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Neurodegenerative Disease: A Perspective On Cell-Based Therapy In The New Era Of Cell-Free Nano-Therapy

Su M Metcalfe*,

Cambridge University Hospitals Trust, Addenbrooke's Hospital, Cambridge UK a/so LIFNanoRx Ltd, Cambridge UK

Sean Bickerton, and

Yale School of Engineering and Applied Science and Yale School of Medicine, 55 Prospect Street, New Haven, CT, 06511, USA

Tarek Fahmy

Yale School of Engineering and Applied Science and Yale School of Medicine, 55 Prospect Street, New Haven, CT, 06511, USA

Abstract

Neurodegenerative diseases (NDD) result in irreversible loss of neurons. Dementia develops when disease-induced neuronal loss becomes sufficient to impair both memory and cognitive functioning and, globally, dementia is increasing to epidemic proportions as populations age. In the current era of regenerative medicine intense activity is asking, can loss of endogenous neurons be compensated by replacement with exogenously derived cells that have either direct, or indirect, neurogenic capacity? But, more recently, excitement is growing around an emerging alternative to the cell-based approach - here nanotechnology for targeted delivery of growth factor aims to support and expand resident central nervous system (CNS) stem cells for endogenous repair. The concept of a high volume, off-the-shelf nano-therapeutic able to rejuvenate the endogenous neuroglia of the CNS is highly attractive, providing a simple solution to the complex challenges posed by cell-based regenerative medicine. The role of inflammation as an underlying driver of NDD is also considered where anti-inflammatory approaches are candidates for therapy. Indeed, cell-based therapy and/or nanotherapy may protect against inflammation to support both immune quiescence and neuronal survival in the CNS - key targets for treating NDD with the potential to reduce or even stop the cascading pathogenesis and disease progression, possibly promoting some repair where disease is treated early. By design, nanoparticles can be formulated to cross the blood brain barrier (BBB) enabling sustained delivery of neuro-protective agents for sufficient duration to reset neuro-immune homeostasis. Proven safe and efficacious, it is now urgent to deliver nano-medicine (NanoMed) as a scalable approach to treat NDD, where key stakeholders are the patients and the global economy.

*Corresponding Author: smm1001@cam.ac.uk, Phone : 44 - 1223 570716.

CONFLICT OF INTEREST

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1. INTRODUCTION

Neurodegenerative disease (NDD) has become the greatest unmet clinical need of our time. The immense value of cell-based research in the field of NDD has opened up new visions for treatment [1–7]. Moreover, the recent discoveries that transplanted neural stem cells can be replaced by growth factor alone in disease models [8, 9] has raised the question, can cell-free therapy provide a successful surrogate to cell-based therapy? [10] Cell-based research is also probing disease-specific molecular pathogenesis in man using the disease-in-a-dish iPSC approach where patient-derived fibroblasts are engineered to become neurons - an approach empowered by knowledge of genetic links to the disease. Advances in having identified such genetic links also underpin clinical trials aimed at detecting disease before it becomes clinically manifest, thus identifying biomarkers for early diagnosis and treatment. [11] The sooner the treatment, the better the outcome - a well recognised fact for all diseases but especially relevant to NDD where capacity to repair is finite: once exceeded, efficacy of therapy is reduced.

Predictions of how to address the NDD crisis have become theoretically possible based on such cell-based studies. But for treatment of patients, the overwhelming numbers argue against cell therapy on logistical grounds: the massive scale-up to provide sufficient cells using suitable clinical grade facilities will be too expensive and complex: in practice cell-based therapy will fail to meet the global need. The option of synthetic biology able to provide a cell-free bulk production of biodegradable nanoparticles loaded with neurotrophic factors provides a practical solution. Co-development of future strategies able to reset and maintain neuro-immune homeostasis will be conducive to neuroprotection and brain health, shifting the population demographics away from increasing dementia.

It is urgent to push boundaries when tackling the escalating incidence of global neurodegenerative disease. Realisation of three core needs may turn the tide:

- Biomarkers for early diagnosis
- Targeted Neurotrophic Factors for early treatment, available in bulk and of universal applicability at low cost, to meet the scale of need
- Therapy-induced recovery of neuro-immune homeostasis and immune quiescence within the CNS

Each of these needs is advancing towards being met today, and their coordination will converge towards a practical global solution to the problem of dementia-linked neurodegenerative disease.

2. BACKGROUND

The endogenous reparative capacity of the adult human brain is low and chronic neurodegenerative disorders of the central nervous system represent one of the greatest areas of unmet clinical need in the world. [12] In the US, as the older adult population increases, those with dementia will also increase with AD alone predicted to increase by 50% by 2030 - afflicting 7.7 million sufferers. In the UK treatment and care of people with AD costs the nation £17 billion annually: although treatments such as cholinesterase blockers that amplify signals between surviving nerves reduce symptoms initially, nothing stops progression. Other NDD diseases include Frontotemporal Dementia (FTD) - the third most common of the cortical dementias - with frontal and/or temporal cortical atrophy often accompanied by personality change. After Alzheimer's Disease, Parkinson's Disease (PD) is the second commonest neurodegenerative disorder of the CNS where the disease process critically involves the nigrostriatal dopaminergic (DA) neurons resulting in their loss with decreased dopamine levels in the dorsal striatum. NDD is not limited to the aging population: multiple sclerosis (MS), caused by autoimmune inflammatory attack against myelin in the CNS, starts around 30 years of age with 2.5 million sufferers worldwide. Following diagnosis of MS the average life expectancy is 63.5 years, and the lifetime economic impact per patient is US\$1.2m whilst the annual global economic impact is US\$94 billion. Figure 1 shows the increasing incidence of dementia and the global costs of Alzheimer's Disease based on analyses published by The World Alzheimer Report 2015 (www.alz.co.uk/worldreport2015). The profound economic and societal costs of NDD are predicted to escalate dramatically in the absence of new therapies able to reduce or stop the disease process. But for most NDD types the cause is unknown other than links to aging and the development of an underlying chronic inflammatory state sometimes referred to as "inflammaging". [13–18] Specific questions arise:

- Can cell-based therapy help?

Although experimental evidence together with some clinical successes suggest yes, the next question is,

- Will cell-based therapy be able to meet global need?

Putting this into perspective, for clinical translation of stem cell-based therapies, commercial support will be essential, raising a further question,

- Who will own and sell the cell-based product?

