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Review

Human trials exploring anti-aging medicines

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SUMMARY

Here, we summarize the current knowledge on eight promising drugs and natural compounds that have been tested in the clinic: metformin, NAD^+ precursors, glucagon-like peptide-1 receptor agonists, TORC1 inhibitors, spermidine, senolytics, probiotics, and anti-inflammatories. Multiple clinical trials have commenced to evaluate the efficacy of such agents against age-associated diseases including diabetes, cardiovascular disease, cancer, and neurodegenerative diseases. There are reasonable expectations that drugs able to decelerate or reverse aging processes will also exert broad disease-preventing or -attenuating effects. Hence, the outcome of past, ongoing, and future disease-specific trials may pave the way to the development of new anti-aging medicines. Drugs approved for specific disease indications may subsequently be repurposed for the treatment of organism-wide aging consequences.

INTRODUCTION

Over the past three decades, aging has been studied using the same advanced tools of genetics and molecular biology that have shed light on other fundamental processes in biology, such as embryonic development and control of cell growth. This research has identified numerous aging-relevant mechanisms and pathways that constitute legitimate targets for pharmacological and lifestyle interventions. This review will summarize the status of translating this knowledge into interventions that have the potential to slow the aging process and extend the healthy, active period of life. Since it is now clear that slowing aging can also delay the onset and progression of diseases in numerous rodent models, aging interventions may offer novel ways to forestall aging-related diseases and even treat them once they have begun.

The eight interventions/pathways discussed here are either synthetic drugs or defined natural compounds, the latter of which have a speedier path to the marketplace since they are viewed separately from drugs by the Food and Drug Administration (FDA). These interventions were chosen based on several criteria. First, they are well represented in completed or ongoing human trials, for example, in the published literature or in https://clinicaltrials.gov, as the number of trials on human aging have increased dramatically in the past decade. Second, they have a solid track record of slowing aging in preclinical studies in model organisms, including rodents. Third, they promise sufficient safety in humans to be viable as long-term interventions. Fourth, similar to true gerotherapeutics, they work by attenuating the major hallmarks of aging. In this review, we have avoided discussing non-pharmacological interventions, such as diet, exercise, or so-called antioxidants, since they have been widely discussed elsewhere in the scientific literature and in public forums and are less likely to be relevant as medical treatments.

We have also avoided recent, potentially exciting strategies that may face significant barriers to their utility in humans. Prominent among these is the genetic intervention first shown to turn adult somatic cells into embryonic stem cells using the "Yamanaka factors," a cocktail of genes encoding four different transcription factors.[1] Constitutive expression of the Yamanaka factors in genetically altered mice causes cancer, but controlled, intermittent expression of three or four of them forestalls aging phenotypes and extends mouse lifespan, evidently by slowing or reversing cellular aging.[2] Expressing the Yamanaka factors through viral vectors in mice shows promise in targeted maladies, such as age-related decrements in vision,[3] but the path toward safe, systemic intervention in humans will be more difficult. More recently, chemical reprogramming cocktails that reverse aspects of aging in old and senescent cells, have opened the possibility of reprograming cells *in vivo* to rejuvenate tissues by injecting or ingesting small molecules.[4] Another approach that has less data but shows promise is SGLT2 inhibition, which seems to prevent heart and kidney diseases, reduce overall mortality in diabetic mice and reduce cardiovascular death in humans.[5]

RESEARCH HISTORY OF EIGHT INTERVENTIONS AND THE PROCESSES THEY AFFECT

The eight interventions emerge from basic research on aging, from drugs used to treat diabetes, and from studies of the gut microbiome, and a schematic on their mechanisms of action is depicted in Figure 1. Note that the interventions have important metabolic effects, which relate to caloric intake and its consequences. We first give a brief scientific history of each of the

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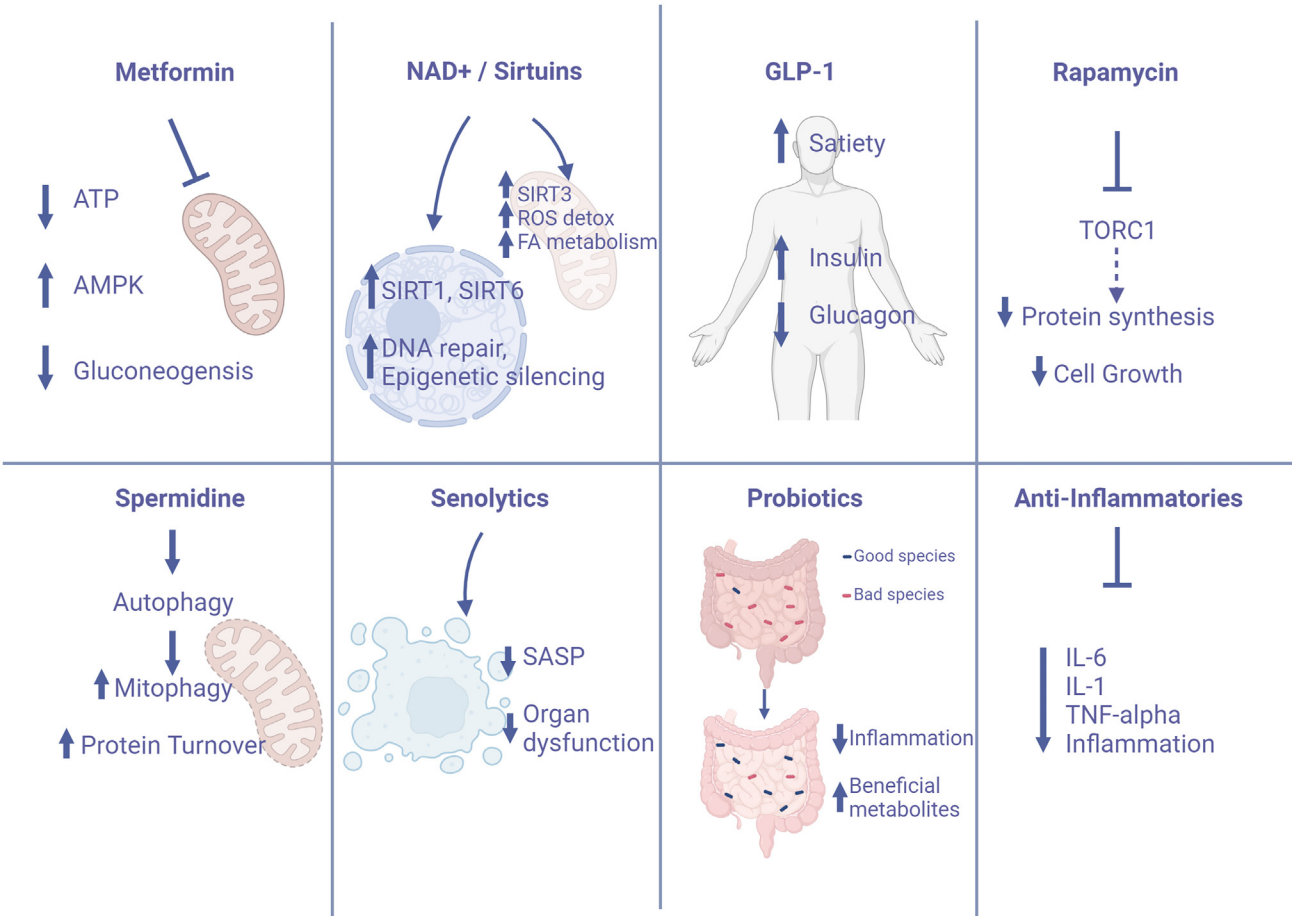

Figure 1. Eight interventions in human trials for aging and its attending diseases

Metformin, a drug to treat diabetes, may work by a mechanism involving inhibition of complex I of the mitochondrial electron transport chain, leading to a reduction of ATP levels, activation of AMP-activated protein kinase (AMPK) and a repression of gluconeogenesis in the liver. NAD^+ precursors restore youthful NAD^+ levels in cells, thus activating nuclear and mitochondrial sirtuins to promote DNA repair and epigenetic silencing in the nucleus and detoxification of reactive oxygen species (ROS) and fatty acid (FA) catabolism in mitochondria. Youthful NAD^+ levels will also foster activity of poly(ADP-ribose) polymerase (PARP) and several metabolic enzymes. GLP-1R agonists activate insulin production and glucagon repression in liver and the food satiety response in the hypothalamus (H). Rapamycin represses activity of TORC1 and thus protein synthesis and cell growth. Spermidine promotes autophagy, which turns over damaged mitochondria as well as denatured proteins. Senolytics kill senescent cells and thus blunt the senescence-activated secretory response (SASP), which can trigger organ dysfunction. Probiotics enhance the growth of beneficial bacterial species in the gut at the expense of toxic species. Anti-inflammatories blunt the activities of pro-inflammatory cytokines, which are a part of the adaptive immune response, and thus reduce systemic inflammation.

interventions, and then describe recent, published results in human studies.

