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Towards precision nanomedicine for cerebrovascular diseases with emphasis on Cerebral Cavernous Malformation (CCM)

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Abstract

Introduction: Cerebrovascular diseases encompass various disorders of the brain vasculature, such as ischemic and hemorrhagic strokes, aneurysms, and vascular malformations, and may also affect the central nervous system leading to a large variety of transient or permanent neurological disorders. They represent major causes of mortality and long-term disability worldwide, and some of them can be inherited, including Cerebral Cavernous Malformation (CCM), an autosomal dominant cerebrovascular disease linked to mutations in CCM1/KRIT1, CCM2 or CCM3/PDCD10 genes.

Areas covered: Besides marked clinical and etiological heterogeneity, some commonalities are emerging among distinct cerebrovascular diseases, including key pathogenetic roles of oxidative stress and inflammation, which are increasingly recognized as major disease hallmarks and therapeutic targets. This review provides a comprehensive overview of the different clinical features and common pathogenetic determinants of cerebrovascular diseases, highlighting major challenges, including the pressing need for new diagnostic and therapeutic strategies, and focusing on the emerging innovative features and promising benefits of nanomedicine strategies for early detection and targeted treatment of such diseases.

Expert opinion: Specifically, we describe and discuss the multiple physico-chemical features and unique biological advantages of nanosystems, including nanodiagnostics, nanotherapeutics and nanotheranostics, that may help improving diagnosis and treatment of cerebrovascular diseases and neurological comorbidities, with an emphasis on CCM disease.

Keywords: Central nervous system (CNS); cerebrovascular diseases; cerebral cavernous malformation (CCM); inflammation, oxidative stress, nanosystems; nanodiagnostics; nanotherapeutics; nanotheranostics; nose-to-brain delivery.

Article highlights

1. Cerebrovascular diseases, including strokes, aneurysms and vascular malformations, are leading causes of mortality and adult long-term disability worldwide
2. A few types of cerebrovascular diseases can be inherited, including Cerebral Cavernous Malformation (CCM)
3. Oxidative stress and inflammation are increasingly recognized as major disease hallmarks and therapeutic targets of distinct of cerebrovascular diseases
4. Nanomedicine is emerging as a promising and effective strategy for early detection and targeted treatment of cerebrovascular diseases and neurological comorbidities
5. This review critically describes the physico-chemical features and biological advantages of nanosystems, including nanodiagnostics, nanotherapeutics and nanotheranostics, that may help improving diagnosis and treatment of cerebrovascular diseases and neurological comorbidities

1. Introduction

1.1. Cerebrovascular diseases

1.1.1. Overview

Cerebrovascular diseases are an heterogeneous group of either congenital or acquired disorders of the blood vessels in the central nervous system (CNS), which are classified on the basis of their unique pathophysiologic mechanisms, hemodynamic features and clinical manifestations [1]. Major cerebrovascular diseases include stroke, brain aneurysms, and cerebrovascular malformations (Table 1), which can be detected by an array of imaging technologies, including cerebral angiography, computerized tomography (CT), magnetic resonance imaging (MRI), and magnetic resonance angiography (MRA) [2,3].

Stroke is the the second leading cause of death worldwide, with 5·5 million deaths attributed to this cause in 2016, as well as the second most common cause of disability-adjusted life-years (DALYs) [4-6]. About 80% of strokes are ischemic,

caused by a blood clot formed either in cerebral vessels with dysfunctional endothelium and atherosclerotic plaques (thrombotic stroke), or at other locations in the circulatory system (embolic stroke). Current guidelines for the early management of acute ischemic stroke recommend the fastest possible use of revascularization procedures, which consist of intravenous thrombolysis with alteplase and endovascular treatment within a few hours after the onset of symptoms [7]. The remaining 20% of stokes are hemorrhagic. Hemorrhagic stroke may be further subdivided into subarachnoid hemorrhage (SAH), which occurs when a swollen artery located on the outer surface of the brain bursts and leaks blood into the subarachnoid space, and intracerebral hemorrhage (ICH), which occurs when a blood vessel bursts and leaks into the brain parenchyma [4,8]. ICH is potentially devastating, with long-term functional independence achieved in only 12–39% of cases and mortality rates of 40% at 1 month and 54% at 1 year [9]. The STICH I and II trials confirm that early surgery does not increase the rate of death or disability at 6 months for patients with ICH. Surgery may have a small but clinically relevant survival advantage for patients with spontaneous superficial ICH without intraventricular hemorrhage [10,11]. Due to the high morbidity and mortality rates after ICH, both early diagnosis and the identification of relevant clinical and radiographic characteristics and molecular biomarkers that may serve as predictors of poor prognosis would enable improved disease outcomes [12,13]. While ischemic and hemorrhagic stroke are generally caused by blood clotting disorders and blood vessel ruptures, respectively, their molecular pathogenesis is complex and largely multifactorial, including the crucial contribution of oxidative stress and inflammatory events, susceptibility factors, and genetic determinants [14,15]. Furthermore, both ischemic and hemorrhagic strokes can result in either localized or widespread brain injury. Despite several generations of both interventional approaches and pharmacological therapies have been developed to improve stroke outcomes and reduce mortality [16], their efficacy is often limited, and there is still an urgent need of new approaches for more effective diagnosis and targeted therapies. In fact, whereas the real complexity of risk factors and pathogenetic mechanisms of stroke begins to take shape [14,15], the aging population, imbalanced lifestyles and environmental causes are expected to increase its incidence and associated mortality, particularly in developing countries [4,17].

A brain aneurysm (also called intracranial or cerebral aneurysm) is a saccular or fusiform bulging of a cerebral artery, which results in a localized weakening of the blood vessel wall and may eventually burst causing life-threatening SAH. Prior to rupture, an aneurysm is usually clinically silent, unless it compresses a nerve or leaks small amounts of blood [18]. Indeed, despite affecting 3–5% of the adult population, most brain aneurysms do not rupture or cause symptoms, and often are incidentally discovered during imaging diagnostic tests performed for other clinical conditions [19]. Their etiology is unknown, but various risk factors have been identified [20]. In particular, it has been demonstrated that oxidative stress and inflammation play a major pathogenetic role in both their formation and their rupture [21,22]. Moreover, epidemiological studies have demonstrated a familiar occurrence, as well as an association with several heritable conditions, suggesting the potential contribution of genetic determinants [19,23]. Neurosurgical clipping and endovascular coiling are the current standard treatments for ruptured intracranial aneurysms, with no statistically significant differences in long-term clinical outcomes between the two treatments [24,25].

Cerebrovascular malformations are divided into low-flow and high-flow lesions, based on their hemodynamic characteristics, and are commonly further subdivided in four major groups, namely arteriovenous malformation (AVM), cerebral cavernous malformation (CCM), venous malformation (VM), and brain capillary telangiectasia (BCT). Moreover, they include also others specific types of cerebrovascular anomalies, such as arteriovenous fistula (AVF), vein of Galen malformation (VOGM), and mixed vascular malformations [26-28]. In contrast to vascular tumors (hemangiomas), which grow by cellular hyperplasia, vascular malformations consist of localized defects in vascular morphogenesis [29-31]. MRI is the primary noninvasive and effective diagnostic tool for the assessment of cerebrovascular malformations due to its ability to highlight their extent and anatomic relationships as well as to differentiate high-flow from low-flow lesions by dynamic post-contrast sequences [32]. VMs, also called developmental venous anomalies (DVAs), are the most frequent cerebrovascular malformations, but they are mostly benign and clinically silent [33,34]. BCTs are small, angiographically occult and clinically benign lesions, which are usually found incidentally by MRI [35] and should not be treated. In contrast, both AVMs and CCMs can cause a wide range of severe clinical symptoms, including severe headaches, seizures, progressive neurologic deficits,

and hemorrhagic stroke, which may become debilitating or even life-threatening at any age [36]. AVMs are high-flow lesions characterized by a nidus of feeder arterioles that shunt directly to veins without intervening capillaries [36-38]. They can form virtually anywhere in the brain or spinal cord, with a prevalence of about 1% in the general population, a risk of hemorrhage of 4% per year, and a 15% chance of consequent stroke or death [37]. In addition to rupturing, AVMs can undergo growth, remodeling, and regression [39-42]. Both congenital and acquired forms of AVMs have been observed to occur either sporadically or in the context of large hereditary syndromes, including hereditary hemorrhagic telangiectasia (HHT, also known as Rendu-Osler-Weber syndrome). Despite these and other observations have suggested potential pathogenetic mechanisms, including a crucial role of oxidative stress and inflammatory events [43-45], the etiology of AVMs is still largely unclear, and no direct therapeutic approaches are available so far besides neurosurgery or stereotactic radiosurgery [36,38,46]. However, a recent study showed that conservative medical management alone resulted superior to interventional therapy for the prevention of death or symptomatic stroke in patients with an unruptured brain AVM, while the long-term risks and differences between the two approaches remains uncertain [47]. On the other hand, compelling evidence accumulated over the last two decades has consistently shown a clear genetic basis and detailed molecular mechanisms for the pathogenesis of CCM disease, a major cerebrovascular disorder that affects capillaries in the brain and spinal cord, and may cause severe neurological signs and symptoms, including recurrent headaches, focal seizures, visual, sensory and motor abnormalities, and life threatening hemorrhagic stroke [48]. CCMs are low-flow lesions consisting of clusters of dilated and dysplastic capillaries forming cavernous sinusoids. Like AVMs, CCMs may form virtually anywhere in the brain or spinal cord, and can cause neurological damage by either compressing/displacing parts of the surrounding tissues or leading to ICH. Modern imaging indicates that the prevalence of CCM lesions in the general population is higher than 0.5%, thus affecting approximately 35 million people worldwide [48,49]. Furthermore, genetic studies have demonstrated that this cerebrovascular disease is caused by loss-of-function mutations in any of three known CCM genes, *KRIT1*/*CCM1*, *CCM2* and *CCM3*, and may arise sporadically or is inherited as an autosomal dominant condition with incomplete penetrance and highly variable expressivity, including wide inter-individual differences in lesion

number, size, and susceptibility to ICH [48,50,51]. CCM genes encode for intracellular proteins that play major pleiotropic functions, including the coordination of key redox-sensitive pathways and mechanisms that govern endothelial cell homeostasis and defenses against oxidative stress and inflammation, suggesting the crucial implication of such pathways and mechanisms in the pathogenesis of CCM disease [52-58]. Consistently, accumulated evidence from animal models and patient cohorts has demonstrated that loss-of-function mutations of CCM genes only predispose to the development of CCM disease, leading to an increased susceptibility to endothelial dysfunction and blood-brain barrier (BBB) disruption induced by oxidative stress and inflammatory events [48,50,51,59-61]. In addition, the amazing progress in the knowledge of molecular mechanisms underlying CCM disease pathogenesis has led to the identification of potential risk factors [15,51,62,63], and prompted the development of targeted preventive and therapeutic strategies, including promising innovative approaches based on multifunctional nanocarriers [48,64-70].

