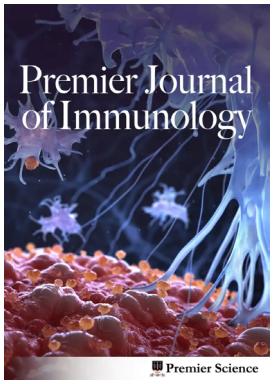

Anti-Aging the Immune System: Hype or Hope? A Mechanistic Review

Ambreen Ilyas

ABSTRACT

Aging is a natural yet highly complex biological phenomenon that affects all organisms, from single-celled yeast to complex humans and plants. Aging should not be confused with a single disease, but rather an overarching, systemic change over the period of an organism's life. This process is characterized by a gradual decline in functions at the cellular, molecular, and organismic levels and increased risk of several diseases, such as cardiovascular abnormalities, neurodegenerative disorders, diabetes, and cancer. Every living thing gradually deteriorates and breaks down as a result of the aging process caused by a complex web of biological cascades. This normal development can be crippling and raises the risk of many diseases. In an effort to reduce clinical load, restore functionality, and increase longevity, researchers in academia and industry have long worked to slow down or even reverse aging. Despite extensive research, the absence of rigorous study design and limited experimental validation has made it difficult to uncover effective treatments. In this immune-focused mechanistic review, we examine our current understanding of the biological mechanisms underlying aging and how this knowledge both helps and hinders the interpretation of results from experimental models that rely on these mechanisms. We also go over a few therapeutic approaches that have shown encouraging results in these model systems and may have clinical applications. Finally, we suggest a unified strategy that is required to thoroughly screen present and potential treatments and direct assessment toward effective treatments.

Keywords: Aging biology, Immune aging, Immunosenescence, Inflammaging, Cellular senescence, Oxidative stress, Stem cell exhaustion, Epigenetic clocks, Anti-aging therapeutics, Longevity interventions

School of Biological Sciences, University of the Punjab, Lahore, Pakistan

Correspondence to:
Ambreen Ilyas
ambreen2.phd.sbs@pu.edu.pk

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Introduction

The natural process of aging involves systemic changes in the human body that eventually lead to decreased functionality, an increased risk of age-related diseases, and mortality. Even after decades of observation and study, human intervention has not yet successfully changed this process. However, the industry for anti-aging treatments and cosmetics is currently valued at around US$60 bn and is predicted to grow by 50% over the next five years. Despite this intense interest, the field is still in its early stages and is motivated by lofty goals, and not much has been done to slow it down.

One of the main issues facing the anti-aging therapeutics sector is the absence of standards to identify effective treatments. Even though the biological effects of aging affect many different regions, treatment candidates are frequently assessed in a limited way, creating excitement and financial investments, but eventually failing to deliver the desired outcome. In this review, the author provides an overview of the current status of aging research, examines a few interventions that are being studied and the models that are now available to examine their effectiveness, and talks about the models' applicability, limitations, and breadth. Lastly, the author provides a methodology for assessing these treatments and for guiding the upcoming studies on aging to drive significant advancements in this area.

What Is Aging?

The body naturally ages during the course of a person's life. It is the outcome of both the acquisition of harmful features and the loss of youthful, rejuvenating qualities, which eventually tips the scales in favor of a degenerative process. Both the quantity and quality of life are impacted by aging. Quality, or health span, is the amount of time a person can live without experiencing the devastation of illness, whereas quantity, or lifespan, is a measure of the time they live. Every organ system is affected by aging, and getting older is a known risk factor for illness (Figure 1). The observed variety in lifespans is believed to be caused by changes in biological age brought on by environmental influences or innate characteristics, such as exposure to chemicals,[1] reproductive capacities,[2] metabolic changes,[3] and factors influencing epigenetic expression.[4] In fact, as modern medicine has developed over the past few decades, we have witnessed how new therapies and improvements in healthcare have made it possible for people to live longer in the developed world. The maximum increase in longevity that can be achieved with this method is currently unclear. Ultimately, the best therapeutic candidates should interfere with the fundamental processes of aging, which may increase longevity.

Advanced age is a major risk factor for a wide spectrum of degenerative and neoplastic conditions affecting multiple organ systems. The figure illustrates key age-associated diseases, including neurodegenerative disorders (e.g., Alzheimer's disease, Parkinsonian syndromes), cardiovascular disease, chronic obstructive pulmonary disease (COPD), type 2 diabetes mellitus, cancer, chronic kidney disease, osteoarthritis, osteoporosis, and ocular disorders such as cataracts and macular degeneration. These conditions collectively account for a substantial proportion of global morbidity and mortality in older populations. Therapeutic strategies in aging research often prioritize targeting

Data Availability: All data supporting the conclusions of this review are derived from published literature sources cited within the manuscript and supplementary materials. Supplementary tables and figures are available with the article.

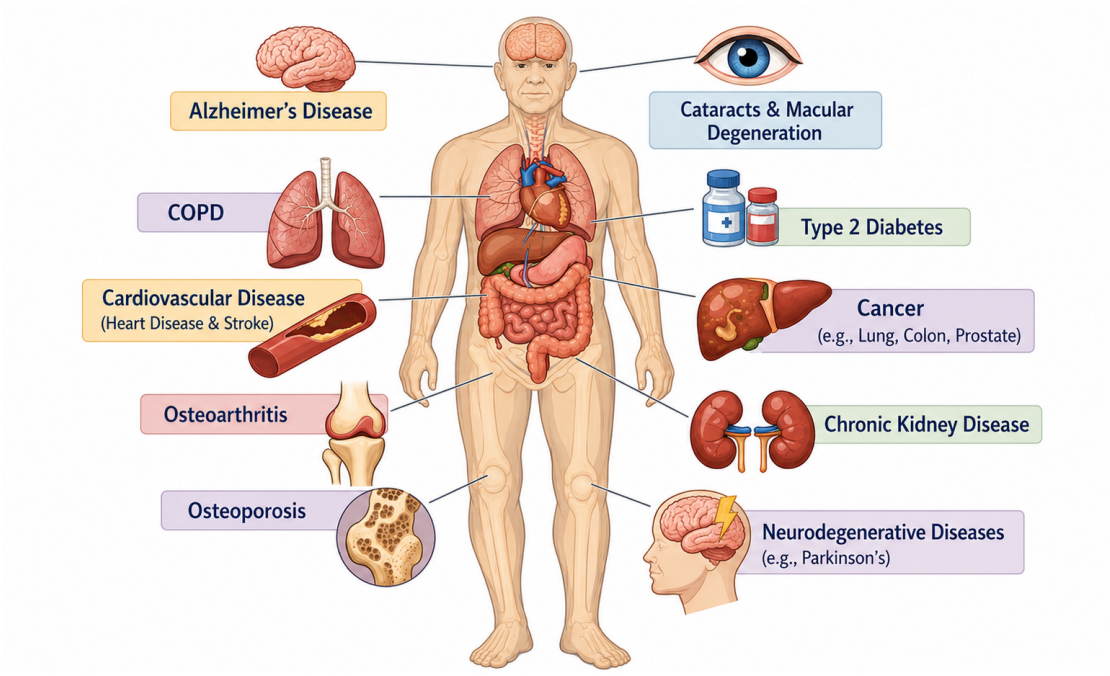

Fig 1 | Common diseases associated with advanced age

disease-specific pathways to mitigate progression rather than directly modifying the underlying aging phenotype.

Between the Hype and Hope: the Anti-Aging Market

Although the anti-aging market has expanded rapidly, scientific validation of many interventions remains limited. This review, therefore, focuses specifically on immune-targeted mechanisms and evidence-based translational strategies rather than commercial claims. The vicinity of many providers to the surroundings of "biohacking"—a movement that enables self-optimization through technology and biology—is mainly outstanding. In this scenario, aging is considered a "bug" in the system; a technical bug in the journey of life that needs to be repaired. However, while these descriptions are attractive in media and economic context, many of their statements remain scientifically questionable. There is really a shortage or lack of clinical data that goes beyond animal models and cell cultures.

Methods and Literature Search Strategy

This narrative, immune-focused, mechanistic review was conducted using a structured and reproducible literature search strategy. Electronic databases, including PubMed, Scopus, and Web of Science, were systematically searched for studies published between January 2010 and March 2025, with the final literature search executed on March 28, 2025.

The search strings included: ("immunosenescence" OR "immune aging" OR "inflammaging") AND ("rapamycin" OR "metformin" OR "senolytics" OR "immune rejuvenation" OR "thymic regeneration" OR "vaccine response aging").

Inclusion criteria comprised: (i) peer-reviewed original research, (ii) randomized controlled trials (RCTs), (iii) clinical cohort studies, and (iv) high-impact mechanistic reviews with relevance to immune aging.

Exclusion criteria included: non-English articles, case reports, editorials, and studies lacking immune-specific outcomes.

Study selection was performed through title/abstract screening followed by full-text evaluation for relevance to immune mechanisms, biomarkers, or interventions targeting aging. Priority was given to human studies, followed by translational animal models.

Throughout the review, nomenclature was standardized according to current immunology and geroscience conventions, including NF-κB, IL-1β, mechanistic target of rapamycin (mTOR)C1, $p16^{INK4a}$, and $p21^{CIP1}$. Units, biomarker reporting formats, cytokine notation, and immune-cell terminology were harmonized across all main and supplementary figures, tables, and text.

To improve methodological transparency, study prioritization followed a predefined hierarchy that emphasized randomized controlled trials (RCTs), longitudinal cohort studies, and mechanistic translational studies with direct relevance to immune aging. Priority was assigned to investigations reporting validated immune biomarkers, functional immune outcomes, or clinically meaningful endpoints such as vaccine responsiveness, infection incidence, hospitalization risk, or immune-cell restoration.

A qualitative risk-of-bias overview was additionally performed for major clinical studies included in this review. Key parameters considered included randomization procedures, blinding strategies, attrition rates, participant selection, endpoint ascertainment, and reproducibility of immune measurements. Studies with

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rigorous placebo-controlled designs and validated immune endpoints were prioritized when interpreting translational significance. Common limitations identified across trials included small cohort sizes, short follow-up duration, biomarker heterogeneity, and inconsistent reporting of immune-functional outcomes.

A concise qualitative risk-of-bias summary for pivotal human clinical studies, including randomization status, blinding procedures, attrition reporting, and immune-endpoint ascertainment, is additionally provided in Supplementary Table S1.

Given the narrative nature of this review, formal meta-analysis and quantitative bias assessment were not conducted. However, emphasis was placed on consistency of findings, reproducibility across models, and translational relevance.

A schematic overview of study selection is provided in Supplementary Figure S1.

The final study-selection counts presented in Supplementary Figure S1 were reconciled against the narrative text and supplementary tables to ensure consistency across included records, excluded studies, and eligibility assessment categories.

A detailed description of the search strategy, eligibility criteria, and study prioritization framework is provided in Supplementary Table S2.

Hallmarks and Processes of Aging

The mechanisms underlying advanced biological age and the aging phenotype are intricate, multifaceted, and linked. Different frameworks of aging have concentrated on cellular and genetic trends observed throughout the aging process, and it was first suggested that this process started with the accumulation of somatic mutations in cells.[5] However, there are still a lot of unanswered questions about this approach, and DNA damage has not yet been established as a unifying theory. It is now evident that several changes to various molecular pathways that control homeostatic activities are responsible for the aging phenotype. In a previous study,[6] the mechanism and the signs of aging were studied, and nine major alterations were found to appear during normal aging, whose manipulation may have an impact on the aging process. Anti-aging treatments now target many of these characteristics mechanistically. However, the aging phenotype emerges individually through several hallmarks at each biological scale in an organism, and therapies intended to target a single hallmark are frequently investigated within a narrow scope (Table 1; Figure 2). An advanced aging phenotype in its whole is not often produced by inducing a single aging signature. For instance, in progeroid animal models, single genetic changes cause different health patterns but generally normal lifespans.[7–10] Similarly, focusing on a certain signature could not slow down aging.

