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.

P2X7R Inhibitors

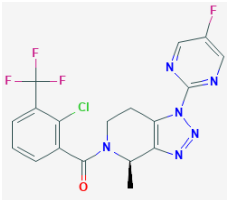
Evidence Summary

P2X7R inhibitors may reduce NLRP3 activation and IL-1β mediated inflammation, but could be subject to genotype effects, and clinical efficacy has not been established thus far.

Neuroprotective Benefit: May reduce microglial inflammation and oxidative stress, and promote phagocytic clearance, but more work is needed to establish the contribution of P2X7R in human neurodegenerative diseases.

Aging and related health concerns: P2X7R inhibition may benefit neuropathic pain, cardiovascular disease, and some cancers, but efficacy is likely dependent on P2X7R genotype.

Safety: Clinically tested inhibitors showed good short-term safety, with headache and gastrointestinal problems reported. Establishing a safe therapeutic window has been a challenge. Long-term safety and genotype-specific effects are unknown.

Availability: In clinical trials	**Dose**: Not established	**JNJ-54175446** **Chemical formula:** $C_{18}H_{13}ClF_4N_6O$ **MW**: 440.8 g/mol  Source: PubChem
CNS penetrant inhibitors: JNJ-54175446, JNJ-55308942		
Half-life: 30.7–37.4 hours for JNJ-54175446	**BBB**: JNJ-54175446 and JNJ-55308942 penetrant	
Clinical trials: P2X7R inhibitors CE-224,535, AZD9056 have been tested in autoimmune disease (rheumatoid arthritis, Crohn's disease) but were ineffective. Anti-nfP2X7Rs BIL010t, BIL06v have been tested in cancer in Phase 1 studies. JNJ-54175446 will be tested for depression.	**Observational studies**: P2X7R genetic polymorphisms influence susceptibility to a variety of diseases.	

What is it?

P2X purinoceptor 7 (P2X7R) is an ATP-activated cation channel that mediates the influx of Na^+ and Ca^{2+}, as well as the efflux of K^+. It is only activated in response to high levels of ATP (0.05–1 mM), as its affinity for ATP is 10 to 100 times lower than other P2XRs [1]. Therefore, it tends to have limited activity under physiological conditions, where ATP levels are low, and becomes more relevant during pathological conditions. With chronic activation, P2X7R forms a pore in the membrane which can allow the passage of large organic ions, and under some conditions, can trigger ATP-mediated apoptosis. P2X7R is widely expressed, and in the CNS, it is primarily expressed in glial cells. Although its effects can be context dependent, **P2X7R's role in promoting pro-inflammatory pathways via the activation of the NLRP3 inflammasome and secretion of mature IL-1β** is well established. P2X7R plays a role in immune system function in the clearance of pathogens, but can also drive pathological inflammation in a variety of injury and disease states. Consequently, a variety of P2X7R inhibitors have been tested in clinical trials for autoimmune disease (rheumatoid arthritis, Crohn's disease), liver disease (NASH), and cancer [2]. However, they have failed to show significant clinical efficacy thus far. Because therapeutic efficacy is expected to require at least 90% inhibition, many compounds are not able to be dosed within the therapeutically effective range due to safety concerns. Two CNS penetrant P2X7R inhibitors have been developed by Janssen, JNJ-54175446, JNJ-55308942, and safely tested in Phase 1 trials, and trials are ongoing or expected for patients with major depressive disorder. Antibodies (BIL010t, BIL06v) targeted to a specific non-functional form of P2X7R, that is highly expressed by some cancers have been developed and are being tested for cancer.

One of the key challenges of P2X7R modulating drugs, is that P2X7R is highly polymorphic, including many variants that confer either increased or decreased function of the receptor [3]. Therefore, there could be a genotype effect to the therapeutic profile of P2X7R inhibitors. (SEE TABLE AT END OF REPORT)

Neuroprotective Benefit: May reduce microglial inflammation and oxidative stress, and promote phagocytic clearance, but more work is needed to establish the contribution of P2X7R in human neurodegenerative diseases.

Types of evidence:

- Two P2X7R PET studies ([11C]SMW139 in AD brain tissue and [11C] JNJ-54173717 in PD patients)
- Numerous laboratory studies

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

P2X7R inhibitors have not yet been clinically tested for neurodegenerative diseases, largely because, aside from failing to show efficacy in peripheral indications, the early clinical candidates were not blood brain barrier (BBB) penetrant. There are currently two BBB penetrant clinical candidates projected to be suitable for CNS indications that have undergone Phase 1 testing (JNJ-54175446, JNJ-55308942), and are in development for major depressive disorder [2].

There is a high degree of polymorphisms (SNPs) for P2X7R, including loss of function and gain of function variants. Gene association studies indicate that these variants alter susceptibility to a variety of diseases, including Alzheimer's disease (AD). This suggests that P2X7R may be a relevant target for AD and that genetics may influence the efficacy of P2X7R targeted therapies, leading to heterogeneity in the therapeutic response. The 1513A>C substitution (rs3751143; E496A) is located in the C-terminus cytoplasmic tail of P2X7R, and is associated with decreased P2X7R function in heterozygotes (AC) and a near loss of function in homozygotes (CC) [4]. This variant is associated with increased risk for infection. In a gene association studying containing 84 Caucasian late onset AD patients and 148 elderly controls, the distribution of this variant was not significantly different between the AD and control populations [4]. However, there was a decreased probability of AD in individuals with the 1513A>C substitution in combination with the 489C>T substitution (Odds ratio OR: 0.25, 95% Confidence Interval CI 0.09 to 0.71, p=0.007). The 489C>T variant (rs208294; H155Y) is localized to the P2X7R ectodomain, which has phagocytic functions, and shows gain of function properties. This variant was less frequent in AD

patients, and is thought to confer protection by enhancing Aβ phagocytosis. Overall, the 1513A>C/489C>T variant combination appears to be protective for AD by mitigating pathological neuroinflammation, while also enhancing Aβ clearance.

