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Chapter 11

Systemic Neural Stem Cell-Based Therapeutic Interventions for Inflammatory CNS Disorders

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Additional information is available at the end of the chapter

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1. Introduction

Regenerative processes occurring under physiological (*maintenance*) [1-3] and pathological (*reparative*) [4-6] conditions are a fundamental part of life, and vary greatly among different species, individuals, and tissues. Despite the central nervous system (CNS) has been considered for years as a perennial tissue, it has recently become clear that both physiological and reparative regeneration occur also within the CNS to sustain tissue homeostasis and repair. Importantly, the proliferation and differentiation of endogenous neural stem cells (NSCs) residing within the healthy CNS, or surviving injury, are considered crucial in sustaining these events. However, these processes are not robust enough to promote a functional and stable recovery of the nervous system architecture. Thus, the development of cell-based therapies designed to promote functional (direct *vs*. indirect) neural cell replacement was anticipated [7]. Nevertheless, most of the experimental cell therapies with neural lineage-committed progenitors have failed to foster substantial repair in disease models where the anatomical and functional damage is widespread and an inflamed and/or degenerative microenvironment coexists. Conversely, the systemic injection of *in vitro* expanded neural stem/precursor cells (NPCs) – both as neurospheres as well as plastic-adherent monolayers - has provided a remarkable amelioration of the clinico-pathological features of rodents affected by experimental inflammatory CNS disorders that include experimental autoimmune encephalomyelitis (EAE), cerebral ischemic/haemorrhagic stroke, spinal cord injury (SCI) and traumatic brain injury (TBI). This has been shown to be dependent on the capacity of transplanted NPCs to engage multiple mechanisms of action within specific microenvironments *in vivo* [8]. Among a wide range of potential therapeutic actions – and in addition to the expected cell replacement – this phenomenon may also occur via several *bystander effects*. These effects are heterogeneous

and likely exerted by undifferentiated NPCs releasing immune regulatory and neuroprotective molecules within specific microenvironments in response to local stimuli elicited by inflammatory cells (*therapeutic plasticity*). The molecular and cellular mechanism(s) that sustain the multifaceted therapeutic plasticity of NPCs remain far from being fully characterized [9].

The transplantation of undifferentiated exogenous NPCs very efficiently protects the CNS from experimental chronic degeneration induced by inflammation both in small rodents (mice and rats) [10-14] as well as in non-human primates [15]. Specific homing of systemically injected NPCs is shown, so far, in experimental models of multiple sclerosis (MS), ischemic/haemorrhagic stroke, SCI and TBI, and epilepsy. I*n vitro* and *in vivo* data provide extensive evidence of the molecular mechanisms behind the ability of NPCs to cross the blood-brain barrier (BBB) and specifically accumulate at the sites of inflammation/tissue damage [16-18]. After entering the CNS using constitutively functional cell adhesion molecules and inflammatory chemokine receptors, systemically injected NPCs accumulate at the level of perivascular CNS areas, where they establish *atypical ectopic perivascular niches* [16, 19]. In these areas, a much likely active cell-to-cell communication takes place between transplanted NPCs and the different cells of the *atypical niche*. As consequence of this, transplanted NPCs survive while displaying undifferentiated features, and promote neuroprotection by releasing immune modulatory molecules and neurotrophic factors *in situ*. Further evidence exists about an additional peripheral immune-modulatory effect exerted by NPCs [20, 21]. Systemically injected NPCs, in fact, enter also peripheral organs (e.g. draining lymph nodes and spleen) were they accumulate at the boundaries of blood vessels and interact closely with lymphocytes and professional antigen presenting cells (APCs), impairing their maturation and functional activation [15, 22, 23].

NPC-based therapies have been therefore considered a plausible alternative strategy for the treatment of neurological inflammatory disorders. However, some urgent and still unclear questions have to be solved prior to straightforwardly translate most of these exciting experimental observations into clinical medicines, such as: (i) the ideal stem cell source, whether it has to be derived from pluripotent or multipotent sources; (ii) the ideal route of cell administration, whether it has to be focal or systemic; (iii) the optimal time point for cell administration, depending on the disease characteristics; (iv) the ideal balance between differentiation and persistence of stem cells into the targeted tissue and (v) the ideal mechanism of tissue repair to foster, whether it has to be cell replacement or tissue protection/healing. Further, while some encouraging efforts are being devoted towards the development of guidelines and establishment of explorative phase I clinical trials, still one of the major constraints to the easy translation into human medicines is represented by the immunogenicity of allogeneic stem cells, and the modest expandability of somatic human NPCs *in vitro*. Within this scenario, the emerging figure of induced pluripotent stem (iPS) cells [24], induced neuronal (iN) cells [25] and/or induced neural stem cells (iNSCs) [26] holds a new exciting promise.

In this chapter we will describe the most recent evidence of the remarkable therapeutic plasticity of transplanted NPCs, when injected systemically in inflammation-driven CNS degeneration experimental models. We will first focus on the evidence that inspired the modern stem cell experimental therapies and then elaborate on the mechanisms regulating the

cross talk between somatic NPCs and the dysfunctional microenvironment, both at the outer and inner endothelial sides, and their clinico-pathological impact. Finally, we will discuss the rationale of the most recent explorative trials that are bringing neural stem cell therapies into the clinic.

2. Adult neural stem cells

2.1. A change in the dogma

Stem cells (SCs) possess the unique ability to self-renew and differentiate into different cell types in the body. Their contribution is essential during embryonic and early post-natal life, where they regulate morphogenesis and development by properly balancing proliferation and differentiation. Though their number is destined to decrease with time, their presence in adult organisms is still required to ensure *homeostasis* and *repair*. While the regenerating properties of some tissues (e.g., the skin) and organs (e.g., the liver) are undisputed, the brain with its unique organization and complexity was considered for long time an exception. In fact, the dogmatic concept '*no new neurons after birth*' (1913) expressed by Santiago Ramon y Cajal, sustaining the limitation of neurogenesis to prenatal life, has been resonating for decades within the scientific community, finally becoming an established belief. NSCs were thought to be present within the brain only during the developmental stage. It was only in the late 60's, thanks to the availability of new techniques and advanced tools of investigation, that the picture of the brain as an immutable organ started to be reviewed. Altman and colleagues, using (^{3}H)-thymidine pulses and autoradiographs, first demonstrated the presence of proliferating neurons in different regions of the post-natal brain in rats [1, 2, 27]. However, the turning point was marked later in 1983 when Goldman and Nottebohm at Rockfeller University (USA) described newly generated neurons at the level of the hyperstriatum ventrale, pars caudalis (HVc), of the ventricular zone in intact adult female canaries [3] Subsequently, numerous pioneering experiments contributed in demonstrating that specific regions of the mammalian CNS undergo a continuous, though moderate, level of neurogenesis throughout adult life [28].

2.2. Adult neurogenesis in physiological conditions

Today it is widely accepted that in the adult mammalian brain, newly generated cells derive from NSCs residing in two regions [29], the ventricular-subventricular zone (V-SVZ) of the forebrain lateral ventricles [2, 27, 30] and the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus [1, 31, 32] (Figure 1). Because of the peculiar cellular organization and exclusive microenvironment, these neurogenic regions are commonly referred to as *germinal-like niches* [33, 34]. Although different, these two areas share an extremely organized and specialized microenvironment where NSCs can strategically interact with a rich vascular plexus [35, 36], while communicating with their progeny and neighbouring NSCs as well as with differentiated neural cells trough specialized structures (e.g. primary cilium, basal and apical processes). Altogether, these cellular components provide a unique milieu of extracel-

lular matrix proteins and growth factors other than electrical stimuli, which define the dynamic characteristic of the adult brain stem cell *niches*. In here, a strictly regulated balance between proliferation and differentiation of NSCs ensure the maintenance of a constant, though quantitatively modest, pool of progenitor cells throughout lifetime [37].

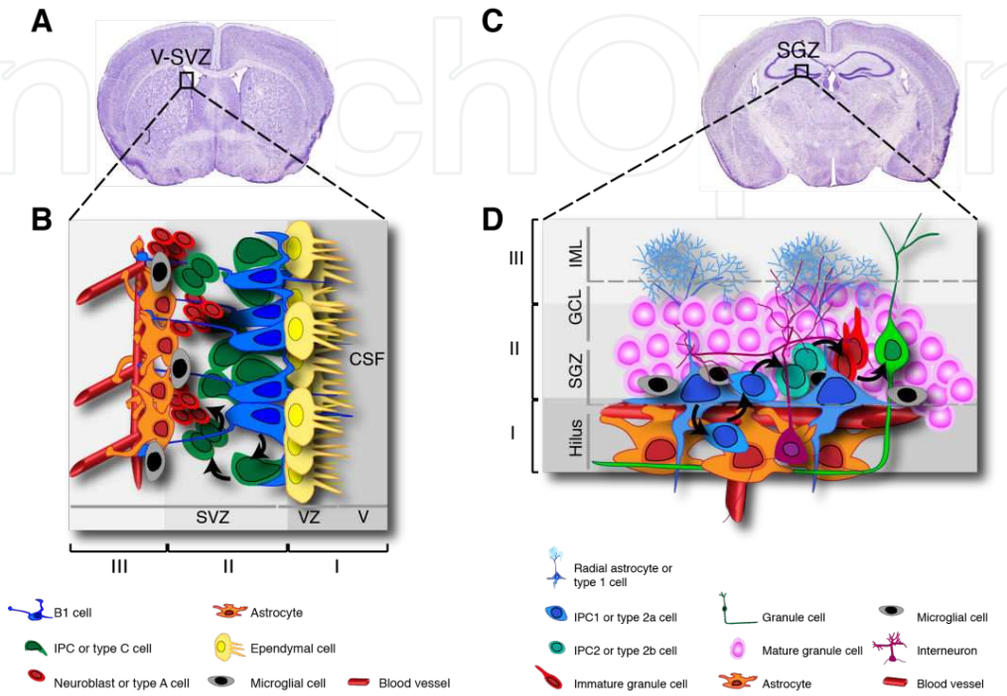

Figure 1. Schematic representations of the adult V-SVZ and SGZ neurogenic compartments.A and **C**, coronal sections of the adult mouse brain showing the localization of the V-SVZ and SGZ of the hippocampus. **B** and **D**, cytoarchitecture of the V-SVZ (**B**), and of the SGZ of the DG of the hippocampus (**D**) in the adult mammalian brain. **B**, Composition of the B1 cell domain into the V-SVZ. NSCs or type B1 cells (blue) extend from the proximal domain (domain I, dark grey) to the distal domain (domain III, light grey). At the level of the ventricles, B1 cells contact the CSF with their primary cilium extruding in the centre of a rosette of multi-ciliated ependymal cells (yellow), forming the typical pinwheel-like structures on the ventricular surface. Here, NSCs can sense different signals circulating into the CSF. In the distal domain, type B1 cells contact the blood vessels (red) with their specialized end-foot terminations. In the intermediate domain (or domain II) type B1 cells give rise to IPCs (or type C cells, green), which are transit-amplifying cells generating neuroblasts (or type A cells, red). In this domain they are also in contact with their progeny, neighbouring cells and neuronal terminations. **D**, Composition of the RA domain at the level of the DG of the SGZ. RAs (or type 1 cells, blue) extend from the hilus of the hippocampus (domain I, dark gray) to the IML (distal domain or domain III, light gray). At the level of domain I, RAs sense the hilus microenvironment with their primary cilium and contact other RAs, IPCs and blood vessels (red). RAs extend, trough their main shaft, into the distal domain where their arborisations receive signals from glial cells and neuronal terminations. RAs give rise to IPCs that mature (trough blue IPC1 or type 2a cells, and light green IPC2 or type 2b cells) and differentiate into immature granule cells (IGC, red). During their maturation, IPCs move from the proximal domain to the intermediate domain (or domain II, composed by SGZ and GCL), where RAs receive signals from the progeny, neighbouring NSCs, interneurons (purple) and microglia (grey). Finally IGC differentiate into mature GC (green), which extend their axons into the hilus and arbores dendrites into the distal domain. Only few new-born neurons survive and become a long-lasting GC (pink).

