Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

Rapamycin (and rapalogs)

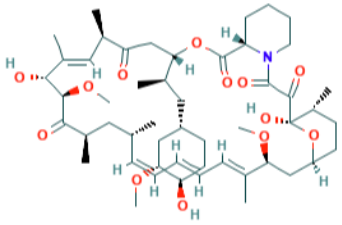
Evidence Summary

Rapamycin extends lifespan in rodents, but healthspan benefits in higher animals have been limited. Human use will likely require a precise dosing regimen guided by biomarkers that don't currently exist.

Neuroprotective Benefit: Cognitive benefits have not yet been observed in humans, and the degree mTOR inhibitors are active in the brain is unclear. There is mixed evidence whether rapamycin can prevent or treat Alzheimer's disease in animal studies.

Aging and related health concerns: Rapamycin consistently extends lifespan in mice, but whether this is due to a slowing of aging is controversial. Healthspan benefits with low doses in higher species have been limited to date.

Safety: Mouth ulcers, hyperglycemia, and hyperlipidemia are common in organ transplant/cancer patients. Minimal adverse events have been observed with low doses in healthy adults to date, but it is still not clear if these lower doses will be safe long-term.

Availability: Rx	**Dose**: Optimal dosing for longevity has not been established. Rapamycin: 1 mg/day Everolimus: 0.5 mg/day (Most common doses used in studies in healthy older populations)	**Chemical formula**: $C_{51}H_{79}NO_{13}$ **Molecular Weight**: 914.2g/mol **Source**: Pubchem  Rapamycin
Half-life: Rapamycin: 57-63 hours Everolimus: ~30 hours Temsirolimus: 17.3 hours (for prodrug; 54.6 hours for active sirolimus)	**BBB**: Probably modestly penetrant, depending on dose (penetrant in rodents)	
Clinical trials: Hundreds of trials completed and ongoing. Approved in organ transplant and cancer. Ongoing trials include low/intermittent dosing for aging related outcomes.	**Observational studies**: Rapamycin/rapalog use has been associated with increased risk for diabetes and dyslipidemia in transplant and cancer patients, but the translatability to a healthy population is unclear.	

What is it?

Rapamycin (also called sirolimus), and its analogs, referred to as rapalogs, are inhibitors of mammalian target of rapamycin (mTOR), a serine/threonine protein kinase found in two protein complexes mTORC1 and mTORC2. mTORC1 senses amino acids, glucose, and oxygen, and controls cellular processes including protein translation, ribosomal biogenesis, and autophagy. mTORC2 is less well-understood, but it functions downstream of insulin/IGF-1 through PI3K and controls cellular processes including metabolism and stress resistance. mTORC1 is acutely sensitive to rapamycin whereas mTORC2 can be inhibited by rapamycin after chronic exposure [1]. In addition, it has been proposed that most of the longevity benefits of rapamycin treatment come from its inhibition of mTORC1 signaling while some but not all common side effects, such as metabolic dysfunction, are due to mTORC2 inhibition [2].

Although rapamycin consistently promotes longevity, more so in females than in males, the mTOR complexes lie in the midst of complicated signaling pathways. For instance, mTORC1 can signal through S6K (to regulate ribosomal biogenesis, protein translation, and lipogenesis), 4E-BP1 (protein translation), and ULK1 (autophagy). In addition, rapamycin and the first generation rapalogs, everolimus and temsirolimus, do not directly bind to mTORC1's catalytic site. Rather it binds to a nearby site and may affect mTORC1 substrates (S6K, 4E-BP1, and ULK1) to different degrees [3]. Adding to the complexity, rapamycin can interact with multiple FKBP proteins which may alter the way it signals through mTORC1 or mTORC2 [4]. Although rapamycin extends lifespan in many animal models, it has been proposed that it does so differently depending on the model (e.g. slowing the aging rate in worms but pushing back the onset of aging in mice) [5].

The complexity of mTOR signaling and its inconsistencies in relation to lifespan and side effects mean that although manipulation of mTOR signaling provides consistent lifespan extension in many animal models, it will be very difficult to find the right treatment paradigm to promote longevity in humans with few side effects.

Approved uses:

Rapamycin (sirolimus): The oral formulation of rapamycin (i.e. Rapamune® from Pfizer) is approved for use as an immunosuppressant to prevent organ rejection in patients aged ≥13 years receiving renal transplants and for the treatment of patients with lymphangioleiomyomatosis (FDA label).
Intravenously administered albumin-bound Sirolimus (Fyarro™ from Aadi Bioscience) is approved for the treatment of adult patients with locally advanced unresectable or metastatic malignant perivascular epithelioid cell tumor (PEComa) (FDA label).
A topical formulation (Hyftor™ from Nobelpharma) is approved for the treatment of facial angiofibroma associated with tuberous sclerosis in adults and pediatric patients 6 years of age and older (FDA label).

Raplogs: Two first generation rapalogs, everolimus (RAD001) and temsirolimus (CCI-779) are also approved for several indications.
Everolimus: The oral formulation of everolimus (Afinator® from Novartis) is approved for the treatment of postmenopausal women with advanced HR+, HER2- breast cancer in combination with exemestane, after failure of treatment with letrozole or anastrozole; adult patients with progressive neuroendocrine tumors of pancreatic origin (PNET) with unresectable, locally advanced or metastatic disease; adult patients with progressive neuroendocrine tumors (NET) of gastrointestinal or lung origin with unresectable, locally advanced or metastatic disease; and adult patients with advanced renal cell

carcinoma after failure of treatment with sunitinib or sorafenib (FDA label). The oral Afinitor Disperz® formulation from Novartis is approved for patients ≥ 1 year of age with tuberous sclerosis (TSC)-associated subependymal giant cell astrocytoma (SEGA) who require therapeutic intervention but cannot be curatively resected; adult patients with TSC-associated renal angiomyolipomas not requiring immediate surgery; and patients ≥ 2 years of age with TSC-associated partial-onset seizures (FDA label). The oral Zortress® formulation from Novartis is approved for the prophylaxis of organ rejection in patients with kidney transplant at low-moderate immunological risk in combination with other drugs, such as basiliximab, cyclosporine (reduced doses) and corticosteroids, and in patients with liver transplant in combination with tacrolimus (reduced doses) and corticosteroids (FDA label).
Temsirolimus: The intravenous formulation of temsirolimus (Torisel® from Pfizer), a prodrug of sirolimus, is approved for the treatment of patients with advanced renal cell carcinoma (FDA label).

Neuroprotective Benefit: Cognitive benefits have not yet been observed in humans, and the degree mTOR inhibitors are active in the brain is unclear. There is mixed evidence whether rapamycin can prevent or treat Alzheimer's disease in animal studies.

Types of evidence:

- 1 pilot trial of rapamycin in healthy older adults
- 1 Phase 1b/2a trial of RTB101 in patients with Parkinson's disease
- Several anatomic/pathology studies in Alzheimer's patients
- Numerous laboratory studies with mixed results

Human research to suggest prevention of dementia and cognitive aging:

To date, there are no completed human studies to support a role for mTOR inhibitors in the preservation of cognitive function with aging. Cognition or brain health-related outcomes have been/are included in some studies testing the effects of mTOR inhibitors on aging-related outcomes in generally healthy older populations. The blood-brain-barrier (BBB) penetrance of rapamycin and other mTOR inhibitors in humans has not been conclusively determined [6]. Rapamycin has low and variable oral bioavailability, a high molecular weight, and can serve as a substrate for the P-glycoprotein efflux pump, but is also highly lipophilic, suggesting it has the potential to be weakly penetrant, as has been observed in rodents [6]. Consequently, low doses may not reach the brain at therapeutically active concentrations. Higher brain levels may occur under conditions of BBB breakdown, as is common with

neurodegenerative disease. Next generation mTORC1 inhibitors with better CNS penetration are currently in development by Aeovin Pharmaceuticals, such as AV078, which recently initiated clinical development for seizures associated with Tuberous Sclerosis Complex.

A pilot randomized, placebo-controlled trial (NCT02874924) assessing the effect of low-dose rapamycin (1 mg/day) in generally healthy older adults (aged 70 to 95) (n=25) for eight weeks did not observe any significant changes on cognitive measures [7]. The study assessed cognition using the Executive Interview-25 (EXIT25), the Saint Louis University Mental Status Exam (SLUMS), and the Texas Assessment of Processing Speed (TAPS).

The effect of mTOR inhibitors (everolimus) on cerebral blood flow in older adults (aged 55 to 80) with insulin resistance will be assessed as a secondary outcome in the randomized, placebo-controlled double-blind Everolimus Aging Study (EVERLAST) (NCT05835999). The study will test both daily (0.5 mg/day) and weekly (5 mg/week) dosing schedules for 24 weeks.

