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David Devos, Z Ioav Cabantchik, Caroline Moreau, Véronique Danel, Laura Mahoney-Sanchez, Hind Bouchaoui, Flore Gouel, Anne-Sophie Rolland, James A Duce, Jean-Christophe Devedjian

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Conservative iron chelation for neurodegenerative diseases such as  
Parkinson’s disease and...

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# Conservative iron chelation for neurodegenerative diseases such as Parkinson’s disease and amyotrophic lateral sclerosis

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## Abstract

Focal iron accumulation associated with brain iron dyshomeostasis is a pathological hallmark of various neurodegenerative diseases (NDD). The application of iron-sensitive sequences in magnetic resonance imaging has provided a useful tool to identify the underlying NDD pathology. In the three major NDD, degeneration occurs in central nervous system (CNS) regions associated with memory (Alzheimer's disease, AD), automaticity (Parkinson's disease, PD) and motor function (amyotrophic lateral sclerosis, ALS), all of which require a high oxygen demand for harnessing neuronal energy. In PD, a progressive degeneration of the substantia nigra pars compacta (SNc) is associated with the appearance of siderotic foci, largely caused by increased labile iron levels resulting from an imbalance between cell iron import, storage and export. At a molecular level,  $\alpha$ -synuclein regulates dopamine and iron transport with PD-associated mutations in this protein causing functional disruption to these processes. Equally, in ALS, an early iron accumulation is present in neurons of the cortico-spinal motor pathway before neuropathology and secondary iron accumulation in microglia. High serum ferritin is an indicator of poor prognosis in ALS and the application of iron-sensitive sequences in magnetic resonance imaging has become a useful tool in identifying pathology. The molecular pathways that cascade down from such dyshomeostasis still remain to be fully elucidated but strong inroads have been made in recent years. Far from being a simple cause or consequence, it has recently been discovered that these alterations can trigger susceptibility to an iron-dependent cell-death pathway with unique lipoperoxidation signatures called ferroptosis. In turn, this has now provided insight into some key modulators of this cell-death pathway that could be therapeutic targets for the



NDD. Interestingly, iron accumulation and ferroptosis are highly sensitive to iron chelation. However, whilst chelators that strongly scavenge intracellular iron protect against oxidative neuronal damage in mammalian models and are proven to be effective in treating systemic siderosis, these compounds are not clinically suitable due to the high risk of developing iatrogenic iron depletion and ensuing anaemia. Instead, a moderate iron chelation modality that conserves systemic iron offers a novel therapeutic strategy for neuroprotection. As demonstrated with the prototype chelator deferiprone, iron can be scavenged from labile iron complexes in the brain and transferred (conservatively) either to higher affinity acceptors in cells or extracellular transferrin. Promising preclinical and clinical proof of concept trials has led to several current large randomized clinical trials that aim to demonstrate the efficacy and safety of conservative iron chelation for NDD, notably in a long-term treatment regimen.

AQ1

AQ2

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## Keywords

Parkinson's disease  
Amyotrophic lateral sclerosis  
Conservative iron chelation  
Ferroptosis  
Iron metabolism

Flore Gouel, Anne-Sophie Rolland, James A. Duce, Jean-Christophe Devedjian contributed equally.

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## Introduction

The development of disease-modifying therapies for slowing down progression of neurodegenerative disorders remains a major clinical challenge. A pathognomonic component of Parkinson's disease (PD) and to a lesser extent, amyotrophic lateral sclerosis (ALS) is the formation of labile iron, respectively, in the substantia nigra pars compacta (SNc) or motor neurons and microglia of the central and peripheral motor pathways. A very recent metanalysis confirmed that iron concentration was constantly increased in the substantia nigra of PD patients (18 studies 211 Parkinson's disease, 215 control) (Sian et al. 2019). A less obvious iron accumulation in ALS is likely to be due to the severity and speed in progression of neurodegeneration in the disease and this observation is substantiated by the accumulation of iron in the remaining phagocytic microglia late in the disease. The presence of such a catalytically active and chelatable

form of iron has been implicated in an increased production of noxious reactive oxygen species (ROS) and ensuing oxidative damage of dopaminergic neurons. Since cells normally regulate their iron levels by safely diverting excess cytosolic metal towards ferritin-iron shells or effluxing excess iron out of the cell, the generation of oxidative cell damage proximal to foci of iron accumulation reflects a state of disrupted iron and/or redox homeostasis. The chemical damage is attributed to a combination of labile iron propensity for ROS formation and the biochemical as well as chemical antioxidant capability of a given cell to cope not only with intracellular iron levels but also ROS. In this context, cells with high aerobic metabolic profiles (e.g. dopaminergic neurons, motor neurons and hippocampus in the CNS) are among the most susceptible to iron-mediated damage proximal to the siderotic foci that are present in the SN of PD and neuromotor pathways of ALS. Thus, focal iron detoxification by metal chelation has been considered as a potential therapeutic strategy in neurodegenerative diseases for some time, provided the treatment compromises neither healthy brain cells nor the systemic iron status of the organism. These factors led to the concept and eventual strategy of conservative chelation, namely the scavenging of labile iron by chelating agents endowed with an ability to recycle the chelated metal back into circulation via the physiological carrier transferrin.

### AQ3

In early studies with animal models of neurodegeneration, chelators were designed originally to treat systemic siderosis but found serendipitously to cross the BBB conferred neuroprotection from oxidative damage. However, for coping with these disorders in a clinical setting, it is imperative that chelation should not result in iatrogenic ID and ensuing anaemia. This consideration led to the development of a more “moderate” iron chelation modality that conserves systemic iron, offering a novel (safe) therapeutic strategy for neuroprotection whilst not causing anaemia.

## Overview of iron homeostasis in the brain

As a more detailed review of iron homeostasis has recently been provided (Ward et al. 2014), a synopsis of brain iron transport is as follows: when required by the brain, peripheral iron is able to cross the vascular endothelial cells of the blood–brain barrier, predominantly by import into the endothelial cells through the diferric transferrin receptor 1 (TFR1) complex system and export into the intracerebral space via ferroportin (Fpn); the only known exporting pore protein for cellular iron (Ward et al. 2014). The other cell types within the brain, such as oligodendrocytes, astrocytes, microglia and neurons, obtain their required iron by extraction from the brain interstitial compartment using a range of cell-dependent

import mechanism (predominantly not only TFR1 and Divalent metal transporter 1 but also other members of the metal transporter ZIP (SLC39A) family). The flux of iron between these cell types is continuous through mechanisms that are still being elucidated. Neurons are thought to predominantly acquire their iron via TFR1, and efflux via Fpn is facilitated by its stabilization to its functional location on the cell surface by  $\beta$ -amyloid precursor protein (APP) (Duce et al. 2010; McCarthy et al. 2014; Wong et al. 2014) and ceruloplasmin (CP) through both non- and an autonomous cellular processes. In healthy ageing, several brain regions are susceptible to small deposits of iron, with this iron safely bound within ferritin, neuromelanin and in some cases hemosiderin. However, a greater accumulation of iron than that reported in healthy ageing occurs in specific brain regions of many neurodegenerative diseases and may contribute to neurodegenerative processes.

