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Achieving stable human stem cell engraftment and survival in the CNS: is the future of regenerative medicine immunodeficient?

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Abstract

There is potential for a variety of stem cell populations to mediate repair in the diseased or injured CNS; in some cases, this theoretical possibility has already transitioned to clinical safety testing. However, careful consideration of preclinical animal models is essential to provide an appropriate assessment of stem cell safety and efficacy, as well as the basic biological mechanisms of stem cell action. This article examines the lessons learned from early tissue, organ and hematopoietic grafting, the early assumptions of the stem cell and CNS fields with regard to immunoprivilege, and the history of success in stem cell transplantation into the CNS. Finally, we discuss strategies in the selection of animal models to maximize the predictive validity of preclinical safety and efficacy studies.

Keywords

clinical translation; engraftment; human stem cells; immunodeficient; immunosuppression; neurological disease; neurotrauma; regenerative medicine; stereology; transplantation; xenograft

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Stem cell treatments for neurological disease and injury: animal models

The loss of cells of the CNS is a hallmark of neurological disorders and traumatic neural injury, such as Parkinson's disease, Huntington's disease, Alzheimer's disease, multiple sclerosis, amyotropic lateral sclerosis, spinal cord injury (SCI), traumatic brain injury and stroke. Currently, there are very few therapeutic interventions to ameliorate these conditions in the human patient population. However, stem cell therapy has potential to provide methods of repairing, regenerating or protecting the damaged CNS. Successful efficacy using human stem cells in animal models has already led to multiple human clinical trials [1–3], which have been sponsored by several sources (Geron, StemCells, Inc., and Advanced Cell Technology).

A stem cell is a cell that possesses the ability to both self-renew and differentiate into multiple cell types. Embryonic stem cells are pluripotent, capable of differentiating into all cell types of the body [4]. Recent advances in the stem cell field have resulted in the development of induced pluripotent stem cells reprogrammed from somatic cells, which have been shown to have a remarkably similar fate potential to embryonic stem cells [5]. Proceeding down the lineage tree of development, later stem cell populations become more restricted to the tissue from which the cells were derived. For example, neural stem cells of the CNS have a restricted fate potential and are capable of only differentiating into neurons and glia [6]. For the purposes of our review of the neurological disease and injury literature (Tables 1–11), we will focus on human embryonic, fetal and adult-derived cells, in particular highlighting the issues associated with neurotransplantation of these populations.

Transplantation of stem cells into the diseased or injured CNS allows a unique replacement therapy not afforded by pharmacological therapeutics. Stem cells can provide benefit by differentiating and integrating into the host to restore functional and behavioral deficits that result from the loss of host CNS cells [2,7,8]. However, stem cells can also provide trophic support or deficient factors to the host tissue, reducing cell loss or potentially promoting host regeneration/plasticity mechanisms to restore function [9,10]. In some cases, the benefits of stem cell transplantation may derive from the short-term neurotrophic/neuroprotective effects during the acute phase postinjury/transplantation. However, the risk: benefit ratio of a cellular therapeutic that is neurotrophic/neuroprotective in nature in a human clinical population may not be advantageous due to increased risk factors to the patient deriving from this method of delivery. These may include tumorigenesis and graft rejection. This is especially true when alternatives that offer similar mechanistic recovery are available, such as conditioned media, neutralizing antibodies, or neuroprotective pharmacological approaches. Thus, we focus here on mechanisms of action in which long-term stem cell engraftment and survival are of critical importance to the regenerative medicine field.

The goal of this review is to broadly look at the role of the choice of animal models in testing proof of concept and safety for the clinical translation of stem cell transplantation strategies, with a particular focus on the CNS. We discuss the available animal model systems for stem cell transplantation into the diseased or injured CNS, identify and discuss key criteria for engraftment and cell survival that should be considered in the experimental setting in the evaluation of efficacy and/or safety studies, and review a brief history of the animal models selected by different arms of the regenerative medicine field. Finally, we review the existing CNS stem cell transplantation literature in the context of cell engraftment and survival criteria, summarizing findings in the field of regenerative medicine and addressing the critical need for more refined criteria of success in animal models utilizing xenotransplants.

Transplantation paradigm & predictive preclinical models

The success of laboratory and clinical transplantation is largely defined by the immunological compatibility between donor and host tissue/cells. Autografts are transplants where the donor tissue comes from the recipient. A common example of an autograft is when skin or bone is taken from one part of a patient's body to reconstruct another [11]. Syngeneic transplants, or isografts, are those in which the donor and recipient are either genetically identical or sufficiently identical to allow for complete immunological compatibility. An example of syngeneic graft is a kidney transplant between identical twins [12]. Allografts are transplants where the donor and recipient are nongenetically identical members of the same species. Differences in MHC antigens, specifically HLA in humans, determine the successful integration of the graft. In the clinical setting this is a complicated issue, as HLA/androgen-binding proteins match stringency can be dependent on the organ/tissue transplanted. However, it is known from organ transplant research that MHC matches, or near matches, reduce the likelihood of graft rejection [13]. Regardless of the level of histocompatibility, immunosuppression is required to overcome the immune response against alloantigens. Finally, a xenograft is a transplant in which the donor and recipient are from different species. Transplantation between closely related species, such as mice and rats, is classified as a concordant xenograft. Transplantation between distantly related species, such as humans and rodents, is classified as a discordant xenograft. The level of immune response, and hence the risk of an immunorejection response, increases in magnitude when moving from an autologous transplant, to a syngeneic transplant, to a matched allograft, to a near-matched allograft, to an unmatched allograft, to a concordant xenograft and finally to a discordant xenograft (Figure 1). Therefore, the type of transplantation paradigm is a crucial factor in establishing a clinically relevant model system for stem cell therapy.

Informative model systems that have good predictive value of the human clinical transplantation setting are necessary to increase the chances of success in translating advances in stem cell therapy of neurological diseases from bench to bedside. From a stem cell transplantation perspective, there are two major components of a model system: the host species in which the neurological disease is being studied and the host species from which the stem cells are derived. Clearly, from both an ethical and regulatory standpoint, human subjects are not an appropriate starting point for testing stem cell therapies. However, there are a wealth of animal models that closely mimic some of the pathological and behavioral deficits associated with different neurological disorders and types of neurotrauma. While some groups have performed experiments in larger animal models such as nonhuman primates [14–16], the vast majority of preclinical research using human stem cells is conducted in rodent models due to the lower cost, lack of nonhuman primate models of neurological disease and injury (versus the abundance of transgenic/knockout mice), and difficulty of achieving adequate immunosuppressive regimens in nonhuman primates. In fact, the latter may be such a significant barrier that, in many cases, stem cell transplantation into nonhuman primate models cannot practically be employed to inform clinical translation. For the purposes of this article, we will focus on rodent models of neurological disease and trauma.

If we accept rodent models as the basis for the study of neurological disease/trauma, what is the most appropriate stem cell source: human stem cells or animal stem cells? A large advantage to using specifically rodent stem cells for preliminary proof-of-concept studies is that researchers can perform syngeneic or allotransplants, which can bypass many of the immunological hurdles of xenografts and more closely mimic some aspects of the clinical setting (i.e., matched human tissue grafted into a human patient). However, while nonhuman cells can provide preliminary proof-of-concept data, the proposed population of human cells

should be tested in preclinical studies for regulatory submission in optimal and appropriate animal models. Furthermore, long-term safety data must be established using the clinical grade of stem cells in the target tissue and in potentially both naive and disease/injury conditions. Consequently, there is a need in the regenerative medicine field for preclinical model systems with good predictive value; this need necessitates the use of human stem cells.

In the case of a discordant xenograft, such as human stem cells into a mouse host, the main hurdle is avoiding or sufficiently minimizing the rejection response by the host immune system in order to achieve successful engraftment and survival of the transplanted human stem cells. Moreover, the presence of an active immunorejection response itself may significantly alter the efficacy, and critically, the safety profile of the cell therapy candidate. In this regard, a valid assessment of the safety profile can be argued to be particularly dependent on conditions that enable or encourage maximal theoretical engraftment and cell survival. In the absence of maximal theoretical engraftment conditions, a valid analysis of the tumorigenic potential of a cell therapy candidate may not be possible. An example of this would be the failure to develop teratomas after embryonic stem cell transplantation into immunocompetent versus immunodeficient hosts [17,18]. By employing animal models that recapitulate the key elements of human pathogenesis, permit sensitive evaluation of disease-modifying activity and permit successful engraftment and long-term survival of transplanted human stem cells, researchers can improve the predictive validity of preclinical safety and efficacy studies, and the likelihood of success of translation to clinical trials.

Lessons learned from tissue, organ & hematopoietic grafting

Recognition of the difficulty of achieving significant long-term engraftment and survival after transplantation is not new; by contrast, it is an issue grounded in the history of skin and organ grafting, which provided the foundations for our understanding of immunological tolerance, as well as the trial-and-error history of hematopoietic cell transplantation [19,20]. Although now regarded as a clinically-acceptable therapy for leukemia, the evolution of hematopoietic cell transplantation spans half a century and culminates in the conclusion that addressing the multidimensionality of the immune response is critical in order to achieve successful cell engraftment and survival.

Human hematopoietic cell transplantation originated from a number of preliminary animal experiments that demonstrated the importance of histocompatibility between donor and host. Murine studies have shown that successful engraftment of allogeneic marrow cells could trigger an immune reaction against the host [21] (reviewed in [22]), now termed graft-versus-host disease and known to be mediated by T cells derived from donor tissue. Critically, the severity of the immune reaction was regulated by genetic factors [23]. Successive animal experiments revealed the importance of histocompatibility between donor and host tissue [24–29]. These data suggested that T-lymphocyte-mediated immune responses could be triggered as a result of MHC mismatch. Accordingly, subsequent experiments employing methotrexate [26] and cyclophosphamide [30], both of which attenuate T-cell responses, achieved more favorable outcomes in animal models of bone marrow transplantation. However, the success rate in translating these advancements for the treatment of human leukemias via hematopoietic cell transplantation remained low [31]. Conversely, hematopoietic cell transplantation in cases where patients exhibited severe combined immunological deficiency [32–34] resulted in high levels of engraftment, and in some cases, patient survival for more than 25 years [35]. With the discovery of a spontaneous mutation in mice leading to a similar form of severe combined immunodeficiency (SCID) [36], efforts were undertaken to recapitulate the human findings in this new mouse model. Approximately 5 years later, Mosier and colleagues performed the

first successful hematopoietic stem cell xenograft using the recently discovered immunodeficient SCID mouse, in which the human hematopoietic stem cell engrafted successfully and constituted long-term repopulation of the mouse immune system [37]. As a result of this and later experiments using immunodeficient models developed to target multiple components of immunorejection (see later), the use of immunodeficient animals in the field of hematopoietic stem cell transplantation permitted increased engraftment, cell survival and rapid advancement of knowledge in this area [38–40].

Mechanisms of allogeneic & xenogeneic rejection

Based in large part on work in the hematopoietic cell transplantation field, the role of T lymphocytes in immunorejection has become much better understood over the past 50 years. T-cell activation is dependent on the recognition of MHC class I and II antigens. MHC I is expressed on almost all cells, while MHC II is generally expressed in association with antigen-presenting cells (APCs), including dendritic cells, B cells and macrophages, as well as microglia and astrocytes in the CNS. MHC class I is principally associated with CD8 cytotoxic T-cell activation, whereas MHC class II is principally associated with CD4 T-cell activation; both lymphocyte subtypes participate in immunorejection responses. In addition to a requirement for the recognition of an MHC/antigen complex displayed by an APC by the T-cell receptor, T-lymphocyte activation also depends on exposure to an array of costimulatory ligands (e.g., B7). If T-cell receptor binding to an antigen/MHC complex takes place in the absence of a costimulatory activation signal, a T-cell can be rendered unable to respond to that antigen (anergic), a mechanism that has been suggested as a means of achieving tolerance [41].