Indeed, this latter is a fraught question applicable in general to the commercial stem cell arena where there remains a key unmet need for a high-volume, off-the-shelf, frozen cell therapy that can be used by many different patients.

Urgent consideration of a NanoMed solution is warranted, since NanoMed may both reduce disease pathology and also suppress neuro-inflammation so protecting the CNS from the insults that trigger pathogenesis. Industry's needs are also met, being defined in composition and utilising materials with a long history of safe use in the clinic, nanomedicinals provide a cell-free solution at low cost, universal applicability, and scalable in production [19,20] where design to treat NDD is a realistic way forward.

3. CELL-BASED THERAPY FOR NEURODEGENERATIVE DISEASES

I first consider current approaches to treatment of NDD using cell-based therapies where objectives typically center on either cellular replacement, or providing environmental enrichment, and has been the subject of several recent reviews recommended to the reader [2–6] and in particular Lunn et al [2]. In addition to these, the EuroStemCell website is a valuable source of information: <http://www.eurostemcell.org/stem-cell-factsheets>

3.1. Cell Replacement

Cellular replacement involves the derivation of specific neuronal subtypes lost in disease. These are then grafted into affected areas of the nervous system, as for example use of dopaminergic cells (DA) to treat Parkinson's disease. Although in principle simple, in practice complex as exemplified by DA cell grafting. Human dopaminergic neurons are especially vulnerable to stress due to the high energy demand required to meet their massive unmyelinated axonal arbor that involves more than one million synapses per cell: the calculated total axonal length of a single DA neuron exceeds 4m. [21] Thus, for the *transplanted* DA cell, the level of cell integration required to replace lost endogenous dopaminergic activity is profound: the situation is further compounded by the DA cell's inherent vulnerability to stress once integrated into the host where the grafted DA neurons need to migrate within the host tissue and their axons must extend to reach their targets. That dopaminergic precursor cell transplants *have* been successful in treating Parkinson's disease is remarkable testament to the potential of the brain to respond to, and accommodate, cell-based therapy.

3.2. Environmental Enrichment

Alternatively to lineage-specific neuronal functions requiring grafts of specific cell types, cell-based therapy may act non-specifically to provide environmental enrichment to support host neurons at several levels including (i) by producing neurotrophic factors; (ii) by scavenging toxic factors; (iii) by creating auxiliary neural networks. Many strategies for environmental enrichment utilize stem cells to provide de novo synthesis and delivery of neuroprotective growth factors at the site of disease. Growth factors such as glial-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), insulin-like growth factor-I (IGF-I) and vascular endothelial growth factor (VEGF) are protective in neurodegenerative disease models and provide in situ support at the main foci of disease. The actual graft site also needs to be considered for each neurodegenerative disease, based on the specific neuronal pathology of each disorder. For example, both spinal muscular atrophy and amyotrophic lateral sclerosis - also known as motor neuron disease due to selective death of motor neurons - are most likely to benefit from cellular therapies that enrich the local spinal cord environment and thus support surviving motor neurons. This contrasts to Alzheimer's disease and Huntington's disease caused by widespread loss of neurons in the brain. Nerve cell death is a primary event in the neurodegenerative diseases with the exception of multiple sclerosis where it is secondary to autoimmune attack within the glia of the CNS causing demyelination of nerve axons with loss of electrical insulation required for efficient nerve conductivity. Denuded axons, although initially able to be

repaired, eventually succumb and die due to loss of nutrient support normally derived from their myelin sheath.

In summary, cell-based therapy for NDD can be identified as having two aims:

- to replace lost and diseased neurons with healthy neurons. Replacement may require acquisition of the specific neuronal subtypes lost in the disease and their subsequent grafting to the effected area of the brain: thereafter the newly transplanted neurons - for example neural precursor cells (NPC) or stem cells of the DA lineage - need to integrate within the host neuroglia, form synapses, and recapitulate the original healthy neuronal network.
- to provide environmental enrichment. Here growth factors released by the grafted cells - for example mesenchymal stem cells (MSC) - support surviving neurons and delay disease progression.

The above considerations are illustrated further below for Parkinson's disease, Alzheimer's disease, and Multiple Sclerosis.

3.3. Parkinson's Disease

PD affects around 1 in 800 people worldwide. Characterised by movement disorder due to progressive loss of nigral dopaminergic neurons, PD can be partially corrected by dopaminergic drugs, but overtime efficacy fails and side effects develop including dyskinesia and for some PD progresses to dementia. The disease process critically involves the nigrostriatal DA neurons resulting in their loss with decreased dopamine levels in the dorsal striatum. Here cell replacement therapy requires acquisition of the specific neuronal subtypes lost in the disease. As previously outlined, following DA precursor cell grafting to the effected area of the brain, the newly transplanted neurons need to integrate within the host neuroglia, form synapses, and recapitulate the original healthy neuronal network. The concept of replacing lost DA neurons with healthy cells of the same lineage underpins one major clinical approach that uses allografts of human fetal ventral mesencephalic (hfVM) tissue. Although inconsistent, this strategy has shown significant long-term benefits in some patients with PD and cell-based therapy for PD is the subject the current clinical TRANSEURO trial [22] to define a reproducible successful protocol using hfVM cells.

In the longer term, a stem cell approach to replace need for hfVM cells will be required to provide sufficient numbers of DA precursor cells. To this end embryonic stem cells (ESC) and possibly also mesenchymal stem cells (MSC) offer sources for large-scale production of neurons which can be manipulated to acquire a midbrain DA phenotype. Grafting both ES- and MSC-derived DA neurons into rat PD models results in functional recovery. The ability to produce patient-specific DA neurons has recently been demonstrated using induced pluripotent cells (iPS). Transplantation of these cells into a rodent PD model improved functional deficits and demonstrated cell integration in the host tissue as reviewed in [2] demonstrating a therapeutic use for iPS cells in a neurodegenerative disease. Two main criteria for ESC or iPSC based therapy are safety and identity where safety questions include what is the cell's proliferation potential? How stable is its phenotype? Is microbial sterility ensured? A key efficacy question is, are the cells fully committed to the dopaminergic

lineage? Even where safety and identity are confirmed, the transplanted cells in the striatum will need to have the capacity to integrate into and innervate the striatal tissue of the patient. [6]

Human ESC-derived DA cells: The recent successful preparation of highly efficient dopamine-producing neurons from human embryonic stem cells is a major breakthrough. After transplantation of these hESC-derived DA cells, including into non-human primates, safety and efficacy of the cells has been demonstrated. For use in PD patients the challenges now are to produce sufficient DA cells from hESC to be effective in the much larger human brain whilst under clinical GMP conditions. Once regulatory compliance is achieved, clinical trials are anticipated in 2018. [1]

Taking a different tack, a further breakthrough achieved in parallel with the TRANSEURO trial came from use of a surrogate stroma for the hfVM cell grafts [23]: this showed increased survival and integration of human DA precursor cells in the striatum of the nude rat as a model to measure human graft survival in the relevant environment of a recipient brain. The surrogate nano-stroma was created ex vivo by attaching biodegradable nanoparticles preloaded with leukaemia inhibitory factor (LIF), or alternative growth factors, to the donor hfVM cells prior to grafting. For cell attachment the nanoparticles were functionalised on their surface to target the Thy-1 antigen expressed on hfVM cells and this work is further described in the NanoMed section.

