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.

GPR39 Agonists

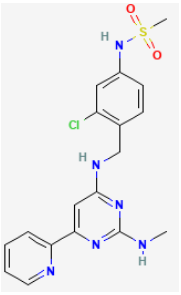
Evidence Summary

Reduced GPR39 activity may drive neuronal dysfunction with zinc deficiency. GRP39 agonists may restore neuronal homeostasis, but may also potentiate cancer growth. More *in vivo* studies are needed.

Neuroprotective Benefit: A reduction in GPR39 activity stemming from a zinc deficiency may disrupt regulation of the neural excitatory-inhibitory balance, increasing the risk for seizures or depression. GPR39 agonists may reduce this risk.

Aging and related health concerns: GPR39 signaling can drive tumor growth and migration, but may have protective anti-inflammatory effects in the context of arthritis and cardiovascular disease.

Safety: Safety data are limited, and more *in vivo* studies are needed. Side effect profile will likely depend on the specificity for GPR39 and which downstream pathways are targeted by the biased agonist. Some agonists may potentiate cancer.

Availability: Research use	**Dose**: N/A	**TC-G-1008** **Chemical formula:** $C_{18}H_{19}ClN_6O_2S$ **MW**: 418.9 g/mol  Source: PubChem
Half-life: Varied	**BBB**: Varied (TC-G-1008 penetrant)	
Clinical trials: None	**Observational studies**: GPR39 expression is decreased in the context of zinc-deficiency and several neurological disorders, but is increased in several cancers, where it is associated with poor prognosis.	

What is it?

GPR39 is an orphan G-protein coupled receptor (GPCR). The endogenous ligand(s) have not yet been identified, but it has been shown to be regulated by zinc [1]. Zinc can serve as an activator of GPR39 and as a positive allosteric modulator for ligand-mediated activation. GPR39 can drive both constitutive and ligand-dependent GPCR signaling, and the level of zinc can regulate this balance. The expression of GPR39 is also sensitive to zinc levels, such that zinc-deficiency can reduce the expression and activity levels of GPR39, in some tissues. GPR39 is coupled to several different G proteins, including Gαq, Gαs, and Gα12/13. These can lead to the activation of different downstream signaling cascades, including PI3K/AKT, ERK1/2, and PLCβ. Therefore, the effects of GPR39 activation are highly context dependent based on whether it is constitutive or ligand-dependent, the identity of the coupled G-protein, and the associated signaling cascade that is triggered in a given cell. One of the most prominent effects of GPR39 activation is the release of calcium from intracellular stores, leading to the regulation of calcium-sensitive cellular programs [2]. Consequently, GPR39 activity is also sensitive to calcium levels, which is particularly relevant in the nervous system, and allows for neuronal activity-related changes in GPR39 activation. GPR39 plays various roles in the maintenance of cellular homeostasis. In the nervous system, loss of GPR39 can prevent the restoration of imbalances in excitatory and inhibitory activity. Due to its ability to promote pathways involved in cell survival and migration, overexpression of GPR39 can promote tumor growth and metastasis. Therefore, GPR39 agonists have been proposed for neurological disorders, while GPR39 inhibitors have been proposed for cancer. Disruptions in GPR39 activity may be

one of the drivers of pathophysiology in the context of zinc dyshomeostasis, thus GRP39 modulators may be necessary for the clinical benefit of zinc-modulating therapies. Similarly, the efficacy of GPR39 modulators may be dependent on the zinc status of a given tissue or individual.

Neuroprotective Benefit: A reduction in GPR39 activity stemming from a zinc deficiency may disrupt regulation of the neural excitatory-inhibitory balance, increasing the risk for seizures or depression. GPR39 agonists may reduce this risk.