Metformin

Metformin is a dimethylbiguanide used as a first-line oral blood-glucose-lowering agent to manage type 2 diabetes (T2D). Its history traces to the French lilac, a traditional herbal medicine in Europe, rich in guanidine, which, in 1918, was shown to lower blood glucose. Guanidine derivatives, including metformin, were synthesized by Jean Sterne in the 1950s. Metformin's general acceptance was slowed by association with a related compound, phenformin, which proved toxic. Gradually, the safety and efficacy of metformin became clear, and it has been widely used as a frontline treatment for diabetes since the 1990s. Metformin was subsequently shown to extend health span in worms,[6] and health span and lifespan in mice,[7,8] though another study did not find extension in murine lifespan.[9]

The mechanism of action of metformin in mitigating diabetes is not certain, but one plausible pathway stems from its weak inhibition of mitochondrial respiratory complex I, which activates AMP-activated protein kinase (AMPK) due to reduced ATP synthesis. AMPK activation lowers glucose production in the liver of insulin resistant diabetics and stimulates mitochondrial biogenesis. Other studies raise possibilities for different mechanisms. For example, a metformin dimer called supformin exhibits superior copper-chelating activities and mediates strong anti-inflammatory effects.[10] Other possible mechanisms include a reduction in reactive oxygen species (ROS) due to a partially weakened respiratory chain, and the decreased release of tissue-damaging factors by senescent cells (senescence-associated secretory phenotype or "SASP"), leading to a decrease in inflammation and reduced senescent cell accumulation.[11,12]

In humans, metformin may also work by altering the microbiome to increase the abundance of bacterial strains that

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produce the health-promoting short-chain fatty acids (SCFAs), butyrate and propionate,[13] and a metabolite called agmatin.[14] Consistent with this idea, oral but not intraperitoneal injection of metformin is associated with changes in the gut microbiome and reductions in tumor cell growth in mice fed a high-fat diet, an effect that can be recapitulated by fecal transplant from metformin-treated mice to uncreated mice.[15] More recently, changes in levels of gene expression and circulating factors have also been found, including the release of glucagon-like peptide-1 (GLP-1), GDF15, and MIC-1.[16]

NAD$^+$/sirtuins

This pathway emerged from studies in the 1990s showing that yeast Sir2 and later homologs in higher organisms (sirtuins) slow aging processes in yeast, worms, flies, and mice and extend their lifespan.[17] Notably, rare variants in human SIRT6 that either increase gene expression or alter amino acids of the protein were recently reported to be enriched in centenarians.[18] Sirtuins were found to be NAD$^+$-dependent histone deacetylases and function and in epigenetic regulation,[19] providing the first link between NAD$^+$, epigenetics, and aging. NAD$^+$ levels decline with normal aging in mice and humans.[7,20] However, supplementing the diet of aging mice with the precursors in NAD synthesis, nicotinamide riboside (NR),[21] and nicotinamide mononucleotide (NMN),[22] can replenish NAD$^+$ to youthful levels. This restored NAD$^+$ activates mitochondrial SIRT3 to facilitate mitochondrial maintenance and fatty acid (FA) metabolism and activates nuclear SIRT1 and SIRT6 for DNA repair and epigenetic silencing. Activation of the other four sirtuins by NAD$^+$ precursors is also presumed to occur. The organismal result is health benefits and longevity.[23] These beneficial NR effects on normal mice, as well as the rescue by NAD$^+$ precursors of mouse models of ataxia telangiectasia and Cockayne syndrome,[24,25] required SIRT1. In addition to NAD$^+$ boosters, sirtuin activating compounds (STACs) that directly activate the mammalian sirtuin, SIRT1, such as resveratrol, quercetin, and the natural flavonol fisetin,[26] extend the lifespan of worms[27] and extend the healthspan of normal or diabetic mice.[28,29] While resveratrol itself has been inconsistent in human trials, natural analogs or novel chemical activators appear to perform better.[30]

GLP-1

GLP-1 is an incretin hormone produced in the intestines in response to food intake, which plays a critical role in glucose homeostasis by stimulating insulin secretion and inhibiting glucagon secretion (see the excellent review by Drucker et al. for all but the reference at the end of this section).[31] Differential processing of proglucagon gives rise to the secreted hormones; glucagon in pancreatic islets and GLP-1 in the intestine and brain. Glucagon and GLP-1 are distinct but related peptides, and lie in different regions of proglucagon. While glucagon is produced in islets in response to fasting, GLP-1 is produced in intestine in response to feeding. Consistent with their opposite regulation by diet, the functions of GLP-1 and glucagon are antagonistic—GLP-1 turns off glucagon in islet alpha cells and stimulates insulin synthesis and secretion in islet beta cells. GLP-1 has effects in intestine, pancreas, heart, brain, and liver, where its receptor is also found. Importantly, GLP-1 mediates satiety by binding to its receptor in the hypothalamus of the brain. Because circulating GLP-1 is proteolytically destroyed by the DPP-4 peptidase, its blood levels upon exogenous administration are difficult to control. A newer class of GLP-1 drugs used today are GLP-1 receptor (GLP-1R) agonists, such as semaglutide, dulaglutide, albiglutide, exenatide, liraglutide, lixisenatide, and tirzepatide, which have been FDA approved and are of great interest because they promote weight loss in humans, as discussed below. In mice, GLP-1R agonists appear to slow or even reverse aging processes affecting the brain.[32]

Rapamycin/TORC1

Rapamycin, a compound first isolated from *Streptomyces hygroscopicus* found on Easter Island, became a highly used drug for immunosuppression. Rapamycin represses activity of a kinase complex, TORC1, which was first identified in yeast,[33] and then mammals,[34] and functions as a nutrient sensor and driver of cell growth.[35] When food is scarce, TORC1 activity is repressed by cellular factors, and this inhibition halts cell growth and activates autophagy, a process that was first dissected in yeast and which degrades and recycles defective cellular proteins or even whole mitochondria (mitophagy).[36] In the 2000s and 2010s, it was shown that genetic inhibition of TORC activity in metazoans extends the lifespan.[37] Rapamycin also extends mouse lifespan, even when dosing is initiated in old animals.[38] Chemical analogs of rapamycin, or rapalogs, have recently been sought to fine-tune mTOR inhibition (i.e., inhibit TORC1 but not TORC2 to prevent unwanted side effects of lowering TORC2), and, perhaps, also weaken immunosuppressive activity. Similar to metformin, rapamycin and rapalogs have the benefit of inhibiting release of SASP factors by senescent cells.[39,40]

Spermidine/autophagy

The natural metabolite, spermidine, is the sole polyamine that increases the lifespan of yeast, worms, flies, and mice.[41,42] In addition, spermidine prevents high-fat-diet-induced metabolic syndrome and obesity in mice[43] and delays age-related cognitive decline.[44] One of the most studied effects of spermidine is its ability to induce autophagy, and its protective effects are causally linked to this ability. For example, knockout of the essential autophagy gene *Atg5* in cardiomyocytes abolishes the capacity of oral spermidine supplementation to avoid age-associated cardiac failure in mice.[42] In addition, spermidine reverses age-associated phenotypes of murine B and T lymphocytes *in vivo* and lymphocytes from human donors *in vitro*, and these effects require activation of the pro-autophagic transcription factor EB (TFEB).[45,46] TFEB activation requires attachment of hypusine (Nε-[4-amino-2-hydroxybutyl]-lysine) to a lysine of the eukaryotic translation initiation factor 5A (eIF5A), and spermidine is required for the synthesis of hypusine.[45,46]

Aging is accompanied by a decline in autophagy, and experimental inhibition of autophagy is sufficient to accelerate aging in mice,[47] while genetic manipulations designed to stimulate autophagy extend the health span and lifespan of mice.[48,49] These beneficial effects of autophagy are thought to be mediated by the selective destruction and recycling of potentially harmful cytoplasmic structures including aggregates of misfolded proteins and dysfunctional organelles including depolarized mitochondria.[50] Due to the tendency of defective mitochondria to

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produce ROS and accumulative damage, one might expect robust mitophagy to contribute to successful aging. Indeed, autophagy is one component of the lifespan extension afforded by dietary restriction or TOR inhibition. Interestingly, other pathways or compounds that can extend lifespan, NAD^+/sirtuins and urolithin A, are also inducers of autophagy and mitophagy.[51–53] Hence, by activating autophagy, spermidine may activate a central mechanism that normally limits the aging process.

