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.

XPro1595

Evidence Summary

XPro1595 has the benefit of inhibiting soluble TNF specifically without affecting transmembrane TNF. Safety and efficacy will be determined when data from clinical trials become available.

Neuroprotective Benefit: XPro1595 treatment improved cognitive function and showed neuroprotective effects in many animal models. XPro1595 is being tested in clinical studies for Alzheimer's disease.

Aging and related health concerns: XPro1595 shows benefit in mouse models of cancer, cerebral ischemia, glaucoma, neuropathic pain, and spinal cord injury, but effects depend on the timing and route of treatment. There have not been any studies in humans.

Safety: A Phase 1b study is ongoing. No safety data from clinical trials in humans have been published.

Availability: in clinical development	**Dose**: A phase 1b open-label study is testing XPro1595 doses of 0.3, 1.0, and 3.0 mg/kg, subcutaneously, once a week (NCT03943264).	**Chemical formula:** not publicly disclosed **MW**: not publicly disclosed
Half life: not published	**BBB**: penetrant based on animal studies	
Clinical trials: Several are ongoing, but no results have been published to date.	**Observational studies**: none available	

What is it? Tumor necrosis factor (TNF) comes in two forms, the transmembrane TNF (binding to TNFR2, playing a role in cell-cell contact) and soluble TNF (binding to TNFR1, producing action at a distance). Transmembrane TNF plays a role in neuroprotection, while soluble TNF is involved in pro-inflammatory functions, demyelination, and neurodegeneration. Anti-TNF antibodies (e.g., etanercept or Enbrel) block both the neuroprotective and neurodegenerative forms of TNF and have been associated with learning and memory impairment along with decreased neurogenesis (Yli-Karjanmaa et al., 2019). XPro1595 is a protein biologic under development by INmune Bio Inc., which selectively sequesters the soluble TNF by forming heterotrimers with native soluble TNFα (Steed et al., 2003). It is a PEGylated human TNF variant devoid of TNF receptor-binding activity. It is being developed for Alzheimer's disease, treatment-resistant depression, and cancer (inmunebio.com).

Neuroprotective Benefit: XPro1595 treatment improved cognitive function and showed neuroprotective effects in many animal models. XPro1595 is being tested in clinical studies for Alzheimer's disease.

Types of evidence:

- Numerous laboratory studies

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

Human research to suggest benefits to patients with dementia:
None available. A phase 1b study is currently ongoing in mild to moderate Alzheimer's patients (NCT03943264).

Mechanisms of action for neuroprotection identified from laboratory and clinical research:
XPro1595 has been studied in various animal and *in vitro* models. Subcutaneous XPro1595 treatment (10 mg/kg, s.c., every 3 days) achieved plasma levels of 1-8 µg/ml and CSF levels of 1-6 ng/mL, suggesting that XPro1595 reaches the brain (Barnum et al., 2014).

Aging model: In aged rats, treatment with XPro1595 (0.08 mg/kg/day into the lateral ventricle) for 4-6 weeks significantly improved cognitive function (measured by the Morris water maze), reduced microglial activation (decreased Iba-1 protein levels), reduced susceptibility to hippocampal long-term depression, increased protein levels of the GluA1 type glutamate receptor, and lowered L-type voltage-sensitive calcium channel activity in the hippocampal CA1 neurons (Sama et al., 2012). Protein levels for TNFR1 are elevated in the hippocampus relative to TNFR2 in aged but not young adult rats, suggesting that selective alterations in TNF signaling may drive age-related cognitive decline and synaptic dysfunction.

Alzheimer's disease models: XPro1595 has been tested in 3 mouse models of Alzheimer's disease. In 5xFAD mice, treatment with XPro1595 (10 mg/kg, twice weekly, subcutaneously) for 2 months reduced the age-dependent increase in activated immune cells, while decreasing the overall number of CD4+ T cells and decreasing Aβ plaques in the subiculum (MacPherson et al., 2017). In brain slices of 5xFAD mice, XPro1595 treatment rescued the impairment in long-term potentiation. Treatment with XPro1595 for 2 months started at 5 months of age (onset of cognitive impairment) decreased the expression of pro-inflammatory genes in both 5xFAD mice and non-transgenic mice. CCL2 and TGFβ are significantly decreased in 5xFAD mice, but IL-1β and TNF failed to reach statistical significance.

