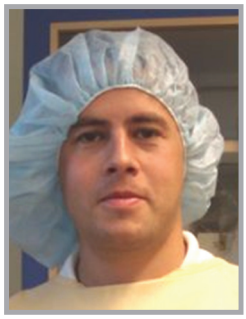
Future NEUROLOGY

EDITORIAL

Stem cell therapy for neurodegenerative diseases: mind the gap



Sergey V Anisimov*

"Only with the gap between fundamental studies and actual clinical applications closed can we hope for efficient and large-scale inculcation of stem cell-based therapy approaches into many clinical disciplines, including neurology."

Stem cells possess a unique spectrum of biological features that provide a foundation of growth, development, adaptation and regeneration of the organism, starting from the embryonic stage and continuing through adult life. It is currently believed that senescence is firmly associated with a decline of stem cell self-renewal [1,2]. Numerous subtypes of stem cells are currently available, including embryonic stem cells (ESCs) [3], various populations of adult/somatic stem cells (ASCs) and, finally, induced pluripotent stem cells (iPSCs) [4]. Taken together, stem cell-based therapy is now rightfully viewed by the clinical society as an emerging novel approach suitable for a treatment of numerous diseases, including those previously considered incurable. A list of diseases currently considered to be targets for future stem cell therapy-based approaches includes neurodegenerative diseases, namely, Parkinson's disease (PD) and secondary Parkinsonism, Alzheimer's disease, multiple system atrophy, amyotrophic lateral sclerosis, stroke, brain trauma and spinal trauma, among others. It should be stressed that in contrast to the majority of other neurodegenerative disorders, the motor symptoms in PD are primarily caused by the loss of dopaminergic neurons in the substantia nigra. Similarly, the pathology of stroke and brain/spinal trauma is, in many cases, associated with lesions within a single known anatomical localization. This factor makes PD and some other neurodegenerative disorders highly suitable for cell replacement strategies.

By October 2013, the ClinicalTrials.gov database listed over 4700 clinical studies associated with stem cells [101]. Many of these studies aim to test the safety and efficiency of stem cells, particularly stem cell-derived cells in various neurodegenerative disorders. For example, PD [5,6], a progressive supranuclear palsy (also Steele-Richardson–Olszewski syndrome) [7], amyotrophic lateral sclerosis [8,9], multiple system atrophy [10] and spinocerebellar ataxia [11] became targets of stem cell-based therapeutic approaches. It is worth

KEYWORDS
• adult stem cells • cell therapy • clinical trials • embryonic stem cells • fundamental research • induced pluripotent stem cells • risks • stem cells • transplantation

*Research Unit of Cellular & Genetic Engineering, VA Almazov Federal Center for Heart, Blood & Endocrinology, Akkuratova Street 2, Saint Petersburg, 197341, Russia
and
Department of Intracellular Signaling & Transport, Institute of Cytology, Russian Academy of Sciences, Saint Petersburg, Russia
Tel.: +7 812 702 3700; Fax: +7 812 702 3701; biobank@almazovcentre.ru

Future Medicine part of fsg

noting that various cell substrates and methods of delivery are tested in the trials. For example, even in a limited group of clinical trials based on stem cell therapy for PD, both autologous [5] (unpublished trials with ClinicalTrials.gov identifiers NCT01446614 [102] and NCT00976430 [103]) and allogenic bone marrow mesenchymal stem cells (MSCs) [6], and autologous adipose-derived MSCs (NCT01453803 study) were tested [104]. Unilateral [5] and bilateral [6] stereotax transplantation into the subventricular zone or striatum (NCT00976430 study [103]), intravenous (NCT01446614 [102] and NCT01453803 [104] studies) and intra-arterial (NCT01453803 study [104]) means of cell delivery were assessed. Results of the key study suggest allogenic bone marrow MSC may be used as a disease-modifying therapeutic strategy in treating PD [6]. These and many other clinical trials are based on the body of experimental data generated through the decades of *in vitro* and animal model-based *in vivo* studies. Systematical research of stem cell biology was able to dissect key molecular mechanisms involved in stem cell proliferation, differentiation and senescence. It is evident, however, that there is an apparent gap between two large groups of studies within an entire field of stem cell research: namely, between experimental and clinical studies – such as between bench and bedside. This gap is supposed to be filled with so-called 'translational studies'. In the context of stem cell therapy, the translational studies aim to create, adapt and troubleshoot techniques established in laboratory conditions for actual clinical applications. In particular, a recently launched journal entitled '*Stem Cells Translational Medicine*' (its first issue dated January 2012) aims to fill the gap outlined above.