Aging is a multifaceted process that manifests differently across hierarchical levels of biological organization. At the organismal (individual) level, it is characterized by frailty, functional decline, metabolic dysregulation, and cognitive impairment. At the tissue level, aging is associated with stem cell exhaustion, reduced regenerative capacity, tissue atrophy, and loss of structural integrity. At the cellular level, hallmark features include cellular senescence, accumulation of damaged proteins and aggregates, and mitochondrial dysfunction. At the molecular level, aging is driven by genomic instability, epigenetic alterations, and telomere attrition. Together, these interconnected changes illustrate the multiscale nature of aging and provide key targets for therapeutic intervention (Table 2).

Here, we draw attention to this research vacuum for three such hallmarks of aging: (i) cellular senescence, (ii) generation of reactive oxygen species (ROS), and (iii) exhaustion of stem cells. These three hallmarks are highly interconnected and are potential targets for therapeutic intervention. They produce reciprocal expression, have similar characteristics, and are generated by similar methods. Researchers can better understand the effects of anti-aging interventions, identify gaps in the field, and clarify future therapeutic avenues to ultimately influence the aging process by untangling their intertwined mechanisms and interpreting experimental results in the context of this cycle.

Cellular Senescence

Replicative function is halted in cellular senescence, a condition that occurs after serial replication, although the cell retains viability and resistance to apoptosis.[11,12] Negative cellular signaling from these reprogrammed cells causes what were once quiescent and productive cellular components of tissues to change into harmful phenotypes that are hypothesized to contribute to the aging process.[13–17]

Numerous factors, including exposure to protein aggregates, advanced glycosylation end products, DNA damage, ROS mediators, and inflammatory cytokines, might result in this cellular destiny, as shown in Figure 3.[12] NF-κB, p38 MAPK, and related signaling pathways that are believed to contribute to the establishment of the senescent phenotype may be further amplified by pro-inflammatory signaling via interleukins and

Table 1 | Interaction Between Key Hallmarks (Conceptual Network)

Primary Process	Interacts with	Nature of Interaction	Outcome
Senescence	ROS	ROS induces senescence	Tissue damage
Senescence	Stem cells	SASP impairs stem cells	Regeneration decline
ROS	DNA damage	Oxidative damage	Genomic instability
Stem cells	Immune system	Aging niche affects immunity	Reduced repair
Immune system	Inflammation	Cytokine feedback loop	Inflammaging

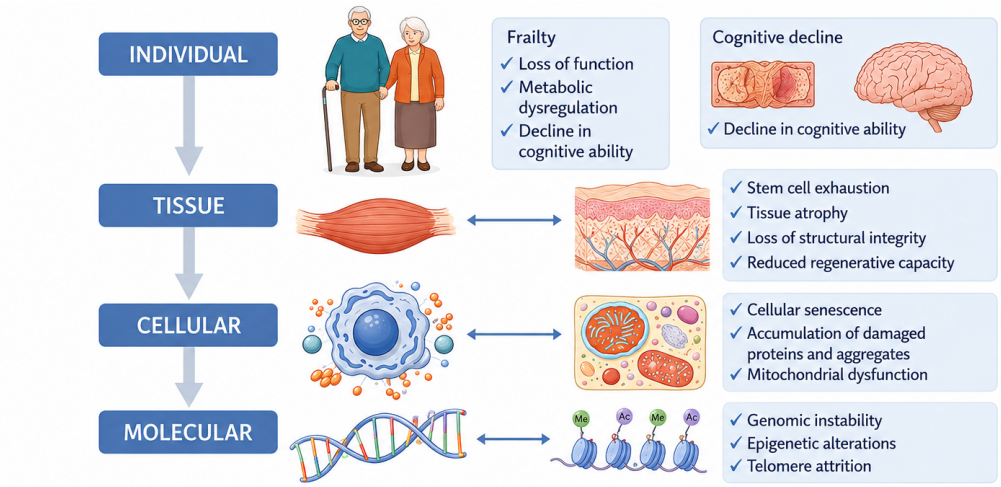

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Fig 2 | Manifestations of the aging phenotype across scales of organized biology

Table 2 | Integrated Hallmarks of Aging and Immune Interactions

Hallmark / Process	Core Mechanism	Immune System Link	Key Molecules / Pathways	Clinical Relevance	Therapeutic Targets
Cellular senescence	Irreversible cell cycle arrest	SASP drives chronic inflammation (inflammaging)	p53, p21, p16, NF-κB	Cancer, fibrosis, tissue dysfunction	Senolytics (dasatinib, quercetin)
Oxidative stress (ROS)	Accumulation of reactive oxygen species	Activates inflammatory signaling and immune dysregulation	NADPH oxidase, mitochondrial ROS	CVD, diabetes, neurodegeneration	Antioxidants, mitochondrial therapies
Stem cell exhaustion	Reduced regenerative capacity	Impaired immune cell renewal	IGF-1, mTOR, Wnt, TGF-β	Poor healing, immune decline	Stem cell niche restoration
Immunosenescence	Decline in immune cell function	Reduced pathogen defense, vaccine response	T-cell depletion, thymic involution	Infections, cancer	Immune modulators, vaccines
Inflammaging	Chronic low-grade inflammation	Central driver of systemic aging	IL-6, TNF-α, CRP	Atherosclerosis, Alzheimer's	Anti-inflammatory drugs
Genomic instability	DNA damage accumulation	Triggers immune activation	DNA repair pathways	Cancer, aging syndromes	DNA repair enhancers
Epigenetic alterations	Changes in gene regulation	Alters immune gene expression	DNA methylation	Biological age acceleration	Epigenetic reprogramming

cytokines.[18–20] Cell cycle arrest and the distinctive replicative-inert senescent hallmark result from the activation of p53/p21$^{WAF1/CIP1}$ in response to DNA damage.[21] Ectopic production of senescence-associated markers does not fully recapitulate this phenotype, suggesting mechanisms beyond DNA damage alone, even though the DNA damage response can directly result in the expression of senescent markers.[21] However, this senescent state is maintained by persistent p16^{INK4a} expression, and the development of age-related patterns has been delayed when cells expressing this marker are removed.[13,21]

Cellular senescence is initiated by environmental (e.g., DNA damage, oxidative stress, chemotherapy) and intrinsic factors (e.g., telomere shortening, oncogene activation), which activate DNA damage response and oncogenic signaling pathways. These signals converge through key regulators, including NF-κB, mTOR, p38 MAPK, and GATA4 to establish the senescent phenotype characterized by cell cycle arrest (p16^{INK4a}, p21^{CIP1}). Senescent cells subsequently develop the senescence-associated secretory phenotype (SASP), marked by the secretion of pro-inflammatory cytokines, chemokines, growth factors, matrix metalloproteinases

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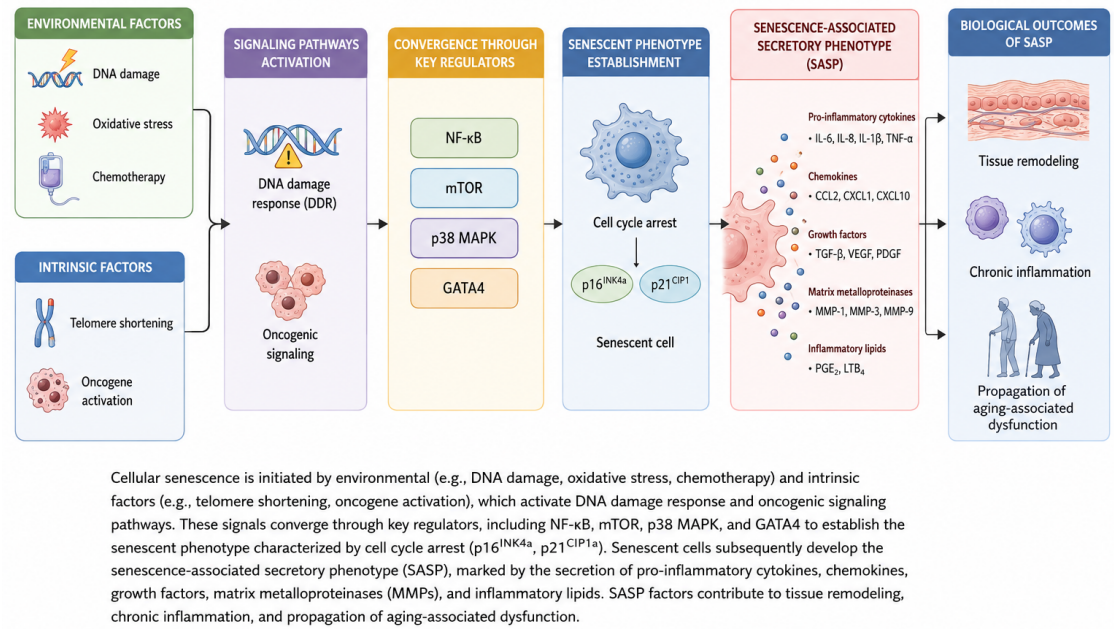

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Fig 3 | Development and features of the senescence-associated secretory phenotype (SASP)

(MMPs), and inflammatory lipids. SASP factors contribute to tissue remodeling, chronic inflammation, and propagation of aging-associated dysfunction.

Through SASP, which has been connected to telomeric shortening and other age-related disorders, including cancer, senescent cells affect tissue homeostasis.[15,22,23] This phenotype causes senescent cells to secrete more nitric oxide, growth factors, chemokines, inflammatory cytokines, and MMPs.[12,24] Furthermore, these cells secrete more stem cell-dysregulating proteins, profibrotic factors, and other harmful biological mediators.[12] In the end, SASP cells may cause ROS damage via directing stem cell dysregulation and cytokine signaling, which might result in the appearance of other characteristic signs of aging.

Stem Cell Exhaustion

The aging phenotype is believed to result from a decreased capacity for replication in tissue-specific stem cells.[5] To replace lost or injured cells, stem cells transition from a quiescent to a proliferative state in response to stimuli. Stem cells occupy niches in tissues mainly for routine tissue maintenance or in response to injury.[25] Their prolonged presence raises the likelihood of damage over time, leading to diminished tissue maintenance and healing in elderly organisms, inadequate functionality and replication, and a dulled response to tissue injury.[25]

This function is lost when the stem cell populations' micro-environment is dysregulated.[26] DNA buildup can be caused by external factors such as inflammatory signaling,[27] ROS-mediated damage,[25,28] and metabolic disruptions[29–31] may cause DNA damage to build up, which could cause stem cell senescence and replicatory arrest.[27] Stem cell exhaustion in aging is influenced by metabolic disruptions caused by insulin-like growth factor 1 (IGF-1), mTOR, Wnt, and TGF-β.[29–31] These disruptions can result from metabolic syndromes and lipodystrophy.[28,32] Due to increased cytokine signaling and decreased frequency of supporting stromal cells, hematopoietic stem cells in elderly mice showed decreased proliferation.[33] This unfriendly milieu is linked to a higher failure rate of stem cell engraftments and transplants in older populations.[33–35] As a rejuvenation strategy, modeling the effects of the young versus old micro-environment and developing strategies to restore the young micro-environment have drawn attention.