Human research to suggest benefits to patients with dementia: None

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

Discerning the contribution of P2X7R to neurodegenerative disease has been challenging until the recent development of specific P2X7R inhibitors, as historically most studies have used the non-selective inhibitor brilliant blue G, which also targets P2X1, P2X2, P2X3, and P2X4 [1]. P2X7R mouse knockout studies have also been problematic because the two major lines developed by GlaxoSmithKline and Pfizer, respectively, do not fully eliminate all splice variants/isoforms of P2X7R [5]. Additionally, many commonly used mouse strains contain a polymorphism that reduces the function of P2X7R [6].

Alzheimer's disease: P2X7R ELEVATED IN AD BRAIN; POTENTIAL BENEFIT (Preclinical)

P2X7R expressing Iba1+ microglia and GFAP+ astrocytes have been detected in postmortem temporal (n=4) and frontal cortex (n=5) brain tissue from AD patients, and the P2X7R immunoreactive cells are primarily localized surrounding amyloid plaques [7; 8]. The expression of P2X7R in microglia was found to be increased by 70% in AD patients [9].

P2X7R plays a critical role in microglial activation in response to Aβ, by which it leads to the activation of the NLRP3 inflammasome and secretion of the pro-inflammatory cytokine IL-1β [1]. Based on rodent studies, the activation of P2X7R stimulates the migration of reactive microglia to plaques, however, **chronic P2X7R activation reduces the phagocytic capacity of the microglia**, so they are ineffective at clearing the plaques [7]. Ca^{2+} influx through the P2X7R channel also contributes to the regulation of Ca^{2+} homeostasis, so chronic P2X7R activation can disrupt Ca^{2+} homeostasis, leading to oxidative stress and altered neurotransmission [10]. P2X7R inhibitors (brilliant blue G, P2X7R siRNA, GSK 1482160A) and P2X7R deficiency have been found to be protective against synaptic loss, neuronal loss, oxidative stress and neuroinflammation, while enhancing microglial Aβ clearing phagocytic capacity in rodent and cell culture AD models [7; 8; 11; 12; 13; 14; 15; 16]. P2X7R has also been shown to be involved in emotional conditions over the lifespan of mice, likely through immune modulation [17]. Consequently, P2X7R inhibition has led to memory impairment related to anxiogenic responses, though these were not done in AD models [1], and thus could potentially reduce anxiety/agitation behaviors in AD patients.

However, P2X7R activity is known to be dependent on the *in vivo* cellular environment, so the transability of these findings is unclear [3]. Purinergic signaling has been shown to be dysregulated in AD, and it is not clear how much benefit would be derived from modulation of a single purinergic receptor. Additionally, as the various purinergic receptors are activated in response to the different purines (ADP, ATP, etc.) to varying degrees, there could be compensatory effects by another purinergic receptor. In particular, there is a close association and overlapping functions between P2X7R and P2X4R [18].

In AD model mice (9-month-old P301S tau), the P2X7R PET tracer [123I]TZ6019, which is an analogue of GSK1482160, and has an IC_{50}= 9.49 ± 1.4 nM, showed 35% higher binding relative to wild type mice [19]. Meanwhile, the P2X7R PET tracer [11C]SMW139 did not show significant differences in labelling between postmortem AD and control tissue, despite showing good uptake in the rat brain *in vivo* [20]. It is unclear whether the discrepancy is related to the use of different tracers, differences in P2X7R *in vivo* vs in postmortem tissue, or species-related. The expression and impact of P2X7R may also be influenced by disease stage, and more human studies are needed to determine the relevance of P2X7R in AD patients, and the optimal stage for therapeutic intervention. P2X7R genotype may also impact the therapeutic efficacy of potential P2X7R inhibitors.

Parkinson's disease: POTENTIAL MINOR/UNCLEAR BENEFIT (Preclinical)

Preclinical models for Parkinson's disease (PD), show discrepancies in whether P2X7R is significantly elevated. Acute models that involve oxidative stress damage are associated with increases in P2X7R, while more chronic models, such as human alpha-synuclein overexpression, fail to show significant increases in P2X7R [21]. Similarly, a PET imaging study involving a CNS-penetrant radiolabeled P2X7R inhibitor [11C] JNJ-54173717 with nanomolar affinity for human P2X7R (K_D = 1.6 nM), showed no significant difference in the total volume of distribution in PD patients (3.3 ± 0.7) relative to controls (3.4 ± 0.8) [22]. The lack of a detectable difference on PET imaging may be because the magnitude of change is below the sensitivity for PET, as a study using postmortem tissue from idiopathic PD patients, found only an approximately two-fold change in P2X7R in the striatum at the mRNA level [23]. The PET study also suggests that there may be a genotype effect for the P2X7R targeted PET ligands, which could limit their clinical utility.

There is evidence to suggest that P2X7R genetic variants may also influence susceptibility for PD. In a Chinese Han population consisting of 285 sporadic PD patients, and 285 controls, the 1513A>C SNP (rs3751143) was associated with risk for PD in men [24]. The 1729T>A variant (rs1653624) was also examined, but no association was found in this population because none of the sampled individuals

carried this variant. It has not been established whether any of the other common P2X7R variants modify PD risk or severity.

Consistent with the increase in P2X7R in acute oxidative stress-driven PD rodent models, primarily 6-OHDA, treatment with the non-selective P2X7R inhibitor brilliant blue G, has been found to be neuroprotective [25; 26; 27; 28; 29]. However, brilliant blue G was not protective against MPTP-mediated dopaminergic neuron loss in an *in vivo* mouse model, despite showing neuroprotective properties *in vitro* [30]. While it is unclear whether P2X7R influences dopaminergic neuron and afferent degeneration in a meaningful way, one study in rats suggests that P2X7R may play a role in L-DOPA induced dyskinesia, such that P2X7R inhibition may prevent dyskinesia-related remodeling of dopaminergic circuitry [29]. Since this study used the 6-OHDA PD model, which impacts P2X7R levels, and the non-selective inhibitor brilliant blue G, it will be necessary to repeat the study using different models and P2X7R inhibitors, to determine whether this is a reproducible effect with translational potential, or is model dependent.

