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.

Fractalkine Modulators

Evidence Summary

Fractalkine (CX3CL1) has context-dependent effects. Levels that are too low may promote neurodegeneration, while high levels may drive inflammatory and pain-related disorders. A clinically tested inhibitor had good safety in trials.

Neuroprotective Benefit: The decline in fractalkine with disease progression may exacerbate disease pathology, however, fractalkine has context-dependent effects, such that the impact of modulation may vary over the disease course.

Aging and related health concerns: Therapies inhibiting fractalkine may benefit inflammatory conditions such as rheumatoid arthritis, some types of cancer, and neuropathic pain. Clinical efficacy of tested antibodies has been modest thus far.

Safety: An antibody inhibiting fractalkine has been well-tolerated with no clear drug-specific adverse effects in clinical trials. Therapeutics specifically boosting fractalkine have not yet been tested.

Availability: E6011 (anti-CX3CL1) is being tested in clinical trials. Tet34 is in preclinical research use.	**Dose**: Not established	**Tet34 peptide** **Sequence:** RKMAGEMAEGLRYIPR SCGSNSYVLVPV + HLNILSTLWKYRC (ref. PMID: 36162508)
Half-life: Fractalkine serum half-life is ~ 30 minutes. E6011 elimination serum half-life is 349 hours (at 400 mg dose).	**BBB**: Not established	
Clinical trials: E6011 has been tested in Phase 1 clinical trials in healthy male volunteers (n=64) rheumatoid arthritis (RA)(n=37), and Crohn's disease (n=28), and in Phase 2 trials in RA (n=64; n=190).	**Observational studies**: CX3CL1 levels vary with disease progression in Alzheimer's disease (AD) and are associated with prognosis in various cancers. Genetic variants in CX3CL1 are associated with atherosclerotic disease and AD progression.	

What is it?

Fractalkine (CX3CL1) is a chemokine. It has four domains, an extracellular chemokine domain, an extracellular mucin stalk-like domain, a transmembrane domain, and an intracellular domain. CX3CL1 is localized to the cell membrane, but can be cleaved by a variety of proteases to release soluble fragments containing the extracellular domains, which can act as paracrine factors [1]. CX3CL1 can also be cleaved in a manner which releases the intracellular fragment, resulting in changes in intracellular signaling and gene expression. CX3CL1 is constitutively expressed on epithelial cells, dendritic cells, macrophages, neurons, renal mesangial cells, and smooth muscle cells [2]. Additionally, it has inducible expression on endothelial cells, fibroblasts, and astrocytes, especially in the context of inflammation. CX3CL1 interacts with the receptor CX3CR1, which is primarily expressed by cytotoxic effector cells and cytokine producing cells, such as CD8 T cells, natural killer (NK) cells, monocytes, dendritic cells, and microglia. CX3CR1 may also be expressed on some populations of neurons. The CX3CL1-CX3CR1 axis plays roles in chemotaxis, leukocyte adhesion/migration, and cell survival [1]. The downstream effects are highly context dependent and can play different roles in different tissue compartments [2]. In part, this is due to the fact that CX3CL1 forms gradients, such that it can have very localized effects depending on the local concentration. Changes in the CX3CL1-CX3CR1 axis are associated with both

neurodegenerative disease and inflammatory autoimmune conditions. Preclinical studies suggest that augmenting levels of CX3CL1 may be neuroprotective [3], but a clinically viable therapeutic approach has not yet been tested. In contrast, for peripheral inflammatory conditions, the inhibition of the CX3CL1-CX3CR1 axis has shown benefit in preclinical models [4]. E6011 is a humanized monoclonal antibody targeting CX3CL1 that is being developed by Eisai, which has undergone clinical testing in patients with rheumatoid arthritis and in patients with Crohn's disease.

Neuroprotective Benefit: The decline in fractalkine with disease progression may exacerbate disease pathology, however, fractalkine has context-dependent effects, such that the impact of modulation may vary over the disease course.

Types of evidence:

- 3 studies of CX3CL1/CX3CR1 expression in postmortem brain tissue in AD
- 4 studies of CX3CL1 levels in CSF in AD
- 3 studies of CX3CL1 levels in blood in AD
- 1 study of CX3CL1 levels in CSF in PD
- 1 gene association study for CX3CR1 variants in AD
- 2 gene association studies for CX3CR1 variants in ALS
- Numerous laboratory studies

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function:

There is currently no evidence regarding whether exogenously administered fractalkine-based therapeutics can influence cognitive function or brain aging trajectories. However, there is evidence from gene association studies suggesting that changes to the CX3CL1-CX3CR1 axis can impact the trajectory of neurodegenerative disease. The CX3CL1-CX3CR1 axis is involved in immune regulation, and there is increasing evidence linking the immune response to disease-related pathology as a key modifier of disease progression. Several genetic variants in CX3CR1 have been identified, and some of them have been characterized as hypofunctional, including the V249I (rs3732379) and T280M (rs3732378) variants [1]. These variants are associated with a reduction in CX3CL1-CX3CR1 density on peripheral blood mononuclear cells (PBMCs) [5], but the functional implications toward various downstream signaling and functional capacities in different tissues has not yet been established. The V249I-T280M haplotype