In an amyloid transgenic mouse model (TgCRND8 mice), treatment with XPro1595 (10 mg/kg, s.c.) started at 1 month of age for 4 weeks prevented synaptic deficits 4 months later at the age of 6 months (Cavanagh et al., 2016). In the inhibitory avoidance task, TgCRND8 mice treated with XPro1595 had latencies that were significantly lower than untreated TgCRND8 mice. In this mouse model, initiating treatment to block TNF-α prior to the onset of amyloid plaque formation prevented the hyperexcitability of glutamate synapses.

In another mouse model of Alzheimer's (3xTgAD mice), XPro1595 treatment (0.1 mg/kg/day, delivered to hippocampal CA1 with an osmotic pump) for 4 weeks significantly decreased the LPS-induced accumulation of Aβ in the hippocampus, cortex, and amygdala (McAlpine et al., 2009).

Parkinson's disease models: In a rat model of Parkinson's disease (6-OHDA hemiparkinsonian rats), XPro1595 treatment (10 mg/kg, s.c., every 3 days) for 35 days significantly reduced microglia and astrocyte number in substantia nigra pars compacta region, whereas loss of nigral dopaminergic neurons was attenuated when XPro1595 treatment was initiated 3, but not 14 days after the 6-OHDA lesion (Barnum et al., 2014).

In a monkey model of Parkinson's disease (escalating MPTP treatment), XPro1595 treatment (10 mg/kg, s.c., every 3 days) started on week 11 of MPTP lesion and continued onto week 40 failed to protect against nigrostrial dopaminergic neuron loss (Joers et al., 2020). Other effects of XPro1595 were mixed and difficult to interpret due to sexually dimorphic responses and small sample sizes. For example, female monkeys displayed less reactive circulating cytokines compared to males, making it difficult to assess the immunomodulatory effects of XPro1595. Neutralization of soluble TNF with XPro1595 may have attenuated inflammation in biofluids and reduced CD68 expression (expressed by cells in the monocyte lineage, e.g., macrophages) in the colon. But there was early and robust increase in neuroinflammation as measured by PET TSPO prior to initiation of XPro1595 treatment, suggesting that the window for therapeutic benefit may have been missed in these monkeys. It remains to be seen whether an earlier intervention with XPro1595 can afford more neuroprotection.

Huntington's disease models: In a mouse model of Huntington's disease (R6/2 mice), intracerebroventricular XPro1595 treatment (0.08 mg/kg/day, into the lateral ventricle) for 28 days starting at the age of 7 weeks decreased levels of TNF-α in the cortex and striatum, improved motor function, reduced caspase activation, decreased the amount of mutant HTT aggregates, increased neuronal density, and decreased gliosis (Hsiao et al., 2014). Systemic injection of XPro1595 (30 mg/kg, twice weekly, i.p.) at the ages of 7-15 weeks old improved the impaired motor function of R6/2 mice but did not affect caspase activation.

In vitro experiments showed that XPro1595 suppressed the inflammatory responses of primary astrocyte-enriched culture isolated from R6/2 mice and human astrocyte-enriched culture derived from induced pluripotent stem cells of Huntington's disease patients evoked by lipopolysaccharide and cytokines, respectively, while also protecting from cytokine-induced toxicity.

Multiple sclerosis models: In a mouse model of multiple sclerosis (experimental autoimmune encephalomyelitis; EAE), treatment with XPro1595 (10 mg/kg, every 3 days, s.c.) for 39 days (started on Day 16 of EAE when half of the mice reached a clinical score at or over 2 and until Day 55) significantly improved clinical outcome, preserved axons, improved myelin compaction, increased expression of axon-specific molecules (e.g., neurofilament-H), and decreased a protein associated with axon damage (non-phosphorylated neurofilament-H)(Brambilla et al., 2011). XPro1595-treated mice showed remyelination accompanied by increased numbers of oligodendrocyte precursors. GFAP protein, IFN-γ, TNF, IL-1β, IL-6, CCL-2, CCL-5, CXCL-10, and CCR-2 were significantly decreased with XPro1595 treatment compared to the vehicle-treated. These findings suggest that selective inhibition of soluble TNF improves recovery following EAE, and that signaling pathways mediated by transmembrane TNF drives the repair process. This is in contrast to non-selective TNF inhibitors, which are associated with demyelination.