Senolytics

These are compounds that selectively kill senescent cells while not damaging normal cells. The cell senescence field can be traced back to the original finding in the 1960s that primary human cells undergo senescence after 40–60 population doublings *in vitro*.[54] Senescent cells were then shown to occur *in vivo* in aging organisms and to be reduced by a low-calorie diet, which is known to extend lifespan.[55] Senescent cells have possible non-cell-autonomous effects due to their secretion of pro-inflammatory cytokines[56] and are resistant to apoptosis.[57] Senescent cells were causally linked to aging, by genetically ablating them in live mice, which forestalled aging phenotypes and extended lifespan.[58] These findings led to the quest to discover senolytic agents that would selectively eliminate senescent cells in 2004[59,60] resulting in the discovery of the first senolytics using a mechanism-based drug discovery approach.[61] Examples of senolytics include the natural flavonol fisetin, the drug dasatinib (a Src kinase inhibitor in clinical use since 2005) used in combination with the flavonol quercetin (D + Q), the natural grape seed extract polyphenol procyanidin C1, certain heat shock protein inhibitors, and the drugs zoledronate (used for treating osteoporosis) and navitoclax (ABT263, an investigational chemotherapy drug).[61–67]

Probiotics/gut microbiota

The human gut contains a concentrated and diverse population of microbial species that can actually favor or disfavor good health.[68] Probiotics are bacteria that colonize the gut and are associated with favorable health outcomes. The differences between beneficial and harmful microbial species may relate to differing immune responses elicited by differing bacterial metabolites that cross from the gut to the bloodstream, differential bacterial metabolism of ingested food, or differential effects on the intestinal barrier. Favorable bacterial species may also produce metabolites that are beneficial to the host organism. Aging may be accompanied by a shift in the microbiome toward relatively unfavorable species. Studies employing fecal transplants of old donor mice and young recipients or vice versa suggest that the inflammation and health status of donors can be transferred to the recipients, at least temporarily. For example, the transfer of youthful gut microbiota from normal to progeroid mice can extend their life span, and this lifespan-increasing effect can be mimicked by oral administration of one single bacterial species, *Akkermansia muciniphila*.[69] Thus, the regular, oral provision of probiotics may have the potential to lower systemic inflammation and yield salutary effects.

Anti-inflammatories

Anti-inflammatory drugs include corticosteroids, analgesics such as aspirin and ibuprofen, and more recent monoclonal antibodies against pro-inflammatory cytokines or their receptors, and decoys to block cytokine signaling. Since systemic, chronic inflammation that accrues with aging, termed inflammaging, appears to be an important driver of aging phenotypes,[70] anti-inflammatories hold potential to mitigate aging-related diseases. Possible causes of inflammaging include poor diets and/or lack of exercise leading to metabolic syndrome, defective mitochondria in aging immune cells triggering cytokine release, the infiltration of pro-inflammatory metabolites of gut microbiota across a weakened intestinal barrier, and the systemic accumulation of senescent cells, which can secrete pro-inflammatory cytokines.

Two mouse studies suggest that inflammaging may be caused, in part, by an aging and dysfunctional immune system and pro-inflammatory cytokines. In the first, the DNA repair gene, ERCC1, was knocked out in hematopoietic cells triggering a wide variety of aging-like phenotypes in non-lymphoid tissues.[71] In the second, knocking out the mitochondrial factor TFAM in cytotoxic T cells weakened the resident mitochondria and triggered inflammation and aging phenotypes.[72] In this case, aging phenotypes could be mitigated by intervention with the NAD^+ precursor NR or an antibody that blocked the tumor necrosis factor alpha (TNF-α) receptor. It will also be interesting to see whether reducing inflammaging impacts DNA methylation clocks,[73] which aid in assessing the rate of biological aging.

HUMAN STUDIES ON THE EIGHT INTERVENTIONS

This section will focus on human trial results that have been published. While, we can only see the tip of the iceberg at present, a blueprint for improved health may begin to emerge with the completion of ongoing trials in the near future. The published literature on PubMed shows roughly 100–400 papers found by searching any one of these interventions by "intervention, human, aging, trial." Selected published human studies for the eight interventions are discussed below, and ongoing studies are listed in Table 1. (Unless otherwise indicated, trials were placebo controlled and blinded.)

Metformin

Metformin appears to be perhaps the frontrunner in experimental anti-aging interventions. While the focus on this drug has traditionally related to diabetes, it appears to have other positive effects on health, which *in toto* suggest that it may impact aging favorably. Four areas are highlighted based on human trials over the past decade or so.

Aging and diabetes

Metformin is not only a frontline treatment for diabetes but reportedly also reduces the incidence of other diseases including cardiovascular disease (CVD), cancer, frailty, and inflammatory conditions.[74] Some of these effects may be indirect consequences of treating the diabetes. For example, cancer protection may be due to mitigating hyperinsulinemia, since insulin has tumor growth-stimulating properties. However, other salutary effects may be unrelated to diabetes. A seminal retrospective analysis published in 2014 indicated that diabetes patients treated with metformin monotherapy over a 5-year period had a lower all-cause mortality rate than matched, non-diabetic

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Table 1. Examples of interventional, ongoing clinical trials on age-associated cardiovascular diseases, cancer prevention, neurodegeneration, and other age-related conditions

Category	Molecule	Indication	Status	Phase	Principal endpoint(s)	NCT no.
Metformin	metformin	atherosclerosis in prediabetes	recruiting	IV	time to death, non-fatal myocardial infarction, stroke, hospitalization for unstable angina, or symptom-driven coronary revascularization during 4.5 years	02915198
		heart failure without diabetes	recruiting	II	change in minute ventilation to CO_2 production slope after 6 months	03331861
		atrial fibrillation	recruiting	IV	atrial fibrillation burden during 1 year	03603912
		atrial fibrillation	not yet recruiting	IV	hospitalization due to atrial fibrillation within 1 year	05878535
		HFpEF in obesity without diabetes	not yet recruiting	II	mean early diastolic mitral annular velocity assessed by echocardiography during 6 months	05847244
		HFpEF in patients >60 years	recruiting	II	peak oxygen consumption during exercise after 20 weeks	05093959
		acute coronary syndrome in patients without diabetes	recruiting	III	need for unscheduled coronary stenting or bypass after 30 months	05305898
		cardioprotection during percutaneous coronary interventions	active, not recruiting	III	plasma levels of creatine kinase MB and cardiac troponin I	05708053
		prevention of cardiovascular events after acute myocardial infarction	recruiting	III	time to next major cardiovascular event (infarction, heart failure or stroke)	05182970
		prevention of recurrence after surgical removal of early-stage breast cancer	active, not recruiting	III	survival without invasive ipsi- or contralateral breast cancer within subgroups defined by hormone receptors in 5 years	01101438
		prevention of breast cancer in patients with atypical hyperplasia or *in situ* breast cancer	active, not recruiting	III	cytological atypia in random periareolar fine-needle aspirates after 1 and 2 years	01905046

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Table 1. ***Continued***

Category	Molecule	Indication	Status	Phase	Principal endpoint(s)	NCT no.
		prevention of progression in low-risk prostate cancer	recruiting	III	time to progression of prostate cancer within 3 years	01864096
		prevention of oral cancer in patients with premalignant lesions	active, not recruiting	II	complete or partial response to treatment for 14 weeks	02581137
		Idem	recruiting	II	complete or partial reversal of dysplasia or hyperplasia in 24 weeks	05237960
		Idem	recruiting	I	3-year transformation-free-survival rate	05536037
		prevention of leukemia in patients at risk	recruiting	II	change in variant allele frequency, gene expression, DNA methylation i n 12 months	04741945
		prevention of multiple myeloma in patients at risk	recruiting	II	change in M-protein and light chain concentrations in 6 months	04850846
		prevention of lung cancer in overweight and obese individuals at risk	recruiting	II	programmed death-1 (PD-1) expression on pulmonary regulatory T cells before and after 26 weeks of treatment	04931017
		prevention of gastric cancer in metaplasia	recruiting	IV	rate of reversal and progression of gastric intestinal metaplasia in 6 months	05288153
		inherited macular dystrophy due to ABCA4 mutation	recruiting	I/II	loss of the ellipsoid zone band measured by optical coherence tomography at 24 months	04545736
		C9orf72 amyotrophic lateral sclerosis/frontotemporal dementia	recruiting	II	RAN protein levels in cerebrospinal fluid at 24 weeks and function outcome up to 52 weeks	04220021
		mild cognitive impairment	recruiting	II/III	memory tests after 18 months, cortical thickness measured by MRI	04098666
		prevention of cognitive decline	recruiting	III	memory and cognitive tests after 3 years, MRI-measured brain volume	04511416
		mild cognitive impairment in overweight or obese >60 years without diabetes	recruiting	II	improved outcome of lifestyle intervention measured by neuro-psychological tests after 1 and 2 years	05109169
		Huntington's disease	recruiting	III	cognitive tests and motor function after 1 year	04826692

