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.

Iron Chelators

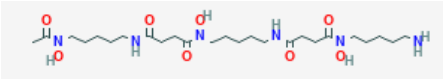
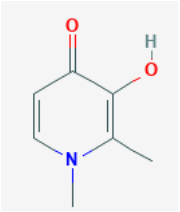
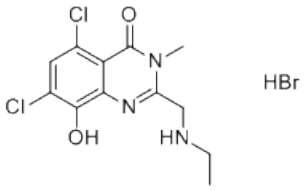
Evidence Summary

Conservative iron chelation may protect against cognitive decline in early stage neurodegenerative disease, but safer, orally bioavailable forms are needed for chronic use.

Neuroprotective Benefit: Elevated brain iron is associated with cognitive decline. Conservative iron chelation with a highly BBB penetrant drug may slow the rate of neurodegeneration, but benefits are likely to be modest.

Aging and related health concerns: Moderate iron chelation may reduce oxidative stress during aging, and help reduce the risk for cancer and cardiovascular disease, but has not yet been tested in humans due to lack of safe chelators.

Safety: Conservative iron chelation through low affinity or moderate dose high affinity orally bioavailable chelators is well-tolerated in clinical trials, but may still pose risks for mild anemia or neutropenia.

Availability: Rx (Deferoxamine, Deferiprone) Clinical trials (PBT434)	**Dose**: Therapeutic dose not established Moderate dose oral deferiprone: 30 mg/kg/day (tested in clinical trials for neurodegenerative diseases)	**Deferoxamine** Chemical formula: $C_{25}H_{48}N_6O_8$ MW: 560.7 g/mol  (Source: PubChem)
Half-life: Deferoxamine 6 hours Deferiprone 1.9 hours PBT434 ~3 hours	**BBB**: Deferiprone and PBT434 penetrant	**Deferiprone** Chemical formula: $C_7H_9NO_2$ MW: 139.15 g/mol  (Source: PubChem)
Clinical trials: PBT434 (Phase 1 healthy adults n=18); Moderate dose deferiprone (Phase 2 PD (n=22, 37), (Phase 2 ALS n=23)	**Observational studies**: Elevated brain iron associated with cognitive decline in AD, elevated serum iron biomarkers associated with CVD, diabetes, cancer.	**PBT434** Chemical formula: $C_{12}H_{14}BrCl_2N_3O_2$ MW: 383.067 g/mol  Source (ProbeChem)

What is it?

Iron is an essential mineral that is used by all cells of the body, and is critical for the maintenance of metabolic capacity. Most of the iron in the body is bound to hemoglobin in red blood cells. **The levels of iron in cells must be tightly regulated**. When iron levels in cells become too high the iron can promote the production of reactive oxygen species (ROS) leading to oxidative stress damage, and can induce a

form of programmed cell death called ferroptosis [1]. However, mitochondrial function is impaired when iron levels are too low, which limits energy production and growth. Iron chelators bind iron, which prevents it from accumulating in cells, and are typically used in the context of iron overload disorders. The pool of iron that is available for chelation by a given chelator depends on the affinity of the chelator for iron relative to the affinity of endogenous iron binding proteins. Therefore, chelators with weak affinity will only be able to bind free iron, while strong chelators can also liberate iron that is bound to iron-binding proteins such as transferrin and ferritin.

Iron homeostasis becomes dysregulated during aging, leading to the accumulation of iron in certain tissues, which may promote pathological processes [1]. Therefore, iron chelation has been proposed as a potential therapeutic strategy for neurodegenerative diseases, cardiovascular disease, and cancer. Some pilot clinical trials with existing iron chelators have been conducted and are ongoing for neurodegenerative diseases, however, safer alternatives with high bioavailability will be needed for iron chelation to be a viable prevention strategy for age-related diseases [2].

Deferoxamine is a high affinity (Kd= 10^{-31}) iron chelator approved for acute iron toxicity or chronic iron overload. It can also bind aluminum, although at lower affinity, and is used off-label for acute aluminum toxicity. It is a bacterial siderophore used to transport iron across cell membranes. It is typically given in an intramuscular formulation, and is not suitable for chronic conditions due to its poor bioavailability, short half-life, extremely limited blood brain barrier (BBB) penetration, and high potential for acute toxicity. Efforts are underway to develop an intranasal formulation that would mitigate the risks for systemic toxicity and enhance brain levels.

Deferiprone is a high affinity (Kd= 10^{-35}) iron chelator approved for thalassemia related iron overload. It is orally available and BBB penetrant, and is being tested in a delayed release formulation at moderate doses as a method of conservative metal chelation for neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease (PD), and Amyotrophic lateral sclerosis (ALS).

PBT434 is a quinazolinone compound with moderate affinity iron chelator (Kd=10^{-10}), which also has modest affinity for copper (Kd= 10^{-10}) and zinc (Kd=10^{-7}) [3]. Since its affinity is less than endogenous iron binding proteins (transferrin Kd=10^{-23}) it only targets labile (un-bound, free) iron, and does not lower central iron stores. It is orally bioavailable and BBB penetrant. PBT434 is being developed by Alterity Therapeutics for Parkinson's related diseases, including an atypical form of Parkinson's called Multiple Systems Atrophy.

Neuroprotective Benefit: Elevated brain iron is associated with cognitive decline. Conservative iron chelation with a highly BBB penetrant drug may slow the rate of neurodegeneration, but benefits are likely to be modest.

