarizes current knowledge on RAGE structure, ligands, and signaling, highlighting its involvement in diabetes, cardiovascular, neurodegenerative, and inflammatory diseases, as well as therapeutic strategies targeting RAGE. By nominating RAGE as a central receptor of garb-aging, we outline new perspectives for combating age-associated pathologies.

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# The role of cellular senescence in cardiovascular disease

The incidence of cardiovascular diseases rises significantly with age, making it one of the leading causes of death and disability worldwide, and cellular senescence plays a crucial role in this process. Cellular senescence constitutes a salient feature of organismal aging and stands as an independent risk factor for a range of cardiovascular diseases, encompassing hypertension, atherosclerosis, myocardial infarction, heart failure, and arrhythmia. This comprehensive review endeavors to comprehensively delineate the intricate regulatory mechanisms underlying cellular senescence and its attendant biological implications, while elucidating the profound implications of this process on the initiation and progression of cardiovascular diseases. Finally, we will delve into a spectrum of targeted interventions aimed at cellular senescence, specifically focusing on eliminating the accumulation of senescent cells during disease progression or inhibiting the inherent cellular senescence processes. Our ultimate goal is to mitigate or postpone the onset of diseases that are intricately linked to cellular senescence. A profound comprehension and rigorous investigation into the regulatory mechanisms of cellular senescence and their intricate interrelationships hold significant potential to furnish invaluable scientific evidence for the prevention and therapeutic strategies against cardiovascular diseases.

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# Translating cellular senescence research into clinical practice for metabolic disease

Translational research on cellular senescence has led to numerous early-phase clinical trials targeting senescent cells to treat, prevent or alleviate multiple disorders and diseases, including metabolic diseases and their comorbidities. Cellular senescence is a cell fate that occurs in response to stressors, including metabolic disruptions, and is one of the hallmarks (or pillars) of ageing. In their senescent state, cells cease proliferation and can develop a senescence-associated secretory and metabolic phenotype that contributes to the pathogenesis of metabolic dysfunction associated with obesity and ageing. Metabolic stress, which is central to the development of metabolic diseases, can trigger cellular senescence, thereby enabling a vicious cycle that exacerbates metabolic dysfunction. Therapies targeting senescent cells (senotherapeutics), either alone or in combination with other gerotherapies or lifestyle interventions, hold great promise for addressing the ongoing obesity epidemic and the need for improved therapies to prevent and treat metabolic diseases and their complications and comorbidities. In this Review, we discuss novel senotherapeutics, including challenges related to the translation of these therapies and the need to establish gerodiagnostic biomarkers to track the elimination of senescent cells, define eligibility and measure efficacy, as well as considerations for clinical trial design and execution.

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# SenoTAC: An Emerging Senotherapy for Combating Aging

Alexis B. Cruickshank-Taylor [a †], Jacob S. Kozora [a †], Jennifer S. Carew [b], Steffan T. Nawrocki [b], Wei Wang [a b c d]

Cellular senescence, a persistent state of cell cycle arrest, accumulates in aged organisms, contributes to tissue dysfunction, and drives aging-related phenotypes. Clearance of senescent cells decreases chronic, low-grade inflammation and restores tissue repair capacity, thus improving human health and lifespan. Senolytics that selectively eliminate senescent cells have become a promising anti-aging strategy. To date, current senolytics are largely developed by repurposing anticancer agents. Therefore, senolytics usually possess various on- and off-target toxicities. These toxicities could preclude their clinical use as anti-aging agents, as elderly people are more susceptible to adverse drug effects than young individuals. Proteolysis-targeting chimeras (PROTACs) as senolytics, termed "SenoTACs", are attractive for more effective treatment of aging-related diseases. In comparison to small molecule inhibitors, SenoTACs can eliminate senescent cells by degrading targeted proteins in a substoichiometric manner, providing better target ability, longer-lasting therapeutic effect, broadened target capability, and decreased drug resistance. Recent efforts have led to the development of several senescence-targeting PROTACs, including ARV825, PZ15227, 753B, Gal-ARV-771, and Gal-MS99 which exhibit selective senolytic activity and improved safety and efficacy profiles when compared to small molecule inhibitors. In this minireview, we summarize the development of the emerging field.