Oxidative Stress

The aging phenotype is influenced by ROS signaling. It's interesting to note that different consequences may result depending on the amount and duration of ROS expression. For example, knockdown of nicotinamide adenine dinucleotide phosphate (NAPDH) oxidase leads to chronic, low levels of ROS expression, which in turn activate compensatory mechanisms and promote longevity.[36,37] However, because of cumulative damage over time, persistent production of elevated ROS levels is a known risk factor linked to the aging phenotype.[38] This buildup results from both a rise in pro-oxidative stimuli and a loss of intrinsic antioxidative systems.[39] Numerous factors, including mitochondrial malfunction,[40,41] inflammatory processes,[38,42–44], and external stressors such as UV light,[45] can cause ROS expression. ROS acts as a polyfunctional signaling moiety, influencing cellular senescent pathways,[38,39,46] metabolic activity, DNA damage response, and repair.[47–49] Each of these signaling pathways affects SASP acquisition and stem cell aging. Numerous aging-related illnesses, including cancer,[38,50] diabetes,[50–55] and cardiovascular

disease,[56,57] have been connected to increased ROS generation. Targeting ROS buildup and stress is an appealing area for aging models and anti-aging therapies due to its important role in various cellular processes and illnesses.

The Immune System Aging

Immunosenescence and Inflammatory Aging

Aging profoundly alters immune homeostasis through interconnected dysfunctions in innate immunity, adaptive immunity, and chronic systemic inflammation. In innate immunity, aging impairs macrophage phagocytosis, dendritic cell antigen presentation, neutrophil chemotaxis, and natural killer cell cytotoxicity, leading to reduced frontline defense against infections. Adaptive immune aging is characterized by thymic involution, reduced naïve T-cell output, restricted T-cell receptor diversity, accumulation of senescent memory T cells, and diminished B-cell responsiveness, collectively weakening antigen-specific immunity. These changes are accompanied by chronic low-grade inflammation, often termed inflammaging, driven by persistent activation of inflammatory pathways, such as NF-κB, NLRP3 inflammasome signaling, and SASP mediators. Together, these mechanisms form the biological basis for targeted interventions aimed at rejuvenating immune competence in older adults.

The human immune system undergoes age-related functional and anatomical changes like all other biological systems. The process is known as immunosenescence and characterizes the qualitative and quantitative decrease in immune functions over time. Meanwhile, the immune system exhibits a chronic increase in its basic activity, which manifests itself in a massive release of its pro-inflammatory substances, a condition known as "inflammaging".

Additional hallmarks of immune aging include persistent latent viral burden, particularly cytomegalovirus (CMV), which drives oligoclonal expansion of late-differentiated T cells and accelerates immune exhaustion. Thymic involution reduces the production of naïve T-lymphocytes, thereby narrowing immune repertoire diversity with age. Emerging biomarkers such as immune clocks integrate inflammatory cytokines, epigenetic markers, and immune-cell composition to estimate biological immune age more accurately than chronological age. Furthermore, dysregulation of innate immune sensors, including the NLRP3 inflammasome and cGAS-STING pathways, contributes to chronic inflammatory activation and tissue dysfunction in aging individuals. These pathways are increasingly recognized as therapeutic targets for interventions aimed at restoring immune resilience.

Immunosenescence affects both the innate and adaptive immune systems. In the innate part, a loss of function in white blood cells, i.e., macrophages, natural killer cells, and neutrophils. Their capacity to recognize pathogens, phagocytes, and foreign invaders is reduced. Alongside, the aging innate immune system generates more pro-inflammatory cytokines such as IL-6, TNF-α, and leads to inflammatory states, even though no acute infection is seen.

In the adaptive immune system, the function of T-lymphocytes is decreased. The thymus shrinking caused the decrease in naïve T-cells, while memory cells against the previous antigen accumulate. The selection of the immune system is thus restricted. The same is the situation with B-cells; antibody affinity decreases, and their production becomes less reliable alongside weaker vaccine responses. These alterations explain why older people are more prone to infectious diseases like influenza, cancer, and pneumonia. Vaccine efficacy also reduces with age. Moreover, chronic inflammation takes part in several age-related diseases such as atherosclerosis, Alzheimer's disease, diabetes, and cancer.

Importantly, immune aging is modulated by multiple host and environmental factors. Sex-based differences influence immune responses, with females generally exhibiting stronger adaptive immunity but a higher inflammatory burden in aging. Frailty status further stratifies immune decline beyond chronological age. Persistent CMV infection significantly alters T-cell repertoire composition and reduces vaccine responsiveness. Additionally, socio-economic and environmental exposures—including nutrition, pollution, and chronic stress—contribute to heterogeneity in immune aging trajectories. These variables should be incorporated into future clinical trial design and biomarker interpretation.

These modifiers should be explicitly incorporated into clinical trial stratification and scoring frameworks. For example, CMV seropositivity significantly alters T-cell composition and may influence intervention response, while sex-specific immune differences necessitate sex-stratified analyses.

Inflammaging is a novel concept in modern gerontology, as it no longer states purely aging as a cellular or genetic process but as a systemic dysregulation of the immune system (Figure 4).

Existing composite immune aging metrics, such as IMM-AGE, integrate longitudinal immune profiling to estimate biological immune age. While these approaches provide valuable predictive insights, they often require extensive datasets and lack direct applicability to interventional trials.

The IMM-AGE framework was originally developed using longitudinal systems-level immune profiling to model trajectories of human immune aging and predict all-cause mortality risk. Unlike intervention-focused translational scoring systems, IMM-AGE primarily functions as a predictive immune-age estimation platform based on multidimensional immune datasets. The framework proposed here differs by emphasizing actionable, intervention-linked biomarkers, enabling prospective validation in clinical settings.

Unlike longitudinal immune-aging models such as IMM-AGE, which primarily estimate biological immune age using large multidimensional datasets, the present framework is specifically designed for translational intervention assessment. The proposed scoring system

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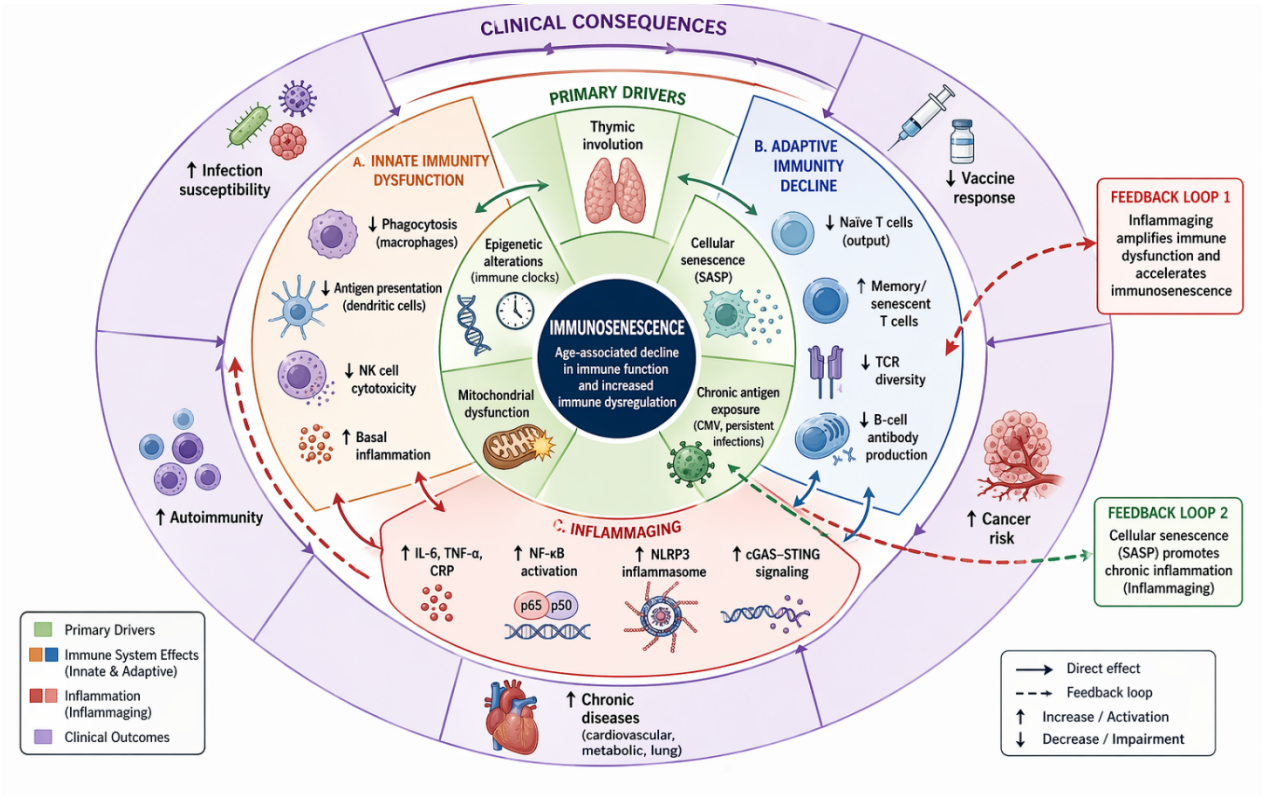

Fig 4 | Mechanistic landscape of immune aging and immunosenescence

prioritizes clinically actionable biomarkers, functional immune endpoints, and therapeutic responsiveness, making it more applicable to prospective gerotherapeutic trials. These approaches should therefore be viewed as complementary rather than competing tools, with IMM-AGE offering predictive biological-age estimation and the present framework emphasizing intervention evaluation and clinical implementation.

Key drivers, including thymic involution, cellular senescence, mitochondrial dysfunction, chronic antigen exposure, and epigenetic alterations, collectively disrupt innate and adaptive immunity (Table 3). These changes promote chronic low-grade inflammation (inflammaging) through pathways such as NF-κB and NLRP3, leading to impaired immune function and increased susceptibility to infections, reduced vaccine responses, and age-related diseases. Feedback interactions between senescence and inflammation further amplify immune decline.

Vaccine Development

Special vaccines are intended to help stimulate the aging immune system in a targeted manner, increasing the efficacy of vaccines, which are known as adjuvanted vaccines designed for older people. Overall, immune rejuvenation is still in its initial stages. Nevertheless, a large number of interdisciplinary research approaches and a close link between immunological aging processes and almost all severe diseases make this area one of the most promising areas of action in modern geriatric medicine.

Recent approval and clinical validation of respiratory syncytial virus (RSV) prefusion F protein (RSVpreF) vaccine platforms for older adults further demonstrate

Table 3 | Immune System Changes Across Aging

Immune Component	Age-Related Change	Functional Consequence	Molecular Basis	Clinical Impact
Innate immunity	Reduced phagocytosis	Poor pathogen clearance	Macrophage dysfunction	Increased infections
Neutrophils	Impaired chemotaxis	Delayed immune response	ROS imbalance	Sepsis risk
NK cells	Reduced cytotoxicity	Tumor escape	Cytokine imbalance	Cancer risk
T-cells (adaptive)	Reduced naïve T-cells	Limited antigen response	Thymic involution	Weak vaccine response
B-cells	Reduced antibody affinity	Poor humoral immunity	Clonal expansion defects	Reduced vaccine efficacy
Cytokine profile	Increased pro-inflammatory cytokines	Chronic inflammation	IL-6, TNF-α elevation	Multimorbidity

the translational potential of immune-optimized adjuvant systems capable of enhancing both humoral and cellular immunity in aging populations. In particular, AS01E-adjuvanted RSVPreF3 vaccines showed strong efficacy against RSV-associated lower respiratory tract disease in adults ≥60 years, supporting the broader application of adjuvanted vaccine technologies designed specifically for immunosenescent populations.[58,59]

Aging System Modeling

Representative models of the aging physiology are necessary to find the best treatment candidates. An ideal aging model should (a) summarize the characteristics of aging and (b) offer useful information as readouts to properly assess the effectiveness of treatments. There are already several important model designs that show promise for assessing the effectiveness of anti-aging treatments. These models fall into three main categories: (i) in silico, (ii) in vitro, and (iii) in vivo. They differ in their methodological approach yet offer distinct advantages and limits (Table 4).