1.1.2. Differences, commonalities and challenges

Overall, accumulated evidence shows both differences and commonalities in the development of cerebrovascular diseases, highlighting the major influence of a complex interplay between genetic and environmental risk factors, as well as significant links between cerebrovascular and neurodegenerative diseases [75-79]. In particular, it is now clearly established that tightly linked abnormal oxidative stress and inflammatory responses underlay the most severe phenotypes of cerebrovascular diseases, including BBB destabilization and breakdown [59,80], and participate actively also in the development of neurological disorders, suggesting that these pathogenetic determinants can eventually bridge the boundary between cerebrovascular and neurodegenerative diseases [15,75,81]. Consistently, besides CCM disease, oxidative stress and inflammation, which can uniquely be referred to as oxy-inflammation due to their established tight interdependence [59], play key pathogenetic roles in other major cerebrovascular diseases and associated neurological disorders, including stroke [15,75,82-85], brain aneurysms [21,22,86], and AVMs [44,87], thus constituting major therapeutic targets for preventing or limiting the most severe clinical outcomes of such diseases.

Molecular determinants of oxidative stress and inflammatory events, such as reactive oxygen species (ROS), pro-inflammatory cytokines, matrix metalloproteinases (MMP), and cyclooxygenase 2 (COX-2), have been shown to cause BBB disruption by affecting all cellular constituents of the neurovascular unit (NVU), including endothelial cells, pericytes, and astrocytes, as well as the interposed extracellular matrix [80,88,89]. In turn, BBB disruption can promote vicious circles of intertwining oxy-inflammatory molecular responses, causing further molecular and cellular dysfunctions, and exacerbating the pathogenesis of both acute and chronic cerebrovascular diseases. Consistently, whereas oxy-inflammation pathways are clearly implicated in most of the different pathogenetic mechanisms that lead to BBB dysregulation and consequent clinical outcomes, potential therapies for cerebrovascular diseases have been directed toward restoring the integrity of the NVU and BBB by modulating such pathways [89-91]. However, the restoration of the BBB may represent a limit for the delivery of diagnostic and therapeutic agents to the damaged neuronal tissues during the recovery phase of cerebrovascular diseases, such as strokes. Therefore, suitable carrier systems should be engineered in such a way as to ensure the targeting of injured brain tissues in both acute and recovery phases of cerebrovascular damage. Furthermore, cerebrovascular diseases should be assessed at different tissue levels by taking advantage of the key developments in clinical vascular imaging in the brain that occurred in the last decade, including the recent availability of high-sensitive and high-resolution imaging techniques, such as 3-T and 7-T MRI [3,92]. Overall, despite the great progress in the understanding of pathogenesis and treatment of cerebrovascular diseases, there are still major challenges and difficulties, including the identification of biomarkers for early diagnosis, prognosis, prediction, and monitoring, the specific and targeted delivery of diagnostic and therapeutic molecules to pathological sites, the overcoming of biological barriers, such as BBB, and the avoidance of fast clearance and undesirable side effects.

Considering the emerging multifactoriality, complexity and challenges of cerebrovascular diseases and associated neurological comorbidities, the scientific community is exploring the promising field of nanomedicine, as a major alternative to existing diagnostic and therapeutic strategies [71,93,94]. Indeed, the recent rapid development of biomedical nanotechnology discoveries applied to the biomedical field has provided scientists with a rich toolbox of complex nanosystems that can be

harnessed to overcome biological barriers to pharmacological treatments and other shortcomings of traditional diagnostic and therapeutic approaches, such as unspecific drug delivery to healthy tissues and off-target toxicity, and uncontrolled drug release, and unfavorable drug retention and adverse effects of drugs in disease sites. Furthermore, innovative approaches have been geared towards the development of various targeted combination therapy and precision nanomedicine strategies, including those based on composite biomimetic and bioresponsive drug delivery nanosystems, for more specific, safe and effective treatment of multifactorial diseases. In particular, nanosystems can facilitate the delivery of drugs through the BBB owing to either passive or active targeting mechanisms [95]. Moreover, they offer the unique possibility to target emerging key pathogenetic determinants, including oxy-inflammatory mechanisms, both simultaneously and synergistically, thus avoiding the failures of traditional approaches [96]. Consistently, recent results demonstrate that innovative biomimetic and bioresponsive nanomedicine strategies can be very effective in treating complex diseases directly or indirectly connect to oxy-inflammatory mechanisms, such major cardiovascular, neurodegenerative and neoplastic diseases [97-104].

In this review, we critically describe the recent advances in development of nanosystems with either diagnostic (nanodiagnostics), therapeutic (nanotherapeutics) or combined (nanotheranostics) properties, and their potential advantageous applications and targeted administration via alternative routes for a more safe and effective diagnosis, therapy and outcome monitoring of cerebrovascular diseases and neurological comorbidities.

1.2 Nanomaterials: manufacture and biomedical applications

According to the European Commission Recommendation (2011/696/EU), a nanomaterial is defined as any form of a material that is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less. However, the mean size of nanoparticulate systems frequently exceeds 100 nm (Figure 1), as larger particles in the submicron size range can still offer improved biological properties due to higher superficial energy, despite having a lower capacity to penetrate through biological structures [105]. Indeed, the existence of nanotechnology-based products for pharmaceutical applications (nanomedicines) with a size greater than 100 nm

induced the European Medicines Agency (EMA) to include within the official definition of nanomedicines all “structures” with a size lower than 1000 nm that are designed to have specific properties and can improve site-specific drug delivery and/or significantly alter toxicological profiles [106,107].

Nanomedicines can be obtained using several types of matrixes (inorganic, metal, polymeric, lipid, surfactant) and various supra-molecular structures, such as micelles, dendrimers, vesicles (liposomes and niosomes), nanoemulsions, and nanoparticles (NPs), which can be further subdivided in core-shell nanocapsules and nanospheres (Figure 1) [108,109]. Different preparation methods can be employed to form the nanostructure, including bottom-up and top-down approaches where the building blocks are added onto a substrate or removed from it, respectively [110]. Moreover, recent advances in the preparation and characterization of nanostructures have given the possibility to strictly control their physico-chemical properties, to draw structure-function relationships in complex biological systems, and to design hybrid and composite nanobiomaterials. Eventually, these technological advancements have facilitated the development of innovative nanomedicine strategies, including the creation of highly engineered biomimetic and bioresponsive nanosystems that allow a fine-tunable, stimuli-responsive and on-demand targeted delivery and release of therapeutic compounds, thus overcoming the major shortcomings of traditional therapeutic approaches mentioned above. Remarkably, distinct nanomedicine-based breakthrough approaches that overcome multiple biological barriers to pharmacological treatment of complex diseases are nowadays moving quickly to clinical trials [97,98,102,111].

1.3. Nanotechnology for diagnosis and therapy of diseases affecting the CNS

The extensively growing field of nanomedicine offers excellent solutions for site-specific targeting, biological imaging, controlled drug delivery and release, and efficient therapy for a wide range of CNS diseases, including cerebrovascular, neurological and neoplastic [112-120]. Compared with conventional medicines, nanomedicines have many physical and biological advantages, such as improved solubility and pharmacokinetics, enhanced efficacy, reduced toxic side effects, and increased tissue selectivity, thus overcoming the major shortcomings of conventional therapeutic approaches [121]. In recent years, multiple nanosystems have indeed been developed for advanced therapeutic and diagnostic applications, including

controlled targeting of nanoengineered therapeutic agents to pathological sites (nanotherapeutics) and visualization/quantitation of pathophysiological processes (nanodiagnostics), as well as for combinatorial approaches (nanotheranostics). In particular, the latter combine diagnostic and therapeutic properties within the same nanocarrier, thus providing innovative and effective solutions for advanced personalized nanomedicine interventions, including the possibility of simultaneous imaging, monitoring, and therapy [116,122,123]. Furthermore, the emerging integration of nanotechnology with stem cell therapy and tissue engineering is providing novel promising options for treatment of brain and spinal cord injury associated with cerebrovascular diseases [94,124].

Several therapeutic agents with either distinct or complementary properties can be assembled in nanomaterials as a single platform, thus allowing a multimodal therapy for combinatorial and synergistic treatment of specific diseases [125,126]. Accordingly, combination nanotherapy approaches have been proved effective in rescuing major pathological features in complex diseases [69,127]. Furthermore, drugs can be associated with imaging contrast agents in a theranostic probe for simultaneous investigation and therapy of the disease [128]. The ultra-small sizes and high surface-to-volume ratios of nanomaterials increase the solubility and circulation half-time of loaded drugs, and may also reduce their potential systemic toxicity by targeted delivery and controlled release. Moreover, nanomaterials can be engineered using molecules capable of responding to various stimuli, including light, magnetic field, ultrasound, temperature, pH, and oxy-inflammatory factors, thus improving their selective and efficient homing to specific tissues and biomolecular targets with appropriate spatial and temporal resolution [129,130]. Specifically, among the most auspicious medical applications, nanomedicine is emerging as a very promising strategy for improved diagnosis and non-invasive treatment of major cerebrovascular diseases and associated neurological comorbidities [71,93,94,131,132]. Despite the current great advances and emerging advantages of nanomedicine, there are still major challenges and difficulties in the pharmacological targeting of cerebrovascular diseases and neurological comorbidities. In particular, a limiting step may be the loading of the drug into the nanocarriers. Indeed, some nanoparticulate systems are associated with low drug payload, implying that only potent drugs can be used effectively, whereas stressful synthetic conditions, such as heat, extreme pH, solvents, and highly reactive components, can be harmful to the

stability of sensitive drugs [133]. Moreover, rapid blood clearance and premature burst drug release are inherent drawbacks of conventional nanoparticles that can affect the effective tissue targeting of loaded drugs [134]. On the other hand, substantial concerns have been raised regarding the safety of some engineered nanomaterials used for drug delivery into the brain, including especially metal NPs [135-137]. In particular, it has been reported that NPs can cause neurotoxicity via several possible mechanisms, including oxidative stress, autophagy, and lysosome dysfunction, and the activation of signaling pathways involved in inflammation and cell death, and there is indeed evidence that high exposure to the CNS can cause effects on neurotransmission, redox homeostasis and behavior [135,136,138]. Furthermore, even if preclinical evaluation of prototype nanosystems proved their safety and efficacy, translation to the humans is highly limited by scale-up issues and high costs. Therefore, NPs prepared with feasible and easy to scale-up techniques and biocompatible matrixes should undergo an easier translation to the humans. The high volume of NPs required for administration in human patients is also a relevant drawback, implying that NPs loaded with potent drugs that are effective at low therapeutic doses should be optimal candidates for human translation [139]. However, as a matter of fact, the approval rate for novel nanomedicines is currently below 10%, mainly because of safety and efficacy profile failures during preclinical and clinical studies [140].

