Strategies and Challenges in Clinical Trials Targeting Human Aging

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Abstract

Interventions that target fundamental aging processes have the potential to transform human health and health care. A variety of candidate drugs have emerged from basic and translational research that may target aging processes. Some of these drugs are already in clinical use for other purposes, such as metformin and rapamycin. However, designing clinical trials to test interventions that target the aging process poses a unique set of challenges. This paper summarizes the outcomes of an international meeting co-ordinated by the NIH-funded Geroscience Network to further the goal of developing a translational pipeline to move candidate compounds through clinical trials and ultimately into use. We review the evidence that some drugs already in clinical use may target fundamental aging processes. We discuss the design principles of clinical trials to test such interventions in humans, including study populations, interventions, and outcomes. As examples, we offer several scenarios for potential clinical trials centered on the concepts of health span (delayed multimorbidity and functional decline) and resilience (response to or recovery from an acute health stress). Finally, we describe how this discussion helped inform the design of the proposed Targeting Aging with Metformin study.

Keywords: Aging—Clinical trials—Metformin—Rapamycin—Acarbose

This paper is an outcome of the National Institutes of Health (NIH)-funded Geroscience Network, a consortium of 18 aging centers and academic groups across the United States, in partnership with groups in the European Union (Supplementary Table 1). Here, we summarize and expand upon discussions held during a R24 Geroscience retreat that followed a Zing Conference on the Biology of Human Aging in September, 2014 in Oropesa, Spain. This retreat brought together geriatric leaders who have carried out intervention studies in the elderly people, and gerontologists and basic scientists who are working on strategies to extend health span and life span in model systems (Supplementary Material).

At the preceding conference, each of the retreat participants gave a focused lecture on their experience related to the mission of the retreat, “To design studies to delay age-related dysfunction and diseases using drugs approved for human use.” The retreat participants brainstormed and prioritized ideas as a group. This retreat approached clinical trials broadly; a later retreat further developed ideas specifically around early-stage proof-of-concept clinical trials, which is presented separately in Justice et al. in this issue. Here, we summarize the ideas developed in Oropesa on the conduct of clinical trials of aging interventions, including support for further prioritization, and discuss an outline of strategies for translating biological findings into treatments to delay, prevent, alleviate, or treat age-related dysfunction and diseases.

Goals of Clinical Trials That Target Aging Processes

The promise of interventions that target the aging process is that they might have an outsized impact on major outcomes that matter deeply to patients—ability to maintain independence and quality of life—through broad effects on many different age-related diseases,
syndromes, and age-related declines in physiological function. Age is not only the leading risk factor for many common chronic diseases such as diabetes, cardiovascular disease (CVD), and Alzheimer’s disease but is also the major risk factor for geriatric syndromes such as falls, immobility, frailty, and incontinence (1). Loss of independence due to geriatric syndromes may not be due to a single disease process but to the accumulation of physiological dysfunctions in multiple systems from many underlying processes. However, they may ultimately have their genesis in a more limited number of fundamental aging mechanisms that predispose to multiple chronic diseases and affect the physiological function of many organ systems. The goal of interventions targeting aging processes is not to prolong life span per se, although this may be a desirable side effect, but rather to extend health span. We define health span as the number of years of life that is relatively free from disease and morbidity, during which the daily activities and independence of the individual are maintained. Data from preclinical animal studies and the limited human studies of the interventions described in the section titled Candidate Drugs That Affect the Aging Process indicate that they may be effective for slowing age-related decline in a variety of settings.

Determining the effect of such interventions on delaying or alleviating aging processes in humans will ultimately require one or more randomized, controlled clinical trials conducted over a sufficient time period in a large, heterogeneous older adult population testing “hard” outcomes such as burden of chronic diseases, functional dependence, and/or mortality. As we describe in this paper, trials of this sort would be a major effort. However, such a successful trial would be groundbreaking, with enormous implications not only for medical practice and policy but for society in general (2). The first step on a path to large clinical trials is a series of smaller clinical trials that can provide evidence for the concept that aging processes can be affected in humans, provide biological data for reverse translation studies of the effects of interventions targeting basic aging mechanisms in humans, suggest or validate intermediate outcomes such as biomarkers or surrogate clinical endpoints, and inform the design and scale of larger trials. Challenges encountered in randomized clinical trials are well described and include protocol adherence by study participants or study sites; dropouts leading to missing data (3); result interpretation of the “intention to treat” analysis, where everyone in the treatment group is analyzed together regardless of their adherence to the study intervention (4); participant diversity (gender, ethnicity, geography, and age) (5); and generalizability. In the following sections, we will describe candidate drugs that affect the aging process, offer demonstrative examples of potential studies, and discuss issues and challenges in the design of such studies that test interventions targeting fundamental aging mechanisms.

Candidate Drugs That Affect the Aging Process

In the decades that have passed since the first single genes were identified that regulate longevity in an organism (6,7), a variety of pathways have been discovered that, if manipulated, are associated with life-span and health-span extension. Alongside pathway discovery has come the identification of a number of candidate drugs that inhibit or activate proteins within these pathways to regulate their function. Some of this has been through traditional translation of basic science findings, but much discovery to date has been “reverse translation”: mapping existing drugs with relevance to aging processes onto specific mechanisms and pathways. Metformin is one prominent example, in use for decades without a clear understanding of its mechanism of action, but now thought to exert its broad effects on health span and aging at least in part as a calorie restriction mimetic through inhibition of mitochondrial complex 1 and activation of AMP-activated protein kinase (AMPK) (8).

A growing number of drugs have been rigorously demonstrated to extend life span in laboratory rodents though multicenter testing with genetically heterogeneous mice in the National Institute on Aging (NIA) Interventions Testing Program (ITP), including rapamycin (9,10), acarbose (11), aspirin (12), 17-β-estradiol (11), and nordihydroguaiaretic acid (11,12). Other drugs that have been shown to extend life span in non-ITP studies include metformin (13), two sirtuin activators (14,15), angiotensin converting enzyme inhibitors (16,17), and aldosterone receptor blockers (ARBs) (18). In some cases, these studies examined health-span outcomes as well, such as cognitive function, metabolic health, and motor performance. Other compounds, such as resveratrol, did not extend life span in nonobese rodents but appear nonetheless to have pleotropic effects on age-related diseases and physiological function.

Many novel agents are under development to target the cellular processes that are now understood to affect fundamental aging (19,20). These include new molecules related to rapamycin that may have better side effect profiles, including less gastrointestinal (GI) irritation and less glucose intolerance (21,22). Growth and differentiation factor-11–related agents (23,24), drugs that selectively clear senescent cells—senolytic agents (25–27), drugs that protect against the proinflammatory senescence-associated secretory phenotype (28,29), drugs that are related to mitochondrial function (30), agents that impact protein synthesis or autophagy (31,32), and caloric restriction mimetics (33) are among the types of compounds that are currently being developed or tested. A robust and standardized preclinical pipeline, discussed in Huffman et al. in this issue, will be essential for developing novel interventions that might target new Food and Drug Administration (FDA) indications related to aging, frailty, functional decline, or multimorbidity. Preclinical studies will be critical for testing various concepts of outcomes, establishing dosing and biomarkers within broad ranges, and studying initial safety.