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Table 1. ***Continued***

Category	Molecule	Indication	Status	Phase	Principal endpoint(s)	NCT no.
		Parkinson's disease	recruiting	II	motor function and serum biomarkers (BDNF) after 3 months	05781711
		muscle health in healthy adults >60 years	active, not recruiting	I	MRI-quantified muscle mass after 5 days of bed rest at the end of a 14-day treatment period	03107884
		prevention of frailty in obese veterans >65 years	recruiting	III	improved fitness induced by exercise + diet measured by physical performance test after 6 months	04221750
		knee osteoarthritis	recruiting	I/II	pain and osteoarthritis scores, serum cytokines after 12 weeks	04767841
		osteoarthritis	recruiting	N/A	tibiofemoral cartilage volume, pain, and osteoarthritis index up to 24 months	05034029
		prevention of frailty in older adults >65 years	active, not recruiting	II	frailty composite measure up to 2 years	02570672
NAD^+ precursors	nicotinic acid (niacin)	Parkinson's disease	recruiting	N/A	motor skills and disease rating scale, mental state, inflammatory blood markers	03808961
		mitochondrial myopathy	recruiting	II	6-min walk test at 6 and 12 months, muscle mass and strength, maximal oxygen uptake	05590468
	nicotinamide mononucleotide	muscle recovery and physical capacity in 20 to 49 years old	active, not recruiting	N/A	wingate anaerobic test after 21 and 38 days	04664361
		diminished ovarian reserved in 20–40 years old	recruiting	N/A	ovarian volume and number of antral follicles measured by transvaginal ultrasonography over 12 weeks	05485610
	nicotinamide riboside	elevated systolic pressure and arterial stiffness in >50 years old	recruiting	II	resting systolic blood pressure and carotid-femoral pulse wage velocity after 3 months	03821623
		systolic heart failure	recruiting	I	myocardial NAD^+ levels and mitochondrial function, structure, and omics for up to 14 days	04528004
		heart failure induced by breast cancer therapy	recruiting	II	left ventricular ejection fraction measure by ecography for 12 months	05732051
		brain vascular age in >65 years	recruiting	IV	neurovascular coupling (NVC) responses after 8 weeks	05483465
		mild cognitive impairment >60 years	recruiting	I/II	cognitive function and brain blood flow after 12 weeks	03482167

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Table 1. ***Continued***

Category	Molecule	Indication	Status	Phase	Principal endpoint(s)	NCT no.
		mild cognitive impairment and mild Alzheimer's disease in patients >55 years	recruiting	I	changes in brain NAD^+, CK/ATPase activity, and glutathione after 12 weeks	04430517
		cognitive impairment	not yet recruiting	N/A	sleep quality and cognitive functions at 12 weeks	05500170
		early Parkinson's disease	recruiting	N/A	clinical rating scale and blood levels of NAD metabolites at 52 weeks	03568968
		Friedreich ataxia	not yet recruiting	II	clinical rating of ataxia for 1 year	03761511
		neurological manifestations of ataxia teleangiectasia	active, not recruiting	II	motor function for 2 years	04870866
		bone, muscle, and metabolic functions in women >65 years	recruiting	N/A	maximum oxygen uptake, fitness test, and muscle biopsy after 6 months	03818802
		muscle function in veterans >65 years	not yet recruiting	N/A	maximum oxygen uptake, muscle strength, and gait speed at 12 weeks	04691986
GLP-1R agonists	dulaglutide, liraglutide, or semaglutide	cardiac steatosis in type 2 diabetes >50 years	recruiting	IV	MRI assessment of heart steatosis for 6 months	03498001
	exenatide	ischemic stroke	recruiting	II	effect on cerebral blood flow velocity up to 3 h after drug injection	02829502
		ischemic stroke	recruiting	II	neurological outcome after 7 days	03287076
		cerebral small vessel disease	recruiting	II	brain MRI after 78 weeks	05356104
		Parkinson's disease	active, not recruiting	II	fluorodeoxyglucose (FDG)-PET network analysis after 21 months	04305002
		Parkinson's disease	active, not recruiting	III	functional outcome after 2 years	04232969
		early Parkinson's disease	active, not recruiting	II	functional outcome after 36 weeks	04154072
	liraglutide	minor ischemic stroke	recruiting	N/A	avoidance of other stroke events within 90 days	03948347
		coronary artery disease	recruiting	IV	expression of pro-inflammatory genes in epicardial fat and plasma IL-6 and TNF-α levels after 4–12 weeks	03260881
		atrial fibrillation	active not recruiting	IV	MRI-assessed size of left atrial epicardial adipose tissue after 3 months	03856632
		mild cognitive impairment	recruiting	N/A	memory testing after 1 year	05313529
	semaglutide	peripheral arterial disease in T2D	active, not recruiting	III	walking distance without and with pain on a constant load treadmill test for 52 weeks	04560998

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Table 1. ***Continued***

Category	Molecule	Indication	Status	Phase	Principal endpoint(s)	NCT no.
		cardiovascular disease in obesity	recruiting	III	time to major cardiovascular event (infarction, stroke, and death) up to 163 weeks or more	05669755
		cardiovascular disease in T2D or obesity	recruiting	IV	frequency of circulating $ALDH^{hi}SSC^{low}$ primitive progenitor cells after 6 months	05870462
		atrial fibrillation in overweight	not yet recruiting	III	atrial fibrillation recurrences up to 75 weeks	04885634
		HFpEF	recruiting	III	clinical rating, body weight, and 6-min walking distance, for 52 weeks	04916470
		HFpEF	recruiting	II	pulmonary capillary wedge pressure after 12 months	05371496
		coronary atheroma plaque	not yet recruiting	III	plaque burden measured by coronary CT after 18 months	05071417
		acute coronary syndrome in T2D	recruiting	IV	reduction in coronary plaque necrotic core identified by CT	05322200
		prevention of CVD and kidney disease in T2D	recruiting	IV	cardiovascular, kidney, and death events for 3 years	05390892
		stroke or transient ischemic attack in obesity	not yet recruiting	IV	time to major cardiovascular events (death, infarction, stroke, transient ischemic attack, and coronary revascularization)	05441267
		intracranial blood flow and blood brain barrier function in T2D	not yet recruiting	IV	intracranial blood flow and blood brain barrier function measured by dynamic contrast-enhanced MRI after 12 months	05780905
		acute ischemic stroke	recruiting	II	functional outcome and cardiac or cerebral events for 90 days	05630586
		ischemic stroke treated by reperfusion	recruiting	II	degree of disability/dependence after 90 days	05920889
		Parkinson's disease	not yet recruiting	II	motor function after 4 years	03659682
		Alzheimer's disease	recruiting	III	scRNA-seq of cells in cerebrospinal fluid and blood for 12 weeks	05891496
		early Alzheimer's disease	active, not recruiting	III	cognitive and memory tests for 2 years	04777396; 04777409
		chronic kidney disease in T2D	recruiting	III	MRI-based measurement of kidney oxygenation, perfusion, and inflammation until week 52	04865770
		knee osteoarthritis in obese subjects	active, not recruiting	III	pain and functional outcome until week 68	05064735
TORC1 inhi-bitors	everolimus	aging in adults >55 years	recruiting	II	improvement of age-related biomarkers after up to 28 weeks	05835999
	rapamycin	heart failure in frail older adults	active, not recruiting	I	peak oxygen consumption velocity at 6 months	04996719

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Table 1. ***Continued***

Category	Molecule	Indication	Status	Phase	Principal endpoint(s)	NCT no.
		cardiovascular function at >70 years	recruiting	II	cardiac and aortic MRI measurements after 8 weeks	04742777
		prevention of breast cancer in postmenopausal women with *in situ* cancer or hyperplasia	recruiting	II	reduction of histological biomarkers of breast cancer progression after 10 days	02642094
		mild cognitive impairment	active, not recruiting	I	clinical evaluation of dementia, reduction of amyloid β42 in cerebrospinal fluid after 8 weeks	04200911
		ovarian aging in women 38–45 years	recruiting	II	ovarian reserved measured by transvaginal ultrasound for up to 1 year	05836025
		aging in adults >50 years	active, not recruiting	II	body composition (visceral fat) and liver and kidney function for 12 months	04488601
Spermidine	spermidine	hypertension	recruiting	III	blood pressure after 20 weeks	04405388
		HFpEF	recruiting	II	cardiorespiratory exercise capacity after 12-week-long aerobic exercise training with or without 16-week spermidine treatment	05128331
		vaccination (65–90 years)	recruiting	N/A	longevity of antibody and T cell responses against SARS-CoV2 after vaccination for 37 weeks	05421546
Senolytics	dasatinib and quercetin	adults at risk of Alzheimer's disease	recruiting	I/II	mental and physical functions; cerebral blood flow measured by eco-doppler during cognitive task for 14 weeks	05422885
		Alzheimer's disease	recruiting	II	clinical and MRI assessments, frequency of senescent cells in blood, PET-CT measurement of tau levels in the brain after 12 or 48 weeks	04685590
		accelerated aging in major depression disorder and schizophrenia	not yet recruiting	II	MRI scans, questionnaires, and blood sampling for 10 weeks	05838560
		sedentary healthy adults	not yet recruiting	I	SASP in adipose tissue measure by snRNA-seq after 16 weeks	05653258
		fibrotic NAFLD in adults	recruiting	I/II	liver biopsy after 21 weeks and fibroscan	05506488
		survivors of hematopoietic stem cell transplantation	recruiting	N/A	frailty and senescence markers up to 180 days	02652052