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# The geroprotective potential of chalcones

Didac Carmona-Gutierrez ✉, Andreas Zimmermann, Guido Kroemer & Frank Madeo ✉

Aging is the most important risk factor for multiple pathologies including cardiovascular, neoplastic, metabolic and neurodegenerative diseases. Potential geroprotective strategies involve lifestyle-related, nutritional and pharmacological interventions. Recently, chalcones, a subgroup of secondary plant metabolites, have gained attention. 4,4'-dimethoxychalcone was the first chalcone to be shown to mediate geroprotection and lifespan extension across different species. Several other chalcones also exert anti-aging effects at the cellular and organismal levels. Defined mechanistic routes that are causally involved in these protective effects have been delineated. Here, we summarize current evidence supporting the potential of 4,4'-dimethoxychalcone and other chalcones as geroprotective agents.

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# $NAD^+$ precursor supplementation in human ageing: clinical evidence and challenges

Kasper T. Vinten, Maria M. Trętowicz, Evrim Coskun, Michel van Weeghel, Carles Cantó, Rubén Zapata-Pérez, Georges E. Janssens & Riekelt H. Houtkooper

Nicotinamide adenine dinucleotide ($NAD^+$) is an essential molecule involved in cellular metabolism, and its decline has been implicated in ageing and age-related disorders. However, evidence for an age-related decline in $NAD^+$ levels in humans has been consistently observed only in a limited number of studies. Similarly, although preclinical studies support the idea that supplementation with $NAD^+$ precursors is a promising therapeutic strategy to promote healthy ageing, human clinical trials have shown limited efficacy. Therefore, an increasing understanding of how $NAD^+$ metabolism is affected in different tissues during disease and following $NAD^+$ precursor supplementation is crucial to defining the therapeutic value of $NAD^+$-targeted therapies. In this Review, we evaluate the clinical evidence supporting the notion that $NAD^+$ levels decline with age, as well as the tissue-specific effects of $NAD^+$ precursor supplementation. Viewed in perspective, the published body of data on $NAD^+$ dynamics in human tissues remains sparse, and the extrapolation of rodent-based data is not straightforward, underscoring the need for more clinical studies to gain deeper insights into systemic and tissue-specific $NAD^+$ metabolism.

# Healthy Aging at Moderate Altitudes: Hypoxia and Hormesis

Johannes Burtscher [1], Michele Samaja [2]

**Background:** Aging is associated with cellular and tissue responses that collectively lead to functional and structural deterioration of tissues. Poor tissue oxygenation, or hypoxia, is involved in such responses and contributes to aging. Consequently, it could be speculated that living at higher altitude, and therefore in hypoxic conditions, accelerates aging. This assumption is indeed supported by evidence from populations residing at very high altitudes (>3,500 m). In contrast, accumulating evidence suggests that living at moderate altitudes (1,500-2,500 m) is protective rather than injurious, at least for some body systems.

**Summary:** In this review, we critically evaluate the hypothesis that the physiological responses to mild hypoxic stress associated to life at moderate altitudes provide protection from many hypoxia-related diseases through hormesis. Hormesis means that a low dose of a stressor (here hypoxia) elicits beneficial outcomes, while a higher dose can be toxic and might explain at least in part the dose-dependent contrasting effects of hypoxia on the aging processes. The lack of well-designed longitudinal studies focusing on the role of the altitude of residence, and difficulties in accounting for potentially confounding factors such as migration, ethnicity/genetics, and socioeconomic and geoclimatic conditions, currently hampers translation of related research into uncontroversial paradigms.

**Key messages:** Deeper investigations are required to understand the impact of altitude-related hypoxia on age-related diseases and to develop molecular markers of ageing/senescence in humans that are linked to hypoxia. However, the presented emerging evidence supports the view that hypoxia conditioning has the potential to improve life quality and expectancy.

