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.

O-GlcNAcase Inhibitors

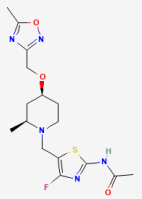
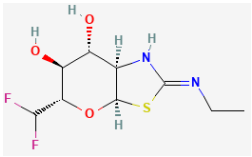
Evidence Summary

The loss of dynamic cycling of O-GlcNAcylation is a feature of several age-related diseases. The long-term benefits and risks of altering levels of O-GlcNAcylation without restoring cycling are unclear.

Neuroprotective Benefit: Reductions in O-GlcNAcylation may contribute to neurodegenerative proteinopathies, including tau, alpha-synuclein, and TDP-43, but the long-term impacts of targeting this pathway are unclear and may be context dependent.

Aging and related health concerns: Broad increases in O-GlcNAcylation are associated with insulin resistance with aging and may contribute to metabolic dysfunction. Hyper O-GlcNAcylation may also contribute to cancer progression.

Safety: OGA inhibitors show good safety in short-term Phase 1 trials, however, the safety of chronic dosing has not been established. High brain to plasma levels are likely needed to mitigate potential peripheral impacts to metabolism.

Availability: In clinical trials	**Dose**: No therapeutic doses have been established.	**LY3372689** **Chemical formula:** $C_{16}H_{22}FN_5O_3S$ **MW**: 383.4 g/mol  Source: PubChem **ML-8719** **Chemical formula:** $C_9H_{14}F_2N_2O_3S$ **MW**: 268.28 g/mol  Source: PubChem
Half-life: LY3372689: ~6 hours (in Ph1 study) ASN90: 2.8 hours (in rodents) ASN51: >40 hours (in Ph1 study)	**BBB**: Penetrant	
Clinical trials: LY3372689, ASN90, ASN51, MK-8719, and BIIB13 have been tested in small Phase 1 trials in healthy volunteers.	**Observational studies**: O-GlcNAcylation is generally increased in aging in conjunction with insulin resistance, though brain levels of O-GlcNAcylation may be decreased in neurodegenerative disease in conjunction with brain hypometabolism.	

What is it?

O-GlcNAcylation is a posttranslational protein modification in which the monosaccharide N-acetylglucosamine (GlcNAc) is attached to serine or threonine residues via an O-linked glycosidic bond [1]. In contrast to most types of glycosylation which attach sugar moieties extracellularly, O-GlcNAcylation is primarily an intracellular modification, and acts in a variety of subcellular compartments, including in mitochondria, where it impacts respiration and cellular metabolism [2]. O-GlcNAcylation is controlled by two enzymes, O-linked N-acetylglucosaminyltransferase (OGT), which adds GlcNAc, and O-GlcNAcase (OGA), which removes it. O-GlcNAc cycling is an important feature of this modification, as it allows dynamic responses to changing cellular conditions. It serves as a nutrient sensing mechanism to couple cellular responses with metabolic states [3]. GlcNAc is produced by the hexamine biosynthesis pathway (HBP). About 2-5% of cellular glucose is shuttled to the HBP based on estimates in adipocytes, leading to the production of alpha uridine diphosphate-N-acetylglucosamine

(UDP-GlcNAc) [4]. OGT activity is sensitive to levels of UDP-GlcNAc, such that conditions of high glucose tend to result in higher levels of O-GlcNAcylation, though there is variation across tissues and proteins. Levels of O-GlcNAcylation have been found to be altered in the context of neurodegenerative diseases, particularly in aggregation prone pathological proteins, such as tau [2]. This spurred the development of several OGA inhibitors, which are designed to increase levels of O-GlcNAcylation [2]. Various OGA inhibitors have been tested in preclinical models, with Thiamet-G as the most widely used, however, most of these inhibitors lack the properties required for clinical use. Several novel OGA inhibitors have been developed and tested in Phase 1 clinical trials in healthy volunteers with planned use in Alzheimer's disease and other tauopathies.

LY3372689 is an OGA inhibitor in clinical development by Eli Lilly, and has been tested in Phase 1 studies in healthy volunteers. It is in clinical development for Alzheimer's disease.

