The FEBS Journal

FEBS PRESS science publishing by scientists

A GUIDE TO...

A guide to ferroptosis, the biological rust of cellular membranes

Geraldine Veeckmans[1], Emily Van San[1] and Tom Vanden Berghe[1,2,3]

1 Department of Biomedical Sciences, University of Antwerp, Belgium
2 VIB-UGent Center for Inflammation Research, Belgium
3 Department of Biomedical Molecular Biology, Ghent University, Belgium

Keywords
ferroptosis; FSP1; GPX4; iron; lipid; lipid peroxidation; metabolism; radical trapping antioxidant; redox

Correspondence
T. Vanden Berghe, Department of Biomedical Sciences, University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium
Tel: +3232659250
E-mail: tom.vandenberghe@uantwerpen.be

(Received 21 July 2023, revised 19 October 2023, accepted 3 November 2023)

doi:10.1111/febs.16993

Unprotected iron can rust due to oxygen exposure. Similarly, in our body, oxidative stress can kill cells in an iron-dependent manner, which can give rise to devastating diseases. This type of cell death is referred to as ferroptosis. Generally, ferroptosis is defined as an iron-catalyzed form of regulated necrosis that occurs through excessive peroxidation of polyunsaturated fatty acids within cellular membranes. This review summarizes how ferroptosis is executed by a rather primitive biochemical process, under tight regulation of lipid, iron, and redox metabolic processes. An overview is given of major classes of ferroptosis inducers and inhibitors, and how to detect ferroptosis. Finally, its detrimental role in disease is briefly discussed.

Introduction

The past two decades of cell death research have revealed the existence of several modes of regulated necrosis [1]. Ferroptosis as a distinct form of regulated, iron-catalyzed cell death driven by excessive lipid peroxidation (LPO) within cellular membranes was first conceptualized by Stockwell's lab [2]. However, research studying the toxicity of compounds, toxins, and transition metals already demonstrated the existence of lipid peroxidation-driven cell death far before ferroptosis was discovered [3,4]. Furthermore, identification of the commonly used ferroptosis-inducing compounds, erastin and RLS3, as well as findings that genetic modulation of genes controlling redox metabolism drives non-apoptotic cell death, was also reported in the pre-ferroptosis era [5–7]. Cell membrane rupture during ferroptotic cell death is characterized by hydrogen abstraction and oxygenation of polyunsaturated fatty acids (PUFAs) of phospholipids (PLs), which is catalyzed by redox-active iron. This subsequently leads to cell death due to disruption of membrane stability and the accumulation of lipid hydroperoxides to lethal levels [8]. Although the process of lipid peroxidation

Abbreviations
4HNE, 4-hydroxy-2-nonenal; ACSL, acyl-CoA synthetase long-chain family member; BH4, tetrahydrobiopterin; CoA, coenzyme A; CoQ10, ubiquinone; CoQ10H2, ubiquinol; DMT1, divalent metal transporter 1; D-PUFA, deuterium-polyunsaturated fatty acid; Fe2+, ferrous iron; Fe3+, ferric iron; Fer-1, ferrostatin-1; FIN, ferroptosis inducing compound; FPN, ferroportin; FSP1, ferroptosis suppressor protein 1; GLN, glutamine; GPX4, glutathione peroxidase 4; GSH, glutathione; HMOX1, heme oxygenase 1; IKE, imidazole ketone erastin; IRI, ischemia–reperfusion injury; LIP, labile iron pool; Lip-1, liproxstatin-1; LOX, lipoxygenase; LPO, lipid peroxidation; MDA, malondialdehyde; MUFA, monounsaturated fatty acid; NCOA4, nuclear receptor coactivator 4; NRF2, nuclear factor erythroid 2-related factor 2; PL, phospholipid; PLOOH, phospholipid hydroperoxide; POR, cytochrome P450 oxidoreductase; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; RSL3, RAS-selective lethal molecule; RTA, radical trapping antioxidant; SFA, saturated fatty acid; TFR1, transferrin receptor 1.

has been linked to several regulated cell death modalities, ferroptosis is exclusively driven by excessive lipid peroxidation [9]. By this, ferroptosis-induced cell death relies on alterations of factors contributing to iron metabolism, antioxidant defense, and lipid metabolism.

In recent years, ferroptosis has been increasingly explored in diseases related to ischemia–reperfusion injury (IRI) or iron toxicity, including neurological disorders, single or multiorgan injury, infarction, and stroke [10]. Intriguingly, extensive studies also suggest a pivotal role of ferroptosis in tumor suppression [11]. As such, pharmacological modulation of ferroptosis either via inhibition or induction may hold great promise for the treatment of a multitude of diseases [12]. The detection of ferroptosis in pathophysiology remains challenging; however, a snapshot of the most important detection techniques is made [13]. In this review, we discuss the main regulatory mechanisms of ferroptosis, different classes of inhibitors and inducers, and current available detection tools. In addition, the role of ferroptosis in some generally accepted pathologies is briefly summarized. Essentially, this review provides a guide to ferroptosis as it exists today.

Ferroptosis execution

Excessive lipid peroxidation of PUFAs containing PLs within cellular membranes is the major executioner mechanism of ferroptosis [2]. Although LPO events also occur in other modes of regulated cell death, an extended oxidative lipidomic approach showed only excessive phospholipid peroxidation during ferroptosis [9]. PUFA-containing PLs have weak C–H bonds in between adjacent carbon–carbon double bonds, which makes them susceptible to LPO [14]. Furthermore, PUFA incorporation into cellular membranes PLs is required for the initiation of ferroptosis [15,16]. Oxidative damage of PUFA-PLs can be initiated either through non-enzymatic free-radical chain reactions involving Fenton chemistry [17] or enzyme-mediated processes catalyzed by iron-dependent lipoxygenases (LOXs) or cytochrome P450 oxidoreductase (POR) (Fig. 1, left panel) [8,18]. The subcellular membranes essential for ferroptosis and the sequence of their peroxidation remains a topic of debate and is likely dependent on how ferroptosis is induced. Different cellular organelles including endoplasmic reticulum [15,19], mitochondria [20], and lysosomes [21–23], all seem to be able to initiate ferroptosis.

Fenton chemistry refers to a series of iron-catalyzed reactions in which oxygen-centered radicals such as hydroxyl radicals are produced [24]. These highly reactive free radicals initiate LPO by abstracting labile hydrogen atoms from PUFAs, producing phospholipid radicals which subsequently react with molecular oxygen during the propagation phase. As a result, PL peroxyl radicals further attack adjacent PUFAs yielding new phospholipid radicals along PL hydroperoxides (PLOOH), which in the presence of redox-active labile iron is further converted to phospholipid alkoxyl radicals (Fig. 1, middle panel). All newly formed PL radicals as well as singlet oxygen molecules generated via the Russell mechanism can re-enter the chain reaction to further amplify PLOOH [25]. Enzymatic lipid peroxidation, which is mediated by LOXs or POR, directly catalyzes the deoxygenation of free and esterified PUFAs producing PLOOH (Fig. 1, left panel) [26,27]. The crucial role of LOXs during ferroptosis is still a matter of debate, due to lack of genetic evidence. Phosphatidylethanolamine binding protein 1 (PEBP1) was suggested to associate with 15LOX to acquire specificity for the phosphatidylethanolamine phospholipids that are key to ferroptosis [28]. Whereas radical-trapping antioxidants (RTAs) can rescue cells from ferroptosis by interfering with the autoxidation process, the inhibition of LOX cannot reverse ferroptotic cell death [17].

The propagation of this free radical chain reaction within cellular membranes eventually leads to the formation of secondary toxic aldehydes such as 4-hydroxy-2-nonenal (4HNE) and malondialdehyde (MDA) [4]. Both 4HNE and MDA can form adducts with proteins and DNA, which in turn results in biomolecular damage [29]. Finally, the membrane becomes thinner and forms curvatures followed by the increased accessibility to oxidants and eventually cell membrane rupture [30], likely involving mechanosensing channels [31] and osmotic processes [32,33].

The auto-amplifying chain reaction can be terminated when lipid hydroperoxides and lipid peroxyl radicals decompose into inactive non-radical products (Fig. 1, right panel). For this, the cell depends on endogenous RTAs including vitamin E [34], vitamin K [35], tetrahydrobiopterin (BH4) [36,37], ubiquinol ($CoQ_{10}H_2$) [38,39], hydropersulfides [40,41], vitamin A and its active derivates [42], 7-dehydrocholesterol [43] and squalene [44]. RTAs intervene directly in the chain reaction by scavenging unpaired electrons thereby counteracting LPO. Furthermore, the selenium-dependent glutathione peroxidase 4 (GPX4), an essential enzyme of the glutathione (GSH) system, impedes the execution of ferroptosis by detoxifying PLOOHs to their corresponding non-toxic PL alcohol forms [7].

