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RESEARCH ARTICLE

Discovery and clinical translation of ceperognastat, an O-GlcNAcase (OGA) inhibitor, for the treatment of Alzheimer's disease

William Kielbasa[1] | Paul Goldsmith[1] | Kevin B. Donnelly[1] | Hugh N. Nuthall[1] | Sergey Shcherbinin[1] | Adam S. Fleisher[1] | Jörg Hendle[1] | Susan L. DuBois[1] | Stephen L. Lowe[1] | Feiyu Fred Zhang[1] | Eric M. Woerly[1] | Nicolas J.-F. Dreyfus[1] | David Evans[1] | Jeremy Gilmore[1] | Michele Mancini[1] | Cristian C. Constantinescu[2] | Roger N. Gunn[3] | David S. Russell[2] | Emily C. Collins[1] | Miroslaw Brys[1] | Michael L. Hutton[1] | Dustin J. Mergott[1]

[1]Eli Lilly and Company, Indianapolis, Indiana, USA

[2]Invicro LLC, Boston, Massachusetts, USA

[3]Invicro, London, UK

Correspondence
Dustin J. Mergott, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, USA.
Email: mergottdu@lilly.com

Funding information
Eli Lilly and Company

Abstract

INTRODUCTION: The aggregation and spread of hyperphosphorylated, pathological tau in the human brain is hypothesized to play a key role in Alzheimer's disease (AD) as well as other neurogenerative tauopathies. O-GlcNAcylation, an important post-translational modification of tau and many other proteins, is significantly decreased in brain tissue of AD patients relative to healthy controls. Increased tau O-GlcNAcylation has been shown to reduce tau pathology in mouse in vivo tauopathy models. O-GlcNAcase (OGA) catalyzes the removal of O-GlcNAc from tau thereby driving interest in OGA inhibition as a potential therapeutic approach to reduce tau pathology and slow the progression of AD.

METHODS: A multidisciplinary approach was used to identify ceperognastat (LY3372689) as a potent OGA inhibitor, including an extensive discovery effort with synthetic chemistry, structure-based drug design, and in vivo OGA enzyme occupancy studies. Preclinical studies assessed the target engagement, inhibition of OGA enzyme activity, OGA enzyme occupancy, and changes in tau O-GlcNAc. Four clinical Phase 1 studies of ceperognastat in healthy participants were performed to assess clinical safety and tolerability, pharmacokinetics (PK), and enzyme occupancy.

RESULTS: Ceperognastat is a potent, central nervous system (CNS)-penetrant, low-dose inhibitor of OGA, which can achieve > 95% OGA enzyme occupancy in animal and human brain. Overall, ceperognastat had an acceptable safety profile in Phase 1 clinical studies with no serious adverse events reported following single and multiple

dosing. The PK, enzyme occupancy, and safety profile supported Phase 2 development of ceperognastat.

DISCUSSION: Ceperognastat is an orally available, highly potent, CNS-penetrant OGA inhibitor that achieved high (> 80%) OGA enzyme occupancy and increased brain O-GlcNAc-tau preclinically. Ceperognastat demonstrated > 95% OGA enzyme occupancy in Phase 1 trials. These occupancy data informed the dose selection for the Phase 2 clinical program.

KEYWORDS
Alzheimer's disease, enzyme occupancy, O-GlcNAcase inhibition, O-GlcNAcylation, pharmacokinetics, tau

Highlights

- Ceperognastat is a highly potent, CNS-penetrant OGA inhibitor.
- Ceperognastat is both orally available and CNS-penetrant even when given at low doses.
- Ceperognastat can achieve > 95% OGA enzyme occupancy in the animal and human brain.
- Ceperognastat had an acceptable safety profile in Phase 1 clinical studies.

1 | INTRODUCTION

Alzheimer's disease (AD) is an age-related, neurodegenerative disorder characterized by progressive decline in cognitive function and ability to perform activities of daily living. Pathologic hallmarks of AD identified at autopsy include the presence of neuritic amyloid-β plaques, neurofibrillary tangles,[1] and neuronal loss in brain regions important for cognition, such as the hippocampus, parietal lobe, and temporal cortex.[2,3]

Tau is predominantly an axonal microtubule-binding protein that promotes microtubule assembly and stability. Spread of tau pathology is highly correlated with neuronal loss and clinical progression in AD, and in other tauopathies such as progressive supranuclear palsy.[4] This correlation has driven strong interest in developing tau-targeting agents as potential disease-modifying treatments for these neurodegenerative diseases.

Tau is regulated by post-translational modifications, including O-GlcNAcylation (OGN) which involves the attachment of an N-acetylglucosamine (GlcNAc) moiety onto the hydroxyl group of serine and threonine residues. This attachment is catalyzed by O-GlcNAc transferase and, conversely, O-GlcNAcase (OGA) catalyzes the removal of O-GlcNAc. The exact function of tau OGN is uncertain; however, studies with human brain tissue from AD patients have shown marked reductions in tau OGN levels in these tissues compared to healthy control brain tissue, and those reductions correlate with increased levels of hyperphosphorylated tau in the brains of AD patients.[5] Additional studies have also demonstrated that overall O-GlcNAc levels are globally reduced in Alzheimer's disease brains.[6]

