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Translational challenges in advancing regenerative therapy for treating neurological disorders using nanotechnology

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ABSTRACT

The focus of regenerative therapies is to replace or enrich diseased or injured cells and tissue in an attempt to replenish the local environment and function, while slowing or halting further degeneration. Targeting neurological diseases specifically is difficult, due to the complex nature of the central nervous system, including the difficulty of bypassing the brain's natural defense systems. While cell-based regenerative therapies show promise in select tissues, preclinical and clinical studies have been largely unable to transfer these successes to the brain. Advancements in nanotechnologies have provided new methods of central nervous system access, drug and cell delivery, as well as new systems of cell maintenance and support that may bridge the gap between regenerative therapies and the brain. In this review, we discuss current regenerative therapies for neurological diseases, nanotechnology as nanocarriers, and the technical, manufacturing, and regulatory challenges that arise from inception to formulation of nanoparticle-regenerative therapies.

Contents

1. Introduction

Regenerative therapy for neurodegenerative disease historically has three major aims: 1) replace diseased or lost neurons with healthy ones; 2) provide environmental enrichment *via* growth factors released by newly grafted cells, which supports remaining neurons and slows degeneration; and 3) restore neural tissue or organs (*e.g.* retina) that are

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damaged due to disease or aging. These aims rely on two major hypotheses: 1) loss of endogenous neurons can be compensated for by replacement with exogenously derived cells that have either direct or indirect neurogenic capacity [1]; and 2) investigation of endogenous stem cell regenerative capacity based on research in regenerative lower vertebrates (*e.g.* zebrafish, drosophila, salamander) indicates the potential for reactivation of endogenous, dormant stem cells in the human central nervous system (CNS) [2–4]. Nanotherapeutic approaches, in which nanocarriers can be formulated to cross the blood brain barrier (BBB) and target diseased cells at the site of injury, have complemented the field of regenerative medicine and address these aims by safely and effectively facilitating the delivery of growth factors, neuroprotective drugs or restorative cells to sites of CNS damage [1].

For a regenerative medicine approach, it is necessary to fully understand disease systems and the cellular interactions within those systems before designing therapies to enhance or treat these systems [5]. It is also necessary to have a clear understanding of which patients may have the best response to a particular therapy in order for these approaches to be of highest yield. Thus, an ideal situation is when the molecular cause of the disease is known, and when reliable clinical biomarkers exist that reflect the pathophysiology. To achieve success in producing a therapy, creativity and collaboration are required between clinicians, neuroscientists, and bioengineers to recall disease aspects and clinical demand while actively designing therapies for a patient population. Development of regenerative therapies is expensive and often requires public funding as well as cooperation between academics, medical researchers, regulatory agencies, and industry. Furthermore, combining regenerative medicines with the required nano-therapy expertise adds a layer of complexity, time, and cost. After product development, there is a need for reproducibility of the product itself and reproducibility in scaling so the therapy can be distributed using the most cost-effective means [5]. Finally, depending on the technique or cell types used to derive these therapies, there is unpredictability of the impact and social acceptance of the approach [6].

2. Cell-based regenerative medicine therapies in neurodegenerative diseases

The main goals of cell-based regenerative therapy in the CNS are to provide neuroprotection, compensate for lacking cellular function, and facilitate tissue repair. In this section, we describe current investigations in cell therapy and their posed challenges such as administration, delivery vector, engraftment, targeting, and BBB permeability, that may, as discussed later, be ameliorated by a nano-therapeutic approach. Investigations into cell-based therapies are in progress for common neurodegenerative diseases, including multiple sclerosis, Alzheimer's disease, and Parkinson's disease. These are well-characterized diseases each with an abundance of pre-clinical research data and drug candidates, and are feasible targets for combined nano-based regenerative therapies.

Multiple Sclerosis (MS) is an autoimmune neurodegenerative disease of the CNS characterized by either relapsing and remitting or progressive disease with auto-immune driven inflammatory demyelination and progressive neuronal cell death. The mainstay of drug treatment modalities is immunosuppression, which reduces relapses but does not offer neuroprotection nor stop disease progression. Cell-based therapies for MS include autologous bone marrow grafts to reconstitute the immune system and to promote self-tolerance to myelin. One such study, an ongoing Canadian clinical trial, harnesses the anti-inflammatory and neurogenic properties of mesenchymal stem cells (MSCs) for neuroprotection in MS; however, eligibility is low, the risk of adverse effects including mortality is high, and the cost is up to $60,000 CAD per treatment [7].