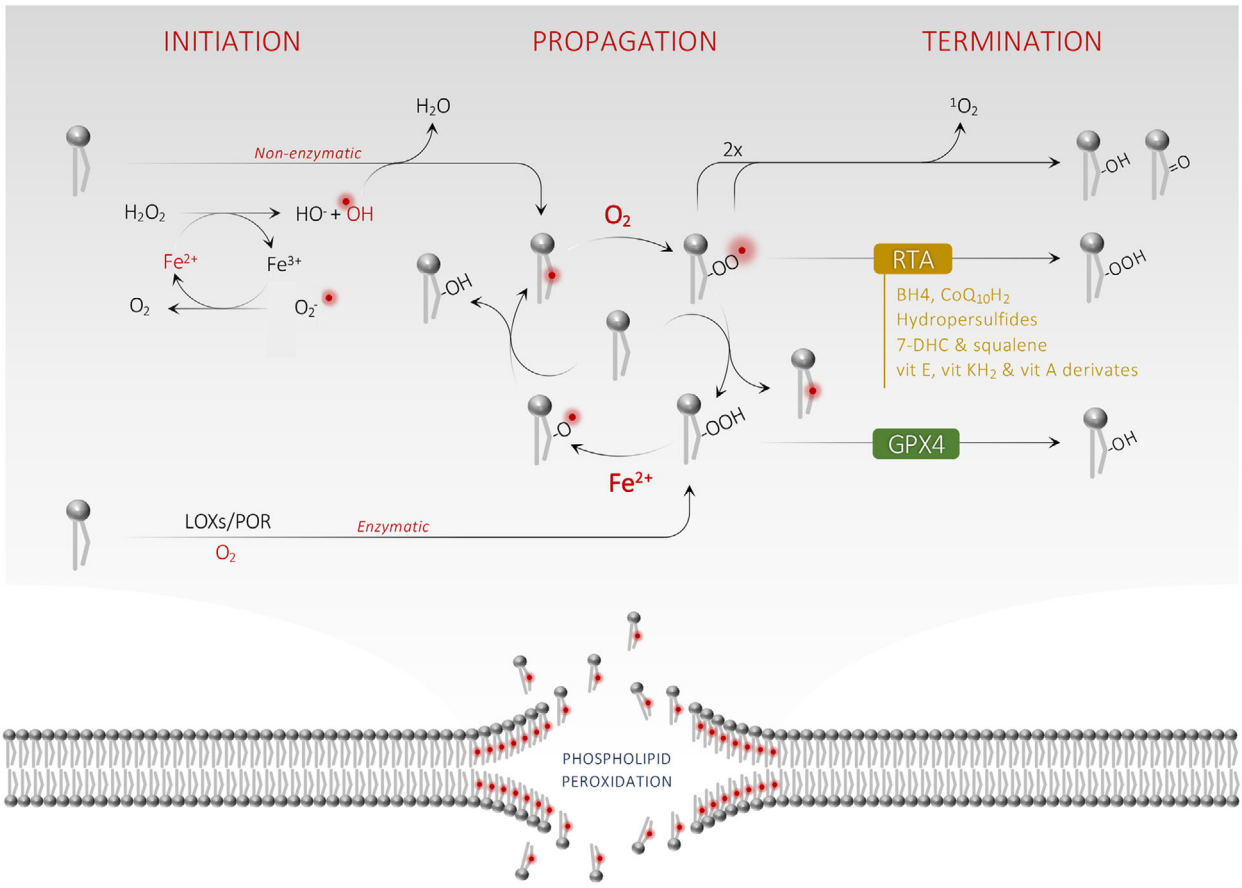

Fig. 1. Phospholipid peroxidation process. LPO initiation step includes the formation of phospholipid radicals catalyzed by redox-active labile iron generated during the Fenton reaction. Additionally, LOXs oxygenate PUFAs directly via an enzymatic process. In the subsequent propagation phase, phospholipid radicals react with molecular oxygen forming phospholipid peroxyl radicals which in turn form phospholipid hydroperoxide and new phospholipid radicals. In the presence of iron, phospholipid hydroperoxide decomposes into phospholipid alkoxyl radicals which refuel the chain reaction by attacking another PUFA. During the termination phase, damaging phospholipids can be neutralized either through the reaction between two phospholipid peroxyl radicals, endogenous RTAs, or peroxidase activity of GPX4. $^1O_2$, singlet oxygen; BH4, tetrahydrobiopterin; $CoQ_{10}H_2$, ubiquinol; Fe^{2+}, ferrous iron; Fe^{3+}, ferric iron; GPX4, glutathione peroxidase 4; H_2O_2, hydrogen peroxide; HO^-, hydroxyl; LOX, lipoxygenase; LPO, lipid peroxidation; O_2, oxygen; $O_2^{\cdot-}$, superoxide radical anion; $OH^{\cdot}$, hydroxy radicals; PL, phospholipid; POR, cytochrome P450 oxidoreductase; PUFA, poly-unsaturated phospholipids; RTA, radical trapping antioxidants; Vit E, KH_2 or A, vitamin E, KH_2, or A.

Ferroptosis induction

Four major classes of ferroptosis-inducing compounds (FINs) have been described to modulate the sensitivity towards ferroptosis (Table 1). Class I, II, and III FINs induce ferroptosis by interfering with redox metabolism, whereas class IV FINs overrule these redox protective mechanisms by directly targeting iron metabolism.

Unleashing ferroptosis redox brakes

Class I ferroptosis inducers downregulate GSH required for the proper functioning of GPX4 by depleting directly or indirectly intracellular cysteine for example by inhibiting cystine/glutamate antiporter system X_C^-. Subsequent GPX4 inactivation results in PLOOH accumulation and ferroptotic cell death. Erastin was the first small molecule identified as a class I ferroptosis inducer [2]. Later, imidazole ketone erastin (IKE), piperazine erastin (PE), and the FDA-approved compounds sulfasalazine and sorafenib were added [45,46]. In addition, excessive concentration of glutamate is also classified as a class I inducer [47]. Noteworthy, PE, IKE, and sorafenib are the only compounds suitable for *in vivo* use due to their high inhibitory potential, stability, and pharmacokinetic profile [47]. Class II inducers directly inhibit GPX4 function by covalent interaction with

Table 1. Ferroptosis-inducing compounds. $(NH_4)_2Fe(SO_4)_2$, ferrous ammonium sulfate; DPI, diphenyleneiodonium; FIN, ferroptosis-inducing compound; FSP1, ferroptosis suppressor protein 1; GPX4, glutathione peroxidase 4; GSH, glutathione; HMOX1, heme oxygenase-1; IKE, imidazole ketone erastin; PE, piperazine erastin; SQS, squalene synthase.

Ferroptosis inducers			
Class	Target	Mechanism	Compounds
FIN I	GSH	Direct or indirect depletion of intracellular cysteine by inhibiting e.g. System Xc^-	Erastin, IKE, PE, sorafenib, sulfasalazine, glutamate
FIN II	GPX4	Covalently binding and inhibition of GPX4 activity	RSL3, ML162, withaferin A, DPI compounds
FIN III	CoQ_{10}	FSP1 inhibition or CoQ_{10} depletion via SQS activation	FIN56, iFSP1, icFSP1, Statins
FIN IV	Redox-active iron	Iron loading, iron oxidation and increase in LIP by HMOX1	FINO2, withaferin A, artemisinin, artesunate, hemin, hemoglobin, $(NH_4)_2Fe(SO_4)_2$

the nucleophilic active-site selenocysteine [48] and include RSL3, ML162, a variety of diphenyleneiodonium compounds [8], and the medicinal plant anticancer agent withaferin A [49]. Class III inducers essentially downregulate mevalonate-derived ubiquinone (CoQ_{10}), which acts as an endogenous lipophilic radical trap. Ferroptosis inducer 56 (FIN56) was the first compound discovered with this mode of action, along inactivation of GPX4 [50]. Recently, the discovery of first-generation (iFSP1) and second-generation (icFSP1) inhibitors of ferroptosis suppressor protein 1 (FSP1) showed the potential to suppress ferroptosis independently of GPX4 activity [38,51]. Finally, different statins have been classified as class III inducers since it inhibits HMG-CoA reductase enzyme which in turn blocks CoQ_{10} biosynthesis [52,53].

Overruling redox protective mechanisms

Class IV FINs are grouped for their ability to increase the levels of cytosolic non-chelatable redox-active iron, often referred to as the labile iron pool (LIP). Iron loading using hemin [54], hemoglobin [55] or ferrous ammonium sulfate ($(NH_4)_2Fe(SO_4)_2$) [49] have shown to trigger ferroptosis *in vitro* as well as in preclinical models of intracerebral hemorrhage. Furthermore, ferritinophagy induced by salinomycin, artemisinin, and its prodrug artesunate triggers ferroptosis in cancer cells by increasing the LIP [56,57]. Ferroptosis-inducing endoperoxides such as $FINO_2$ directly oxidize ferrous iron (Fe^{2+}) but also inactivate GPX4 indirectly in cells [58]. Similarly, withaferin A also induces ferroptosis by exerting dual effects involving LIP increase through heme oxygenase 1 (HMOX1)-mediated degradation of heme and GPX4 inactivation [49].

Ferroptosis regulation

Lipid metabolism

Peroxidation of specific membrane phospholipids and subsequent cell membrane damage ultimately drives cells to death. PLs acylated with PUFAs are the main target of lipid peroxidation since weak bis-allylic protons of PUFAs are more easily abstracted in comparison with the hydrogens in monounsaturated fatty acids (MUFAs) or saturated fatty acids (SFAs) [14]. PUFAs containing the heavy hydrogen isotope deuterium (D-PUFA) are much less susceptible to ferroptosis. As such, treating cells with D-PUFAs suppresses ferroptosis sensitivity, underscoring the importance of PUFA peroxidation in the execution of ferroptosis [8]. In addition, the lipid composition of the cellular membrane as well as the abundance of PUFAs determine the extent of LPO and thus also ferroptosis sensitivity (Fig. 2, brown panel) [15,17]. Although free fatty acids are substrates for the synthesis of lipid signaling mediators, the incorporation of esterified PUFAs in cellular membranes is necessary to exert lethal effects upon oxidation [15,16]. Acyl-CoA synthetase long-chain family member 4 (ACSL4) and lysophosphatidylcholine acyltransferase 3 (LPCAT3) are two key enzymes involved in the biosynthesis and remodeling of PUFAs in cellular membranes. Thus, the deletion of both genes suppresses ferroptosis by depleting LPO substrates [59]. In contrast, supplementing cells with exogenous PUFAs enhanced erastin-induced ferroptosis [8]. Unlike PUFAs, MUFAs induce a ferroptosis resistance state in cells by blocking the formation of lipid reactive oxygen species (ROS) and displacing oxidizable PUFAs from the phospholipid membrane. The protective role of MUFAs relies on the activity of acyl-coenzyme A synthetase long-chain family member 3 (ACSL3) protein, which catalyzes the esterification

