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.

EGX358

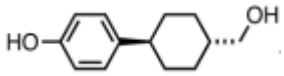
Evidence Summary

EGX358 improved cognitive function and reduced vasomotor symptoms in ovariectomized mice. Efficacy and safety in menopausal women have not been tested to date.

Neuroprotective Benefit: EGX358 treatment in ovariectomized mice improved spatial memory and object recognition, though effects were less robust with chronic treatment. No studies have tested EGX358 in humans yet.

Aging and related health concerns: Preclinical studies suggest that targeting ERβ may be safer than traditional HRT for menopausal symptoms by not promoting breast cell cancer. However, EGX358 has not been tested in menopausal women yet.

Safety: Studies in mice suggest that EGX358 does not cause overt safety issues for the heart, kidney, and liver. Cell culture studies suggest EGX358 does not increase proliferation of breast cancer cells. Safety in humans is currently unknown.

Availability: in clinical development	**Dose**: Laboratory studies have tested a dose of 0.5 mg/kg via oral gavage.	**Chemical formula:** $C_{13}H_{18}O_2$ **MW**: 206  Source: Hanson et al., 2018
Half-life: not documented	**BBB**: penetrance not established experimentally	
Clinical trials: EGX358 has not been tested in a clinical trial to date.	**Observational studies**: No studies of EGX358 exist. Carriers of minor ESR2 alleles showed lower cognitive performance and higher Alzheimer's risk.	

What is it?

EGX358 (formerly called ISP358-2) is an estrogen receptor β (ERβ) agonist that has around 750-fold selectivity for ERβ over ERα and is the most selective synthetic ERβ agonist to date (Hanson et al., 2018). EGX358 is under development by Estrigenix Therapeutics Inc. for the treatment of hot flashes and memory decline in menopausal women.

Menopause is characterized by the cessation of menstrual cycles and the loss of ovarian hormone production, signaling the end of a woman's reproductive phase. The menopausal transition is accompanied by symptoms such as memory problems and hot flashes. Estrogen-based hormone therapies are often used for the treatment of menopausal symptoms including hot flashes, but hormone replacement therapies (HRTs) are associated with risks of breast and uterine cancer, blood clots, stroke, deep vein thrombosis, pulmonary embolus, and gallbladder disease (CognitiveVitality Estrogen rating; 2022 NAMS Position Statement). Some studies suggest that the harmful effects of HRTs may be due to the activation of ERα, and not ERβ (Fleischer et al., 2021). Thus, efforts are underway to selectively target ERβ (e.g., via phytoestrogens such as soy isoflavones).

In humans and other primates, ERβ is abundant in the hippocampus and frontal cortex (Hara et al., 2015). During aging, ERβ expression declines but its affinity for estrogen remains (reviewed in Sekikawa et al., 2022). ERβ activation improves performance in hippocampus-dependent memory tasks and is implicated in the regulation of genes involved in the maintenance of hippocampal function (via phospho-MAPK/ERK, Akt/TrkB, and CREB expression/phosphorylation).

Neuroprotective Benefit: EGX358 treatment in ovariectomized mice improved spatial memory and object recognition, though effects were less robust with chronic treatment. No studies have tested EGX358 in humans yet.

Types of evidence:

- Numerous observational studies examining ESR2 SNPs and risk of cognitive decline/dementia
- 1 review
- 2 laboratory studies

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

No studies have tested EGX358 treatment for the prevention of dementia or age-related cognitive decline.

Several studies have examined the relationships between single nucleotide polymorphisms (SNPs) in the ESR2 gene that encodes ERβ and risks of cognitive decline or dementia.

In a prospective population-based study of 834 elderly women in West Germany (SALIA cohort), carriers of minor ESR2 alleles (rs1256062, rs10144225, and rs2274705) had lower cognitive performance, as measured by the CERAD total score, with the most pronounced deficits in semantic memory (rs1256062, rs10144225, and rs2274705) and executive function (rs1256062)(Fehsel et al., 2016). The minor allele effects of one of the ESR2 SNPs (rs1256062) were stronger in APOE4 carriers for the cognitive domain 'executive function' (p=0.023). None of the SNPs of ESR1 (encoding ERα) were associated with lower cognitive performance in this cohort.