is relatively common and is found in approximately 25-30% of the population [6]. A case-control gene association study from Spain including 205 pathologically confirmed Alzheimer's disease (AD) cases and 270 controls found that patients homozygous for the 249I allele had an earlier age of disease onset and were at higher risk for disease progression (Odds ratio [OR]: 3.967, 95% Confidence Interval [CI] 1.271 to 12.391) [7]. They showed higher levels of tau pathology (Braak's stages V and VI) at the time of death. Meanwhile, individuals heterozygous at V249I had less neocortical pathology and a lower degree of tau tangle pathology at time of death (OR: 0.42, 95%CI 0.23 to 0.74), indicative of a lower degree of disease progression. The association with tau is consistent in what has been seen in preclinical AD models, where CX3CR1 deficiency promotes tau accumulation [3]. This pattern is also similar to the associations with cardiovascular disease for which heterozygosity is protective while homozygosity for these hypofunctional variants is associated with higher risk [8]. The CX3CL1-CX3CR1 axis has been associated with both protective and deleterious activities in preclinical models, depending on the type of brain insult and stage of disease. It is hypothesized that having two different CX3CR1 alleles which have different degrees of signaling and immune modulating capacity may be protective by allowing for the maintenance of neuroprotective activity while preventing potentially harmful immune overactivation [7].

Human research to suggest benefits to patients with dementia:

Fractalkine modulators have not yet been tested in dementia patients.

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

Alzheimer's disease: CX3CL1 AND CX3CR1 LEVELS VARY WITH DISEASE PROGRESSION
Biomarker studies indicate that changes in the CX3CL1-CX3CR1 axis follow the course of disease progression. Within the CNS, this axis is a mediator of neuron-microglial crosstalk, such that the changes indicate a breakdown in the communication between these cell types [3]. CX3CL1 generally promotes a homeostatic surveillance state of the microglia, which limits their activation in response to inflammatory stimuli. Thus, the loss of this signaling can lead to unrestrained microglial activation. Due to changes in the roles of microglia over time, modulation of this pathway may have different effects at different times in the disease course. Early on, in an amyloid-dominant environment, having more active phagocytic microglia may be beneficial, but over time, this can lead to the rise of a chronic inflammatory state which exacerbates pathology. Additionally, CX3CL1 can have both intracellular neuroprotective

and extracellular immunomodulatory effects. As a result, the augmentation of different aspects of CX3CL1 signaling may be preferentially beneficial at certain stages.
Postmortem brain tissue: Membrane bound and soluble CX3CL1 levels were found to be elevated in early Braak stages (II-III), but not at later Braak stages (V-V1) [9]. The expression of CX3CR1 was found to increase in the cortex in the context of AD progression, which is thought to be a compensatory response to a reduction in levels of its ligand, CX3CL1 [10]. The increase in CX3CR1 expression tracks with the progression of tau pathology and Braak staging [11].

CSF: Circulating (soluble) CX3CL1 levels were found to be elevated in the cerebrospinal fluid (CSF) of patients with AD relative to age-matched controls [12]. The levels of CX3CL1, as measured by ELISA, were highest in patients with mild cognitive impairment (MCI) (n=18) (0.173 ng/mL; interquartile range 0.14 to 0.23). Levels decreased with AD progression (n=42) (0.135 ng/mL; interquartile range 0.04 to 0.18), but were still elevated relative to controls (n=20) (0.070 ng/mL; interquartile range 0.05 to 0.12). Higher levels of CSF CX3CL1 and YKL-40 in controls were associated with increased risk for MCI (OR: 2.683, 95% CI 1.346 to 5.348), while in MCI patients, a decrease in levels of CSF CX3CL1 was associated with greater risk for conversion to AD. In a separate study, CSF levels of CX3CL, measured by ELISA, were found to be comparable between controls (n=14) and MCI (n=14), but were reduced in AD patients (n=14) relative to MCI [13]. Another study found that CSF CX3CL1 levels, measured by ELISA, were also elevated in AD patients (n=28) relative to patients with non-AD-related dementia/cognitive impairment [14].
In a CSF proteomic analysis, levels of CX3CL1, along with other proteins involved in chemotaxis and cytokine production, were found to be upregulated in patients with AD (n=29) or infectious disease-related delirium (n=15), relative to infectious disease patients without delirium (n=30) or controls (n=15) [15].

Blood: Circulating levels of CX3CL1 could be derived from numerous sources, and thus are not necessarily reflective of changes in CX3CL1 levels or signaling in the CNS. One study found no differences in plasma levels of CX3CL1 between AD and controls, despite detecting a difference in CSF in the same cohort [13]. Another study found a similar pattern of elevation in CSF and blood CX3CL1 in AD patients relative to controls (0.31 ng/mL, interquartile range 0.21 to 0.49), with levels decreasing with the progression from MCI (0.58, interquartile range 0.41 to 0.76) to AD (0.49 ng/mL, interquartile range 0.23 to 0.65), though the degree of separation among the groups was lower relative to the CSF [12]. The increased levels of CX3CL1 could be indicative of BBB dysfunction. A study assessing serum CX3CL1 levels found that patients with vascular dementia (n=18) had the highest levels (794.7 ± 302.6 pg/mL),

relative to AD (n=42) and controls (n=20) (562.8 ± 109.3 pg/mL) [16]. Levels of CX3CL1 also declined with disease progression from mild (698.1 ± 261.7 pg/mL) to moderate (673.4 ± 258.2 pg/mL) to severe (518.2 ± 114.7 pg/mL) AD. In a BBB model using patient derived peripheral blood mononuclear cells (PBMCs), the expression of CX3CL1 was found to be increased in endothelial and abluminal compartments in PBMCs from mild AD patients relative to controls [17].

