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.

S6K1 Inhibitors

Evidence Summary

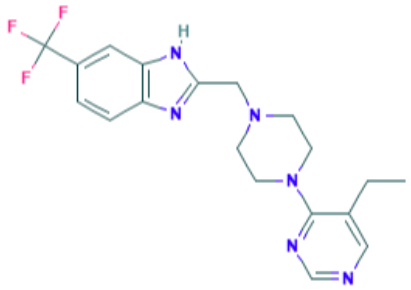
The safety of the available inhibitors is unknown but inhibiting S6K1 may provide some of the benefits for age-related diseases without the immune or all the metabolic effects of rapalogs.

Neuroprotective Benefit: Multiple preclinical studies suggest a potential benefit in neurological conditions, though the direct evidence that an inhibitor would be beneficial for Alzheimer's disease is lacking.

Aging and related health concerns: S6K1 inhibitors may be beneficial in certain cancers associated with an increased expression of S6K1, and the potential of S6K1 inhibitors as a geroprotector is interesting.

Safety: Preclinical studies suggest some side effects on metabolism, but effects in humans are not known.

PF-470861

Availability: Not available as a drug (research grade only)	**Dose:** Up to 75mg/kg in mice	**Chemical formula:** $C_{19}H_{21}F_3N_6$ **MW:** 390.4g/mol  **Source:** Pubchem
Half life: Not reported	**BBB:** Penetrant (in animals)	
Clinical trials: None	**Observational studies:** None	

FS-115

Availability: Not available	**Dose:** Up to 250mg/kg per day in mice	**Chemical formula:** Not disclosed **MW:** Not disclosed
Half life: 0.83 (IV in mice)	**BBB:** Penetrant (in animals)	
Clinical trials: None	**Observational studies:** None	

What is it?

S6K1 is a protein kinase downstream of mTOR that feeds back onto the insulin signaling pathway – two pathways implicated in longevity. Activation of mTOR activates S6K1 which targets the S6 ribosomal protein. S6K1 regulates cell growth and proliferation by modulating protein translation. Additionally, activation of S6K1 also phosphorylates insulin receptor substrate (IRS) 1 and IRS 2, decreasing insulin signaling (Selman et al, 2009). In addition to longevity, researchers have implicated the S6K1 pathway in Alzheimer's disease, cancer, and metabolic diseases.

There are two forms of S6K1, p70S6K1 and p85S6K1. p85 is located in the nucleus, p70 is cytoplasmic. This report focuses on the role of p70S6K1, and throughout this report, S6K1 will refer to p70S6K1. S6K1 can be activated by phosphorylation at eight sites: Thr (T) 229, Ser (S) 371, T389, S404, S411, S418, T421,

and S424. Its activation begins with phosphorylation of S411, S418, S421, and S424, which exposes an internal region allowing for the phosphorylation of T389 (by mTOR) and T229 (by PDK1). Multiple proteins are reported to regulate S6K1 (Tavares et al, 2015).

There are multiple mechanisms through which S6K1 activation may be directly related to Alzheimer's disease. S6K1 may directly phosphorylate tau. In addition, since S6K1 regulates protein translation, inhibiting S6K1 may reduce the expression of BACE1 (which cleaves amyloid precursor protein at the start of the beta-amyloid pathway) and tau.

PF-4708671 is the first tool compound that is a specific S6K1 inhibitor. FS-115 is a more brain penetrant S6K1 inhibitor and is under development for cancer from Sentinel Oncology.

Neuroprotective Benefit: Multiple preclinical studies suggest a potential benefit in neurological conditions, though the direct evidence that an inhibitor would be beneficial for Alzheimer's disease is lacking.

Types of evidence:

- Five studies of S6K1 expression in post-mortem tissue
- Two genetic reduction studies for cognition or Alzheimer's disease
- Multiple preclinical studies of PF-4708671 in different neurological conditions

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function?

None

Human research to suggest benefits to patients with dementia

SK61 phosphorylation and activity (but not S6K1 levels) were increased in post-mortem tissue from Alzheimer's patients (Caccamo et al, 2015). Additionally, S6K1 phosphorylation at T421/S424 and T389 was increased in Alzheimer's patients, phosphorylation at T421/S424 correlated with Braak staging tau pathology, and S6K1 phosphorylation may increase before the formation of tau tangles (An et al, 2003; Sonoda et al, 2016; Pei et al, 2006). Another study reported moderate S6K1 phosphorylation at S240/S244 in neurons with ptau staining in the hippocampus of patients with Alzheimer's disease (Klingebiel et al, 2017).

However, other studies found reduced S6K1 phosphorylation at T389 in an Alzheimer's animal model and in Alzheimer patients' lymphocytes. S6K1 phosphorylation at T421/S424 may also be linked to ERK1/ERK2 signaling rather than mTOR signaling (Lafay-Chebassier et al, 2005), or may be mediated through the PI3K pathway (Zhou et al, 2008).