Increasing evidence suggests that other aspects of the immune system contribute to both allogeneic and xenogenic rejection, including natural killer (NK) cells, the complement cascade and the lymphatic system [42–44]. In this regard, it is not only the expression, but also the lack of expression, of MHC I that can invoke an immune response by NK cells [45,46]. As the immunological barrier grows, the role of these additional mechanisms of rejection may increase, becoming greater for xenotransplantation than in the case of allotransplantation [47]. Immunorejection in the allograft setting is predominantly mediated by the adaptive immune response via T cells and B cells. By contrast, xenorejection in discordant species combinations occurs at three relatively distinct phases. Within minutes of transplantation, the humoral immune response is activated during hyperacute rejection [48]. Within days, infiltration of inflammatory mediators, such as host mononuclear cells and neutrophils, mediate acute rejection. Finally, in the delayed xenograft rejection response, cellular mechanisms (both NK and T cell-mediated) modulate xenograft rejection [49]. Understanding these cellular mechanisms is critical, because while T-cell expansion can be controlled by conventional immunosuppressant agents that target calcineurin signaling, such as cyclosporin A [50] and FK506 (Prograf®/tacrolimus) [51], these agents do not affect NK cells or other rejection mechanisms. In fact, transplantation of human cells into immunosuppressed rodents often results in eventual graft failure within the first few weeks after injection [52–57], suggesting that pharmacological immunosuppression is ineffective at preventing the delayed xenograft rejection response. These data suggest that immunorejection in the xenotransplantation setting is complex and requires multidimensional intervention (i.e., the administration of immunosuppressive agents targeting T-cell expansion combined with anti-NK cell), and/or anti-costimulation factor agents, to sufficiently attenuate the immune response in order to obtain long-term engraftment in immunocompetent animal models [58,59].

Achieving transplant engraftment & survival across the xenobarrier

In accordance with the immunological barrier presented by xenotransplantation, several approaches have been utilized in an attempt to achieve high levels of transplant engraftment and cell survival in the organ and bone marrow transplantation fields. First, high-dose combinatorial treatment with multiple conventional pharmacological immunosuppressant drugs. High dose and combinatorial pharmacological immunosuppression has associated toxicity concerns in human [60,61] and animal models [62–64]. A key question to be considered is whether there may be exogenous, alternative and/or unanticipated effects of conventional immunosuppressant agents on transplanted cell populations. In this regard, multiple studies have also shown that immunosuppressive agents can interact with, and influence, various cell populations, altering cell proliferation, fate, migration and perhaps secretomes [65–67].

Second, humanized rodent models have been developed to lower the xenotransplantation barrier. Humanized mice or mouse–human chimeras are immunodeficient animals that are reconstituted with human-derived hematopoietic cells or tissues to minimize HLA mismatches with subsequent human-derived cell populations [68]. While in many ways this model may be considered the ultimate goal for regenerative medicine research, its use is significantly confounded by several major constraints, including the low efficacy of immune reconstitution, the time required for generation of animals, specialized equipment and significant cost. Thus, humanized rodent models of cell transplantation are rarely utilized.

Third, constitutively immunodeficient animal models, in which components of adaptive and/or innate immunity are compromised or deficient, can be utilized to improve the success of xenotransplantation. Immunodeficient animals provide an environment in which the immune response is suppressed endogenously, rather than via exogenous treatment, which allows for direct hypothesis evaluation without complications that may arise from the immunosuppressive treatments necessary to support sufficient engraftment in immunocompetent animals. Historically, evidence for long-term (up to 6 months post-transplant) tolerance of cellular xenografts in immunodeficient animal models is supported by experiments demonstrating prolonged survival of human fetal tissue and blood cells [37,69], and later, pig and human islet cells in constitutively T-cell deficient mice and rats [70,71], suggesting that a lack of functional T cells at least partially circumvents the barriers of chronic rejection. However, the survival of at least mouse–mouse allografts of embryonic stem cells transplanted into heart [72] or muscle [73], and human–rat xenografts of neural stem cells transplanted into the spinal cord [7] has also been shown to be significantly greater in immunodeficient models compared with immunosuppressed models. While a wide variety of immunodeficient/immunocompromised rodents are available for xenotransplantation studies [74–76], not all constitutively immunodeficient animal models achieve equal levels of immunodeficiency; identification of a model that is ‘sufficiently immunodeficient’, meaning that it achieves 100% engraftment and long-term survival, is therefore essential.

Owing to a loss-of-function mutation in the mouse *PRKDC* gene preventing full T- and B-cell development [77], CB-17 SCID mice, which were used in the original hematopoetic stem cell xenografts performed by Mosier *et al.* in 1988 [37], lack functional T- and B-cells [76]. However, SCID mice retain high levels of innate (NK cell) immunity [76], which precludes complete avoidance of immune rejection; the increase in graft failure in initial hemopoietic stem cell transplant studies highlights this limitation [78]. To avoid the shortcomings observed in these early SCID models, alternative immunodeficient animal strains have been generated to further improve graft survival [79]. Nonobese diabetic (NOD)-SCID mice, which in addition to the T- and B-cell deficiencies of CB-17 SCID

models, also display reduced hemolytic complement levels, reduced dendritic cell function and defective macrophage function [76], as well as reduced NK cell activity [80], have been used extensively in a multitude of different stem cell and transplantation studies with great success [74]. Additional SCID variants include β2 microglobulin-deficient (B2Mnull) mice, which display limited amounts of MHC class I (classical and nonclassical) on the cell surface and therefore prevent CD8 T-cell development [81], and recombinase activating gene 1- and 2-deficient (Rag1null and Rag2null) mice, which, similar to PRKDC mutant mice, do not have the ability to generate fully mature T- and B-cell lymphocytes due to failure of DNA strand break V(D)J recombination [82,83]. Furthermore, recent development [84,85] of genetic variants with nearly complete ablation of T-, B-, and NK cell activity offer even more effective options in a xenograft transplantation setting [75]. These include NOD-SCID IL2RG, and Rag2null IL2RG mice, which include a null mutation in the gene encoding the IL-2 receptor γ chain (IL2Rγ), which prevents cell surface signaling to several interleukins as well as NK cell differentiation [86]. Additionally, larger rodent models lacking certain components of the immune response also exist and may be utilized in experiments where smaller laboratory mice are not an appropriate choice; the principle example is the athymic nude rat, which lacks a normal thymus and functionally mature T cells [87]. However, caution should be exercised when considering nude rodent models, as evidence suggests normal to increased levels of NK cell activity [88], which may be sufficient to induce graft rejection [87,89]. Accordingly, selection of an immunodeficient mouse (or rat) model should be considered based on the known combination of deficits in the immune response and resulting engraftment characteristics.

Stem cells & neurotransplantation: the inordinate influence of ‘immunoprivilege’

In contrast to the hematopoietic transplantation field, in which constitutively immunodeficient animal models rapidly gained widespread use because they enabled the study of both normal and malignant hematopoietic repopulation [90], the neurotransplantation field has not followed this path. In fact, neurotransplantation research was heavily directed in its early foundations by a small body of data suggesting that the CNS is immunoprivileged, which led to the widespread belief that achieving engraftment in the CNS was a relatively easy task. Billingham and Boswell first suggested the term ‘immunologically privileged’ in 1953 [91], in a paper in which they described evidence of longer tissue graft survival in some sites (e.g., the cornea) in comparison to others (e.g., skin). The concept of the CNS as an ‘immunoprivileged’ site was extended later based on similar tissue grafting studies using brain tissue [92]. Several mechanisms explaining the relative immunoprivilege of the CNS were hypothesized, including the tight nature of the blood–brain barrier, the absence of professional APCs (which are required to mount a T-cell-mediated adaptive immune rejection response) in the CNS, the lack of MHC expression in the CNS, reports of high levels of factors with immunomodulatory properties in the CNS (e.g., TGF-β), and the absence of traditional lymphatic drainage in the brain as an organ [41,93]. Combined, these factors were thought to render the immune system incapable of mounting an effective rejection response within the CNS.

More recent data has made it clear that, while the CNS may be ‘immunologically quiescent’ [93], it is not at all immunologically incompetent. In fact, the ability of T cells to conduct surveillance via migration across even an intact blood–brain barrier is now recognized as a normal part of immune system function [94]. Similarly, we now know that there is breakdown of the blood–brain barrier and evidence for MHC I/II upregulation in CNS injury/disease, the capacity for microglia, infiltrating macrophages/monocytes, and astrocytes to express MHC II and function as APCs [95], and lymphatic drainage from the

CNS to the cervical lymph nodes, which may serve as sites for antigen-mediated activation of the adaptive immune response [96].

It is not only the CNS itself that has been assumed to possess an immunoprivileged status. Like mesenchymal cells, embryonic stem cells and their derivatives, as well as neural stem cells, have been ascribed a mix of immunoprivilege, immunosuppressive and immunomodulatory properties [97], and have even been reported to fail to induce an immune response in immunocompetent animals [98,99]. Initial reports from work with embryonic stem cells suggested that these cells express very low levels of MHC I and II [100,101], which were not sufficient to stimulate T-cell proliferation *in vitro*, and that their inherent immunoprivileged state could contribute instead to the induction of host tolerance to embryonic stem cell-derived tissues [102]. Similarly, neural stem cells have been reported to express low levels of MHC [103,104], and to lack the expression of co-stimulatory molecules required for T-cell activation [105]. The combination of CNS and stem cell immunoprivilege led to the assumption, in some cases, that immunosuppression would be unnecessary for clinical stem cell allograft transplantation, or even for proof-of-concept xenograft studies in animal models.

However, as in the case of the immunoprivileged status of the CNS, there is a wealth of more recent data contradicting the existence of immunoprivilege in stem cell populations [41,106–109], from which several common themes emerge. First, embryonic stem cells, their differentiated progeny and fetal neural precursor populations can all upregulate MHC expression *in vitro* and upon *in vivo* transplantation [108,110], and thus, permit immunorejection [111]. Second, MHC I expression in both embryonic and neural stem cells is dramatically upregulated following cytokine exposure, notably exposure to IFN-γ [101,112]. IFN-γ is expressed at high levels in many CNS disease and injury states, a point that should be noted in combination with the potential role of disease and/or trauma-induced inflammatory responses to augment immunorejection [113]. Third, NK cells exhibit the capacity to target both embryonic and neural stem cells *in vivo* and/or *in vitro* [45,112]. Taken together, the current literature for human embryonic and neural stem cells does not support the capacity to transplant these cell populations with immunological impunity.

Engraftment & cell survival for xenogeneic transplantation of stem cells in animal models of neurological disease and injury

Up to this point, we have discussed the necessity of starting with an animal model that mimics the clinical pathology for the neurological disease of interest and supports engraftment as well as long-term survival, explored lessons learned from the hematopoietic transplantation field and mechanisms of allogenic and xenogeneic rejection, and reviewed early classifications of the CNS, as well as embryonic and neural stem cells, as 'immunoprivileged'. We now present a comprehensive review of the literature regarding achieving engraftment and cell survival with xenografts in the CNS. We focused our literature review on studies xenotransplanting human cells into rodent models of neurological diseases/trauma or into the normal brain. For purposes of this review, we define 'engraftment' as the percentage of animals that demonstrate surviving cells (total number of animals at sacrifice with human cells still present divided by the total number of initially transplanted animals × 100). In parallel, we define 'cell survival' as the total number of cells present at sacrifice. The total number of cells must have been assessed either via unbiased stereology (typically via optical fractionator) or bioluminescence for the percentage cell survival report to be included in our tables. Unbiased stereological techniques permit rigorous quantitative analysis of tissue, including accurate volume-corrected estimates of cell number; because changes in tissue and structure volume due to disease/injury pathogenesis can be a significant experimental confound, stereological analysis is the gold

standard for determining cell number, lesion volume and other variables vulnerable to these artifacts (see [114,115] for a review of the use of stereology in neuroscience). A limitation to the cell survival data generated by stereological analysis in the CNS is how the region of interest is defined. If the analysis is confined to an anatomically defined region (e.g., the striatum) [116], but the transplanted cell population has migrated beyond this artificial boundary, the determination of estimated cell number will only be accurate within the striatum, and the total number of surviving cells will be underestimated. Because of this limitation, papers that have this constraint in stereological data collection have been included in the overall tables, indicated by a symbol, and in the calculation of percentage engraftment, but have not been included in the calculation of percentage cell survival. Bioluminescence also permits quantitative analysis of cell survival within tissue, however, there are two critical limits for cell detection. First, sensitivity; while stereology permits an estimate of total cell number based on the detection of every cell visualized by immunoperoxidate, immunofluorescence, or promotor driven fluorescence in a transplanted tissue, bioluminescence has a clear threshold for detection that is affected by many factors, including transplantation site/depth. In the neural stem cell transplantation field, at least one study has demonstrated that the number of luciferase-expressing cells necessary to generate a detectable bioluminescence signal is in the order of 1000 [117]. Coupled with this detection threshold limit, the propensity of transplanted cell populations for migration will significantly affect the accuracy of total cell survival quantification by this method. Finally, the long-term stability of luciferase expression has not been established, and decrements in signal may, in some cases, result from promoter downregulation [117]. Owing to the limitations of bioluminescence in providing accurate total cell survival quantification, papers using this method of quantification have been included in the overall tables, indicated by a symbol, and in the calculation of percentage engraftment, but have not been included in the calculation of percentage cell survival.

