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.

Irosustat (also known as STX64)

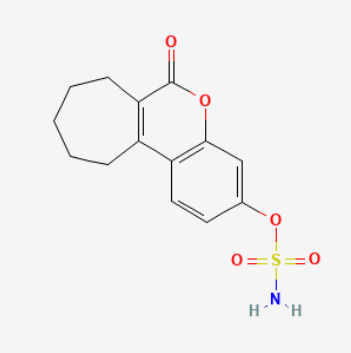
Evidence Summary

Irosustat has been tested in hormone-sensitive cancers, but this line of work has been discontinued. One study in a mouse model of Alzheimer's reported improved memory and reduced amyloid plaques.

Neuroprotective Benefit: One study in a mouse model of Alzheimer's disease has reported that irosustat improved memory and reduced amyloid plaques in the brain. No studies have tested whether irosustat has neuroprotective effects in humans.

Aging and related health concerns: Irosustat has been tested in small early-phase trials of hormone-sensitive breast cancer and endometrial cancer, but these lines of work have been discontinued. Irosustat increases lifespan in *C. elegans.*

Safety: Adverse events are common with irosustat. The most common is dry skin, but others include asthenia, fatigue, anorexia, nausea/vomiting, and changes in hematological measures (e.g., hyponatremia). Long-term safety is not established.

Availability: not approved for any indication	**Dose**: not established for any indication; in phase 2 trials, 40 mg once daily, orally, has been tested	**Chemical formula:** $C_{14}H_{15}NO_5S$ **MW**: 309.34  Source: PubChem
Half-life: 24-30 hours	**BBB**: not documented	
Clinical trials: The largest clinical trial to date enrolled 71 women with endometrial cancer, of whom 36 received irosustat.	**Observational studies**: none available	

What is it?

Irosustat (also known as STX64) irreversibly inhibits steroid sulfatase, an enzyme that converts inactive hormones like estrone sulfate, estradiol sulfate, or 5-androstenediol, to estrone, estradiol, and androstenediol, respectively. Steroid sulfatase is expressed in the placenta, uterus, testis, thyroid, liver, lung, heart, mammary gland, and prostate (Miki et al., 2002). Steroid sulfatase is overexpressed in hormone-dependent tumors (e.g., breast, ovarian, and prostate cancer) as well as other types of cancer (e.g., bladder and colorectal cancer)(Anbar et al., 2021). Consequently, irosustat has been tested in clinical trials of hormone-dependent cancers, such as breast cancer, endometrial cancer, and prostate cancer.

Neuroprotective Benefit: One study in a mouse model of Alzheimer's disease has reported that irosustat improved memory and reduced amyloid plaques in the brain. No studies have tested whether irosustat has neuroprotective effects in humans.

Types of evidence:

- 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:

None available.

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

In a mouse model of Alzheimer's disease (15-month-old APP-PS1 mice), STX64 treatment (dissolved in drinking water at 0.005 mg/ml) for 3-4 weeks significantly reduced Aβ plaque density and area occupied by plaques in the frontal cortex and hippocampus (Pérez-Jiménez et al., 2021). Average plaque size was also significantly decreased with irosustat treatment in the frontal cortex but not in the hippocampus.

In 15-month-old APP-PS1 mice, vehicle treatment led to a deficit in passive avoidance test, but STX64 treatment improved cognitive function, measured by short-term and long-term memory sessions on the passive avoidance test (Pérez-Jiménez et al., 2021). STX64-treated APP-PS1 mice performed at levels comparable to wild-type mice younger than 15 months of age.

In a *C. elegans* worm model of Alzheimer's disease (expression of Aβ protein in muscle cells), sul-2 mutation and treatment with STX64 delayed paralysis and immobility (Pérez-Jiménez et al., 2021).

In a *C. elegans* worm model of Parkinson's disease (human α-synuclein expression in muscle cells), sul-2 mutation or STX64 treatment significantly improved mobility (Pérez-Jiménez et al., 2021). Loss of function of sul-2 decreased the number of α-synuclein aggregates. In a different *C. elegans* worm model of Parkinson's disease (expressing α-synuclein in dopaminergic neurons), sul-2 mutants showed increased neuronal survival compared to control worms, suggesting that reduced steroid sulfatase activity is associated with neuroprotection.