AQ4

## Cellular mechanisms implicated in iron redistribution in PD and ALS

Elevated pro-oxidant labile iron in the SNc of PD or motor neurons and microglia of ALS, in particular within the mitochondrial subcellular compartment, has been proposed to result from an altered ability of cells to regulate iron levels and distribution. This may arise from impaired iron influx/efflux, altered iron storage or from deranged utilization (Hare and Double 2016).

### Impaired iron release

Fpn in the SNc is depleted in several parkinsonian models including intoxication with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA) (Wang et al. 2007; Lee et al. 2009; Finkelstein et al. 2017). Levels of this protein are less clear in ALS models, where Fpn is decreased in only one pathological region of a transgenic mouse model with the G93A familial mutation in SOD1 (Halon et al. 2014; Gajowiak et al. 2016) and increased in an alternative model with the familial SOD1<sup>G37R</sup> mutation. However, the authors suggest that the Fpn increase in the SOD1<sup>G37R</sup> is not sufficient enough to counter the dramatic increase in expression of proteins involved in iron import and thus conclude that this has little effect on the overall iron accumulation in this model (Jeong et al. 2009).

Depletion of either APP or tau (required for the transport of APP to the cell surface (Lei et al. 2012) causes cellular iron retention as well as iron-dependent nigral cell loss. As historically recognised neuropathogenic proteins, APP and tau are not just altered in AD but markedly decreased in the SNc in PD (Lei et al.

2012; Ayton et al. 2013). Tau is often also identified to be post-translationally modified in motor neurons from ALS (Stevens et al. 2019). Several rare variants of APP predispose individuals to PD<sup>10</sup> and some familial Alzheimer's disease (AD) patients that carry mutations in APP present with a parkinsonian phenotype and Lewy body pathology.

Cellular iron egress by ferroportin is assisted by ceruloplasmin, particularly in glia. The physiological importance of ceruloplasmin in the brain is exemplified by aceruloplasminemia; caused by loss of function mutations in CP that results in phenotypic characteristics similar to PD. Indeed, ceruloplasmin deficient mice develop age-dependent parkinsonism and the nigral iron elevation in the MPTP model of parkinsonism can be rescued by peripheral ceruloplasmin infusion (Ayton et al. 2013). Low ceruloplasmin activity has been identified in the SN, cerebrospinal fluid (CSF) and serum of patients with PD (reviewed in 1). Point mutations in the ceruloplasmin-encoding gene (especially the D544E mutation) are significantly associated with parkinsonism<sup>11</sup> and R793H has been found to segregate with SN hyperechogenicity in PD (Hochstrasser et al. 2005; Ayton et al. 2013; Barbariga et al. 2015). In SOD1 models of ALS, initial observations with CP are consistent with Fpn whereby the SOD1<sup>G93A</sup> mutation gives rise to no change to CP levels in most pathological regions but the SOD1<sup>G37R</sup> mutation elevates CP levels (Jeong et al. 2009; Halon et al. 2014; Gajowiak et al. 2016). Of relevance, more recently, it has been determined that whilst total levels are elevated in the SOD1<sup>G37R</sup> model, CP accumulates in a copper-deficient inactive form that is unable to facilitate iron efflux through Fpn (Hilton et al. 2018). This disparity between total levels and the enzymatic activity of CP is similarly observed in ALS patients (Conti et al. 2008).

### Altered iron storage

The limited capacity of select neurons to sequester surplus iron into ferritin molecules, (Ward et al. 2014; Belaidi and Bush 2016) can be countered by neuromelanin being used as an alternative “iron sink”(Zucca et al. 2017). However, such capacities may still be exceeded in both PD and ALS [reviewed in (Belaidi and Bush 2016)]. Importantly, the number of ferritin-immunoreactive microglia is markedly increased in the SN of PD patients, especially in close vicinity to neuromelanin-containing neurons. These microglia contain enhanced amounts of ferritin (Wu et al. 2017) and the ferritin cores in the SN of PD patients harbour relatively greater levels of iron compared to those of healthy subjects. As elevated levels of densely iron-loaded ferritin may promote free radical formation, this metastable reservoir of iron over time may contribute to age-related neurodegeneration (Ward et al. 2014; Belaidi and Bush 2016; Zucca et al. 2017). Whilst little has been reported on ferritin levels in ALS patient brain

tissue, it is a strong peripheral indicator in CSF and serum (see below) and has been identified to be altered in various models of ALS. In the SOD1<sup>G93A</sup> rat model, ferritin is elevated with disease progression (Halon et al. 2014) and an elevated ubiquitination of this protein may lead to an inability of it to control the labile iron pool (Halon et al. 2010). In aged SOD<sup>G37R</sup> transgenic mice, an increased cytosolic ferritin expression only occurs in glia, mainly microglia and some astrocytes, but not in neurons. The presence of the iron-positive inclusions in motor neurons of this model with the lack of cytosolic ferritin response suggests that glia and neurons may retain and accumulate iron via different mechanisms (Jeong et al. 2009).

### Increased iron uptake

Analysis of single nucleotide polymorphisms in PD case–control studies have identified a protective role of genetic variations in transferrin (Tf) and its receptor (TfR) required for iron-bound Tf incorporation into the cell (Rhodes et al. 2014). Clinical studies have also shown increased transferrin saturation in ALS patient cohorts but a significant decrease in serum transferrin levels (Nadjar et al. 2012; Veyrat-Durebex et al. 2014). The iron transporter lactoferrin (Lf) and its receptor (LfR) may also play an important role as it has a higher affinity for iron than Tf (due to its off rate) and is increased during inflammation. The expression Lf and its receptor are both increased in PD and ALS (Leveugle et al. 1994; Hirsch 2006). As LfR expression is not regulated by intracellular iron concentration, the Lf/LfR system may well be non-autonomously responsible for increased iron uptake during neurodegeneration.(Faucheux et al. 1995).

