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# Current Trends in Anti-Aging Strategies

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## Keywords

aging, biological clocks, senescence, anti-aging, rejuvenation, progeria

## Abstract

The process of aging manifests from a highly interconnected network of biological cascades resulting in the degradation and breakdown of every living organism over time. This natural development increases risk for numerous diseases and can be debilitating. Academic and industrial investigators have long sought to impede, or potentially reverse, aging in the hopes of alleviating clinical burden, restoring functionality, and promoting longevity. Despite widespread investigation, identifying impactful therapeutics has been hindered by narrow experimental validation and the lack of rigorous study design. In this review, we explore the current understanding of the biological mechanisms of aging and how this understanding both informs and limits interpreting data from experimental models based on these mechanisms. We also discuss select therapeutic strategies that have yielded promising data in these model systems with potential clinical translation. Lastly, we propose a unifying approach needed to rigorously vet current and future therapeutics and guide evaluation toward efficacious therapies.

## Contents



## INTRODUCTION

Aging is the natural process in which the human body undergoes systemic changes leading to decreased functionality and increased risk for age-related diseases, eventually resulting in death. Despite decades of observation and research, this process has yet to be effectively altered by human intervention. Yet the market for anti-aging therapies and cosmetics is nearly $60 billion USD and is expected to increase by 50% in the next 5 years. Despite this great interest, the field is still in its infancy and driven by big promises, with little impact made in slowing this process.

A major problem plaguing the field of anti-aging therapeutics is a lack of standardization with which to determine efficacious therapies. Although the biological effect of aging impacts many areas, therapeutic candidates are often evaluated within a narrow scope, generating hype and capital investments, but ultimately unable to provide the promised effect. In this review, we present an overview of the current state of aging research; review select interventions under investigation and the models currently available to study their efficacy; and discuss the scope, utility, and limitations of these models. Finally, we propose a framework to evaluate these therapies and future aging-related research to produce meaningful progress in this field.

### What Is Aging?

Aging is the normal change to the body throughout the human life cycle. It is the result of the combination of loss of youthful, rejuvenation properties and gain of detrimental features, tipping the balance between these factors toward a degenerative process over time. Aging affects both the quantity and quality of life. The quantity, or lifespan, is a measure of the time alive, whereas the quality, or healthspan, is the duration of life whereby an individual can function fully without the ravages of disease. The effects of aging spare no organ system, and advanced age is a well-established risk factor for diseases (**Figure 1**). It is thought that changes in biological age due to environmental factors or intrinsic properties, such as exposure to chemicals (1), reproductive capabilities (2), metabolic changes (3), and factors influencing epigenetic expression (4), result in the observed variation in individuals' lifespans. Indeed, in recent decades, as modern medicine has advanced, we have seen how new treatments and advances in healthcare enable prolonged longevity in the civilized world. The upper limit of extending longevity through this approach is still unknown. Ultimately, ideal therapeutic candidates should impede the underlying mechanisms of aging that, in turn, may promote longevity.

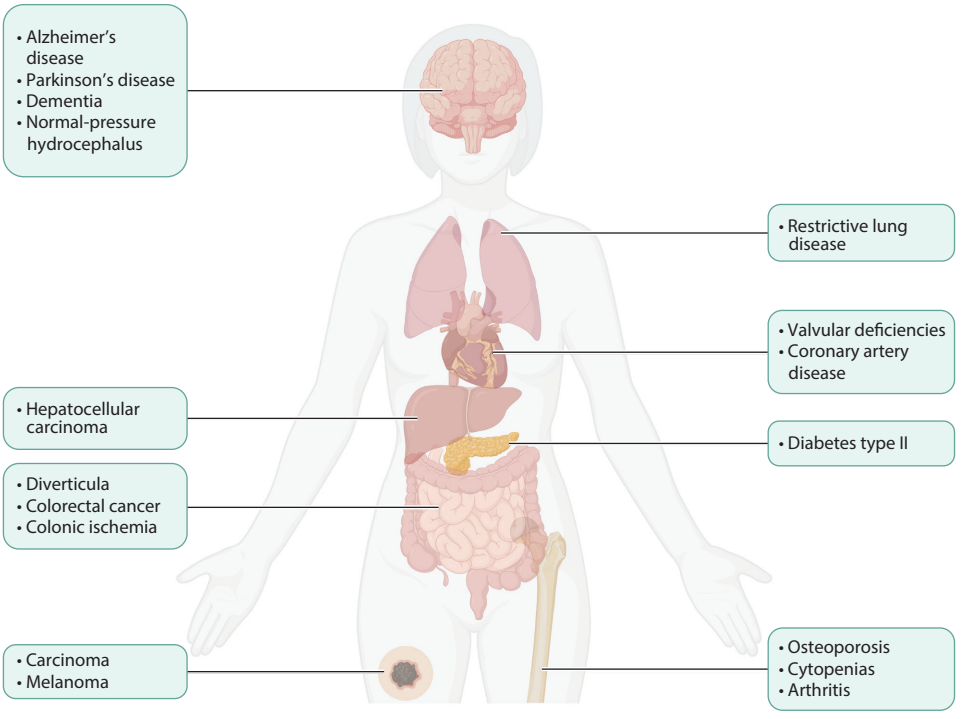



**Figure 1**

Common diseases associated with advanced age. Advanced age is a known risk factor for degenerative diseases and cancer in nearly every organ system. Some of the most common diseases, which account for the greatest mortality, are depicted. A direction often taken for anti-aging strategies is to target disease progression rather than the aging phenotype in that organ system. Figure adapted from Internal Organs with Callouts (Layout) (2) by BioRender.com, retrieved from **https://app.biorender.com/biorender-templates**.