# LINE-1 retrotransposon activation drives age-associated inflammation *via* cytoplasmic cDNA-STING/type I interferon signalling: therapeutic potential of reverse transcriptase inhibition

Retrotransposable elements are harmful at several levels, and host surveillance systems fail to consider all these elements in severe effects. The key role of retrotransposon in aging and age-associated diseases remains unclear. We summarise whether LINE-1 retrotransposable elements transcriptio-nally derepress and activate type-I interferon response during cellular senescence. Type-I interferon response is the late senescence phenotype that maintains the senescence-associated secretory phenotype. Cytoplasmic LINE-1 cDNA activates type-I interferon response, while LINE-1 reverse transcriptase inhibitors suppress it. The nucleoside reverse transcriptase inhibitor lamivudine downregulates activation of type-I interferon and age- associated inflammation in tissues in the treatment of aging. Activation of retrotransposons is a key factor in sterile inflammation, which is a hallmark of aging, and LINE-1 reverse trans-criptase is an important target for the treatment of age-associated diseases. Nucleoside reverse transcriptase inhibitor lamivudine downregulates activation of type-I interferon and age-associated inflammation.

# Targeting DNA damage in ageing: towards supercharging DNA repair

Arturo Bujarrabal-Dueso [1] [2], George A Garinis [3] [4], Paul D Robbins [5], Jan Vijg [6], Björn Schumacher [7] [8] [9]

Ageing is the most important risk factor for many common human diseases, including cancer, diabetes, neurodegeneration and cardiovascular disease. Consequently, combating ageing itself has emerged as a rational strategy for addressing age-related multimorbidity. Over the past three decades, multiple genetic and pharmacologic interventions have led to substantial extension of lifespan and healthspan in model organisms. However, it is unclear whether these interventions target the causal mechanisms of ageing or downstream consequences. Ample evidence suggests that DNA damage to the somatic genome is a major causal mechanism of ageing, which compromises essential cellular functions such as transcription and replication, and leads to cellular senescence, apoptosis and mutations. Recently, new concepts have emerged to target the main consequences of DNA damage and enhance DNA repair capacities, thereby extending maintenance of the genome. Here, we review advances in this field and discuss approaches to pharmacologically mitigate the adverse effects of DNA damage to delay ageing, prevent mutation-driven cancer and mitigate age-related degenerative diseases.

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# Aging on Chip: Harnessing the Potential of Microfluidic Technologies in Aging and Rejuvenation Research

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Aging is a complex process and the main risk factor for many common human diseases. Traditional aging research using short-lived animal models and two-dimensional cell cultures has led to key discoveries, but their relevance to human aging remains debatable. Microfluidics, a rapidly growing field that manipulates small volumes of fluids within microscale channels, offers new opportunities for aging research. By enabling the development of advanced three-dimensional cellular models that closely mimic human tissues, microfluidics allows more accurate investigation of aging processes while reducing costs, resource use, and culture time. This review explores how microfluidic systems, particularly organ-on-chip models, can improve our understanding of aging and age-related diseases, bridge the gap between animal models and human biology, and support the discovery of rejuvenation therapies. We highlight their role in monitoring aging biomarkers, analyzing functional cellular changes, and identifying longevity-promoting compounds. The ability of microfluidics to detect, analyze, and remove senescent cells is also discussed, along with emerging applications such as partial reprogramming for cellular rejuvenation. Furthermore, we summarize how these devices support single-cell analysis and recreate specific tissue microenvironments that influence aging. Insights from microfluidic approaches hold promise for developing therapeutic strategies to extend healthspan and promote longevity.

