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Translational Geroscience: From invertebrate models to companion animal and human interventions

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Abstract

Translational geroscience is an interdisciplinary field descended from basic gerontology that seeks to identify, validate, and clinically apply interventions to maximize healthy, disease-free lifespan. In this review, we describe a research pipeline for the identification and validation of lifespan extending interventions. Beginning in invertebrate model systems, interventions are discovered and then characterized using other invertebrate model systems (evolutionary translation), models of genetic diversity, and disease models. Vertebrate model systems, particularly mice, can then be utilized to validate interventions in mammalian systems. Collaborative, multi-site efforts, like the Interventions Testing Program (ITP), provide a key resource to assess intervention robustness in genetically diverse mice. Mouse disease models provide a tool to understand the broader utility of longevity interventions. Beyond mouse models, we advocate for studies in companion pets. The Dog Aging Project is an exciting example of translating research in dogs, both to develop a model system and to extend their healthy lifespan as a goal in itself. Finally, we discuss proposed and ongoing intervention studies in humans, unmet needs for validating interventions in humans, and speculate on how differences in survival among human populations may influence intervention efficacy.

Keywords

Translational geroscience; healthspan; longevity; aging; rapamycin; metformin; mTOR; companion animals

Introduction

What is Translational geroscience?

The hope of dramatically extending our lifespan has captivated humanity for millennia. Over the last two decades, the biology of aging has matured as a field of study and led to greater engagement and investment in aging as a biological problem that can be understood at the molecular level [1–3]. In addition to the functional decline and loss of vigor associated with age, a generalized increase in disease susceptibility is now regarded as a consequence of biological aging [4, 5]. Numerous chronic diseases manifest during aging. In fact, of the ten leading causes of mortality in high income countries (as of 2016, the last year with available

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data), eight have advanced age as their greatest predisposing factor [6]. Developing interventions that target the molecular mechanisms of aging (or “hallmarks of aging”, as they’re commonly referred) should not only add vigorous years to our lives, but also reduce overall human disease burden. Translational geroscience is an emerging, interdisciplinary field descended from basic gerontology that seeks to identify, validate, and clinically apply interventions to maximize healthy, disease-free lifespan [7–9].

Why drug aging?

In some ways, the secrets of healthy aging are not enigmatic. Proper diet with care to include necessary micronutrients, exercise, adequate sleep, and effective management of stress are all well-known and intuitive ways to add to our healthy years. An important question to address in light of this is why we should focus on developing interventions if lifestyle management alone is sufficient to extend healthy lifespan? One answer is that many people do not have the resources (either in terms of money, time, or both) to proactively invest in maintaining their health. For some, making sure there is enough to eat is a priority over eating healthy. Instead of exercise after a long day of work, many instead prioritize family time and relaxation. This situation is common, even in economically-thriving countries. Those people, whose limited resources keep them focused on day-to-day survival, are as equally deserving of living long, disease-free, lives as those with resources to invest in their long-term health. All of this is not to say that lifestyle choices should not be pursued, or even prioritized, as healthy aging strategies, only that pharmacological interventions that increase healthy lifespan are an option to address an important inequity that exists in human health.

Another reason to consider pharmacological intervention to maintain our health as we age comes from early successes in preclinical geroscience to identify compounds that can impressively extend lifespan in model systems. Several compounds are now known to extend lifespan across broad evolutionary distances [10–14]. For example, rapamycin, among the most promising current interventions, can extend lifespan in yeast, worms, flies, and multiple mouse models [15–20]. While the magnitude of effect varies between organisms and genetic backgrounds, experiments in genetically heterogeneous mice show average lifespan extension between 10–25% [19]. Applied to human populations, a 15% increase in average life expectancy at birth in the US would change from 78.8 (as of 2015, the last year with available data) to 90.6 years [21]. This would be a dramatic improvement in human survival. In addition to improving lifespan in wild type model systems, rapamycin also promotes extended lifespan in multiple mouse disease models [22, 23], including heart disease [24] and cancer models [25–28]. This bolsters the hypothesis that there are broad clinical applications for rapamycin and other mTOR inhibitors. In a first of its kind test, short-term rapamycin administration improved measures of cardiac function in pet dogs [29]. While no lifespan data are available for humans, the short-term treatment with mTOR inhibitors appear to broadly improve immune function in the elderly [30, 31].

In addition to rapamycin, multiple other compounds show promise as healthspan and lifespan extending interventions. Metformin, typically used to treat type II diabetes, extends lifespan in worms and mice [32, 33]. In humans, numerous meta analyses suggest an

association between metformin use and lowered cancer incidence [34–42]. Further tests in non-diabetic individuals, like the proposed Targeting Aging with Metformin (TAME) study, will better establish metformin's potential to broadly reduce cancer and other age-related disease incidence in humans [10]. Nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN) are NAD(+) precursors that improve several features of aging, including muscle and cognitive function and vascular aging, and treatment with NR beginning at midlife is reported to increase lifespan in mice [43–45]. Senolytics, compounds that specifically target senescent cells for destruction, are important therapies that could increase lifespan and reduce age-related disease burden, particularly cancer and diseases driven by chronic inflammation [46–48]. Other compounds commonly used by humans, including caffeine, aspirin, and ibuprofen have successfully extended lifespan in model systems [12, 14, 49–51]. This raises the question: what other commonly consumed and FDA-regulated compounds alter physiology in such a way as to promote longevity?

How do we identify and validate lifespan promoting compounds?

What other molecules extend healthspan and lifespan? What combinations of molecules can be designed such that they target multiple pathways associated with aging and increased disease risk? Most importantly, how does individual genetic variation influence success of these interventions? We are only beginning to explore this "intervention space" and understand what is possible with regards to healthspan and lifespan intervention. In addition to broader options with regard to mTOR inhibition, compounds that target other pathways known to regulate aging, or stated more precisely, other nodes in the "aging network", are critical to develop and validate. Breakthroughs in interventions that extend healthy longevity can lead the way to a precision medicine like approach to maximize individual health by utilizing combinatorial treatment strategies coupled with individualized dosing based on genotype. In what follows, we describe a translational geroscientific workflow to identify and validate lifespan-extending interventions. We envision this as a translational pyramid with identification of compounds in invertebrate systems, like yeast, forming the base of our translational research pipeline and leading to experiments in other invertebrate systems, in models of genetic diversity, into wild type and genetically diverse vertebrate systems, then finally, in companion pets and humans (Figure 1).

Invertebrate systems

For basic biology, single-celled yeast and invertebrate systems (referred to hereafter simply as "invertebrates") boast unrivaled benefits. These organisms are inexpensive to culture in large populations and have a variety of phenotypes and disease models that can be analyzed using multiple techniques. There are also well-developed genetic tools, models of genetic diversity, and cost-effective genome sequencing methods that are broadly utilized. Taken together, invertebrate systems are optimal for discovery-driven research. For biology of aging in particular, most invertebrates are short-lived, which allows experiments to be conducted in a reasonable time frame. Using multiple invertebrate models to identify and validate interventions provides assessment of evolutionary translatability that is important to consider when developing mammalian interventions [52]. Beyond these general

characteristics, each of the three major invertebrate genetic model systems (yeast, worms, and flies) have unique strengths and weaknesses.

1 Wild type (WT) lab models

The term wild type (WT) originally described the collective traits of organisms isolated from the wild, namely flies [53]. Today, the term is more commonly used to denote the genetic background of laboratory organisms used in a given experimental system. There is growing appreciation that these organisms really no longer represent the ancestral state but are instead adapted to conditions in the lab [54, 55]. Laboratory conditions often alter developmental and reproductive life history traits and not always the same way depending on the model system [54, 56, 57]. These adaptations may be particularly important in studies of longevity, where factors like environmental nutrient status and reproductive timing strongly influence aging. It remains an open and important question whether genetic and environmental interventions that increase longevity in lab-evolved backgrounds have similar effects in genotypes that are not domesticated to the lab (discussed further below).

Within each model organism the WT strain used in one study may differ substantially from the WT strain used in a different study, even among studies from the same laboratory. Such strain background differences have the potential to confound interpretation if not carefully considered and addressed by the researchers performing the studies. For example, understanding the relationship between Sir2 and caloric restriction (CR) in budding yeast was complicated by the fact that Sir2 overexpression was shown to increase lifespan in one yeast WT strain (W303–1A)[58], while CR extended lifespan in a second yeast WT strain (PSY316) [59, 60]. Although a model was initially proposed that Sir2 and CR act within the same genetic pathway, it was not appreciated for several years that neither intervention extended lifespan in the other strain background [61, 62]. In a third WT strain background (BY4742), however, both interventions significantly extend lifespan allowing for more formal analysis of their genetic relationship [62].