ASN90, also called ASN120290, is an OGA inhibitor developed by Asceneuron. It has been tested in Phase 1 trials in healthy volunteers. Target engagement measures in clinical trials from human peripheral blood mononuclear cells (PBMCs) indicated an EC_{50} of 209 nM, while rat studies indicated a brain-to-plasma ratio ~1 [5]. In 2023, it was licensed to Ferrer for clinical development in Progressive supranuclear palsy (PSP) (Press release).

ASN51 is a next-generation OGA inhibitor developed by Asceneuron that is designed to be longer lasting to allow for once daily dosing. It has been tested in Phase 1 studies in healthy volunteers and is in clinical development for Alzheimer's disease. Target engagement measures in clinical trials indicate an EC_{50} of 9.4 ng/mL [6].

BIIB113 is an OGA inhibitor in clinical development by Biogen. It was recently tested in a Phase 1 study in healthy volunteers (NCT05195008). The study was also designed to examine OGA occupancy with PET imaging as a measure of target engagement. No results have been posted to date.

MK- 8719 is an OGA inhibitor developed by Alectos and Merck. It was tested in a Phase 1 study in healthy volunteers. In preclinical studies it showed a Kd value of 3.1 nM towards human OGA, and a brain-to-plasma ratio ~1.84 in rats [7]. Clinical development of this compound appears to have been discontinued, and instead Alectos is working on developing novel OGA inhibitors.

Neuroprotective Benefit: Reductions in O-GlcNAcylation may contribute to neurodegenerative proteinopathies, including tau, alpha-synuclein, and TDP-43, but the long-term impacts of targeting this pathway are unclear and may be context dependent.

Types of evidence:

- 1 study relating O-GlcNAcylation and tau phosphorylation with cognition in MCI
- Several studies of O-GlcNAcylation proteome profiling in AD
- Numerous laboratory studies

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

O-GlcNAcylation is a dynamic modification related to the nutrient sensing pathway because the synthesis of GlcNAc utilizes intracellular nutrient-derived molecules [8]. Consequently, metabolic dysfunction, particularly glucose dyshomeostasis, can lead to aberrant O-GlcNAcylation, which, in turn, can further exacerbate metabolic dysfunction. As such, altered O-GlcNAcylation is a feature of several age-related diseases, including dementia. Chronic alterations to the O-GlcNAcylation profile stemming from a loss of dynamic cycling can result in a loss of proteostasis [2]. There is preclinical evidence to suggest that in the context of cerebral metabolic dysfunction, modulating O-GlcNAcylation levels may help slow the progression of pathologies related to cognitive decline. However, O-GlcNAc cycling is a mechanism allowing for the regulation of important cellular functions to various stimuli, and pharmacologically altering the balance of O-GlcNAcylation in an otherwise healthy individual could be detrimental, and thus may not be well-suited for prevention [9].

Human research to suggest benefits to patients with dementia:

Clinical trials testing different O-GlcNAcase inhibitors in patients with cognitive impairment are planned or ongoing, however, to date, there is no clinical evidence related to potential cognitive benefits in this population. LY3372689 is currently being tested in a Phase 2 trial in patients with early symptomatic Alzheimer's disease (NCT05063539). A Phase 1 trial testing ASN51 in patients with Alzheimer's disease was terminated due to Covid-related recruitment challenges (NCT04759365).