# Advancing Single-Cell Transcriptomic Analysis to Reveal Age-Related Skeletal Muscle Changes: A Systematic Review

Population aging has become a widespread health problem that leads to huge socioeconomic burden. Skeletal muscle as an important component of motor system, gradually degenerates with age. Age-related muscle disorders, such as sarcopenia is associated with higher risks of falls, fracture, disability, and mortality in old people. As there is still no Food and Drug Administration (FDA) approved drug to treat sarcopenia, conducting research of in-depth mechanisms is warranted to develop novel treatments. The cutting-edge techniques single-cell and single-nuclei RNA sequencing can help to address this issue by discovering age-related changes of muscle at the single-cell level. This review aims to systematically explore current evidence of age-related muscle changes during normal aging, regeneration, and after treatments at the single-cell level. 29 studies were eligible and included in the current review according to the PRISMA guideline. The muscle cell composition was altered with age, such as diminished muscle stem cells (MuSCs), vascular cells, Schwann cells, and increased myocytes as well as some types of immune cells. Inflammation levels, collagen and extracellular matrix (ECM) signaling, protein catabolism, TGFβ signaling, apoptosis, and autophagy of MuSCs, myocytes, fibro-adipogenic progenitor cells, vascular cells, or immune cells were regulated with age. Delayed muscle regeneration of aged muscle was relied on disorders of cell-specific immune response, myogenesis, angiogenesis, and ECM remodeling. Three treatments involved in this review could reverse age-related dysfunction of muscle cells to some extent. Further research targeting age-related changes of muscle at the single-cell level is an important tool in assisting development of more effective treatments for sarcopenia.

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# Biophysical insights into the molecular mechanisms of beta amyloid aggregation and its toxic effects in Alzheimer's disease

Alzheimer's disease is recognized as the most common neurodegenerative disorder, characterized by the presence of amyloid plaques, which have consistently garnered significant attention. Since the disease was first identified, extensive research has been devoted to investigating these plaques. As our understanding of the disease has progressed, the detrimental role of plaques has been questioned, leading to the hypothesis that amyloid oligomeric aggregates are the main culprits. Nevertheless, subsequent research indicated that the concentrations of amyloids employed in the experiments were considerably elevated compared to physiological conditions, and that at physiological concentrations, amyloids do not exhibit significant accumulation or toxicity. This article aims to offer a detailed biophysical perspective on the formation of amyloid aggregates under physiological conditions and their impact on membranes, providing valuable insights for researchers in this field.

# OTHER RESEARCH & REVIEWS

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# Precise gene editing of pathogenic Lamin A mutations corrects cardiac disease

Mutations in the Lamin A (*LMNA*) gene, which encodes the Lamin A and C proteins, cause severe human diseases collectively known as laminopathies. These conditions are often devastating and lack effective therapies. In this study, we developed precise base editing (BE) strategies targeting the human *LMNA* gene variants L35P and R249Q, which cause congenital muscular dystrophy (CMD) and dilated cardiomyopathy with conduction defects (DCM-CD), respectively. Induced pluripotent stem cell–derived cardiomyocytes (iPSC-CMs) carrying the R249Q mutation displayed nuclear aberrations, DNA damage, and abnormal $Ca^{2+}$ transients. Similarly, L35P iPSC-CMs exhibited abnormal contraction, DNA damage, and reduced Lamin A/C protein expression. We also generated "humanized" mouse models carrying these pathogenic human mutations. R249Q homozygous mice exhibited cardiac conduction abnormalities, cardiac arrhythmias, and premature death. Mice with the homozygous L35P mutation displayed severe muscle-wasting and reduced lifespan, while heterozygous L35P mice displayed DCM. We developed an adenine base editing (ABE) approach for correcting the R249Q mutation and a cytosine base editing (CBE) strategy for the L35P variant. Precise correction of these mutations in iPSC-CMs successfully rescued all of the in vitro abnormalities. Furthermore, delivery of the BE components using adeno-associated virus prevented the pathological phenotypes and extended longevity of mice carrying the *LMNA* L35P and the R249Q mutations. These results demonstrate the efficacy of ABE and CBE in correcting pathogenic *LMNA* mutations that cause cardiac disease, highlighting BE as a promising therapeutic approach for human laminopathies.