Alzheimer's Disease (AD) is characterized by loss of neurons and synaptic connectivity throughout the cortex, hippocampus, amygdala, and basal forebrain, leading to memory and cognitive impairment. One strategy to improve these deficits is through the enhancement of cholinergic function, which can be achieved by using stem cell therapies to replace lost neurons or promote neurogenesis [8]. Brain-derived neurotrophic factor (BDNF) levels can be enhanced to counteract the naturally-occurring loss of BDNF with aging. Models of AD in which rodents received neural precursor cell grafts showed an increase in hippocampal synaptic density and cognitive function associated with local production of BDNF [9]. A critical drawback of cell-based therapy for AD is the recognition that the odds of promoting a successful therapeutic neural network will decrease over time. For many neurodegenerative diseases, it is understood that there are windows of opportunity for therapeutic efficacy based on the disease stage. In the early stage, neuroprotection and/or cell-based repair may be achieved through growth factor infusion [10] or delivery of stem cells [11], but at advanced stages, replacement with tissue or organ prosthesis may be necessary. In these cases, even the healthy grafted cells may eventually succumb to disease due to the spread of pre-existing pathology, namely extracellular amyloid beta plaques and intracellular neurofibrillary tangles made of hyper-phosphorylated tau, a microtubule protein that enables intracellular transport along nerve axons. More recently, it has been determined that early AD pathogenesis is facilitated by disturbed BBB function, which is likely triggered by early neuroinflammation [12]. This provides a possible new target for early stage pathogenesis in AD [12,13] and early work using polymeric nanoparticles to target amyloid beta plaques in the cerebral vasculature of APP transgenic mice is underway [14].

Parkinson's Disease (PD) is a movement disorder caused by a progressive loss of dopaminergic (DA) neurons in the substantia nigra, resulting in decreased dopamine levels in the dorsal striatum and resultant dyskinesia and dementia. Current PD therapies aimed at protecting or enhancing DA neurons are known to produce side effects, and the efficacy of these therapies fades over time, often leading to the inevitable progression of motor and cognitive disease. In this disease context, cell replacement therapy requires acquisition and grafting of the specific neuronal subtypes lost in the disease, namely the DA neurons. Once DA precursor cells are grafted to the brain region, they must integrate and re-create the original healthy neuronal network. As with many cell-based therapies, there are problems with inconsistency and reproducibility of this protocol [15,16]. Furthermore, human embryonic stem cell-derived DA cells have been safe and effective in non-human primate models but it is unclear if the cells can be produced in sufficient quantities to demonstrate similar efficacy in the human brain.

3. Cell types in regenerative medicine

There are a variety of cell types currently being studied for applications in regenerative medicine, including for the neurodegenerative diseases, each with its own unique set of limitations. In designing nanotherapeutic agents, understanding cell-specific biology and limitations is paramount to combination therapeutic approaches. Mouse and human embryonic stem cells (ESCs) were first isolated in the 1980s and 1990s, respectively [17], and both have the capacity to differentiate into lineages of the three germinal layers (endoderm, mesoderm, and ectoderm) [18]. As opposed to embryonic stem cells, some adult stem cells, such as hematopoietic or mesenchymal stem cells, can be removed from a subject, used in a tissue or organ construct and transplanted back in an autologous fashion eliminating the need for immunosuppression. Despite the utility for autologous replacement, these cells are hard to access, and have a limited differentiation capacity and poor growth once differentiated relative to ESCs [19].

Mesenchymal stem cells (MSCs) are precursors of connective tissue cells, and like fibroblasts, are easier to culture *in vitro* than human ESCs. They derive well into mesodermal tissues and the type of tissue can be directed by changing the composition of their growth medium [20]. There are a growing number of studies confirming safety and efficacy of adult MSCs in degenerative brain diseases, such as stroke, traumatic brain injury, and Huntington's disease [21]. A recent investigation into

the possibility of using intra-arterial delivery of adult MSCs to treat cerebral ischemia in rats found effective neuroprotection 24 h after administration of the maximum tolerated dose [22]. A drawback of adult MSCs is the finite number of divisions (based on donor age) and the acquisition of genetic changes with time. Furthermore, some believe that nonhuman primate models are essential for clinical modeling in brain disorders before any therapies progress to translation [21].