2. Nanosystems for cerebrovascular diseases

2.1. Features of nanocarrier matrices

NPs for nanomedicine applications can be synthesized from various organic or inorganic materials, such as synthetic and natural polymers, lipids, proteins, and metals. Among the most commonly used matrices there are synthetic polymers [141], including poly(lactic acid) (PLA) [142], poly(lactic-co-glycolic acid) (PLGA) [143], poly(ethylenimine) (PEI) [144], poly(vanillin oxalate) (PVO) [145], and poly(amidoamine) dendrimers (PAMAM) [146]. The advantages of synthetic polymeric NPs include easy fabrication and absence of biological contamination. However, nanocarriers useful for nanomedicine applications can also be prepared by using other materials, including natural biopolymers, inorganic materials, and lipids. Furthermore, two or more types of materials can be integrated to obtain

nanosystems with specific combinations of desired physicochemical properties, such as tailored sizes, shapes and surface functionality, improved drug loading capacity, enhanced stability, solubility and biocompatibility, as well as targeted delivery and controlled release of various drugs and theranostic agents. Indeed, distinct multimaterial nanosystems capable of targeting human diseases, including cerebrovascular diseases and cancers, and serving as early diagnostic and therapeutic agents, have been already developed into different safety systems that simultaneously improve prognosis and therapy [147-152].

As compared to polymeric NPs, potential advantages, including *in vivo* multimodality imaging and therapy, are offered by inorganic NPs composed of pure metals or metal oxide, such as gold [153,154], silver [155], platinum [156,157], palladium [158], iron oxide [93], manganese dioxide [159], and silicon dioxide [160]. Such NPs are easy to prepare and functionalize, and can be synthesized in various controllable sizes and geometric shapes. Moreover, metal-based composite NPs can easily be tracked by different diagnostic techniques, including MRI, transmission electron microscopy (TEM), and inductively coupled plasma mass spectrometry (ICP-MS) [154], as well as by innovative approaches, such as Sputtering-Enabled Intracellular X-ray Photoelectron Spectroscopy (SEI-XPS) [161]. In particular, metallic NPs with magnetic and super-magnetic properties (MNPs) have attracted great attention due to the possibility of combining elective diagnostic imaging studies by MRI, with accurate targeted delivery of drugs toward specific regions of the human body guided by external magnetic fields [123,162,163].

Importantly, a new class of nanomaterials defined as nanozymes is emerging as a major tool in nanomedicine applications for the treatment of oxidative stress-related diseases, including vascular diseases [164-166]. Among such nanozymes, there are noble metal-based NPs, in particular platinum (Pt) and palladium (Pd) NPs, which are endowed with intrinsic antioxidant properties, mimicking the catalytic activity of first line defense antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) [165,167-169]. Indeed, both Pt- and Pd-NPs have been reported to produce positive biological effects by reducing intracellular ROS levels and oxidative stress, as well as by combining this intrinsic activity with that of various carried drugs, thereby integrating antioxidant nanozyme and nanocarrier functions [66,157,169,170]. Remarkably, distinct metal-based nanosystems with antioxidant properties have been demonstrated to rescue

endothelial dysfunction induced by oxidative stress and inflammation, thus emerging as major candidates for combination therapy of oxy-inflammatory vascular diseases [66,69,169,171,172].

Along with synthetic polymers and inorganic materials, also natural polymers, such as chitosan, and natural lipids are increasingly employed for nanodrug formulations with either diagnostic, therapeutic or theranostic purposes due to their particular physicochemical and biological properties, including biocompatibility, biodegradability and simplicity of functionalization [173-176]. In particular, lipid-based nanoformulations, such as micelles, liposomes, nanoemulsions and solid lipid nanoparticles (SLN), have become very attractive for their unique size dependent properties, as well as for their potential to improve performance of pharmaceuticals, and reach the goal of controlled and site specific drug delivery to the brain. Indeed, they are highly biocompatible and can enhance drug transport through the BBB by targeting specific transport processes in the brain vasculature [177,178].

For drug delivery applications, the conjugation between distinct NPs and drugs can be achieved through either adsorbing, covalent binding or encapsulating methods, whereas the subsequent drug release into target sites may occur by diffusion or desorption [177].

Overall, NPs composed of a specific combination of distinct materials have been shown to be well suited for advanced therapeutic strategies based on targeted delivery of antioxidant and anti-inflammatory agents to treat various human disease [145,162,179-181].

2.2. Physico-chemical requirements

Various nanoparticle features, such as size, shape, zeta potential, material composition, and lag time of drug release, have significant roles in the design of efficient therapeutic and theranostic systems for CNS diseases [182,183]. Particles with a size bigger than 200 nm can be rapidly opsonized and massively cleared by Kupffer cells or other phagocytic cells, while smaller hydrophilic particles have a lower opsonization rate, and show prolonged biodistribution and high site-specific targeting properties. On the other hand, nanosystems with a very small size (less than 6 nm) are quickly removed from the body by renal filtration. However, hydrophilic and biodegradable polymers or surfactants can be applied to increase the NP retention [184]. Notably, accumulated evidence has proved inverse

correlation between particle size and BBB penetration [177], thereby the size of NPs must be optimized according to the final goal. The most studied nanosystems for the treatment of brain diseases are 50-100 nm size [177]. Shilo et al. reported that the size of gold NPs (GNPs) has strong effect on their intracellular uptake, demonstrating that 70 nm GNPs show maximum accumulation in the brain and 20 nm GNPs offer the maximum free surface [185]. In capsulated nanosystems, both capsule size and thickness play an important role for increasing the therapeutic capacity. The core-to-surface ratio changes upon size manipulation, and the low core-to-surface ratio of small nanosystems may cause drug release once their membrane is broken [186]. Moreover, a key factor in cellular uptake and body distribution of NPs is the shape of the particle. Among differently shaped NPs, including spherical, cubic, rod-, disk- and star-like, spherical NPs have exhibited the fastest internalization rate [187]. However, rod shaped NPs showed higher accumulation in the brain compared with spherical counterpart [188], further highlighting the complex interplay between size, shape and surface property of NPs. Indeed, another critical factor to be considered in the formulation of NPs for drug delivery to the brain is the electrokinetic potential, also known as zeta potential, which has an important effect on the BBB permeation. For sufficient electrostatic repulsion of NPs, a zeta potential of at least ±30 mV is needed [189]. On the other hand, it has been shown that an high positive zeta potential causes toxicity to the BBB [177], as well as that the NP surface charge affects the therapeutic properties of the loaded drug [190]. Formulation of NPs with proper zeta potential values is therefore critical for nanomedicine applications [191-194].

2.3. Targeted delivery strategies

2.3.1 Passive targeting

The enhanced BBB permeability that occurs after acute ischemic stroke may enable both blood substances and nanosystems to cross the BBB and enter the brain parenchyma by passive diffusion [80]. In such pathological condition, major therapeutic objectives should be neuroprotection, reduced disruption of the NVU, and prevention of secondary injuries. Enhanced permeation and retention (EPR)-like effects can be achieved depending on chemico-physical features of nanocarriers [195], which regulate their biological interactions [196-198]. Indeed, nanosystems

should be preserved from the reticulo-endothelial system (RES) and have prolonged plasma circulation time. To this aim, hydrophilic surface and reduced particle size are the most relevant parameters. Furthermore, compared to EPR effect of leaky vessel in tumor/inflamed tissue, additional size restrictions apply to disrupted BBB [199], and brain accumulation depends also on injection time and the spatial location of the brain injury [200]. Only nanosystems below 100 nm showed relevant accumulation in the damaged brain [143]. Indeed, small size dendrimers helped to disclose the size effect on overcoming BBB: dendrimer uptake relies on disease severity, extent of BBB disruption, and glial cell activation [201-205].

2.3.2 Active targeting

In the presence of an intact BBB, active targeting approaches are required to delivery nanosystems into injured brain tissues (Figure 2) [206]. Targeting moieties include ligands for carrier-mediated transporters (CMT) and/or receptor-mediated transporters (RMT) overexpressed in the BBB [207]. Most of the ligands belong to protein/peptide category. Transferrin [208], anti-transferrin receptor antibodies [209-211], lactoferrin [212], Angiopep-2 [213], and T7 peptide [214] are the commonest ligands employed to target the BBB. Moreover, specific targeting to injured brain can be performed. Chlorotoxin, a 36-amino acid peptide, can be used to target matrix metalloproteinase 2 (MMP-2), which is up-regulated in the ischemic microenvironment in the brain [215]. CAQK peptide [216] and stroke homing peptide [214] bind to specific sites of brain injury. Neurons can be targeted by Rabies Virus Glycoprotein (RVG-acetylcholine receptor) [217] and antibodies against NMDA (N-methyl-D-aspartate) receptor 1 (NR1) [218]. Fas ligand recruits microglia to injured regions for compensatory repair, suggesting that Fas ligand antibody may be used for injured brain targeting [211]. Alternatively, targeting to ischemic brain can be achieved through neutrophil-mediated inflammatory migration after Pro-Gly-Pro (PGP) functionalization [219]. Nonetheless, different critical issues are associated with protein mediated targeting, including immunogenicity. Indeed, most of the RMT are present also in non-target tissues: the presence of a “spacer” between the nanoparticle surface and the grafted protein improves selectivity for the BBB, as well as a low amount of grafted protein [220]. Moreover, transferrin-functionalized nanocarriers undergo binding competition with the corresponding endogenous protein [221], whereas employment of monoclonal antibodies against transferrin

receptors raises safety concerns [222]. Lactoferrin, instead, is a cationic iron transporting glycoprotein, whose receptor in the BBB has two binding sites with a K_d higher than its plasmatic concentration, avoiding the competitive inhibition with endogenous ligand [223]. Polysorbate 80 surfactant, instead, adsorbs endogenous serum apolipoproteins on NP surface, allowing low density lipoprotein receptor (LDLR)-mediated targeting [224].