Mechanisms of action for neuroprotection identified from laboratory and clinical research:
Genetic reduction of S6K1 in an Alzheimer's animal model improved LTP and synaptic density, improved cognition, reduced amyloid and tau levels, and reduced BACE1 levels and activity (Caccamo et al, 2015). However, S6K1 regulates protein synthesis which is important for memory formation. In healthy animals, complete genetic elimination of S6K1 impaired memory formation (Antion et al, 2008). Therefore, similar to inhibition of mTOR, the proper amount of S6K1 inhibition will have to be determined.

In Alzheimer's animal models, pS6K1 has both been reported to be increased (at T421/S424) and decreased (at T389) (Zhou et al, 2008; Lafay-Chebassier et al, 2005; Damjanac et al, 2008).

In cell culture studies, zinc-induced phosphorylation of S6K1 increased the accumulation of phosphorylated tau (An et al, 2003), and pS6K1 phosphorylated tau at S262, T212 and S214, may directly interact with tau, and may increase the expression of tau (Pei et al, 2006). Cell culture studies have also suggested that exposure to amyloid can transiently increase pS6K1 (Zhou et al, 2008).

S6K1 inhibitors in neurological conditions
Pearce et al (2010) reported that PF-4708671 is an inhibitor of S6K1 and suppresses S6K1 activity in response to IGF-1. However, PF-4708671 did promote S6K1 phosphorylation at its activating sites T389 (mTORC1 site) and T229 (PDK1 site) – the implications of which are not currently known (Bilanges and Vanhaesebroeck, 2010). Previous studies examining the role of S6K1 have either relied on knockouts or inhibition of mTOR with rapamycin, and PF-4708671 is the first S6K1-specific inhibitor. Shum et al (2019) reported that PF-4708671 also rapidly increases AMPK activation before S6K1 inhibition and found that this was due to rapid inhibition of mitochondrial complex 1.

PF-4708671 offers protection from several neurological conditions in animal models. It reduced infarct size and blood-brain barrier disruption soon after ischemia-reperfusion injury (Chi et al, 2019) and improved microregional oxygen supply (Weiss et al, 2019). In a Parkinson's disease mouse model, PF-4708671 improved memory but not anxiety- or depression-like behaviors (Masini et al, 2018). In a Fragile X mouse model, a model characterized by abnormal protein translation, PF-4708671 (and FS-115)

normalized protein translation, improved social behavior, and improved altered dendritic spine morphology. PF-4708671 prevented an increase in testicular weight but had no effect on compulsive behavior. FS-115 was also found to have higher brain penetration than PF-4708671 (Bhattacharya et al, 2016). PF-4708671 also improved long-term potentiation in brain slices from a mouse model of Angelman syndrome (Sun et al, 2016). In an animal model of spinal cord injury, cortical injection of PF-4708671 improved axon regeneration and locomotor movement (Al-Ali et al, 2017). Acute treatment with PF-4708671 also reduced neuronal death in a model of cerebral palsy (hypoxia-ischemia and LPS-induced inflammation in newborn mice) (Srivastava et al, 2016).

Beyond its role in reducing translation, PF-4708671 was also reported to increase markers of autophagy in cell culture, and another S6K1 inhibitor, A77 1726 induced autophagy and increased the elimination of superoxide dismutase 1 (SOD1) aggregates (Sun et al, 2018).

APOE4
None Reported

Aging and related health concerns: S6K1 inhibitors may be beneficial in certain cancers associated with an increased expression of S6K1, and the potential of S6K1 inhibitors as geroprotectors is interesting.

Types of evidence:

- Four genetic studies for lifespan extension in mice, worms, and flies
- Three studies (one genetic, two with PF-4708761) for metabolic effects
- One preclinical study for cardiovascular disease
- Four genetic or inhibitor studies for cancer

S6K1 gene deletion (S6K1 -/-) increased median lifespan by 9% and maximum lifespan by 10% in mice. This effect was driven by an increase in lifespan in female mice (median increase = 19%, maximum increase = 10%; no effect in males). This was associated with improvements in several measures of healthspan in female mice including motor performance, immune measures, bone density, insulin resistance, and fat mass (though no change in incidence to macroscopic tumors). However, young female S6K1 -/- mice had impaired glucose tolerance. S6K1 -/- mice were smaller than control mice, despite increased food intake per body weight, though endocrinologically they were normal (normal circulating IGF-1, thyroid-stimulating hormone, and prolactin levels). Hepatic gene expression changes were similar to those seen with caloric restriction, and muscle gene expression changes were similar to

those seen after administration with an AMPK activator, aminoimidazole carboxamide ribonucleotide (AICAR). In S6K1 -/- mice, AMPK activity is increased in white adipose, muscle, and liver tissue. To confirm that the pro-longevity effects of S6K1 knockout might be acting through AMPK, they found that knockout of both AMPK and S6K1 in *C. elegans* prevented the lifespan increasing effect of S6K1 knockout alone (Selman et al, 2009). Another study reported that S6K1 acted through arginine kinase (*argk-1*) as knocking out both S6K1 and ARGK-1 prevented the increase in lifespan in *C. elegans* attained through knocking out S6K1 alone (McQuary et al, 2016).