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Table 1. *Continued*

Category	Molecule	Indication	Status	Phase	Principal endpoint(s)	NCT no.
	dasatinib and quercetin versus fisetin	frail adult survivors of childhood cancer	recruiting	II	walking speed and abundance of senescent cells in the blood for 150 days	04733534
		skeletal health in women >60 years	active, not recruiting	II	C-terminal telopeptide and N-terminal propeptide of type I collagen in plasma after 20 weeks	04313634
	fisetin	frail elderly >70 years	recruiting	II	blood inflammation markers after 7 days	03675724
		frail women >70 years	recruiting	II	gait speed (6-min walk) after 1 month	03430037
		frailty in breast cancer survivors >65 years	recruiting	II	6-min walk distance, grip strength, and physical performance	05595499
Probiotics	*Bifidobacterium* and *Lactobacillus*	Alzheimer's disease	active, not recruiting	N/A	cognition and mental illness after 12 weeks	05145881
	Bifidobacterium adolescentis Bif-038	inflammation in >60 years old	recruiting	N/A	CRP and TNF-α levels after 12 weeks	05529693
	Bifidobacterium triple	post-operative cognitive dysfunction in >65 years old after orthopedic surgery	recruiting	N/A	incidence of post-operative cognitive disorder at 7 days	04017403
		mild or moderate Parkinson's disease	recruiting	IV	motoric function after 12 weeks	04871464
	fecal microbiota transplantation	elderly 70–85 years	not yet recruiting	I	proportion of participants with reduced frailty score at week 96	05598112
	GI Biome #7	elderly >60 years	recruiting	N/A	grip strength and walking speed; microbial and inflammatory markers	05735418
	Lacticaseibacillus paracasei strain Shirota	mild cognitive impairment i n >60 years old	recruiting	N/A	microbial and inflammatory markers; cognitive function	05859230
	Lactobacillus paracasei PS23	mild cognitive impairment in >40 years old	recruiting	N/A	cognitive and memory tests after 12 weeks	04971096
Anti-inflammatories	aspirin	prevention of ovarian cancer in women with BRCA1/2 mutation	active, not recruiting	II	pre- and malignant lesions found in ovarectomy specimen within 5 years	03480776
		prevention of colorectal cancer in Lynch syndrome	not yet recruiting	III	mismatch repair-deficient colorectal cancers within 5 years	02497820
		Idem	recruiting	III	recurrence of adenomas within 4 years	02813824
		prevention of recurrence of Barrett esophagus after its ablation	not yet recruiting	II	expression of CDX2 mRNA and nuclear factor (NF)-κB activation in biopsies after 1 year	02521285
		prevention of gastric cancer after endoscopic submucosal dissection of early gastric cancer	recruiting	III	diagnosis of gastric cancer within 5 years	04214990

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Table 1. ***Continued***

Category	Molecule	Indication	Status	Phase	Principal endpoint(s)	NCT no.
		prevention of disease recurrence after primary treatment of non-metastatic solid cancers	active, not recruiting	III	disease-free and overall survival for up to 10 years	02804815
		prevention of colorectal cancer in patients with colorectal adenoma	active, not recruiting	II	ratio of proliferation and apoptosis in fecal biopsies after 5 years	02965703
		reduction of colorectal cancer risk after adenoma	recruiting	I	expression of intestinal stem cells in scRNA-seq after 2 months	05056896
	canakinumab (anti-IL-1β)	prevention of heart failure after acute myocardial infarction	recruiting	II	peak oxygen consumption after 6 weeks	05177822
		prevention of lung cancer in patients with high-risk pulmonary nodules	recruiting	II	regression of nodules and lung cancer-free survival after 7 months	04789681
		progression to cancer in patients with clonal cytopenias of unknown significance	recruiting	II	time to overt myeloid malignancy up to 6 years	05641831
		mild Alzheimer's disease	recruiting	II	neuropsychological and neuropsychiatric tests; PET-translocator protein TSPO) measurement of neuroinflammation after 24 weeks	04795466
	mesalazine (5-amino-salicylic acid)	prevention of colorectal neoplasia in Lynch syndrome	recruiting	II	occurrence of colorectal neoplasias within 2 years	04920149

Interventional, ongoing clinical trials on age-associated cardiovascular diseases, cancer prevention, neurodegeneration, and other age-related conditions.
Abbreviations: CVD, cardiovascular disease; HFpEF, heart failure with preserved ejection fraction; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; scRNA-seq, single-cell RNA sequencing; snRNA-seq, single-nuclei RNA sequencing; T2D, type 2 diabetes.

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controls (or a second control group of diabetics treated with the insulin secretagogue, sulfonyl-urea).[75] These claims were apparently challenged in a 2014 epidemiological study following >100,000 diabetics over 20 years in Wales. Subjects received a minimum of 6 months of antidiabetic treatments, and metformin did not give rise to lower mortality rates compared with age-matched, disease-free controls over the duration of the trial.[76] However, in the first 3 years of treatment, the metformin group did exhibit lower mortality rates; at later time points, the effects of the drug on diabetes waned and mortality rates soared due to the reappearance of the disease. So, this study actually supports the hypothesis that metformin has potential to slow aging processes, at least in the short term. However, one limitation of retrospective analyses based on electronic health records is they cannot measure patient compliance and are often plagued by confounding factors. Thus, these studies must be corroborated by prospective, interventional trials.

Cognitive decline and heart failure

A positive effect of metformin on cognitive decline in diabetics might be anticipated, since lowering blood glucose should reduce glucose toxicity to neurons. Two retrospective studies to date, indeed, showed reduced cognitive impairment in diabetics treated with metformin,[77,78] but another study actually reported that metformin use was associated with worse cognitive performance in participants with diabetes.[79] As these studies were similarly adjusted for confounding factors including age and level of education, it is not clear why they yielded disparate results. Clearly more studies are needed to assess the effects of metformin on cognitive function. Similarly, metformin may be expected to mitigate heart failure in diabetics by protecting cardiomyocytes against hyperglycemia. A recent meta-analysis of nine randomized clinical trials[80] is consistent with the notion that metformin might protect heart function in diabetics. Metformin use was associated with reduced N-terminal pro-brain natriuretic peptide and low-density lipoprotein levels, which are markers of heart failure. However, the actual cardiological benefit of metformin use remains to be assessed in diabetic and non-diabetic patients.

T cells

A recent 20-week pilot study in non-diabetic older adults queried whether metformin influenced the effects of influenza vaccination on the immune system. This randomized study, which only enrolled 8 metformin-treated patients and 7 placebo controls, found that the drug reduced exhaustion of $CD4^+$ helper T cells that recognize influenza virus.[81] Pending further confirmation in larger studies, this would imply that vaccination might have a more long-lasting effect in subjects who take metformin, perhaps due to the mitigation of immune-senescence. In favor of this conjecture, it has been found that metformin treatment of obese, prediabetic subjects is associated with an increase in autophagy in $CD4^+$ T lymphocytes, as well as signs of improved organelle clearance.[82]

Gut microbiota

Two studies suggest that metformin affects the relative proportion of bacterial species in gut microbiota.[83,84] It is too early to say whether this shift always tends to enrich for more favorable species, but this is yet another way metformin could affect aging by postponing inflammaging. Conflicting data emerge from a meta-analysis of trials on metformin and inflammation,[85] which found that metformin lowered one marker of inflammation, CRP, but not another, interleukin (IL)-6. The same analysis found that probiotics or omega-3 FAs lowered both markers.

Does metformin have any down sides? Two independent randomized trials of older adults found that metformin slightly countered the benefits of aerobic exercise (on a treadmill, stationary upright cycle ergometer, or an elliptical machine) and those of progressive resistance exercise training (leg press, knee extension, body weight squat progressing to a split squat, calf press, chest press, lat pull-down, biceps curl, and triceps press down) on skeletal muscle by about 5%.[86,87] This reduction appeared to be due to a ceiling on muscle mitochondrial function imposed by the drug, which would be consistent with its known action as a partial inhibitor of complex I. Whether this would be a significant impediment for people who use exercise to prolong their health span remains to be seen. Finally, another trial indicated that metformin increases skeletal muscle production of hydrogen peroxide in healthy older adults, as measured by a fluorescent biosensor on muscle biopsies.[88] However, it is not clear how reducing electron flow through complex I would lead to an increase in hydrogen peroxide, calling for corroboration of these observations. In addition, small amounts of oxidative stress have been shown to induce a beneficial effect on cells and organisms.[89]

In summary, metformin is an extremely promising drug for slowing aging and increasing health span with manageable side effects. Several ongoing trials evaluate the potential of metformin to postpone the development of additional cardiovascular events after a first episode of acute myocardial infarction (Table 1). Similarly, current trials investigate the capacity of metformin to prevent breast, prostate, lung, and oral cancer as well as multiple myeloma in specific at-risk populations. Finally, ongoing studies explore the effects of metformin on the progressive aggravation of neurodegenerative diseases, as well as frailty.

NAD^+ boosters/sirtuins

Three areas of biology will be discussed, which follow preclinical studies showing that aging processes can be slowed by boosting sirtuin activity genetically, or pharmacologically with SIRT1-activating compounds, NR or NMN. The pattern of disease protection bestowed on mice, rats, and non-human primates by these interventions frames the categories of human trials that have been completed.

