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.

Immunoproteasome Inhibitors

Evidence Summary
Immunoproteasome inhibition may protect against chronic inflammatory damage in autoimmune disease, but may need to be coupled with proteostasis activators to be useful for neurodegenerative disease.

**Neuroprotective Benefit:** Immunoproteasome upregulation may be a protective response to inflammation and oxidative stress, but promote neurodegeneration if chronically elevated. Effects of inhibition are likely context dependent.

**Aging and related health concerns:** Immunoproteasome inhibition may limit inflammatory damage in cardiovascular events, and treat blood cancers, but exacerbate other cancers, including breast cancer.

**Safety:** Likely safer than non-selective proteasome inhibitors, but carries a possible risk for immunosuppression. Well-tolerated in Phase 1 studies, but associated with nausea and injection reactions at high doses.
**What is it?**

The proteasome plays a major role in the processing of proteins for degradation, cellular signaling, and antigen processing [1]. It consists of a 20S core particle and a 19S regulatory particle. The 20S core is comprised of structural alpha subunits and catalytic beta (ß) subunits. In addition to the constitutive catalytic subunits, there are three alternative ß subunits (ß1i, ß2i, ß5i, also called LMP2, MECL-1, LMP7, respectively), which are highly expressed in hematopoietic cells, and can be induced in non-hematopoietic cells following exposure to inflammatory cytokines. Proteasomes containing the alternative ß subunits are referred to as the immunoproteasome. The immunoproteasome has altered protein cleavage preferences, thus the balance between constitutive proteasomes and
immunoproteasomes in a given cell can play a major role in regulating protein levels and function in different cell types.

The immunoproteasome has been shown to be better at generating peptides suitable for antigen presentation due to their increased ability to bind MHC class I molecules [2]. The immunoproteasome can promote inflammatory immune responses through its roles in antigen presentation, promoting cytokine production, and promoting pro-inflammatory T helper cell (Th1, Th17) differentiation and survival. Consequently, immunoproteasome inhibitors are being developed for autoimmune diseases. One of these inhibitors, KZR-616 from Kezar Life Sciences, is currently in clinical development for several autoimmune conditions.

Immunoproteasome inhibitors are also being developed for cancer, primarily multiple myeloma, as a potentially safer alternative to non-selective proteasome inhibitors.

**Neuroprotective Benefit:** Immunoproteasome upregulation may be a protective response to inflammation and oxidative stress, but promote neurodegeneration if chronically elevated. Effects of inhibition are likely context dependent.

**Types of evidence:**

- 4 observational studies (Immunoproteasome levels in postmortem brain in AD, PD, HD)
- Several laboratory studies (LMP7 preferential inhibitor ONX-0914)

*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:*

The mix of beta subunits in a proteasome determines its enzymatic properties, and the pool of proteasomes containing particular beta subunits varies in different cell types [3]. There are proteasomes containing all constitutive subunits, all inducible (immunoproteasome) subunits, as well as hybrids containing both. The pool of different proteasomes provides plasticity, and the balance can shift under different conditions. In order to maintain proteostasis, the changes need to be dynamic, and prolonged shifts in composition are associated with cellular dysfunction and pathology.
There is evidence from postmortem tissue that the **immunoproteasome is upregulated in the CNS in the context of various neurodegenerative conditions**, which may be a downstream effect of increased basal levels of oxidative stress and inflammation [4; 5; 6]. It may also be a compensatory response to decreased constitutive proteasome activity, and play a role in directing an immune response toward aggregation-prone proteins associated with neurodegenerative disease. However, as a byproduct, it may also accelerate neuroinflammatory damage, and promote the loss of neurons containing these disease-associated proteins.

The role of the immunoproteasome in neurodegenerative diseases may differ depending on the stage of disease, the cell type(s) in which it is upregulated, and whether proteostasis is impaired. An early increase in immunoproteasome activity in response to increased levels of oxidized and aggregated proteins may be beneficial in limiting the spread of pathology [7]. Immunoproteasome induction in glial cells and monocytes may promote the clearance of the protein aggregates [5; 8]. In neurons, a transient induction of the immunoproteasome may provide resilience against mild oxidative stress and promote protein homeostasis [9]. However, if there is a **prolonged shift in the balance between immunoproteasome and constitutive proteasome subunits**, then it may drive inflammation, impair neuronal function, and ultimately promote neurodegeneration.