Iron import through the non-transferrin family may also be affected in disease. Iron accumulation in the SNc of PD patients and the MPTP mouse model correlates with elevation of the divalent metal transporter 1 (DMT1) (Belaidi and Bush 2016) and also with Nedd4 family-interacting protein1 (Ndfip1); a ubiquitin ligase that regulates DMT1 expression most notably in non-neuronal cell types such as astrocytes (Howitt et al. 2014). In a SOD<sup>G93A</sup> cell model, both DMT1 and TfR1 are unregulated and this is replicated within the spinal cord from SOD<sup>G37R</sup> transgenic mice (Jeong et al. 2009; Khadzhiev et al. 2013).

### Impaired iron redistribution and inflammation

Compounding evidence has identified a close association with the innate immune response and iron regulatory systems. It is, therefore, intriguing that there are multiple occurrences of overlap between these systems in NDD. In-vitro studies show that inflammation [stimulated either by nuclear factor—kappaB, tumour necrosis factor  $\alpha$ , interleukin 6, or lipopolysaccharide (LPS)] results in neuronal and microglial iron accumulation but has no reported effect with astrocytic levels

(Ward et al. 2014). The intraneuronal retention of iron following intracranial injection of LPS also results in microglia activation, oxidative stress and mitochondrial impairment. Whilst there is a direct impact on dopaminergic neurodegeneration within the SN (Zhang et al. 2014) motor neuron degeneration in ALS is thought to occur via the microglial activation induced by inflammation (Frakes et al. 2014). One possible pathway in which a pro-inflammatory response is able to cause neuronal iron retention is via cytokine-induced down-regulation of Fpn. However, heme oxygenase-1 and inducible NO synthase are also up regulated and may contribute to the general mechanism (Zhang et al. 2014). As both inflammation and increased cytokine expression have been reported in the spinal cord of SOD1 transgenic mice (Jeong et al. 2009), this may also be an underlying factor to the increased levels of ferritin and capacity to store iron in microglial. Whilst this not likely to be acutely detrimental to microglia, prolonged elevated levels in iron could lead to iron-mediated toxicity as has been demonstrated with glia forced to markedly increase their ferritin levels to compensate for a loss of efflux iron capability (Jeong et al. 2009). Of note, iron response element transcripts that are not directly regulated by iron can instead be affected by factors such as proinflammatory cytokines and thus may be a contributory factor in the iron associated detrimental changes observed in the spinal cord of SOD1 transgenic mice (Ghezzi and Mennini 2001; Elliott 2001; Hensley et al. 2002; Hensley 2003).

Lastly, hepcidin is a key peripheral regulator of the iron entry into circulation by inhibiting cellular iron efflux via Fpn and is rapidly elevated during inflammation (Ward et al. 2014). As peripherally released hepcidin can easily cross the BBB and select cells in the brain secrete this peptide, it is likely to be an important regulator in provoking decreased ferroportin expressing on the plasma membrane of astrocytes, microglia, and neurons. Despite this being a strong biomarker in other iron overload diseases, to date, there has been no clear relationship in hepcidin levels in the brain and changes to iron or iron response proteins with PD (Ward et al. 2014) or ALS (Jeong 2006).

## Role of iron in neurons

### In dopaminergic neurons

Iron is particularly abundant in the SNc dopaminergic neurons due to it being an integral component of dopamine synthesis through the tyrosine hydroxylase (TH) pathway as well as other enzymatic and non-enzymatic reactions associated with dopamine metabolism (Meiser et al. 2013). The presence of brain labile non-heme high-spin complexes that increase with age (Wofford et al. 2017) might explain the iron catalytic role in the generation of:

- a. Noxious ROS by Fenton chemistry involving hydrogen peroxide; a result in part from the oxidative deamination of dopamine by monoamine oxidase (MAO).
- b. Metastable iron-dopamine complexes that lead to dopamine auto-oxidation and quinone formation.

These, in turn, will generate a variety of potentially toxic products sequestered by neuromelanin (e.g. 6-OHDA) and confer a distinctive pigmentation upon the SNc. However, as the neuromelanin sanctuary for toxins is lost during PD, (Hare and Double 2016; Zucca et al. 2017) free 6-OHDA (the endogenous auto-oxidation product of dopamine) can strongly inhibit mitochondrial complexes I and IV and thereby exert neurotoxicity. The iron enrichment in the SNc is necessary for the high energy demands required by the active dopaminergic neurons with autonomous pace-making activity. But this higher energy demand also renders the SNc more susceptible to an imbalance in labile iron level and ensuing ROS production (Guzman et al. 2010). This may explain the selective contribution to oxidative stress in the SNc that is exerted by the 6-OHDA neurotoxin model of PD, whilst the ventral tegmental area remains relatively unaffected (Hare and Double 2016). Intriguingly, iron levels also influence the density of dopamine receptor D1 and D2 as well as dopamine transporter (DaT) expression (i.e. iron chelation downregulates DaT) (Hare and Double 2016; Belaidi and Bush 2016). Finally, iron is a cofactor of prolyl hydroxylases that regulate hypoxia-inducible factor 1 $\alpha$ , a major transcription factor required for survival (Rajagopalan et al. 2016). In summary, accumulation of SNc iron in the absence of adequate cell protective measures is a major contributory factor in impairing dopaminergic neurophysiology and can exacerbate PD progression.

### In motor neurons

Dysregulation of iron homeostasis has been observed in several pre-clinical and clinical studies of ALS (Jeong et al. 2009; Kwan et al. 2012; Ignjatović et al. 2012; Veyrat-Durebex et al. 2014; Adachi et al. 2015; Lu et al. 2016; Golko-Perez et al. 2017). Indeed, a total iron increase has been measured in SOD1<sup>G93A</sup> transfected cells compared to wild type (WT), associated with higher mRNA expression of TfR1 and DMT1 (Jeong et al. 2009; Kwan et al. 2012; Ignjatović et al. 2012; Veyrat-Durebex et al. 2014; Adachi et al. 2015; Lu et al. 2016; Golko-Perez et al. 2017). Iron accumulation occurs both in motor neurons (MN) and glia in the SOD1<sup>G37R</sup> mice, with a significant increase in mitochondria in both cell population (Jeong et al. 2009). Because of these results, the therapeutic potential of iron chelators has been tested in vivo. Particularly, brain permeable iron-chelating drugs M30 and HLA20 protect the NSC-34 cell line against

oxidative stress and extend lifespan and delay the onset of the disease of SOD1<sup>G93A</sup> mice (Kupersmidt et al. 2009). Similar results are obtained with the VK-28 chelator, associated with limited elevated iron level and decreased TDP-43 aggregation (Wang et al. 2011). More recently, combined administration of M30 chelator with a high-Calorie Energy-supplemented Diet (CED) shows additive protective effect in SOD1<sup>G93A</sup> mice (Golko-Perez et al. 2016). Finally, the co-treatment of SOD1<sup>G93A</sup> mice after the appearance of the symptoms with CED and VAR10303 (a brain permeable chelating-radical scavenging drug) shows an increase of motor performance and lifespan, and limited iron accumulation and MN loss (Golko-Perez et al. 2017).