A variety of other nondrug interventions have been shown to either delay age-related changes or extend life span in rodent models. Together with the hundreds of single-gene mutations that extend life span (34), these include (nonexhaustively) calorie, protein, and methionine restriction (35); visceral fat removal (36); elimination of senescent cells (27,37); exposure to young serum (38); and serum-derived proteins such as GDF-11 (23,24,39); GH and insulin-like growth factor-1 (IGF-1) receptor inhibitors (40); mitochondrial derived peptides (41); activators of AMPK (42); and many others. Some of these are currently the focus of drug-discovery efforts to recapitulate their beneficial effects.

In the sections that follow we will briefly review some of the more promising agents that have emerged from human and animal testing, emphasizing those already approved for use in humans (summarized in Table 1).

Metformin

Metformin, an FDA-approved first-line drug for the treatment of type 2 diabetes mellitus (T2DM), has been used successfully in patients for more than 60 years with an outstanding safety record. Data on both clinical and model organisms suggest that metformin has broad effects on aging (see the recent review (43) and Supplemental Material, for additional references). Metformin is associated with extended longevity in male C57BL/6 mice, and the ITP suggested a synergistic effect with rapamycin. Metformin also extends the life span of nematodes, suggesting that it targets an evolutionarily conserved mechanism. In T2DM, metformin decreases hepatic glucose production by several mechanisms, leading to lower circulating
glucose and insulin levels and improved insulin action. Metformin also decreases IGF-1 signaling, activates AMPK, and may inhibit the proinflammatory senescence-associated secretory phenotype. A recent study extends metformin actions to reduction of oxidative stress and inflammation, together with prolongation of both life span and health span in C57BL/6 males.

In humans, metformin can prevent the progression from impaired glucose tolerance to overt diabetes in overweight patients (44). But if indeed metformin targets human aging processes, its administration should be associated with less age-related disease in general, rather than simply the decreased incidence of a single age-related disease such as T2DM. Notably, in the United Kingdom Prospective Diabetes Study metformin, compared with other anti-diabetes drugs, also demonstrated a decreased risk of CVD—an effect also suggested by meta-analysis of other studies. In addition, numerous epidemiologic studies have shown an association between metformin use and decreased risk of cancer, as well as decreased cancer mortality. There is also evidence from studies performed both in vitro and in vivo of metformin’s role in attenuating tumorigenesis. In fact, metformin’s potential protective effect against cancer has been gaining much attention, with more than 100 ongoing studies registered on the clinicaltrials.gov website. Data on effects of metformin on Alzheimer’s disease are still emerging but controversial. In addition, a recent observational study demonstrated that metformin treatment among older diabetics was associated with improved overall survival that surpassed the survival of matched controls without diabetes. This evidence from human studies, combined with other clinical and basic science investigations, makes metformin a prime candidate for a clinical trial that aims to target aging processes.

Acarbose
Acarbose (ACA) has been approved and used around the world for the treatment of T2DM for more than 15 years. It has an outstanding safety record, although it commonly leads to minor GI side effects. ACA slows processing of starch into disaccharides by inhibiting α-glucosidases in the intestine, thereby reducing peaks in glucose absorption (45). ACA was tested in the ITP based on the hypothesis that spikes in post meal glucose could contribute to biological aging (46). ACA administration increased male mouse median life span by 22%, but increased female median life span by only 5% in the ITP (11). This sexual dimorphism could not be explained by effects of ACA on weight. ACA increased maximum life span by ~10% in both genders. ACA administration resulted in increased food intake, but decreased body weight and glucose, insulin, and IGF-1 levels (11, 47). In humans, the Stop-NIDDM trial (48) showed that ACA can prevent progression of impaired glucose tolerance to T2DM. ACA was also associated with a statistically significant 49% relative risk reduction in the development of CVD events, including myocardial infarction (49). ACA administration was associated with a 34% relative risk reduction in the incidence of new cases of hypertension and risk of “silent” myocardial infarction (50). It is important to note that glucose-lowering efforts using numerous drug interventions to try to prevent cardiovascular effects in T2DM have not been successful. This raises the possibility that ACA targets aging processes through other mechanisms, perhaps through effects on the intestinal microbiome, causing intestinal cells to release other peptides such as GLP1, or, possibly, systemic absorption of ACA leading to direct effects of ACA on cellular processes.

ACEi and ARBs
Angiotensin converting enzyme inhibitors (ACEi) and angiotensin II receptor antagonists (also known as angiotensin receptor blockers or ARBs) are widely used as antihypertensive agents and have a strong safety record. Interestingly, ACEi and (in some cases) ARBs have been shown to reduce mortality in heart failure and after myocardial infarction, and to be renoprotective in diabetes, all at least partially independent of the effect on blood pressure (51). Although ARBs were at first thought to increase cancer risk (52), later studies have found that they are actually associated with a lower risk of cancer (53) and reduced cancer mortality (54). Finally, treatment of hypertension with an ACEi or ARB may help prevent cognitive decline in people with hypertension or CVD (55–57), although this may reflect a general effect of antihypertensive treatment, and the relationship between antihypertensive treatment and cognition remains controversial. Many of these human data were presaged by rodent studies. ACEi increased life span in both healthy (16, 17) and hypertensive rodents (58, 59). Both ACEi and ARB prolong rat life span and improve cardiovascular function (18), as well as protect against age-related declines in kidney and cognitive function (60). One nonantihypertensive mechanism for these benefits may be reduced oxidative stress in the mitochondria through inhibition of the angiotensin system (61). Finally, genetic disruption of the angiotensin II receptor prolongs mouse life span (62), and variations in its gene are associated with exceptional human longevity (63).

Table 1. Clinical Data From Potential Study Drugs

| Drug                | FDA | Current Indication | Safety (adverse reactions) | Effect on Other Age-Related Conditions |
|---------------------|-----|--------------------|---------------------------|---------------------------------------|
| Metformin           | ✓   | T2DM               | +++ (diabetes and GI upset)| Reduced risk of CVD, cancer, and dementia|
| Acarbose            | ✓   | T2DM               | +++ (flatusulence and diarrhea) | Reduced risk of CVD and hypertension |
| Resveratrol/sirtuins | ✓   | None               | Limited data              | No major studies                      |
| Rapamycin/rapalogs  | ✓   | Transplant, cancers | ++ (hyperglycemia and oral ulcers) | Improved response to flu vaccine |
| ACEi/ARB            | ✓   | Cardiovascular     | ++ (hypotension, hyperkalemia, and renal injury) | Reduced risk of cancer, cognitive decline, and dementia |
| Aspirin/salicylic acid | ✓   | Many               | ++ (bleeding and GI ulcers) | Reduced risk of CVD and cancer |
| 17α-Estradiol       | ✓   | Alopecia (Europe)  | Limited data              | No major studies                      |

Note: ACEi = angiotensin converting enzyme inhibitors; ARB = aldosterone receptor blockers; CVD = cardiovascular disease; GI = gastrointestinal; T2DM = type 2 diabetes mellitus; +/+/(++/+++ Qualitative safety ranking for long-term use. *Available OTC. †Includes temsirolimus and everolimus; other rapamycin analogs are in development. FDA indications vary for specific drugs in this group.
Rapamycin and rapalogs

Rapamycin inhibits the eponymous mTOR kinase, which lies in two distinct protein complexes. mTORC1 in particular is an integrative center of cellular energy signaling, activated by glucose and amino acids, and strongly inhibited by rapamycin. Rapamycin is FDA approved as an immunosuppressant for solid organ transplantation, while derivative “rapalogs” are approved for treatment of certain cancers (64). Rapamycin extends life span in yeast, flies, and worms, as well as in C57BL/6, 129/Sv, and genetically heterogeneous mice, even when started late in life (see the recent reviews (65,66) for additional references). Rapamycin has a variety of effects in mice that might contribute to longevity, including modulating stem cell function and inflammation, promoting autophagy, slowing cognitive decline, and alleviating models of heart failure and neurodegeneration. It is not a panacea in rodents, however, with side effects including increased rates of kyphosis and cataracts. The clinical use of rapamycin in humans has permitted detailed examination of human adverse effects, including metabolic dysfunction, impaired wound healing, and aphthous ulcers. Despite these challenges, clinical trials are at various stages of development to test rapamycin or its analogs as an adjuvant to cardiac rehabilitation after cardiac surgery or angioplasty (CARE study, NCT01649960), for cognitive impairment in Alzheimer’s disease and for enhancing the immune response to vaccination in the elderly people (67).