Metabolism

Studies in rodents show that sirtuin activation protects against metabolic stress, such as high-fat diets. Human trials have shown that oral NR and NMN raise NAD^+ levels significantly after 10 days and sustainably.[90] At an appropriate dose of compounds, this increase can roughly replenish the NAD^+ lost due to aging without causing non-physiologically high NAD^+ levels. A study published in 2021 on NMN supplementation in prediabetic women reported a significant increase in insulin sensitivity, which was attributed to improvement in insulin action in muscle.[91] A more recent trial of NMN on middle age adults demonstrated a dose-dependent increase in physical performance and a lowering of biological age, as determined by measuring 19 relevant clinical parameters.[92] Two trials of NR plus a SIRT1 activator, pterostilbene (Pt), showed benefit to liver, by demonstrating a lowering of markers of liver dysfunction. In the first of

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these trials, 2-month administration of NR plus Pt in healthy older adults caused a reduction in blood levels of the liver enzyme alanine transaminase (ALT),[90] while in the second, larger, 6-month trial of patients with non-alcoholic fatty liver disease, administration caused a reduction in ALT and two other liver enzymes, as well as a reduction in the toxic lipid species ceramide 14:0.[93] Since fatty liver not only predisposes to more serious liver disease but is also a marker of metabolic syndrome,[94] these findings point to a systemic benefit from targeting the NAD/sirtuin pathway.

Cholesterol, blood pressure, and physical performance

Administration of MIB-626, a polymorph of NMN, to overweight or obese, middle-aged and older adults at 2 g/day safely increased circulating NAD levels and significantly reduced total low-density lipoprotein (LDL) and non- high-density lipoprotein (HDL) cholesterol, trigelyerides, body weight, and diastolic blood pressure.[95] These data provide the rationale for larger trials to assess the efficacy of NAD augmentation in improving cardiometabolic outcomes in older adults. The study showed a trend toward increased endurance, an effect reminiscent of another study showing nominally significant improvements in gait speed and performance in grip tests.[96] Similarly, 250 mg of NMN taken in the afternoon improved lower limb function assessed by the five times sit-to-stand test and reduced drowsiness in older adults.[97]

Neurodegenerative diseases

Parkinson's disease. Numerous neurodegenerative murine disease models are favorably impacted by genetic overexpression of SIRT1 or NAD^+ boosters. In humans, a phase 1 trial on patients with Parkinson's disease (PD),[98] 1 mg per day of NR for 32 days elevated NAD^+ levels in the cerebrospinal fluid (CSF) and in the brain in most, but not all patients. In patients where an increase in NAD^+ was found, there was an improvement in Parkinson's symptoms measured by the standard test (Movement Disorder Society-Unified Parkinson's Disease Rating Scale). Further, there was a decrease in markers associated with mitochondrial defects in the CSF, as well as in pro-inflammatory cytokines. These findings have prompted the same group in Bergen, Norway to initiate a phase II trial and plan a likely phase III trial in the future.

Alzheimer's disease. A trial in healthy adults looked at the effects of NR on the contents of extracellular vesicles associated with neurons. The authors report an increase in NAD^+ levels in these vesicles and a reduction in amyloid β42 (aβ42), associated with Alzheimer's disease, and kinases associated with pro-inflammatory pathways.[99]

ALS. In a 4-month pilot trial conducted on patients with amyotrophic lateral sclerosis (ALS) in Spain, NR plus Pt was found to be safe, and the treatment group showed a significantly slower decline than the placebo group in ALS symptoms, as scored on the Revised ALS Functional Rating Scale.[100] Because of the promising nature of these findings, a phase II ALS trial is ongoing in Bergen, Norway.

Ataxia telangiectasia. An open-label study of NR was conducted on 24 patients with ataxia telangiectasia, which is due to mutation in ATM kinase reducing DNA repair and having broad phenotypes, including ataxia, cutaneous, and neurological manifestations.[101] The authors reported improvement in the patients' ataxia, as rated by the Scale for the Assessment and Rating of Ataxia and International Cooperative Ataxia Rating Scale ataxia that was lost upon NR withdrawal.[102]

Axonal degeneration. NAD^+ is also known to protect against axonal degeneration, since the Wallerian mutation in mice, which slows degeneration of axons in dorsal root ganglia, is a translocation that upregulates the NAD^+ biosynthetic enzyme, NMNAT1. Along these lines, an NAD^+ degrading enzyme expressed in neurons, sterile alpha and TIR motif containing 1 (SARM1), is under development as a drug target for inhibitors to treat neurodegenerative disorders.[103] It will be interesting to see if trials with NAD^+ boosters show a spectrum of neuroprotective effects that overlap those of SARM1 inhibitors.

Finally, there are other diseases or conditions of aging for which there are preclinical data indicating protective effects of NAD^+ boosters or genetic activation of sirtuins may be effective, including chronic and acute kidney disease, where one phase I study has been done[104] cardiovascular disease, muscular dystrophy, infertility, and hearing loss. These areas, along with other maladies accompanying aging, offer additional opportunities for exploration in ongoing and future human trials (Table 1).

GLP-1R agonists

GLP-1 was first identified as an insulin secretagogue and subsequently shown to function in the brain to mediate satiety. Studies using synthetic GLP-1R agonists are focused on disease outcomes, but the possible links of glycemic control and the avoidance of metabolic syndrome to aging and health, at least for a substantial portion of the population, is hard to overstate. Indeed, blood glucose tests have long been the primary measure used by life insurance companies to assess mortality risk of new clients.

Diabetes/metabolism

It seems clear that GLP-1 drugs are fulfilling their promise as drugs to combat diabetes,[64] and they are now the second line treatment (and rising fast) after metformin. Numerous human trials testify to their efficacy in reducing body fat,[105] controlling hyperglycemia,[106] and reducing HbA1c,[107,108] which is a measure of diabetes-associated protein glycation and a process that also occurs in normal aging. It appears that the GLP-1R agonists (semaglutide and liraglutide) are superior to GLP-1 itself and its peptide analogs because they are orally available and easier to dose.

Aging/weight loss

In 2018, semaglutide was found to promote significant weight loss in T2D patients in the SUSTAIN trial.[109] That same year, semaglutide was also found to promote weight loss in non-diabetics.[110] Roughly one third of Americans who suffer from obesity and its afferent metabolic consequences that ultimately accelerate biological aging might benefit from drugs that activate the GLP-1 pathway.

Cardiovascular disease

Two large trials of diabetic patients have demonstrated that semaglutide[110,111] and liraglutide[112] favorably impact parameters of cardiovascular function; death due to cardiovascular disease, non-fatal myocardial infarctions, and stroke. A meta-analysis of numerous adverse cardiovascular and renal outcomes concludes that GLP-1R agonists are protective in diabetics against both of these outcomes.[113]

PD and cognition

GLP-1 drugs are also being investigated for their possible effects on PD.[114] Two studies published in Lancet investigated GLP-1R

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agonists in PD. In the first, exenatide was found to significantly improve clinical signs of PD compared with the placebo group, as measured by the Movement Disorders Society unified PD rating scale MDS-UPDRS.[115] In a larger multi-center trial in 24 countries, dulaglutide corroborated the earlier trial by showing a similar result, which appeared as a trend in the raw data but reached significance (14% reduction in the risk of cognitive impairment compared with the placebo group) when adjusted for baseline scores.[116] Another trial in prediabetic or diabetic patients showed an improvement in short-term memory along with weight loss in response to the receptor agonist, liraglutide. Finally, a 26-week trial found that liraglutide improved brain metabolism of glucose in patients with Alzheimer's disease.[117] These findings all point to a role of GLP-1 drugs in managing neurodegenerative diseases.

Altogether, it appears that GLP-1R agonists provide a major benefit through their capacity to induce weight loss and combat metabolic syndrome with little or no side effects. The present knowledge on these agents justifies optimism with respect to their potential capacity to broadly increase human healthspan. This optimism is reflected by the larger number of clinical trials in which distinct GLP-1 analogs or receptor agonists are administered to patients with age-related cardiovascular and neurological diseases (Table 1).

Rapamycin/mTOR

Rapamycin and related compounds are strong candidates for interventions targeting aging processes, because they downregulate TORC1 activity, the dysregulation of which is associated with aging.[118] Murine data clearly show that rapamycin can extend lifespan in mixed genetic strains and in multiple animal facilities.[38] We discuss the upsides, as well as potential pitfalls, of TORC1 inhibition below.

