




Multifaceted nanoparticles: emerging mechanisms and therapies in neurodegenerative diseases

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Neurodegenerative diseases are a major global health burden particularly with the increasing ageing population. Hereditary predisposition and environmental risk factors contribute to the heterogeneity of existing pathological phenotypes. Traditional clinical interventions focused on the use of small drugs have often led to failures due to the difficulties in crossing the blood–brain barrier and reaching the brain. In this regard, nanosystems can specifically deliver drugs and improve their bioavailability, overcoming some of the major challenges in neurodegenerative disease treatment.

This review focuses on the use of nanosystems as an encouraging therapeutic approach targeting molecular pathways involved in localized and systematic neurodegenerative diseases. Among the latter, Friedreich's ataxia is an untreatable complex multisystemic disorder and the most widespread type of ataxia; it represents a test case to validate the clinical potential of therapeutic strategies based on nanoparticles with pleiotropic effects.

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Introduction

Neurodegenerative diseases (NDs) are a heterogeneous group of CNS disorders characterized by chronic and selective neuronal cell death, decreased strength, coordination and mobility, respiratory distress and cognitive deficit.¹ Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS) are the major NDs.^{1,2} Although genetic and hereditary predisposition seem to play an important role, especially combined with environmental risk factors,³ NDs differ in pathophysiology and symptomatology.

On the contrary, protein misfolding, aggregation and accumulation of proteins, neuroinflammation, mitochondrial dysfunctions, oxidative stress, dysregulated autophagy and apoptosis⁴ are some of the most important shared biological processes. Most of these processes are also characteristic of Friedreich's ataxia (FRDA), a multi-systemic autosomal recessive degenerative disorder affecting central and peripheral nervous system, heart, skeletal muscle and endocrine pancreas.^{5,6} With onset before 25 years of age, FRDA affects 1 in 30 000–50 000 people, with prominent neurological manifestations including limb ataxia, spinocerebellar ataxia, dysarthria, muscle weakness of lower limbs, loss of tone and

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areflexia, alterations of cardiac function and diabetes mellitus.⁷ The most severe degenerative processes affect the spinocerebellar and pyramidal tracts as well as the dorsal root ganglia (DRGs) and dentate nuclei of cerebellum, resulting in degeneration of large sensory neurons and neuronal cell death.⁸ Cardiac complications and aspiration pneumonia may appear subsequently to neurological symptoms and in most cases are the cause of death of patients affected with FRDA.^{9,10} Other related complications include coma and stroke.¹¹

Most FRDA cases (96–98%) are caused by a homozygous GAA triplet-repeat expansion mutation in the first intron of the frataxin (FXN) gene on chromosome 9. A minority of patients have heterozygous GAA expansions, deletions or point mutations in the FXN gene.^{5,12} In both cases, there is a dramatic reduction of FXN protein expression. Frataxin is a nuclear-encoded mitochondrial protein involved in iron-sulphur (Fe-S) cluster biogenesis, iron homeostasis, maintenance of the redox state, energetic metabolism, purine synthesis and DNA repair.^{13,14} FXN is localized to the mitochondrial inner membrane and its deficiency leads to mitochondrial iron accumulation, mitochondrial dysfunction, dysregulated energetic metabolism, increased oxidative stress with generation of mitochondrial reactive oxygen species (ROS) and mitochondrial network dynamic dysregulations.¹⁵ Furthermore, FXN interacts with mitochondrial electron-transport chain (ETC) complexes affecting mitochondrial respiration and energy production.¹⁶

Recently, life expectancy has increased, but to date there is no cure for FRDA. Therapeutic interventions include treatment with antioxidant compounds to mitigate mitochondrial oxidative stress, β -blockers, and ACE inhibitors to control heart rhythm preventing cardiac failure.^{17,18}

The pharmaceuticals targeting mitochondrial dysfunction and metabolism (Friedreich's Ataxia Research Alliance, <https://curefa.org/index.php>) are promising, but none of them have been approved yet by the Food and Drug Administration (FDA).

The poor understanding of FXN-related complex pathogenesis mechanisms challenges the development of therapies with long term efficacy and low secondary toxicity, which can cause adverse outcomes. Emerging scientific works suggest the possibility to use nanotechnologies such as nanoparticles (NPs) and engineered materials to improve the treatment of NDs. The aim of this review is to discuss the role of NPs and other nanosystems in the treatment of NDs and of FRDA. Data were retrieved mainly from PubMed, from relevant articles published in the last 10 years regarding the use of nanosystems in the management of NDs.

Nanoparticles and their use as therapeutic strategy

NPs are between 1 and 100 nm in size. They are synthesized from metals, polymers or carbon compounds and differ in size, origin, shape and composition.¹⁹ NPs can be categorized as 'organic lipid-based' (micelles, liposomes and nanoemulsions),²⁰ 'polymeric' (polymer micelles and dendrimers)²¹ or 'inorganic' [carbon nanotubes, nanocrystals, gold (Au) NPs, iron oxide NPs]²² (Fig. 1).

NPs are great candidates for ND treatment due to their intrinsic properties, namely the high surface to mass ratio, high stability and both hydrophilic and hydrophobic affinity. They can mimic the lipidic environment of cell membranes, can encapsulate both hydrophilic and hydrophobic pharmaceuticals²³ and may be functionalized with a variety of molecules for biosensing and drug delivery.²⁴ Depending on origin and shape, NPs can be flexible,²⁵ biocompatible²⁶ and suitable for drug delivery across different compartments, including the blood–brain barrier (BBB).^{22,27,28}

The morphological and physico-chemical properties of NPs have a strong impact on their cellular internalization, and they can easily be manipulated to achieve active and passive drug targeting in a controlled fashion and through different routes of administration (oral, transdermal, intravenous, intranasal and pulmonary).^{29,30}

The major internalization pathway is endocytosis (Fig. 2),³¹ an active transport mechanism. The amount of energy that is required for internalization is mainly dependent on the size, surface charge and flexibility of the nanosystem,³² with silica and metal NPs being the ones that require the highest amount of energy. Indeed, lipid NPs are held together by the same hydrophobic interactions that normally occur in cell membranes, hence their fusion and cellular uptake is facilitated compared to silica and metal NPs which have more rigid structures.³¹

Internalization by passive transport is also possible through intramolecular spaces in the membrane (hydrophobic NPs), protein channels (hydrophilic NPs) and protein carriers (hydrophilic NPs). Mechanisms of NP uptake have been previously described in detail by Sabourian *et al.*³¹

Stimuli-responsive NPs can also be designed to increase internalization and drug release by modifying the surface of the nanocarrier in order to make it responsive to a biological stimulus, such as a change in pH, temperature or presence of ROS.³³ pH-sensitive NPs have mainly been used to treat tumours and their microenvironment, as the change in pH is crucial for the development of the lesion and can be exploited for pH-dependent release of drugs. A similar approach is also applicable to the treatment of NDs, since abnormal ROS production, commonly observed in NDs (FRDA, PD, AD), is linked to pH changes in the brains of patients.