Historically, there have been numerous yeast and fly genetic backgrounds commonly used across different labs. In recent years, the yeast aging field has mostly adopted the strain background used for the yeast ORF deletion collection [63, 64]: BY4743 for diploids and the corresponding haploid derivatives BY4741 (MAT**a**) and BY4742 (MATα). In *C. elegans*, the vast majority of research is performed in a single genetic background, Bristol N2.

Yeast

The budding yeast *Saccharomyces cerevisiae* is an important workhorse in geroscience [65]. Some of the most well-established longevity regulating pathways were first identified in yeast, including sirtuins and mTOR signaling [15, 58, 66, 67]. Two major aging paradigms exist in yeast: chronological and replicative [68]. In the chronological model, populations of yeast are grown to quiescence in liquid culture and changes in viability are assessed over time by outgrowth challenge in rich media [69–71]. At the cellular level, yeast chronological aging can be thought of as a model of post-mitotic cell aging [72]. These cells, like neurons in humans, are non-dividing and must maintain cellular function while bearing whatever burden is imposed upon them by the environment and their continued cellular metabolism.

This is particularly important during yeast chronological aging, where media acidification resulting from carbon metabolism is a strong driver of lost cellular viability [73].

Yeast replicative aging, instead of monitoring population viability over time, measures the replicative potential of individual yeast cells [74, 75]. To do this, individual “mother” cells are arrayed on plates using a compound microscope equipped with an optical fiber dissection needle [76]. Cycles of incubation, microdissection of daughter cells, and quantification are then performed until all mother cells cease dividing. This model of aging is akin to stem cell aging, where numbers of divisions that cells undergo before replicative senescence importantly influences tissue-level health and function. Newer methods of quantifying replicative lifespan and age-related changes in cell biology using microfluidics hold promise to increase throughput by decreasing assay time (from three weeks using microdissection to three days using microfluidics) and allow for longitudinal studies of cellular aging to be performed at the single cell level [77, 78].

Worms

The nematode *Caenorhabditis elegans* is one of the simplest animal model systems with tissue-level differentiation, which includes a nervous system, muscle, intestine, and a pharynx whose neuromuscular function resembles that of a mammalian heart. The first breakthrough in molecular genetics of aging that established the role of insulin-like signaling as a key longevity pathway was made using worms, underscoring the importance of this model system [79–82]. As opposed to yeast, measuring worm lifespan is conceptually more straightforward [83]. Synchronized populations of animals are plated on media containing a bacterial lawn (typically *E.coli*) as a food source and monitored until all animals cease moving [84]. The lifespan of a typical wild type animal is around three weeks. Lifespan in worms, however, is highly sensitive to changes in environment, including temperature and food source [85, 86]. As with yeast replicative lifespan, new technologies are utilizing platforms like microfluidics and so-called “lifespan machines”, incubation and culturing systems combined with image capturing to facilitate automated lifespan analysis [87, 88]. Utilizing these standardized technologies to perform lifespan analysis reduces technical hurdles to performing assays while increasing output, allowing for higher-throughput survival analysis.

Flies

The fruit fly *Drosophila melanogaster* is the most physiologically complex of the three major invertebrate aging models [89]. Along with greater complexity comes more opportunities to study tissue level aging, sex-specific lifespan differences, and more complex health measures like age-related behavioral changes and impaired learning [90–93]. This allowed for some of the first targeted studies on the evolution of lifespan characteristics where researchers evolved short- or long-lived fly populations by controlling reproductive age of parents [94, 95]. Early validation, and better understanding, of the role that insulin signaling plays in regulating aging was performed using flies [96–98]. Other processes likely to play a role in mammalian health and aging, like sleep and circadian rhythms, are also effectively studied using fruit flies [99]. An interesting difference from other invertebrate models is that fruit flies do not have a dominant wild type background in which

the majority of research is performed. Multiple genetic backgrounds are utilized, including Oregon R, Canton S, w^{118}, and yw. Importantly, these backgrounds have different survival characteristics [100, 101]. The typical fruit fly lifespan is 2–3 months but, just as with nematodes, this is highly dependent on culture conditions [102, 103]. Again, like nematodes, cohorts of age-matched animals are separately cultured [104, 105]. Fruit flies are typically reared in vials containing a yeast-based food source and transferred to new vials periodically.

Once a lifespan extending intervention is identified using a WT invertebrate system, a next step is to validate its translatability, identify natural genetic variants that influence intervention efficacy, and evaluate how the intervention impacts age-associated and other disease models. There are three common ways these goals are achieved using other invertebrate models and genetic collections.

2 Evolutionary translation between invertebrate model systems

Utilizing short-lived invertebrate models is ideal for rapidly identifying lifespan-extending molecules and extracts. Once an intervention is identified, how do we proceed? We can divide lifespan extending interventions by whether they impact private or public mechanisms of aging [106]. Private mechanism interventions target cellular processes that uniquely contribute to aging in certain model systems. For instance, yeast accumulate a type of genome instability with replicative age marked by proliferation of repeating ribosomal DNA (rDNA) in the form of extrachromosomal circular rDNA [107]. Inhibiting rDNA instability and accumulation of extrachromosomal rDNA circles leads to extended lifespan in yeast [108]. While the role of rDNA instability in some disease states is becoming better appreciated [109], rDNA circle accumulation is likely a private mechanism of yeast aging that is not shared, at least by mammals.

Interventions that target public mechanisms impact cellular mechanisms that contribute to aging in many different organisms. These shared mechanisms comprise the “Hallmarks of Aging” and include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication [3]. Interventions that target fundamental cellular processes that fail as organisms age, or those that elicit evolutionarily conserved cellular stress responses are likely to be public. These interventions have the greatest potential to extend lifespan in multiple organisms, including mammals. One way to validate whether an intervention impacts public or private mechanisms of aging is to test for conserved longevity and/or healthspan-related measures in multiple model systems. Utilizing invertebrate systems to validate interventions harnesses the research power of each system while also testing whether the intervention effect is conserved across hundreds of millions of years of evolutionary divergence (Figure 2) [110, 111]. This strategy is effectively used to validate newly identified and commonly used compounds [12, 14, 33]. Evolutionary conservation of this sort provides an important foundation for choosing interventions to move forward in translational geroscience and compliments traditional genetic and mechanistic approaches.

3 Genetically-diverse collections

Besides testing interventions across invertebrate model organisms, other diverse invertebrate collections and genetic libraries are useful for evaluating lifespan extending interventions. Some of these collections and libraries represent engineered genetic variation, while others capture natural genetic diversity. There are genetically diverse panels and libraries for all three major invertebrate systems that can be utilized to develop a more sophisticated understanding of lifespan and healthspan interventions.

Yeast

The most utilized collection of engineered genetic mutants is the yeast single gene deletion collection [63, 112]. This library of nearly 6000 mutants is constructed by systematic replacement of every non-essential yeast gene with a selectable marker. Using this collection, comprehensive genome wide analyses of genes that modulate both replicative and chronological lifespan have been performed [15, 113–115]. These studies have revealed numerous pathways that regulate cellular aging. In a screen of deletion mutants, the particular benefits of CR in suppressing diminished lifespan in mitochondrial mutant was identified [116]. Insights here led to using the mTOR inhibitor rapamycin as a novel treatment for mitochondrial disease in mice (discussed in detail below). For intervention studies, this collection is particularly valuable as a means of performing epistasis analysis that allows a better understanding of which cellular pathways the intervention interacts. Testing interventions in deletion mutants is also a useful means of identifying new interventions that target known longevity pathways [117].

For natural genetic diversity, the *Saccharomyces* Genome Resequencing Project (SGRP) collects a global assortment of two different *Saccharomyces* species (*S. cerevisiae* and *S. paradoxus*) representing wild and domesticated yeast from multiple ecotypes [118]. Initial characterization of strains from this collection reveal substantial natural variation in replicative lifespan [57]. Given the large evolutionary distance between yeast isolates [119], this collection represents another resource for evolutionary translation approaches. Perhaps more interesting for translational geroscience, this collection captures a substantial amount of natural genetic variation that can be probed in the context of intervention evaluation. Across these strains, there are over 235,000 SNPs [118]. Understanding drug efficacy across a range of genotypes provides insights into safety of the drug when given to genetically diverse populations and how well individual genotypes respond. Such an understanding will be critical for precision medicine approaches to translational geroscience.