Literature searches for this analysis of xenotransplantation in the CNS were performed in January 2011 with the keywords ‘human stem cell’ in combination with other keywords in series: ‘transplantation’, ‘brain’, ‘CNS’, ‘spinal cord’, ‘spinal cord injury’, ‘stroke’, ‘middle cerebral artery occlusion’, ‘ischemia’, ‘brain injury’, ‘brain trauma’, ‘multiple sclerosis’, ‘Parkinson disease’ and ‘Huntington disease’. Additional references were added when found cited in the initial round of papers retrieved via MedLine. No papers were excluded from our analysis, *a priori*. Using these criteria, we found 133 unique, relevant papers. It should be noted that the primary focus of any given paper need not have been the key variables discussed in this article (i.e., engraftment and cell survival). Rather, many papers compared a cell line with and without an experimental treatment or other component in an injured environment, or the effects of a cell line on functional outcome, and did claim to assess either engraftment or quantify total surviving cells. We grouped these papers into three primary categories based on common features of the model: models of normal neonatal or adult brain; models of acute/traumatic injury (SCI, traumatic brain injury or stroke); and models of chronic/atraumatic injury (Parkinson’s disease, Alzheimer’s disease, Huntington’s disease, amyotropic lateral sclerosis, allodynia or demyelination). Within each primary category, references were subdivided into those using immunocompetent animals with immunosuppression, those using immunocompetent animals without immunosuppression and those using immunodeficient animals. A summary of the primary categories and the number of papers using immunocompetent versus immunodeficient animals is shown in Table 1.

We recorded 25 variables for each paper: citation, URL, model, primary category of model, paradigm or method, cell type, host species/sex, immunocompetent or immunodeficient host, location of injury, transplant time (time post-injury), final dose of cells, volume of injection, route/location of transplant, immunosuppressant, dose and duration, detection

method for human cells, quantification method (stereology or number of sections), treatment groups, survival time (post-transplant), total number of transplanted animals in a group, numbers of animals with cells at sacrifice, percentage engraftment, and behavioral outcome. Factors that were not applicable for a given model or not reported were noted as well. In order to accurately assess the influence of immunocompetent (with or without immunosuppression) or immunodeficient models on successful engraftment and cell survival, we included only those studies using cell suspensions and excluded studies using solid grafts where total cell number (dose) or surviving cell number could not be determined. Individual tables are provided for each of the three primary categories (models of normal neonatal or adult brain, models of acute/traumatic injury and models of chronic/atraumatic injury, as defined previously); these primary categories are further subdivided by whether cells in the identified studies were transplanted into immunocompetent animals without immunosuppression, immunocompetent animals with immunosuppression, and immunodeficient animals (Tables 3–11).

Table 1 shows that of the 133 unique papers, six papers examined xenotransplantation in normal brain (5%), 95 papers examined xenotransplantation in acute/traumatic models (71%), and 32 papers examined xenotransplantation in chronic/atraumatic models (24%). A total of 79 papers (59%) reported neither engraftment nor survival, while only 11 papers (8%) reported both engraftment and survival. For example, in the primary category ‘acute/traumatic’, 37 out of 95 papers (40%) used no immunosuppression in immunocompetent animals. Seven of these 37 papers reported the percentage of animals engrafted, only one reported the percentage of cell survival based on stereology, no papers reported both engraftment and percentage of cell survival, and 30 reported neither variable. In this category (acute/traumatic), the one paper to report cell survival (based on stereology) in a stroke model without the use of immunosuppression reported that only 35% of the initial cell dose survived 8 weeks post-transplant across four treatment groups [118] (see Table 7 for additional details).

Several key conclusions can be drawn from Tables 1 & 2. First, the majority of CNS xenografts have used immunocompetent animals coupled with immunosuppression (n = 69 or 52%). The next most common paradigm is to use immunocompetent animals and no immunosuppression (n = 47 or 35%). Only 19 papers (or 14%) used immunodeficient animals in CNS xenograft studies using human cells. Second, the percentage of animals engrafted (when reported) is usually highest in immunodeficient animals. Third, the paucity of studies in the normal CNS and chronic/atraumatic cohorts that have employed immunodeficient models and quantitatively assessed engraftment/cell survival makes acute/traumatic models the only category in which these variables can be compared between immunocompetent and immunodeficient animals, and makes this the most robust category from which to draw relative conclusions for xenotransplantation success. In the acute/traumatic/cohort, the highest percentage of cell survival is in immunodeficient animal models (263%, n = 4). Fourth, although the studies available for comparison are limited, the data suggest that an uninjured niche (normal brain) may be no better than an injured niche in terms of engraftment or cell survival. Finally, caution should be exercised in interpreting papers that report surviving cell numbers. Many papers extrapolate total cell number from a limited number of histological sections. Moreover, even when systematic random sampling and stereological assessment of total cell number is performed, the number of animals assessed can be insufficient to yield interpretable numbers. For example, Suzuki *et al.* report the stereological assessment of total cell number in an amyotropic lateral sclerosis model where 114% of the initial dose of cells was detected 6 weeks post-transplant; but quantification was performed in only one animal [119].

Looking at those papers that reported either human cell engraftment or survival numbers, several interesting issues are evident. First, in immunocompetent animals given immunosuppression, cell survival decreases over time. Yashuhara *et al.* transplanted green fluorescent protein (GFP)-expressing human HB1.F3 fetal-derived neural stem cells in a 6-OHDA toxicity model of Parkinson's disease and quantified GFP^+/human nuclear antibody $(hNuc)^+$ cells in every fifth section through the entire striatum [57]. Animals received 200,000 cells in 3 μl phosphate-buffered saline into the ipsilateral striatum immediately following the 6-OHDA lesion. Sets of animals (n=8 per time point) were sacrificed 3, 7, 14 or 28 days post-transplant. All animals received daily intraperitoneal injections of cyclosporin A (10 mg/kg). Cell survival within the striatum was 11.2, 4.7, 1.7 and 1.1% across the four time points, respectively. Although quantification was not a true stereological assessment (neither random counting frames nor stereological dissectors were used), these cell survival numbers are directly comparable within the study and demonstrate that a host rejection response was likely active and involved in rapidly killing the human cells over time. Notably, it is possible that GFP expression may have also been downregulated over time in conjunction with a rejection response, resulting in the observed reduction in total cell numbers over time; however, antibodies to a human nuclear antigen were also used to detect the transplanted cells [57].

Second, while direct comparisons within a single study between groups of immunocompetent animals receiving either immunosuppression or no immunosuppression are rare, not using immunosuppression in immunocompetent animals significantly reduces successful engraftment. Wennersten *et al.* transplanted 210,000 human fetal-derived neural stem cells into a contusion model of SCI immediately postinjury [120]. Animals received cyclosporin A for 3 or 6 weeks post-transplant (4 mg/kg Monday and Wednesday, and 8 mg/kg on Friday), while a third group received no cyclosporin A post-transplant. The three groups were sacrificed 6 weeks post-transplant and the presence or absence of humans cells was confirmed using hNuc immunohistochemistry. All animals receiving cyclosporin A, regardless of length of administration, were successfully engrafted, but only one of six animals (16.7%) contained human cells in the no cyclosporin A group. A fourth group of animals (n = 8) received cyclosporin A for 3 weeks but was allowed to survive 6 months instead of 6 weeks; five out of eight (62.5%) exhibited successful engraftment at 6 months. Quantification of cell numbers was not performed [120]. While this study suggests that transient immunosuppression may suffice to achieve long-term engraftment, it also demonstrates that using no immunosuppression in immunocompetent animals significantly reduces the rate of successful engraftment. Unfortunately, without quantification of total cell numbers in such a study, it is impossible to ascertain the effect of short versus long-term immunosuppression on cell survival.

Third, in the few studies that conducted direct comparisons, human cell engraftment was shown to be higher in immunodeficient animals than immunocompetent ones. Deng *et al.* transplanted human olfactory ensheathing cells into a hemisection model of SCI using either athymic nude (immunodeficient) or Sprague–Dawley (immunocompetent) rats [121]. Their data demonstrate that survival of human olfactory ensheathing cells transplanted into immunocompetent animals was minimal at 24 h post-transplant and no surviving cells were identified by 7 days post-transplant; robust macrophage infiltration was found at the injection site by 7 days and engraftment was 0%. Conversely, human olfactory ensheathing cells transplanted into athymic nude rats survived and migrated away from the site of injection at 24 h and 7 days post-transplant. Engraftment was observed in 40% of the athymic nude rats, while no engraftment was observed in Sprague–Dawley rats [121]. Although this study transplanted olfactory ensheathing cells rather than a strictly defined stem cell population, the proliferative properties of olfactory ensheathing cells *in vitro* are

well known, suggesting that activation of the host immune system initiates a rejection response.

The goal of this comprehensive literature review was to assess the status of the field in achieving adequate engraftment and survival in xenotransplantation models to predict clinical translation. As noted earlier, it should be acknowledged that the principal end point of a given study may not have been the assessment of cell survival *per se*, and such studies can still contribute meaningful data to the literature. This review of 133 xenotransplantation papers shows that the field of regenerative medicine has focused heavily on the administration of cyclosporin A alone in immunocompetent animals as a strategy for proof-of-concept experiments, resulting in both poor engraftment and low to very low cell survival (when reported). Furthermore, this summary shows that xenografted stem cells retain proliferative capacity in immunodeficient versus immunosuppressed models within the acute/traumatic category. While other differences in the acute/traumatic CNS niche could contribute to differences in the retention of proliferative capacity, it is likely that these differences provide insight into the capacity to initiate immunorejection mechanisms in these conditions. Again, given that acute/traumatic models represent the only category in which multiple studies have quantified engraftment and survival in immunodeficient animals, these data represent the most robust category from which to draw relative conclusions for xenotransplantation success. Combined with our historical survey of the broader xenotransplantation field, this analysis clearly suggests that it will be necessary to administer a multimodal course of pharmacological immunosuppression to achieve meaningful engraftment of a transplanted human cell population when using immunocompetent animals. Alternatively, immunodeficient animals yield much higher engraftment and cell survival numbers than using immunocompetent animals (with or without immunosuppression).

Future perspective

It seems evident that, ideally, preclinical testing of safety and efficacy should have the goal of achieving a human–mouse xenograft that is as comparable as possible to the human clinical setting (i.e., a human–human allograft). In this regard, we begin by considering what features one can reasonably predict to be associated with human–human allografts in the CNS.

First, as we have shown in this article, one would expect that the immunological barrier associated with a human–human allograft would be less than that associated with a human–rodent xenograft, and be principally T-lymphocyte mediated, and therefore require less in the way of multifaceted immunosuppression protocols. Accordingly, the administration of drugs targeting T-cell proliferation and expansion via calcineurin signaling may be sufficient to maintain long-term cell survival in the clinical setting, especially under conditions in which the partial restriction of access of the immune system to CNS parenchyma maintained by the blood–brain barrier is restored over time. By contrast, we know that this is not the case for xenotransplantation, and at least NK cells must also be suppressed (e.g., as in the case of SCID beige mice or athymic nude rats). Second, one would expect that a more successful pharmacological immunosuppression protocol could be achieved in human–human allografts than in human–rodent xenografts. Human immunosuppression protocols are understandably considerably better characterized and designed in terms of the pharmacokinetics of drug delivery and metabolism, and achieving target circulating drug levels in man is more precise, particularly when one considers the side effects of subcutaneous delivery and variability in oral delivery of immunosuppressants in rodent models. Moreover, the pharmacokinetics of immunosuppressant metabolism are almost never monitored or accounted for in rodent models, resulting in both reduced efficacy and

increased toxicity. In this regard, for example, there are known differences in the peak/trough levels of immunosuppressant metabolism and effective dosing strategies [62]. Furthermore, rodent and human T cells can exhibit different levels of responsiveness to calcineurin inhibitors such as cyclosporin A [122]. Third, one can predict that several factors will gain momentum in the future, further reducing the immunological barrier of human clinical allotransplantation [123]: MHC I matching by virtue of the generation of cell banks for candidate clinical cell therapeutics [124,125]; the development of clinical strategies to achieve allograft tolerance [126,127] and/or an eventual shift to autologous cell transplantation, for example using induced pluripotent cells from somatic sources [5,128]. As a result, one would predict both higher levels of engraftment and a greater degree of cell survival in human–human allografts than in human–rodent xenografts under immunosuppressive therapy, particularly under optimal immunosuppression and MHC matching conditions, and certainly in the event of successful immunotolerance strategies and/or autologous transplantation.