Finally, fibroblasts or peripheral blood mononuclear cells from humans and mice can be reprogrammed into induced pluripotent stem cells (iPSCs) [23–25]. In studies of PD, human iPSC-derived dopaminergic neurons survived and functioned as midbrain dopaminergic neurons in a primate model of PD treated with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [26]. While the use of iPSCs can avoid the ethical concerns that ESCs carry and they can be made patient-specific, oncogenic viruses often used to derive them pose safety concerns for clinical translation. Research groups have improved upon the original method, including some virus-free protocols, the use of a combination of vectors, direct use of protein products from reprogramming genes, and a strategy of using a sequence of all the Yamanaka factors separated by spacer elements with a transposome vector [24].

4. Challenges of translating cell-based therapy to clinical practice

A number of logistical challenges hinder wide-spread use of cell-based regenerative therapies. A thoughtful understanding of these challenges and how nanotechnology marries into these challenges will help facilitate the development and production of combination use. Many cell-based therapies begin with conventional 2D static cultures which result in the differentiation of a small number of cells following a process that is time-consuming, cumbersome, labor intensive, and requires clinical grade facilities. Further, the complexity of these cells as therapeutic agents creates challenges for determining their safety and efficacy, including 1) tumorigenicity, specifically what is the cell's proliferation potential and how stable is its phenotype; 2) mutagenesis that may affect cell function; 3) contamination - is microbial sterility ensured? and, 4) immune rejection or immune activation [27–29]. On a much larger scale, the required basic laboratory cell culture practices would be difficult to validate, reproduce, and ensure quality control [30], and production of sufficient numbers of these high-quality cells is anticipated to fall short of the need [1]. Because these cells may require synthetic scaffolding in order to adhere to tissues once in a system, the ideal model involves 3D cultures executed to form a functional tissue [31]. These 3D dynamic cultures imitate an environment similar to the *in vivo* situation [32] and have been made possible through the use of bioreactors that supply nutrients and oxygen, remove catabolites, monitor pH, and apply mechanical stress to facilitate formation of an extracellular matrix [33]. Within a bioreactor, large volumes of encapsulated undifferentiated cells can be maintained indefinitely with growth and differentiation directed by the addition of specific growth agents [34] essentially recreating the physiological environment specific to the tissue being regenerated. Bioreactors on a large-scale could reduce batch numbers and variability, however work needs to be done to assess the economics and strategies for expanding cell production [35]. Similarly, iPSC derived cell therapies will require the production of specific cell types that in turn require precise conditions for growth, transplantation, host integration, and extensive genomic analysis to minimize risk for tumorigenesis. These factors pose enormous obstacles for the successful replacement or enrichment of brain tissue; however, parallel efforts are underway to determine the methods and best practices of stimulating or enhancing endogenous stem cells and their environment for maximum survival [36]. Nanotechnology, as discussed now, offers solutions for many of these issues through enhanced cell targeted delivery, longevity, immune tolerance, and stability.

5. Nanoparticles as nanocarriers

For close to 30 years, the successes of nanodiagnostics and nanotherapies have been demonstrated for the treatment of cancers and systemic disorders [37]; however, such progress has yet to be made for neurological disorders. Because nanoparticle therapies stand to facilitate drug delivery to target cells allowing for lowered drug concentrations, reduced side effects, and fewer applications (also increasing patient compliance), they are particularly well suited for the treatment of complex neurological diseases. The benefits of this platform are achieved through unique nanostructures which interact with their environment in a fashion that is dependent on their surface characteristics, making this an extremely flexible platform. Furthermore, because nanoparticles can transport fairly large cargo, including antibodies and proteins (~100 nm), size of small-molecule pharmaceuticals (~1 nm) is not an issue and most drugs can be attached at a fairly high loading density [38,39]. Several nanoparticles have been approved by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA); however, only a few for CNS disorders. An outline of nanoparticles approved or in clinical trial for CNS disorders are shown in Table 1 [37].

