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.

Vafidemstat (ORY-2001)

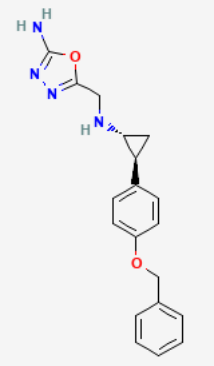
Evidence Summary

Vafidemstat has shown improvements in aggression and agitation in Alzheimer's patients, but not cognition or biomarkers of Alzheimer's pathology. Rates of adverse events are similar to placebo.

Neuroprotective Benefit: In a phase 2 trial in Alzheimer's patients, vafidemstat decreased CSF YKL40, but did not alter CSF Aβ, phospho-tau, S100A9, or others. No cognitive effects were seen, but aggression was ameliorated in moderate/severe patients.

Aging and related health concerns: No studies have examined vafidemstat for age-related diseases.

Safety: Based on a phase I study, adverse events with vafidemstat were not significantly different from placebo and included headache, dry mouth, dizziness, and somnolence. Longer-term phase 2 study results have not been published in peer-reviewed articles.

Availability: in clinical trials	**Dose**: In a phase 2 clinical trial in mild to moderate Alzheimer's disease patients, vafidemstat doses of 0.6 mg and 1.2 mg, orally, were tested (NCT03867253).	**Chemical formula:** $C_{19}H_{20}N_4O_2$ **MW**: 336.4  Source: PubChem
Half life: ~20-30 hours	**BBB**: penetrant	
Clinical trials: The phase I trial enrolled 110 healthy young and older adults. The phase 2a trial in Alzheimer's patients enrolled 150 subjects.	**Observational studies**: none available	

What is it? Vafidemstat (also known as ORY-2001) is a covalent inhibitor of the epigenetic enzyme lysine specific demethylase 1 (LSD1, also known as KDM1A). It is a novel orally bioavailable brain-penetrant LSD1 inhibitor under development by Oryzon Genomix, S.A., a public clinical stage biopharmaceutical company in Spain.

LSD1 is a component of chromatin complexes that localizes to the promoter or enhancer regions of active genes, where it specifically demethylates lysine 4 and 9 of histone 3 (H3K4 and H3K9, respectively), modulating chromatin structure and regulating transcription (Maes et al., 2015). (Demethylation of H3K4me2/1 by LSD1 is associated with repression of expression, while demethylation of H3K9me2/1 by LSD1 is associated with activation of expression). LSD1 has been most studied in the context of cancer, as it is overexpressed in certain cancer types and LSD1 inhibitors are under clinical development for the treatment of certain acute myeloid leukemia subtypes or solid tumors, such as small cell lung cancer (Fang et al., 2019; Maes et al., 2015). In the brain, LSD1 has been implicated in the control of immediate early gene transcription, which is important for neuronal plasticity, memory, and behavior (Zhang et al., 2021). LSD1 is also an important regulator of neural stem cell proliferation. LSD1 is required for the maintenance and proliferation of neural stems through the repression of genes that control cell proliferation. Neurogenesis is achieved by the dimming of the repressive activity of LSD1. In addition, LSD1 plays an important role in response to major insults in the brain, such as in cerebral ischemia (Zhang et al., 2010). It is also worth noting that while LSD1 is expressed ubiquitously

throughout the body, neurons express developmentally regulated LSD1 splice forms that incorporate a small exon (E8a, or DTVK)(Maes et al., 2020). Although the exact mechanisms are not known, the neuron-specific splice form is thought to counteract the ubiquitous splice form and is required for neuronal differentiation and memory.

In vitro studies have shown that vafidemstat also inhibits the monoamine oxidase B (MAOB; enzyme involved in the metabolism of dopamine) (Maes et al., 2020). However, MAOB inhibition has not been detected in clinical trials at the therapeutic doses tested (Antonijoan et al., 2021).

Vafidemstat has been studied in patients with Alzheimer's disease, multiple sclerosis, autism spectrum disorder, borderline personality disorder, and adult attention deficit hyperactivity disorder (Oryzon.com).

Neuroprotective Benefit: In a phase 2 trial in Alzheimer's patients, vafidemstat decreased CSF YKL40, but did not alter CSF Aβ, phospho-tau, S100A9, or others. No cognitive effects were seen, but aggression was ameliorated in moderate/severe patients.