## Hallmarks and Mechanisms of Aging

The forces driving the aging phenotype and advanced biological age are complex, multifactorial, and mechanistically interconnected. It was originally proposed that this process began through the accumulation of somatic mutations in cells, and varying frameworks of aging have focused on cellular and genetic trends seen throughout the aging process (5, 6). Yet significant gaps remain in this hypothesis, and DNA damage as a unifying theory has yet to be proven. It is now becoming clear that the aging phenotype is the result of numerous alterations to several molecular pathways that regulate homeostatic functions. In 2013, López-Otín et al. (5) proposed a new paradigm to view the mechanisms of aging, dubbed the hallmarks of aging, and identified nine key changes that manifest during the normal aging process and whose manipulation can potentially influence the aging process. Many of these hallmarks have become the mechanistic targets of anti-aging therapies. Yet these hallmarks are highly interconnected, and interventions meant to target a single hallmark are often studied within a narrow scope, while the aging phenotype manifests uniquely through multiple hallmarks at each biological scale in an organism (**Figure 2**). Induction of a single hallmark of aging does not necessarily yield an advanced aging phenotype in totality. For example, single mutations at the genetic level in progeroid animal models result in altered patterns of health but overall normal lifespans (7–10). Likewise, targeting a specific hallmark may not attenuate aging.

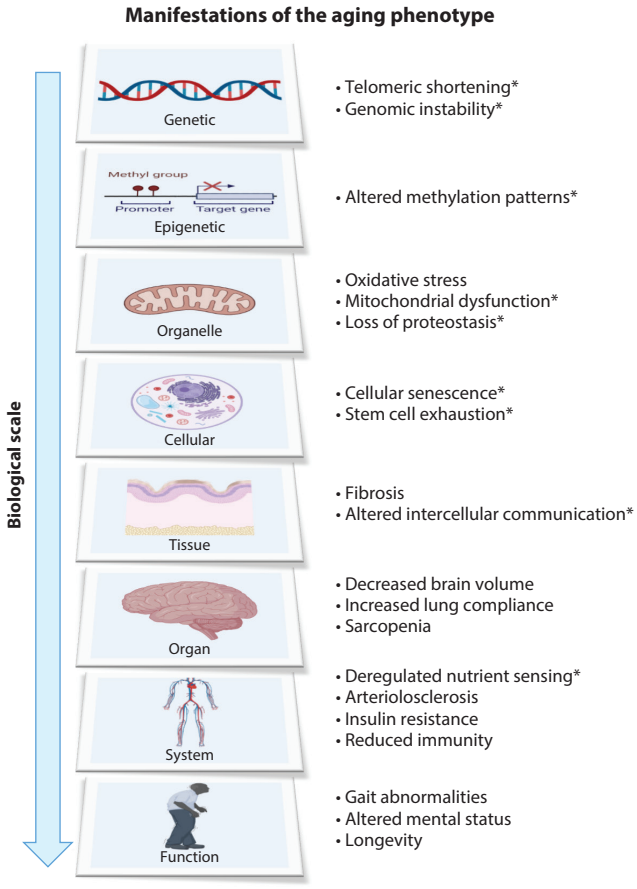



**Figure 2**

Manifestations of the aging phenotype across scales of organized biology. Aging presents with differing features, depending on the biological level at which it is observed. This figure outlines such levels and the common manifestations of the aging phenotype at each point in the biological scale. Figure adapted from images created with BioRender.com.

Here we highlight this gap in the research approach for three such hallmarks of aging: (*a*) cellular senescence, (*b*) reactive oxygen species (ROS) production, and (*c*) stem cell exhaustion, which are highly interdependent and are emerging targets for therapeutic intervention. They manifest with similar features, are induced through similar mechanisms, and result in reciprocal expression. Untangling their intertwined mechanisms, and understanding experimental results in the context of this cycle, can help researchers further interpret the effects of anti-aging interventions, identify shortcomings in the field, and elucidate future therapeutic avenues to ultimately influence the aging process.

**Cellular senescence.** In cellular senescence, a phenotype following serial replication, replicatory function is arrested, but the cell maintains viability and resistance to apoptosis (11, 12). What were once quiescent and productive cellular components of tissue switch to detrimental phenotypes that are thought to contribute to the aging process by negative cellular signaling coming from these reprogrammed cells (13–17).