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

Alzheimer's disease: O-GLCNAC DYNAMICS ARE DISRUPTED IN THE AD BRAIN

O-GlcNAcylation levels are sensitive to tissue glucose levels, such that glucose hypometabolism may result in a reduction in O-GlcNAcylation. While there are discrepancies across studies regarding whether overall O-GlcNAcylation is increased or decreased in Alzheimer's disease (AD), depending on the brain fraction, brain region, and proteins examined, there is evidence to indicate that O-GlcNAcylation of pathological proteins is reduced in AD and other tauopathies, and the lack of this modification may contribute to disease progression [9]. O-GlcNAcylation exhibits crosstalk with other post-translational modifications, most notably phosphorylation [10]. In many cases, the modifications compete for the same sites. Several O-GlcNAcylation sites on tau have been identified *in vitro*, particularly S400 [11]. There is evidence to suggest that there may be an interaction between the O-GlcNAcylation and phosphorylation of tau [2], though the nature of this interaction has not been fully elucidated. Some studies suggest that higher O-GlcNAcylation results in lower levels of tau phosphorylation, however, others suggest that tau can be simultaneously O-GlcNAcylated and phosphorylated [12]. In patients with type 2 diabetes (n=48), having a higher ratio of global O-GlcNAcylation relative to ptau-212 in blood was associated with lower odds of mild cognitive impairment (MCI) (Odds Ratio [OR]: 0.028, 95% Confidence Interval [CI] 0.002 to 0.388) [13]. Relative to type 2 diabetics with normal cognition, those with MCI tended to have lower levels of O-GlcNAcylation in relation to tau phosphorylation at multiple residues including Ser396, Ser404, Thr212, and Thr231. However, in mice, higher levels of tau O-GlcNAcylation were associated with increased tau phosphorylation at specific residues [12]. This was seen in the context of SIRT1 deficiency, a deacetylase involved in glucose regulation that is decreased in the AD brain. SIRT1 negatively regulates O-GlcNAcylation via the induction of OGA, and yet low SIRT1 is associated with increased aggregation of phosphorylated tau. This suggests that the regulation of O-GlcNAcylation and interaction between the posttranslational modifications is complex and likely context dependent. In the rodent study, the loss of SIRT1 primarily affected the O-GlcNAcylation and phosphorylation of synaptic tau, suggesting that there may be differences to the regulation and impact of O-GlcNAcylation depending on the subcellular compartment.

Amyloid precursor protein (APP) also undergoes O-GlcNAcylation, and the presence of this modification is associated with non-amyloidogenic processing, such that the loss of O-GlcNAcylation promotes β-secretase activity and production of pathological Aβ [14].

The O-GlcNAcylation of TDP-43 was shown to suppress its toxic aggregation in cell culture by reducing its hyperphosphorylation and regulating its mRNA splicing [1]. Additionally, the O-GlcNAcylation of TDP-43 could mitigate deficits to motor function and lifespan in TDP-43 overexpressing flies.

While altered O-GlcNAcylation may be a byproduct of altered brain metabolism, the loss of O-GlcNAc cycling can further exacerbate metabolic dysfunction. O-GlcNAcylation of mitochondrial proteins plays an important role in the regulation of metabolism. Proteome profiling following treatment with O-GlcNAcylation modulators indicates that mitochondrial and other metabolic proteins are among the most highly impacted by differential O-GlcNAcylation [15; 16]. Alteration of OGT activity, and thus O-GlcNAcylation was found to impact metabolic processes in astrocytes, which may in turn affect neuronal-glial metabolic coupling [17].

O-GlcNAcylation is an important posttranslational modification of synaptic proteins, such that altering the dynamics of O-GlcNAcylation can disrupt the mechanisms of synaptic plasticity that underlie learning and memory. In transcriptional analysis following 10 weeks of treatment with the OGA inhibitor Thiamet G in male mice, the top upregulated genes were related to learning, cognition, and behavior, while the top downregulated genes were related to inflammation [18]. In Drosophila, OGA is involved in cognition and synaptic morphology, such that the loss of OGA activity affects the number of synaptic boutons at the neuromuscular junction and leads to deficits in habituation learning [19]. Elevated O-GlcNAcylation in the context of SIRT1 deficiency led to altered synaptic protein distribution and synaptic dysfunction [12]. In a mouse model of streptozotocin-induced cognitive impairment, treatment with the OGA inhibitor Thiamme2-G (2 mg/kg 3x/week, orally) influenced levels of some synaptic proteins, such as PSD95. Similarly, treatment with the OGA inhibitor Thiamet G increased levels of particular synaptic proteins, including PSD95 and syntaxin 1A in the Ts2Cje mouse model of Down Syndrome [20]. The balance of O-GlcNAcylation is involved in the regulation of ERK signaling, and chronic OGA inhibition was shown to potentiate ERK phosphorylation [21]. Notably, knockdown of either OGA or OGT could increase ERK phosphorylation, suggesting that the maintenance of the balance of O-GlcNAcylation is the critical aspect. In wildtype male mice, short-term treatment (one month) with the OGA inhibitor Thiamet-G did not have an effect, but longer-term inhibition of six months led to an increase in the amplitude of phosphorylated-ERK and APP levels in the brain. In the 5XFAD mouse model, one month of OGA inhibition led to the amplification of phosphorylated-ERK, suggesting that chronic OGA inhibition could potentially exacerbate the dysregulation of ERK signaling.

