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.

TLR2 Inhibitors

Evidence Summary
TLR2 dysfunction in aging-related diseases may be due to lifetime infection burden and microbiome-derived components. Better to target disease specific TLR2 interactions than to broadly inhibit TLR2.

Neuroprotective Benefit: TLR2 signaling is dysfunctional in neurodegenerative disease, which may promote pathological inflammation and the accumulation of toxic proteins, but functional restoration may be more useful than total inhibition.

Aging and related health concerns: TLR2 is elevated in atherosclerosis and metabolic disease, but response to intervention may be impacted by the microbiome. Inhibition may be best suited to protect against inflammatory ischemic injury.

Safety: TLR2 inhibitors may increase the risk for infections, particularly for Gram+ bacteria. Short term use of anti-TLR2 was well-tolerated, but long-term safety is unknown. Effects may be inhibitor type and disease type specific.
**What is it?**

Toll-like receptor 2 (TLR2) is a transmembrane receptor that belongs to the class of pattern recognition receptors. TLRs respond primarily to pathogen-associated molecular patterns (PAMPs), and serve an important role in the innate system to recognize pathogens and initiate processes which facilitate their clearance [1]. TLRs also respond to damage-associated molecular patterns (DAMPs), also known as alarmins, which are endogenous molecules released from damaged or dying cells. The TLR response to DAMPs can initiate inflammatory processes associated with many chronic diseases. Since Aβ and alpha-synuclein have been identified as DAMPs for TLR2, it is hypothesized that inhibiting TLR2 may reduce deleterious inflammation in the context of various dementias [2; 3].

Inhibition of TLR signaling has emerged as a therapeutic target. There are at least 11 TLRs in humans, which are activated in response to distinct and overlapping sets of PAMPs and DAMPs. Although, the response to a specific molecular pattern may be driven primarily by a particular TLR, most pathogens and cell stress-inducing agents release a variety of molecules which may collectively activate an array of TLRs [4]. Depending on the conditions, the different TLRs could activate synergistic or antagonistic signaling pathways [5]. Therefore, TLR responses tend to be highly complex, and difficult to predict. TLR2 tends to be co-activated with TLR4, particularly in the CNS.

TLR signaling requires complexes of co-receptors and adaptor proteins, thus is typically cell-type and context dependent [6]. With the exception of TLR3, all TLRs require the adaptor protein MyD88 for downstream signaling. TLR2 forms heterodimers with TLR1 or with TLR6. The best characterized downstream response of TLR2 heterodimer activation involves the activation of pro-inflammatory MAPKs (p38 and JNK) and NF-κB nuclear translocation/ inflammatory gene transcription. However, TLR2 signaling can also be anti-inflammatory under certain conditions, which may involve the activation of the P13K/AKT pathway [7]. Since broad TLR2 inhibitors have pleiotropic effects, more research is needed.
to understand the particular ligands, complexes, and conditions that specifically trigger the pro-inflammatory pathway, so that it can be specifically targeted. A humanized anti-TLR2 antibody, OPN-305, was in clinical development for myelodysplastic syndrome, but development has been discontinued.

**Neuroprotective Benefit:** TLR2 signaling is dysfunctional in neurodegenerative disease which may promote pathological inflammation and the accumulation of toxic proteins, but functional restoration may be more useful than total inhibition.

**Types of evidence:**
- 11 Observational biomarker studies (TLR2 expression/activity in the brain or blood)
- Numerous laboratory studies

**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:**
TLR2 acts as a regulator of inflammatory signaling, but **whether it exerts pro- or anti-inflammatory signaling is cell type and context dependent** [7]. The downstream effects of TLR2 activation depend on which signaling pathways are activated, which in turn depends on its association with its various co-receptors and adaptor proteins, as well as whether there is a simultaneous activation of other TLRs. Many TLRs have synergistic or antagonistic effects, so **the particular combination of TLRs activated influences the outcome** [5]. Discerning the contribution of TLR2 to neurodegenerative diseases through the use of animal knockout studies is challenging and potentially problematic because TLRs are important for neurogenesis and brain development [8]. As a result, TLR2 knockout animals have underlying neurological deficits and compensatory responses from related TLRs [9; 10], which makes it difficult to interpret the phenotypes that emerge in the context of disease models. Additionally, the lifelong absence of TLR2 is not reflective of the age-related impairments and alterations to TLR2 function which may drive disease.
The use of TLR2 agonists and antagonists avoids the developmental pitfalls of TLR2 knockout animals, however, the results of studies using these agents have also been contradictory, and reflects the context dependency of TLR2 signaling. Broad TLR2 inhibition will block TLR2 signaling systemically, which will have different effects in different cell types, some of which may be antagonistic, thus it is possible to observe opposite effects under different conditions.