Increasing OGN in vitro using recombinant tau promotes a more soluble conformation and reduces aggregation.[7] Inhibition of OGA in animal tauopathy models increases tau OGN, slows accumulation of aggregated, hyperphosphorylated tau,[7–9] and reduces pathological changes associated with tau aggregation, such as brain atrophy and tau accumulation in cerebrospinal fluid.[10] Importantly, analysis of brains from the transgenic tau mouse, rTg4510, indicated that OGN is not detected in aggregated tau protein but is found in normal, soluble tau.[9] Accordingly, OGA inhibition represents a potential therapeutic approach to reduce the spread of tau pathology and thus slow the clinical progression of AD and other tauopathies.[11]

Herein, we report the discovery and clinical translation of ceperognastat (LY3372689), a low-dose, central nervous system (CNS)-penetrant OGA inhibitor that achieves > 95% enzyme occupancy in animal and human brain. Ceperognastat was the first OGA inhibitor tested in AD patients in a Phase 2 study (NCT05063539) and was one of the few small-molecule, oral, anti-tau therapeutics in clinical development.

2 | MATERIALS AND METHODS

2.1 | Study design

The experiments and studies herein were performed to demonstrate and characterize the safety, tolerability, pharmacokinetic (PK)/pharmacodynamic effects of ceperognastat in animals and healthy human subjects. All animal studies were conducted by LabCorp, Inc., an American Association for Accreditation of Laboratory

Animal Care—accredited facility and were approved by their Institutional Animal Care and Use Committee. All studies were conducted in accordance with the National Institutes of Health Guide to the Care and Use of Laboratory Animals and the US Animal Welfare Act. A detailed description of the discovery and preclinical methods are in the Supplemental Methods section. All clinical studies were conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonization Good Clinical Practice guideline, and local regulatory requirements. Study protocols were approved by an independent ethics committee or institutional review board at each study site. All participants provided written consent before the start of the study.

2.2 | Design—clinical trials

The data contained herein were obtained from a single ascending dose (SAD) study (NCT03819270), a multiple ascending dose (MAD) study (NCT04106206), a single-dose positron emission tomography (SD-PET) study (NCT03944031) and a multiple-dose PET (MD-PET) study (NCT04392271).

Both the SAD and MAD studies were single-center, double-blind, placebo-controlled, randomized clinical trials. In the SAD study healthy male volunteers and women with no child-bearing potential were enrolled. In the MAD study, males who underwent vasectomy and women with no child-bearing potential were enrolled. The SAD study was conducted in two alternating cohorts (Cohort 1, $N = 11$; Cohort 2, $N = 12$) of participants who participated in up to three study periods. Participants were randomized to one of three treatment sequences in each cohort, with each sequence including two doses of ceperognastat and one of placebo over the three study periods in a complete crossover manner. Placebo and doses of 0.15, 0.6, 2, 5, 10, and 16 mg ceperognastat were administered orally. For the MAD study, participants were randomized to one of three cohorts that participated in a single period. Participants were randomly assigned and received placebo ($N = 10$) or 1 mg ($N = 9$), 3 mg ($N = 12$) or 7 mg ($N = 9$) ceperognastat, orally once daily (QD) for 14 consecutive days.

The SD-PET and MD-PET studies were single-center, open-label and non-randomized that used a PET imaging ligand, LY3316612, to assess enzyme occupancy in the brain after ceperognastat administration. The SD-PET study included doses of 0.25, 1, and 5 mg across four cohorts ($N = 4$ subjects per cohort; 1 mg ceperognastat was administered in two cohorts). Each participant underwent a baseline PET scan and two post-dose PET scans; scans were conducted at approximately the following times post-dose; 2 and 24 h at 0.25 and 5 mg; and 2 and 24 h or 30 and 54 h at 1 mg. The MD-PET study included a dose of 1 mg QD ceperognastat given to four participants for 14 days. Each participant had a baseline PET scan and two post-dose PET scans; scans were conducted at approximately 24 h after the 1st and 14th administration of ceperognastat. Serial dynamic PET with arterial sampling was performed over a total duration of 120 min. The primary imaging outcome was the total distribution volume V_T determined using a two-tissue compartment model in multiple brain regions.

RESEARCH IN CONTEXT

1. **Systematic review**: Despite significant discovery and development investment, therapeutic efforts to slow the aggregation and spread of tau have been unsuccessful to date. Inhibition of O-GlcNAcase (OGA) represented a potential approach that could reduce development of tau pathology and thus slow progression of Alzheimer's disease (AD) and other tauopathies.
2. **Interpretation**: We identified ceperognastat (LY3372689) as a highly potent, CNS-penetrant, orally available OGA inhibitor which can achieve > 95% OGA enzyme occupancy in the animal and human brain. ceperognastat had an acceptable safety profile in Phase 1 clinical studies following single and multiple dosing.
3. **Future directions**: To our knowledge, ceperognastat was one of the few small molecules in development intended to slow progression of AD pathology and, as of writing, was the only OGA inhibitor to advance to Phase 2 testing in patients with AD (NCT05063539).

2.3 | Statistical analysis

In vivo enzyme occupancy in rats and mice were reported as mean and standard error.

Treatment-emergent adverse events (TEAEs) were summarized by treatment, severity, and relationship to the study drug. The frequency (the number of adverse events [AEs], the number of subjects with an AE, and the percentage of subjects with an AE) of TEAEs was summarized by treatment, Medical Dictionary for Regulatory Activities (MedDRA) version 21.1 System Organ Class and Preferred Term.