Types of evidence:

- 2 studies examining GPR39 expression in postmortem brain tissue
- Numerous laboratory studies

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

Human research to suggest benefits to patients with dementia:

Metal dyshomeostasis is a prominent feature of Alzheimer's disease (AD) and other neurodegenerative disorders [3]. The essential trace element, zinc, is one of the metals that shows altered localization and functional patterns in the context of dementia. Some small underpowered clinical trials showed mixed benefit for zinc supplementation. Clinical trials testing the zinc ionophore PBT2 were also underpowered and showed mixed cognitive benefit, but the clinical program was terminated due to lack of efficacy. The history of zinc-related therapy for AD suggests that zinc modulation may offer modest benefit, but due to the multifaceted nature of zinc dysregulation in AD, it may need to be targeted in multiple ways. Altered activity at GPR39 may be one of the mechanisms by which zinc dyshomeostasis impacts cognitive function, thus biased GPR39 agonists may offer benefit as part of a zinc-targeted therapy [4]. Thus far, direct modulation of GPR39 has not been clinically tested.

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

Alzheimer's disease: GPR39 ACTIVITY MAY BE ALTERED IN ASSOCIATION WITH ZINC DYSREGULATION

GRP39 has both ligand-independent constitutive activity and ligand-dependent activity [1]. GPR39 activity can have different downstream effects depending on the G-protein and associated signaling cascade it is coupled to in a given cell. Although the endogenous ligand(s) for GRP39 has not been identified, zinc has been established as an activator and/or allosteric modulator of GPR39, and the

expression and activity of GPR39 is sensitive to the concentration of zinc in a given tissue. One of the key pathways downstream of GPR39 activation is the release of Ca^{2+} from internal stores [2]. The affinity of zinc for GPR39 is influenced by the Ca^{2+} levels, such that changes in neuronal activity can influence the level and activity of synaptic zinc. Consequently, the zinc dyshomeostasis found in the AD brain may lead to altered GPR39 signaling, which could lead to synaptic dysfunction, altered excitatory/inhibitory balance, increased risk for seizures, and depression/anxiety.

Neuronal GRP39 activity plays a role in the maintenance of homeostatic mechanisms. Zinc/GPR39 can modulate neurotransmission, and plays a role in the restoration of excitatory/inhibitory balance, particularly in the context of an excitotoxic insult [5]. GPR39 also plays a role in the homeostatic adaptation to changes in pH to maintain neuronal activity [6]. Prolonged neuronal activity can lead to intracellular acidification. GPR39 activation upregulates the Na+/H+ exchanger in hippocampal neurons to facilitate recovery from intracellular acidification, through ERK1/2 mediated signaling.

In the context of AD, Aβ may be a primary driver of zinc dyshomeostasis. Aβ binds zinc, and this binding can promote Aβ aggregation. In the presence of Aβ, synaptically released zinc binds to Aβ instead of to GPR39 [7]. This prevents the activation of zinc-dependent GPR39 signaling which leads to the Ca^{2+} release from intracellular stores, and the induction of downstream Ca^{2+}-dependent signaling cascades. One such zinc/GPR39-dependent signaling cascade involves the activation of ERK1/2 and the upregulation of the chaperone protein clusterin, which is implicated in AD pathophysiology. Zinc deficiency in immune cells has also been shown to promote pathological pro-inflammatory signaling, including NRLP3 inflammasome activation, in an AD mouse model [8]. Aβ essentially acts to sequester zinc from synaptic sites into aggregate deposits, leading to a functional zinc deficiency, coupled with a toxic accumulation of zinc.