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# Lamin A/C loss promotes R-loop-mediated genomic instability and poor survival in small-cell lung cancer

Lamin A/C (*LMNA*), a key component of the nuclear envelope, is essential for maintaining nuclear integrity and genome organization [W. Xie *et al.*, *Curr. Biol.* **26**, 2651–2658 (2016)]. While *LMNA* dysregulation has been implicated in genomic instability across cancer and aging, the underlying mechanisms remain poorly understood [S. Graziano *et al.*, *Nucleus* **9**, 258–275 (2018)]. Here, we define a mechanistic role for *LMNA* in preserving genome stability in small-cell lung cancer (SCLC), a malignancy marked by extreme genomic instability [N. Takahashi *et al.*, *Cancer Res. Commun.* **2**, 503–517 (2022)]. *LMNA* depletion promotes R-loop accumulation, transcription-replication conflicts, replication stress, DNA breaks, and micronuclei formation. Mechanistically, *LMNA* deficiency disrupts nuclear pore complex organization, specifically reducing phenylalanine-glycine (FG)-nucleoporin incorporation, resulting in impaired RNA export and nuclear retention of RNA. *LMNA* expression is repressed by EZH2 and reexpressed during SCLC differentiation from neuroendocrine (NE) to non-NE states, and low *LMNA* levels correlate with poor clinical outcomes. These findings establish *LMNA* as a key regulator of nuclear transport and genome integrity, linking nuclear architecture to SCLC progression and therapeutic vulnerability.

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# Chemotherapy awakens dormant cancer cells in lung by inducing neutrophil extracellular traps

Disseminated tumor cells (DTCs) can remain in a non-proliferative, dormant state for years in distant organs, but the exogenous causes triggering their reactivation and metastatic colonization are unclear. Here, we demonstrate that chemotherapeutic drugs, including doxorubicin and cisplatin, enhance proliferation and lung metastasis of dormant breast cancer cells. Using a recombinase-based dormancy tracing system, DormTracer, we confirm chemotherapy-induced reactivation of dormant DTCs leading to metastatic relapse. Mechanistically, chemotherapy induces fibroblast senescence, which promotes formation of neutrophil extracellular traps (NETs) through secreted proteins. NETs promote dormant DTC proliferation through extracellular matrix remodeling. Importantly, combining senolytic drugs, dasatinib and quercetin, with doxorubicin inhibits post-therapy DTC reactivation and suppresses metastatic relapse. This study provides direct evidence of dormancy awakening and reveals a mechanism underlying detrimental effect of chemotherapy on metastasis, highlighting potential strategies to improve cancer treatment.

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# Lung Cancer in Nonsmoking Individuals
## A Review

**Importance** Lung cancer in nonsmoking individuals (defined as people who have smoked fewer than 100 cigarettes in their lifetime) accounts for 15% to 20% of all lung cancer cases worldwide. In the US, the annual incidence of lung cancer in nonsmoking individuals is 14.4 to 20.8 per 100 000 person-years in females and 4.8 to 12.7 per 100 000 person-years in males.

**Observations** Most lung cancers in nonsmoking individuals are histologically adenocarcinomas (60%-80%) with the remainder being squamous or adenosquamous (10%-20%) and rarely small cell lung cancer (<10%). Risk factors include exposure to passive smoking, radon exposure, air pollution, asbestos, and history of lung cancer in a first-degree family member. Therapeutically targetable genomic variants, such as *EGFR* mutations or *ALK* gene rearrangements, are more common in tumors from nonsmoking individuals compared with those with a smoking history (defined as people who currently or formerly smoked) (43% vs 11% for *EGFR* and 12% vs 2% for *ALK*). In contrast, tumor mutation burden, the number of somatic mutations in a tumor cell, is lower in lung cancer among nonsmoking individuals (0-3 mutations/megabase [Mb] vs 0-30 mutations/Mb). Similar to individuals with a history of smoking, nonsmoking individuals with lung cancer may present with wheeze, chest pain, dyspnea, hemoptysis, or symptoms attributable to metastatic disease (eg, bone pain and headache) or be diagnosed with incidentally detected disease. The US Preventive Services Task Force does not currently recommend lung cancer screening with low-dose computed tomographic scans for nonsmoking individuals, although screening guidelines vary globally. Treatment typically involves a combination of surgery, radiotherapy, and systemic therapies depending on stage, performance status, and molecular features of the tumor. Comprehensive next-generation sequencing should be performed on stage Ib to IIIa lung cancer tumor tissue from nonsmoking individuals because actionable genomic alterations, such as *EGFR* mutations or *ALK* gene rearrangements, are treated with targeted therapy such as the tyrosine kinase inhibitors osimertinib or lorlatinib, respectively. Median survival among nonsmoking individuals with advanced non–small cell lung cancer (stage IIIb or higher) and actionable genomic alterations can exceed 3 to 5 years, while survival without these genomic alterations is similar to lung cancer in people with a history of smoking (1-2 years).