Preclinical models

CX3CR1 deficiency: The impact of CX3CR1 deletion has been shown to have disparate effects on AD pathology in AD mouse models [3]. In general, the absence of CX3CR1 reduces levels of amyloid pathology while exacerbating tau pathology. This is thought to relate to how microglial activation and phagocytic activity differentially impacts their clearance and propagation. The activation of CX3CR1 on microglia by CX3CL1 is generally considered to maintain the microglia in a quiescent, surveillance state. CX3CL1-CX3CR1 signaling in response to inflammatory stimulation, such as lipopolysaccharide (LPS), has been shown to dampen the inflammatory cytokine response. Thus, the lack of CX3CR1 may lead to a chronic activation phenotype with enhanced phagocytic capacity. This may promote the early clearance of amyloid, however, it may also promote neuroinflammation and foster the uptake and spreading of tau. The deletion of CX3CR1 from human induced pluripotent stem (iPS) cell-derived microglia-like cells was also shown to increase their inflammatory profile and phagocytic activity [18]. Alternatively, there is some evidence to suggest that tau may be able to bind to CX3CR1, and this interaction fosters the uptake and degradation of tau [11]. This interaction appears to be weakened by the phosphorylation of tau (S396). Tau clearance was impaired in mouse primary microglial culture lacking CX3CR1.
There have also been efforts to look at the impact of CX3CR1 haploinsufficiency, which may be more physiologically relevant, particularly in relation to individuals with hypofunctional CX3CR1 alleles. In the APPswe, PSEN1dE9 (APP-PS1) mouse model, a partial deficiency in CX3CR1 was associated with a reduction in Aβ levels and plaque load from ages 5 to 24 months, which was accompanied by an increase in levels of insulin degrading enzyme, in male mice [19]. A separate study using the APP-PS1 model found that Aβ plaque load was impacted by CX3CR1 haploinsufficiency in an age and sex-specific manner [20]. Aβ plaque loads were not impacted by CX3CR1 status in young mice, but in 12-month-old males, CX3CR1 haploinsufficiency was associated with a modest increase in plaques, along with a decrease in the expression of amyloid degrading enzymes. The disparate finding between these two studies highlights the context dependency of CX3CL1-CX3CR1 signaling, such that differences in environment may impact immune regulation.

Soluble CX3CL1: Membrane bound CX3CL1 can be cleaved by a variety of proteases, including ADAM10, ADAM117, MMP-2, and cathepsin S to generate soluble forms of CX3CL1 containing fragments of the extracellular domain, which can act in a paracrine manner [1]. While both membrane bound and soluble forms of CX3CL1 can interact with CX3CR1, they can have different effects, depending on the environment. The membrane bound form tends to play a larger role in the recruitment and adhesion of leukocytes, while the soluble form plays a larger role in chemoattraction. Numerous studies have been conducted to try to disentangle the neuroprotective effects of CX3CL1 in relation to its membrane bound and soluble forms. Soluble CX3CL1 has more consistently been shown to exert neuroprotective effects, however, the overall impact of supplementing with membrane bound or soluble CX3CL1 is largely context dependent. Additionally, the type of soluble form can also make a difference. CX3CL1 can be cleaved to a form containing the entire extracellular domain consisting of both the chemokine domain and the mucin-like stalk domain, or it can be cleaved into a smaller fragment containing only the chemokine domain. The soluble form containing the mucin-like stalk domain was found to be ten times more potent in stimulating β-arrestin recruitment downstream of CX3CR1, which appears to be involved in promoting anti-inflammatory responses [21]. This suggests that the different variants may differentially impact downstream signaling such that differences in the local concentrations of the variants could result in different responses. Furthermore, at high concentrations (>1 nM), soluble CX3CL1 may interact with another, not yet identified, receptor, which increases pro-inflammatory responses [21]. Since human plasma levels of CX3CL1 are normally in the range of 1-2 ng/mL, and CX3CL1 typically forms local concentration gradients, local tissue concentrations reaching into the mid nanomolar range are physiologically relevant, particularly under inflammatory conditions. In microglia from aged (15 months old) rats, the anti-inflammatory response of CX3CL1 was blunted, while the pro-inflammatory response of high concentration CX3CL1 was exacerbated [21]. This suggests that the production or supplementation with high levels of soluble CX3CL1 could potentially exacerbate rather than ameliorate chronic low-grade aging-related inflammation. It may also account for discrepancies across studies using exogenous CX3CL1 supplementation at different concentrations.