It is worth noting that all of the examined ESR SNPs are present in the introns and therefore not affecting the receptor structure itself (Fehsel et al., 2016). The authors speculate that these SNPs may be within target sequences for regulatory proteins or microRNAs that modulate receptor transcription and/or methylation. How these SNPs influence cognitive outcomes is not known.

In an observational study of 1,686 women from the Washington Heights Inwood Columbia Aging Project (WHICAP), four ESR2 SNPs (rs944045, rs1256062, rs10144225, and rs2274705) were associated with

increased risk for Alzheimer's disease (OR range, 1.6-1.9, empiric p-value range, 0.002-0.004)(Janicki et al., 2014). In women who identified as Black, the rs10137185 SNP was associated with a lower risk for Alzheimer's disease (OR=0.6). When vascular risk factors were included in the model, a different SNP (rs1256059) was associated with increased risk for Alzheimer's disease in women of Hispanic ancestry (OR=1.5).

In a longitudinal study of 3,799 non-demented community-dwelling elderly women (3C Study in France) who were followed for 7 years, the A allele of rs1256049 was associated with an increased risk of substantial decline in visual memory (HR=1.64; 95% CI, 1.23 to 2.18; p=0.0007), psychomotor speed (HR=1.43; 95% CI, 1.12 to1.83; p=0.004), and on the incidence of mild cognitive impairment (HR=1.31; 95% CI, 1.05 to 1.64; p=0.02) (Ryan et al., 2013). There was also an association between the A allele of rs4986938 and a decreased risk of decline in psychomotor speed. In this cohort, the two ESR1 SNPs (rs2234693 and rs9340799) were not significantly associated with the risk of cognitive decline.

In an observational study of 2,527 community-dwelling elders from the Health ABC Cohort, two of the ESR1 SNPs (rs8179176, rs9340799) and two of the ESR2 SNPs (rs1256065, rs1256030) were associated with likelihood of developing cognitive impairment (decline of five or more points on the Modified MMSE over 4 years) in women after multivariate adjustment (age, education, and MMSE score), although the association for rs8179176 was of trend level significance (Yaffe et al., 2009). For the rs1256065 SNP, the CC genotype had a significantly lower OR of 0.56 (95% CI, 0.35 to 0.88) compared to AA or AC (reference), though further adjustment for race resulted in a lessening of association (OR=0.69; 95% CI, 0.43 to 1.12). In men, after adjustment for age, education, and MMSE score, cognitive impairment was significantly associated with 2 ESR2 SNPs: rs1255998 (GG vs. CC or CG, OR=1.68; 95% CI, 1.06 to 2.65) and rs1256030 (TT vs. CC or CT, OR=1.56; 95% CI, 1.07 to 2.29), though further adjustment for race made the association with rs1255998 non-significant but the association with rs1256030 stronger (TT vs. CC or CT, OR=1.91; 95% CI, 1.28 to 2.83). No associations were found between any of the ESR SNPs and level of bioavailable estradiol, though testosterone levels varied among two of the SNPs (ESR2 rs1256065 for women and ESR2 rs1255998 in men; $p<0.05$).

Human research to suggest benefits to patients with dementia:

No studies have tested EGX358 in patients with dementia.

In a postmortem immunohistochemistry study, ERβ immunoreactivity in the hippocampus was increased in cellular and extracellular locations in Alzheimer's disease cases compared to age-matched controls (Savaskan et al., 2001).

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

EGX358 has been shown to be selective for activating ERβ relative to 7 other nuclear hormone receptors, with a 750-fold selectivity for ERβ over ERα and with EC50 values of 20-30 nM in cell-based and direct binding assays (Hanson et al., 2018).

In female ovariectomized mice, intrahippocampal infusion of EGX358 (100 pg or 1 ng per hemisphere) resulted in a significantly better performance on the object placement test (significantly more time spent with the moved object) compared to the vehicle-treated group, suggesting that EGX358 infusion enhanced object placement memory consolidation (Hanson et al., 2018). However, the lowest EGX358 dose tested, 10 pg, did not affect object placement test performance. Intrahippocampal infusion of EGX358 at 100 pg or 1 ng (but not 10 pg) per hemisphere also improved object recognition performance (significantly more time spent with the novel object) compared to vehicle treatment, suggesting that EGX358 infusion enhanced object recognition memory consolidation.