Types of evidence:

- 1 meta-analysis/ systematic review based on 43 observational studies of iron levels in AD patients
- 6 clinical trials (1 pilot RCT for intramuscular deferoxamine in AD, 2 Phase 2 RCT for oral PBT2 in AD, 2 Phase 2 RCT for oral moderate dose deferiprone in PD, 1 Phase 2 RCT for oral moderate dose deferiprone in ALS)
- 3 observational studies (Relationship between brain iron levels or biomarkers with cognitive decline in AD).
- 1 genetic study of iron-regulating gene SNPs and AD
- Numerous laboratory studies

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

Iron dyshomeostasis is one of the hallmarks of unhealthy aging. Iron deposition in the brain is positively correlated with age, though the iron is not evenly distributed such that some regions have overload, while others are deficient [4]. A meta-analysis of 43 studies looking at the relationship between iron perturbation and Alzheimer's disease (n=1,813 AD and n=2,401 controls) found that levels of serum iron were significantly reduced in AD patients (effect size −0.31; 95% Confidence Interval (CI) −0.52 to −0.10; $p < 0.001$), though the heterogeneity was high [5]. Nineteen of the studies examined brain iron content across twelve brain regions, and meta-analyses of these studies indicated that **AD patients had significantly elevated levels of iron** in the frontal lobe, parietal lobe, temporal lobe, amygdala, putamen, cingulate, globus pallidus, and caudate. There was also a trend to iron overload in the hippocampus. In an MRI based study (n=60), patients with mild to moderate AD had increased brain iron levels in the caudate and putamen, and the iron content of the caudate was correlated with the severity of cognitive decline based on the Mini-Mental State Examination (MMSE) score (r=-0.52; P=0.0046) [6].

The discrepancy between circulating serum levels of iron and brain tissue stores relates to changes in the expression and binding properties of iron transport proteins [7]. The decrease in serum iron has been attributed to a decrease in the binding of iron to transferrin, which transports free iron throughout the body [8]. Ferritin binds iron within cells, so increased levels of ferritin are associated with increased

intracellular stores. In the AD brain, there appears to be a downregulation of cellular iron export, so it accumulates within tissues [7].

A study examining the association between baseline cerebrospinal fluid (CSF) ferritin levels and cognitive performance was assessed in patients from the ADNI cohort (n=91 cognitively normal, n=144 mild cognitive impairment (MCI), n=67 AD) [9]. Based on tertile analysis, those with high levels of CSF ferritin (>7.2 ng/mL) had 3 point worse cognition scores using the ADAS-Cog13, relative to those with low ferritin (<5.4 ng/mL). **Individuals with higher baseline CSF ferritin were also more likely to convert from MCI to AD** (Odds ratio OR: 1.36, 95%CI 1.17 to 1.58). Over a 6-year period, higher CSF ferritin was also associated with a higher rate of brain atrophy in the hippocampus and expansion of the lateral ventricles.

The association between increased brain iron levels and cognitive decline was confirmed in a follow-up study using postmortem tissue from participants in the Memory and Aging Project study (n=209) whose cognitive trajectory was assessed for 12 years prior to death [10]. Iron was found to be elevated in the inferior temporal cortex only in people with both a clinical diagnosis of dementia and high AD pathology. **Iron could be used to distinguish patients with clinical AD from those with prodromal AD**, who had AD pathology, but were asymptomatic. Iron had a moderate association with tau tangles, and tau pathology may induce altered iron trafficking. It is not yet clear whether the iron accumulation plays a causative role in cognitive decline, or if it is simply an epiphenomenon. It is hypothesized that iron accumulation follows AD proteinopathy, and then can potentiate neurodegeneration through the induction of oxidative stress and ferroptosis.

This suggests that iron chelation therapy may be most beneficial in preventing the conversion from prodromal-AD or MCI to AD, by slowing the rate of cognitive decline.

Human research to suggest benefits to patients with dementia:

Iron chelation therapy has been tested in pilot trials in patients with AD, PD, and ALS. While not conclusive, due to small sample sizes and short time scales, the results from these trials suggest that iron chelators may help modestly stabilize or slow neurodegenerative disease progression. It will likely be necessary to combine chelation therapy with another type of neuroprotective/reparative disease modifying therapy to see sustained clinically meaningful benefits. Since neurodegenerative diseases are associated with an iron imbalance consistent with iron dyshomeostasis, rather than excess systemic iron, a therapeutic that improves iron regulation would likely be more beneficial than a pure iron chelator.

Alzheimer's disease: Potential minor benefit at early stages

Deferoxamine was found to decrease the rate of decline in activities of daily living by half in Caucasian patients with probable (clinically diagnosed) AD in a small (n=48) proof-of-principle trial [11]. In this two-year single-blind trial, patients received 125 mg intramuscular injections of deferoxamine 5 days per week. This is not an ideal administration route for this population, and this preparation also suffers from poor bioavailability, poor BBB penetration, and risks for acute toxicity, thus itself is not a viable therapeutic option for AD patients. However, the results from this trial have served as a justification for later efforts to use metal chelation therapy for AD.

PBT2 is an orally bioavailable transition metal ionophore that can reduce cellular levels of iron, zinc, and copper. It is a clioquinol derivative developed by Prana Biotechnology, now called Alterity Therapeutics, and was tested in two Phase 2 clinical trials for AD. The first double-blind, placebo-controlled 12-week RCT in early AD patients (n=78) (NCT00471211) showed reductions in CSF Aβ levels and improvement on the Trail Making Test Part B measuring executive function at the highest dose (250 mg) [12]. However, the 12 months IMAGINE trial (n=40) did not significantly improve cognitive scores, although there were non-significant reductions in brain amyloid burden and hippocampal atrophy [13]. The lack of significance on outcome measures may stem from high patient heterogeneity and low sample size. Due to toxicity findings in a preclinical dog study, the FDA placed a cap on the maximum clinical dose. As a result, Prana has discontinued clinical development of PBT2, though it remains available for licensing.

These results suggest that improving metal ion homeostasis may be beneficial for AD, but that it **may be most useful in early stage patients**, and that the efficacy of a therapeutic may depend on its relative affinity for different metals.

Parkinson's disease: Potential benefit at early stages

Moderate dose deferiprone (20 to 30 mg/kg oral solution) has been tested in two Phase 2 small pilot trials for patients with early stage PD. These were proof-of-concept studies for conservative iron chelation which aims to reduce pathological accumulation of iron in the brain without significantly impacting peripheral stores necessary for hemostasis.