17-α-Estradiol

17-α-Estradiol is a nonfeminizing endogenous estrogen that has lower affinity for estrogen receptors than 17-β-estradiol (68). It was demonstrated to have neuroprotective properties in animal models of Parkinson’s (69), Alzheimer’s (70), and cerebrovascular diseases (71). Its protection may be mediated, in part, via anti-oxidant effects. However, the anti-oxidant effects of 17-α-estradiol are likely not mediated through estrogen receptors (72). It has been shown to activate multiple signaling proteins within the mitogen-activated protein kinase pathway, including B-Raf and extracellular signal-regulated kinase (73). Like metformin, 17-ε-estradiol increases AMPK activity and, like rapamycin, it reduces mTOR activity (74). In mice, 17-α-estradiol alleviates age-related metabolic and adipose tissue dysfunction (74). Rodent studies using 17-ε-estradiol conducted through the NIA ITP demonstrated a significant extension in median life span in males by 12%, but not in females (11), albeit with a wide variation among the three ITP testing sites. 17-α-estradiol appears to have a good safety profile in humans, although the data are limited. No adverse events were documented in a Phase I clinical study conducted with orally administered 17-α-estradiol at various doses in a group of postmenopausal females of an average age of 60 years (75). It has been approved for topical use in Europe for treatment of alopecia (76) as well as an element of postmenopausal estrogen replacement (77).

Sirtuin agonists

Sirtuins are a class of NAD-dependent deacetylases related to the budding yeast gene Sir2, one of the first longevity genes identified in this workhorse model organism (see the review (78) and Supplementary Materials for additional references). Genetically increasing the activity of Sir2 or its homolog increases life span in yeast and, controversially, in fruit flies and round worms. There are seven mammalian Sir2 homologs. Overexpression of SIRT6 increases longevity in otherwise normal male mice and protects mice against the deleterious metabolic effects of high-fat feeding, possibly through repression of IGF signaling. The other mammalian sirtuins have not been shown to extend life span when overexpressed, but they do have beneficial functions in a variety of age-related physiological parameters and diseases. SIRT1, when overexpressed in mice, reduces cancer incidence and osteoporosis, while improving glucose tolerance and wound healing. The regulation of sirtuins is complex, but it is hoped that sirtuin-activating compounds might recapitulate some of these health benefits. Resveratrol, a plant phenol that activates sirtuins among other biochemical functions, extends life span in yeast and flies. Two newer sirtuin activators, SRT2104 and SRT1720, both extend health span and life span in mice. Resveratrol itself does not affect life span in normally fed, nonobese mice, but it does, along with the other sirtuin activators, improve health and life span in mice made obese by high-fat feeding. Resveratrol appears to have similarly beneficial metabolic effects in rhesus monkeys fed a high calorie diet and possibly obese humans, although there are conflicting data about whether sirtuin activators alleviate glucose intolerance in humans. The diversity of effects of sirtuin activators on age-related diseases and evidence of life-span extension in certain model organism studies—especially when obese or under metabolic stress—suggest that these drugs might eventually turn out to hold promise for targeting age-related processes.

Aspirin and salicylic acid

Aspirin is the most widely used medication in the world, popular as an analgesic and anti-inflammatory agent as well as an antiplatelet thrombosis inhibitor. It is an acetylating agent, with many of its clinical effects thought to be due to irreversible acetylation of a serine residue in the active site of cyclooxygenase enzymes. Interestingly, aspirin appears to reduce cancer risk in humans (79–81). When tested in the ITP, aspirin was found to increase the median, but not maximum life span of genetically heterogeneous mice (12). Additional mechanisms of action may explain these diverse effects. Salicylic acid is a metabolite of both aspirin and the related anti-inflammatory drug salicylate and has been shown to be an allosteric activator of AMPK (82). In mice, salicylic acid improves lipid utilization via AMPK, but also improves glucose homeostasis through an independent and currently unknown mechanism (82). In a small clinical trial, salicate improved glucose control in diabetics (83). Subsequently, aspirin was found to inhibit mTOR and activate AMPK in human colorectal cancer cells (84) and to extend life span in worms through activation of AMPK and Daf-16/FOXO3 (85). Together, these results from both human and model organism studies suggest that aspirin and related compounds may broadly regulate effects of aging and age-related diseases through several distinct mechanisms.

Designing Clinical Trials That Target Aging Processes

There are two broad strategies for an intermediate trial of an intervention targeting fundamental aging processes, aimed at the related concepts of health span and resilience (Figure 1). Health span might be gradually limited by slow physiological decline, together with the accumulation of multiple chronic diseases and geriatric syndromes that all together result in morbidity and functional dependence (Figure 1A). Alternatively, health span may be truncated by an acute event that causes sudden, severe morbidity and functional dependence with limited or no recovery (Figure 1B). The ability to rebound back to baseline functional and health status after an acute event is termed resilience. Older, frail individuals are less able to cope with major physiological stressors such as surgery or myocardial infarction. Acute illnesses have more severe presentations in the elderly people, recovery times are longer, risk of death is higher, and likelihood of recovering to the prior level of function and independence
The selection of the study population would determine whether the order to demonstrate its efficacy as a drug that targets aging processes. Systems, the therapeutic intervention must exhibit multisystem effects in the cardiovascular, renal, neurologic, musculoskeletal, and endocrine age-related health events of interest involve multiple systems, including delaying disease and disability is a relatively novel concept. Because the idea of a clinical trial that targets the aging process as a means of wealth of data available from a variety of clinical trials on the effect of interviews at follow-up study visits or through telephone interviews semiannually, or at other designated frequencies through structured interviews at follow-up study visits or through telephone interviews with the participants. Such a design is not without precedent; there is a wealth of data available from a variety of clinical trials on the effect of function dependence threshold (black dashed line), where the individual may remain after recovery from the stressor. The intervention may enhance the individual's ability to recover back to independence (blue solid line).

is much lower than when the same stress occurs in a young person (86). This lack of resilience is a major cause of morbidity, functional dependence, and death.

Principles of Study Design for a Clinical Trial of Health Span

Scenario 1: Extension of health span

Example: A longitudinal double-blind placebo-controlled trial of older adults at high risk for age-related diseases testing the effect of metformin on prevention of age-related diseases or significant functional limitation over a 5-year period.