The tunable characteristics of NPs contribute to improved pharmacokinetics compared with conventional drugs and allow some major problems in the treatment of NDs to be overcome, such as the difficulty in reaching the target tissue or compartment while also avoiding local and systemic toxicity. Small molecule drugs frequently show poor bioavailability at the desired site of treatment due to hepatic metabolism and clearance. For this reason, the administered dose of drug is higher than the effective dose, with consequent accumulation and potentially toxic side effects. Instead, nanosystems can be modified to increase drug bioavailability, reduce the administered dose and increase retention times. More recently, new nanomaterials with further improved characteristics have been developed, such as nanoemulsions and nanocrystals.

Nanoemulsions are nano-sized emulsions, typically in the range of 20–200 nm, in which two immiscible liquids are mixed to form a single phase by means of an emulsifying agent. They differ from standard emulsions due to their reduced size that makes nanoemulsions easier to be administered, absorbed and engrafted. Nanocrystals instead are solid carriers, usually spheres, with amorphous and lipophilic surfaces, which are often negatively charged. These systems are manufactured to facilitate the delivery of active pharmaceutical ingredients. As a matter of fact, nanoemulsions have already been used to deliver compounds to the CNS,^{34,35} demonstrating higher efficacy than intravenous administration of small-molecule drugs.^{36,37} Gold nanocrystal suspensions have been tested in preclinical studies in animal models of AD,³⁸ MS³⁹ and PD,⁴⁰ showing improvement in neurorepair processes and neuronal resistance. Phase I clinical trials showed that gold nanocrystals (CNM-Au8) treatment is safe and well-tolerated,⁴¹ leading to its approval for phase II clinical trials in patients with early symptoms of ALS (Table 1).⁴²

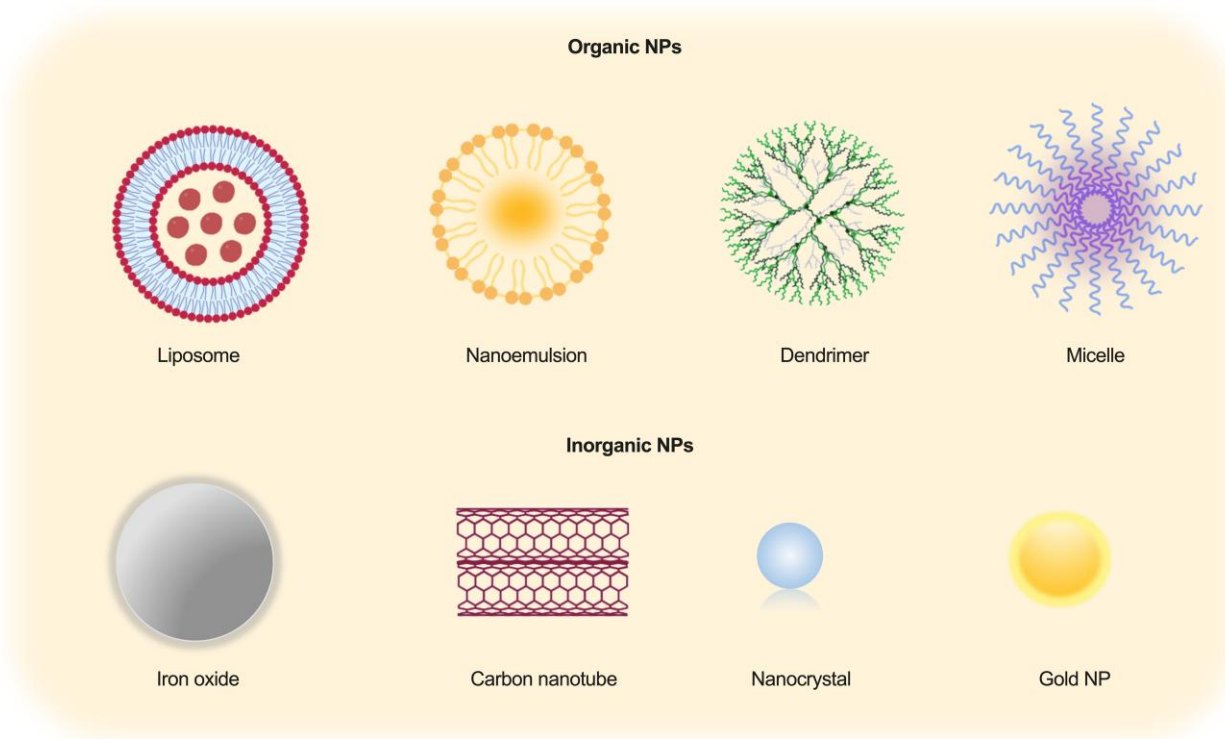


Figure 1 NP classification. Schematic representation of organic and inorganic NPs.

The most common disadvantages of NPs are: (i) the tendency to create aggregates with potential toxicity; (ii) the abnormal tissue internalization and excretion from the body; (iii) the interaction and adhesion to other cellular components, such as lipids, proteins and nucleic acids.^{68–70} Mostly, these disadvantages are dependent on NP size, material and route of administration. The inhalation of NPs may lead to their accumulation in the alveoli, where they can cause oxidative stress-mediated lung inflammation at both acute and chronic stages. Inhalation may also lead to NPs accumulation in the brain through the olfactory bulb,⁷¹ where they can exert neurotoxicity and cause neuronal cell death due to oxidative damage.^{72–74} Systemically injected NPs entering the bloodstream to reach distant organs may instead get entrapped into filter organs such as spleen and kidneys, where they accumulate and may determine inflammation by triggering ROS mediated inflammatory pathways.^{75,76} To overcome these issues, future optimization processes will aim at improving clearance from the body. For instance, NPs with high targeting capacity can be coated with different molecules depending on the compartment of interest to promote receptor-mediated transcytosis and endocytosis, which also improves drug delivery and residual time at the specific site.^{77,78} This strategy is particularly well suited for those pathology characterized by the overexpression of a receptor, whose ligand can be functionalized on the NP surface.⁷⁹ Furthermore, ‘stealth-coating’ of NPs with polymers (such as polyethylene glycol and polyzwitterions) and signalling proteins (ex. CD47) prevent opsonization and clearance through the mononuclear phagocyte system or complement system, thus improving blood circulation half-life.⁸⁰

Other strategies include using surfactants as stabilizers to avoid the formation of high dimension aggregates and limit unwanted interaction with cellular components, therefore reducing ROS

formation and preventing local toxicity. Such modifications are more applicable to NPs rather than small-molecule drugs due to their versatility in terms of physical-chemical properties, biological functions and synthetic processes.

Nanoparticles for neuroinflammation and protein aggregation

Neuroinflammation is one of the primary mechanisms triggering neurodegeneration. The neuroinflammatory response in CNS is initiated by microglial cells that activate the production of pro-inflammatory cytokines when they recognize a harmful substance threatening the brain⁸¹ (Fig. 3). In addition, neuroinflammation can also be determined by insoluble protein aggregation and dysregulated protein degradation, such as tau, A β in AD, huntingtin in HD and α -synuclein in PD, which cause the continuous activation of inflammatory cells.