2.4. Administration routes

Traditional strategies for targeted delivery of nanosystems rely mainly upon intravenous administration to reach injured tissues. However, such strategies are often not suitable for chronic diseases requiring repeated administrations. Furthermore, intravenously injected ingredients, such as surfactants, may cause off-target toxicity, including toxicity to the BBB. Therefore, alternative administration routes have been investigated, including cochlear and nose-to-brain routes. The first entails the demonstrated communication between labyrinthine perilymph, in the inner ear, and cerebrospinal fluid (CSF) through the cochlear aqueduct [225]. Owing to this communication, the intra-tympanic injection is becoming of increasing interest for the delivery of nanosystems for the treatment of cerebrovascular diseases [226], with a size exclusion limit of 1-3 microns [227]. Avoidance of systemic exposure is a relevant advantage for cochlear route. However, whereas effective drug delivery to the inner ear relies on the retention in the site of administration, unfortunately large portions of the administered drugs are usually eliminated through the Eustachian tube, leading to unpredictable pharmacokinetic profiles. NPs provide certain advantages over conventional drug delivery methods in terms of increased retention and targeted drug delivery to specific cells in the cochlea [228].

So far, nose-to-brain delivery is the most popular alternative method to target the brain [229]. In particular, nose-to-brain delivery works due to the olfactory and trigeminal nerves, protruding respectively in the olfactory and the respiratory epithelium [230]. It can occur by slow intra-axonal (intra-neuronal or intracellular) transport, or by fast transfer into the CSF along the perineural clefts (extra-neuronal or paracellular transport), which is also associated to systemic absorption through the highly vascularized lamina propria [231]. Systemic uptake, avoiding first pass effect, is given by transcellular transport through epithelial cells, and it can reach the injured brain in the case of BBB disruption (Figure 3). However, main limitations of

nose-to-brain delivery are: I) the small volume administered, meaning that only potent drugs can be employed successfully; II) the individual variability of nasal uptake, as well as the susceptibility to pathological conditions (rhinitis, etc.). Nonetheless, the advantages in terms of patient compliance, fast onset of action and efficient BBB bypassing, overcome the existing drawbacks [174,232,233].

Despite the utility of nanosystems in nose-to-brain delivery is controversial [234,235], some potential advantages can be foreseen [236]. Indeed, nanocarriers protect the encapsulated drug from biological and/or chemical degradation, and from extracellular transport by P-glycoprotein efflux [237]. Moreover, bioadhesive nanosystems can increase nasal retention, preventing muco-ciliary clearance, and transiently open the tight junctions of the mucosal epithelium, because of the surfactants used in the formulation [238]. Thus, they can act as nasal absorption promoters, particularly useful for peptides and proteins, whose nasal delivery is prevented by molecular weight. Drug payload can be released at the mucous layer, and/or entire nanosystems can move along the axon up to the olfactory bulb, via pinocytosis and clathrin- (20–200 nm) or caveolae- (200–1000 nm) mediated endocytosis [239]. However, since axon diameter is about 100–700 nm, the transport of colloidal systems within this size range might be highly limited [240]. Major parameters influencing nose-to-brain uptake of nanosystems include surface charge (positively charged are easily taken up by intracellular transport), reduced particle size, presence of muco-adhesive polymers, such as polysaccharides (including positively charged chitosan) and surface functionalization (cell-penetrating peptides, lactoferrin, lectins, etc.). The role of PEGylation is more controversial, because its mucus-penetrating ability depends upon coating density and chain length [238], although the intravenously injection of PEGylated nanoparticles has been demonstrated to increase the blood circulation of conjugated-drugs and enhance their accumulation within specific tissues [241,242]. Finally, biocompatible nanomaterials should be used, because the extended contact with nasal mucosa can lead to irritation, ciliotoxicity and damage of the primary olfactory nerves [243,244].

2.5. Preclinical and clinical studies

2.5.1. Nanotherapies for cerebrovascular diseases

Owing to the targeting mechanisms described above, nanocarriers offer a versatile platform for treating acute and chronic cerebrovascular diseases. Intravenous thrombolytic agents, such as t-PA, can be administered within 4.5 h from stroke onset for reducing infarct size and neuronal death. This approach can be successful due to effective nanocarrier-mediated targeting to the ischemic region and fast clot dissolution, even if it is associated to a high risk of hemorrhage [245]. However, nanocarrier-based drug delivery is mainly addressed towards NVU function recovery after acute events, such as stroke, as well as in the case of chronic diseases [206]. In particular, given that oxidative stress and inflammation have emerged as major causes and consequences of NVU dysfunctions underlying both acute and chronic cerebrovascular diseases and neuronal comorbidities [59,81,246,247], antioxidant and anti-inflammatory nanotherapies have been recognized as promising strategies for an effective treatment of such diseases [104,248]. Over the past decade, significant advances have been made in the development of antioxidant and anti-inflammatory nanosystems based on a number of natural and synthetic materials, such as carbon, metals, nanocrystals, lipids, and polymers [104]. Such nanotechnology approaches have significantly overcome the shortcomings of conventional administration of antioxidant compounds, which showed limited *in vivo* effects owing to their non-specific distribution and low release and retention in disease sites, thus improving pharmacokinetics properties and decreasing side effects of therapeutic drugs. Specifically, the emergence of various ROS-responsive nanocarrier systems, consisting of ROS-responsive functional moieties integrated with either ROS-scavenging inorganic NPs, organic NPs with intrinsic antioxidant activity, or NPs loaded with antioxidant and anti-inflammatory drugs or activatable prodrugs, allows a spatiotemporally controlled release of drugs for a more effective therapy [104,249-252]. Consistently, the increasing availability of various ‘smart’ bioresponsive materials that are sensitive to biological signals or to pathological abnormalities are expanding the opportunities for the development of next-generation precision nanomedicines [253,254].

Besides the most widely used antioxidant and anti-inflammatory compounds of the polyphenol family, other types of materials have been used in the formulation of

antioxidant and anti-inflammatory nanosystems, including ceria oxide [213,255], carbon-based compounds [256-258], and antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT) [218,219,256,258-262]. Furthermore, the emerging multiple properties and potential benefits of antioxidant nanozymes, including metal-based NPs mimicking SOD and CAT enzymes, have attracted extensive attention for both therapeutic and theranostic applications, yielding promising results in various experimental models of human diseases associated with oxidative stress and inflammation, including cerebrovascular diseases [66,69,104,263].

Several protein/peptide growth factors have been used for stroke therapy due to their ability to promote angiogenesis/neurogenesis, and to inhibit apoptosis and inflammatory cascade [264]. In addition, approaches for *ex vivo* gene therapy employing genetically engineered mesenchymal stem cells (MSCs) have also been proposed [265,266]. Also in this regard, nanocarriers have become a realistic alternative to traditional approaches for achieving better efficacy in both drug and gene therapy for cerebrovascular diseases as well as for other human diseases, including the use of NPs as non-viral vectors for encapsulation and targeted delivery of either plasmid DNA (p-DNA), mRNA, small interfering RNA (siRNA), or microRNA (miRNA) [133,267].

Special attention should be paid to secondary prevention of both acute and chronic cerebrovascular diseases in sensitive subjects. Apart from the aforementioned neuroprotective substances, anti-hypertensive drugs have been loaded in nanocarriers to prevent stroke, with particular regards to calcium channel blockers (such as nimodipine), which also show promising inhibition of atherosclerotic plaque deposits [268]. Similarly, lipid lowering statins are being investigated to reduce the risk of stroke in dyslipidemic patients [269]. Furthermore, accumulated evidence indicates a significant relationship between defective autophagy and abnormal inflammatory responses in the pathogenesis of both ischemic stroke and CCM disease, thus pointing to autophagy inducers, including mammalian target of rapamycin (mTOR) inhibitors, as potential therapeutic compounds for such diseases [54,270]. For secondary prevention purposes, nanocarrier-mediated nose-to-brain delivery is an emerging approach, as well as co-delivery within the same nanocarrier of drugs acting towards multiple mechanisms.

A great variety of matrixes and supramolecular structures have been employed for drug delivery in cerebrovascular diseases (Table 2). The most promising are lipid and polymeric systems, because of a long history of safe use in pharmaceutical products [271,272]. Among other emerging approaches, cell mediated delivery is gaining importance. Indeed, it can be achieved by exploiting internalization within MSCs, which may mediate targeting to the injured tissue [273], or by wrapping NPs with platelet cell membranes [274].

2.5.2. Nanodiagnostics and nanotheranostics for advanced imaging of cerebrovascular diseases

Taken together, the growing understanding of pathogenetic mechanisms and the rapid development of nanotechnologies have provided promising possibilities for advanced imaging of cerebrovascular lesions, as well as for the production of nanotheranostics with multiple functions, including targeting, multimodal imaging and monitoring, and synergistic therapies [253]. In particular, smart nanodiagnostics and nanotheranostics responsive to key components in the pathogenesis of cerebrovascular diseases, such as oxidative stress and inflammation (oxy-inflammation), have emerged as innovative nanosystems with advanced diagnostic and therapeutic properties. Major evidence concern CT and MRI nanodiagnostics. Among smart nanodiagnostics, there are ferumoxytol, an iron oxide nanoparticle coated by a carbohydrate shell that is used in MRI studies as an inflammatory marker, and di-5-hydroxytryptamide of gadopentetate dimeglumine, a myeloperoxidase (MPO)-specific paramagnetic MRI contrast agent [93]. Indeed, these nanosystems may allow noninvasive assessment of the inflammatory status of cerebral aneurysms and arteriovenous malformations by contrast-enhanced MRI, with the potentiality to differentiate lesions that require early intervention [93]. Consistently, MRI paired with ultrasmall superparamagnetic iron oxide NPs (USPIOs) injection has demonstrated great potential for inflammation imaging, whereby USPIOs serve as contrast agent and tracking system [275,276]. In particular, it has been demonstrated that USPIO-enhanced MRI could constantly monitor therapeutic effects of minocycline treatment in cerebral ischemia [275]. Furthermore, Park et al. have designed poly(ethylene glycol)-coated cross-linked iron oxide NPs (PCIONs) as a therapeutic approach and simultaneous tracking system for MSCs via MRI. Magnetic MSCs can be precisely visualized *in vitro* and *in*