This suggests that inhibition of the immunoproteasome alone is likely to be of limited benefit, and could potentially promote the accumulation of pathological proteins in contexts where the primary cellular protein degradation processes, the constitutive proteasome and autophagy, are impaired. [**Immunoproteasome inhibition is likely to be most beneficial when coupled with agents that can promote the constitutive proteasome**, as part of a mechanism to restore the balance in neurons.]

**Evidence from human postmortem tissue**

**Alzheimer’s disease (AD):** The levels of the immunoproteasome subunits LMP7 (β5i) and LMP2 (β1i) were found to increase with age in the hippocampus, as they were present in neurons, astrocytes, and endothelial cells in elderly individuals (mean age 70), but barely detectable in younger adults (mean age 42) [10]. Polymorphisms in LMP2 can alter proteasome activity in the brain, but it is not yet clear if or how these changes contribute to AD risk. Postmortem hippocampal tissue showed evidence of a shift in proteasome activity in Braak staged brains from AD patients, with a decrease in the constitutive β5 subunit and increases in immunoproteasome subunits [4; 11]. The expression of immunoproteasome subunits was increased primarily in astrocytes. [**The increase in immunoproteasome subunit activity was correlated with amyloid pathology** (Pearson’s’ r: β5i 0.536, P < 0.0001; β2i 0.543, P < 0.0001; β1i 0.283, P = 0.054) and **tau pathology** (Pearson’s’ r: β5i 0.554, P < 0.001; β2i 0.630, P < 0.001) [4]].
**Parkinson’s disease (PD):** The protein levels of the immunoproteasome subunit LMP7 (β5i) were found to be 3-fold higher in the brains of Lewy body dementia patients relative to controls, and LMP7 levels were also elevated in the substantia nigra and ventral tegmental area of patients with PD [5]. Notably, the immunoproteasome subunit was increased in both neurons and glia of the substantia nigra, which is the most vulnerable to dopamine neuron loss, but only increased in glia in the less vulnerable ventral tegmental area.

**Huntington’s disease:** In patients with Huntington’s disease, levels of LMP7 (β5i) and LMP2 (β1i) were found to be 500% and 80% higher in the cortex, and 350% and 150% higher in the striatum, respectively, relative to age-matched controls [6]. The increase in immunoproteasome subunits was associated with a corresponding loss of constitutive proteasome subunits (β1 and β5) in the striatum, but not in the cortex. The *induction of the immunoproteasome subunits in neurons was associated with signs of neurodegeneration*, and may be a secondary response to elevated neuroinflammation.

**Evidence from preclinical animal models**

**Alzheimer’s disease:** Unclear

The ratio of immunoproteasome to proteasome subunits was found to increase with age in the hippocampus of male rats [12], which likely stems from increased levels of inflammation and advanced glycated end products [13]. Expression of the LMP7 (β5i) subunit decreased temporarily in response to spatial memory training, suggesting that changes in proteasome subunit composition could impact learning and memory [12]. APP/PS1 mice deficient in LMP7 (β5i) had reduced levels of pro-inflammatory cytokines (TNFα, IL-6) and modest improvements in spatial memory, but no change in Aβ plaque burden relative to AD mice that express LMP7 [14]. However, in the 5XFAD model, the recruitment of monocytes expressing high levels of LMP7 (β5i) facilitated the clearance of Aβ in the brain [15].

In the AD11 mouse model of sporadic AD, the expression profile of proteasome subunits diverges over time from age matched controls, leading to differences in proteasome function [16]. The differential recruitment of proteasome subunits likely stems from differences in the neuro-environment, such as the cytokine milieu, and the presence of the pathological proteins, Aβ and tau. The change in proteasome subunit composition could impact neuronal function by altering the preference for which proteins are degraded, as well as the antigenicity and signaling capacity of the cleaved peptides.
Parkinson’s disease: Potential harm for inhibitors at early stages (preclinical)

The upregulation of the immunoproteasome was found to be protective at early stages in preclinical PD models. The immunoproteasome subunits were induced in response to oxidative stressors rotenone and 6-OHDA, which limited the extent of dopamine neuron loss [9; 17]. Knockdown or inhibition of the immunoproteasome subunits exacerbated neurotoxicity, while overexpression was protective. The immunoproteasome may also help protect against alpha-synuclein pathology by promoting its clearance [5]. In contrast to the constitutive proteasome, cleavage of alpha synuclein fibrils by the immunoproteasome results in the production of peptides within the antigenic region, which may help direct an immune response toward alpha-synuclein. This suggests that at least early in the disease course, the immunoproteasome may be responding to elevations in oxidative stress and alpha synuclein fibrils, and acting to mitigate damage by promoting the degradation of oxidized proteins and aggregation prone alpha-synuclein.