The effects of O-GlcNAcylation are highly context dependent, such that in many cases the effects of acute O-GlcNAcylation in a physiological setting are the opposite of chronic O-GlcNAcylation in a pathological setting. Sex differences in O-GlcNAcylation have also been observed in multiple species, which may be related to sex-dependent glycomes [15]. In mice, strong sex-dependent differences were observed on the interactions between O-GlcNAcylation, mitochondrial function, and mitochondrial

quality control. In wild type mice, there is a large degree of heterogeneity in baseline O-GlcNAcylation as well as in the response to OGA inhibitors. Within the same sex, up to twofold differences were observed in levels of OGA, citrate synthase, mitochondrial electron transport chain activities, autophagy, and neurological disease-related proteins [15]. Chronic treatment with the OGA inhibitor Thiamet-G for 10 weeks resulted in a high degree of heterogeneity in behavioral responses in male mice [18]. Chronic OGA inhibitor treatment led to adaptive remodeling of the O-GlcNAcylation pathway, including compensatory increases in the expression of OGA and decreases in OGT protein levels.

Overall, these studies suggest that a reduction in the O-GlcNAcylation of aggregation-prone neurodegenerative disease-related proteins in the brain may contribute to disease progression. These effects are part of a broader dysregulation of O-GlcNAcylation stemming from changes in glucose homeostasis and brain metabolism. Depending on the protein, brain region, and cellular compartment, O-GlcNAcylation could be aberrantly increased or decreased, such that promoting a global increase in O-GlcNAcylation through OGA inhibitors could mitigate some of these deficits, while exacerbating others. Influencing O-GlcNAcylation may help alleviate some of the neurological impairments stemming from metabolic dysfunction, however, due to the highly context-dependent nature of O-GlcNAcylation and propensity toward compensatory responses, the viability of this approach as a long-term therapeutic strategy is unclear.

Tauopathies: POTENTIAL BENEFIT (Preclinical)
OGA inhibitors have shown benefit in rodent tauopathy models. In the rTg4510 tauopathy model, in which mice overexpress human mutant tau P301L, MK-8719 administered at 100 mg/kg BID from 8 to 32 weeks of age reduced CSF levels of total tau, attenuated hyperactivity, and mitigated the decline in hippocampal volume [7]. Similarly, treatment with ASN90 (100 mg/kg) for six months starting at 3.5 months of age increased O-GlcNAcylated tau levels and improved survival time in this model [5]. In contrast, ASN90 was not effective in the faster progressing P301S model with treatment starting at two months of age because the pathology was already too advanced, suggesting that O-GlcNAcase inhibitors may have a defined therapeutic window confined to the earliest stages of disease.

Synucleinopathies: POTENTIAL BENEFIT (Preclinical)
In Line 61 human alpha-synuclein overexpressing mice, treatment with ASN90 (30 or 100 mg/kg) starting at four weeks of age led to a reduction in motor impairments, based on the beam walk test, and in astrogliosis, based on GFAP immunoreactivity at 12 and 24 weeks of age [5]. This was accompanied by an increase in levels of O-GlcNAcylated alpha synuclein.

Sleep deprivation: POTENTIAL BENEFIT TO ACUTE SLEEP DEPRIVATION-INDUCED COGNITIVE IMPAIRMENT (Preclinical)

Studies in preclinical models suggest that sleep deprivation can contribute to AD pathology and memory impairment by disrupting O-GlcNAcylation. This appears to be a byproduct of dysregulated glucose metabolism in response to sleep deprivation, as other contexts that depress brain glucose metabolism, such as hypoxia, lead to a similar disruption in O-GlcNAcylation [22]. In zebrafish, chronic sleep deprivation led to a reduction in O-GlcNAcylation in the hippocampus stemming from a decrease in levels of OGT and an increase in levels of OGA [23]. These changes were associated with memory impairment, neuroinflammation, and accumulation of Aβ in the zebrafish brain. Glucosamine-6P, an HBP metabolite, also generated via the modification of exogenous glucosamine, allows for increased HBP flux that can help restore the buffering capacity of the O-GlcNAcylation system. Treatment with glucosamine attenuated sleep deprivation-related neuroinflammation and Aβ production. Similar neuroprotective effects were observed in this model by overexpressing OGT to boost O-GlcNAcylation. In mice, REM sleep deprivation reduced HBP flux in the mouse brain, which was accompanied by a downregulation of OGT and upregulation of OGA, resulting in an overall reduction in O-GlcNAcylation [24]. These animals exhibited impaired contextual fear memory stemming from deficits in hippocampal synaptic plasticity, including reduced dendritic spine density, long-term potentiation, and CREB-mediated signaling. Treatment with glucosamine restored cognitive function to the level of non-sleep deprived mice, while treatment with the OGA inhibitor Thiamet G restored synaptic plasticity, suggesting that reduced O-GlcNAcylation plays a role in sleep deprivation-related cognitive impairment.