In another mouse model of multiple sclerosis (cuprizone-fed mice), XPro1595 treatment (10 mg/kg, twice weekly, s.c.) did not prevent toxin-induced oligodendrocyte loss and demyelination, but resulted in early remyelination due to improved phagocytosis of myelin debris by CNS macrophages and prevention of disease-associated decline in motor performance (Karamita et al., 2017). Because these benefits with XPro1595 were absent in TNF-deficient mice and replicated in transmembrane TNF knock-in mice, transmembrane TNF appears to be sufficient for the maintenance of myelin and remyelination. XPro exerts neuroprotective effects by promoting early resolution of microgliosis in demyelinated lesions and increasing the clearance of myelin debris by phagocytic CNS macrophages, while promoting the synthesis of new myelin in demyelinated axons.

Other models: In a mouse model of type 2 diabetes (fed a high-fat high carbohydrate diet for 14 weeks), XPro1595 treatment (10 mg/kg, every 3 days, s.c.) for 11 weeks ameliorated hepatic metabolic disturbances, insulin resistance, and behavioral deficits (sociability deficits and anxiety-like behavior), while decreasing hepatic and intestinal levels of lipocalin-2, a marker of inflammation, ischemia, and kidney damage (de Sousa Rodriguez et al., 2019; Moschen et al., 2017).

APOE4 interactions: Unknown.

Aging and related health concerns: XPro1595 shows benefit in mouse models of cancer, cerebral ischemia, glaucoma, neuropathic pain, and spinal cord injury, but effects depend on the timing and route of treatment. There have not been any studies in humans.

Types of evidence:

- Numerous laboratory studies

XPro1595 has been studied in numerous animal models of age-related diseases. No data in humans have been published in peer-reviewed journals.

Cancer: BENEFIT IN MOUSE MODELS
In a mouse model of cancer (3-methylcholanthrene-induced carcinogenesis), XPro1595 treatment (200 µg/mL, i.p. twice weekly) for 12 weeks decreased tumor incidence and growth, prolonged survival of the mice, while decreasing IL-1α and increasing IL-1β, IL12p70, and IL-17 in the blood (Sobo-Vujanovic et al., 2016). In addition, XPro1595 treatment prevented accumulation of myeloid-derived suppressor cells, STAT3 phosphorylation, and immunosuppression in these mice. Based on studies of other mouse models (TNF-, TNFR1-, and TNFR2-deficient mice), soluble TNF is both an essential promoter of carcinogenesis and a pivotal regulator of myeloid-derived suppressor cells.

In a rat model of bone cancer pain (intratibial inoculation of Walker 256 mammary gland carcinoma cells), XPro1595 treatment (5, 10, and 20 mg/kg, intrathecal, every other day) from postoperative day 1-13 suppressed bone cancer-evoked glial activation and neuroinflammation (Zhou et al., 2019). These effects of XPro1595 were, at least partly, mediated by a reduction in the phosphorylation of p38 MAPK in spinal glial cells.

Cerebral ischemia: BENEFIT IN MOUSE MODEL WITH IMMEDIATE AND LOCALIZED TREATMENT
In a mouse model of focal cerebral ischemia, (permanent middle cerebral artery occlusion), XPro1595 treatment (intravenously once, at a dose of 10 mg/kg) 30 minutes after occlusion significantly improved functional outcomes, altered microglial responses, decreased granulocyte infiltration into the brain, and modified the acute phase response, spleen T cell and microvesicle numbers, but without decreasing infarct volumes (Clausen et al., 2014). The protective effects of XPro1595 were comparable to those of etanercept, suggesting that soluble TNF is principally involved in the peripheral inflammation after stroke.