Amyotrophic lateral sclerosis (ALS): Potential harm for inhibitors (preclinical)

In the SOD1G93A mouse model, there is an induction of immunoproteasome subunits in microglia and astrocytes in the spinal cord in response to increased levels of inflammatory cytokines as the disease progresses [8; 18; 19]. Degenerating motor neurons show impairment of the ubiquitin proteasome system. Treatment with a drug blocking the immunoproteasome induction decreased survival by 15% (20 days) [19], thus the immunoproteasome induction may be part of a compensatory response.

APOE4 interactions: Unknown

Aging and related health concerns: Immunoproteasome inhibition may limit inflammatory damage in cardiovascular events, and treat blood cancers, but exacerbate other cancers, including breast cancer.

Types of evidence:

- 4 observational studies (Immunoproteasome levels in heart failure, breast cancer, stroke)
- Several laboratory studies

Lifespan: Dynamic immunoproteasome induction associated with longevity

Longevity in primates was associated with elevated 20S proteasomal activity ($R^2 = 0.21$, $P = 0.03$) and increased expression of the LMP7 (85i) immunoproteasome subunit ($R^2 = 0.29$, $P = 0.04$) in fibroblasts.
The fibroblasts from long-lived primates had better induction of immunoproteasome subunits in response to IFN-γ, and incurred less protein damage in response to oxidative stress, relative to shorter-lived primates. Additionally, both constitutive and immunoproteasome expression was found to increase in the livers of male mice exposed to drugs associated with lifespan extension (rapamycin, nordihydroguaiaretic acid, 17-α-estradiol). This suggests that the dynamic induction of the immunoproteasome is protective, and dysregulation of this process could impair cellular resiliency.

**Cancer**

As metabolically active cells, cancer cells require high levels of proteasome activity, and thus are vulnerable to proteasome inhibitors [1]. However, proteasome inhibitors also target metabolically active healthy cells, leading to side effects. Specifically targeting the immunoproteasome could improve the therapeutic window, however, there is variation in the expression of the immunoproteasome and its contribution to disease progression across different types of cancer. Therefore, for some cancers inhibition of the immunoproteasome may be beneficial, whereas it may be detrimental for other types.

**Multiple Myeloma: Immunoproteasome inhibition has potential benefit (preclinical)**

Since the immunoproteasome is normally most highly expressed in cells of hematopoietic origin, immunoproteasome subunits are highest in myeloid cell and lymphoid cell cancers [21]. Immunoproteasome inhibitors are primarily being developed for these blood cell cancers, especially multiple myeloma, for which non-selective proteasome inhibitors are currently used. In multiple myeloma cell culture and/or mouse xenograft models, treatment with the LMP7 (β5i) selective inhibitor M3258, LMP7 (β5i) preferential inhibitor PR-924, or LMP2 (β1i) preferential inhibitor IPSI-001 was effective at reducing tumor cell growth, improving survival, and/or overcoming chemoresistance [22; 23; 24]. M3258 will be tested in a Phase 1 trial for multiple myeloma (NCT04075721).

**Breast Cancer and Colon Cancer: Immunoproteasome expression associated with better outcome**

The immunoproteasome plays a role in processing proteins for MHC class I antigen presentation, and can promote the activation of the immune system towards tumor antigens [25]. The downregulation of the immunoproteasome is associated with the loss of antigen presentation and fosters tumor evasion for breast and colon cancer. High expression of LMP7 (β5i) was associated with higher levels of tumor infiltrating lymphocytes and better disease-free survival (Hazard Ratio HR: 0.483, 95% CI 0.239-0.978, p=0.043) [26]. Breast cancer patients in the top-third of immunoproteasome expression were found to have better ten-year survival relative to those in the bottom third (61.9% vs 36.1%) [21]. The LMP7 Q/Q genotype is associated with a lower risk of colon cancer (Odds Ratio OR: 0.10, P = 5.97 × 10⁻¹³), relative
to those with the K/Q genotype (OR: 8.10, P = 1.10 × 10⁻¹¹), which may stem from the lower LMP7 induction capacity and stability of the K polymorphism, leading to reduced tumor antigen presentation [27].

