The future of stem cell therapy for stroke rehabilitation

Cesar V Borlongan[*,1], **Jukka Jolkkonen**[*,2], and **Olivier Detante**[3,4,5]

[1]Department of Neurosurgery and Brain Repair, Morsani College of Medicine, University of South Florida, 12901 Bruce B Downs Blvd, Tampa, FL 33612 USA [2]University of Eastern Finland, Institute of Clinical Medicine – Neurology, Yliopistonranta 1 C, 70210 Kuopio, Finland [3]University Hospital of Grenoble, Stroke Unit, Department of Neurology, CS 10217, 38043 Grenoble, France [4]Inserm, U 836, BP 170, 38042 Grenoble, France [5]University Grenoble Alpes, Grenoble Institute of Neurosciences, BP 170, 38042 Grenoble, France

Currently what are the biggest challenges in the field of stem cell therapy for stroke rehabilitation?

Borlongan: The biggest challenge is the coalescing cell therapy with rehabilitation therapy in an effort to realize improved outcomes in stroke patients. Indeed, in the recent Stem Cell Therapeutics as an Emerging Paradigm for Stroke or STEPS 3 meeting, a key theme that was advanced by the group is to examine stem cell therapy vis-à-vis with rehabilitation therapy to reveal not only stand-alone effects of each therapy [1, 2], but to potentially assess the concept of combination therapy for stroke. Although stroke rehabilitation is well recognized in the clinic [3], most of the preclinical studies on stem cell therapy have not incorporated this rehabilitation effect in the experimental design. Accordingly, this lack of rehabilitation control arm in the laboratory represents as a major disconnect between the preclinical arena and the clinical setting, limiting the recognition of the potential benefits of stem cell therapy to stroke rehabilitation and vice versa. Notwithstanding, if we define rehabilitation as 'exercise', there are excellent studies in the laboratory that have assessed the beneficial role of exercise in influencing the fate of endogenous stem cells. The concept that exercise enhances neurogenesis was popularized by Rusty Gage and colleagues [4]. In particular, in this paper and several other subsequent papers from this group and others showed that new cells almost doubled in the hippocampus after just 12 days of daily running. A paper by Mark Mattson [5], fittingly titled 'take away my food, and let me run'

[*]Authors for correspondence: Tel: +1 813 974 3154; Fax: +1 813 974 3078; cborlong@health.usf.edu Tel: +358 40 355 2519; Fax: +358 17 163539; jukka.jolkkonen@uef.f.

discusses the benefit of running, as well as good diet! Over the next decade or so since the milestone publication by Gage and colleagues, exercise has been demonstrated to reverse some of the symptoms of age-related disorders, such as AD [6], PD [7], stroke [8] and TBI [9]. Fast forward to our paper in 2007 [10], we show that we could capture the adverse effects of withholding exercise using the hindlimb suspension model, in that animals with no access to exercise over a 2-week period displayed a reduction in neurogenesis in the hippocampus and in the other neurogenic niche SVZ. I think our reverse paradigm of 'lack of exercise' closely mimics the scenario in the aging population whereby there is a challenge for these individuals to access daily/ regular exercise. Interestingly, in the young animals (i.e., newly weaned rats as opposed to our adult rats), when a similar model of hindlimb suspension was employed, hippocampal neurogenesis was not severely affected (i.e., newly generated neurons only slightly decreased by hindlimb suspension) [11]. Our model of hindlimb suspension leading to impaired neurogenesis in hippocampus has been similarly replicated using a 'restraint stress' protocol (animal is placed into a conical tube, which prevents forward or backward movement) [12,13], with females more prone to such hippocampal alterations [14]. I envision that future research, as we noted in our recent paper [15], will need to combine this lack of exercise paradigm with established models of age-related disorders (e.g., AD, PD, stroke, TBI) to fully capture whether such lifestyle of absence of physical activity will further deteriorate neurogenesis, thereby providing insights on functional interaction between stem cell therapy and rehabilitation.