2.2.1. Defining the cellular composition of the V-SVZ

The V-SVZ is situated in proximity of the lateral ventricles and contains slow-cycling SCs with astroglial properties that express glial-fibrillary acidic protein (GFAP), called type B1

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cells. These cells give rise to intermediate progenitor cells (IPCs) or type C cells, which lose GFAP immunoreactivity and acquire the expression of the distal-less homeobox (Dlx)-2. These cells finally give origin to a pool of neuroblasts (type A cells) expressing the polysialylated form of neural cell adhesion molecule (PSA-NCAM) and the early neuronal marker doublecourtin (DCX) [38]. Within rodent's brain these neuroblasts form chains of migration along the rostral migratory stream (RMS) to reach the olfactory bulb (OB), where they terminally differentiate into at least six different subtypes of OB interneurons, depending on their origin along the axes of the V-SVZ [39-41]. The V-SVZ niche (Figure 1 A-B) can be divided in three differently organized domains where self-renewing B1 cells receive different signals: *proximal* (or apical, I), *intermediate* (II) and *distal* (or basal, III) [37]. Type B1 cells retain the typical apical-basal bi-polarity of their embryonic predecessors (radial glia) [42] extending their processes along the three different domains and spanning the cerebrospinal fluid (CSF) and the blood stream. In the proximal domain (composed by VZ and part of the SVZ) Type B1 cells are enclosed within a cluster of ependymal (type E) cells, which sense the CSF by means of motile cilia and create an appropriate gradient of molecules within the VZ [43]. Type B1 cells are therefore physically separated from the ventricles. Nevertheless, their contact with the CSF is still made possible by a single apical primary cilium extruding in the centre of a rosette of type E cells. Typically, these apical end-foot terminations cluster together to finally arise in the middle of a layer of E cells forming a characteristic *pinwheel structure* resembling the embryonic forebrain germinal zone [36, 42]. Recently, it has been shown that the expression of the adhesion and signalling molecule vascular cell adhesion molecule (VCAM)-1 is critical for the correct positioning of these protrusions and the preservation of this complex structure [44]. The small apical surface of B1 cells gives them the chance to sense the CSF which contains soluble factors, such as insulin-like growth factor (IGF)-2, bone morphogenetic proteins (BMPs) and Noggin, Wnts, Sonic hedgehog (Shh) and retinoic acid, able to modulate NSCs behaviour [45]. At the same time a long basal process from the opposite pole (distal domain), bridges B1 cells to the surrounding vascular plexus that runs in the parenchymal side of the V-SVZ. Here, with a specialized end-foot termination, type B1 cells contact endothelial cells (ECs) of the blood vessels, thus being influenced from soluble factors released from ECs and/or possibly by molecules produced far away from the niche and released in the blood stream. The intermediate domain (composed by the SVZ) contains B1 cell progeny, such as IPCs and neuroblasts, which participate in the maintenance of the niche equilibrium perhaps trough mechanisms of direct feedback on NSCs providing information about the number of new neurons already generated. This balance, seems to be regulated on one side by canonical Notch signalling trough ligands released or expressed by both IPCs and neighbouring B1 cells [46, 47] and on the other side by neurotransmitters [e.g. gamma-aminobutyric acid (GABA)] secreted by neuroblasts [48]. Importantly, while many studies have focussed on the role of the microenvironment on the functionality of NSCs [49], much less is known about the role that NSCs themselves exert on the definition of the niche. Recently it has been shown that NSCs in the germinal niches do secrete a multitude of factors, among which some with immune modulatory potentials that may influence the behaviour of the surrounding cells, including microglia [50]. In parallel to the rodent CNS, the lateral wall of the lateral ventricles (and the hippocampus) of the human brain contains NSCs that generate

new neurons throughout adult life [51-53]. A total of four layers have been observed forming the human lateral ventricular wall, which comprise a monolayer of ependymal cells, a hypocellular gap, a ribbon of astrocytes, and a transitional zone into the brain parenchyma [52, 54]. Unlike the rodent and non-human primate brain [55], SVZ astrocytes of the human brain are separated from the ependyma by a hypocellular gap [52]. The presence of prominent neurogenesis in the V-SVZ as well as of a RMS of migrating neuroblasts in the human brain has been, however, intensively debated (for a preview, see [56]). Initially it was reported the existence of a ribbon of astrocytes in the adult human V-SVZ that function as multipotent NSCs in culture although, only few proliferating cells and no evidence of chains of migratory (β-III tubulin positive) immature neurons were observed [52]. In contrast, a later report evidenced a robust cell proliferation in adult human V-SVZ and the presence of a RMS of neuroblasts along a lateral ventricular extension that connects the lateral ventricle to the OB [51]. Finally, two recent studies have provided evidence of a small ventricular lumen connecting the lateral ventricles to the OB that is observed only in the foetal [57], but not adult, human brain [55, 58]. Interestingly, the absence of this ventricular extension has been confirmed even in the postnatal infant human brain [58], whereas a new medial migratory stream (MMS) targeting the prefrontal cortex has been observed. Altogether these findings suggest a dynamic evolution in human SVZ neurogenesis throughout life; with the infant human SVZ, RMS and MMS activity, undergoing a progressive extinction at ages older than 18 months post-natal [58].

2.2.2. Defining the cellular composition of the SGZ

The second putative progenitor cell compartment is located in the SGZ of the DG of the hippocampus (Figure 1C-D), namely the region of the brain involved in learning and memory [1, 31, 32]. In this area, NSCs residing at the interface of the hilus and dentate gyrus are called type-1 progenitors or radial astrocytes (RAs) [59] and they mainly correspond to astroglial cells [60]. They mature in dentate granule cells and migrate towards the granule cell layer (GCL) to finally integrate into hippocampal circuitry [59]. RAs, unlike B1 cells of the V-SVZ, are found deeper into the brain parenchyma, surrounded by neurons, neighbouring RAs and other glial cells but without any chance to contact the CSF [37]. However, B1 cells and RAs share some key features: they both express astroglial markers, have ultrastructural characteristics of astrocytes [41] and possess long processes reaching different compartments of the niche far away from where the cell bodies reside [37]. RAs function as the primary precursors for the generation of new dentate granule neurons, either directly or via the generation of IPC1 (type 2a cells) and IPC2 (type 2b cells) [61]. Similarly to the V-SVZ, also the SGZ can be subdivided in a proximal, intermediate and distal domain along which RAs, with their polarized structure (apical-basal), span from the hilus interface (proximal domain) to the inner molecular layer (IML, distal domain) [37]. The proximal domain contains the primary cilium (important for Sonic hedgehog (Shh) signalling), which sense the hilus microenvironment, and lateral processes contacting other RAs and IPCs and, importantly, blood vessels. Here ECs release vascular endothelial growth factor (VEGF), IGF and brain-derived neurotrophic factor (BDNF) responsible for the regulation of the balance between proliferation and differentiation. RAs have their cell bodies in the SGZ and extend their main shaft along the GCL, which compose

the intermediate domain. In this area astrocytes receive inputs from their progeny, including immature and mature granule neurons, IPCs and different neuronal and glial (e.g. microglia) cell types. Type 2a cells expressing Achaete-scute homolog (Ascl)-1 (also known as Mash-1) - a transcription factor important for neuronal commitment - are likely to originate in the proximal domain and then rapidly migrate into the intermediate one, where they divide once before differentiating into type 2b cells that will express DCX [62]. Similarly to V-SVZ, feedback mechanisms from the progeny, such as canonical and non-canonical Notch signalling are responsible for the quiescence of RAs or their transition to IPCs [63, 64]. In the IML, RAs terminate with an elaborate and branched structure contacting glial cells, neuronal processes and synapses. Although the contacts taking place in this area are still not completely understood, it seems probable that the GABAergic and glutamatergic inputs coming from interneurons and mossy cells, are important for the regulation of NSCs [65]. These astrocyte-like cells of CNS germinal areas work as real pacemakers of adult neurogenesis, as they receive internal and external inputs from their main shaft as well as from the end-foot of their radial processes that contact ECs in the V-SVZ [42], or embedded into the molecular layer in the SGZ [41]. However, despite the relatively high rate of neurogenesis, only a minority of new born cells eventually survive, mature and integrate within the existing circuitries at the level of the GCL of the hippocampus [66]. In parallel, postnatal SGZ neurogenesis in the human brain has been demonstrated to occur across the lifespan [55]. Although the role of new born neurons generating in the SGZ is not yet fully understood, increasing evidence suggest a possible role in learning and memory function [55].

3. CNS inflammation effects on endogenous adult NSC niches

3.1. Switching from an immune-privileged to an immune-specialized state

Protection and homeostasis are fundamental keystones for the proper maintenance of the CNS. Hence, brain and spinal cord must be kept under an extreme security state to ensure their fully functionality and, ultimately, the survival of an organism. However, in the past the CNS has been often regarded as an immune *privileged* site, where immune cells were not supposed to enter and interact with cells of the nervous system. This common belief was strongly supported by observations showing lack of lymphatic vessels, absence of parenchymal APCs, low expression of constitutive major histocompatibility complex (MHC) class I and II molecules within the brain parenchyma, as well as poor rejection of transplanted allo- or xeno-graft. In the last decades this historical concept has been extensively revised, and there is now convincing evidence that the CNS is instead an immune *specialized* site, where a complex regimen of immune surveillance does occur under physiological as well as pathological conditions and is essential to guarantee its optimal functionality [67]. It is now clear that cells of both the innate (microglia and monocyte-derived macrophages) and the adaptive (mainly $CD4^+$ cells) immune system are present within the brain parenchyma and exert beneficial effects on adult brain plasticity and neurogenesis, as well as on the spontaneous attempt of the CNS to self-repair following an injury [68].

3.2. Effects of inflammation on neurogenesis

Studies conducted over the last decade have extensively proved that the immune and nervous systems interact by engaging an active bidirectional crosstalk. Indeed, the expression of receptors able to recognize inflammatory mediators released by activated immune cells allows endogenous progenitor cells to increase their proliferation rate and specifically home to the site of inflammation after a trauma. As a consequence, both acute [69, 70] and chronic CNS inflammation [6, 71] has been shown to perturb the anatomical architecture and functional activity of adult germinal niches.

Work on EAE mice, the most widely accepted model of MS, has shown that chronic CNS inflammation in myelin oligodendrocyte glycoprotein $(MOG)_{35-55}$-immunized mice causes a transient decrease in the proliferation rate of both C and B1 type cells and a contemporary increased accumulation of neuroblasts within the V-SVZ [6]. This effect, observed during the peak of the disease, was attributed to cell non-autonomous factors, such as pro-inflammatory (Th1) cytokines [e.g. interferon (IFN)- and its intracellular effector Stat-1]. However, these data contrast with other studies showing how inflammatory demyelination in $MOG^{+/-}$ mice immunized with purified mouse myelin increased proliferation and mobilization of neural progenitor cells from the V-SVZ of adult mice. Surprisingly, while new born cells generated at the level of V-SVZ commonly intended to differentiate into neurons, in response to EAE, these cells were able to generate astrocytes and oligodendrocytes as well, thus suggesting that inflammation can diverge (at least partially) their intrinsic nature [4]. Increased proliferation, measured in terms of BrdU-positive cells, has been found also at the level of the hippocampus both during the acute and chronic phases of the disease in MOG35-55 immunized mice. Similarly to the observed accumulation of neuroblasts in the V-SVZ, autoimmune inflammation leads to increased numbers of immature DCX-positive cells in the DG of the hippocampus [72]. Even though some alterations in the Notch, Wnt/β-catenin, Shh, and BDNF signalling pathways have been observed, their real contribution to the deregulation of hippocampal neurogenesis in the course of chronic autoimmune neuroinflammation needs to be further confirmed [72]. In addition, magnetic resonance imaging (MRI) techniques revealed structural alterations in the hippocampus, evidencing marked hippocampal atrophy [73], which may correlate with deficits in attention, information processing capacity and long-term memory observed in the majority of MS patients. Enhanced proliferation during the acute phase of the disease has been observed in proteolipid protein $(PLP)_{139-151}$-induced relapsing EAE in SJL mice. However, during both the relapsing and chronic phase of the disease, the number of SVZ progenitors cells decreased, without changes in the ultrastructural features of the type B, C or A cells, but accompanied by an impaired maturation of oligodendrocyte progenitor cells (OPCs). This suggests that the chronic activation of glial cells (namely microglia and astrocytes) might be deleterious for the repair potential of endogenous brain stem/progenitor cells. Indeed, minocycline-induced inactivation of microglia during the chronic phase in relapsing-remitting EAE mice was associated with an improvement in the number of proliferating Sox2/Bromodeoxyuridine $(BrdU)^{+}$ neural stem cells [74]. Finally, models of targeted focal EAE, obtained by stereotactic injection of cytokines [e.g. tumor necrosis factor (TNF)-α and INF-γ] in rodents

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pre-immunized with a sub-clinical amount of myelin peptides, allowed to better analyse the time course effect of auto-immune inflammation in the neurogenic areas. In this experimental model a decreased proliferation in the proximity of the V-SVZ was observed at 3 days, followed by an increase at 7 days after the injection of the cytokines, suggesting a regenerative attempt at the level of the V-SVZ area. Interestingly, the concomitant death of neuroblasts, the decreased type C cell proliferation, and the reduction of type A migrating cells, during the initial phase, might explain the impaired long-term olfactory memory observed by means of behavioural analysis [75]. Altogether, these findings suggest the existence of a compensatory mechanism of the injured brain in its attempt to counteract neuronal injury and disturbed conductivity resulting from T cell attack to the myelin sheaths wrapping the axons, which is among the most accepted causes of EAE and MS [76].

In agreement with what described in animal models, SVZ activation and expansion have been found at the level of periventricular active and chronic active lesions in MS patients, thus suggesting that the repetitive exposure to inflammatory insults does not completely exhaust the proliferative potential of the SVZ [77]. V-SVZ from post-mortem brains shows an altered balance between neurogenesis and gliogenesis, likely related to these inflammation effects within the neurogenic niche of MS patients [78]. Interestingly, the majority of MS patients show deficits in attention, information processing capacity and long-term memory, thus suggesting that neuronal damage in MS can result not only in motor and sensory deficits but also cognitive impairment. In support of these MRI techniques revealed structural alterations in the hippocampus, evidencing marked hippocampal atrophy [73].