Prior therapeutic attempts toward zinc modulation may have failed because they did not adequately address the multiple levels of zinc dyshomeostasis [3]. Zinc supplementation may allow for increased receptor binding, as *in vitro* studies suggest that increasing zinc levels can partially overcome Aβ sequestering [7]. However, the supplemented zinc must be properly localized, otherwise it will simply contribute to increased Aβ oligomerization and toxicity. Similarly, chelation strategies can exacerbate functional deficiencies [4]. The ionophore PBT2 was intended to disrupt the interaction between Aβ and zinc. The mixed clinical trial results suggest that there may have been some benefit to this approach, but that it was insufficient as a monotherapy [4]. Dysregulation of GPR39 may be a key feature of zinc dyshomeostasis-related pathophysiology that needs to be corrected to see cognitive benefit. However, the extent to which GPR39 expression and/or activity is altered in the AD brain, remains to be elucidated. Since GPR39 expression is impacted by zinc levels, and its activation in response to agonists

is potentiated by zinc, it is unclear whether GPR39 agonists alone would offer significant benefit in the context of an overall zinc dyshomeostatic environment. In the context of AD, GPR39 agonists may need to be combined with another zinc-modulating therapy to achieve clinical efficacy. Additionally, GPR39 agonists are generally designed to activate GPR39 in a biased manner based on the nature of their binding, thus they will preferentially activate certain G-protein mediated downstream signaling pathways. The optimal agonist for boosting cognitive function in the context of AD has not yet been established.

Vascular dementia: REDUCED GPR39 MAY INCREASE SUSCEPTIBILITY

GPR39 is highly expressed in the circulatory system, and single nucleotide polymorphisms (SNPs) in the GPR39 gene are associated with several vascular-related diseases [9]. In the dorsolateral prefrontal cortex, GPR39 expression was found to be primarily localized within CD68+ microglia and capillary-associated pericytes in postmortem human brain tissue [10]. There were no differences in ratios of individuals who were non-carriers (n=26 vs n=26) or heterozygous (n=10 vs n=11) for SNPs (rs13420028 and rs10188442) in GPR39 with high linkage disequilibrium previously associated with vascular disease between controls and individuals with mild cognitive impairment (MCI). However, individuals who were homozygous for GPR39 SNPs were exclusively in the MCI group (n=5). The homozygotes had significantly higher white matter hyperintensity burden (38.03 ± 13.46 mL) relative to the non-carriers (15.87 ± 2.59 mL) or heterozygote carriers (11.90 ± 2.91 mL). These SNPs appear to reduce the expression and/or activity of GPR39, suggesting that GPR39 function may be important for the maintenance of vascular integrity. Preservation of GPR39 activity may be beneficial in the context (prevention) of vascular dementia. In other vascular conditions, both zinc and GPR39 appear to be important, thus it remains to be established whether zinc dyshomeostasis also plays a role, such that therapeutic GPR39 agonists may need to be paired with an additional zinc-modulating therapy.

Epilepsy: GPR39 INFLUENCES SUSCEPTIBILITY TO SEIZURES (Preclinical)

GPR39 plays a role in the maintenance of excitatory/inhibitory balance in the CNS. Zinc released at the synapse can modulate neurotransmitters, and thus influence neural activity. Zinc is released from synaptic vesicles in an activity-dependent manner. The co-release of zinc by glutamatergic neurons (gluzinergic) can reduce further glutamate release by binding to and inhibiting the NMDA receptor, and serve as a homeostatic mechanism to restore excitatory/inhibitory balance in the context of an excitotoxic event, such as a seizure [11]. Zinc can also enhance inhibitory drive through a mechanism dependent on the activity of GPR39. Synaptic activation of GPR39 drives a Gαq-PLCβ-ERK1/2 signaling cascade that leads to increased surface expression and activity of the neuronal K+/Cl− co-transporter,

KCC2 [5]. Kainic acid provokes seizure activity, leading to enhanced synaptic zinc release, and the induction of a GPR39-dependent increase in KCC2 as part of a homeostatic response. Mice lacking GPR39 have enhanced susceptibility to seizures and seizure-related excitotoxic damage, due to the lack of this homeostatic mechanism to restore excitatory/inhibitory balance.