Extension of human health span, the number of years of life that are spent free of functional limitations, morbidity, and chronic pain, is a key goal of new clinical interventions that target aging processes (Figure 1A). If aging is associated with a gradual accumulation of multiple chronic diseases (multimorbidity), geriatric syndromes, and functional decline that leads to limited health span, then a study targeting the aging process would test if an intervention can slow or prevent these adverse health events over a relatively long period of time. Such a study should be longitudinal, double blinded, and placebo controlled, with careful attention to the adjudication of events. Longitudinal design is essential to evaluate the effect of an intervention on the incidence of new events or conditions. In a longitudinal study, participants are followed prospectively and information is collected about the occurrence of new clinical events of interest. Event information might be collected yearly, semiannually, or at other designated frequencies through structured interviews at follow-up study visits or through telephone interviews with the participants. Such a design is not without precedent; there is a wealth of data available from a variety of clinical trials on the effect of drugs and lifestyle interventions on multiple health outcomes. However, the idea of a clinical trial that targets the aging process as a means of delaying disease and disability is a relatively novel concept. Because the age-related health events of interest involve multiple systems, including the cardiovascular, renal, neurologic, musculoskeletal, and endocrine systems, the therapeutic intervention must exhibit multisystem effects in order to demonstrate its efficacy as a drug that targets aging processes. The selection of the study population would determine whether the intervention might be thought of as primary or secondary prevention. At one extreme, a study of healthy, community-dwelling older adults might require a large study population for adequate power and a fairly benign intervention given the low likelihood of a poor outcome. At the other extreme, a study of frail, already multimorbid or partially dependent older adults might be more analogous to a secondary prevention trial where the likelihood of poor outcomes is greater. For a secondary prevention trial, a smaller study population can provide adequate power. Greater risk might be tolerated from the intervention in the hope of altering the poor natural course. The study proposed here should strike a balance, discussed in the sections that follow.

Figure 1. Schematic of study designs. (A) The natural course of health span is often a gradual decline in function (red dotted line) that results in disability or death when a certain threshold is crossed (black dashed line). Interventions that target aging processes and delay health span would delay the occurrence of disability or death by slowing this decline (blue solid line). (B) Health span may also be interrupted by an acute stressor, which may push the individual's function (red dotted line) below the disability/death threshold (black dashed line), where the individual may remain after recovery from the stressor. The intervention may enhance the individual's ability to recover back to independence (blue solid line).

Considerations for Trials That Target Either Health Span or Resilience

As outlined in this paper, many unique challenges accompany the design of clinical trials that target aging processes, some of which will be addressed in the following sections in the context of the two described scenarios.
Population selection
Inclusion criteria would ideally be as broad as possible within the context of the trial design. The practice of excluding very elderly people or patients with extensive comorbidities would be counterproductive, because these people are those who would be most likely to benefit from interventions targeting basic aging mechanisms and for whom the interventions may ultimately be used. In many circumstances, it may make sense to stratify by frailty, function, comorbidities, or biomarkers upon enrollment and preferentially target those patients at higher risk for the primary outcome to increase power and the likelihood of benefit.

A key consideration in selecting the study population is the degree of risk the population has for primary outcomes such as age-related morbidity and disability. This, in turn, affects the study size, study duration, and tolerable safety profile of the intervention. The extension of health-span study proposed earlier (Scenario 1) can illustrate these considerations. The study design would favor selection of older individuals (eg, age ≥ 65 years) who already manifest at least one age-related disease or condition, who are therefore at increased risk for age-related morbidity and disability. Study participants could manifest conditions that are considered risk factors for disease or disability, such as metabolic syndrome, mild cognitive impairment, or subclinical vascular disease or diseases that are not yet associated with significant dysfunction, such as hypertension and early stages of chronic kidney disease (CKD Stages 1 and 2). A study of a more intensive intervention might include patients with one or two overt age-related diseases, such as ischemic heart disease, congestive heart failure, peripheral arterial disease, stroke, T2DM, mild dementia, cancer in remission, or more advanced stages of renal failure (CKD Stages 3 and 4). Optimizing the population selection in this way decreases the time needed to carry out the study and increases the probability of observing the effect of the study intervention. Going too far along the risk spectrum in either direction might be counterproductive. For example, one might not include individuals with multiple major comorbidities or functional impairment, because at that point it may be too late to intervene in the aging process and the use of study drug may be contraindicated by the individual diseases. Conversely, selection of older adults without any age-related diseases, disabilities, or preclinical conditions might make the study difficult to be carried out because the yearly incidence rate of disease or disability may be low and healthy older adults may actually possess factors that protect them from developing age-related conditions. Thus, individuals living independently in the community who do not have significant disease burden, but have some manifestations of aging, would be the population of choice for a drug intervention trial. This selection strategy would also facilitate individuals’ participation in the study because they ideally would travel to study visits. Studies should also exclude individuals with limited life expectancy (<5 years), who would be unlikely to complete the relatively long follow-up period.

Intervention
Selecting a drug that is already in clinical use and that demonstrates a good safety profile with pleiotropic effects on multiple systems may be ideal. Metformin is one such drug. It has been extensively studied and was shown to delay T2DM, reduce CVD, and improve overall survival (44,89,90). Alternatively, a new drug specifically developed to target the known pathways implicated in aging, such as mTOR-inhibiting rapalogs or senescent-cell-clearing senolytics could be considered. The disadvantages of using a novel agent are several, including the need for pharmacokinetic data in older individuals, drug safety information, and the lack of knowledge about its effects on age-related dysfunction and diseases in humans. Combination therapy involving multiple drugs with nonoverlapping mechanisms is a therapeutic mainstay of many common diseases, including tuberculosis, coronary artery disease, HIV, and most cancers. Combination therapies targeting aging are already being tested in preclinical models and might be considered for human studies as well.

When deciding on the most effective dose, safety, tolerability, and efficacy must be balanced. In the case of metformin, the maximum recommended daily dose is 2,550 mg per day. However, the risk of GI side effects is proportional to the dose, with most people tolerating a daily dose of 1,500–2,000 mg without serious side effects (91). Thus, the dose can be titrated up to a maximum of 2,000 mg per day in those who tolerate it and those who do not may be maintained on the maximum tolerated dose. Rapamycin may also need to be titrated based on tolerance, although there is evidence that some analogs of rapamycin are effective at low doses without significant adverse effects (67). In fact, it is not entirely certain that higher doses, or the doses used for treatment of overt disease (such as metformin for diabetes), will be more effective as an intervention targeting fundamental aging processes than lower doses. Preclinical studies can help inform initial dose selection, and doses can potentially be titrated to achieve similar biological effect (eg, AMPK activation) between preclinical and human pilot studies. The ease of administration of the drug must also be considered. A drug taken orally, for example, may be preferable to one only available as an injection. Some drugs may have specific contraindications that must be considered in the study design. For example, metformin is contraindicated in renal impairment, and so would not be suitable for a health-span study enrolling many patients with advanced CKD. In addition, metformin is presently indicated for the treatment of T2DM, so participants with T2DM at baseline will need to be excluded from randomized trials using metformin. If a patient with T2DM is randomized to the placebo group, it would be ethically untenable to withhold an effective therapy from them, while experimentally unsound to permit them to receive the study drug “off-study.” As another example, rapamycin may inhibit wound healing and may not be appropriate for a resilience study of postsurgical recovery. Drug interactions should also be considered, especially in older individuals who are often treated with multiple prescription or over-the-counter drugs and supplements. One advantage of metformin is that it is not known to have serious interactions with commonly prescribed medications.