Acute events, occurring in non-autoimmune diseases such as stroke, have been similarly proved of giving rise to increased proliferation of endogenous NSCs in the V-SVZ. These cells migrate from the neurogenic niche towards the ischemic boundary regions of the striatum and cerebral cortex, where they differentiate into mature striatal neurons [79-81]. During this (injury-reactive) site-specific homing, newly generated neuroblasts form chain-like structures in association with reactive astrocytes and blood vessels in the striatum, a reminiscence of the embryonic migration of type A cells along the RMS [82, 83]. Initially, this potential self-repair mechanism was supposed to happen only during the acute post-stroke phase. However, subsequent studies showed that stroke-induced neurogenesis is an extensive and long-lasting (up to 2 weeks) event, with continuous production of mature striatal neurons for several months after the insult [84]. Unfortunately, the vast majority of migrating new born neurons die within few weeks after the ischemia, and only few damaged cells (about 0.1%) are replaced by newly generated neurons [85]. Similar evidence of stroke-induced neurogenesis has been reported in post-mortem brains, where new born neurons are present in the ischemic penumbra surrounding cerebral cortical infarcts, preferentially localized in the vicinity of blood vessels [80]. The identification of those factors able to influence NSCs proliferation, homing and survival after stroke may have a great therapeutic impact. Several cytokines and growth factors that may be released by injured cells are thought to play a substantial role in promoting the observed neurogenic response after stroke. Among these, ciliary neurotrophic factor (CNTF) [86], transforming growth factor (TGF)-α [87], VEGF [88], fibroblast growth factor (FGF)-2 [89] and erythropoietin (Epo) [90] have been proposed.

Much less is known about the presence of adult neurogenesis after SCI. Most likely, this can be ascribed to a more diffuse scepticism concerning the existence of stem cells within the spinal cord. Indeed, even if the spinal cord is generally considered a non-neurogenic tissue, multipotent precursors can be isolated and propagated *in vitro* [91, 92]. In addition, spinal neurogenesis has been shown to occur to a limited extent in response to several types of trauma [93-95]. In a very recent study, the modulation of neurogenesis in the more canonical niches of the adult brain has been investigated following SCI in rats. Interestingly, $BrdU^+$ positive cells were found to be significantly decreased both at the level of the V-SVZ and the SGZ in subacute [15 days post injury (dpi)] condition. However, while V-SVZ proliferation returns to normal levels at 90 dpi, this does not happen at the hippocampal level. This could be equally explained by either a higher plasticity in the V-SVZ or a higher sensibility of the SGZ [96]. Alterations in adult neurogenesis have been extensively observed in a multitude of models of other neurodegenerative diseases. Rats suffering by pilocarpine-induced temporal lobe epilepsy (TLE), exhibit increased neurogenesis in the V-SVZ [97] as well as in the SGZ [98] after a period of latency and then it lasts for several weeks following prolonged seizures activity. Further, status epilepticus (SE) seems to accelerate the maturation and integration of adult new born DG cells [99]. However, chronic TLE induces a decrease of neurogenesis, as children affected by frequent seizures show decreased numbers of newly generated neurons and proliferating cells [100]. Impaired (cell type specific) proliferation of V-SVZ as well as SGZ progenitors has been observed also in experimental models of Alzheimer's disease (AD) [101]. While it is not clear whether this is reflected in an increased [102] or decreased neurogenesis [103], mainly because of the high number of different models used [104, 105], a recent study suggested that abnormalities at the level of both the neurogenic niches might precede the onset of amyloid deposition and memory impairments [106]. Interestingly, in post-mortem brains of patients with AD, it has been observed an increase of neurogenesis into the SGZ accompanied by depletion in the V-SVZ [107].

3.3. The double face of inflammation

According to what described, it is suggestive that the CNS is able to start a beneficial, though limited, process of self-repair. However, most of the new born cells generated following injury are destined to die within few weeks, maybe due to a failure in their integration or due to the inflammatory milieu. Even if these cells have been shown to fully differentiate into mature neurons [82], the very low rate of occurrence imposes logical concerns regarding the therapeutic value of this regenerative response to brain injury [81, 108]. Because of the rearrangements occurring in the neurogenic niches after an inflammatory event, the immune system has been accredited as one of the major responsible of this failure. This assumption is further corroborated by the observation that the increasing complexity of the immune system over the evolutionary process has been accompanied by a concomitant loss of regenerative capacity [109]. Also, several findings link inflammation to the pathogenesis of neurodegenerative disorders and anti-inflammatory drugs seem to be promising candidates for their treatment. Recent studies suggest that inflammation may indeed have a neuroprotective effect [110]. Nevertheless, the real effect of inflammation in several of these pathologies still needs to be completely clarified.

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The early (acute) post-traumatic phase of a neuroinflammatory process involves the action of resident microglial cells. These cells of myeloid origin are usually present in a resting but dynamic state, ready to shift their activity and undergo morphological and functional transformations in response to any kind of brain damage or injury [111, 112]. While on one side the selective ablation of microglia has been shown to exacerbate the ischemic injury in a mouse model of focal cerebral ischemia [113], on the other side mounting evidence indicates that chronic microglial activation may also contribute to the development and progression of neurodegenerative disorders, mainly through the release of pro-inflammatory cytokines such as interleukin (IL)-1, IL-6, and TNF-α [114]. As an example, infiltrating blood-derived macrophages have been shown to exert a beneficial role in an experimental model of SCI, where they contribute to limit the action of activated resident microglia, whose prolonged presence would finally lead to detrimental consequences [115]. Indeed, when the activity of microglia is not properly contained, their action may lead to a prolonged (chronic) inflammation eventually culminating in the formation of fibrotic tissue.

Endogenous T cells are key components of the protective immunity process. As such, physiological trafficking of lymphocytes through the CNS is required to support the essential function of immune surveillance [116]. Cellular composition analysis of the CSF of healthy patients has revealed that up to 80% of the total number of cells is represented by central memory and effector memory T-cells. This atypical composition, which is very different from the one of the blood, also suggests a major role for the CSF in the defence of the CNS [117]. Indeed, the CSF drains the interstitial fluid of the CNS and brings CNS antigens to the cervical lymph nodes, thus supplying for the absence of a proper lymphatic drainage [118, 119]. Following the seminal observation that self-specific T-cells recognizing myelin basic protein were able to protect injured CNS neurons from secondary degeneration in a rat model of optic nerve crash injury [120], several studies further supported the idea that T cell-dependent autoimmunity might promote recovery from CNS injuries [121, 122]. These studies finally culminated with the idea that boosting T-cell response to CNS antigens by means of immunization with CNS myelin-associated self-antigens could have enhanced this therapeutic potential [123-125]. Also, myelin-reactive T-cells possess neuroprotective effects, which may be essentially attributed to their ability to release neurotrophic factors such as BDNF, nerve growth factor (NGF) and CNTF [126]. Importantly though, auto-reactive T-cells showing this protective effect may turn out to be harmful if escaping the control exerted by the immune system, finally resulting into the development of autoimmune diseases such as MS. Therefore, a strict control is required to finely tune the balance between the *good* and the *bad* [76].

To further complicate the scene, several inflammatory mediators, such as TNF-α, TGF-β, IL-1, IL-6, IL-10 and IL-12 may have contrasting effects (e.g neuroprotective *vs.* neurotoxic) depending on the overall context. As an example, the role of IL-6 is crucial for the induction of EAE [127], and its overexpression exacerbates tissue injury in experimental models of SCI [128, 129]. Also, high levels of this inflammatory marker in the blood of patients undergoing inflammatory response after stroke correlates with the disease severity and poor clinical outcome [130]. Accordingly, the use of monoclonal antibody directed towards IL-6 proved to be beneficial in the treatment of acute SCI and MOG_{35-55}-induced EAE [131, 132]. However,

IL-6 knockout mice showed significantly increased of chronic (but not acute) lesion volumes and worse long-term functional outcome after stroke [133]. This may imply the need of a finely tuned regulation, most likely depending on their precise timing and location other than on the specific nature of the disease. Indeed, while some pathologies such as MS, SCI, stroke, TBI and are characterized by an acute inflammatory event followed by secondary neurodegeneration, others, such as epilepsy, Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD) are instead caused by primary neurodegeneration subsequently leading to secondary reactive inflammation [19].

As described in this paragraph, the CNS is able to regulate the proliferation rate within the adult neurogenic niches as an extreme attempt to respond to damages in both primary and secondary inflammatory neurodegenerative diseases. Nevertheless, this process is not robust enough to effectively re-establish the complex functionality of the CNS. Therefore, protocols aiming at pharmacological manipulation of endogenous precursors from germinal niche(s), *in vivo,* might be therapeutically inefficacious in inflammatory CNS disorders. Thank to the development of protocols allowing *in vitro* growth and large scale-up of brain-derived NPCs [134], innovative therapies, for both acute and chronic CNS inflammatory disorders, based on stem cell transplants have been proposed [7]. Transplantation of adult exogenous NPCs represents, in fact, an alternative, and possibly more efficacious, therapeutic approach that might overcome the limited endogenous repair. Motivated by the ambitious expectation to achieve CNS repair (and/or regeneration) via functional neural cell replacement, many different preclinical studies have evidenced a potential benefit of NPC-based treatments in experimental animal models of several neurological diseases [8].

4. Exogenous NPC-based therapies: The systemic administration

4.1. Systemic injection and functional recovery

Following the seminal observation that systemically delivered NPCs were able to target an intracranial tumour in rodent (both mice and rats) model of experimental brain tumours [135], numerous studies started to investigate the validity of this administration route in a variety of CNS disorders. Over the last decade, data have been provided on the feasibility of systemic NPC transplants via either intravenous (i.v.) cell injection into the blood stream, or interacerebroventricular (i.c.v.)/intrathecal (i.t.) into the CSF, in experimental CNS disease models [10-13, 16, 136-138]. Adult somatic mouse NPCs, administered 22 days post immunization (dpi) greatly reduced the functional impairment observed in chronic MOG_{35-55}-immunized EAE mice [11]. Also, rat NPC neurospheres, administered i.c.v. or i.t. in rats affected by acute EAE, attenuate the clinical symptoms when administered at the same day of disease induction (0 dpi) [136]. Intravenous injected mouse NPCs were proved to be efficacious in $PLP_{139-151}$ immunized relapsing EAE mice [16]. Indeed, mice, treated with NPCs at the disease onset or at the time of the first relapse, recovered faster and showed a decrease in the relapse rate compared to controls. At the end of the follow-up (90 dpi) both treatments resulted in a lower relapsing remitting EAE cumulative score [16]. Finally, MOG_{35-55}-immunized chronic EAE

mice receiving either mouse neurospheres (i.c.v) or single cell NPCs (i.v.), at 6 and 8 dpi respectively also showed significant clinical amelioration [20, 139].

The therapeutic efficacy of systemically administered NPCs has been later observed in a different pathological context, such as stroke. Mouse somatic NPCs, systemically transplanted 3 days after middle cerebral artery occlusion (MCAo), resulted in a better recovery, significantly improving the neurological severity score starting from 18 days post transplantation (dpt) until the end of the follow-up (30 dpt) [140]. Similar neurological improvements were observed in rats subjected to MCAo and common carotid artery occlusion (CCAo) after i.t. transplantation (7 days after stroke) of rat NPCs [141]. Significant locomotor recovery was also observed after acute systemic NPC transplant in mice suffering from contusion SCI [13].

Similarly to rodent cells, human NPCs have been proved to be therapeutically efficacious. Foetal NPCs administered either i.v. or i.t. at the disease onset, reduced the severity of MOG_{1-125}-induced chronic EAE in common marmosets [15]. Further, human embryonic stem cell (ESC)-derived NPCs have been shown to reduce disease severity of chronic EAE mice [142]. Human immortalized NPCs have been widely enrolled in stroke models. The systemic injection of the HB1.F3 NPC line resulted in neurological improvements of rats treated 1 day after MCAo or intracerebral hemorrhage (ICH) stroke models [12, 137, 138]. The same line resulted therapeutically effective also in quinolinic acid-induced experimental HD in rats, where NPCs administered intravenously at 7 days after disease induction, significantly ameliorated the behavioural outcome [14].

All these evidences showing behavioural recovery upon systemic injection of NPCs in different CNS inflammatory models, led to the investigation of the molecular mechanisms standing behind, since this capacity bears the hope of developing less invasive surgical techniques to implant therapeutic adult human stem cells into patients affected by highly debilitating CNS disorders, such as MS, stroke, SCI, epilepsy, PD, AD and HD.

4.2. Homing capacity: NPCs breach the CNS barriers

Brain and spinal cord are protected by a complex control system, composed by tight barriers shielding the action of a unique troop of immune cells. Indeed, to access the brain and spinal cord parenchyma, circulating cells have to breach through all the barriers that closely seal the CNS from the surrounding environment. Namely, these are the blood-brain barrier (BBB) at the level of parenchymal capillaries and post-capillary venules, the blood-cerebrospinal fluid barrier (BCSFB) at the level of the choroid plexus in the brain ventricles, and the blood-leptomeningeal barrier (BLMB) at the level of the leptomeningeal/subarachnoid space. The main role of these barriers is to maintain the chemical composition of the CNS microenvironment, thus ensuring the proper functionality of neuronal circuits, synaptic transmission and remodelling, angiogenesis, and neurogenesis in the adult brain, while their rupture is involved in many neuroinflammatory disorders [143]. Because of the existence of such barriers, the access of systemically injected NPCs to the CNS parenchyma seems quite unlikely.