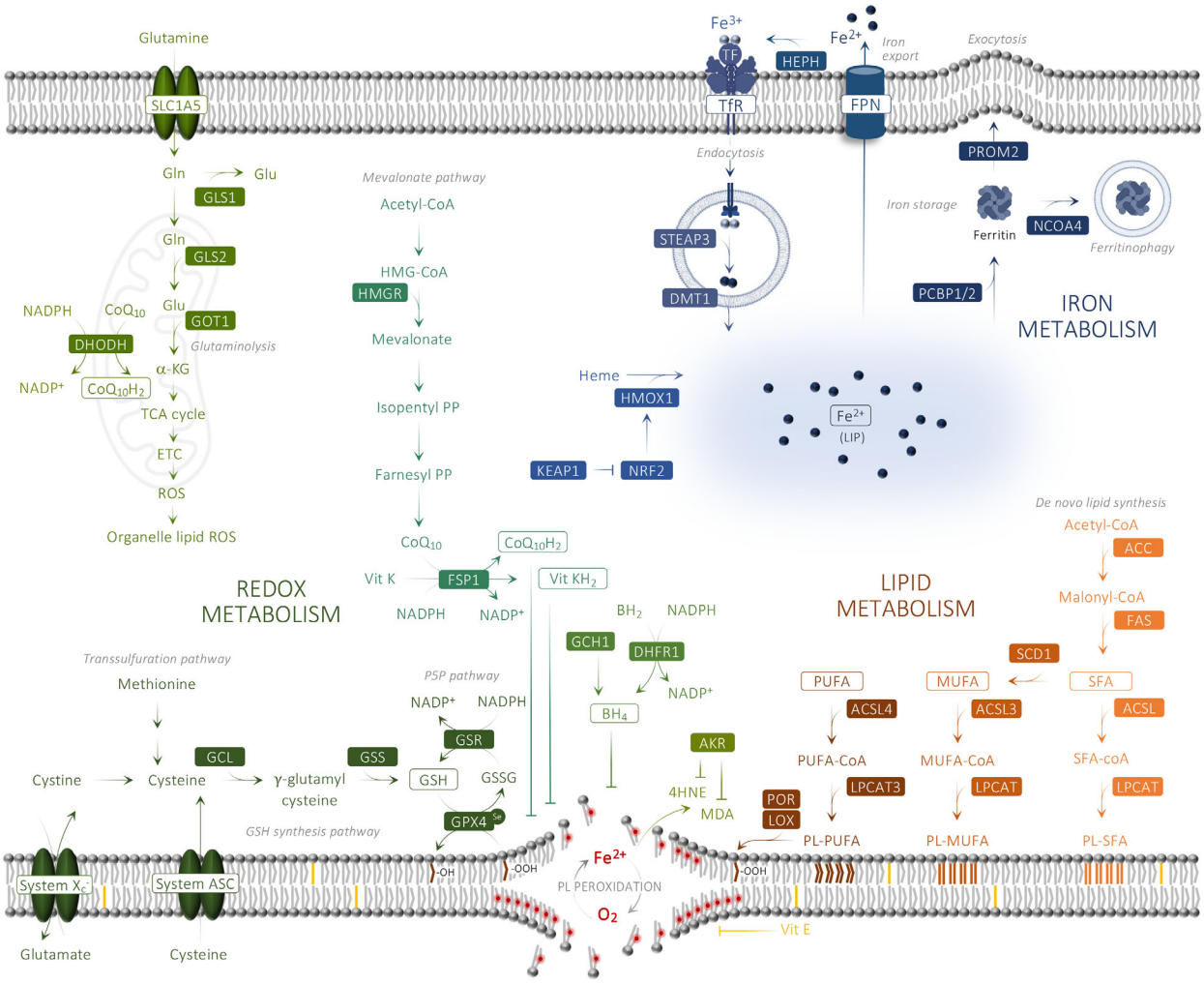

Fig. 2. Metabolic regulation of ferroptosis. Ferroptosis is tightly regulated by three key elements: redox, iron, and lipids. The different antioxidant defense mechanisms implicated in the ferroptosis pathway include the XC- GSH-GPX4 pathway, transsulfuration pathway, mevalonate pathway, FSP1-Vitamin K (vit K)/CoQ10 pathway, glutaminolysis, DHODH-CoQ10H2 pathway, GCH1-BH4 pathway, and aldo-keto reductases (redox metabolism displayed in green). Fluctuations in the labile iron pool (Fe^{2+}) are mainly controlled by TFR, FPN, DMT1, NCOA4, NRF2, and HMOX1 (iron metabolism displayed in blue). The peroxidation of PUFA-containing phospholipids (PUFA-PLs) within cellular membranes is mainly regulated by ACSL4, LPCAT3, LOX, POR and Vitamin E (vit E), (lipid metabolism displayed in orange). An imbalance between the production of endogenous oxidants and antioxidants and the presence of excess free labile iron and oxidizable phospholipids acylated with PUFAs are both required for ferroptosis execution. 4HNE, 4-hydroxy-2-nonenal; ACC, acetyl CoA-carboxylase; ACSL, acyl-CoA synthetase long-chain family member; AKR, aldo-keto reductases; BH2, dihydrobiopterin; BH4, tetrahydrobiopterin; CoQ10, ubiquinone; CoQ10H2, ubiquinol; DHFR1, dihydrofolate reductase; DHFR1, dihydrofolate reductase; DHODH, dihydroorotate dehydrogenase; DMT1, divalent metal transporter 1; ETC, electron transport chain; FAS, fatty acid synthase; Fe^{2+}, ferrous iron; FPN, ferroportin; FSP1, ferroptosis suppressor protein 1; GCH1, guanosine triphosphate cyclohydrolase 1; GCL, glutamate-cysteine ligase; Gln, glutamine; GLS1, glutaminases; Glu, glutamate; GOT1, glutamic-oxaloacetic transaminase 1; GPX4, glutathione peroxidase 4; GSH, glutathione; GSR, glutathione–disulfide reductase; GSS, glutathione synthetase; GSSG, oxidized glutathione; HEPH, hephaestin; HMOX1, heme oxygenase 1; KEAP1, Kelch-like ECH-associated protein 1; LOX, lipoxygenase; LPCAT, lysophosphatidylcholine acyltransferase; MDA, malondialdehyde; MUFA, monounsaturated fatty acid; NCOA4, nuclear receptor coactivator 4; NRF2, nuclear factor E2-related factor 2; O_2, oxygen; PCBP1/2, poly(rC)-binding protein 1/2; PL, phospholipid; POR, cytochrome P450 oxidoreductase; PP, pyrophosphate; PROM2, prominin2; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; SCD1, stearoyl-CoA desaturase 1; SFA, saturated fatty acid; STEAP3, transmembrane epithelial antigen of the prostate 3; TCA cycle, tricarboxylic acid cycle; TFR1, transferrin receptor 1; α-KG, alpha-ketoglutarate.

of MUFAs with coenzyme A (CoA) [60]. Downregulating the expression of ACSL3 in cells treated with exogenous MUFAs showed less protection against ferroptosis [61]. In addition, stearoyl-CoA desaturase 1 enzyme, which converts SFA into MUFAs, also showed to sensitize cells to ferroptosis upon inhibition [62].

Iron metabolism

Iron homeostasis is kept under exquisite control by many cellular processes. Increasing levels of unbound redox-active iron or LIP triggered by dysregulation of either iron import, export, storage, or turnover impacts the sensitivity towards ferroptosis (Fig. 2, blue panel). Under physiological conditions, circulating ferric iron (Fe^{3+}) is internalized into cells as a transferrin-iron complex through the membrane-bound transferrin receptor 1 (TFR1) [63]. Once released in endosome compartments, Fe^{3+} is reduced to Fe^{2+} via the endosomal metalloreductase enzyme six-transmembrane epithelial antigen of the prostate 3 (STEAP3) [64]. Subsequently, Fe^{2+} fuels the LIP in the cytoplasm through divalent metal transporter 1 (DMT1) [65]. Genetic inactivation of TFR1 has previously been shown to prevent ferroptosis upon erastin treatment or cystine deprivation [6]. Conversely, treatment with transferrin enhanced erastin-induced cell death [66]. Excess of cellular Fe^{3+}, which is not needed for metabolic functions such as synthesis of iron-containing enzymes, is sequestered within the iron storage protein complex ferritin [67]. Not surprisingly, autophagy-mediated degradation of ferritin, referred to as ferritinophagy promotes accumulation of lipid ROS by increasing the LIP [68]. Consistently, the knockdown of selective cargo receptor nuclear receptor coactivator 4 (NCOA4), which recruits ferritin to autophagosomes, blocks ferroptosis [69]. Ferrous iron can also be released from heme by HMOX1, which is controlled by the nuclear factor E2-related factor 2 (NRF2). Under condition of oxidative stress, NRF2 unleashes from the Kelch-like ECH associated protein 1 (KEAP1) bond, allowing nuclear translocation and activation of target genes with mainly anti-ferroptotic function [70]. However, in certain contexts, excessive activation of HMOX1 along an insufficient buffer capacity of ferritin upon NRF2 upregulation has been shown to promote ferroptosis by increasing the LIP [49]. Cellular iron export can be mediated through the transmembrane protein ferroportin (FPN). Downregulation of FPN either genetically or pharmacologically increases ferroptosis sensitivity by limiting iron export [71,72]. Alternatively, upregulation of the prominin2-mediated ferritin exocytosis pathway promotes resistance to ferroptosis [28]. Thus, modulation of the intracellular redox-active iron levels by the cellular iron homeostatic network is key in regulating ferroptosis sensitivity.

Redox metabolism

The thiol-containing tripeptide glutathione is an essential intracellular antioxidant that is synthesized from cysteine via two ATP-dependent enzymes, glutamate-cysteine ligase (GCL) and glutathione synthetase (GSS) [73]. The importance of cystine and cysteine to maintain GSH biosynthesis was previously shown by findings that cells supplemented with cystine-free medium die due to GSH depletion and this death could be prevented by the administration of lipophilic antioxidants such as alpha-tocopherol [74]. The intracellular cysteine pool mainly relies on the system X_c^- which takes up extracellular cystine, the oxidized form of cysteine, in exchange for intracellular glutamate (Fig. 2, green panel) [73]. Indeed, glutamate serves as a trigger for ferroptosis induction since high extracellular glutamate concentrations inhibit system X_c^- [2]. Cellular cysteine can also be provided via the alanine-serine-cysteine (ASC) system or synthesized from methionine via the transsulfuration pathway [75,76]. Conditions that hinder intracellular cysteine and consequently GSH levels [2,77] or selenium/selenocysteine uptake mechanisms via low-density lipoprotein receptor-related protein 8 (LRP8), directly impact the activity of the GPX4 enzyme. GPX4 converts GSH to oxidized GSSG, which is then recycled back by glutathione reductase (GSR) at the expense of $NADPH/H^+$ [78]. Obviously, pharmacological or genetic inactivation of GPX4 is an often-used strategy to induce ferroptosis [79–81].

Along the X_C^--GSH-GPX4 axis, cells can also counteract lipid peroxidation by promoting endogenous radical trapping antioxidant systems. FSP1, formerly known as AIFM2, maintains the reduction of CoQ_{10} using NADPH [38]. Upon activation, FSP1 is recruited to the plasma membrane where it generates $CoQ_{10}H_2$ which in turn traps PL peroxyl radicals and prevents subsequent phospholipid peroxidation [38,82]. Like the function of FSP1 in the extramitochondrial membrane, dihydroorotate dehydrogenase (DHODH) reduces mitochondrial CoQ_{10} to $CoQ_{10}H_2$ independently of GPX4 or FSP1 [83]. BH4 is another endogenous RTA that protects against ferroptosis independently from GPX4 [82]. Treating BH4-deficient cells with exogenous dihydrobiopterin (BH2), the dehydrogenated product of BH4 that is converted by dihydrofolate reductase (DHFR), protected against

RSL3- and ML162-induced cell death. Consistently, genetic deletion of the rate-limiting enzyme guanosine triphosphate cyclohydrolase 1 (GCH1) reduced intracellular levels of BH4 and decreased the antioxidant capacity of the cells [37]. Noteworthy, a class of aldo-keto reductases was also classified as endogenous RTA since it has been shown to detoxify oxidative lipid breakdown products such as 4HNE [4,84]. Finally, the ferroptotic pathway is also tightly linked to the glutamine (Gln) metabolism, known as glutaminolysis. The Gln transporter SLC1A5, glutaminases 2 (GLS2), and glutamic-oxaloacetic transaminase 1 (GOT1) are required for Gln import and the conversion to glutamate and α-ketoglutarate. Genetic knockdown of these genes showed to counteract ferroptosis [20,66].

Ferroptosis detection

Considering the high clinical relevance of ferroptosis, understanding its core molecular machinery is paramount for disease prevention, diagnosis, treatment, and prognosis. Since it remains challenging to detect (per) oxidized PLs and redox-active iron in biofluids, a variety of biomarkers related to lipid and iron metabolism are needed to differentiate ferroptosis [48,85–87]. Considering the central role of excessive LPO in ferroptosis, different tools have been described to study how (per) oxidized lipids are involved in the dying process by detecting and quantifying the extent of LPO or lipid ROS. Both C11-BODIPY and LiperFluo are widely used probes to measure lipid ROS. Upon oxidation, the fluorescent switch of these probes can easily be detected by fluorescent microscopy or flow cytometry [15,88]. Toxic lipid degradation products, such as MDA and 4HNE, can be determined by thiobarbituric acid reactive substances (TBARS) approach [89], western blotting, or staining procedures [90]. Another approach to detect oxidized phospholipids involves the use of an E06 antibody that specifically labels oxidized phosphatidylcholines [91,92]. The most sophisticated and specific approach for detection of PLOOHs is the use of liquid chromatography with tandem mass spectrometry (LC–MS/MS) analysis, referred to as oxidative lipidomics [15,93].