## Pivotal interplay between iron and canonical proteins involved in PD and ALS

### $\alpha$ -synuclein

The aggregation of  $\alpha$ -synuclein that contributes to intracellular inclusions (i.e. Lewy bodies) in dopaminergic neurons is a common neuropathological feature in PD. Iron can markedly induces to replace by induce aggregation of  $\alpha$ -synuclein and enables redox cycling as well as oxidative catalysis of lipids and dopamine metabolites (Duce et al. 2017). Moreover, the apparent increase in magnetic susceptibility used to measure iron deposition with Magnetic Resonance Imaging (MRI) sequence of Quantitative Susceptibility Mapping (QSM), follows a pattern of tight concordance with the distribution of  $\alpha$ -synuclein pathology in the dorsal SN, basal ganglia and cortex of PD (Acosta-Cabronero et al. 2017). Accordingly, iron chelation can reduce the amount of insoluble  $\alpha$ -synuclein deposits in the brains of murine synucleinopathy models (Ayton et al. 2015; Finkelstein et al. 2016).

Functionally,  $\alpha$ -synuclein has been strongly implicated in neurotransmitter storage and release at the synapse. By binding to the synaptic plasma membrane via vesicle-associated membrane protein 2 (Synaptobrevin-2/VAMP2),  $\alpha$ -synuclein is able to modulate neurotransmitter release controlled by the fusion and clustering of SNARE (Soluble N-ethylmaleimide-sensitive-factor attachment protein receptor)-associated vesicles (Duce et al. 2017). An interaction with vesicular monoamine transporter 2 (VMAT2), involved in vesicle filling, as well as the dopamine transporter (DAT) required for dopamine reuptake, also indicate that  $\alpha$ -synuclein might normally modulate dopamine recruitment and homeostasis (Butler et al. 2015). This is supported by evidence that  $\alpha$ -synuclein is a rate limiting factor in dopamine synthesis by tyrosine hydroxylase (TH). Since iron can regulate protein translation of  $\alpha$ -synuclein through its promoter region, (Duce et al. 2017) a role for  $\alpha$ -synuclein in modulating iron homeostasis can also be suggested. Depletion of  $\alpha$ -synuclein's functional role with the



membrane impairs the capacity for TfR to import iron and indicates that  $\alpha$ -synuclein could modulate clatherin-mediated endocytosis (Duce et al. 2017). Recently, neonatal iron-feeding of a transgenic mouse model overexpressing human  $\alpha$ -synuclein bearing the A53T mutation has been shown to exacerbate both PD-related motor and non-motor phenotypes, and that the deficits could be rescued by iron chelation. Although these observations were not accompanied by alterations in the  $\alpha$ -synuclein aggregation state, this does support an interaction between mutated  $\alpha$ -synuclein and iron homeostasis (Carboni et al. 2017).

## Tdp43

Misfolded or mislocalized RNA-binding proteins (and consequently altered mRNA processing) can cause neuronal dysfunction and even lead to neurodegeneration. A prominent example is the TAR DNA-binding protein of 43 kDa (TDP-43). Aggregates of TDP-43 are incorporated in ubiquitinated inclusions of the cytoplasm of motor neurons in ALS. SOD1 interact with TDP-43 (Higashi et al. 2010). Cai and colleagues (Cai et al. 2015) demonstrated that TDP-43 modification, including phosphorylation and truncation, was increased in the spinal cord of hSOD1G93A ALS mice and that SOD1 initiated the modification and accumulation of TDP-43 (Zeineddine et al. 2017; Jeon et al. 2019). In hSOD1G93A ALS mice, iron chelators reduced TDP-43 aggregation (Wang et al. 2011) but since oxidative stress-mediated accumulation of ROS promotes the TDP-43 aggregation (Cohen et al. 2012), the effect of iron chelator on TDP-43 aggregation may be indirect through a reduction in iron induced oxidative stress.

## Ferroptosis-a new iron-dependent cell-death pathway that may yield further therapeutic options

A new iron-dependent cell-death pathway that has recently come to light has strong implications in neuropathology. Ferroptosis appears to be selectively triggered by an iron-dependent mechanism with key features including lipid peroxidation, specific depletion of glutathione peroxidases-4 (Gpx4) to alter glutathione protection, mitochondriopathy and distinct morphological modifications that are independent from other cell-death pathways (e.g. apoptosis, necrosis and autophagy) (Dixon et al. 2012; Friedmann Angeli et al. 2014; Doll and Conrad 2017). Inhibition of the xCT cystine/glutamate antiporter during ferroptosis consequentially prevents cystine uptake into the cell and leads to lower levels of GSH synthesis and increases cellular availability of labile iron to catalyse lipid peroxidation (Fig. 2) (Dixon et al. 2012). Ferroptosis is associated with pathogenic changes observed in PD, including nigral iron elevation, mitochondriopathy, GSH depletion, lipid peroxidation, elevated ROS generation and oxidation of dopamine ( [Van Do](#) To replace by DoVan for all the

references

et al. 2016; Guiney et al. 2017). Ferroptosis has been identified to be present in vitro, using non-oncogenic dopaminergic neurons, ex vivo, on organotypically cultured striatal slices and in vivo in the MPTP mouse model (Van Do et al. 2016). Ferroptosis can be rescued by iron chelation (e.g. with DFP), (Dixon et al. 2012; Torii et al. 2016; Van Do et al. 2016) supporting the requirement for iron in the initiation of this cell-death pathway. Importantly, a range of inhibitors with greater specificity to ferroptosis (e.g. ferrostatin-1 and liproxstatin-1) have recently been designed with promising future implications in disease modification.