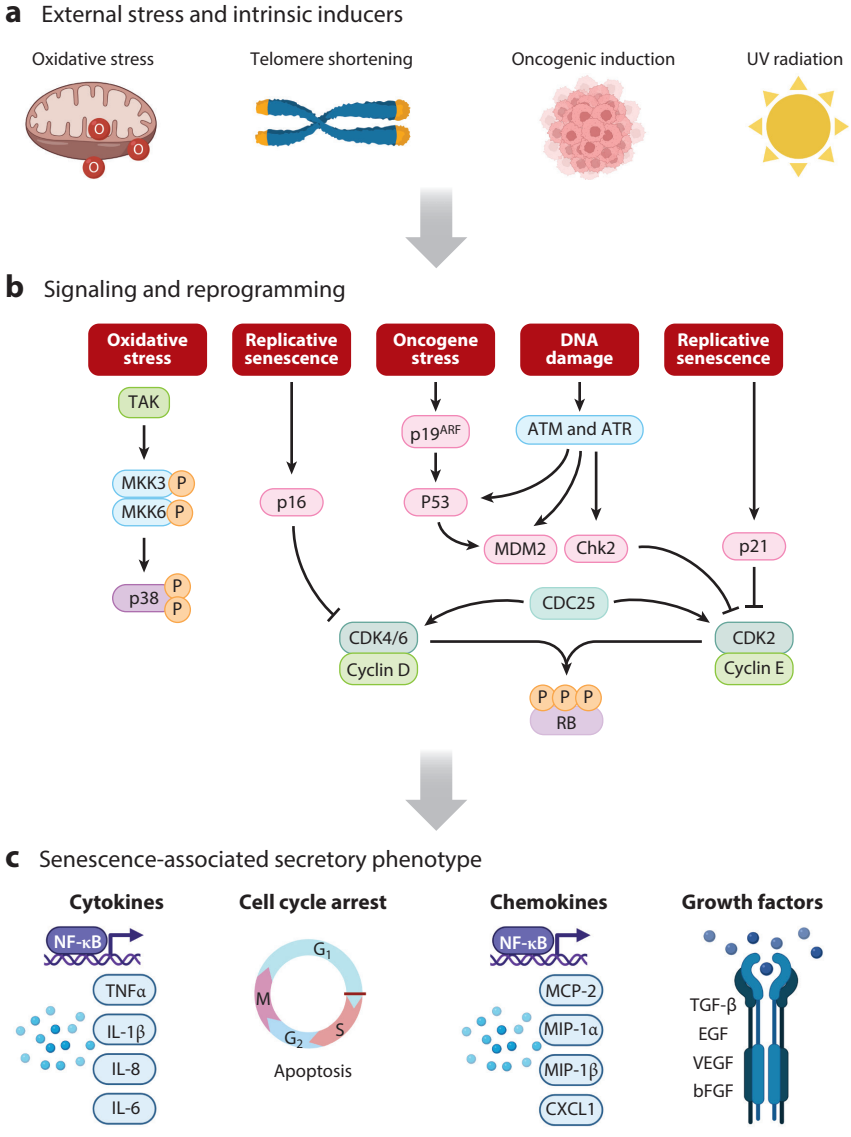



**Figure 3**

Development and features of the senescence-associated secretory phenotype. The senescent phenotype can be induced through (*a*) signaling generated by environmental or intrinsic cues. These signaling pathways (*b*) lead to the senescence-associated secretory phenotype, which (*c*) produces a wide range of intercellular mediators. Figure adapted from images created with BioRender.com.

As depicted in **Figure 3**, many conditions, such as exposure to protein aggregates, advanced glycosylation end products, DNA damage, ROS mediators, and inflammatory cytokines, may lead to this cellular fate (12). Proinflammatory signaling through interleukins and cytokines may further amplify NF-κB, p38MAPK, and associated signaling pathways that are thought to play a role in the development of the senescent phenotype (18–20). In response to DNA damage, p53/p21$^{WAF1/CIP1}$ is activated, leading to cell cycle arrest and the characteristic replicative-inert senescent feature

(21). While the DNA damage response can directly lead to expression of senescent markers, ectopic induction of senescence-associated markers does not recapitulate this phenotype in its totality, indicating mechanisms beyond DNA damage alone (21). However, sustained $p16^{INK4a}$ expression maintains this senescent state, and removal of cells expressing this marker has resulted in the delayed onset of age-related patterns (13, 21).

Senescent cells influence tissue homeostasis through the senescence-associated secretory phenotype (SASP), which has been linked to many age-related conditions, particularly cancer, and telomeric shortening (15, 22, 23). As a result of this phenotype, senescent cells increase secretion of inflammatory cytokines, chemokines, growth factors, matrix metalloproteinases, and nitric oxide (12, 24). In addition, these cells increase secretion of profibrotic factors, stem cell–dysregulating factors, and other detrimental biological mediators (12). Ultimately, SASP cells may induce ROS damage through cytokine signaling and direct stem cell dysregulation, leading to the manifestation of other hallmark features of aging.

**Oxidative stress.** ROS signaling influences the aging phenotype. Interestingly, the magnitude and duration of ROS expression may yield differing effects. For example, knockdown of NAPDH oxidase leads to chronic, low levels of ROS expression, which in turn activate compensatory mechanisms and promote longevity (25, 26). However, chronic expression of high levels of ROS is a well-established risk factor associated with the aging phenotype, in part due to the accumulated damage over time (27). This accumulation occurs from loss of intrinsic antioxidative mechanisms coupled with increased pro-oxidative stressors (28). ROS expression is induced due to multiple sources, including mitochondrial dysfunction (29, 30), inflammatory processes (27, 31–33), and exogenous stressors such as UV light (34). ROS acts as a polyfunctional signaling moiety, influencing cellular senescent pathways (27, 28, 35), DNA damage response and repair, and metabolic activity (36–38). All these signaling pathways influence stem cell aging and acquisition of the SASP. Increased ROS production has been linked to many aging-associated diseases, such as cardiovascular disease (39, 40), diabetes (41–46), and cancer (27, 42). This central functionality in multiple cellular processes and diseases has made targeting ROS accumulation and stress an attractive area for modeling aging and for anti-aging interventions.

**Stem cell exhaustion.** Decreased replicative function of tissue-specific stem cells is thought to contribute to development of the aging phenotype (5). Stem cells occupy niches in tissues primarily for regular tissue maintenance or in response to injury, and upon stimulatory cues, the stem cells shift from a quiescent to a proliferative state to replace damaged or lost cells (47). Their long-term presence increases the probability of damage over time, which results in ineffective functionality and replication, a blunted response to tissue injury, and decreased healing and maintenance of tissues in aged organisms (47).

Dysregulation of the microenvironment for stem cell populations induces loss of this function (48). External factors such as inflammatory signaling (49), ROS-mediated damage (47, 50), and metabolic perturbations (51–53) may result in accumulation of DNA damage, leading to senescence and replicatory arrest in stem cells (49). Metabolic perturbations through insulin-like growth factor 1 (IGF-1), mTOR, Wnt, and TGF-β influence stem cell exhaustion in aging (51–53) and can occur from metabolic syndromes and lipodystrophy (50, 54). Hematopoietic stem cells in aged mice displayed reduced proliferation due to increased cytokine signaling and reduction of the supportive stromal cell population (55). An increased failure rate of stem cell transplantations and engraftments in elderly populations is attributed to this hostile microenvironment (55–57). Modeling the effects of the young versus old microenvironment, and deriving methods to restore the young microenvironment, has gained interest as a rejuvenation strategy.

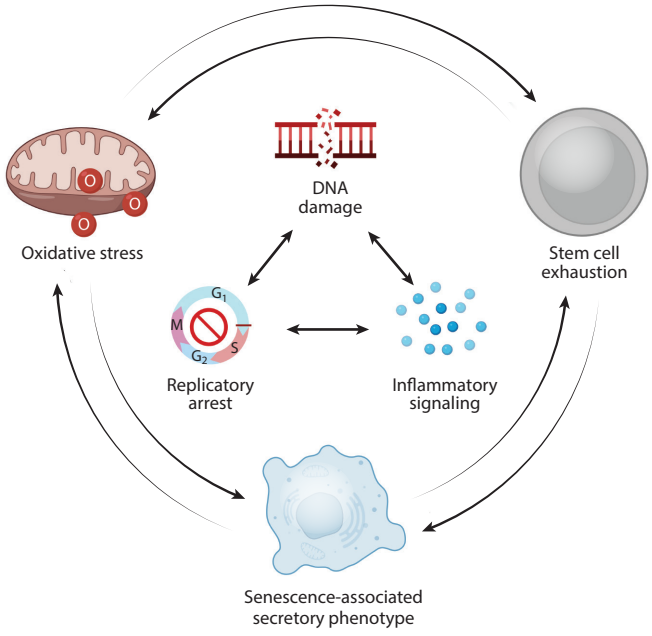



**Figure 4**

Overlapping characteristics of three key hallmarks of aging: oxidative stress, stem cell exhaustion, and the senescence-associated secretory phenotype. These three hallmarks share common features such as replicatory arrest, DNA damage, and inflammatory signaling. Induction of one hallmark can yield the manifestation of the other two hallmark features. Figure adapted from images created with BioRender.com.