Furthermore, alterations in iron homeostasis have shown to control the sensitivity towards ferroptosis. For instance, increased expression of iron influx proteins TFR1 and DMT1, or contrary, decreased expression of efflux proteins FPN, ceruloplasmin, and hephaestin enhance ferroptosis induction [94]. Recently, TFR1 was proposed as a biomarker to detect ferroptosis *in vitro* and *in vivo* [95]. However, the use of TFR1 antibodies is dependent on cell type, tissue, and condition since iron influx may be orchestrated by several influx transporters in different cell types [94]. Additionally, altered expression levels of NCOA4 [96], HMOX1 [49], NRF2 [97,98], and heat shock protein beta-1 (HSPB1) [99] have also been linked to ferroptosis. However, many of these suggested biomarkers are often context-dependent and therefore considered as rather bystander effects of ferroptosis. Next to the analysis of protein and/or gene expression, several commercial assays are available to measure the iron content such as calcein-AM assay [100], FeRhoNox, FerroOrange, Mito-FerroGreen probes [49,101,102], and Perl's Prussian Blue staining with or without DAB-enhancement [103]. However, a more reliable technique to measure the total iron content, ferrous iron as well as ferric iron, is capillary electrophoresis coupled plasma mass spectrometry with dynamic reaction cell (CE-ICP-DRC-MS) [104–106].

Pharmacological ferroptosis inhibition

The high clinical relevance of ferroptosis in a variety of diseases has boosted the development of novel therapeutics (Table 2). Here, we will only give a snapshot of the most potent ferroptosis inhibitors.

Iron chelators

Considering the central role of iron in ferroptosis execution, iron-chelating therapies such as deferasirox

Table 2. Ferroptosis inhibiting compounds. $(NH_4)_2Fe(SO_4)_2$, ferrous ammonium sulfate; DPI, diphenyleneiodonium; FIN, ferroptosis-inducing compound; FSP1, ferroptosis suppressor protein 1; GPX4, glutathione peroxidase 4; GSH, glutathione; HMOX1, heme oxygenase-1; IKE, imidazole ketone erastin; PE, piperazine erastin.

Ferroptosis inhibitors			
Class	Characteristics	Mechanism of action	Compounds
I	Iron chelators	Remove excess iron	Deferasirox, deferiprone, deferoxamine, ciclopirox, CN128
II	Lipophilic radical traps	Trapping chain-carrying radicals in phospholipid bilayer	Vitamin E, BHT, Fer-1, Lip-1, XJB-5-131, CoQ_{10}, UAMC-3203
III	Deuterated PUFAs	Prevent initiation and propagation of lipid peroxidation	D4-arachidonic acid, D10-docosahexaenoic acid
IV	Lipoxygenase inhibitors	Prevent LOX-induced LPO	CDC, baicalein, PD-146176, AA-861, zileuton, FerroLoxin-1/2

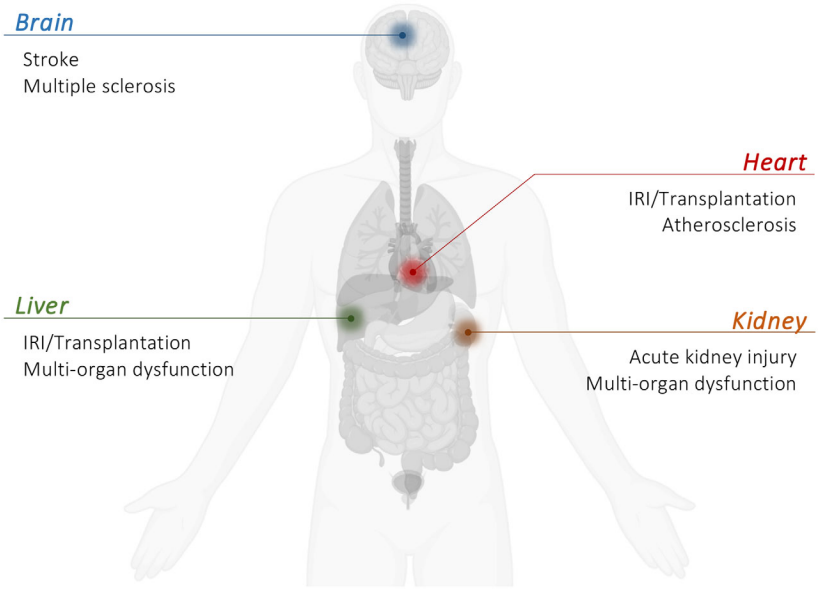

Fig. 3. Ferroptosis-driven pathologies and ferroptosis-associated therapeutic benefits in experimental rodent models. Although the number of ferroptosis-driven pathologies is increasing, only a few pathologies have shown a convincing ferroptosis signature in patients along with a therapeutic benefit in representative experimental preclinical models. To date, evidence of a ferroptosis signature in human disorders is based on the presence of elevated levels of redox-active iron, peroxidized phospholipids and lipid degradation products in either biofluids or injured tissue. Therapeutic targeting of ferroptosis using mainly lipophilic radical trapping agents in experimental rodent models highlights its therapeutic potential.

[107], deferiprone [108], deferoxamine [2], ciclopirox olamine [2] and CN128 [109] have been widely considered as potential therapeutic agents in the treatment of ferroptosis-driven diseases. Indeed, iron chelators have already been shown to mitigate ischemia–reperfusion injury (IRI) in a variety of experimental animal models [110–112], as well as the severity of several neurodegenerative diseases in both animal models and human clinical trials [113–116]. Although these compounds show different pharmacokinetic and metabolic properties, the mechanism of action relies on free iron chelation thereby avoiding the formation of highly reactive hydroxyl radicals [117]. However, side effects related to the essential role of iron in many metabolic processes are discouraging this treatment strategy.

Lipophilic radical traps

Since ferroptosis is driven by a radical chain reaction within cellular membranes, different strategies that halt this process have been developed. Small molecules that react with chain-carrying radicals, and thus inhibit phospholipid autooxidation are identified as lipophilic radical trapping antioxidants. Extensive high-throughput screenings using cell-based ferroptosis assays identified novel synthetic RTAs such as Ferrostatin-1 (Fer-1) and Liproxstatin-1 (Lip-1) [118–120]. These lipophilic RTAs scavenge unpaired electrons at the level of toxic phospholipid radicals [121]. Although Fer-1 has been shown to be a potent inhibitor of ferroptosis in multiple *in vitro* settings, it is not well-suited for *in vivo* use because it suffers from solubility and metabolic stability problems [122]. Consequently, different improved analogues were developed showing highly improved *in vivo* efficacy such as SRS16-86 [123] or UAMC-3203 [81,122]. Additionally, other lipophilic RTAs have been suggested as potential ferroptosis inhibitors for both *in vitro* (e.g. butylated hydroxytoluene (BHT) [2] and CoQ_{10} [38,39]) and *in vivo* use (e.g. XJB-5-131 [124,125]).

Other ferroptosis inhibitors

PUFAs containing the heavy hydrogen isotope deuterium at the peroxidation site have also been found to suppress ferroptosis induced by RLS3 and erastin [8]. Apart from agents interfering with iron metabolism or harboring anti-oxidant properties, a range of ferroptosis inhibitors directed to the lipoxygenase enzyme have been described [8,15,126–129]. However, since genetic targeting of LOXs failed to prevent ferroptotic cell death, this class of inhibitors is still controversial [17,121].

Ferroptosis in disease

Emerging evidence indicates the involvement of ferroptosis in many human disorders. Therefore, pharmacological modulation of ferroptosis, by either inhibiting or inducing it, may represent a possible avenue for treating multiple pathologies. Here, an overview of the main diseases in which a ferroptosis signature in humans as well as its therapeutic potential in preclinical models have been explored is briefly discussed (Fig. 3). How infectious agents regulate ferroptosis to

promote their replication, dissemination, and pathogenesis [130], and how ferroptosis induction might be a promising novel anti-cancer therapy [12] is reviewed elsewhere. The immune response to ferroptotic cells is still debated requiring further clarification and is out of the scope of this review. Essentially, current knowledge suggests that ferroptosis boosts innate immunity [49,80], in which M1, but not M2, macrophages seemed to survive this oxidative stress environment [131], while suppressing adaptive immunity, at least in vaccination anti-cancer setup [132].

Organ injury

Ischemia–reperfusion injury is a complex pathophysiological condition induced by an imbalance between oxygen/nutrient needs and supply upon vascular occlusion of the organ during the ischemic event. Paradoxically, subsequent reperfusion exacerbates the injury of the affected organ through destructive inflammatory responses and massive cell death [133,134]. Eventually, IRI can lead to devastating diseases ranging from single to multiple organ-injury. Mounting evidence suggests ferroptosis as a major contributor to IRI-associated cell death and multiple preclinical studies have already shown the beneficial effects of targeting ferroptosis during IRI [135]. For instance, kidney tubular cells are sensitive to ferroptosis in response to IRI, a leading cause of acute kidney injury (AKI). Consistently, different ferroptosis inhibitors were able to mitigate kidney tubular cell death using preclinical models of AKI induced by IRI, genetic deletion of GPX4, and folic acid [81,136]. Furthermore, increased levels of ferroptotic oxygenated PLs were found in urinary cell pellets obtained from patients with AKI who did not recover [28]. Beyond the kidney, the liver has also shown to be dependent on the functionality of GPX4, since inducible knockout of this enzyme leads to massive hepatocyte cell death [81]. Moreover, lipophilic RTAs protect liver parenchyma from IR-induced injury in a preclinical mouse model [80]. The importance of monitoring ferroptosis during hepatic IRI has also been investigated in a transplantation clinical setting since higher levels of circulating MDA were observed in non-surviving patients undergoing liver transplantation when compared to those who survived [137]. Pharmacological inhibition of ferroptosis significantly reduced myocardial infarct size using an *in vivo* mouse heart model mimicking IRI [138] as well as injury during heart transplantation in mice [139]. Note that a beneficial effect of inhibiting LPO for organ preservation during heart transplantation in dogs was already shown in the nineties [140]. Lastly, a case report revealed a ferroptosis signature in myocardial tissue derived from patients suffering from COVID-19. Although this highlights the importance of ferroptosis monitoring in COVID-19 cardiac damage, further research is required [141].

Ferroptosis has also been implicated in plaque destabilization during atherosclerosis. For example, erythrophagocytosis as a key feature of advanced human atherosclerosis, induced ferroptosis *in vitro* and was characterized by increased HMOX1 and ferritin expression. In line with this finding, ferroptosis inhibition decreased HMOX1 and ferritin expression observed in erythrocyte-rich plaque regions derived from a mouse model of advanced atherosclerosis [142]. Moreover, several studies reported upregulation of important ferroptosis-related genes [143–145] as well as increased levels of iron in atherosclerotic lesions from humans [146].

Interestingly, multiple organ dysfunction syndrome (MODS), which refers to the critical illness that causes 30% of deaths worldwide has been linked to ferroptosis [147,148]. A prospective cohort study involving plasma samples of 176 critically ill adult patients revealed that the extent of organ dysfunction, reflected in the patient's sequential organ failure assessment (SOFA) score, is positively correlated to increased levels of MDA and catalytic iron. Furthermore, an excess amount of iron sulfate proved to be sufficient to overrule the systemic buffer capacity and induce MODS in mice. In this experimental model, a rapid increase of MDA in plasma and tissue was observed. Additionally, the administration of a highly soluble lipophilic RTA UAMC-3203 attenuated ferroptosis-driven multi-organ injury and death [81]. These findings suggest ferroptosis inhibition as a possible strategy to prevent MODS in critical care settings.