Depression: GPR39 AGONISTS HAVE ANTI-DEPRESSANT ACTIVITY (Preclinical)

Zinc deficiency is a common feature of major depression, and a low serum zinc level has been proposed as a biomarker [12]. The etiology of the zinc deficiency is unclear, but may be related to poor nutritional status, which is also common in the context of depression. Zinc deficient diets can also lead to the induction of depressive-like behaviors in rodent models [13]. Since GPR39 expression is sensitive to zinc levels, zinc deficiency decreases the expression of GPR39 in key brain regions, such as the hippocampus and cortex. Postmortem analysis of suicide victims with depression indicated that GPR39 expression was decreased by 19% in the hippocampus and by 17% in the cortex [14]. In male mice, zinc deficiency leads to a 50% decrease in GPR39 in the hippocampus, along with a 39% decrease in both the transcription factor CREB and the neurotrophic factor BDNF in this brain region [14].

The efficacy of antidepressants is known to take several weeks, which is thought to be related to changes in CREB and BDNF. In zinc-deficient mice with depressive-like phenotypes, chronic treatment with monoamine modulating anti-depressants (imipramine, escitalopram, reboxetine, bupropion) increased levels of GPR39, CREB, BDNF, and its receptor TrkB [13]. Studies conducted in male mice suggest that GPR39 and its downstream activity, such as induction of BDNF, may play a role in depressive phenotypes and anti-depressant efficacy. Mice lacking GPR39 show depressive-like and anxiety-like phenotypes, and are less responsive to treatment with monoamine-modulating anti-depressants, such as imipramine [11; 15]. A similar effect is not seen with anti-depressant agents acting through other mechanisms, such as glutamate regulation [16]. This suggests that the effects may be at least partially mediated through an interaction between GPR39 and the monoaminergic systems. GPR39 has been shown to form heterodimers with the serotonergic 5-HT1A receptor, and the formation of these complexes is influenced by zinc levels [12]. GPR39 knockout mice have aberrant activity patterns in brain regions where GPR39 is normally expressed, such as the hippocampus and amygdala, which are involved in emotional regulation [15].

Some GPR39 agonists, such as TC-G-1008, show anti-depressant-like and anti-anxiety-like responses in mice, and in some paradigms, this is accompanied by an increase in hippocampal levels of BDNF [17; 18]. These studies suggest that GPR39 agonists may be a useful adjunct in treatment-refractory depression,

and help alleviate depression associated with other neurological conditions where GRP39 expression/signaling is altered.

Alcohol abuse: GPR39 ACTIVATION MAY REDUCE THE ABUSE POTENTIAL OF ALCOHOL (Preclinical)

GPR39 was found to be responsive to heavy alcohol consumption. In macaques, heavy alcohol use led to the hypermethylation, and thus the downregulation of GPR39 expression in the brain [19]. Through its role in maintaining homeostatic excitatory/inhibitory balance, the decline in GPR39 may contribute to an imbalance in the reward center, the nucleus accumbens, that promotes substance abuse. In male mice, treatment with the GPR39 agonist, TC-G-1008 (7.5 mg/kg i.p.) reduced alcohol intake and preference, in the context of either an acute challenge, or a chronic paradigm [20]. These effects were associated with increased GRP39 expression and BDNF levels in the nucleus accumbens, which promoted excitatory neurotransmission. As alcohol use drives the system toward an inhibitory GABAergic bias, increased excitatory glutamatergic neurotransmission could offset this bias, and restore a homeostatic state.

APOE4 interactions: Not established

Aging and related health concerns: GPR39 signaling can drive tumor growth and migration, but may have protective anti-inflammatory effects in the context of arthritis and cardiovascular disease.

Types of evidence:

- 1 study assessing association between zinc and vascular calcification
- 1 study assessing GPR39 expression in rheumatoid arthritis
- 5 primary tumor tissue analysis studies showing a relationship between GRP39 and cancer
- Numerous laboratory studies

Cancer: HIGH GPR39 IS ASSOCIATED WITH POOR PROGNOSIS

GPR39 has been shown to promote tumor cell proliferation and migration through the induction of cell survival and cell motility pathways in a variety of cancers [2]. GPR39 expression serves as a biomarker for poor prognosis. Thus, in the context of cancer, GPR39 inhibitors could be of potential therapeutic value, while agonists would likely promote tumor aggressiveness.