Structurally, the main component of the BBB is represented by specialized ECs characterized by the absence of fenestrae, low pinocytotic activity and by the presence of intercellular tight

junctions (TJs) [144]. This clutched arrangement prevents the free passage of molecules, while the transport of nutrients into the CNS and the discard of toxic molecules into the circulation is ensured by active mechanisms, thus guarantying a proper neuronal activity [145]. Moreover, the BBB is an essential constituent of the so-called *neurovascular unit*, a boundary zone defined on one side by the *endothelium basement membrane* (located in the abluminal side of the vasculature) and on the other by the *parenchymal basement membrane*, which establishes the ultimate border between the perivascular space and the CNS parenchyma. In post-capillary venules these two membranes lay in close proximity, leaving just a narrow perivascular space in between, which becomes more significant at the level of arteries and veins. In this area, occasional APCs (leptomeningeal mesothelial cells) reside and play a major role in the immunosurveilance program of the CNS. Finally, the inner and outer sides of the parenchymal basement membrane are juxtaposed to the *glia limitans*, whose crossing seems to be crucial for the effective triggering of a neuroinflammation process [146, 147]. The functionality of the BBB in clinical situations, such as those occurring in some neurodegenerative disorders like MS, ischemic stroke, sub-arachnoid haemorrhage, TBI and AD is markedly reduced, leading to an increased permeability and trafficking of immune cells into the CNS parenchyma [148-152].

Most of the knowledge about the mechanisms that allow circulating cells to breach the barrier(s) [117, 153, 154] and move into the CNS parenchyma comes from observations conducted with models of CNS inflammation. Initial studies showed how intravenously injected radioactively labelled encephalitogenic T cells were able to cross the BBB of healthy recipients [153]. It was also shown that, while activation is mandatory for T-cells to cross the endothelial barrier and reach perivascular spaces, antigen specificity is dispensable to further cross the glia limitans and invade the CNS parenchyma after having encountered the appropriate APCs [155].

The extravasation of specific T cells requires a multistep process [156]. The first step, known as *capture* (in non-inflamed endothelia) or *tethering* (in inflamed endothelia) *and rolling* is represented by an initial, transient contact promoted by the specific interaction between members of the selectin and integrin families expressed by the activated endothelium with their respective ligands on circulating immune cells. It has been shown how the recruitment of inflammatory cells across the BBB involves α4-integrin and its ligands of the immunoglobulin (Ig) superfamily, namely vascular cell adhesion molecule (VCAM)-1 and mucosal addressin cell adhesion molecule (MAdCAM)-1 [147]. Upon this initial contact, circulating cells decrease their initial speed and resist the shear stress created by the blood flow, mainly through endothelial intercellular adhesion molecule (ICAM)-1 and VCAM-1, but not ICAM-2 [157]. Elegant studies have consistently shown that the inhibition of the dimeric α4β1-integrin and its cognate receptor VCAM-1 on the activated endothelium prevented the accumulation of leukocytes in the CNS and the development of EAE [158]. Interestingly, when the inflammatory process is started, α4β1-integrin is no more dispensable for T-cell capture or rolling [159].

The following step requires the *firm adhesion* and *crawling* of T-cells along the vascular wall. This is orchestrated by some chemokines and chemoattractants, such as stromal cell-derived factor (SDF)-1α/CXCL12 [160], monocyte chemoattractant protein (MCP)-1/CCL2 [161],

regulated and normal T cell expressed and secreted (RANTES)/CCL5 [162], EBI1 ligand chemokine (ELC)/CCL19, secondary lymphoid-tissue chemokine (SLC)/CCL21 released by the inflamed endothelia and CCR7 [163]. By binding to their G-coupled receptor (e.g.: C-X-C chemokine receptor -CXCR- type 4 for SDF-1 and CCR2 for MCP-1), these chemokines transmit an inside out signalling to T-cell surface integrins, which undergo dramatic conformational modifications thus increasing their *avidity* (specificity for the ligand). Once engaged in such a firm adhesion, T-cells need to make their way through the endothelium. To this purpose they start probing the vasculature to find the optimal site to breach the wall. Following adhesion to blood vessel walls, leukocytes undergo a series of actin rearrangements that eventually mark their transition to a more flatten and polarized shape [164]. Finally, T-cells cross the border either by paracellular or transcellular diapedesis or by creating pores through the cells - *transcellular diapedesis* -. While the former require the disassembly of the intercellular junction structure, the latter involves the formation of "cell-in-cell" interactions through the arrangement of docking structures or *transmigratory cups* enriched in ICAM-1 and VCAM-1, which partially embrace migrating leukocytes [165].

A very similar sequential process has been shown being recapitulated when systemically injected NPCs specifically home to the site of damage. In fact, NPCs posses the ability to reach the cerebral parenchyma where they eventually induce recovery in animal models of neurodegenerative diseases such as EAE [10, 11] stroke [12, 166], SCI [167, 168], epilepsy [138, 169], HD [14], other than glioblastoma [135, 170]. The first studies showing the extravasation capacity of NPCs [10-12] clearly demonstrated that this capacity was strictly related to the activation of the ECs by an inflammation process occurring within the brain. NPCs administered either i.c.v. or i.v. to healthy animals were, in fact, never observed inside the CNS, while mainly accumulating in peripheral organs, or remaining confined in the ventricles or subarachnoid space. Only after activation of endothelial cells, exogenous NPCs were observed to accumulate into the CNS. Systemically injected NPCs are, in fact, able to follow a gradient of chemoattractants (e.g. pro-inflammatory cytokines and chemokines) released by the inflammatory lesions into the blood stream and CSF. Following these signals, NPCs rapidly reach the source of pro-inflammatory molecules within and interact with the activated endothelial/ependymal cells around inflamed CNS tissues. At this level, NPCs and endothelial cells start an organized sequence of events resembling those described for T cell extravasation that allow the selective entrance and specific *homing* of transplanted cells in multifocal inflammatory CNS areas [16]. Interestingly enough, only small percentages (between 1-5%) of the systemically administered NPCs actually infiltrate and integrate within the CNS [11, 13, 140]. Mouse SVZ-derived adult NPCs transplanted in a subacute model of brain inflammation were shown to adhere to the CD49d counterligand VCAM-1 [16]. Further *in vivo* experiments showed that migration of mouse NPCs towards the site of damage is dependent on the CXCR4-SDF-1α signalling in mouse models of MS and brain tumour [17, 171]. In stroke models, the up-regulation of VCAM-1 on the surface of endothelial cells facilitates the targeting and the subsequent extravasation of VLA-4 expressing NPCs to the site of injury [18]. In line with this, mouse NPCs sorted via FACS for the presence of VLA-4 revealed a more efficient transendothelial migration in a mouse model of stroke after intracarotid injection [18]. More recently it

was shown that also the CCR2/CCL2 interaction is substantially involved in the recruitment of systemically delivered NPCs in a mouse model of stroke [172].

In vitro experiments confirmed that mouse NPCs express many functional receptors on theirs surfaces, among which the α4 subunit of the integrin VLA-4 [16], the SDF-1α receptor CXCR4 [173] and CD44, a cell-surface glycoprotein that binds to hyaluronic acid (HA) and is expressed also in activated T cells [174, 175]. Interestingly, NPCs led to the formation of transmigratory cups, enriched in multiple adhesion molecules such as ICAM-1 and VCAM-1, on the surface of endothelial cells [175] as previously shown for T lymphocytes diapedesis [176].

Similarly, also immortalized human NPC lines express CD44 [175] and CXCR4 [173]. However, in a recent study, human NPCs were shown to interact with activated ECs through integrins α2, α6 and β1 rather than CXCR4 [177]. Further, human NPCs express the receptors CXCR1 and CXCR5, which mediate their *in vitro* migration across a monolayer of human brain ECs in response to IL-8/CXCL8 and B lymphocyte chemoattractant (BLC)/CXCL13, chemokines previously known to favour the trans-endothelial migration of immune cells [178].

All these evidences suggest that systemically injected mouse and human NPCs share the expression of a variety of functional immune-like receptors, such as functional cell adhesion molecules (e.g. CD44 and VLA-4) and inflammatory chemokine receptors (e.g. CCR2, CCR5 and CXCR4), giving them a unique leukocyte-like molecular signature. This characteristic, allowing NPC interaction with activated endothelial and ependymal cells, represents an essential requirement in the therapeutic paradigm of systemic delivery. Therefore, the discovery of the specific homing ability of NPCs across the BBB opened new frontiers for the treatment of CNS diseases, in particular for those diseases characterized by disseminated damage.

4.3. NPC interaction with the dysfunctional CNS microenvironment: The establishment of ectopic niches

Consistent data exists reporting the ability of i.v. injected NPCs to cross the BBB and accumulate into the CNS. Here, exogenous NPCs co-exist with different host components, such as ECs, infiltrating inflammatory cells, activated macrophages/microglia and reactive astrocytes [19]. In this context, the intimate association with ECs, the physical proximity to the vasculature and the enhanced expression of stem cell regulators and growth factors involved both in angiogenesis and neurogenesis has been described to play a major role in defining a molecular architecture reminiscent of prototypical germinal stem cell niches [16]. Within these *atypical ectopic perivascular niches*, in addition to hierarchical (mother-to-daughter) communication, a sophisticated level of cell-to-cell horizontal communication takes place between transplanted NPCs and resident cells. Recent evidences confirm that NPCs are able to communicate with host cells via cellular contacts. For instance, functional gap junction formation has been shown to allow exogenous NPCs to rescue host neurons and their projections in animal models of Purkinje neurodegeneration. Gap junctions permitted the trans-cellular delivery of homeostasis-modulating molecules, as well as directly influenced the coordinated activity of the host network via Ca^{++} waves. Moreover, hypoxic preconditioning of NPCs before their *in vitro* engraftment increased Connexin 43 expression and improved subsequent communication

with host cells [179]. Possible mechanisms of communication include also secretion of growth factors, hormones, cytokines, chemokines and small molecular mediators [180], cell-to-cell interactions via tunnelling nanotubes [181] and secretion of circular membrane vesicles [182], other than cell-to-cell contacts [183].

Correlative evidence suggest that, depending on local inflammatory milieu, transplanted NPCs may either remain in the niche while maintaining an undifferentiated state, or move out from the niche, finally acquiring a terminally differentiated phenotype [16]. When systemically injected in chronic EAE mice, syngenic NPCs were found almost exclusively in areas of CNS damage, mainly within the submeningeal space in close proximity to subpial inflammatory foci (after i.t. stem cell injection), or around post-capillary venules (after i.v. stem cell injection) [11]. Ten days after transplantation, relatively few cells were found in the CNS parenchyma and at 30 dpi many of the surviving donor cells were localized deeply within the brain parenchyma and displayed a marked distribution pattern: most of them were confined within areas of demyelination and axonal loss, and only very few cells were found within regions where the myelin architecture was preserved [11]. Similar results were obtained after i.c.v NPC injection at the peak of EAE in rats: cells entered into the brain or spinal cord parenchyma and mostly accumulated at sites of inflamed white matter but not into adjacent grey matter regions. In line with the previous study, after 2 weeks cells had migrated into distant white matter tracts but, on the contrary, most of them had acquired specific markers of the astroglial and oligodendroglial lineages [184]. Mouse NPC transplants in rodents affected by EAE are also associated with significantly reduced glial scar formation [11] and an increased survival and recruitment of endogenous neural cells participating to the naturally occurring brain reparative response upon myelin damage [10, 15, 16, 185, 186].

Human NPCs have shown a higher rate of cell integration after being administered in different animal models. In particular, the HB1.F3 immortalized cell line, i.v. injected in a model of ischemic stroke, were shown to enter the ischemic area and differentiate into neurons and astrocytes, similarly to what observed with focal injected cells [12, 187]. Transplanted cells seemed to adapt their fate accordingly to the region of engraftment, showing the appropriate neuronal and glial markers. $NeuN^+$ and NF^+ cells were identified primarily in the CA1 area of the hippocampus and in the dentate gyrus, mixed with $GFAP^+$ cells. Vimentin, GFAP and NF markers showed a progressive expression during the first 2-3 weeks after transplantation, suggesting a step-by-step maturation of the cells [187]. The very same line of cells, injected in a rat model of ICH [137], was observed to infiltrate the brain, survive and migrate towards the peri-hematomal areas. The cells were found mainly differentiating into $GFAP^+$ and $NeuN^+$ cells. However, the rapid behavioural recovery observed in ICH rats as soon as 2 weeks after transplantation, suggested that the NPC therapeutic effect was mainly related to neuroprotection, rather than to integration into neuronal circuitry [137, 187], since the latter would require longer time to produce clinical ameliorations. A similar trend towards human NPC differentiation has been observed in animal models of SCI, SE and HD. HB1.F3 hNPCs administered in mice subjected to compression SCI, were observed to differentiate into βIII-$tubulin^+$ neurons at 21 days after transplantation [167]. GABA-immunoreactive interneurons were, instead,

observed originating from HB1.F3 when systemically administered the day after lithium-pilocarpine induction of experimental SE in rats [138]. Further, HB1.F3 cells injected 7 days after unilateral quinolinic acid (QA)-induced model of HD in rats were found to stay confined around blood vessels, mostly in the damaged hemisphere and only partially differentiating in $GFAP^+$ and $NeuN^+$ cells at 3 weeks post transplantation [14].