Liposomes are one of the most frequently employed carriers due to their usefulness in the transportation of water-soluble drugs and were one of the first carrier platforms approved by the FDA and EMA. Liposomes are circular vesicles composed of at least one lipid bilayer and a hollow core and are favored due to their low inherent toxicity and

Table 1
FDA/EMA approved nanoparticles for CNS disorders (top) and nanoparticles currently undergoing clinical trial (bottom).

Approved nanoparticles for CNS disorders

CNS indication	Year approved	Name	Particle and function (developer)
Multiple sclerosis	1996	Copaxone®	Polymer nanoparticle immunomodulator to reduce the frequency of relapse, but not disability, in MS. (Teva)
Schizophrenia	2009	Invega Sustenna®	Nanocrystal formulation for the short-term mood stabilization for schizophrenia and schizoaffective disorder. (Janssen Pharms)
Multiple sclerosis	2014	Plegridy®	Pegylated-Interferon beta-1α used to treat relapsing MS. (Biogen)

Nanoparticles for CNS disorders in clinical trial

CNS indication	Clinicaltrials.gov identifier	Name	Particle and function (developer)
Multiple sclerosis	NCT02511028	Ferumoxytol	Ultrasmall Superparamagnetic Ion Oxide Nanoparticle used as imaging agent of neuroinflammation in MS
Parkinson's disease	NCT03815916	CNM-Au8	Oral gold nanocrystal to enhance cellular bioenergetics in PD. Clene Nanomedicine
Leigh syndrome	NCT03747328	ABI-009	Albumin nanoparticle bound rapamycin for reduction of mTOR activity in Leigh Syndrome. Aadi, LLC
Alzheimer's disease	NCT03806478	APH-1105	Alpha secretase modulator. Aphios
Adrenoleukodystrophy	NCT03500627	OP-101	Dendrimer for immunomodulation in cerebral adrenoleukodystrophy

propensity for self-assembly [40,41]. Similar encapsulating carriers, micelles, formed by amphiphilic block copolymers, contain a hydrophobic core suitable for poorly soluble (lipophilic) compounds [42]. Due to the unstable nature of drug encapsulation, as discussed later, improvements on these basic structures have emerged to further enhance both the stability and functionality of these carriers. This has led to liposomal compounds such as *Doxorubicin* (Janssen, Teva UK) and *Verteporfin* (Novartis), and micellar nanoparticles including *Paclitaxel* (Bristol Myers Squibb), *Estradiol hemihydrate* (Novavax/Graceway), and *Docetaxel* (Sanofi-Aventis).

Inorganic magnetic iron oxide nanoparticles were FDA approved as early as the 1970s, however became more widely used in the 1990s and are commonly tailored or "functionalized" for enhanced, or bifunctional performance [43]. These and other magnetic nanoparticles are particularly useful for prognostic imaging assays as they show accumulation in tumor sites both through phagocytosis by tumor associated macrophages and through magnetic localization to the affected areas [43,44]. Despite the successful use of magnetic iron oxide nanoparticles for imaging of angiogenesis and lymph nodes, especially in the context of cancer, these nanoparticles have suffered setbacks on their way to clinical trial following irreproducible and varied results in test. Adding to this, the return on investment for imaging-only particles is low, challenging its wide-spread use in favor of other multifunctional agents that are better suited for active targeting therapeutic benefits [45]. Successful inorganic nanoparticles include *Ferumoxytol* (AMAG pharmaceuticals), Ferrlecit® (Sanofi Avertis), and Venofer® (Luitpold Pharmaceuticals).

Polymeric nanoparticles are polymeric colloidal particles that can be present as nanospheres or nanocapsules. Several polymeric nanoparticles have been approved by the FDA and EMA, and in addition to clinical trials for cancers, inflammatory diseases, and MS, polymeric conjugate SER-214 has recently entered clinical trials as a dopaminergic agonist for the treatment of PD (clinicaltrials.gov, Identifier NCT02579473; [46]). Dendrimer nanoparticles which are highly branched, symmetrical compounds can be functionalized with additional polymers or ligands for improved payload and directed biodistribution for targeted release. Dendrimers, ranging from 3 to 10 nm are much smaller than many organic counterparts, and because they are made of concentric monomers around a central core, these "generations" enable precise control of size and shape [47]. With high surface area, water solubility, and neutral surface charge, dendrimer nanoparticles offer an advantage that make them particularly suited for brain targeting across the BBB for CNS disorders and are considered one of the most "useful" platforms to date [39,47,48]. FDA approved polymeric nanoparticles include Adagen® (Sigma-Tau Pharmaceuticals), Cimzia® (UCB), Pegasys® (Roche), and Plegridy® (Biogen), among others [49].