In the rTg450 tauopathy mouse model, AAV4-mediated overexpression of soluble CX3CL1 containing both the chemokine domain and mucin-like stalk into the lateral ventricles at five months of age did not reduce tau pathology or major markers of microglial activation, but had modest protective effects on some cognitive tests [22]. AAV-mediated expression of membrane bound or soluble CX3CL1 differentially impacted performance on different cognitive tests in CX3CL1 knockout mice. The membrane bound form partially restored performance on the Barnes maze, an assessment of hippocampal-dependent spatial learning and memory, but had no effect on hippocampal neurogenesis or performance on a fear conditioning task. The soluble form enhanced neurogenesis and showed a

trend toward improvement on long-term memory in the fear conditioning task. Although CX3CL1 tends to act locally, it may also have distal effects. In 15-month-old male mice, intraperitoneal (i.p.) injection of soluble CX3CL1 containing the chemokine domain led to an increase in the number of hippocampal neural progenitor cells, brain derived neurotrophic factor (BDNF) levels, and enhanced cognitive performance on a novel object recognition task [23]. The effect appeared to be mediated by the activity of CX3CL1 locally on the vagus nerve in the peritoneal space, resulting in an increase in the production of BDNF within the hippocampus. Consistent with its context-dependent activity, the presence or absence of other factors in the environmental milieu may differentially impact the neuroprotective potential of CX3CL1. Intracerebroventricular injection of mesenchymal stem cells (MSCs) expressing CX3CL1 attenuated neuroinflammation and synaptic loss in APP/PS1 mice, but did not impact cognitive function. Meanwhile, MSCs expressing both CX3CL1 and Wnt3a were able to enhance neurogenesis and improve cognitive performance in these mice.

CX3CL1-intracelllular domain: In addition to the production of a circulating soluble extracellular fragment, the cleavage of membrane bound CX3CL1 can also generate an intracellular fragment [24]. It was recently demonstrated that CX3CL1 can be cleaved by α and β secretase (BACE1). The intracellular C-terminal fragment can be subsequently cleaved from the membrane by gamma secretase, leading to the release of the intracellular fragment into the cytoplasm. This intracellular fragment of CX3CL1 can then translocate to the nucleus, where it is involved in the transcriptional regulation of a subset of genes involved in cell growth and differentiation, particularly TGFβ2/β3-Smad2 signaling, and may play a role in the promotion of neurogenesis. Ectopic expression of the membrane-bound C-terminal CX3CL1 fragment in the neurons (using the CaMKIIα promoter) of 5XFAD mice led to a reduction in amyloid plaques and neuronal loss at nine months of age [24]. Transgenic expression of the intracellular fragment in neurons enhanced neurogenesis in the subgranular and subventricular zones of mice, in conjunction with an elevation in TGF-β2/β3-Smad2 signaling [25]. It also led to the induction of several pathways that promote cell survival including an upregulation of insulin/insulin-like growth factor-1 receptor signaling and Akt, as well as a reduction in levels of Foxo-regulated cyclin-dependent kinase inhibitors p21 and p27 [26]. In the P19 tauopathy mouse model, overexpression of the CX3CL1 intracellular fragment in neurons reduced neurodegeneration and cognitive deficits, while extending survival time, and increasing the synaptic density of neurons [25]. The effects appear to be mediated within neurons, in the enhancement of survival and neurogenesis, rather than through crosstalk with glia, as there were no detectable effects on microglia, astrocytes, or tau pathology. A synthetic peptide, Tet34, containing the CX3CL1 intracellular domain coupled to a 13-amino acid tetanus sequence at the N terminus, which binds to GT1b ganglioside receptors, in order to facilitate neuronal uptake

(RKMAGEMAEGLRYIPRSCGSNSYVLVPV + HLNILSTLWKYRC) was found to induce similar signaling pathways in mouse neuroblastoma Neuro-2A cells, relative to the overexpression of the CX3CL1 intracellular domain in mice [26]. Tet34 was effectively taken up by Neuro-2A cells and primary mouse hippocampal neurons, and translocated to the nucleus where it activated TGF-β2/β3. The Tet34 peptide protected the cells against Aβ-induced cell death via the activation of Akt and Foxo.
The relevance of this protective pathway endogenously, particularly in humans, is not yet clear.

Overall, CX3CL1 and CX3CR1 change in their expression profile and possibly also in their activity profile over the disease course. These changes may be a reflection of other disease-related changes taking place in the brain. The pattern of change in the levels of soluble CX3CL1 could be a reflection of changes in the expression or activity of the enzymes which cleave CX3CL1 from the membrane. Low levels of the metalloproteases ADAM10 and ADAM17 are associated with increased levels of amyloid precursor protein (APP) and AD risk [27], such that a decline in the activity of these proteases may contribute to the decline in soluble CX3CL1 with disease progression. Additionally, there could be a shift in the distribution of the pool of CX3CL1 forms toward those containing only the chemokine domain, which may have lower capacity to promote anti-inflammatory and neurogenic responses [21], due to increased levels of cathepsin S [28]. Norepinephrine can increase the production of CX3CL1 in neurons, thus the loss of norepinephrine may contribute to the decline in neuronal CX3CL1 production, and compensatory upregulation of microglial CX3CR1 [10]. The dysregulation of GSK-3β may also impact the sorting and trafficking of CX3CL1 [9]. Post-translational modifications also affect the activity of CX3CL1, including the modification of its N-terminal glutamine residue to a pyroglutamate by glutaminyl cyclase, which enhances downstream ERK1/2, Akt, and p38 MAPK signaling by increasing the stability of the ligand-receptor interaction [1; 29]. Pyroglutamation of Aβ enhances its aggregation potential, and glutaminyl cyclase has been shown to decrease with AD progression [30]. High levels of glutaminyl cyclase were associated with higher levels of vascular adhesion markers, suggestive of increased immune cell trafficking [30]. This suggests that changes to glutaminyl cyclase levels/activity could impact the downstream signaling associated with CX3CL1-CX3CR1 and the associated immune response.
Chemokines, such as CX3CL1 also have the capacity to interact with glycoaminoglycans (GAGs) on the extracellular matrix and cell surface, which facilitates the formation of concentration gradients [1]. The binding of CX3CL1 to GAGs such as heparan sulfate, which is implicated in AD [31], could influence where these gradients form and where local hot spots of CX3CL1 occur. Recent *in vitro* evidence suggests that very high, but physiologically reasonable, levels of CX3CL1 levels can induce pro-inflammatory signaling in microglia, and this pathway appears to become more prominent with age [21]. These findings suggest that the dysregulation in the CX3CL1-CX3CR1 axis is likely a byproduct of disease-