Choice of study drug is also influenced by the planned duration and intensity of the study. Whereas it may be acceptable to conduct a study of short duration or secondary prevention with an agent that is associated with more potential side effects or greater risk of toxicity, longer-term or primary prevention studies should be conducted with a drug that is safe and well tolerated to ensure participant compliance throughout the study period and to minimize the risk for cumulative toxicity. The duration of the study depends on its goals. Ultimately, a longitudinal study of long duration that can capture delay or prevention of age-related disease and disability would be the ideal test of effects on health span, potentially with a decade or more of follow-up. Before committing to a long-term health-span study, it would be prudent to carry out a shorter-duration or a pilot study that would not only help solidify the hypothesis for the longer study, but would also help resolve some of the issues surrounding participant, intervention, and outcomes selections. The duration of such a pilot study would be between 1 and 5 years. Studies of resilience could be designed to be shorter, as the outcomes of interest occur relatively soon after the acute event. The length of exposure to potential toxicity or side effects is therefore shorter as well.
The intervention itself might be as simple as a single drug or might be more complex. Single-drug simplicity may be helpful for trials where a key goal is reverse translation studies to identify molecular mechanisms. However, additional complexity may ultimately be inevitable and desirable for trials intended to change clinical practice. Combinations of drugs acting on complementary pathways may maximize benefits and minimize side effects, and efficacy of drugs might be potentiated by simultaneous nondrug interventions (eg, exercise). For example, combining metformin with rapamycin may not only provide additional effects on fundamental aging processes but may also alleviate the insulin resistance and hyperglycemia related to rapamycin (92,93). If a two-drug combination is considered, a 2×2 trial design may be most informative, where the four treatment arms would include metformin alone, rapamycin alone, metformin with rapamycin, or placebo.

A randomized, double-blind placebo controlled trial is the gold standard. The placebo in this case would likely include an inactive pill, but the current standard of care will also need to be incorporated into all study arms. In a health-span study (Scenario 1), diet and exercise counseling might be offered to all participants. In Example C of the resilience scenario (Scenario 2, rehabilitation after sepsis), the standard of care might include admission to an Acute Care of Elders unit and home-based rehabilitation after hospital discharge. Indeed, existing geriatric-focused care programs both in and out of the hospital might provide an ideal framework for conducting these trials. Investigators should be comfortable with multifactorial interventions (94) and pragmatic study design (95) to reflect the current standard of care in geriatrics and the nature of the population being targeted.

Measureable outcomes
The main outcome of interest would be whether an intervention can target fundamental aging processes as measured by age-related diseases and functional disability. Using longevity itself as an outcome may not be feasible within a reasonable timeframe, and longevity with poor health or compromised function may not be a desirable outcome anyway. Therefore, disease-free survival or disability-free survival would be the primary outcomes of interest in clinical trials of agents that target fundamental aging processes. In a longitudinal study of health span, this might be operationalized as time to incidence of a second or third age-related disease or impairment of a first activity of daily living. Although life span by itself may not be the outcome of choice, for reasons discussed earlier, mortality should be included among a composite of outcomes. Time between disability and death, end-of-life functional trajectory, and quality of death could be included in longer trials. Trials of resilience should also measure outcomes directly related to the stressor, such as antibody titers following vaccination or length of stay after elective surgery. For both trial scenarios, quantitative intermediate or secondary outcomes might include gait speed, grip strength, mobility stress test (Walking while Talking Test) (96,97), activities of daily living (ADLs), instrumental ADL (IADLs), and number of chronic prescription medications. Cognitive tests such as the Digit Symbol Substitution Test or Montreal Cognitive Test can be used to assess executive cognitive function (96,98).

Particularly for larger studies, the community of older adults can be involved in determining high-priority outcomes—perhaps, for example, independence in ADLs or avoiding institutionalization. This can be facilitated by academic–community partnership programs such as the Los Angeles Community Academic Partnership for Research in Aging (99) or through institutional community advisory boards. Such outreach could help build broader public support, enhance recruitment, assist in advocacy for funding, and ensure the relevance of the studies to the general population.

Recording clinical events like new onset of disease requires careful and systematic adjudication. After an event is reported, it must be reviewed and confirmed by experienced physicians. This involves obtaining the participants’ medical records, after receiving their informed consent, from medical institutions where the event was documented and/or treated. Adjudication of events is usually based on a set of prespecified criteria and is performed by a designated committee that is blinded to the intervention in order to ensure objectivity.

Longitudinal data collection would be helpful in studies of both health span and resilience. Resilience studies should collect baseline data prior to the intervention and/or stressor if possible, as well as at several time points after the stressor. All studies might collect biological samples at each of several time points, including blood and, where feasible, muscle, adipose tissue, and skin biopsies. Such biological samples would be crucial for reverse translation, biomarker validation, and possibly risk stratification. A patient registry could be maintained to facilitate long-term follow-up of age-related outcomes. Furthermore, links to national or local clinical databases, with participant consent, would provide opportunity for additional collection of outcomes data even after the completion of the intervention study.

In certain situations, primary outcomes of clinical trials can be surrogate endpoint biomarkers that are highly predictive of an actual clinical event, such as high blood sugar, which is predictive of diabetes mellitus, or hypertension and increased circulating lipids, which are predictive of CVD. Such biomarkers can be used as endpoints for trials, but extensive testing and validation are required before they are accepted by the medical community and regulators in place of actual clinical events. At present, there are no validated, easily measurable biomarkers that predict age-related morbidity and disability in large populations. Potential biomarker candidates that have been linked to function and health span in older adults in limited studies include interleukin-6, IGF-1, and IGF binding proteins (96). Development of reliable biomarkers or surrogate markers could dramatically accelerate aging research and should be one goal of initial pilot studies. As demonstrated earlier, multiple factors individually or in combination may be considered as outcomes, with the concept of a composite outcome potentially being most representative of the complex physiology that accompanies aging.

Challenges
The aging population is diverse ethnically, genetically, socioeconomically, and physically. It is also heterogeneous in terms of diseases, co-morbidities, and disabilities. This creates great challenges in designing a study, yet these challenges must be tackled to address the needs of the aging population. Many clinical trials have excluded older individuals precisely for these reasons, but in order for the clinical trial intervention to target the aging process itself and not only individual diseases, it must embrace this diversity across the older population.

Human physiology is complex, with perturbations in one system having effects on multiple systems. If an individual with T2DM subsequently develops CVD, cancer, or Alzheimer’s disease, should these subsequent diseases be considered as independent outcomes of the aging process or did T2DM through hyperinsulinemia, hyperglycemia, dyslipidemia, and inflammation increase the risk for these diseases? Epidemiological data support the association between T2DM...
and increased risk of other diseases, and this would need to be considered in designing a trial.

A trial incorporating all the necessary diversity of the aging population would need to be powered sufficiently. This may pose a great challenge, because more homogenous studies have not always had sufficient power to detect multiple outcomes, despite enrolling thousands of patients. However, one advantage of a study targeting aging processes may be the very age of the participants enrolled. Age is a major risk factor for most diseases and functional disability, thus the incidence of these events is expected to be greater in this population, which would translate into greater power to detect an effect. In addition, both sexes have to be represented adequately in a clinical trial. The numbers of individuals of each sex may need to be large enough so that the study will have sufficient power to detect sex-specific effects. This is important to consider, because several pathways implicated in the biology of aging appear to be sex specific, such as the GH/IGF-1 axis (100).

The ultimate goals of clinical trials that test an aging intervention will include obtaining FDA approval for an aging-related indication. “Aging” is not currently an FDA indication, but plausible indications representative of aging-associated morbidity might include combinations of age-related diseases (multimorbidity), ADL or IADL function, or defined geriatric syndromes such as frailty. A key goal of the first large clinical trials will be to define outcomes that are representative of fundamental aging processes as well as acceptable to regulatory agencies as potential indications. Furthermore, imperative to this goal is to identify outcomes that are clinically meaningful and not only statistically significant.