In a different set of ovariectomized mice, acute systemic EGX358 treatment (0.5 and 5 mg/kg, i.p.) resulted in more time than chance spent with the moved object in the object placement test; however, posthoc tests showed that only the 0.5 mg/kg dose of EGX358 showed a significant difference in performance compared to vehicle treatment. Intraperitoneal EGX358 treatment at 0.5 mg/kg and 5 mg/kg doses also significantly improved object recognition performance compared to vehicle treatment.

Finally, a single EGX358 treatment via oral gavage (0.5 or 5 mg/kg) in ovariectomized mice resulted in significantly better object placement test performance compared to vehicle treatment, suggesting that oral treatment also improved spatial memory consolidation. Oral EGX358 treatment at 0.5 mg/kg and 5 mg/kg doses also significantly improved object recognition performance compared to vehicle treatment.

Unlike mice treated with EGX358 within 1 month of ovariectomy, intraperitoneal EGX358 treatment 4 months post-ovariectomy failed to enhance spatial memory consolidation, and this was likely due to the significantly reduced levels of ERβ at this time point.

In a subsequent study, long-term treatment with EGX358 was tested in female ovariectomized mice (Fleischer et al., 2021). Ovariectomized mice were oral-gavaged with EGX358 (0.5 mg/kg, daily; dissolved in 10% DMSO in 0.9% saline), estradiol (E2; 0.2 mg/kg), ERβ agonist DPN (0.05 mg/kg), or vehicle (10% DMSO in 0.9% saline) for 9 weeks. In the object placement task, EGX358, E2, and DPN treatments led to significantly more time than chance spent with the moved object. However, there was no main effect of treatment ($p=0.186$). In the object recognition test, EGX358, E2, and DPN treatments led to significantly more time than chance spent with the novel object, but again, the main effect of treatment was not significant ($p=0.5972$). The authors speculate that the reason for the weaker effect of chronic EGX358 treatment compared to the acute treatment effects seen in Hanson et al., 2018, may be due to treatment timing, the stress of repeated oral gavage, or timing of testing relative to ovariectomy surgery.

APOE4 interactions: No studies have evaluated whether EGX358 treatment would have differential effects based on APOE4 carrier status. Observational studies have reported the relationship between ESR2 SNPs and greater risk for cognitive decline, and in one of those studies, APOE4 carriers were more susceptible to the negative effects of the ESR2 minor allele (rs1256062) than non-carriers, for executive function (Fehsel et al., 2016). But how these findings on ESR2 SNPs translate to efficacy with EGX358 based on APOE4 status remains to be tested.

Aging and related health concerns: Preclinical studies suggest that targeting ERβ may be safer than traditional HRT for menopausal symptoms by not promoting breast cell cancer. However, EGX358 has not been tested in menopausal women yet.

Types of evidence:

- 2 laboratory studies

Menopausal symptoms: VMS IMPROVED IN FEMALE OVARIECTOMIZED MICE
Menopause-related vasomotor symptoms (VMS; e.g., hot flashes) can be modeled in ovariectomized mice by administering senktide, which binds to tachykinin 3 receptors in the median preoptic area of the hypothalamus, activating the warm-sensitive neurons and eliciting vasodilation and an increase in tail skin temperature (Krull et al., 2017). In female ovariectomized mice, EGX358 treatment (0.5 mg/kg/day, oral gavage) for 2.5 weeks significantly reduced senktide-induced rise in tail skin temperature at 5-9, 16, and 19 minutes post-senktide injection compared to vehicle treatment ($p<0.05$)(Fleischer et al., 2021). Treatment with estradiol (E2) as well as the ERβ agonist DPN also reduced senktide-induced rise in tail

skin temperature. However, only estradiol treatment for 9 weeks, but not treatments with ERβ agonists EGX358 and DPN for 9 weeks, reduced the final tail skin temperature (i.e., baseline temperature), when compared to vehicle treatment.

Breast cancer: MAY BE SAFER THAN ESTRADIOL BASED ON CELL CULTURE STUDIES
In MCF-7 human breast cancer cells, estradiol (E2) administration significantly increased proliferation at doses of 10, 100, and 1000 nM, but EGX358 did not result in any significant proliferation at any concentrations tested, compared to untreated control cells (Hanson et al., 2018). Treatment with the other ERβ agonist, DPN, also did not affect MCF-7 cell proliferation.