In both trials, deferiprone treatment for 6 months reduced iron content in PD-affected brain regions based on MRI. In the single-blind FAIRPARK trial (n=37) (NCT00943748) there was a significant decrease in iron levels in the substantia nigra for treated versus placebo in PD patients [14]. Meanwhile, in a small double-blind RCT (n=22) (NCT01539837) iron was significantly reduced in the dentate gyrus and caudate nucleus in treated patients relative to placebo, but only a small subset of patients had significant

reduction of iron in the substantia nigra [15]. The iron in the substantia nigra may be less susceptible to conservative chelation because here it is primarily bound to neuromelanin, which has a higher binding affinity than ferritin. In the FAIRPARK trial, treated patients showed relative improvement on the UPDRS-motor score (−2.3±0.6 vs +1.0±0.73) [14], and while a similar trend toward improvement was seen in the smaller 6-month RCT there was no change in cognitive function in this trial [15]. Additionally, the rate of motor improvement waned in the extension phase of the FAIRPARK trial [14]. Deferiprone was most effective in patients with low serum ferritin levels (<100 ng/mL) [15], suggesting that high levels of peripheral iron stores may serve as a sink for deferiprone that prevents it from effectively reducing brain iron stores.

ALS: Potential benefit

Conservative iron chelation using deferiprone (30 mg/kg oral solution) was tested in a pilot study in ALS patients (n=23) in the SAFEFAIRALS trial (NCT02164253) [16]. Treated patients were found to have significantly decreased iron in the cervical spinal cord, medulla oblongata, and motor cortex, which was accompanied by lower oxidative stress markers and neurofilament light levels in the CSF. The ALSFRS-R score was lower following 3 months of treatment compared to the prior 3-month untreated period. This suggests modest iron chelation may help slow disease progression, though larger trials are needed to confirm potential benefits.

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

Iron chelation is thought to be neuroprotective by reducing iron-mediated induction of oxidative stress, and preventing neuron loss due to iron toxicity induced apoptosis, called ferroptosis. This type of therapy would be expected to be most beneficial in preventing or slowing the onset of pathology, since it cannot repair synaptic dysfunction or restore lost neurons.

PBT434:

Due to its moderate binding affinity for iron, PBT434 cannot outcompete with endogenous iron-binding proteins, and thus just **targets the labile pool of iron**. It has been tested in animal models for PD (6-OHDA, MPTP, A53T) [3] and multiple systems atrophy (PLP-αSyn) [17] and shown to protect against α-synuclein mediated pathology. α-synuclein can directly bind to iron, which can affect its conformation and solubility. Thus, preventing α-synuclein from binding to iron via chelation could potentially reduce its aggregation. Since PBT434 has a higher binding affinity for iron than α-synuclein, it is expected to outcompete. In the 6-OHDA model, 30 mg/kg PBT434 administered for three days after toxin exposure protected against further neuron loss by preserving 75% of the remaining neurons in the substantia

nigra [3]. While PBT434 one day after MPTP improved neuronal survival, reduced motor deficits, and prevented an elevation in brain iron and oxidative stress markers (8-isoprotane). PBT434 was also shown to reduce levels of CSF α-synuclein in PD models, as well as in wild-type animals. In the multiple systems atrophy model, PBT434 reduced levels of insoluble α-synuclein, reduced neuron loss, and improved motor function [17]. These studies suggest that PBT434 exerts neuroprotective effects by binding and redistributing labile iron, which in turn, protects against protein aggregation and oxidative stress.

Intranasal Deferoxamine:

Systemically administered forms of deferoxamine have poor pharmacokinetic properties, poor BBB penetration, and are associated with the risk for acute toxicity [2]. Intranasal deferoxamine has been proposed as an alternative administration route to enhance brain levels and limit systemic exposure. In mice, intranasal administration led to micromolar concentrations of deferoxamine in the CNS after 30 minutes [18]. The highest levels were in the meninges (21-54 uM) with concentrations of 0.4 to 1 uM in most brain regions [19]. Relatively high levels were found in the frontal cortex (1.1-1.7 uM) and hippocampus (0.8 uM), however these were still lower than in some peripheral areas (kidney 8.5 uM, blood 5.2 uM). Additionally, as a hydrophilic small molecule, deferoxamine has poor cell permeability, which may still limit its efficacy [2].

Intranasal deferoxamine has been tested in several AD and PD rodent models, and while it has been shown to have beneficial effects, these **effects are typically modest and have been inconsistent** across studies. Several studies have shown cognitive benefits for working and spatial memory using the radial maze or Morris water maze tests in AD models [19; 20; 21; 22]. Deferoxamine was also protective against motor impairments in PD models, but the strength of the protection was variable depending on the model, the timing of treatment administration, and the motor assessment used [23; 24; 25]. In some studies, deferoxamine decreased A levels, tau phosphorylation, synaptic loss (based on synaptophysin), oxidative stress markers, or neuronal loss, however, other studies showed no protective effects on these measures [19; 20; 21; 22; 23; 24; 25; 26; 27].

Intranasal deferoxamine did not decrease overall levels of brain iron (Fe^{3+}), but rather may have altered its distribution, leading to decreases in susceptible brain areas such as the cortex and hippocampus [26]. In PD models, it was most effective as a preventative against acute oxidative stress prior to toxin exposure, and since it cannot repair preexisting damage, it was less effective when administered in chronic models. In addition to its iron chelation properties, part of deferoxamine's neuroprotective

effect appears to be related to its ability to stabilize HIF-1α and influence expression of its downstream targets, including neurotrophic factors and iron-regulating genes [20; 25; 26].

Deferoxamine was found to be protective against postoperative cognitive dysfunction when given systemically as a preventative six days prior to surgery in aged (18-month-old) rats [28]. It prevented surgery induced increases in hippocampal iron and ferritin, and changes to iron-regulating genes. Although not tested in this study, intranasal deferoxamine may be a more practical option.

Overall, these studies suggest that while intranasal administration may help improve the ability of the metal chelator to act on brain iron stores rather than systemic stores, due to its poor bioavailability, deferoxamine is a suboptimal chelator for neurodegenerative diseases.