Recently, the FDA has increasingly utilized a process called “accelerated approval,” particularly for oncology drugs (101,102), which may provide a model for approval of repurposed drugs that target fundamental aging mechanisms while building evidence to support larger studies. In this route, the FDA approves drugs that show promising results in Phase 2, with the condition that the drug sponsor must still perform confirmatory Phase 3 studies. If the Phase 3 study eventually indicates that the results did not meet the expectations, that is, failure to reach the primary endpoint, or has previously unknown safety issues, the FDA would revoke its approval. For instance, bevacizumab was approved for metastatic breast cancer in 2008 under the FDA's accelerated approval program. After the accelerated approval of bevacizumab for breast cancer, the drug's sponsoring pharmaceutical company completed two additional clinical trials and submitted the data from those studies to the FDA. These data showed a very modest effect on cancer without evidence of improved survival or improvement in quality of life compared with taking standard chemotherapy alone—thus the FDA revoked the approval in 2011 (103).

For studies that target fundamental aging processes, if a Phase 2 clinical trial shows efficacy with proof of concept, and an excellent safety profile is demonstrated, then the trial may possibly lead to accelerated approval. The rationale for this conditional approval on the basis of a Phase 2 trial would be twofold. First, if efficacy is established in a major clinical outcome (eg, impact on the risk of age-related chronic diseases such as dementia), then the benefits would greatly outweigh the risks given the impact on lessening chronic disease risk on an individual's morbidity and quality of life (as well substantial cost savings both at the individual and public health levels). Second, a large Phase 3 trial might take a decade to complete, so the results of Phase 2 trials can help establish many therapeutic avenues in a shorter time period.

**Funding sources**

Valuable small pilot and proof-of-concept studies might be carried out within traditional funding mechanisms. These might test safety and dose ranges of interventions, correlate drug interventions with tissue biomarkers (eg, S6K activation from rapamycin), and provide preliminary data for effect sizes on physiological (eg, gait speed), disease (eg, CVD incidence), and functional (eg, ADL dependency) outcomes. Initially, larger clinical studies in either the health-span or resilience schemas that robustly test effects on disease can be funded in part by private, not-for-profit organizations, some of which have provided initial support. Although these studies are in the interest of the NIA, it may be difficult to cover the entire cost from NIA grants. As aging is a universal human condition and an inherently multi-disciplinary field that affects a wide range of diseases, funding for such studies may turn out to be shared by other disease-focused NIH Institutes. If successful, a large landmark study is likely to be a one-time effort, because once a precedent is established with the FDA to accept the notion that multimorbidities of aging can be targeted, larger studies will probably be led by the pharmaceutical industry, realizing this opportunity for drug development or repurposing in Phase 3 trials.

**Example of an Intervention Study to Delay Aging Processes: Targeting Aging With Metformin**

One tangible outcome of the NIH-sponsored retreat was the concept of the Targeting Aging with Metformin (TAME) study hypothesizing that metformin will delay the onset of several major age-related diseases, thereby indicating its potential to extend health span and increase active life expectancy (104). Initial support for the study is currently from the American Federation for Aging Research, and it is co-ordinated with the FDA to approve multicomposite of age-related diseases as an accepted drug target. The TAME study is planned as a double-blind, placebo-controlled multicenter trial, enrolling approximately 3,000 individuals aged 65 years and older and will exclude variety of pre-existing conditions. The primary outcome for this trial is the time to occurrence of any component of a multimorbidity composite, which includes coronary heart disease, stroke, congestive heart failure, peripheral arterial disease, cancer (driven mainly by breast, colorectal, prostate, and lung), T2DM, cognitive impairment, and mortality. The study proposes to use a metformin dose of ~1,500 mg per day. It is designed as a 6-year study, with a mean follow-up time of more than 3.5 years.

**Summary**

Clinical trials that target fundamental aging processes in humans are a novel concept that presents unique challenges and enormous opportunities. Challenges include selection of appropriate study populations, study designs, interventions, and outcomes. We presented two models that conceptualize trial designs for interventions that target fundamental aging processes in long-term and acute settings, defined by extension of health span and resilience to acute stressors, respectively. However, in order to gain the full support of federal and private sectors for development of therapeutics that target aging in humans, it is important to have “aging” or aging-associated outcomes such as frailty, functional decline, and multimorbidity designated as conditions eligible for registration by the FDA. Evidence from human studies is emerging that indicates certain interventions can target multiple age-related conditions simultaneously, potentially by interfering with
the aging process itself. With the aging population projected to grow exponentially in the near future, clinical studies that can demonstrate the protective effect of these therapeutics during acute and chronic perturbations in aging humans are more timely than ever. Thus, delaying or preventing the disabilities that occur as a consequence of the aging process would result not only in tremendous cost savings for the healthcare system but also in gains for society on the whole from the increase in productive contributions from older members of society [2].

**Supplementary Material**

Please visit the article online at [http://gerontologist.oxfordjournals.org/](http://gerontologist.oxfordjournals.org/) to view supplementary material.