Breast cancer: In estrogen receptor (ER) negative breast cancer, the expression of GRP39 was found to be elevated with tumor aggressiveness, such that grade 3 tumors had higher expression than grade 2

tumors [21]. This pattern was not seen with ER-positive breast cancer cases. In the context of ER-negative breast cancer, altered zinc distribution is associated with disease progression and tamoxifen resistance. In these tumor cells, activation of GRP39 upregulates the cell-survival promoting P13K/AKT and MAPK pathways. One of the functional consequences of this signaling is the activation of the K+/Cl– co-transporter, KCC3, which promotes tumor cell proliferation and migration through the activation of the matrix metalloproteases MMP-2 and MMP-9 [22; 23].

Hepatocellular carcinoma: The microRNA miR-1914 inhibits tumor cell proliferation by inhibiting GPR39 and its downstream PI3K/AKT/mTOR signaling [24]. The expression of miR-1914 was found to be reduced in accordance with histological tumor grade and size, while the opposite effect was seen for GPR39. Patients with low miR-1914 and high GPR39 showed worse overall survival and disease-free survival.

Esophageal squamous cell carcinoma: GPR39 was found to be overexpressed at the mRNA and protein levels in primary tumors [25]. High GPR39 was also associated with lymph node metastasis and clinical stage. The induction of GPR39 in esophageal squamous cell carcinoma cell lines promoted tumor formation when injected into mice, and increased cell mobility through promotion of the epithelial-mesenchymal transition.

Gastric adenocarcinomas: GPR39 was found to be aberrantly expressed in primary gastric adenocarcinomas relative to healthy stomach tissue, and this was associated with de-differentiation of the tumor tissue and promotion of the epithelial-mesenchymal transition [26]. The expression of GPR39 promotes the cytoskeletal remodeling necessary to facilitate tumor cell migration.

Oral squamous cell carcinoma: GPR39 was found to be overexpressed in primary tumors, which was associated with malignant progression and poor survival [27]. The increase in GPR39 was coupled with an increase in YAP, and important regulator of cell size and growth. GPR39 was shown to activate YAP through a Gαq/11-RhoA–dependent signaling pathway.

Metabolic disease: GPR39 HAS MILD VARIED EFFECTS ON METABOLIC PARAMETERS (Preclinical)

GPR39 was originally implicated in metabolic regulation when it was misidentified as the receptor for obestatin, a regulator of appetite [28]. A lack of expression of GPR39 in the hypothalamus suggests that central regulation of food intake is unlikely to be a primary function of GPR39, however, mild alterations in metabolic parameters in GPR39 knockout mice, suggests that GPR39 does play a role in metabolic function [29]. The effects appear to be context dependent, suggesting that GPR39 is not a major player, but that GPR39 modulators could have minor impacts on metabolic function. GPR39 expression was

found to be altered in two different rat models of metabolic syndrome, diet-induced and genetic, but the effects were both tissue and model specific [30]. Under basal conditions, GPR39 knockout mice generally show normal body weight, food intake, and insulin secretion [31]. However, mild phenotypes emerge under challenge conditions. When aged to 52 weeks, male GPR39 knockout mice had decreased insulin levels following a glucose challenge [31]. A separate study found that cholesterol levels were increased in fasted male and female 24-week GPR39 knockout mice [29].

Due to the context dependent nature of GPR39 signaling, and the biased signaling of GPR39 agonists, different agonists may have different metabolic effects. The first discovered orally bioavailable GPR39 agonist, TC-G-1008 was shown to stimulate GLP-1 secretion in mice and rats, suggesting it may promote glucose homeostasis [32]. The drugs AZ7914, AZ4237, and AZ1395 were found to have GPR39 agonist activity in cell-based screens, however, they failed to lower glucose or promote insulin in lean mice, diet-induced obese mice, or genetically obese rats [33]. Additionally, these agonists led to hyperglycemia in obese mice, which could be severe at high doses. These studies suggest that the development of GPR39 agonists may require monitoring for metabolic side effects.