Safety: Studies in mice suggest that EGX358 does not cause overt safety issues for the heart, kidney, and liver. Cell culture studies suggest EGX358 does not increase proliferation of breast cancer cells. Safety in humans is currently unknown.

Types of evidence:

- 2 laboratory studies

EGX358 has not been tested in humans yet, so safety for humans is currently unknown.

EGX358 shows no inhibition of drug-metabolizing enzymes CYP1A2 or CYP2D6, but shows a weak inhibition of CYP2C9 (EC50=34±4.7 µM) and CYP3A4 (EC50=89±18 µM)(Hanson et al., 2018). EGX358 does not bind to the heart potassium ion channel hERG, and nephelometry showed no significant aggregation. EGX358 does not show significant off-target activity with 7 other nuclear hormone receptors.

In female ovariectomized mice, a single intraperitoneal injection of EGX358 (0.5 mg/kg) did not result in overt safety concerns for the heart, kidney, and liver (Hanson et al., 2018). Heart tissues were unremarkable, the ventricular walls were intact and of normal thickness, and the atrial walls were intact with normal thickness. No evidence of congenital defects or ischemic heart disease/injury was found. Kidneys were also unremarkable, with intact glomeruli and normal-appearing tubules, with no evidence of inflammation. The liver was intact and normal-appearing. There was a generalized appearance of low grade mild ischemic injury in all samples, which was not specific to treatment, and may have occurred postmortem. There was no evidence of acute inflammation composed of neutrophils or damage to the liver structures. Blood chemistry and hematology analysis did not show significant deviations from

reference ranges, relative to vehicle; however modest effects due to hemolysis was likely due to sample collection from cardiac puncture.

While estradiol (E2) significantly increased proliferation of MCF-7 breast cancer cells at doses of 10, 100, and 1000 nM, EGX358 (and DPN) did not show any significant proliferation relative to untreated control cells (Hanson et al., 2018).

In female ovariectomized mice, EGX358 treatment (0.5 mg/kg/day, oral gavage) for 9 weeks did not adversely affect overall health relative to vehicle treatment (Fleischer et al., 2021). EGX358 treatment did not prevent the ovariectomy-induced weight gain, which has been observed in the past with some hormone treatments. However, the absence of an effect on body weight may be due to the rodent chow, which contained 350-650 mg/kg of phytoestrogen, thus potentially minimizing the effects of estrogenic interventions.

EGX358 treatment also did not significantly affect locomotor activity (distance traveled), bouts spent grooming/barbering or rearing, compared to vehicle treatment. Also, no treatment effects were seen for anxiety-like behaviors (measured by elevated plus maze test) and depression-like behaviors (tail suspension and forced swim test).

Drug interactions: Drug interactions have not been investigated.

Sources and dosing:

EGX358 is under development by Estrigenix Therapeutics Inc. for the treatment of hot flashes and memory decline in menopausal women. The dose has not been established for humans. In ovariectomized mice, oral doses of 0.5 and 5.0 mg/kg have been tested (Hanson et al., 2018; Fleischer et al., 2021).

Research underway:

Estrigenix Therapeutics Inc. is optimizing and developing EGX358 as a therapeutic to treat hot flashes and memory decline in menopausal women (R43AG079715). This NIA-funded Small Business Innovation Research program has 3 aims: 1) to demonstrate that EGX358 can reduce hot flashes and enhance memory in middle-aged mouse model of menopause; 2) to demonstrate minimal toxicity and favorable

pharmacokinetic stability for EGX358, and 3) to develop process chemistry scaleup synthesis of EGX358 suitable for method transfer to a cGMP laboratory.

Other ERβ agonists (e.g., PhytoSERM derived from soy isoflavones) have progressed into human clinical trials for different indications, including schizophrenia (Eli Lilly's LY500307; NCT01874756), Fragile-X syndrome (NCT01855971), and menopausal symptoms (PhytoSERM; NCT01723917; NCT05664477).

Search terms:

Pubmed, Google:

- EGX358

Websites visited for EGX358:

- Clinicaltrials.gov (0)
- NIH RePORTER (1)
- DrugAge (0)
- Geroprotectors (0)
- Drugs.com (0)
- WebMD.com (0)
- PubChem (0)
- DrugBank.ca (0)
- Cafepharma (0)
- Pharmapro.com (0)