Gpx4 is also essential for motor neuron health and survival. In vivo, conditional ablation of Gpx4 in neurons of adult mice results in rapid onset and progressive paralysis and death. Spinal motor neuron degeneration induced by Gpx4 ablation exhibited features of ferroptosis (including lipid peroxidation) but not from apoptosis (no caspase-3 activation, no TUNEL staining). Supplementation with vitamin E, another inhibitor of ferroptosis, delayed the onset of paralysis and death induced by Gpx4 ablation (Chen et al. 2015).

Gpx4 is important in the protection against lipid peroxidation because of its ability to reduce hydroperoxides in lipids. Conversely, ACSL4 and LOX activities contribute to the cellular pool of lipid hydroperoxides that initiate ferroptosis. A robust anti-ferroptotic effect relies on a strongly reduced incorporation of long gamma-6 polyunsaturated fatty acids (such as arachidonic and adrenic acid) into phospholipids thus dramatically lowering the susceptibility to lipid peroxidation events in membranes. Trostchansky and colleagues showed that 12-hydroxyeicosatetraenoic acid (12-HETE), an LOX-derived oxidation product, increases with disease progression in SOD1G93A mice. Moreover, they demonstrated a protective role of Nitro-Oleic Acid in this ALS model due to its ability to cross the BBB and lower the observed increase in brain 12-HETE levels (Trostchansky et al. 2018).

## Iron deposits: advancements in an imaging biomarker

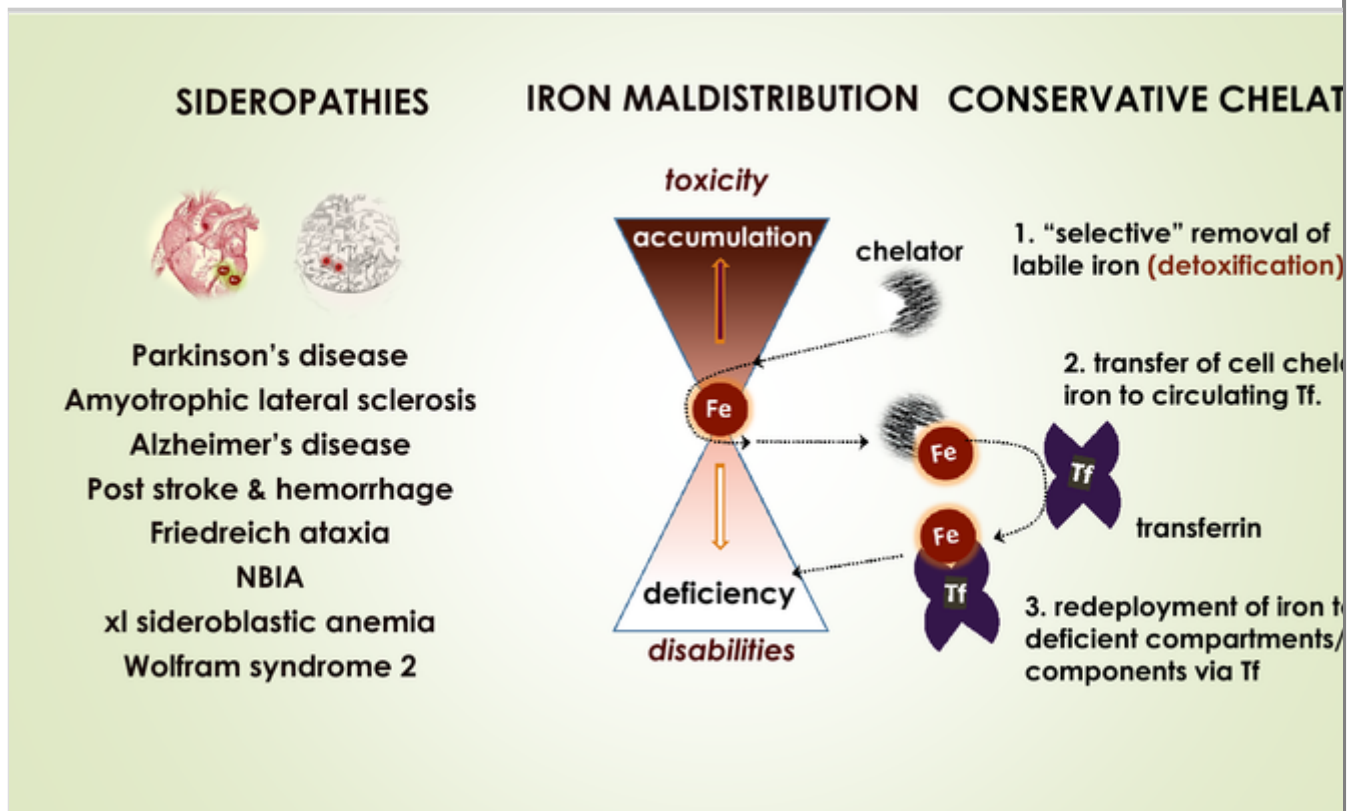
The association of ageing with elevated iron levels across PD brain regions is a risk factor most prominently found in the basal ganglia (caudate nucleus, putamen and globus pallidus) (Ramos et al. 2014). In post-mortem SNc of patients (Ayton et al. 2013, 2015; Ward et al. 2014; Belaidi and Bush 2016) as well as all Parkinsonian animal models (Kaur et al. 2003; Ayton et al. 2013, 2015; Devos et al. 2014; Ward et al. 2014; You et al. 2015; Lei et al. 2015; Belaidi and Bush 2016) relatively high iron accumulation has been observed and this has been confirmed by iron-sensitive high-field MRI (3 and 7 Tesla) with the

quantitative weighted T2\* sequence showing a higher  $R2^*$  value ( $R2^* = 1/T2^*$ ) of the SNc (Fig. 1) (Ulla et al. 2013; Rossi et al. 2014; Aquino et al. 2014; Hopes et al. 2016; Langley et al. 2017). Both longitudinal and meta-analysis studies in PD patients have shown iron overload with disease progression in the SNc and to a lesser extent in the putamen and caudate nucleus (Ulla et al. 2013; Hopes et al. 2016; Wang et al. 2016). Although hyperintensity of the dorsolateral SNc in PD has been noted by susceptibility weighted imaging (SWI) (Acosta-Cabronero et al. 2017; Nam et al. 2017), a recent meta-analysis of SWI data (Mahlknecht et al. 2017) demonstrated: (a) visual assessment of dorsolateral nigral hyperintensity to have excellent diagnostic accuracy for PD versus controls; a loss in hyperintensity could be a diagnostic marker of nigral pathology (i.e. nigrosome 1 degeneration) in PD. (b) An ability to differentiate neurodegenerative from non-neurodegenerative parkinsonian syndromes.

