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.

NLRP3 Inhibitors

Evidence Summary
NLRP3 inhibitors have beneficial effects in several different diseases, including Alzheimer’s and longevity.

Neuroprotective Benefit: Preclinical evidence suggests that NLRP3 inhibitors are effective across a range of neurodegenerative diseases.

Aging and related health concerns: Preclinical evidence suggests that NLRP3 inhibitors may be useful for several age-related diseases.

Safety: No human evidence yet for potential side effects, but no indication from most preclinical studies.
**MCC950 (also known as CP-456773) – NLRP3 tool compound**

| Availability: Not available, several NLRP3 inhibitors in different stages of development | Dose: N/A | Molecular Formula: $\text{C}_{20}\text{H}_{24}\text{N}_{2}\text{O}_{5}\text{S}$  
Molecular weight: 404.5 g/mol |
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<td>Half life: N/A</td>
<td>BBB: Penetrant in animals</td>
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<td>Clinical trials: 0 completed for MCC950. 1 phase 1 study of another NLRP3 inhibitor.</td>
<td>Observational studies: 0</td>
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**What is it?**

The NLRP3 inflammasome is a central figure in the innate immune system – the non-specific immune response that occurs in the presence of pathogen-associated molecular patterns (PAMPS) and damage-associated molecular patterns (DAMPS). PAMPS are small conserved molecular motifs on classes of infectious pathogens while DAMPs are molecular patterns from the host itself (e.g. DNA, HMGB1, etc.).

NLRP3 is a supramolecular complex made up of a sensor molecular, the adaptor apoptosis-associated speck-like protein containing a CARD (ASC), and an effector protease, caspase-1. NLRP3 is usually present at low concentrations but can be primed by molecules such as PAMPS or DAMPS that bind to immune receptors and activate transcription factors that increase the expression of NLRP3. In addition, NLRP3 is typically held in an inactive state by post-translational modifications.

There are several mechanisms by which NLRP3 can be activated. Once activated, NLRP3 cleaves caspase-1 which can then lead to the release of pro-inflammatory mediators such as IL-1β, IL-18, high-mobility group protein B1 (HMGB1), leukotrienes, and prostaglandins (Mangan et al, 2018). On the other hand, TNFα and IL-6 are released through other pathways and usually not expected to change with NLRP3 inhibition.
NLRP3 can be activated through multiple mechanisms:

- **Pore formation and redistribution of ions**: opening of pores in the plasma membrane leading to potassium efflux with concomitant calcium influx or the opening of chloride channels (e.g. opening of the volume-regulated anion channel (VRAC)) can activate NLRP3.
- **Lysosomal disruption**: phagocytosis of crystalline structures can cause lysosomal disruption and ATP release leading to extracellular ATP-dependent NLRP3 activation.
- **Metabolic dysfunction**: inhibition of glycolysis and mitochondrial NADH oxidase (complex I) with depolarization of lysosomes can activate NLRP3.
- **Mitochondrial dysfunction**: increased mtROS or externalization of mitochondrial cardiolipin can activate NLRP3.

These are some of the canonical NLRP3 activation pathways and are the most prevalent drug targets (Mangan et al, 2018).

There are several inflammasomes with overlapping functions, which is why targeting NLRP3 does not necessarily increase the risk of infections (as opposed to drugs such as IL-1β inhibitors). In addition, NLRP3 seems to be especially involved with sterile inflammation.

**Neuroprotective Benefit**: Preclinical evidence suggests that NLRP3 inhibitors are effective across a range of neurodegenerative diseases.

**Types of evidence:**
- 2 postmortem dementia studies
- 4 Alzheimer’s preclinical studies
- 11 preclinical studies in other neurodegenerative diseases

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

**Human research to suggest benefits to patients with dementia**
Heneka et al (2013) reported increased expression of cleaved caspase-1 in the hippocampus and frontal cortex in patients with Alzheimer’s disease. Elevated levels of cleaved caspase-1, ASC, and IL-1β were also reported in the cortex of patients with frontotemporal dementia (FTD) (Ising et al, 2019).
Mechanisms of action for neuroprotection identified from laboratory and clinical research

Heneka et al, 2013 reported increased expression of cleaved caspase-1 and IL-1β in the brain of an Alzheimer’s animal model. The expression was reduced to control levels when the animals were crossed with NLRP3 knockout animals. Similarly, NLRP3 or Caspase-1 knockout animals crossed with Alzheimer’s animals were protected from memory deficits and long-term potentiation (LTP) impairment. The reduction in spine density in Alzheimer’s animals (which was protected in NLRP3 or Caspase-1 knockout animals) was small, but significant, suggesting that deficits may be due to functional, rather than structural, changes in synapses. NLRP3 knockout was also associated with reductions in amyloid levels, amyloid plaque size, and an increase in phagocytic capacity of microglia in vivo. Finally, NLRP3 knockout animals also had increased levels of an amyloid degrading enzyme, insulin degrading enzyme (IDE).