Worms

Worms, like yeast, boast a large genetic library that allows for whole genome analysis at the single gene level. In contrast to yeast, however, this library is not composed of genetic mutants, but is instead made up of *E. coli* housing unique vectors, each of which targets a worm gene for post-transcriptional knockdown using feeding-based RNA interference (RNAi) [120–124]. Several large-scale screens have identified lifespan extending mutants using RNAi in WT and other mutant backgrounds [125–129]. The important roles of the mitochondria and mRNA translation in determining longevity were conclusively established

in these early screens. While there are challenges when comparing RNAi results across labs, primarily due to methodological and RNAi induction variability, these screens are a particularly useful means of generating hypotheses that can be independently validated.

A new program to investigate lifespan extending interventions in nematodes utilizes natural genetic diversity not just within *C. elegans*, but also in closely related species. The *Caenorhabditis* Interventions Testing Program (CITP) is a multi-site effort meant to validate longevity enhancing treatments using worms [130, 131]. This project is modeled after another NIA-supported Interventions Testing Program (ITP) which evaluates well-studied interventions for increased lifespan and healthspan in mice (see below). In addition to their efforts to develop standardized protocols for worm lifespan studies, the CITP is notable for its use of multiple independent strains of genetically diverse *Caenorhabditis* species [131–133]. In addition to eight different *C. elegans* isolates, the CITP utilizes eight isolates of *C. briggsae* and six isolates of *C. tropicalis*. This is particularly remarkable given historical difficulties culturing *Caenorhabditis* species from the wild [134, 135]. Whole genome sequences of the CITP strains will be an important resource for investigating intervention efficacy among this set of animals.

Flies

Cost-effective genome sequencing methods have allowed expansion of genetic model systems from a few wild type lab organisms to large panels of organisms representing genetically diverse natural populations. An example of this is the *Drosophila* Genetic Reference Panel (DGRP) [136]. The DGRP is a collection of ~200 inbred *Drosophila melanogaster* lines descended from a population of flies isolated at a farmer's market in Raleigh NC, USA. Even though the parental lines were all isolated from the same location, the DGRP represents a wealth of genetic diversity [137]. Amongst these 205 lines, over 4.8 million SNPs and 1.2 million larger polymorphisms are captured. Disease phenotypes are screened in the DGRP to identify naturally segregating genetic variants that modify human disease states [138, 139]. The population structure of the DGRP makes this collection well-suited for genome-wide association studies (GWAS) [140]. While there is substantial variation in survival amongst the DGRP, a GWAS for longevity failed to statistically validate variants associated with longevity within this collection [141]. This likely rules out alleles of large effect segregating in this population. Epistatic interactions between multiple alleles, however, may account for a larger amount of variation in aging than currently appreciated [142]. These researchers suggested expanding the number of lines within the collection to increase power to resolve longevity associated effects of segregating SNPs. Another GWAS using 800 lines from a different genetically diverse population, the *Drosophila* Synthetic Population Resource (DSPR), identified multiple quantitative trait loci (QTLs) that explain up to 22% of lifespan variation [143–145]. Capturing broader genetic variation in sexually isolated populations may reveal novel longevity associated alleles of greater effect [146, 147]. Studies addressing the efficacy of lifespan extending compounds on survival and health have not been completed with the DGRP, but this collection holds promise as a model to investigate how natural genetic variation influences intervention outcomes.

4 Age associated disease models

The promise of targeting aging is not only that we can extend the quantity of our lifespan, but that we can enhance the quality. This is not possible without decreasing the burden of age-associated disease. The core hypothesis of translational geroscience is that there is a fundamental connection between aging and age-associated disease such that, delaying aging will consequently delay the onset of disease. In particular, a major emphasis of translational geroscience is compression of morbidity, meaning that the functional decline and chronic diseases associated with age are pushed back as far as possible toward the end of life [148]. A key test of this hypothesis is showing that lifespan extending interventions broadly reduce age-associated disease. There are numerous invertebrate models that attempt to recapitulate aspects of human age-associated diseases. Testing compounds of interest in these disease models is useful for understanding the full utility of lifespan interventions. Interestingly, in some cases, lifespan extending compounds have proven to be effective in disease models not obviously linked to normative aging, such as the ability of rapamycin to suppress severe mitochondrial disease in mice. This lends support for additional studies that screen lifespan extending compounds widely across disease models.

Yeast

Yeast provide a window into fundamental features of normal and disease cell biology that translate to humans in many cases [149]. In terms of age-related diseases, many models of protein aggregation diseases have been developed using yeast [150–152]. These have helped elucidate basic mechanisms of the disease state. Yeast are very well-suited for measuring cellular phenotypes associated with a given disease, like mitochondrial dysfunction or protein aggregation [153]. Fundamental drivers of cancer, like genome instability and mutagenesis, are effectively modeled using mutator yeast that are defective for mismatch repair and polymerase proofreading [154–158]. With few complex behaviors that can be tracked with disease progression, however, yeast is limited beyond understanding cell-intrinsic aspects of basic molecular and cellular biology of disease.

Worms

Multicellularity and tissue differentiation in *C. elegans* provide a more relevant context to study the effects of lifespan extending interventions on age-associated disease. Like yeast, worms are also a popular model system to study protein aggregation diseases [159, 160]. With a simple neuromuscular system, age-related paralysis and other behavioral changes provide healthspan-related phenotypes that can be measured in the context of these disease models [161]. Without a circulatory system, worms are less amenable to studying vascular aging. However, the pharynx, an appendage in the head that grinds bacteria as they are ingested, has been described as similar to the heart in its pumping activity and regulation [162]. Age-associated changes in pharyngeal pumping are another commonly used functional measure of organismal health [163, 164]. Mitochondrial disease and mtDNA genome instability are modeled using Pol gamma proofreading or polymerase dead mutator worms, multiple mutants defective for components of the electron transport chain, and mutants for other critical mitochondrial functions [165–167]. Worms also provide useful models of conditions involved in dysregulated lipid metabolism, like obesity and related

metabolic disorders [168, 169]. While they do not possess a kidney, worms have even proven useful in understanding kidney disease, particularly as its pathogenesis relates to defective ciliary function [170, 171].

Flies

Combining genetic disease models with pharmacological interventions is well established in flies [172]. Flies are well-suited for studying similar neurodegenerative and protein aggregation diseases as those of yeast and worms [173–175]. Fundamental connections between mitochondrial fission and fusion cycles and Parkinson's disease onset were identified in flies [176, 177]. Additionally, thanks to their more advanced nervous system and complex behavior, flies allow other neurological and psychiatric diseases to be studied [178, 179]. Mitochondrial disease models that impact the electron transport chain (ETC) and mitochondrial energetics are also well characterized [180]. Interestingly, rapamycin improves survival in a fly model of Leigh syndrome, a disease driven by defects in ETC function [17]. Flies also provide useful models of cardiac aging, cancer, and even as a model for immunoaging [181–183].

Vertebrates

Validating interventions in vertebrate systems

Invertebrate systems are a discovery engine for longevity interventions. The goal, however, is to translate these interventions into vertebrate systems. Short-lived vertebrate systems are essential for validating interventions in a timely manner, but few options are available. An emerging model for aging research is the African turquoise killifish (*Nothobranchius furzeri*) [184]. With a median lifespan between 4–6 months, the African turquoise killifish is the shortest-lived vertebrate that can currently be bred in the lab [185, 186]. In addition to survival, many age-associated phenotypes, including enhanced cognitive decline, can be studied using the killifish [187]. While few longitudinal studies have been reported, resveratrol and low temperature both extend lifespan in this model [188, 189]. Interestingly, transfer of gut microbiome from young to old fish extends lifespan, showing the importance of gut flora in aging [190]. With more genome editing and analysis resources now available [191, 192], we can hope to see the killifish develop as a vertebrate discovery and validation engine for lifespan interventions.

While non-mammalian fish models and rats are occasionally used, mouse models are the major vertebrate workhorse in translational geroscience [193, 194]. Wild type mouse models are genetically well-established and age-associated pathologies are deeply understood. Ever increasing disease models provide complimentary approaches to test interventions. The NIA-sponsored Interventions Testing Program (ITP) is a multi-site effort utilizing genetically heterogeneous mice to validate longevity interventions. These resources establish mice as the major mammalian system for intervention validation.