ALS: MIXED PRECLINICAL BENEFIT; CLINICAL BENEFIT UNLIKELY

Although P2X7R inhibitors generally indicate a minor level of benefit, the results across preclinical studies using the SOD1 G93A mouse model have been inconsistent, depending on the dosing regimen, timing of therapeutic intervention, and sex of the animals. Most studies used the non-selective P2X7R inhibitor brilliant blue G. When administered prior to disease onset (45.5 mg/kg i.p. 3x/week starting day 62), treatment reduced body weight loss and prolonged survival by 4.3% in female mice, but had no effect on motor neuron loss, or other clinical parameters [31]. Meanwhile another study using the same concentration, but starting dosing at day 90 found that there was motor improvement in males, but no effect on survival [32]. A study using either 50 or 250 mg/kg at different stages ranging from asymptomatic (40 days) to disease onset (135 days), found that treatment was only effective at reducing motor neuron loss and delaying the onset of motor impairments, but did not impact survival, when administered during the late pre-symptomatic stage (around 100 days), which is consistent with the other study showing motor improvements [33]. A study with the CNS penetrant selective P2X7R antagonist, JNJ-47965567 (30 mg/kg i.p. 3x/week), suggests that the lack of efficacy following disease onset is not limited to brilliant blue G, but is likely to be a feature of P2X7R inhibitors in general, which suggests that these drugs are unlikely to have clinical utility in ALS [34].

APOE4 interactions: Not known

Aging and related health concerns: P2X7R inhibition may benefit neuropathic pain, cardiovascular disease, and some cancers, but efficacy is likely dependent on P2X7R genotype.

Types of evidence:

- 2 clinical trials (BIL010t in cancer, and SGM-1019 in NASH)
- P2X7R polymorphism association studies for cancer, pain, diabetes, cardiovascular events, osteoporosis, lifespan
- Numerous laboratory studies

Lifespan: REDUCED FUNCTION P2X7R VARIANTS ASSOCIATED WITH LONGER LIFESPAN IN CAUCASIANS IN EUROPE AND THE USA

One gene association study including 34 worldwide studies (11,858 subjects) found that specifically in European Caucasian cohorts (7,241 subjects) there was significant increase in 1513CC frequency with age (Correlation coefficient 0.589, p = 0.027) [4]. In an analysis of Caucasian cohorts from the USA, there was also an increased frequency of P2X7R hypomorphic variants with age (1513CC p = 0.0055 and 489CC p= 0.0019), which is projected to be an anti-inflammatory phenotype. This suggests that Caucasians living in high income countries with this anti-inflammatory P2X7R genotype tend to have longer life expectancy.

Cancer: POTENTIAL BENEFIT/CANCER TYPE DEPENDENT

P2X7R was originally projected to be a cell death receptor, mediating ATP-driven apoptosis, due to its ability to form large pores in the membrane in the context of high extracellular ATP [35]. P2X7R can also promote cell growth and proliferation under lower ATP concentrations. Since the tumor microenvironment tends to have high levels of ATP, it was thought that the apoptotic function would predominate in cancer cells and that P2X7R agonists would promote cancer cell death. Similarly, activation of P2X7R on immune cells can potentially promote an immunological anti-cancer response. However, it was subsequently discovered that much of the P2X7R on cancer cells is modified in manners that reduces its pore-forming capacity, either by altering its subcellular localization, or by inhibiting pore-forming activity, while preserving growth promoting activity. In this context then, inhibition of the modified P2X7R is expected to be therapeutically beneficial. One of the major forms of altered P2X7R is called non-functional P2X7R (nfP2X7R) because it lacks pore-forming capacity, thus it can promote cancer cell growth, but not trigger cell death in response to ATP [36]. An anti-cancer vaccine targeting nfP2X7R called BIL06v/ Alhydrogel® (subcutaneous injection) is currently being tested in an open label trial in patients with advanced solid tumors (n=29) (ACTRN12618000838213).

Prostate Cancer: P2X7R ELEVATED

P2X7R mRNA expression was found to be elevated in tumor tissue relative to non-tumor prostate tissue (1.28 ± 0.13 vs 0.78 ± 0.09), and correlated with known drivers of cancer cell proliferation, including ERα ($r=0.50$, $p<0.001$) and EGFR ($r=0.82$, $p<0.0001$) [37]. nfP2X7R, recognized by the E200 epitope, has been detected in prostate cancer biopsies, and increased levels of nfP2X7R, based on immunohistochemistry were found to be **correlated with increased prostate specific antigen** (PSA) levels (avg PSA in absence of nfP2X7R 0.81± 0.1 ng/ml, avg PSA in presence of nfP2X7R 13.7± 1.1 ng/ml) [38]. These studies suggest that in conjunction with PSA, P2X7R may be a biomarker for prostate cancer, and may be a good candidate for nfP2X7R targeted therapies.

Lung Cancer: ELEVATED P2X7R ASSOCIATED WITH METASTASIS

In a small study comparing bronchoalveolar lavage fluid from patients with non-small-cell lung cancer (NSCLC) (n=21) with patients with chronic obstructive pulmonary disease (COPD) (n=21), there was a trend toward higher P2X7R expression in NSCLC patients [39]. There was, however, a significant increase in the expression of P2X7R and several other ATP responsive purinergic receptors (P2Y1R, P2X4R) in NSCLC patients with metastasis, suggesting that increased purinergic receptor expression, likely driven by elevated extracellular ATP concentrations may contribute to metastasis, and worse prognosis in NSCLC.

Kidney Cancer: HIGH P2X7R ASSOCIATED WITH WORSE PROGNOSIS

A study examining tumor tissues from patients with clear-cell renal cell carcinoma (n=273) found that high intratumoral P2X7 expression was a prognostic factor for worse postoperative cancer-specific survival (HR: 1.693, 95% CI 1.051 to 2.728, $p = 0.034$) [40].

Colorectal Cancer: HIGH P2X7R ASSOCIATED WITH METASTASIS AND WORSE SURVIVAL

The level of P2X7R expression depends on the subtype of colorectal cancer, however, high P2X7R expressing cancers were associated with worse prognosis. One study (n=116 tumor samples) found that high P2X7R expression correlated with tumor size, lymph node metastasis, and TNM stage, and was an independent prognostic factor for worse survival (HR: 2.038, 95%CI 1.187 to 3.498, $p=0.010$) [41]. A separate study examining colorectal tumor samples (n=97) found that patients with high P2X7R had more lymphatic invasion and distant metastasis relative to those with low P2X7R. High P2X7R was also associated with worse overall survival (HR: 24.476, 95% CI 3.196 to 187.475, $p=0.002$) [42].