In Silico Models

In aging research, computational models that estimate biological age from large-scale biomarker datasets have become effective tools that connect data science with experimental biology. These methods depend on biologically produced data (such as genomes, transcriptomics, proteomics, and clinical biomarkers) to create prediction "aging clocks" that have a significant correlation with chronological age and can evaluate the effectiveness of interventions, even though they are not entirely in silico. The most common method for determining biological age is to measure longevity-related biomarkers and monitor how they change over time. Nevertheless, a significant obstacle is the lack of agreement on a common definition or set of biomarkers. Ideal aging biomarkers are being non-invasive, outperforming chronological age in predicting functional deterioration, and estimating remaining lifespan—criteria that no single marker has yet met.[60] As a result, many features are included in current models, including telomere length,[61] transcriptomics,[62] DNA methylation,[63,64] proteomics,[65] and physiological metrics. DNA methylation is the most reliable predictor because of its strong correlation with age (Pearson >0.9) and sensitivity to temporal and environmental influences.[5,63–67]

These models have a lot of drawbacks despite their potential. The ability of early DNA methylation clocks to capture inter-individual variability was limited by their reliance on linear regression; however, developments in deep learning and artificial intelligence (AI) increasingly incorporate multi-omics data to increase prediction flexibility.[68]

More recent immune-aging clocks additionally integrate single-cell immune transcriptomics, inflammatory proteomics, and AI-assisted multi-omics modeling to improve prediction of frailty, multimorbidity, mortality risk, and immune resilience beyond chronological age alone. Emerging systems-biology approaches combining proteomic aging signatures with machine-learning algorithms have further enhanced biological age estimation and identification of heterogeneous aging trajectories across populations.[69–71]

While inflammatory indicators indicating "inflammaging" have predictive promise but lack standardized biomarker panels,[17,65,72–76] telomere length, formerly thought to be a critical biomarker, has inconsistent

Table 4 | Summary of Models Implied to Evaluate Anti-Aging Therapeutics

Category	Definition / Concept	Methodological Approach	Key Features in Aging Research	Typical Readouts / Outputs	Advantages	Limitations	Examples in Aging Studies
In silico models	Computer-based simulations using mathematical and computational frameworks	Use of algorithms, systems biology models, AI, and big data integration	Model multi-scale aging processes (molecular → organism level), simulate disease progression, predict intervention outcomes	Predictive outputs (lifespan, disease risk), virtual biomarkers, system dynamics	– Cost-effective and rapid; – Can integrate large datasets; – Enables hypothesis generation and predictive modeling; – Reduces need for animal/human testing (PMC)	– Dependent on quality of input data; – May not fully replicate biological complexity; – Requires validation with experimental models (PMC)	Aging trajectory simulations, AI-based drug discovery, virtual clinical trials
In vitro models	Experiments conducted outside living organisms (e.g., cell cultures, organoids)	Cell cultures, 2D/3D systems, organ-on-chip, molecular assays	Study cellular senescence, oxidative stress, inflammation, and ECM remodeling	Gene/protein expression, senescence markers (e.g., SA-β-gal), cytokine levels	– High experimental control; – Cost-efficient and reproducible; – Reduces animal use; – Suitable for mechanistic studies (NCBI)	– Lack of whole-organism complexity; – Limited cell–cell and system interactions; – Short-term modeling of aging processes (PMC)	Fibroblast senescence assays, skin aging models, and anti-aging compound screening
In vivo models	Studies conducted on whole living organisms (animals or humans)	Animal models (mice, zebrafish), clinical studies	Capture systemic aging, organ interactions, and physiological decline	Lifespan, functional decline, pathology, pharmacokinetics	– Most physiologically relevant- Reflects complex biological interactions; – Essential for translational validation (PMC)	– Ethical concerns- Expensive and time-consuming; – Species differences limit human translation (PMC)	Mouse models of aging, longevity studies, and intervention trials

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relationships with aging across studies.[77,78] Crucially, aging clocks demonstrate heterogeneity between tissues and even within individuals,[79,80] are frequently population-specific, and are impacted by environmental and socio-economic factors.[66,79] These features restrict generalizability and mechanistic interpretability.[67] Despite difficulties with complexity and data requirements, DNA methylation clocks are still useful for estimating lifespan, assessing environmental impacts on health, and evaluating anti-aging therapies.[69,79,81–85] Their integration with high-throughput screening platforms further advances their use in drug discovery.

In Vitro Models

In vitro systems are crucial for analyzing the cellular and molecular causes of aging as well as for testing anti-aging treatments. They fall into two general categories: models of normal aging and models of accelerated aging. To study intrinsic aging features like telomere shortening,[86] stem cell exhaustion,[87] and pathway-specific effects (e.g., Wnt signaling–induced senescence),[88] normal aging models rely on replicative senescence, where cells undergo progressive decline through serial passaging as described by the Hayflick limit.[11] Nevertheless, these models are low-throughput, resource-intensive, and time-consuming.[89] On the other hand, accelerated aging models use stressors such as ROS, typically by exposure to hydrogen peroxide or D-galactose, to cause aging-like phenotypes.[90–92] These models generate characteristics such as elevated cytokine expression and senescence markers (p53, p21),[91] but their usefulness is restricted because they frequently represent acute stress reactions rather than actual physiological aging, and the phenotype's durability is questionable.[93–95]

In Vivo Models

Because they capture systemic, organ-level, and physiological connections, in vivo models offer the most thorough understanding of aging. Yeast, worms, flies, fish, rats, and non-human primates are only a few of the many creatures that have been used.[96] The main drawback is lifetime variability, whereas short-lived models like mice (about two to three years) are frequently employed for mechanistic and therapeutic research, long-lived species like rhesus monkeys provide practical and budgetary difficulties. In particular, murine models have proved crucial in understanding the mechanisms underlying progeroid disorders, aging, and the creation of rejuvenation techniques, making them indispensable for translational aging research.[34,97–104]

Therapeutic Strategies

Human clinical evidence for immune-targeted gerotherapeutics remains promising but heterogeneous. Rapalog compounds have shown the ability to improve vaccine responsiveness and reduce infection rates in older adults by modulating mTOR signaling, though long-term safety data remain limited. Metformin has demonstrated anti-inflammatory and immunometabolic benefits, but evidence for direct immune rejuvenation in non-diabetic aging populations is still emerging. Senolytic agents such as dasatinib and quercetin show potential for reducing senescent immune cell burden; however, robust clinical validation in elderly cohorts is lacking. IL-7 and thymic regeneration strategies may improve lymphopoiesis and T-cell repertoire restoration, yet these approaches remain largely experimental. In parallel, optimized vaccine adjuvants designed for older adults have demonstrated improved immunogenicity, underscoring the translational value of immune-specific intervention strategies (Table 5).

In the biotechnology industry, anti-aging treatments have drawn more attention, and several pharmaceutical and biotechnology businesses have made this research and development a key focus. The three main goals of anti-aging therapy are to avoid damage over time (geroprotection), restore youthful features (rejuvenation), and regenerate damaged tissue.[105–113] Attenuating one of the signs of aging[5] or focusing on a particular age-related illness[114] are central to many of these anti-aging tactics. Here, we highlight four new areas of interest in the realm of anti-aging therapies (Figure 5) and discuss their limitations and supporting data.

Quantitative Clinical Outcomes Across Major Gerotherapeutic Trials

Where quantitative immune-functional outcomes were available, rapalog-based interventions demonstrated some of the most reproducible clinical effects in older adults. Low-dose everolimus-based TORC1 inhibition trials reported approximately 20%–40% improvement in influenza vaccine antibody responses alongside reductions in self-reported respiratory tract infections. In contrast, the large Phase 3 RTB101 trial failed to demonstrate statistically significant reductions in clinically symptomatic respiratory infections despite earlier mechanistic promise. Vaccine adjuvant platforms such as AS01E and MF59 consistently demonstrated enhanced seroconversion rates and improved T-cell-mediated immunity in elderly populations compared with non-adjuvanted formulations.

For senolytic therapies, currently available human studies remain underpowered and heterogeneous, with modest reductions in SASP-associated cytokines and limited functional improvement data. Similarly, metformin studies have primarily demonstrated indirect anti-inflammatory benefits rather than direct restoration of immune competence. These findings collectively emphasize the need for larger standardized trials integrating both mechanistic biomarkers and clinically meaningful immune-functional outcomes.

Human clinical evidence remains heterogeneous and occasionally contradictory. While rapalogs and vaccine adjuvants show consistent immunological benefits, other interventions, such as senolytics and metformin, lack robust large-scale validation in aging populations. Importantly, negative or null findings (e.g., RTB101 Phase 3) highlight the need for cautious interpretation and balanced reporting. Future trials should prioritize

Table 5 | Clinical Evidence for Immune-Targeted Gerotherapeutics in Older Adults

Intervention	Study Design	Population	Dose/ Duration	Immune Endpoints	Clinical Outcomes	Effect Size	Durability	Adverse Events	Notes
Rapalogs (everolimus, RTB101)	Phase 2/3 RCTs	≥65 yrs, some CMV+	Low-dose, weeks–months	↑ IFN response, ↑ vaccine response	↓ respiratory infections	Moderate	Short-term benefit	Mild cytopenia, metabolic effects	RTB101 Phase 3 negative
Metformin	Observational + TAME (ongoing)	Aging, non-diabetic	Chronic	↓ IL-6, CRP	↓ multimorbidity (indirect)	Modest	Long-term unclear	GI effects, B12 deficiency	No direct immune RCT endpoint yet
Senolytics (D+Q)	Pilot trials	IPF, elderly	Intermittent dosing	↓ SASP cytokines	↑ physical function	Small–moderate	Unknown	Thrombocytopenia risk	Small cohorts
IL-7 / thymic regeneration	Early trials	Lymphopenic adults	Short-term	↑ naïve T cells, ↑ TCR diversity	Immune restoration	Moderate	Transient	Injection-related	Experimental
Vaccine adjuvants (AS01, MF59)	RCTs	Elderly	Per vaccine	↑ antibody titers, ↑ T-cell response	↑ vaccine efficacy	Strong	Months	Local/systemic reactogenicity	Clinically validated
GDF11-related interventions	Preclinical + limited human	Mixed	Variable	Conflicting immune effects	Unclear	Inconsistent	Unknown	Unknown	Controversial
RTB101 (Phase 3)	Large RCT	Elderly	Oral	No significant immune improvement	No reduction in infections	Null			

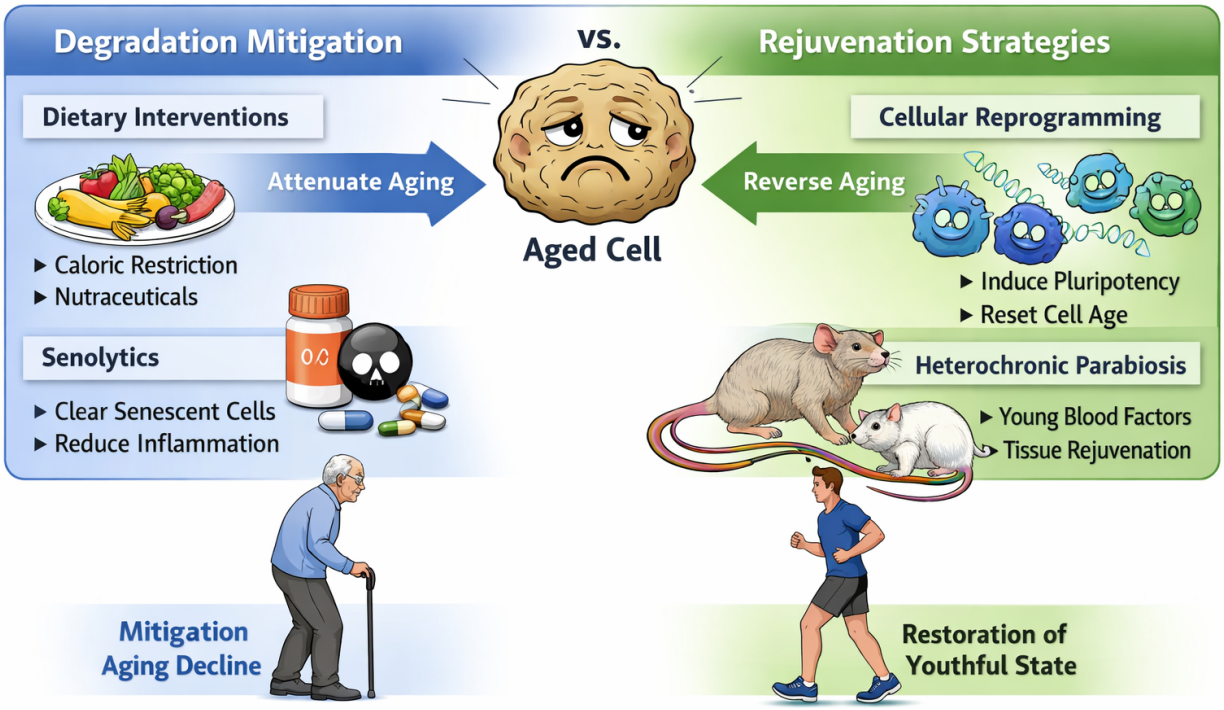

Fig 5 | Therapeutic strategies to attenuate the aging phenotype

standardized immune endpoints, long-term durability, and clinically meaningful outcomes such as infection-related morbidity and vaccine responsiveness.

