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

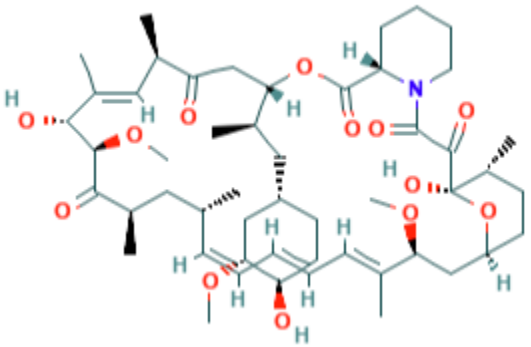
Evidence Summary

Rapamycin can extend lifespan in rodent species and could either protect or harm the brain. Treatment in humans will likely require a precise dosing regimen guided by biomarkers that don't currently exist.

Neuroprotective Benefit: 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 worms, flies and mice, but whether these effects are due to a slowing of aging is controversial.

Safety: Adverse events are common in organ transplant and cancer patients, but it is still not clear if lower doses in healthy patients will prevent common side-effects or will be safe long-term.

Availability: Available with a prescription	**Dose**: Rapamycin – 1mg/day Everolimus – 0.1-.05mg/day (doses used in non-transplant studies)	**Chemical formula**: $C_{51}H_{79}NO_{13}$ **Molecular Weight**: 914.2g/mol **Source**: Pubchem  Rapamycin
Half life: 57-63 hours (rapamycin)	**BBB**: Possibly penetrant (in animals)	
Clinical trials: Many ongoing for cancer	**Observational studies**: None	

What is it?

Rapamycin (and the related molecules, rapalogs) is an inhibitor 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 (Arriola Apelo and Lamming, 2016) 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 (Lamming 2012).

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 they bind to a nearby site and may affect mTORC1 substrates (S6K, 4E-BP1, and ULK1) to different degrees (Xie et al, 2016). Adding to the

complexity, rapamycin can interact with multiple FKBP proteins which may alter the way it signals through mTORC1 or mTORC2 (Schreiber et al, 2015). 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) (Garratt et al, 2016).

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.

Neuroprotective Benefit: There is mixed evidence whether rapamycin can prevent or treat Alzheimer's disease in animal studies.

Types of evidence:

- No meta-analyses & no clinical trials or observational studies
- Several anatomic/pathology studies in Alzheimer's patients
- Numerous laboratory studies with opposing results

Human research to suggest prevention of dementia and cognitive aging:
None.

Clinical research to suggest benefits to patients with dementia or cognitive aging.
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) (An et al, 2003; Ma et al, 2010; Li et al, 2005; Yates 2013; Sun 2014), while phosphorylated mTOR was reported to be decreased in the lymphocytes of Alzheimer's patients (Paccalin et al, 2006). Tramutola et al (2015) looked at post-mortem tissue from patients with preclinical Alzheimer's, mild cognitive impairment (MCI), and Alzheimer's disease. Increase activation of mTOR was only present in patients with MCI or Alzheimer's, and this increased activation correlated with the degree of amyloid accumulation (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.

Mechanisms of action for neuroprotection identified from laboratory and clinical research.
In Alzheimer's animal models, signaling through mTOR is increased (Caccamo et al, 2010). Long-term treatment with rapamycin or genetic reduction of mTOR signaling in both amyloid and tau Alzheimer's models partially reduced levels of amyloid beta and phosphorylated tau, reduced cerebral amyloid angiopathy, improved brain tissue integrity, improved vasculature and cerebral blood flow, prevented blood-brain barrier breakdown, increased glucose uptake, reduced vasculature expression of inflammation (cyclophilin A), prevented or rescued cognitive deficits, increased levels of synaptophysin (a synaptic marker), and reduced astrogliosis (a measure of inflammation) (Spilman et al, 2010; Lin et al, 2013; Ozcelik et al, 2013; Caccamo et al, 2014; Lin et al, 2015; Van Skike et al, 2017; Lin et al, 2017). Long-term treatment with rapamycin also prevented blood-brain barrier breakdown and fibrinogen extravasation in a mouse model of vascular cognitive impairment (Van Skike et al, 2017). Short-term treatment (10 weeks or less) also reduced levels of amyloid beta and phosphorylated tau (Caccamo et al, 2010; Ozcelik et al, 2013). 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 (Siman et al, 2015).

Two studies (Jiang et al, 2014a; Jiang et al, 2014b) 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 hyperphosphorylated tau, and reduced memory impairment.

Vartak et al (2019) found that some aged Alzheimer's mice performed poorly on cognitive tests while some performed as well as controls. 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 Alzheimer's mice. This suggest that mTOR may be a promising target for cognitive decline, but it may have to be given to individuals with excessive mTOR activation (which cannot currently be measured in the brain).

Many mechanisms have been proposed to mediate these benefits: 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 (Caccamo et al, 2010; Spilman et al, 2010; Lin et al, 2013; Ozcelik et al, 2013; Lelegren et al, 2016; Galvan and Hart, 2015). Given mTORs central role in many cellular processes, this wide range of potential mechanisms is not surprising.