5 WT vertebrate models

If all roads in the ancient world led to Rome, then the mouse, *Mus musculus*, is truly the Rome of the translational geroscientific research pipeline. Mice are ubiquitous in aging

research and translational research in general. All of the most promising longevity interventions are being actively researched using mouse models [195–198]. Mice are among the shortest-lived mammals and have a typical lifespan in the lab of 2–3 years [199]. There are multiple common genetic backgrounds that are used for general research [200]. Mice are anatomically and physiologically very well characterized. This allows for detailed pathological analyses to be conducted, even though reporting of this data is sometimes sparse [201, 202]. Efforts to standardize pathological grading of aged tissues will allow detailed assessment of organismal aging that can be applied to testing intervention efficacy [203]. Assessing cause of death in mice is difficult given care and maintenance requirements, but the most common pathologies that contribute to mortality are cancer, kidney disease, and inflammation [204]. Animal care and maintenance is rigorous [205, 206]. This is necessary to maintain healthy and humane conditions and it provides a measure of standardization across research sites that is underappreciated. Compared to humans, it is estimated that 9 days is approximately equivalent to a human year for a mouse [200]. This is interesting to consider in the context of drug studies when thinking about dosing windows and comparing to humans.

As described above for invertebrate models, it is important to carefully consider genetic background when designing and interpreting geroscience studies in mice. Several different WT mouse strains are utilized for such studies, with the most common being inbred C57BL/6J and genetically heterogeneous UMHET3. A comparison of longevity across 31 different mouse strains at The Jackson Laboratory found substantial differences in morbidity and mortality [207, 208]. Also, as mentioned above, multiple generations of breeding under laboratory conditions is likely to have selected for genotypes that develop rapidly and produce large litter sizes among inbred mouse strains.

6 Genetically-diverse collections

The relative complexity of mice slows the development of genetic tools compared to invertebrate systems. Efforts are underway to systematically construct the knockout mouse collection, a genome wide set of null mutants for every non-essential mouse gene [209]. This will unlock similar genetic tools for intervention epistasis studies currently used in yeast. Many recombinant lines exist that are produced typically by mating lab strains and establishing diverse lines from progeny. The ILSXISS are a set of recombinant inbred lines generated by mating mutants isolated within the lab [210]. These lines provided key insights into the importance of genetic background in modifying the effect of CR on lifespan [211]. The largest effort to create diverse genetic models using mice is the Collaborative Cross, a genetic reference panel of recombinant inbred mice descended from an eight-way cross and mating scheme utilizing five lab mouse models and three mouse models representing natural genetic variation [212, 213]. By mating lines from the Collaborative Cross, researchers are now constructing the Diversity Outbred (DO) mouse populations [214, 215]. This set of lines is different than the other genetically diverse organism collections discussed because these lines are kept as heterozygotes instead of being inbred to homozygosity [216]. This makes the DO population the closest model to human genetic variation available to biomedical researchers. This will be an important population for testing intervention robustness in genetically diverse mammals.

A key resource for translational geroscience is the NIH-sponsored Interventions Testing Program (ITP). This is an NIA-sponsored multi-site effort bringing together labs from University of Michigan, The Jackson Laboratory, and University of Texas-San Antonio to test proposed pro-longevity interventions [217, 218]. The ITP uses a heterogeneous genetic mouse model constructed from crosses of multiple unique mouse strains (in this case, a four-way cross between common lab strains) [219–221]. Interventions are selected for ITP testing based on proposal and then evaluated by committee. To date, 42 compounds have been tested, nine of these at different doses, in combination with other compounds, or tested in different aged mice (Table 1) [19, 20, 51, 199, 222–225]. Of these, two (rapamycin and acarbose) extend lifespan in both sexes and another four (aspirin, 17αEstradiol, nordihydroguaiaretic acid, and protandim®) extend only in males [226].

7 Disease models

A particular strength of mouse research is the variety of disease models that can be utilized. Multiple reviews are devoted to documenting and comparing the wealth of models for different pathologies available, including age-associated diseases [227–229]. Of particular interest in biology of aging research are mouse progeria models, like the *Zmpste*$^{-/-}$ and *Lmna*$^{L530P/L530P}$ mice (Hutchinson Guilford Syndrome and laminopathy models), *Ercc1*$^{-/-}$ mice (Xeroderma pigmentosum model), and *Bubr1*$^{-/-}$ mice [230–235]. Mouse models of elevated oxidative stress, like the Cu/Zn superoxide dismutase knockout mouse, are an emerging progeroid model that shows accelerated frailty and molecular damage, especially in the liver, lungs, and kidneys [236, 237].

One notable example for the use of lifespan extending interventions beyond impacting age-related survival comes from research into mitochondrial disease that began using yeast. In a screen for yeast deletion mutants that differentially respond to low glucose CR, multiple mitochondrial mutants responded best to the intervention, with some seeing their lifespans more than double [116]. This led to the hypothesis that CR, or a CR mimetic, may have clinical application in the context of mitochondrial disease. To test this, researchers utilized the *Ndufs4* knockout mouse model that recapitulates many aspects of Leigh syndrome, a human childhood mitochondrial disease [238]. Due to the frail nature of the *Ndufs4* knockout mice and the early onset of disease, rapamycin was administered beginning at postnatal day 10 instead of CR. Impressively, rapamycin treatment nearly doubled median lifespan in this disease model [23]. This groundbreaking study opened up new interest in utilizing mTOR inhibitors as treatments for mitochondrial disease [22, 239]. It is exciting to consider that this study began in yeast and ended with a successful mammalian disease intervention in little more than a few years. Interestingly, more recent work has shown that hypoxia increases lifespan of *Ndufs4* knockout mice even more robustly than rapamycin [240, 241]. Like rapamycin, hypoxia increases lifespan in *C. elegans* [242, 243] and may explain longevity among people living at high altitude [244]. These observations suggest that this mouse model of severe mitochondrial disease can be used as a short-lived discovery platform for interventions likely to extend lifespan in WT mice and perhaps people.

Reaching the goal

Applying interventions in companion animals and humans

Using the translational geroscience pipeline, we can go from identifying interventions in diverse invertebrate model systems to validating interventions using mice. From here, where do we go? In studies of CR, the first well-studied longevity extending intervention, substantial efforts were made to validate findings in a non-human primate, the rhesus monkey [245]. Parallel studies lasting more than 30 years each at the University of Wisconsin-Madison and NIA ultimately converged on CR having positive effects on primate health and lifespan, despite differences in study design and early controversies about CR having inconsistent effects between sites [245–247]. Given the exceptionally long lifespan and correspondingly high costs of rhesus monkeys, however, it seems unlikely that additional longevity studies will be performed in these animals. More recently, efforts have been made to establish the common marmoset (*Callithrix jacchus*) as a shorter-lived non-human primate model in geroscience [248–250]. This small primate has an average lifespan in the lab of 4–6 years and a maximum lifespan upwards of 16 years [251]. Efforts are underway now to establish whether rapamycin extends lifespan in this primate [252, 253]

While primate studies are the intuitive next step for testing putative geroscience interventions from an evolutionary point of view (Figure 2), they lack an important component for translational validation: the human environment. In this context an exciting approach is to utilize companion animals for understanding aging biology and intervention testing.

8 Companion animals

Companion animals as a non-laboratory model system for translational geroscience represent both a unique scientific model and potentially a realization of the goals of geroscience itself. Companion animals share a number of similarities with humans that are not recapitulated in lab organisms, including their environment and microbiota [254]. These non-genetic factors are critically important to model and understand in translational geroscience as it is estimated they account for approximately 80% of human lifespan variation [255–259]. In terms of pathology, companion animals share many age-associated diseases with humans, and we have a sophisticated understanding of disease progression for many species through veterinary records [260–262]. It is even suggested that studies in companion animals be built into the FDA drug approval process to span the “valley of death” between basic biomedical research and successful human disease treatment [260, 263]. Beyond their utility as it relates to developing interventions for ourselves, delaying disease and extending lifespan in companion animals is an important end in itself. Considering the important role of pets in our lives and our efforts to care for them to our fullest abilities, it is clear that we generally regard these animals with the same deference as we do other people. Investing in the healthy life of companion animals, then, is an important way that we fulfill an obligation to them that is similar, if not identical, to the obligation we have in biomedical research to help each other.