Emerging evidence suggests the potential efficacy of NPs in targeting neuroinflammation and protein aggregation. Phosphatidic acid and cardiolipin immune-polyethylene glycol (PEG), and poly lactic-co-glycolic acid (PLGA) liposomes can promote the reduction of neuroinflammation and A β aggregates in AD animal models.^{44–48} In this work, lipidic nanocarriers administered intraperitoneally could reach the lymphatic system, and eventually the bloodstream. Here, the liposomes sequestered free A β due to their high avidity and affinity, thus shifting the equilibrium and drawing A β from the CNS, a phenomenon that has previously been reported and referred to as the ‘sink-effect’.^{82,83} Moreover, PEG and PLGA are biocompatible polymers that provide a steric barrier for the nanocarriers, preventing opsonization and allowing longer retention times in the blood stream. Polyethylenimine (PEI) loaded NPs have shown

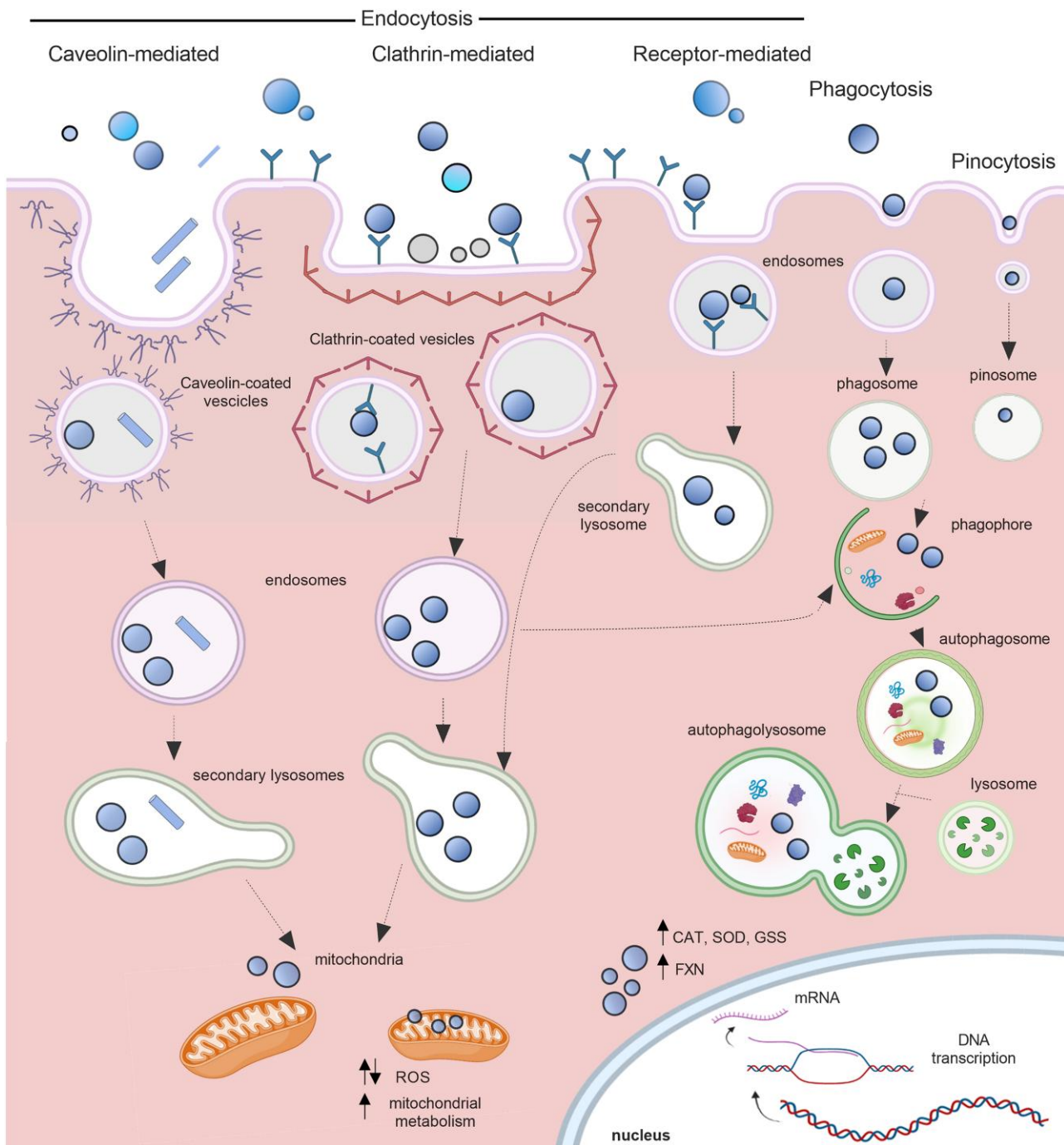


Figure 2 Internalization and target pathways of NPs. NPs are internalized by different mechanism, including endocytosis mediated by caveolin vesicles, clathrin vesicles, specific receptors along with phagocytosis and pinocytosis. NPs can enter lysosomes before interacting with other cellular components, such as mitochondria. Finally, NPs can exert their effect on ROS production, mitochondrial metabolism and gene expression.

similar results, as PEI is a suitable material for improving delivery of different types of molecules.⁵¹ Effective reduction in neuroinflammation and protein aggregate formation has been achieved by using AuNPs as a stabilizing surface for chiral peptide inhibitors. In this regard, the enantioselectivity of the inhibitors was proven to inhibit A β protein aggregation⁶⁵ more effectively than free peptide inhibitors and with very low, if any, cytotoxicity. Moreover, AuNP administration showed significant reduction in A β ₄₂

fibrillation *in vitro*, showing shorter, amorphous fibrils.³⁸ When administered intravenously in mice models, chiral AuNPs were able to exit blood circulation and penetrate the BBB to exert their effect in the brain. After the treatment, AD animal models showed reduced A β deposition and significant improvement in spatial learning and memory. Therefore, the use of NPs is effective in reducing A β deposition by increasing the affinity, avidity and selectivity for free A β peptides.