Aging interventions broadly target either the mitigation of age-related decline or the reversal of aging-associated damage. Mitigation strategies include dietary interventions (e.g., caloric restriction and nutraceuticals) and senolytics, which aim to reduce cellular damage, clear senescent cells, and suppress inflammation. In contrast, rejuvenation strategies focus on restoring youthful function through approaches such as cellular reprogramming, which resets epigenetic age, and heterochronic parabiosis, which leverages systemic factors from young organisms to promote tissue regeneration. Together, these approaches represent complementary pathways to delay or reverse the aging phenotype (Table 6).

Geroprotective Strategies

Geroprotection is a popular anti-aging strategy. By reducing exposure to aging inducers, these therapies aim to slow down the rate of deterioration over time.

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Dietary Modifications

Dietary adjustments and the ensuing metabolic alterations show promise as an accessible way to slow down aging. Aging has been linked to changes in metabolic pathways, particularly the transition from anabolic to catabolic states, which causes cell stress and ROS generation and signaling.[48,49,115] In many non-vertebrate model species, altering metabolic pathways such as IGF signaling,[116,117] growth hormone (GH) signaling,[117,118] and mTOR pathways[119] enhances longevity. Indeed, certain genetic loci involved in these metabolic processes, like TOMM40/APOE, have been identified by investigation of human centenarian tissues[120–122] and FOXO3a,[123,124] are associated with longer lifespans. As a result, many approaches that alter metabolic pathways are being investigated as an anti-aging tactic.

Calorie restriction may increase longevity, according to studies.[125] The technique of lowering calorie consumption below baseline levels while preserving adequate nutritious intake is known as calorie restriction. This process, which was first thought to be caused by a passive mechanism of decreasing metabolic processing, is now known to cause metabolic alterations that impact aging.[126,127] DNA methylation, post-translational histone modifications, and other epigenetic changes are brought about by calorie restriction.[128] Calorie restriction extended the lifespans of yeasts, flies, worms, fish, and rats, according to a systematic assessment of calorie restriction interventions in human and animal trials.[129] However, different species experienced this effect to varying degrees, and a universal mechanism between species is contested.[130]

An increasing amount of research indicates that intermittent fasting, or altering the schedule of calorie intake rather than the amount consumed, may have positive effects akin to those of calorie restriction.[131–134] Periods of fasting cause metabolic changes similar to those observed in calorie restriction, which have the advantageous effects of averting illness and deterioration.[135] In fact, lengthy durations of fasting are necessary to generate the critical molecular and metabolic consequences seen in calorie-restricted diets.[136]

Since many commercial products now use the label "antioxidative" as a marketing tactic, antioxidative diets have also become more popular as a way to slow down the aging process. There isn't much data, nevertheless, to support an impact on human longevity.[137–140]

The simplest way to lessen the impacts of aging and other disease processes is to combine lifestyle changes like diet and exercise. Dietary changes are challenging to sustain and call for consistent effort and discipline, while being extensively researched and showing great promise. As a result, different approaches utilizing pharmaceutical mimetics are being explored. Numerous widely used drugs have been shown to modulate metabolism, including aspirin, metformin, rapamycin, and resveratrol.[141–143] On the basis of four distinct epigenetic clocks, it was even stated that patients receiving recombinant human GH showed signs of epigenetic de-aging.[144]

However, interpretation of thymus regeneration, immunorestoration, and insulin mitigation (TRIIM)-related

Table 6 | Anti-Aging Therapeutics and Immune Modulation

Strategy	Primary Immune Mechanism	Composite Immune Score (0–3)	Key Supporting Evidence	Translational Confidence	Major Limitations
Rapalogs (rapamycin, everolimus)	mTORC1 modulation, enhanced antiviral signaling	3	Improved vaccine responses and reduced respiratory infections in older adults	High	Cytopenias, metabolic adverse effects
Vaccine adjuvants (AS01E, MF59)	Enhanced antigen presentation and T-cell activation	3	Strong RCT evidence in elderly vaccine populations	High	Reactogenicity
Caloric restriction	Metabolic and inflammatory regulation	2	Reproducible anti-inflammatory effects across models	Moderate	Long-term adherence
Intermittent fasting	Immunometabolic modulation	2	Moderate improvement in inflammatory biomarkers	Moderate	Heterogeneous protocols
Senolytics (D+Q, fisetin)	Clearance of senescent immune cells	2	Early human and preclinical immune benefits	Moderate	Limited large-scale clinical validation
Metformin	AMPK activation and inflammatory suppression	2	Consistent reduction in inflammatory mediators	Moderate	Limited direct immune-functional endpoints
IL-7 / thymic regeneration	Restoration of naïve T-cell output	2	Early improvement in T-cell repertoire diversity	Experimental–Moderate	Transient effects
Cellular reprogramming	Epigenetic rejuvenation	1	Primarily experimental/preclinical evidence	Experimental	Tumorigenic risk
Young plasma factors / parabiosis	Systemic rejuvenation signaling	1	Mixed and controversial findings	Low	Mechanistic uncertainty
GDF11-related interventions	Putative regenerative signaling	0–1	Contradictory evidence across studies	Low	Poor reproducibility
RTB101 Phase 3	TORC1 inhibition	0	No significant infection reduction in Phase 3 trial	Low	Failed primary endpoint

epigenetic findings should remain cautious given the small cohort size, absence of randomized placebo-controlled validation, and uncertainty regarding the long-term durability and clinical significance of observed methylation-age changes.

While antioxidant-rich diets are widely promoted, current human evidence does not consistently demonstrate significant effects on longevity or immune rejuvenation, emphasizing the need for controlled clinical studies.

Senolytics

In a transgenic mouse model, Baker et al.[13] showed that removing senescent cells increased longevity and a youthful phenotype. Since then, many biotech firms have looked for pharmaceutical ways to lower this phenotype's expression, and research on senotherapeutics, or drugs targeting the cellular senescence pathway, is a highly active area.

There are only a few medications that are specifically classified as senolytics. Dasatinib, quercetin, navitoclax, A1331852, A1155463, and fisetin are some of them. In pre-clinical models, these medications have demonstrated encouraging outcomes in reducing senescent cells, hence postponing and avoiding diseases associated with aging and senescence.[14] Recent human trials for idiopathic pulmonary fibrosis (NCT02874989) utilizing a combination of dasatinib and quercetin revealed improved functional changes upon pulmonary function testing, although the results regarding these drugs' senolytic capabilities were equivocal.[145] To properly evaluate the possible advantages of these medications for organ function and longevity, more placebo-controlled, double-blind, randomized clinical trials are required.

Repurposed medications with senolytic qualities are the most promising prospects in this field. Through a variety of potential processes, metformin, a commonly used type-2 diabetes drug, has been shown to increase rates of autophagy of pathogenic cells while decreasing rates of DNA damage, cellular senescence, and mitochondrial oxidation.[146] As a preventive measure for age-related illnesses, metformin is presently being studied in FDA-approved clinical trials [targeting aging with metformin (TAME) trials]. The senolytic candidate medication UBX0101, which was intended to lessen the joint stiffness and discomfort associated with osteoarthritis, attracted the attention of Unity Biotechnology through billionaire investments; however, trial findings did not demonstrate significance. Other well-known treatments that target the mTOR pathway, including sirolimus or rapamycin, have shown senolytic qualities and are currently under investigation.[12,147]

Recent early-phase human senolytic investigations continue to demonstrate reductions in SASP mediators and inflammatory burden; however, substantial heterogeneity persists regarding dosing regimens, biomarker selection, durability of response, and reproducibility across patient populations. Larger randomized trials with standardized immune-functional endpoints remain necessary before broad clinical translation can be justified.[115,116]

Strategies for Rejuvenation

Rejuvenation, or the restoration of young qualities, is one strategy to fight aging. This method takes advantage of our knowledge of stem cell biology and embryonic development to eliminate signs of age-related damage.

Reprogramming of Cells

The ability to generate fully differentiated cells from a patient into an embryonic state with pluripotent differentiative capacity and infinite self-renewal was shown in 2006.[148] OCT4, SOX2, KLF4, and C-MYC (OSKM factors) are four essential transcription factors that can be induced to return cells to an embryonic state, where they can develop into any type of cell or tissue from the three germ layers: ectoderm, mesoderm, and endoderm.[149] The idea behind this tactic is that during the reprogramming process, cells revert to their youthful condition, giving the organism youthful traits. The induction process resets the epigenetic DNA methylation clock and lowers levels of the senescence markers $p16^{INK4a}$ and $p21^{CIP1}$.[150,151] Given the vast quantity of age-associated DNA methylation sites (about 500,000), this may be a better approach than directly targeting methylation sites.[152] This process seems to be controlled by IL-6 signaling and a cytokine signaling cascade that converges through NF-κB.[153] Reduced ROS, restored telomeric lengths, restored mitochondrial activity, and reversal of other aging markers are all shown in patient-derived iPSCs.[97,151,154] Therefore, cellular reprogramming has potential uses in the management of age-related illnesses. However, there are hazards associated with creating this youthful phenotype. Teratomas[155] and malignancies arising from reprogramming[156,157] have been reported as a result of persistent expression of OSKM genes. This restriction seems to have been circumvented by cyclical expression of OSKM genes, as studies using this strategy have not reported any cases of malignancy.[157–159]

In a mouse model, transient production of OSKM factors revealed young epigenetic methylation patterns in the blood, liver, spleen, and pancreas.[160] In fact, partial cell reprogramming in a mouse model of Hutchinson–Gilford progeria syndrome showed a delayed beginning of aging, marked by a longer median lifespan (from 18 to 24 weeks) and a decrease in age-related traits at the tissue level.[16,161]

These findings have motivated businesses to employ partial reprogramming as a human rejuvenation technique.[162,163] There have been numerous in vitro demonstrations of this technique;[164] there have been no documented human clinical trials utilizing cellular reprogramming. The practical use of this approach is beset with numerous obstacles, raising concerns about its potential future uses. Genetically edited organisms with designed promoters to ectopically express the OSKM factors—which cannot be utilized in humans—are the models used to illustrate their impact. Transfection of plasmids may provide transient expression; this strategy presents additional difficulties in obtaining efficient transduction to have a significant effect.