***Clinical research to suggest benefits to patients with dementia or cognitive aging*:**

The Cognition, Age, and Rapamycin Effectiveness – Down Regulation of the mTOR Pathway (CARPE_DIEM) trial was completed, but limited results are currently available (NCT04200911). This open-label pilot study included 10 older adults (55 to 85 years old) with a diagnosis of mild cognitive impairment (MCI) or Alzheimer's disease (CDR 0.5-1) treated with oral rapamycin 1 mg/day for eight weeks. The primary outcome was the BBB penetrance, based on CSF levels, while secondary outcomes included safety, AD biomarkers, and cognition. The reported level of rapamycin in the CSF is listed as 0, suggesting that it was not detected. Statistical analysis was not provided, but numerical levels of AD biomarkers in the CSF tended to increase from baseline, while some plasma biomarkers modestly decreased from baseline. The reported results of inflammatory biomarkers were mixed, with some showing no change, others increasing, and some decreasing, but the directions were not necessarily consistent between CSF and plasma. There were no meaningful changes on cognitive measures.

There are ongoing/planned clinical studies designed to assess the potential of rapamycin or other mTOR inhibitors for the prevention of cognitive decline or neurodegenerative disease.

The Evaluating Rapamycin Treatment in Alzheimer's Disease using Positron Emission Tomography (ERAP) study is a single-arm, open-label, Phase 2a biomarker-driven trial (NCT06022068) designed to assess

whether rapamycin improves cerebral blood flow and cerebral glucose metabolism [6]. The study aims to treat 15 patients with early AD with rapamycin (7 mg weekly) for six months. The primary endpoint is the change in cerebral glucose uptake, based on [^{18}F]FDG PET imaging. Secondary endpoints include changes in cognitive measures, levels of Aβ and tau in the CSF, and MRI-based measures of cerebral blood flow.

The Rapamycin - Effects on Alzheimer's and Cognitive Health (REACH) study is a randomized, placebo-controlled, double-blind trial (NCT04629495) testing 1 mg/day rapamycin (or placebo) for 12 months in older adults (55 to 89 years old) diagnosed with MCI or AD (CDR 0.5-1) (estimated n=40). The primary outcomes include safety (adverse events) and metabolic parameters (changes in glucose level, albumin level, carbon dioxide, and calcium). Secondary outcomes include the BBB penetrance of rapamycin, cognitive outcomes (PACC5, CDR-SoB), functional status (activities of daily living, gait speed, grip strength), neuropsychiatric symptoms, CSF amyloid, cerebral glucose metabolism using FDG-PET, and MRI assessments of brain volume.

Numerous studies report that signaling through mTORC1, but not mTORC2, is upregulated in the postmortem brain tissue of Alzheimer's patients. The degree of overactivity correlates with the severity of disease and distribution of neurofibrillary tangles (and phosphorylated tau) [8; 9; 10; 11; 12], while phosphorylated mTOR was reported to be decreased in the lymphocytes of Alzheimer's patients [13]. One study looked at post-mortem tissue from patients with preclinical Alzheimer's, MCI, and Alzheimer's [14]. Increased activation of mTOR was only present in patients with MCI or Alzheimer's, and this increased activation correlated with the degree of amyloid (R^2=0.36). Similarly, activation of downstream mTOR targets (p-p70S6K and p-4EBP1) was only present in patients with MCI or Alzheimer's. On the other hand, expression of markers of autophagy was reduced in all three patient groups.

A large single-cell transcriptomics study including 2.3 million cells from the prefrontal cortices of 427 individuals with varying degrees of AD pathology and cognitive impairment assessed sex-dependent molecular changes, and identified RPTOR (regulatory associated protein of MTOR complex 1), a key regulator of mTOR as under expressed in the astrocytes from males with Alzheimer's disease [15]. RPTOR can serve as a negative regulator of mTOR kinase activity under low nutrient conditions. Since metabolic stress is commonly observed in the context of neurodegenerative disease, these cells may be chronically over activating mTOR.

A Mendelian randomization study found evidence to support a causal relationship between mTOR-related signaling with the risk for Alzheimer's disease, however, the direction of the associations for the

different mTOR-activated signaling pathways were not always consistent, suggesting interplay with other regulators/pathways [16]. For example, higher levels of Akt (Odds Ratio [OR]: 0.910, 95% Confidence Interval [CI] 0.840 to 0.986) and RP-S6K (OR: 0.910, 95% CI 0.840 to 0.986) were associated with decreased risk, while elevated levels of eIF4E levels (OR: 1.805, 95% CI 1.002 to 1.174) were associated with increased risk.

Parkinson's disease: The catalytic mTOR inhibitor RTB101 (also known as BEZ235) was tested in a clinical trial for Parkinson's disease, sponsored by ResTORbio. The randomized, double-blind, placebo-controlled Phase 1b/2a trial was designed to test the safety and tolerability of 300 mg RTB101 alone or in combination with escalating doses of rapamycin (2 mg, 4 mg and 6 mg) administered once weekly for four weeks in patients with Parkinson's disease. Interim results were reported by the company in 2020, which included data for participants from three cohorts (300 mg of RTB101 alone, 2 mg of rapamycin alone, or a combination of 300 mg RTB101 plus 2 mg of rapamycin) (Press release). They reported the doses were well-tolerated and that 300 mg RTB101 reached the CSF at levels predicted to influence autophagy, based on preclinical studies. The full results have not been published. In 2020, ResTORbio merged with Adicet Bio (Press release), and clinical development of RTB101 appears to have been discontinued.

Mechanisms of action for neuroprotection identified from laboratory and clinical research:

mTOR is involved in a wide variety of cellular processes, and thus many mechanisms have been proposed to mediate the neuroprotective effects of mTOR inhibitors, including the clearance of misfolded proteins by the upregulation of autophagy, improved vasculature by the increase in nitric oxide production, an increase in the translation of chaperone proteins, and regulation of vascular smooth muscle cell proliferation [17; 18; 19; 20; 21; 22].
Consistent with the pleiotropic effects of mTOR signaling, the inhibition of mTOR can also have negative impacts on cognition, by disrupting processes involved in learning and memory. As a result, the impact of modulating mTOR can depend on the baseline status and degree of dysfunction in the various pathways that mTOR regulates. For example, one preclinical study found that some aged Alzheimer's model mice performed poorly on cognitive tests while some performed as well as controls [23]. These cognitive differences were associated with variation in mTOR-mediated autophagic processes. They reported an inverse correlation between amyloid plaque load and mTOR activation with cognition and a positive correlation between expression of autophagy genes and cognition in the Alzheimer's mice. This suggests that the cognitive benefits of mTOR inhibitors may be limited to individuals with excessive

mTOR activation related to particular downstream targets, but this cannot currently be measured *in vivo* in the human brain.
Different mTOR regulated pathways may be differentially impacted by mTOR inhibitors based on dosage and disease state. Consistent with this, the effects of mTOR inhibitors in Alzheimer's disease models vary across studies depending on the model, age, stage of disease, dosing, timing of intervention, and outcomes measured.

In most of the studied Alzheimer's animal models, signaling through mTOR is generally increased [17]. Long-term treatment with rapamycin or genetic reduction of mTOR signaling in both amyloid and tau Alzheimer's models has been shown to partially reduce levels of amyloid beta and phosphorylated tau, reduce cerebral amyloid angiopathy, improve brain tissue integrity, improve vasculature and cerebral blood flow, prevent blood-brain barrier breakdown, increase glucose uptake, reduce vasculature expression of inflammation (cyclophilin A), prevent or rescue cognitive deficits, increase levels of synaptophysin (a synaptic marker), and reduce astrogliosis (a measure of inflammation) [10; 18; 19; 20; 24; 25; 26]. Long-term treatment with rapamycin also prevented blood-brain barrier breakdown and fibrinogen extravasation in a mouse model of vascular cognitive impairment [26]. Short-term treatment (10 weeks or less) also reduced levels of amyloid beta and phosphorylated tau [17; 20]. In a model of gene delivery of htau in the hippocampus, rapamycin treatment reduced neuronal and synaptic loss, activated microglia, but had no effect on tau levels. It also reduced transsynaptic expression of htau [27]. Two studies [28; 29] reported that treatment of two Alzheimer's mouse models (APP/PS1 and P301Stau) with temsirolimus, a rapalog and mTORC1 inhibitor, every two days for 60 days also increased autophagy, decreased amyloid beta and hyper-phosphorylated tau, and improved memory impairment. In non-transgenic adult and aged mice, long-term treatment with rapamycin was reported to improve spatial learning and memory [30; 31]. Treated mice had lower levels of IL-1β (an inflammatory marker) and increased levels of NMDA signaling and phosphorylated CREB (markers of memory). However, rapamycin treatment started at old age failed to improve cognition [31].

Despite these promising results, rapamycin may be a double-edged sword for memory formation. One study reported that two weeks of rapamycin increased levels of amyloid beta in an Alzheimer's mouse model [32]. In addition, signaling through mTORC1 is important for protein translation which is important for memory formation. In support of this, rapamycin treatment of non-diseased control brain slices impaired long-term potentiation (LTP – a measure of synaptic strength) to levels found in untreated Alzheimer's mouse brain slices [9]. Sleep deprivation was also reported to decrease mTORC1 signaling and memory in mice, and increasing mTORC1 signaling prevented memory deficits from sleep

deprivation [33]. mTORC1 signaling was also reported to be reduced in an Alzheimer's mouse model despite its increase in late-stage Alzheimer's post-mortem tissue [9].
One potential explanation is that mTORC1 is changed in different ways in mouse models compared to Alzheimer's patients, which casts doubt on the translational relevance of the mouse models for this pathway [34].