## Summary of Mechanisms of Aging

The molecular mechanisms of aging form a complex and highly interconnected network, leading to the emergence of aging phenotypes. Their role as truly causal mechanisms of the aging phenotype is muddled by their overlapping features and reciprocal induction (27, 28, 35) (summarized in **Figure 4**). To identify truly efficacious anti-aging therapeutics, it is imperative to study the effects across multiple hallmarks of aging and biological scales in relevant model systems. Identifying efficacious drug candidates therefore requires methods that can capture these features.

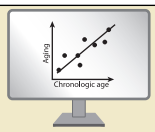
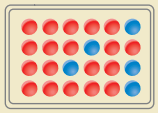
# MODELING AGING SYSTEMS

Identifying ideal therapeutic candidates requires representative models of the aging physiology. An ideal model of aging should (*a*) recapitulate the hallmarks of aging and (*b*) provide practical and actionable data as readouts to adequately evaluate therapeutic efficacy. Several key model designs with promise in determining efficacy of anti-aging therapeutics have emerged. These models, which vary in their methodological approach but provide unique benefits and limitations, fall under three major categories: (*a*) in silico, (*b*) in vitro, and (*c*) in vivo (see **Table 1**).

## In Silico Models

A popular approach to characterize biological age from chronological age is through computationally derived predictions from large datasets of biomarkers. These models are not completely in silico, as they rely on biologically derived experimental data to develop the basis of their predictions. However, the application of these models beyond their training dataset and in clinical trials makes them powerful tools to characterize the effects of anti-aging interventions.

**Table 1 Summary of models used to evaluate anti-aging therapeutics**

| |   **In silico** |  **In vitro** |  **In vivo** |
|---|---|---|---|
| **Key readouts** | Predictions of biological age | Molecular and genomic signatures | Functional activity, whole-tissue histology |
| **Biological scale** | Genetic, epigenetic, functional | Genetic, epigenetic, organelle, cellular | All |
| **Benefits** | Large datasets integrating complex interactions | Single-pathway and cell type analysis | Lifespan measurements, whole-organism reactions |
| **Limitations** | Requires high-throughput data collection, rigid training structures | Limited scope of biological scales, low throughput | Multifaceted interactions, difficult to discern causal mechanisms, low throughput |

Implementation is simple: Measure biomarkers that predict longevity, and use changes in the levels of these markers to determine therapeutic efficacy. However, a major challenge in the field of aging research is a lack of consensus on the definition of biological age and its associated biomarkers. Butler et al. (58) proposed a definition of aging biomarkers as measurable features that (*a*) can be used to predict the outcome of an age-sensitive test better than chronological age, (*b*) can be used to predict an individual's remaining longevity, and (*c*) can be measured without influencing life expectancy. Such an ideal marker for aging and lifespan has yet to be identified (58–60).

Models have instead used biomarkers that correlate with time; such biomarkers have provided accurate predictions of chronological age, thereby creating aging clocks. Biological age estimators have been trained using biomedical data on different features, including genetics, epigenetics, function (e.g., spirometry), neuroimaging, telomere length (61), transcriptomics (62), DNA methylation (63, 64), proteomics (65), and blood-based biomarkers.

One of the most reliable data sources to predict chronological age is DNA methylation. DNA methylation is considered to be a hallmark of aging (5) and is susceptible to changes from interactions with the environment, such as interactions with stress, as well as to changes through the passage of time alone (64, 66). The first DNA methylation models were reported in 2013, and time-dependent features from methylation datasets were extracted and regressed to chronological age to generate age estimators, reaching a high correlation (>0.9) of Pearson scores (63–65, 67). These predictive models have intrinsic features with serious limitations to their utility. Due to the linear nature of these methylation clocks, they lack flexibility to accurately predict the intrinsic variability in biological age seen between individuals in whom variance is most prevalent and, critically, has the highest yield for its application. Efforts have been made to circumvent this limitation by leveraging advances in artificial intelligence using deep neural networks to overcome the rigidity of linear regression models, and these artificial intelligence models have been applied to predict biological age by integrating transcriptomics, methylation, and proteomic arrays (68–70). Leveraging artificial intelligence for DNA methylation clocks continues to be an active area of research (71). Yet this increasing complexity and the need for larger datasets are additional hurdles for these clocks' widespread application in the drug discovery sector.

It was originally thought that telomere length could be a predictive biomarker for aging. However, its use has been limited due to high rates of variability across epidemiological studies, casting doubt as to telomere length being a cause or result of aging (72, 73). Vaiserman & Krasnienkov (73) provide a highly detailed and thorough recent review of telomeres in aging research.

Many studies in aging have demonstrated enrichment of inflammatory pathways with age (65, 74), indicating chronic inflammation develops over time and is associated with many age-related diseases and increased mortality (75, 76). It is unclear whether this low-grade chronic inflammatory state, dubbed inflammaging, is a mechanism or a feature of the aging process, but it can be used to predict features of aging (17), and serum levels of proinflammatory cytokines have been used to predict mortality (32, 77). Biological age clocks based on inflammaging are still in the early stages of development since identifying the relevant biomarkers has been challenging (78). One clock developed uses a guided autoencoder method to nonlinearly model the inflammatory burden and predict chronological age (17).