Despite these evidences showing the ability of exogenous NPCs to survive and differentiate into multiple derivatives according to local cues, the majority of the data provided has substantially failed to show convincing relevant differentiation and integration of transplanted NPCs *in vivo*. It is now quite evident that NPCs (and more generally somatic adult SCs) might protect the CNS trough mechanisms alternative to direct cell replacement, which imply the interaction of NPCs with both resident neural and immune cells [7, 188]. Cell replacement is therefore only one of the multiple ways by which transplanted NPCs can promote tissue repair, and a much more complex therapeutic scenario should be foreseen. The concept of *stem cell therapeutic plasticity* (or *functional multipotency*) has therefore emerged, describing the different way(s) NPCs use to interact with tissue-resident *vs.* infiltrating immune cells, at the level of the inflammatory tissue context in which they are either transplanted or migrate to after transplantation. These bystander effects, are mainly represented by *neuroprotection,* which might occur both through secretion of soluble factors and cell-to-cell contact interactions and *immunomodulation,* intended as the capacity of NPCs to influence the activity of the immune system in the CNS and/or in the periphery, at the level of secondary lymphoid organs [5, 19].

4.3.1. Tissue trophic effects

NPCs may exert their neuroprotective effect by increasing *in situ* bioavailability of several molecules, such as neurotrophins, growth factors and developmental stem cell regulators, thus promoting the survival and function of endogenous glial and neuronal progenitors that escaped the primary insults [19].

Mouse NPCs systemically injected in mice affected by middle cerebral artery occlusion (MCAo) were observed to mostly maintain an undifferentiated phenotype, while accumulating at the boundaries of the lesioned area [140, 141]. Tissue survival was associated with a down regulation of inflammation, glial scar formation and neuronal apoptotic cell death at both mRNA and protein levels [140]. Increased levels of BDNF, NGF and neurotrophin (NT)-3 were found in the CSF of stroke-affected rats after intra-cisterna magna administration of NPCs. In addition, immunohistochemical analysis of the injured brain revealed an increase of MHC class I levels in treated rats [141]. Interestingly, this neuroprotective effect in the ischemic microenvironment seems to start very soon after the systemic administration of cells. In fact, data have been provided showing an increase in the gene expression levels of IGF-1, VEGF, TGF-1β, BDNF and CXCL12/SDF1-α in the NPC-transplanted MCAo brain, as soon as 24 hours after the acute i.c.v. injection [189]. Further, NPCs have been proved to increase *in vivo* vascularisation when administered after stroke, most likely due to their ability to increase the presence of VEGF, FGF, BDNF and chemoattractant factors (such as SDF-1α), which promote angiogenesis and mobilization of endogenous endothelial progenitors [18, 190]

More recently, adult mouse NPCs systemically injected in mice (3 injections few hours after the injury) suffering from acute contusion SCI, showed an undifferentiated morphology (similarly to what observed in EAE) at the level of the damaged CNS. *Ex vivo* RT-PCR analysis showed NPC-driven up regulation of BDNF, NT-3, NGF, leukemia inhibitory factor (LIF) and TNF- α only at 48h after treatments, while no differences were observed neither at 24h or 7 days after transplantation [13]. Similarly to what observed by indirect evidence *in vivo*, real-time PCR gene expression analysis directly revealed high levels of NGF, BDNF and NT-3 and glial cell line-derived neurotrophic factor (GDNF) in the transcripts of cultured rat NPCs [141, 191]. In addition, in line with the observed pro-angiogeneic effect *in vivo* after transplantation of mouse NPCs in stroke models [18, 190], human NPCs were proved to secrete VEGF *in vitro* [192].

All these evidences suggested that the underlying molecular mechanisms by which transplanted NPCs instruct tissue protection effects are partly related to increased *in vivo* bioavailability of major neurotrophins [11, 138, 139, 193] able to modulate the host environment resulting more permissive to regeneration. Neurotrophins exert important roles as mediators in cell cycle regulation, cell survival, and differentiation during development and adulthood. The delivery of diffusible proteins to the CNS has been seen as a possible therapeutic weapon for neurological diseases. However, because the CNS is likely impenetrable for many of these diffusible proteins, NPCs might be envisaged as carrier of neurotrophic factors. To this aim, NPCs have been genetically modified to act as *Trojan horses* to deliver the desired diffusible molecules at the site of injury, thus fostering their innate capacity to secrete neurotrophic and growth factors [194]. Among all the candidate neurotrophic factors to be delivered, GDNF has shown a potent neuroprotective effect on a variety of neuronal inflammatory models, such as stroke and PD [195-197]. However, its effects are generally transient and need consecutive administrations to obtain long-standing results. NPCs over-expressing GDNF can instead provide durable neuroprotective effects, as shown with mouse NPCs, transplanted i.c.v in rats 3 days after MCAo [198]. The exogenous cells resulted in an overall increase of cell survival of endogenous cells after the insult, which in turn was associated to a partial functional recovery. Interestingly, treated rats also displayed a significant increase of the synaptic proteins synaptophysin and post-synaptic density protein (PSD)-95, suggesting an enhanced neuronal function and a possible reconstruction of endogenous neural circuitries after the grafting [198]. Finally, a recent study showed that the intravenous administration HB1.F3 human NPCs transduced with INF-β and cytosine deaminase (CD), was able to interfere with toll-like-receptor (TLR)-4 (up-regulated into the site of injury) suppressing the SCI-induced proliferation of reactive astrocytes and promoting functional recovery [199]. Other neurotrophic factors, such as BDNF and VEGF, have been over-expressed in NPCs and mainly tested upon intraparenchymal injection in models of SCI [200] or ICH [201, 202]. Taken together, these data suggest that the clinical amelioration observed in CNS disease animal models are, at least in part, mediated by a multilayered NPC neurotrophic signature.

4.3.2. Regulation of the immune system

Considerable evidence of the immune modulatory capacity of NPCs has derived from transplantation studies through different routes in the EAE model. As mentioned, transplanted NPCs are consistently found around inflamed blood vessels, in close contact with both endogenous neural cells (e.g. astrocytes and neurons) and CNS-infiltrating blood-borne $CD45^+$ immune cells [185]. Also, i.c.v. administered NPCs were found to attenuate brain inflammation primarily through a reduction of perivascular infiltrates and $CD3^+$ T cells with a concomitant increase of $CD25^+$ and $CD25^+/CD62L^+$ regulatory T cells [136]. Interestingly, i.v. injection of NPCs also protects against chronic neural tissue loss as well as disease-related disability in EAE, via induction of apoptosis of blood-borne CNS-infiltrating encephalitogenic T cells [185]

NPCs have been shown to possess immune modulatory capacity also in models of stroke, where T cells do not have a major role in the disease pathology. Irrespectively of the route of administration (systemically *vs.* intraparechimally), transplanted NPCs migrate towards the site of infarct in MCAo and ICH models [12, 137, 138, 203-207] and once reached the ischemic boundary zone (IBZ), grafted NPCs interact with the inflammatory environment. The subacute (*delayed*) i.v. injection of mouse NPCs after MCAo in mice, significantly down-regulates multiple RNA species involved in inflammation, including IFN-γ, TNF-α, IL-1β, IL-6 and leptin receptor [140]. Therefore, NPCs may exert an immune modulatory action, causing a profound down regulation of inflammatory *lymphoid* (T cells) and *myeloid* (macrophages) cells within inflamed brain areas.

While the inhibition of the T cell responses by NPCs is a quite established concept [208], the effect of the interaction between transplanted NPCs and microglia/macrophages is still controversial, mainly because of the non-univocal data regarding the role sustained by professional phagocytes under CNS inflammatory conditions. *In vivo* studies have in fact produced opposite evidences that might underline once more the bimodal action of some immune regulators [209]. NPC transplantation promote the infiltration of $CD11b^+$ myeloid cells into the brain of MCAo mice, thus suggesting that myeloid cell activation might be required for transplanted NPCs to exert part of their neuroprotective action [189]. Indeed, MCAo mice in which $CD11b^+$ microglia have been selectively ablated showed exacerbation of the ischemia-dependent brain injury [113]. However, several studies have showed a significant reduction of microglia/macrophages in the brain of mice, with either ischemic or haemorrhagic stroke, together with improved neuronal survival and locomotor functions after NPC transplantation [22, 140]. Also in this case, NPCs have been engineered in order to increase their immune modulatory capacity. Recently, mouse NPCs were transduced with IL-10, which has been proved to efficiently suppress EAE symptoms and promote survival of neurons and oligodendrocytes [210-212]. Mouse NPCs, transduced with a lentiviral vector encoding IL-10, showed enhanced ability to induce remyelination, neuronal repair and immune suppression after systemic injection in EAE mice compared to control NPCs [213].

In vitro, NPCs are shown to increase the apoptosis of $PLP_{139-151}$-specific Th1 pro-inflammatory (but not Th2 anti-inflammatory) cells through the engagement of death receptors, including FasL, TNF-related apoptosis-inducing ligand (TRAIL), and APO3L, on the surface of NPCs [16]. Mouse and rat NPCs also inhibit T cell activation and proliferation in response to T cell recep-

tor (TCR)-mediated stimuli (e.g., concanavalin A and anti-CD3/anti-CD28) [136, 214]. NPC/T lymphocyte co-culture experiments suggest that part of the anti-proliferative effect of NPCs might depend on the inhibition of IL-2 and IL-6 signalling on T lymphocytes [214]. In addition, NPCs have shown a selective pro-apoptotic effect on Th17 cells *in vitro* via a FasL (CD95L)-dependent mechanism, identifying the axis Fas-Birc3 as an additional survival pathway for NPCs [215]. Mouse C17.2 NPCs also suppress T-cell proliferation, at least in part, by reactive production of the soluble mediators nitric oxide (NO) and prostaglandin E2 (PGE2). High levels of NO and PGE2 are in fact induced in T cells when co-cultured with NPCs. In addition, inducible NO synthase (iNOS) and microsomal type 1 PGES (mPGES-1) are detected in NPCs in co-culture with T-cells, suggesting that NO and PGE2 production in NPCs is induced by exposure to activated T cells [216].

Human NPCs have been proved to suppress the proliferation and alter the cytokine secretion profiles of activated T cells on both xenogeneic antigen-specific T cells derived from EAE induced non-human primates (common marmosets), and allogeneic mitogen-activated T cells. Co-culture of human NPCs with T cells, revealed their immune modulatory capacity through both direct cell-to-cell contacts as well as via the release of soluble mediators into the culture medium [15]. Notably, in contrast to the mouse counterpart, human NPCs have a limited cytotoxicity against T cells *in vitro*, given that FasL is only barely detectable on their surface. However, human NPCs exposed to cytokines express high levels of TNF-α, resulting in a higher cytotoxic potential against monocytes and macrophages [217]. In line with this, immortalized human NPCs were also proved, trough direct *in vitro* experiments, to reduce T cell activation and proliferation. Conditioned media collected from human NSCs (HB1.F3 line) *in vitro*, directly suppress the proliferation of activated human T cells through both induction of apoptosis and cell cycle arrest. Nonetheless, human NPC-released mediators alter the cytokine production pattern of T lymphocytes, increasing the expression of IL-4, IL-10, TNF-α, and IFN-γ and decreasing IL-2, thus affecting the overall activation [200].

4.4. NPC interactions with the dysfunctional non-CNS microenvironment

In parallel to the observed immune modulation and neuroprotection into the CNS, other studies have shown that systemically injected cells may act also outside the injured CNS. Different studies, in fact, have reported the capacity of NPCs to target and synergize with immune cells at the level of secondary lymphoid organs (e.g. draining lymph nodes) and the spleen, resulting in the attenuation of the inflammatory response following EAE, stroke and SCI.