Environmental Enrichment for PD Although environmental enrichment is relatively non-specific compared to the need for DA lineage cells in PD, in experimental models, significant benefits are gained by exogenous growth factor delivery, or de novo synthesis of growth factor, at the site of disease where the factors include BDNF, GDNF, VEGF, IGF-1, and LIF. Prolonged growth factor delivery either direct, via virus, or via genetically engineered NPC or MSC grafts producing growth factors such as BDNF, VEGF, GDNF, and/or IGF-1 in situ, protects against neuronal decay in PD - a promising approach to achieve both cellular replacement benefits plus environmental enrichment.

3.4. Alzheimer's Disease

Memory loss and cognitive decline characterise AD with widespread loss of neurons and synaptic connectivity throughout the cortex, hippocampus, amygdala, and basal forebrain. Current treatment options for AD are centered on regulating neurotransmitter activity. Enhancing cholinergic function improves AD behavioral and cognitive defects [1] and targeting the cholinergic system using stem cell therapies may enhance neurogenesis or replace lost neurons and so delay the progression of AD. Enhancing BDNF levels, to meet the increasing loss of BDNF that occurs with age and more so in AD, promotes neurogenesis and protects neuronal function and rodent AD models receiving NPC grafts demonstrate increased hippocampal synaptic density and increased cognitive function associated with local production of BDNF. Similarly, BDNF upregulation along with neural precursor cell (NPC) transplants also improves cell engraftment and functional outcomes in an AD rat model. Nerve growth factor (NGF) production is another mechanism of cellular therapy efficacy.

A notable consideration in use cell-based therapy for AD is the need to accommodate the complexity of the disease, where, once AD has set in, the chances of therapeutic neural networks succeeding will decrease over time since the healthy grafted cells will themselves become diseased due to spread of the pre-existing pathology. The pathologic hallmarks include extracellular amyloid β ($A\beta$) plaques and intracellular neurofibrillary tangles made up of hyper-phosphorylated tau - a microtubule protein normally involved in enabling intracellular transport along the length of nerve axons. No replacement cell therapy for AD has been successful in the longer term, where grafted cells (i) need to locate to where damage has occurred; (ii) need to produce the many different types of neurons required to replace the damaged or lost cell populations; and (iii) need to integrate effectively into the brain. However recent recognition that AD pathogenesis links to both BBB function and circulation of the cerebral spinal fluid (CSF) - that normally removes $A\beta$ and toxins from the CNS - provide potential new targets aimed at early stage pathologies [24, 25]

As indicated above, the BBB itself represents a therapeutic target [24–26]. The recent excellent review of Banks [24] has integrated a wealth of data that reveals the BBB as much more than a barrier. The BBB is in fact a complex and dynamic interface that adapts to the needs of the CNS, responding to physiological changes, and in turn being vulnerable to disease. For example, the pathogenesis of AD is discussed in the context of disturbed BBB function where reduced bulk flow of CSF in aging is linked to $A\beta$ accumulation in the CSF together with albumin and neurotoxic substances as production of CSF decreases. The reduced production and bulk flow of CSF in both aging and AD is likely to be triggered by neuro-inflammation - again emphasising the central role of neuro-inflammation in NDD. The recent demonstration that pharmaceutical targeting of CFS1R is able to reduce AD-type pathology by inhibiting microglial proliferation [25] further highlights the BBB as a target for treatment in NDD.

3.5. Multiple sclerosis

Multiple sclerosis (MS) is an incurable autoimmune demyelinating disease of the CNS. It afflicts more than 2.5 million people worldwide with an estimated 700,000 people in Europe with MS. It is the main cause of non-traumatic disability in young adults, being mainly diagnosed between the ages of 20 and 40 with 70% being diagnosed during prime working years. For the patient, with onset around 30 years of age and lasting up to 45 years, the quality-of-life burden is profound. The suicide rate in MS is upto 14-fold higher compared to average and euthanasia is increasing. Relapsing or progressive MS is cruel, variably affecting different areas of the CNS during each relapse whilst also accumulating irreversible nerve damage involving motor, autonomic, cognitive, and sensory function. Symptoms reflect the involved CNS areas and include pain and fatigue. The underlying disease process is auto-immune-driven inflammatory demyelination of CNS nerve axons. Demyelination not only reduces conduction efficiency along nerve axons, but also - *and critically* - metabolic support of that axon, eventually leading to death of the nerve and irreversible neurodegeneration. Current MS treatments are immunosuppressive and may modify disease in terms of reducing relapses but fail to halt disease progression; most are cost ineffective; and most cause side-effects or have potential high risk side effects which

can be severe, so that an estimated 50% of patients receive no treatment at all due in part to non-compliance or concern about potential dangerous side effects.

Current cell-based therapies for MS include autologous bone marrow grafts harvested prior to myelo-ablative drug therapy. The bone marrow graft aims to reconstitute the immune system together with self-tolerance to myelin. This procedure has risks with 1–2% mortality and approved clinical trials are restricted to those who have failed alternative therapies. MESCAMS is a new Canadian clinical trial using autologous MSC to treat MS patients suffering from progressive MS: here the anti-inflammatory, neurogenic properties of MSC are anticipated to be neuro-protective for MS patients and the MESCAMS trial is linked to an international effort to reach safe protocols exploiting MSC for MS. Of note only 5% of MS patients are currently eligible for the MESCAMS trial where the risk remains high and the cost is upto \$60,000 per treatment excluding treatment of any complications. [27]

4. NANOTHERAPY: CELL-FREE DELIVERY OF NEURO-REPARATIVE FACTORS

Nanomedicine (NanoMed) is a new era in therapeutics enabled by fundamental advances in biology combined with progress in biocompatible nanotechnology [19,20, 28–34]. As a synthetic biology approach, nanotechnology permits design of therapeutic devices with defined structure and molecular composition. Modular design options employing surface-bound ligands provide highly specific homing devices for loaded cargo; biocompatible and biodegradable constructs provide surrogate temporary micro-environments. As an emerging alternative to cell-based therapy to treat NDD, nanomedicinals meet the need for a high volume, off-the-shelf therapeutic able to rejuvenate the endogenous neuroglia of the CNS and suppress neuro-inflammation. The NanoMed approach is the only route that provides targeted delivery of therapeutic cargo in paracrine manner, providing high therapeutic index and high potency for the desired effect.