For PET studies, enzyme occupancy data were summarized by treatment and dose time point. Enzyme occupancy was obtained using graphical analysis of regional V_T values according to the occupancy plot.[12] A mixed-effect repeated measures model was used when the study included multiple cohorts, whereas a paired *t*-test was conducted when the study included only one cohort.

The relationship of ceperognastat plasma concentrations and enzyme occupancy was characterized with nonlinear regression analysis using a Hill equation to estimate maximum enzyme occupancy (E_{MAX}), the plasma concentration eliciting 50% and 80% maximum enzyme occupancy (EC_{50} and EC_{80}), and the Hill slope.

3 | RESULTS

3.1 | Discovery of ceperognastat

A combination of high throughput, fragment, and virtual screening efforts were used to identify molecules with OGA inhibitory activity,

which were then triaged using mechanism-of-action assays to identify competitive, active-site inhibitors. This screening campaign identified aminothiazole 1 as an OGA inhibitor that demonstrated moderate potency (OGA IC_{50} = 124 nM) (structure shown in Figure S1A). A multidisciplinary discovery approach, including synthetic chemistry, structure-based drug design, in vitro pharmacology, and in vivo OGA enzyme occupancy studies, was used to identify ceperognastat as a potent OGA inhibitor. An x-ray structure of ceperognastat in complex with human OGA displays how this molecule binds in the enzyme active site (Figure S1B generated using parameters outlined in Table S1). Ceperognastat exhibits properties consistent with an oral CNS drug, including high solubility (1.36 mg/mL in pH 7.5 phosphate buffer), low P-glycoprotein efflux (Madin-Darby canine kidney [MDCK] cell line mechanistic net efflux ratio of 2.2), and high permeability (MDCK passive permeability of 50.1×10^{-6} cm/sec).

3.2 | In vitro activity of ceperognastat

Competition radioligand-binding assays using the tritiated analog 2, a non-fluorine analog of the OGA PET imaging ligand [^{18}F]LSN3316612,[13] showed that ceperognastat binds OGA from different species with high affinity (1.8–2.4 nM) and displayed no difference in binding to brain homogenates from either AD or age-matched control brains (Table 1). More detailed binding characteristics of ceperognastat were assessed by a surface plasmon resonance (SPR) assay. Ceperognastat demonstrated binding to human OGA at an affinity (K_D) of 133 pM with a fast on-rate (k_{on}) of 1.968e+5 $M^{-1}s^{-1}$ and slow dissociation kinetics with an off-rate (k_{off}) of 2.622e-5 s^{-1}. In vitro OGA residence $t_{1/2}$ was 7.3 h. Ceperognastat demonstrated potent inhibition of purified human and mouse OGA and was highly selective against the functionally related lysosomal hexosaminidase enzymes HexA and HexB (Table 1 and Figure S2). Ceperognastat also demonstrated high potency in a cellular assay in which treatment with ceperognastat resulted in a concentration-dependent increase in O-GlcNAc modified proteins (Table 1). Ceperognastat showed no important in vitro binding activity across an industry standard selectivity panel of receptors, ion channels, transporters, and enzymes, and exhibited weak inhibition of the human ether-à-go-go (hERG) channel (IC_{50} = 81 μM).

3.3 | In vivo brain enzyme occupancy of ceperognastat

Frontal cortex enzyme occupancy of ceperognastat was determined in Sprague Dawley (SD) rats after single oral doses ranging from 0.001 to 3 mg/kg. Table S2, Part A shows the dose-dependent effect of ceperognastat on enzyme occupancy and Figure 1A shows the relationship between ceperognastat plasma concentration and enzyme occupancy (E_{max} = 98%, EC_{50} = 3.8 nM, EC_{80} = 5.9 nM and Hill coefficient = 3.1). The ceperognastat exposures resulting from the low doses of 0.001, 0.003, and 0.01 mg/kg were below the quantifiable limit of the liquid chromatography (LC)-tandem mass spectrometry (MS) (LC-MS/MS) bioanalytical assay. Therefore, exposures at these doses were estimated based on extrapolation of exposure at higher doses.

Frontal cortex enzyme occupancy of ceperognastat was determined in a P301S mouse model with ongoing tau pathogenesis[14] across a dose range of 0.05 to 10 mg/kg using continuous subcutaneous administration for 10 days to enable steady state plasma concentrations. Table S2, Part B shows the dose-dependent effect of ceperognastat on enzyme occupancy, and Figure 1B shows the relationship between ceperognastat plasma concentration and enzyme occupancy (E_{max} = 98%, EC_{50} = 2.2nM, EC_{80} = 5.2 nM, Hill coefficient = 1.6).

TABLE 1 Summary of Ki values for displacement of analog 2 by ceperognastat and relative IC50 values in human embryonic kidney cells.

Displacement of analog 2 by ceperognastat	Value
Source of OGA	**Ki (nM)**
Human recombinant. Enzyme	2.4 ± 0.1
Mouse cortical homogenate	2.2 ± 0.2
Rat cortical homogenate	1.8 ± 0.2
Monkey cortical homogenate	2.2 ± 0.4
Human control cortical homogenate	1.8 ± 0.4
Human AD cortical homogenate	2.0 ± 0.4
OGA inhibition and protein O-GlcNAc increase in cells by ceperognastat	
Assay type	**Relative IC_{50} or EC_{50} (nM)[a]**
hOGA	2.4 ± 0.8
mOGA	1.8
Hex	> 30,000
Protein O-GlcNAc (cellular)	21.9 ± 7.3

Note: Data shown as geometric mean ± standard error of the mean. Ki values represent the geometric mean ± SEM from Ki values calculated in each individual experiment. Analog 2 is a tritiated non-fluorine analog of the OGA PET ligand LY3316612.