Atherosclerosis: IN COMBINATION WITH ZINC, GPR39 REGULATES VASCULAR HOMEOSTASIS (Preclinical)

Preclinical studies suggest that GPR39 may play a role in the regulation of vascular inflammation and calcification. GPR39 expression was found to be decreased by 30% in calcified human aortic valves relative to those without calcific aortic valve disease [34]. This was accompanied by a decrease in serum zinc levels, such that higher serum zinc was protective in the context of calcific aortic valve disease (Odds ratio [OR]: 0.135, 95% Confidence Interval [CI] 0.022 to 0.815; P = 0.029). In cell culture, zinc supplementation upregulates expression of zinc transporters as well as GPR39 in human valve interstitial cells. Zinc prevents calcification through the activation of GPR39-mediated ERK1/2 signaling, and the inhibition of TNFα. GPR39 influences vascular endothelial cell dynamics in human coronary artery endothelial cells [35]. Extracellular zinc promotes the activation of cell survival and cell mobility, as well as the regulation of inflammatory mediators through the activation of GPR39-mediated signaling pathways, including AKT.

In human aortic endothelial cells, treatment with the GPR39 agonist, TC-G 1008, protects against oxidized LDL-mediated oxidative stress and inflammation, by inhibiting p38 and NF-kB-mediated signaling [36]. In the mouse heart, GPR39 expression was primarily localized to microvessels, and was found to regulate microvascular tone in response to eicosanoids, the microvascular vasoconstrictor, 15-HETE, and the microvascular vasodilator, 14,15-EET [37]. These studies suggest that GPR39 plays a role

in vascular homeostasis. Further studies are needed to determine whether GPR39 agonists have therapeutic value for vascular pathologies *in vivo*.

Osteoporosis: GPR39 STIMULATES BONE CELLS IN CELL CULTURE

In cell culture, GPR39 expression was shown to promote osteoblast differentiation [38]. Osteoblast differentiation media induces the expression of GPR39 in cultured MC3T3-E1 cells. Treatment with the GPR39 agonist, TC-G 1008, promotes the induction of osteoblast markers, including alkaline phosphatase, osteocalcin, and type I collagen expression, and calcium deposition. The effect is mediated by the activation of an AMPK-mediated signaling cascade by GPR39 which leads to the production of nitric oxide, and the induction of the Runx-2 transcriptional program for osteoblast differentiation. Due to the context-dependent nature of GPR39 signaling, it remains to be determined whether the activation of GPR39 can stimulate bone growth or protect against bone loss under different physiological conditions *in vivo*.

Arthritis: GPR39 SHOWS ANTI-INFLAMMATORY PROPERTIES IN CELL CULTURE

The expression of GPR39 was found to be decreased in synoviocytes from patients with rheumatoid arthritis, relative to controls [39]. In cultured human fibroblast-like synoviocytes, treatment with the GPR39 agonist, TC-G 1008, reduced markers of oxidative stress, mitochondrial dysfunction, pro-inflammatory cytokines (IL-16, IL-6, MCP-1), and metalloproteases (MMP-1, MMP-3, MMP-13). The inhibition of TNF-α proinflammatory signaling appears to involve the inhibition of NF-kB-mediated signaling. Similarly, in human SW1353 chondrocytes exposed to advanced glycation end products, an *in vitro* model of osteoarthritis, treatment with the GPR39 agonist, TC-G 1008, reduced expression of the collagen-degrading enzymes matrix metalloproteinases MMP-3 and MMP-13 [40]. These effects were mediated through the inhibition of p38 MAPK and NF-kB signaling. This suggests that some GPR39 agonists may have the potential to reduce inflammatory joint damage, though *in vivo* studies are needed.