In a *C. elegans* worm model of Huntington disease (expressing polyglutamine repeats fused to YFP), sul-2 mutation and treatment with STX64 reduced the number of aggregates (Pérez-Jiménez et al., 2021).

APOE4 interactions: Unknown.

Aging and related health concerns: Irosustat has been tested in small early-phase trials of hormone-sensitive breast cancer and endometrial cancer, but these lines of work have been discontinued. Irosustat increases lifespan in *C. elegans*.

Types of evidence:

- 3 phase II clinical trials and 2 phase I trials
- Numerous review articles on steroid sulphatase inhibition
- Several laboratory studies

Lifespan: INCREASED IN WORMS

In *C. elegans* worms, loss of function of sul-2, which encodes steroid sulfatase, raises the pool of sulfated steroid hormones and increases longevity (Pérez-Jiménez et al., 2021). In wild-type *C. elegans*, treatment with irosustat reproduced the longevity phenotype of sul-2 mutant worms. Deletion of the other two sulfatase genes, sul-1 and sul-3, did not increase lifespan in *C. elegans*.

Breast cancer: INCONCLUSIVE

Estrogen receptor is a key therapeutic target in breast cancer, with ~80% of breast cancers expressing estrogen receptor. Endocrine therapy is a key treatment for estrogen receptor-positive breast cancer, including aromatase inhibitors that inhibit estrogen production. In estrogen receptor-positive breast cancer, higher steroid sulfatase mRNA is associated with poorer prognosis (Miyoshi et al., 2003). It is thought that high steroid sulfatase levels can lead to a high intratumoral estrogen concentration, promoting tumor growth. The use of aromatase inhibitors, a class of drugs used to treat estrogen receptor-positive breast cancer, can upregulate steroid sulfatase levels (Chanplakorn et al., 2010), suggesting that steroid sulfatase inhibition may have a therapeutic role in this cancer.

In a phase I trial of 14 postmenopausal women with breast cancer, STX64 treatment significantly inhibited steroid sulfatase activity by 98% in peripheral blood lymphocytes and by 99% in biopsied breast tumor tissue at the end of the 5-day dosing period (Stanway et al., 2006). STX64 was administered orally, with 9 patients taking a 5 mg dose and 5 patients taking a 20 mg dose initially, followed by 3 biweekly cycles, with each cycle consisting of daily dosing for 5 days followed by 9 days off treatment. Serum concentrations of estrone, estradiol, androstenediol, and DHEA decreased

significantly from pretreatment levels. Four out of the 14 patients, all of whom had previously progressed on aromatase inhibitors, had stable disease for 2.75 to 7 months.

In an open-label phase I dose escalation study of 50 postmenopausal women with estrogen receptor-positive breast cancer, the maximum tolerated dose of irosustat was not reached and the 40 mg dose was established as the recommended dose (Coombes et al., 2013). This dose escalation study tested 5 doses of irosustat (1, 5, 20, 40, and 80 mg). First, a single dose of irosustat was administered with a 7-day observation period, followed by a daily oral dose of irosustat for 28 days. There was also an extension phase in which the daily oral irosustat was continued at the discretion of the investigator as long as the patient was benefiting from the treatment. After 7 days of irosustat treatment, all the patients in the 1, 5, and 80 mg groups, 5 out of 6 patients in the 20 mg group, and 5 out of 7 patients in the 40 mg group achieved over 95% steroid sulfatase inhibition in peripheral blood mononuclear cells. After 28 days of irosustat treatment, all evaluated patients (in the 5, 20, 40, and 80 mg groups) achieved over 95% steroid sulfatase inhibition in peripheral blood mononuclear cells. After 28 days of irosustat administration, geometric mean ratios of estradiol levels were reduced by 28-75% relative to baseline in the 1, 5, 20, 40, and 80 mg groups; however, the 40 mg dose group was the only group that showed a statistically significant reduction in estradiol levels. The geometric mean ratios of androstenediol were also reduced by 59-81% relative to baseline and was statistically significant in all dose groups except in patients receiving the 1 mg dose. There were no clear relationships between irosustat dose and plasma estradiol or androstenediol levels. There were no complete or partial responses observed during the study. Disease stabilization was achieved in 5 patients (10%), where they remained progression-free for at least 24 weeks (33.1 weeks in 1 patient receiving 20 mg, 72.3, 28.4, and 27.1 weeks in 3 patients receiving 40 mg, and 30.7 weeks in 1 patient receiving 80 mg). The median time to progression was: 5.1 (range 5.1-22) weeks for patients receiving the 1 mg dose; 5.6 (range 4.9-21.7) weeks in the 5 mg group; 13.1 (range 4.9-33.1) weeks in the 20 mg group; 10.1 (3.0-72.3) weeks in the 40 mg group; and 5.0 (4.9-11.2) weeks in the 80 mg group. Of the 6 patients in the 40 mg cohort, 3 patients showed combined significant median decreases in maximum value (SUVmax) and hypermetabolic tissue volume on the FDG-PET, while 3 patients did not.