It was initially showed *in vitro* that NPCs strongly inhibited the ability of EAE-derived lymphocytes to produce pro-inflammatory Th1 cytokines in response to MOG_{35-55} stimulation. In addition, specifically activated T cells isolated from EAE mice treated with NPCs, were deficient in their ability to adoptively transfer EAE (to a naïve host), thus suggesting a long-lasting inhibition of encephalitogenicity of T cells [20]. Further data have been provided about a specific and almost exclusive targeting of the peripheral immune system in SJL mice with PLP-induced EAE, in which NPCs had been injected subcutaneously (s.c.) at 3 and 10 dpi [21]. This alternative administration protocol showed a significant clinical improvement in EAE mice despite injected cells were never been consistently found into the inflamed CNS. Sub-cutaneous-

ly injected, s.c.-injected cells were mainly found accumulating and persisting (up to 2 months) at the level of the perivascular areas of the draining lymph nodes, where they interacted with resident cells. Similarly to what observed in the CNS parenchyma, NPCs accumulated as focal clusters around blood vessels of the hilum and medullary/paracortical areas. Here they established close interactions with endothelial cells and cell-to-cell contacts with $CD11c^+$ DCs, $F4/80^+$ professional phagocytes and MHC class II^+ immune cells [21]. Further, *ex vivo* analyses of lymph nodes isolated from NPC-treated EAE mice, showed hampered activation and maturation of myeloid DCs. This was associated, according to both *in vivo* and *in vitro* analyses, to the release of BMP-4 and TNF-α or TLR agonists. The BMP-dependent effect is highly specific for immune regulatory NPCs and, in turn, leads to the restraint of encephalitogenic T cell expansion at sites of antigen presentation. In addition to BMP-4, transplanted NPCs modulated the local increase of major stem cell fate determinants, including BMP-7, the extracellular matrix protein tenascin C, Shh and Noggin. The pattern of NPC accumulation, the secretion of extracellular matrix proteins and stem cell regulators, and the lack of expression of neural lineage antigens (e.g. PSA-NCAM, class III β–tubulin, NeuN, NG-2 and GFAP) once more suggest the establishment of perivascular *atypical ectopic niche-like* areas in the peripheral lymph nodes, similarly to what already seen in the CNS [15]. Supported by these experiments, a successive step forward was undertaken to test NPCs therapeutic capacity in a non-human primate model of EAE. Systemically injected foetal human NPCs into MOG_{74-96}-immunized common marmosets delivered at either the clinical onset of the disease or at subclinical occurrence of MRI detectable brain lesions, were found not only at the level of perivascular inflammatory CNS areas but also in secondary lymphoid organs. In parallel to these observations, human NPCs interfere *in vitro* with a number of key functions, such as the differentiation of myeloid precursor cells (MPCs) into immature DC (iDC) and the maturation of iDC to functional mature DCs. A significant impairment of the differentiation of $CD14^+$ MPCs into $CD1a^+$ iDCs has been reported when MPCs were cultured with granulocyte macrophage colony-stimulating factor (GM-CSF) and IL-4 in presence of NPCs [15]. In the same study, NPCs influenced the up regulation of the co-stimulatory molecules CD80, CD86 and MHC-II on LPS-treated DCs, thus impairing their capacity to induce a proliferative allogeneic response in mixed leukocyte reaction *in vitro*. Clinically wise, the i.v. NPC injection resulted more efficacious than the i.t. NPC treatment. This might be related to either the higher number of surviving cells or to an additional peripheral effect. Systemic NPCs were, in fact, subjected to selective capturing into cervical lymph nodes where they persisted up to 3 months while establishing close contacts with blood-borne inflammatory cells [15].

Similarly to what observed in EAE models, i.v. administered human NPCs, in a rat model of ICH, revealed a peripheral therapeutic function in attenuating the inflammatory response to the insult [22]. In line with previous studies, cells were rarely observed into the injured brain while the majority of NPCs were found distributed within the systemic organs. In particular, few NPCs were observed in mesenteric lymph nodes while large numbers were detected in the spleen, especially in the marginal zone area, which is typically enriched in macrophages. Once again NPCs were found in close contact with immune cells and some of them were establishing cell-to-cell contact interactions with $CD11b^+$ spleen macrophages. This result was probably due to the existence of a link between brain and spleen inflammation, called "brain-spleen inflammatory coupling". Remarkably, splenectomy prior to ICH has been shown to

reduce the initial cerebral oedema and inflammatory cell infiltration caused by stroke [22]. NPC accumulation into the spleen, in this case, modulated brain inflammation by reducing the level of major inflammatory mediators in stroke, such as TNF-α, IL-6 and nuclear factor-kappa B (NF-κB), and consequently improved neurologic outcome.

It has been recently shown that mouse NPCs (from fully mismatched C57BL/6 mice) co-transplanted with pancreatic islet under the kidney capsule of Balb/c diabetic mice prevents acute islet allograft rejection. This effect was related to a significant reduction of $CD4^+$ T cells and with a concomitant enrichment of $CD4^+/CD25^+/FoxP3^+$ regulatory T cells in the spleen, inducing active tolerance. These data suggest that the peripheral immune-modulation exerted by NPCs could alleviate the immune reaction leading to organ rejection. Unfortunately this condition appeared strictly associated with the development of NPC-derived tumours mainly sustained by insulin secretion from the co-transplanted islets [218].

Whether most of the immune regulatory effects of systemically injected NPCs act mainly into the CNS or in the periphery is still under debate. Peripheral lymphoid organs have been demonstrated to play an important role in the regulation of the immune responses to myelin antigens in EAE and a very sophisticated modulation of T-cell self-reactivity is known to take place [219-221]. A very recent study proposes a molecular mechanism sustaining NPCs immune modulation capacity in EAE. The preventive (0 dpi) or therapeutic (10 dpi) i.v. administration of NPCs resulted in their accumulation in lymph nodes and spleen, with rare cells observed into the CNS and without any evidence of myelin repair. Nevertheless, treated mice showed partial clinical recovery. Remarkably, the authors achieved the same results even transplanting NPC conditioned-medium or minimally irradiated NPCs (unable to differentiate but capable of secreting cytokines and neurotrophins), evidence sustaining a true peripheral function of NPCs. In particular, the observed clinical amelioration seem to be related to the selective inhibition of encephalitogenic Th17 cell differentiation through secreted factors. LIF has been identified as the key factor responsible for the observed inhibition of Th17 cell differentiation and the authors elucidated the signalling pathway behind this novel mechanism of action, where LIF antagonizes IL-6-induced Th17 cell differentiation through ERK-dependent inhibition of STAT3 phosphorylation [23]. Further studies will be needed to establish the absolute relevance of these pre-clinical data in EAE, where peripheral lymphoid organs play an important role in the regulation of the immune responses to myelin antigens, and their potential for future applications in MS. All the preclinical data describing NPC therapeutic effect upon systemic administration are summarized in Table 1.

Disease Model	**Species**	**Transplant Features**				**Observed Effect(s)**	**Proposed Mechanism(s)**	**Refs**
		Cell type	*Cell no./ animal*	*Route*	*Time*			
Experimental Autoimmune Encephalomyelitis (EAE)								
Acute EAE	Rat	Rat neurospheres	1.5-2x10^4	i.c.v. or i.t.	Disease peak	Cell differentiatio	None	[10]

Disease Model	Species	Transplant Features				Observed Effect(s)	Proposed Mechanism(s)	Refs
		Cell type	*Cell no./ animal*	*Route*	*Time*			
						n (neuronal and glial and tissue trophism		
Acute EAE	Rat	Rat neurospheres	$2x10^4$	i.c.v.	0 dpi	Immune regulation (central)	None	[136]
Chronic EAE	Mouse	Mouse NPCs	$1x10^6$	i.v. or i.t.	22 dpi	(Low) cell differentiation and tissue trophism	Inhibition of reactive gliosis	[11]
Chronic EAE	Mouse	Mouse neurospheres	$2.5x10^3$	i.c.v.	6 dpi	Immune regulation (local)	Reduction of CNS inflammatory infiltrates, increase of regulatory T cells	[139]
Chronic EAE Passive EAE	Mouse	Mouse NPCs	$1x10^6$	i.v.	8 dpi	Immune regulation (peripheral)	Suppression of encephalitogenic T cells	[20]
Chronic EAE	Mouse	Human ES cell-derived NPCs	$5x10^5$	i.c.v.	7 dpi	Immune regulation (local)	Suppression of encephalitogenic T cells	[142]
Chronic EAE	Mouse	IL-10-transduced mouse NPCs	$1.5x10^6$	i.v. or i.c.v.	10, 22 or 30 dpi	Immune regulation (local, peripheral) and cell differentiation	Induction of T cell apoptosis, promotion of myelin debris clearance	[213]
Chronic EAE	Mouse	Mouse and human ES cell-derived NPCs	$2x10^6$	i.v.	0 or 10 dpi	Immune regulation (peripheral)	LIF-mediated inhibition of Th17 cell differentiation	[23]

Disease Model	Species	Transplant Features				Observed Effect(s)	Proposed Mechanism(s)	Refs
		Cell type	*Cell no./ animal*	*Route*	*Time*			
Chronic EAE	Mouse	Mouse MSC-derived NPCs	$3.5x10^4$-6. $1x10^6$	i.t.	21, 28 and 35 dpi	Tissue trophism	None	[258]
Chronic EAE Passive EAE	Mouse	CCR5-transduced mouse BM-derived NPCs	$1.5x10^6$	i.v.	22 dpi (peak)	Immune regulation	None	[259]
Chronic EAE	Common Marmoset	Human NPCs	$2\text{-}6x10^6$	i.t. or i.v.	Disease onset	Immune regulation (central)	Suppression of encephalitogenic T cells, imparment of dendritic cell maturation	[15]
Relapsing EAE	Mouse	Mouse NPCs	$1x10^6$	i.v.	Disease onset or first relapse	Immune regulation (central)	Induction of T cell apoptosis	[16]
Relapsing EAE	Mouse	Mouse NPCs	$0.5x10^6$	s.c.	3 and 10 dpi, or 10 dpi only	Immune regulation (peripheral)	BMP-4-dependent hindrance of dendritic cell maturation	[21]
Relapsing EAE	Mouse	Mouse NPCs and Olig2-transduced NPCs	$1.5x10^5$	i.c.v.	Disease onset or first relapse	Immune regulation (central) and Tissue trophism	None	[260]
				Stroke				
MCAo	Rat	Rat NPCs	$1x10^5$	i.t.	2 dpi	Cell differentiation (neuronal)	None	[204]
MCAo (10' or 90')	Rat	Human NPCs	$5x10^6$	i.v.	1 dpi	Cell differentiation (neuronal, glial)	None	[12, 187]
MCAo (180')	Rat	Rat NPCs	$1x10^5$	i.t.	2 dpi	Tissue trophism	Increased angiogenesis	[190]

Disease Model	Species	Transplant Features				Observed Effect(s)	Proposed Mechanism(s)	Refs
		Cell type	*Cell no./ animal*	*Route*	*Time*			
MCAo (180'	Rat	Human NPCs	$1x10^6$	i.v.	2 dpi	Tissue trophism	None	[261]
CCAo + global hypoxia-ischemia	Mouse	Mouse 17.2 NSCs	$3x10^5$	i.ca.	2 dpi	Tissue trophism	Increased angiogenesis	[18]
MCAo (120')	Rat	GDNF-transduced rat NPCs	$5x10^5$	i.c.v.	3 dpi	Tissue trophism, Cell differentiation (neuronal)	None	[198]
MCAo (45')	Mouse	Mouse NPCs	$1x10^6$	i.v.	3 dpi	Immune regulation (local) and Tissue trophism	Reduction of microglial activation and neuronal death	[140]
MCAo and CCAo	Rat	Rat NPCs	$1.5x10^5$	i.t.	7 dpi	Immune regulation and tissue trophism	Neuroprotection mediated by NGF and modulation of class I MHC expression	[141]
MCAo (90')	Rat	HIF-1α-transduced rat NPCs	$1x10^6$	i.c.v.	1 dpi	Tissue trophism	Promotion of angiogenesis	[227]
MCAo (45')	Mouse	TAT-Hsp70-transduced mouse NPCs	$1x10^6$ or $5x10^5$	i.v. or i.p.c.	Acute	Tissue trophism, reduction of ROS formation and BBB leakage	Neuroprotection and enhanced neurogenesis	[232]
ICH	Rat	Human NPCs	$5x10^6$	i.v.	1 dpi	Tissue trophism, Cell differentiation	Neuroprotection and integration in endogenous	[137]

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Disease Model	Species	Transplant Features				Observed Effect(s)	Proposed Mechanism(s)	Refs
		Cell type	*Cell no./ animal*	*Route*	*Time*			
						n (glial and neuronal)	neuronal circuitries	
Spinal Cord Injury (SCI)								
Contusion (T8)	Mouse	Mouse NPCs	$1x10^6$ or$1x10^5$	i.v. or i.p.c.	Acute	Tissue trophism	Reduction of apoptosis and modulation of TNF-α expression	[13]
Compression (T8)	Mouse	Human NPCs	$1x10^7$	i.v.	7 dpi	Cell differentiation (neuronal, glial)	None	[167]
Contusion (T12)	Mouse	Mouse NPCs and MOG_{35-55} immunization	$5x10^5$	i.c.v.	7 dpi	Immune regulation (local) and Tissue trophism	T-cell mediated activation of microglia with a protective phenotype	[226]

BBB: blood-brain barrier; BM: bone marrow; BMP-4: bone morphogenetic protein 4; CCAo: common carotid artery occlusion; CCR5: C-C chemokine receptor type 5; dpi: days post immunization/injury; ES cells: embryonic stem cells; GDNF: glial-derived neurotrophic factor; HIF-1α: hypoxia-inducible factor 1α; i.ca.: intracarotid; ICH: intracerebral haemorrhage; i.c.v.: intracerebroventricular; i.p.c.: intraparenchymal (perilesional); i.t.: intrathecal; i.v.: intravenous; LIF: leukemia inhibitory factor; MCAo: middle cerebral artery occlusion; MSC: mesenchymal stem cells; ROS: reactive oxygen species; s.c.: subcutaneous; TAT-Hsp70: TAT-heat shock protein.

Table 1. Neuro-immune interaction following systemic neural stem cell transplantation in experimental disease models.