5.1. Nanoparticle encapsulation and conjugation

Encapsulating drug carriers allow compounds to be carried to target sites without molecular or chemical modification of the drug itself. Encapsulating particles protect drug activity from degradation, dilution, or premature release, relying on the degradation of the encapsulating membrane to slowly make the load available. Although encapsulation offers protection and maintains drug integrity, these nanocarriers are often unstable and thus not capable of reliable drug delivery [50]. As a remedy, modified PEGylated liposomes (a hydrophilic polymer coating of polyethylene glycol, PEG) are sterically stabilized and have improved circulation times and reduced clearance by the reticuloendothelial system. These compounds stay in circulation longer; however, the comparatively larger molecule formed by PEGylation limits travel and uptake by the brain. Chemical conjugation of drugs to encapsulating vesicles (such as lysosomes) is also possible; however, encapsulation is more suited for targeted drug delivery and release, as it requires a detethering of the drug from the carrier to be active, making premature release less likely [42]. Despite these advances in stability and circulation, nanoparticle systems are still subject to other phenomena, including: the Enhanced Permeability and Retention (EPR) Effect, in which liposomes accumulate in off-target areas, the Accelerated Blood Clearance (ABC) phenomenon, in which repeated dosing of PEGylated liposomes are cleared more rapidly, and innate immune activation [42,51,52]. These issues and others have resulted in the somewhat slow transition of nanoparticles to clinical use.

Nanoparticles with covalently conjugated drugs rely on surface characteristics, size, and charge for the transport of compounds to target sites. If the drug lacks an appropriate linkage site, chemical alterations may be necessary to link drugs to nanocarriers; however, these modified surface groups or linkages allow for a tailored "plug and play" strategy which enables a high level of flexibility through the precise control over surface groups, charge, and size [40,53–55]. Non-encapsulating particles include many varieties of nanoparticles, and surface "plug and play" linkages for enhancement is not limited to non-encapsulating particles, as liposomes can be modified in similar fashion [51]. Although these surface linkers enhance tissue localization and cellular uptake, large-scale production can be challenging, especially for magnetic nanoparticle carriers. Favored methods of magnetic nanoparticle synthesis require steps to prevent agglomeration, and often complex and dangerous high temperature thermal reactions – which may contribute to complexity, production variability, and limit mass and low-cost production [43].

6. Nanoparticles and regenerative medicine

The use of nanoparticles to modulate and/or enhance cell therapies maximizes the benefits of regenerative medicine as the strengths of nanoparticle delivery redress many of the inherent weaknesses of cell-based therapies. Human and mouse stem cells have shown compatibility with nanotechnology and, for example, take up magnetic iron oxide particles without effects on cell viability or differentiation capacity [56]. Therapeutic benefit necessitates survival and integration of transplanted stem cells which can be confirmed *via* MRI imaging of nanoparticle-labeled cells. To date, magnetically-labeled nanoparticles have been used to label and track stem cells to assess migration and viability and the safety and feasibility of these nanoparticles has been demonstrated in a handful of human studies which track the short- and long-term activity as well as mobility of these cells within the brain [57]. Although these studies are few and show promise, several factors including signal persistence after cell death or engulfment, slow clearance, and artifact hypointense signals may complicate and confound the interpretation of true contrast agent signal [58]. Similarly, different nanoparticle compounds have been used to protect and prolong fragile stem cells while simultaneously improving the homing of these cells to their desired location [56,59,60]. Quantum dots, small light emitting nanocrystals, also frequently used in different imaging modalities have been shown to work with stem cells; however, less is known about their interactions with stem cells and there is some concern regarding toxicity of accumulated quantum dots [56,61].