Table 1 Applications of nanosystems in NDs

Nanosystem	Loaded compound	Disease	Preclinical studies	Clinical studies (ClinicalTrials.gov identifier)
Liposomes				
PEG-LPs	Anti-A β antibodies	AD	Ordóñez-Gutiérrez <i>et al.</i> ⁴³	–
LPs	Phosphatidic acid and cardiolipin	AD	Ordóñez-Gutiérrez <i>et al.</i> ⁴⁴	–
Lipid NPs	FXN mRNA	FRDA	Nabhan <i>et al.</i> ⁴⁵	–
LPs	Polyphenols	PD	Marino <i>et al.</i> ⁴⁶	–
Polymeric NPs				
PLGA NPs	Vitamin D binding protein	AD	Jeon <i>et al.</i> ⁴⁷	–
PEG-PLGA NPs	Pioglitazone	AD	Silva-Abreu <i>et al.</i> ⁴⁸	–
Silk fibroin NPs	Curcumin	FRDA	Xu <i>et al.</i> ⁴⁹	–
PLGA NPs	Nicotine	PD	Tiwari <i>et al.</i> ⁵⁰	–
PEI NPs	siRNA against α -synuclein	PD	Helmschrodt <i>et al.</i> ⁵¹	–
Nanoemulsions				
Curcumin NE	–	PD	Ramires Júnior <i>et al.</i> ⁵²	–
Rutin NE	–	PD	Sharma <i>et al.</i> ⁵³	–
Naringenin NE	Vitamin E	PD	Gaba <i>et al.</i> ³⁷	–
Resveratrol NE	Vitamin E	PD	Pangeni <i>et al.</i> ³⁶	–
Dendrimers				
Polyamidoamine dendrimers	–	AD, PD	Klajnert <i>et al.</i> , ⁵⁴ Rekas <i>et al.</i> , ⁵⁵ Igartúa <i>et al.</i> ⁵⁶	–
Hydroxyl polyamidoamine dendrimers	2-(phosphonomethyl)-pentanedioic acid	MS	Hollinger <i>et al.</i> ⁵⁷	–
Cation carbosilane dendrimers	–	PD	Ferrer-Lorente <i>et al.</i> ⁵⁸	–
Carbon nanotubes				
Single-walled CNTs (SWNT)	–	AD	Xue <i>et al.</i> ⁵⁹	–
Single-walled CNTs (SWNT)	Dopamine	PD	Guo <i>et al.</i> ⁶⁰	–
Biopolymer-coated carboxylated SWNTs	Levodopa	PD	Tan <i>et al.</i> ⁶¹	–
Metal oxide NPs				
Cerium oxide NPs	–	AD	Dowding <i>et al.</i> ⁶²	–
fibrin γ 377–395–conjugated Fe ₂ O ₃ NPs	–	AD	Glat <i>et al.</i> ⁶³	–
Nanocrystals				
Gold nanocrystals	–	PD	n/a	NCT03815916 (phase 2, completed)
Gold nanocrystals	–	ALS	n/a	NCT04098406 (phase 2, completed)
Gold nanocrystals	–	MS	Robinson <i>et al.</i> ³⁹	NCT04414345 (phase 3) NCT03993171 (phase 2) NCT04626921 (phase 3)
AuNPs				
PEG-AuNPs	Anthocyanins	AD	Kim <i>et al.</i> ⁶⁴	–
AuNPs	Hybrid peptide inhibitors	AD	Xiong <i>et al.</i> ⁶⁵	–
AuNPs	–	AD, PD	Hou <i>et al.</i> , ³⁸ Dos Santos Tramontin <i>et al.</i> ⁶⁶	–
AuNPs	FM19G11	ALS	Marcuzzo <i>et al.</i> ⁴¹	–
Gold quantum clusters				
Au8pXs	–	FRDA	Villa <i>et al.</i> ⁶⁷	–

A β = amyloid- β ; CNT = carbon nanotube; LP = liposome; NE = nanoemulsion.

Nanoparticle targeting of reactive oxygen species production and oxidative stress in NDs

Oxidative stress results from the imbalance of oxidant substances, and the ability of the biological system to detoxify cells of toxic and dangerous compounds.⁸⁴ ROS are produced through different physiological intracellular processes,⁸⁵ and redox homeostasis is strictly coordinated by the nuclear factor erythroid 2-related factor 2 (Nrf2), which stimulates the antioxidant response through the transcriptional activation of antioxidant genes. Brain has a very low level of antioxidant defences, such as superoxide dismutase

(SOD1), glutathione peroxidases and catalases, becoming highly sensitive to oxidative damage and overproduction of ROS.⁸⁶ Moreover, some areas of the brain are rich in iron, which can contribute to ROS overproduction.⁸⁷ It is important to notice that ROS production can also result in secondary effects in other tissues, such as lipid peroxidation, DNA damage leading to tissue degeneration and accelerated ageing,⁸⁸ which might be limited with the use of nanosystems. Emerging preclinical studies investigated potential therapeutic interventions by using antioxidant-loaded nanocarriers. In an *in vitro* model of PD, polyphenol-loaded