In non-transgenic adult and aged mice, long-term treatment with rapamycin was reported to improve spatial learning and memory (Halloran et al, 2012; Majumder et al, 2012). 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 (Majumder et al, 2012).

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 (Zhang et al, 2010). 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 (Ma et al, 2010). Sleep deprivation was also reported to decrease mTORC1 signaling and memory in mice, and increasing mTORC1 signaling prevented memory deficits from sleep deprivation (Tudor et al, 2016).

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 (Ma et al, 2010). The authors of this paper speculated that perhaps mTORC1 signaling is downregulated in early Alzheimer's disease but increases at later stages of the disease. This idea, while not yet confirmed, casts doubt on the ability of rapalogs to prevent Alzheimer's when given at early stages of disease. Another interpretation is that mTORC1 is changed in different ways in mouse models vs Alzheimer's patients, casting doubt on the translational relevance of the mouse models for this pathway.

It may be possible to tailor mTOR inhibition, seeking a "window" of mTOR activity that walks the line between the potential benefit vs harm (Halloran et al, 2012; Bove et al, 2011). However, in the brains of living human beings, we do not have the ability to measure mTOR activity or various the cellular pathways targeted by mTOR (e.g. autophagy).

APOE4 interactions:
No studies to-date suggest that rapamycin activity will depend on E4 status. One study reported the relationship between mTOR activity and cognitive status in Alzheimer's patients does not depend on E4 status (eg. Yates 2013).

Aging and related health concerns: Rapamycin consistently extends lifespan in worms, flies and mice, but whether these effects are due to a slowing of aging is controversial.

Types of evidence:

- Several RCTs on short-term use for vaccine efficacy
- 1 pilot study in non-human primates
- Multiple mouse studies on lifespan

Lifespan/Healthspan

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 (Arriola Apelo and Lamming, 2016; Kennedy and Lamming, 2016).

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. Neff et al, (2013) conducted a comprehensive assessment of aging-related phenotypes (over 150 molecular, cellular, histopathological, and functional aging phenotypes in more than 25 different tissues) in inbred male mice. 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, Neff et al, (2013) concluded, rapamycin does not "slow aging", per se, and instead extends lifespan in an aging-independent manner.

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 (Leslie 2013, Wilkinson et al, 2012). Some argue that some of the negative results in Neff et al, (2013) 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 (Lamming et al, 2013). 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 (Lamming 2016).

Chronic rapamycin started in mid-life in mice was reported to improve mitochondrial DNA quality (Bielas et al, 2018). 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 (Correia-Melo et al, 2019).

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 a non-human primate model, the marmoset. 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. 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). However, longevity was not studied (Ross et al, 2015; Tardif et al, 2015; Lelegren et al, 2016; Sills et al, 2018).

One Novartis-funded RCT in healthy, elderly individuals reported that 0.5mg/day, 5mg weekly, or 20mg weekly of everolimus over 6 weeks enhanced the immune response to a flu vaccine by about 20% (Mannick et al, 2014). ResTORbio, took over Novartis's assets to continue development of the mTOR inhibitors.

A phase 2a study in 264 elderly individuals tested combinations of an mTOR catalytic inhibitor (BEZ235, RTB101) and an allosteric inhibitor (RAD001, everolimus). Patients were treated for 6 weeks (RAD001, 0.1mg/day; RAD001, 0.5mg/day; BEZ235, 10mg/day; RAD001, 0.1mg/day + BEZ235, 10mg/day), were drug-free for 2 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 (Mannick et al, 2018). A phase 2b study in 652 older adults also suggested that 16 week treatment with BEZ235 at 10mg/day (but not BEZ235, 5mg/day; BEZ235, 10mg bid; or BEZ235, 10mg/day + RAD001, 0.1mg/day) also reduced the incidence of respiratory tract infections (OR: 0.601; p=0.025) (presentation). However, a large phase 3 study in 1,024 patients failed to show an effect (press release). In the phase 2 studies, serious adverse events were balanced between treatment groups. Hyperglycemia and hypercholesterolemia were lower in the treatment groups while diarrhea was more common in the treatment group.

ResTORbio has shifted its focus to Parkinson's disease and is currently running a 4-week phase 1b/2a study in Parkinson's patients with different combinations of BEZ235 and rapamycin.

Preclinical studies also suggest rapamycin might also slow the progression of cancer, improve age-related vascular dysfunction, and reduce markers of cellular senescence (Xie et al, 2016; Lesniewski et al, 2016). Other age-related conditions like gonadal atrophy, metabolic dysfunction, and cataracts might be accelerated by rapamycin. More detail on rapalogs' effects on some of these different conditions are below.