DNA methylation clocks have been utilized to predict lifespan by integrating organ-functional data to estimate age-related physiological decline (69) and to determine changes in the rate of biological aging to evaluate anti-aging therapies (69, 79, 80) and rejuvenation strategies (81). These clocks have also been used to explore how environmental and health risk factors, such as socioeconomic status (66, 82), smoking (82), ischemic stroke (83), and even COVID-19 infection (84), might influence mortality. Yet the predictions from these clocks must be understood in the context of their limitations. These clocks are often highly population specific, owing to the influence of the external environment on these epigenetic expression patterns (82), and lose external validity when applied to alternative populations. Furthermore, there is no consensus on which methylation sites to measure (82), making these clocks mechanistically uninterpretable (67). There are variations in the clinical data integrated into the models, and they aggregate population data to apply to individuals, thereby reducing the interpersonal variability in the mean error in the model. These predictions are even limited between samples within the same individual. DNA methylation can vary across tissue types, thereby limiting the predictive value when one is using samples derived from locations not included in the training of the model (85).

Even with these limitations, DNA methylation clocks have the potential to be implemented in conjunction with high-throughput screenings of therapeutic candidates to further characterize their anti-aging effects.

## In Vitro Models

In vitro modeling provides the means to elucidate underlying aging mechanisms, the effects of drug candidates, and the efficacy of anti-aging therapeutics. In vitro models are broadly characterized into normal aging models or accelerated aging models.

Normal aging models subject cells to the mercy of time. Cell culture models intrinsically achieve a senescent state, as defined by the Hayflick model of cellular aging (11). Ultimately, the characteristic changes in these cell models that occur from serial passaging can be used to describe the intrinsic properties of aging cells (86). This approach has yielded a wealth of findings on telomere biology and its impact on cellular senescence (87), as well as stem cell aging through telomeric shortening (88). Methodology using pathway perturbations with serial passaging can elucidate mechanistic associations of individual proteins and pathways that manifest in aging phenotypes, such as induction of cellular senescence through constant Wnt signaling (89). However, these models have obvious limitations; most notably, they are time consuming and expensive, using large quantities of reagents, and have a low overall throughput. These models would be best utilized to identify mechanisms of specific drug candidates.

Accelerated aging models have emerged as an alternative to normal aging models. These models induce aging in vitro using ROS-mediated damage (90). The reducing sugar D-galactose induces ROS production in vitro and has been used to generate aging models across numerous cell types of all primordial germ layers. Cells treated with D-galactose exhibit increased cytokine production, reduced proliferation, and increased expression of senescent genes such as those encoding

p53 and p21, indicating an aged phenotype (91). Other reducing sugars have also demonstrated increased ROS production and similar aging phenotypes (92). Models utilizing hydrogen peroxide to directly induce ROS damage yielded differing results relative to in vivo data (93). Interpretation of the results from these models is limited, as they are not truly models of aging, but rather models of ROS damage. Limited exposure to ROS-inducing agents [e.g., 48 h (94) to 7 weeks (95)] may yield expression of aging markers. However, the durability of this phenotype, and whether it truly represents aging or a transient stress response, is poorly validated.

## In Vivo Models

Whole organisms provide the greatest insight into the aging process. Many organisms, including yeasts, flies, worms, fish, rodents, and nonhuman primates, have been used to study aging (96). The primary challenge of in vivo studies of aging is the lifespan of the model organism used. For example, rhesus monkeys can live up to 40 years, making their ubiquitous use in therapeutic trials untenable. In comparison, murine models have relatively short lifespans (~2–3 years) and can be invaluable for untangling the mechanisms and therapeutic targets for aging. Murine models have already provided insight into the mechanisms and pathophysiology of progeroid diseases and the potential application of rejuvenation strategies.

**Progeria and accelerated aging models.** Murine models of accelerated aging provide mechanistic insight into the features of aging. Single-mutation models have demonstrated features of advanced and accelerated aging of multiple tissue types, such as in models of human progeroid syndromes or other mutations (10, 89). Human progeroid syndromes are a group of diseases that constitute an early-onset advanced-age phenotype. Typically induced by defects in DNA damage repair, these progeroid syndromes are also a promising source for in vitro models to study age-related diseases by using inducible pluripotent stem cells (iPSCs) that can recapitulate organ-specific progeroid disease states. These models have illuminated our understanding of age-related diseases across multiple organ systems, including brain, heart, lung, and kidneys (97). Several mouse models of progeroid syndromes have been established and can be readily utilized to test therapeutic candidates (**Table 2**).

**Heterochronic parabiosis.** Heterochronic parabiosis is the process through which two organisms of differing aged individuals (*hetero* denotes different, and *chronic* denotes ages) are surgically

**Table 2 Summary of progeroid models**

| Model organism | Key mutations | Phenotypic features | Disease modeling | Reference(s) |
|---|---|---|---|---|
| Mouse | BubR1 overexpression | Decreased cancer deaths<br>Extended lifespan<br>Increased muscle function | Not applicable | 98 |
| Mouse | WRN | Reduced cellular proliferation<br>Increased sensitivity to p53 knockout<br>No premature aging | Werner syndrome | 7, 8, 10 |
| Mouse | LMNA | Muscular dystrophy<br>Reduced growth rate<br>Normal lifespan | Hutchinson–Gilford progeria syndrome | 99, 100 |
| Mouse | Zmptste24 | Growth retardation<br>Reduced lifespan<br>Muscular dystrophy | Hutchinson–Gilford progeria syndrome | 9 |

joined, forming an anastomosis (parabiosis) between their circulations to share blood contents (101). This model of aging is implemented to study the response of tissues to altered environments due to age-related factors, although very few studies have utilized this approach (101).

In 2005, Conboy et al. (102) reported a heterochronic parabiosis study to investigate whether biological factors in young blood might enhance pathways associated with progenitor cell proliferation and rejuvenate old stem cells. They found that parabiosis of young to old mice facilitated muscle regeneration through activation of resident progenitor cells via upregulation of the Notch ligand Delta, whose activity is lost with age in muscle satellite cells (102, 103). Furthermore, liver regeneration increased twofold in old parabiont mice. A secondary pathway potentially mediating muscle repair was through reduction of an age-dependent increase in tissue fibrosis that accompanies loss of muscle regeneration via canonical Wnt signaling pathways (51). These rejuvenation properties may also be attributed to growth differentiation factor 11 (GDF11) activity, which normally declines with age (104). This rejuvenation effect is thought to extend to epigenetic modifications, specifically in the central nervous system (105, 106), where parabiosis may induce cAMP signaling and CREB activation in hippocampal neurons, promoting increased learning and memory (107). Heterochronic parabiosis models have also observed variations in the quantity of senescent cells, with increased senescent cells in younger mice and decreased senescent cells in older mice (108). Single-cell RNA sequencing of these models revealed that introduction of young blood facilitated expression of metabolic genes in old counterparts across multiple tissues (109). In fact, young heterochronic mice have significantly lower mitochondrial fitness relative to young isochronic controls (110).