APOE4 interactions: The impact of the ApoE4 allele on OGA inhibitor efficacy has not been established, but theoretically, OGA inhibitors could offer preferential benefit, as cerebral hypometabolism tends to be more prominent in the context of ApoE4.

Aging and related health concerns: Broad increases in O-GlcNAcylation are associated with insulin resistance with aging and may contribute to metabolic dysfunction. Hyper O-GlcNAcylation may also contribute to cancer progression.

Types of evidence:

- 2 reviews of studies examining the role of O-GlcNAcylation in cardiovascular disease
- 1 review studies examining the role of O-GlcNAcylation as a prognostic marker in cancer
- 1 study assessing O-GlcNAcylation in liver steatosis
- Numerous laboratory studies

Lifespan: O-GLCNACYLATION MAY REGULATE NUTRIENT-RELATED LONGEVITY PATHWAYS
Altered O-GlcNAcylation is a hallmark of metabolic dysfunction, a common feature of age-related diseases. As a result, the maintenance of regulated O-GlcNAcylation cycling is essential for the prolongation of healthspan. O-GlcNAcylation is essential during development such that the loss of OGT, which adds O-GlcNAc, or its counterpart O-GlcNAcase, which removes it, is lethal in mice, and these enzymes have context-dependent effects in different tissues throughout the lifespan [25]. As a result, the loss of OGT or OGA, which lead to decreased or increased O-GlcNAcylation, respectively, can differentially affect lifespan depending on the species, tissues impacted, and environmental conditions. In *Drosophila* O-GlcNAcylation is involved in organismal growth such that the loss of OGA in adulthood results in flies that are larger and have shorter (~15%) lifespans [26]. In contrast, *C. elegans* lacking OGA have longer median lifespans (~33%), which is related to the regulation of insulin-mediated signaling. These worms exhibit an elevated tolerance to oxidative stress [27].
Metabolic profiling in mice suggests that mitochondrial O-GlcNAcylation may play a role in the nutritional regulation of longevity [16]. O-GlcNAcylation generally increases with aging, due to elevated blood glucose levels related to altered insulin dynamics and sensitivity [28]. In the mouse liver, carbamoyl phosphate synthetase 1 (CPS1) was found to be the most abundant O-GlcNAcylated protein upregulated with aging [16]. This protein accounts for about 20% of mitochondrial matrix mass and is involved in the regulation of the urea cycle and ammonia detoxification. The elevation of O-GlcNAcylated CPS1 may impair the ability of the body to keep fluctuations of blood ammonia within the physiological range. This elevation in O-GlcNAcylation is reversed in the context of calorie restriction-mediated life extension.

Liver steatosis: ELEVATED HEPATIC O-GLCYNACYLATION IS ASSOCIATED WITH LIVER STEATOSIS

Elevated hepatic O-GlcNAcylation has been observed in patients with non-alcoholic fatty liver disease (NAFLD) as well as in animal models of liver steatosis [29]. The increase in O-GlcNAcylation was observed primarily in hepatocytes and was found to be positively correlated with steatosis stage (r=0.67), fibrosis stage (r=0.77) and NAFLD activity score (r=0.65) in NAFLD patients [29]. Liver fibrosis is associated with the increased O-GlcNAcylation of mitochondrial proteins, which is associated with impairments in mitochondrial respiration. Preclinical models suggest that the increase in O-GlcNAcylation may be both a symptom and driver of hepatic metabolic dysfunction [29]. In rodent models, hepatic knockdown of OGT reduced the elevation in circulating liver transaminases such as alanine transaminase (ALT), as well as the expression of fibrotic markers such as collagen type 1, and smooth muscle actin. It also reduced hepatic lipid content, hepatic macrophage infiltration, and the expression of inflammation markers, including TNFα, TGFβ, and IL-6. Additionally, the knockdown of OGT also restored mitochondrial respiration, suggesting that the hepatic lipid accumulation may be a byproduct of altered mitochondrial function. These effects were independent of changes to ER stress, cell proliferation or cell death. The AIMP2–PARP1 axis may be a driver of O-GlcNAc-associated hepatic steatosis [30]. This pathway was found to be elevated in the livers of old mice (20 months) relative to young mice (4 months), and in response to hepatic OGA deficiency.