related changes in the brain, while various preclinical studies suggest that the dysregulation of this chemokine axis further exacerbates disease pathology. Due to the dynamic context-dependent effects of CX3CL1 activity, the impact of modulating this axis may vary over the course of the disease in relation to corresponding changes in interacting partners/pathways.

Parkinson's disease: CSF CX3CL1 LEVELS REDUCED

The studies assessing the role of the CX3CL1-CX3CR1 axis in Parkinson's disease (PD) models highlight the context dependency of this pathway in the modulation of disease progression. In the MPTP model, deletion of CX3CR1 was partially protective against dopamine neuron loss with intranasal MPTP administration, but exacerbated neuronal loss with i.p. MPTP administration [32]. The differential effects may be related to differences in the degree of astrogliosis in the substantia nigra. CX3CR1 deficiency protected against astrogliosis with intranasal MPTP, but not with i.p. MPTP. This may be related to differences in astrocyte-mediated CX3CL1-CX3CR1 signaling, since astrocytes can express CX3CL1 in an inducible manner in response to inflammatory stimuli. In an alpha-synuclein overexpressing model (AAV2: human alpha-syn), CX3CR1 deficient mice had a reduced inflammatory response toward alpha-synuclein, but did not significantly impact the degree of neuronal loss [33]. In another study overexpressing alpha-synuclein via AAV9, co-infection with a virus expressing different forms of CX3CL1 under neuronal or astrocytic promoters found that expression of the soluble form, but not the membrane bound form, protected against dopamine neuron loss [34]. CSF levels of soluble CX3CL1 were found to be decreased in PD patients (n=31) relative to controls (n=12), particularly in patients with freezing of gait [35]. The role of fractalkine in disease progression in PD is not clear. It is also unclear whether the impact of the CX3CL1-CX3CR1 axis is differentially impacted by the genetic or environmental factors associated with disease pathogenesis.

Amyotrophic lateral sclerosis: IMPACT OF FRACTALKINE IS UNCLEAR

Genetic variants in CX3CR1 have been associated with survival duration in patients with amyotrophic lateral sclerosis (ALS), though the findings across studies are contradictory. One study found that the 249I allele was associated with faster disease progression (OR: 2.58, 95% CI 1.32 to 5.07), while the 249I-280M haplotype was associated with shorter survival in a study including 187 ALS patients and 378 controls in Spain [6]. A study using a separate cohort in Italy including 755 ALS patients and 369 controls found that the patients with spinal onset ALS homozygous for the wildtype V249 allele (VV) had an 11-month reduction in mean survival time, relative to those with at least one copy of the 249I variant [36]. The presence of the 249I-280M haplotype was also associated with longer survival in this cohort. As a

result, the impact of these CX3CR1 variants, and the CX3CL1-CX3CR1 axis in general on disease progression in ALS is unclear.
The results of preclinical studies modulating this pathway suggest that it may have a context-dependent protective effect. In the SOD1G93A model, male, but not female, mice lacking CX3CR1 had more aggressive disease, greater microglial activation, and shorter survival [37]. The loss of CX3CL1-CX3CR1 signaling was associated with an impairment in the maturation of autophagosomes. The expression of CX3CL1 and CX3CR1 was found to change in the spinal cord over the course of the disease in this mouse model [38]. CX3CL1 levels peaked early then declined over time, while CX3R1 levels remained persistently elevated. The decline in CX3CL1 was associated with a transition in the microglial activation phenotype from an M2-like to a more pro-inflammatory M1-like state. Additionally, CX3CL1 has been identified as one of the main features of the mesenchymal stem cell (MSC) secretome associated with the neuroprotection of SOD1 mutant motor neurons in cell culture [39; 40].

