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.

AZ-67

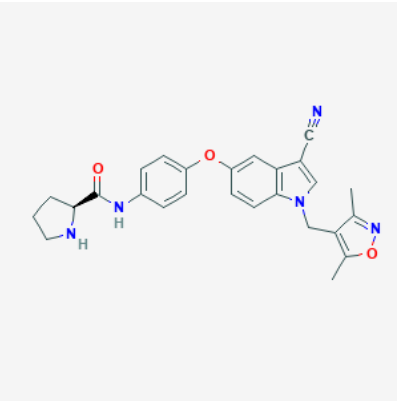
Evidence Summary

AZ-67 showed neuroprotective benefits in a preclinical model of stroke, but globally inhibiting PFKFB3 (particularly astrocytic PFKFB3), would impair glycolysis and lead to energy failure in the brain.

Neuroprotective Benefit: A single preclinical study showed neuroprotective benefits in a stroke model, but inhibiting glycolysis, particularly in astrocytes, would likely lead to energy failure and increased apoptosis of neurons and astrocytes.

Aging and related health concerns: No studies have tested AZ-67 for age-related conditions. Although PFKFB3 inhibition has been studied as a target for treating cancer, so far, no inhibitors have been approved yet for treatment of cancer.

Safety: Animal toxicity studies have not been reported and AZ-67 has never been tested in humans. So far, a single preclinical study reported that there were no overt toxicities in cell culture and with a single injection in a mouse model of ischemia.

Availability: Research grade only	**Dose:** Not established. In a mouse model of transient ischemia, a dose of 60 mg/kg, i.v. was used.	**Chemical formula:** $C_{26}H_{25}N_5O_3$ **MW:** 455.5  Source: PubChem
Common/preferred brand: Under development by Gero Discovery		
Half life: Not reported	**BBB:** Not reported	
Clinical trials: None to date	**Observational studies:** None to date	

What is it? AZ-67 is a small molecule that selectively inhibits 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3), an enzyme that promotes glycolysis (Burmistrova et al., 2019). In neurons, glucose metabolism via glycolysis is normally very low, as it is mainly metabolized through the pentose–phosphate pathway (PPP), a metabolic route that contributes to the maintenance of neuronal redox status. Astrocytes, in contrast, mainly generate energy from glycolysis, producing lactate for neurons as an oxidizable metabolic fuel. A key molecular switch governing metabolic pathways is PFKFB3, a pro-glycolytic enzyme that is normally absent in neurons but abundant in astrocytes. PFKFB3 levels are very low in neurons because they are continuously degraded after ubiquitylation (by APC/C-Cdh1). However, under certain neuropathological conditions, such as during excitotoxic stress, the activity of APC/C-Cdh1 in neurons is inhibited, increasing PFKFB3 protein stabilization. Active neuronal PFKFB3 then stimulates glucose consumption through glycolysis, which results in redox stress and neuronal apoptosis.

PFKFB3 inhibition has been most well-studied in cancer as it results in suppression of the growth of tumor cells by downregulation of glycolytic flux (Lu et al., 2017).

Neuroprotective Benefit: A single preclinical study showed neuroprotective benefits in a stroke model, but inhibiting glycolysis, particularly in astrocytes, would likely lead to energy failure and increased apoptosis of neurons and astrocytes.

Types of evidence:

- 1 laboratory study testing AZ-67
- A few laboratory studies studying PKFKB3 inhibition

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function?
None.

Human research to suggest benefits to patients with dementia:
None.

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

There has only been one preclinical study testing AZ-67 as a potential therapy for stroke (Burmistrova et al., 2019). No studies have tested AZ-67 for Alzheimer's models, though in a mouse model of Alzheimer's (TgCRND8 mice), an increase in PFKFB3 protein expression is observed at 9 months of age, significantly later than when Aβ deposition and reactive astrogliosis are observed (2 and 4 months, respectively)(Fu et al., 2015).

Stroke leads to excitotoxicity, mitochondrial dysfunction, and increased oxidative stress, contributing to infarct and neurological deficits. The authors hypothesized that pharmacological inhibition of PFKFB3 in neurons would prevent the redox stress associated with glycolytic activation and may protect neurons from apoptosis upon excitotoxic insults.

In mouse cortical primary neurons, AZ-67 prevented apoptosis that was induced by Aβ(25-35) in a dose-dependent manner (Burmistrova et al., 2019). When neurons were subjected to excitotoxicity (a short-term incubation with glutamate or NMDA), PFKFB3 protein was stabilized and glycolysis was activated, but these effects were dose-dependently reversed by incubation of neurons with AZ-67. The minimum concentration of AZ-67 that was effective at inhibiting glycolysis was 1 nM. AZ-67 treatment also prevented the increase in mitochondrial reactive oxygen species induced by excitotoxicity.

In an *in vitro* model of oxygen and glucose deprivation, primary neuronal cultures had increased glycolysis, increased hydrogen peroxide levels, increased mitochondrial reactive oxygen species, a loss of mitochondrial membrane potential, and increased apoptosis, all of which were dose-dependently attenuated by AZ-67 (10 nM; immediately after deprivation, during the reoxygenation period).