The Dog Aging Project is a research program designed to develop companion dogs as a model system for aging and test the efficacy of rapamycin as a healthy longevity intervention in pets [9]. This national study is enrolling dogs for longitudinal and intervention studies. In addition to survival, an important measure of intervention success in the rapamycin trials will be delayed disease onset. Veterinary records provide a good resource to establish breed specific age associated disease risk and life expectancy [261, 262, 264–266]. This allows a comparison of types of morbidities and comorbidities and when they occur between rapamycin treated and historical populations. Middle aged mid and large breed dogs (≥ 40 pounds) were selected for the first rapamycin experiments. Pilot studies with these dogs identified higher rates of asymptomatic heart valve degeneration than previously understood and provided evidence that short-term rapamycin treatment may improve age-associated cardiac decline [29, 267].

9 Humans

The last translational step to humans is the most difficult. The ideal intervention, if started between 40–55 years old, would take decades to be clearly assessed in terms of delayed morbidity and mortality. This adds to the already substantial challenges associated with longitudinal research in humans [268]. Despite its difficulty, clinical research that firmly establishes intervention efficacy in humans is critical to obtain. Proposed and ongoing human studies with metformin and rapamycin analogs (rapalogs) use more proximal measures of health and resilience in older individuals to assess efficacy. A further challenge is that no validated biomarker of biological age currently exists that can be used to validate and measure intervention success in human populations.

Initial clinical studies

The two best studied pharmacological longevity interventions to date are metformin and rapamycin. Metformin is a biguanide that was initially synthesized in the early 1920's for its potential antidiabetic properties. Interest in biguanides as a diabetes medication originates from early use of French lilac extract as a diabetes treatment [269]. This extract is rich in guanidine, an otherwise toxic compound with long-known antidiabetic properties [270]. Metformin is FDA-approved for type-II diabetes treatment [271, 272]. There is increasing evidence that metformin also broadly reduces cancer risk [42, 273–278]. Epidemiological studies and meta-analysis also suggest that diabetics taking metformin have greater survival than age-matched non-diabetic controls [279, 280]. In the near future, Targeting Aging with Metformin (TAME), a study led by researchers at Albert Einstein College of Medicine, will test metformin as a healthy aging intervention in the elderly [10]. TAME intends to recruit thousands of non-diabetic individuals 65–79 years of age across the US for a randomized, placebo-controlled trial with metformin. Survival, as well as morbidity and comorbidities, will be monitored during and after treatment.

Rapamycin was originally identified as an antifungal compound secreted by soil *Streptomyces* species on Easter Island (Rapa Nui) [281]. These effects were known long before the mechanistic target of rapamycin (mTOR) was identified as a kinase complex that acts as a signaling hub in nutrient sensing [282–285]. In the clinic, rapamycin is used in high

doses as an immunosuppressive, particularly during organ transplant [286, 287]. Like metformin, rapamycin and related analogs (rapalogs) are being explored for their anticancer potential [288, 289]. Current efforts to establish the benefits of rapamycin during human aging are focused on delaying age-associated immunosenescence [30]. Over 200 elderly (≥65 years old) individuals participated in an initial study that tested whether administration of the rapalog everolimus improved immune response to an annual flu vaccination. Those treated with everolimus showed greater antibody response to flu vaccine than placebo treated controls with only minor side effects (mouth ulceration or headache). In another study by this group, combined treatment of everolimus with a catalytic mTOR inhibitor (BEZ235) also improved immune function and largely avoided these side effects [31]. Interestingly, a short-term (6 weeks) treatment with BEZ235 alone resulted in reduced annual rate of infection in the elderly compared to control over at least one year. This suggests that immune changes associated with mTOR inhibition persist long after treatment is stopped.

Unmet needs

A standing challenge for human studies of healthspan intervention is measuring successful intervention. Unlike a traditional clinical trial, geroscience interventions are not aimed at treating or curing a specific disease after diagnosis. Instead, they should keep people healthy and alive longer, more akin to a preventative therapy. Due to the long lifespans of humans, this means that the length of time needed to assess efficacy is much longer than a typical clinical trial. As seen in the rapalog tests in elderly patients more proximal measures, like immune function, can be used to assess intervention success. While this can be an effective path to FDA approval, it does not really demonstrate that an intervention is impacting the biological aging process.

Perhaps the greatest unmet need for translational geroscience are clinically validated biomarkers of aging that can be measured to assess changes in biological age (which is in contrast to chronological age, typically measured as the time since birth). The American Federation for Aging Research propose that ideal biomarkers would be non-invasive, accurately measure cellular health as it relates to age-related physiological decline, and be translatable to other systems in such a way that basic research can be performed utilizing the marker [290]. Identifying such biomarkers has proven difficult; however, the advent of relatively inexpensive -omics technologies combined with powerful computational approaches such as machine learning have given new hope to finding predictive biomarkers [291]. Recent efforts have focused on developing panels of multiple biomarkers that can be assayed together to meet these proposed guidelines [292, 293]. Overall, however, there remains no consensus on what marker(s) reliably predict age at this time.

An emerging biomarker of aging is the epigenetic clock. The epigenetic clock is composed of a set of methylated genomic CpG residues that, taken together, are highly predictive of biological age [294]. DNA methylation (DNAm) patterns are predictive of age in multiple tissues and respond to healthy lifestyle and diet [295–298]. Epigenetic clocks have been characterized for mice (under normal and caloric restricted conditions) and domesticated dogs [299, 300]. Understanding methylation age, particularly in mice, provides new means of validating age-associated changes of other interventions that can be related to humans.

The epigenetic clock is accelerated in progeroid conditions like Werner syndrome [301]. Interestingly, the epigenetic clock is found to be accelerated in conditions not typically thought of as accelerating aging, such as HIV infection and Down syndrome [302, 303]. Lifestyle factors, like stress, are also being recognized as driving accelerated epigenetic aging [304–306].

What to expect

Increased disease-free lifespan is without doubt a great benefit to individuals, but what are the broader societal impacts of extending our healthy lifespan? One answer to this is found in the concept of the longevity dividend [307, 308]. The longevity dividend refers to economic and other societal benefits that result from wide improvements in healthy aging [309]. Healthcare expenditure, for instance, will be fundamentally changed by breakthroughs in extended healthspan. In 2016, healthcare in the US cost $3.3 trillion dollars and 37% of that was paid through Medicare or Medicaid [310]. A great deal of these costs (37.9% as of 2013) are generated by treating chronic disease in elderly individuals, like heart disease, hypertension, periodontal disease, and diabetes [311–313]. Breakthroughs in healthspan interventions that delay or prevent these conditions will likely save considerable money for individuals as well as local, state, and federal governments. Due to the similarities in healthcare between humans and companion animals [314], a key test of the longevity dividend concept as it relates to healthcare economics may be provided by successful geroscience intervention in pet dogs.

Beyond the question of whether interventions will extend human healthspan and lifespan, a related question that is less frequently asked is whether interventions will work the same for everyone. As seen in mice, many interventions preferentially extend lifespan in males [20, 51, 226]. In invertebrate CR studies using genetically diverse models, shorter lived strains tend to reap the largest benefits from the intervention [116, 315]. Additionally, in the studies that revealed genotype-dependent responses to CR using the ILSXISS mice lines, the best responders to CR were also among the shorter lived [316]. An explanation proposed for this is that, the closer a population is to its maximum lifespan, the harder it will be to improve upon its lifespan [317]. While this is certainly true, it is unclear if organisms studied in the lab are close to their physiological maximum lifespan. In yeast, for instance, even the longest lived-strain tested (*sgf73Δ* median RLS = 42, WT median RLS = 25) showed a small increase in lifespan under CR [116]. It seems, instead, that interventions like CR are inherently equitable, meaning that those who get the largest benefit from the intervention are those that need it the most.