vivo and it is conceivable to monitor their translocation from infusion site to cerebrovascular ischemic area. Moreover, PCIONs can mediate aggregation and retention resulting from magnets at the target site, which also improve the precision of *in vivo* cell monitoring [273]. Besides iron oxide, also manganese-enhanced MRI might be used, as it enhances neuroarchitecture visualization. Joen et al. developed hollow manganese oxide NPs (HMONs) as a T1 MRI contrast agent. The large water-accessible surface of HMONs facilitate and enhance the relaxation of target site, thus producing a positive contrast [277]. Since the quick imaging improve the outcome of the therapy, the demand for advanced imaging in cerebrovascular diseases is daily increasing. In this regard, IONs are able to upgrade the microwave images to rapidly distinguish emergent ischemic stroke from hemorrhagic stroke [278]. Furthermore, CT is used for time-critical decision in stroke, having been applied by Kim et al. for thrombolytic treatment with t-PA [153]. This study showed that GNPs sensitively and quantitatively allowed high-resolution *in vivo* micro CT imaging of *in situ* thrombosis, as well as optimized managing of thrombolytic therapy. In addition, a single administration of nanosystems for imaging allowed to monitor patients up to 3 weeks. This approach offers also the possibility for personalized therapy and stratification of patients, thus preventing excessive or dangerous treatments [153]. Distinct approaches have been used for monitoring and preventing complications of SAH as well as of surgical and endovascular treatments for intracranial aneurysms [279,280], including the use of a label-free cellulose surface-enhanced Raman spectroscopy (SERS) biosensor chip with pH-functionalized, GPN-enhanced localized surface plasmon resonance (LSPR) effects for early diagnosis of SAH-induced complications [279]. Finally, the recent development of ROS-responsive diagnostic imaging nanosystems has also been described [251].
Besides CT and MRI, a growing number of studies is emerging on the potential use of positron emission tomography (PET) and single photon emission computed tomography (SPECT) emitter isotopes integrated into NPs and/or linked to NPs surface for advanced brain imaging [281]. Indeed, through clinical PET imaging it is possible to investigate the contribution of neuroinflammation and the evolution of microglial activation, and evaluate the effects of pharmacological intervention over an extended period after stroke events [282]. Notably, many PET ligands have been developed and clinically used as biomarkers of neuroinflammation to image the accumulation of activated microglia and astrocytes in various diseases, and a better

signal-to-noise ratio and sensitivity have been achieved with second-generation PET ligands [283-285]. On the other hand, SPECT investigation can detect cerebral perfusion changes during the management for preventing the delayed cerebral ischemia after aneurysmal SAH [286]. Alongside the promising benefits, both PET and SPECT imaging techniques also harbor potential pitfalls and shortcomings, including the use of radioactive tracers and the limited spatial resolution relative to the biological process [287].

Also photoacoustic (PA) tomography/imaging (PAT/PAI) and optical imaging, based on the differences in light absorption of various biological tissues, are alternative noninvasive, nonionizing modalities to track changes in cerebral vasculature with excellent contrast and great spatial resolution, and have indeed contributed to a better knowledge of the brain microvasculature and cerebrovascular diseases in mouse models [288-290]. Specifically, PAI is a hybrid imaging modality that integrates high optical contrasts with high ultrasonic spatial resolution in deep tissues, and may combine the intrinsic hemoglobin contrast PA neuroimaging with the specific properties of distinct NPs, including near-infrared (NIR) absorbing NPs, which greatly enhance the vascular contrast in deep-brain PAI, and multifunctional NPs, which allow comprehensive brain examination through multimodal imaging [289]. Different types of NPs can be employed, including porphysomes, perfluorocarbon nanodroplets and perylene-3,4,9,10-tetracarboxylic diimide NPs. However, many clinically approved NPs, such as iron oxide NPs, do not exhibit strong absorption in the NIR region, therefore encapsulation of light absorption materials may be required. Moreover, acoustic distortion from skull must be minimized by mapping the bone profile through another modality, such as X-ray or ultrasound CT, and incorporating it into PAI reconstruction [289].

Optical brain imaging, including fluorescence-based imaging in the visible and traditional near-infrared regions (400-900 nm), is an alternative methodology that can provide real-time and high-resolution assessment of blood flow anomaly in mouse models of cerebrovascular diseases, but currently requires craniotomy, cranial windows and skull thinning techniques, and the penetration depth is limited to 1-2 mm due to light scattering [290]. Nevertheless, higher spatial resolution and larger penetration depth have been achieved by fluorescence bioimaging in the second NIR spectral region (NIR-II, 900-1700 nm). In particular, fluorescence imaging of cerebral vasculature in mouse was obtained using the inherent photoluminescence

property of single-walled carbon nanotubes in the 1.3-1.4 micrometer NIR window. In this spectral range, decreased photon scattering enables fluorescence imaging to exceed a depth of 2 mm in the mouse brain with high resolution without craniotomy [290]. Accordingly, fluorescence bioimaging in the NIR-II spectral region and its related imaging-guided therapy based on biocompatible fluorescence NPs are considered as a promising nanotheranostic method for cerebrovascular imaging and disease treatment in clinical practice [291].

Overall, nanotheranostics offer a versatile platform to merge advanced imaging and targeted drug delivery in the same NP, thus overcoming the multiple shortcoming of conventional diagnostic and therapeutic approaches mentioned above, and facilitating the development of precision medicine strategies based on smart, multifunctional NPs [289,292]. Furthermore, a major advantage of such delivery systems is the ability to monitor disease progression along with targeted drug administration [293]. Specifically, multiple aspects of cerebrovascular diseases and neurological comorbidities, including tissue injuries and oxy-inflammatory responses, can be treated and monitored simultaneously [150,294,295]. For instance, there is evidence that plain liposomal citicoline, a well-known neuroprotective drug, can serve as a multifunctional nanotheranostic tool in the treatment of ischemic stroke, owing to its recently disclosed inherent chemical exchange saturation transfer (CEST) MRI signal [296].

3. Nanosystem-based therapy for CCM disease

3.1. CCM disease: pathogenic mechanisms and treatment

Despite the widespread use of improved diagnostic imaging techniques, including MRI, allows a clear diagnosis of CCM disease, to date there are no direct therapeutic approaches besides the neurosurgical removal of accessible lesions in patients with recurrent hemorrhage or intractable seizures. However, the identification of CCM genes and the characterization of their physiopathological functions have suggested distinct promising pharmacological strategies for preventing or limiting symptomatic disease onset and severity in susceptible individuals [48]. Indeed, compelling evidence accumulated over the last decade has clearly demonstrated that loss-of-function mutations of CCM genes affect major mechanisms of cellular antioxidant and anti-inflammatory defenses, including redox homeostasis and signaling, and

autophagy, pointing to a major role for oxy-inflammation in the pathogenesis of CCM disease [48,59]. Specifically, loss-of-function of CCM proteins induces an increase in intracellular levels of dysfunctional mitochondria and ROS through the downregulation of major antioxidant mechanisms, including the signaling pathway involving forkhead box protein O1 (FoxO1) and superoxide dismutase 2 (SOD2/MnSOD) [52,64], the glutathione redox buffer system [58], and autophagy [54]. In turn, the altered redox homeostasis causes the upregulation of the JNK/c-Jun pathway and the consequent induction of COX-2, a major oxidative stress and inflammatory biomarker involved in vascular dysfunctions [52-54]. Furthermore, there is also an abnormal and sustained activation of the major antioxidant transcription factor Nrf2, which results in a chronic adaptive redox homeostasis that sensitizes cells to additional stressful events [56,57]. Conversely, growing evidence in cellular and animal models demonstrates that limiting oxidative stress and inflammatory responses via distinct approaches may contribute significantly in preventing or reversing CCM disease phenotypes [48]. In particular, it is noteworthy that all of the different therapeutic candidates for CCM disease proposed so far are endowed with either antioxidant or autophagy-inducing properties or both [48]. Specifically, among the major therapeutic candidates for CCM disease there are statins [368,369], which have been shown to exert powerful antioxidant and pro-autophagic activities, as well as significant benefits in the treatment of other cerebrovascular diseases [370-372]. Furthermore, rapamycin and Torin1, two well-known autophagy stimulators that resulted effective in rescuing defective autophagy in cellular models of CCM disease [54], have been also shown to reduce mitochondrial dysfunction and oxidative stress [69,373-375]. Recently, two other compounds known to exert multiple health benefits due to their established antioxidant, anti-inflammatory and pro-autophagic activities, such as vitamin D and avenanthramides [15,65,68], have been demonstrated to either prevent or rescue pathological phenotypes in mouse models of CCM disease [60,64], thus emerging as promising therapeutic candidates.

3.2. Nanomedicine approaches for CCM disease

Given the established major role of oxy-inflammatory mechanisms in the pathogenesis of CCM disease, it was tempting to hypothesize that the development of specific nanosystems endowed with combined and synergistic antioxidant and anti-inflammatory properties may represent a promising therapeutic strategy for its

treatment [59,62,69]. Consistently, we demonstrated the reliability and effectiveness of both Pt- and Pd-NPs in rescuing increased intracellular ROS levels and oxidative stress in cellular models of CCM disease [66,69,169]. Furthermore, this possibility was strongly supported and extended by the demonstration that a multitargeted therapy approach based on a composite nanosystem endowed with intrinsic antioxidant activity and carrying a pro-autophagic drug was effective in rescuing major molecular and cellular mechanisms of CCM disease pathogenesis, suggesting its potential for the treatment of this and other oxidative stress-related diseases [69]. Specifically, this composite multifunctional nanosystem combined the intrinsic ROS scavenging activity of Pt nanozymes with the autophagy-stimulating activity of rapamycin (Rapa), and was tested in distinct cellular models of CCM disease, including KRIT1 knockout mouse embryonic fibroblasts (MEF) [52] and KRIT1-silenced human endothelial cells [54]. The experimental outcomes highlighted the advantages of composite platinum/rapamycin nanosystems (Pt@Rapa NPs), including the enhancement of rapamycin physicochemical properties, such as solubility, permeability, stability and bioavailability. Furthermore, they showed that cellular uptake of Pt@Rapa NPs occurred via endocytosis and resulted in synergistic biological effects, including the rescue of established hallmarks of CCM disease, such as altered redox homeostasis and signaling [52,53], mitochondrial and autophagy dysfunctions [52,54,55,67], and endothelial to mesenchymal transition (EndMT) [54,376] (Figure 4). Overall, the outcomes of these *in vitro* studies stimulate their further implementation for precision nanomedicine approaches in animal models of CCM disease, thus paving the way for the development of advanced combinatorial nanotherapeutic and nanotheranostic strategies to overcome current diagnostic and therapeutic limitations [69].