Issues of safety and biocompatibility must be considered. In general terms, the technology currently favoured for NanoMed encapsulates the medicinal cargo into a matrix of nanospheres or microspheres, which slowly degrade in situ and release cargo into circulation in a paracrine manner to mimic physiological low level cargo dose that is sustained and can be adjusted over days. The structural matrix of the microsphere is composed of a medical-grade biodegradable polymer called poly-(D,L-lactide-co-glycolide) (PLGA). These polymers have been used in surgical sutures, bone plates, and orthopedic implants for decades, and in microsphere form as a long-acting drug delivery system since 1984. Degradation of the PLGA polymer occurs by natural (i.e., non-catalyzed) hydrolysis of the ester linkages into lactic acid and glycolic acid, which are naturally occurring substances that are easily eliminated as carbon dioxide and water. NanoMed is currently approved by the FDA for phase II clinical trials after phase I safety trials to target delivery of drugs to treat cancer: see BIND Therapeutics (<http://www.businesswire.com/news/home/20160107005225/en/BIND-Therapeutics-Presents-Complete-Data-Clinical-Activity>). These clinical trials employ the proprietary technology in PLGA-based NanoMed developed by Yale University's school of Engineering and product lock-down plus bulk production with

defined physico-chemical characteristics that are established for clinically-compliant nanomedicinals. The ability to target delivery of cargo is a key advance with profound impact on the therapeutic index and therapeutic safety.

4.1. NanoMed Technology

There are a number of recent reviews concerning technology behind nanotherapy for clinical use and this is not to be further covered in this review. Mitragotri et al [19] covers formulation and delivery strategies for biopharmaceuticals; Patel et al [31] use of polymeric nanocarriers for drug delivery to the CNS; Danhier et al [29] on biomedical applications of PLGA nanoparticles; Tosi et al [28] on drug delivery across the BBB; and Metcalfe and Fahmy [20] on nanotherapy for induction of therapeutic immune responses.

It has to be emphasised that delivery of biologically active cytokines and growth factors using biocompatible nanocarriers is a significant challenge. Cargo-specific formulations are required to meet physico-chemical constraints with adjustments for pH for example to maintain desired properties of the polymeric carrier whilst also preserving biological activity of the cargo. Quality controls include cargo release rate, consistent particle size within a tight size range, cargo load, and successful incorporation of any linker or coupling agent (eg avidin) required for attachment of the nanoparticle to its targeting moiety. Any change in one component will affect the behaviour of the rest. The Fahmy Laboratory at Yale University have not only pioneered successful delivery of biologics to treat disease using sophisticated single and dual-purpose particles able to manipulate endogenous control mechanisms - for example to treat Lupus [30] - but have also prepared reporter constructs able to deliver cargo and simultaneously report on responses of the target to the cargo - creating in effect intelligent nano-robots. Figure 2 shows a diagram of a biodegradable nanoparticle developed by the Fahmy laboratory.

Development of nanocarriers for targeted therapy is a skilled art: thereafter scale up to meet clinical need, ensuring full compliance with Regulatory Authorities and meeting FDA requirements, is a major undertaking. Commercial development is essential if nanotherapy is to become realised as a treatment for NDD: although great, the risk is far less than cell-based therapies on many fronts not least that, once locked down with successful production, the nano-therapeutic is a defined product suitable to global-scale manufacture over many years of use. Cell therapy being by definition living and thus continuously changing will never achieve product lock-down.

Crossing the BBB is of special relevance to treatment of NDD by nanotherapy. Here Tosi and Cattaneo [35] recently demonstrated beyond all doubt that PLGA nanoparticles are able to deliver therapeutic cargo across the BBB, showing that not only do the particles cross the BBB, but the delivered cargo within the CNS improved synaptic activity in the stringent R6/2 mouse model of HD. Critically, this therapeutic success followed intra-*peritoneal* delivery of cholesterol-loaded PLGA nanoparticles - whilst the confirmed benefit was within the CNS. PLGA-based nanoparticles are recognised as the best prospect for systemic delivery through the BBB without forcibly opening or damaging it: some 12% PLGANanoparticles delivered intravenously cross the BBB [29]. Means to enhance BBB transport have been probed Tosi [28, 36–38], comparing different delivery routes -

intravenous, intraperitoneal, intranasal, and per oral. The outcome showed that uptake across the BBB using all routes where the amount of nanoparticle uptake was proportional to the uptake that entered the circulation.

4.2. Development of the LIF-Nano therapeutic to treat MS

LIF plays a fundamental role in the CNS, regulating homeostasis, myelination, and neurogenesis in addition to being neuroprotective. [39–47] Following the discovery that LIF is tolerogenic and functions as the polar opposite to IL-6 for control of lymphocyte lineage maturation, [48–50] LIFNano was invented as a therapeutic agent to oppose inflammatory immunity. [49, 50] LIFNano is being developed to treat MS, given the compound properties of not only switching off autoimmune attack but also promoting myelin repair and being a neuroprotective cytokine [51, 52]. LIFNano is now being produced for a first-in-man trial to treat early stage MS. It has already been shown that an imbalance in the LIF/IL-6 occurs in clinical MS [53], adding to the evidence that therapeutic LIF may reset the balance for endogenous recovery of homeostasis.

LIFNano provides an example of how NanoMedicine allows neurogenic reparative properties of growth factors to be harnessed, tapping into endogenous repair pathways. The future implications for treatment of NDD are profound, especially if a single, or few, nanotherapeutic devices are able to treat a wide variety of NDD.