"...no single stem cell type distinguishes as a 'universal' cell therapy substrate for all – or at least for a majority of diseases."

Clearly, factors such as stem cell source availability, stem cell stability in culture, efficiency of stem cell differentiation *in vitro* into the functional target cells, donor cell survival and finally functional effects following a transplantation of particular stem cells or stem cell-derived cellular populations all play roles in selecting optimal transplantation substrates. While certain stem cell types demonstrate preponderance over others in some of these features, no single stem cell type distinguishes as a 'universal' cell therapy substrate for all – or at least for a majority of diseases. For example, both ESCs and iPSCs have the highest proliferative activity and plasticity, superior to those of ASCs. Currently, effective expansion of stem cells and directed differentiation of the latter to the functional target cells (e.g., neurons) stopped being a limiting step of cell therapy approaches [12]. At the same time, ASCs (namely MSCs of various origins) and iPSCs allow authological applications, thus escaping many ethical issues and a necessity for immunosuppressive therapy. In exchange to lower proliferative activity, the ASC/MSC karyotype is highly stable *in vitro*, while the ESC and, especially, iPSC karyotype is unstable at prolonged culture *in vitro*, causing a risk of cell transformation. Moreover, even a few of the residual undifferentiated cells of ESC and iPSC types can cause teratoma in the sites of transplantation [13]. In fact, any cells that are not pluripotent but still mitotic, including committed stem cell derivatives, can cause tumor formation in sites of transplantation. Even a limited cell division can cause 'graft overgrowth', leading to the overproduction of specific hormones or to dyscirculatory alterations in the surrounding tissues.

Taken together, the risks associated with clinical applications of stem cells include oncological risks [14], and infection transmission risks (in particular, note a risk of xenozoonosis transmission [15]), as well as immunological, genetical and some other risks [16]. Numerous adaptations should be introduced into the optimized stem cell expansion and differentiation protocols to effectively reduce the risks listed above. For example, establishing and expanding human stem cells (ESCs, iPSCs and ASCs) in xeno-free conditions is already possible [17,18], with organic/xenogenic material being replaced with xeno-free ones in virtually every step of the cell culturing protocol. A traditional method of ESC isolation from the inner cell mass of the blastocysts with pronase is now replaced with Tyrode's solution application, mechanical/laser-based cell isolation or utilization of spontaneously hatched blastocysts. Some of the organic compounds traditionally used to cover cell culture surfaces (laminin, poly-L-lysine, fibronectin, gelatine and collagen) may be derived from both animal and human sources; moreover, novel highly adhesive plastic materials reduce the need for the organic compounds. Similarly, numerous growth factors used to induce stem cell differentiation *in vitro* and maintain cell survival *in vivo* are currently available in 'natural' and human recombinant forms. Finally, animal serum, a key component

of cell culture media, could be replaced with either entirely synthetic formulations or with human serum, including autologous serum, although reportedly with reduced efficiency of cell expansion [19]. Contrary to the development outlined above, stem cell differentiation into functional neurons still appears to be relatively less effective and significantly more costly when it utilizes solely human recombinant growth factors compared with methods relying on coculturing with animal stromal cells, or a combination of both [20,21]. It is obvious that available protocols aiming to provide stem cell application for clinical purposes should be adapted/evolved to completely xeno-free regimens to be accepted for cell therapy.