Many candidate clinical cell therapeutics under current investigation are partly committed stem/progenitors, rather than terminally differentiated replacement cells; accordingly, transplanted cells retain some proliferation capacity. Taking the transplantation of human neural stem cells into constitutively immunodeficient animals as an example, it is clear that these cells, at least initially, retain the capacity for proliferation. At the simplest level, this is evident because there a greater number of surviving cells at the time of sacrifice than initially transplanted, albeit in the absence of tumorigenesis (Tables 1 & 3–11). We suggest that preclinical animal models in which the number of surviving cells is a fraction of the number contained in the initial transplant cannot provide informative data regarding proliferation potential, and therefore, cannot provide adequate data for predictive validity in the human clinical setting. The limits of such data are particularly relevant for establishing a risk versus safety profile for a candidate clinical cell therapeutic, as tumorigenesis is known to be dependent on both dose and cell survival, and is greatly attenuated in immunosuppressed immunocompetent versus constitutively immunodeficient animal models [17,18]. However, while it might be argued that efficacy data would only be enhanced by increased cell survival, therefore supporting the use of immunosuppressed immunocompetent animal models, we suggest that this is an assumption with little or no supporting empirical data. Cells respond to both the microenvironment into which they are transplanted, and the conditions of that microenvironment. The potential effects of a microenvironment in which there is an active immune response, or conversely, an increase in cell survival due to a lack of immune-mediated cell death, on cell fate and migration are essentially unknown, and are likely to be different for each candidate clinical cell therapeutic. Furthermore, the potential for an active immunorejection response to contribute to disease modifying activity cannot be ruled out. Owing to the known effect of dose and immune rejection on tumorigenesis, and the unknown effect of dose and immune status on fate, migration and/or disease modifying activity, simply scaling up the initial cell dose administered in an immunosuppressed immunocompetent animal model is not adequate to address either tumorigenesis or efficacy. Accordingly, preclinical studies should be designed to reduce immunorejection of transplanted cells in order to optimize cell engraftment and survival, and preclinical transplantation models should seek to achieve maximal theoretical engraftment in order to provide informative safety and efficacy information. We therefore suggest that immunodeficient animal models should be the model of choice for preclinical testing of safety and efficacy for candidate clinical cell therapeutics.

There are two principal issues in considering this approach. One key question is whether there are exogenous, alternative and/or unanticipated effects of conventional immunosuppressant agents on the transplanted cell population. For example, on overall cell engraftment, or cell fate/migration that could be critical in a clinical human–human

allotransplantation setting where these agents will have to be administered post-transplantation, at least for a period of time. Many investigators have used this rationale to continue with experiments in xenograft models using pharmacological immunosuppressive therapy. However, we suggest that it is time for the field of regenerative medicine to re-evaluate this concept. We suggest that a more informative approach would be to establish data from two models. First, investigation of engraftment, tumorigenesis, fate and migration in immunodeficient animal models treated with the planned clinical immunosuppressive therapy. In this paradigm, any potential direct effects of immunosuppressant treatment on the candidate clinical cell population would be more likely to be revealed, given the increased overall cell survival, and reduced likelihood of the inadvertent selection against either the primary stem cell population, or precursor/progenitor populations, providing an improved assessment of tumorigenesis and safety. Second, the effect of immunosuppressant withdrawal on target organ integrity, engraftment, tumorigenesis, fate and migration in an immunocompetent animal model treated with the planned clinical immunosuppression therapy for a transient period following cell transplantation.

A second key question is whether predictive models of neurological disease/injury for testing cellular therapeutics can be established in immunodeficient versus immunosuppressed models. It is increasingly clear that there is a role for both the innate and adaptive immune responses in many types of neurological disease and injury. In the case of autoimmune diseases of the CNS, this role can be pivotal to the development of pathology (e.g., the generation of autoreactive T cells to myelin epitopes in multiple sclerosis); while the specific role of adaptive immune responses and T-cell activation are less clear, it appears certain that Alzheimer's disease, Parkinson's disease, stroke, traumatic brain injury and SCI all include an inflammatory component in their pathogenesis [129–134]. Furthermore, in the case of traumatic injury models such as SCI, access of the immune system to the CNS is greatly enhanced by breakdown in the blood–brain barrier, and there may be profound, prolonged and diverse effects of immune activation on pathogenesis and functional outcome [135]. Understandably, the generation of transgenic neurological disease models backcrossed onto constitutively immunodeficient mouse strains is a difficult process. Furthermore, the effect of constitutive ablation or attenuation of T-cell and NK cell responses associated with immunodeficient models, such as the NOD-SCID mouse, on innate immune responses, inflammation and the essential characteristics of lesion pathogenesis would have to be tested as a part of validating a predictive animal model [136]. At least in the case of traumatic SCI, the host macrophage/microglia response, neutrophil response and evolution of the central lesion are overtly unaltered in comparison with other mouse strains [136]. As noted earlier, however, the immune and inflammatory microenvironment, composed of a host of complement proteins, cytokines and chemokines, may affect transplanted cell populations in ways that have yet to be recognized, and the potential for an immunorejection response to influence stem cell populations is equally as great as the potential for alteration of the immune microenvironment presented by the disease state to do so. Critically, however, in order to achieve engraftment of a candidate therapeutic cell population at any level for the purpose of assessment of safety/efficacy in animal models, it is necessary to impair the functional immune status of the host, particularly with regard to activation of T-cell- and NK cell-mediated immune responses; the difference between immunosuppressed and immunodeficient lies then in the fact that, at least under some conditions, treatment with immunosuppressants is not sufficient to achieve levels of xenoengraftment that may be comparable to human–human allotransplantation.

In summary, we suggest the following criteria for preclinical safety and efficacy studies for the application of human stem cell populations in a clinical setting:

- At a minimum, reproducible long-term engraftment and cell survival should be quantitatively assessed no earlier than 4 weeks post-transplantation, a time at which both the acute and delayed immunorejection phases would have been initiated. Engraftment should be reported for the entire study cohort (i.e., animals should not be excluded from analysis of these variables).
- We suggest that in a niche amenable to cell engraftment and survival, the number of animals demonstrating engraftment should approach 100% of those transplanted. At present, at least in acute/traumatic models, this percentage of xenoengraftment can only be achieved in immunodeficient models.
- For safety/tumorogenesis studies, post-transplantation analysis should be extended based on the disease indication planned for therapy; in the absence of clinical data with an identical candidate cell population, the concept of maximal theoretical engraftment should include long-term studies encompassing the lifespan of the animal model. While some immunodeficient animal models exhibit a shortened lifespan, others can be maintained to establish long-term safety data.
- While in actual human clinical trials, the maximal theoretical number of surviving transplanted cells is unknown, we suggest that a microenvironment that enables maximal theoretical survival, as well as proliferation of transplanted stem cell populations, will be most informative, particularly for safety and tumorogenesis, but also for disease modifying activity (efficacy) and mechanism of action. At present, this type of survival in preclinical neurotransplantation studies, at least in acute/traumatic models, has only been achieved in immunodeficient models.
- It should be noted that evaluation of survival is complex, and quantification of the number of surviving cells at the end of a study is not informative about how that number came to be; the relative contributions of cell death and cell proliferation at different intervals post-transplantation will clearly both play a role in the end result. Accordingly, the initial number of transplanted cells, the number of surviving cells at an early timepoint (days) post-transplantation, and the number of surviving cells at the termination of the experiment should all be considered. In some cases it may also be appropriate to carefully consider the accuracy of the initial cell bolus delivered and the degree of early loss of transplanted cells (e.g., via assays for colocalization with apoptotic markers such as activated caspase-3).
- We suggest that studies testing the direct effects of planned clinical immunosuppressive therapy on candidate therapeutic stem cell populations be conducted in an immunodeficient model, to rule out possible unexpected interactions under conditions of maximal theoretical engraftment.
- Bystander effects resulting from immune activation/diversion via activation of immunorejection mechanisms may provide part or all of the beneficial effects yielded from a given cell transplantation strategy. Accordingly, if immunosuppressed immunocompetent models are used, we suggest that assessment of host-mediated rejection mechanisms should be considered in addition to quantification of engraftment and cell survival. However, *in vivo* assessment of immunorejection responses is complex, as multiple cell types, timing and locations (e.g., within the target tissue, adjacent lymph nodes or systemic immune effects) can all play a role, and an established protocol to assay for immunorejection in animal models has not as yet been developed.

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Executive summary

- The choice of animal models of neurological disease and injury, and consideration of immunological compatibility between donor and host tissues are critical for testing cell therapies.
  - The tissue, organ and hematopoietic grafting fields can provide historical insight into donor cell engraftment and immunological rejection mechanisms.
  - Immunological rejection mechanisms and issues are different for xenogeneic and allogenic transplantation.
  - Neither stem cells nor the CNS are truly immunoprivileged.
- Most stem cell transplantation studies in the CNS have used short-term assessments and reported minimal or no assessments of cell engraftment/survival.
  - Assessment of the survival of transplanted cell populations should be conducted using unbiased methods (e.g., stereology).
  - Of 133 papers in which stem cells were transplanted into the naive, acute or chronic models of CNS disease/injury, few conducted thorough assessment of engraftment or survival of donor cells in the host.
  - The majority of these 133 papers were conducted using pharmacological immunosuppression of otherwise immunosufficient mice/rats, and achieved low levels of cell engraftment and poor cell survival.
- Transplantation into immunodeficient animal models results in dramatically greater cell engraftment and survival.
  - Immunodeficient models can provide a more realistic assessment of safety and efficacy.
  - Recommendations for stem cell transplantation studies are presented based on these data.