### **Fig. 1**

Conservative iron chelation. Maldistribution of iron is a feature common to several sideropathies, whereby excessive accumulation of the metal in particular loci results in the release of labile-toxic metal but also generates focal deficiencies and ensuing cell malfunctions. Removal of labile iron as means of detoxification by iron chelators is beneficial in diseases of systemic iron overload, but could be detrimental for disorders of focal iron accumulation in discrete brain or heart cells. The application of chelators endowed with the ability to transfer chelated iron to circulating transferrin (Tf) provides not only a safety tools to conserve iron systemically but also redeploy the metal to iron-deficient compartments or components. We define that property as conservative chelation

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These observations have been corroborated by a technique that is proposed to detect ferritin-iron, independent of hemosiderin spin dephasing, based on measurements of reduced transverse relaxation rates (RR2) (Bunzeck et al. 2013). A novel MRI approach with QSM has also recently demonstrated superior sensitivity for mapping the whole-brain landscape of magnetostatic alterations as a surrogate for changes in iron levels. In the dorsal SNc an increase in magnetic susceptibility is consistent with non-heme iron deposition and clinical PD status (Acosta-Cabronero et al. 2017; Wang et al. 2017). In addition, hyperechogenicity of the SNc visualized by transcranial ultrasound is also an established supplementary marker for PD diagnosis and can detect tissue that has increased iron levels and alterations in iron metabolism genes (Berg et al. 2002, 2006; Zecca et al. 2005). Local neuromelanin density is reduced in the SNc (predominantly the lateral-ventral tier), in concordance with pathology, as detected by a new sequence of magnetization transfer contrast (Huddleston et al. 2017). As neuromelanin in the SNc increases with age and decreases in PD, this suggests a neuroprotective role in which neuromelanin chelates metals and xenobiotics.

Iron accumulation was also observed in ALS patients decades ago (Ishikawa et al. 1993; Oba et al. 1993) using T2-weighted imaging in the motor cortex.

Recent advances in MRI sequences and post-processing images using T2\*-weighted gradient echo imaging (T2\*), Quantitative Susceptibility Mapping

(QSM) and Susceptibility-Weighted Images (SWI) have allowed the detected low signal intensity to be correlated with postmortem analysis and clinical data. With T2\*, hypointensities to the deeper layers of the motor cortex have been described in ALS patients and these have corresponded with an iron accumulation in microglial cells from these areas (Kwan et al. 2012) as well as a correlation between increased area of hypointensities and decreased ALSFRS (Ignjatović et al. 2013). In the same study, no significant difference was observed between bulbar or limb onset, although patients with bulbar onset did tend to have higher MRI scores. On SWI images, lower signal intensity of the precentral cortex was detected in ALS patients and this correlated with post-mortem analysis showing ferritin-positive staining in microglia and macrophages (Adachi et al. 2015). More recently, Vazquez-Costa and collaborators (Vázquez-Costa et al. 2018) found no differences in iron accumulation between genetic and non-genetic ALS, which suggest that genetic factors do not influence hypointensities in the motor cortex.

## A therapeutic strategy of conservative chelation based on iron scavenging and redeployment

The implication of siderosis and iron toxicity in NDD, notably PD and ALS, has largely been based on the protective effects of iron chelation in cell and animal models (Kaur et al. 2003; Jeong et al. 2009; Ayton et al. 2013, 2015; Weinreb et al. 2013; Ramos et al. 2014; Devos et al. 2014; Ward et al. 2014; Workman et al. 2015; Matak et al. 2016; Evans et al. 2016; Belaidi and Bush 2016; Golko-Perez et al. 2017; Zhu et al. 2017). However, for any chelator to be of clinical value in disorders of regional siderosis they must be endowed with a requisite accessibility to the relevant sites and differential specificity so as to spare unaffected areas of the organism from scavenging this essential element (Cabantchik et al. 2013). Multiple agents with iron-chelating features have been assessed preclinically in NDD models. In PD, this includes:

- Deferoxamine (DFO),
- 8-Hydroxyquinolines analogs such as clioquinol, VK28, M30 (a multimodal iron chelator that sequesters iron and inhibits MAO-A and MAO-B) and M10 (containing a peptide NAPVSIPQ and an iron-chelating moiety),
- Prochelators such as SIH-B and BSIH (derived from salicylaldehyde isocotinoyl hydrazine which is then converted to the active non-specific iron chelator SIH during oxidative stress),
- Aroylhydrazones (Youdim et al. 2005; Whitnall and Richardson 2006; Perez et al. 2008; Gal et al. 2010),