4.3. Nanoparticles as Surrogate Stroma for Cell-Based Therapy

Cell based therapy today does have a role for treatment of NDD especially where there is a need to produce lineage-specific cell replacement therapy such as DA cells for Parkinson's Disease. Here benefit from nanotherapy can be gained with support individual cells - on a cell-by-cell basis - provided by attaching growth factor-loaded nanoparticles directly to the cell prior to grafting: this creates surrogate stromal support for each cell over the first few weeks post grafting. Successful outcome has been confirmed during preclinical development of the TransEuro Trial for PD, with nanostroma providing some fourfold increase in vivo survival of hfVM cells assessed in vivo at three months following transplantation into the striatum of nude rats. The human stem cells differentiated in situ to DA following grafting, showing that the surrogate nanostroma supported natural lineage progression in vivo.

Preclinical studies [54] plus TransEuro preclinical data [22] testing therapeutic value of the surrogate nanoparticle stroma showed

- Safety - no adverse effects have been observed including after direct injection of stroma/cell constructs into the brain
- Efficacy - the delivered stromal cargo induced cargo-specific responses
- When encapsulated within a stroma at the single cells level, cell survival is increased:
 - hfVM cell survival *in vivo* was increased four-fold
 - increased survival of both mature DA cells, and of DA-precursor cells
 - hfVM supported by LIF-Nano differentiate in vivo to mature DA cells

- The Neuro-Glial Ratio was unaltered
- The ratio of DA to 5HT (serotonergic) cells remains constant - obviating the risk of graft-induced dyskinesia
- Extensive neurite outgrowth from the grafted DA cells
- High potency and specificity of cargo delivered by targeted delivery where both targeting and cargo were essential for therapeutic benefit.

5. INFLAMMATION AND AGING LINKS THE BRAIN TO THE PERIPHERY

The role of inflammation as an underlying driver of NDD is a core consideration since and-inflammatory approaches are candidates for therapy. Indeed, cell based therapy and/or nanotherapy may protect against inflammation to support both immune quiescence and neuronal survival in the CNS - key targets for treating NDD with the potential to reduce or even stop the cascading pathogenesis and disease progression, possibly promoting some repair where disease is treated early.

Aging itself is a fixed, chronologically-driven process. However, the physiological events linked to aging are not fixed. These may be influenced to reduce progression of adverse consequences. The excellent recent review of Ostan et al. [56] discusses how, during aging, the inflammatory response becomes chronic and low grade, leading to tissue degeneration. Inflammaging is characterized by a general increase in plasma levels of pro-inflammatory cytokines such as Interleukin-6 (IL-6), Interleukin-1 (IL-1), and Tumour Necrosis Factor- α): thereafter increases in the main in-inflammatory markers include C-reactive protein (CRP) and serum amyloid A (A-SAA). This generalized pro-inflammatory status, interacting with the genetic background and environmental factors, potentially triggers the onset of the most important age-related diseases, such as cardiovascular diseases, atherosclerosis, metabolic syndrome, type 2 diabetes, obesity, neurodegeneration, arthrosis and arthritis, osteoporosis and osteoarthritis, sarcopenia, major depression, frailty and cancer. Thus ability to oppose chronic in-inflammation is a key target for therapy and especially for NDD given the finite ability of repair within the CNS.

Inflammation is discussed further below and includes recent experimental proof of concept from the authors. Pioneered by Fahmy, amongst the first to develop NanoMed to treat immune-based diseases, and Metcalfe together with Fahmy to treat NDD, the work has given rise to spin out companies (eg Toleragen INC and LIFNanoRx Ltd) with the aim to bring such advances in NanoMed to the benefit of patients. For NDD, leukaemia inhibitory factor (LIF) in nano-formulation is selected for LIF's combined neuroprotective and anti-inflammatory properties. Indeed, LIF itself is a stem cells factor with "stemness" properties and is required to expand stem/precursor cell populations for cell-based therapies. The option of use of a surrogate "stem cell"- enabled by synthetic biology as nano-formulated LIF - would avoid need and costs for scale up of therapeutic cells. In the context of LIF, we also high-light that certain cell-based therapies can increase inflammaging.

Inflammation is linked to molecular pathogenesis in the CNS with involvement of the BBB as reviewed by Banks [24]. Several other recent discussions concern inflammation more

generally [13–18], and here I highlight that of Gage and colleagues [18] who provide a unifying mechanistic sequence applicable across the different neuro-inflammatory diseases: (i) inducers (ii) sensors (iii) transducers and (iv) effectors. Each progressively contributes to neuronal dysfunction and death. Although inducers of inflammation may be generated in a disease specific manner, there is evidence for a remarkable convergence in the mechanisms responsible for thereafter sensing, transducing, and amplifying the inflammatory processes that results in the production of neurotoxic mediators that in turn set the vicious cycle of progression and aging. The study concludes with the question, can pharmacological inhibition of inflammation pathways safely reverse or slow the course of disease? Rather than drugs, an alternative solution might be proposed, namely biological inhibition of inflammation where targeted delivery of neurogenic growth factor to the CNS is safe whilst promoting (in the case of LIF) an endogenously controlled anti-inflammatory microenvironment that is also neurogenic.

A second highlight publication plus accompanying N&V article provides evidence of how inflammation in the periphery is sensed by the brain during aging [13, 14]. Inflammaging describes the close relationship between low grade chronic inflammation and aging and leads to the questions, does the brain itself orchestrate systemic aging across all organ systems? If so, how? The finger points to the hypothalamus and the transcription factor NF- κ B where the level of NF- κ B is highest compared to the rest of the brain during aging. Increased levels of NF- κ B set up a pro-inflammatory feed forward loop in the hypothalamus, giving rise to a new homeostatic state biased toward inflammation and repression of gonadotrophin-releasing hormone (GnRH). Reduced GnRH in turn results in multiple systemic attributes of aging.

The brain's link to the aging process brings to the fore how immune and endocrine responses are integrated in the hypothalamus to drive accelerated aging. NF- κ B signalling in the hypothalamus, and subsequent decline in GnRH production, could be activated locally in response to increased inflammatory cytokines produced by microglia within the hypothalamus. Alternatively - and remarkably - the hypothalamus may respond to circulating systemic signals in the blood since dendrites of GnRH-producing neurons extend through the blood-brain-barrier and so are positioned to sense both pro-inflammatory and metabolic signals in the blood, conjuring up an image of the brain constantly "sampling" the blood and setting homeostasis accordingly. If the homeostatic balance becomes chronically inflammatory albeit low level, then epigenetic lockdown of inflammaging ensues. Early intervention supported by biomarkers to suppress pro-inflammatory signals may revert homeostasis away from the aging signature in favour of normal activity and a more healthy CNS.