Jolkkonen, Detante: Stroke is the second leading cause of death worldwide and the most common source of severe disability in adults. With the exception of early thrombolysis, thrombectomy and craniectomy for large strokes, or admission to a stroke unit and rehabilitation, no treatment currently exists to reliably improve the chances of a functional recovery. It is plausible that cell therapy may represent a breakthrough in the treatment of stroke, since not only can it confer tissue neuroprotection but at the same time it can also facilitate endogenous neuronal repair [16]. This approach has the major advantage of acting over an extended therapeutic time period after the stroke and, unlike the present thrombolytic therapy, it might be possible to provide therapy to patients many hours, even days, after the stroke. However, we need to clarify the mechanisms through which stem cells act in order to optimize the current treatment protocols. Another major challenge will be to validate safety and efficacy of this kind of therapy in large multicenter clinical trials, although there have been encouraging results emerging from preclinical experiments and small-scale patient studies.

What is the most attractive source of stem cells?

Borlongan: I always refer to almost 10 years or so (during the early 2000s) of a federal moratorium to use NIH funds for embryonic stem cell research. This period of lack of NIH funding significantly put the ES field behind adult stem cells. It would appear that the adult stem cells have a 10-year head start over ES cells. Thus, if I have to point a finger on which is an attractive stem cell source with potential clinical application, my choice will be the adult stem cells, especially the bone marrow-derived mesenchymal stromal cells. It is not surprising that the adult stem cells are already being tested in stroke patients under limited clinical trials. We have a good handle on the effective cell dose, route and timing of delivery

and already established a respectable safety profile of these cells. However, we are still trying to understand the mechanism of action of these cells, which I believe will further guide us in optimizing the clinical transplant regimen. With this knowledge we gained from adult stem cells, we may be able to modify the transplant parameters to better cater to clinical application of ES cells, as well as other cell sources such as iPSCs. In parallel, we need to be open-minded to other potential cell sources such as the very small embryonic-like (VSELs) [17], multilineage-differentiating stress-enduring (Muse) cells [18], and the recently reported region-selective pluripotent stem cells (rsPSCs) [19]. While I understand the controversies associated with these latter cells, my choice of 'attractiveness of stem cells' is based on the cells' therapeutic potential. So although I put emphasis on the need for well-defined population of stem cells as initial criterion to qualify as transplantable stem cells, ultimately we need to show that the cells are safe and effective. We may have a well-defined population of stem cells, but in the end the cells are not safe and effective, thereby negating their potential clinical application. A vis-à-vis comparison among these available stem cells may offer insights on the optimal cell type for a particular stroke-targeted disease stage and pathology.

Detante, Jolkkonen: The great variety of available cell types and sources represent a therapeutic treasure trove for stroke treatment, but this demands that we need to make a careful evaluation of the options with regard to their safety and efficacy profiles, mechanisms of action, delivery routes and *in vivo* biodistribution properties [20]. Three main therapeutic cell categories can be distinguished: first, mesodermal-derived stromal/stem cells; second, ectodermal-derived neural stem/progenitor cells; and third, hematopoietic/endothelial stem cells. Mesenchymal stromal/stem cells (MSC) originating from bone marrow or adipose tissue and umbilical cord stem cells have been the cells most commonly utilized in clinical trials, since they are readily available, are not subject to ethical problems and have a good safety profile. However, it is obvious that the therapeutic potential of these cells is far from optimal; one could envisage that in the future their properties will be improved, for example to improve their homing efficiency, to increase cell survival and possibly also to ease their integration into the host tissue. Pluripotent cells such as embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) from adult tissue cannot be used directly due to their high tumorigenicity risk, although this can be reduced when the cells are derived in more differentiated cells. In the future, we may well have access to a virtually unlimited supply of multipotent or mature derived cells suitable for cell therapy.

What is the most promising administration route for transplantation?

Borlongan: The choice for the most promising cell delivery route for transplantation needs to be based on the disease target. This is where the pre-clinical science becomes the driver of the initial choice of delivery route. In experimental stroke, we realize that the chemokine signal of CXCR4-SDF1 is utilized by stem cells when delivered from the periphery to reach the ischemic brain.

This chemokine signal, while elevated over time, appears to peak within days after stroke, suggesting that peripheral routes of administration (intravenous or intra-arterial) may be

indicated for acute stroke. At the chronic stroke stage, however, with the chemokine signal not as robust as the early stage, the preferred route of administration will be the intracerebral (IC) approach [21]. T w o caveats that I want to note here. First, stroke is now recognized as a chronic disease, with several secondary cell death events such as inflammation, progressing over time long after the initial insult. With this in mind, repeated or booster transplants may be required. To this end, repeated transplants will need to be examined for its feasibility, safety and efficacy. Second, while the conventional concept is to facilitate the entry of the cells to the ischemic brain, we demonstrated that CNS bioavailability may be required for functional recovery in stroke [22], and that stem cell targeting of the spleen to abrogate the secondary inflammation plagued cell death, may render equally effective therapeutic benefits [23]. Accordingly, several factors need to be considered in determining the choice of stem cell transplant route.