Breast Cancer: nfP2X7R ELEVATED

nfP2X7R expression is generally absent from healthy breast tissue, and greatly increased in breast cancer cells [43]. The localization of nfP2X7R may be stage specific, as membrane (surface) nfP2X7R is primarily found in invasive tumors, suggesting that it may be a marker for disease progression and worse prognosis. Levels of pore functional P2X7R may decrease on breast cancer cells, however, this remains unclear due to a lack of specificity for the antibodies used in these studies [44].

Carcinoma: nfP2X7R ELEVATED; POTENTIAL BENFIT (for anti-nfP2X7R)

nfP2X7R has been found to be expressed in basal cell carcinoma tumor tissue. In a Phase 1 clinical trial, patients with basal cell carcinoma (n=21) were treated with a polyethylene glycol (PEG)- based ointment containing nfP2X7-targeted antibodies called BIL010t (50-100 mg 2x/day for 28 days) [45]. 65% of patients showed a reduction in lesion area, 20% showed no change and 15% showed an increase. In end-of-treatment biopsies, there appeared to be histological tumor clearance in three patients and partial tumor regression in nine patients. Treatment enhanced the infiltration of tumor infiltrating lymphocytes, suggesting it boosted the endogenous immunological anti-cancer response.

Leukemia: P2X7R ELEVATED AND ASSOCIATED WITH WORSE PROGNOSIS IN AML

In a study examining bone marrow mononuclear cells from patients with leukemia or myelodysplastic syndrome (n=97), P2X7R was found to be significantly higher in acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), and myelodysplastic syndrome (MDS) groups [46]. Expression of P2X7R varied in different subtypes of AML, and high P2X7R was associated with less complete remission (17%) relative to low P2X7R (78%) or absent P2X7R (79%) groups, suggesting that high P2X7R is associated with worse prognosis in AML. High P2X7R was also found to be associated with disease severity in B-cell chronic lymphocytic leukemia (n=21) [47].

Neuroblastoma: HIGH P2X7R ASSOCIATED WITH WORSE PROGNOSIS AT LATE STATE

High P2X7R expression was found to be associated with poor prognosis in advanced-staged (stage 4) neuroblastoma patients (n=56), but there was no significant association of P2X7R with survival in earlier stages (1, 2, 3, 4s), suggesting that P2X7R may be a relevant driver of tumor growth and progression only in aggressive, advanced-stage neuroblastoma [48].

Thyroid Cancer: ELEVATED nfP2XYR ASSOCIATED WITH METASTASIS

P2X7R and nfP2X7R expression has been found to be elevated in papillary thyroid cancer, and P2X7R expression was associated with lymph node metastasis (OR: 6.1, 95% CI: 1.5 to 24.4, p=0.007) [49]. The loss-of-function P2X7R variant 1513A>C (E496A, rs3751143) was found to be positively correlated with tumor size (Rho = 0.22; P = 0.02) and TMN stage that indicates degree of tumor spread (Rho = 0.38; p< 0.001) in patients with papillary thyroid carcinoma (n=121) [50]. Additionally, the minor allele was more common in patients with lymph node metastases, suggesting that individuals with a predominance of P2X7R forms that have reduced apoptotic pore forming capacity have a higher chance for tumor growth and spread, so these patients might benefit from selective inhibition of non-functional forms or activation of fully functional forms.

Hepatocellular carcinoma: LOW FUNCTION P2X7R VARIANTS ASSOCIATED WITH INCREASED RISK

In a case-control study in the Han Chinese population (n=646), there were increased risks for hepatocellular carcinoma with the loss-of-function P2X7R variants, 1513A>C (OR:1.37, 95%CI 1.05 to 1.79, p=0.021) (rs3751143) and 946G>A (rs28360457) (OR:1.48, 95%CI 1.09 to 2.01, p=0.013), and a decreased risk for the gain of function 1068G>A variant (rs1718119) (OR: 0.68, 95%CI 0.51 to 0.91, p=0.010) [51]. Since hepatocellular carcinoma is most common in people with underlying liver disease, such as hepatitis infection [52], the effect of P2X7R on immune cell function and pathogen clearance may play a role in the risk associations, as those with reduced function variants may also have reduced immune function and a higher lifetime burden of infection [53].

Glioma: HIGH P2X7R ASSOCIATED WITH BETTER SURVIVAL

P2X7R promotes ATP-induced cell death in glioma cells, and accordingly, high P2X7R expression is associated with better prognosis in terms of radiosensitivity and survival [54]. In a small sample of glioma patients (n=18), high P2X7R expressing patients had a longer median survival time (>80 months), relative to those with low P2X7R (14 months), and a similar effect on survival time was seen in a separate analysis of tissue from 324 glioma patients. Glioblastoma patients, which have the highest-grade glioma, were more likely to have downregulation of P2X7R, suggesting that this change may play a role in malignant transformation.

Bladder cancer: HIGH P2X7R ASSOCIATED WITH BETTER SURVIVAL

In an analysis of differentially expressed proteins in resected tissue from patients with bladder urothelial carcinoma (n=74), high P2X7R expression was found to be associated with better survival (HR: 0.301,

95% CI 0.116 to 0.785, p= 0.014), suggesting that P2X7R may drive ATP mediated cell death in bladder cancer cells [55].