5. Pros and cons of NPC systemic administration

In parallel to the investigation concerning the principal mechanism(s) sustaining NPC therapeutic efficacy, other questions, such as (i) the ideal administration route, (ii) the amount of cells to be transplanted and (iii) the optimal time point for cell delivery need to be answered. Among the different possible routes of cell administration, intravenous cell delivery represents one of the most attractive because of its technical simplicity and clinical practicability. However, i.v. and i.t. administrations result in lower numbers of cells infiltrating the CNS, compared to local stereotaxic-driven intracerebral injections, a reason why local injections of cells are commonly preferred in clinical trials (see next section) despite the higher invasiveness of the procedure. Even though initially investigated for multifocal disorders (e.g. MS), in order

to deliver exogenous cells to all the disseminated inflammatory foci, all the previous experimental data suggest that intravenous or intrathecal administration routes could be desirable even for focal damages, such as those occurring in stroke and spinal cord injury [222]. In experimental animal studies, i.p.c [223-225], i.v. [137, 185] i.a. [222], i.t. [204, 226] and i.c.v. [142, 227] protocols have been tested so far. However, only few comparative studies have been conducted, testing pros and cons of the different administration routes. These studies (mainly in animal models of stroke) evidenced the obvious capacity of intraparenchymal injection to deliver higher numbers of cells *in situ*, compared to i.c.v. and i.v. [228]. By contrast, systemic injections are thought to lead to a wider distribution of cells around the focal lesioned area. This aspect is extremely important if we consider that human stem cells (and in particular hNPCs) are still a limited resource [229]. Intravenously injected NPCs are firstly delivered to peripheral organs, such as lungs, liver, spleen and kidney [16, 230]. This whole-body distribution of exogenous systemic injected NPCs significantly reduces cell homing to the injured brain [222]. To avoid this problem, at least partially, intra-arterial administration could be a valid alternative (possibly coupled with pre-interventional imaging-based planning) to selectively cover an injured volume supplied by several target vessels. Intracarotid injection has already been proved to be functional for delivering stem cells in models of stroke, TBI and SCI, resulting in higher numbers of extravasating cells (20%) compared to i.v. injections [18]. Nevertheless, although the number of cells infiltrating the CNS has been sometimes described as fundamental, or at least proportional to their therapeutic effect [231], others have shown that very low numbers of cells [140] can result into similar outcomes (in term of functional recovery) compared to higher numbers of locally injected cells. This effect may be explained by the fact that cell replacement is unlikely the only mechanism sustaining stem-cell therapeutic potential. Higher starting numbers of cells, in fact, increase the therapeutic potential of intracerebral administered cells, but did not affected the efficacy of the i.v. injected cells. This again suggests that the number of cells is much more important for focal than systemic injections [232].

Importantly, when evaluating the optimal protocol, we should consider the procedure itself, so that the risk should not outweigh the benefits of the treatment. From this point of view, i.e. cell injection might be accompanied by increased mortality during cell delivery, probably due to further ischemia or thrombosis [233, 234]. By contrast, cell transplantation trough the vertebral artery, into patients affected by SCI, showed no adverse effects [235].

Another important unsolved issue for experimental stem cell therapies is the ideal time point of transplantation. As described, the inflammatory activation of the CNS, characterizing MS, stroke, SCI, epilepsy, AD, PD, HD is necessary for the homing of systemically injected cells. Because of the rapid and dynamic changes occurring into the CNS during these inflammatory conditions, the time of transplantation should be evaluated carefully. In fact, cell death, excitotossicity, reactive oxygen species accumulation, inflammatory cell infiltrations and glial scar formation, cause a rapid evolution in the damaged tissue, while creating an hostile microenvironment for the engraftment of exogenous cells. This is important irrespectively to the route of administration. For example, the acute focal transplantation of cells into the ischemic brain or the injured spinal cord reduces the therapeutic efficacy of the cells, which

are subjected to highly inflammatory conditions causing cell death [205, 236]. On the contrary, the sub acute phase (few days after insult, in rodents) of the injury seems to be characterized by better conditions for stem cell survival and a permissive microenvironment for tissue repair/healing [237]. Although higher inflammation generally correlates with higher number of cells infiltrating the CNS, it has been shown that greater numbers of cells accumulated into the spinal cord after i.v. injection at 7 dpi compared to 3 and 10 dpi [167]. However, the optimal time window for cell transplantation is still elusive and depends mainly on the type of pathology and aim of the treatment. While neuroprotection should be addressed in the early stage of the inflammatory disease, just after the initial insult, cell replacement and neuroregeneration should be targeted in a later stage, when the lesion has stabilized. Indeed, administration route, number of cells and time window and seem intimately related and it is not so difficult to envisage a future in which a combination of early i.v. and late i.p.c administration of different stem cell sources will be enrolled for the treatment of so far incurable CNS disorders.

6. Clinical trials

In the last two decades the clinical potential of stem cells in the field of regenerative/restorative medicine has been often matter of debate, mainly because of its inconsistent outcome. As an example, the first attempt to treat a CNS disorder by means of stem cell transplantation took place in the '80s: autologous adrenal medulla cells were intracerebrally transplanted into the striatum of PD patients to provide a local source of catecholamine. The study was proved safe although with minimal beneficial effect. Further, the first intrastriatal grafts of human foetal ventral mesencephalic (neuronal preparations) tissue have provided proof-of-concept that cell therapy can work in patients affected by PD [238]. However, subsequent randomized, double-blind, placebo-controlled trials brought to much more sceptical conclusions because of patients showing functional decline (post transplantation) due to dyskinesias (graft-induced involuntary movements), originated by excessive graft function [239, 240].

Prospectively, many factors can be contended to (partially) justify these patchy results. First, it is now clear that different cell types are needed for different diseases. If on one side PD and amyotrophic lateral sclerosis (ALS) patients will require cells with dopaminergic and motor neuron properties respectively, on the other side, cell replacement in AD patients is much more complicated by the necessity to replace a large variety of cell populations lost in different brain areas. Second, even though initially expected and long-term envisaged, neuronal replacement and circuitry integration of transplanted NPCs have been poorly proved. Third, it as to be considered that pre-clinical animal studies only represent models of human conditions, and, as such, they offer an exceptionally homogeneous platform, where the genetic background, age, and environment are all alike. Clearly, this is not the case with patients. Further, even if multiple models have been established to investigate different aspects of a given disease, none of them can faithfully emulate the human pathology in its complexity [241, 242]. This is particularly challenging considering the rate of progression and lack of validated surrogate disease markers typical of many neurodegenerative disorders. While these aspects are most

likely destined to remain unsolved pitfalls, others (including the amount of cells to be transplanted, the manipulation protocols used, the time of transplantation, the route of cell delivery and the statistics adopted to analyse the data) need to be ameliorated through the establishment of common guidelines. In particular, the International Society for Stem Cell Research (ISSCR) composed with a group of international experts (scientists, surgeons, ethicists and patient advocates) "The ISSCR Guidelines for the Clinical Translation of Stem Cells" [243] to trace a roadmap guiding the application of experimental stem cell therapeutics in patients. Importantly, when translating into clinical trials, the choice of the "ideal patient" imposes major scientific and ethical constraints. Indeed, if on one side the treatment of the most chronic/severe patients who were not able to respond to previous treatment lowers the blame for a possible ineffectiveness of a therapy, on the other side the scenario offered by such a compromised tissue may hinder the potential effect of the treatment.

The primary importance of patient's care dramatically impacts also on the choice of the best route of cell delivery. Indeed, if one side the intravenous injection allows for a less invasive procedure, on the other, the number of cells delivered to the site of interest is lower compared to local injections. Further, the intracerebral transplantation has been widely accepted, by both clinicians and patients, after years of clinical applications and technical improvements. These, together with the relatively limited availability of human NPCs explains why most of the clinical trials started so far have nevertheless favoured the adoption of more invasive procedures, such as intraparenchymal/intracerebroventricular ones (see Table 2). However, as discussed, the correlation between the number of cells entering the CNS and their efficacy still need to be confirmed.

Sponsor and place	**Disease**	**Trial phase**	**Patients (no)**	**Age at enrolment (y)**	**Follow up (months)**	**Transplant Features**					**Status**	**Principal Investigator**	**Trial Identifier**	**Outcome and Notes**
						Cell type	*Cell no./ patient*	*Route*	*Time after disease/ injury*	*Immune suppression*				
StemCells, Inc. at University Hospital Balgrist-Uniklinik Zurich, (Switzerland)	Thoracic spinal cord injuries (SCI)	I/II	12	18-60	12	HuCNS-SC® (Foetal, Brain-derived, Allogeneic, single donor)	$2x10^7$	Multiple injections, Single dose, Intramedullar	≥ 3 months	Y (9 months pt)	AR	Armin Curt, MD	NCT01321333	NA

Sponsor and place	Disease	Trial phase	Patients (no)	Age at enrolment (y)	Follow up (months)	Transplant Features					Status	Principal Investigator	Trial Identifier	Outcome and Notes
						Cell type	*Cell no./ patient*	*Route*	*Time after disease/ injury*	*Immune suppression*				
ReNeuron, Ltd. at Glasgow Southern General Hospital, Glasgow (UK)	Stable Ischemic Stroke (PISCES)	I	12	60-85	24	CTX0E303 (Foetal, Brain-derived, c-myc immortalized, Allogeneic, single donor)	2-20x10^6	Single injection, Four Ascending doses, Intracerebral (putamen)	0.5-5 years	NA	AR	Keith Muir, MD	NCT01151124	NA
Neuralstem, Inc. at Emory University, Atlanta (USA)	Amyotrophic Lateral Sclerosis (ALS)	I	18	> 18	48	NSI-566RSC (Foetal, Spinal cord-derived, Allogeneic, single donor)	0.5-1x10^6	Multiple injections, Intraspinal	≥ 1.5 years	Y (≥ 4 months pt)	AnR	Eva Feldman, MD, PhD	NCT01348451	[248, 249]
Azienda Ospedaliera Santa Maria, Terni (Italy)	ALS	I	18	20-75	36	Foetal, Brain-derived, Allogeneic, single donor	2.5x10^5/ injection	Multiple injections, Single dose, Intraspinal	> 6 months	NA	AR	Angelo Vescovi, PhD	NCT01640067	NA
StemCells, Inc. at University of California, San Francisco (USA)	Pelizaeus Merzbacher disease (PMD)	I	4	0.5-5	12	HuCNS-SC®	3x10^8	Multiple injections, Single dose, Intracerebral	NA	Y (9 months pt)	AnR	Stephen Huhn, MD	NCT01005004	[250]

Sponsor and place	Disease	Trial phase	Patients (no)	Age at enrolment (y)	Follow up (months)	Transplant Features					Status	Principal Investigator	Trial Identifier	Outcome and Notes
						Cell type	*Cell no./ patient*	*Route*	*Time after disease/ injury*	*Immune suppression*				
StemCells, Inc. at Oregon Health and Science University, Portland (USA)	Neuronal Ceroid Lipofuscinosis (NCL)	I	6	1.5-12	13	HuCNS-SC®	0.5-1x10^9	Multiple injections, Single dose, Intracerebral	NA	Y (12 months pt)	C	Robert Steiner, MD	NCT00337636	[247]
StemCells, Inc. Retina Foundation of the Southwest, Dallas (USA)	Age-related Macular Degeneration (AMD)	I/II	16	> 50	12	HuCNS-SC®	0.2-1x10^6	Single injection, Single dose, Subretinal	NA	Y (3 months pt)	AR	David Birch, PhD	NCT01632527	NA

pt: post-transplant; NA: information not available. AR: Active Recruiting; AnR: Active not Recruiting; C: Completed.

Table 2. Active clinical trials with neural stem/precursor cells.

Other challenging problems that need to be faced when approaching the clinic, are related to safety, product potency, and manufacturing quality of the cell source. Indeed, principles of good tissue practice (GTP) and good manufacturing practice (GMP) are mandatory requirements, especially when dealing with cells of human origin [244].

Last, but not least, some major issues related to the long-term safety of the cellular product need to be solved. It is important to stress how, differently from the classical drug-therapy, a cell-based treatment cannot be discontinued, since once the cells are within the patient they cannot be removed. Therefore, long-term pre-clinical data need to be collected before translating from the bench to the bed-side to avoid the occurrence of dramatic outcomes, such as the one involving a young patient suffering of Ataxia Telangiectasia who developed a donor-derived brain tumour following neural stem cell transplantation [245].