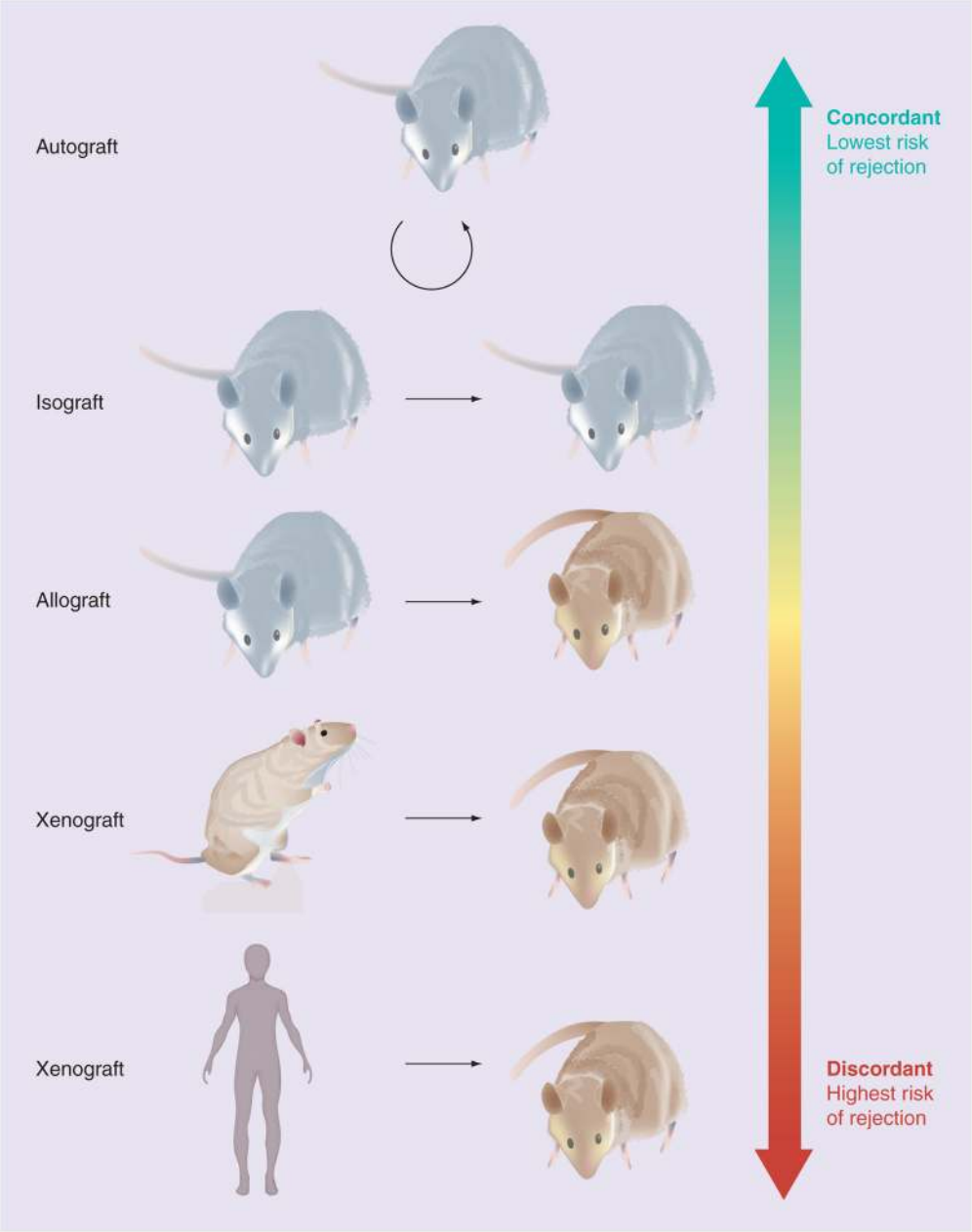

Figure 1. Diagram of the types of transplantation, from autografts to xenografts

As the level of donor to host increases (from transfer between littermate mice [isograft], to mice of different strains [allograft], to rat into mice [concordant xenograft] and finally from human into mice [discordant xenograft]), so does discordance and associated increased risk of immunorejection.

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Table 1

Analysis of 132 unique papers reporting xenotransplantation of human stem cells into the CNS.

	Normal brain (n = 6 papers)			Acute/traumatic (n = 94 papers[‡])			Chronic/atraumatic (n = 32 papers)		
	IC with IS	***IC with no IS***	***ID model***	***IC with IS***	***IC with no IS***	***ID model***	***IC with IS***	***IC with no IS***	***ID model***
Total number of papers	3	2	1	45	37	14	21	7	4
Number reporting animals engrafted (%)	3	0	1	12	7	9	6	4	1
Number reporting cell survival (%)	2	1	1[†]	9	1	4	6	2	1
Number reporting engraftment and survival	2	0	1	1	0	4	1	1	1
Number reporting neither	0	1	0	28	30	5	9	2	3
Average number of animals engrafted (%)	78 (n = 3)	NR	100 (n = 1)	85 (n = 12)	71 (n = 7)	95 (n = 9)	81 (n = 6)	89 (n = 4)	87 (n = 1)
Average cell survival (%)	53 (n = 2)	8 (n = 1)	NA[†]	17 (n = 9)	35 (n = 1)	263 (n = 4)	83 (n = 6)	34 (n = 2)	NA[§]

[†]One immunodeficient paper reported a 10% cell survival via bioluminescence, but detection sensitivity is not comparable to histology.

[‡]94 unique papers, one of which includes IC animals with and without IS, and one includes IC animals in one group and ID animals in another group. These duplications are subtracted from the total. Hence, 45 + 37 + 14 = 96 − 2 = 94.

[§]One paper quantified survival stereologically within a restricted region while cells had migrated beyond that region, hence total cell survival is unknown.

IC: Immunocompetent; ID: Immunodeficient; IS: Immunosuppression; NA: Not applicable; NR: Not reported.

Table 2

Proportion of papers from Table 1 that use immunocompetent or immunodeficient models.

Total immunocompetent models with immunsuppression (%)	Total immunocompetent models without immunosuppression (%)	Total immunodeficient models (%)
69 (51)	46 (34)	19 (14)

Table 3

Normal CNS: immunocompetent animals with immunosuppression.

Author (year)	Model	Paradigm	Cell type	Host species/ sex	Time post-injury	Final cell dose	IS	Quantification method	Groups	Final time point	No. animals explanted	No. animals with cells	Animals engrafted (%)	Cell survival (%)	Ref.
Fricker *et al.* (1999)	Normal adult brain	Brain	6–9-week fetal hNPC	Female Sprague–Dawley Rats	NA	100,000	CsA	Visualization of $BrdU^+$ cells		2 and 6 weeks	54	54	100	NR	[137]
Kishi *et al.* (2005)	Normal young brain	Brain	9-week fetal hNSCs	8–9 week old male C57BL6 mice	tx cells 4 days after control or β-estradiol pellet	20,000	CsA	hNuc not via stereology	4 days after control pellet	4 weeks	6	4	67	1.0	[138]
Kishi *et al.* (2005)									4 days after estradiol pellet	4 weeks	6	4	67	1.0	[138]
Englund *et al.* (2002)	Normal adult brain	Brain	9-week fetal hNPC	Female Sprague–Dawley rats	NA	150,000	CsA	Stereology (other)	Striatum	4 weeks	24	22	92	190.0	[139]
Englund *et al.* (2002)									Hippo-campus	4 weeks	18	13	72	20.0	[139]
Englund *et al.* (2002)									Sub-ventricular zone	4 weeks	20	14	70	NR	[139]

CsA: Cyclosporine A; BrdU: Bromodeoxyuridine; hNPC: Human neural progenitor cell; hNSC: Human neural stem cell; hNuc: Human nuclear antibody; IS: Immunosuppression; NA: Not applicable; NR: Not reported; tx: Transplantation.

Table 4

Normal CNS: immunocompetent animals with no immunosuppression.

Author (year)	No.	Class	Cell type	Host species/ sex	IC or ID	Final cell dose	Time post-injury	Final cell dose	IS	Quantification method	Final time point	No. animals explanted	No. animals with cells	Ref.
Kallur *et al.* (2006)	3	Normal	6–9-week fetal hNSCs	P2–3 Sprague–Dawley rats	IC	150,000	NA	150,000	No	Stereology (other)	4 months	22	NR	[140]
Kallur *et al.* (2008)	4	Normal	6–9-week fetal hNSCs (GFP and GFP:Pax6)	P3–5 Sprague–Dawley rats	IC	100,000	NA	100,000	No	Stereology (other)	1 month	13	NR	[141]

GFP: Green fluorescent protein; hNSC: Human neural stem cell; IC: Immunocompetent; ID: Immunodeficient; IS: Immunosuppression; NA: Not applicable; NR: Not reported.

Table 5

Normal CNS: immunodeficient animals.

Author (year)	Model	Paradigm	Cell type	Host species/ sex	Time post-injury	Final cell dose	IS	Quantification Groups method	Final time point	No. animals explanted	No. animals with cells	Animals engrafted (%)	Cell survival (%)	Ref.
Ogawa *et al.* (2009)	Normal young brain	Brain	8-week fetal hNSC p25	Nonobese diabetic/severe combined immunodeficiency mice	NA	2,000,000	No	Biolumin	6 months	16	16	100	10.0	[142]

hNSC: Human neural stem cell; IS: Immunosuppression; NA: Not applicable.

Table 6

Acute/traumatic: immunocompetent animals with immunosuppression.

Author (year)	Model	Paradigm	Cell type	Host species/ sex	Time post-injury	Final cell dose	IS	Quantification method	Groups	Final time point	No. animals explanted	No. animals with cells	Animals engrafted (%)	Cell survival (%)	Ref.
Al Nimer *et al.* (2004)	TBI	TBI via weight-drop model	7-week fetal hNSC neurospheres (passage 10)	Male SD rats	10 min	210,000	CsA	Not performed		6 weeks	16	16	100.0	NR	[143]
Alexanian *et al.* (2011)	SCI	NYU Impactor	hNPCs as neurospheres or as a monolayer	Adult female SD rats	7 days	100,000	FK506	NR		8 weeks	20	NR	NR	NR	[144]
Cho *et al.* (2010)	Stroke	MCAO	EPO gene transduced hMSCs (EPO-hMSCs)	Male SD rats	14 days	600,000	CsA	NR		5 weeks	40	NR	NR	NR	[145]
Cloutier *et al.* (2006)	SCI	Contusion (IH) 50 or 200 kD	hESC-OPCs	Adult female SD rats	7 days	2,000,000	CsA	NR	50 kD	2 months	10	10	100.0	NR	[146]
Cloutier *et al.* (2006)									200 kD	2 months	7	7	100.0	NR	[146]
Daadi *et al.* (2008)	Stroke	MCAO	H9 line hESC-derived hNSCs (SD 56)	Adult male SD rats	7 days	100,000	CsA	Stereology (MBF)		9 weeks	10	NR	NR	37.0	[147]
Darsalia *et al.* (2007)	Stroke	MCAO	hNSC from fetal striatal and cortical tissue	Male Wistar rats	7–14 days	300,000	CsA	Stereology (CAST-Grid)		5–6 weeks	18	NR	NR	30.0	[148]
Darsalia *et al.* (2011)	Stroke	MCAO	hNSC from fetal striatum	Male Wistar rats	48 h or 6 weeks	300,000, 750,000 or 1,500,000	CsA	Stereology (CAST-Grid)	48 h post-injury	52 h to 11 weeks	52	NR	NR	58.0	[149]
Darsalia *et al.* (2011)									6 weeks post-injury		52	NR	NR	27.0	[149]
Darsalia *et al.* (2011)									Intact striatum		52	NR	NR	31.0	[149]
Dasari *et al.* (2007)	SCI	NYU Impactor	hUCB	Adult male Lewis rats	7 days	3,000,000	CsA	NR		2 weeks	NR	NR	NR	NR	[150]
Dasari *et al.* (2008)	SCI	NYU Impactor	hUCB	Adult male Lewis rats	7 days	3,000,000	CsA or none	NA		3 weeks	5	NR	NR	NR	[151]
Eaton *et al.* (2007)	SCI	Excitotoxic lesion	Human neuronal cell line (hNT2.17 derived)	Adult male Wistar rats	14 days	1,000,000	CsA	Stereology (MBF)		8 weeks	NR	3	NR	0.3	[152]
Gao *et al.* (2006)	TBI	TBI via parasagittal FP	bFGF primed K048 line of hNSCs (8-week human fetus)	Male SD rats	1 day	100,000	CsA	Not performed		2 weeks	9	3 out of 3 animals examined	100.0	NR	[153]
Erceg *et al.* (2010)	SCI	Transection	hESC-OPCs and/or H9-GFP-MNP	5–7 week old female rats	0 day	2,000,000	CsA	Three sections per block	hESC-OPCs	4 months	14	14	100.0	<1	[154]
Erceg *et al.* (2010)									H9-GFP-MNP	4 months	14	NR	NR	<1	[154]
Erceg *et al.* (2010)									OPCs + MNPs	4 months	14	NR	NR	<1	[154]