O-GlcNAcylation is disrupted in various tissues, in a tissue-specific manner, in the context of overnutrition-related metabolic disease. Hyperglycemia can lead to hyper O-GlcNAcylation of numerous proteins due to increased HBP flux. O-GlcNAcylation is an important regulator of insulin signaling [28]. The increase in O-GlcNAcylation in response to increased nutrient consumption is an important mechanism to stimulate insulin secretion and prevent hyperglycemia/diabetes, since acute OGT activity, and thus O-GlcNAcylation, promotes insulin secretion. However, the chronic elevation of O-GlcNAcylation stemming from chronic overnutrition can lead to pancreatic cell death, ultimately resulting in impaired insulin secretion and hyperglycemia [31]. This suggests that chronic OGA inhibition could potentially drive insulin resistance. O-GlcNAcylation is also an important regulator of gluconeogenesis in the liver such that OGT activity drives gluconeogenesis under fasting conditions [28]. This process includes the modification of proteins involved in mitochondrial biogenesis, including PGC1α. These pathways may provide the mechanisms by which chronic elevation of OGT activity and O-GlcNAcylation drive hepatic mitochondrial dysfunction and aberrant lipogenesis. Elevated O-GlcNAcylation has also been shown to reduce lipolysis in adipocytes, leading to insulin resistance and fat mass retention.
All together these studies suggest that the dynamic cycling of O-GlcNAc is an important regulator of metabolism, and that chronic increases in O-GlcNAcylation are a feature of overnutrition-related

metabolic disease. This suggests that in the context of individuals with some degree of insulin resistance, chronic treatment with peripheral acting OGA inhibitors could potentially exacerbate glucose dys-homeostasis and metabolic dysfunction.

Cardiovascular disease: CHRONIC ELEVATIONS IN O-GLCNACYLATION ASSOCIATED WITH CARDIOVASCULAR DYSFUNCTION

As part of a dynamic response to stress-inducing stimuli, O-GlcNAcylation can promote cell survival and thus shows an acute protective benefit, however, chronic upregulation of O-GlcNAcylation in the cardiovascular system becomes maladaptive [32]. The elevation in O-GlcNAcylation can be considered both a biomarker and a contributor to cardiovascular dysfunction. The increase in O-GlcNAcylation stems from metabolic abnormalities leading to increased HBP flux. The increase in O-GlcNAcylation may compete with other posttranslational modifications on cardiovascular-associated proteins, resulting in altered functionality [10]. O-GlcNAcylation has been associated with the impairment of endothelial nitric oxide synthase, as well as vascular smooth muscle cell hypercontractility, de-differentiation, calcification, and inflammation [10]. Altered O-GlcNAcylation may also disrupt mitochondrial function and impair cardiac energy production. The O-GlcNAcylation of cardiac proteins may also serve as biomarkers of cardiac disease [32]. For example, the O-GlcNAcylation of c-Myc, troponin I, and troponin T is associated with poor cardiac remodeling and cardiac hypertrophy. O-GlcNAcylation reduces the stability of proBNP, resulting in lower levels of NT-proBNP, a biomarker of heart failure [32]. In mice, cardiomyocyte hypertrophy and elevated O-GlcNAcylation could be reversed through the use of AMPK activators, such as metformin, and these cardioprotective effects were blocked by the use of OGA inhibitors [33].