Osteoporosis: REDUCED FUNCTION VARIANTS ASSOCIATED WITH HIGHER FRACTURE RISK AND MORE BONE LOSS

P2X7R is a key regulator of bone mass, with roles in bone formation and reabsorption. A gene association study involving 1694 women ages 45 to 58 participating in the Danish Osteoporosis Prevention Study, found that **P2X7R gain of function genotypes** (H155Y and A348T) **were associated with a low rate of bone loss, while loss of (reduced) function genotypes** (151+1g->t, G150R, R276H, R307Q, E496A, and I568N) **were associated with a high rate of bone loss** [56]. Women, not taking hormone replacement therapy, with the loss of function R307Q variant (G>A) had a greater decrease in bone mineral density over ten years (hip change in bone mineral density GG −0.89 ± 0.02% per year vs. GA −1.20 ± 0.13, p=0.029). Notably, although hormone replacement therapy reduced bone loss irrespective of P2X7R genotype, women with the R307Q variant (GA) taking hormone replacement therapy experienced higher rates of bone loss than those without this variant (GG). The association between the R307Q variant bone loss was replicated in the Aberdeen Prospective Osteoporosis Screening Study (n=506 postmenopausal women over 6-7 years). Meanwhile, the increase in bone mineral density for those with the gain of function variants resulted in decreased fracture incidence. Women homozygous for the A348T (G>A) variant (AA) had a 70% reduction in vertebral fractures compared to women homozygous for the wildtype allele (GG). Similar associations between P2X7R high and low functioning variants with bone density and fracture risk have been replicated in other studies [57; 58; 59].

Efforts to understand the molecular mechanisms underlying bone mass regulation by P2X7R have been complicated by the presence of loss of function P2X7R gene variants in several of the most commonly used mouse strains, including C57bl/6 [6]. This variant may confound studies using P2X7R inhibition or deletion, as these animals already have reduced P2X7R activity, so depending on the magnitude, a further reduction may or may not show a clinically meaningful effect [60]. Mouse strains with the wildtype P2X7R allele (P451), such as BALB, NOD, NZW, and 129, had both stronger bones and higher levels of bone reabsorption markers relative to strains with the reduced function variant P451L [6]. P2X7R regulates the lifespan and activity level of bone reabsorbing osteoclasts, however, the functional impact appears to be context dependent [61]. Low levels of P2X7R activation are expected to promote osteoclast formation and bone reabsorbing activity, while chronic exposure to high concentrations of ATP, or elevated P2X7R activation can lead to the formation of large membrane pores leading to the apoptosis of osteoclasts [60]. Under physiological conditions, strains of mice that have fully functional

P2X7R show increases in bone mineral density and bone strength when P2X7R is knocked out, due to a decrease in osteoclast activity [60]. However, under menopausal-like conditions (estrogen deficiency), P2X7R deficient mice had greater bone loss [61], which mimics the genetic data in humans, likely due to a decrease in osteoclast elimination under inflammatory conditions. Meanwhile, in strains that have reduced pore-forming capacity, P2X7R activation is more likely to promote osteoclast activity, though to a lesser degree than those with the fully functional variant, and thus P2X7R deficiency is more likely to show protection against inflammatory bone loss in these animals.

Neuropathic pain: POTENTIAL BENEFIT (Preclinical)

P2X7R is involved in pain processing and tolerance. Several studies have found associations between P2X7R genetic variants and pain tolerance. Although the specific variants and haplotypes varied, there was a general trend that variants with reduced P2X7R pore function were associated with higher pain tolerance, while those with increased P2X7R pore function were associated with lower pain tolerance, suggesting that the pore function of P2X7R is critical for its effect on pain. In a cohort of women who underwent mastectomy (n=346), those with the gain-of-function H155Y (rs208294) variant reported more pain (β= 1.17, p=0.003) while those with the loss-of-function R270H (rs7958311) variant reported less pain (β=-1.19, p=0.006) [62]. In a cohort of patients with osteoarthritis pain (n= 743), the R270H (rs7958311) variant was also associated with less pain intensity (OR: 0.79, 95% CI 0.65 to 0.95, p=0.015). In a meta-analysis of the two cohorts, the pore disabling loss-of-function R270H (rs7958311) variant was significantly associated with reduced chronic pain ($P = 3.3 \times 10^{-4}$).

In a model of experimental pain, the R270H (rs7958311) variant was also associated with higher cold pain tolerance (HR: 0.895, 95% CI 0.77 to 1.02, p=0.059), and reduced cold pain intensity (β=-1.83± 0.55, p=0.006) in the combined Tromsø and BrePainGen cohorts (n=2460), which contain a higher percentage of females [63]. Carriers of this minor allele also had less multisite pain (OR: 0.75, 95% CI 0.57 to 0.99), and homozygotes of the R270H variant (AA), had less postoperative pain.

The presence of the gain-of-function P2X7R variants, H155Y (rs208294) and Ala348Thr (rs1718119) were associated with higher pain intensity in diabetic neuropathy, however, the effect was only seen in females, suggesting there may be a sex-dependent effect, with females being more affected by P2X7R variants [64].

Mouse strains containing reduced function P2X7R variants (i.e. P451L) also show reduced mechanical allodynia in a nerve injury model [62]. In rats, P2X7R activity induces microglial reactivity in response to morphine, which ultimately leads to a reduction in antinociceptive activity, and the development of

morphine tolerance [65]. Use of a peptide that specifically blocks P2X7R within the C-terminus (Y382–384) prevented morphine tolerance in rats, and this type of targeted response is expected to be safer and more effective than systemic P2X7R inhibition for this indication.

In peripheral blood lymphocytes and monocytes, the expression of P2X7R was found to be increased 1.6-fold in individuals with neuropathic pain, but not in those with chronic nociceptive back pain [66]. Serum levels of IL-1β were also elevated 1.4-fold in those with neuropathic pain, which may stem from increased P2X7R activation. In preclinical models, P2X7R inhibitors are effective against inflammatory and neuropathic pain, without impairing physiological nociception [67]. Inhibition of P2X7R reduces microglial activation, which can generate reactive oxygen species (ROS) and facilitate abnormal sensory afferent firing. The protective effects appear to be at least partially mediated by inhibition of the NLRP3 inflammasome. Neuropathic pain is anticipated to be a key clinical indication for P2X7R inhibitors; however, genotype effects are likely to impact efficacy. Individuals with gain-of-function P2X7R variants are more likely to benefit from P2X7R inhibitors, and there may be different efficacy in males and females. There are P2X7R inhibitors in preclinical development for this indication.