Deferiprone:

Oral deferiprone was found to have protective effects in a variety of neurodegenerative animal models (AD, PD, Huntington's, ALS), though the benefits were typically modest. In a rabbit model of cholesterol-diet induced AD pathology, deferiprone reduced levels of Aβ and phosphorylated tau, but did not significantly affect brain iron levels, oxidative stress levels (ROS, H_2O_2), or correct iron-related protein dysregulation [29]. Deferiprone was protective against Fe^{3+} accumulation, microglia proliferation, and dopamine neuron necrosis when administered prior to 6-OHDA exposure, but did not affect neuronal survival or α-synuclein aggregation in the A53T transgenic PD model [30]. In Huntington's disease mouse models (R6/2, YAC128), deferiprone at the lowest tested dose (50 mg/kg) reduced levels of mitochondrial iron and improved mitochondrial function [31]. Deferiprone also reduced spinal cord iron levels and improved survival (by 56%) in the SOD^{G86R} ALS mouse model [16].

The discrepancies in the efficacy of deferiprone in reducing CNS iron and associated pathology in these different models may be related to differences in peripheral iron levels and the accessibility of the iron in the brain regions most affected in these different diseases. Since deferiprone is administered orally, its **efficacy in the brain may be limited in those with high easily accessible peripheral iron**.

APOE4 interactions:

Studies suggest that there may be a connection between iron metabolism and lipoprotein metabolism such that iron dysregulation may drive lipoprotein related pathology. A genetic study (n=765) found that single nucleotide polymorphisms (SNPs) in iron regulation associated genes could act as modifiers of ApoE4 associated AD risk and severity [32]. The presence of the HFE 282Y allele was associated with a 3-fold reduced risk for AD, and an interaction analysis indicated that the presence of the HFE 282Y allele

reduced the ApoE4 allele associated risk. High ferrum hemochromatosis (HFE) is involved in iron absorption. Additionally, ApoE4 carriers with a high burden of iron alleles showed the greatest degree of cognitive decline, suggesting that the iron dysregulation potentiates ApoE4-associated negative effects.

In the study of CSF ferritin levels in the ADNI cohort, CSF ferritin was associated with CSF ApoE4 (R^2=0.34, P=1.52 X 10^{-29}), with carriers having 22% higher levels of CSF ferritin [9]. There was, however, no effect of ApoE4 status with plasma ferritin, which is consistent with the reported poor correlation between circulating iron biomarkers with brain iron levels. However, another study found that there was no relationship between brain iron stores and ApoE4 status [10].

These studies suggest that ApoE4 carriers with iron dysregulation may be at highest risk for cognitive decline, and thus may preferentially benefit from iron chelation therapy, however, the supporting evidence is weak.

Aging and related health concerns: Moderate iron chelation may reduce oxidative stress during aging, and help reduce the risk for cancer and cardiovascular disease, but has not yet been tested in humans due to lack of safe chelators.

Types of evidence:

- 8 observational studies: Studies examining relationship between iron biomarkers with cancer risk (3), cardiovascular disease (3), type 2 diabetes (2)
- Several laboratory studies

Cellular senescence: Senescent cells accumulate iron

Senescent cells have been shown to accumulate large amounts of intracellular iron due to impaired ferritin degradation, therefore, the increase in **senescent cells during aging contributes to the accumulation of iron in tissues** with age [33]. Unlike normal cells, senescent cells do not experience ferroptosis, or iron-mediated cell death, following this iron accumulation. The excess iron can promote oxidative stress in these tissues. As an alternative strategy to the clearance of senescent cells with senolytics, it may be possible to target specific pathological features of senescent cells [34]. Consequently, iron chelation could potentially be used to reduce iron-mediated oxidative stress damage. However, this strategy will only be considered feasible with the development of chelators with a good safety profile.

Cancer: Elevated iron biomarkers associated with cancer risk

Cellular iron is essential for replication, metabolism, and cell growth. Therefore, cancer cells require high levels of iron to sustain their proliferation and survival [35].

Elevated iron has been identified as a risk factor for cancer and is correlated with cancer progression. In the National Health and Nutrition Examination Survey in the US (n=8556) transferrin saturation was associated with cancer risk, such that those with baseline transferrin saturation >40% had elevated risk [36]. In a prospective cohort study in Taiwan (n=309,443), high serum iron (≥120 ug/dL) was associated with increased risk incidence for all cancers (Hazard Ratio HR: 1.25; 95% CI 1.16 to 1.35), and increased risk for mortality from all cancers (HR: 1.39; 95% CI 1.23 to 1.57) [37]. Liver and breast cancer had the most significant associations with elevated serum iron. There was a 4% increase in cancer risk for every 10 ug/dL above 80 ug/dL, but there was also an increased risk in people with iron deficiency (<60 ug/dL), suggesting that **iron dysregulation in either direction is associated with adverse effects**. The EPIC-Heidelberg prospective case-cohort study in Germany (n=2738) found that elevated ferritin was inversely associated with breast cancer risk (HR: 0.67; 95% CI 0.49 to 0.92), and that ferritin levels were also inversely associated with cancer mortality [38]. The contradictory results may reflect differences in methodology, highlight the high intraindividual variability of these iron biomarkers, and indicate that iron needs to be regulated within a narrow range. The effects of iron may also be related to the tumor microenvironment.

In cell culture, iron chelators have been shown to have anti-cancer activity by inhibiting their proliferation [39]. One study also found that iron metabolism may play a role in the maintenance of cancer stem cells [40]. Iron chelators have been proposed as a potential therapeutic agent for cancer, however, new formulations would be necessary due to the acute toxicity and short half-life of currently approved chelators.

Cardiovascular disease/Diabetes: Elevated iron biomarkers associated with CVD

Epidemiological studies have found associations of iron levels with cardiovascular disease and diabetes, suggesting that iron overload is a risk factor for these conditions [41].