Brain injury: POTENTIAL MIXED ROLES ACUTE AND LONGTERM (Preclinical)
Preclinical studies examining the role of the CX3CL1-CX3CR1 axis in acute neurological injury have indicated that this pathway can have opposite effects on acute and long-term neurological damage and recovery. CX3CL1 or CX3CR1 deficiency has been associated with neuroprotection and a reduction in pro-inflammatory mediators in mouse models of ischemic injury [3]. However, inhibiting CX3CR1 after the induction of global cerebral ischemia was associated with worse neurological outcomes. The different effects across studies highlight the context-dependent nature of CX3CL1-CX3CR1 signaling, in that its functional consequences can differ depending on the time in relation to the injury, as well as the extent and location of the injury. In rodent models of traumatic brain injury, CX3CR1 deficient mice initially showed lower levels of neuronal loss and neurological injury, but over the long term, showed greater neuronal loss and neurological deficits [6; 41]. This suggests that early on this signaling axis may promote damaging inflammatory responses, but later on contribute to healing and repair processes. Additionally, the early upregulation of CX3CR1 on neurons may sensitize them toward cell death in response to injury-inducing stimuli such as ischemia or glutamate toxicity [42]. Together, these studies suggest that the roles of CX3CL1-CX3CR1 signaling are multi-faceted, time-dependent, and complex, which makes it difficult to determine a viable CX3CL1-based therapeutic approach that would be broadly applicable in the context of brain injury.

APOE4 interactions: Not established.

Aging and related health concerns: Therapies inhibiting fractalkine may benefit inflammatory conditions such as rheumatoid arthritis, some types of cancer, and neuropathic pain. Clinical efficacy of tested antibodies has been modest thus far.

Types of evidence:

- 1 meta-analysis of case-control gene association studies for CX3CR1 variants and atherosclerotic disease
- 3 CX3CR1 variant association study for cardiovascular outcomes
- 1 CX3CR1 variant case-control study for carotid intima-media thickness
- 3 clinical trials for E6011 in rheumatoid arthritis
- Numerous laboratory studies

Cardiovascular disease: CX3CR1 VARIANTS ASSOCIATED WITH ATHEROSCLEROSIS

Gene variants in CX3CR1 have been associated with risk for atherosclerosis, and coronary artery disease (CAD), although there has been a lot of variability across studies. A meta-analysis of 49 case-control studies including 7,732 cases and 5,905 controls for the T280M polymorphism, as well as 7,952 cases and 6,035 controls for the V249I polymorphism, analyzed the association between CX3CR1 variants and atherosclerotic diseases [8]. For T280M variant, the heterozygous TM genotype was associated with protection for CAD (OR: 0.67, 95% CI 0.52 to 0.87), while the homozygous MM genotype was associated with higher risk for ischemic cerebrovascular disease (OR: 2.88, 95% CI 1.65 to 5.04). The heterozygous VI genotype for the V249I variant was also associated with lower risk for CAD (OR: 0.72, 95% CI 0.59 to 0.90). The V249I-T280M (VITM) genotype was also associated with lower risk for atherosclerotic disease (OR: 0.64, 95% CI 0.50 to 0.82). A study examined the relationship between chemokine variants, including the CX3CR1 V249I and T280M variants, and myocardial infarction using the CARDIoGRAMplusC4D (n=184,305), MIGen Exome array (n=120,575), and PROMIS MI (n=17,437) datasets, and did not find any significant associations [43]. Similarly, the study did not find an association of CX3CR1 variants with glucometabolic traits. A study including 111 patients with type 2 diabetes and 109 controls in Mexico also found that classic glucometabolic parameters such as circulating lipid and glucose levels were not impacted by CX3CR1 genotype [44]. But, consistent with the findings of the meta-analysis, individuals with the T280M MM genotype were more likely to have increased carotid intima-media thickness, while type 2 diabetics heterozygous for the V249I variant (VI genotype) were less likely to have increased carotid intima-media thickness.

Some studies also suggest that elevated levels of CX3CL1 could be a biomarker associated with unstable plaques. Levels of CD16+ monocytes, which are known to express high levels of CX3CR1, have been

found to be elevated in patients with carotid stenosis, and higher levels of these monocytes were associated with greater risk for stroke [45]. The expression of CX3CL1 and CX3CR1 was found to increase with the degree of atherosclerosis in carotid artery tissues in conjunction with the degree of macrophage and T cell infiltration [46]. One study assessing 105 symptomatic plaques and 95 asymptomatic plaques from 197 patients found that fractalkine, MCP-1 and IL-6 were the most abundant pro-inflammatory cytokines/chemokines in the plaques. Additionally, there was a correlation between the plaque and plasma levels of fractalkine. Elevated plasma levels of fractalkine, MIP-1β, and TNF-α were associated with the presence of high inflammatory plaques. High levels of fractalkine were also associated with higher risk for a cerebrovascular transient ischemic attack (Hazard Ratio [HR]: 12.7, 95% CI 1.2 to 136.6). Patients with unstable angina pectoris (n=60) were found to have higher levels of fractalkine, MCP-1, and RANTES, relative to those with stable angina pectoris (n=60) [47]. Additionally, in patients with unstable angina pectoris (n=120), higher plasma levels of fractalkine and CD36 were associated with more severe lesions and higher risk for experiencing a major adverse cardiovascular event (MACE) [48]. These studies suggest that elevated CX3CL1-CX3CR1 signaling is associated with increased atherosclerotic plaque progression and vulnerability through the promotion of pro-atherosclerotic immune responses.