Fenamate NSAIDs (e.g. flufenamic acid, meclovenamic acid, and mefenamic acid) were reported to be stronger inhibitors of NLRP3 than other NSAIDs and reduced the release of IL-1β in vitro due to their inhibition of VRACs. Mefenamic acid improved cognition in an acute model of intracerebroventricular amyloid injection and in an aged Alzheimer’s mouse model. It also reduced the number of inflammatory microglia and IL-1β levels. However, mefenamic acid also inhibits COX enzymes, and there may be off-target effects (Daniels et al, 2016).

Yin et al (2018) reported upregulation of multiple components of the NLRP3 inflammasome (NLRP3, ASC, cleaved caspase-1) in another Alzheimer’s animal model and a reduction of caspase-1 after one-month treatment with JC-124, an NLRP3 inhibitor. JC-124 also reduced levels of soluble amyloid-beta, amyloid oligomers, plaques, and plaque size. In addition, JC-124 also reduced levels of microgliosis (but increased astrogliosis), oxidative stress, and increased synaptic markers.

In vitro, MCC950, an NLRP3 inhibitor, reduced IL-1β release, caspase-1 expression, and increased phagocytosis of Aβ from LPS- and Aβ-challenged microglia but had no effect on TNFα or IL-6 (suggesting it was specific for NLRP3). In aged Alzheimer’s mice, treatment with MCC950 improved cognition, reduced Aβ, IL-1β, and microglial activation (Dempsey et al, 2017).

Other neurological diseases

FTD: Ising et al (2019) reported increased levels of cleaved caspase-1 and ASC in cortical samples of old FTD (tau) mice compared to younger mice. There were also increased levels of extracellular ASC specks in old FTD mice compared to controls. FTD mice crossed with ASC or NLRP3 knockout mice had reduced levels of ASC speck formation, cleaved caspase-1, p-tau, and misfolded tau. Knockout mice also displayed improved spatial memory.
NLRP3 knockout mice had reduced expression of several kinases and a phosphatase that regulate tau phosphorylation, reductions of some genes involved in inflammation, and increases in genes involved in synapse formation. Injection of brain homogenates from amyloid Alzheimer’s mice can induce tau phosphorylation in FTD mice, and the investigators found that phosphorylation of tau was reduced when amyloid brain homogenates were injected into FTD/NLRP3-/- mice.

In a tau seeding model (intracerebral injection of aggregated tau in a tau transgenic model), both ASC knockout and treatment with MCC950 reduced the spreading of tau and reduced microgliosis (Stancu et al, 2019).

**Traumatic Brain Injury (TBI):** In a mouse model of TBI, NLRP3 expression was increased after injury. Treatment with MCC950 improved neurological outcomes, reduced the infiltration of macrophages into the brain, reduced lesion size, reduced cell death, improved blood brain barrier integrity and the expression of tight junction proteins, and reduced cerebral edema (Xu et al, 2018).

**Multiple Sclerosis:** In a mouse model of multiple sclerosis, pretreatment with MCC950 before LPS injection reduced the serum levels of IL-1β and IL-6, but not TNFα. It also improved clinical symptoms in the model (Coll et al, 2015).

**Parkinson’s:** Cleaved caspase-1 and ASC were upregulated in the substantia nigra in Parkinson’s patients and in mouse models of Parkinson’s disease. *In vitro* exposure of microglia to alpha synuclein increased expression of IL-1β and ASC. Oral administration of MCC950 crossed the blood brain barrier and in multiple Parkinson’s models was neuroprotective, reduced the expression of IL-1β and ASC, improved behavioral symptoms, and reduced the aggregation of alpha synuclein (Gordon et al, 2018).

**ALS:** Cleaved caspase-1 and IL-1β were upregulated in microglia from ALS mouse models. Expression of both was reduced after *in vitro* administration of MCC950 (Deora et al, 2019).

**Stroke:** In a model of ischemic stroke, NLRP3 (but not other inflammasomes) was increased 24 hours after stroke. However, treatment with MCC950 and NLRP3 genetic knockout had no effect on the extent of lesion volume (Lemarchand et al, 2019). On the other hand, other studies have suggested that treatment with MCC950 reduced the extent of lesion volume and improved neurological outcomes in stroke models (Wang et al, 2019; Ward et al, 2019; Luo et al, 2019; Ismael et al, 2018).

**APOE4 interactions:** None Reported
Aging and related health concerns: Preclinical evidence suggests that NLRP3 inhibitors may be useful for several age-related diseases.

Types of evidence:
- 4 lifespan preclinical studies
- 3 cardiovascular preclinical studies
- 1 review and 2 preclinical cancer studies

Lifespan: MIXED/POTENTIAL BENEFIT
Genetic knockout of NLRP3 increased mean lifespan in mice by 34% and maximum lifespan by 29% without a change in food intake or body weight. Old NLRP3 knockout mice also had reduced levels of cholesterol, fasting glucose, IGF-1, lactate dehydrogenase, several liver enzymes, improved insulin sensitivity, and reduced leptin/adiponectin ratio. Serum IL-1β was not detected, but tissue levels of IL-1β and caspase-1 were reduced in knockout mice. There were no significant changes in other inflammatory markers such as TNFα, IL-6, and IL-8.