In an open-label phase 2 trial of 10 postmenopausal women with untreated estrogen-receptor-positive early breast cancer, irosustat treatment (40 mg once daily, orally) for 2 weeks significantly reduced tumor cell proliferation, measured by 3'-deoxy-3'-[18F] fluorothymidine uptake measured by PET scanning (FLT-PET) and Ki67 (Palmieri et al., 2017). Defining response as decreases of ≥20% in standardized uptake value (SUV) or ≥30% in Ki67, 1 (12.5%) and 3 (43%) patients, respectively, responded. Six out of 7 patients (85%) had a Ki67 reduction and the median percentage difference in

Ki67 was 52.3% (p=0.028). In one patient with a low baseline steroid sulfatase expression, a 19.7% increase in Ki67 was recorded, and this same patient had an increase in FLT-PET (from 2.73 to 3.09). At baseline, 57% of patients had high steroid sulfatase expression (scores ≥ 4) and these cases all had notable decreases in steroid sulfatase after irosustat treatment. These findings suggest that baseline expression of steroid sulfatase may be a biomarker predictive of sensitivity to irosustat. With regards to immunointensity data, 71% (5 of 7) of patients had a reduction in aromatase, 57% (4 of 7) had a reduction in steroid sulfatase, 57% (4 of 7) had a reduction in 17β-HSD2, and 29% (2/7) had a reduction in 17β-HSD1.

In an open-label phase 2 trial of 23 postmenopausal women with estrogen receptor-positive locally advanced or metastatic breast cancer (who had benefitted from aromatase inhibitor but subsequently progressed), irosustat treatment (40 mg daily, orally) added to an aromatase inhibitor resulted in a clinical benefit rate of 18.5% on an intent-to-treat analysis and 21.7% with per-protocol analysis (Palmieri et al., 2017). In patients who achieved clinical benefit (n=5), the median (interquartile range) duration was 9.4 months (8.1-11.3) months. The median progression-free survival time was 2.7 months (95% CI, 2.5-4.6 months) in both the intent-to-treat and per-protocol analyses. At study entry and at all subsequent time points, circulating levels of estradiol and estrone were below the threshold of detection in all patients. There were significant decreases in androstenedione, DHEA (p<0.01), and testosterone (p=0.03) with irosustat treatment, as well as significant increases in DHEAS (p=0.02) and DHEA:DHEAS ratio (p<0.01) at 3 months.

There have not been any clinical trials testing irosustat in breast cancer since the 2017 publications.

Endometrial cancer: NO BENEFIT COMPARED TO MEGESTROL ACETATE

In an open-label phase 2 randomized trial of 71 patients with advanced endometrial cancer with estrogen receptor positivity, irosustat treatment (40 mg/day) was compared against megestrol acetate, an antiprogesterone drug that is standard of care for advanced endometrial cancer (160 mg/day), but the study was stopped prematurely after futility analysis (Pautier et al., 2017). At 6 months, 36.1% and 54.1% of patients receiving irosustat or megestrol acetate, respectively, had not progressed or died. There were no statistically significant differences between irosustat and megestrol acetate in treatment response or overall survival rates. Irosustat-treated patients had a median progression-free survival of 16.1 weeks (90% confidence interval, 9.0-31.4) versus 40.1 weeks (90% confidence interval, 16.3-64.0) in patients treated with megestrol acetate. Clinical benefit was achieved in 57.1% of irosustat-treated patients and 70.6% of megestrol acetate- treated patients. The overall response rate (35.3% versus

8.6%) and disease control rate (64.7% versus 57.1%) were numerically higher in the megestrol acetate arm compared with the irosustat arm. With regards to hormone levels, significant reductions in circulating estrone (by a median of 14 ng/dL, p=0.017) and DHEA (by a median of 82 ng/dL, p=0.043) were observed on day 14. No significant effects of irosustat treatment were observed for DHEAS/DHEA ratio, estrone sulphate, androstenedione, testosterone, estrone sulfate/estrone ratio, or estradiol.