Abbreviations: AD, Alzheimer's disease; EC_{50}, half-maximal effective concentration; Hex, human lysosomal β-hexosaminidase A/B; hOGA, human O-linked N-acetyl glucosaminidase; IC_{50}, half-maximal inhibitory concentration; Ki, equilibrium dissociation constant; mOGA, mouse O-linked N-acetyl glucosaminidase; OGA, O-linked N-acetyl glucosaminidase; PET, positron emission tomography; protein O-GlcNAc, protein O-GlcNAc modification.

[a]The concentration corresponding to a response midway between the estimates of the lower and upper plateaus.

3.4 | Effect of ceperognastat on brain protein O-GlcNAc and tau O-GlcNAc

The effect of ceperognastat on brain protein O-GlcNAc, a pharmacodynamic measure of OGA inhibition, was also explored in P301S mice[14] using continuous subcutaneous administration for 10 days. Table S3, Part A shows the dose-dependent effect of ceperognastat on increases in protein O-GlcNAc. To assess the impact of OGA inhibition by ceperognastat on tau, which is central to the therapeutic hypothesis, we also

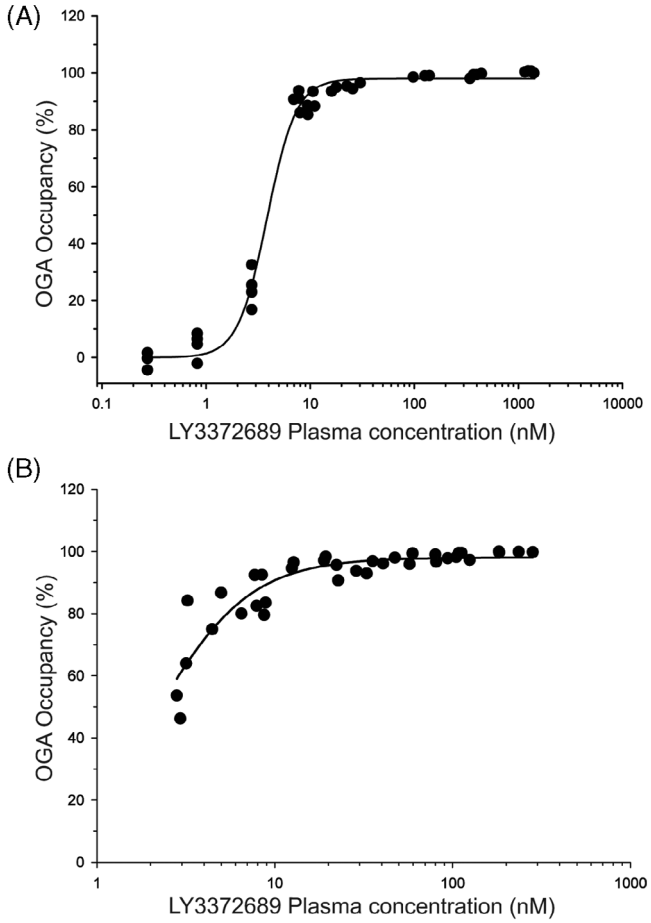

FIGURE 1 Relationship between ceperognastat plasma concentration and enzyme occupancy in Sprague-Dawley rats following a single oral dose of ceperognastat (A) and in P301S mice after 10-day administration of ceperognastat (B). OGA, O-GlcNAcase.

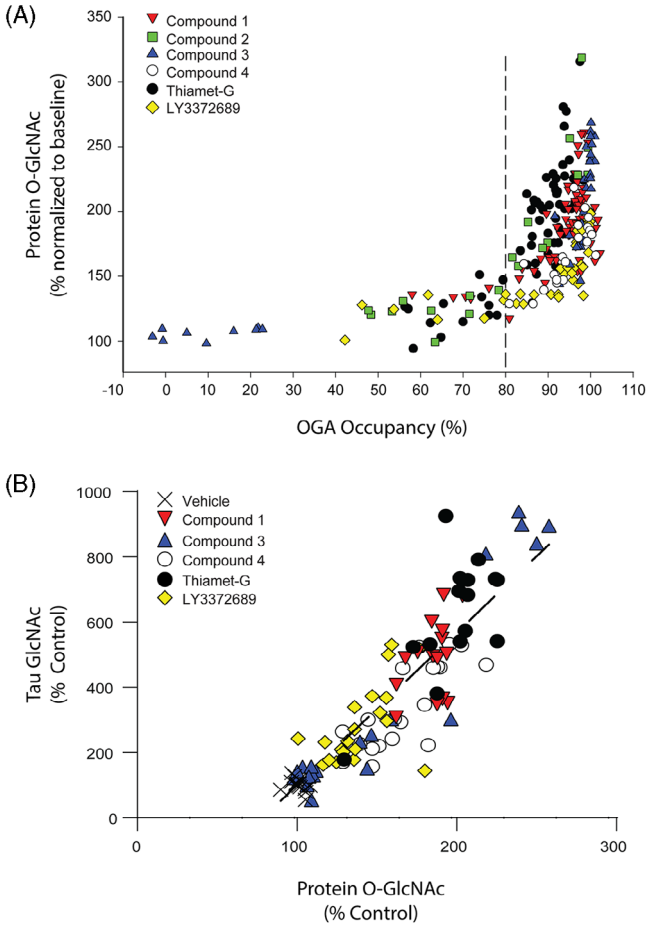

FIGURE 2 Relationship of frontal cortex O-GlcNAc with enzyme occupancy (A) and with tau O-GlcNAc (B) and for OGA inhibitors. Dashed line in (A) denotes 80% enzyme occupancy and in (B) denotes a linear regression fit. OGA,O-GlcNAcase; O-GlcNAc, O-GlcNAc modification.

measured changes in tau O-GlcNAc at serine S400. Table S3, Part B shows the dose-dependent effect of ceperognastat on increases in tau O-GlcNAc.