Cardiovascular: REDUCED FUNCTION VARIANTS AND REDICED P2X7R EXPRESSION ASSOCIATED WITH REDUCED RISK OF CARDIOVASCULAR EVENTS

In a prospective observational study in Chinese individuals with acute myocardial infarction (n= 79 vs. n=48 controls), the mRNA levels of P2X7R on peripheral blood mononuclear cells (PBMCs) was 2.85-fold higher in the group with myocardial infarction relative to controls (2.81 ± 0.15 vs. 0.99 ± 0.06, $p < 0.01$) [68]. P2X7R expression levels were positively correlated with C-reactive protein (CRP) levels (r= 0.46), serum LDL levels (r = 0.31) and angiographic Gensini scores (r=0.36), a measure of atherosclerotic burden. Additionally, **higher P2X7R expression was associated with a higher risk for having a major adverse cardiovascular event** (MACE) within 360 days (univariate hazard ratio HR: 2.87, 95% CI 1.11 to 7.42 and multivariate HR: 3.28, 95% CI 1.12 to 9.65).

In a gene association study, a loss of function P2X7R variant was associated with reduced risk for adverse cardiovascular events [69]. The study involved 244 ischemic heart disease cases and 2488 controls as well as 5969 individuals with cardiovascular risk factors. An additional comparison involved 4138 individuals with ischemic stroke and 2528 controls. The loss of function variant E496A (rs3751143) was associated with a decreased risk of ischemic heart disease only in smokers (OR: 0.77, 95% CI 0.61 to 0.97, p = 0.03), as well as decreased risk of ischemic stroke in the total population (OR: 0.89, 95% CI 0.81 to 0.97, p = 0.012). The protective effect for reduced P2X7R function is thought to be due to a decrease in vascular inflammation.

The stronger protective effect for reduced P2X7R function with respect to ischemic stroke may stem from evidence that P2X7R is strongly tied to systemic inflammation in this population. One study found a strong correlation between blood CRP levels and blood levels of shed P2X7R (r=0.645) (n=10). Shed P2X7R was also associated with elevated CRP levels in individuals with infectious disease, but not for those with cancer or trauma, suggesting that P2X7R is only a strong driver of inflammation under particular pathological contexts [70].

Rodent models indicate that P2X7R inhibitors can protect against cardiac dysfunction and fibrosis, largely through the inhibition of NLRP3 and IL-1β [71]. Prolonged P2X7R activation can alter Ca^{2+} homeostasis, and promote pro-inflammatory cytokines and oxidative stress. However, P2X7R-related therapeutic intervention for ischemic injury is likely to be complex, dependent on the timing and severity of the injury. Preclinical studies indicate that P2X7R is involved in preconditioning, and possibly postconditioning, so weak P2X7R agonists may be protective prior to injury, while P2X7R antagonists have been shown to limit neuronal damage after ischemic injury [72]. Further studies are necessary to determine the optimal point of intervention.

Atherosclerosis: POTENTIAL BENEFIT (Preclinical)

The infiltration of activated macrophages to the vasculature is a key driver of endothelial remodeling and atherosclerotic plaque formation. P2X7R has been shown to be expressed higher in carotid arteries with plaques relative to non-atherosclerotic arteries in *ex vivo* cultures [73]. The increase in P2XR7 may drive IL-1β production, as treatment with the P2X7R inhibitor A740003 reduced IL-1β content. Reducing IL-1β could have clinical utility, as high IL-1β levels were associated with unstable carotid plaques, whereas levels were barely detectable in stable plaques (10 pg/mg protein < IL-1β < 260 pg/mg protein). Similarly, in an atherosclerosis model (LDLr-/- with high cholesterol diet) P2X7R deficient mice have reduced lesional macrophages and smaller atherosclerotic lesions [74]. This suggests that P2X7R inhibition could protect against atherosclerosis, but the timing of therapeutic intervention necessary for a clinically meaningful benefit has not been established.

Diabetes/Metabolic syndrome: POTENTIAL MIXED BENEFIT (Preclinical)

P2X7R inhibition may impair glucose tolerance; however, in a metabolically unfavorable (obesogenic) environment, P2X7R inhibition may protect against adipose tissue inflammation [75]. Additionally, P2X7R inhibition may protect against diabetes-related complications, such as diabetic nephropathy, by mitigating oxidative stress damage.

In a gene association study involving a cohort from the Prevalence, Prediction, and Prevention of Diabetes in Botnia (n = 3504), P2X7R variants that modulate P2X7R function [rs17525809 (V76A), rs208294 (Y155H), rs7958311 (R270H), rs1718119 (A348T), and rs2230912 (Q460R)] were associated with glycemic traits [76]. The gain-of-function variant rs1718119 (A348T), which enhances pore function by 2.5-fold *in vitro*, was associated with improved insulin sensitivity, while variants with reduced pore function were associated with impaired glucose homeostasis.

P2X7R has been found to influence metabolic rate in mice, and to be involved in the function of pancreatic beta cells, including insulin secretion [75]. Some studies in P2X7R knockout mice have shown that these mice had elevated serum triglyceride and cholesterol levels as well as increased lipid accumulation in the liver, due to a reduction in lipid metabolism genes and shift from lipid oxidation toward carbohydrate oxidation for energy [77; 78]. Data from P2X7R knockouts can be difficult to interpret since not all splice variants are eliminated, so depending on the cell type, P2X7R may or may not be expressed, and the function could potentially be altered if other normally present P2X7R variants are missing. Data using the P2X7R inhibitor A804598 (90 mg/kg i.p. for 7 days) also led to an impairment in energy homeostasis, but the effect was marginal compared to the knockout [78]. It is not clear whether more clinically meaningful changes to metabolism would take place with chronic use, or whether P2X7R would further impair glucose homeostasis in diabetic patients.