5.1. Opposing Neuro-Inflammation at the level of the LIF / IL-6 Axis

LIF and IL-6 are structurally related cytokines that differ in that LIF signaling requires the LIF-specific receptor subunit "gp190". In the immune system a clearly defined regulatory axis between LIF and IL-6 has been discovered [48–50]: here IL-6 drives inflammatory immunity via TH17 cells, whilst LIF promotes tolerant immunity via Treg cells where Treg in turn release more LIF providing an autocrine tolerogenic micro-environment. The

mechanism of the LIF/IL-6 axis has also been elucidated: IL-6 activity suppresses transcription of gp190 thus reducing the cell's ability to respond to LIF. [48, 50]

An important question is, in the nervous system, is there also a regulatory axis between LIF and IL-6? If yes, this will be fundamental to our understanding of NDD. If neuro-immune crosstalk involving IL-6 perpetuates low-grade inflammation, can this IL-6-linked inflammation be opposed by LIF? If so, then shifting the homeostatic balance towards sustained LIF-linked neuro-immune interactions that are not only non-inflammatory but also provide a neurogenic prosurvival environment (via LIF) will provide a universal approach to reducing inappropriate neuro-inflammation. Here the nanotherapeutic use of LIF would provide compound therapeutic benefits, resetting endogenous homeostasis. Indeed, do Treg lymphocytes play a central role in neuro-immune homeostasis via their release of LIF ? [10]

There is a cautionary note to cell-based therapy based on the LIF/IL-6 axis. Human bone marrow-derived mesenchymal stem cells (hBM-MSC) have been found to produce high levels of IL-6 [8]. When hBM-MSC were used to treat EAE, the disease worsened, correlating with TH17 activity. In contrast to hBM-MSC, human embryonic stem cell-MSC (hES-MSC) lack IL-6 and treatment with hES-MSC improved disease state in the EAE model. Thus, caution is urged when selecting a cell source for grafting so as to avoid inadvertent inflammatory immune activation. [10] Clinical programs using autologous grafts that require hBM-MSC might benefit from including the nanostromal approach using LIFNano, both to support the graft and oppose any inflammatory potential of the graft.

In addition to physiological levels of endogenous IL-6, viral-derived IL-6 (vIL-6) can promote inflammation. For example Epstein Barr Virus (EBV) encodes an IL-6 gene and EBV-IL-6 contributes to overall IL-6 activity since it is able to directly activate the human IL-6 receptor. Moreover, EBV infects B lymphocytes where IL-6 is a B cell growth factor, underpinning the massive B cell proliferation associated with EBV infection. Since MS is associated with universal positivity for EBV, and since B cell-depleting regimens are effective in MS, an underlying commonality may be IL-6 as a therapeutic target. If so, targeting IL-6 is further warranted as a means to reset self-tolerance and the ability of LIFNano to oppose IL-6 with high potency [49] is notable.

A further molecular lesion in NDD and inflammatory immunity is oxidative damage in neurons where mitochondrial dysfunction and energy deficits play a central role in the pathogenesis. These pathogenic mechanisms represent key targets for neuroprotective therapies and LIF is again highlighted as an anti-inflammatory neuroprotective agent with the very recent finding that LIF upregulates expression of superoxide dismutase 3 (SOD3), an endogenous anti-oxidant able to reduce stroke damage in animal models [46].

CONCLUSION

The rising toll of NDD on the economy continues [55]. There are two outstanding issues: one is the *expanding scale* of the need to treat. The other is that treatment needs to be *early*, when homeostasis in the CNS might be reset. Here we bring to the fore a causal role for

neuro-inflammation as a common target for treatment across all the neurodegenerative diseases.

We argue that we have the tools today to treat NDD and that these need to be developed as a matter of urgency in an efficient and coordinated manner by bringing together the following aims:

- To identify biomarkers for early diagnosis.
- To exploit the new era of nanomed to provide a bulk, off-the-shelf, targeted therapeutic at low cost and of universal applicability.
- To treat neuro-inflammation and to recover neuro-immune homeostasis within the CNS.

Combined, these aims become permissive for inflammatory insult within the CNS to be suppressed before irreversible cascading damage sets in. Although genetic causes of disease, or disease resulting from loss of specific cell function, will remain, their severity may be reduced since these are also linked to neuroinflammation. The role for cell-based therapy will become more focussed within an overall coordinated approach to NDD.

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References

1. <http://www.eurostemcell.org>
2. Lunn JS, Sakowski SA, Hur J, Feldman EL. Stem cell technology for neurodegenerative diseases. *Ann Neurol*. 2011; 70(3):353–61. [PubMed: 21905078]
3. Lindvall O, Kokaia Z, Martinez-Serrano A. Stem cell therapy for human neurodegenerative disorders-how to make it work. *Nat Med*. 2004; 10(Suppl):S42–50. [PubMed: 15272269]
4. Taguchi A. Cell-based therapy for patients with vascular dementia. *Psychogeriatrics*. 2011; 11(2): 113–5. [PubMed: 21707859]
5. Lindvall O, Kokaia Z. Stem cells in human neurodegenerative disorders – time for clinical translation? *K Clin Invest*. 2010; 120(1):29–40.
6. Barker RA. Developing stem cell therapies for Parkinson's disease: waiting until the time is right. *Cell Stem Cell*. 2014; 15(5):539–42. [PubMed: 25517462]
7. Marchesi VT. Alzheimer's dementia begins as a disease of small blood vessels, damaged by oxidative-induced inflammation and dysregulated amyloid metabolism: implications for early detection and therapy. *FASEB J*. 2011; 25(1):5–13. [PubMed: 21205781]
8. Wang X, Kimbrel EA, Ijichi K, et al. Human ESC-derived MSCs outperform bone marrow MSCs in the treatment of an EAE model of multiple sclerosis. *Stem Cell Reports*. 2014; 3(1):115–30. [PubMed: 25068126]
9. Cao W, Yang Y, Wang Z, et al. Leukemia inhibitory factor inhibits T helper 17 cell differentiation and confers treatment effects of neural progenitor cell therapy in autoimmune disease. *Immunity*. 2011; 35(2):273–84. [PubMed: 21835648]
10. Metcalfe SM. Stem cell therapy versus T lymphocytes: friend or foe? *Stem Cells*. 2015; 33(2):622–5. [PubMed: 25303042]
11. Dominantly inherited Alzheimer Network Trial Completes Participant Enrollment for Two Drugs in a Phase II/III Study of Amyloid Therapies [Enrollment completion announcement]. Washington University in St. Louis School of Medicine; Released 2015 Dec 3. Available from: <https://>

www.alz.org/documents_custom/DIANTU_EnrollmentCompletionAnnouncement_03Dec2015_FINAL_v2.pdf