3.5 | Relationship between enzyme occupancy, protein O-GlcNAc, and tau O-GlcNAc for ceperognastat and additional OGA inhibitors

Figure 2A shows the relationship between enzyme occupancy and protein O-GlcNAc in the P301S mouse model after 10 days administration of Thiamet-G,[15] ceperognastat, and four Lilly proprietary OGA inhibitors. These composite data demonstrate that > 80% enzyme occupancy results in increased protein O-GlcNAc. Furthermore, literature reports of OGA inhibitors have indicated that a minimum of 80% enzyme occupancy is needed for protein O-GlcNAc increases and a corresponding reduction in tau pathology.[7,9,16] Figure 2B shows the relationship between brain protein O-GlcNAc and brain tau O-GlcNAc in the P301S mouse model after 10 days administration of Thiamet-G,[15] ceperognastat, and three Lilly proprietary OGA inhibitors. The increase in brain protein O-GlcNAc following OGA inhibition correlated with the increase in tau O-GlcNAc, which suggested that the former can be used as a surrogate marker for tau O-GlcNAcylation. These data collectively supported the use of enzyme occupancy as a translational biomarker with a minimum trough target occupancy of 80%.

3.6 | Predicting the ceperognastat human dose—enzyme occupancy relationship

The aforementioned preclinical and literature data informed the clinical OGA trough occupancy target of > 80%. Based on a total EC_{80} of 5.2 nM and unbound fraction for plasma protein binding in mice ($f_{u,\,mice}$ = 0.684), the unbound EC_{80} in mice was 3.6 nM. Assuming that mice and humans have the same unbound EC_{80} and adjusting for the unbound fraction for plasma protein binding in human ($f_{u,\,human}$ = 0.45), the total human EC_{80} was estimated to be 7.9 nM. As such, a plasma concentration of 7.9 nM is predicted to achieve 80% enzyme occupancy in the human brain.

TABLE 2 Predicted human OGA enzyme occupancy following a single dose of ceperognastat.

	Time post dose		
Dose (mg)	2 h	12 h	24 h
0.15	64% (49%–76%)	< 5%	< 5%
0.75	95% (92%–96%)	41% (1%–79%)	< 5%
3	> 95%	85% (13%–96%)	< 5%
10	> 95%	> 95%	28% (0.003%–95%)
20	> 95%	> 95%	60% (0.008%–97%)

Note: Data shown as median (90% prediction interval).
Abbreviation: OGA, O-GlcNAcase.

Human apparent clearance (CL/F = 12 L/h) and apparent volume of distribution (V/F = 48 L) of ceperognastat were predicted by allometry using PK data from rat, dog, and monkey. A 1-compartment linear PK model with first-order oral absorption (absorption rate = 1.1 h^{-1}) was used to predict the human PK profile of ceperognastat following single oral doses of 0.15, 0.75, 3, 10 and 20 mg. The time to maximum plasma concentration (t_{max}) and the elimination half-life ($t_{1/2}$) of ceperognastat were predicted to be approximately 2 h and 2–3 h, respectively. Based on the predicted relationship of ceperognastat plasma concentrations and enzyme occupancy in humans, the PK profile of ceperognastat was used to predict the time—course of enzyme occupancy in humans (Table 2). These analyses demonstrated the potential for ceperognastat to achieve 80% enzyme occupancy in human and supported the evaluation of safety, PK, and enzyme occupancy in clinical studies.

3.7 | Clinical safety and tolerability of ceperognastat

There were no serious adverse events (SAEs) reported following dosing of ceperognastat in Phase 1 clinical studies. In the SAD study, 23 participants received at least one dose of ceperognastat (15 males, 8 females, aged 22–63 years), of whom 18 completed the study. Forty TEAEs were reported by 13 ceperognastat-treated participants (56.5%), mostly mild in severity. The most common TEAEs were headache, nausea, pain in extremity, pain of skin, vessel puncture site pain, and limb discomfort. In the MAD study, there were 40 participants (5 males, 35 females, aged 29–65 years), of whom 39 participants completed the study. Forty-two TEAEs were reported, all mild in severity. The most common TEAEs were headache, abdominal pain, diarrhea, back pain, nausea, constipation, dizziness, medical device site irritation, and feeling cold.

In the SD-PET study, there were 18 participants (18 males, aged 30–58 years), of whom 16 participants completed the study. Sixteen participants received a single oral dose of ceperognastat, and 8 participants (50%) reported at least one TEAE irrespective of causality. All TEAEs were of mild or moderate severity, and the most common TEAEs were ecchymosis, headache and nausea.