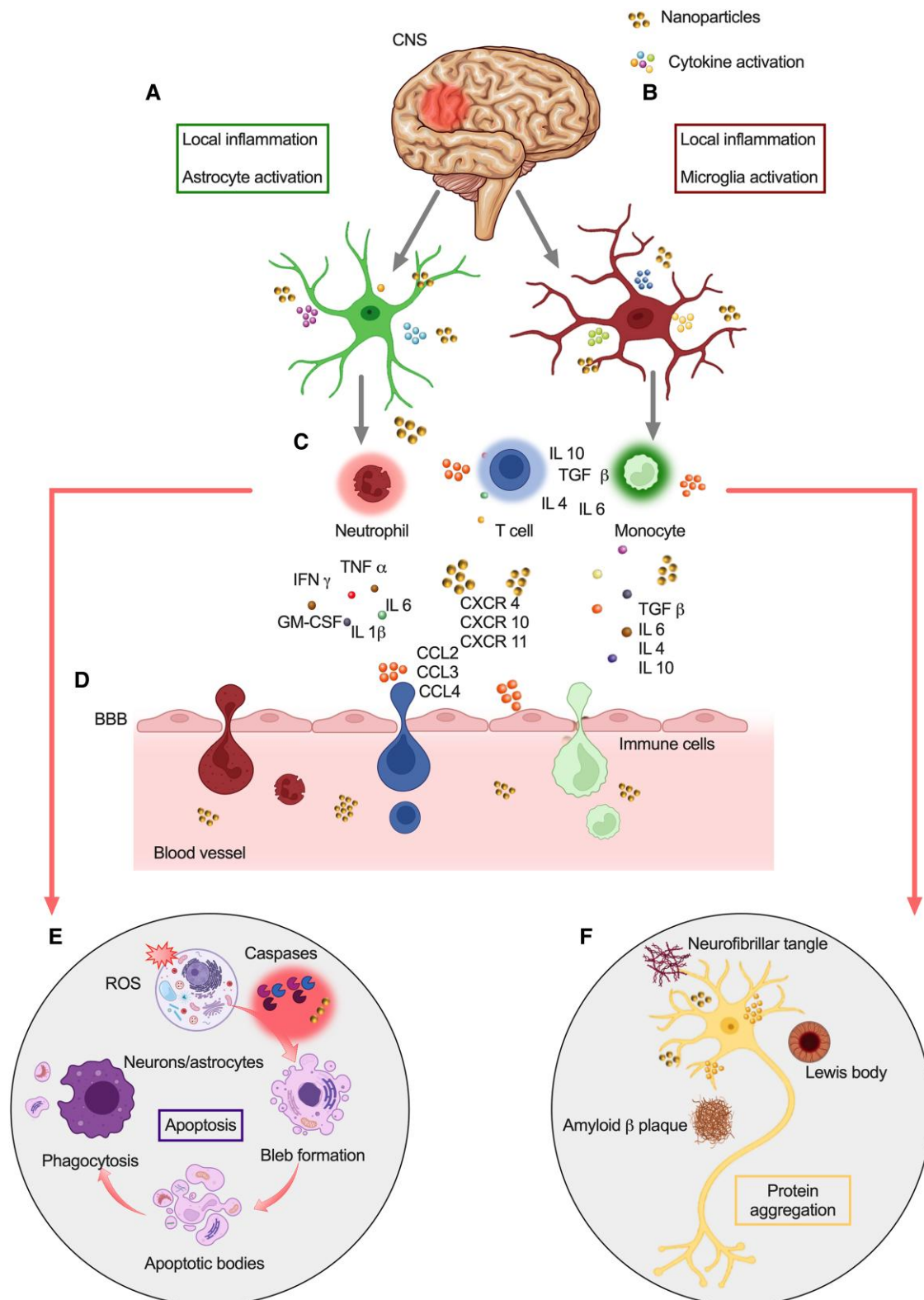


Figure 3 Action of NPs on neuroinflammation. (A and B) After CNS injury, astrocyte and/or microglia cells activate and promote local inflammation. (C) Persistent inflammation leads to stimulation of cytokine/chemokine storm and activation of inflammatory cells (lymphocytes and monocytes). (D) Inflammatory cells (monocytes, neutrophils, T cells) penetrate the BBB and cause neuroinflammation. NPs might mitigate neuroinflammatory response acting on local inflammation by penetrating the BBB. (E) Neuroinflammation, oxidative stress and excitotoxicity affect neuronal survival and enhance apoptosis by stimulating caspases hyperactivation. NPs can reduce neuronal cell death mitigating caspases hyperactivation. (F) Neuronal dysfunction and inflammatory environment stimulate misfolded protein production and protein aggregate (neurofibrillary tangles, A β plaques, Lewy bodies) formation. NPs reduce protein and peptide aggregation and inflammation reducing neuronal toxicity and improving neuronal network and behaviours.

nanoliposomes were able to rescue the ROS concentration and expression of genes involved in antioxidant responses, such as catalase (CAT), superoxide dismutase 1 and 2 (SOD1 and SOD2) and glutathione synthetase (GSS),⁴⁶ to physiological levels. The protein expression of α -synuclein and phospho- α -synuclein, whose accumulation is typical in Lewy bodies upon ROS damage, was also rescued. Normally, free antioxidants show scarce brain accumulation and may exhaust their antioxidant power before reaching the brain. Antioxidant incorporation into nanosystems could instead effectively increase brain localization and ROS scavenging activity by protecting them from undesired oxidation from the extracellular environment. In this work, the authors also improved the structure of these nanoliposomes by using brain lipids as a shell, functionalized with an anti-transferrin receptor antibody, to ameliorate BBB penetration and cellular uptake through pinocytosis.

Other successful examples of compounds tested on PD models include vitamin E-loaded resveratrol and naringenin nanoemulsions,^{36,37} which have been shown to reduce oxidative stress and ROS production more efficiently compared to their free suspensions. This is due to the synergistic effect of vitamin E, the nanoemulsion incorporation and the intranasal route of administration that ameliorated untargeted brain localization, while also avoiding first pass metabolism. Although the mechanism of action is still unclear, it is likely that ROS reduction also restores dopamine levels in PD models.

Rutin is another molecule with ROS-scavenging activity, which binds to antioxidant enzymes, causing their activation and consequently protecting neurons from neurodegeneration and apoptosis. Rutin also prevents lipid peroxidation and protein and DNA denaturation, thus preventing oxidative damage. As disadvantage, this molecule has poor aqueous solubility, requiring the incorporation into nanoemulsions for enhancement of bioavailability at the desired site of action over an extended period in rat models of PD.⁵³

Furthermore, in a recent work AuNPs were found effective for mitigating cognitive impairment and oxidative stress in AD animal models given the intrinsic antioxidant power of this gold nanosystem.⁶⁶ Collectively these preclinical results underline the benefits of nanosystems on pharmacokinetics and seem promising for clinical translation.

Nanoparticle action on autophagic flux and mitophagy

Autophagy is a multi-stage recycling process required for cellular homeostasis.^{89,90} Neuronal autophagy controls the correct balance of anti-inflammatory responses, myelination, modulation of neurotransmission, synaptic pruning, and as neuroprotective mechanism it is involved in removing toxic proteins from the neural network.^{91,92} Defective autophagy is found in many different NDs, including AD, PD and FRDA, as dysregulation of this mechanism increases neuron susceptibility to neurotoxicity, leading to cell death.^{93–95} Aggregate-prone proteins such as huntingtin, α -synuclein and misfolded peripheral myelin protein 22 could all be degraded by autophagy.⁹⁶ For this reason, the stimulation of autophagy in neurons is a potential therapeutic approach. *In vitro* studies demonstrated that uncoated and oleic acid-coated NPs are internalized by brain-derived human endothelial cells where they aggregate, thus stimulating the activation of lysosomal proteases and autophagy.⁹⁷

Autophagy is also responsible for general mitochondria quality control and for the removal of dysfunctional mitochondria through engulfment in autophagosomes and degradation in lysosomes.

This process of selective autophagy is known as mitophagy and uses the same core autophagy machinery, largely encoded by Autophagy-related Genes.⁹⁸ While basal mitophagy is a physiological process that is required for maintaining neuronal health, its upregulation is often observed in NDs, as demonstrated by the post-mortem analysis of PD patient brains, showing autophagocytosed mitochondria. Furthermore, genetic mutations in mitophagy key proteins, like PINK1 or Parkin, occur in several forms of PD and AD.

Protein aggregation and ROS-mediated neuroinflammation can upregulate mitophagy and dysregulate mitochondrial dynamics, thus promoting mitochondrial fragmentation processes.^{99–101} Indeed, mitophagy is also responsible for maintaining the physiological morphology of mitochondria.¹⁰² The accumulation of fragmented mitochondria can block both anterograde and retrograde axonal transport, reducing neurite growth and branching, eventually leading to cell death.

Cerium oxide NPs have been proved efficient in reducing A β -induced mitochondrial fragmentation in a cellular model of AD.⁶² These NPs can switch between Ce³⁺ and Ce⁴⁺ and scavenge ROS and peroxynitrite, a mediator of A β aggregate formation and tau protein accumulation. Peroxynitrite can also induce mitochondrial fragmentation, thus cerium oxide NPs could exert a double function which is protective against neuronal cell death.

The use of NPs to target autophagy in NDs has shown promising results, but it is still controversial.¹⁰³ When interacting with subcellular components, NPs can also stimulate autophagy through the upregulation of ROS signalling, which is normally considered a side effect of NP treatments.^{104,105} *In vivo* studies in wild-type animal models demonstrated that the intravenous administration of titanium dioxide NPs caused increased ROS formation and inflammatory responses, suggesting a neurotoxic effect.^{106,107}

From a therapeutic standpoint, it is important to consider the context of therapeutic intervention: for some patients, the upregulation of autophagic flux might elicit beneficial clearance of aggregates and damaged mitochondria,^{108,109} whereas other patients might experience excessive mitochondrial depletion and axodendritic shrinkage.

The outcome of autophagy or mitophagy modulation in specific pathological contexts is hardly predictable, and the therapeutic strategy in terms of dosage and administration should be tested experimentally and tailored to the patient in order to avoid dangerous side effects.