The induction of fractalkine on vascular endothelial cells under inflammatory conditions can facilitate the entry of pro-inflammatory immune cells across vascular membranes [4]. CX3CL1-CX3CR1 signaling can also contribute to plaque stability via the enhancement of fibrotic processes. Fractalkine can promote the proliferation of vascular smooth muscle tissue and collagen deposition [1]. Overall, these studies suggest that a local reduction in vascular CX3CL1-CX3CR1 signaling could be protective in the context of atherosclerosis, as this may underlie the protective effect of a heterozygous hypofunctional variant (V249I). However, the pathway likely plays both vascular protective and detrimental roles, since the lack of a fully functional CX3CR1 (i.e. homozygous for a hypofunctional variant) appears to be associated with greater risk for some adverse cardiovascular events.

Rheumatoid arthritis: POTENTIAL BENEFIT WITH ANTI-FRACTALKINE THERAPY

C3XCL1-CX3CR1 plays a role in the movement of patrolling immune cells into inflamed tissues [4]. CX3CR1 is highly expressed on non-classical CD16+ monocytes, which have been implicated in the pathophysiology of rheumatoid arthritis (RA). The induction of CX3CL1 expression on vascular endothelial cells in response to inflammation facilitates the adhesion and entry of CX3CR1 expressing immune cell subsets, such as CD16+ monocytes into inflamed tissues. A humanized monoclonal antibody against fractalkine, E6011, has been clinically tested in patients with RA [4]. Since chemokines form gradients which facilitate localized responses based on local concentrations, E6011 is expected to

work in a localized, rather than a systemic manner. In a Phase 1/2 trial, E6011 was tested via subcutaneous injection at doses of 100, 200, and 400 mg during weeks 0, 1, and 2, and then every two weeks for 12 weeks in patients with RA (n=37) (NCT02196558) [49]. American College of Rheumatology (ACR) 20 responses at week 12 were 75%, 66.7%, and 60%, in the 100, 200, and 400 mg cohorts, respectively. In a placebo-controlled Phase 2 RCT in patients with active RA with an inadequate response to methotrexate (n=169) (NCT02960438), E60011 was administered subcutaneously at 100 or 200 mg on weeks 0, 1, and 2 and then every two weeks until week 22, or at 400 mg on weeks 0, 1, 2, 4, 6, 8, and 10 and then 200 mg once every two weeks [50]. The study did not meet its primary endpoint of ACR20 response rate at week 12, with response rates of 37.0%, 39.3%, 48.1%, and 46.3% for the placebo, 100 mg, 200 mg, and 400/200 mg groups, respectively. However, the 200 mg and 400/200 mg groups did show significant improvement on the ACR20 at week 24, which was a secondary endpoint, with responses of 53.7% and 57.4% respectively, relative to a 35.2% response rate in the placebo group. These responses were maintained during a long-term extension phase to week 84 [51]. Patients with higher baseline levels of CD16+ monocytes tended to show greater responses. Another placebo-controlled Phase 2 RCT testing E6011 in patients with RA inadequately responding to biological disease-modifying antirheumatic drugs (n=64) (NCT02960490) failed to show efficacy in this population [52]. E6011 was dosed at 400 mg at weeks 0, 1, 2, and then every two weeks until week 10, at which point patients received either 200 mg or 400 mg every two weeks until week 22. The study did not meet its primary endpoint of ACR20 at week 12, nor its secondary endpoints of ACR50 or ACR70. A significant reduction in CD16+ monocytes was sustained during the treatment period, despite a lack of clinical efficacy. Since CX3CL1-C3XCR1 signaling can have context dependent effects, the overall immune milieu may be different in patients based on their prior exposure and refractoriness to different types of antirheumatic therapies, which could account for different degrees in responsiveness across the trials. Due to the localized effects of this pathway, it may be suitable for use in combination with other more systemic immunomodulatory therapies [4].

Cancer: CX3CL1 IS ASSOCIATED WITH PROGNOSIS BUT DIRECTION VARIES ACROSS TYPES
The CX3CL1-CX3CR1 axis can play dual roles in cancer, depending on the tumor type and tumor environment. In some cases, elevated CX3CL1 can promote the migration of tumor-infiltrating lymphocytes, while in other contexts it can promote the infiltration of tolerogenic tumor associated macrophages or promote tumor cell growth and migration [2; 53]. It may depend on local concentrations of CX3CL1 as well as receptor expression of CX3CR1 on cells in the tumor microenvironment. CX3CL1 signaling may also promote tumor angiogenesis.

Elevated CX3CL1 has been shown to be pro-oncogenic and associated with poor prognosis in breast cancer, B-cell lymphoma, gastric cancer, glioma, pancreatic cancer, and prostate cancer [2; 54]. Whereas high CX3CL1 is associated with better prognosis in colorectal cancer, stemming from an increased infiltration of cytotoxic T cells and NK cells into the tumor. Elevated CX3CL1 has also been found to be beneficial in some subtypes of lung cancer (lung adenocarcinoma), but not in others (lung squamous cell carcinoma), highlighting the context-dependent effects of CX3CL1 in different tumor microenvironments [1].
Modulation of CX3CL1-CX3CR1 signaling could represent a therapeutic strategy, in a tumor-type specific manner. Augmentation of this pathway may be beneficial in combination with immunotherapy in tumor types where CX3CL1 is associated with better prognosis.