The microbiome may be one of the key elements influencing TLR2 mediated inflammatory signaling, which ties into the microbial hypothesis for neurodegenerative disease [11]. The presence of microbial processes may be needed to trigger a pathological immune response [12]. TLRs primarily respond to PAMPs, and are important for mediating tolerance to commensal bacteria [13]. Some TLRs, including TLR2, have also been shown to respond to a variety of endogenous molecules, known as DAMPs, which are associated with cell stress or damage. Many of these endogenous activators, including Aβ, are very similar to pathogen derived ligands (i.e. bacterial amyloids), so the composition of microbiome-derived compounds within a given individual, and which ones reach the brain may impact the TLR immune response to these neurodegenerative disease-associated proteins [11]. TLR2 signaling has been shown to influence microglial polarization and the inflammatory response profile [14]. In the context of aging, blood-derived innate immune cells were shown to have elevated basal secretion of pro-inflammatory cytokines, but impaired response following stimulation with TLR2 ligands [15]. This may reflect a form of tolerance based on lifetime burden of infections and exposure to microbiome-derived compounds. This could promote neurodegeneration, by promoting a damaging pro-inflammatory milieu, while also preventing microglia from becoming phagocytically active in response to pathogenic proteins such as Aβ or alpha synuclein. Overall, the data suggests that TLR2 dysfunction contributes to neurodegenerative processes, but a targeted approach aimed at ameliorating the dysfunction may be more therapeutically beneficial than broad-based inhibition.

Alzheimer’s disease: TLR2 ELEVATED BUT DYSFUNCTIONAL, POTENTIAL BENEFIT FOR TLR2 NORMALIZATION OR INHIBITION, BUT MAY BE MICROBIOME DEPENDENT

TLR2 and MyD88 expression have been found to be elevated in postmortem prefrontal cortex brain tissue from patients with Alzheimer’s disease (AD) [16]. The levels of TLR2 (Spearman’s rank order correlation 0.463, P = 0.007) and MyD88 (0.371, P = 0.033) were also correlated with Braak staging. TLR2 and CD14+ cells were observed around Aβ plaques, with a greater number surrounding diffuse Aβ plaques compared to dense-core Aβ plaques [17]. The expression of TLR2 mRNA and protein was also found to be elevated in peripheral blood mononuclear cells (PBMCs) from AD patients, with an inverse correlation between TLR2 level and mini-mental status exam (MMSE) score (TLR2: r = −0.32; P = 0.01)
Single nucleotide polymorphisms (SNPs) that affect TLR2 function have also been associated with AD in some populations in gene association studies [19; 20; 21]. Since these SNPs primarily alter pattern recognition ability and responses [22], they may differentially prime the immune response toward Aβ.

In AD animal models, elevated TLR2 signaling was associated with pathogenic inflammation and cognitive impairment, which could be alleviated by inhibiting TLR2 via anti-TLR2 antibodies, TLR2 antagonists, or TLR2-MyD88 interaction blocking peptides [16; 23; 24; 25]. However, TLR2 agonists have also been found to be neuroprotective in some models, particularly those using Aβ injection/infusion in non-aged animals where TLR2 function is relatively intact [26; 27]. In this context, the protective effects of TLR2 activation are primarily related to the clearance of Aβ by phagocytically active microglia. **Once TLR2 signaling becomes dysfunctional, activation of TLR2 no longer stimulates the clearance of Aβ**, and instead appears to be detrimental by promoting pathogenic inflammation, suggesting that once significant pathology is present, TLR2 inhibition is likely to be the more therapeutically useful strategy [2; 14].