Safety: Adverse events are common with irosustat. The most common is dry skin, but others include asthenia, fatigue, anorexia, nausea/vomiting, and changes in hematological measures (e.g., hyponatremia). Long-term safety is not established.

Types of evidence:

- 3 phase II clinical trials and 2 phase I trials
- A few laboratory studies

The largest clinical trial to date of irosustat was an open-label phase 2 randomized trial of 71 patients with advanced estrogen receptor-positive endometrial cancer; irosustat treatment (40 mg/day) was compared against megestrol acetate, and treatment-related adverse events occurred in 20 (55.6%) and 13 (37.1%) patients receiving irosustat or megestrol, respectively (Pautier et al., 2017). Overall, 179 treatment-emergent adverse events were observed in 32 (88.9%) patients in the irosustat arm, and 186 events were observed in 29 (82.9%) patients in the megestrol acetate arm. Of these, 154/179 (86%) were mild to moderate with irosustat and 169/186 (91%) were mild to moderate with megestrol acetate. There were 18 (10.1%), 2 (1.1%), and 1 (0.6%) grade 3, 4, and 5 treatment-emergent adverse events, respectively, in the irosustat arm. The most frequent treatment-related adverse event in both study arms was dry skin. Grade 3 or higher treatment-related adverse events in the irosustat arm included dry skin, asthenia, hyponatremia, and hypertension (n=1 for all). Four patients discontinued from the study due to 5 treatment-emergent adverse events, 3 patients in the irosustat arm (1 patient with grade 3 acute myocardial infarction considered not related to study drug, 1 patient with grade 3 hyponatremia considered related to study drug, and 1 patient with grade 2 tumor hemorrhage considered not related to study drug) and 1 patient in the megestrol acetate arm (grade 3 dyspnea and grade 3 pleural effusion considered not related to study drug). Serious adverse events were reported in 9 patients in the irosustat arm and 6 patients in the megestrol acetate arm, of which there were 2 deaths, 1 patient in the irosustat group with lower respiratory tract infection and lung metastases from endometrial cancer, assessed as not related to study drug, and 1 patient in the megestrol acetate group with a pulmonary embolism assessed as related to study drug. The serious adverse events in the

irosustat arm, each occurring in a different patient, included grade 3 inadequate control of diabetes; grade 3 acute renal failure; grade 3 vomiting and grade 2 asthenia; grade 2 hematuria; grade 2 anemia; grade 3 upper abdominal pain; grade 4 anemia; grade 2 nephrolithiasis; and grade 2 renal colic. None of these serious adverse events were considered related to the study drug. However, 1 patient had grade 3 asthenia, grade 3 hyponatremia, grade 2 decreased appetite, and grade 2 constipation, which were all considered related to study drug. With regards to hematological values, a non-clinically significant grade 4 abnormal potassium value was reported in 1 patient in the irosustat group. Four patients in the irosustat group and 4 patients in the megestrol acetate group experienced grade 4 hematological values. Among these patients, decreased lymphocyte counts were seen in 2 patients, 1 in each drug group, and were judged by the investigator as clinically significant. Three of 10 grade 4 biochemistry values in the irosustat group and 6 of 13 in the megestrol acetate group were considered to be clinically significant.

In an open-label phase 2 trial of 23 postmenopausal women with estrogen receptor-positive locally advanced or metastatic breast cancer (who had benefitted from aromatase inhibitor but subsequently progressed), irosustat treatment (40 mg daily, orally) added to an aromatase inhibitor resulted in all patients experiencing treatment-emergent adverse events, 91% of which were grade 1 or 2 (Palmieri et al., 2017). The most common adverse events were dry skin (77%), nausea (48%), and fatigue (40%). Grade 2 ECG abnormalities (QT prolongation) were reported in 1 patient, which were considered unrelated to study drug. Three patients discontinued the study due to adverse events; urinary tract infection, possible renal toxicity (not reported as an adverse event), and dry skin. The most frequently reported grade 3/4 toxicities were dry skin (28%), nausea (13%), fatigue (13%), diarrhea (8%), headache (7%), anorexia (7%), and lethargy (7%). There were 9 serious adverse events which occurred in 6 patients (22%); one was considered probably related (nausea and vomiting) and another possibly related (nausea and vomiting). All other serious adverse events were considered as unlikely to be related (sepsis, urinary tract infection, breast pain) or not related (cellulitis secondary to an animal bite, symptoms of morphine toxicity, pneumonia). There was one death due to bronchopneumonia during the study that was unrelated to the study medication.