12. Prince, M., Wimo, A., Guerchet, M., Ali, G-C., Wu, Y-T., Prina, M. World Alzheimer Report 2015. London: Alzheimer's Disease International (ADI); Aug. 2015
13. Gabuzda D, Yankner BA. Physiology: Inflammation links ageing to the brain. *Nature*. 2013; 497(7448):197–8. [PubMed: 23636321]
14. Zhang G1, Li J, Purkayastha S, Tang Y, Zhang H, Yin Y, Li B, Liu G, Cai D. Hypothalamic programming of systemic ageing involving IKK- β , NF- κ B and GnRH. *Nature*. 2013; 9(7448): 211–6. 497.
15. Dufek M, Rektorova I, Thon V, Lokaj J, Rektor I. Interleukin-6 may contribute to mortality in Parkinson's disease patients: A 4-year prospective study. *Parkinsons Dis*. 2015; 2015:898192. [PubMed: 26351617]
16. Golde TE, Petrucelli L. "What kills neurons in neurodegenerative diseases?", a review series in an open access journal. *Mol Neurodegener*. 2009; 4:7. [PubMed: 19193221]
17. Amor S, Peferoen LA, Vogel DY, et al. Inflammation in neurodegenerative disease--an update. *Immunology*. 2014; 142(2):151–66. [PubMed: 24329535]
18. Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. *Cell*. 2010; 140(6):918–34. [PubMed: 20303880]
19. Mitragotri S, Burke PA, Langer R. Overcoming the challenges in administering biopharmaceuticals: formulation and delivery strategies. *Nat Rev Drug Discov*. 2014; 13(9):655–72. [PubMed: 25103255]
20. Metcalfe SM, Fahmy TM. Targeted nanotherapy for induction of therapeutic immune responses. *Trends Mol Med*. 2012; 18(2):72–80. [PubMed: 22172276]
21. Pissadaki EK, Bolam JP. The energy cost of action potential propagation in dopamine neurons: clues to susceptibility in Parkinson's disease. *Front Comput Neurosci*. 2013; 7:13. [PubMed: 23515615]
22. <http://www.transeuro.org.uk>
23. Zhao JW, Dyson SC, Kriegel C, et al. Modeling of a targeted nanotherapeutic 'stroma' to deliver the cytokine LIF, or XAB939, a potent inhibitor of Wnt- β -catenin signaling, for use in human fetal dopaminergic grafts in Parkinson's disease. *Dis Model Mech*. 2014; 7(10):1193–203. [PubMed: 25085990]
24. Banks WA. From blood-brain barrier to blood-brain interface: new opportunities for CNS drug delivery. *Nat Rev Drug Discov*. 2016; 15:275–92. [PubMed: 26794270]
25. Olmos-Alonso A, Schettters ST, Sri S, et al. Pharmacological targeting of CSF1R inhibits microglial proliferation and prevents the progression of Alzheimer's-like pathology. *Brain*. 2016; 139(Pt 3):891–907. [PubMed: 26747862]
26. Pardridge WM. Drug transport in brain via the cerebrospinal fluid. *Fluids Barriers CNS*. 2011; 8:7. [PubMed: 21349155]
27. Atkins HA, Bowman M, Allan D, et al. Immunoablation and autologous haemopoietic stem-cell transplantation for aggressive multiple sclerosis: a multicentre single-group phase 2 trial. *The Lancet*. Jun 9.2016
28. Tosi G, Bortot B, Ruozi B, et al. Potential use of polymeric nanoparticles for drug delivery across the blood-brain barrier. *Curr Med Chem*. 2013; 20(17):2212–25. [PubMed: 23458620]
29. Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Préat V. PLGA-based nanoparticles: an overview of biomedical applications. *J Control Release*. 2012; 161(2):505–22. [PubMed: 22353619]
30. Look M, Stern E, Wang QA, et al. Nanogel-based delivery of mycophenolic acid ameliorates systemic lupus erythematosus in mice. *J Clin Invest*. 2013; 123(4):1741–9. [PubMed: 23454752]
31. Patel T, Zhou J, Piepmeyer JM, Saltzman WM. Polymeric nanoparticles for drug delivery to the central nervous system. *Adv Drug Deliv Rev*. 2012; 64(7):701–5. [PubMed: 22210134]
32. Fornaguera C, Dols-Perez A, Calderó G, García-Celma MJ, Camarasa J, Solans C. PLGA nanoparticles prepared by nano-emulsion templating using low-energy methods as efficient nanocarriers for drug delivery across the blood-brain barrier. *J Control Release*. 2015; 211:134–43. [PubMed: 26057857]