In the MD-PET study, there were four participants (four females, aged 43–52 years), and all participants completed the study. Four out of four participants enrolled in this study reported at least one TEAE. All TEAEs were of mild or moderate severity, and the most common TEAEs were ecchymosis and vessel puncture site hemorrhage (each reported by two participants). A summary of the safety results following single and multiple dosing clinical studies can be found in the supplement (Tables S4 and S5). Across all clinical studies, no significant findings with other safety measures such as vital signs, electrocardiograms, laboratory tests (including fasting glucose), or neurological exams were identified.

TABLE 3 OGA enzyme occupancy after administration of ceperognastat.

Dose regimen	OGA occupancy (%)			
Single dose	**2 h**	**24 h**	**30 h**	**54 h**
0.25 mg	25.6 (13.4)	46.0 (16.2)	-	-
1 mg	97.3 (2.6)	80.6 (5.6)	68 (11.5)	30.3 (19.9)
5 mg	98.2 (2.0)	92.5 (3.3)	-	-
Multiple dose	**1st dose, 24 h**		**14th dose, 24 h**	
1 mg QD	84.1 (1.2)		83.7 (5.2)	

Note: Data are shown as mean (standard deviation). $N = 4$ per dose regimen.
Abbreviations: OGA, O-GlcNAcase; QD, once daily.

3.8 | Clinical PK of ceperognastat

Figure 3A shows the mean ceperognastat PK profiles following administration of single doses ranging from 0.15 to 16 mg in the SAD study. Overall, the ceperognastat median t_{max} was 1 h, the geometric mean $t_{1/2}$ was 6 h, CL/F was 12 L/h and V/F was 112 L. The MAD study evaluated doses of 1, 3, and 7 mg given QD for 14 days. The ceperognastat PK profiles were similar on days 1 and 14 of dosing, which indicated minimal accumulation with QD dosing.

3.9 | Clinical evaluation of enzyme occupancy

Figure 3 shows the PET-based voxel-wise map of the distribution volume (V_T) for the SD-PET study (Panel A) and MD-PET study (Panel B). Table 3 shows the frontal cortex enzyme occupancy values across dose