Aβ has been shown to activate TLR2 [28], though it is controversial whether the endogenous ligands are true ligands or whether they are agonists that enhance TLR2 stimulation to PAMPs [29]. Highly purified forms often fail to activate TLR2 *in vitro*, and these endogenous ligands are known to contain motifs that interact with PAMPs, suggesting that Aβ and other DAMPs may need to interact with circulating microbial-derived compounds to trigger a TLR2-mediated immune response *in vivo*. However, there is evidence to suggest that elevated TLR2 activation driven by a pathogen during an infection is distinct from elevated TLR2 in the context of a chronic inflammatory disease. A soluble form of TLR2 (sTLR2) is generated from the transmembrane form, and acts as an endogenous inhibitor of TLR2 signaling by binding to the microbial ligands, and it is typically generated in the context of elevated TLR2 activation, as part of a negative feedback loop [30]. sTLR2 levels have been found to be elevated in a variety of chronic inflammatory conditions, however, the increase is significantly less than what is seen in the context of an infection [31]. This suggests that DAMPs/endogenous ligand stimulation may not drive sTLR2 generation to the same degree as PAMPs, and/or that insufficient activation of the negative feedback loop following TLR activation may drive chronic inflammatory processes. Cerebrospinal fluid (CSF) levels of sTLR2 and sTLR4 were found in SIV-infected macaques and HIV-infected humans with neurological impairments, and was associated with neuroinflammation [32]. Meanwhile sTLR2 and sTLR4 levels were not elevated in AD patients, which could be due to a lack of TLR mediated neuroinflammation, or the lack of the sTLR inhibitory response. It suggests that activation or infusion of this endogenous TLR2 inhibitor (sTLR2) could have protective anti-inflammatory effects.
**Parkinson’s disease: TLR2 ELEVATED AND DYSFUNCTION, POTENTIAL BENEFIT FOR INHIBITION IN NEURONS, BUT MAY BE MICROBIOME DEPENDENT**

TLR2 expression has been found to be increased in postmortem brain tissue from Parkinson’s disease (PD) patients [33; 34; 35]. While TLR2 is expressed on neurons and immune cells, in PD, the increased expression in the brain appears to be driven by an increase in neuronal levels. Increasing TLR2-immunoreactivity in neurons was found to be associated with increasing disease stage (\( p = 0.87, p = 0.003 \)) [33]. Alpha-synuclein has been shown to act as an endogenous ligand, or at least associated with the activation of TLR2 [36]. In neurons, the activation of TLR2 has been shown to inhibit autophagy via regulation of the AKT/mTOR pathway [37]. In model systems, inhibition of neuronal TLR2 enhances autophagic clearance of alpha-synuclein [35; 37]. These findings may be neuronal specific, and/or related to the particular downstream signaling pathways triggered by alpha-synuclein mediated activation of TLR2. AKT is one of several downstream targets that can be activated by TLR2, suggesting that there is a specific TLR2 receptor complex that mediates this effect [1]. In immune cells, TLRs are classically understood to be activators of autophagy to facilitate pathogen clearance and/or negatively regulated by autophagy [38]. Consequently, TLR2 inhibitors should not be classified broadly as autophagy activators, since this effect may be restricted to particular cell types or contexts.

Meanwhile, similar to AD, PD-related dementia may involve changes to TLR signaling on myeloid cells. PD patients at high risk for dementia were found to have higher TLR2 on monocytes and macrophages, and these patients also had higher circulating levels of bacterial endotoxins [39]. There is evidence that the TLR2 response in blood-derived immune cells is also dysfunctional in PD patients. The induction of TNFα in response to TLR2 stimulation is dampened in PD blood cells [36; 40]. This could be due to the development of tolerance stemming from repeated exposure to alpha-synuclein, ultimately resulting in a blunted immune response, hampering alpha-synuclein clearance. Anti-TLR2 function blocking antibodies have been shown to be protective in PD mouse models, but efficacy may be dependent on the timing of administration with respect to the onset of pathology [35; 37; 41].

Similar to AD, gene association studies have found that SNPs in the TLR2 gene are associated with PD risk in certain populations [42; 43]. Disparities in TLR-related risk are thought to stem from important interactions of TLR2 with the microbiome, which tends to vary in different populations due to ethnicity, diet, location, and other factors [44]. Differences in lifetime infection burden across individuals may also play a role.
**APOE4 interactions:**
It is not known whether the efficacy of TLR2 inhibitors will vary with ApoE4 status, but the ApoE genotype is known to modulate cytokine production, and *ex vivo* studies have shown that immune cells from E4 carriers have a stronger proinflammatory cytokine response to TLR2 agonists [45].

**Aging and related health concerns:** is elevated in atherosclerosis and metabolic disease, but response to intervention may be impacted by the microbiome. Inhibition may be best suited to protect against inflammatory ischemic injury.

**Types of evidence:**
- 1 systematic review based on 17 studies (8 human studies) for exercise and TLR expression
- 2 Observational biomarker studies (TLR2 expression/activity in blood cells)
- Numerous laboratory studies

**Inflamm-aging and senescence: TLR2 SIGNALING DYSFUNCTIONAL WITH AGING**
TLR2 signaling is altered in the context of aging. The expression levels of TLR2 has not been found to be consistently altered with age, but this could be tissue type dependent and stem from differences across cell types. While the expression appears relatively unaffected, the function and activation capacity of TLR2 is affected by age [46]. In a veterans aging study (n=554 men), there was an increase of 2.5% per year in basal secretion of TNFα from blood cells with aging, but a decreased response to TLR2 (2.1 to 2.6% decrease/year) and TLR4 (1.9% decrease/year) agonists with age [15]. The decreased response to TLR2 stimulation may stem from a deficit in TLR1/2 signaling, as a separate study found that aging led to an impairment in TLR1/2 stimulation, whereas TLR2/6 stimulation appeared to be intact [46]. This could lead to an imbalance in TLR2 signaling and drive some of the age-related dysfunction. However, this study looked at blood cells, and TLR2 signaling may be differentially impacted with age in different cell types.