Oncological risks are clearly most important in cell therapy applications. As mentioned above, even a few residual undifferentiated pluripotent stem cells (ESCs or iPSCs) are a heterogeneous cell population that serves transplantation substrate and could cause teratoma formation in the sites of transplantation. For intracranial cell deliveries/grafts, such a complication will, in a majority of cases, cause fatal consequences [13]. Completely eliminating residual undifferentiated cells is not an easy task as mature cells differentiated *in vitro* for a prolonged time (in particular, mature neurons with long processes) have significantly lower chances of surviving a transplantational procedure [22]. Promising approaches based on cell selection *in vitro* by exposing heterogeneous cell populations to low-dose γ-irradiation [14], ceramide/ceramide analogs [23] or human/bovine α-lactalbumin made lethal to tumor cells [24] are not highly effective, possibly owing to the difference between rodent and human stem cell biology/molecular signaling mechanisms. At the same time, genetic engineering represents the most promising strategy for selective ablation of undifferentiated pluripotent cells. Those approaches are based on the introduction of so-called 'suicide genes' (e.g., genes encoding *HSV-TK*/*HSVtk*, tetracycline-inducible form of the diphtheria toxin or bacterial cytosine deaminase) into the stem cell genome under the control of promoters of the genes characteristic of 'embryonic stemness' [25]. Furthermore, straightforward cell sorting based on the advanced FACS or magnetic-activated cell sorting technology may also be effective in selective cell sorting [14]. Indeed, the consequences of donor cell-derived tumor development in stem cell therapy recipients may be severe, risking compromising a whole field. Therefore, ongoing translational studies should aim to not just reduce oncological risks associated with stem cells, but also to effectively nullify these risks.

It is worth noting that in many neurological disorders, even a minor improvement of motor and cognitive functions may significantly improve a patient's quality of life and contribute to survival. However, one should not underestimate challenges and risks related to stem cell applications. Among those, oncological risks and xenozoonosis transmission risks are most important. Rapid progress in all areas of stem cell research promises to revolutionize clinical medicine, particularly neurology. It is obvious that certain neurodegenerative disorders, including PD and secondary Parkinsonism, represent attractive (although not easy) and important targets of cell therapy. With the gap described above bridged and key risks eliminated, stem cell therapy will amplify the currently available arsenal of therapeutic methods. In many clinical situations, transplantation of autologous or allogenic stem cell-derived neural/neuronal cells – specific populations of postmitotic neural/neuronal progenitor cells in particular – would be able to induce a significant functional improvement in the recipients. In conclusion, it can be stressed that developing safe and efficient protocols for stem cell-based therapies of many disorders should now become the aim of concentrated efforts of the clinical and research communities, further supplemented by the technological advances becoming available annually. Only with the gap between fundamental studies and actual clinical applications closed can we hope for efficient and large-scale inculcation of stem cell-based therapy approaches into many clinical disciplines, including neurology.

"Numerous adaptations should be introduced into the optimized stem cell expansion and differentiation protocols to effectively reduce the risks..."

Financial & competing interests disclosure

This work was supported by the Ministry of Healthcare and Social Development of the Russian Federation (State Contract K-32-NIR/111-3) within the framework of the 'Stem Cell' program of the State, and by the Molecular and Cellular Biology Program, Russian Academy of Sciences. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

1 Drummond-Barbosa D. Stem cells, their niches and the systemic environment: an aging network. *Genetics* 180(4), 1787–1797 (2008).

2 Baker DJ, Weaver RL, van Deursen JM. p21 both attenuates and drives senescence and aging in BubR1 progeroid mice. *Cell Rep.* 3(4), 1164–1174 (2013).

3 Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. *Nature* 292(5819), 154–156 (1981).

4 Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126(4), 663–676 (2006).

5 Venkataramana NK, Kumar SK, Balaraju S *et al.* Open-labeled study of unilateral autologous bone-marrow-derived mesenchymal stem cell transplantation in Parkinson's disease. *Transl. Res.* 155(2), 62–70 (2010).

6 Venkataramana NK, Pal R, Rao SA *et al.* Bilateral transplantation of allogenic adult human bone marrow-derived mesenchymal stem cells into the subventricular zone of Parkinson's disease: a pilot clinical study. *Stem Cells Int.* 2012, 931902 (2012).

7 Litvan I, Bhatia KP, Burn DJ *et al.* Movement Disorders Society Scientific Issues Committee report: SIC Task Force appraisal of clinical diagnostic criteria for Parkinsonian disorders. *Mov. Disord.* 18(5), 467–486 (2003).

8 Blanquer M, Moraleda JM, Iniesta F *et al.* Neurotrophic bone marrow cellular nests prevent spinal motoneuron degeneration in amyotrophic lateral sclerosis patients: a pilot safety study. *Stem Cells* 30(6), 1277–1285 (2012).

9 Riley J, Federici T, Polak M *et al.* Intraspinal stem cell transplantation in amyotrophic lateral sclerosis: a phase I safety trial, technical note, and lumbar safety outcomes. *Neurosurgery* 71(2), 405–416 (2012).