In a mouse model of focal cerebral ischemia (permanent middle cerebral artery occlusion), XPro1595 treatment into the infarct core (2.5 mg/ml/1 µL/hour, mini-osmotic pump implanted 30 minutes after occlusion) for 1 or 3 consecutive days reduced infarct volume (Yli-Karjanmaa et al., 2019). However, intracerebroventricular treatment (1.25 mg/kg/0.5 ml, single dose) immediately after occlusion failed to reduce infarct volume. XPro1595 increased gene expression of P2RY12 and Trem2, while decreasing CX3CR1 expression after occlusion, suggesting a shift in microglial activation toward a phagocytic phenotype.

Glaucoma: BENEFIT IN A RAT MODEL
In a rat model of glaucoma, XPro1595 injection into the vitreous chamber (total volume of 5 µL at 50 µg/µL) protected the retinal ganglion cell soma and axons (Cueva Vargas et al., 2015). Retinal ganglion cell soma and axon/optic nerve survival were 94% and 81% with XPro1595 treatment, respectively, relative to intact control, whereas in vehicle-treated rats, survival was 66% and 46%, respectively. In this model, glia-derived soluble TNF-α, downregulation of GluA2 subunit of AMPA receptors, and upregulation of calcium-permeable AMPA receptor play a critical role in glaucomatous neurodegeneration.

Neuropathic pain: BENEFIT IN MALE MOUSE MODEL
In a mouse model of neuropathic pain (chronic peripheral nerve constriction injury), XPro1595 treatment (10 mg/kg, s.c., every 2 days) for 5 weeks significantly accelerated recovery from neuropathic pain in males, but not females (Del Rivero et al., 2019). In male mice, XPro1595 treatment reduces elevated NMDA receptor levels in the brain after injury, whereas in female mice, NMDA receptor levels decrease after nerve injury. It appears that estrogen inhibits the therapeutic response of XPro1595 in females, as ovariectomized mice that underwent the same injury showed reduction in allodynia, similar to responses observed in males.

Spinal cord injury: BENEFIT IN RODENT MODELS WHEN APPLIED DIRECTLY AND IMMEDIATELY AFTER INJURY
In a mouse model of moderate spinal cord injury (laminectomization between vertebrae T8 and T10, and an approximate displacement of 500 µm), XPro1595 treatment (10 mg/kg, every 3 days, s.c., started 30 minutes after injury) for 8 weeks failed to improve locomotor performance (Novrup et al., 2014). In contrast, in the same mouse model, central administration of XPro1595 (2.5 mg/mL/1 µL/hour, started immediately after injury) for 3 days resulted in improved locomotor function, decreased anxiety-related behavior, and reduced damage to the lesioned spinal cord. Central administration of etanercept (non-selective anti-TNF) showed no therapeutic benefits. Improvements in XPro1595-treated mice were

accompanied by increases in Toll-like receptor 4 and TNFR2 protein levels and changes in Iba1 protein expression in microglia/macrophages. Thus, protection is observed only when selective inhibition of soluble TNF is achieved directly to the lesioned cord immediately after injury.

After a severe, high-level spinal cord injury, heightened spinal sympathetic reflex activity can detrimentally impact peripheral organ systems, causing episodes of autonomic dysreflexia, a life-threatening condition characterized by sudden hypertension and reflexive bradycardia following below-level sensory inputs (O'Reilly et al., 2021). The frequency and severity of such events increase greatly beginning several weeks post-injury. People who have experienced spinal cord injury are susceptible to cardiovascular disease and infections, which are leading causes of morbidity and mortality.