It has also been suggested that mTORC1 signaling is down regulated in early Alzheimer's disease but increases at later stages of the disease [9; 34]. At early stages of disease, lower levels may be beneficial to promote the clearance of pathological misfolded proteins, while at later stages, higher levels of mTORC signaling may help promote neurogenesis and synaptic plasticity [34].

This suggests that similar to what has been observed in other conditions, such as cancer, there may be a limited therapeutic window for intervention. Work in animal models suggests that very early intervention may be needed, but this may be at an earlier stage than when most patients get diagnosed. It is unclear whether it is possible to tailor mTOR inhibition, seeking a "window" of mTOR activity that walks the line between the potential benefit vs harm [30; 35]. Especially because, in the brains of living human beings, we do not have the ability to measure mTOR activity or various cellular pathways targeted by mTOR (e.g. autophagy). Without these biomarkers to guide dosing, it is difficult to see how this will be a promising therapeutic to prevent or treat Alzheimer's patients.

APOE4 interactions: No studies to-date suggest that rapamycin activity will depend on ApoE4 status. One study reported the relationship between mTOR activity and cognitive status in Alzheimer's patients does not depend on ApoE4 status [11].

Aging and related health concerns: Rapamycin consistently extends lifespan in mice, but whether this is due to a slowing of aging is controversial. Healthspan benefits with low doses in higher species have been limited to date.

Types of evidence:

- 5 meta-analyses of adverse health effects of rapamycin/rapalog use in organ transplant/cancer
- 1 meta-analysis of studies assessing musculoskeletal effects of rapamycin/rapalogs
- 4 RCTs on short-term use for vaccine efficacy
- 2 pilot studies of low dose rapamycin in healthy older adults

- 1 pilot study of rapamycin in coronary artery disease
- 1 observational study of off-label rapamycin use
- 2 studies in non-human primates
- 2 studies in client-owned dogs
- Multiple mouse studies on lifespan

Lifespan/Healthspan: BENEFIT IN ANIMAL MODELS BUT TRANSLATABILITY TO HUMANS REMAINS UNCLEAR

Rapamycin extends lifespan in worms, flies, and mice. It was first reported to extend the lifespan of wild-type mice in the NIA's Interventions Testing Program in 2009. Since then, rapamycin or genetic mouse models of decreased mTOR signaling have extended lifespan in many different genetic strains of wild-type mice and have provided benefit in many animal disease models [1; 36]. Whether mTOR inhibitors extend lifespan in higher order species is currently unclear, though there are ongoing studies, such as those in dog and marmoset, aimed at addressing this question. To date, studies using relatively low doses of mTOR inhibitors, primarily rapamycin, have not provided strong evidence for health benefits in these species, though this could stem from suboptimal timing, dosing, or study duration.

Mice: LIFESPAN EXTENSION

Rapamycin is an anti-cancer molecule, and it has been argued that it does not slow aging, per se, but rather reduces the incidence of cancer in rodents. A comprehensive assessment of aging-related phenotypes (over 150 molecular, cellular, histopathological, and functional aging phenotypes in more than 25 different tissues) was conducted in inbred male mice [37]. Young, middle aged, and old mice were treated with rapamycin for 12 months, and many age-related phenotypes were unaffected. In addition, other age-related phenotypes in young animals treated for a brief period also improved. Thus, the study authors concluded, rapamycin does not "slow aging", per se, and instead extends lifespan in an aging-independent manner. However, to date, rapamycin has generally failed as a cancer therapeutic in humans despite intense investment.

Other groups, however, disagree. Just because a drug improves function in young mice, does not mean it is not an anti-aging drug. After all, caloric restriction improves metabolic outcomes in young animals as well as old [38; 39]. Some argue that some of the negative results in Neff et al, (2013)[37] might be due to an inbred strain of male mice used. This idea has not been tested, but it has been reported that the effects of rapamycin on insulin resistance are strain-dependent [40]. Although one study reported

that everolimus and temsirolimus reduce glucose intolerance in male mice, these two rapalogs have not been studied for their effects on longevity [41].

Chronic rapamycin started in mid-life in mice was reported to improve mitochondrial DNA quality [42]. In a mouse model of inflammaging and chronic liver disease (CLD) (NF-κB knockout), chronic rapamycin treatment had no effect on inflammation, CLD, or lifespan. However, it did improve many aspects of healthspan such as reduced frailty, improved long-term memory, neuromuscular coordination, forelimb grip strength, and tissue pathology. In addition, rapamycin reduced the number of senescent cells in the lung and liver. This suggests that rapamycin's beneficial effects on healthspan are uncoupled from its role suppressing inflammation [43].

A meta-analysis of 23 mouse studies compared the impact of rapamycin and dietary restriction on pathogen control, based on short-term survival following an acute infection [44]. Rapamycin was associated with increased post-infection survival (natural logarithm of Hazard Ratio [lnHR]: -0.72, 95% CI − 1.17 to -0.28), while dietary restriction was associated with reduced post-infection survival. However, there was variability across the individual rapamycin studies, which may have been related to differences in timing and dosing.

Whether an intermittent dosing schedule can replicate the benefits to healthspan and lifespan observed with chronic administration while avoiding side effects remains unclear. A study in C3B6F1 hybrid mice found that intermittent dosing with 42 mg/kg rapamycin (in food) administered on an alternating weekly schedule starting at six months of age had less of an impact on health outcomes/age-related pathologies compared to a continuous dosing (42 mg/kg) schedule [45]. Brain levels of rapamycin were much lower with intermittent dosing compared to continuous dosing. Maximum lifespan was similarly extended with both dosing schedules. Median lifespan in males increased by 18% and 28% with intermittent and continuous dosing, respectively, while median lifespan in females increased 14% and 25%, respectively. Many of the outcomes on other health parameters, such as cardiac function, liver pathology, organ fibrosis, and inflammation, varied by sex, with chronic dosing generally having a more pronounced effect. This may be related to sex differences in tissue concentrations of rapamycin. While the impacts on these outcomes may depend on the specific intermittent dosing schedule, dosing level, and species tested, this study suggests that intermittent dosing may not fully replicate the aging-related health benefits observed with chronic dosing. Pilot studies using intermittent and/or low-dose rapamycin in other species (see below) suggest a similar lack of clear benefit.

Dogs: POSSIBLE CARDIAC BENEFIT/NO CLEAR HEALTHSPAN BENEFITS TO DATE

The Dog Aging Project is a research program designed to study aging and longevity in client-owned dogs, co-directed by Matt Kaeberlein, PhD and Daniel Promislow, DPhil.

A pilot study including 24 healthy dogs (aged 6.6 to 12.7 years, weighing 21 to 44 kg) testing rapamycin at a dose of 0.1 mg/kg or 0.05 mg/kg three times weekly for 10 weeks found that rapamycin, but not placebo treatment, was associated with significant changes in systolic and diastolic function, including an improvement in fractional shortening [46]. However, a similar benefit was not observed in a longer duration follow-up study. A masked, placebo-controlled, randomized clinical trial from the Dog Aging Project including 17 healthy client-owned dogs (aged 6 to 10 years, weighing 18 to 36 kg) tested the effects of low-dose intermittent rapamycin (0.025 mg/kg) or placebo administered three times per week for six months [47]. Physical examinations, cardiology examinations, and clinicopathology were assessed at baseline, six, and 12 months. Overall, there were no significant differences in echocardiogram measures, blood pressure changes, or the development of insulin resistance (HOMA-IR scores) between the treatment groups. The small number of participants and variability with included breeds may have limited the ability to detect subtle changes. The dose may also have been too low. There were also no clinically significant adverse events in the dogs following this low-dose rapamycin regimen.

The Test of Rapamycin in Aging Dogs (TRIAD) is a clinical trial designed to test the effects of rapamycin on health and aging in dogs meeting health and behavioral criteria that are at least seven years old (44 to 120 lbs). This trial will test the effects of a higher cumulative dose (0.15 mg/kg) administered once weekly.

Primates: NO CLEAR LIFESPAN/HEALTHSPAN BENEFITS TO DATE

A long-term study testing the effects of rapamycin on longevity and aging-related outcomes in the common marmoset (*Callithrix jacchus),* a non-human primate model*,* is being conducted at the Marmoset Aging Center at UT Health San Antonio. Treatment is initiated around middle age.

One pilot study (n=13) tested the effects of rapamycin over 14 months at 1 mg/kg/day (equivalent to blood levels of 5.2 ng/ml) in the marmosets (initiated at 7.1 to 9.1 years of age). Rapamycin was well-tolerated with no evidence of clinical anemia, fibrotic lung changes, mouth ulcers, metabolic dysfunction, or changes in body weight, daily activity, or most hematological markers [21; 48; 49]. Animals exhibited a small decrease in fat mass and a small increase in the expression of mitochondria-targeted protein chaperones and autophagy in a tissue specific manner (i.e. in skeletal muscle but not liver). A longer-term controlled study assessed the effect of 1 mg/kg/day rapamycin (6 male and 6

female) relative to untreated control (5 male and 5 female) marmosets (initiated at 4.2 to 10.1 years of age) [50]. Hematological analysis following nine months of treatment indicated only small, non-meaningful changes. There were no changes in total white blood cell (WBC) count or most leukocyte subsets (except basophils), fasting blood glucose, cholesterol or triglycerides, or the systemic inflammation marker CRP. Notably, blood levels of rapamycin varied by sex, such that blood levels in males (8.4 ± 1.7 ng/mL) were higher than in females (4.4 ± 0.6 ng/mL).