Understanding aging using the findings from this model system has significant limitations. In general, these studies suffer from using one time point with little or no investigation into whether the effect is sustained over time. Furthermore, in heterochronic parabiosis, organisms share the same organs, and functional changes may be explained due to processing blood in younger organs, and not necessarily due to the presence or absence of age-related blood-borne factors. These studies also involve little follow-up analysis to discern causative features and have few controls in place to rule out alternative hypotheses, such as weight differences, volume considerations between parabionts, and bullying between parabiont animals (111). In fact, heterochronic organ transplants influence the fitness of the organ and function as an independent predictor of graft success (112). Rebo et al. (113) addressed this limitation by performing heterochronic blood exchanges rather than pairings, thereby removing the effect of shared organs, and studied the effects on muscle regeneration, liver proliferation, and hippocampal neurogenesis. These researchers found that, following ~90% homogenization of blood, there was a moderate increase in muscle fiber regeneration and a decrease in fibrosis (113).

A critical area needed to move this model and other aging models forward is standardization of methods. Assays measuring proliferation, by using nuclear markers that merely correlate with proliferation, are poor indicators of stem cell rejuvenation, and little functional analysis follows this initial measurement. Many features of stem cell exhaustion—including cytoplasmic accumulation of toxic macromolecules; aberrant activity of Wnt, Notch, and Hedgehog pathways; and epigenetic alterations—could be measured (114). An increased emphasis must be placed on investigating effects across multiple biological scales, and while histopathological evidence is a starting point, true functional evaluation of organisms must also be performed.

## THERAPEUTIC STRATEGIES

Anti-aging therapeutics have gained increased attention in the biotechnology sector, with several pharmaceutical and biotechnology companies making such development a top priority of

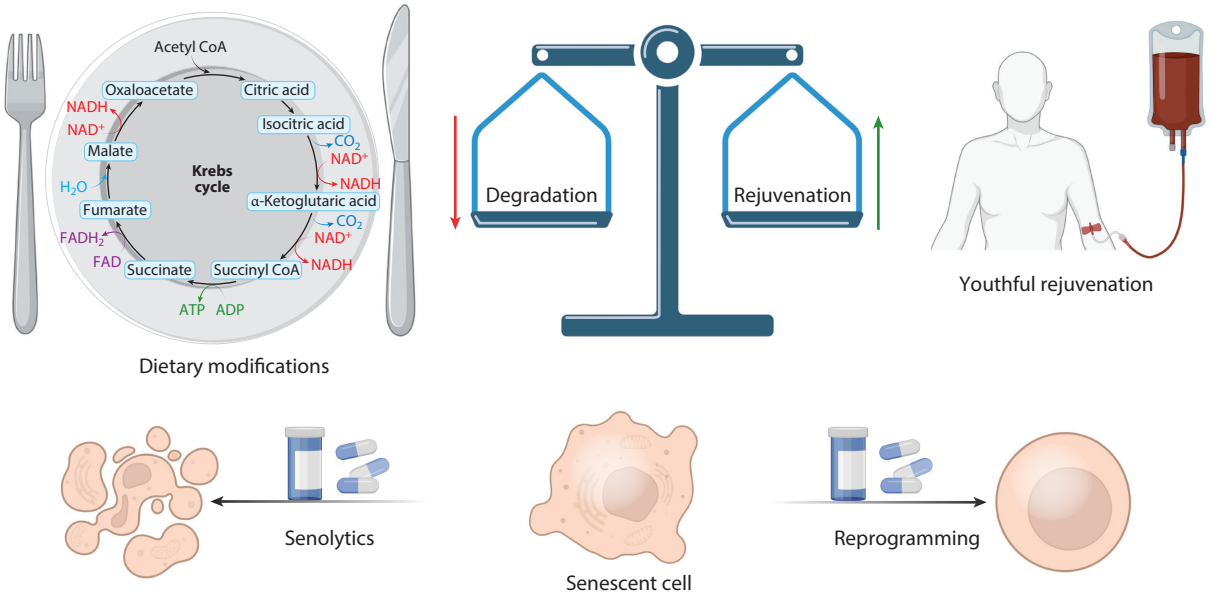



**Figure 5**

Therapeutic strategies to attenuate the aging phenotype. Emerging therapeutic strategies focus on mitigating degradation over time (e.g., dietary modifications and senolytics) or reversing the effects of aging through rejuvenation mechanisms (e.g., cellular reprogramming and heterochronic parabiosis for youthful rejuvenation). Figure adapted from images created with BioRender.com.

their research and development. Anti-aging treatments focus on prevention of damage over time (geroprotection), restoration of youthful features (rejuvenation), and the ability to replace tissue following injury (regeneration). Many of these anti-aging strategies revolve around attenuating one of the hallmarks of aging (5) or targeting a specific age-related disease (115). Here we highlight four emerging areas of interest in the field of anti-aging interventions (**Figure 5**) and discuss the evidence supporting their implementation and their limitations.

## Geroprotective Strategies

A common approach of anti-aging strategies is through geroprotection. These interventions focus on impeding the rate of degradation over time by minimizing exposure to inducers of aging.

**Dietary modifications.** Dietary modifications and resulting metabolic changes hold promise as a readily available method to attenuate aging. Alterations to metabolic pathways, specifically the shift from an anabolic to a catabolic state, have been implicated in the aging process, leading to cell stress and ROS signaling and production (37, 38, 116). Modulation of metabolic pathways such as IGF signaling (117, 118), growth hormone (GH) signaling (118, 119), and mTOR pathways (120) increases longevity in various nonvertebrate model organisms. In fact, analysis of human centenarian tissues has found that specific genetic loci involved in these metabolic processes, such as *TOMM40/APOE* (121–123) and *FOXO3a* (124, 125), are linked with increased longevity. Therefore, various methods modulating metabolic processes are being explored as an anti-aging strategy.