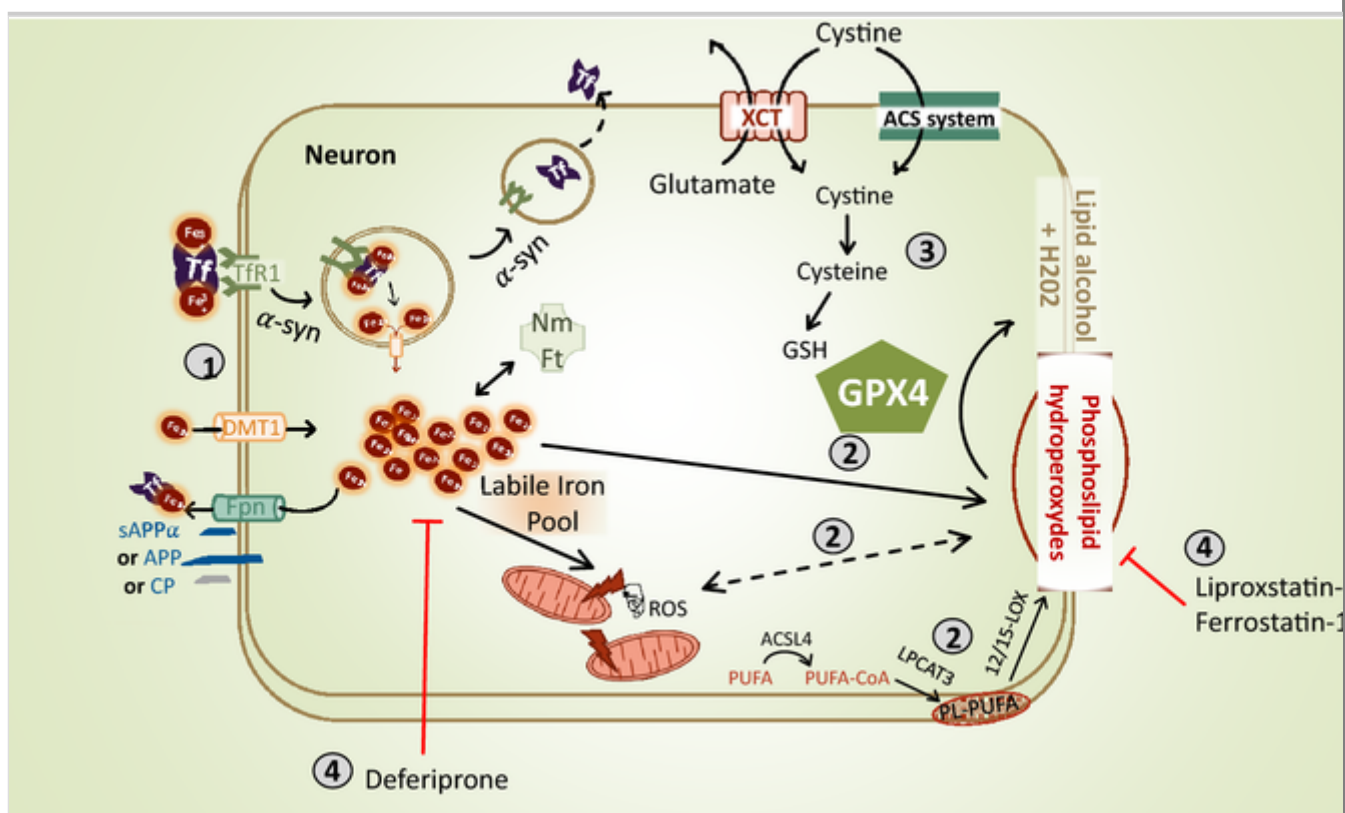
- Natural plant-derived polyphenol flavonoids,
- New multimodal iron chelators with multifunctional characteristics (Nuñez and Chana-Cuevas 2018).

As yet none of these compounds have progressed to clinical trial for PD and ALS apart from deferiprone (DFP). DFP is considered exceptional among iron chelators in its ability to cross membranes, including the blood brain barrier (BBB), (Cabantchik et al. 2013) and to chelate components of the cellular labile iron pool in brain tissue (Devos et al. 2014). DFP has the remarkable ability to rescue transfusional hemosiderosis in the heart of  $\beta$ -thalassemia patients without inducing anaemia. This capability of DFP is largely attributable to the redeployment of captured iron to extracellular iron free transferrin and then subsequent distribution (e.g. for uptake to iron-sulphur cluster and heme biosynthetic machineries) (Fig. 2) (Cabantchik et al. 2013).

## Fig. 2

Ferroptosis as a therapeutic target in Parkinson's disease. Alterations in the iron-regulatory pathway and phospholipid oxidation are implicated in Parkinson's disease pathology. **1** Increased intracellular iron occurs by enhanced import of iron within transferrin (Tf) through Transferrin receptor (TfR) endocytosis that is promoted by  $\alpha$ -synuclein ( $\alpha$ -syn), and increased import of  $\text{Fe}^{2+}$  through the divalent metal transporter 1 (DMT1). In addition, iron export is impaired through the destabilization of ferroportin (Fpn) on the cell surface by  $\beta$ -amyloid precursor protein (APP) or ceruloplasmin (CP). **2** When the storage protein neuromelanin (Nm) and ferritin (Ft) are no longer able to safely store intracellular iron, the labile pool of iron is elevated and catalyses the formation of phospholipid hydroperoxides. Free cytosolic polyunsaturated fatty acids (PUFA) are conjugated to coenzyme-A (CoA) by acyl-CoA synthetase long-chain family member 4 (ACSL4) allowing PUFA-CoA to be incorporated into the phospholipids in the plasma membrane. Phospholipid-PUFA are oxidised by lipoxygenases 12/15, contributing to the accumulation of phospholipid hydroperoxides at the plasma membrane level. Mitochondrial dysfunction, as reported in PD pathology result in increased ROS production which may also contribute to lipid peroxidation in the plasma membrane. Lipid peroxidation may also accumulate in mitochondrial membrane further disrupting mitochondrial function. **3** Cystine uptake through the  $X_c^-$  antiporter (in oxidative conditions) or the alanine, serine, cysteine–preferring (ASC) system (in reducing conditions) is required for biosynthesis of glutathione (GSH). Glutathione peroxidase 4 (Gpx4) uses 2 GSH molecules to safely reduce phospholipid hydroperoxides to their corresponding lipid-alcohols, producing  $\text{H}_2\text{O}$  and glutathione disulphide (GSSG) as byproducts. Elevated levels of intracellular iron with depletion of Gpx4, as evidenced in models of PD, promotes the

accumulation of phospholipid hydroperoxides leading to a disruption in membrane integrity through a ferroptotic pathway. **4** Reducing the labile iron pool (i.e. deferiprone) or depleting the phospholipid hydroperoxides (i.e. liproxstatin-1 or ferrostatin-1) are thus promising targets for inhibiting ferroptosis in PD pathology



This conservative repositioning strategy to subserve iron scavenging and redeployment has now been applied to both PD and ALS using DFP at the oral dose of 30 mg/kg/day (Devos et al. 2014; Moreau et al. 2018). In PD, an initial study used 40 early-stage patients with a disease duration of less than 3 years that were enrolled in a delayed start paradigm (6 months DFP or placebo pretreatment followed by 12 months DFP for all). A significant reduction in SNc and putamen siderosis was observed, particularly in the group that started early with DFP. Compared to placebo this remained stable until completion (18 months). A concomitant clinical benefit was noted at 6 months with a three-point improvement in the unified Parkinson's disease rating scale (UPDRS) for motor skills in the early start group ( $21.6 \pm 8$ ) versus the delayed start group ( $24 \pm 6$ ). Importantly, at 12 months, these 'early start' patients retained a significantly lower motor handicap (1 point on the motor UPDRS:  $21.3 \pm 8$ ) compared to the delayed start group ( $22.8 \pm 6$ ), signifying a disease modifying effect (Devos et al. 2014). Interestingly, an independent trial in 22 early-onset PD patients receiving DFP at 20 or 35 mg/kg or placebo for 6 months also showed promising results (Martin-Bastida et al. 2017). The dose of 30 mg/kg/day



and a treatment period of 12 months appeared yet more efficient than 20 mg and 6 months, respectively (Devos et al. 2014).