An epigenetic clock for marmosets was developed by the Horvath lab [51]. This epigenetic clock was used to assess the impact of rapamycin on changes in blood DNA methylation status in middle-aged marmosets. At the time of blood collection, the marmosets (n=17) were, on average, 10 years of age, and had been treated with rapamycin for about 2 to 3.5 years [51]. Compared to control, untreated middle-aged marmosets (n=20), rapamycin was not associated with significant changes in the DNAmAge epigenetic clock. Similarly, an epigenome-wide association study found that none of the individual CpGs sites showed significant differential methylation related to treatment status, after adjusting for multiple comparisons. In general, rapamycin tended to be associated with hypermethylation. The top hypermethylated transcription-related motif with rapamycin was SP1, which is a regulator of the mTORC1/P70S6K/S6 signaling pathway. It is unclear whether the lack of effect on the epigenome was related to biology or experimental design. The authors indicate that it is possible that rapamycin alters the epigenome of particular cell types, as observed in other studies, but not in the blood. Alternatively, the animals may not have been old enough for differences in aging-related genes to become apparent.

The impact of rapamycin on measures related to age-related osteoarthritis was assessed in a subset of marmosets using postmortem fixed limb tissue [52](preprint). The marmosets (n=24) were treated with rapamycin (1 mg/kg/day) starting at 9.2±3.0 years of age, until death (an average of 2.1±1.5 years) and compared to control, untreated marmosets (n=41). Similar to what was observed in prior studies, there were no significant differences in peak body mass, lean and fat mass, blood glucose, LDL cholesterol, or triglyceride levels in rapamycin-treated animals relative to control. Rapamycin treatment had no significant effects on age-related increases in microCT or cartilage osteoarthritis scores. While rapamycin lowered lateral synovium scores primarily in males, it also worsened age-related meniscus calcification in female marmosets. It also tended to decrease subchondral bone thickness in the lateral tibia. The authors speculate that in the context of a proinflammatory environment, mTORC1 inhibition may lead to aberrant calcification and osteogenesis. Rapamycin treatment reduced mTORC1 signaling, based on the reduction of p-RPS6Ser235/36 by approximately 50% relative to controls in the cartilage, meniscus, and fat pad. However, it also led to an increase in mTORC2 signaling, based on a two-fold increase in p-Akt2Ser473 in these tissues, suggesting rapamycin may have led to feedback mTORC2

activation in joint tissues. Overall, this study does not support the use of rapamycin for long-term joint or bone health in primates.
Collectively, these studies do not support a protective role for chronic low-dose rapamycin on aging-related parameters in middle-aged non-human primates, as most parameters have remained unchanged with treatment. However, it remains to be seen whether rapamycin impacts lifespan or health outcomes in late-life marmosets.

Humans
IMMUNE FUNCTION: While rapamycin and everolimus are used chronically as part of immunosuppressant regimens to prevent organ transplant, some clinical studies in older adults suggest that acute/intermittent use of mTOR inhibitors may potentiate/rejuvenate immune function.
One Novartis-funded RCT in healthy, elderly individuals reported that 0.5 mg/day, 5 mg weekly, or 20 mg weekly administration of oral everolimus over six weeks enhanced the immune response to a flu vaccine by about 20% [53]. Many of the mTOR-related assets from Novartis, were licensed to resTORbio for further clinical development.

A phase 2a study in 264 elderly individuals tested combinations of a mTOR catalytic inhibitor (BEZ235, RTB101) and an allosteric inhibitor (RAD001, everolimus). Patients were treated for six weeks (RAD001, 0.1 mg/day; RAD001, 0.5 mg/day; RTB101, 10 mg/day; RAD001, 0.1 mg/day + RTB101, 10 mg/day), were drug-free for two weeks, then were given a flu vaccination. Antibody titers for several strains of influenza were increased after several of the treatments, and infections and respiratory tract infections decreased for two of the treatments [54].
A phase 2b study (NCT03373903) in 652 older adults (≥65 years old) found that treatment with once daily 10 mg RTB101 for 16 weeks during winter led to a significant reduction in the proportion of patients with one or more laboratory-confirmed respiratory tract infections (RTIs) (34/176 [19%] vs 50/180 [28%]) (OR: 0.601, 90% CI 0.391 to 0.922) [55]. However, treatment with RTB101 at 5 mg/day, RTB101 10 mg b.i.d., or RTB101 10 mg/day in combination with RAD001 0.1 mg/day, was not associated with significant reductions in the incidence of RTIs, relative to placebo. Treatment with RTB101 was also not associated with a reduced number of RTI- associated symptoms, though treatment with RTB101 10 mg/day was associated with a reduction in the proportion of participants with laboratory-confirmed severe RTI symptoms (8/176 [5%] vs 17/180 [9%]), and showed greatest benefit in the oldest participants (≥85 years old).
A Phase 3 trial (ACTRN12619000628145) including 1,024 older adults (≥65 years old) treated participants with RTB101 10 mg/day or placebo for 16 weeks during winter [55]. The trial failed to meet its primary

outcome of the proportion of patients with at least one clinically symptomatic respiratory illness, defined as symptoms consistent with an RTI, irrespective of whether an infection was laboratory-confirmed (134/511 [26%] vs 12/510 [25%]). The study authors suggest that the failure to meet this endpoint may have been related to a high proportion of symptoms unrelated to infectious RTIs. The incidence of laboratory confirmed RTIs also did not differ (13% vs 14%), however, the overall incidence of laboratory confirmed RTIs was lower than expected in this study (14% vs 28% in the placebo group of the Phase 2b study), such that the study was underpowered for this endpoint. Gene expression analysis indicated that treatment with RTB101 was associated with the increased expression of interferon (IFN)-induced antiviral genes in the Phase 2b and 3 trials. RTB101 also appeared to exert more protection against RTIs associated with suppressing host IFN immune responses, including coronavirus, and influenza [55]. These trials suggest that intermittent dosing with mTOR inhibitors may modestly potentiate immune responses associated with pathogen control, but the clinical relevance has not been supported thus far. Clinical development of RTB101 for the prevention of RTIs was discontinued following these trials.

Most immune-related parameters were unchanged following administration of rapamycin at a dose of 1 mg/day in 25 healthy older adults in a pilot study [7]. There were some minor decrements in erythrocyte-related parameters, including hemoglobin and hematocrit, to levels slightly below normal, while some other parameters decreased, but remained within the normal range. Rapamycin also led to an increase in a small myeloid subset (CD11b+ $CD3^{low}$) and an increase in circulating T regulatory (Foxp3+ CD4+) cells.

Rates of respiratory illness were similar between rapamycin (equivalent to 1.43 mg or 2.86 mg weekly for 48 weeks) or placebo treated groups in the PEARL trial (NCT04488601) in healthy older adults (n=114) [56].

In a survey of self-reported health outcomes from adults using rapamycin off-label for health/longevity purposes for at least 90 days (n=245), there was a non-significant trend toward higher rates of infection in users relative to non-users (n=172) [57]. There were similar rates of reported covid-19 infections between the groups, and possible differences in the severity of illness depending on the window of use relative to the time of infection. Those who took rapamycin starting prior to the infection tended to have more mild cases, while rates of moderate/severe illness were not impacted in groups that didn't start rapamycin until after the onset of covid-19.

Overall, these studies suggest that prophylactic use of mTOR inhibitors may modestly boost some aspects of immune function, such as interferon responses, that could reduce the risk for severe symptoms of certain subsets of pathogen-borne illness.

PEARL Trial: The Participatory Evaluation of Aging with Rapamycin for Longevity (PEARL) study was a randomized, double-blind, placebo-controlled trial (NCT04488601) testing intermittent, low-dose compounded 5 mg and 10 mg rapamycin (equivalent to 1.43 mg or 2.86 mg of generic formulations) administered weekly in healthy older adults (aged 50 to 85) (n=114) for 48 weeks [23]. The primary outcome was visceral adiposity, as measured by DXA scan. There were non-significant trends toward improvements with rapamycin (5 mg: OR: 1.95, 95% CI 0.66 to 5.79; 10 mg: OR: 1.43, 95% CI 0.48 to 4.2), particularly in females. There were no significant changes on blood panels, epigenetic aging analysis, gut microbiome analysis, or on self-reported surveys of health and well-being. In sex-stratified analysis, women in the 10 mg rapamycin group reported improvements in pain, while there was a trend toward improved bone mineral density in men treated with rapamycin. Overall, the health impacts of this very low dose rapamycin regimen were very minor.