4. Expert opinion

The incidence of major diseases affecting the CNS, such as cerebrovascular, neurodegenerative and neoplastic diseases, is increasing with the rising life expectancy, posing a heavy burden not only on patients and their families but also on society and governments, through enormous use of health care services and resources. Therefore, there is a pressing need for new diagnostic and therapeutic strategies that can be used in patients with CNS diseases, including the

development of tailor-made drug delivery systems that allow a more precise and selective targeting of the pathological sites without harmful side-effects on normal tissues and cellular processes. The urgent need for innovative diagnostic and therapeutic approaches is further highlighted by new research outcomes providing strong evidence for a causal relationship between cerebrovascular disorders, including stroke and cerebrovascular malformations, and the onset and progression of major neurodegenerative diseases associated with high morbidity rates, such as Alzheimer's disease, multiple sclerosis and Parkinson disease.

Altered BBB is a major common feature of the major cerebrovascular diseases, such as ischemic and hemorrhagic stroke, and cerebrovascular malformations, and represent therefore a primary therapeutic target [80]. However, reconstitution of BBB restraint can drastically hamper the delivery of therapeutic and diagnostic agents into the brain in the recovery phase. Therefore, suitable drug carrier systems should be engineered in such a way as to be able to target the brain both in the acute and in the recovery phase of cerebrovascular damage. In light of this and other difficulties, including the multifactoriality and complexity of the pathogenetic mechanisms underlying cerebrovascular disorders nanomedicine has rapidly and powerfully emerged as one of the most dynamic and promising technological frontiers for the development of integrated, precise and effective diagnostic and therapeutic strategies aimed at detecting and counteracting the disease phenotypes. Indeed, the growing understanding of key pathogenetic mechanisms underlying the onset, progression and severity of major cerebrovascular diseases and associated comorbidities has disclosed novel opportunities for multimodal diagnostic imaging, controlled drug targeting and release, and combined and synergistic therapies. Specifically, despite being complex, multifactorial disorders characterized by marked clinical and etiological heterogeneity, the most severe cerebrovascular diseases, including stroke, brain aneurysms, AVMs and CCMs, share oxidative stress and inflammation as major triggers of disease pathogenesis and severity, suggesting that oxy-inflammatory phenotypes may represent crucial nanomedicine targets for monitoring and counteracting distinct cerebrovascular diseases (Figure 5). In particular, the development of smart nanosystems responsive to physical, chemical, and biological triggers, including pathological stimuli such as altered pH, oxidative stress, inflammation, and reactive biomolecules [253,254,377], may allow their

selective homing to specific tissues and biomolecular targets, thus increasing their effectiveness and reducing their potential systemic toxicity.

Notably, various nanosystems developed so far for the prevention and treatment of cerebrovascular diseases are endowed with antioxidant and anti-inflammatory properties [378-380], and could be further implemented according to recent innovative advances in nanomedicine strategies. Furthermore, given the emerging molecular links between cerebrovascular and neurological diseases [79,247], additional biological benefits may result from multitargeted, combinatorial nanomedicine approaches that simultaneously and synergistically target cerebrovascular diseases and associated neurological comorbidities in response to pathological stimuli. Consistently, whereas the effectiveness of multidisciplinary strategies of precision medicine and combination therapies based on targeted delivery of stimuli-responsive nanotherapeutics has been clearly demonstrated [381-387], the combinatorial targeting of oxidative stress and defective autophagy by a composite nanosystem endowed with both antioxidant and pro-autophagic activities has recently emerged as a potential nanomedicine strategy for CCM disease [69]. Nonetheless, several relevant hurdles are still limiting the clinical translation of nanotechnology for cerebrovascular diseases. Indeed, the term “nanocarriers” include a great variety of nanomaterials, with different size and biological properties. To this regard, while metal NPs are characterized by a very small size that favors BBB overcoming owing to passive targeting mechanisms, they have raised major neurotoxicity concerns [137]. Indeed, the assessment of the quality, safety, and efficacy profiles of a new nanomedicine is the main limiting step to its clinical translation [388]. On the other hand, polymeric and lipid NPs are made up of biocompatible materials or physiological lipids, and have a safe history of clinical use. However, such NPs have often a large size and are associated to rapid blood clearance. To overcome this issue, surface functionalization can be optimized in order to avoid RES uptake and/or to achieve active targeting to the BBB and the injured brain [80,389-391], taking into account that this procedure is usually associated with high costs and scale-up issues. It is worth to stress that the high heterogeneity of NP synthetic methods, sizes, shapes, purity, and the variability of experimental conditions (i.e. cell types used for toxicity assessments) make a clear picture of the toxic profile of NPs difficult. Furthermore, the surface properties of NPs (coating/functionalization), along with protein corona effects, govern the interactions

with tissues and cells, significantly influencing their biological fate. Nonetheless, the presence of potential contaminants in solution, such as residual solvents, reaction by-products and endotoxins, could play a considerable role in the cellular toxicity of NP preparations. Therefore, the following procedures should be recommended in order to achieve safe and biocompatible NP preparations: 1) complete batch-to-batch physico-chemical assessment of NP properties (size distribution, stability, zeta potential, aggregation state, protein corona formation), in particular in complex environment like cell culture media; 2) extensive purification procedures, crucial to minimize the presence of contaminants, that can easily overcome the benefits of the NP pure material and of the coatings; 3) assessment of the toxicological effect of each reagent employed for NPs preparation, including solvents; 4) control of the absence of endotoxin and bacterial contamination: 5) Evaluation of the toxicity of the coating materials *per se* [392].

Within this context, nose-to-brain offers a new paradigm to target cerebrovascular diseases, being suitable for chronic administration and allowing a rapid BBB overcoming. Bioadhesive nanosystems can increase nasal retention, preventing muco-ciliary clearance, and transiently open the tight junctions of the mucosal epithelium, due to the surfactants used in the formulation [238], while a 100–700 nm size limit can be hypothesized for intra-axonal transport [240]. On the other side, relevant achievements can be obtained in the near future by employing NPs as non-viral vectors for gene therapy of cerebrovascular diseases. Indeed, along with current applications relying on gene over-expression (p-DNA), or gene silencing (siRNA, miR) in stroke, the usage of novel mRNA-based technologies allows interesting translational perspectives [393]. Unlike p-DNA, mRNA does not need to overcome nuclear membrane to achieve transfection, and circumvents the need of a specific promoter. Moreover, although protein expression arising from mRNA transfection is more transient than from p-DNA, mRNA does not integrate into the genome and thus poses no risk of insertional mutagenesis [394]. In this light, mRNA complexed NPs could be suitable as a novel potential therapeutic approach also in cerebrovascular disorders of genetic origin, such as CCM disease.

Taken together, the great progress toward a comprehensive characterization of disease mechanisms, and the rapidly growing variety of effective therapeutic/diagnostic nanosystems and alternative administration routes allow to foresee significant improvements of existing diagnostic and therapeutic approaches

for CCM and other cerebrovascular diseases, pointing to precision risk stratification and personalized nanomedicine strategies. With this growing trend, the future for an early and more effective treatment of cerebrovascular diseases and associated comorbidities looks bright.

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Table legends

Table 1. Clinical features of major cerebrovascular diseases. AVM: arteriovenous malformation; BCT: brain capillary telangiectasia; CCM: cerebral cavernous malformation; CT: computerized tomography; CTA: CT angiography; DVA: developmental venous anomaly; ICH: intracerebral hemorrhage; MRI: magnetic resonance imaging; MRA: magnetic resonance angiography; SAH: subarachnoid hemorrhage.

Table 2. Nanoparticulate systems employed for cerebrovascular diseases. ADSC: adipose-derived stem cells; Apo E: apoliprotein E; AQP-1: aquaporin-1; AVM: Arterio Venous Malformations; bFGF: Basic fibroblast growth factor; BBB: blood brain barrier; Bcl-2 B-cell lymphoma 2; CAT: catalase; CCM: Cerebral Cavernous Malformation; CT: computer tomography; EC: endothelial cells; EPCs: Endothelial progenitor cells; EV: extracellular vesicles; HSP70: Heat Shock Protein 70 kilodaltons; EC: Endothelial cell, FK506: tacrolimus; ICH: intracerebral haemorrhage;

iNOS: inducible Nitric Oxide Synthase; ION: iron oxide nanoparticles; I/R: ischemia-reperfusion; LDC: Lipid Drug Conjugates; LNC: Lipid nanocapsules; MCAO: middle cerebral artery occlusion; MEKi: inhibitor of the Mitogen-activated protein kinase; MESC: embryonic stem cell; miR: microRNA; MRA: magnetic resonance angiography; MRI: magnetic resonance imaging; MRP: magnetic resonance perfusion; MSC: multipotent mesenchymal stromal cells; mTOR: mammalian target of rapamycin; MV: microvesicles; NEP1-40: Nogo-66 receptor antagonist peptide; NDMA R1: N-methyl-D-aspartate receptor 1; NGF: nerve growth factor ; NIRF: near-infrared fluorescence; NPs: nanoparticles; NSC: Neural stem cells; OX26: anti trasnferrin receptor antibody; PAMAM: Hydroxyl polyamidoamine; pDNA: plasmid DNA; PEDF: pigment epithelium-derived factor; PEG: polyethylene glycol; PELG: poly(ethylenediamine L-glutamate; PET: Positron Emission Tomography; PGP: Pro-Gly-Pro; PLGA: poly(lactic-co-glycolic acid); PLL: poly(L-lysine); RVG: rabies virus glycoprotein; SHp: stroke homing peptide ; siRNA : small interfering RNA; SLNs: Solid Lipid Nanoparticles; SOD: superoxide dismutase; SPION: paramagnetic iron oxide nanoparticles; sPirB: soluble PirB (Paired immunoglobulin-like receptor B) ectodomain; T7: T7 peptide; TBI: traumatic brain injury; TfR: transferrin receptor; TNF: tumor necrosis factor; t-PA: tissue plasminogen activator; USPION: ultrasmall paramagnetic iron oxide nanoparticles; VEGF: Vascular Endothelial Growth Factor; ZL006: neuroprotectant drug ZL006.

Figure legends

Figure 1. Schematic representation of the main available nanoparticulate systems. LUVs: large unilamellar vesicles; MLVs: multi-lamellar vesicles; MVVs: multi-vesicular vesicles; NPs: nanoparticles; PLA: poly(lactic acid); PLGA: poly(lactic-glycolic acid); SUVs: small unilamellar vesicles.