The connection was first described in a cohort of Finnish men (n=1931), which showed that men with serum ferritin levels ≥ 200 ug/L had a 2.2-fold higher risk for acute myocardial function relative to men with lower serum ferritin [42]. Similar results were found in a study of Dutch post-menopausal women (n=11,471) in that women with the highest tertile of serum ferritin had 2.23-fold higher risk for ischemic stroke than those with lowest levels [43]. In both men and women, **those with both high ferritin and**

lipoproteins (LDL) had the greatest risk for ischemic cardiovascular events. Due to its established role in ROS production, elevated iron may promote the formation of oxidized LDL [41]. Similar results regarding the association between lipoproteins and ferritin levels were found with carotid atherosclerosis in subjects from the Bruneck Ischemic Heart Disease and Stroke Prevention Study (n=847) [44], and with acute myocardial infarction in the Nutrition Canada Survey cohort (n=9920) [45]. However, there have been other studies that failed to replicate these results, which may stem from the fact that the biomarker surrogates often do not reliably reflect body stores of iron [41; 46]. For example, transferrin saturation is frequently used, but it does not correlate well with body iron stores. Furthermore, the negative effects of excess iron may depend on where it is stored, which would not be reflected in systemic iron-related serum measures. Iron chelation has not yet been tested for the prevention of iron-related cardiovascular disease due to the historic lack of chelators that have good oral bioavailability and are non-toxic.

Hemochromatosis, a disease involving iron overload, is associated with increased risk for type 2 diabetes, suggesting that elevated iron stores may be involved in the pathogenesis of diabetes. A prospective nested case-control study of healthy women who did (n=698) and did not develop diabetes (n=716) during the 10-year follow-up period found that the multivariate **relative risk for incident type 2 diabetes increased with levels of serum ferritin**, such that those with the highest quintile had 2.68 fold higher risk [47]. A similar association between higher baseline serum ferritin and risk for diabetes was also found in men and women in the prospective case-cohort EPIC-Potsdam study (n=1969) [48]. These studies suggest that elevations in ferritin are not simply a compensatory or downstream effect of diabetes, but that elevated iron stores may precede disease pathology and contribute to disease onset.

Wound healing: Potential benefit (preclinical)

Deferoxamine has been found to prevent the formation and promote the healing of ulcers in diabetes models and aged mice [49]. The accelerated rate of wound healing and enhanced neovascularization is attributed to the ability of deferoxamine to stabilize HIF-1□ and promote VEGF. A transdermal formulation of deferoxamine enclosed in nanoscale revers-micelles was found to be as effective as systemic (intravenous) deferoxamine in promoting wound healing, and has a stronger benefit to risk profile.

Safety: Conservative iron chelation through low affinity or moderate dose high affinity orally bioavailable chelators is well-tolerated in clinical trials, but may still pose risks for mild anemia or neutropenia.

Types of evidence:

- 5 clinical trials (1 Phase 1 for PBT434; 3 Phase 2 for moderate dose deferiprone in PD, ALS; 1 Phase 3 for moderate dose deferiprone in PKAN)
- Numerous laboratory studies

PBT434

PBT434 was tested in healthy volunteers (n=18) in a Phase 1 RCT (doses between 50 mg to 600 mg) [50]. The oral formulation was **well-tolerated in single doses up to 300 mg**. All adverse events were mild, and there were no serious adverse events or abnormalities on ECG or routine laboratory tests. It had proportional pharmacokinetic properties over the tested dose range, and was rapidly absorbed after oral administration.

Intranasal deferoxamine

Systemically administered high dose deferoxamine (intramuscular or intravenous) can cause blurred vision, difficulty breathing, seizures, headaches, and nausea (Drugs.com). Rapid intravenous administration can also cause flushing, hypotension, or shock (FDA label). It is also associated with musculoskeletal growth retardation. It is contraindicated in people with kidney disease, and due to increased risk for side effects is not recommended in people with heart disease, liver disease, asthma, or vision problems. Vitamin C increases the availability of iron for chelation, and is often given as an adjuvant in patients with iron overload. There is a drug interaction with the antipsychotic prochlorperazine which can impair consciousness. Deferoxamine led to loss of appetite and weight loss in AD patients [11].

Intranasal deferoxamine has not yet been clinically tested in humans, but is expected to have a better safety profile. It was well-tolerated in preclinical animal models that were treated several times a week for up to 3 to 5 months [19; 20; 27]. Treated mice and rats did not show any overt physical side effects or changes in white blood cell counts.

Deferiprone

High dose deferiprone (75 mg/kg/day) for iron overload is not recommended for people with liver disease or weak immune systems (Drugs.com). It is associated with an **increased risk for infections**. The most common side effects are nausea, vomiting, joint pain, and abnormal liver tests. Deferiprone has a warning for neutropenia, liver enzyme elevations, and zinc deficiency (FDA label). It should not be used in combination with other drugs that increase the risk for neutropenia or with UGT1A6 inhibitors.

Moderate dose deferiprone (30 mg/kg/day) used for conservative iron chelation in neurodegenerative diseases was **well-tolerated in clinical trials**. In Parkinson's patients treated for 6 to 12 months, side effects were mild including joint pain, mild gastrointestinal events, increased liver enzymes, and reduced neutrophils which all resolved upon drug cessation [14; 15]. There were no significant effects on hemoglobin, transferrin, serum iron, copper, or zinc levels. In ALS patients, patients treated up to 12 months had a normal hematological profile [16].

Moderate dose deferiprone (30 mg/kg/day) was also tested in a double blind, placebo-controlled RCT for children with Pantothenate kinase-associated neurodegeneration (n=88, mean age 8), which is a rare genetic disorder causing progressive dystonia and brain iron accumulation [51]. Deferiprone was well-tolerated in the 18-month trial with similar levels of adverse events between treatment and placebo arms. Treated children had higher levels of anemia (21%) and some developed mild iron deficiency requiring dietary supplementation. Three patients discontinued due to moderate neutropenia.

These studies suggest that moderate dose deferiprone has a safety profile that is more acceptable for use in chronic conditions.