Cancer: ELEVATED O-GLCNACYLATION IS ASSOCIATED WITH POOR PROGNOSIS

O-GlcNAcylation is observed in numerous cancers, and is associated with several hallmarks of cancer, including metabolic reprogramming, proliferation, invasion, metastasis, and angiogenesis [34]. The expression of OGT and OGA varies based on tumor type and stage. In a study analyzing 33 cancer types, OGT expression was found to be associated with higher grades of liver hepatocellular carcinoma, and with lower tumor stages of bladder urothelial carcinoma, testicular germ cell tumors, and lung adenocarcinoma [34]. The expression of OGA was associated with higher grades of kidney renal clear cell carcinoma, and brain lower grade glioma, and with lower grades of head and neck squamous cell carcinomas, and stomach adenocarcinoma. OGA expression was also associated with higher tumor stages in mesothelioma and tenosynovial giant cell tumor, as well as with lower stages in adenoid cystic

carcinoma, rectum adenocarcinoma, and stomach adenocarcinoma. O-GlcNAcylation sites have been identified in several cancer-associated genes, including TP53, TTN, MUC16 and SYNE1.
Hyper O-GlcNAcylation appears to be a byproduct of Warburg metabolism, which drives increased glucose and HBP flux. It frequently serves as an indicator of poor prognosis of patients, shorter disease-free survival, and tumor recurrence. The level of O-GlcNAcylation can serve as a biomarker of poor prognosis, and has been shown to act as a biomarker to predict poor prognosis in lung adenocarcinoma, laryngeal carcinoma, cholangiocarcinoma, squamous cell carcinoma, gastric cancer, colorectal cancer, renal cell carcinoma, acute myeloid leukemia, diffuse large B cell lymphoma, and testicular cancer [34]. Preclinical studies suggest that OGT inhibitors may be beneficial adjuncts for cancer therapy, however, specific OGT inhibitors with good drug-like properties are not yet available [34].

Safety: OGA inhibitors show good safety in short-term Phase 1 trials, however, the safety of chronic dosing has not been established. High brain to plasma levels are likely needed to mitigate potential peripheral impacts to metabolism.

Types of evidence:

- 6 Phase 1 clinical trials in healthy volunteers
- Numerous laboratory studies

Several OGA inhibitors have been tested in Phase 1 clinical trials in populations of healthy volunteers. Thus far, the inhibitors have generally been found to be safe and well-tolerated, at least with short-term use. The safety of long-term use has not been established.

LY3372689 was generally well tolerated in Phase 1 single ascending dose (SAD) (NCT03819270) and multiple ascending dose (MAD) (NCT04106206) studies in healthy volunteers [35]. Drug accumulation was not seen with multiple dosing, and food intake did not appreciably affect the pharmacokinetics. The pharmacokinetics and adverse event profile was similar between Japanese and non-Japanese participants. No serious adverse events were reported, and treatment-emergent adverse events (TEAE) were primarily of mild severity. There were no clinically significant changes in markers of inflammation, muscle injury, hormones, hepatoxicity, vital signs, neurological examinations, or ECGs, including QTc and PR prolongation. In the SAD study, the maximum tolerated dose is projected to exceed the highest tested oral dose of 16 mg. The most common TEAEs were headache, nausea, pain in extremity, pain of skin, vessel puncture site pain, and limb discomfort. In the MAD study with oral dosing up to 7 mg once

daily for 14 days, the most common TEAEs were headache, abdominal pain, diarrhea, back pain, nausea, constipation, dizziness, medical device site irritation, and feeling cold.

ASN90 was well-tolerated in a Phase 1 SAD study at doses up to 1000 mg in healthy young and elderly adults, and in a MAD study at doses up to 500 mg BID in healthy elderly volunteers (n=61) [36]. No serious adverse events or dose-limiting toxicities were reported. Most adverse events were of mild severity. Based on CSF sampling in a subset of elderly participants in the MAD study, ASN90 is BBB penetrant, with CSF drug concentrations similar to free concentrations in plasma. ASN90 has a short half-life of 2.8 hours in rodents, such that frequent dosing may be required to reach clinically therapeutic levels in humans [5].

ASN51 was well-tolerated in a Phase 1 SAD/MAD study in healthy volunteers (n=24) [6]. Single doses were tested in healthy young adults at 20 mg or 50 mg, while multiple doses of 20 mg were tested in healthy elderly adults. There were no serious adverse events, or clinically relevant changes on physical exams, vital signs, telemetry, ECGs, laboratory studies, or neurological exams. The most common adverse events were headache and musculoskeletal. Adverse events were primarily mild, with the exception of one moderate case of transient dizziness. ASN51 was tested in a Phase 1 MAD study designed to test safety and target engagement in healthy volunteers (n=12) [37]. Participants received oral doses of 10 mg/day or 20 mg/day of ASN51 for 14 days. There were no serious adverse events or withdrawals. There was one case of mild lethargy that was considered possibly drug related. There were no clinically relevant changes in vital signs, laboratory tests, physical exams, neurological exams, or ECGs.