Figure 2. Schematic representation of different strategies for drug delivery to the brain. BBB: blood brain barrier; CMT: carrier mediated transport; CTX: chlorotoxin; EPR: enhanced permeability and retention; LDL: low density lipoprotein; Lf: lactoferrin; MMP: matrix metalloproteinase; NMDA R1: N-methyl-D-aspartate receptor 1; R: receptor; RMT: receptor mediated transport; T7: T7 peptide; Tf:

transferrin.

Figure 3. Scheme of nose-to-brain uptake pathway of nanoparticulate systems. BBB: blood brain barrier.

Figure 4. Schematic representation of the synergistic rescue effects of composite platinum/rapamycin nanosystems (Pt@Rapa NPs) in KRIT1-deficient cells. Red and green arrows indicate the detrimental effects of KRIT1 loss-of-function, and the beneficial multifunctional biological activities of platinum/rapamycin nanosystems, respectively. KRIT1 loss-of-function impairs redox homeostasis, mitochondrial function, and autophagy, leading to major molecular and cellular hallmarks of CCM disease pathogenesis, including the aberrant accumulation of intracellular ROS and autophagy markers, such as the p62/SQTSM1 protein, and the induction of endothelial to mesenchymal transition (EndMT), which are synergistically rescued by Pt@Rapa NPs [69].

Figure 5. Schematic representation of major cerebrovascular diseases that might be targeted by nanodiagnostic, nanotherapeutic and nanotheranostic precision medicine approaches.

Disease	Disease subtype	Predominant vessel types	Causes	Pathological and clinical outcomes	Diagnostic Tests	Treatment	References
Stroke	Ischemic	Mixed	Cerebral thrombosis or embolism. Multifactorial etiology. Oxy-inflammation	Brain ischemia. Neurological disorders. Temporary or permanent disabilities	CT; MRI	Pharmacological therapies and interventional mechanical devices aimed at restoring cerebral blood flow. Nanotechnology	[4,14-17,71]
	Hemorrhagic	Mixed	Rupture or leakage of a weakened blood vessel. Multifactorial etiology. Oxy-inflammation	SAH or ICH. Neurological disorders. Severe morbidity and high mortality	CT; MRI	Surgical or endovascular treatments. Strategies to reduce the secondary injury	
Aneurysm	Saccular Non-saccular	Arterial	The exact etiology remains unclear. Oxy-inflammation	Brain aneurysms can remain clinically silent or cause life-threatening hemorrhagic stroke (SAH)	CTA; MRA; Cerebral Angiography	Preventive treatments (endovascular or surgical aneurysm repair), or conservative management with follow-up imaging	[19,20,23]
AVM		Arteriovenous	The exact etiology remains unclear. Oxy-inflammation	SAH or ICH. Neurological damage related to lesion location	Cerebral angiography; CTA	Conventional surgery, endovascular embolization, and radiosurgery	[36-42,46]
DVA		Venous	The exact etiology remains unclear	Mostly benign and clinically silent. Often found associated with sCCM	MRI	Conservative management	[33,34]
CCM	Sporadic (sCCM) Familial (fCCM)	Capillary	Proven genetic origin. Oxy-inflammation	Recurrent headaches, neurological deficits, seizures, ICH.	MRI	Surgery or conservative management with follow-up imaging	[15,48,59,72-74]
BCT		Capillary	The exact etiology remains unclear	Mostly benign and clinically silent	MRI	Conservative management	[35]

Nanosystem		Drug/probe delivered	Use	Outcomes	Ref.
Micelles		Neuroprotective drug edaravone	Therapeutic: stroke	Enhancements of ischemic brain targeting; improved neuroprotection	[297]
Nanoemulsions		Triolein	Diagnostic	Increased vascular permeability with minimal risk of cerebral edema	[298]
		Antioxidants	Therapeutic: stroke, ICH	Enhancement of bioavailability by nose-to-brain (thymoquinone); Improvement in the motor skills and haematoma size decrement (quercetin)	[299,300]
		Statins (simvastatin)	Therapeutic: brain injury	Enhancement of the nose-to-brain transport	[269]
		Calcium channel antagonist nimodipine	Therapeutic: stroke	Decreased toxicity and irritation after i.v. administration	[301]
Lipid NPs	LDC	Neuroprotective squalenoil-adenosine	Therapeutic stroke	Improved brain bioavailability	[302]
	SLN	Neuroprotective drugs	Therapeutic: stroke	Ischemic brain targeting by FAS-ligand antibody conjugation (3-n-butylphthalide); BBB overcoming (andrographolide); Nose-to-brain delivery of muco-adhesive SLNs (vinpocetine)	[303-305]
		Polyphenols (antioxidants)	Therapeutic: stroke	Brain targeting by OX26 conjugation, and improved relieve of neuronal injury (baicalin); Reduced clearance by PEGylation (daidzein); Increased oral bioavailability and brain delivery (hydroxysafflor yellow A); Protection from ischemia injury by up-regulation of Bcl-2 and HSP70 expression and down-regulation of Caspase-3 expression (puerarin); Improved brain bioavailability (epigallocatechin gallate)	[210,306-309]
	LNC	Calcium channel antagonist nimodipine	Therapeutic: stroke	Brain targeting through lactoferrin functionalization and reduced clearance by PEGylation	[212]
		Polyphenols: baicalin and salvianolic acid B	Therapeutic: stroke	Brain targeting by OX26 conjugation	[211]
Liposomes	PEGylated	Antioxidant enzyme SOD	Therapeutic: stroke	Improved drug pharmacokinetics and brain delivery through anti-NDMA R1 receptor antibody conjugation	[218]
		Thrombolytics, vasodilators		Improved neuroprotection due to co-delivery of dexamethasone and t-PA; Accumulation in the ischemic area with amelioration of I/R injury and motor score; Accumulation in the ischemic area with amelioration of I/R injury and motor score; (fasudil): Extended therapeutic time window (t-PA and fasudil)	[310-312]
		Statins (simvastatin)		Improved brain bioavailability (simvastatin)	[313]
		mTOR inhibitors		enhanced therapeutic efficacy of FK506	[314]
		Anti-inflammatory cyclosporine A		Significant effective dose reduction	[315]
		Neuroprotective drugs	Theranostic: stroke	Enhanced accumulation in the ischemic area of T7 and SHp dual targeting with improved therapeutic outcomes (ZL006); Improved motor function and reduced water retention and protection from cognitive impairment (hemoglobin); Improved drug delivery of the MRI-traceable PEG-immuno-liposomes in the ischemic area with significantly reduction of lesion volumes (citicoline);	[95,214,296,316]
	Plain	Antioxidant panax notoginsenoside	Therapeutic: stroke	Brain edema inhibition, reduction of infarct volume and increased SOD level	[259]
		Neuroprotective drugs & peptides		Improved neurologic deficit score and locomotor activity by intranasally administered gelatin-coated nanoliposome (bFGF); Improvement of neuroprotective outcomes and disclosure of novel neuroprotection mechanism by regulating iron metabolism in ischemic brain (lycopene); Improved brain delivery, promotion of neuroprotection and vascular regeneration in the chronic stage of cerebral infarction due to	[208,317-319]

				TfR-targeted liposomes (VEGF) Improved drug pharmacokinetics and increased neuroprotective effects (acetate);	
		Neuroprotective drugs	Theranostic: stroke	Accumulation in the ischemic regions and improved ischemic stroke recovery (sPirB); Accumulation in the ischemic regions detected by PET (hemoglobin)	[294,295]
Dendrimers		Neuroprotective drugs	Therapeutic: brain injury	Active targeting of microglia and damaged neurons in injured brain: potential role of PAMAM dendrimers in brain delivery; Attenuation of the inflammatory response and improvement in myelination and in the Therapeutic time window: potential delivery system for neonatal brain injury treatment; Effective brain delivery and accumulation in injured areas of neutral dendrimers: possible role of dendrimers as biomarkers for disease phenotypes; Significant reduction of the adverse side effects of N-acetyl cysteine and valproic acid	[201-205]
Exosomes, MV. EV	MSC-derived	miR	Therapeutic: stroke, ICH	Enhancement in the efficacy of miR based therapy; Improvement in functional recovery, neurite remodeling, neurogenesis and angiogenesis (miR-133b-overexpressing and miR-17–92 cluster–enriched); Improved safety profile	[320-326]
	ADSC-derived	miR		Prevention of cerebral injury by inhibiting autophagy-mediated microglial polarization (enriched with miR-30d-5p); Ameliorated cerebral I/R injury by autophagy activation and neuronal apoptosis suppression (PEDF-overexpressing exosomes);	[327,328]
	MESC-derived	Antioxidant curcumin		Improvement in neurovascular restoration	[329]
	NSC-derived			Alteration of the systemic immune response with a neuroprotective effect; improved neural tissue preservation and functional levels	[330]
	EC-derived			Modulation of astrocyte functions, BBB integrity and cerebral blood flow with a neuroprotective effect	[331]
	Surface fucntionalized	miR		Efficient RVG targeted delivery of gene drugs (miR-124) to the brain; Efficient targeted brain delivery associated at a large-scale production	[332,333]
Polymeric NPs	Polystyrene		Therapeutic: stroke	Colloidal stability determines overcoming of disrupted BBB	[199]
	Chitosan	Anti-oxidative and anti-inflammatory silymarin	Therapeutic: stroke	Prevention of oxidative/inflammatory brain damage by I/R after oral administration	[334]
	Gelatin	iNOS siRNA	Therapeutic: stroke	Increased therapeutic potency of intranasal NPs in the postischemic brain	[335]
	dendrigraft PLL	Antioxidant enzyme CAT	Therapeutic: stroke	PGP funtionalization allows neutrophil mediated delivery to inflamed injured brain	[219]
	PEG-b-(PELG-g-PLL)	Neuroprotective TNF-α	Therapeutic: stroke	enhanced bioavailability, reduced oxidative stress, inflammation, and apoptosis in I/R	[336]
	PLGA	Antioxidant enzymes CAT and SOD	Therapeutic: stroke	CAT/SOD loaded NPs co-administered with t-PA mitigated inflammatory response, induced neuroprotection, and inhibited edema formation in I/R injury	[261]
		Neuroprotective drugs		Significantly reduced infarct volumes and enhanced survival with lexiscan and NEP1-40 loaded NPs	[215]
		Antioxidants		Curcumin loaded PEGylated NPs induced neuroprotection in I/R injury by reducing oxidative damage neuronal apoptosis	[337]
				Increased quercetin oral bioavailability and remarkable mitochondrial localization post I/R injury	[338]
		800CW imaging agent	Diagnostic: stroke	Smaller (100-nm) PEG-coated NPs penetrated deeper into the mouse brain than large containing NPs (800nm)	[143]
	PLGA-chitosan	Antioxidant thymoquinone	Therapeutic: stroke	Intranasally administered NPS reduced ischemia infarct volume and enhanced locomotor activity and grip strength in MCAO	[339]
	Platelet	selective spleen tyrosine kinase	Theranostic:	Platelet membrane coating allows delivery of	[274]