Types of evidence:

- 1 phase 2a double-blind randomized controlled trial in Alzheimer's patients
- 1 phase I double-blind randomized controlled trial of healthy young and older adults
- 1 open-label study in moderate to severe Alzheimer's patients
- Several laboratory studies

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

Human research to suggest benefits to patients with dementia:
Although not published yet in a peer-reviewed journal, during the 2020 AD/PD conference, some of the results were presented from the phase 2 double-blind randomized controlled trial of 140 mild to moderate Alzheimer's patients (ETHERAL study; NCT03867253)(2020 AD/PD slide deck). Vafidemstat treatment (0.6 mg or 1.2 mg/day, orally) for 6 months did not result in any differences across treatment arms for cerebral spinal fluid (CSF) biomarkers of Aβ ratio, total tau, phospho-tau, and S100A9 (inflammation marker). However, CSF levels of YKL40, a secreted inflammatory chitinase, was

significantly lower in the 0.6 mg vafidemstat group compared to the placebo group ($p<0.01$). The 1.2 mg group also had a numerically lower level of CSF YKL40, but this was not statistically significant ($p=0.06$). This effect on YKL-40 appears to be driven by moderate Alzheimer's patients. Vafidemstat treatment did not affect levels of CSF neurogranin, a synaptic marker. For CSF neurofilament light (NFL) levels, the 1.2 mg vafidemstat group had significantly lower levels compared to the 0.6 mg vafidemstat group, but no differences with treatment were seen compared to placebo (CSF NFL levels were similar between placebo and 1.2 mg group and elevated in the 0.6 mg group). No statistically significant differences were expected or observed at 6 months in cognition (measured by the ADAS-Cog14) between treatment arms. Oddly, the placebo group fared better than vafidemstat 0.6 and 1.2 mg groups. In a follow-up, results from the 6-month open-label extension of the study above (ETHERAL study) were presented at the 2021 AD/PD conference (2021 AD/PD poster). The patients receiving vafidemstat (0.6 mg or 1.2 mg/day) continued with their treatment while the placebo arm switched over to vafidemstat (0.6 mg or 1.2 mg/day), for 6 months. The reduction in CSF YKL40 seen at the 6-month point was maintained after 12 months of treatment.

Complete analysis of the data, as well as other endpoints including the CogState Brief Battery and volumetric MRI are still ongoing.

A single-center, open-label, single-arm, 24-week study aimed to evaluate the effects of vafidemstat on agitation and aggression in 12 patients with moderate to severe Alzheimer's disease (REIMAGINE-AD study)(2020 AD/PD slide deck). In moderate to severe Alzheimer's patients, vafidemstat treatment (1.2 mg/day, orally) for 24 weeks significantly improved agitation and aggression scales, including the Clinical Global Impressions Improvement (CGI-I), Cohen-Mansfield Agitation Inventory (CMAI), and Neuropsychiatric Inventory Agitation/Aggression (NPI-A/A). Total NPI as well as the NPI emotional distress scores were also improved after 6 months of treatment. An improvement in cognitive functions (measured by the MMSE) was detected by the second month of treatment, but this improvement did not persist in the following months. Because this clinical study was an open-label, uncontrolled study, placebo effects cannot be excluded.

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

Human data: In a phase I double-blind randomized controlled trial of 110 healthy young and older adults, vafidemstat (0.2, 0.6, 1.0, 1.5, 2.5, and 4.0 mg, orally) was administered in a single- and multiple (5-day) ascending dose paradigm (Antonijoan et al., 2021). In the CSF substudy, single doses of 2 and 4 mg were tested and showed that vafidemstat penetrated the central nervous system, based on the CSF-

to-plasma unbound ratio (mean of 0.81). The plasma pharmacokinetic profiles of vafidemstat after single and multiple dosing showed rapid oral absorption with a Tmax of 0.5-2 hours, a relatively long half-life of ~20-30 hours, and approximately dose-proportional exposures. Although vafidemstat was initially characterized as a dual inhibitor of LSD1 and monoamine oxidase B (MAOB; involved in dopamine metabolism), MAOB inhibition was not detected in the dose ranges tested (based on lack of MAOB inhibition in platelets). LSD1 inhibition was confirmed in a pharmacodynamics study using a chemoprobe-based immunoassay in peripheral blood mononuclear cells. For example, with a single 4 mg dose, a maximum LSD1 target engagement was around 80% at 12 hours and substantial binding remained 72 hours after dosing. In the multiple ascending dose study, maximum target engagement values of up to 90% were reached at 108 hours post-dose. LSD1 target engagement was 85.6% in the older adult population, comparable with that observed in young adult subjects. Because LSD1 PET tracer is unavailable, LSD1 target engagement in the brain could not be confirmed. No significant treatment or dose effects of vafidemstat were observed for neuropsychological assessments, including sleeping or awakening patterns, somnolence, Wisconsin Card Sorting Test, or the Sternberg test of working memory. This phase I study was not powered to study cognitive benefits of vafidemstat.