Studies suggest that caloric restriction may extend lifespan (126). Caloric restriction is the process of reducing caloric intake below baseline levels while maintaining sufficient nutritional intake.

Originally hypothesized to be due to a passive mechanism of reducing metabolic processing, this process is now understood to induce metabolic changes to influence aging (127, 128). Caloric restriction induces changes in DNA methylation, posttranslational histone modifications, and other epigenetic alterations (129). A systematic review of caloric restriction intervention in human and animal trials found that caloric restriction increased the lifespans of yeasts, flies, worms, fish, and rodents (130). However, the extent of this effect varied greatly between species, and a universal mechanism between species is contested (131).

In humans, a 2-year clinical trial investigating caloric restriction found no change in circulating levels of IGF-1, GH, or other caloric restriction–sensitive biomarkers but did find favorable changes in body weight, glucoregulatory function, and markers for cardiovascular disease (132). However, studies in humans have been relatively short term, relying on metabolic biomarkers as a gauge of efficacy with an undetermined effect on longevity (133). These results are further complicated by the inclusion criteria: Many of the studies conducted on humans use an initially overweight cohort, which raises the question as to whether the mechanism of effect is directly on aging or indirectly due to modifying disease progression. Ultimately, it is estimated that caloric restriction has the potential to extend human lifespan by 1–5 years, depending on the age at which this modification is implemented (134).

A growing body of evidence suggests that intermittent fasting, or changing the pattern of calorie intake, rather than the quantity of calories taken in, may induce favorable benefits similar to those of caloric restriction (135). Fasting periods trigger metabolic alterations like those seen in caloric restriction, which has the favorable benefits of preventing disease and degradation (136). In fact, prolonged periods of fasting are necessary to induce the key molecular and metabolic effects seen in caloric restriction diets (137).

Antioxidative diets have also gained popularity to attenuate the rate of aging, with many commercial products now using the antioxidative term as a marketing strategy. However, little evidence exists to justify an influence on longevity in humans. Dietary supplementation of antioxidative molecules demonstrated cognitive benefits in mammalian models but failed to increase lifespan (96). Several studies looking at gain-of-function mutations in superoxide dismutase and catalase, two antioxidant enzymes, found that these mutations did yield an increase in *Drosophila* lifespan (96), and in a dose-dependent manner (138). Interestingly, alterations to other antioxidant enzymes, such as glutathione reductase, did not yield an increase in lifespan, suggesting that distinct ROS signaling pathways influence the aging process (139). These mutations had the greatest effect on adult motor neurons, indicating a site-specific benefit (140). In fact, oxidative stress has the greatest detrimental effect on cognition and neuronal functionality in mammalian models (96, 141).

Collectively, lifestyle modifications, such as diet and exercise, are the most straightforward method to attenuate the effects of aging and other disease processes. While widely studied and highly promising, dietary modifications are difficult to maintain and require sustained effort and discipline. Therefore, alternative strategies using pharmaceutical mimetics are under consideration. Many common medications, such as aspirin, metformin, rapamycin, and resveratrol, have demonstrated metabolic modulation (142–144). It was even reported that patients receiving recombinant human GH displayed evidence of epigenetic deaging on the basis of four different epigenetic clocks (145).

**Senolytics.** In 2011, Baker et al. (13) demonstrated that removal of senescent cells in a transgenic mouse model promoted longevity and a youthful phenotype. Since then, numerous biotech companies have sought pharmaceutical means to reduce expression of this phenotype, and research on senotherapeutics, or drugs targeting the cellular senescence pathway, is a highly active area.

Only a handful of pharmaceuticals are categorized specifically as senolytics. These include dasatinib, quercetin, navitoclax, A1331852, A1155463, and fisetin. These drugs have shown promising results in preclinical models to reduce senescent cells, resulting in delaying and preventing age- and senescence-related conditions (14). Recent trials in humans for idiopathic pulmonary fibrosis (NCT02874989) using a combination of dasatinib and quercetin showed inconclusive results about these compounds' senolytic properties but did find improved functional changes upon pulmonary function testing (146). Additional placebo-controlled, double-blinded, randomized clinical trials are needed to adequately assess the potential benefits of these pharmaceuticals in organ function and longevity.

The most advanced candidates in this field are repurposed pharmaceuticals with senolytic properties. Metformin, a widely prescribed type II diabetes medication, has been found to decrease rates of DNA damage, cellular senescence, and mitochondrial oxidation while increasing rates of autophagy of pathogenic cells through a wide array of possible mechanisms (147–149). Metformin is currently under investigation in FDA-approved clinical trials [the TAME (Targeting Aging with Metformin) trials] as a prevention strategy for age-related diseases. Unity Biotechnology gained attention through billionaire investments for its senolytic candidate drug UBX0101, which was hoped to reduce the joint stiffness and pain associated with osteoarthritis, although trial results failed to show significance. Other well-established therapeutics targeting the mTOR pathway, such as rapamycin or sirolimus, have demonstrated senolytic properties and are currently under investigation (12, 150).

Despite sparse data showing real-world efficacy of senolytics in humans, senolytics continue to be highly sought after among pharmaceutical companies and academic centers (150). Companies are searching for novel senolytic targets, leveraging advances in artificial intelligence, to prevent cellular senescence and promote regenerative growth. The majority of senolytics under investigation are small-molecule or peptide-based pharmaceuticals. However, recently (18) a cell-based approach using senolytic CAR T cells targeting urokinase-type plasminogen activator receptor, a cell surface protein induced during senescence, extended the survival of mice with lung adenocarcinoma that were treated with a senescence-inducing combination of drugs, and this approach restored tissue homeostasis in mice in which liver fibrosis was induced chemically or by diet.

## Rejuvenation Strategies

One approach to combat aging is through restoration of youthful properties, or rejuvenation. These strategies leverage our understanding of embryonic development and stem cell biology to remove markers of damage accumulated with age.

**Cellular reprogramming.** In 2006, it was demonstrated that fully differentiated cells derived from a patient could be induced into an embryonic state with pluripotent differentiative capacity and indefinite self-renewal (151). Through induction of four key transcription factors, OCT4, SOX2, KLF4, and C-MYC (OSKM factors), cells can be brought back to an embryonic state, with the potential to differentiate into any cell type or tissue among the three germ layers: ectoderm, mesoderm, and endoderm (152). These cells, iPSCs, have become a bedrock of the modern field of stem cell engineering, with promising applications in modeling and treating a wide array of human diseases, including age-related diseases (152, 153).