33. Loureiro JA, Gomes B, Coelho MA, do Carmo Pereira M, Rocha S. Targeting nanoparticles across the blood-brain barrier with monoclonal antibodies. *Nanomedicine (Lond)*. 2014; 9(5):709–22. [PubMed: 24827845]
34. Alexis F, Pridgen E, Molnar LK, Farokhzad OC. Factors affecting the clearance and biodistribution of polymeric nanoparticles. *Mol Pharm*. 2008; 5(4):505–15. [PubMed: 18672949]
35. Valenza M, Chen JY, Di Paolo E, et al. Cholesterol-loaded nanoparticles ameliorate synaptic and cognitive function in Huntington's disease mice. *EMBO Mol Med*. 2015; 7(12):1547–64. [PubMed: 26589247]
36. Vilella A, Tosi G, Grabrucker AM, et al. Insight of the fate of CNS-targeted nanoparticles. Part I: Rab5-dependent cell-specific uptake and distribution. *J Control Release*. 2014; 174:195–201. [PubMed: 24316476]
37. Tosi G, Vilella A, Chhabra R, et al. Insight on the fate of CNS-targeted nanoparticles. Part II: Intercellular neuronal cell-to-cell transport. *J Control Release*. 2014; 177:96–107. [PubMed: 24417968]
38. Tosi G, Vilella A, Veratti P, et al. Exploiting bacterial pathways for BBB crossing with PLGA nanoparticles modified with a mutated form of diphtheria toxin (CRM197): In vivo experiments. *Mol Pharm*. 2015; 12(10):3672–84. [PubMed: 26312414]
39. Bauer S, Patterson P. Leukemia Inhibitory Factor Promotes Neural Stem Cell Self-Renewal in the Adult Brain 2006. *J. Neurosci*. 26(46):12089–12099. [PubMed: 17108182]
40. Deverman, Patterson.
41. Laterza C, Merlini A, De Feo D, et al. iPSC-derived neural precursors exert a neuroprotective role in immune-mediated demyelination via the secretion of LIF. *Nat Commun*. 2013; 4:2597. [PubMed: 24169527]
42. Reynolds AD, Stone DK, Hutter JA, Benner EJ, Mosley RL, Gendelman HE. Regulatory T cells attenuate Th17 cell-mediated nigrostriatal dopaminergic neurodegeneration in a model of Parkinson's disease. *J Immunol*. 2010; 184(5):2261–71. [PubMed: 20118279]
43. Jeong CH, Lee HJ, Cha JH, et al. Hypoxia-inducible factor-1 alpha inhibits self-renewal of mouse embryonic stem cells in Vitro via negative regulation of the leukemia inhibitory factor-STAT3 pathway. *J Biol Chem*. 2007; 282(18):13672–9. [PubMed: 17360716]
44. Kubota Y, Hirashima M, Kishi K, Stewart CL, Suda T. Leukemia inhibitory factor regulates microvessel density by modulating oxygen-dependent VEGF expression in mice. *J Clin Invest*. 2008; 118(7):2393–403. [PubMed: 18521186]
45. Moidunny S, Vinet J, Wesseling E, et al. Adenosine A2B receptor-mediated leukemia inhibitory factor release from astrocytes protects cortical neurons against excitotoxicity. *J Neuroinflammation*. 2012; 9:198. [PubMed: 22894638]
46. Davis SM, Collier LA, Leonardo CC, et al. Leukemia Inhibitory Factor Protects Neurons from Ischemic Damage via Upregulation of Superoxide Dismutase. *Mol Neurobiol*. 2016; 3
47. Metcalfe SM. Multiple sclerosis: One protein, two healing properties. *Nature*. 2011; 477(7364): 287–8. [PubMed: 21921909]
48. Metcalfe SM. LIF in the regulation of T-cell fate and as a potential therapeutic. *Genes Immun*. 2011; 12(3):157–68. [PubMed: 21368774]
49. Park J, Gao W, Whiston R, Strom TB, Metcalfe SM, Fahmy TM. Modulation of CD4+ T lymphocyte lineage outcomes with targeted nanoparticle-mediated cytokine delivery. *Mol Pharm*. 2011; 8(1):143–52. [PubMed: 20977190]
50. Gao W, Thompson L, Zhou Q, et al. Treg versus Th17 lymphocyte lineages are cross-regulated by LIF versus IL-6. *Cell Cycle*. 2009; 8(9):1444–50. [PubMed: 19342884]
51. Rittchen S, Boyd A, Burns A, et al. Myelin repair in vivo is increased by targeting oligodendrocyte precursor cells with nanoparticles encapsulating leukaemia inhibitory factor (LIF). *Biomaterials*. 2015; 56:78–85. [PubMed: 25934281]
52. Metcalfe SM, Strom TB, Williams A, Fahmy TM. Multiple sclerosis and the LIF/IL-6 axis: Use of nanotechnology to harness the tolerogenic and reparative properties of LIF. *Nanobiomedicine*. 2015; 2:5.doi: 10.5772/60622

53. Janssens K, Van den Haute C, Baekelandt V, et al. Leukemia inhibitory factor tips the immune balance towards regulatory T cells in multiple sclerosis. *Brain Behav Immun*. 2015; 45:180–8. [PubMed: 25514345]
54. Dyson S, Fahmy TM, Metcalfe SM, Barker RA. Evaluation of PLGA nanoparticles carrying leukaemia inhibitory factor for stromal-like support of rat fetal dopaminergic cells. *J Nanomater Mol Nanotechnol*. 2014; S2:003.
55. <http://www.bloomberg.com/view/articles/2015-07-29/the-inadequate-war-on-alzheimer-s>
56. Ostan R, Lanzarini C, Pini E, et al. Inflammaging and Cancer: A Challenge for the Mediterranean Diet Nutrients. 2015; 7:2589–2621. DOI: 10.3390/nu7042589

Figure 1. Global Costs of Dementia

Alzheimer's disease is, in a sense, synonymous with dementia. Analyses published by The World Alzheimer Report 2015 (www.alz.co.uk/worldreport2015) shows AD-related dementia in terms of prevalence, incidence, cost and trends. Under Costs, the report states: *"...Excluding informal care costs, total direct costs account for 0.65% of global GDP. Cost estimates have increased for all world regions, with the greatest relative increases occurring in the African and in East Asia regions (largely due to higher prevalence estimates for these regions)."*

The Report's recommendations include... *"that dementia risk reduction should be an explicit priority in work led by the World Health Organization that research investment for dementia should be scaled up, proportionate to the societal cost of the disease; and that this investment should be balanced between prevention, treatment, care and cure."*

The left panel shows predicted increase in dementia on a global scale, where low and middle income countries make significant impact. The right panel shows predicted costs relative to research funding in the US.

Generic NanoTherapeutic

- Programmable Targeting including to cross Blood Brain Barrier
- Programmable Cargo Release Rate
- Non-Immunogenic
- Biodegrades to CO₂ and H₂O

Nanoparticles
 1 billion particles per mg
 100 – 200nm diameter
biodegradable
biocompatible

Cargo (red)
 neurogenic growth factor
 embedded in PLGA
 nanoparticle for targeted,
 paracrine delivery to target

Targeted Delivery
 functionalised surface using
 antibody to target specific
 cell types

The Platform Technology is in Phase II Clinical Trials for Drug Delivery with proven safety

Figure 2. The NanoMedicinal

The new era of cell-free NanoMed provides a platform for targeted delivery of neurotrophic growth factors to the CNS. Unlike cell-based therapy, NanoMed provides an off-the-shelf, chemically defined therapeutic suitable for bulk production and storage, that is applicable to a wide range of different NDD, and that can be transported around the globe. These properties are compatible with commercial translation by industry: only industry can meet the size of investment to achieve the necessary scale of production for approved clinical use.