Rodent data: In a mouse model of accelerated aging and Alzheimer's disease (SAMP8 mice), vafidemstat treatment (doses ranging from 0.11 to 3.2 mg/kg/day, in drinking water) for 2 to 4 months dose-dependently restored novel object recognition memory, with the lowest dose of 0.11 mg/kg/day providing ~50% rescue (Maes et al., 2020). A 5-day treatment of vafidemstat at a dose of 0.96 mg/kg/day was equally effective in rescuing novel object recognition memory as a one-month treatment. Also, in a two-period crossover study, SAMP8 mice that received vafidemstat treatment for 1 month and then received vehicle the following month still remained significantly improved relative to the mice treated with vehicle for 2 months. Brain compound and target engagement levels return to baseline in 1 and 3 days, respectively, suggesting that the therapeutic effect of vafidemstat outlasts the target engagement. The cognitive benefits of ORY-2001 was attributed to inhibition of LSD1, based on a comparison with a selective LSD-1 inhibitor (ORY-LSD1) and a selective MAOB inhibitor (rasagiline). In aged SAMP8 mice (8-month-old), vafidemstat treatment for 4 months also fully rescued novel object recognition memory.

SAMP8 mice exhibit aggressive behavior, but treatment with vafidemstat (0.32 or 0.96 mg/kg/day) for 5 weeks significantly reduced aggressiveness, measured using the resident-intruder test, without any sign of sedation (Maes et al., 2020). Vafidemstat treatment (0.96 mg/kg/day, orally) for 4 months also improved social interactions in 12-month-old female aged mice in the Three-Chamber Test. Vafidemstat

treatment (0.32 or 0.96 mg/kg/day, orally) for 2 months in SAMP8 mice did not have anxiolytic or sedative activity, measured by the open field and elevated plus maze tests.

A microarray-based analysis of hippocampal tissue from vafidemstat-treated SAMP8 mice revealed that some of the genes expressed at higher levels in SAMP8 mice (compared to SAMR1 controls) were downregulated with vafidemstat treatment (Maes et al., 2020). These genes included: Mela, Iap, Agxt2l1 and genes involved in neuroinflammation including S100a9, T-cell receptor beta genes, Twist, Cd3d, and Ly6c. S100A9, an amplifier of inflammation, is upregulated in the hippocampus of Alzheimer's patients and is expressed in microglia next to Aβ plaques (Wang et al., 2014; Horvath et al., 2016). Genes that were upregulated by vafidemstat treatment in the SAMP8 hippocampus included Baiap3, Prph, Fabp7 and Doc2a (genes required for cognitive function and memory), and others. Some immediate-early genes including Egr1/2, cFos, Npas4, Dusp1 and Arc were upregulated mildly by vafidemstat treatment.

A microarray analysis of prefrontal cortex samples from SAMP8 mice showed that vafidemstat treatment downregulated genes overexpressed in SAMP8 mice compared to the control SAMR1 mice, suggesting that vafidemstat treatment partially rebalanced gene expression profiles (Maes et al., 2020). For example, Pcdh21, Doc2g, and Pbx3 were downregulated with treatment in the prefrontal cortex, along with immediate-early genes Fos, Npas4, Tac1, and Egr1/2; GABAergic genes relevant to synaptic plasticity such as Calb2 and Gad1; and genes involved in signal transduction such as Gng4 and Doc2g. Some of these genes dysregulated in SAMP8 mice, such as the immediate-early genes, GABAergic genes, and synaptic plasticity genes were also dysregulated in postmortem prefrontal cortex samples from people who had late onset Alzheimer's disease (LOAD), when compared with normal samples. In LOAD samples, immediate early genes including ERG1, ERG2, NPAS4, CALB2, GAD1, GNG4, and DOC2A were decreased while FOS was increased compared to control samples.

APOE4 interactions: None available.

Aging and related health concerns: No studies have examined vafidemstat for age-related diseases.

Types of evidence:

- 0 clinical trials
- 0 laboratory studies

Vafidemstat has not been tested in age-related diseases, as it is optimized for central nervous system indications. Other LSD1 inhibitors including ORY-1001 (iadademstat), bomedemstat (IMG-7289), CC-90011, and INCB059872 are undergoing clinical testing in oncology and hematology, as LSD1 inhibitors can inhibit proliferation, invasion, and migration of cancer cells (Oryzon.com; Dai et al., 2020; Fang et al., 2019). Many oncogenes and tumor suppressor genes are regulated by multiple factors. Recently, LSD1 demethylase-independent mechanisms have also been implicated in the development of cancer (Dai et al., 2020). Thus, a combination of LSD1 inhibitors with other drugs that target these other mechanisms may be most promising for cancer therapy.