In an open-label phase 2 trial of 10 postmenopausal women with untreated estrogen-receptor-positive early breast cancer, irosustat treatment (40 mg once daily, orally) for 2 weeks was generally well tolerated with all adverse events being grade 2 or lower (Palmieri et al., 2017). Sixty-four adverse events were reported, of which 40 (62%) were unrelated to irosustat. Of the 8 (12.5%) adverse events that were definitely or probably related to study drug, the majority of these were dry skin. There was one serious adverse event which was an allergic reaction to the dye used for sentinel lymph node biopsy and was unrelated to irosustat.

In an open-label phase I dose escalation study of 50 postmenopausal women with estrogen receptor-positive breast cancer, irosustat treatment (1, 5, 20, 40, and 80 mg, daily) for up to 28 days led to treatment-related adverse events in 38 (76%) patients, with dry skin being the most frequent (Coombes et al., 2013). Dry skin was experienced by 3 patients receiving the 1 mg dose, 7 patients receiving the 5 mg dose, 6 patients receiving the 20 mg dose, 7 patients receiving the 40 mg dose, and 6 patients receiving the 80 mg dose. One patient (receiving a single 5 mg dose) discontinued the study due to QTc prolongation; however, the patient had a history of QTc prolongation, and the event was unlikely related to the study drug. In the chronic dosing group, 2 patients discontinued treatment: disease progression was observed after 4.9 weeks in 1 patient receiving the 5 mg dose, and after 3 weeks in 1 patient receiving the 40 mg dose. Six patients experienced a grade 3 treatment-related adverse event: fatigue (2 patients receiving the 40 mg dose); metastatic pain (1 patient receiving the 40 mg dose); anorexia (1 patient receiving the 40 mg dose); dry skin, exfoliation, and fatigue (1 patient receiving the 40 mg dose); and anorexia and fatigue (1 patient receiving the 80 mg dose). There were 4 serious adverse events that were thought to be related to the study drug in 2 patients: 1 patient receiving the 80 mg dose who was hospitalized for fatigue after 114 days of treatment and 1 patient receiving the 40 mg dose who reported anorexia (grade 3), nausea, and vomiting requiring hospitalization after 30 days of treatment. Serious adverse events in 2 other patients were not considered to be treatment-related. There were 2 deaths due to disease progression during the study, and neither of these deaths were thought to be related to the study medication.

In a phase I trial of 14 postmenopausal women with breast cancer, STX64 treatment (5 or 20 mg, three 2-week cycles) was well tolerated with only minor drug-related adverse events (Stanway et al., 2006).

Irosustat has a half-life ranging from 24.44 to 29.75 hours, depending on the dose (5-80 mg)(Coombes et al., 2013).

Drug interactions: Drug interactions have not been well studied or documented.

Sources and dosing:

Irosustat was under development by Sterix Ltd and Ipsen for the treatment of hormone-sensitive cancers including breast cancer, endometrial cancer, and prostate cancer, but this research has been discontinued (NIH NCATS). In cancer patients, irosustat dose of 40 mg daily, orally, has been tested (Palmieri et al., 2017; Palmieri et al., 2017 ; Pautier et al., 2017).

ONeSTX Ltd. is developing STX64 (ONESTX-1) for the treatment of Alzheimer's disease, Parkinson's disease, and Huntington's disease (onestx.bio/pipeline).

Research underway:

There are no ongoing clinical trials testing irosustat based on ClinicalTrials.gov. Based on the ONeSTX website, clinical planning is ongoing for Alzheimer's disease, Parkinson's disease, and Huntington's disease (onestx.bio/pipeline).

Search terms:

Pubmed, Google: irosustat, STX64, BN83495

Websites visited for irosustat, STX64:

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

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