Neuropathic pain: POTENTIAL BENEFIT WITH FRACTALKINE INHIBITION (Preclinical)
CX3CL1 has been implicated in the induction of neuropathic pain following injury. Preclinical studies suggest that in the context of nerve injury, membrane bound CX3CL1 is cleaved by cathepsin S to generate a soluble fragment containing only the extracellular chemokine domain [6]. The production of this fragment appears to facilitate the induction of hypersensitivity. Intrathecal administration of full-length CX3CL1 or of the soluble form containing only the chemokine domain has been shown to promote allodynia and thermal hyperalgesia, whereas these processes can be reduced following intrathecal administration of CX3CL1 or CX3CR1 blocking antibodies [6]. The reduction in pain thresholds stemming from CX3CL1-CX3CR1 activation may be mediated by IP3/p38MAPK signaling [6]. In a rat model of spinal cord injury, CX3CL1 and CX3CR1 were elevated following injury, with prominent CX3CR1 expression on microglia and astrocytes [55]. Treatment with the CX3CR1 inhibitor, AZD8797 (80 µg/kg i.p.), once per day following spinal cord injury reduced the inflammatory response and facilitated behavioral recovery to a similar degree as methylprednisolone. These studies suggest that inhibition of the CX3CL1-CX3CR1 axis could help reduce hyperalgesia associated with nerve injury. However, due to the localized effects of this chemokine system, it is unclear whether a systemic treatment would be effective or whether, as was most commonly used in animal models, intrathecal delivery would be necessary for therapeutic benefit.

Safety: An antibody inhibiting fractalkine has been well-tolerated with no clear drug-specific adverse effects in clinical trials. Therapeutics specifically boosting fractalkine have not yet been tested.

Types of evidence:

- 5 clinical trials for E6011 (healthy volunteers, rheumatoid arthritis, Crohn's disease)
- Numerous laboratory studies

Fractalkine-based therapeutics which mimic fractalkine or boost its signaling have not yet been clinically tested. Most preclinical studies use either genetic manipulation, such as knockout animals, transgenic overexpression, or viral mediated overexpression of membrane bound or soluble CX3CL1 [3]. Fractalkine itself is not a practical therapeutic because it has a serum half-life of around 30 minutes [23]. The administration of fractalkine-based peptides for neuroprotection poses a challenge in terms of ensuring sufficient distribution to the relevant brain regions at therapeutically beneficial concentrations.

E6011 is a humanized monoclonal antibody targeting fractalkine. In a Phase 1 single ascending dose study in healthy men (n=64) (NCT01731275), single intravenous doses up to 10 mg/kg were generally well-tolerated [56]. The most common treatment-emergent adverse events were infusion-related reactions, including pruritus, feeling hot, puncture-site hemorrhage, and puncture-site pain, in both the E6011 and placebo groups. In a Phase 1/2 study in patients with rheumatoid arthritis (n=37) (NCT02196558) testing subcutaneous doses up to 400 mg every two weeks, adverse events included nasopharyngitis (16.2%), injection site erythema (8.1%), headache, and oropharyngeal pain (5.4%) [49]. There were two serious adverse events deemed to be not related to E6011. In one 24-week Phase 2 study in rheumatoid arthritis patients (n=190) (NCT02960438), adverse events that were more common with E6011 relative to placebo were stomatitis (5.1% vs 1.9%), bronchitis (4.4% vs 1.9%), back pain (4.4% vs 1.9%), and dental caries (2.2% vs 0%) [50]. The safety profile was similar in the 104-week extension of this trial, with nasopharyngitis as the most common adverse event overall [51]. Similarly, the most common adverse events with E6011 in another Phase 2 trial in rheumatoid arthritis (n=64) (NCT02960490) were stomatitis, injection site erythema, and nasopharyngitis [52]. In a Phase 1 single ascending dose study testing intravenous E6011 up to 15 mg/kg in patients with Crohn's disease (n=28) (NCT02039063) adverse events included nasopharyngitis (18%), headache (7%), disease progression (7%), and anal abscess (7%) [57]. E6011 was generally well-tolerated across studies, with no abnormalities on laboratory tests, ECG, chest X-rays, or neurological findings. The adverse event profile was generally non-specific and consistent with conditions common in the tested populations.

Drug interactions: Not established. Since fractalkine has immunomodulatory properties, fractalkine modulating therapies may interact with other immunomodulatory drugs.

Sources and dosing:

Fractalkine-based peptides, such as Tet34 [26], have been used in preclinical research studies, but have not been tested in humans and are not available for clinical use. E6011 is being developed by Eisai, and has been tested in clinical trials for rheumatoid arthritis primarily at doses of 200 mg and 400 mg every two weeks with subcutaneous administration [50; 52] and in Crohn's disease with intravenous administration up to 15 mg/kg every two weeks [57].

Research underway:

E6011 is currently being tested in a Phase 2 trial for Crohn's disease with an estimated completion date in late 2024 (NCT03733314).

Search terms:

Pubmed, Google: Fractalkine, CX3CL1

- Alzheimer's disease, neurodegeneration, Parkinson's disease, ALS, neurogenesis, aging, genetic variant, cardiovascular, atherosclerosis, cancer, inflammation, clinical trial

Websites visited for Fractalkine modulators:

- Clinicaltrials.gov (E6011)

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