P2X7R expression has been shown to be elevated in renal tissue from patients with diabetes. Diabetics with elevated renal interstitial P2X7R had more severe nephropathy, including more interstitial fibrosis and a lower estimated glomerular filtration rate [79]. Preclinical studies in rodents suggest that inhibition of P2X7R could protect against diabetic nephropathy. P2X7R inhibition has also been found to be protective against kidney damage in a variety of other models of inflammatory and fibrotic kidney injury. In male rats with streptozotocin-induced diabetes, infusion of P2X7R siRNA to the kidney via an osmotic pump decreased measures of oxidative stress (TBARS, NO) and improved measures of renal function [80; 81]. The protective effect for reducing P2X7R expression in the kidney may stem from its association with klotho. The kidneys of the diabetic animals had increased levels of P2X7R and decreased levels of klotho, such that there was an inverse correlation between them (Pearson r = 0.5944) [82]. P2X7R silencing increased kidney klotho levels around 70% relative to the untreated diabetic animals, and also increased circulating klotho to control levels [81]. Treatment with the P2X7R inhibitor AZ11657312 (50 mg/kg i.p. 2x/day, 4 weeks) was also protective in reducing renal macrophage infiltration in a mouse model of streptozotocin-induced diabetes [79]. Notably, treatment with the P2X7R inhibitors did not adversely affect glucose homeostasis in these diabetic animal models.

It remains to be established whether P2X7R variant genotype influences the response to P2X7R-directed therapy, as those with decreased pore function variants would be expected to get less benefit.

Liver disease: POTENTIAL BENEFIT (Preclinical)/ UNCLEAR (Clinical)

P2X7R was found to be expressed in inflammatory immune cells in liver biopsies from patients with non-alcoholic steatohepatitis (NASH), and the expression on these populations was increased from 2.9 to 10 fold relative to healthy liver tissue [83]. The increased P2X7R in NASH livers was associated with increased NLRP3 inflammasome activation and mature IL-1β. Treatment of human liver monocytes with the selective P2X7R inhibitor SGM-1019 (also known as EVT-401), led to a reduced production of the pro-inflammatory cytokine IL-1β, which contributes to inflammatory cell death of hepatocytes and fibrosis of hepatic stellate cells. In a liver fibrosis model (CCl_4-induced) in cynomolgus monkeys (*Macaca fascicularis*), treatment with SGM-1019 (5, 15, and 30 mg/Kg for 4 weeks, starting at week 2) partially reduced profibrotic genes, inflammatory markers, hepatic stellate cell activation, serum levels of the liver enzyme alanine aminotransferase (ALT), and histological signs of liver pathology [83]. This suggests that P2X7R can have anti-fibrotic and anti-inflammatory effects in the liver. However, SMG-1019 is not a suitable P2X7R inhibitor for this indication, as a Phase 2a clinical trial (n=100) testing pharmacokinetics and safety of SGM-1019 in NASH patients was terminated due to a safety event that indicates the risk/benefit profile is not favorable in this population (NCT03676231). It is not clear if these safety issues are specific for SGM-1019, or would be applicable to other P2X7R inhibitors.

Safety: Clinically tested inhibitors showed good short-term safety, with headache and gastrointestinal problems reported. Establishing a safe therapeutic window has been a challenge. Long-term safety and genotype-specific effects are unknown.

Types of evidence:

- 8 clinical trials (CE-224,535, AZD9056, SGM-1019, GSK1482160, BIL010t, JNJ-54175446)
- Numerous laboratory studies

Several P2X7R inhibitors have been tested in clinical trials, and were generally found to be well-tolerated, however, it is expected that greater than 90% inhibition of P2X7R activity is necessary for efficacy, and **the development of many clinical candidates has been terminated because the dose required for this degree of inhibition is too close to the maximum tolerated dose** to have a viable therapeutic efficacy-safety window. Since P2X7R is widely expressed, there is a risk for side effects with systemic administration of P2X7R inhibitors. Since P2X7R is highly expressed in immune cells, and

individuals with loss-of-function variants have been shown to be at higher risk for infection by some types of pathogens, chronic use of P2X7R inhibitors could potentially increase the risk for infection, though that has not been reported in any of the clinical trials conducted thus far.

Another potential caveat for the clinical development of P2X7R inhibitors is the fact that P2X7R is highly polymorphic, thus both the therapeutic efficacy and side effect profile could be determined by the genetic makeup of each individual. The influence of genotype has not yet been assessed in the published clinical trials conducted thus far. Targeting a specific form of P2X7R, such as the effort to specifically target nfP2X7R in cancer, is likely to have the best safety profile.

CE-224,535, from Pfizer, was tested in a Phase 2a RCT in patients with active rheumatoid arthritis taking methotrexate (n=100) for 12 weeks (NCT00628095). The dose tested, 500 mg orally BID, exceeded the estimated IC_{90} for inhibition of IL-1β release, the pharmacodynamic measure of P2X7R inhibition, but was clinically ineffective in this population [84]. The most common treatment-emergent adverse events were nausea (11.3%, CE-224,535 vs. 4.3%, placebo) and diarrhea (7.5%, CE-224,535 vs. 4.3%, placebo). The discontinuation rate due to an adverse event was 9.4% for CE-224,535 and 6.4% for placebo. Notably, there was a similar incidence of infections between the groups (24.5% CE-224,535 vs. 21.3% placebo).

AZD9056, from AstraZeneca, was tested in Phase 2a (n=75) and 2b (n=383) (NCT00520572) RCTs in patients with active rheumatoid arthritis taking methotrexate or sulphasalazine, for 4 weeks and 6 months, respectively at doses from 100 to 400 mg/day, orally [85]. AZA9056 was not clinically effective in this population. AZD9056 was well tolerated, though gastrointestinal events were common (21.7% vs 18.5% placebo) and dose related such that nausea, vomiting and diarrhea were reported more frequently at the 400 mg dose of AZA9056. There was one serious adverse event involving a patient receiving 400 mg AZD9056 who was hospitalized after 3 days of treatment due to nausea and vomiting, though the condition resolved with drug discontinuation. There were no serious infections reported.

AZA9056 was also tested in a Phase 2a RCT for active Crohn's disease (n=34) at a dose of 200 mg/day for 28 days, to try to minimize gastrointestinal side effects (Eudra-CT Number: 2005-002319-26) [86]. The primary endpoint of this study was met, but it was not accompanied by a reduction in inflammatory markers, as would be expected based on the projected mechanism of action, and was not pursued further for this indication. Most adverse events were related to the underlying condition, though gastrointestinal adverse events were more common in the AZA9056 group relative to placebo. Three patients in the AZA9056 group discontinued study treatment due to adverse events which included, reduced vision, diarrhea, vertigo, and worsening of Crohn's disease.