Safety: Safety data are limited, and more *in vivo* studies are needed. Side effect profile will likely depend on the specificity for GPR39 and which downstream pathways are targeted by the biased agonist. Some agonists may potentiate cancer.

Types of evidence:

- 5 primary tumor tissue analysis studies showing a relationship between GRP39 and cancer
- Numerous laboratory studies

GPR39 agonists are in preclinical development. Most of the compounds with GPR39 agonist activity identified thus far have shown GPR39 promoting activity in the context of *in vitro* screens [1; 41]. The GPR39 agonist, TC-G-1008, has been the most widely tested in a variety of cell types *in vitro,* as well as in rodent studies *in vivo*, due to its oral bioavailability [32]. However, it has primarily been used in acute studies for efficacy, with limited data regarding safety. Many of the other GPR39 agonists identified from screens have additional activity at other targets, which may increase their side effect profile, and limit therapeutic utility. The screen-identified agonists, AZ7914, AZ4237, and AZ1395 showed hyperglycemic effects in rodents, and the severity of the hyperglycemia led to mortality in some animals at high doses (100 mg/kg) [33].

Due to the association between elevated GPR39 expression and tumor aggressiveness [2], GPR39 agonists are likely to aggravate some types of cancer, thus cancer screenings would likely be necessary before starting treatment with a GPR39 agonist.

More studies regarding the expression pattern and function of GPR39 in human tissues are needed. There are discrepancies in expression studies for GPR39 in brain tissue between rodents and humans, which calls into question the potential translatability of GPR39 agonists tested in rodent models [4].

GPR39 couples to several G proteins, and thus facilitates G-protein coupled signaling in a context-dependent manner. Most of the GPR39 agonists identified thus far are biased agonists which favor the activation of particular G-proteins and downstream targets [1]. The use of biased agonists is likely to mitigate the potential for on-target side effects, however, different conditions may preferentially benefit from the activation of different GPR39-mediated signaling cascades, thus the agonist will likely need to be tailored to the condition. Therefore, it will be necessary to understand which GPR39-related signaling pathways need to be augmented in a given condition in order to develop the appropriate therapeutic. The side effect profile of a given GPR39 agonist will also depend on which pathways it preferentially targets.

Since zinc acts as a positive allosteric modulator for the majority of GPR39 agonists identified to date, the potential therapeutic profile of GPR39 is likely to be influenced by the physiological status of zinc in a given individual [18]. A major outstanding question is whether the therapeutic benefit, activity, level, and/or G-protein/pathway signaling bias is impacted by zinc status, such that zinc deficient or zinc dyshomeostatic conditions may alter the clinical effects.

Sex effect: The majority of *in vivo* rodent studies conducted thus far assessing the physiological functions of GPR39 and the effects of GPR39 agonists have been done in male animals. The few studies conducted with female animals suggest that GPR39 may act similarly in males and females, but more studies need to be done including both males and females to confirm whether GRP39 would have similar utility in all sexes.

Drug interactions: The potential interactions are likely to vary with different GPR39 agonists, but they may interact with other drugs which regulate the PI3K/AKT, ERK1/2, or PLCβ - related signaling pathways [2]. Due to the role of zinc in the activation/augmentation of GPR39, metal chelating, or other zinc-modulating drugs are likely to interact with GPR39 agonists.

Sources and dosing:

GPR39 agonists are available for research use from commercial suppliers. TC-G-1008, also called GPR39-C3, is the most well-characterized, and is suitable for *in vivo* use in rodents due to its oral bioavailability.

Research underway:

GPR39 agonists are currently in the preclinical development phase.

Search terms:

Pubmed, Google: GPR39 agonists

- Alzheimer's disease, neurodegeneration, depression, cancer, cardiovascular, aging, metabolism

Websites visited for GPR39 Agonists:

- PubChem

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