Sources and dosing:

PBT434 is still in clinical testing by Alterity Therapeutics, and the therapeutic dose for this orally available formulation has not yet been established.

Deferoxamine is marketed by Novartis under the trade name Desferal®. It is FDA approved for acute iron toxicity and chronic iron overload (FDA label). It can be administered intravenously, intramuscularly, or subcutaneously, but intramuscular is the recommended route of administration. It is typically given at doses of 500 to 1000 mg for patients with iron overload. It is not currently approved for clinical use in an intranasal formulation.

Deferiprone is marketed as an oral solution by ApoPharma under the trade name Ferriprox®. It is FDA approved for use in transfusional iron overload due to thalassemia as a second line treatment (FDA label). It is typically given as an 80 mg/mL oral solution administered three times daily for a total dose of 75 mg/kg/day. A delayed release oral formulation used at lower doses (15 mg/kg/dose twice a day) is currently in clinical testing for several neurodegenerative diseases.

Research underway:

PBT434: The Phase 1 clinical trial (ACTRN12618000541202) for PBT434 in healthy volunteers was recently completed in July 2019 (Press Release). It is the lead clinical candidate of Alterity Therapeutics, and they are initially focusing on developing PBT434 for Multiple Systems Atrophy, and have received an Orphan Drug Designation for this indication.

Deferiprone: Moderate dose deferiprone in a delayed-release tablets (600 mg) is currently being tested in several proof-of-concept clinical trials.

3D study for AD (Deferiprone to Delay Dementia) is a clinical proofof -concept study. It is a 54 week Phase 2 RCT for patients with MCI, prodromal AD, or mild AD sponsored by Neuroscience Trials Australia (NCT03234686). It has an expected completion date of December 2021.

SKY study for PD (Study of Parkinson's Early Stage) is for newly diagnosed Parkinson's patients in Canada, France, Germany, and the UK. It is being sponsored by ApoPharma (NCT02728843). It has enrolled 140 patients and is expected to conclude in December 2019.

FAIRPARK II study for PD is a five-year EU-funded research project involving the collaboration of 15 partners in a multicenter trial testing conservative iron chelation for PD. Patients will be treated for 9 months. There is an estimated enrollment of 338 and the estimated completion date is February 2021 (NCT02655315).

FAIR-ALS II study for ALS is a Phase 2/3 12-month RCT testing conservative iron chelation for ALS. It is sponsored by the University Hospital, Lille/ France Ministry of Health (NCT03293069). It has an estimated enrollment of 240 and is expected to be completed in April 2022.

Search terms:

Pubmed, Google: PBT434, PBT2, Deferiprone, Intranasal deferoxoamine, 'iron chelator'

- Alzheimer's disease, Parkinson's disease, neurodegeneration, cogntive decline, aging, cardiovascular, diabetes, cancer, clinical trials, safety

Websites visited for Iron chelators:

- Clinicaltrials.gov (Deferiprone, PBT2)
- ANZCTR.org.au (PBT434)
- WebMD.com (Deferoxamine, Deferiprone)
- PubChem (Deferoxamine, Deferiprone)
- Drugs.com (Deferoxamine, Deferiprone)
- DrugBank.ca (Deferoxmaine, Deferiprone

References:

1. Xu J, Jia Z, Knutson MD *et al.* (2012) Impaired iron status in aging research. *Int J Mol Sci* 13, 2368-2386.https://www.ncbi.nlm.nih.gov/pubmed/22408459 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3292028/

2. Liu J-L, Fan Y-G, Yang Z-S *et al.* (2018) Iron and Alzheimer's Disease: From Pathogenesis to Therapeutic Implications. *Front Neurosci* 12, 632-632.https://www.ncbi.nlm.nih.gov/pubmed/30250423 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6139360/

3. Finkelstein DI, Billings JL, Adlard PA *et al.* (2017) The novel compound PBT434 prevents iron mediated neurodegeneration and alpha-synuclein toxicity in multiple models of Parkinson's disease. *Acta Neuropathol Commun* 5, 53-53.https://www.ncbi.nlm.nih.gov/pubmed/28659169 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5490188/

4. Ashraf A, Clark M, So P-W (2018) The Aging of Iron Man. *Front Aging Neurosci* 10.https://www.frontiersin.org/article/10.3389/fnagi.2018.00065

5. Tao Y (2014) Perturbed iron distribution in Alzheimer's disease serum, cerebrospinal fluid, and selected brain regions: a systematic review and meta-analysis. *Journal of Alzheimer's disease* 42, 679-690

6. Du L, Zhao Z, Cui A *et al.* (2018) Increased Iron Deposition on Brain Quantitative Susceptibility Mapping Correlates with Decreased Cognitive Function in Alzheimer's Disease. *ACS Chemical Neuroscience* 9, 1849-1857.https://doi.org/10.1021/acschemneuro.8b00194

7. Belaidi AA, Bush AI (2016) Iron neurochemistry in Alzheimer's disease and Parkinson's disease: targets for therapeutics. *Journal of Neurochemistry* 139, 179-197.https://doi.org/10.1111/jnc.13425

8. Hare DJ, Doecke JD, Faux NG *et al.* (2015) Decreased Plasma Iron in Alzheimer's Disease Is Due to Transferrin Desaturation. *ACS Chemical Neuroscience* 6, 398-402.https://doi.org/10.1021/cn5003557

9. Ayton S, Faux NG, Bush AI *et al.* (2015) Ferritin levels in the cerebrospinal fluid predict Alzheimer's disease outcomes and are regulated by APOE. *Nat Commun* 6, 6760-6760.https://www.ncbi.nlm.nih.gov/pubmed/25988319 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4479012/

10. Ayton S, Wang Y, Diouf I *et al.* (2019) Brain iron is associated with accelerated cognitive decline in people with Alzheimer pathology. *Molecular Psychiatry*.https://doi.org/10.1038/s41380-019-0375-7

11. McLachlan DRC, Kruck TPA, Kalow W *et al.* (1991) Intramuscular desferrioxamine in patients with Alzheimer's disease. *The Lancet* 337, 1304-1308.http://www.sciencedirect.com/science/article/pii/014067369192978B