Safety: Based on a phase I study, adverse events with vafidemstat were not significantly different from placebo and included headache, dry mouth, dizziness, and somnolence. Longer-term phase 2 study results have not been published in peer-reviewed articles.

Types of evidence:

- 1 phase I study
- Several laboratory studies

Several phase 2a clinical trials have tested vafidemstat in patients with relapsing-remitting multiple sclerosis, secondary-progressive multiple sclerosis, mild to moderate Alzheimer's disease (NCT03867253), and in several psychiatric diseases, with vafidemstat treatment lasting up to 18 months. Detailed data and results from these studies have not been published in peer-reviewed articles, as of March 2022.

During the 2020 AD/PD conference, some of the safety results were presented from the phase 2 double-blind randomized controlled trial of 140 mild to moderate Alzheimer's patients (ETHERAL study)(2020 AD/PD slide deck). Vafidemstat treatment (0.6 mg or 1.2 mg/day, orally) for 6 months resulted in no significant differences across treatment arms with regards to adverse events (100% in placebo, 97.4% in 0.6 mg, 96.9% in 1.2 mg). Drug-related adverse events were also similar across arms: 62.5% in placebo,

63.2% in 0.6 mg vafidemstat group, and 65.6% in 1.2 mg vafidemstat group. No differences were observed between study arms in any of the vital signs, electrocardiogram, or laboratory safety evaluations. No clinically relevant hematological impact was observed after 6 months of treatment.

In a follow-up, results from the 6-month open-label extension of the study above (ETHERAL study) were presented at the 2021 AD/PD (2021 AD/PD poster). The patients receiving vafidemstat (0.6 mg or 1.2 mg/day) continued with their treatment while the placebo arm switched over to vafidemstat (0.6 mg or 1.2 mg/day), for 6 months. There were only 2 drug-related severe adverse events reported in the placebo and 2 in the vafidemstat arms (details of the nature of the severe adverse events are not noted). No clinically relevant differences were observed on the number of drop-outs, adverse events or severe adverse events between study arms.

In a phase I double-blind randomized controlled trial of 110 healthy young and older adults, vafidemstat doses (0.2, 0.6, 1.0, 1.5, 2.5, and 4.0 mg, orally) administered in a single- and multiple ascending dose resulted in adverse events that were not significantly different in proportion between vafidemstat and placebo (Antonijoan et al., 2021). No severe adverse events or adverse events leading to treatment discontinuation were reported during the study. A total of 120 adverse events were reported during the single ascending dose stage, multiple ascending dose stage, and the study with older adults: 90 adverse events were experienced by subjects receiving vafidemstat and 30 adverse events by subjects receiving placebo, which was the same proportion as the vafidemstat treatment to placebo randomization (3:1). Of these, 108 adverse events were classified as treatment-emergent adverse events (TEAEs), possibly or probably related to the study drug: 79 TEAEs were reported in subjects given vafidemstat and 29 in subjects treated with placebo.

During the single ascending dose study in healthy young male volunteers (N=40), 15 adverse events were reported, of which 13 were considered to be treatment-related (12 in subjects receiving vafidemstat and 1 in the placebo arm)(Antonijoan et al., 2021). In people receiving vafidemstat, the most common adverse events were headache, followed by euphoric mood. However, none of the adverse events occurred in a dose-dependent manner.

During the multiple ascending dose study in healthy young male/female volunteers (N=44), a total of 69 adverse events were reported (51 adverse events with vafidemstat and 18 with placebo)(Antonijoan et al., 2021). The most common adverse events were headache, followed by dry mouth, asthenia, postural dizziness, and somnolence. All headaches experienced with vafidemstat, which were considered to be

moderate adverse events, were reported in dose levels: 0.2 mg/day, 1.0 mg/day, and 1.5 mg/day, but not in the highest dose levels (2.5 and 4 mg/day). The rest of the adverse events were considered mild.

In the multiple ascending dose study in older adult volunteers, vafidemstat dose of 4.0 mg/day was tested (N=4; 3 active, 1 placebo). A total of 23 adverse events were reported: 12 with vafidemstat and 11 with placebo (Antonijoan et al., 2021). The most common adverse events were postural dizziness (in a subject receiving placebo) followed by headache, blunted affect, and vertigo. Most reported adverse events were mild. A total of 21 treatment-related TEAEs were reported in this group (10 with vafidemstat and 11 with placebo).