Another important longevity dividend produced by successful healthy aging intervention may be decreased health disparities between different human populations. In the US, human survival is stratified based on sex, race, and ethnicity (Figure 3) [318]. As of 2014, there is a 12-year difference in median survival between the longest-lived group, Hispanic women (median lifespan = 88) and the shortest lived, black men (median lifespan = 76). Black men and women, along with white men, are the three shortest lived groups. We can model idealized human survival by assuming that the maximum human lifespan is close to what we've already witnessed [319], ~125 years, and that age-associated survival dynamics will

be similar to the longest-lived subgroup (Hispanic males and females) (Figure 3a and 3b). To achieve this ideal, an intervention (or combinations of interventions) would need to extend median lifespan by ~30% in the longest-lived populations and by closer to 40% in the shortest-lived populations (black males and females). In terms of age-associated morbidities, comparing black and white populations (data for other groups is less reliable), the biggest difference in causes of death between these groups is due to diabetes [320]. If healthspan interventions are similarly equitable in human populations as they are in model systems, we may see the largest benefits in health among those with the shortest lifespans, particularly black populations. Decreased health disparities that result from healthspan intervention can be thought of as another important longevity dividend.

Conclusions

Identifying and utilizing lifespan and healthspan extending interventions holds particular promise in extending lifespan and reducing human and companion animal disease burden. Common invertebrate systems provide models for discovery of lifespan extending compounds. Using diverse invertebrate systems, as well as models of genetic diversity and disease models, treatment efficacy can be evaluated in genetically diverse and evolutionarily distant organisms. Vertebrate model systems, particularly mice, provide a mammalian system to validate interventions and understand efficacy as it relates to genetic diversity and disease models. Translating successful interventions to companion pets, particularly dogs, provides a large, genetically diverse mammalian model to better evaluate interventions while potentially extending healthy lifespan of these animals, which is desirable and a biomedical breakthrough in itself. Finally, human healthspan interventions, while promising for all, may yield particular benefits for those already predisposed to shorter lifespan.

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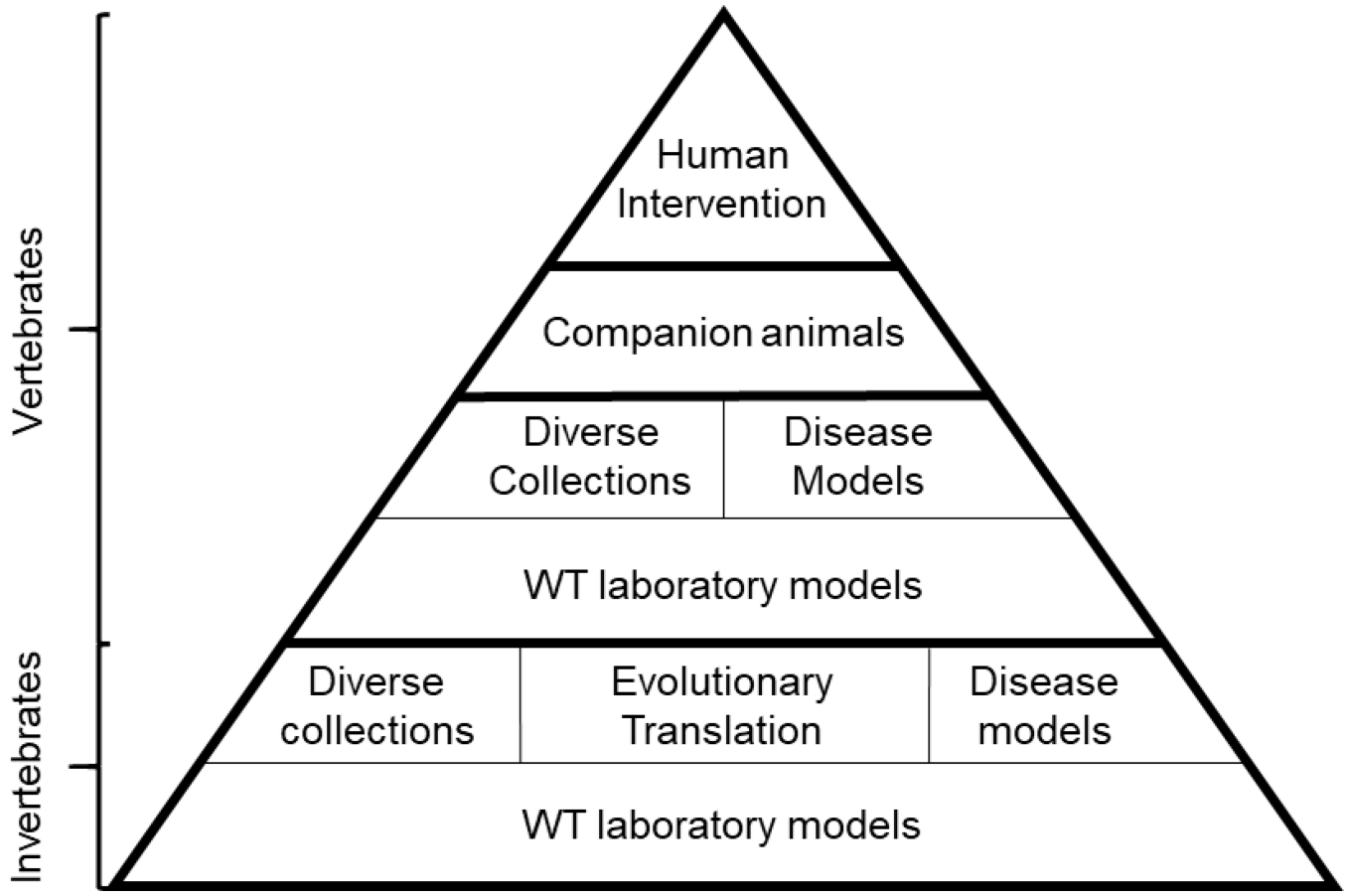

Figure 1. A translational pyramid for longevity intervention from invertebrates to companion animals and humans.

Interventions are first screened using common laboratory models (either invertebrate or vertebrate) in wild type (WT) laboratory genetic backgrounds. Successful interventions can then be studied for their efficacy among genetically-diverse collections. Disease models and other short-lived backgrounds can also be utilized to identify particular translational opportunities for interventions. Studies in genetically-diverse companion animals can validate interventions that are then tested in humans.