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**References**

1. Hazzard WR, Halter JB. Hazzard’s Geriatric Medicine and Gerontology. 6th ed. New York: McGraw-Hill; 2009.
2. Goldman DP, Cutler D, Rowe JW, et al. Substantial health and economic returns from delayed aging may warrant a new focus for medical research. *Health Aff.* 2013;32:1698–1703. doi:10.1377/hlthaff.2013.0052
3. Little RJ, D’Agostino R, Cohen ML, et al. The prevention and treatment of missing data in clinical trials. *N Engl J Med.* 2012;367:1355–1360. doi:10.1056/NEJMr1203730
4. Detry MA, Lewis RJ. The intention-to-treat principle: how to assess the true effect of choosing a medical treatment. *JAMA.* 2014;312:85–86. doi:10.1001/jama.2014.7523
5. Sun X, Ioannidis JP, Agoritsas T, Alba AC, Guyatt G. How to use a subgroup analysis: users’ guide to the medical literature. *JAMA.* 2014;311:405–411. doi:10.1001/jama.2013.285063
6. Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R. A C. elegans mutant that lives twice as long as wild type. *Nature.* 1993;366:461–464. doi:10.1038/366461a0
7. Friedman DB, Johnson TE. Three mutants that extend both mean and maximum life-span of the nematode, *Caenorhabditis elegans*, define the age-1 gene. *J Gerontol.* 1988;43:B102–B109.
8. McCarty MF. AMPK activation–protein potential for boosting healthspan. *Age.* 2014;36:641–663. doi:10.1007/s11357-013-9595-y
9. Harrison DE, Strong R, Sharp ZD, et al. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature* 2009;460:392–395. doi:10.1038/nature08221
10. Miller RA, Harrison DE, Astle CM, et al. Rapamycin-mediated lifespan increase in mice is dose and sex dependent and metabolically distinct from dietary restriction. *Aging Cell.* 2014;13:468–477. doi:10.1111/acer.12194
11. Harrison DE, Strong R, Allison DB, et al. Acabose, 17-alpha-estradiol, and norgrihydroguaiaretic acid extend mouse lifespan preferentially in males. *Aging Cell.* 2014;13:273–282. doi:10.1111/acer.12170
12. Strong R, Miller RA, Astle CM, et al. Nordihydroguaiaretic acid and aspirin increase lifespan of genetically heterogeneous male mice. *Aging Cell.* 2008;7:641–650. doi:10.1111/j.1474-9726.2008.00414.x
13. Martin-Montalvo A, Mercken EM, Mitchell SJ, et al. Metformin improves healthspan and lifespan in mice. *Nat Commun.* 2013;4:2192. doi:10.1038/ncomms13192
14. Mitchell SJ, Martin-Montalvo A, Mercken EM, et al. The SIRT1 activator SRT1720 extends lifespan and improves health of mice fed a standard diet. *Cell Rep.* 2014;6:836–843. doi:10.1016/j.celrep.2014.01.031
15. Mercken EM, Mitchell SJ, Martin-Montalvo A, et al. SRT2104 extends survival of male mice on a standard diet and preserves bone and muscle mass. *Aging Cell.* 2014;13:787–796. doi:10.1111/acer.12220
16. Santos EL, de Picoli Souza K, da Silva ED, et al. Long term treatment with ACE inhibitor enalapril decreases body weight gain and increases life span in rats. *Biochim Biophys Acta.* 2009;78:951–958. doi:10.1016/j.bcp.2009.06.018
17. Ferder L, Inserna F, Romano L, Ercole L, Pszenny V. Effects of angiotensin-converting enzyme inhibition on mitochondrial number in the aging brain. *Am J Physiol.* 1993;265.C15–C18.
18. Baso N, Cim R, Pietrelli A, Ferder L, Terragno NA, Inserna F. Protective effect of long-term angiotensin II inhibition. *Am J Physiol Heart Circ Physiol.* 2007;293:H1351–H1358. doi:10.1152/ajpheart.00393.2007
19. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell.* 2013;153:1194–1217. doi:10.1016/j.cell.2013.05.039
20. Kennedy BK, Berger SL, Brunet A, et al. Geroscience: linking aging to chronic disease. *Cell.* 2014;159:709–713. doi:10.1016/j.cell.2014.10.039
21. Pallet N, Legendre C. Adverse events associated with mTOR inhibitors. *Expert Opin Drug Saf.* 2013;12:177–186. doi:10.1517/14740338.2013.752814
22. Vergès B, Walter T, Carriou B. Endocrine side effects of anti-cancer drugs: effects of anti-cancer targeted therapies on lipid and glucose metabolism. *Eur J Endocrinol.* 2014;170:R43–R35. doi:10.1530/EJE-13-0586
23. Katsimpardi L, Litterman NK, Schein PA, et al. Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. *Science.* 2014;344:630–634. doi:10.1126/science.1251141
24. Sinha M, Jung YC, Oh J, et al. Restoring systemic GDF11 levels reverses age-related dysfunction in mouse skeletal muscle. *Science.* 2014;344:649–652. doi:10.1126/science.1251152
25. Kirkland JL, Tchkonia T. Clinical strategies and animal models for developing senolytic agents. *Exp Gerontol.* 2015;68:19–25. doi:10.1016/j.exger.2014.01.012
26. Zhu Y, Tchkonia T, Purtshkalava T, et al. The Achilles’ heel of senescent cells: from transcriptome to senolytic drugs. *Aging Cell.* 2015;14:644–658. doi:10.1111/acer.12344
27. Zhu Y, Tchkonia T, Fuhrmann-Strossnigg H, et al. Identification of a novel senolytic agent, navitoclax, targeting the Bcl-2 family of anti-apoptotic factors. *Aging Cell.* 2016;15:428–435. doi:10.1111/acer.12445
28. Tchkonia T, Zhu Y, van Deursen J, Campisi J, Kirkland JL. Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. *J Clin Invest.* 2013;123:966–972. doi:10.1172/JCI64098
29. Xie M, Tchkonia T, Ding H, et al. JAK inhibition alleviates the cellular senescence-associated secretory phenotype and frailty in old age. *Proc Natl Acad Sci USA.* 2015;112:E6301–E6310. doi:10.1073/pnas.1513586112
30. Romano AD, Greco E, Vendemiale G, Serviddio G. Bioenergetics and mitochondrial dysfunction in aging: recent insights for a therapeutic approach. *Curr Pharm Des.* 2014;20:2978–2992.
31. Otero AM, Gustafsson ÅB. Therapeutic targeting of autophagy: potential and concerns in treating cardiovascular disease. *Circ. Res.* 2015;116:489–501. doi:10.1161/CIRCRESAHA.116.303791
32. Joachim J, Jiang M, McKnight NC, Howell M, Tzoe SA. High-throughput screening approaches to identify regulators of mammalian autophagy. *Methods.* 2015;75:96–104. doi:10.1016/j.ymeth.2015.02.002
33. Testa G, Biasi F, Poli G, Chiarpotto E. Calorie restriction and dietary restriction mimetics: a strategy for improving healthy aging and longevity. *Curr Pharm Des.* 2014;20:2950–2977.
34. Kenyon CJ. The genetics of ageing. Nature. 2010;464:504–512. doi:10.1038/nature08980
35. Fontana L, Partridge L. Promoting health and longevity through diet: from model organisms to humans. Cell. 2015;161:106–118. doi:10.1016/j.cell.2015.02.020
36. Muzzumdar R, Allison DB, Huffman DM, et al. Visceral adipose tissue modulates mammalian longevity. Aging Cell. 2008;7:438–440. doi:10.1111/j.1474-9726.2008.00391.x
37. Baker DJ, Wijshake T, Tchkonia T, et al. Clearance of p16INK4a-positive senescent cells delays aging-associated disorders. Nature. 2011;479:232–236. doi:10.1038/nature10600
38. Villeda SA, Plambeck KE, Middeldorp J, et al. Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. Nat Med. 2014;20:659–663. doi:10.1038/nm.3569
39. Loffredo FS, Steinhauser ML, Jay SM, et al. Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. Cell. 2013;153:828–839. doi:10.1016/j.cell.2013.04.015
40. Jumilla RK, List EO, Berryme DE, Murrey JW, Kopchick JJ. The GH/IGF-1 axis in ageing and longevity. Nat Rev Endocrinol. 2013;9:366–376. doi:10.1038/nrendo.2013.67
41. Lee C, Yen K, Cohen P. Humanin: a harbringer of mitochondrial-derived peptides? Trends Endocrinol Metab. 2013;24:222–228. doi:10.1016/j.tem.2013.01.005
42. Burkwitz K, Zhang Y, Mair WB, AMPK at the nexus of energetics and aging. Cell Metab. 2014;20:10–25. doi:10.1016/j.cmet.2014.03.002