Regulating apoptosis: the neuroprotection strategy

Neuronal apoptosis is a highly conserved mechanism that takes place during the development of the nervous system and acts as a regulatory mechanism to maintain tissue homeostasis.¹¹⁰ Biochemical signals initiating apoptosis include excitotoxicity, glutamatergic hyperactivation, brain-derived neurotrophic factor (BDNF) deficiency, oxidative stress and toxic insults to the brain.¹¹¹ However, once development is complete, the CNS has very limited regenerative capacity and abnormal cell death occurring through apoptosis and necrosis is indeed predominant in NDs, though with some differences among the affected neurons. For instance, in PD, nigral dopaminergic neurons are the most susceptible to cell death; in HD, striatal neurons expressing GABA and enkephalin die more than those expressing GABA and substance P; whereas in ALS, motor neurons innervating fast muscles are more affected compared to those innervating slow muscles.¹¹²

The death of one cell can negatively affect the dynamics of neighbouring cells by secreting proapoptotic factors that can trigger caspase-mediated apoptosis in the neighbours. Therefore, it is crucial to limit the apoptotic damage to ameliorate the disease progression.

Superparamagnetic Fe₃O₄ NPs have been used to target apoptosis in PD models.¹¹³ In this work, NPs were coated with oleic acid, loaded with a shRNA against α -synuclein synthesis, and nerve growth factor was absorbed to ensure NP uptake in neurons through nerve growth factor-mediated endocytosis. This strategy allowed specific targeting of α -synuclein synthesis, whose aggregation in brain leads to apoptosis upregulation in PD affected patients, hence preventing cell death and ameliorating the pathological phenotype. Despite the promising results, clinical translation of iron oxide NPs is controversial because of the potential induction of oxidative stress, iron accumulation and protein aggregation in the brain.⁷³

Another approach exploiting NPs is neuroprotection, a therapeutic strategy aimed at defending the CNS from both acute and chronic injuries, maintaining viability of neuronal progenitor cells and ameliorating neuronal functional recovery.¹¹⁴ Neuroprotection in NDs aims to regulate apoptosis and autophagy, as well as repair and regenerate damaged neuronal cells to change the course of the disease. Emerging evidence supports the hypothesis that natural products, some food-additives, neurotrophic factors and growth factors provide neuroprotection.

It has been proven that PEG-coated AuNPs loaded with anthocyanin are able to target A β plaques and provide neuroprotection via inhibition of the NF- κ B/JNK/GSK3 β pathway in both *in vitro* and *in vivo* AD models.⁶⁴ Anthocyanins have antioxidant and anti-inflammatory properties that were found to be neuroprotective against A β -induced oxidative stress neuroinflammation and neurodegeneration. Indeed, upon intravenous injection of PEG-coated AuNPs loaded with anthocyanin, the expression of markers of activated astrocytes and microglia (GFAP, Iba-1, RAGE), inflammation (p-NF- κ B, iNOS) and apoptosis (Bax, cytochrome c, caspase 3) were found to be reduced *in vitro* and *in vivo*. Tau hyperphosphorylation, which is associated with neurodegeneration, was also reduced. Collectively, treatment with anthocyanin-loaded NPs had a strong positive effect on neuron survival and rescue in the CA2 and DG regions of hippocampus and cortex of AD mouse models. Moreover, these NPs were able to penetrate the BBB and localize in the brain up to 14 days after treatment, due to the PEG coating that increased retention times. Intranasal administration of fibroblast growth factor-conjugated NPs showed a neuroprotective effect, mitigating AD progression and memory deficits in AD animal models, through the trophic activity of basic fibroblast growth factors on neurons and the activation of antioxidant pathways that mitigate the neurotoxicity induced by A β .¹¹⁵ These NPs were optimized by adding lectins to enhance brain permeability via endocytosis. Similar results were obtained with nicotine-encapsulated PLGA NPs in PD models⁵⁰ [nicotine is neuroprotective against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism, as is nanocurcumin in EAE mice (a mouse model of MS)], which were able to stimulate neuroprotection and improve myelin repair, due to anti-inflammatory activity.¹¹⁶ Moreover, treatment with curcumin-loaded nanoemulsions was found to be effective at improving motor deficits and antioxidant defences in PD models.⁵² Neuroprotection is an encouraging strategy, because it allows neuron survival to be stimulation and employs natural compounds, which have properties that can be optimized with the use of nanosystems and whose use does not fall under FDA drug regulations.

Mitochondrial and metabolic dysfunctions: bioenergetics as a novel NDs therapeutic target

Defective mitochondrial metabolism has been observed in NDs, but it has often been considered a consequence of the disease. Recently, nuclear and mitochondrial DNA mutations leading to reduced oxidative metabolism have been found in hereditary forms of NDs, suggesting that metabolic dysfunctions might also be causative of NDs. However, the same mutation leading to mitochondrial dysfunction can cause very different pathological phenotypes, as compensatory effects arise.¹¹²

Mitochondria are involved in a network of metabolic pathways and are present in multiple copies within the cell, so that healthy mitochondria can compensate for defective ones. Defective energetic metabolism and ATP synthesis can originate from dysfunctional ETC complexes and proton pumps in mitochondrial membranes, mistaken diversion of the electron flow to other cellular components leading to ROS formation and oxidative stress, uncoupling, and defective fuelling of the ETC from the Krebs cycle and glycolysis. Although it is difficult to demonstrate the causative role of metabolic defects in NDs, their effect in these diseases is well documented and suggests that bioenergetics can be a new suitable target.

Clean-surfaced, faceted nanocrystals of gold have been used to promote remyelination by enhancing glycolytic metabolism. Indeed, myelination is a process, mediated by oligodendrocytes, that is highly dependent on glycolysis, the primary energetic source of oligodendrocytes.³⁹ Compared to bulk gold, which is inert, gold nanocrystals have biological catalytic properties, and they can catalyse the oxidation of nicotinamide adenine dinucleotide hydride (NADH) to NAD⁺, a critical energetic cofactor and sensor of energy levels. In this work, a suspension of gold nanocrystals (named CNM-Au8) was first used to enhance the glycolytic activity in neuronal-glia cocultures, as confirmed by the increased extracellular lactate and non-mitochondrial ATP production, and then administered to animal models of MS by gavage, showing prominent remyelination and amelioration of movements. These encouraging results have led to the approval of CNM-Au8 in clinical trials (<https://clinicaltrials.gov/ct2/results?cond=Neuro-Degenerative+Disease&term=CNM+Au8&cntry=&state=&city=&dist=>).

Exploiting the pleiotropic effects of nanoparticles in Friederich's ataxia

Most of the nanosystems previously discussed have several effects: acting on ROS reduction, autophagy and exerting their function both in the CNS and distant organs, showing pleiotropic characteristics. In pharmacology, pleiotropy refers to all of the effects that a drug can deploy, including the ones for which it was not specifically developed. For ND treatment, it is of particular importance to find pleiotropic drugs in order to target primary pathways of early pathogenesis directly, while also acting on reversing secondary effects.¹¹⁷ A good example to test the importance of this concept is FRDA, as it is a multisystem disease affecting central and peripheral nervous systems, the musculoskeletal system, the heart and endocrine pancreas. Together with gait and limb ataxia, FRDA is often associated with cardiomyopathy and diabetes mellitus. Furthermore, in FRDA, not all the organs and tissues are affected to the same extent of degeneration and severity of pathologic pathways,¹¹⁸ despite the same genetic background and frataxin expression.