	membrane wrapped PLGA	inhibitor piceatannol and SPION	stroke	piceatannol to adherent neutrophils, detaching them into circulation, and thus decreasing their infarct infiltration	
	PEG-PLA	siRNA	Therapeutic: stroke	Inhibiting microglial neurotoxicity	[340]
	PEG-PLGA	nanoceria	Therapeutic: stroke	Reduction of focal ischemia by 60% and brain edema by 78% in MCAO	[255]
	Acrylate	SPION and t-PA	Theranostic: embolic stroke	Accelerated thrombolysis and reduced infarct area in cerebral embolism model	[341]
	PAMAM	MRI and NIRF probes	Theranostic: diabetic stroke	αvβ3 integrin-targeted NPs allow early detection of angiogenesis and therapy in photothrombotic stroke	[342]
	Peptide	siRNA	Therapeutic: TBI	Accumulation into the injured site and downregulation of a therapeutic candidate	[343]
	Albumin	Neuroprotective NGF, MEKi U0126 and USPION	Theranostic: stroke	Infarct size reduction	[276]
	Gd-conjugated oxygen reactive polymer		Theranostic: TBI	MRI guided 3-fold reduction of H_2O_2 levels	[344]
Carbon NPs	silicon–graphene oxide core–shell	siRNA	Therapeutic: injured brain	RVG targeted NPs caused 2-fold greater cellular uptake and gene silencing	[217]
	Fullerene	Glucosamine conjugated fullrenol	Therapeutic: stroke	Reduced immunoreactivity, infarct volume and cerebral inflammation	[345]
	Carbon		Therapeutic stroke	Antioxidant activity acting as a biomimetic SOD; Restored balance between nitric oxide and superoxide in MCAO	[256,258]
	Fullerenol		Therapeutic stroke	Reduced neurological dysfunction, brain edema and infarction of ischemic brain due to ROS quenching	[257]
Inorganic NPs	Silver	siRNA.	Therapeutic: TBI	Gene silencing in injured brain parenchyma	[216]
	Gold		Theranostic: stroke	Visualize cerebrovascular thrombi in CT and guide thrombolytic therapy	[153]
			Therapeutic: AVM	Radiation dose enhancers in radiosurgery	[346]
			Diagnostic: ICH	Raman spectroscopy	[279]
			Diagnostic: AVM	MRA contrast agent	[347]
	ION: SPION		Diagnostic: stroke	Contrast for Microwave Imaging Resonance; Early detection of endothelial activation and neuroinflammation by P-selectin targeted SPION; MRI of BBB alteration by PEG-SPION; No post-stroke passive targeting by certain types of SPION; Neuroinflammation detection via MRI in absence of lesions and symptoms;	[278,348-351]
		Fluorescent Sulphorhodamine B linked to valylalanylaspartic acid fluoromethyl ketone (caspase inhibitor)	Theranostic: stroke	Strong platform for non-invasive imaging and targeting delivery to apoptotic cells	[352,353]
	ION: USPION		Diagnostic: stroke	USPION-enhanced MRI: Passive diffusion of USPION after BBB disruption and by intravascular trapping; Non-invasive monitoring of macrophage recruitment into ischemic brain lesions	[275,354-357]
	ION: SPION-labeled MSC		Theranostic: stroke	Therapeutic role of stem cells conjugated with SPION to monitor efficacy	[358]
		Nucleic acids		Translocations of loaded VEGF-pDNA into MSC, allowing MRI tracking due to combined PEG-SPION; Improved therapeutic efficacy and imaging tracking of transplanted EPCs due to siRNA-loaded SPION	[273,359]
	Platinum	mTOR inhibitor rapamycin	Therapeutic: CCM	Antioxidant and pro-autophagic activity	[66,69]
	Manganese oxide		Diagnostic: stroke	Determine and monitor apoptotic area via MRI after hypoxic-ischemic injury	[277]
	Ceria	Neuroprotective drug edavarone	Therapeutic: stroke	Highly effective BBB crossing by angiopep-2 targeted NPs and synergistic ROS elimination by both the loaded edaravone and ceria NPs	[213]
	Hydrophobic		Diagnostic:	Improve diagnostic efficiency of acute	[360]

	$NaYF_4$:Yb/Er		stroke	ischemia and stroke via MRI, MRA and MRP	
	Copper/zinc	SOD	Therapeutic: stroke	Decreased infarct volume and improved sensorimotor in MCAO and I/R injury	[260]
Perfluorocarbon NPs			Diagnostic: stroke	Visualize inflammatory processes by MRI; Identify ischemic penumbra and propose; Enhance sensitivity of MRI to detect penumbra in acute stroke patients	[361-363]
			Theranostic: stroke	Treatment based on metabolic status of the brain tissue, independent of time from stroke onset	[364]
			Therapeutic: stroke	Thrombolytic agent for treating acute stroke; Decrease ischemic stroke infarct volume and protect brain	[365-367]

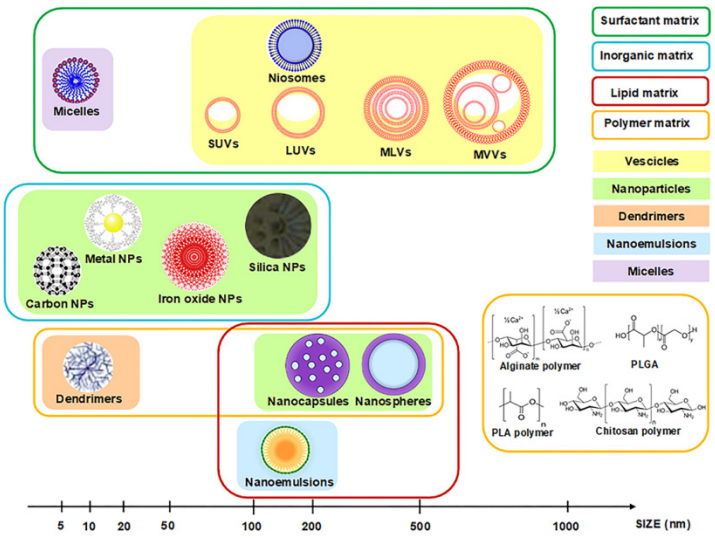

Figure 1

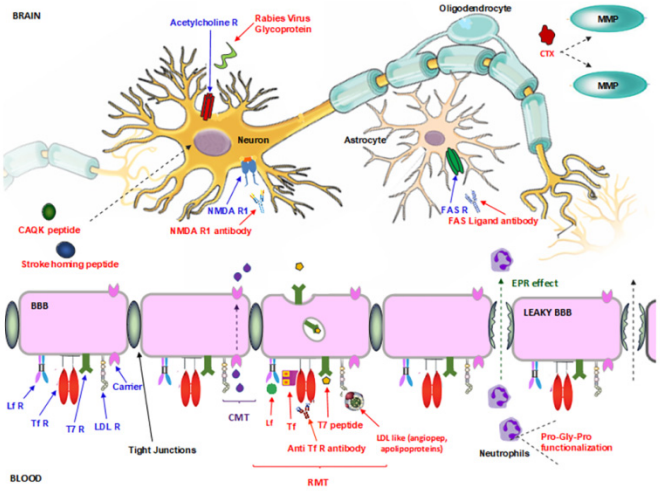

Figure 2

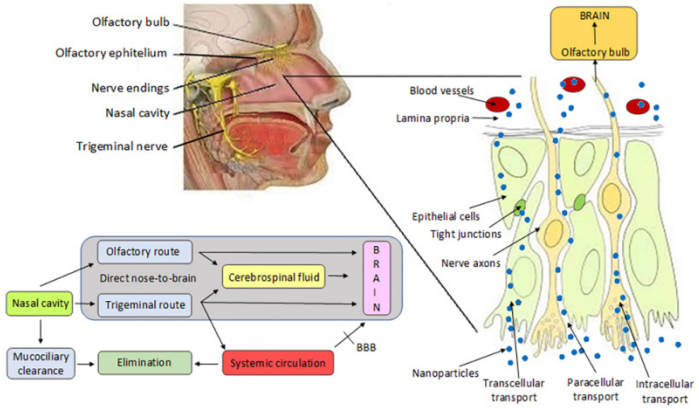

Figure 3

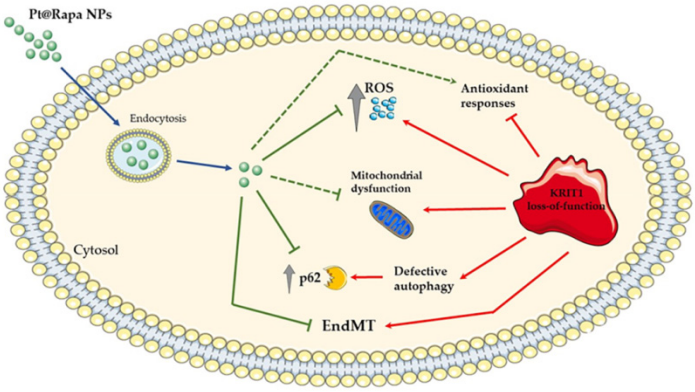

Figure 4

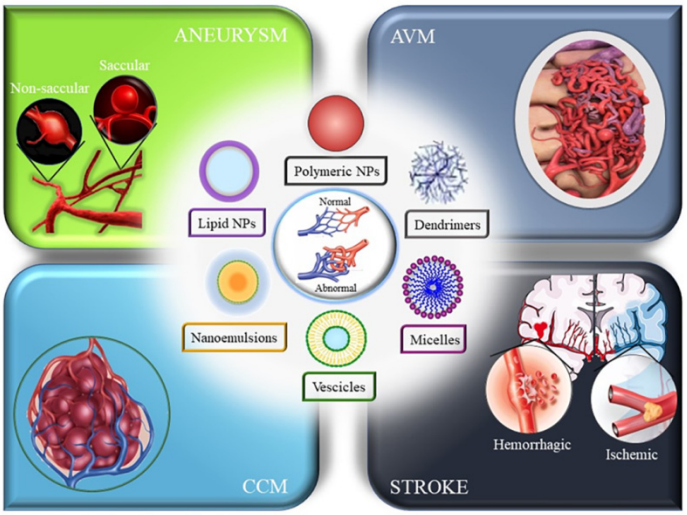

Figure 5