Aging-related conditions: MIXED

When it comes to specific age-related diseases or symptoms, mTOR inhibitors (rapamycin and rapalogs) may either protect or harm. Preclinical studies suggest rapamycin might slow the progression of cancer, improve age-related vascular dysfunction, and reduce markers of cellular senescence [3; 58]. Meanwhile, the development of other age-related conditions like gonadal atrophy and metabolic dysfunction, might be accelerated by rapamycin.

Cancer: POTENTIAL BENEFIT
mTOR inhibitors have been the most intensely tested in the context of cancer. mTOR signaling controls cell growth, proliferation, and survival. The overactivation of PI3K/Akt/mTOR signaling is commonly observed in cancer tissue. Two rapalogs (temsirolimus & everolimus) are approved for the treatment of breast and renal cancer and rapamycin's lifespan extension effects in mice are purportedly due primarily to effects on slowing cancer progression [59]. However, rapamycin/rapalogs have not been as promising as expected as anti-cancer therapeutics, leading to the development of more aggressive drugs [60] including dual mTORC2 & mTORC1 inhibitors. Rapamycin and first generation rapalogs are allosteric inhibitors of mTORC1, and can result in feedback activation of Akt [61]. They also do not inhibit all downstream targets of mTOR to a similar degree, such that they only partially block 4E-BP-dependent

translation and do not reliably inhibit the pro-survival pathways regulated by mTORC2–Akt. This led to the development of second generation ATP-competitive mTOR inhibitors (dual mTORC1 and mTORC2 inhibitors), as well as third generation inhibitors, such as RapaLink, which is a conjugation of rapamycin with second generation mTOR inhibitors, and dual PI3K/mTOR inhibitors [61]. To date, the use of these more potent next generation inhibitors has been limited by their toxicity profiles.
The clinical experience with mTOR inhibitors in the context of overactivated dysregulated mTOR signaling in cancer does not, however, provide insight into whether they may have utility prophylactically for the prevention of cancer. Cancer prevention is thought to be a predominant mechanism by which mTOR inhibitors promote longevity in rodents, however, the evidence to support a similar role in humans is limited. A meta-analysis of 20 RCTs and two observational studies including 39,039 kidney transplant patients found that rapamycin use was associated with a lower overall cancer incidence (incidence rate ratio [IRR]: 0.71, 95% CI 0.56 to 0.90) [62]. However, this effect was primarily driven by the reduced incidence of nonmelanoma skin cancer (IRR: 0.49, 95% CI 0.32 to 0.76), stemming from RCTs comparing rapamycin use with cyclosporine (a known carcinogenic agent). In the full dataset, incidence rates for other cancers were not significantly impacted by rapamycin use, though there were trends of a reduction in risk for kidney cancer (IRR: 0.31, 95% CI 0.08 to 1.23) and increased risk for prostate cancer (IRR: 1.84, 95% CI 0.97 to 3.49). Due to the increased baseline risk of organ transplant patients and confounding use of other therapeutic agents that impact cancer risk, these findings are difficult to interpret, and the translatability to healthy aging populations is unclear.

Diabetes & metabolic dysfunction: POTENTIAL HARM/UNCLEAR
Chronic use of doses of rapamycin/rapalogs used for approved indications, such as transplant and cancer, have been associated with glucose intolerance and insulin resistance. The link with newly-onset diabetes and health implications, however, remains controversial. For example, it has been reported that up to 38% of patients treated with rapalogs after renal transplantation develop new-onset diabetes [63], while other studies find that this trend is not statistically significant (RR: 1.32, 95% CI 0.92 to 1.87) [64]. The risk that stems from use of rapalogs alone is difficult to tease apart from other confounding risk factors in these populations. Renal transplant patients are at heighted risk for both transplant-associated hyperglycemia and newly-onset diabetes, particularly in the first year following the transplant [65]. Additionally, the reported risk related to post-transplant diabetes stems from the combination of mTOR inhibitors with other immunosuppressant agents, many of which are known to be diabetogenic. Thus, the effect may stem from drug-drug interactions. Indeed, other studies find that shifting from some of these other immunosuppressant agents to rapamycin protects against diabetes in

transplant patients. Furthermore, the Grade 1-2 hyperglycemia observed in some cancer patients with the use of rapamycin/rapalogs is reversible and does not negatively impact drug efficacy [65].

Although limited to date, evidence of clinically meaningful diabetes-like metabolic changes has not emerged in studies assessing the effect of low dose daily or intermittent (i.e. weekly) rapamycin or rapalogs (everolimus and RTB101) in healthy older adults [55]. A trend toward an increase in HbA1c (+0.19, 95% CI 0.02 to 0.35) was observed in a pilot study with eight weeks of rapamycin (1 mg/day) use including 25 healthy older adults, but this finding was not accompanied by significant changes on other glucose-related measures [7]. Ultimately, the effects may depend on the baseline metabolic state of the population.

Preclinical models highlight how the effect of mTOR inhibitors on metabolic parameters can depend on the metabolic context [66]. Baseline beta cell function may play a role on these different metabolic outcomes. mTOR inhibitors have been shown to negatively impact pancreatic beta cell mass, impair glucose-induced insulin secretion, and promote glucose intolerance and insulin resistance. However, mTOR inhibitors have also been found to be protective in some diabetes models.

It has been hypothesized that rapamycin-induced hyperglycemia and other metabolic changes are not necessarily pathological, but instead can be beneficial, in certain contexts [65]. These changes may be a mimic of starvation/calorie restriction-induced metabolic adaptions as a reflection of low-nutrient status conditions [65]. Notably, both mTOR inhibitors and prolonged fasting are associated with hyperglycemia and dyslipidemia during periods of feeding. These effects are also reversible with drug cessation, suggesting that they are adaptations to the on-drug state ("pseudo-diabetes"), and not evidence of persistent pathological processes.

Mechanistically, a reduction in Akt signaling can lead to beta cell loss and impair glucose-stimulated insulin secretion [66]. It also leads to the upregulation of gluconeogenic genes in the liver. The negative effects on beta cells primarily stem from the loss of mTORC-mediated Akt signaling. In mice, these effects on insulin production and sensitivity appear to be driven by the inhibition of mTORC2. Since acute rapamycin/rapalog treatment inhibits mTORC1, while chronic treatment inhibits both mTORC1 and mTORC2, it has been hypothesized that use of an intermittent dosing schedule may prevent the induction of insulin resistance and glucose intolerance. However, the relative contributions of mTORC1 and mTORC2 inhibition on these effects in humans remain unclear. Another proposed strategy to mitigate these effects on glucose and insulin is to combine rapamycin with metformin, an antidiabetic medication with purported longevity properties. This strategy has been shown to mitigate rapamycin-related glucose intolerance in some mouse models, without negatively impacting rapamycin's effect on lifespan [67; 68].

The Clinical Evaluation of mTORC1 Inhibition for Geroprotection (Everolimus Aging Study or EVERLAST) (NCT05835999) study is a randomized, placebo-controlled double-blind trial assessing the impacts of everolimus on hallmarks of aging in older adults (ages 55 to 80 years) with insulin resistance (HOMA-IR ≥ 1.5). Participants will be treated with everolimus 0.5 mg/day plus weekly placebo, everolimus 5 mg weekly plus daily placebo, or double placebo for 24 weeks. Baseline measures will be conducted in young healthy untreated participants to be used as a reference comparison. The primary outcome is the change in peripheral insulin sensitivity (metabolic function). Secondary outcomes include safety, mTOR signaling, cardiac function, and cerebral blood flow. Additional exploratory analyses include changes in physical function, cognitive function, and metabolic function.

Cardiovascular disease: POTENTIAL MIXED

Elevations in circulating lipid levels are a common adverse event associated with mTOR inhibitor use. The clinical implications of these changes are unclear, and may depend on the presence or absence of comorbidities and overall cardiovascular risk.

In transplant patients, rapalogs can cause hypercholesterolemia (RR 2.15, 95% CI 1.35-3.41 in kidney transplant patients; for grade 3-4 hypercholesterolemia, RR 6.51, 95% CI 1.48-28.59 in cancer trials) [64], with dyslipidemia reported in up to 66% of patients and hypertension in up to 17% [63]. Evidence of hyperlipidemia has also been observed in clinical trials testing mTOR inhibitors for other conditions, including rheumatoid arthritis, lupus, and tuberous sclerosis [69; 70; 71]. Meanwhile, these effects on lipid parameters have generally not been observed in studies using low dose mTOR inhibitors in healthy older populations [72]. Trends toward increases in VLDL and triglycerides were observed in an eight-week pilot study testing rapamycin (1 mg/day) in older adults [7], but there were no significant differences in blood lipid measures in other studies testing mTOR inhibitors in healthy older adults for up to one year [56]. Significant changes in lipid parameters were also not observed in middle-aged marmosets treated with daily low-dose rapamycin for approximately two to three years [50; 51]. It has been suggested that similar to the observed reversible hyperglycemia, mTOR inhibitor-associated dyslipidemia may be related to the fasting mimicking attributes of these drugs [65]. As such, the potential of these lipid changes to drive adverse cardiovascular outcomes likely depends on baseline cardiometabolic health and other risk factors.