Cell delivery through encapsulation is a strategy that has been achieved and tested widely to improve islet transplantation/grafting for the long-term treatment of type 1 diabetes mellitus. Encapsulation of islets or insulin-expressing cells in biocompatible materials (such as hydrogels or polyethylene glycol and its derivatives) reduces the need for immune-suppression while enhancing graft function and survival. Nanoencapsulation creates a fine immune-isolation layer surrounding the cell surface which outperforms micro- and macro-encapsulation in terms of nutrient access, glucose response times, and the ability to easily modify layer permeability and composition [62]. Additional modifications allow for enhanced oxygen flow, reducing encapsulation induced hypoxia, and layer-by-layer approaches enable greater control over timing and release of cell loads. ESCs and MSCs show sensitivity to carrier composition, but under ideal conditions continue to proliferate and differentiate well within encapsulation and can even be exposed to specific environments to drive differentiation appropriate for the target

tissue [62]. Liposome encapsulation offers similar promise for cell delivery, and studies using inverse-emulsion techniques demonstrate preserved cell viability and protection from harsh microenvironments [63]. Finally, collagen microspheres encapsulating oligodendrocyte progenitor cells [64] or astrocytes [65] have been shown to differentiate and mature during encapsulation, suggesting these non-immunogenic carriers may be useful for the delivery of live cell material.

The synthesis of polymeric nanoparticles can be highly regulated and reproducible, thus the delivery of genes and other biomaterials is most favorable on the polymer platform [66]. Because gene transfection efficiency, reproducibility, and toxicity have historically been factors for some polymeric nanoparticles in terms of effective gene delivery [56,66], work has progressed to define variables, such as composition and core characteristics, for the optimization of these nanoparticles as reliable carriers in regenerative medicine [56,67,68]. Intracellular delivery and release does not stop at genetic material, as growth factors, chemicals, and other biomolecules that need protection from harsh environments on their way to target tissue can be delivered in this fashion [56,60]. Other carriers, such as lipid nanoparticles, have shown *in vitro* and *in vivo* promise for the delivery of genetic material using a two-step process, in which a targeting sequence directs localization followed by release of a charged PEGylated layer in that new microenvironment. The release of PEG shifts the lipid nanoparticle surface charge, favoring interaction with target cells for the release of siRNA [69] or plasmid DNA [70] to the brain, tumor sites, or areas of high inflammation.

Other fields of nanoparticle aided regenerative medicine are burgeoning as well. These include nanopatterned surfaces for cell adhesion, nanoscaffolds and other nanotopographical features, nanofibers for cell targeting, and biomimetic cellular environments [56,60,71]. These topics are worthy of mention and are fully discussed elsewhere in this article.

7. Barriers to translation

7.1. Nanoparticle challenges

The landscape of nanoparticle formulations has matured in the last decade, and with the commercial and clinical success of several nanocompounds, a significant expansion of the complexity, sophistication, and therapeutic potential of the next generation of nanoparticles is expected. With growth and maturation of the nanoparticle field comes the need for heightened preparation for the increased demand and production of nanotherapeutics- especially for neuro-applications [72]. These nanoparticles will likely be more sophisticated and multifunctional, requiring manufacturing, regulation, and standardization that leaves little room for error. While preclinical and industrial production of nanotherapeutics does not differ much on the technical level, controlling large batch production- and batch-to-batch variability while maintaining product sterility, product life (storage and freezing) and integrity to ensure a standardized product becomes difficult [5,53,73]. Due to the critical nature of the structural complexity, structural integrity, and surface chemistry of nanoparticles on their therapeutic efficacy, slight variations in reproducibility can have tremendous effects.

With a wide understanding of the current challenges of nanoparticle production, administration, and pharmacokinetics, along with an appreciation for the complexity of neurological disease, it is important that there is careful consideration of the initial “form follows function” nanoparticle design. With little current focus on designing nanotherapies to suit specific patient populations, this advocates for designing nanoparticle characteristics to suit a need, rather than retrofitting therapies or nanotherapies to disease(Fig. 1) [5]. Many challenges facing pre-existing nanotherapies can be remedied; for example, ensuring robust linkages between the carrier and the drug simplifies the production process and reduces toxicity and variability through ensuring the