Influenza vaccination

Several trials by Mannick et al. indicate that everolimus (RAD001), an allosteric TORC1-specific inhibitor, helps the immune response to influenza vaccination in older adults compared with a control group. In the first pilot trial, RAD001 showed a 20% improvement in efficacy of the vaccine against flu infections and a reduction in T cell exhaustion, as measured by reduction in the PD-1 receptor.[119] In a second, larger trial, RAD001 again increased the efficacy of influenza vaccination in older adults, increasing antibody titer and reducing the infection rate over a 1-year period post vaccination.[120] These findings suggest an important application for TORC1 inhibitors, since vaccines are limited in their effectiveness in older adults because of their weaker immune systems. A third trial to query whether TORC1 inhibition reduced overall respiratory tract infections irrespective of vaccination status was negative.[121]

It might seem surprising that an immune suppressant has positive effects on immune function. It is possible that the newer TORC1 inhibitors may cause only a partial inhibition of TORC1 compared with its blockade by rapamycin. Thus, the effects on the immune system in these trials may be more selective as to which immune cells are targeted, for example by increasing the function of T effector cells by lowering their exhaustion.

Autoimmunity

A single study looked at rapamycin in lupus erythematosus and found a reduction in pro-inflammatory T helper 17 (Th17) cells and an increase in T regulatory cells.[122] A mechanism for the reduction in Th17 cells is not presently clear. If future trials confirm and expand these findings, TORC1 inhibition may find a general application in auto-immune diseases, which would be more consistent with its traditional role as an immune suppressant.

Skin aging

Skin aging is partly caused by long-term sun exposure, which causes photoaging and the appearance of age spots due to increased melanin production, and other changes related to accumulating senescent cells, as well as normal aging triggering a thinning of the dermal and subcutaneous fat layers.[123,124] A single placebo-controlled trial found that topically applied rapamycin increased dermal volume and collagen and reduced senescent cells, as assessed by histological quantification of p16 in skin biopsies.[125]

A two-edged sword?

It is important to note that trials to date suggest that rapamycin might be a two-edged sword, conferring the benefits of slower aging and delayed diseases, but prone to side effects that must be carefully weighed against these benefits. Two human trials illustrates this point. An 8-week placebo-controlled trial of 25 older adults reported five adverse events along with a general reduction in red blood cells and hematocrit in the rapamycin-treated arm.[126] On the positive side, there was an increase in T regulatory cells, which can dampen the activity of cytotoxic T cells and lower inflammation. A different trial followed patients after bladder surgery and found that there was a benefit to the immune system due to a reduction in T cell inactivation by exhaustion, but an increased rate of weakened wound healing at the surgical site.[127] Many of the favorable and deleterious effects of rapamycin can be explained by its inhibition of TORC1 and cell growth; i.e., fewer cell divisions could forestall exhaustion of T cells, but that same inhibition might slow cell growth required for wound healing or hemoglobin production.

Exercise results in a strengthening of skeletal muscle and concomitantly bone structure by the activation of TORC1, protein synthesis, and muscle growth, resulting in a mitigation of frailty. Several trials report that these effects on skeletal muscle in response to exercise are inhibited by rapamycin and its analogs.[128–131] Again, these findings are not surprising given the known inhibitory effects of rapamycin on protein synthesis and cell growth and are analogous to the effects of metformin on muscle, as discussed above. This considerable body of data from human trials suggest that rapalogs and metformin might be disadvantageous for individuals who use exercise to prolong their health span. It has not yet been demonstrated that TORC1-specific analogs (e.g., everolimus) also block the anabolic effects of exercise, but this seems likely given the known effects of this complex.

In summary, TORC1 inhibition likely has high promise for health by normalizing the elevated level of its expression in an aging population. However, some concerns due to blocking efficacious cell growth have also arisen. It seems that the trick here might be to calibrate the system in a way that TORC1 inhibition is partial but optimal for reversing the aging effects of elevated TORC1 levels without interfering with necessary cell growth. It remains to be seen whether such a partial inhibitor for TORC1 will be identified, which can function at a constant dose and across different cell types. In ongoing clinical trials, rapamycin

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and rapalogs are being further evaluated for the capacity to ameliorate age-related biomarkers and disorders (Table 1).

Spermidine/autophagy

Human clinical trials on spermidine are sparse at this point, but some results have been reported, which are summarized below.

Cognition

Cognitive function is an obvious endpoint in spermidine trials, since autophagy may clear aggregates such as aβ in the brain. In an exploratory study, 90 older adults were stratified for dietary spermidine intake by a questionnaire. A positive correlation was reported between spermidine intake and cortical thickness and hippocampal volume.[132] In a different blinded but placebo-less trial of older adults in Austria, spermidine was provided in two concentrations in wheat rolls.[133] The group receiving the higher spermidine rolls performed significantly better in cognitive tests. A placebo-controlled trial enrolling a group of 30 older adults also found improvement in cognitive tests in the spermidine arm.[134]

Mortality

A retrospective study in Northern Italy followed 829 subjects aged 45–84 for 20 years and reported a lower mortality for the group with highest dietary spermidine, and this result was confirmed in an independent study performed in Austria.[135] Of course, this study cannot pinpoint spermidine as the causative agent of longevity in the diet or lifestyle of participants. Another study found high levels of circulating spermidine catabolites (in particular N_1-acetylspermidine and N_1,N_8-diacetylspermidine) correlated with patients who experience the most severe form of COVID-19.[136] Enhanced spermidine catabolism has also been found in patients with acute kidney damage[137] and patients with various malignancies.[138] If acetylation promotes spermidine turnover, one can speculate that the non-acetylated, active pool of spermidine may be somehow depleted in patients with the most severe manifestation of COVID-19 and other diseases.

Thus, spermidine has the potential to promote healthy aging by ramping up autophagy, but the extent to which it will play a major role in improving health awaits more testing in human trials. Clinical trials evaluating the capacity of spermidine to reduce hypertension, to treat heart failure with preserved ejection fraction (HFpEF) and to improve vaccination in older adults are underway (Table 1).

Senolytics

Human study results of senolytics are only now emerging, but there are over 30 studies in progress and planned for this promising approach for numerous disease states, such as osteoarthritis, age-related osteoporosis, Alzheimer's disease, diabetes/obesity, fatty liver, COVID-19, chronic HIV syndrome, sepsis, peripheral vascular disease, bone marrow transplant survivors, brain cancer, and tissue dysfunction caused by cancer therapy, which can leave a wake of senescent cells. The published studies below are mainly observational.

Reduction of senescent cells in humans

An important milestone was an open-label study of patients with diabetic kidney disease showing that dasatinib, a tyrosine kinase inhibitor used to treat cancer, and quercetin, a plant polyphenol (D + Q) reduced the number of senescent cells in adipose tissue, as shown by a reduction in levels of p16 and β-galactosidase-positive cells, together with reduced inflammation and fibrosis and a decreased blood SASP factor composite score.[139] Another trial then found that exercise reduced senescence in T cells, as determined by lowering p16, p21, and TNF-α in those cells.[140] Finally, a third study reported that exercise reduced p16 levels in peripheral blood mononuclear cells (PBMCs).[141]

Idiopathic pulmonary fibrosis

A pilot safety trial of patients with idiopathic pulmonary fibrosis (IPF), a debilitating then fatal disease that causes progressive fibrosis in lungs, found promising signs of a possible alleviation of frailty symptoms by D + Q.[142] Urine analysis found that IPF reduced levels of the anti-aging protein α-klotho,[143] which was restored by treatment with D + Q in each of 20 subjects.[144] A pilot study on D + Q for Alzheimer's disease also suggested safety.[145]

The potential of senolytics is ultimately tied to their specificity in killing senescent but not normal cells. Data so far suggest that this therapeutic window is wide enough to unleash their considerable potential for improving human health span, but the full depth and breadth of their promise will be revealed in the next few years as the result of ongoing clinical trials (Table 1).

Probiotics/gut microbiota

More trials on probiotics have been described compared with the above interventions, although the results are still early and generalizations are not certain. In most of these trials, the probiotic bacterial strains used were a *Lactobacillus* and a *Bifidobacterium*, although some trials use other strains. The three categories below represent a majority of completed studies, and a large number of additional trials in these and other categories are ongoing. Unless otherwise specified, the trials discussed below were all placebo controlled and blinded.

Immune function

As mentioned above, one clear way gut microbiota might influence health is by their effects, whether beneficial or deleterious, on the immune system. An early trial in 2013 enrolling 30 healthy older volunteers gave the first indication that probiotics boosted the activity of natural killer cells.[146] While one meta-analysis of four trials tended to corroborate the early study,[147] a separate meta-analysis found either no or negligible effects.[148] A different study of subjects over 75 years found an increase in T cells in the probiotic arm, which one imagines might improve immunity by replenishing a population diminished by aging.[149] An open-label study[150] found that a combination of probiotics and diet lowered homocysteine, a potentially harmful metabolite that that may predispose to heart, renal and cognitive disease.[151]

Another way to assess effects of probiotics on immunity is through studies to measure susceptibility to infectious diseases. In this regard, a study enrolling 50 older adults found a multi-strain probiotic combination lowered the number and duration of common infectious diseases.[152] Another study with 58 young and 58 old volunteers found an increase in B cells producing IgG in young but not old subjects. Finally, a study of 43 children in day care centers found *Lactobacillus* reduced infections in the upper but not the lower respiratory tract.[153] Overall, the majority of these studies suggest some positive effect on the immune system, but more validation is needed.