S6K1 knockout was reported to increase *C. elegans* lifespan by 22% , while also slowing development, reducing fecundity, and increasing resistance to starvation (Pan et al, 2006). Additionally, expression of a dominant-negative form of S6K1 in *Drosophila* increased lifespan by 22% (Kapahi et al, 2004).

Metabolism
S6K1 knockout mice on a normal diet weighed less than control mice (despite eating more after controlling for body weight), had reduced fat mass and increased lipolysis and metabolic rate. Similar results were seen on a high fat diet. On a high fat diet, S6K1 knockout mice had increased levels of circulating free fatty acids, suggesting insulin resistance, and they were hypoinsulinemic. However, on both a normal and high fat diet, S6K1 knockout mice remained more insulin sensitive, shown by faster glucose clearance. They suggest that knocking out S6K1 modulates insulin signaling through feedback mechanisms on IRS (Um et al, 2004).

In vitro, PF-4708671 increased phosphorylation of AKT and reduced glucose production in hepatic cells while increasing glucose uptake in muscle cells. In mice fed a high fat diet, 7-day treatment with PF-4708671 improved glucose tolerance (though it did not improve gluconeogenesis), independent of insulin levels, and slightly lowered triglycerides (while rapamycin impaired glucose tolerance) (Shum et al, 2016). Shum et al (2019) later reported that PF-4708671 also rapidly increases AMPK activation before S6K1 inhibition and found that this was due to rapid inhibition of mitochondrial complex 1.

Cardiovascular
In a mouse model of myocardial infarction (MI), there was an upregulation of pS6K1 after MI. Treatment with PF-4708671 improved heart function and reduced aberrant cardiac remodeling (Di et al, 2012).

Cancer

Since the mTOR pathway is implicated in multiple cancers, S6K1 inhibitors are also being developed as anti-cancer agents. S6K1 is expressed in various progenitor cells in the bone marrow, and S6K1 knockout mice have altered cell populations in the bone marrow. The mTOR pathway is overactive in some patients with acute myeloid leukemia (AML), and pS6K1 is overexpressed in cells from patients with AML. Transplantation of AML cells in S6K1 knockout mice did not improve survival time, but transplantation of cancer cells from S6K1 knockout mice into new recipients did increase survival compared to control cells (Ghosh et al, 2015).

In about 10% of breast cancer patients, the chromosomal region 17q23, which contains the gene for S6K1, is amplified and S6K1 is overexpressed and associated with poor prognosis. S6K1 expression in breast cancer was also associated with breast cancer reoccurrence. In mice inoculated with triple-negative breast cancer cells, FS-115, an S6K1 inhibitor, reduced tumor uptake, size, metastasis rate, and increased survival (Segatto et al, 2016). PF-4708671 inhibited tumor growth in a mouse model of non-small cell lung cancer (Qui et al, 2016) and increased survival in a model of breast cancer (Segatto et al, 2013).

Safety: Preclinical studies suggest some side effects on metabolism, but effects in humans are not known.

Types of evidence:

- Multiple preclinical studies

SK61 inhibitors have not been studied in humans. Although S6K1 inhibition appears safe in animals, no proper toxicology studies have been conducted. Studies in S6K1 knockout mice suggest that S6K1 inhibition may reduce weight and affect insulin signaling (see studies above). It is not clear what the effects of long-term S6K1 inhibition might be.

There are some theoretical concerns with S6K1 inhibitors. Similar to the potential effects with rapamycin, S6K1 is important for protein translation, and the potential side effects with inhibiting protein translation excessively are unknown (e.g. protein translation is important for memory formation). It is also unclear whether inhibiting S6K1 may have the same immune effects as rapamycin. Finally, similar to mTOR, S6K1 is located at a hub, with feedback mechanisms around insulin signaling. So long-term S6K1 inhibition may have unintended side effects.

Drug interactions:
Unknown – though theoretically it would interact with rapalogs and potentially other drugs that affect insulin signaling.

Sources and dosing:
Not available. In animal studies, FS-115 has been used up to 250mg/kg per day, and PF-4708671 has been used up to 75mg/kg per day.

Research underway:
No clinical studies are underway. Sentinal Oncology was developing FS-115 for cancer.

Search terms:
s6k1 + alzheimer, cardiovascular, longevity, lifespan, hypotension, osteoaritis, cancer
s6 kinase + alzheimer
PF-4708671
FS-115

Websites:
- Clinicaltrials.gov
- Pubmed
- Longecity
- Pubchem
- Drugbank.ca