10 Lee PH, Lee JE, Kim HS *et al.* A randomized trial of mesenchymal stem cells in multiple system atrophy. *Ann. Neurol.* 72(1), 32–40 (2012).

11 Jin JL, Liu Z, Lu ZJ *et al.* Safety and efficacy of umbilical cord mesenchymal stem cell therapy in hereditary spinocerebellar ataxia. *Curr. Neurovasc. Res.* 10(1), 11–20 (2013).

12 Kozhukharova IV, Fridlyanskaya II, Zemel'ko VI *et al.* Generation of dopamine neurons from human embryonic stem cells *in vitro*. *Cell Tissue Biol.* 4(5), 411–418 (2010).

13 Brederlau A, Correia AS, Anisimov SV *et al.* Transplantation of human embryonic stem cell-derived cells to a rat model of Parkinson's disease: effect of *in vitro* differentiation on graft survival and teratoma formation. *Stem Cells* 24(6), 1433–1440 (2006).

14 Anisimov SV, Morizane A, Correia AS. Risks and mechanisms of oncological disease following stem cell transplantation. *Stem Cell Rev.* 6(3), 411–424 (2010).

15 Anisimov SV. Risks of the xenogenic origin in stem cells applications. *Tsitologiia* 54(4), 289–297 (2012).

16 Anisimov SV. Cell therapy for Parkinson's disease: IV. Risks and future trends. *Adv. Gerontol.* 22(3), 418–439 (2009).

17 Swistowski A, Peng J, Han Y, Swistowska AM, Rao MS, Zeng X. Xeno-free defined conditions for culture of human embryonic stem cells, neural stem cells and dopaminergic neurons derived from them. *PLoS ONE* 4(7), e6233 (2009).

18 Kim HT, Lee KI, Kim DW, Hwang DY. An ECM-based culture system for the generation and maintenance of xeno-free human iPS cells. *Biomaterials* 34(4), 1041–1050 (2013).

19 Rajala K, Hakala H, Panula S *et al.* Testing of nine different xeno-free culture media for human embryonic stem cell cultures. *Hum. Reprod.* 22(5), 1231–1238 (2007).

20 Correia AS, Anisimov SV, Li JY, Brundin P. Growth factors and feeder cells promote differentiation of human embryonic stem cells into dopaminergic neurons: a novel role for fibroblast growth factor-20. *Front. Neurosci.* 2(1), 26–34 (2008).

21 Morizane A, Darsalia V, Guloglu MO *et al.* A simple method for large-scale generation of dopamine neurons from human embryonic stem cells. *J. Neurosci. Res.* 88(16), 3467–3478 (2010).

22 Anisimov SV, Correia AS, Li JY, Brundin P. Being realistic about human embryonic stem cell-based therapy of Parkinson's disease. In: *Parkinson's Disease and Movement Disorders (5th Edition).* Jankovic JJ, Tolosa E (Eds). Lippincott Williams & Wilkins, Philadelphia, PA, USA, 642–652 (2006).

23 Bieberich E, Mackinnon S, Silva J, Yu RK. Regulation of apoptosis during neuronal differentiation by ceramide and b-series complex gangliosides. *J. Biol. Chem.* 276(48), 44396–44404 (2001).

24 Svensson M, Håkansson A, Mossberg AK, Linse S, Svanborg C. Conversion of alpha-lactalbumin to a protein inducing apoptosis. *Proc. Natl Acad. Sci. USA* 97(8), 4221–4226 (2000).

25 Schuldiner M, Itskovitz-Eldor J, Benvenisty N. Selective ablation of human embryonic stem cells expressing a 'suicide' gene. *Stem Cells* 21(3), 257–265 (2003).

• Websites

101 ClinicalTrials.gov database. http://clinicaltrials.gov

102 Mesenchymal Stem Cells Transplantation to Patients With Parkinson's Disease. http://clinicaltrials.gov/ct2/show/NCT01446614

103 Autologous Mesenchymal Stem Cell Transplant for Parkinson's Disease. http://clinicaltrials.gov/ct2/show/NCT00976430

104 Study to Assess the Safety and Effects of Autologous Adipose-Derived Stromal in Patients With Parkinson's Disease. http://clinicaltrials.gov/ct2/show/NCT01453803