On the other hand, rapamycin has been associated with cardioprotection in a variety of preclinical studies, including the improvement of cardiac function in aged mice [73].

Rapamycin (0.5 mg, 1 mg, or 2 mg/day) was clinically tested in the Mayo Clinic CARE trial (NCT01649960) in older adults (n=13) with coronary artery disease undergoing cardiac rehabilitation for 12 weeks. The

effects on inflammatory markers were mixed and there was no significant effect on frailty in this small pilot study [74].

The effects of low dose rapamycin (1 mg/day for 8 weeks) is being tested in healthy older adults in an open label sub-study of the Rapa & cMRI to Evaluate Cardiac Function trial (NCT04742777). The primary outcome measures are changes in systolic function, diastolic function, aortic cross-sectional area, and aortic distensibility from baseline to eight weeks.

Stents: mTOR inhibitors are used as part of drug-eluting stents that keep narrowed arteries open [75]. mTOR inhibitors are used in this capacity for their antiproliferative properties to prevent restenosis. They do, however, carry a risk for neoatherosclerosis, or the development of atherosclerosis within the stent [76]. There are several active clinical trials assessing the safety and efficacy of this type of intervention. For example, the SORT OUT X (NCT03216733) study is a randomized, open-label trial comparing the safety and efficacy of a combined rapamycin (sirolimus)-eluting and endothelial progenitor cell COMBO Stent with a sirolimus-eluting OSIRO stent in patients treated with percutaneous coronary intervention (n= 3,148). The primary outcomes are device failure and lesion revascularization.

Musculoskeletal health: POTENTIAL MIXED

A systemic review including 14 studies assessing the effect of rapamycin on age-related musculoskeletal diseases found that rapamycin was associated with reduced bone turnover in postmenopausal women, but led to some negative effects on skeletal muscle metabolism in healthy young men [69]. The positive effects on bone health were related to a reduction in osteoclastogenesis (i.e. bone reabsorption). Meanwhile, rapamycin was found to inhibit contraction-induced skeletal muscle protein synthesis, but short-term administration did not have significant effects on basal skeletal muscle protein metabolism. Long term administration of rapalogs in cancer patients was associated with a decrease in skeletal muscle mass. In the context of rheumatoid arthritis, participants that experienced a reduction in disease activity with rapamycin showed an increase in the proportion of anti-inflammatory T regulatory cells. The ability to translate these findings to the potential impact on musculoskeletal aging in healthy populations, however, is unclear. The disruption of mTOR signaling is a common feature of cancer, such that the effects of the disease state and concomitant medication use may impact the effect profile of mTOR inhibitors. Additionally, baseline mTOR activity varies with age, such that the effects of its inhibition on a given tissue, like skeletal muscle, may vary with age and as a function of baseline signaling activity.

The rapamycin study through the Marmoset Aging Center found that long-term (~2 years) use of 1 mg/kg/day rapamycin in late middle-aged marmosets did not meaningfully protect against age-related bone loss or osteoarthritis, but did worsen age-related meniscal calcification in older females [52].

Overall, it appears that the effects on musculoskeletal health are likely to be mixed, with outcomes varying based on population, dose, duration, and context.

The effect of rapamycin (1 mg/day for 16 weeks) in combination with exercise training (unilateral resistance training for 14 weeks) in older men is being assessed in the randomized, single-blind, placebo-controlled Impacts of mTOR Inhibition on Aged Human Muscle (NCT05414292) trial. The primary outcome is change in muscle mass, while secondary outcomes include changes in muscle strength, power, function, protein synthesis, and protein breakdown.
Another study aimed at assessing the impact of rapamycin (6 mg/week for 13 weeks) in combination with an exercise (exercycle based) program is a randomized, double-blind, placebo-controlled, investigator-led (Dr. Brad Stanfield, New Zealand) trial (ACTRN12624000790549) [77]. The study plans to include older sedentary adults (aged 65 to 85). The rationale for weekly dosing is that it will allow for alternating cycles of mTOR activation (needed for protein synthesis) and inhibition (to facilitate autophagy) with the goal of improving muscle health. The primary outcome is change in muscle strength and endurance, assessed by the 30-second chair-stand test. Secondary outcome measures include the 6-minute walk test, handgrip strength, and participant-reported outcomes using the SF-36 survey.

***Fatigue**:* POTENTIAL HARM MAY BE POPULATION SPECIFIC
A meta-analysis of 56 clinical trials in cancer patients reported that mTOR inhibitors (everolimus or temsirolimus) were associated with all-grade fatigue (RR: 1.22, 95% CI 1.08 to 1.38) and high-grade fatigue (RR: 1.82, 95% CI 1.24 to 2.69) [78]. Similarly, another meta-analysis of 18 trials also found that rapalog use was associated with increased risk for all-grade (RR: 1.26, 95% CI 1.09 to 1.46) and high-grade (RR: 1.49, 95% CI 0.99 to 2.24) fatigue in cancer patients [79]. However, fatigue is a common symptom of other cancer medications that may have been used prior to or alongside the mTOR inhibitors, and can be a symptom associated with cancer itself. Clinically significant fatigue has not been reported as an mTOR inhibitor-related adverse event in shorter term studies in generally healthy populations using low/intermittent dosing, thus far.
There is emerging evidence to suggest that mTOR inhibitors may be beneficial in the context of chronic fatigue syndrome through the induction of autophagy. The ability of mTOR inhibition to promote autophagy, reduce fatigue, and improve well-being is being tested in a pilot prospective observational trial (NCT06257420) in patients with Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS), Long-Covid, or other infection-associated chronic conditions. Participants will be prescribed a dose (up to 6 mg/week) of weekly rapamycin and followed for a year.

Gonadal dysfunction: POTENTIAL HARM MAY DEPEND ON DOSE
In men, rapamycin and everolimus have caused erectile dysfunction, testosterone deficiency, and abnormal testes function. In women, they have caused ovarian cysts and abnormal menstrual cycles. The effects appear to be reversible but are not well studied [80]. In rodents, rapamycin has been shown to inhibit spermatogenesis and induce testicular degeneration [59; 81]. The damage was found to be reversible at low doses, but only partially reversible following drug cessation when higher doses were used. Another study found a similar degree of gonadal dysfunction in male mice following the use of either continuous (daily) and intermittent (weekly) rapamycin (42 mg/kg) (from 10 to 24 months of age) [45]. However, this study did not assess the reversibility of these effects and whether that differed based on the dosing scheme.

Ovarian aging: POTENTIAL BENEFIT
Interestingly, rapamycin is now being tested for its potential protective effects on ovarian aging. Short term use of rapamycin led to temporary ovarian dysfunction in young and middle-aged female mice, but subsequently led to a preservation of ovarian lifespan [82]. However, a study in non-human primates found that treatment with rapamycin (0.02mg/kg twice daily, i.m.) for 10 months did not increase primordial and primary follicle pools, and had mixed effects on follicular senescence markers in old (17 to 21-year-old) female macaques [83]. This suggests that rejuvenating capacity may differ by species and depend on the dosing protocol.
A pilot randomized, double-blind, placebo-controlled study (NCT05836025) conducted in 50 women (aged 35-45) in stage 3a of reproductive aging (just prior to perimenopause) were administered 5 mg of weekly rapamycin or placebo for 12 weeks. The study authors reported that this regimen was able to delay ovarian aging up to 20% based on ovarian reserve and hormone levels, though full results have not yet been formally published. Based on these results, a larger trial (VIBRANT II) is being planned, with the goal of including around 1,000 women (Columbia press release).

Oral health**:** POTENTIAL BENEFIT
Rapamycin use has been associated with the development of mouth ulcers, however, recent studies suggest it may also have positive effects on oral health. A survey of health outcomes in adults using off-label rapamycin found that while the majority of participants reported no significant changes to oral health status, 28 (out of 245) reported positive changes [84]. Dental x-rays from a small sample of participants found no evidence of periodontal bone loss over 1 to 4 years of rapamycin use, suggesting that, at least, it does not harm dental health.

A study in mice found evidence of oral rejuvenation with short-term rapamycin use. Old mice (20 months of age) treated with rapamycin (42 ppm in food) for eight weeks exhibited an increase in periodontal bone relative to baseline pretreatment levels and untreated controls [85]. This was accompanied by a decrease in levels of an osteoclast marker (TRAP), suggesting rapamycin reduced bone reabsorption. Rapamycin also altered the inflammatory profile of the oral cavity, leading to a reduction in levels of NF-κB-mediated pro-inflammatory signaling. There were also some changes in the oral microbiome directionally consistent with a more youthful state.
Based on these preclinical results, rapamycin will be tested for its effects on human oral health in the RAPID (Rapamycin in Older Adults with Periodontal Disease) clinical trial. This trial plans to enroll adults over age 50 with periodontal disease.

Safety: Mouth ulcers, hyperglycemia, and hyperlipidemia are common in organ transplant/cancer patients. Minimal adverse events have been observed with low doses in healthy adults to date, but it is still not clear if these lower doses will be safe long-term.