The plasma pharmacokinetic profiles of vafidemstat after single and multiple dosing showed rapid oral absorption with a Tmax of 0.5-2 hours, a relatively long half-life of ~20-30 hours, and approximately dose-proportional exposures (Antonijoan et al., 2021). A tendency for more than proportional increase in plasma vafidemstat was found at and above the 2.5 mg/day dose in the multiple ascending dose cohort, which could be from drug absorption saturation and/or elimination at higher dose levels. A moderate systemic accumulation was observed after 5 days of vafidemstat treatment but reached steady state by day 5. Pharmacokinetic profiles at 2.5 mg/day did not differ significantly between the young and older adult cohorts.

No significant changes were observed over time in hematological, biochemical, or urinalysis parameters or in vital signs or electrocardiogram values in the single- and multiple-ascending dose studies (including both young and older adults)(Antonijoan et al., 2021). Platelet levels remained stable at all of the single-ascending doses and multiple-ascending doses up to the 1.0 mg/day. At the multiple ascending dose of 1.5 and 2.5 mg/day, platelet counts were within the normal range, but a tendency for lower counts was emerging when compared with the placebo arm. At 1.5 and 2.5 mg/day, a slight reduction (−11 and −21%) of the mean was observed by 192 hours after the first administration and a small rebound (+15 and +18%) was observed in the follow-up visit. These changes likely reflect the first indication of hematopoietic engagement. A protocol amendment incorporated an additional multiple ascending dose level (4 mg/day) to investigate the hematopoietic impact. At the 4 mg/day dose level, a transient reduction of platelet levels was observed by 192 hours, and platelets dropped below 50% of baseline levels in two of three vafidemstat-treated subjects by 216 hours after the first treatment administration and in all subjects at 240 hours and 264 hours. These levels recovered during the follow-up visits. Based on these data, the minimum intolerable dose was established at 4 mg/day, and the maximum tolerated dose was established at 2.5 mg/day. For phase II studies, doses were established at 0.6 and 1.2 mg/day,

which were estimated to be sufficient to reach ~60-80% LSD1 target engagement while avoiding the effects on platelets.

The no observed adverse effect level (NOAEL) in nonclinical good laboratory practices toxicology studies in rats and dogs were 0.2 and 0.054 mg/kg, respectively (Antonijoan et al., 2021). In rodents, vafidemstat treatment provided a 30-fold window between the therapeutic effect and the hematopoietic effect.

Drug interactions: Drug interactions for vafidemstat have not been well-studied. Based on the phase I trial showing a slight reduction in platelet counts with higher doses of vafidemstat (1.5 and 2.5 mg/day) (Antonijoan et al., 2021), vafidemstat may not be compatible with other drugs that decrease platelet counts.

Sources and dosing: Vafidemstat is under clinical development by Oryzon Genomix, S.A., a public clinical stage biopharmaceutical company in Spain. In a phase 2 clinical trial in mild to moderate Alzheimer's disease patients, vafidemstat doses of 0.6 mg and 1.2 mg, orally, were tested (NCT03867253).

Research underway: Based on ClinicalTrials.gov, there is a phase 2 clinical trial testing the safety and efficacy of vafidemstat in adults with borderline personality disorder (NCT04932291). In this double-blind, randomized, placebo-controlled 14-week trial, 156 participants will be enrolled, and vafidemstat at an oral dose of 1.2 mg will be tested. The estimated study completion date is noted as December 30, 2022. Based on Clinicaltrialsregister.eu, there are 3 additional clinical trials testing vafidemstat. A single-center, open-label, single-arm, 24-week study is evaluating the efficacy, safety, and tolerability of vafidemstat in aggression in Alzheimer's disease (REIMAGINE-AD study). A phase 2 trial in severely ill COVID-19 patients (ESCAPE study) is evaluating vafidemstat's efficacy to prevent progression to acute respiratory distress syndrome (EudraCT number: 2020-001618-39). A phase 2b double-blind randomized placebo-controlled adaptive 24-week trial is investigating vafidemstat's efficacy on negative symptoms and cognitive impairment in schizophrenia.

Search terms:
Pubmed, Google: Vafidemstat, ORY-2001

Websites visited for Vafidemstat, ORY-2001:

- Clinicaltrials.gov
- Clinicaltrialsregister.eu
- NIH RePORTER (0)
- DrugAge (0)
- Geroprotectors (0)
- Drugs.com (0)
- WebMD.com (0)
- PubChem
- DrugBank.ca
- Cafepharma (0)
- Pharmapro.com (0)