Author (year)	Model	Paradigm	Cell type	Host species/ sex	Time post-injury	Final cell dose	IS	Quantification method	Groups	Final time point	No. animals explanted	No. animals with cells	Animals engrafted (%)	Cell survival (%)	Ref.
Hatami *et al.* (2009)	SCI	Lateral hemisection	hESC-NPCs	Adult male Wistar rats	6 days	200,000–300,000	CsA	NA		5 weeks	15	NR	NR	NR	[155]
Hicks *et al.* (2009)	Stroke	MCAO	hESC-derived neural precursors (hNPCs): HS181	Male Wistar rats	7 days	800,000	CsA	Stereology (MBF)	hNPCs + EE	67 days	10	5	50.0	1.0	[156]
Hicks *et al.* (2009)									hNPCs + ST	67 days	10	5	50.0	0.3	[156]
Hicks *et al.* (2009)									Sham + hNPCs	67 days	11	5	45.5	0.6	[156]
Himes *et al.* (2006)	SCI	MASCIS Impactor	BM-derived hMSC	Adult female SD rats	7 days	500,000	CsA	NR		11 weeks	15	NR	NR	NR	[157]
Honma *et al.* (2006)	Stroke	MCAO	Immortalized hMSCs with human telomerase gene (hTERT-MSCs)	Adult male SD rats	12 h	10,000–1,000,000	Yes	NR		7 and 25 days	NR	NR	NR	NR	[158]
Horita *et al.* (2006)	Stroke	MCAO	hMSCs transfected with a GDNF gene	Adult female SD rats	3 h	1,000,000	CsA	NR		31 days	46	NR	NR	NR	[159]
Hwang *et al.* (2009)	SCI	NYU Impactor	Fetal hNSCs and Olig2 over-expressing fetal hNSCs	Adult female SD rats	7 days	200,000	CsA	Stereology (only two sections/1 mm region)	Fetal hNSCs	2 weeks for survival	12	4	33.3	~1	[160]
Hwang *et al.* (2009)									Olig2 fetal hNSCs	2 weeks for survival	12	4	33.3	~4	[160]
Kamada *et al.* (2010)	SCI	NYU Impactor	BM-derived hMSC and BM-derived hMSC-derived Schwann cells	9 week old male Wistar rats	7 days	2,000,000	CsA + Dex	NR		6 weeks	9	NR	NR	NR	[161]
Keirstead *et al.* (2005)	SCI	Contusion (IH)	hESC-OPCs	Adult female SD rats	7 days and 10 months	2,000,000	CsA	Three sections/1 mm tissue block	7 days	2 months	8	8	100.0	NR	[162]
Keirstead *et al.* (2005)									10 months post-injury		6	6	100.0	NR	[162]
Kelly *et al.* (2004)	Stroke	MCAO	Human CNS-derived neurospheres	Adult male SD rats	7 days	300,000	CsA	Stereology (MBF)		5 weeks	13	NR	NR	33.4	[163]
Kerr *et al.* (2010)	SCI	NYU Impactor	hESC-OPCs	6–8 week old female SD rats	3 and 24 h	800,000	CsA	Not clear		8 days	5	NR	NR	NR	[164]
Kim *et al.* (2009)	SCI	Contusion (IH)	Fetal hNSCs (F3) and VEGF-fetal hNSCs	Adult female SD rats	7 days	200,000	CsA	Stereology (CAST-Grid)	hNSC	6 weeks	NR	5	NR	9.1	[165]
Kim *et al.* (2009)									hNSC + VEGF		NR	5	NR	11.6	[165]
Koh *et al.* (2008)	Stroke	MCAO	UC-derived hMSCs	SD rats (male/female?)	14 days	600,000	CsA	NR		5 weeks	18	NR	NR	NR	[166]

Author (year)	Model	Paradigm	Cell type	Host species/ sex	Time post-injury	Final cell dose	IS	Quantification method	Groups	Final time point	No. animals explanted	No. animals with cells	Animals engrafted (%)	Cell survival (%)	Ref.
Kurozumi *et al.* (2004)	Stroke	MCAO	BM-derived hMSCs transfected with BDNF	Male Wistar rats	24 h	500,000	CsA	NR		8 and 16 days	14	NR	NR	NR	[167]
Kurozumi *et al.* (2005)	Stroke	MCAO	BM-derived hMSCs transfected with GDNF	Male Wistar rats	24 h	500,000	CsA	NR		15 and 16 days	27	NR	NR	NR	[168]
Longhi *et al.* (2004)	TBI	TBI via controlled cortical impact	NGF-NT2N or untransduced NT2N	C57BL/6 mice/male	24 h	20,000	CsA	Not performed		1 month	~20	NR	NR	NR	[169]
Omori *et al.* (2008)	Stroke	MCAO	BM-derived hMSCs	Adult female SD rats	Multiple	1,000,000–3,000,000	CsA	NR		2–3 weeks	54	NR	NR	NR	[170]
Park *et al.* (2010)	SCI	MASCIS Impactor	BM-derived hMSCs	Adult SD female rats	9 days	NR	CsA	Total cells/section		4 and 7 weeks	22	NR	NR	NR	[171]
Rossi *et al.* (2010)	SCI	Contusion (IH)	hESC-motor neuron progenitors	Adult SD female rats	7 days	100,000 and 500,000	CsA	NR		12 weeks	15	15	100.0	NR	[172]
Samdani *et al.* (2009)	SCI	Dorsolateral funiculotomy	BM-derived hMSCs	Adult female SD rats	0 days	150,000	CsA	Graft size by thresholding		3 weeks	6	NR	NR	NR	[173]
Saporta *et al.* (2003)	SCI	Clip compression	hUCB	Adult male SD rats	1 and 5 days	1,000,000	CsA	NR		3–4 weeks	NR	NR	NR	NR	[174]
Sasaki *et al.* (2009)	SCI	Transection	BM-derived hMSCs	Adult female SD rats	0 days	120,000	CsA	Flow cytometry		5 weeks	6	NR	NR	NR	[175]
Sharp *et al.* (2010)	SCI	Contusion (IH)	hESC-OPCs	Adult female SD rats	7 days	2,000,000	CsA	Three sections/1 mm tissue block		9 weeks	NR	All	100.0	NR	[176]
Skardelly *et al.* (2010)	TBI	TBI via controlled cortical impact	Fetal hNPCs (passage 8–12)	Adult male SD rats	1 day	100,000 (locally) 500,000 (systemic)	CsA + Pred	Not performed		12 weeks	52	NR	NR	NR	[177]
Stroemer *et al.* (2009)	Stroke	MCAO	Fetal hNSCs (CTX0E03 line)	Male SD rats	1 month	4500, 45,000 or 450,000	CsA + Pred	NR		16 weeks	30	NR	NR	NR	[178]
Tarasenko *et al.* (2007)	SCI	Contusion (IH)	Fetal hNSCs	Adult male SD rats	0, 3 and 9 days	200,000	CsA	Stereology (other)	hNSC in 9 days	3 months	25	NR	NR	0.5	[179]
Tarasenko *et al.* (2007)									Primed hNSC in 9 days	3 months	28	NR	NR	0.8	[179]
Watson *et al.* (2003)	TBI	TBI via controlled cortical impact	NGF-expressing and control-untransduced NT2N neurons	Male C57BL/6 mice	1 day	15,000–20,000	CsA	Not performed		1 month	12	NR	NR	NR	[180]
Wennersten *et al.* (2004)	TBI	TBI via weight-drop model	10-week fetal hNSC (passage 13)	Male SD rats	0 days	200,000	CsA	Four sequential sections spaced 400 μm apart		2 and 6 weeks	12	12	100.0	NR	[181]
Wennersten *et al.* (2006)	TBI	TBI via weight-drop model	7-week fetal hNSC (passage 10)	Male SD rats	0 days	210,000	CsA	Four sequential sections spaced 400 μm apart	CsA for 3 weeks	3 or 6 weeks, or 6 months	19	19	100.0	0.2	[182]

Author (year)	Model	Paradigm	Cell type	Host species/ sex	Time post-injury	Final cell dose	IS	Quantification method	Groups	Final time point	No. animals explanted	No. animals with cells	Animals engrafted (%)	Cell survival (%)	Ref.
Wennersten *et al.* (2006)									CsA for 6 weeks	6 weeks	11	11	100.0	0.2	[182]
Xiao *et al.* (2005)	SCI	Dorso lateral funiculotomy	Olfactory neuroepithelium derived neurosphere-forming cells	Adult female SD rats	7 days	200,000	CsA	Seven sections up to 2 mm away from injury		2 weeks for survival	6	6	100.0	0.3	[183]
Zhang *et al.* (2005)	TBI	TBI via lateral FP	NT2N neurons	Male SD rats	4 weeks	66,000	CsA	Not performed		4, 8 and 12 weeks	27	27	100.0	NR	[184]
Zhang *et al.* (2009)	SCI	Transection	Human Wharton's jelly cell NSCs	Adult female SD rats	0 days	200,000	CsA	Not clear		8 weeks	15	NR	NR	NR	[185]
Zhao *et al.* (2002)	Stroke	MCAO	BM-derived hMSCs	Adult male SH rats	7 days	75,000	CsA	NR		7 weeks	15	NR	NR	NR	[186]
Zheng *et al.* (2010)	Stroke	Global cerebral ischemia (via cardiac arrest)	BM-derived hMSCs	Male Wistar rats	3 h	1,000,000	CsA	NR		5 h, and 1, 3, 7 and 9 days	9	NR	NR	NR	[187]

Mesenchymal stem cell and BM stromal cell populations have been referred to by the general term multipotent stomal cell.

bFGF: Basic FGF; BM: Bone marrow; CAST: Computer-assisted stereological toolbox (Olympus stereology); CsA: Cyclosporine; Dex: Dexamethasone; EE: Enriched environment; EPO: Erythropoietin; FP: Fluid percussion; hESC: Human embryonic stem cell; hMSC: Human multipotent stomal cell; hNPC: Human neural progenitor cell; hNSC: Human neural stem cell; hUCB: Human umbilical cord blood; IH: Infinite horizons; IS: Immunosuppression; MASCIS: Multicenter Animal Spinal Cord Injury Study; MBF: MicroBrightField; MCAO: Middle cerebral artery occlusion; MNP: Motor neuron progenitor; NA: Not applicable; NPC: Neural progenitor cell; NR: Not reported; NSC: Neural stem cell; NT2N: Ntera-2 neuron; NYU: New York University; OPC: Oligodendrocyte progenitor cell; Pred: Prednisone; SCI: Spinal cord injury; SD: Sprague–Dawley; SH: Spontaneously hypertensive; ST: Standard cages; TBI: Traumatic brain injury; UC: Umbilical cord.

Table 7

Acute/traumatic: immunocompetent animals with no immunosuppression.

Author (year)	Model	Paradigm	Cell type	Host species/ sex	Time post-injury	Final cell dose	IS	Quantification method	Groups	Final time point	No. animals explanted	No. animals with cells	Animals engrafted (%)	Cell survival (%)	Ref.
Chen *et al.* (2003)	Stroke	MCAO	BM-derived hMSCs	Adult male Wistar rats	1 days	1,000,000	None	NR		15 days	12	NR	NR	NR	[188]
Chen *et al.* (2009)	TBI	TBI via PBBI	Human amnion-derived AMP cells (2 or fewer passages) – Stemnion cells	Male SD rats	0 days	2,000,000	None	Not performed		1, 2, 3, and 4 weeks	34	NR	NR	NR	[189]
Chen *et al.* (2011)	TBI	TBI via PBBI	Collagen scaffolds (Millipore) seeded with human amnion-derived AMP cells (2 or fewer passages)	Male SD rats	0 days	2,000,000	None	Not performed		2 weeks	20	NR	NR	NR	[190]
Deng *et al.* (2006)	SCI	Hemi-section	hOECs	Adult ATN rats and adult SD rats	0 days	Unclear	None	12–16 sections per cord	SD rats	1 week	4	0	0.0	NR	[191]
Ding *et al.* (2007)	Stroke	MCAO	Wharton's Jelly/umbilical cord-derived hMSCs	Adult male SD rats	7 days	1,000,000	None	NR		10, 14, 21 or 35 days	6	NR	NR	NR	[192]
Fang *et al.* (2010)	SCI	Weight drop (NYU)	Immortalized hMSCs ± PACP	Adult female SD rats	7 days	200,000	None	NR		31 days postinjury	NR	NR	NR	NR	[193]
Fatar *et al.* (2008)	Stroke	ICH	Human processed lipoaspirate hMSC	Adult male Wistar rats	1 day	3,000,000	None	NR		29 days	14	NR	NR	NR	[194]
Hagan *et al.* (2003)	TBI	TBI via cortical contusion	7 week old fetal hNSC (passage 11)	SD rats/sex NR	0 days	210,000	None	Five 25 and 100× fields per subject		6 days	6	5	83.3	0.12–0.95	[195]
Heile *et al.* (2009)	TBI	TBI via controlled cortical impact	hTERT immortalized GLP-1 expressing, alginate encapsulated BM-derived hMSCs	Male SD rats	0 days	64,000	None	NP		2, 7 and 14 days	13	NR	NR	NR	[196]
Hu *et al.* (2010)	SCI	Weight drop	UC-derived hMSCs	Female SD rats	1 day	400,000	None	NR		8 weeks	12	NR	NR	NR	[197]
Hung *et al.* (2010)	TBI	TBI via dragonfly FP device	Immortalized hTERT/RFP cord blood-derived hMSC (with and without RFP)	Male SD rats	3 days	100,000	None	NP		2, 7, and 14 days	NR	NR	NR	NR	[198]
Jeong *et al.* (2003)	Stroke	ICH	Fetal brain hNSC (HB1.F3)	Male SD rats	1 day	5,000,000	None	NR		8 weeks	12	NR	NR	NR	[199]
Kim *et al.* (2010)	TBI	TBI via controlled cortical impact	BM-derived hMSCs (supplied by FCB Pharmicell)	Male SD rats	1 day	2,000,000	None	NP		1, 2, 8, 15, 22 and 29 days after TBI	40	NR	NR	NR	[200]
Lee *et al.* (2009)	Stroke	ICH	Fetal brain hNSC (HB1.F3) overexpressing Akt1	F3.Akt1 mice	7 days	200,000	None	Stereology (CAST-Grid)	F3.Akt1, 2 weeks	9 weeks	9	NR	NR	53.9	[201]
Lee *et al.* (2009)									F3.Akt1 and 8 weeks	9 weeks	9	NR	NR	32.4	[201]