**Melanoma: Immunoproteasome expression associated with disease progression**

However, for melanoma, the MART-1\_126-35 antigen is optimally produced through different proteasome subunits, and high levels of immunoproteasome subunits can prevent effective antigen production, thus facilitating melanoma immune evasion and progression [28].

**Cardiovascular disease: Immunoproteasome inhibition has potential benefit (preclinical)**

The upregulation of immunoproteasome subunits in the blood and heart may serve as a biomarker for worse cardiovascular outcomes, and signify an increase in inflammation-associated damage. Partial inhibition of the immunoproteasome may help limit inflammation-associated damage in cardiovascular tissue.

**Heart failure:** Increased levels of the immunoproteasome subunit LMP7 (β5i) in the heart (n=6) and serum (n=76) were found to be associated with heart failure (OR: 12.123, 95% CI 2.38-62.08, p=0.003) [29]. LMP7 (β5i) expression is also elevated in cardiac tissue from patients with aortic abdominal aneurysms and atrial fibrillation [30; 31]. In mouse models of angiotensin-II induced heart disease, LMP7 (β5i) expression is upregulated, and knockdown or inhibition of LMP7 has been shown to reduce cardiac inflammation and fibrosis [29; 30; 31]. In addition to promoting inflammatory immune cell infiltration, the immunoproteasome can contribute to pathological processes by promoting the degradation of protective proteins [29; 31].

**Stroke:** Elevated levels of immunoproteasome subunits (LMP2 ≥ 988.3 pg/mL, MECL-1 ≥ 584.7 pg/mL, LMP7 ≥ 509.0 pg/mL) were found to be associated with ischemic stroke severity and serve as prognostic factors for hemorrhagic transformation risk [32]. The induction of immunoproteasome subunits occurred under conditions of high inflammation, as marked by elevated levels of Hs-CRP and IL-1β. In the mouse MCAO stroke model, treatment with the LMP7 inhibitor ONX-0914 reduced T cell infiltration, inflammatory cytokine levels, and infarct volume [33].
Safety: Likely safer than non-selective proteasome inhibitors, but carries a possible risk for immunosuppression. Well-tolerated in Phase 1 studies, but associated with nausea and injection reactions at high doses.

Types of evidence:

- 2 clinical trials [KZR-616: Phase 1 (n=72) in healthy volunteers, Phase 1b/2 (n=72) in lupus]
- Numerous laboratory studies

Non-selective proteasome inhibitors which inhibit both the constitutive and immunoproteasome catalytic subunits by approximately 80%, such as bortezomib, which is used for multiple myeloma, have a narrow therapeutic window due to the high risk for side effects, especially peripheral neuropathy [3]. Immunoproteasome specific inhibitors are expected to have a better safety profile because they only target cells with high levels of immunoproteasome activity, presumably pathogenic cells, while preserving the essential functions of the constitutive proteasome in healthy cells. However, the improved safety is dependent on the inhibitors having strong selectivity for the immunoproteasome subunits over the constitutive subunits.

ONX-0914 (PR-957) has been the most extensively tested immunoproteasome inhibitor used in preclinical studies, but due to its relatively low selectivity for the immunoproteasome, is not a good candidate for clinical translation. It has been found to be effective in animal models of autoimmune diseases including rheumatoid arthritis, diabetes, multiple sclerosis, colitis, and lupus, without significantly impacting normal immune function [34]. The efficacy appears to depend on the ability of ONX-0914 to inhibit multiple immunoproteasome subunits, as one study found that a highly selective LMP7 (ß5i) inhibitor was not effective in these autoimmune models. While ONX-0914 maximally inhibits LMP7, at efficacious doses, it also inhibits LMP2 (ß1i) by 60%, with additional minor effects on MECL-1 (ß2i). The good safety exhibited in these models may be due to the short duration of treatment in these studies. Cellular toxicity stems from the inhibition of the constitutive proteasome [35], and since ONX-0914 is only 20 to 40-fold more selective for the ß5i immunoproteasome subunit over the ß5 constitutive subunit [36], its long-term use could lead to gradual inhibition of the constitutive proteasome and poses a risk for toxicity.