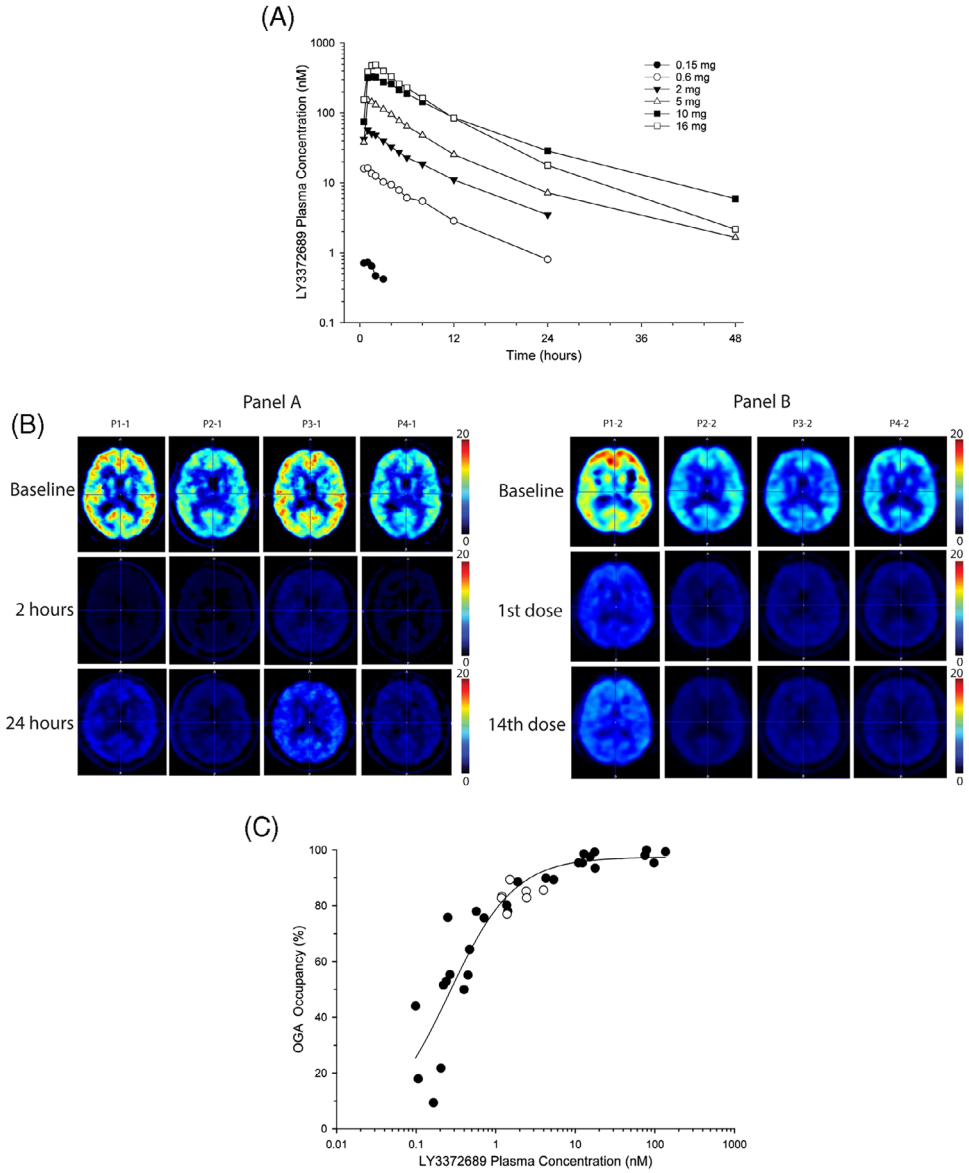

FIGURE 3 (A) Arithmetic mean plasma concentration—time profiles following a single dose of ceperognastat from the SAD study ($N = 5$-8 per dose group). (B) PET-based voxel-wise maps of the distribution volume (VT); Panel A (SD-PET study): single dose of 1 mg with PET images at baseline, 2-h, and 24-h post-dose. Panel B (MD-PET study): PET images at baseline and 24 h after the 1st and 14th administration of 1 mg ceperognastat. $N = 4$ per dose regimen. (C) Relationship between enzyme occupancy and ceperognastat plasma concentration from SD-PET and MD-PET studies. Solid line is the median model response. Circles correspond to individual participant values from the SD-PET (black) and MD-PET study (white). MD, multiple dose; OGA, O-linked N-acetyl glucosaminidase; P, participant; PET, positron emission tomography; SAD, single ascending dose; SD, Sprague Dawley.

and time from the SD-PET and MD-PET studies. In the SD-PET study there was generally a dose- and time-dependent change in enzyme occupancy. To note for 1 mg, the enzyme occupancy declined from 97% at 2 h to 30% at 54 h. Following 1 mg QD in the MD-PET study, the enzyme occupancy at 24 h post-dose following the 1st and 14th administration was 84%, which indicated no apparent loss of target engagement with this dosing regimen.

Figure 3C illustrates the relationship between ceperognastat plasma concentrations and enzyme occupancy across the SD-PET (black circles) and MD-PET (white circles) studies, with an $E_{max} = 97\%$, $EC_{50} = 0.26$ nM, $EC_{80} = 0.91$ nM and Hill slope = 1.1. The data collected 2 h post-dose at 0.25 mg were excluded from the pharmacodynamic parameter estimations because the enzyme occupancy at 2 h was less than 24 h post-dose despite a higher plasma concentration at 2 h (0.97 nM) compared to 24 h (0.29 nM). This observation could not be explained by operational or bioanalytical factors.

4 | DISCUSSION

Despite significant discovery and development investment, therapeutic efforts to slow the aggregation and spread of tau have been

unsuccessful to date. Several monoclonal antibodies and small molecule tau aggregation inhibitors have failed to demonstrate a reduction of tau pathology and show efficacy in clinical trials, even when administered at high doses.[17,18] An alternative approach has therefore been to target enzymes, including OGA, that post-translationally modify tau to slow the accumulation of pathological tau.[19] Previous studies in non-clinical transgenic models have consistently shown that increasing tau O-GlcNAc levels by OGA inhibition reduces the relative level of tau pathology compared with control animals.[9] While OGA inhibitors have been identified,[20] they have proven challenging to develop. OGA is known to be involved in post-translational modification of many proteins, which has raised potential safety concerns. Additional drug discovery hurdles have included low permeability[21] and poor PK properties, which have led to high human doses.[22,23] As of this writing, Asceneuron has indicated their intent to bring an OGA inhibitor to Phase 2 trial for AD, while Biogen has reportedly advanced an OGA inhibitor to Phase 1 clinical trials for AD. Ferrer has advanced to Phase 2 trials with their molecule for progressive supranuclear palsy.[24–26]

Ceperognastat was identified as an orally available, highly potent, CNS-penetrant OGA inhibitor that both achieved high (> 80%) brain enzyme occupancy, and increased brain O-GlcNAc-tau and O-GlcNAc-total-protein in preclinical studies. These data informed the selection of dose regimens in the clinical program that could maintain 80% enzyme occupancy at trough as a minimum level of target engagement.

Ceperognastat demonstrated an acceptable safety profile with no SAEs or dose limiting TEAEs up to 16 mg in the SAD and 7 mg QD for 2 weeks in the MAD. The plasma concentrations of ceperognastat exceeded the predicted human EC_{80} for brain enzyme occupancy (7.9 nM) and informed the dose range for the SD-PET and MD-PET clinical studies. Results from the SD-PET and MD-PET identified an EC_{80} for brain enzyme occupany of 0.91 nM indicating a more potent effect in humans compared to preclinical data. In addition, prediction of human PK prior to Phase 1 clinical studies indicated the potential for a $t_{1/2}$ of 2–3 h, but the observed $t_{1/2}$ in humans was determined to be about 6 h. These findings allowed for > 80% enzyme occupancy at trough with much lower QD doses than that predicted preclinically.

OGA inhibition has been reported to cause OGA protein upregulation within 24 h,[14] raising the risk of enzyme occupancy loss upon sustained OGA inhibition. Our preclinical data show that the EC_{80} for enzyme occupancy is similar when ceperognastat is administered acutely in rats (5.9 nM) and over 10 days in mice (5.2 nM), which suggests that enzyme occupancy was not affected under these experimental conditions. In the human MD-PET study, a dose of 1 mg QD for 14 days allowed maintenance of enzyme occupancy at trough of 84%, thus alleviating concerns about the risk for loss of enzyme occupancy due to protein upregulation with repeat administration of ceperognastat in humans.