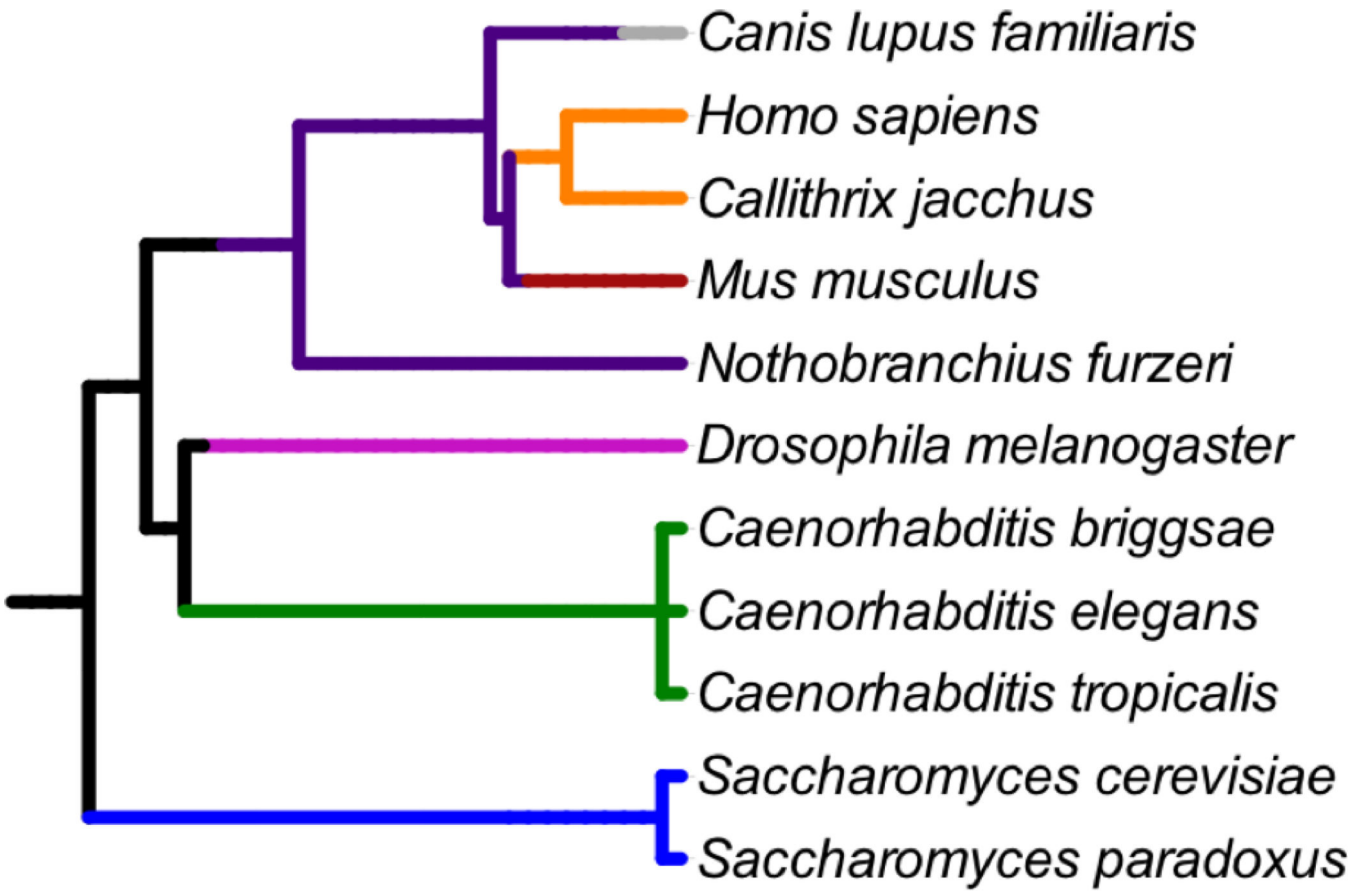

Figure 2. Phylogenetic relationships of key organisms used in the translational geroscience research pyramid.

Fungi = blue, nematodes = green, Arthropods = magenta, vertebrates = dark purple, rodents = dark red, primates = orange, and canines = gray. Phylogenetic tree contructed using NCBI phyloT and Interactive Tree of Life (iTOL) v3 [111].

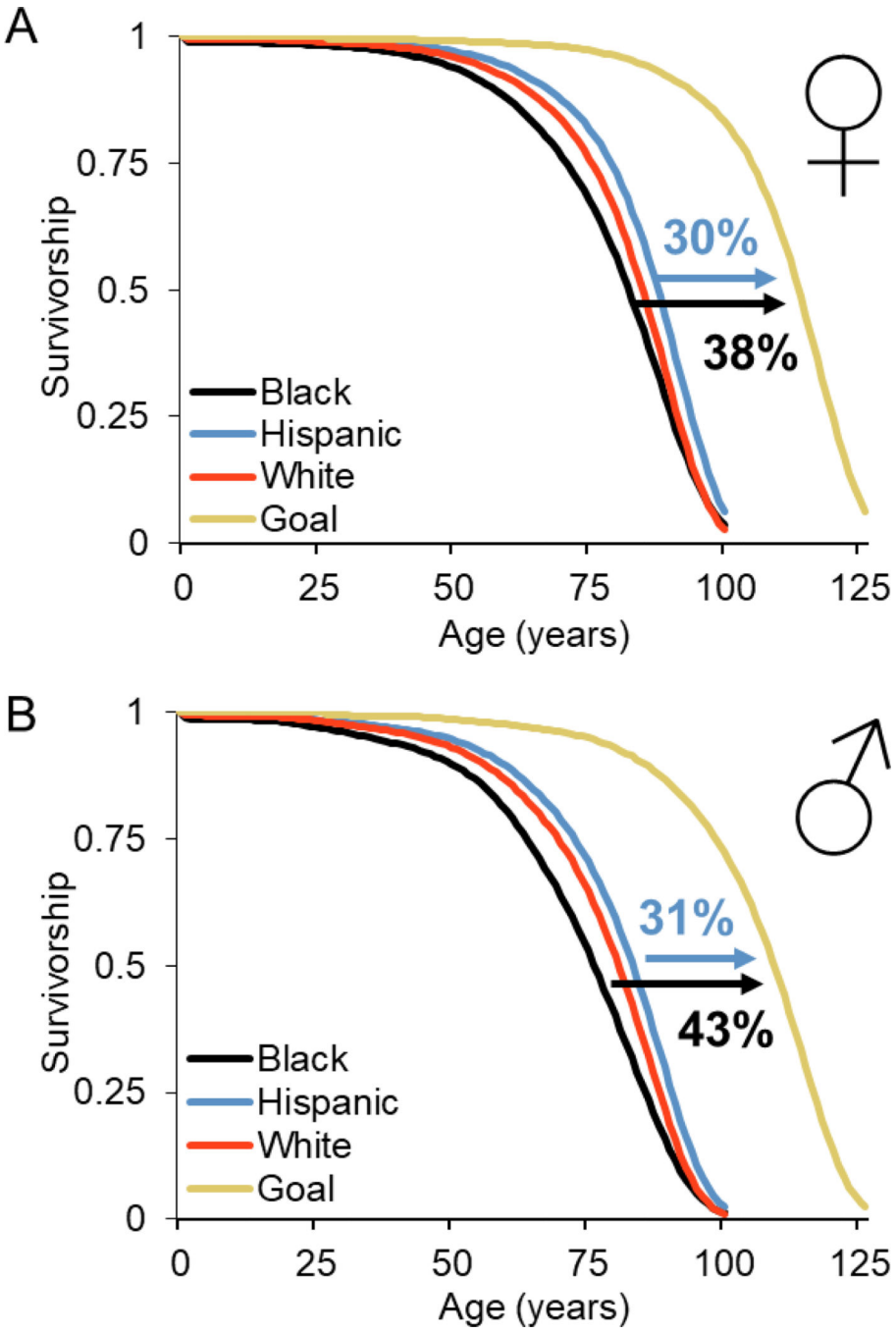

Figure 3. Human survival curves separated by sex, race, and Hispanic origin.

A) females B) males. Gold curves represent idealized survival based on maximum human lifespan. Data from US life tables, 2014 [318].

Table 1.

Summary of lifespan data from the NIA Interventions Testing Progam by Cohort.

Interventions and concentrations tested, age treament started, median lifespan with 95% confidence intervals (CIs), number of animals enrolled (n), and percent change (compared to cohort controls) separated by sex, year enrolled (Cohort), and reference for compounds screened and in progress by the Interventions Testing Program, 2004–2018.

			Median lifespan and 95% CIs (days)							
Intervention	**Concentration**	**Age started**	**Females**	**n**	**% change**	**Males**	**n**	**% change**	**Cohort**	**Reference**
Control (combined)	NA	NA	886 (875–894)	2269	NA	803 (792–812)	2599	NA	Compiled	NA
Control	NA	NA	892 (860–916)	288	NA	790 (765–819)	379	NA	2004	Strong et al., 2008
4-OH-PBN	315 ppm	4 months	850 (827–905)	144	−4.7	813 (773–866)	180	2.9	2004	Strong et al., 2008
Aspirin	20 ppm	4 months	851 (835–878)	144	−4.6	852 (825–889)	180	7.8*	2004	Strong et al., 2008
Nordihydroguaiaretic acid (NDGA)	2,500 ppm	9 months	895 (862–923)	149	0.3	883 (847–911)	180	11.8*	2004	Strong et al., 2008
Nitrofluribprofen (NFP)	200 ppm	4 months	884 (845–915)	144	−0.9	815 (774–855)	181	3.2	2004	Strong et al., 2008
Control	NA	NA	887 (862–905)	296	NA	797 (769–835)	378	NA	2005	Harrrison et al., 2009
caffeic acid phenethyl ester (CAPE)	30 ppm	4 months	884 (855–920)	144	−0.3	817 (774–875)	180	2.5	2005	Harrrison et al., 2009
caffeic acid phenethyl ester (CAPE)	300 ppm	4 months	926 (886–965)	148	4.4	815 (748–852)	183	2.3	2005	Harrrison et al., 2009
Enalapril Maleate	120 ppm	4 months	868 (839–934)	144	−2.1	849 (807–875)	180	6.5	2005	Harrrison et al., 2009
Rapamycin	14 ppm	20 months	1005 (988–1058)	144	13.3*	952 (902–1004)	177	19.4*	2005	Harrrison et al., 2009
Control	NA	NA	891 (873–911)	272	NA	834 (800–869)	342	NA	2006	Miller et al., 2011
Rapamycin	14 ppm	9 months	1052 (1001–1112)	144	18.1*	902 (854–966)	171	8.2*	2006	Miller et al., 2011
Resveratrol	300 ppm	12 months	906 (870–939)	144	1.7	830 (794–879)	165	−0.5	2006	Miller et al., 2011
Resveratrol	1200 ppm	12 months	905 (867–944)	144	1.6	884 (845–908)	168	6.0	2006	Miller et al., 2011
Simvastatin	12 ppm	10 months	916 (883–952)	143	2.8	858 (809–888)	167	2.9	2006	Miller et al., 2011
Simvastatin	120 ppm	10 months	898 (869–933)	144	0.8	793 (753–853)	168	−4.9	2006	Miller et al., 2011
Control	NA	NA	866 (835–892)	281	NA	786 (749–827)	294	NA	2007	Strong et al., 2013
Curcumin	2000 ppm	4 months	905 (845–936)	132	4.5	808 (758–840)	158	2.8	2007	Strong et al., 2013
Green tea extract	2000 ppm	4 months	923 (898–944)	130	6.6	822 (778–881)	153	4.6	2007	Strong et al., 2013
Medium Chain Triglyceride Oil	60000 ppm	4 months	882 (852–931)	136	1.8	777 (734–853)	153	−1.1	2007	Strong et al., 2013
Oxaloacetic acid	2200 ppm	4 months	892 (855–940)	134	3.0	819 (760–858)	153	4.2	2007	Strong et al., 2013