The latter portrays the complexity of FRDA, both in understanding the tissue-specific biomolecular mechanisms of pathology and

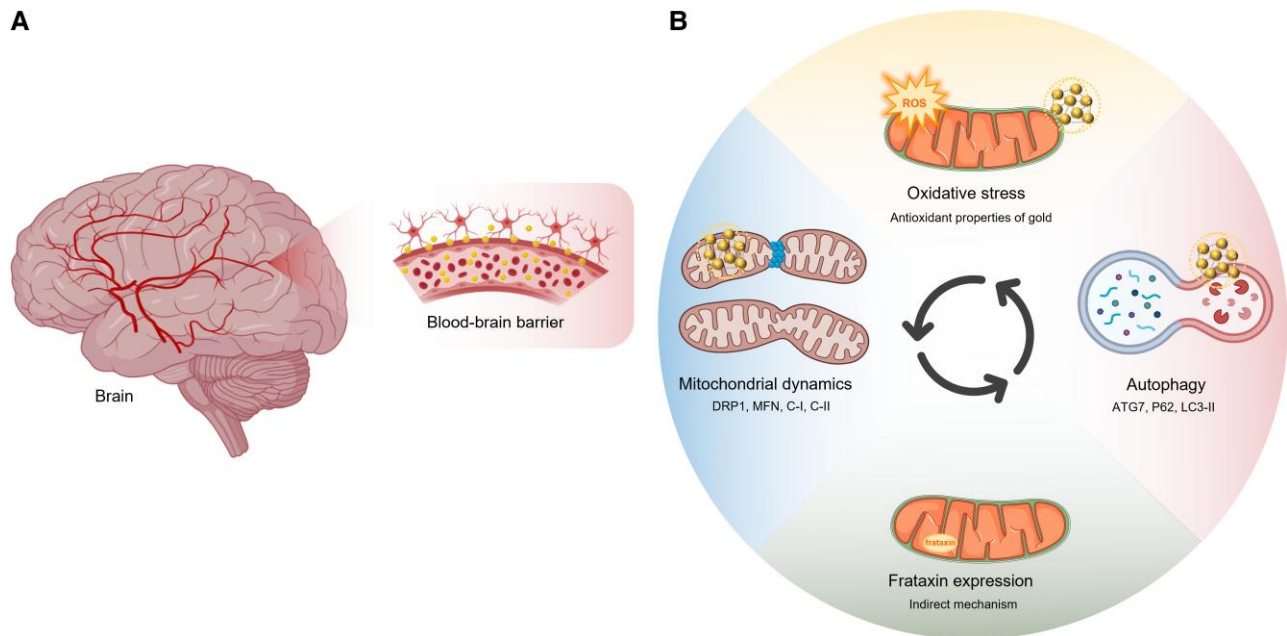


Figure 4 Gold nanoclusters effect in CNS. (A) Au₈pX are able to migrate across the BBB acting in different biological pathways (B) such as oxidative stress, autophagy and mitochondrial dynamics. These processes have an indirect effect on frataxin expression.

in finding suitable and broad-spectrum therapeutic intervention strategies.

The most common strategies targeting the pathological mechanisms driving FRDA onset aim at increasing frataxin expression, since its reduction in FRDA patients causes pathologic oxidative stress and defective mitochondrial metabolism. Indeed, frataxin is involved in several mitochondrial functions, including respiration, iron-sulphur cluster biogenesis, haem synthesis and redox status,¹¹⁹ as well as mitochondrial dynamics and autophagy.¹²⁰ Typically, FXN replacement via gene therapy or protein supplementation is coupled with the use of iron chelators (deferiprone) and antioxidants (vitamin E + coenzyme Q10).¹²¹ For instance, one of the most widely studied approaches to improve FRDA symptoms, in particular for the amelioration of cardiac functionality, is the administration of adeno-associated virus (AAV) carrying human FXN.^{122,123} However, even though a single injection could be effective, AAV-mediated gene therapy is hindered by the stimulation of immune responses and difficult virus delivery strategies. More recently, a list of FDA approved drugs has been screened in yeast to find compounds that can increase frataxin levels by post-translational regulation.¹²⁴ The most efficient were dibenzoylmethane (DBM), 4,4'-diisothiocyano-2,2'-stilbenedisulfonic acid (DIDS), bifonazole and fipronil. These compounds are likely to act on mitochondrial iron metabolism and ROS formation, although the mechanism of action needs to be elucidated fully. Likewise, the glucagon-like peptide-1 (GLP-1) analogue exenatide, which is already employed in the management of type 2 diabetes mellitus, was found to be effective at increasing frataxin expression and improving mitochondrial function.¹²⁵

Despite the variety of tested therapeutic approaches, results from clinical trials have been modest.¹²⁶ The use of NPs may be the solution to overcome some of the difficulties causing the inefficiency of treatments, as they offer the possibility of treating different systems with a single nanocarrier.

In 2016, Nabhan *et al.*⁴⁵ delivered lipid NPs loaded with FXN mRNA to DRG. Lipid NPs encapsulating FXN mRNA were generated and administered intravenously in adult mice, revealing efficient uptake in hepatocytes and correct maturation into the mature form 24 h post-injection. Finally, FXN lipid NPs were administered intrathecally into the lower lumbar region of the mouse spine and the recombinant human FXN protein was detected at high concentrations in DRG.

NPs loaded with curcumin have been effective in alleviating FRDA symptoms in mouse models.⁴⁹ Curcumin is known to reduce oxidative stress through Nrf2 activation, and it can also chelate iron, which are both characteristics that can be useful to reducing the symptoms of FRDA, which are often caused by mitochondrial dysfunction, abnormal iron metabolism and increased oxidative stress. However, curcumin is poorly soluble and permeable; therefore, it has been loaded in silk fibroin (SF) to generate Cur@SF NPs. The safety of Cur@SF NPs was evaluated in lymphocytes derived from FRDA patients. Results showed no effect on viability, but iron content was reduced in mitochondria because of chelation. Moreover, the activity of antioxidant enzymes (SOD and catalase) increased, along with the activity of complexes I and II and ATP production. The injection of Cur@SF NPs in mouse models of FRDA for 1 month improved functional performance (strength, coordination, holding times). Furthermore, heart iron, whose accumulation is one of the main causes of myocardial dysfunction in FRDA patients, was significantly reduced.

Recently, a new non-genetic approach for FRDA treatment based on gold quantum clusters (Au₈pXs) has shown promising results. Conversely to the other works discussed in this review, Villa and colleagues⁶⁷ have exploited the intrinsic characteristics of gold nanostructures (Au₈pXs), their excellent ROS scavenger potential, in bone-marrow mesenchymal stem cells (BMSCs) derived from patients with FRDA and in YG8sR mice, which are considered a good animal models of FRDA.¹²⁷ The YG8sR mice received a single dose of Au₈pXs injected into the tail vein without the help of other

molecules already used in FRDA treatment.⁶⁷ Interestingly, Au₈pXs reached many tissues, including cerebellum and cortex, thus demonstrating the ability to cross the BBB, and accumulated in the liver, where they were retained for more than 30 days before being cleared slowly. Of note, the Au₈pXs have been proven effective in recovering mitochondrial function, counteracting oxidative stress and rescuing neuromuscular and cardiac function.⁶⁷ Moreover, Au₈pX treatment prevented neuronal loss in the cerebellar dentate nuclei and improved neuronal firing properties.⁶⁷ Mechanistically, the Au₈pX treatment was able to restore the antioxidant pathways, autophagic flux, mitochondrial dynamics and complex I and II activities while indirectly increasing FXN expression (Fig. 4). Taken together, these data encourage the use of Au₈pXs as a potential treatment option for FRDA patients. The key advantages of this system include the long retention time, which allows a single administration, and the possibility to cross the BBB and reach distant tissues, including muscles and heart, where they can exert their effects on multiple processes, thus showing a pleiotropic effect.