Author (year)	Model	Paradigm	Cell type	Host species/ sex	Time post-injury	Final cell dose	IS	Quantification method	Groups	Final time point	No. animals explanted	No. animals with cells	Animals engrafted (%)	Cell survival (%)	Ref.
Lee *et al.* (2009)									F3 control, 2 weeks	9 weeks	9	NR	NR	39.2	[201]
Lee *et al.* (2009)									F3 control, 8 weeks	9 weeks	9	NR	NR	16.3	[201]
Lee *et al.* (2008)	Stroke	ICH	Fetal brain hNSC H1 clone	Male SD rats	2 or 24 h	1,000,000 ic. 5,000,000 iv.	None	NR		7 weeks	179	NR	NR	NR	[202]
Liang *et al.* (2009)	SCI	Transection	Human amniotic membrane-derived hMSCs	Adult female Wistar rats	0 days	500,000	None	NR		12 weeks post-injury	12	NR	NR	NR	[203]
Liao *et al.* (2009)	Stroke	ICH	UC-derived hMSCs	Male SD rats	1 day	20,000	None	NR		15 days	20	NR	NR	NR	[204]
Liu *et al.* (2010)	Stroke	ICH	UC-derived HGF-transduced hMSCs	Male SD rats	7 days	600,000	None	NR		5 weeks	40	NR	NR	NR	[205]
Liu *et al.* (2006)	Stroke	ICH	PIGF-transduced BM-derived hMSCs	Adult male SD rats	7 days	600,000	None	NR		7 weeks	91	NR	NR	NR	[206]
Lu *et al.* (2007)	TBI	TBI via controlled cortical impact	Scaffolds seeded with BM-derived hMSCs (passage 3)	Male Wistar rats	4 days	3,000,000	None	Five equally spaced slides		5 weeks	12	12	100.0	NR	[207]
Lundberg *et al.* (2009)	TBI	TBI via weight-drop model	BM-derived hMSCs (isolated from 12 and 26 year old patients; used at passage 3)	Female SD rats	1 day	500,000 or 2,500,000	None	Six equally spaced slides		1 or 5 days post-transplant	32	NR	NR	NR	[208]
Mahmood *et al.* (2003)	TBI	TBI via controlled cortical impact	BM-derived hMSCs	Male Wistar rats	1 day	1,000,000 and 2,000,000	None	Five equally spaced slides	1,000,000	4 weeks	9	9	100.0	0.05	[209]
Mahmood *et al.* (2003)									2,000,000	4 weeks	9	9	100.0	0.06	[209]
Mahmood *et al.* (2005)	TBI	TBI via controlled cortical impact	BM-derived hMSCs	Male Wistar rats	1 day	2,000,000, 4,000,000 and 8,000,000	None	Three equally spaced slides		3 months	30	NR	NR	NR	[210]
Muir *et al.* (1999)	TBI	TBI via lateral FP	hNT cells (human postmitotic neurons)	Male SD rats	1 day	100,000	None	NP		2 weeks	28	23	82.1	NR	[211]
Nomura *et al.* (2005)	Stroke	MCAO	BDNF-hMSCs	Adult male SD rats	6 h	1,000,000	None	NR		1 week	44	NR	NR	NR	[212]
Onda *et al.* (2008)	Stroke	MCAO	Angiopoietin-1 gene-modified BM-derived hMSCs	Adult male SD rats	6 h	1,000,000	NR	NR		1 and 4 weeks	41	NR	NR	NR	[213]
Philips *et al.* (1999)	TBI	TBI via lateral FP	Human NT2N	Male SD rats	1 day	100,000	None	NP		2 and 4 weeks post-injury	24	21	87.5	NR	[214]
Qu *et al.* (2009)	TBI	TBI via controlled cortical impact	Scaffolds seeded with BM-derived hMSCs (passage 3)	Male C57BL/6 mice	7 days	300,000	None	Five equally spaced slides		5 weeks post-injury	24	NR	NR	NR	[215]
Shyu *et al.* (2007)	Stroke	MCAO	Immortalized BM-derived hMSC labeled with Gd-DTPA	Male adult SD rats	7 days	1,000,000	None	NR		5 weeks	NR	NR	NR	NR	[216]
Skvortsova *et al.* (2008)	Stroke	MCAO	BM-derived hMSCs	Male Wistar rats	NS	6,000,000	NR	NR		3 months	10	NR	NR	NR	[217]

Author (year)	Model	Paradigm	Cell type	Host species/ sex	Time post-injury	Final cell dose	IS	Quantification method	Groups	Final time point	No. animals explanted	No. animals with cells	Animals engrafted (%)	Cell survival (%)	Ref.
Song *et al.* (2009)	Stroke	MCAO	Human ferumoxide-labeled neural stem cells (HB1. F3)	Male SD rats	1 day	4,000,000 iv.; 400,000 ic.	NR	NR		4 weeks	3	NR	NR	NR	[218]
Toyama *et al.* (2009)	Stroke	MCAO	Angiopoietin-1 and VEGF gene-modified BM-derived hMSCs	Adult male SD rats	6 h	1,000,000	NR	NR		1 and 2 weeks	77	NR	NR	NR	[219]
Wakabayshi *et al.* (2010)	Stroke	MCAO	BM-derived hMSCs (HM3. B10 line)	Adult male Wistar rats	1 day	3,000,000	None	NR		4, 8 and 15 days	5	NR	NR	NR	[220]
Wennersten *et al.* (2006)	TBI	TBI via weight-drop model	7 week old fetal hNSC (passage 10)	Male SD rats	0 days	210,000	None	Four equally spaced slides	No IS	6 weeks	6	1	16.7	0.03	[182]
Yang *et al.* (2008)	SCI	Transection	Wharton's jelly-derived hMSCs	Adult female SD rats	0 days	100,000	None	NR		16 weeks post-tx	NR	NR	NR	NR	[221]
Zhang *et al.* (2009)	Stroke	MCAO	Embryonic hNSC	Adult male SD rats	1 day	50,000	None	NR		8, 15 or 29 days	30	NR	NR	NR	[222]
Zhu *et al.* (2009)	Stroke	MCAO	CX3CR1 shRNA transduced BM-derived hMSCs	Adult male SD rats	1 day	2,000,000	NR	NR		2, 4, or 8 days	45	NR	NR	NR	[223]

Mesenchymal stem cell and BM stromal cell populations have been referred to by the general term multipotent stomal cell.

AMP: Amnion-derived multipotent progenitor; ATN: Athymic nude rat; BM: Bone marrow; CAST: Computer-assisted stereological toolbox (Olympus stereology); FP: Fluid percussion; Gd: Gadolinium; GLP: Glucagon like peptide; hMSC: Human multipotent stomal cell; hNSC: Human neural stem cell; hOEC: Human olfactory ensheathing cell; ic.: Intracutaneously; ICH: Intracerebral hemorrhage; IS: Immunosuppression; iv.: Intravenously; MCAO: Middle cerebral artery occlusion; NP: Not performed; NR: Not reported; NS: Not specified; NT2N: Ntera-2 neuron; NYU: New York University; PACP: Pituitary adenyate cyclase-activating polypeptide; PBBI: Penetrating ballistic-like brain injury; RFP: Red fluorescent protein; SCI: Spinal cord injury; SD: Sprague–Dawley; TBI: Traumatic brain injury; tx: Transplantation; UC: Umbilical cord.

Table 8

Acute/traumatic: immunodeficient animals.

Author (year)	Model	Paradigm	Cell type	Host species/ sex	Time post-injury	Final cell dose	IS	Quantification method	Groups	Final time point	No. animals explanted	No. animals with cells	Animals engrafted (%)	Cell survival (%)	Ref.
Akesson *et al.* (2001)	SCI	Aspiration lesion	Human embryonic spinal cord tissue	Adult female ATN rats	0 days and 6–7 weeks	Solid graft	No	NR	0 days TT	6 weeks and 6 months	16	15	93.8	NR	[224]
Akesson *et al.* (2001)									6–7 weeks post-injury TT	6 weeks and 6 months	17	16	94.1	NR	[224]
Akesson *et al.* (2007)	SCI	Clip compression	Spinal cord-derived human fetal NSs	Adult female ATN rats	14 days	10–12 NSs	No	NA		6 weeks	7	5	71.4	NR	[225]
Cummings *et al.* (2005)	SCI	Contusion (IH)	Fetal hNSCs	Adult female NOD-SCID mice	9 days	75,000	No	Stereology (MBF)		17 weeks	15	15	100.0	194.0	[226]
Deng *et al.* (2006)	SCI	Hemisection	hOECs	Adult ATN rats and adult SD rats	0 days	Unclear	No	12–16 sections per cord	ATN rats	1 week	15	6 out of 6 animals examined	100.0	NR	[191]
Eaton *et al.* (2008)	SCI	NYU Impactor	Human neuronal cell line (5HT hNT2.19 derived)	Adult female nude rats	7 days	1,000,000	No	NR		8 weeks	6	NR	NR	NR	[227]
Emgard *et al.* (2009)	SCI	Clip compression	Human fetal NSs	Adult ATN female rats	14 days	12 NSs (~70,000 cells)	No	Every 20th section and several photos per section (~17 per animal)		3 weeks	32	29	90.6	NR	[228]
Gorrie *et al.* (2010)	SCI	MASCIS Impactor	hOECs	8–9 week old ATN female rats	7 days	1,000,000	No	NR		6 weeks	NR	NR	NR	NR	[229]
Hooshmand *et al.* (2009)	SCI	Contusion (IH)	Fetal hNSCs	Adult female NOD-SCID mice	9 days	75,000	No	Stereology (MBF)		17 weeks	15	15	100.0	194.0	[230]
Salazar *et al.* (2010)	SCI	Contusion (IH)	Fetal hNSCs	Adult female NOD-SCID mice	30 days	75,000	No	Stereology (MBF)		16 weeks	15	15	100.0	288.0	[231]
Sasaki *et al.* (2009)	SCI	Weight drop	Human blood-derived $CD133^+$	Adult male ATN rats	0 days	100,000	No	NR		6 weeks	NR	NR	NR	NR	[232]
Sheth *et al.* (2008)	SCI	NYU Impactor	BM-derived hMSCs	Adult female nude rats	7 days	600,000	No	NR		6 weeks	29	29	100.0	NR	[233]
Sundberg *et al.* (2011)	SCI	Contusion (IH)	hESC-NPCs	Adult ID female rats	8 days	100,000	No	NR		12 weeks	6	NR	NR	NR	[234]
Takeuchi *et al.* (2007)	SCI	Weight drop	Immortalized hNSC line	Adult female nude (KSN) mice	3, 7 and 10 days	1,000,000	No	Every 3rd section in a 0.25 mm^2 grid		1 week	24	NR	NR	NR	[235]
Yan *et al.* (2007)	SCI	Excitotoxic lesion	Fetal hNSCs	Adult ATN rats	14 days	400,000	No	Stereology (MBF)		6 months	12	12	100.0	375.0	[236]

Mesenchymal stem cell and BM stromal cell populations have been referred to by the general term multipotent stomal cell.

ATN: Athymic nude rat; BM: Bone marrow; hESC: Human embryonic stem cell; hMSC: Human multipotent stomal cell; hNSC: Human neural stem cell; hOEC: Human olfactory ensheathing cell; IH: Infinite horizons; ID: Immunodeficient; IS: Immunosuppression; MASCIS: Multicenter Animal Spinal Cord Injury Study; MBF: MicroBrightField; NA: Not applicable; NOD: Nonobese diabetic; NPC: Neural progenitor cell; NR: Not reported; NS: Neurospheres; NYU: New York University; SCI: Spinal cord injury; SCID: Severe combined immunodeficiency; SD: Sprague–Dawley; TT: Transplant time.