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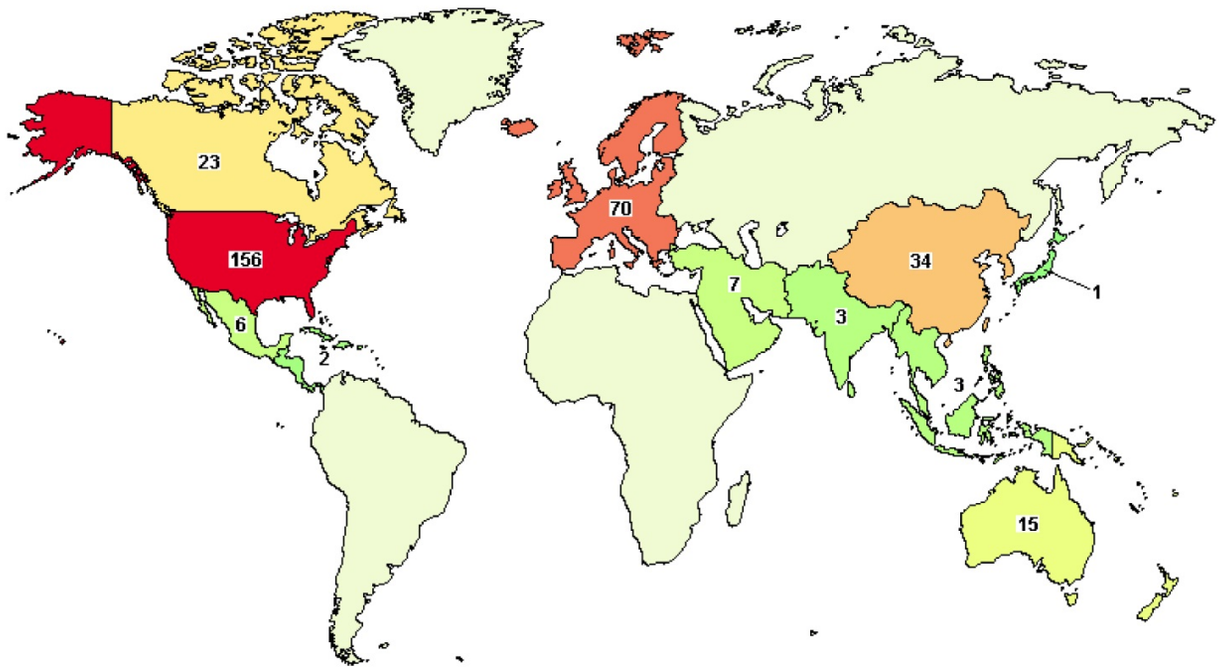

Figure 2. Worldwide open experimental clinical trials involving the use of stem cells for CNS disorders. The map shows the distribution of open stem cell-based clinical trials enrolling patients affected by CNS pathologies and treated with different source of SCs. Figure has been generate by clinicaltrials.gov, using the keywords *stem cells* AND *CNS diseases*; the inclusion criteria were only open studies; while the exclusion criteria where studies with unknown status. Stem cells in the figure include: neural stem/precursor cells, mesenchymal stem cells from the bone marrow and the adipose tissue, hematopoietic stem cells, embryonic stem cells.

Nevertheless, translational research did not (and should not) stop. As a matter of fact, the huge amount of data collected so far led to the development of numerous early stage clinical trials. There are currently 1750 studies employing the use of stem cells in interventional clinical trials, among which 280 are testing NPCs. Within the total number of clinical studies, 277 worldwide open trials involve SCs and patients affected by CNS disorders (Figure 2), such as MS, stroke, SCI, epilepsy, ALS, PD, AD, HD, neuronal ceroid lipofuscinose (NCL), Pelizaeus–Merzbacher disease (PMD), age-related macular degeneration (AMD) [246]. Several phase I and II clinical studies with NPCs (9 in highly debilitating CNS disorders, Table 2) have now been started with the primary aim to verify the safety (mainly in terms of toxicity) and feasibility - rather than the efficacy - of the treatment. Further, clinical studies are often accompanied by non-official secondary end-points usually concerning the potential impact in the clinical outcomes. These explorative trials have certainly a key role in stem cell medicine development, as both phase II dose-escalation studies and the inclusion of non-fatal diseases with larger population bases will definitely be facilitated once human safety will be established.

In May 2006 at Oregon Health and Science University (OHSU, Portland, OR, USA) the first human study involving the transplantation of allogeneic NPCs was started on a fatal rare neurometabolic syndrome, such as the NCL Batten's disease. In this open-label dose-escalating phase I trial, a total of 6 subjects with infantile and late-infantile NCL were transplanted in a single-stage procedure. StemCell, Inc. proprietary, single donor allogeneic free-floating

cultured, foetal-derived brain human NPCs (HuCNS- SC®) were directly administered to the cerebral hemispheres and lateral ventricles. Immune suppression was administered for 12 months after transplantation. This study has now been completed with one out of 6 patients died for disease progression, 11 months after treatment. The cell transplantation and combination with prolonged immune suppression were both well tolerated [247].

In September of 2009, NeuralStem, Inc. sponsored a phase I trial in ALS at the Emory University School of Medicine (Atlanta, GA, USA), using proprietary single donor allogeneic, adherent cultured, foetal-derived spinal NPCs (NSI-566RSC). NSI-566 cells were surgically implanted on a total of 12 patients via multiple injections directly into the thoracic spinal cord (either unilateral or bilateral). The clinical assessments demonstrated no evidence of acceleration of disease progression with the planned 18 months post-transplantation follow up [248, 249]. StemCell, Inc. is also sponsoring other two phase I trials with HuCNS-SC® in X chromosome linked connatal leukodistrophy PMD (in which oligodendrocytes cannot myelinate axons) and AMD. With the PMD trial at the University of California, San Francisco (UCSF, San Francisco, CA, USA), HuCNS-SC® were directly delivered through multiple injections into the brain of a total of 4 male patients (clinicaltrials.gov identifier no. NCT01005004). Data regarding this clinical trial has been recently published [250]. The transplantation procedure, the immunosuppression and the cells were well tolerated by all the 4 patients. No adverse effects related to the implant were detected. MRI investigation before and after the transplantation of cells, revealed, after 9 months, a consistent donor cell-derived myelination *in situ*, in three of the patients. However, these data are just published and under intense scientific discussion. With the AMD trial at the Retina Foundation of the Southwest (Dallas, TX, USA), HuCNS-SC® are being delivered directly into the subretinal space of one eye in a single transplant procedure in a total 16 patients. The estimated completion date of this study is March 2014 (clinicaltrials.gov identifier no. NCT01632527).

In June 2012, the Glasgow Southern General Hospital (Glasgow, Scotland) enrolled the first patient (of 12 total) of the dose-escalating Pilot Investigation of Stem Cells in Stroke (PISCES) phase I trial to be transplanted in a single-stage procedure with direct cerebral (intraparenchymal) delivery of Reneuron, Ltd. proprietary single donor allogeneic adherent cultured, c-myc immortalized foetal-derived brain human NPCs (CTX0E03) (clinicaltrials.gov identifier no. NCT01151124).

In March 2011, the University Hospital Balgrist (Zurich, Switzerland) enrolled the first patient (of 12 total) with chronic thoracic (T2–T11) SCI (3 to 12 months after complete and incomplete cord injuries) to be transplanted with HuCNS-SC® in a further StemCell, Inc. sponsored phase I/II clinical trial estimated to be concluded in March 2016. A single dose ($20x10^6$ cells) of HuCNS-SC® has been directly implanted through multiple injections into the thoracic spinal cord, and immune suppression administered for 9 months after transplantation (clinicaltrials.gov identifier no. NCT01321333). In November 2012 started the consequent long-term follow up of the 12 patients subjected to HuCNS-SC® transplantation that will last until March 2018 (clinicaltrials.gov identifier no. NCT01725880).

In June 2012, the Azienda Ospedaliera Santa Maria (Terni, Italy) enrolled the very first of total 18 ALS patients to treat with intraspinally implanted allogeneic free-floating cultured, foetal-derived brain NPCs. (clinicaltrials.gov identifier no. NCT01640067).

Importantly, there are not yet clinical trials with NPCs in MS. However, a consensus paper has recently been produced by a group of experts to define the uniform guidelines on the development of haematopoietic and non-haematopoietic stem cell therapies for MS [9]. All the current clinical trials envolving NPCs for CNS disorders are described in Table 2.

While in this paragraph we offer an overview of the current clinical trials involving solely human NPCs, it has to be said that, in the light of the neuroprotective/immunomodulatory (rather than cell replacement) properties attributed to stem cells, the therapeutic plasticity of cells of non-neural origin are being tested as well. Among these, MSCs are emerging as a good potential candidate, mainly because of their great accessibility and remarkable proliferation. Also, growing evidence suggests that other than giving origin to multiple derivatives of the mesodermal lineage (from which they derive), under particular conditions MSCs seem able to *transdifferentiate* into neuro-ectodermal cells *in vitro* [251-254]. However, this ability to convert from one lineage to another is still highly questionable and opened to different interpretations. Several studies have also proved the ability of MSCs to survive, migrate and eventually bring about functional recovery when transplanted into the CNS of different experimental models of neurological diseases (for a review see [255]). However, the mechanisms yielding to such rescue are unlikely ascribable merely to cell replacement.

Since 2006, the advent of induced pluripotent stem cells (iPSCs, [24]) technology has brought new excitement in medical research and clinical therapy, since these cells provide a valuable alternative without being constrained by ethical issues and immunological incompatibility [256]. Although still under debate about their long-term safety, the methods for iPSC generation, reprogramming and differentiation efficiency, iPSCs represent a break-trough for both study of disease mechanisms and investigation of potential new treatments (for a perspective analysis, see [257]).

The potential impact of this technological platform has been further boosted by the scientific stream emerged from iPSC technology that is the "direct reprogramming" from one somatic lineage to another. In fact, the direct conversion of fibroblasts to functional neurons (iN cells) or iNSCs [25, 26], for example, represents one of the most exciting, ultimate technologies for future application in CNS pathologies. Thanks to these next generation techniques, it will be possible to derive virtually unlimited numbers of specific neural/neuronal population bypassing the pluripotent stage, thus likely eliminating the potential presence of unwanted undifferentiated cells. However, many issues, such as the purity of the cell preparation, the use of virus-based technologies and the proper *in vivo* integration and differentiation still need to be better addressed. Importantly, the availability of such a high number of cells will release the intravenous protocol from one of its major limit, thus casting new light on its clinical potentiality.

Abbreviations

AD: Alzheimer's disease

ALS: Amyotrophic lateral sclerosis

APC: Antigen presenting cell

ASCL1: Achaete-scute homolog 1

BBB: Blood brain barrier

BCSFB: Blood-cerebrospinal fluid barrier

BDNF: Brain-derived neurotrophic factor

BLMB: Blood-leptomeningeal barrier

BMP: Bone morphogenetic protein

BMSC: Bone marrow-derived stem cell

CCAo: Common carotid artery occlusion

CCL: Chemokine (C-C motif) ligand

CCR: C-C chemokine receptor

CNS: Central nervous system

CNTF: Ciliary neurotrophic factor

CSF: Cerebrospinal fluid

CXCR: C-X-C chemokine receptor

DCs: dendritic cells

DCX: Doublecortin

DG: Dentate gyrus

DGC: Dentate granule cell

Dlx: Distal-less homeobox

d.p.t.: days post transplantation

EAE: Experimental autoimmune encephalomyelitis

EC: Endothelial cell

ES cells: Embryonic stem cells

FACS: Fluorescence-activated cell sorting

FGF: Fibroblast growth factor

GABA: Gamma-aminobutyric acid

GCL: Granule cell layer

GDNF: Glial-derived neurotrophic factor

GFAP: Glial-fibrillary acidic protein

GF-CSF: granulocyte macrophage colony stimulating factor

GMP: Good manufacturing practice

GTP: Good tissue practice

HD: Huntington's disease

hNPC: Human neural stem/precursor cell

Hsp70: Heat shock protein 70

HuCNS-SC: Human CNS stem cell

HVc: hyperstriatum ventrale, pars caudalis

i.a.: Intraartery

i.c.v.: Intracerebroventricular

i.p.c.: Intraparenchyma

i.t.: Intrathecal

i.v.: Intravenous

ICAM: Intercellular adhesion molecule

ICH: Intracerebral hemorrhage

Ig: Immunoglobulin

IGF: Insulin-like growth factor

IL: Interleukin

IML: Inner molecular layer

iN cells: Induced neuronal cells

INF: Interferon

iNOS: inducible nitric oxide synthase

iNSC: Induced neural stem cell

IPC: Intermediate progenitor cell

iPS: Induced pluripotent stem cell

LFA: Leukocyte-function associated antigen

LIF: Leukemia inhibitory factor

LPS: Lipopolysaccharide

MAdCAM: Mucosal addressin cell adhesion molecule

MCAo: Middle cerebral artery occlusion

MCP: Monocyte chemoattractant protein

MHC: Major histocompatibility complex

MMS: Medial migratory stream

MOG: Myelin oligodendrocyte glycoprotein

MPC: Myeloid precursor cell

MRI: Magnetic resonance imaging

MS: Multiple sclerosis

MSC: Mesenchymal stem cell

NCL: Neuronal ceroid lipofuscinose

NeuN: Neuronal nuclei

NF: Neurofilament

NF-kB: Nuclear factor-kB

NGF: Nerve growth factor

NO: Nitric oxide

NPC: Neural stem/precursor cell

NSC: Neural stem cell

OB: Olfactory bulb

OPC: Oligodendrocyte progenitor cells

PD: Parkinson's disease

PGE2: prostaglandine 2

PLP: Proteolipid protein

PSA-NCAM: Polysialylated neural cell adhesion molecule

PSGL: P-selectin glycoprotein ligand

pt: Post-transplantation

RA: Radial astrocyte

RMS: Rostral migratory stream

s.c.: Subcutaneous

SCF: Stem cell factor

SCI: Spinal cord injury

SC: Stem cell

SDF: Stromal cell-derived factor

SE: Status epilepticus

SGZ: Subgranular zone

Shh: Sonic hedgehog

SIDS: Stroke-induced immune depression syndrome

TCR: T cell receptor

TGF: Transforming growth factor

TJ: Tight junction

TLE: Temporal lobe epilepsy

TLR: Toll-like receptor

TNF: Tumor necrosis factor

V-SVZ: Ventricular-subventricular zone

VCAM: Vascular cell adhesion molecule

VEGF: Vascular endothelial growth factor

VLA: Very late antigen

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