The immunoproteasome is highly expressed in immune cells, and affects immune system function through its roles in promoting antigen presentation, cytokine secretion, and T cell differentiation [37]. Inhibition of immunoproteasome subunits limits the diversity of generated antigenic and signaling peptides, which could compromise the ability of the immune system to clear some types of pathogens.
Preclinical studies may also underestimate the potential risk of immunosuppression because the immune system is typically not challenged in these models. In a mouse model of fungal infection (Candida albicans), treatment with the immunoproteasome inhibitor ONX-0914, increased susceptibility to infection and fungal burden, leading to increased neutrophil recruitment and immunopathology [39]. This suggests that chronic immunoproteasome inhibition could increase risk for some types of infections.

KZR-616 is an LMP7 (β5i) and LMP2 (β1i) inhibitor being developed for autoimmune diseases [34] which has been shown to be well-tolerated at doses up to 45 mg in Phase 1 trials in healthy volunteers and systemic lupus erythematosus patients. At a dose of 45 mg injected subcutaneously, KZR-616 inhibited LMP7 (β5i) by 95% and LMP2 (β1i) by 70% in blood cells from healthy volunteers [40], and inhibited LMP7 (β5i) >80% and LMP2 (β1i) >40% in lupus patients (MISSION Study Data Presentation). Meanwhile, the activity of the β5 constitutive proteasome subunit was largely spared, with study subjects showing levels of inhibition between ~ 0 to 60%. A couple of patients showed >60% β5 inhibition at the 60 mg dose, which is a dose that was also associated with a higher number of adverse events, including nausea and vomiting.

In the Phase 1 study in healthy volunteers (n=72), the most common adverse events were mild, transient injection site reactions, with no evidence of peripheral neuropathy, or clinically significant laboratory or ECG abnormalities [40]. At the 60 mg dose, 4 subjects experienced a systemic drug reaction including chills, elevated heart rate and nausea. As a result, the protocol was changed for all other cohorts to escalate from an initial 30 mg dose and to include prophylactic treatment with antihistamines and prednisone. There was a similar safety profile found in the ongoing Phase 1b/2 study (n=72) in lupus patients (NCT03393013). There were two serious adverse events of thrombotic microangiopathy and herpes zoster virus, however, since these are associated with both protease inhibitors and lupus, it is unclear whether they are drug related (MISSION Study Data Presentation).

Non-selective protease inhibitors have interactions with drugs that act as immunosuppressants and QT prolonging agents (WebMD), and although it has not yet been established, immunoproteasome inhibitors may have similar drug interactions.

Sources and dosing:

Immunoproteasome inhibitors, such as ONX-0914, are available for research use from commercial suppliers. KZR-616 is currently being developed by Kezar Life Sciences for autoimmune diseases. It is being clinically tested at a dose of 45 mg subcutaneously once weekly (with dose escalation from 30 mg)
in combination with prednisone in a Phase 2 study in patients with lupus, but a clinically therapeutic
dose has not yet been established.

**Research underway:**

KZR-616 is currently being tested in a Phase 1b/2 RCT for lupus with and without nephritis
([NCT03393013](https://clinicaltrials.gov/ct2/show/NCT03393013)), a Phase 2 RCT for autoimmune hemolytic anemia and immune thrombocytopenia
([NCT04039477](https://clinicaltrials.gov/ct2/show/NCT04039477)), and a Phase 2 RCT for polymyositis and dermatomyositis ([NCT04033926](https://clinicaltrials.gov/ct2/show/NCT04033926)).

M3258 is recruiting for a Phase 1 open label trial in patients with multiple myeloma ([NCT04075721](https://clinicaltrials.gov/ct2/show/NCT04075721)).


The currently used immunoproteasome inhibitors have a peptide epoxyketone backbone and act as irreversible inhibitors by covalently modifying proteasome subunits. There are efforts underway to develop reversible inhibitors that have a high degree of specificity and selectivity toward immunoproteasome subunits relative constitutive subunits [41].

**Search terms:**

Pubmed, Google: Immunoproteasome inhibitor, LMP7 inhibitor, LMP2 inhibitor, ONX-0914, KZR-616, UK-101, IPSI-001, M3258 +

- Alzheimer’s disease, Parkinson’s disease, neurodegeneration, aging, cancer, cardiovascular, inflammation, safety, clinical trials

Websites visited for Immunoproteaseome Inhibitors:

- Clinicaltrials.gov ([KZR-616](https://clinicaltrials.gov/ct2/show/NCT03393013), [M3258](https://clinicaltrials.gov/ct2/show/NCT04039477))

**References:**

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