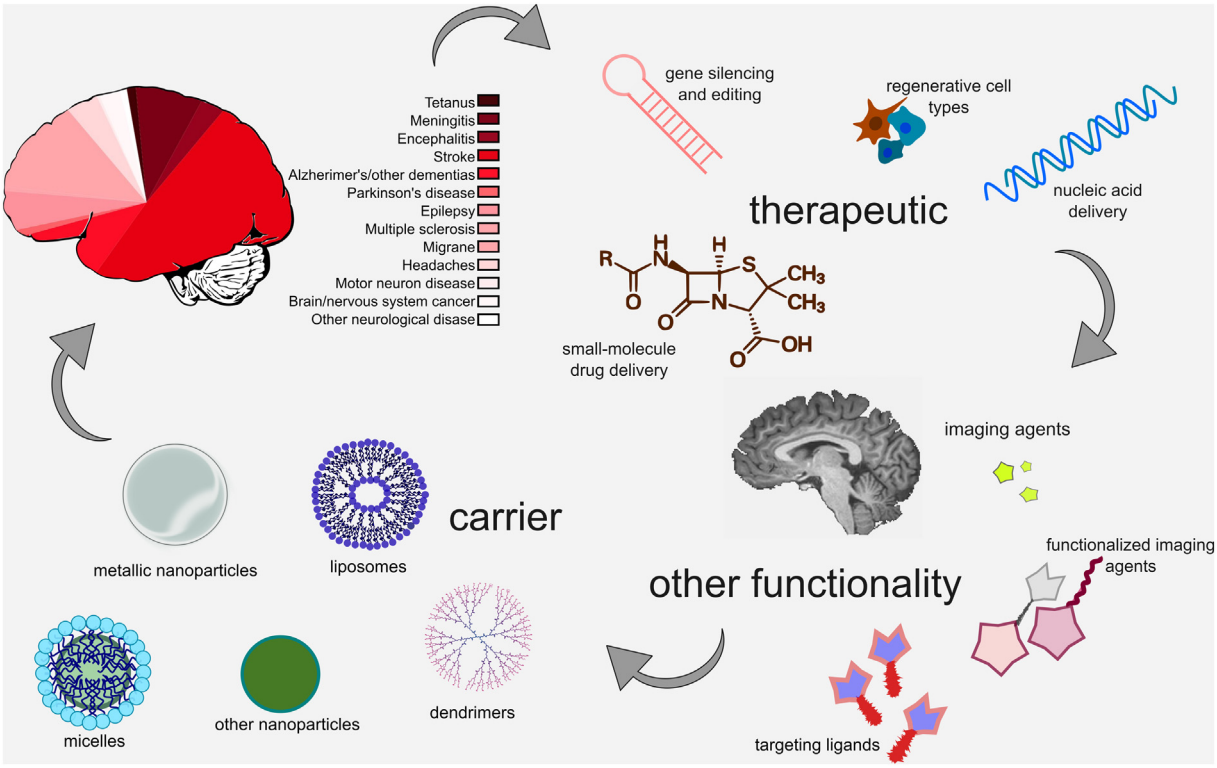

Fig. 1. Targeting CNS disorders is especially difficult due to the diverse and complex nature of these diseases. Advanced regenerative therapies will need to be tailored to the specific pathology, include targeting or enhancing mechanisms, and be precisely delivered to the region of interest by an appropriate carrier system. Despite successes at each stage of development, sustained targeting, immunological responses, as well as technical, manufacturing, and regulatory challenges exist as barriers to translation.

nanodrug is one entity, rather than fragments each subject to independent accumulation, degradation, and toxicity. Employing efficient and robust chemical transformations such as click chemistry can simplify production while increasing specificity of chemical reactions and reducing biproducts and reaction requirements [74]. Consideration of these factors and employment of these methods will greatly reduce the time and cost of production and better facilitate the path to translation. Finally, production is not the only barrier to success in translation, as a considerable increase in transparency and communication with funding agencies is critical to improve pharmaceutical industry collaboration. Academics can focus on building incentive, expertise, and the fundability of nanotherapeutics. This, in turn, appeals to pharma partners and creates shared goals that can help expedite the transition to clinical testing [48].