FRDA patients show very different clinical manifestations and onset due to the genetic variability of FXN mutations and protein expression. This heterogeneity will likely strongly affect the efficacy of the treatment and should be considered in view of a clinical trial. In line, with Villa et al.,⁶⁷ BMSCs were derived from two patients with FRDA carrying different GAA repeats sizes and treated with different Au₈pX molarities, 5 μM and 10 μM. Despite both cell lines showed an overall autophagic flux recovery and mitochondrial machinery restoration to physiological levels, BMSCs from the patient carrying the smaller GAA expansion were more vulnerable to FRDA degenerative pathways and more prone to largely benefit from Au₈pX treatment.⁶⁷ In addition, BMSCs derived from the same patients exhibited different levels of Au₈pX activity, in a dose related manner. Administration of 5 μM Au₈pX resulted in faster and more effective ROS scavenging activity, despite slightly lower mitochondrial accumulation. Conversely, treatment with 10 μM Au₈pXs primed a robust post-translational expression of the mature form of FXN, suggesting a multi-target intervention strategy tunable by Au₈pX dose.⁶⁷

The analysis of different patient-derived cells to study the transcriptional regulation of FXN expression, ROS production and autophagy upon different dosage of Au₈pXs is therefore needed to determine the patient-specific beneficial concentration of gold quantum clusters and facilitate the clinical translation of this treatment.

These examples underline the advantage in the use of nanosystems over conventional small molecule drugs. The pleiotropic effect of NPs would be beneficial for the treatment of other NDs, such as AD and PD, because it would allow treatment of multiple targets, including oxidative stress, autophagy and protein aggregation, with a single nanosystem.

Conclusions

Neurodegenerative diseases are unfortunately very common, with more than 600 different neurologic disorders, and approximately 50 million people affected (one in six of the world population). In our modern era, these numbers are expected to increase as the population grows and ages.¹²⁸ The recent advanced strategies to prevent and manage at least the most common neurological disorders such as AD, PD, HM, MS and ALS are still not completely effective in counteracting the variety of symptoms and guaranteeing long-term patient quality of life. New therapies are therefore urgently

needed to cover the heterogeneity and temporal complexity of NDs and reduce the world-wide health burden.

Among the common mechanisms occurring in NDs, none seems to play a primary role in neurodegeneration on its own. Rather, the synergistic action of their complex interactions contributes to the onset and pathogenesis of NDs. So far, the challenge of finding effective pharmacological treatments and resolutive therapies is unsolved. In many cases, addressing and specifically targeting only a unique dysregulated process results into unsatisfactory outcomes and rather negatively impacts on other biological pathways. The multifactorial nature of NDs requires the development of therapeutic interventions and new compounds simultaneously targeting several mechanisms, risk factors and features shared among ND spectra. Among others, therapeutic targets include neuronal cell apoptosis and neurodegeneration, as well as neuroinflammation caused by oxidative stress, protein aggregation, autophagic flux impairment and mitochondrial dysfunction. However, current therapeutic approaches are limited at mitigating disease progression and preserving surviving neuronal cells and their function,^{64,113,114} whereas strategies that can definitively arrest neurodegeneration and neuroinflammation progression, or even prevent the early pathological degenerative pathways, are still unavailable.

One of the major obstacles in developing efficient treatments is provided by the restrictive nature of BBB, a highly selective semi-permeable membrane formed by endothelial cells and surrounding cerebral microcirculation. An intact BBB creates a physical barrier hindering the freely diffusion and passive entrance of cells, particles and macromolecules, such as drugs, into the brain.¹²⁹ While there may be challenges in obtaining effective doses of traditional pharmacotherapies for adequate cell repair and treatment,¹³⁰ novel strategies based on nanomaterials can be designed to transport drugs across the BBB or to develop self-healing nanosystems.

In comparison with conventional drugs, NPs are therefore good candidates as drug carriers crossing the BBB, leading to a faster rate of drug release and higher bioavailability. Furthermore, NPs can be engineered through various biological reactions, such as receptor–ligand interaction and antibody–antigen interaction, to conjugate specific biomolecules, which allow targeted delivery of NPs *in vivo*. Similarly, NPs can cross the blood–nerve barrier (BNB), showing the same advantages and site specificity obtained when treating the CNS. In this regard, nerve growth factors conjugated with or encapsulated in NPs have already been used for the treatment of peripheral nerve damage, resulting in efficient reconstruction of axons and promotion of remyelination.¹³¹ However, despite the longer retention times compared with conventional drugs, the relatively frequent injections make it difficult to translate in clinic. Furthermore, most NDs are characterized by stronger CNS pathologic phenotypes, rather than peripheral nerve damage, hence treating peripheral nerves would be beneficial just for some NDs, for example FRDA.

In the framework of the complex genetics and symptomatology, and the multifaceted causative mechanisms characterizing NDs, the largest clinical feasibility will be exerted by nanosystems displaying a wide efficacy in several neurodegenerative pathways.

In addition to carrier properties, the physicochemical characteristics of NPs contribute to their beneficial effects in NDs to dampen neuroinflammation and oxidative stress, while controlling mitochondrial bioenergetics and metabolism.

These processes are particularly jeopardized in FRDA, due to the pivotal role of frataxin in many mitochondrial functions. Indeed, FRDA is a complex multisystemic disorder, with a wide range of clinical manifestations, which also differ among patients, making

this disease a good candidate for treatment approaches based on nanosystems. Traditional clinical interventions have often proved to be unsuccessful, whereas nanosystem-based therapeutic strategies are showing encouraging results in preclinical studies.

Despite the numerous advantages, the clinical translation of most nanosystems is limited by their intrinsic properties, such as size, shape, stability, and physico-chemical surface features, that affect their adsorption time and biodistribution after administration. There is not a golden route for nanosystem delivery. Comparison studies of pharmacokinetic must be performed in animal models to avoid nanosystem adsorption from filter organs, blood or body tissues different from the target area in order to ensure effective dose delivery and treatment efficacy, while preventing their toxic accumulation and aggregation. In line with this, a clinically relevant application of nanosystems should be founded on controlled biodistribution, reduced toxicity, less frequent administration and an easy delivery method aimed at increasing patient benefits and compliance. In this sense, the development of standardized toxicological studies is also crucial for the improvement of NP-based applications. Accordingly, patients suffering from NDs, who differ in their genetic backgrounds and clinical phenotypes, bring about a demand for personalization of therapeutic doses and administration strategies.

In summary, NPs have led to promising preclinical results and might represent a huge opportunity and offer broader prospects for curing NDs in the near future.

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Competing interests

C.V. and Y.T. are listed as inventors on a patent application (n. IT102019000020724) for Au8-pXs mediated therapy for FRDA. The other authors report no competing interests.

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