Jolkkonen, Detante: The delivery route to be chosen depends on two factors – what cells are going to be delivered and what is the therapeutic indication. It is likely that intracerebral delivery would be preferred for cell replacement while intravenous (IV) or intra-arterial (IA) injections would provide systemic and trophic support [24]. The IV administration leads to a widespread cell distribution into vascularized and viable areas of the brain, not only into localized graft sites. Since cytokine, chemokine and inflammatory signals such as SDF-1 are released by compromised brain tissue, it is believed that IV-injected mesenchymal stem cells (MSCs) will migrate preferentially to the damaged brain regions even if they are initially subjected to transient entrapment in the lungs. However, IV cell infusions have been utilized for the majority of clinical trials since this route is relatively non-invasive. However, perhaps the next step will be to further boost cell efficacy by combining transplantation with rehabilitation or some kind of restorative drug treatment.

Currently, what are the most relevant animal models for research?

Borlongan: The small rodent models of stroke have been instrumental in the translation of stem cell therapeutics from the laboratory to the clinic. However, we are aware of the mediocre record of stroke 'neuroprotective' therapeutics as evidenced by hundreds of positive laboratory findings only to fail in the clinic. Many are quick to point to the limitation of rodent stroke models, owing in part to the small white matter which is not representative of the human brain. Recent studies have addressed this white matter injury, by creating models specifically designed to produce white matter injury [25,26]. My rule of thumb to increase the success of clinical translation of stem cell based products is to pursue alternative models, even larger animals, if there are outstanding issues that cannot be addressed in the rodent stroke models.

Jolkkonen: Experimental stroke models have been available for the last 20–30 years. All models have their own advantages and drawbacks and these need to be taken into account when planning experiments [27]. Perhaps the most common model is still the filament model. This allows reperfusion after a predetermined occlusion time (60–120 min) and it evokes extensive corticostriatal damage. In human stroke patients, such a huge lesion would be fatal. For this reason, researchers are actively seeking alternative models, for example, administration of endothelin-1, which produces a more limited lesion in a predefined

location. Unfortunately, in most of the studies conducted so far, the effect of comorbidities has been ignored. Age, diabetes and hypertension are extremely common in stroke patients, but their presence is not being taken into account when assessing efficacy of some drug or cell therapy in the experimental setting. This is thought to be one of the major reasons for the many failures to duplicate in the clinic the results of successful neuroprotective studies conducted in animal models.

How can functional recovery be measured in animal models?

Borlongan: Many research teams, including ours, have initially focused on motor function as primary functional outcomes of stem cell therapy. The reason behind this, at least from our end, is the cost–effectiveness in terms of the manpower hours required to complete the motor task testing of animals. We subsequently incorporated cognitive function, as this behavioral deficit is equally manifested in the clinic by stroke patients. Compared with motor function, this requires tedious work for both animals and investigators for training and retesting stages of the experiments. But if we truly want to capture the full range of therapeutic potential of stem cells or any other novel therapy, one must be willing to invest in a battery of motor and cognitive tests to assess the safety and efficacy of cell therapy. One caveat here is we need to cater the choice of behavioral tests to the stroke model, as some models (for example, the distal MCA ligation model that creates localized cortical damage) may not show robust cognitive deficits.

Jolkkonen: Just as there are many stroke models, so too there are numerous behavioral outcome measures available with which to measure functional impairment and recovery, ranging from an assessment of gross motor functions to fine motor skills and cognitive functions [27]. What is somewhat unusual about behavioral testing in rodents after an experimentally induced stroke is that the animals try to disguise or hide their impairment or they resort to a variety of compensatory tricks to complete the given task and this can lead to false-positive results [28,29,28]. Therefore, in order to monitor a true behavioral recovery one needs to pay attention to the way that the animals perform the task rather than simply counting the number of successfully completed tests. Alternatively, behavioral tasks can be selected that do not allow the use of compensatory strategies. Of course, one additional major challenge is how to compare quadrupedal versus bipedal behavior. One might predict that quadrupedal rodents would have more possibilities for compensation by adjusting their body balance and gait.