In female rats subjected to autonomic dysreflexia in high-level spinal cord injury (dorsal laminectomy of T3), XPro1595 treatment (intrathecal, 60 µg/day via osmotic minipumps at the lesion epicenter) decreased the frequency and severity of autonomic dysreflexia episodes (measured by autonomic dysreflexia events and peak blood pressure and duration per autonomic dysreflexia event) (Mironets et al., 2018). This was mediated by decreased sprouting of nociceptive primary afferents and activation of the spinal sympathetic reflex circuit. pNF-kB p65 is significantly elevated in rats subjected to spinal cord injury, but intrathecal XPro1595 treatment lowered this to levels comparable to naïve rats. XPro1595 treatment also slightly decreased TNFR1 levels in the lumbar spinal cord. XPro1595 treatment did not affect basal hemodynamics (baseline mean arterial pressure or baseline heart rate).

In rats subjected to high thoracic spinal cord injury, intrathecal XPro1595 treatment started at 2 weeks post-injury and continued for 42 days failed to attenuate the severity or intensification of sympathetic hyperreflexia compared with vehicle-treated controls (O'Reilly et al., 2021). These findings suggest that central soluble TNF-α signaling needs to be inhibited sooner after injury in order to decrease sympathetic hyperreflexia.

Safety: A Phase 1b study is ongoing. No safety data from clinical trials in humans have been published.

Types of evidence:

- Numerous laboratory studies

A phase Ib study of XPro1595 in patients with mild to moderate Alzheimer's disease is ongoing (NCT03943264). It is an open-label study including 18 participants designed to determine the optimal

dose (0.3, 1.0, and 3.0 mg/kg via subcutaneous injection once a week for 12 weeks). Results from this phase I study have not been published as of September 2021.

In a rat model of spinal cord injury (T9 contusive injury), XPro1595 treatment (10 mg/kg, every 3 days, s.c.) started at the time of injury caused an exacerbation of depressive phenotype (assessed by forced swim immobility) seen in greater than 50% of female rats subjected to spinal cord injury (Farrell and Houle, 2019). There was a positive correlation between increased TNF levels in the dorsal raphe nucleus after thoracic spinal cord injury, but targeting this increase failed to reduce the depressive phenotype, and instead, exacerbated the incidence of depression. Whether or not XPro1595 treatment produces similar effects in people is not yet known.

Drug interactions: Drug interactions have not been studied to date. Based on XPro1595's mechanism of action, it is likely to interact with medications that target the TNF or other immune modulators.

Sources and dosing: XPro1595 is under development by INmune Bio Inc. It is being developed for Alzheimer's disease, treatment-resistant depression, and cancer (inmunebio.com). A phase 1b open-label study is testing 0.3, 1.0, and 3.0 mg/kg doses of XPro1595, subcutaneously, once a week, in patients with Alzheimer's disease (NCT03943264). In mouse studies, the typical dose is 10 mg/kg, subcutaneously, every 3 days (de Sousa Rodriguez et al., 2019; Yli-Karjanmaa et al., 2019).

Research underway: There are currently 2 clinical trials testing XPro1595. One is a biomarker-directed phase I study of XPro1595 in patients with mild to moderate Alzheimer's disease (NCT03943264). It is an open-label study including 18 participants designed to determine the optimal dose (0.3, 1.0, and 3.0 mg/kg via subcutaneous injection once a week for 12 weeks). The estimated study completion date was December 2020. Results from this phase I study have not been published as of September 2021. The other ongoing clinical trial is testing XPro1595 to determine whether it can prevent the progression of respiratory complications in COVID-19 patients (NCT04370236). This study is enrolling 366 participants and is a double-blind randomized placebo-controlled trial testing XPro1595 at 1 mg/kg, up to two once per week subcutaneous injections. The estimated study completion date was February 2021. Results from this study have not been published as of September 2021.

NIH is funding an R44 (SBIR) grant to Christopher Barnum at INmune Bio, Inc. to study XPro1595 on inflammation-related deficits in reward circuitry and motivation in depression (R44MH125480-01).

Search terms:
Pubmed, Google: XPro1595, INB03, XENP1595, DN-TNF, XENP345

Websites visited for XPro1595:

- Clinicaltrials.gov
- NIH RePORTER
- DrugAge (0)
- Geroprotectors (0)
- Drugs.com (0)
- WebMD.com (0)
- PubChem (0)
- DrugBank.ca (0)
- Cafepharma (0)
- Pharmapro.com (0)