Central nervous system

The high content of PUFAs, dependency on iron, and limited antioxidant defense, make the central nervous system (CNS) highly vulnerable to damage by lipid peroxidation [149]. Before the conceptualization of ferroptosis in 2012, oxytosis, glutamate toxicity, or excitotoxicity were described to be involved in neuronal cell death in the context of several neurological disorders including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS) [150]. Later, it was suggested that cell death by oxytosis and ferroptosis have overlapping pathways [151]. Iron chelation or therapeutic intervention with ferrostatins prevented disease development in preclinical models of AD [152] and PD

[153], while healthy neurons were rescued in HD [154]. Recently, the detrimental role of ferroptosis was revealed in multiple sclerosis, a chronic inflammatory disorder of the CNS. A prominent ferroptosis signature was reflected by the accumulation of ferrous iron and an increase of oxidized phospholipids in the lesions and cerebrospinal fluid of patients. In addition, therapeutic intervention with the lipophilic RTA UAMC-3203 delayed relapse and ameliorated disease progression in a preclinical model for relapsing–remitting multiple sclerosis [155]. Strokes, including ischemic and hemorrhagic stroke, are the second leading cause of death after coronary heart disease and are characterized by acute focal CNS injury [156]. Both iron accumulation, as well as lipid peroxidation, are involved in the pathogenesis of stroke, indicating a role for ferroptotic cell death. The neurotoxic effects of MDA and 4HNE have been reported extensively, and 4HNE has been studied as a potential biomarker for ischemic stroke [157]. Thereby, it was demonstrated that intracerebroventricular treatment with Fer-1 after hemorrhage exhibited marked brain protection and improved neurologic function in mice [55]. Consequently, intranasal delivery of Fer-1 and Lip-1, as an easy method to pass the blood–brain barrier, attenuated neurological deficits after ischemic stroke [158]. This is further underscored by the use of α-Tocotrienol, one of the eight fat-soluble chemicals in vitamin E, which decreases stroke size in animal models [159] and is currently validated in clinical trials as a neuroprotectant in stroke (NCT01578629). Signatures of altered lipid metabolism [160] and GPX4 depletion in post-mortem samples of ALS patients [161] highlight the importance of ferroptosis targeting. In line with these recent findings, neuron-specific delivery of GPX4 and Fer-1 treatment ameliorated motoric impairment in a classical preclinical model for ALS (SOD1G93A) [162], suggesting GPX4 as an interesting therapeutic target. Note that data related to the *in vivo* use of Fer-1 should be interpreted with caution considering its metabolic instability *in vivo*.

Conclusion and perspectives

Although ferroptosis has only been conceptualized in 2012, it was supposedly already studied since the 50s or earlier. Since its conceptualization, ferroptosis has been a flourishing field with cutting-edge discoveries of novel regulatory genes, its involvement in disease, and novel pharmacological intervention tools. It is tempting to speculate that ferroptosis might be a very ancient cell death process, which evolved 2.5 billion years ago during the great oxygenation event. To create life, one had to find ways to protect biological membranes against excessive lipid peroxidation catalyzed by oxygen and iron. The genetic pathways discovered to date, likely reflect these protective mechanisms, rather than proactive signaling towards cell death as is the case in apoptosis, necroptosis, or pyroptosis. The clinical relevance of ferroptosis as a detrimental factor in a multitude of diseases as well as its tumor-suppressing efficacy has boosted translational ferroptosis research. The *in vivo* effectiveness of novel small molecule ferroptosis inhibitors and/or inducers in experimental disease models gives hope for future novel treatment options related to targeting ferroptosis.

Acknowledgements

We thank the following institutions for funding the Vanden Berghe lab: Excellence of Science MODEL-IDI and CD-INFLADIS; Consortium of excellence at University of Antwerp INFLA-MED; Industrial Research Fund and BOF-IMPULS from University of Antwerp; Foundation Against Cancer FAF-C/2018/1250 and F/2022/2067; Charcot Foundation; VLIRUOS TEAM2018-01-137; Research Foundation Flanders FWO-SBO S001522N.

Conflict of interest

TVB holds patents related to ferrostatin-1 analogs (US9862678, WO2016075330, EP3218357, WO2019 154795).

Author contributions

GV wrote the manuscripts. EVS and TVB revised the manuscript.

References

1 Vanden Berghe T, Linkermann A, Jouan-Lanhouet S, Walczak H & Vandenabeele P (2014) Regulated necrosis: the expanding network of non-apoptotic cell death pathways. *Nat Rev Mol Cell Biol* **15**, 135–147.
2 Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, Patel DN, Bauer AJ, Cantley AM, Yang WS *et al.* (2012) Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* **149**, 1060–1072.
3 Hirschhorn T & Stockwell BR (2019) The development of the concept of ferroptosis. *Free Radic Biol Med* **133**, 130–143.
4 Ayala A, Muñoz MF & Argüelles S (2014) Lipid peroxidation: production, metabolism, and signaling

mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid med Cell Longev* **2014**, 1–31.
5 Dolma S, Lessnick SL, Hahn WC & Stockwell BR (2003) Identification of genotype-selective antitumor agents using synthetic lethal chemical screening in engineered human tumor cells. *Cancer Cell* **3**, 285–296.
6 Yang WS & Stockwell BR (2008) Synthetic lethal screening identifies compounds activating iron-dependent, nonapoptotic cell death in oncogenic-RAS-harboring cancer cells. *Chem Biol* **15**, 234–245.
7 Seiler A, Schneider M, Förster H, Roth S, Wirth EK, Culmsee C, Plesnila N, Kremmer E, Rådmark O, Wurst W *et al.* (2008) Glutathione peroxidase 4 senses and translates oxidative stress into 12/15-lipoxygenase dependent- and AIF-mediated cell death. *Cell Metab* **8**, 237–248.
8 Yang WS, Kim KJ, Gaschler MM, Patel M, Shchepinov MS & Stockwell BR (2016) Peroxidation of polyunsaturated fatty acids by lipoxygenases drives ferroptosis. *Proc Natl Acad Sci USA* **113**, E4966–E4975.
9 Wiernicki B, Dubois H, Tyurina YY, Hassannia B, Bayir H, Kagan VE, Vandenabeele P, Wullaert A & Vanden Berghe T (2020) Excessive phospholipid peroxidation distinguishes ferroptosis from other cell death modes including pyroptosis. *Cell Death Dis* **11**, 922.
10 Stockwell BR, Jiang X & Gu W (2020) Emerging mechanisms and disease relevance of ferroptosis. *Trends Cell Biol* **30**, 478–490.
11 Zhang C, Liu X, Jin S, Chen Y & Guo R (2022) Ferroptosis in cancer therapy: a novel approach to reversing drug resistance. *Mol Cancer* **21**, 47.
12 Hassannia B, Vandenabeele P & Vanden Berghe T (2019) Targeting ferroptosis to iron out cancer. *Cancer Cell* **35**, 830–849.
13 Hadian K & Stockwell BR (2021) A roadmap to creating ferroptosis-based medicines. *Nat Chem Biol* **17**, 1113–1116.
14 Wagner BA, Buettner GR & Burns CP (1994) Free radical-mediated lipid peroxidation in cells: oxidizability is a function of cell lipid bis-allylic hydrogen content. *Biochemistry* **33**, 4449–4453.
15 Kagan VE, Mao G, Qu F, Angeli JP, Doll S, Croix CS, Dar HH, Liu B, Tyurin VA, Ritov VB *et al.* (2017) Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. *Nat Chem Biol* **13**, 81–90.
16 Doll S, Proneth B, Tyurina YY, Panzilius E, Kobayashi S, Ingold I, Irmler M, Beckers J, Aichler M, Walch A *et al.* (2017) ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nat Chem Biol* **13**, 91–98.
17 Shah R, Shchepinov MS & Pratt DA (2018) Resolving the role of lipoxygenases in the initiation and execution of ferroptosis. *ACS Cent Sci* **4**, 387–396.
18 Zou Y, Li H, Graham ET, Deik AA, Eaton JK, Wang W, Sandoval-Gomez G, Clish CB, Doench JG & Schreiber SL (2020) Cytochrome P450 oxidoreductase contributes to phospholipid peroxidation in ferroptosis. *Nat Chem Biol* **16**, 302–309.
19 von Krusenstiern AN, Robson RN, Qian N, Qiu B, Hu F, Reznik E, Smith N, Zandkarimi F, Estes VM, Dupont M *et al.* (2023) Identification of essential sites of lipid peroxidation in ferroptosis. *Nat Chem Biol* **19**, 719–730.
20 Gao M, Yi J, Zhu J, Minikes AM, Monian P, Thompson CB & Jiang X (2019) Role of mitochondria in ferroptosis. *Mol Cell* **73**, 354–363.e3.
21 Torii S, Shintoku R, Kubota C, Yaegashi M, Torii R, Sasaki M, Suzuki T, Mori M, Yoshimoto Y, Takeuchi T *et al.* (2016) An essential role for functional lysosomes in ferroptosis of cancer cells. *Biochem J* **473**, 769–777.
22 Gao H, Bai Y, Jia Y, Zhao Y, Kang R, Tang D & Dai E (2018) Ferroptosis is a lysosomal cell death process. *Biochem Biophys Res Commun* **503**, 1550–1556.
23 Solier S, Muller S, Caneque T, Versini A, Mansart A, Sindikubwabo F, Baron L, Emam L, Gestraud P, Pantos GD *et al.* (2023) A druggable copper-signalling pathway that drives inflammation. *Nature* **617**, 386–394.
24 Li J, Cao F, Yin H-L, Huang Z-J, Lin Z-T, Mao N, Sun B & Wang G (2020) Ferroptosis: past, present and future. *Cell Death Dis* **11**, 88.
25 Miyamoto S, Martinez GR, Medeiros MHG & Di Mascio P (2003) Singlet molecular oxygen generated from lipid hydroperoxides by the Russell mechanism: studies using ^{18}O-labeled linoleic acid hydroperoxide and monomol light emission measurements. *J Am Chem Soc* **125**, 6172–6179.
26 Conrad M & Pratt DA (2019) The chemical basis of ferroptosis. *Nat Chem Biol* **15**, 1137–1147.
27 Helberg J & Pratt DA (2021) Autoxidation vs. antioxidants – the fight for forever. *Chem Soc Rev* **50**, 7343–7358.
28 Wenzel SE, Tyurina YY, Zhao J, St Croix CM, Dar HH, Mao G, Tyurin VA, Anthonymuthu TS, Kapralov AA, Amoscato AA *et al.* (2017) PEBP1 wardens ferroptosis by enabling lipoxygenase generation of lipid death signals. *Cell* **171**, 628–641.e26.
29 Milkovic L, Zarkovic N, Marusic Z, Zarkovic K & Jaganjac M (2023) The 4-hydroxynonenal–protein adducts and their biological relevance: are some proteins preferred targets? *Antioxidants* **12**, 856.
30 Agmon E, Solon J, Bassereau P & Stockwell BR (2018) Modeling the effects of lipid peroxidation during ferroptosis on membrane properties. *Sci Rep* **8**, 5155.

31 Hirata Y, Cai R, Volchuk A, Steinberg BE, Saito Y, Matsuzawa A, Grinstein S & Freeman SA (2023) Lipid peroxidation increases membrane tension, Piezo1 gating, and cation permeability to execute ferroptosis. *Curr Biol* **33**, 1282–1294.e5.

32 Riegman M, Sagie L, Galed C, Levin T, Steinberg N, Dixon SJ, Wiesner U, Bradbury MS, Niethammer P, Zaritsky A *et al.* (2020) Ferroptosis occurs through an osmotic mechanism and propagates independently of cell rupture. *Nat Cell Biol* **22**, 1042–1048.

33 Pedrera L, Espiritu RA, Ros U, Weber J, Schmitt A, Stroh J, Hailfinger S, von Karstedt S & Garcia-Saez AJ (2021) Ferroptotic pores induce Ca(2+) fluxes and ESCRT-III activation to modulate cell death kinetics. *Cell Death Differ* **28**, 1644–1657.