In ALS, 23 consecutive sporadic patients (22 limb onset and 1 bulbar onset), enrolled at time of diagnosis, showed a significant decrease in iron (by R2\*) following treatment with DFP in the cervical spinal cord, medulla oblongata and motor cortex, but not in areas outside the motor system (i.e. the cerebellum and the occipital cortex). Levels of iron, oxidative stress and the neurofilament light chains were also lowered after DFP treatment in the cerebrospinal fluid. A decrease in the ALS Functional Rating Scale score was significantly smaller for the first 3 months of DFP treatment than for the 3-month treatment-free period (5 versus 2 points). Likewise, the decrease in the Body Mass Index (BMI) was significantly altered, with a decrease of about 1 kg during the first 3 months but a small increase during the treatment period (BMI:  $26.3 \pm 4$  versus  $25.9 \pm 4$  and back to  $26.0 \pm 4$  under DFP), upon which BMI remained unchanged for a further 9 months. The reduction in manual muscle testing scores was lower in patients on DFP than placebo matched patients from the Mitotarget study, although this difference did not reach statistical significance (Moreau ARS). In all, DFP trials have a good safety profile, despite the requirement for weekly blood counts during the first 6 months to monitor reversible neutropenia that could occur in 1–3% (agranulocytosis in 0.8%) of patients.

The conservative mode of chelation was reflected by an absence of systemic iron loss, with patients showing normal iron indices that were unaltered after DFP treatment for 18 or 24 months in PD and 12 months in ALS. Interestingly, for compassionate reasons 3 ALS patients followed deferiprone for more than 50 months and one atypical PD patients for 5 years and none showed abnormal iron indices (i.e. anaemia). The only modification identified was a mild ferritin reduction in blood and CSF from PD patients that persisted long term but still remained within a range considered normal in patients. In ALS patients, the reduction of ferritin was slight and very transient (only 3 months) with a subsequent return to normal levels in the long term. Thus, it appears that iron homeostasis is able to be maintained under small doses of DFP (equal or below 30 mg/kg/day) for at least for a few years. This important finding in a small patient population requires confirmation on larger ALS cohorts treated longer term.

These promising results have now led to several large phase II clinical trials: a European multicentre, parallel-group, placebo-controlled, randomized clinical trial (FAIRPARK-II [www.fairpark2.eu](http://www.fairpark2.eu) (Nuñez and Chana-Cuevas 2018)) on 372 de novo PD patients for 9 months, and a French multicentre, parallel-group, placebo-controlled, randomized clinical trial on 240 ALS patients at the



diagnosis for 12 months (FAIRALS-II). Importantly, Apopharma has launched the clinical development in PD with the SKY study; a DFP dose-ranging study of efficacy, safety and pharmacokinetics using delayed release tablets in 140 early PD patients for 9 months (300 mg vs 600 mg vs 900 mg vs 1200 mg vs placebo).

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### Author contribution

(1) The research project: A: conception; B: organization; C: execution. (2) The manuscript: A: writing of the first draft, B. review and critical comment. DD: A1, B1, C1, A2, B2. IC: B1, C1, A2, B2. CM: C1, B2. VD: C1, B2. LMS: C1, B2. HB: C1, B2. FG: C1, A2, B2. ASR: B1, C1, A2, B2. JD: B1, C1, A2, B2. JCD: B1, C1, A2, B2

### Compliance with ethical standards

**Conflict of interest** The authors have no financial disclosures to make or potential conflicts of interest to report in relation to this study. The paper is referring to four academic studies including two translational studies already published : FAIRPARK-I (Efficacy and Safety of the Iron Chelator Deferiprone in Parkinson's Disease Protocol ID: 2008-006842-25; ClinicalTrials.gov: NCT00943748) and SAFEFAIR-ALS (Efficacy and Safety of the Iron Chelator Deferiprone in Amyotrophic lateral sclerosis Protocol ID: 2013-001228-21; ClinicalTrials.gov: NCT02164253) and two in progress : FAIRPARK-II (with the French NS-Park network, which is funded by a grant from the European Commission Horizon 2020 PHC13 2014-2015 (N° 633190): “Conservative iron

chelation as a disease-modifying strategy in Parkinson's disease: a multicentre, parallel-group, placebo-controlled, randomized clinical trial of deferiprone" Protocol ID: 2015\_22; Clinical trial: NCT02655315 <http://fairpark2.eu>) and FAIRALS-II (Conservative Iron Chelation by Deferiprone as a Disease-modifying Strategy for Amyotrophic Lateral Sclerosis using a Multicentre, Parallel-group, Placebo-controlled, Randomized Clinical Trial on 240 patients. Protocol ID: 2017-003763-35; ClinicalTrials.gov: NCT03293069 funded by the French Ministry of Health, PHRC-N2017). ApoPharma provided deferiprone and advices on the molecule for the four investigator drive studies. Caroline Moreau has received grants from the France Parkinson charity. She has received various honoraria from pharmaceutical companies for consultancy and lectures on Parkinson's disease at symposia such as Aguetant, Abbvie, Medtronic, Novartis. James Duce has received research funding from Alzheimer's Society, Alzheimer's Research UK, European Commission, Parkinson's UK and NHMRC. He serves as a scientific advisor on the FAIRPARK II but has no financial disclosures. Ioav Cabantchik consults for Aferrix Ltd (Israel) and Hinoman (Ltd) Israel and has been an invited speaker in meetings organized by Apopharma (Canada) for which he received lecturer honoraria. David Devos has received PHRC grants from the French Ministry of Health and research funding from the ARSLA charity, France Parkinson charity, Credit Agricole Foundation. He has led two pilot investigator driven studies with DFP provided for free by ApoPharma (FAIRPARK-I and SAFE-FAIR ALS-I). He is leading two large investigator driven studies with DFP provided for free by ApoPharma (FAIRPARK-II and FAIR ALS-II). He served on advisory boards, served as a consultant and given lectures for pharmaceutical companies such as Orkyn, Everpharma, Abbvie, Boston Scientific, Lundbeck. Jean-Christophe Devedjian, Véronique Danel, Laura Mahoney-Sanchez, Hind Bouchaoui, Anne-Sophie Rolland have nothing to declare.

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