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Modifying the Habitat for Stem Cells

Exposure to young serum has been shown to cause rejuvenation and regeneration in elderly parabionts, causing histological organ-level alterations in all primordial germ layers across many tissue types.[165] The results of this research have led to the investigation of young blood components as a possible rejuvenation method, even if the precise mechanisms mediating these effects differ between organs and are now poorly defined (Table 7).[166]

According to parabiosis studies, the accumulation of harmful substances is reduced when old serum is diluted, whereas youthful blood may carry rejuvenation-promoting elements that diminish with age. To answer this question, Mehdipour et al.[167] performed blood exchanges in mice using either albumin-rich saline or isochronic pairs. The authors propose that improved characteristics of muscle regeneration, neurogenesis, and decreased liver obesity and fibrosis can be induced by a single exchange of plasma with albumin-rich saline. These results imply that the main cause of heterochronic parabiosis may be the dilution of harmful elements rather than the advantages of youth. On the other hand, GDF11 was reported to be raised in young serum and to contribute to olfactory neurogenesis and muscle regeneration by Sinha et al.[103] and Katsimpardi et al.[168] Before being used in humans, these seemingly contradicting findings from parabiosis experiments need more research.

Importantly, substantial uncertainty remains regarding the reproducibility, tissue specificity, and mechanistic role of GDF11 in mammalian aging. Reported rejuvenation-associated effects have not been consistently replicated across laboratories, and methodological differences in protein quantification and experimental design likely contribute to conflicting conclusions.

To improve translational relevance, evaluation of anti-aging interventions should incorporate immune-specific biomarkers alongside conventional aging metrics. Key parameters include the CD4/CD8 ratio, naïve-to-memory T-cell balance, inflammatory mediators such as IL-6 and TNF-α, markers of thymic output, vaccine responsiveness, and immune age indices derived from immune-cell profiling. For example, an intervention that reduces inflammatory cytokines while improving naïve T-cell frequencies and vaccine responsiveness may be considered to provide meaningful immune rejuvenation benefits. Integrating such biomarkers into the evaluation framework allows for a more precise assessment of whether interventions truly restore immune competence rather than merely alter general aging phenotypes (Figure 6).[169,170]

A semi-quantitative scoring system (0–3 scale) integrating cellular, inflammatory, and functional immune biomarkers. Scores were assigned based on reported effects in human and preclinical studies, considering study design, population, intervention dose, and duration. A score of 0 indicates no evidence of effect, 1 indicates minimal or inconsistent evidence, 2 indicates moderate improvement, and 3 indicates strong and reproducible immune benefit.

Immune Scoring Framework: Methodological Clarification

To enhance transparency and reproducibility, the immune scoring system follows a structured evidence hierarchy. Scores are assigned on a 0–3 scale based on:

- *Evidence weighting*: Human randomized trials > cohort studies > pre-clinical models
- *Effect consistency*: Reproducibility across independent studies
- *Biomarker robustness*: Use of validated immune endpoints
- *Clinical relevance*: Inclusion of functional or disease outcomes

Two independent evaluations of the scoring framework were performed by the contributing authors using predefined evidence-ranking criteria. Discrepancies in assigned scores were discussed collectively and resolved through consensus review based on study quality, reproducibility of findings, biomarker robustness, and clinical relevance.

Formal inter-rater statistical analysis was not performed because the framework is intended as a semi-quantitative translational tool rather than a validated meta-analytic scoring system. Future prospective applications may incorporate formal inter-rater reliability assessment to further strengthen reproducibility.

Sensitivity analyses indicate that weighting inflammatory biomarkers (e.g., IL-6, CRP) versus cellular markers (e.g., naïve T-cells) can shift scores by ~10%–20%, emphasizing the need for standardized biomarker prioritization.

Weighting approaches prioritized clinically validated immune-functional outcomes over isolated molecular changes. Functional endpoints such as vaccine responsiveness, T-cell restoration, and infection reduction were weighted more heavily than isolated cytokine fluctuations. Exploratory sensitivity analyses demonstrated that emphasizing inflammatory

Table 7 | Key Gaps and Future Directions in Aging Research

Challenge	Description	Impact	Suggested Solution
Lack of standardization	No unified evaluation framework	Poor comparability	Multi-scale standardized models
Over-reliance on single models	Narrow experimental scope	Misleading conclusions	Integrate in silico + in vitro + in vivo
Limited human validation	Most data from animals	Low translational value	More clinical trials
Biomarker inconsistency	No universal aging marker	Unreliable predictions	Multi-omics integration
Immune-aging disconnect	Immune role under-integrated	Incomplete models	Immune-centric frameworks

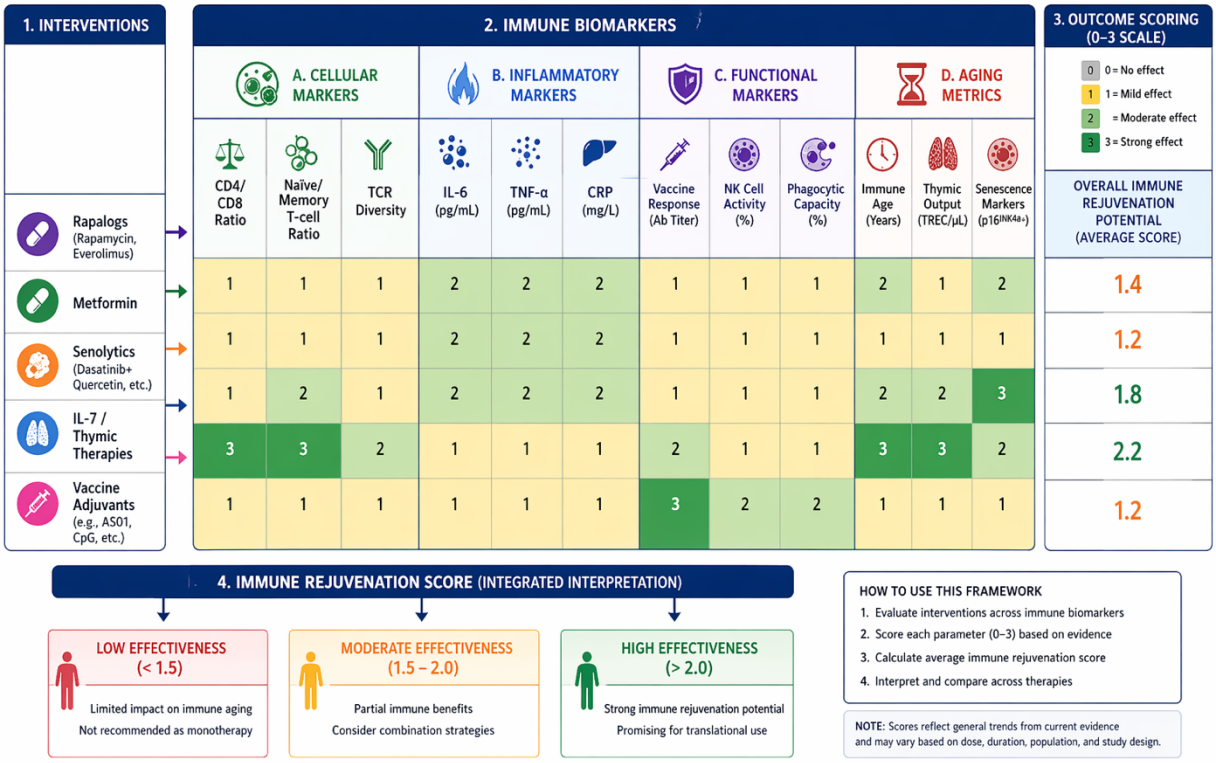

Fig 6 | Immune-specific evaluation framework for anti-aging interventions

biomarkers alone could moderately alter composite intervention scores, highlighting the importance of balanced multi-domain immune assessment.

To ensure consistency across the study, all composite intervention scores presented in Figure 6, Table 6, Supplementary Table S3A, and Supplementary Table S3B were calculated using a unified evidence-weighted framework. Final composite scores were derived from four predefined domains: (i) quality of evidence, (ii) reproducibility across studies, (iii) robustness of immune biomarkers, and (iv) demonstration of clinically meaningful functional outcomes. Human randomized controlled trials and interventions demonstrating reproducible vaccine responsiveness, infection reduction, or restoration of immune-cell functionality received the highest weighting.

Using this harmonized framework, rapalog-based interventions and clinically validated vaccine adjuvants (AS01E, MF59) demonstrated the strongest and most reproducible immune benefits. Senolytic therapies and metformin showed moderate but heterogeneous evidence, whereas GDF11-related interventions and RTB101 Phase 3 outcomes remained inconsistent or insufficiently validated. All intervention scores shown throughout the manuscript now correspond directly to the evidence-to-score mapping provided in Supplementary Table S3B.

Worked examples were used to standardize the interpretation of the 0–3 immune-benefit scale. A score of 0 represented no measurable improvement in immune biomarkers or clinical outcomes. A score of 1 represented minimal or inconsistent findings, such as modest reductions in inflammatory cytokines without functional immune improvement. A score of 2 represented moderate but reproducible effects, including partial restoration of naïve T-cell frequencies, moderate reductions in IL-6 or CRP, or modest enhancement of vaccine antibody responses. A score of 3 represented strong and reproducible immune benefits across multiple studies or endpoints, including significant enhancement of vaccine responsiveness, improved interferon signaling, restoration of immune-cell functionality, and clinically meaningful reduction in infection burden.

For example, rapalog interventions demonstrating improved influenza vaccine antibody responses and reduced respiratory tract infections in placebo-controlled trials were categorized as high-confidence immune-modulating interventions. Conversely, interventions demonstrating biomarker-only improvements without consistent clinical or functional immune effects were assigned lower scores.

Detailed scoring criteria are provided in Supplementary Table S3A, while the evidence-to-score mapping linking each intervention to supporting studies and biomarker outcomes is provided in Supplementary Table S3B.

Recent clinical and translational advances (2023–2025) have further refined the field, particularly in mTOR modulation, innate immune pathway targeting, and vaccine optimization in older adults. However, conflicting findings—including debates surrounding GDF11 and negative RTB101 outcomes—underscore the importance of balanced interpretation and continued validation (Table 8).

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Safety, Ethical, and Regulatory Considerations

Translation of geriatric therapies into clinical practice requires careful evaluation of safety, particularly in older adults with frailty and polypharmacy. Rapalogs may induce metabolic dysregulation and cytopenias, while metformin carries risks of gastrointestinal intolerance and vitamin B12 deficiency. Senolytics raise concerns regarding off-target effects and hematological toxicity (Table 9).

Drug–drug interactions represent a critical consideration in elderly populations, necessitating stratification based on comorbidity burden. From a regulatory perspective, the qualification of immune biomarkers as surrogate endpoints remains a key challenge. Agencies such as the FDA and EMA increasingly emphasize clinically meaningful outcomes, including infection rates and vaccine responsiveness, over molecular readouts alone.

Ethical considerations include equitable access to gerotherapeutics, especially in low- and middle-income countries, and the responsible communication of anti-aging claims.

Limitations

Despite substantial progress in the field, important limitations remain in evaluating immune-targeted anti-aging strategies. Many interventions are supported primarily by preclinical or early-phase clinical studies, with limited long-term evidence in diverse aging populations. Variability in biomarker selection, intervention protocols, and outcome measures complicates direct comparison across studies. In addition, the multifactorial nature of immunosenescence makes it difficult to isolate the effects of individual therapies. Future studies should prioritize standardized immune biomarkers, longitudinal validation, and clinically meaningful endpoints to strengthen translational applicability.

Future geroscience-guided clinical trials must also address ethical and regulatory considerations, particularly in defining appropriate endpoints. Clinically meaningful outcomes should include infection incidence, vaccine responsiveness, immune risk profiles, and healthspan-associated functional measures rather than reliance solely on molecular biomarkers.