In a mouse model of ischemia (middle cerebral artery occlusion followed by reperfusion), AZ-67 treatment (60 mg/kg via jugular vein) significantly improved neurological deficit, prevented motor discoordination (to ~60% performance), and decreased the infarcted brain volume to 27%, compared to the significant neurological deficit, ~40% motor performance, and 43% infarct volume observed in untreated ischemic mice (Burmistrova et al., 2019).

Oddly, AZ-67 did not inhibit glycolysis in an astrocytic culture (Burmistrova et al., 2019). Selective inhibition of PFKFB3 in neurons, but not astrocytes, during excitotoxic and other insults is desirable, but this finding will need to be validated in *in vivo* models. In human fetal astrocyte culture, PFKFB3 inhibition with PFK-15 resulted in increased reactive astrocytes, increased accumulation of Aβ within and around astrocytes, greater vulnerability of these cells to Aβ toxicity, and increased apoptosis (Fu et al., 2015).

APOE4 interactions: Unknown.

Aging and related health concerns: No studies have tested AZ-67 for age-related conditions. Although PFKFB3 inhibition has been studied as a target for treating cancer, so far, no inhibitors have been approved yet for treatment of cancer.

Types of evidence:

- No studies testing AZ-67
- A few clinical and preclinical studies testing other PFKFB3 inhibitors

No studies have tested AZ-67 for age-related health conditions. However, PFKFB3 has been evaluated as a potential target for treating cancer, as PFKFB3, and therefore glycolytic activity, is upregulated in a variety of tumor cells even in the presence of oxygen (referred to as the "Warburg effect"; Warburg, 1956). PFKFB3 inhibition results in suppression of the growth of tumor cells by downregulation of glycolytic flux (Lu et al., 2017). PFKFB3 is overexpressed in a wide variety of cancers, including breast, prostate, colon, astrocytoma, and ovarian cancers, and its expression and/or activity is found to correlate strongly with aggressiveness/poor prognosis in colon, breast, ovarian, and thyroid tumors (Boyd et al., 2015). Recent studies have also shown that the efficacy of PFKFB3 inhibition in tumor cells is not only related to glycolysis, but also to autophagy. It is speculated that PFKFB3 inhibitors are able to downregulate intracellular reactive oxygen species, which helps to maintain genomic stability and

prevent drug resistance and disease progression. Preclinical studies have shown that small molecule inhibitors of PFKFB3, such as 3PO and PFK-15, inhibits tumor growth (Clem et al., 2008; 2013).

Advanced Cancer Therapeutics was evaluating a different PFKFB3 inhibitor, PFK-158, for treatment of skin, lung, colon, breast, and pancreatic cancers at the University of Louisville, Georgetown University, and MD Anderson Cancer Center. In 2017, results were published from a phase I study testing PFK-158 in advanced solid malignancies (Redman et al., 2018), which suggested there was no dose-limiting toxicity. However, later that year, the CEO announced that results in the clinical trials did not produce enough of an effect to retain interest from pharmaceutical companies (InsiderLouisville.com).

Safety: Animal toxicity studies have not been reported and AZ-67 has never been tested in humans. So far, a single preclinical study reported that there were no overt toxicities in cell culture and with a single injection in a mouse model of ischemia.

Types of evidence:

- 1 laboratory study

In mouse cortical primary neurons, AZ-67 lacked toxicity in the range of 0.01 to 100 nM for 24 hours (Burmistrova et al., 2019). In mice that received sham operation, AZ-67 administration (60 mg/kg, i.v.) did not cause any signs of neurological deficit, motor discoordination, or brain injury after 24 hours.

However, no formal toxicity studies have been reported, and therefore, no information is currently available on AZ-67's acute toxicity, subacute toxicity, subchronic/chronic toxicity, immunotoxicity, reproductive toxicity, genotoxicity/mutagenicity, carcinogenicity, or other laboratory animal toxicology measures.

Drug interactions with AZ-67 have not been studied.

Sources and dosing: Gero Discovery LLC (Moscow, Russia) is developing AZ-67 and other PFKFB3 inhibitors. The company holds intellectual property covering small molecule inhibitors of PFKFB3 and their therapeutic applications. Of the authors of the 2019 AZ-67 preclinical paper (Burmistrova et al., 2019), one author is a shareholder and three authors are employees of Gero Discovery LLC.

The effective dose of AZ-67 is not established. In a mouse model of transient ischemia, a dose of 60 mg/kg was given through the jugular vein during reperfusion.

Research underway: There are no clinical trials ongoing for AZ-67 based on clinicaltrials.gov or clinicaltrialsregister.eu. There are no NIH-funded programs currently studying AZ-67.

Search terms:
Pubmed, Google: AZ-67, PFKFB3, AZ-PFKFB3-67

Websites visited for AZ-67:

- Clinicaltrials.gov (0)
- Examine.com (0)
- DrugAge (0)
- Geroprotectors (0)
- Drugs.com (0)
- WebMD.com (0)
- PubChem
- DrugBank.ca (0)