12. Lannfelt L, Blennow K, Zetterberg H *et al.* (2008) Safety, efficacy, and biomarker findings of PBT2 in targeting Ab; as a modifying therapy for Alzheimer's disease: a phase IIa, double-blind, randomised, placebo-controlled trial. *The Lancet Neurology* 7, 779-786.https://doi.org/10.1016/S1474-4422(08)70167-4

13. Adlard PA (2018) Metals and Alzheimer's Disease: How Far Have We Come in the Clinic? *Journal of Alzheimer's disease* 62, 1369-1379

14. Devos D, Moreau C, Devedjian JC *et al.* (2014) Targeting chelatable iron as a therapeutic modality in Parkinson's disease. *Antioxid Redox Signal* 21, 195-210.https://www.ncbi.nlm.nih.gov/pubmed/24251381 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4060813/

15. Martin-Bastida A, Ward RJ, Newbould R *et al.* (2017) Brain iron chelation by deferiprone in a phase 2 randomised double-blinded placebo controlled clinical trial in Parkinson's disease. *Sci Rep* 7, 1398-1398.https://www.ncbi.nlm.nih.gov/pubmed/28469157 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5431100/

16. Moreau C, Danel V, Devedjian JC *et al.* (2018) Could Conservative Iron Chelation Lead to Neuroprotection in Amyotrophic Lateral Sclerosis? *Antioxid Redox Signal* 29, 742-748.https://www.ncbi.nlm.nih.gov/pubmed/29287521 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6067092/

17. Finkelstein D, Stefanova N, Adlard P *et al.* (2019) PBT434 Prevents α-synuclein Aggregation, Neuron Loss, Motor Dysfunction and Reduces Glial Cell Inclusions in a Transgenic Mouse Model of Multiple System Atrophy (P5.8-006). *Neurology* 92, P5.8-006

18. Hanson LR, Roeytenberg A, Martinez PM *et al.* (2009) Intranasal deferoxamine provides increased brain exposure and significant protection in rat ischemic stroke. *J Pharmacol Exp Ther* 330, 679-686.https://www.ncbi.nlm.nih.gov/pubmed/19509317 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2729791/

19. Hanson LR, Fine JM, Renner DB *et al.* (2012) Intranasal delivery of deferoxamine reduces spatial memory loss in APP/PS1 mice. *Drug Delivery and Translational Research* 2, 160-168.https://doi.org/10.1007/s13346-011-0050-2

20. Fine JM, Baillargeon AM, Renner DB *et al.* (2012) Intranasal deferoxamine improves performance in radial arm water maze, stabilizes HIF-1α, and phosphorylates GSK3β in P301L tau transgenic mice. *Experimental Brain Research* 219, 381-390.https://doi.org/10.1007/s00221-012-3101-0

21. Fine JM, Renner DB, Forsberg AC *et al.* (2015) Intranasal deferoxamine engages multiple pathways to decrease memory loss in the APP/PS1 model of amyloid accumulation. *Neuroscience Letters* 584, 362-367.http://www.sciencedirect.com/science/article/pii/S0304394014008805

22. Fine JM, Forsberg AC, Stroebel BM *et al.* (2017) Intranasal deferoxamine affects memory loss, oxidation, and the insulin pathway in the streptozotocin rat model of Alzheimer's disease. *Journal of the Neurological Sciences* 380, 164-171.http://www.sciencedirect.com/science/article/pii/S0022510X17304628

23. Febbraro F, Andersen KJ, Sanchez-Guajardo V *et al.* (2013) Chronic intranasal deferoxamine ameliorates motor defects and pathology in the α-synuclein rAAV Parkinson's model. *Experimental Neurology* 247, 45-58.http://www.sciencedirect.com/science/article/pii/S0014488613001106

24. Fine JM, Forsberg AC, Renner DB *et al.* (2014) Intranasally-administered deferoxamine mitigates toxicity of 6-OHDA in a rat model of Parkinson's disease. *Brain Research* 1574, 96-104.http://www.sciencedirect.com/science/article/pii/S0006899314007793

25. Guo C, Hao L-J, Yang Z-H *et al.* (2016) Deferoxamine-mediated up-regulation of HIF-1α prevents dopaminergic neuronal death via the activation of MAPK family proteins in MPTP-treated mice. *Experimental Neurology* 280, 13-23.http://www.sciencedirect.com/science/article/pii/S0014488616300590

26. Guo C, Zhang Y-X, Wang T *et al.* (2015) Intranasal deferoxamine attenuates synapse loss via up-regulating the P38/HIF-1α pathway on the brain of APP/PS1 transgenic mice. *Front Aging Neurosci* 7, 104-104.https://www.ncbi.nlm.nih.gov/pubmed/26082716 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4451419/

27. Guo C, Wang P, Zhong M-L *et al.* (2013) Deferoxamine inhibits iron induced hippocampal tau phosphorylation in the Alzheimer transgenic mouse brain. *Neurochemistry International* 62, 165-172.http://www.sciencedirect.com/science/article/pii/S0197018612003956

28. Pan K, Li X, Chen Y *et al.* (2016) Deferoxamine pre-treatment protects against postoperative cognitive dysfunction of aged rats by depressing microglial activation via ameliorating iron accumulation in hippocampus. *Neuropharmacology* 111, 180-194.http://www.sciencedirect.com/science/article/pii/S0028390816303793

29. Prasanthi JRP, Schrag M, Dasari B *et al.* (2012) Deferiprone reduces amyloid-β and tau phosphorylation levels but not reactive oxygen species generation in hippocampus of rabbits fed a cholesterol-enriched diet. *J Alzheimers Dis* 30, 167-182.https://www.ncbi.nlm.nih.gov/pubmed/22406440 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3645354/