Based on the safety, PK, and enzyme occupancy obtained from Phase 1 studies, the program was advanced into a Phase 2 study (PROSPECT-ALZ) to evaluate the safety and efficacy of ceperognastat in patients with early symptomatic AD (NCT05063539). The top dose in the Phase 2 study was selected to achieve enzyme occupancy > 95% at trough, above the targeted threshold (> 80%) identified preclinically for enzyme occupancy. The low dose was selected based on a minimum criterion of 80% enzyme occupancy at trough. This study was intended to test the hypothesis that OGA inhibition would slow cognitive decline in early symptomatic AD patients.

The Prospect-ALZ study results ultimately demonstrated that ceperognastat did not show evidence of clinical benefit in early AD despite the high levels of predicted enzyme occupancy at the doses selected. To our knowledge, ceperognastat is the only OGA inhibitor that has been tested in patients with AD, and this study represents an important test of the OGA hypothesis. A report describing the clinical and biomarker outcomes of the Prospect-ALZ study will be published separately.

AUTHOR CONTRIBUTIONS

Conception: William Kielbasa; Paul Goldsmith; Hugh N. Nuthall; Stephen L. Lowe; Nicolas J.-F. Dreyfus; David Evans; Michele Mancini; Emily C. Collins; Michael L. Hutton; Dustin J. Mergott. ***Design***: William Kielbasa; Paul Goldsmith; Kevin B. Donnelly; Hugh N. Nuthall; Susan L. DuBois; Stephen L. Lowe; Feiyu Fred Zhang; Nicolas J.-F. Dreyfus; David Evans; Jeremy Gilmore; Roger N. Gunn; David S. Russell; Emily C. Collins; Michael L. Hutton; Dustin J. Mergott. ***Acquisition of data***: Paul Goldsmith; Hugh N. Nuthall; Jörg Hendle; Susan L. DuBois; Feiyu Fred Zhang; Nicolas J.-F. Dreyfus; Jeremy Gilmore; Michele Mancini; Roger N. Gunn; David S. Russell; Emily C. Collins. ***Analysis of data***: William Kielbasa; Paul Goldsmith; Kevin B. Donnelly; Hugh N. Nuthall; Jörg Hendle; Susan L. DuBois; Feiyu Fred Zhang; Eric M. Woerly; Nicolas J.-F. Dreyfus; David Evans; Jeremy Gilmore; Cristian C. Constantinescu; Roger N. Gunn; Emily C. Collins; Dustin J. Mergott. ***Interpretation of data***: William Kielbasa; Paul Goldsmith; Kevin B. Donnelly; Hugh N. Nuthall; Sergey Shcherbinin; Adam S. Fleisher; Jörg Hendle; Stephen L. Lowe; David Evans; Jeremy Gilmore; Michele Mancini; Roger N. Gunn; David S. Russell; Emily C. Collins; Miroslaw Brys; Michael L. Hutton; Dustin J. Mergott. ***Drafting of the manuscript***: William Kielbasa; Paul Goldsmith; Kevin B. Donnelly; Hugh N. Nuthall; Susan L. DuBois; Nicolas J.-F. Dreyfus; David Evans; Michele Mancini; Emily C. Collins; Michael L. Hutton; Dustin J. Mergott. ***Critical revision of the manuscript***: William Kielbasa; Paul Goldsmith; Hugh N. Nuthall; Sergey Shcherbinin; Adam S. Fleisher; Jörg Hendle; Stephen L. Lowe; Feiyu Fred Zhang; Eric M. Woerly; Nicolas J.-F. Dreyfus; David Evans; Jeremy Gilmore; Michele Mancini; Cristian C. Constantinescu; Roger N. Gunn; David S. Russell; Emily C. Collins; Miroslaw Brys; Michael L. Hutton; Dustin J. Mergott. All authors provided input and gave final approval for the work to be published.

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CONFLICT OF INTEREST STATEMENT

William Kielbasa, Paul Goldsmith, Kevin B. Donnelly, Hugh N. Nuthall, Sergey Shcherbinin, Adam S. Fleisher, Jörg Hendle, Susan L. DuBois, Stephen L. Lowe, Feiyu Fred Zhang, Eric M. Woerly, Michele Mancini, Emily C. Collins, Miroslaw Brys, Michael L. Hutton and Dustin J. Mergott are employees and minor shareholders at Eli Lilly and Company. Nicolas J.-F. Dreyfus, David Evans and Jeremy Gilmore were employed by Eli Lilly and Company during this work. Eli Lilly and Company are developing patents relevant to this research. David Evans is currently employed by Google DeepMind, has received Alphabet stock as part of this employment, and is an author on a published patent for Exscientia WO2022106857A1. Cristian C. Constantinescu and David S. Russell are current employees at Invicro, whereas Roger N. Gunn was an employee at Invicro during this work. Author disclosures are available in the Supporting Information.

DATA AVAILABILITY STATEMENT

Lilly provides access to all individual participant data collected during the trial, after anonymization, with the exception of PK or genetic data. Data are available to request 6 months after primary publication acceptance. No expiration date of data requests is currently set once data are made available. Access is provided after a proposal has been approved by an independent review committee identified for this purpose and after receipt of the signed data. sharing agreement. Data and documents, including the study protocol, statistical analysis plan, clinical study report, and blank or annotated case report forms, will be provided in a secure data sharing environment. For details on submitting a request, see the instructions provided at www.vivli.org.

CONSENT STATEMENT

All participants in the clinical trials reported herein provided informed written consent for study participation.

ORCID

Dustin J. Mergott https://orcid.org/0000-0003-1802-0758

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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