# Next-generation T cell immunotherapy: overcoming exhaustion, senescence, and suppression

T-cells are a core component of tumor immunotherapy because of their potent ability to identify and kill cancer cells. Yet efficacy is limited by exhaustion, senescence, metabolic dysregulation, an immunosuppressive tumor microenvironment (TME), and limited persistence. This review analyzed these key issues and proposed targeted improvement strategies. Emerging approaches encompass pharmacological modulation of T cell activation and survival pathways, epigenetic reprogramming to reverse exhaustion and senescence, metabolic engineering, combinatorial targeting of immunosuppressive TME components and advanced genetic tools, notably CRISPR-Cas9-based CAR-T optimization, which exemplifies how precise genome editing can enhance therapeutic efficacy. We review the progress and prospects of T-cell improvement strategies in tumor immunotherapy, emphasizing the need for further exploration to enhance the broader application and long-term efficacy of T-cell therapies. This review highlights recent advances and future directions in T-cell engineering, metabolic modulation, and microenvironment targeting, aiming to translate innovations into effective cancer immunotherapies.

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# GRAIL PATHFINDER 2 Results Show Galleri® Multi-Cancer Early Detection Blood Test Increased Cancer Detection More Than Seven-Fold When Added to USPSTF A and B Recommended Screenings

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# CarD-T: An Automated Pipeline for the Nomination and Analysis of Potential Human Carcinogens

The identification and classification of carcinogens is critical in cancer epidemiology, necessitating updated methodologies to manage the burgeoning biomedical literature. We introduce the Carcinogen Detection via Transformers (CarD-T) framework, combining transformer-based machine learning with probabilistic analysis to efficiently nominate potential carcinogens from scientific texts. Trained on 60% of established carcinogens, CarD-T correctly identifies all remaining known carcinogens and nominates ~1,600 potential new carcinogens. Comparative assessment against GPT-4 reveals CarD-T's comparable precision (0.896 vs 0.903), and superior recall (0.853 vs 0.757), implying an improved ability to nominate potential carcinogens for further evaluation. CarD-T associates each nominated entity with relevant scientific literature, allowing for additional analysis of conflicting implications over time through a Bayesian Probabilistic Carcinogen Denomination (PCarD) analysis. The framework also provides rich insights into carcinogenesis associated research, revealing significant shifts in research focus on carcinogenic agents over time, from chemical carcinogens to broader categories including biological agents, environmental factors and lifestyle choices. We establish the CarD-T framework as a locally deployable, computationally inexpensive, and robust tool for identifying and nominating potential carcinogens from vast biomedical literature. This framework enhances the agility of public health responses to carcinogen identification, setting a new benchmark for automated, scalable toxicological investigations.

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# New Cryoprotectants for Cell Therapies

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## Abstract

This chapter explores the role of cryoprotective agents (CPAs) in the cryopreservation of cells, with a particular focus on those relevant to cell therapy. It provides an overview of new classes of CPAs, including nanomaterials, metabolites, and polymers. In addition, the chapter highlights innovative research strategies used to discover novel CPAs, such as modeling and machine learning.