43. Barzilai N, Crandall JP, Kritchevsky SB, Espeland MA. Metformin as a tool to target aging. Cell Metab. 2016;23:1060–1065. doi:10.1016/j.cmet.2015.06.011
44. Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med. 2002;346:393–403. doi:10.1056/NEJMoa012512
45. Ballfourt JA, McTavish D. Acarbose. An update of its pharmacology and therapeutic use in diabetes mellitus. Drugs. 1993;46:1025–1054.
46. Archer VE. Does dietary sugar and fat influence longevity? Am J Cardiol. 2002;89:504–508. doi:10.1016/S0002-9149(02)02647-6
47. Pasternak B, Svanström H, Callréus T, Melbye M, Hviid A. Use of angiotensin receptor blockers and risk of dementia in a predominantly male population: prospective cohort analysis. BMJ. 2010;340:b5465. doi:10.1136/bmj.b5465
48. Linz W, Heitsch H, Schöllens BA, Wiemer G. Long-term angiotensin II type 1 receptor blockade with fonsartan doubles lifespan of hypertensive rats. Hypertension. 2000;35:908–913.
49. Linz W, Jessen T, Becker RH, Schöllens BA, Wiemer G. Long-term ACE inhibition doubles lifespan of hypertensive rats. Circulation. 1997;96:3164–3172.
50. Basso N, Paglia N, Stella L, et al. Protective effect of the inhibition of the renin-angiotensin system on aging. Regul Pept. 2005;128:247–252. doi:10.1016/j.reg pep.2004.12.027
51. de Cavanagh EM, Insera F, Ferder M, Ferder L. From mitochondria to disease: role of the renin-angiotensin system. Am J Nephrol. 2007;27:545–553. doi:10.1159/000010775
52. Benigni A, Corna D, Zoga, C, et al. Disruption of the Ang II type 1 receptor promotes longevity in mice. J Clin Invest. 2009;119:524–530. doi:10.1172/JCI36703
53. Benigni A, Orisio S, Noris M, et al. Variations of the angiotensin II type 1 receptor gene are associated with extreme human longevity. Age. 2013;35:993–1005. doi:10.1186/11137-012-9408-8
54. Li J, Kim SG, Blenis J. Rapamycin: one drug, many effects. Cell Metab. 2014;19:373–379. doi:10.1016/j.cmet.2014.01.001
55. Lamming DW, Ye L, Sabatini DM, Baur JA. Rapalogs and mTOR inhibitors as anti-aging therapeutics. J Clin Invest. 2013;123:980–989. doi:10.1172/JCI64099
56. Kennedy BK, Lamming DW. The mechanistic target of rapamycin: The grand conductor of metabolism and aging. Cell Metab. 2016;33:990–1003. doi:10.1016/j.cmet.2016.05.009
57. Mannuck JB, Del Giudice G, Lattanzio M, et al. mTOR inhibition improves immune function in the elderly. Sci Transl Med. 2016;8:326ra179. doi:10.1126/scitranslmed.3009892
58. Lillfeld BA, Gurpide E, Markiewicz L, McKinley B, Hochberg RB. A simple and sensitive microtiter plate estrogen bioassay based on stimulation of alkaline phosphatase in Ishakawa cells: estrogenic action of delta 5 adrenal steroids. Endocrinology. 1996;127:2757–2762. doi:10.1210/endo-127-6-2757
59. Chiasson JL, Josse RG, Gomis R, et al. Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. Lancet. 2002;359:2072–2077. doi:10.1016/S0140-6736(02)08905-9
60. Chiasson JL, Josse RG, Gomis R, et al. Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. JAMA. 2003;290:486–494. doi:10.1001/jama.290.4.486
61. Zeymer U, Schwarzmair-D’assie A, Petzinna D, Chiasson JL; Group S-NTR. Effect of acarbose treatment on the risk of silent myocardial infarctions in patients with impaired glucose tolerance: results of the randomised STOP-NIDDM trial electrocardiography substudy. Eur J Cardiovasc Prev Rehabil. 2004;11:412–415.
62. Bakris G. Are there effects of renin-angiotensin system antagonists beyond blood pressure control? Am J Cardiol. 2010;105(1 Suppl):2A–29A. doi:10.1016/j.amjcard.2009.10.010
63. Sipahi I, Debonne SM, Rowland DJ, Simon DJ, Fang JC. Angiotensin-receptor blockade and risk of cancer: meta-analysis of randomised controlled trials. Lancet Oncol. 2011;12:627–636. doi:10.1016/S1470-2045(11)70106-6
64. Huang CC, Chan WL, Chen YC, et al. Angiotensin II receptor blockers and risk of cancer in patients with systemic hypertension. Am J Cardiol. 2011;107:1028–1033. doi:10.1016/j.am jcard.2010.11.026
65. Panaretak B, Svanström H, Callréus T, Melbye M, Hvid A. Use of angiotensin receptor blockers and the risk of cancer. Circulation. 2011;123:1729–1736. doi:10.1161/CIRCULATIONAHA.110.007336
66. Hanon O, Berrou JP, Negre-Pages L, et al. Effects of hypertension therapy based on eprosartan on systolic arterial blood pressure and cognitive function: primary results of the Observational Study on Cognitive function And Systolic Blood Pressure Reduction open-label study. J Hypertens. 2008;26:1642–1650. doi:10.1097/01.hjh.0b013e28301a2b0
87. Guarante L, Franklin H. Epstein Lecture: sirtuins, aging, and medicine. N Engl J Med. 2011;364:2235–2244. doi:10.1056/NEJMra1100831
88. Burn J, Gerdes AM, Macrae F, et al. Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomised controlled trial. Lancet. 2011;378:2081–2087. doi:10.1016/S0140-6736(11)61049-0
89. Chang ET, Froslev T, Sorensen HT, Pedersen L. A nationwide study of aspirin, other non-steroidal anti-inflammatory drugs, and Hodgkin lymphoma risk in Denmark. Br J Cancer. 2011;105:1776–1782. doi:10.1038/bjc.2011.443
90. Flossmann E, Rothwell PM, British Doctors Aspirin T, the UKTIAAT. Effect of aspirin on long-term risk of colorectal cancer: consistent evidence from randomised and observational studies. Lancet. 2007;369:1603–1613. doi:10.1016/S0140-6736(07)60747-8
91. Hawley SA, Fullerton MD, Ross FA, et al. The ancient drug salicylate directly activates AMP-activated protein kinase. Science. 2012;336:918–922. doi:10.1126/science.1215327
92. Goldfine AB, Fonseca V, Jablonski KA, et al. Salicylate (salsalate) in patients with type 2 diabetes: a randomized trial. Ann Intern Med. 2013;159:1–12. doi:10.7326/0003-4819-159-1-201307020-00003
93. Din FV, Valancute A, Houde VP, et al. Aspirin inhibits mTOR signaling, activates AMP-activated protein kinase, and induces autophagy in colorectal cancer cells. Gastroenterology. 2012;142:1504–1515. doi:10.1053/j.gastro.2012.02.050
94. Wan QL, Zheng SQ, Wu GS, Luo HR. Aspirin extends the lifespan of C. elegans via AMPK and DAF-16/FOXO in dietary restriction pathway. Exp Gerontol. 2013;48:499–506. doi:10.1016/j.exger.2013.02.020
95. Covinsky KE, Pierluissi E, Johnston CB. Hospitalization-associated disability in frail, older persons: a consensus report. J Gen Intern Med. 2011;36:1782–1793. doi:10.1001/jama.2011.1556
96. Zulman DM, Sussman JB, Chen X, Cigolle CT, Blaum CS, Hayward RA. Examining the evidence: a systematic review of the inclusion and analysis of older adults in randomized controlled trials. J Gen Intern Med. 2011;26:783–790. doi:10.1007/s10874-010-1629-x
97. Ferrucci L, Guralnik JM, Studenski S, et al. Designing randomized, controlled trials aimed at preventing or delaying functional decline and disability in frail, older persons; a consensus report. J Am Geriatr Soc. 2004;52:625–634. doi:10.1111/j.1532-5415.2004.52174.x
98. Bannister CA, Holden SE, Jenkins-Jones S, et al. Can people with type 2 diabetes love longer than those without? A comparison of mortality in people initiated with metformin or sulphonylurea monotherapy and matched, non-diabetic controls. Diabetes Obes Metab. 2014;16:1165–1173. doi:10.1111/dom.12354
99. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med. 2008;359:1577–1589. doi:10.1056/NEJMoa0806470
100. Dornan TL, Heller SR, Peck GM, Tattersall RB. Double-blind evaluation of efficacy and tolerability of metformin in NIDDM. Diabetes Care. 1991;14:342–344.