Old NLRP3 knockout mice had a reduced heart rate and a lack of cardiac hypertrophy and reduced cardiac fibrosis. Old NLRP3 knockout mice had other measures of improved cardiac fitness and fewer deaths from cancer, so the investigators still do not know the cause of death in these mice. Cardiac tissue from old NLRP3 knockout mice also had longer telomeres, fewer lipofuscin inclusions, though similar levels of senescent markers. There was a reduced expression of phosphorylated mTOR in old NLRP3 knockout mice and fewer autophagosomes, suggesting that autophagy may not be impaired in these mice.

Gene expression studies from the hearts of old NLRP3 knockout and WT mice suggested that the greatest difference in gene expression was increased expression of *Nampt*, the rate limiting step in NAD+ synthesis. After 15 weeks on a high fat diet, old NLRP3 mice had increased NAD+ levels and SIRT1 expression (Marin-Aguilar, 2019).

Genetic knockout of several components of the NLRP3 inflammasome (NLRP3 -/-; Asc -/-) improved several aspects of the aging phenotype including improved glucose levels, a reduction in IL-1β, improved thymic function, a reduction of bone-loss, a reduction of astrogliosis, an increase in BDNF, and improved cognition (Youm et al, 2013; Youm et al, 2012).
In aged mice, treatment with MCC950 reduced glucose and IGF-1 levels and reduced the weight of fatty liver. It reduced cholesterol and triglycerides. It was reported to inhibit mTOR and induce autophagy in the liver (Marin-Aguilar et al, 2019).

Youm et al (2015) reported that beta-hydroxybutyrate (BHB), but not acetoacetate, was an NLRP3 inhibitor by preventing K+ efflux and reducing ASC oligomerization and speck formation. Administration of BHB or a ketogenic diet prevented activation of NLRP3.

**Cardiovascular disease**

In a mouse model of atherosclerosis, 4-week treatment with MCC950 slightly reduce atherosclerotic plaque size and volume. It reduced macrophage plaque infiltration but had no effect on collagen content or the size of the necrotic core. VCAM1 and ICAM1, but not MCP-1, were also reduced (Van der Heijden et al, 2017). Additionally, genetic elimination of multiple components of the NLRP3 inflammasome reduced atherosclerotic lesion size in multiple models of atherosclerosis (Duewell et al, 2010).

In a mouse model of myocardial infarction (MI), 14-day treatment with MCC950 improved heart function, improved cardiac remodeling, and reduced myocardial fibrosis. MCC950 also reduced levels of cleaved IL-1β, NLRP3, and cleaved IL-18, and reduced the infiltration of inflammatory cells into the injured site (Gao et al, 2019).

**Cancer**

The role of NLRP3 in cancer is still controversial. Given the inflammatory nature of many cancers, NLRP3 is often upregulated, giving a potential for NLRP3 inhibitors in cancer. However, much remains to be known (Moossavi et al, 2018). One preclinical study suggested that NLRP3 knockout may exacerbate liver cancer metastasis (Dupaul-Chicoine et al, 2015), though an in vitro study suggested NLRP3 knockout or inhibition of NLRP3 can reduce metastasis (Lee et al, 2019).

**Safety:** No human evidence yet for potential side effects, but no indication from most preclinical studies.

**Types of evidence:**
- No studies in humans
- Many preclinical studies
No reported safety issues have been reported from preclinical studies. In humans, nothing is known about potential safety issues. IL-1β antibodies, such as anakinra and canakinumab, are potentially associated with severe infection risks. However, there are several redundant inflammasomes that all have anti-infective properties. Preclinical studies have suggested that NLRP3 may be important for resistance to a couple of infective agents (e.g. *Burkholderia pseudomallei*, *Toxoplasma gondii*). Future clinical studies will be required to understand the potential side effects of NLRP3 inhibitors.

Regarding MCC950, given that it has poor bioavailability, it was reported that Pfizer dosed rheumatoid arthritis patients at high doses, but the trial was stopped early, rumored to be because of elevated liver enzymes (Mallard 2019). However, this could be due to a drug-specific, rather than a class effect.

**Drug interactions:**
None known, but potentially with other anti-inflammatory therapies, especially IL-1β antibodies.

**Sources and dosing:**
None of the NLRP3 inhibitors are currently available. Several companies have drugs in development.

**Research underway:**
Several companies currently developing NLRP3 inhibitors for CNS diseases are at various stages of development including IFM Therapeutics, Inflazome, and NodThera.

Currently one NLRP3 inhibitor is in early stage clinical trials.
- Inflazome’s IZD334 in a phase 1 study in 77 patients with Cryopyrin Associated Periodic Syndrome.

**Search terms:**
- NLRP3 + alzheimer, lifespan, aging, cancer
- MCC950

**Websites:**
- Clinicaltrials.gov
- Pubmed
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