30. Zhu Y, Wang B, Tao K *et al.* (2017) Iron accumulation and microglia activation contribute to substantia nigra hyperechogenicity in the 6-OHDA-induced rat model of Parkinson's disease. *Parkinsonism & Related Disorders* 36, 76-82.http://www.sciencedirect.com/science/article/pii/S1353802017300032

31. Agrawal S, Fox J, Thyagarajan B *et al.* (2018) Brain mitochondrial iron accumulates in Huntington's disease, mediates mitochondrial dysfunction, and can be removed pharmacologically. *Free Radic Biol Med* 120, 317-329.https://www.ncbi.nlm.nih.gov/pubmed/29625173 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5940499/

32. Tisato V, Zuliani G, Vigliano M *et al.* (2018) Gene-gene interactions among coding genes of iron-homeostasis proteins and APOE-alleles in cognitive impairment diseases. *PLOS ONE* 13, e0193867.https://doi.org/10.1371/journal.pone.0193867

33. Masaldan S, Clatworthy SAS, Gamell C *et al.* (2018) Iron accumulation in senescent cells is coupled with impaired ferritinophagy and inhibition of ferroptosis. *Redox Biol* 14, 100-115.https://www.ncbi.nlm.nih.gov/pubmed/28888202 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5596264/

34. Masaldan S, Belaidi AA, Ayton S *et al.* (2019) Cellular Senescence and Iron Dyshomeostasis in Alzheimer's Disease. *Pharmaceuticals* 12, 93.https://www.mdpi.com/1424-8247/12/2/93

35. Gaur K, Vázquez-Salgado AM, Duran-Camacho G *et al.* (2018) Iron and Copper Intracellular Chelation as an Anticancer Drug Strategy. *Inorganics* 6, 126.https://www.mdpi.com/2304-6740/6/4/126

36. Stevens RG, Graubard BI, Micozzi MS *et al.* (1994) Moderate elevation of body iron level and increased risk of cancer occurrence and death. *International Journal of Cancer* 56, 364-369.https://onlinelibrary.wiley.com/doi/abs/10.1002/ijc.2910560312

37. Wen CP, Lee JH, Tai Y-P *et al.* (2014) High Serum Iron Is Associated with Increased Cancer Risk. *Cancer Research* 74, 6589-6597.https://cancerres.aacrjournals.org/content/canres/74/22/6589.full.pdf

38. Quintana Pacheco DA, Sookthai D, Graf ME *et al.* (2018) Iron status in relation to cancer risk and mortality: Findings from a population-based prospective study. *International Journal of Cancer* 143, 561-569.https://onlinelibrary.wiley.com/doi/abs/10.1002/ijc.31384

39. Fryknäs M, Zhang X, Bremberg U *et al.* (2016) Iron chelators target both proliferating and quiescent cancer cells. *Sci Rep* 6, 38343-38343.https://www.ncbi.nlm.nih.gov/pubmed/27924826 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5141479/

40. Katsura Y, Ohara T, Noma K *et al.* (2019) A Novel Combination Cancer Therapy with Iron Chelator Targeting Cancer Stem Cells via Suppressing Stemness. *Cancers (Basel)* 11, 177.https://www.ncbi.nlm.nih.gov/pubmed/30717462 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6406536/

41. Basuli D, Stevens RG, Torti FM *et al.* (2014) Epidemiological associations between iron and cardiovascular disease and diabetes. *Front Pharmacol* 5, 117-117.https://www.ncbi.nlm.nih.gov/pubmed/24904420 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4033158/

42. Salonen JT, Nyyssönen K, Korpela H *et al.* (1992) High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. *Circulation* 86, 803-811.https://www.ahajournals.org/doi/abs/10.1161/01.CIR.86.3.803

43. der DLv, Grobbee DE, Roest M *et al.* (2005) Serum Ferritin Is a Risk Factor for Stroke in Postmenopausal Women. *Stroke* 36, 1637-1641.https://www.ahajournals.org/doi/abs/10.1161/01.STR.0000173172.82880.72

44. Kiechl S, Aichner F, Gerstenbrand F *et al.* (1994) Body iron stores and presence of carotid atherosclerosis. Results from the Bruneck Study. *Arteriosclerosis and Thrombosis: A Journal of Vascular Biology* 14, 1625-1630.https://www.ahajournals.org/doi/abs/10.1161/01.ATV.14.10.1625

45. Morrison HI, Semenciw RM, Mao Y *et al.* (1994) Serum Iron and Risk of Fatal Acute Myocardial Infarction. *Epidemiology* 5, 243-246.http://www.jstor.org/stable/3702367

46. de Valk B, Marx JJM (1999) Iron, Atherosclerosis, and Ischemic Heart Disease. *JAMA Internal Medicine* 159, 1542-1548.https://doi.org/10.1001/archinte.159.14.1542

47. Jiang R, Manson JE, Meigs JB *et al.* (2004) Body Iron Stores in Relation to Risk of Type 2 Diabetes in Apparently Healthy Women. *JAMA* 291, 711-717.https://doi.org/10.1001/jama.291.6.711

48. Montonen J, Boeing H, Steffen A *et al.* (2012) Body iron stores and risk of type 2 diabetes: results from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study. *Diabetologia* 55, 2613-

2621.https://www.ncbi.nlm.nih.gov/pubmed/22752055
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3433660/

49. Bonham CA, Rodrigues M, Galvez M *et al.* (2018) Deferoxamine can prevent pressure ulcers and accelerate healing in aged mice. *Wound Repair and Regeneration* 26, 300-305.https://onlinelibrary.wiley.com/doi/abs/10.1111/wrr.12667

50. Stamler D, Bradbury M, Wong C *et al.* (2019) A First in Human Study of PBT434, a Novel Small Molecule Inhibitor of α-Synuclein Aggregation (S4.001). *Neurology* 92, S4.001

51. Klopstock T, Tricta F, Neumayr L *et al.* (2019) Safety and efficacy of deferiprone for pantothenate kinase-associated neurodegeneration: a randomised, double-blind, controlled trial and an open-label extension study. *The Lancet Neurology* 18, 631-642.https://doi.org/10.1016/S1474-4422(19)30142-5