- ***Cancer***: Two rapalogs (temsirolimus & everolimus) are approved for the treatment of breast and renal cancer. Rapalogs have not, however, been as promising as expected, leading the development of more aggressive drugs (Feldman & Shokat 2010) including dual mTORC2 & mTORC1 inhibitors
- ***Diabetes & metabolic dysfunction***: Up to 38% patients treated rapalogs after renal transplantation develop new-onset diabetes (Shihab & Kaplan 2014). The risk in kidney transplant patients is similar but not quite significant (RR 1.32, 95% CI 0.92-1.87) (Murakami 2014). Studies at doses lower than those used for organ transplant do not currently suggest it may increase diabetes risk.
- ***Cardiovascular disease:*** In transplant patients, rapalogs can cause hypercholesterolemia (eg. 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) (Murakami 2014), with dyslipidemia reported in up to 66% of patients and hypertension in up to 17% (Shihab & Kaplan 2014). On the other hand, mTOR inhibition was proposed to delay or prevent atherosclerosis, possibly when given in combination with statins or metformin (Martinet 2014).
- ***Sarcopenia***: Short clinical trials report that rapamycin can block protein synthesis in muscles (eg. Dickinson 2011). Sarcopenia has not been reported as a side effect of rapamycin but it might not have been measured and the fatigue caused by rapalogs might be related to muscle function. Sarcopenia is associated with defects in mTOR signaling (Sakuma 2015).
- ***Fatigue****:* Clinical trials in cancer patients report that mTOR inhibitors cause all-grade fatigue (RR 1.22, 95% CI 1.08-1.38) and high-grade fatigue (1.82, 1.24-2.69). The risk might be higher with everolimus than temsirolimus (Peng 2015) but another meta-analysis did not find this difference (Abden 2015).
- ***Gonadal dysfunction***: 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 (Kaplan 2014). Mouse studies also report testicular degeneration (Ehninger 2014)
- ***Tendon health***: One rodent study reported that rapamycin improved tendon health in old mice (reviewed in Enhinger 2014).
- ***Cataracts***: Long-term rapamycin extended lifespan but caused cataracts in mice (Wilkinson 2012).

Safety: Adverse events are common in organ transplant and cancer patients, but it is still not clear if lower doses in healthy patients will prevent common side-effects or will be safe long-term.

Types of evidence:

- Many clinical studies and related meta-analyses in patients with organ transplantation and/or cancer.
- 1 RCT in healthy elderly
- Several RCTs with short-term use for vaccine efficacy
- 2 unpublished open-label studies in elderly individuals
- 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 (Kaplan et al, 2014; Murakami et al, 2014). 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 (Leslie 2013). 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 (Johnson and Kaeberlein, 2016).

One study tested the effect of 8 weeks of daily rapamycin (1mg/day) in elderly individuals. There were no changes in lipid levels, glucose levels (though there was a trend for increased 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 remains to be explored. Blood concentration of rapamycin ranged between 4.7ng/ml-11.8ng/ml (Kraig et al, 2018).

In one Novartis-funded RCT, 218 healthy, elderly individuals were given 0.5mg/day, 5mg weekly, or 20mg weekly of everolimus over 6 weeks before given a flu shot. The low dose (0.5 mg/day) was relatively safe with the most common adverse events including mouth ulcers (11% 0.5mg/day vs. 5.1%

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% (Mannick et al, 2014). In subsequent phase 2 and phase 3 studies, side effects were generally balanced between placebo and drug groups with an increase in diarrhea in the mTORC inhibitor groups – though the full results of these studies are not published.

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, or what side-effects would persist with more chronic dosing. One preclinical study reported that higher doses led to greater increase in longevity, especially in females (Miller 2014).

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 3-8ng/ml in the blood for everolimus (Shihab et al, 2014). Organ transplant patients are generally started on 2mg/day of rapamycin and 0.75mg 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. 5-10mg/day for everolimus) (Sosongko et al, 2016, Abdel-Rahman and Fouad, 2015).

In addition, 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 drug interactions (e.g. 214 major drug interactions with rapamycin).

Lower doses, such as those taken in the ResTORbio/Novartis or CAD trial (0.5mg/day of everolimus or rapamycin, respectively) appear to only cause mild side effects. However, the course of treatment in these studies was short, and we do not know yet whether these doses will provide the longevity benefits that occur with lower species.

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 (Lamming et al, 2012). 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 (Chen et

al, 2009; Bitto et al, 2016), and intermittent treatments (Arriola Apelo et al, 2016) reduced some of the negative metabolic side effects of chronic rapamycin treatment.

Future research:
Rapamycin is being tested in canine through the Dog Aging Project. Companies working on rapalogs for anti-aging and/or Alzheimer's conditions include: Rapamycin Holdings (primarily for dogs) and Aeovian PHarmaceuticals, resTORbio (currently in clinical trials for Parkinson's), and Navitor Pharmaceuticals.

One study (NCT04200911) is testing the blood brain barrier penetrance of rapamycin in patients with Alzheimer's disease.

Search terms:
Pubmed:
Rapamycin with aging, longevity, alzheimer

Clinicaltrials.gov
Rapamycin, everolimus with aging/Alzheimer/cadiac