Types of evidence:

- 7 meta-analyses of adverse health effects of rapamycin/rapalog use in organ transplant/cancer/TSC
- 1 meta-analysis of studies assessing musculoskeletal effects of rapamycin/rapalogs
- 4 RCTs on short-term use for vaccine efficacy
- 2 pilot studies of low dose rapamycin in healthy older adults
- 1 pilot study of rapamycin in coronary artery disease
- 1 observational study of off-label rapamycin use
- Many animal studies (some conflicting)

Rapamycin and rapalogs are primarily used in organ transplant and cancer patients. Adverse events of everolimus and rapamycin when given to transplant patients include mouth ulcers, wound-healing complications (at higher doses), lymphedema, hyperglycemia, hypercholesterolemia, and hyperlipidemia [64; 80]. However, cancer and organ transplant patients are usually very sick to begin with and might be on multiple drugs, so it is not clear whether these side effects would be seen in healthy individuals at lower doses [38]. Doses used to extend lifespan in mice are usually free from most of these side effects; however, metabolic dysfunction, gonadal atrophy, and increased incidence of cataracts are common [86]. A safety analysis of patients with tuberous sclerosis and/or

lymphangioleiomyomatosis treated with rapamycin found that the most common adverse event in this population was stomatitis [71].

One study tested the effect of 8 weeks of daily rapamycin (1mg/day) in elderly individuals [7]. There were no changes in lipid levels, glucose levels (though there was a trend for increase HbA1c), insulin levels, or HOMA-IR. There were also no changes in cognitive measures, physical performance, or inflammatory markers (except for an increase in TNFα – though > two dozen markers were analyzed, so it could be a statistical chance). However, several hematological changes were noted including significant decreases in hemoglobin, hematocrit, red blood cell count, red blood cell distribution width, mean corpuscular volume, and mean corpuscular hemoglobin. Although statistically significant, they were not deemed clinically significant. Whether long-term rapamycin treatment would continue to reduce these measures is an avenue being explored in other studies. Blood concentration of rapamycin ranged between 2.5 ng/ml-11.8 ng/ml, which is within the range considered physiologically relevant. Rates of adverse events and serious adverse events were balanced between rapamycin and placebo arms in the PEARL trial testing intermittent low dose rapamycin healthy older adults (aged 50 to 85) (n=114) for 48 weeks [56]. Participants in the rapamycin arms received 5 mg or 10 mg of compounded rapamycin per week, which was equivalent to 1.43 mg and 2.86 mg of generic formulations, respectively. Rates of respiratory illness were similar across groups. Significant changes in laboratory blood measures, including metabolic parameters, were not observed. The only adverse event class that was more common with rapamycin was gastrointestinal symptoms (n=8 rapamycin 10 mg, n=7 rapamycin 5 mg, n=4 placebo).
A survey-based assessment of 245 adults using rapamycin off-label for at least three months, primarily for longevity-related purposes, found that relative to non-users (n=172), mouth ulceration was the only adverse event more common with rapamycin use [57]. Most mouth ulcers were transient, and did not track with dose or duration of use [84]. There was also a non-significant trend toward a higher incidence of infections with rapamycin [57].

In one Novartis-funded RCT, 218 healthy, elderly individuals were given 0.5 mg/day, 5 mg weekly, or 20 mg weekly of everolimus over six weeks before given a flu shot [53]. The low dose (0.5 mg/day) was relatively safe with the most common adverse events including mouth ulcers (11% for 0.5mg/day vs. 5.1% for placebo), cholesterol increase (4% vs. 0%), and LDL increase (4% vs. 0%). In addition, everolimus enhanced the response to the flu vaccine by about 20%. In the Phase 2a trial testing RTB101 alone or in combination with everolimus, serious adverse events were balanced between treatment groups [55]. Hyperglycemia and hypercholesterolemia were lower in the treatment groups while diarrhea was more

common in the treatment group. In subsequent Phase 2 and Phase 3 studies testing RTB101, side effects were generally balanced between placebo and drug groups.

An open-label pilot study (n=13) (NCT01649960) tested rapamycin in elderly patients with coronary artery disease at 0.5 mg/day, 1 mg/day, and 2 mg/day for 12 weeks [74]. Side effects restricted enrollment at the higher dose, such that only one patient was treated with 2 mg/day. However, 0.5 mg/day resulted in few side effects (diarrhea being most prominent), and no patients had serious infections. There were trends towards reductions in markers of SASP (such as p16 and interleukins) in adipose tissue and trends towards an increase in physical performance from baseline.

Current evidence suggests that low doses of rapamycin (and rapalogs) seem to avoid many of the side-effects seen in previous preclinical studies and in earlier clinical trials in cancer or transplant patients. However, it is unknown whether these doses will promote longevity. One preclinical study reported that higher doses led to greater increase in longevity, especially in females [87]. Another preclinical study in mice found that intermittent dosing (42 mg/kg every other week) was less effective than chronic dosing for health and longevity-related measures [45]. Additionally, intermittent dosing did not prevent the rapamycin-associated induction of gonadal pathology in male mice.

Drug interactions: Rapamycin and other rapalogs are metabolized by the CYP450 liver enzymes and interact with the P-glycoprotein intestinal drug efflux pump which means that there are numerous (>700) drug interactions (e.g. 182 major drug interactions with rapamycin).

Sources and dosing:
Determining dosing for rapamycin (and rapalogs) is difficult because their clinical use for organ transplants is restricted to a narrow therapeutic index (too little and you might reject the organ, too high and you get more side effects). The dosing for organ transplants is about 5-15 ng/ml for rapamycin and 3-8 ng/ml in the blood for everolimus [63]. Organ transplant patients are generally started on 2 mg/day of rapamycin and 0.75 mg twice/day for everolimus. The same dose can yield different serum levels in different patients, so the dose may be adjusted based on serum measurements. In cancer therapy and tuberous sclerosis, the doses are generally higher (e.g. 5-10 mg/day for everolimus) [79; 88].

Lower doses of mTOR inhibitors used in healthy older populations (commonly around 0.5 mg/day of everolimus or 1 mg/day rapamycin) appear to only cause mild side effects [7; 55; 56]. However, the

course of treatment in these studies was short, and we just do not know yet whether these doses will provide the longevity benefits that occur with lower species. To date, the studies testing low/intermittent dosing of mTOR inhibitors have not shown prominent effects on health-related measures.

Some studies have explored short-term or intermittent treatment to avoid mTORC2 inhibition that appears to underlie some of the negative side effects of chronic rapamycin treatment [2]. Transient rapamycin treatment (for 6 weeks or 3 months) in middle aged mice extended lifespan, restored the self-renewal of hematopoietic stem cells, and improved immune cell functioning [89; 90], and intermittent treatments [1] reduced some of the negative metabolic side effects of chronic rapamycin treatment. However, another study testing intermittent rapamycin dosing in mice found that it still negatively impacted gonadal function in males [45].

At this time, doses/administration schedules of mTOR inhibitors that could be considered safe for long-term use and promote benefits to lifespan/healthspan have not been established. However, there are clinical studies underway aimed at determining these optimized dosing schemes.

The planned RESTOR [Rapamycin and Everolimus Study Towards Older Rejuvenation]: PK/PD mTORi Inhibition in Older Adults study includes three sub-studies with the goal of finding a dose and dose timing that can be used to safely inhibit mTOR to the levels seen in young healthy persons (NCT06658093).

The Safer mTOR Inhibition for Human Geroprotection (Rapalog Pharmacology or RAP PAC) study (NCT05949658) is a non-randomized, open-label dose-finding trial designed to identify safe and effective weekly dose(s) for the mTOR inhibitors sirolimus and everolimus that intervene on the underlying fundamental biology of aging in healthy older adults (ages 55-89 years).

The Clinical Evaluation of mTORC1 Inhibition for Geroprotection (Everolimus Aging Study or EVERLAST) (NCT05835999) study is a randomized, placebo-controlled double-blind trial assessing the impacts of everolimus on hallmarks of aging in older adults (ages 55 to 80 years) with insulin resistance (HOMA-IR ≥ 1.5). Baseline measures will be conducted in young healthy untreated participants to be used as a reference comparison.

Research underway:

There are several companies developing next generation mTOR inhibitors for clinical use:

Tornado Therapeutics is developing next generation rapalogs specifically targeting mTORC1, as well as potent mTORC1/2 inhibitors. Tornado is a subsidiary of Cambrian BioPharma, a longevity biotech that licensed the portfolio of mTOR targeting compounds from Novartis (Press release). TOR-101, which is intended for use to mitigate viral respiratory tract infections and for oncology indications is currently in the IND-enabling stage (Tornado pipeline).

Aeovian Pharmaceuticals has recently initiated a Phase 1 trial (NCT06205381) for their first-in-class CNS penetrant selective mTORC1 inhibitor, AV078, which is intended for the treatment of tuberous sclerosis complex refractory epilepsy. The company has additional mTORC1 inhibitors in earlier stages of their development pipeline, including the CNS-penetrant inhibitor AV805, and the peripheral inhibitor AV505.

Janssen has acquired Anakuria Therapeutics, Inc., a company formed by Navitor Pharmaceuticals to advance the development of their novel mTORC1 inhibitors in 2022 (Press release). AT-20494 is the lead Phase 1 ready asset for autosomal dominant polycystic kidney disease.