34 Carlson BA, Tobe R, Yefremova E, Tsuji PA, Hoffmann VJ, Schweizer U, Gladyshev VN, Hatfield DL & Conrad M (2016) Glutathione peroxidase 4 and vitamin E cooperatively prevent hepatocellular degeneration. *Redox Biol* **9**, 22–31.

35 Mishima E, Ito J, Wu Z, Nakamura T, Wahida A, Doll S, Tonnus W, Nepachalovich P, Eggenhofer E, Aldrovandi M *et al.* (2022) A non-canonical vitamin K cycle is a potent ferroptosis suppressor. *Nature* **608**, 778–783.

36 Kraft VAN, Bezjian CT, Pfeiffer S, Ringelstetter L, Müller C, Zandkarimi F, Merl-Pham J, Bao X, Anastasov N, Kössl J *et al.* (2020) GTP cyclohydrolase 1/tetrahydrobiopterin counteract ferroptosis through lipid remodeling. *ACS Cent Sci* **6**, 41–53.

37 Soula M, Weber RA, Zilka O, Alwaseem H, La K, Yen F, Molina H, Garcia-Bermudez J, Pratt DA & Birsoy K (2020) Metabolic determinants of cancer cell sensitivity to canonical ferroptosis inducers. *Nat Chem Biol* **16**, 1351–1360.

38 Doll S, Freitas FP, Shah R, Aldrovandi M, da Silva MC, Ingold I, Goya Grocin A, Xavier da Silva TN, Panzilius E, Scheel CH *et al.* (2019) FSP1 is a glutathione-independent ferroptosis suppressor. *Nature* **575**, 693–698.

39 Frei B, Kim MC & Ames BN (1990) Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations. *Proc Natl Acad Sci USA* **87**, 4879–4883.

40 Barayeu U, Schilling D, Eid M, Xavier Da Silva TN, Schlicker L, Mitreska N, Zapp C, Gräter F, Miller AK, Kappl R *et al.* (2023) Hydropersulfides inhibit lipid peroxidation and ferroptosis by scavenging radicals. *Nat Chem Biol* **19**, 28–37.

41 Lange M & Olzmann JA (2022) Hydropersulfides are endogenous antioxidants that inhibit ferroptosis. *Cell Chem Biol* **29**, 1661–1663.

42 Jakaria M, Belaidi AA, Bush AI & Ayton S (2023) Vitamin A metabolites inhibit ferroptosis. *Biomed Pharmacother* **164**, 114930.

43 Angeli JPF, Freitas FP, Nepachalovich P, Puentes L, Zilka O, Inague A, Lorenz S, Kunz V, Nehring H, Silva TNXD *et al.* (2021) 7-Dehydrocholesterol is an endogenous suppressor of ferroptosis. *Research Square*. doi: 10.21203/rs.3.rs-943221/v1

44 Garcia-Bermudez J, Baudrier L, Bayraktar EC, Shen Y, La K, Guarecuco R, Yucel B, Fiore D, Tavora B, Freinkman E *et al.* (2019) Squalene accumulation in cholesterol auxotrophic lymphomas prevents oxidative cell death. *Nature* **567**, 118–122.

45 Xie Y, Hou W, Song X, Yu Y, Huang J, Sun X, Kang R & Tang D (2016) Ferroptosis: process and function. *Cell Death Differ* **23**, 369–379.

46 Zhang Y, Tan H, Daniels JD, Zandkarimi F, Liu H, Brown LM, Uchida K, O'Connor OA & Stockwell BR (2019) Imidazole ketone Erastin induces ferroptosis and slows tumor growth in a mouse lymphoma model. *Cell Chem Biol* **26**, 623–633.e9.

47 Feng H & Stockwell BR (2018) Unsolved mysteries: how does lipid peroxidation cause ferroptosis? *PLoS Biol* **16**, e2006203.

48 Stockwell BR, Friedmann Angeli JP, Bayir H, Bush AI, Conrad M, Dixon SJ, Fulda S, Gascon S, Hatzios SK, Kagan VE *et al.* (2017) Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. *Cell* **171**, 273–285.

49 Hassannia B, Wiernicki B, Ingold I, Qu F, Van Herck S, Tyurina YY, Bayır H, Abhari BA, Angeli JPF, Choi SM *et al.* (2018) Nano-targeted induction of dual ferroptotic mechanisms eradicates high-risk neuroblastoma. *J Clin Investig* **128**, 3341–3355.

50 Shimada K, Skouta R, Kaplan A, Yang WS, Hayano M, Dixon SJ, Brown LM, Valenzuela CA, Wolpaw AJ & Stockwell BR (2016) Global survey of cell death mechanisms reveals metabolic regulation of ferroptosis. *Nat Chem Biol* **12**, 497–503.

51 Nakamura T, Hipp C, Santos Dias Mourao A, Borggrafe J, Aldrovandi M, Henkelmann B, Wanninger J, Mishima E, Lytton E, Emler D *et al.* (2023) Phase separation of FSP1 promotes ferroptosis. *Nature* **619**, 371–377.

52 Yao X, Xie R, Cao Y, Tang J, Men Y, Peng H & Yang W (2021) Simvastatin induced ferroptosis for triple-negative breast cancer therapy. *J Nanobiotechnol* **19**, 311.

53 Viswanathan VS, Ryan MJ, Dhruv HD, Gill S, Eichhoff OM, Seashore-Ludlow B, Kaffenberger SD, Eaton JK, Shimada K, Aguirre AJ *et al.* (2017) Dependency of a therapy-resistant state of cancer cells on a lipid peroxidase pathway. *Nature* **547**, 453–457.

54 Alim I, Caulfield JT, Chen Y, Swarup V, Geschwind DH, Ivanova E, Seravalli J, Ai Y, Sansing LH, Ste Marie EJ *et al.* (2019) Selenium drives a transcriptional adaptive program to block ferroptosis and treat stroke. *Cell* **177**, 1262–1279.e25.

55 Li Q, Han X, Lan X, Gao Y, Wan J, Durham F, Cheng T, Yang J, Wang Z, Jiang C *et al.* (2017) Inhibition of neuronal ferroptosis protects hemorrhagic brain. *JCI Insight* **2**, e90777.
56 Mai TT, Hamaï A, Hienzsch A, Cañeque T, Müller S, Wicinski J, Cabaud O, Leroy C, David A, Acevedo V *et al.* (2017) Salinomycin kills cancer stem cells by sequestering iron in lysosomes. *Nat Chem* **9**, 1025–1033.
57 Eling N, Reuter L, Hazin J, Hamacher-Brady A & Brady NR (2015) Identification of artesunate as a specific activator of ferroptosis in pancreatic cancer cells. *Oncoscience* **2**, 517–532.
58 Gaschler MM, Andia AA, Liu H, Csuka JM, Hurlocker B, Vaiana CA, Heindel DW, Zuckerman DS, Bos PH, Reznik E *et al.* (2018) FINO2 initiates ferroptosis through GPX4 inactivation and iron oxidation. *Nat Chem Biol* **14**, 507–515.
59 Dixon SJ, Winter GE, Musavi LS, Lee ED, Snijder B, Rebsamen M, Superti-Furga G & Stockwell BR (2015) Human haploid cell genetics reveals roles for lipid metabolism genes in nonapoptotic cell death. *ACS Chem Biol* **10**, 1604–1609.
60 Rodencal J & Dixon SJ (2023) A tale of two lipids: lipid unsaturation commands ferroptosis sensitivity. *Proteomics* **23**, 2100308.
61 Magtanong L, Ko P-J, To M, Cao JY, Forcina GC, Tarangelo A, Ward CC, Cho K, Patti GJ, Nomura DK *et al.* (2019) Exogenous monounsaturated fatty acids promote a ferroptosis-resistant cell state. *Cell Chem Biol* **26**, 420–432.e9.
62 Tesfay L, Paul BT, Konstorum A, Deng Z, Cox AO, Lee J, Furdui CM, Hegde P, Torti FM & Torti SV (2019) Stearoyl-CoA desaturase 1 protects ovarian cancer cells from ferroptotic cell death. *Cancer Res* **79**, 5355–5366.
63 Ma S, Henson ES, Chen Y & Gibson SB (2016) Ferroptosis is induced following siramesine and lapatinib treatment of breast cancer cells. *Cell Death Dis* **7**, e2307.
64 Song X, Xie Y, Kang R, Hou W, Sun X, Epperly MW, Greenberger JS & Tang D (2016) FANCD2 protects against bone marrow injury from ferroptosis. *Biochem Biophys Res Commun* **480**, 443–449.
65 Zhang S, Xin W, Anderson GJ, Li R, Gao L, Chen S, Zhao J & Liu S (2022) Double-edge sword roles of iron in driving energy production versus instigating ferroptosis. *Cell Death Dis* **13**, 40.
66 Gao M, Monian P, Quadri N, Ramasamy R & Jiang X (2015) Glutaminolysis and transferrin regulate ferroptosis. *Mol Cell* **59**, 298–308.
67 Anderson GJ & Vulpe CD (2009) Mammalian iron transport. *Cell Mol Life Sci* **66**, 3241–3261.
68 Hou W, Xie Y, Song X, Sun X, Lotze MT, Zeh HJ, Kang R & Tang D (2016) Autophagy promotes ferroptosis by degradation of ferritin. *Autophagy* **12**, 1425–1428.
69 Gao M, Monian P, Pan Q, Zhang W, Xiang J & Jiang X (2016) Ferroptosis is an autophagic cell death process. *Cell Res* **26**, 1021–1032.
70 Anandhan A, Dodson M, Schmidlin CJ, Liu P & Zhang DD (2020) Breakdown of an ironclad defense system: the critical role of NRF2 in mediating ferroptosis. *Cell Chem Biol* **27**, 436–447.
71 Brown CW, Amante JJ, Chhoy P, Elaimy AL, Liu H, Zhu LJ, Baer CE, Dixon SJ & Mercurio AM (2019) Prominin2 drives ferroptosis resistance by stimulating iron export. *Dev Cell* **51**, 575–586.e4.
72 Kong Y, Hu L, Lu K, Wang Y, Xie Y, Gao L, Yang G, Xie B, He W, Chen G *et al.* (2019) Ferroportin downregulation promotes cell proliferation by modulating the Nrf2–miR-17-5p axis in multiple myeloma. *Cell Death Dis* **10**, 624.
73 Yan H-F, Zou T, Tuo Q-Z, Xu S, Li H, Belaidi AA & Lei P (2021) Ferroptosis: mechanisms and links with diseases. *Signal Transduct Target Ther* **6**, 49.
74 Bannai S, Tsukeda H & Okumura H (1977) Effect of antioxidants on cultured human diploid fibroblasts exposed to cystine-free medium. *Biochem Biophys Res Commun* **74**, 1582–1588.
75 Liu N, Lin X & Huang C (2020) Activation of the reverse transsulfuration pathway through NRF2/CBS confers erastin-induced ferroptosis resistance. *Br J Cancer* **122**, 279–292.
76 Srivastava MK, Sinha P, Clements VK, Rodriguez P & Ostrand-Rosenberg S (2010) Myeloid-derived suppressor cells inhibit T-cell activation by depleting cystine and cysteine. *Cancer Res* **70**, 68–77.
77 Yang WS, SriRamaratnam R, Welsch ME, Shimada K, Skouta R, Viswanathan VS, Cheah JH, Clemons PA, Shamji AF, Clish CB *et al.* (2014) Regulation of ferroptotic cancer cell death by GPX4. *Cell* **156**, 317–331.
78 Seibt TM, Proneth B & Conrad M (2019) Role of GPX4 in ferroptosis and its pharmacological implication. *Free Radic Biol Med* **133**, 144–152.
79 Conrad M, Lorenz SM & Proneth B (2021) Targeting ferroptosis: new hope for as-yet-incurable diseases. *Trends Mol med* **27**, 113–122.
80 Friedmann Angeli JP, Schneider M, Proneth B, Tyurina YY, Tyurin VA, Hammond VJ, Herbach N, Aichler M, Walch A, Eggenhofer E *et al.* (2014) Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. *Nat Cell Biol* **16**, 1180–1191.
81 Van Coillie S, Van San E, Goetschalckx I, Wiernicki B, Mukhopadhyay B, Tonnus W, Choi SM, Roelandt R, Dumitrascu C, Lamberts L *et al.* (2022) Targeting ferroptosis protects against experimental (multi)organ dysfunction and death. *Nat Commun* **13**, 1046.