Importantly, the proposed immune scoring framework is semi-quantitative and intended to support translational interpretation rather than replace formal meta-analytic evaluation. The framework has not yet undergone prospective validation against hard clinical outcomes such as infection incidence, hospitalization, frailty progression, or vaccine non-response. Future multicenter studies should therefore evaluate cross-cohort reproducibility, longitudinal stability, and predictive performance across diverse aging populations, including stratification by sex, frailty status, CMV seropositivity, and socioeconomic background (Box 1).

Table 8 | Core Immune Biomarker Panel for Clinical Trials

Category	Biomarker	Method	Units	Timepoints
Cellular	CD4/CD8 ratio	Flow cytometry	Ratio	Baseline, 3, 6, 12 months
T-cells	Naïve/memory subsets (CD45RA/CCR7)	Flow cytometry	% cells	Same
Diversity	TCR repertoire	Sequencing	Diversity index	Baseline, 6–12 months
Inflammation	IL-6, TNF-*α*, CRP	ELISA	pg/mL, mg/L	Monthly/quarterly
Thymic output	TREC	qPCR	Copies/μg DNA	Baseline, 6 months
Viral burden	CMV serostatus	Serology	Positive/negative	Baseline
Innate immunity	NK cytotoxicity	Functional assay	% lysis	Baseline, 6 months
Function	Vaccine response	Antibody titers	Fold change	1, 3, 6, 12 months

Table 9 | Safety and Regulatory Considerations for Immune-Targeted Gerotherapeutics

Intervention	Key Adverse Effects	Drug–Drug Interaction Concerns	Monitoring Considerations	Regulatory/Clinical Notes
Rapalogs (rapamycin, everolimus, RTB101)	Cytopenias, hyperlipidemia, impaired wound healing	CYP3A4-mediated interactions common in polypharmacy	CBC, lipid profile, infection monitoring	Immune biomarkers not yet FDA/EMA-qualified surrogate endpoints
Metformin	Gastrointestinal intolerance, vitamin B12 deficiency, lactic acidosis risk	Renal-function-dependent interactions	Renal function, B12 monitoring	TAME trial may inform future geroscience regulatory frameworks
Senolytics (D+Q)	Thrombocytopenia, off-target toxicity	Potential hematologic and kinase-inhibitor interactions	CBC, hepatic function	Clinical evidence remains early-phase
IL-7 / thymic regeneration	Injection-site reactions, transient lymphoid activation	Limited long-term data	Immune-cell monitoring	Experimental immune-restoration strategy
Vaccine adjuvants (AS01, MF59)	Local/systemic reactogenicity	Generally limited interaction burden	Vaccine-response assessment	Clinically validated in older adults

Box 1 | Public Health and Policy Implications

- Optimize vaccine strategies for aging populations (adjuvants, boosters)
- Prioritize immune resilience in long-term care settings
- Integrate immune biomarkers into routine geriatric assessment
- Address disparities in access to gerotherapeutics in LMICs
- Promote preventive strategies targeting inflammaging

Conclusion

Anti-aging treatments are a very active field. Currently, nonetheless, it is distinguished by a growing trend of initial enthusiasm and popularity followed by a decline in interest as a result of disappointing outcomes. There needs to be some reformation if the field is to progress toward meaningful and significant work. In particular, in order to screen for interventions that have the best chance of preventing aging, baseline analyses and standardization of techniques are required for all therapy candidates.

Research across biological scales is one field that requires significant improvement. Many studies restrict their study to a single scale and presume propagation of the effect, even though aging has unique characteristics at every biological scale. Additionally, the information gathered must accurately depict an impact on the aspect of aging at that scale. Instead of measuring surrogate indicators, which may arise from different assumptions, assays should evaluate the traits directly. We provide examples of various assays that could be used at each scale to show therapy efficacy in Table 3. Most importantly, in order to properly contextualize the treatment effect, efficacy must be shown at a functional level for both health span and lifespan. For maximum clinical relevance, research should ideally be carried out in mammalian models.

Moving forward, integrating immune-specific biomarkers with clinically meaningful outcomes will be essential to distinguish true immune rejuvenation from generalized anti-aging effects. A standardized, multi-scale evaluation framework combining molecular, cellular, and functional immune metrics offers a practical path toward translational success (Table 10).

Table 10 | Proposed Standardized Methods to Evaluate Anti-Aging Strategies

Domain	Method / Approach	Key Measurements	Tools / Techniques	Purpose in Evaluation	Advantages	Limitations / Challenges
Biological age estimation	Epigenetic clocks (DNA methylation)	CpG methylation patterns, epigenetic age acceleration	Bisulfite sequencing, Illumina arrays	Quantify biological vs chronological age; assess intervention impact	High predictive accuracy; widely validated	Population specificity; tissue variability; limited mechanistic insight
	Multi-omics aging clocks	Integrated genomics, transcriptomics, proteomics	AI/deep learning models, bioinformatics pipelines	Holistic aging assessment	Captures system-level aging	Requires large datasets; computational complexity
	Telomere length analysis	Telomere shortening rate	qPCR, Southern blot	Indicator of replicative aging	Simple, widely studied	High variability; inconsistent predictive value
Cellular senescence assessment	Senescence-associated markers	SA-β-gal activity, $p16^{INK4a}$, $p21^{CIP1}$, p53 expression	Histochemistry, Western blot, qPCR	Identify senescent cell burden	Direct cellular aging readout	Context-dependent; not universally specific
	SASP profiling	Cytokines (IL-6, IL-1β, TNF-α)	ELISA, multiplex assays	Measure inflammatory aging (inflammaging)	Links aging to disease risk	Biomarker heterogeneity
Functional & physiological testing	Organ/system function	Lung (spirometry), cardiac, cognitive tests	Clinical diagnostics, imaging	Evaluate functional decline/improvement	Clinically relevant	Influenced by external factors
	Physical performance	Grip strength, gait speed, endurance	Functional assays	Reflect systemic aging	Non-invasive; translational	Inter-individual variability
Molecular damage & stress	Oxidative stress markers	ROS levels, lipid peroxidation	Fluorescent probes, biochemical assays	Assess cellular damage	Mechanistic insight	Transient and variable
	DNA damage markers	γ-H2AX foci, mutation burden	Immunofluorescence, sequencing	Quantify genomic instability	Core hallmark of aging	Requires specialized tools
Preclinical model evaluation	In vitro assays	Cell viability, proliferation, senescence	Cell culture systems	Screen drug candidates	Controlled, scalable	Limited systemic relevance
	In vivo lifespan studies	Survival curves, healthspan metrics	Animal models (mouse, zebrafish)	Validate longevity effects	Physiologically relevant	Time-consuming; ethical concerns
Imaging & structural analysis	Neuroimaging & organ imaging	Brain volume, tissue integrity	MRI, CT, PET	Detect structural aging	Non-invasive, detailed	Expensive; limited accessibility
Inflammation & immune profiling	Inflammatory biomarkers	CRP, cytokines, and immune cell profiling	ELISA, flow cytometry	Evaluate inflammaging	Strong disease correlation	Non-specific markers
Data integration & validation	Longitudinal cohort analysis	Time-series biomarker changes	Epidemiological studies, registries	Validate intervention		

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In conclusion, several effective tactics are being developed to counteract the aging phenomenon. However, technique consistency remains crucial for effectively assessing possible candidates. The impact of a drug candidate on (a) organ and physical function in vivo, (b) lifespan duration in vivo, (c) changes to multiple hallmarks of aging by using assays that best represent the aging phenotype, (d) the therapeutic effect across the various scales of biology, and—most importantly—(e) the robustness and longevity of the therapeutic intervention must be reported more frequently. High-impact treatments to increase longevity and lengthen healthspan may be found using this thorough and exacting evaluation framework.

List of Abbreviations

AI: Artificial Intelligence
CMV: Cytomegalovirus
COPD: Chronic Obstructive Pulmonary Disease
CVD: Cardiovascular Disease
GH: Growth Hormone
IGF-1: Insulin-like Growth Factor 1
MMPs: Matrix Metalloproteinases
mTOR: mechanistic Target Of Rapamycin
NADPH: Nicotinamide Adenine Dinucleotide Phosphate
OSKM: OCT4, SOX2, KLF4, and C-MYC
RCTs: Randomized Controlled Trials
ROS: Reactive Oxygen Species
RSV: Respiratory Syncytial Virus
SASP: Senescence-Associated Secretory Phenotype
TAME: Targeting Aging with Metformin
TRIIM: Thymus Regeneration, Immunorestoration, and Insulin Mitigation

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Supplementary Materials

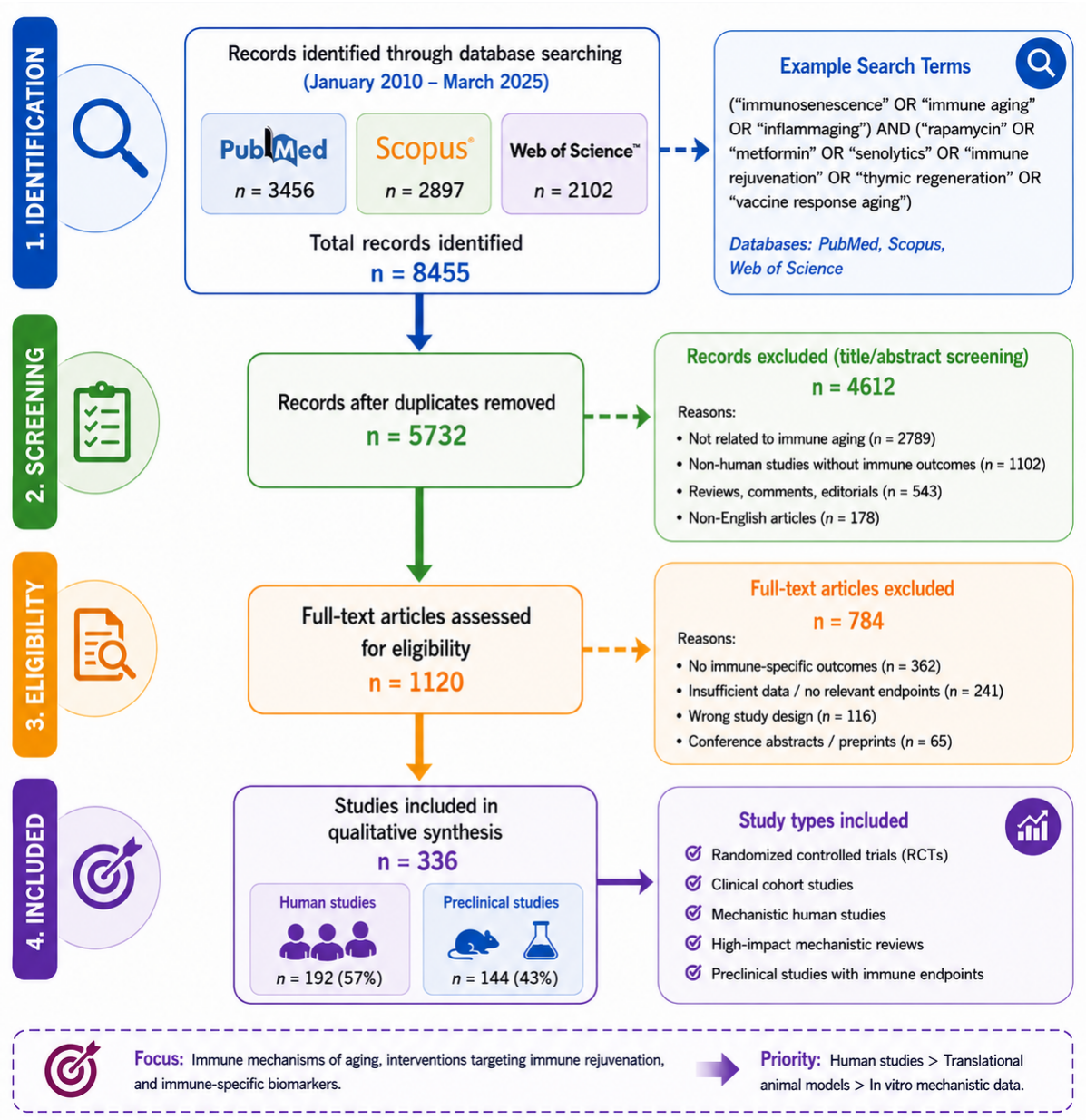

Fig S1 | Literature search and study selection workflow for immune-focused aging studies

Note: A PRISMA-style flow diagram illustrating the structured identification, screening, eligibility assessment, and inclusion of studies evaluating immune mechanisms of aging and anti-aging interventions. Records were retrieved from PubMed, Scopus, and Web of Science between January 2010 and March 2025, with the final literature search executed on March 28, 2025, using predefined search terms related to immunosenescence, inflammaging, immune rejuvenation, and immune-targeted therapeutics. After duplicate removal and title/abstract screening, full-text articles were assessed for relevance based on immune-specific outcomes. Studies meeting the inclusion criteria were incorporated into the qualitative synthesis, comprising both human and preclinical evidence. Final study-selection counts were reconciled across the main text and supplementary materials to ensure consistency. The diagram additionally summarizes key reasons for exclusion at each stage and highlights prioritization of translationally relevant immune biomarkers, vaccine responsiveness, infection outcomes, and clinically meaningful immune-functional endpoints.
Qualitative assessment of pivotal human clinical studies included in this review, focusing on methodological rigor, randomization status, blinding procedures, attrition reporting, and ascertainment of immune-related endpoints.