The principle behind this strategy is that, throughout the reprogramming process, cells return to a youthful state and, as a result, will confer youthful characteristics to the organism. The induction process decreases levels of the senescence markers $p16^{INK4A}$ and $p21^{CIP1}$ and resets the

epigenetic DNA methylation clock (154). This may be a preferable strategy over directly targeting methylation sites, given the sheer number of age-associated DNA methylation sites (~500,000) (155). This process appears to be mediated through a cytokine signaling cascade converging through NF-κB and mediated by IL-6 signaling (156). Patient-derived iPSCs exhibit reduced ROS, restored telomeric lengths, restored mitochondrial function, and reversal of other hallmarks of aging (97, 154, 157). Thus, cellular reprogramming holds promising applications in treatment of age-related diseases, although inducing this youthful phenotype is not without its risks. Chronic expression of OSKM genes has generated reports of teratomas (158) and reprogramming-derived cancers (159, 160). Cyclical expression of OSKM genes appears to have overcome this limitation, with no reports of cancer in studies utilizing this approach (160–162).

Transient expression of OSKM factors in a mouse model demonstrated youthful epigenetic methylation patterns in the pancreas, liver, spleen, and blood (163). Indeed, partial reprogramming of cells in a Hutchinson–Gilford progeria syndrome mouse model demonstrated delayed onset of aging that was characterized by increased median lifespan (from 18 to 24 weeks) and tissue-level reduction in age-related characteristics (161). Researchers also recently reported that activation of three of the main iPSC-deriving factors restored youthful DNA methylation expression patterns in mouse retinal ganglion cells and reversed vision loss (164). Furthermore, short-term expression of OSKM factors in a mouse model showed that expression of these factors during myocardial infarction reduced myocardial damage and improved cardiac function, supporting the use of these factors to facilitate cardiac regeneration following tissue insults (160).

These results have inspired companies to use partial reprogramming as a rejuvenation process in humans (165, 166). Many reports of this process have been demonstrated in vitro (167), but no reports of human clinical trials using cellular reprogramming have been reported. There are many challenges facing the clinical implementation of this strategy, calling into question its future applications. The models used to demonstrate its effect are genetically modified organisms with engineered promoters to ectopically express the OSKM factors, which cannot be implemented in humans. Transient expression may be possible through transfection of plasmids, but that approach incurs the further challenges of achieving effective transduction efficiency to generate a meaningful effect.

**Modulating the stem cell niche.** Reports of rejuvenation and regeneration have been documented in aged parabionts due to exposure to young serum, inducing histological organ-level changes across multiple tissue types, in all primordial germ layers (168). While the exact mechanisms mediating these effects vary between organs and are currently poorly defined, the findings from these studies have prompted the exploration of young blood components as a potential rejuvenation strategy (169).

Parabiosis studies suggest that young blood may contain rejuvenation-promoting factors that decline with age and that the dilution of old serum decreases the accumulation of detrimental factors. Mehdipour et al. (170) investigated this question by performing blood exchanges in mice with either isochronic pairings or albumin-rich saline. The authors suggest that a single exchange of plasma with albumin-rich saline is sufficient to induce enhanced properties of muscle regeneration, neurogenesis, and reduced liver adiposity and fibrosis. These findings suggest that the dilution of detrimental factors, rather than the beneficial effects gained from youth, may be the primary driving force behind heterochronic parabiosis. In contrast, Sinha et al. (104) and Katsimpardi et al. (171) found that GDF11 was elevated in young serum and that this factor contributed to muscle regeneration and olfactory neurogenesis. These seemingly contradictory results from parabiosis studies require additional investigation prior to implementation in humans.

**Table 3 Proposed standardized methods to effectively evaluate anti-aging strategies**

| Biological scale | Representative feature | Example assays | Evidence of efficacy |
|---|---|---|---|
| Genetic | Telomeric shortening | Quantitative PCR of telomere length (172) | ↑ Average length |
| Epigenetic | Altered methylation | Methylation clocks | ↓ Biological age |
| Organellar | Mitochondrial dysfunction | Reactive oxygen species staining | ↓ Reactive oxygen species expression |
| Cellular | Senescence | $p16^{INK4a}$ and $p53/p21^{WAF1/CIP}$ expression | ↓ Expression |
| Tissue | Fibrosis | Collagen quantification (173) | ↓ Extracellular matrix deposition |
| Organ | Organ dependent, e.g., decreased glomerular filtration rate in kidneys | Organ dependent | Attenuation of aging-related decline |
| System/function | System dependent, e.g., liver function testing, pulmonary function testing | Blood tests, plethysmography, etc. | System specific |

## CONCLUSIONS

The field of anti-aging therapeutics is highly active. However, it is currently characterized by an emerging pattern of initial excitement and popularity followed by a loss of interest due to underwhelming results. For the field to advance toward meaningful and impactful work, there must be some reformation. Specifically, there must be standardization of methodologies and baseline analyses for all therapeutic candidates to help screen for interventions that hold the greatest potential to combat aging.

An area in need of major reformation is investigation across biological scales. Despite novel features of aging at each scale of biology, many studies limit their analysis to a single scale and assume propagation of the effect. Furthermore, the data collected must truly reflect an effect on the feature of aging at that scale. Assays should measure the features directly and not measure surrogate markers, which can be the result of alternative hypotheses. In **Table 3**, we outline examples of different assays that could be utilized at each scale to demonstrate therapeutic efficacy. Most critically, efficacy must be demonstrated at a functional level for both healthspan and lifespan to fully contextualize the therapeutic effect, with experiments ideally conducted in mammalian models for the greatest clinical relevance.

In conclusion, several promising strategies are emerging to combat the aging phenotype. Yet there is still a critical need for method standardization to efficiently evaluate potential candidates. Specifically, there is a need to increase reports of a drug candidate's effect 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 differing scales of biology, and most critically (*e*) the robustness and longevity of the therapeutic intervention. With this comprehensive and rigorous evaluation framework, high-impact therapeutics may be identified to promote longevity and extend healthspan.

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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