In cell culture systems, TLR2 has been shown to promote the pro-inflammatory senescence-associate secretory phenotype (SASP) in response to activation by acute-phase serum amyloids, which act as DAMPs [47]. Since TLR10 has also been implicated in mediating senescence, this effect may stem from activation of TLR2/10 heterodimers, which have been detected, but for which the function has not been characterized [1]. The conditions under which TLR2 drives senescence need to be better characterized in order to determine the best way to inhibit this process.
Cardiovascular disease: POTENTIAL BENEFIT FOR ACUTE ISCHEMIC INJURY (Preclinical)
Animal studies investigating the therapeutic benefit of TLR2 inhibition for cardiovascular diseases have shown conflicting results, especially those which involve the use of TLR2 knockout animals, due to the role of TLR2 in cardiovascular system development [48]. Overall, TLR2 inhibition appears to be protective in mitigating deleterious inflammatory responses following ischemic events. In mice, anti-TLR2 (OPN-301 10 mg/kg IV) administered prior to reperfusion reduced infarct size, reduced inflammatory immune cell infiltration and helped preserve systolic function in the context of cardiac ischemia/reperfusion injury [49]. Similar protective benefits were seen with OPN-350, a humanized anti-TLR2 in a pig model of myocardial ischemia/reperfusion injury [50]. The therapeutic response in pigs suggests a higher potential for therapeutic translation to humans. Development of OPN-350 has been discontinued, but these data suggest that TLR2 inhibitors may be beneficial for acute administration during ischemic cardiovascular events.

Atherosclerosis: POTENTIAL BENEFIT (Preclinical)
TLR2 is implicated in the development and progression of atherosclerosis [51]. The activation of TLR2 on vascular endothelial cells promotes inflammatory immune cell infiltration, foam cell formation, and smooth muscle cell proliferation [4]. The evidence suggests that TLR2 signaling in non-bone marrow derived cells is atherogenic, but it acts as a secondary factor to accelerate atheroma formation, and is not a primary driver of atherosclerosis [51]. Infections can accelerate plaque formation, which may stem from infection-related increases in TLR2 signaling [52]. The role of pathogens and associated inflammatory signaling in atherosclerosis has been questioned due to the failure of anti-infective therapy for reducing atherosclerotic cardiovascular events, however, this may be related to the timing of therapeutic intervention [4]. The evidence suggests that preventing infections or inhibiting the induction of associated inflammatory signaling would be most protective, and once TLR signaling has become chronically upregulated or dysregulated, anti-infective therapy may no longer be atheroprotective.

Due to differential expression of TLRs across tissues between mice and humans, it is necessary to have human tissue data in order to determine whether the findings regarding the role of a particular TLR in mice may be applicable to humans [53]. TLR2 has been shown to be expressed in human vasculature, and ex vivo studies have found that TLR2 signaling promotes the production of pro-inflammatory mediators (MCP-1/CCL2, IL-8/CXCL8, IL-6, NF-kB activation), and matrix metalloproteases (MMP-1, MMP-2, MMP-3, MMP-9) [54; 55]. The induction of these factors could be reduced through the use of TLR2 neutralizing antibodies.
**Metabolic syndrome/Diabetes**: POTENTIAL BENEFIT IS MICROBIOME DEPENDENT

Obesity is associated with an abnormal inflammatory response and insulin resistance. TLRs have been implicated in these inflammatory responses. Animal models have found that TLR2 can modulate energy metabolism. TLRs 2 and 4 have been shown to influence autonomic regulation of heart rate, body temperature, and energy metabolism in mice, by parasympathetic tone, and may be adaptive during periods of stress [56]. The activation of TLR2 on hypothalamic neurons has also been shown to maintain energy homeostasis in mice [57]. Whether TLR2 activation promotes or protects against high fat diet induced metabolic disturbances appears to be context dependent, as both outcomes have been reported in rodent studies. The outcome may be dependent upon the composition of the microbiomes of the particular strains or colonies of mice used in the experiments [56]. Therefore, **metabolic regulation may involve a complex interplay between the gut microbiome and TLRs**.