82 Bersuker K, Hendricks JM, Li Z, Magtanong L, Ford B, Tang PH, Roberts MA, Tong B, Maimone TJ, Zoncu R *et al*. (2019) The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis. *Nature* **575**, 688–692.
83 Mao C, Liu X, Zhang Y, Lei G, Yan Y, Lee H, Koppula P, Wu S, Zhuang L, Fang B *et al*. (2021) DHODH-mediated ferroptosis defence is a targetable vulnerability in cancer. *Nature* **593**, 586–590.
84 Dixon SJ, Patel DN, Welsch M, Skouta R, Lee ED, Hayano M, Thomas AG, Gleason CE, Tatonetti NP, Slusher BS *et al*. (2014) Pharmacological inhibition of cystine–glutamate exchange induces endoplasmic reticulum stress and ferroptosis. *eLife* **3**, e02523.
85 Yang WS & Stockwell BR (2016) Ferroptosis: death by lipid peroxidation. *Trends Cell Biol* **26**, 165–176.
86 Hassannia B, Van Coillie S & Vanden Berghe T (2021) Ferroptosis: biological rust of lipid membranes. *Antioxid Redox Signal* **35**, 487–509.
87 Stockwell BR (2022) Ferroptosis turns 10: emerging mechanisms, physiological functions, and therapeutic applications. *Cell* **185**, 2401–2421.
88 Bayır H, Anthonymuthu TS, Tyurina YY, Patel SJ, Amoscato AA, Lamade AM, Yang Q, Vladimirov GK, Philpott CC & Kagan VE (2020) Achieving life through death: redox biology of lipid peroxidation in ferroptosis. *Cell Chem Biol* **27**, 387–408.
89 Gérard-Monnier D, Erdelmeier I, Régnard K, Moze-Henry N, Yadan J-C & Chaudière J (1998) Reactions of 1-methyl-2-phenylindole with malondialdehyde and 4-hydroxyalkenals. Analytical applications to a colorimetric assay of lipid peroxidation. *Chem Res Toxicol* **11**, 1176–1183.
90 Toyokuni S, Miyake N, Hiai H, Hagiwara M, Kawakishi S, Osawa T & Uchida K (1995) The monoclonal antibody specific for the 4-hydroxy-2-nonenal histidine adduct. *FEBS Lett* **359**, 189–191.
91 Bochkov V, Gesslbauer B, Mauerhofer C, Philippova M, Erne P & Oskolkova OV (2017) Pleiotropic effects of oxidized phospholipids. *Free Radic Biol Med* **111**, 6–24.
92 Qin J, Goswami R, Balabanov R & Dawson G (2007) Oxidized phosphatidylcholine is a marker for neuroinflammation in multiple sclerosis brain. *J Neurosci Res* **85**, 977–984.
93 Criscuolo A, Nepachalovich P, Garcia-Del Rio DF, Lange M, Ni Z, Baroni M, Cruciani G, Goracci L, Bluher M & Fedorova M (2022) Analytical and computational workflow for in-depth analysis of oxidized complex lipids in blood plasma. *Nat Commun* **13**, 6547.
94 David S, Jhelum P, Ryan F, Jeong SY & Kroner A (2022) Dysregulation of iron homeostasis in the central nervous system and the role of ferroptosis in neurodegenerative disorders. *Antioxid Redox Signal* **37**, 150–170.
95 Feng H, Schorpp K, Jin J, Yozwiak CE, Hoffstrom BG, Decker AM, Rajbhandari P, Stokes ME, Bender HG, Csuka JM *et al*. (2020) Transferrin receptor is a specific ferroptosis marker. *Cell Rep* **30**, 3411–3423.e7.
96 Fang Y, Chen X, Tan Q, Zhou H, Xu J & Gu Q (2021) Inhibiting ferroptosis through disrupting the NCOA4–FTH1 interaction: a new mechanism of action. *ACS Cent Sci* **7**, 980–989.
97 Sun X, Ou Z, Chen R, Niu X, Chen D, Kang R & Tang D (2016) Activation of the p62-Keap1-NRF2 pathway protects against ferroptosis in hepatocellular carcinoma cells. *Hepatology* **63**, 173–184.
98 Dodson M, Castro-Portuguez R & Zhang DD (2019) NRF2 plays a critical role in mitigating lipid peroxidation and ferroptosis. *Redox Biol* **23**, 101107.
99 Sun X, Ou Z, Xie M, Kang R, Fan Y, Niu X, Wang H, Cao L & Tang D (2015) HSPB1 as a novel regulator of ferroptotic cancer cell death. *Oncogene* **34**, 5617–5625.
100 Tenopoulou M, Kurz T, Doulias P-T, Galaris D & Brunk UT (2007) Does the calcein-AM method assay the total cellular 'labile iron pool' or only a fraction of it? *Biochem J* **403**, 261–266.
101 Hirayama T, Okuda K & Nagasawa H (2013) A highly selective turn-on fluorescent probe for iron(ii) to visualize labile iron in living cells. *Chem Sci* **4**, 1250–1256.
102 Hirayama T (2018) Development of chemical tools for imaging of Fe(II) ions in living cells: a review. *Acta Histochem Cytochem* **51**, 137–143.
103 Hametner S, Wimmer I, Haider L, Pfeifenbring S, Bruck W & Lassmann H (2013) Iron and neurodegeneration in the multiple sclerosis brain. *Ann Neurol* **74**, 848–861.
104 Bernhard Michalke VV (2022) Ex Vivo Analytical Method; International PCT Application at the European Patent Office with the File Number: PCT/EP2022/073848 in pp. 1–24.
105 Michalke B, Willkommen D & Venkataramani V (2019) Iron redox speciation analysis using capillary electrophoresis coupled to inductively coupled plasma mass spectrometry (CE-ICP-MS). *Front Chem* **7**, 136.
106 Michalke B, Willkommen D & Venkataramani V (2020) Setup of capillary electrophoresis-inductively coupled plasma mass spectrometry (CE-ICP-MS) for quantification of iron redox species (Fe(II), Fe(III)). *J Vis Exp*. doi: 10.3791/61055
107 Hu S, Sechi M, Singh PK, Dai L, McCann S, Sun D, Ljungman M & Neamati N (2020) A novel redox modulator induces a GPX4-mediated cell death that is dependent on iron and reactive oxygen species. *J med Chem* **63**, 9838–9855.
108 Zheng J, Sato M, Mishima E, Sato H, Proneth B & Conrad M (2021) Sorafenib fails to trigger ferroptosis across a wide range of cancer cell lines. *Cell Death Dis* **12**, 698.

109 Chen W, Yuan X, Li Z, Lu Z, Kong S, Jiang H, Du H, Pan X, Nandi M, Kong X *et al.* (2020) CN128: a new orally active hydroxypyridinone iron chelator. *J med Chem* **63**, 4215–4226.
110 Lesnefsky EJ, Repine JE & Horwitz LD (1990) Deferoxamine pretreatment reduces canine infarct size and oxidative injury. *J Pharmacol Exp Ther* **253**, 1103–1109.
111 Patt A, Horesh IR, Berger EM, Harken AH & Repine JE (1990) Iron depletion or chelation reduces ischemia/reperfusion-induced edema in gerbil brains. *J Pediatr Surg* **25**, 224–227; discussion 227–8.
112 Paller MS & Hedlund BE (1988) Role of iron in postischemic renal injury in the rat. *Kidney Int* **34**, 474–480.
113 Chen J, Marks E, Lai B, Zhang Z, Duce JA, Lam LQ, Volitakis I, Bush AI, Hersch S & Fox JH (2013) Iron accumulates in Huntington's disease neurons: protection by deferoxamine. *PLoS One* **8**, e77023.
114 Nunez M & Chaná P (2019) New perspectives in iron chelation therapy for the treatment of Parkinson's disease. *Neural Regen Res* **14**, 1905–1906.
115 Devos D, Cabantchik Z, Moreau C, Danel V, Mahoney-Sanchez L, Bouchaoui H, Gouel F, Rolland A-S, Duce J & Devedjian J-C (2020) Conservative iron chelation for neurodegenerative diseases such as Parkinson's disease and amyotrophic lateral sclerosis. *J Neural Transm* **127**, 189–203.
116 Devos D, Moreau C, Devedjian JC, Kluza J, Petrault M, Laloux C, Jonneaux A, Ryckewaert G, Garçon G, Rouaix N *et al.* (2014) Targeting chelatable iron as a therapeutic modality in Parkinson's disease. *Antioxid Redox Signal* **21**, 195–210.
117 Kontoghiorghes GJ & Kontoghiorghe CN (2020) Iron and chelation in biochemistry and medicine: new approaches to controlling iron metabolism and treating related diseases. *Cell* **9**, 1456.
118 Zilka O, Shah R, Li B, Friedmann Angeli JP, Griesser M, Conrad M & Pratt DA (2017) On the mechanism of cytoprotection by ferrostatin-1 and liproxstatin-1 and the role of lipid peroxidation in ferroptotic cell death. *ACS Cent Sci* **3**, 232–243.
119 Miotto G, Rossetto M, Di Paolo ML, Orian L, Venerando R, Roveri A, Vuckovic AM, Bosello Travain V, Zaccarin M, Zennaro L *et al.* (2020) Insight into the mechanism of ferroptosis inhibition by ferrostatin-1. *Redox Biol* **28**, 101328.
120 Sheng X-H, Cui C-C, Shan C, Li Y-Z, Sheng D-H, Sun B & Chen D-Z (2018) O-phenylenediamine: a privileged pharmacophore of ferrostatins for radical-trapping reactivity in blocking ferroptosis. *Org Biomol Chem* **16**, 3952–3960.
121 Angeli JPF, Shah R, Pratt DA & Conrad M (2017) Ferroptosis inhibition: mechanisms and opportunities. *Trends Pharmacol Sci* **38**, 489–498.
122 Devisscher L, Van Coillie S, Hofmans S, Van Rompaey D, Goossens K, Meul E, Maes L, De Winter H, Van Der Veken P, Vandenabeele P *et al.* (2018) Discovery of novel, drug-like ferroptosis inhibitors with in vivo efficacy. *J med Chem* **61**, 10126–10140.
123 Linkermann A, Skouta R, Himmerkus N, Mulay SR, Dewitz C, De Zen F, Prokai A, Zuchtriegel G, Krombach F, Welz PS *et al.* (2014) Synchronized renal tubular cell death involves ferroptosis. *Proc Natl Acad Sci USA* **111**, 16836–16841.
124 Zhao Z, Wu J, Xu H, Zhou C, Han B, Zhu H, Hu Z, Ma Z, Ming Z, Yao Y *et al.* (2020) XJB-5-131 inhibited ferroptosis in tubular epithelial cells after ischemia–reperfusion injury. *Cell Death Dis* **11**, 629.
125 Krainz T, Gaschler MM, Lim C, Sacher JR, Stockwell BR & Wipf P (2016) A mitochondrial-targeted nitroxide is a potent inhibitor of ferroptosis. *ACS Cent Sci* **2**, 653–659.
126 Xie Y, Song X, Sun X, Huang J, Zhong M, Lotze MT, Zeh HJ, Kang R & Tang D (2016) Identification of baicalein as a ferroptosis inhibitor by natural product library screening. *Biochem Biophys Res Commun* **473**, 775–780.
127 Liu Y, Wang W, Li Y, Xiao Y, Cheng J & Jia J (2015) The 5-lipoxygenase inhibitor zileuton confers neuroprotection against glutamate oxidative damage by inhibiting ferroptosis. *Biol Pharm Bull* **38**, 1234–1239.
128 Soriano-Castell D, Currais A & Maher P (2021) Defining a pharmacological inhibitor fingerprint for oxytosis/ferroptosis. *Free Radic Biol Med* **171**, 219–231.
129 Dar HH, Mikulska-Ruminska K, Tyurina YY, Luci DK, Yasgar A, Samovich SN, Kapralov AA, Souryavong AB, Tyurin VA, Amoscato AA *et al.* (2023) Discovering selective antiferroptotic inhibitors of the 15LOX/PEBP1 complex noninterfering with biosynthesis of lipid mediators. *Proc Natl Acad Sci USA* **120**, e2218896120.
130 Bagayoko S & Meunier E (2022) Emerging roles of ferroptosis in infectious diseases. *FEBS J* **289**, 7869–7890.
131 Kapralov AA, Yang Q, Dar HH, Tyurina YY, Anthonymuthu TS, Kim R, St Croix CM, Mikulska-Ruminska K, Liu B, Shrivastava IH *et al.* (2020) Redox lipid reprogramming commands susceptibility of macrophages and microglia to ferroptotic death. *Nat Chem Biol* **16**, 278–290.
132 Wiernicki B, Maschalidi S, Pinney J, Adjemian S, Vanden Berghe T, Ravichandran KS & Vandenabeele P (2022) Cancer cells dying from ferroptosis impede dendritic cell-mediated anti-tumor immunity. *Nat Commun* **13**, 3676.