Table 9

Chronic/atraumatic: immunocompetent animals with immunosuppression.

Author (year)	Model	Paradigm	Cell type	Host species/ sex	Time post-injury	Final cell dose	IS	Quantification method	Groups	Final time point	No. animals explanted	No. animals with cells	Animals engrafted (%)	Cell survival (%)	Ref.
Aharonowiz *et al.* (2008)	MS	EAE (MS)	hESC-derived NPC	6–7 week female C57BL mice	7 day post-EAE	1,000,000	CsA	NR		43 days	15	NR	NR	NR	[237]
Anderson *et al.* (2007)	PD	6-OHDA (PD)	7–9-week fetal hNPC grown for 8 or 20 weeks *in vitro*	Lewis rats	2 week post-lesion	775,000 or 706,000	CsA	Stereology (other)	7–9-week fetal hNPC grown for 8 weeks *in vitro*	6 weeks	NR	NR	NR	52.0	[238]
Anderson *et al.* (2007)									7–9-week fetal hNPC grown for 20 weeks *in vitro*	6 weeks	NR	NR	NR	25.0	[238]
Armstrong *et al.* (2000)	HD	Quinolinic acid (HD)	9-week fetal hNSC	Female adult cluster determinant rats	6 h post-lesion	900,000	CsA	NR		12 weeks	NR	NR	NR	NR	[239]
Behrstock *et al.* (2006)	PD	6-OHDA (PD)	10–15-week fetal hNPC with lenti-GDNF and GFP	Adult male Lewis rat	1 week post-lesion	120,000	CsA	NR		9 weeks	NR	NR	NR	NR	[240]
Brederlau *et al.* (2006)	PD	6-OHDA (PD)	hESC-derived DA neurons	Female SD rats	2 week post-lesion	100,000	CsA	Stereology (other)		13 weeks	38	NR	NR	500.0	[241]
Garbuzova-Davis *et al.* (2003)	ALS	SOD1 (ALS)	Human umbilical cord blood cells	Male SOD1 mice	9.5 weeks old	100,000	CsA	Visualization of hNuc		10–12 weeks	9	NR	NR	NR	[242]
Kerr *et al.* (2003)	ALS	Viral motor neuron death	hEBD-derived cells	3–4 week Lewis rats	8 days after infection	300,000	CsA	Stereology (other)		1 month	NR	NR	NR	1.0	[243]
Klein *et al.* (2005)	ALS	SOD1 (ALS)	22-week fetal hNSCs control or expressing GDNF	SOD1 mice	100 days old	120,000	CsA	hNuc	hNSCs Control	Disease end point	7	6	86	NR	[244]
Klein *et al.* (2005)									hNSCs plus GDNF	Disease end point	7	2	29	NR	[244]
McBride *et al.* (2004)	HD	Quinolinic acid (HD)	12-week fetal hNSC in media with B27 or B27 and CNTF	Adult male Lewis rat	1 week post-lesion	200,000	CsA	Stereology (MBF)	hNSC in media with B27	8 weeks	7	NR	NR	155.0	[245]
McBride *et al.* (2004)									hNSC in media plus CNTF	8 weeks	9	NR	NR	154.0	[245]
Mukhida *et al.* (2007)	Allodynia	L5 and L6 nerve root ligation (allodynia)	10-week fetal hNSC undifferentiated or GABA differentiated	Female Wistar rats	10 day post-ligation	200,000	CsA	Stereology (MBF)	hNSC undifferentiated	7 weeks	7	NR	NR	48.0	[246]
Mukhida *et al.* (2007)									hNSC GABA	7 weeks	7	NR	NR	37.0	[246]
Ostenfeld *et al.* (2000)	PD	6-OHDA (PD)	6–21-week fetal hNSC	Female SD rats	2 weeks post-lesion	200,000 and 1,000,000 and 2,000,000	CsA	Stereology for volume		2, 6 and 20 weeks	15	15	100	NR	[247]
Park *et al.* (2005)	PD	6-OHDA (PD)	hESC-derived DA neurons	Adult male SD rats	3 weeks post-lesion	500,000	CsA	NR		2 weeks	NR	NR	NR	NR	[248]
Roy *et al.* (2006)	PD	6-OHDA (PD)	hESC-derived DA neurons	8 week adult male SD rats	5 weeks post-lesion	500,000	CsA	hNuc over five sagittal sections		10 weeks	6	6	100	NR	[249]
Sanchez-Pernaute *et al.* (2001)	PD	6-OHDA (PD)	7-week fetal hNSC	Adult female SD rats	3 weeks post-lesion	420,000	CsA	hNSE		6 weeks	14	13	93	NR	[250]

Author (year)	Model	Paradigm	Cell type	Host species/ sex	Time post-injury	Final cell dose	IS	Quantification method	Groups	Final time point	No. animals explanted	No. animals with cells	Animals engrafted (%)	Cell survival (%)	Ref.
Suzuki *et al.* (2007)	ALS	SOD1 (ALS)	10–15-week fetal hNSC expressing GDNF	Female SOD1 rats	70 days old	1,000,000	CsA	Stereology (MBF) n = 1		2 and 6 weeks	12	7	58	113 †	[251]
Svendsen *et al.* (1996)	PD	6-OHDA (PD)	13-week fetal hNSC	Adult female Wistar rats	NR	750,000	CsA	NR		4 weeks	NR	NR	NR	NR	[252]
Xu *et al.* (2006)	ALS	SOD1 (ALS)	8-week fetal hNSC	SOD1 rats	62 days old	400,000	FK506	Six sections at 100×		Disease end point (BBB <3)	NR	NR	NR	NR	[253]
Xu *et al.* (2009)	ALS	SOD1 (ALS)	8-week fetal hNSC	SOD1 rats and SD rats	56 days old	160,000	FK506	hNuc		Disease end point	NR	NR	NR	NR	[254]
Yan *et al.* (2006)	ALS	SOD1 (ALS)	8-week fetal hNSC	SOD1 rats	8 weeks old	80,000	Yes: 1) FK506, 2) FK506 and Rapa, 3) FK506 and Rapa and MMF, 4) CD4 Ab	hNuc		1 week and 1 month	70	NR	NR	NR	[255]
Yasuhara *et al.* (2006)	PD	6-OHDA (PD)	15-week fetal hNSC (HB1.F3)	SD rats	Immediate	200,000	CsA	hNuc and GFP every 5th section through striatum	3 day survival	3 days, 1, 2 or 4 weeks	8	NR	NR	11.0	[256]
Yasuhara *et al.* (2006)									1 week survival	3 days	8	NR	NR	5.0	[256]
Yasuhara *et al.* (2006)									2 week survival	1 week	8	NR	NR	2.0	[256]
Yasuhara *et al.* (2006)									4 week survival	2 weeks	8	NR	NR	1.0	[256]
Zeng *et al.* (2004)	PD	6-OHDA (PD)	hESC-derived DA neurons and undifferentiated hESC	Adult Fisher rats	4 weeks	NR	CsA and vibramycin	10 sections/animal stained		5 weeks	15	15	100	NR	[257]

† Only one animal was quantified.

Ab: Antibody; ALS: Amyotrophic lateral sclerosis; BBB: Basso, Beattie, Bresnahan locomotor rating scale; CsA: Cyclosporine; DA: Dopaminergic; EAE: Experimental autoimmune encephalitis; GFP: Green fluorescent protein; HD: Huntington's disease; hEBD: Human embryoid body-derived; hESC: Human embryonic stem cell; hNPC: Human neural progenitor cell; hNSE: Human neuron-specific enolase; hNSC: Human neural stem cell; hNuc: Human nuclear antibody; IS: Immunosuppression; MBF: MicroBrightField; MS: Multiple sclerosis; NPC: Neural progenitor cell; NR: Not reported; PD: Parkinson's disease; SD: Sprague–Dawley; SOD: Superoxide dismutase.

Table 10

Chronic/atraumatic: immunocompetent animals with no immunosuppression.

Citation	Model	Paradigm	Cell type	Host species/ sex	Time post-injury	Final cell dose	IS	Quantification method	Groups	Final time point	No. animals explanted	No. animals with cells	Animals engrafted (%)	Cell survival (%)	Ref.
Aubry *et al.* (2008)	HD	Quinolinic acid	hESC-derived NPC	Adult OFA	1 week post-lesion	125,000	No	NR	4–6 week survival	4–6 weeks	48	48	100	NR	[258]
Aubry *et al.* (2008)									13–21 week survival	13–21 weeks	24	24	100	NR	[258]
Hwang *et al.* (2009)	ALS	SOD1	hNSC expressing VEGF	SOD1 mice	10 weeks old	100,000	No	Stereology (other)		2 and 4 weeks	20	NR	NR	52.0	[259]
Kim *et al.* (2006)	PD	6-OHDA	Fetal hNSCs	Female Sprague–Dawley rats	4 weeks post-lesion	1,000,000	No	Visualization of BGal		4 weeks	12	12	100	NR	[260]
Lee *et al.* (2005)	HD	Quinolinic acid	15-week fetal hNSC	Adult male Sprague–Dawley rats	1 week	5,000,000	No	12 ROIs		3 weeks	16	NR	NR	NR	[261]
Liker *et al.* (2003)	PD	MPTP	12–20-week fetal hNSC	8 week C57BL6	7 days after MPTP	400,000	No	Stereology (other)	7 day survival	7, 30, 60 or 90 days	6	6	100	55.0	[262]
Liker *et al.* (2003)									30 day survival	1 week	6	6	100	28.0	[262]
Liker *et al.* (2003)									60 day survival	30 days	6	6	100	24.0	[262]
Liker *et al.* (2003)									90 day survival	60 days	6	2	33	9.0	[262]
Ryu *et al.* (2004)	HD	3-NP	15-week fetal hNSC	Adult male Lewis rat	1 week before or 12 h after	100,000	No	BGal ICC		2 weeks	NR	NR	NR	NR	[263]
Windrem *et al.* (2004)	Demyelination	Shiverer	Fetal hOPC and adult hOPC	1 day old homo Shi pups mice	1 day old	100,000	No	NA		4, 8, 12 or 16 weeks	44	34	77	NR	[264]

ALS: Amyotrophic lateral sclerosis; BGal: β-galactosidase; HD: Huntington's disease; hESC: Human embryonic stem cell; hNSC: Human neural stem cell; hOPC: Human oligodendrocyte progenitor cell;

ICC: Immunocytochemistry; IS: Immunosuppression; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NA: Not applicable; NP: Nitropropionic acid; NPC: Neural progenitor cell; NR: Not reported; OFA: Oncins France strain A; PD: Parkinson's disease; ROI: Region of interest; SOD: Superoxide dismutase.

Table 11

Chronic/atraumatic: immunodeficient animals.

Author (year)	Model	Paradigm	Cell type	Host species/ sex	Time post -injury	Final cell dose	IS	Quantification method	Final time point	No. animals explanted	No. animals with cells	Animals engrafted (%)	Cell survival (%)	Ref.
Hurelbrink *et al.* (2002)	HD	Quinolinic acid	9–10-week fetal tissue	Female nude rats	4–39 days	500,000	No	Stereology (CAST-Grid)	6 weeks or 6 months	15	13	87	40 in striatum only	[265]
Kim *et al.* (2005)	Glioma tumor	Brain (tumor)	15-week fetal hNSC	6–8 week old male Swiss nude mice	7 days post-tumor	240,000	No	Visualization of DiI (prelabeled)	2 weeks	12	NR	NR	NR	[266]
Kim *et al.* (2006)	Glioma tumor	Brain (tumor)	15-week fetal hNSC	6–8 week old male Swiss nude mice	4 week post-tumor	240,000	No	Visualization of DiI (prelabeled)	2 weeks	4	NR	NR	NR	[267]
Shimato *et al.* (2007)	Cancer	Leptomeningeal medulloblastoma (cancer)	15-week fetal hNSC	BALB/c female nude mice	2 weeks post-cancer induction	50,000,000	No	DiI prelabeled	Disease end point	NR	NR	NR	NR	[268]

CAST: Computer-assisted stereological toolbox (Olympus stereology); HD: Huntington's disease; hNSC: Human neural stem cell; IS: Immunosuppression; NR: Not reported.