SGM-1019 from Second Genome was in-licensed from Evotec was reported to be safe in healthy volunteers with 2x/daily dosing (Press release), however, a trial in patients with NASH had to be terminated due to an undisclosed safety event that precludes the use of the drug in this population (NCT03676231).

In a small Phase 1 study testing the P2X7 receptor allosteric modulator GSK1482160 from GlaxoSmithKline in healthy volunteers (n=29), 20/29 subjects reported adverse events, though most were mild or moderate, with headache being the most common adverse event (NCT00849134) [87]. There were two serious adverse events that led to withdrawal from the study, one of which was considered drug-related. This event involved a single asymptomatic run of accelerated idioventricular rhythm in a 41-year-old male at 1 h 25 min following dosing with 1000 mg (the highest dose tested) of GSK1482160. Based on modeling of the PK/PD data, it was determined that it would not be possible to achieve >90% inhibition of P2X7R for the duration of a dosing period at doses that were within the safety margin, so development was terminated.

The PEG-based 10% ointment containing nfP2X7R-targeted antibodies (BIL010t) from Biosceptre was tested in a Phase 1 open label trial in patients with basal cell carcinoma (n=21) at a dose of 50-100 mg/day for 28 days (NCT02587819) [88]. The treatment was well-tolerated, and the most common adverse events were treatment site erythema, pruritus, dryness and pain. There was no evidence of systemic penetration of the antibody from use of the ointment on the skin.

The brain penetrant P2X7R inhibitor from Janssen, JNJ-54175446, was tested in a Phase 1 trial in healthy volunteers (n=119) (NCT02475148) [89]. The most common adverse events were headache (18.6% vs. 5.6% in placebo), back pain and fatigue (8.5% each vs 5.6% each in placebo), diarrhea (6.8% vs 5.6% in placebo) followed by rhinitis, vomiting and hypercholesterolemia. One participant (50 mg dose, fed state) had an abnormal increase in ALT, and one showed sinus tachycardia. This study also included healthy elderly men and women (mean age 63.2, n=6), who received a lumbar puncture to collect cerebrospinal fluid (CSF). Systemic exposure (at 300 mg) was found to be 30 to 40% higher in the elderly relative to the young adults, suggesting dose titration may be necessary in an elderly population. Passive brain penetration of JNJ-54175446 was confirmed, with similar unbound Cmax for plasma (88.3±35.7 ng/mL) and CSF (114±39 ng/mL), suggesting this compound may have utility for CNS conditions, such as neurodegenerative disease.

Drug interactions: No drug interactions have been reported, but due to its role in regulating immune function, it would be expected to have interactions with other immune modulators or immunosuppressants.

Sources and dosing:

There are currently no P2X7R inhibitors available for clinical use, however, there are a variety of inhibitors available for preclinical research use from commercial suppliers.

Research underway:

There continues to be preclinical efforts to develop new P2X7R inhibitors and PET ligands.

AKP-23494954/RQ-00466479 is a P2X7R inhibitor being developed for neuropathic pain, though it has not yet been tested clinically. It was licensed from RaQualia Pharma to Asahi Kasei Pharma, which now holds the worldwide rights for sale and manufacture (Press release).

A Phase 1 open label trial (n=29) for the anti-cancer vaccine targeting nfP2X7R called BIL06v/ Alhydrogel® for patients with advanced solid tumors dosed its first patient in August 2018 (ACTRN12618000838213), and was projected to complete in 2020.

A trial for the CNS penetrant P2X7R inhibitor JNJ-54175446 in major depressive disorder (n=142) (NCT04116606) has been suspended due to the Covid-19 pandemic.

Substitution			Effect	
1513A>C	E496A	rs3751143	Loss of function	ALZHEIMER'S DISEASE: **Decreased risk** for AD with minor (C) variant only IN COMBINATION with minor (T) variant of 489C>T (G
				OSTEOPOROSIS: Associated with **increased bone loss** in postmenopausal women
				PARKINSON'S DISEASE: **Increased risk** for PD in men in a Chinese Han population
				LIFESPAN: Minor variant (C) found in **increased** proportion of European/American Caucasian population at advanced age
				Hepatocellular carcinoma: Minor allele associated with **increased risk** in Chinese Han population
				Papillary thyroid carcinoma: Associated with **increased tumor size** and worse prognosis
				CARDIOVASCULAR: Associated with **decreased risk** for ischemic heart disease and ischemic stroke
	R270H	rs7958311	Loss of function	NEUROPATHIC PAIN: Associated with **decreased pain**
(G>A)	R307Q	rs28360457	Loss of function	OSTEOPOROSIS: Associated with **increased bone loss** in postmenopausal women
489C>T	H155Y	rs208294	Gain of function	ALZHEIMER' S DISEASE: **Decreased risk** for AD with minor (T) variant
				LIFESPAN: Major allele (C) found in **increased** proportion of European/American Caucasian population at advanced age
				NEUROPATHIC PAIN: Associated with **increased pain**
				OSTEOPOROSIS: Associated with **decreased bone loss** in postmenopausal women
(G>A)	A348T	rs1718119	Gain of function	OSTEOPOROSIS: Associated with **decreased fracture risk**
				NEUROPATHIC PAIN: Associated with **increased pain** in diabetic neuropathy in females
	A348T	rs1718119	Gain of function	DIABETES: Associated with **improved insulin sensitivity**

Search terms:

Pubmed, Google: P2X7R ihibitor

- Alzheimer's disease, Parkinson's disease, neurodegeneration, aging, cardiovascular, cancer, diabetes, polymorphisms, clinical trials, safety

Websites visited for P2X7R Inhibitors:

- Clinicaltrials.gov (JNJ54175446; JNJ55308942; CE-224,535; AZD9056; GSK1482160; SGM-1019)
- PubChem (JNJ-54175446)
- DrugBank.ca (JNJ-54175446; CE-224,535)

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