Intervention	Concentration	Age started	Median lifespan and 95% CIs (days)						Cohort	Reference
			Females	n	% change	Males	n	% change		
Resveratrol	300 ppm	4 months	907 (877–939)	131	4.7	813 (767–869)	153	3.4	2007	Strong et al., 2013
Control	NA	NA	891 (869–915)	280	NA	807 (782–841)	300	NA	2009	Harrison et al., 2014
17α-Estradiol	4.8 ppm	10 months	902 (867–930)	136	1.2	900 (852–935)	156	11.5*	2009	Harrison et al., 2014
Acarbose	1000 ppm	4 months	939 (883–985)	136	5.4*	984 (952–1027)	156	21.9*	2009	Harrison et al., 2014
Methylene Blue	28 ppm	4 months	903 (866–945)	136	1.3	790 (738–848)	156	−2.1	2009	Harrison et al., 2014
Rapamycin	42 ppm	9 months	1131 (1115–1174)	136	26.9*	992 (911–1037)	156	22.9*	2009	Miller et al., 2014
Rapamycin	4.7 ppm	9 months	1037 (977–1079)	136	16.4*	834 (767–897)	156	3.3	2009	Miller et al., 2014
Rapamycin	14 ppm	9 months	1084 (1031–1149)	136	21.7*	909 (820–974)	156	12.6*	2009	Miller et al., 2014
Control	NA	NA	902 (873–940)	280	NA	784 (739–819)	300	NA	2010	Strong et al., 2016
Fish Oil	15000 ppm	9 months	863 (834–920)	136	−4.3	812 (771–876)	156	3.6	2010	Strong et al., 2016
Fish Oil	50000 ppm	9 months	923 (883–947)	136	2.3	734 (696–808)	156	−6.4	2010	Strong et al., 2016
Nordihydroguaiaretic acid (NDGA)	5000 ppm	6 months	880 (849–934)	136	−2.4	852 (818–892)	156	8.7*	2010	Strong et al., 2016
Nordihydroguaiaretic acid (NDGA)	800 ppm	6 months	NA	NA	NA	855 (820–931)	156	9.1*	2010	Strong et al., 2016
Nordihydroguaiaretic acid (NDGA)	2500 ppm	6 months	NA	NA	NA	875 (828–917)	156	11.6*	2010	Strong et al., 2016
Control	NA	NA	874 (862–896)	288	NA	785 (749–810)	306	NA	2011	Strong et al., 2016
17α-Estradiol	14.4 ppm	10 months	893 (858–931)	136	2.2	931 (867–966)	156	18.6*	2011	Strong et al., 2016
Bile Acids (ursodeoxycholic acid)	5000 ppm	5 months	884 (848–913)	136	1.1	839 (775–875)	156	6.9	2011	Strong et al., 2016
Metformin	1000 ppm	9 months	875 (839–922)	144	0.1	847 (800–887)	162	7.9	2011	Strong et al., 2016
Protandim®	600 ppm	10 months	887 (868–930)	136	1.5	853 (791–891)	156	8.7*	2011	Strong et al., 2016
Rapamycin + Metformin	14 ppm + 1000 ppm	9 months	1079 (1037–1123)	144	23.5*	955 (906–999)	162	21.7*	2011	Strong et al., 2016
Control	NA	NA	877 (852–914)	284	NA	821 (789–849)	300	NA	2012	Strong et al., 2016
Acarbose	1000 ppm	16 months	903 (873–950)	136	3.0	880 (814–916)	156	7.2*	2012	Strong et al., 2016
2-(2-hydroxyphenyl)-benzoxazole	1 ppm	15 months	856 (832–900)	136	−2.4	822 (790–853)	156	0.1	2012	NA
INT-767 FXR/TG5R agonist	180 ppm	10 months	868 (840–895)	136	−1.0	793 (762–836)	156	−3.4	2012	NA
Control	NA	NA	NA	NA	NA	NA	NA	NA	2013	In progress
Acarbose	2500 ppm	8 months	NA	NA	NA	NA	NA	NA	2013	In progress
Acarbose	1000 ppm	8 months	NA	NA	NA	NA	NA	NA	2013	In progress

Intervention	Concentration	Age started	Median lifespan and 95% CIs (days)						Cohort	Reference
			Females	n	% change	Males	n	% change		
Acarbose	400 ppm	8 months	NA	NA	NA	NA	NA	NA	2013	In progress
Ursolic Acid	2000 ppm	10 months	NA	NA	NA	NA	NA	NA	2013	In progress
Control	NA	NA	NA	NA	NA	NA	NA	NA	2014	In progress
Aspirin	60 ppm	11 months	NA	NA	NA	NA	NA	NA	2014	In progress
Aspirin	200 ppm	11 months	NA	NA	NA	NA	NA	NA	2014	In progress
Inulin	600 ppm	11 months	NA	NA	NA	NA	NA	NA	2014	In progress
Glycine	80,000 ppm	9 months	NA	NA	NA	NA	NA	NA	2014	In progress
TM5441	60 ppm	11 months	NA	NA	NA	NA	NA	NA	2014	In progress
Control	NA	NA	NA	NA	NA	NA	NA	NA	2015	In progress
17-DMAG	30 ppm	6 months	NA	NA	NA	NA	NA	NA	2015	In progress
Minocycline	300 ppm	6 months	NA	NA	NA	NA	NA	NA	2015	In progress
MitoQ	100 ppm	7 months	NA	NA	NA	NA	NA	NA	2015	In progress
Rapamycin – intermittent	42 ppm	20 months	NA	NA	NA	NA	NA	NA	2015	In progress
β-guanadinopropionic acid	3300 ppm	6 months	NA	NA	NA	NA	NA	NA	2015	In progress
Control	NA	NA	NA	NA	NA	NA	NA	NA	2016	In progress
17α-Estradiol	14.4 ppm	16 + 20 months	NA	NA	NA	NA	NA	NA	2016	In progress
Canagliflozin	180 ppm	7 months	NA	NA	NA	NA	NA	NA	2016	In progress
Candesartan Cilexetil	30 ppm	8 months	NA	NA	NA	NA	NA	NA	2016	In progress
Geranylgeranyl acetone	600 ppm	9 months	NA	NA	NA	NA	NA	NA	2016	In progress
MIF098	240 ppm	8 months	NA	NA	NA	NA	NA	NA	2016	In progress
Nicotinamide riboside	1000 ppm	8 months	NA	NA	NA	NA	NA	NA	2016	In progress
Control	NA	NA	NA	NA	NA	NA	NA	NA	2017	In progress
1,3-butanediol	100,000 ppm	6 months	NA	NA	NA	NA	NA	NA	2017	In progress
Captopril	180 ppm	5 months	NA	NA	NA	NA	NA	NA	2017	In progress
L-Leucine	40,000 ppm	5 months	NA	NA	NA	NA	NA	NA	2017	In progress
PB125	100 ppm	5 months	NA	NA	NA	NA	NA	NA	2017	In progress
Rapamycin + Acarbose	14.7 ppm + 1000 ppm	9 + 16 months	NA	NA	NA	NA	NA	NA	2017	In progress
Sulindac	5 ppm	5 months	NA	NA	NA	NA	NA	NA	2017	In progress
Syringaresinol	300 ppm	5 months	NA	NA	NA	NA	NA	NA	2017	In progress

* = significantly long-lived compared to cohort matched control.