7.2. Nanoparticle-regenerative therapy challenges

The previously discussed challenges facing each regenerative medicine and nanotherapies stand for their combined functional use. For example, the therapeutic output of polymeric nanoparticles, such as dendrimers, depends exclusively on size and surface chemistry and merging such nanoparticles with regenerative therapies may alter characteristics of the compound, thus, standardization and quality control are paramount [75]. Furthermore, with the understanding that production issues (time, cost, and scalability) remains one of the largest barriers to translation, scalability and improved techniques to reduce production time and complexity are already becoming a focus of the field in order to facilitate translation [75–77]. With these improvements in mind, nanoparticles, specifically on the dendrimer platform, have been studied extensively across several pre-clinical models for brain targeting and have entered Phase 1 clinical trials for attenuating cerebral inflammation in neurodegenerative disease (clinicaltrials.gov Identifier NCT03500627). Again, as constructs grow in complexity, the methods for characterization, standardization, and regulation grow proportionately complex, as does the need to minimize variation.

Appropriate administration of these compounds require consideration prior to their formulation, taking in to account the target region of the CNS, the stability of the delivery vehicle and cargo, and patient comfort and compliance. Several routes of nanoparticle administration have been FDA approved, with even more studies focused on optimizing these delivery routes for specific nanocarriers [40,78–81]. Ideally, non-invasive methods would provide optimal brain targeting and accessibility at appropriate doses for one-time or repeated dosing with the understanding that the route of administration may direct certain aspects of compound formulation. Clinical trials currently underway involving nanotherapies employ intravenous (clinicaltrials.gov Identifier NCT03500627), sub-cutaneous (clinicaltrials.gov, Identifier NCT02579473), as well as intranasal (clinicaltrials.gov Identifier NCT03806478) administration for CNS targeting.

With the advent of new nanoparticle technologies and enhanced nanoparticle utility, the need for new and updated strategies for determining treatment safety and efficacy will further develop. In order to achieve the greatest potential for success, clinical trial inclusion and exclusion criteria will need to be refined to reduce variability, and ensure a clean result that maximizes benefits and quality of life for the patients [21,82]. Furthermore, endpoints will need to be clearly defined in terms of both safety and efficacy and considered alongside intermediate markers of efficacy [21,83,84]. These methods for safety and toxicity will further need to be regulated, standardized, and specified to the nanomaterial [84]. Although much more difficult to ascertain, autopsy studies will be necessary for many regenerative medicine trials, as long term differentiation and integration of cells will be paramount to determine overall efficacy [83]. Importantly, control subjects, with careful inclusion and definition of control guidelines are necessary, again with consideration of risk-benefit-ratios, quality of life, and intermediate and endpoint data [82,83]. Randomly assigning study participants to control groups prevents biased results and ensures study participants are exposed to equivalent experimental interventions. Because control and or sham experiences can require significant procedures, cost-benefit ratios and ethical factors will need to be considered [83].

8. Conclusions and outlook

As with many neurodegenerative diseases, including MS, AD, and PD, it is often thought that the therapeutic window is short and that even therapies targeted at replacing damaged tissue can be too little too late. Preclinical attempts using regenerative therapies have shown success in these diseases to prevent the worsening of symptoms and even brain injections of human umbilical cord blood-derived mesenchymal stem cells showed no adverse effects in patients with mild AD [85]. These therapies, however, still lack in their ability to integrate in areas of significant disease and to provide complete and sustained efficacy for many of the reasons discussed within this review. Nanotechnology offers strategies to circumvent these hurdles by targeting, prolonging, and enhancing therapies, to perhaps one day eliminate the idea of a limited window for therapy.

Nanotechnologies for the advancement of regenerative therapies will propel the need for redefined guidelines of therapeutic efficacy, safety, and regulation, along with updated infrastructure for the large-scale manufacturing of these products. Despite the complex nature of these carriers, standardized manufacturing of certain nanoparticles can be accomplished in a regulated fashion, facilitating the translation of these compounds to wide spread use. Large scale manufacturing may be limited by infrastructure and cost; however, the economy of drug delivery using these platforms is favorable in the long-run compared to current practice. Conquering such challenges will allow for the full spectrum of nanoparticle enhanced regenerative therapies and these therapies hold significant promise for the replacement or enrichment of host tissue through the ability to modulate, deliver, and control stem cell integration, differentiation, and survival. Furthermore, these particles can serve as complex vectors for the targeted delivery of genes and other biomaterials offering remarkable therapeutic flexibility. As administration of nanotherapies stands to be relatively non-invasive, these delivery systems would provide support as well as graded, long-term release with little patient discomfort making them particularly well-suited for the treatment of neurodegenerative diseases.

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