133 Eltzschig HK & Eckle T (2011) Ischemia and reperfusion – from mechanism to translation. *Nat Med* **17**, 1391–1401.
134 Wu MY, Yiang GT, Liao WT, Tsai AP, Cheng YL, Cheng PW, Li CY & Li CJ (2018) Current mechanistic concepts in ischemia and reperfusion injury. *Cell Physiol Biochem* **46**, 1650–1667.
135 Yan HF, Tuo QZ, Yin QZ & Lei P (2020) The pathological role of ferroptosis in ischemia/reperfusion-related injury. *Zool Res* **41**, 220–230.
136 Martin-Sanchez D, Ruiz-Andres O, Poveda J, Carrasco S, Cannata-Ortiz P, Sanchez-Niño MD, Ruiz Ortega M, Egido J, Linkermann A, Ortiz A *et al.* (2017) Ferroptosis, but not necroptosis, is important in nephrotoxic folic acid–induced AKI. *J Am Soc Nephrol* **28**, 218–229.
137 Lorente L, Rodriguez ST, Sanz P, Abreu-González P, Díaz D, Moreno AM, Borja E, Martín MM, Jiménez A & Barrera MA (2016) Association between pre-transplant serum malondialdehyde levels and survival one year after liver transplantation for hepatocellular carcinoma. *Int J Mol Sci* **17**, 500.
138 Fang X, Wang H, Han D, Xie E, Yang X, Wei J, Gu S, Gao F, Zhu N, Yin X *et al.* (2019) Ferroptosis as a target for protection against cardiomyopathy. *Proc Natl Acad Sci USA* **116**, 2672–2680.
139 Li W, Feng G, Gauthier JM, Lokshina I, Higashikubo R, Evans S, Liu X, Hassan A, Tanaka S, Cicka M *et al.* (2019) Ferroptotic cell death and TLR4/Trif signaling initiate neutrophil recruitment after heart transplantation. *J Clin Invest* **129**, 2293–2304.
140 Tanoue Y, Morita S, Ochiai Y, Hisahara M, Masuda M, Kawachi Y, Tominaga R & Yasui H (1996) Inhibition of lipid peroxidation with the lazaroid U74500A attenuates ischemia-reperfusion injury in a canine orthotopic heart transplantation model. *J Thorac Cardiovasc Surg* **112**, 1017–1026.
141 Jacobs W, Lammens M, Kerckhofs A, Voets E, Van San E, Van Coillie S, Peleman C, Mergeay M, Sirimsi S, Matheeussen V *et al.* (2020) Fatal lymphocytic cardiac damage in coronavirus disease 2019 (COVID-19): autopsy reveals a ferroptosis signature. *ESC Heart Fail* **7**, 3772–3781.
142 Puylaert P, Roth L, Van Praet M, Pintelon I, Dumitrascu C, Van Nuijs A, Klejborowska G, Guns P-J, Berghe TV, Augustyns K *et al.* (2023) Effect of erythrophagocytosis-induced ferroptosis during angiogenesis in atherosclerotic plaques. *Angiogenesis* **26**, 505–522.
143 Zhou Y, Zhou H, Hua L, Hou C, Jia Q, Chen J, Zhang S, Wang Y, He S & Jia E (2021) Verification of ferroptosis and pyroptosis and identification of PTGS2 as the hub gene in human coronary artery atherosclerosis. *Free Radic Biol Med* **171**, 55–68.
144 Li W, Xu LH, Forssell C, Sullivan JL & Yuan XM (2008) Overexpression of transferrin receptor and ferritin related to clinical symptoms and destabilization of human carotid plaques. *Exp Biol Med (Maywood)* **233**, 818–826.
145 IjäS P, Nuotio K, Saksi J, Soinne L, Saimanen E, Karjalainen-Lindsberg M-L, Salonen O, Sarna S, Tuimala J, Kovanen PT *et al.* (2007) Microarray analysis reveals overexpression of CD163 and HO-1 in symptomatic carotid plaques. *Arterioscler Thromb Vasc Biol* **27**, 154–160.
146 Kockx MM, Cromheeke KM, Knaapen MWM, Bosmans JM, De Meyer GRY, Herman AG & Bult H (2003) Phagocytosis and macrophage activation associated with hemorrhagic microvessels in human atherosclerosis. *Arterioscler Thromb Vasc Biol* **23**, 440–446.
147 Fleischmann-Struzek C, Mellhammar L, Rose N, Cassini A, Rudd KE, Schlattmann P, Allegranzi B & Reinhart K (2020) Incidence and mortality of hospital- and ICU-treated sepsis: results from an updated and expanded systematic review and meta-analysis. *Intensive Care Med* **46**, 1552–1562.
148 Rudd KE, Johnson SC, Agesa KM, Shackelford KA, Tsoi D, Kievlan DR, Colombara DV, Ikuta KS, Kissoon N, Finfer S *et al.* (2020) Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. *Lancet* **395**, 200–211.
149 DeGregorio-Rocasolano N, Marti-Sistac O & Gasull T (2019) Deciphering the iron side of stroke: neurodegeneration at the crossroads between iron dyshomeostasis, excitotoxicity, and ferroptosis. *Front Neurosci* **13**, 85.
150 Maher P, Currais A & Schubert D (2020) Using the oxytosis/ferroptosis pathway to understand and treat age-associated neurodegenerative diseases. *Cell Chem Biol* **27**, 1456–1471.
151 Lewerenz J, Ates G, Methner A, Conrad M & Maher P (2018) Oxytosis/ferroptosis—(re-) emerging roles for oxidative stress-dependent non-apoptotic cell death in diseases of the central nervous system. *Front Neurosci* **12**, 214.
152 Zhang Y-H, Wang D-W, Xu S-F, Zhang S, Fan Y-G, Yang Y-Y, Guo S-Q, Wang S, Guo T, Wang Z-Y *et al.* (2018) α-Lipoic acid improves abnormal behavior by mitigation of oxidative stress, inflammation, ferroptosis, and tauopathy in P301S tau transgenic mice. *Redox Biol* **14**, 535–548.
153 Do Van B, Gouel F, Jonneaux A, Timmerman K, Gele P, Petrault M, Bastide M, Laloux C, Moreau C, Bordet R *et al.* (2016) Ferroptosis, a newly characterized form of cell death in Parkinson's disease that is regulated by PKC. *Neurobiol Dis* **94**, 169–178.
154 Skouta R, Dixon SJ, Wang J, Dunn DE, Orman M, Shimada K, Rosenberg PA, Lo DC, Weinberg

JM, Linkermann A *et al.* (2014) Ferrostatins inhibit oxidative lipid damage and cell death in diverse disease models. *J Am Chem Soc* **136**, 4551–4556.
155 San EV, Debruyne AC, Veeckmans G, Tyurina YY, Tyurin VA, Zheng H, Choi SM, Augustyns K, van Loo G, Michalke B *et al.* (2023) Ferroptosis contributes to multiple sclerosis and its pharmacological targeting suppresses experimental disease progression. *Cell Death Differ* **30**, 2092–2103.
156 Campbell BCV & Khatri P (2020) Stroke. *Lancet* **396**, 129–142.
157 Lee WC, Wong HY, Chai YY, Shi CW, Amino N, Kikuchi S & Huang SH (2012) Lipid peroxidation dysregulation in ischemic stroke: plasma 4-HNE as a potential biomarker? *Biochem Biophys Res Commun* **425**, 842–847.
158 Tuo QZ, Lei P, Jackman KA, Li XL, Xiong H, Li XL, Liuyang ZY, Roisman L, Zhang ST, Ayton S *et al.* (2017) Tau-mediated iron export prevents ferroptotic damage after ischemic stroke. *Mol Psychiatry* **22**, 1520–1530.
159 Park H-A, Kubicki N, Gnyawali S, Chan Y, Roy S, Khanna S & Sen CK (2011) Natural vitamin E α-tocotrienol protects against ischemic stroke by induction of multidrug resistance-associated protein 1. *Stroke* **42**, 2308–2314.
160 Simpson EP, Henry YK, Henkel JS, Smith RG & Appel SH (2004) Increased lipid peroxidation in sera of ALS patients. *Neurology* **62**, 1758–1765.
161 Wang T, Tomas D, Perera ND, Cuic B, Luikinga S, Viden A, Barton SK, McLean CA, Samson AL, Southon A *et al.* (2021) Ferroptosis mediates selective motor neuron death in amyotrophic lateral sclerosis. *Cell Death Differ* **29**, 1–12.
162 Tu L-F, Zhang T-Z, Zhou Y-F, Zhou Q-Q, Gong H-B, Liang L, Hai L-N, You N-X, Su Y, Chen Y-J *et al.* (2023) GPX4 deficiency-dependent phospholipid peroxidation drives motor deficits of ALS. *J Adv Res* **43**, 205–218.