The context dependent nature of TLR function in metabolism may account for the inconsistency across studies in whether TLR plays a role in mediating the anti-inflammatory properties of exercise. In a systematic review of 17 studies, eight of which were in humans (n=271), animal studies showed decreases in TLR2, TLR4, NF-kB, and insulin resistance, but the responses in human studies were more variable [58]. Baseline levels of TLRs were generally higher in the obese and type 2 diabetics. The heterogeneity is thought to stem, at least in part, from differences in the exercise protocols used across studies, highlighting the sensitivity of TLR signaling to specific conditional states. There is cross-talk between the autophagy and TLR pathways in which TLRs can promote autophagy, which then acts in a negative feedback manner to reduce TLR levels [38]. One study assessing how exercise influences autophagy markers in the elderly, found that the expression of autophagy related proteins could be altered with exercise in the absence of detectable changes in TLR2/4 expression, though the duration of the study may have been too short to detect the corresponding change in TLRs [59]. Overall, the ability of TLR2 to productively regulate metabolic homeostasis is dependent on its ability to dynamically respond to changing conditions, such that chronic overactivation, as is present with metabolic disease, leads to metabolic disturbances. In this case, it is the normalization of TLR2 function, rather than its inhibition per se, that would be most beneficial.
Safety: TLR2 inhibitors may increase the risk for infections, particularly for Gram+ bacteria. Short term use of anti-TLR2 was well-tolerated, but long-term safety is unknown. Effects may be inhibitor type and disease type specific.

Types of evidence:
- 2 clinical trials (for OPN-350)
- Numerous laboratory studies

The anti-TLR2 antibody, OPN-350, has been tested in clinical trials to prevent delayed renal graft function in people receiving kidney transplants (n=252) ([NCT01794663](https://clinicaltrials.gov/ct2/show/NCT01794663)), and to improve the hematological response in people with myelodysplastic syndrome (n=96) ([NCT02363491](https://clinicaltrials.gov/ct2/show/NCT02363491)). Only a very limited amount of safety information from these trials has been made publicly available. An interim report of 15 myelodysplastic syndrome patients indicated that monthly IV doses of 5 or 10 mg/kg for a median of 5 cycles (2-17) was not associated with any significant drug-related toxicity or excess infections [60]. All drug-related adverse events were grade 1, with gastrointestinal events (33%) as the most frequent. It was also well tolerated when used in combination with azacitidine.

Infection risk is the primary safety concern for TLR2 inhibitors. TLRs are important for mediating the recognition and clearance of pathogens from the body [1]. If TLRs fail to mount a productive inflammatory response to a pathogen in a timely manner, it can increase the burden of the pathogen, which may lead to a potentially dangerous hyper-inflammatory response later on which can be more detrimental to the host than to the pathogen. The inability to mount an effective TLR response is thought to contribute to aging-related immunosenescence. Since many pathogens activate a variety of different TLRs to varying degrees, it is not clear whether the loss or inhibition of a single TLR (i.e. TLR2) would substantially impair the immune response and increase infection risk. It is likely, that in most cases, other TLRs would be able to compensate to some degree. However, TLR2 is extremely important for responding to gram positive bacteria, and for some species TLR2 activation may be necessary for an adequate response. Overall, it will be necessary to do long-term safety studies on TLR2 inhibitors to determine whether they pose a clinically meaningful risk for infection.

Since TLR2 signaling is highly context dependent and can induce a broad spectrum of effects, therapeutics targeting specific TLR2 interactions to particular co-receptors and adaptor proteins may have the highest therapeutic value and best safety profile. It will be necessary to determine which
interactions and downstream TLR2-mediated signaling pathways are most relevant for a particular disease.

Due to their effects on the immune system, TLR2 inhibitors are projected to have drug interactions with other immunosuppressive agents.

**Sources and dosing:** There are some TLR2 inhibitors in preclinical development, but none are currently in clinical development. The humanized anti-TLR2 antibody, OPN-350, had been tested in clinical trials in an IV formulation, however, development has been discontinued following the liquidation of the parent company, Opsona Therapeutics in 2019 ([Financial article](#)).

**Research underway:** There are currently no clinical trials testing TLR2 inhibitors.

**Search terms:**
Pubmed, Google: TLR2, Anti-TLR2 +
- Inhibitor, Alzheimer’s disease, Parkinson’s disease, neurodegeneration, aging, cardiovascular, diabetes, metabolism, inflammation, autophagy, infection, clinical trial, safety

**Websites visited for TLR2 Inhibitors:**
- [Clinicaltrials.gov](#) (OPN-350)

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