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Supplementary Table S1 | Qualitative Risk-of-Bias Summary for Pivotal Human Clinical Studies

Study / Intervention	Study Design	Randomization	Blinding	Attrition Reporting	Immune-Endpoint Ascertainment	Overall Risk of Bias	Key Notes
Mannick et al. (everolimus / rapalogs)	Phase 2 RCT	Yes	Double-blind placebo-controlled	Adequately reported	Validated vaccine-response and IFN-signaling biomarkers	Low–Moderate	Strong translational immune endpoints but limited long-term follow-up
RTB101 Phase 3 trial	Large multicenter RCT	Yes	Double-blind	Well reported	Respiratory infection outcomes and IFN-related biomarkers	Moderate	Robust design but failed primary efficacy endpoint
TAME / metformin-related studies	Mixed cohort and ongoing RCT framework	Partial / variable	Variable	Incomplete in some observational cohorts	Primarily inflammatory biomarkers (IL-6, CRP)	Moderate	Limited direct immune-functional outcome validation
Justice et al. (Dasatinib + Quercetin)	Pilot clinical trial	Limited	Open-label	Reported	SASP cytokines and physical-function metrics	Moderate–High	Small sample size and limited placebo control
Hickson et al. (senolytics)	Pilot human study	Limited	Open-label	Partially reported	Inflammatory and senescence-associated biomarkers	Moderate–High	Preliminary translational evidence only
Sportès et al. (IL-7 therapy)	Early-phase clinical study	No formal randomization	Non-blinded	Adequately reported	Naïve T-cell recovery and TCR diversity	Moderate	Mechanistically strong but early-stage evidence
TRIIM thymic regeneration trial	Small prospective clinical study	No	Open-label	Reported	Epigenetic age, TREC levels, naïve T cells	Moderate–High	Small cohort and lack of placebo control
AS01E / MF59 vaccine-adjuvant trials	Large RCTs	Yes	Double-blind	Comprehensively reported	Validated antibody titers and T-cell-response assays	Low	Strongest clinical immune-functional evidence among reviewed interventions
CALERIE caloric-restriction trial	Randomized controlled trial	Yes	Partial blinding not feasible	Well reported	Inflammatory biomarkers and metabolic outcomes	Moderate	Behavioral intervention limits blinding feasibility

Risk-of-bias categorization was qualitative and narrative in nature, emphasizing randomization procedures, blinding, attrition transparency, reproducibility of immune measurements, and clinical relevance of immune-functional outcomes. No formal meta-analytic bias scoring tool was applied.

Supplementary Table S2 | Detailed Search Strategy, Inclusion/Exclusion Criteria, and Study Prioritization Framework

Category	Description
Databases searched	PubMed, Scopus, Web of Science
Search period	January 2010–March 2025
Final search execution date	March 28, 2025
Search approach	Boolean keyword combinations targeting immune aging and immune-focused interventions
Example search string	("immunosenescence" OR "immune aging" OR "inflammaging") AND ("rapamycin" OR "metformin" OR "senolytics" OR "immune rejuvenation" OR "thymic regeneration" OR "vaccine response aging" OR "RSV vaccine older adults" OR "immune clock")
Language restriction	English only
Study types included	Randomized controlled trials (RCTs), clinical cohort studies, mechanistic human studies, translational systems-immunology studies, high-impact reviews, and relevant preclinical studies with immune endpoints
Study types excluded	Case reports, editorials, conference abstracts, non-peer-reviewed preprints, and non-immune-focused studies
Population focus	Older adults (≥60 years) prioritized; supporting mechanistic evidence from animal models included
Screening process	Title/abstract screening followed by full-text eligibility assessment
Primary inclusion criteria	Studies reporting immune-related outcomes (e.g., T-cell function, cytokines, vaccine responsiveness, immune clocks, infection outcomes, immune biomarkers)
Exclusion criteria	Lack of immune endpoints, insufficient methodological detail, and inappropriate study design
Priority hierarchy	Human RCT evidence › translational clinical studies › animal models › in vitro mechanistic studies
Outcome emphasis	Immune biomarkers, functional immune responses, infection reduction, vaccine efficacy, and clinically relevant immune endpoints
Review type	Narrative review with structured evidence prioritization; no formal meta-analysis performed
Bias assessment	Qualitative risk-of-bias assessment performed for pivotal human studies, emphasizing randomization, blinding, attrition reporting, reproducibility, and translational relevance

Supplementary Table S3A | Harmonized Scoring Criteria and Biomarker Framework for Immune Rejuvenation Evaluation

A. Semi-Quantitative Scoring Criteria (0–3 Scale)

Score	Definition	Evidence Basis	Interpretation
0	No demonstrated effect	No significant biomarker or clinical improvement across studies	No immune benefit
1	Minimal or inconsistent effect	Limited, conflicting, or poorly reproducible evidence	Low confidence
2	Moderate improvement	Reproducible benefit in ≥1 human study or multiple preclinical studies	Moderate confidence
3	Strong, consistent effect	Multiple human studies or RCT-level evidence demonstrating functional immune benefit	High confidence

B. Biomarker Domains and Measurement Criteria

Domain	Biomarker	Measurement Method	Rationale
Cellular immunity	CD4/CD8 ratio	Flow cytometry	Indicator of immune-system balance
	Naïve/memory T-cell ratio	Immunophenotyping (CD45RA/CCR7)	Reflects thymic output and immune aging
	TCR diversity	High-throughput sequencing	Measures adaptive immune repertoire
Inflammaging	IL-6, TNF-*a*, CRP	ELISA / multiplex assays	Core markers of chronic inflammation
Thymic function	TREC, recent thymic emigrants (RTEs)	qPCR	Proxy for thymic activity
Functional immunity	Vaccine responsiveness / antibody titers	Serology	Clinically relevant immune competence
Immune aging index	Immune clocks (e.g., iAge)	Multi-omics / AI-assisted models	Composite biological immune age

C. Harmonized Intervention-Level Scoring Matrix

Intervention	Quality of Evidence	Reproducibility	Biomarker Robustness	Clinical Functional Outcomes	Composite Score
Rapalogs (rapamycin, everolimus)	3	3	3	3	3
Vaccine adjuvants (AS01E, MF59)	3	3	3	3	3
Senolytics (Dasatinib + Quercetin, fisetin)	2	2	2	2	2
Metformin	2	2	2	2	2
IL-7 / thymic regeneration	2	2	2	2	2
Caloric restriction / fasting	2	2	2	2	2
Intermittent fasting	2	2	2	2	2
RTB101 Phase 3	0	0	1	0	0
GDF11-related interventions	1	0	1	0	0–1
Young plasma / parabiosis	1	1	1	0	1

Scoring Notes:
Composite scores were harmonized across Figure 6, Table 6, Supplementary Table S3A, and Supplementary Table S3B to ensure consistency throughout the manuscript.
Scores were derived using four integrated domains: (i) quality of evidence, (ii) reproducibility across studies, (iii) robustness of immune biomarkers, and (iv) clinically meaningful functional outcomes.
Human randomized controlled trials demonstrating reproducible vaccine responsiveness, infection reduction, or restoration of immune-cell functionality received the highest weighting.

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Supplementary Table S3B | Evidence-to-Score Mapping for Immune Gerotherapeutic Interventions

Intervention	Key Study	Study Type	Population	Immune Biomarkers	Direction of Effect	Clinical Outcome	Evidence Level	Assigned Score	Justification
Rapalogs (everolimus)	Mannick et al.	RCT	≥65 years	IFN signaling, vaccine response	↑	↓ respiratory infections	Human RCT	3	Strong reproducible immune enhancement
RTB101 Phase 3	Phase 3 trial	RCT	Older adults	IFN pathway	No significant improvement	No reduction in infections	Human RCT	0	Failed primary endpoint
Metformin	TAME and related studies	Cohort/RCT	Aging adults	IL-6, CRP	↓ inflammation	Indirect immune benefit	Mixed	2	Consistent anti-inflammatory effects but limited direct immune-functional outcomes
Senolytics (D+Q)	Justice et al.; Hickson et al.	Pilot clinical studies	IPF and diabetic kidney disease	SASP cytokines	↓	Functional improvement	Small human studies	2	Promising but limited sample size
Senolytics (preclinical)	Baker et al.	Animal model	Mouse	Senescence markers	↓	↑ lifespan and immune function	Preclinical	2	Strong mechanistic evidence
IL-7 therapy	Sportès et al.	Early clinical	Lymphopenic adults	Naïve T-cells, TCR diversity	↑	Immune restoration	Early human	2	Mechanistically strong but limited clinical scale
Thymic regeneration	TRIIM trial	Small clinical study	Middle-aged adults	TREC, naïve T cells	↑	Epigenetic age reduction	Small human	2	Innovative but preliminary
Vaccine adjuvants (AS01E, MF59)	Multiple RCTs; RSVPreF3 studies	RCT	Older adults	Antibody titers, T-cell responses	↑	↑ vaccine efficacy	Human RCT	3	Clinically validated immune enhancement
GDF11-related interventions	Mixed studies	Preclinical	Variable	Immune and regenerative markers	Mixed	Unclear	Mixed	0–1	Contradictory and poorly reproducible evidence
Young plasma / parabiosis	Mehdipour et al.	Animal model	Mouse	Inflammatory markers	↓	Partial regeneration	Preclinical	1	Mechanistic uncertainty
Caloric restriction	CALERIE trial	RCT	Adults	IL-6, CRP	↓	Metabolic and inflammatory improvement	Human RCT	2	Consistent but indirect immune effects
Intermittent fasting	Multiple studies	Mixed	Adults	Inflammatory markers	↓	Health improvement	Mixed	2	Moderate consistency across studies

Evidence Mapping Notes:
Evidence hierarchy applied: Human RCTs › Clinical translational studies › Preclinical models.
Consistency scores were determined based on reproducibility across independent studies and validation of immune-functional outcomes.
Negative or neutral trials (e.g., RTB101 Phase 3) were intentionally retained to maintain balanced interpretation and avoid publication bias.
Composite scoring reflects integration of biomarker modulation, immune-functional restoration, translational reproducibility, and clinically meaningful outcomes.
Emerging immune-aging clock methodologies incorporating AI-assisted multi-omics integration and single-cell immune profiling were additionally considered in immune-age assessment domains.