There are several active clinical trials testing approved mTOR inhibitors (rapamycin and everolimus) for use in aging-related conditions:

The Effect of mTOR Inhibition & Other Metabolism Modulating Interventions on the Elderly: Immune, Cognitive, and Functional Consequences is a substudy of the completed Rapa & cMRI to Evaluate Cardiac Function trial (NCT04742777). This open-label study includes 12 adults (70-95 years of age) in generally good health treated with 1 mg/day oral rapamycin for eight weeks. The primary outcome measures are changes in systolic function, diastolic function, aortic cross-sectional area, and aortic distensibility from baseline to eight weeks. The study has an estimated completion date in 2026.

The Rapamycin - Effects on Alzheimer's and Cognitive Health (REACH) study is a randomized, placebo-controlled, double-blind trial (NCT04629495) testing 1 mg/day rapamycin (or placebo) for 12 months in older adults (55 to 89 years old) diagnosed with MCI or AD (CDR 0.5-1) (estimated n=40). The primary outcomes include safety (adverse events) and metabolic parameters (changes in glucose level, albumin level, carbon dioxide, and calcium). Secondary outcomes include the BBB penetrance of rapamycin,

cognitive outcomes (PACC5, CDR-SoB), functional status (activities of daily living, gait speed, grip strength), neuropsychiatric symptoms, CSF amyloid, cerebral glucose metabolism (FDG-PET), and brain volume (MRI). The trial has an expected completion date in 2026.

The Evaluating Rapamycin Treatment in Alzheimer's Disease Using Positron Emission Tomography (ERAP) study is an open-label trial testing a weekly oral dose of 7 mg rapamycin for six months in older adults (50 to 80 years old) diagnosed with MCI or AD (CDR≤1 and MoCA ≥ 18) (n=15) (NCT06022068). The primary outcome is the change in cerebral glucose metabolism, measured using [^{18}F]FDG-PET. Secondary outcomes include safety, CSF AD biomarkers (Aβ42, total tau, ptau), cerebral blood flow, rapamycin levels, and cognition (MoCA). Additional exploratory outcomes include neuropsychological and functional assessments. The trial has an expected completion date in 2025.

The planned RESTOR [Rapamycin and Everolimus Study Towards Older Rejuvenation]: PK/PD mTORi Inhibition in Older Adults study includes three sub-studies with the goal of finding a dose and dose timing that can be used to safely inhibit mTOR to the levels seen in young healthy persons (NCT06658093). The first substudy will include untreated healthy young participants (ages 20-30 years) to measure mTOR activity levels. The second substudy will be an open-label, adaptive dose-finding study that will include healthy older adults (ages 65-90 years) treated with rapamycin or everolimus using daily or intermittent dosing for six weeks to determine the optimal dosing paradigm to restore mTOR to 'youthful' levels. The third substudy will be a randomized, blinded, placebo-controlled trial in healthy older adults (ages 65-90 years) treated with the optimized daily dose or the optimized intermittent dose of an mTOR inhibitor (rapamycin or everolimius), which may differ based on sex, for six months (with an additional six month follow-up period). Primary outcomes include PK and PD (mTORC1 and mTORC2 activity), and levels of serum soluble ICAM-1. Secondary outcomes include effects on adipose tissue and muscle tissue. The trial has an estimated completion date in 2029.

mTOR as Mediator of Insulin Sensitivity Study (NCT05233722) is a non-randomized, blinded, crossover trial assessing the role of mTOR in mediating enhancement of muscle insulin sensitivity following a single bout of exercise in healthy young men (ages 22-35 years). Participants (n=10) will be administered rapamycin (16 mg) or placebo (800 mg calcium tablets), then 2 hours later perform a single bout of knee-extensor exercise for 1 hour. Insulin action towards muscle glucose uptake and protein synthesis will be assessed at four hours post exercise through a 2 hour euglycemic hyperinsulinemic clamp. The primary outcome is insulin stimulated muscle glucose uptake, while secondary outcomes include insulin stimulated muscle protein synthesis (based on incorporation of ^{13}C6-phenylalanine in muscle biopsies),

and posttranslational modification of proteins in muscle biopsies (via mass spectrometry-based phosphoproteomic analysis). The trial has an expected completion date in 2029.

The Characterization of mTOR Inhibitor Pharmacokinetics and Pharmacodynamics in Older Adults study (NCT06727305) is a planned randomized, open-label trial testing mTOR inhibitors in community-dwelling older adults (ages 65 to 80 years). Particpants (n=60) will receive rapamycin or everolimus at a dose of 0.5 mg, 1 mg, or 2 mg per day for two weeks, and then following PK/PD testing, dose adjustments will be made to obtain stable blood levels of 5-7 ng/ml for three months of treatment. Assessments of phenotypic biomarkers of aging based on SASP (senescence-associated secretory phenotype) index score will be assessed at three months follow-up. Exploratory analyses will also measure laboratory biomarkers (ESR, CRP, S6K activity, mitochondrial function, metabolomics) and functional biomarkers of aging (walking speed, chair stand, standing balance, grip strength). The trial has an estimated completion date in 2027.

The Safer mTOR Inhibition for Human Geroprotection (Rapalog Pharmacology or RAP PAC) study (NCT05949658) is a non-randomized, open-label dose-finding trial designed to identify safe and effective weekly dose(s) for the mTOR inhibitors sirolimus and everolimus that intervene on the underlying fundamental biology of aging in healthy older adults (ages 55-89 years). Participants (n=72) will receive rapamycin or everolimus at a dose of 5 mg, 10 mg, or 15 mg once weekly for six weeks. The primary outcome is dose limiting toxicities. Secondary outcomes include PK measures, PD measures (mTOR signaling), and metabolic measures (insulin sensitivity, glucose tolerance, glucose variability, metabolites, lipid species, transcriptomics). The trial has an estimated completion date in 2028.

The Impacts of Mechanistic Target of Rapamycin (mTOR) Inhibition on Aged Human Muscle (NCT05414292) study is a randomized, single-blind, placebo-controlled trial designed to assess the effects of rapamycin (Rapamune) at a dose of 1 mg/day for 16 weeks in combination with a 14 week unilateral resistance exercise training program in men (n=16) over age 50. The primary outcome is change in muscle mass, while secondary outcomes include changes in muscle strength, power, function, protein synthesis, and protein breakdown. The trial has passed its estimated completion date, which was in 2024.

RAPA-EX-01 is a planned investigator led single-center, randomized, double-blind, placebo-controlled, trial aimed at assessing the impact of rapamycin (6 mg/week for 13 weeks) in combination with an exercise (exercycle based) program in older sedentary adults (aged 65 to 85) (n=40)

(ACTRN12624000790549). The primary outcome is change in muscle strength and endurance, assessed by the 30-second chair-stand test. Secondary outcome measures include adverse events, the 6-minute walk test, handgrip strength, and participant-reported outcomes using the SF-36 survey.

The Clinical Evaluation of mTORC1 Inhibition for Geroprotection (Everolimus Aging Study or EVERLAST) (NCT05835999) study is a randomized, placebo-controlled double-blind trial assessing the impacts of everolimus on hallmarks of aging in older adults (ages 55 to 80 years) with insulin resistance (HOMA-IR ≥ 1.5). Participants will be treated with everolimus 0.5 mg/day plus weekly placebo, everolimus 5 mg weekly plus daily placebo, or double placebo for 24 weeks. Baseline measures will be conducted in young healthy untreated participants to be used as a reference comparison. The primary outcome is the change in peripheral insulin sensitivity (metabolic function). Secondary outcomes include safety, mTOR signaling, cardiac function, and cerebral blood flow. Additional exploratory analyses include changes in physical function, cognitive function, and metabolic function. The trial has an expected completion date in 2026.

Low Dose Rapamycin in ME/CFS, Long-COVID, and Other Infection Associated Chronic Conditions (NCT06257420) study is a prospective observational study including ME/CFS and Long-COVID patients with and without serological evidence of autophagy disruption prescribed weekly rapamycin (at a max dose of 6 mg/week). The primary outcomes are questionnaires related to function and well-being after one year, including the Short Form (36) Health Survey, Multidimensional Fatigue Inventory (MFI), and the Bell Activity Scale. The secondary outcome is the change in mTOR activation panel and blood markers involved in autophagy function. The trial has an expected completion date in 2026.

Search terms:

Pubmed, Google: mTOR inhibitor, rapamycin, everolimus, temsirolimus

- Alzheimer's, cognition, neurodegeneration, cardiovascular, metabolism, aging, autophagy, lifespan, healthspan, cancer, diabetes, longevity, aging, intermittent dosing, clinical trials, meta-analysis, safety

Websites visited for: mTOR inhibitors

- Clinicaltrials.gov (rapamycin, everolimus, temsirolimus, BEZ235, AV078)
- DrugAge (rapamycin)
- PubChem (rapamycin, everolimus, temsirolimus, BEZ235)

- Drugs.com (mTOR inhibitors, rapamycin, everolimus, temsirolimus)
- DrugBank.ca (rapamycin, everolimus, temsirolimus, BEZ235)

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