MK-8719 was generally well tolerated in a Phase 1 study testing single oral doses up to 1200 mg. There were no reported laboratory, ECG, or vital sign adverse experiences in this study [38]. The drug also showed no evidence of toxicity in preclinical studies with no appreciable activity (<50% inhibition at 30 µM) against the cardiac and hemodynamic ion channel targets, no changes in hemodynamic parameters in rats at oral doses up to 380 mg/kg, and no changes in EKG parameters in dogs in oral doses up to 300 mg/kg [39]. In a seven-day toxicology study in rats, treatment up to 1000 mg/day resulted in no changes in physical signs, serum chemistry, or histomorphic findings in any tissue.

The OGA inhibitors currently in clinical development are designed to inhibit OGA within the brain for the treatment of neurodegenerative diseases. This necessitates high brain uptake to allow for sufficient target engagement within the CNS. According to preclinical studies, high OGA drug occupancy is

required to increase brain OGA levels in a potentially clinically meaningful manner. In rodents, 80% OGA enzyme occupancy resulted in an approximately1.5-fold elevation in brain O-GlcNAc, while near-maximal occupancy was required to achieve >3-fold increase in brain O-GlcNAc. OGA occupancy studies using PET ligands have been used as a measure of target engagement for the various OGA inhibitors in clinical development. Enzyme occupancy of LY3372689 in healthy volunteers (n=17) was found to be over 90% at 24 hours following the highest tested dose in a Phase 1 study using the OGA PET ligand ^{18}F-LY3316612 (NCT03944031) [40]. The enzyme occupancy of ASN51 was assessed using the ^{18}F-IMA601 PET tracer in healthy volunteers at a dose of 20 mg, and was also found to be over 90%[37]. Receptor occupancy was also assessed with BIIB113 using the tracer ^{11}C-BIO-1819578, though the results have not yet been disclosed (NCT05195008).

The long-term safety of OGA inhibitors has not been established. Although the short-term safety looks quite promising, evidence from preclinical studies suggests that chronic OGA inhibition could have adverse effects, especially in peripheral tissues. Since O-GlcNAcylation is a dynamic posttranslational modification, interventions which lead to chronic increases or decreases could negatively impact the regulation of various homeostatic processes. Most notably, are the impacts to metabolic health, as chronically elevated O-GlcNAcylation is associated with insulin resistance and metabolic dysfunction. Additionally, in the context of neurodegenerative diseases it is clear that there are changes in O-GlcNAcylation in both directions, in different subsets of proteins, thus correcting an imbalance in one direction could exacerbate it in the other direction. More studies are needed to determine the effects of chronic dosing with OGA inhibitors, or if intermittent dosing paradigms may be more effective in terms of restoring O-GlcNAc cycling without inducing compensatory changes or systemic side effects.

Drug interactions: Drug interactions have not been established, but due to the widespread effects of O-GlcNAcylation, interactions are likely.

Sources and dosing:
Oral OGA inhibitors are currently in clinical development from several companies, including LY3372689 from Eli Lilly, ASN90 from Ferrer, ASN51 from Asceneuron, and BIIB113 from Biogen.
While short term safety studies have been performed, no long-term studies in patient populations have been completed to date, so doses that are safe and effective have not yet been established for any indication. The safety of chronic dosing with OGA inhibitors has not been established, and it is unclear whether cycling, intermittent, or some other type of alternative dosing schedule will be required.

Research underway:

There are a variety of novel OGA inhibitors in preclinical development. LY3372689 is currently being tested in a Phase 2 trial in patients with early symptomatic Alzheimer's disease (NCT05063539), which is expected to be completed in 2024.

Search terms:

Pubmed, Google: O-GlcNAcase Inhibitor

- Alzheimer's disease, neurodegeneration, cognition, aging, lifespan, cardiovascular, diabetes, cancer, clinical trial, safety

Websites visited for O-GlcNAcase Inhibitors:

- Clinicaltrials.gov (LY3372689, ASN51, BIIB113)
- PubChem (LY3372689, MK-8719)

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