The immunostimulatory effects of bacteria can also be studied in the context of anticancer immunotherapies. Thus, in an interventional phase I, fecal microbial transplantation of the microbiota from healthy individuals improved the efficacy of immune checkpoint inhibitors against advanced melanoma.[154] The

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abundance of intestinal *A. muciniphila* has been correlated with the efficacy of immune checkpoint inhibitors against non-small cell lung cancer in several studies[155,156] echoing prior results in mice that oral gavage with *A. muciniphila* can improve the outcome of cancer immunotherapy.[157] Thus, it appears possible that some intestinal microbes or microbial communities can be harnessed to improve anticancer immunosurveillance.

Metabolism

This is an area that may be particularly promising, given the strong influence of gut microbiota on metabolic health in rodents. To test whether probiotics mitigated metabolic syndrome, a blend containing *Lactobacillus* and *Bifidobacterium* was provided to 82 obese children, who showed an improvement in several parameters of their lipid profile.[158] Similarly, a 12-week trial in 128 subjects with hypertriglyceridemia found a significant reduction in triglycerides,[159] and a similar finding was reported in a trial using two different *Lactobacillus* strains.[160] Supplementation of overweight and obese volunteers with live or heat-inactivated *A. muciniphila* caused a reduction in insulinemia, an increase in insulin sensitivity, as well as favorable effects on blood markers of liver dysfunction and inflammation.[161]

Inflammation

A trial in middle-aged adults reported a reduction in inflammation, as determined by a lowering the pro-inflammatory cytokines, IL-6, IL-8, and interferon (IFN)-γ in response to a combination of *Bifidobacterium animalis lactis* and fructo-oligosaccharides.[162] Consistent with an effect of probiotics on inflammation, the trial also indicated a reduction in upper respiratory tract infections and a lowering of TNF-α in the probiotics arm.

Cognition

Numerous studies have tested effects of probiotics on cognition in older adults, including patients with Alzheimer's disease. Cognitive improvements have been reported,[163–166] but more corroboration is required, in part because quantitative measurements of cognitive function pose more challenges than, for example, laboratory tests quantifying blood analytes.

Overall, probiotics offer considerable promise for improving human health. The further testing of probiotics for maladies of aging and diseases is well underway (Table 1). Results so far may augur a favorable effect on metabolic disease and inflammation, but no firm conclusions can be drawn for any indication at present.

Anti-inflammatories

As discussed above, inflammation impacts aging in both specific and systemic ways. Three drivers of inflammaging mentioned above, IL-1, IL-6, and TNF-α, are also a part of the medley of factors that are produced in response to infections, and also can be secreted by senescent cells. Inhibition of these factors may therefore be expected to trigger a reduction in inflammaging, but a concomitant reduction in the growth of immune cells in response to infections.

The published data on human trials are most developed for IL-6, which was initially proposed to be the cytokine most associated with phenotypes of aging.[167] Levels of IL-6 in humans typically increase with aging for all the reasons mentioned above. At present, most clinical studies of IL-6, as for the other cytokines, are in progress but not completed. There are clinical data, however, relating to one tissue that is known to be susceptible to deleterious effects of this cytokine, the bowel. An open-label study on patients with irritable bowel disease found olamkicept, a selective inhibitor of the soluble IL-6 receptor (sIL-6R)/IL-6 complex, to be capable of alleviating symptoms.[168] This drug is an injectable decoy protein containing the extracellular domain of the gp130 coreceptor required for IL-6 signaling. Olamkicept short-circuits signaling when IL-6 binds to its receptor on the surface of cells. A second, placebo-controlled trial using this drug in patients with ulcerative colitis also found it to be efficient in a dose-dependent manner.[169] In addition, IL-6 has been found to promote myocyte atrophy,[170] implying that its inhibition may have beneficial effects on skeletal muscle retention.

However, as with metformin and rapamycin, blocking cytokines may have unintended adverse consequences—most obviously, impairment of the ability to fight infections. Indeed, an analysis of trials on patients with rheumatoid arthritis found that the group receiving tocilizumab, an antibody that neutralizes IL-6, had reduced neutrophils compared with the placebo groups, and a corresponding increase in infections.[171] Perhaps for this reason, ongoing clinical trials that are evaluating anti-inflammatories on aging-related disorders mostly rely on aspirin or IL-1β inhibition (Table 1). So far, a large randomized trial has yielded negative effects on overall mortality if aspirin is administered to non-selected patients >70 years,[172] but several trials are now assessing whether aspirin can prevent cancers in at-risk patients.

For the moment, we must patiently await a spate of new data that will emerge in the next few years on anti-inflammatories and aging-related conditions (Table 1). As in the case of GLP-1-related therapies, it seems likely that a new avenue will emerge to treat conditions ranging from diseases of specific tissues to system-wide deficits resulting from aging processes, but care in dosing and treatment regimens may be important to avoid possible unwanted side effects.

Conclusions

Aging research over the past three decades has unveiled numerous pathways that may be targeted for interventions to slow aging processes and their accompanying diseases. This review has sketched out some of the leading candidates under current scrutiny, although it is possible that other approaches will reveal themselves in the future. We believe that the next few years will present a tipping point, when the most viable approaches will become evident and move us toward a more widespread use of interventions targeting aging processes. While aging is not a disease as prescribed by the FDA, one might expect approval of these interventions to treat aging-fostered diseases. Off-label use may allow a more general application to combat aging and its effects (Figure 2). In the future, the burgeoning field of aging biomarkers, such as DNA methylation, glycanation, metabolomics, and proteomics, may lead to FDA approval of these agents for aging processes per se. Given the momentum in human studies targeting aging, we are optimistic that the course of human health could be fundamentally changed. However, at this point no drug can be recommended for human use before its utility has been confirmed in convincing clinical trials.

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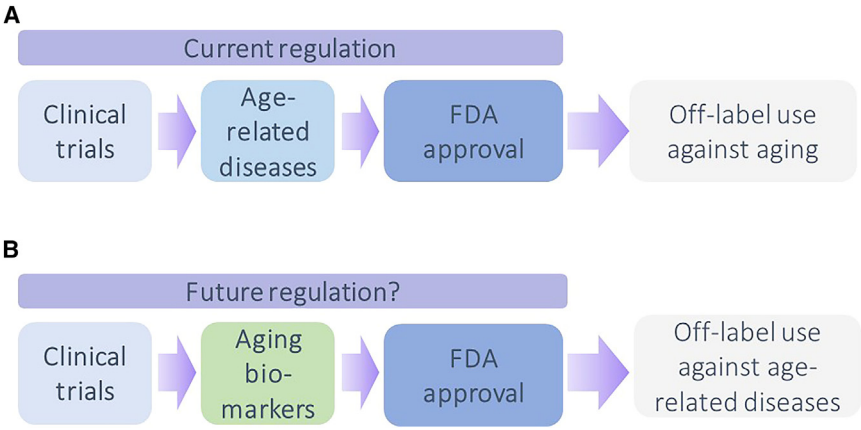

Figure 2. Current and future paths to FDA approval for interventions to slow (or reverse) aging processes

(A) In compliance with current FDA regulations, interventions must be tested for their effects on aging-dependent diseases. If approved, they could be used off label for more widespread applications.

(B) In the future, the acceptance of aging biomarkers would allow drugs to be tested and approved for aging itself, and then be used off label to treat diseases.

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DECLARATION OF INTERESTS

L.G. is a founder of Elysium Health and a founder of Galilei BioSciences. G.K. has been holding research contracts with Daiichi Sankyo, Eleor, Kaleido, Lytix Pharma, PharmaMar, Osasuna Therapeutics, Samsara Therapeutics, Sanofi, Tollys, and Vascage. G.K. is on the Board of Directors of the Bristol Myers Squibb Foundation France. G.K. is a scientific co-founder of everImmune, Osasuna Therapeutics, Samsara Therapeutics, and Therafast Bio. G.K. is in the scientific advisory boards of Hevolution, Institut Servier, Longevity Vision Funds, and Rejuveron Life Sciences. G.K. is the inventor of patents covering therapeutic targeting of aging, cancer, cystic fibrosis, and metabolic disorders. G.K.'s wife, Laurence Zitvogel, has held research contracts with Glaxo Smyth Kline, Incyte, Lytix, Kaleido, Innovate Pharma, Daiichi Sankyo, Pilege, Merus, Transgene, 9 m, Tusk and Roche, was on the on the Board of Directors of Transgene, is a cofounder of everImmune, and holds patents covering the treatment of cancer and the therapeutic manipulation of the microbiota. G.K.'s brother, Romano Kroemer, was an employee of Sanofi and now consults for Boehringer-Ingelheim. The funders had no role in the design of the study; in the writing of the manuscript, or in the decision to publish the results. D.A.S. is a board member, equity owner and inventor on patents licensed to Life Biosciences, an epigenetic reprogramming company and MetroBiotech/EdenRox Sciences and Jumpstart Fertility, both developing molecules for the treatment of diseases by raising NAD levels. Other affiliations are listed https://sinclair.hms.harvard.edu/david-sinclairs-affiliations.

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