What are the challenges of translating animal studies of stem cell therapies to clinical trials?

Borlongan: I alluded to these 'translational lab-to-clinic' challenges above, but to reiterate here, they include the need to examine the role of stem cell therapy in rehabilitation and vice versa, further optimization of the transplant regimen, especially on route of delivery and likely need for repeated/booster transplants, and recognizing that certain animal models and functional end points refect specific stroke stages and cell death events.

Jolkkonen, Detante: Ischemic damage has been a target for numerous neuroprotective studies. Although there are now thousands of positive results emerging from animal experiments, unfortunately, when these compounds have been tested in rigorous clinical trials, the results have been far from impressive. It is important that we learn the lessons from past failures when designing future preclinical/clinical studies, only then do we have a chance of successful clinical translation. For example, preclinical studies should follow a randomized treatment assignment, conduct blinded assessments, and if possible, have a multicenter design with carefully predefined inclusion and exclusion criteria according to the recommendations of the Stroke Therapy Academic Industry Roundtable (STAIR) [30] and the Stem Cell Therapeutics as an Emerging Paradigm in Stroke (STEPS) [31] consortium. In addition, the clinical transfer potential might be improved by adopting computational modeling techniques. These so-called '*in silico*' mathematical stroke models can simulate many of the effects of cell therapy by implementing explicit models of endogenous brain repair mechanisms such as neurogenesis.

Please highlight some of the key clinical trials that have helped to shape the field in recent years

Borlongan: I give credit to our clinician experts who pioneered the Parkinson's disease clinical trials [32–36], which became the basis of the first cell therapy in stroke patients [37, 38]. This initial intracerebral transplantation of NT2N cells in chronic stroke patients has now been used as template for current clinical trials also in chronic stroke patients [39]. Peripheral transplantation of stem cells in acute stroke patients has also been explored [40].

Detante: More than 20 pilot clinical trials have been published [41]. Most commonly the cell source has been autologous bone marrow [42]), followed by NSC/neuron cultures [43]. Several cell products and routes have been investigated: IC or intrathecal transplantation of NSCs or immortalized neurons, IV injection of autologous MSCs, IA infusion of autologous mono-nuclear cells (MNCs). This makes it difficult to make direct comparisons due to the differences in cell products, routes and post-stroke delays among the trials. However, these initial studies of cell therapy for stroke have reported encouraging results with regard to safety and feasibility. Some points can be highlighted from these studies: 1) NSC transplantation requires the use of immunosuppressants; 2) xenografts can induce deleterious inflammatory effects; 3) MSC or MNC injections are more straightforward.

What ethical barriers are currently present in the field?

Borlongan: For the adult stem cells, there are limited, if any, ethical barriers to their use for cell therapy, mostly relegated to the financial rewards associated with scrupulous clinics operating as 'medical tourism' industry. For the ES cells, the ethics of destroying the embryos to generate stem cells still remains as a major obstacle. In general, I view that an ethical dilemma that we will face with this novel therapy (which can cost about $500k), is whether the treatment will be covered by health insurance, and if not, then the question is our ability to offer such treatment to the economically challenged sector of the society.

Detante: The ethical barriers depend on the cell source. If one thinks about the current autologous cell treatment protocols (based on bone marrow cells), then there are no specific ethical concerns. The debate focuses around allogenic sources and cell banking. For adult cells (e.g., bone marrow and adipose tissue), iPS from adult donor and umbilical cord, it is relatively easy to obtain an informed consent from a donor. This procedure and banking can be monitored using national and international labels for stem cell banking. When one considers future translational studies, good cell characterization and safety concerns must be emphasized. Tumorigenicity was clearly identified in using 'native' ESCs or iPSCs and it may also represent a potential, if rare, safety issue for MSCs. Ethical issues are particularly important when one thinks about embryonic stem cells; this is due to questions about the use of frozen embryos and cultures of ESCs not only for cell therapy trials, but also possibly for cloning.

How do you see the field progressing in the next 5 years?

Borlongan: We will be getting soon clinical updates on the ongoing trials of cell therapy in stroke patients. Our initial bench-to-bedside approach in translating stem cell therapy to the clinic will need to be followed by bedside-to-bench investigations for optimization or improvement of the transplant regimen and then to pursue additional clinical trials. This approach to trouble-shoot in the lab and to re-examine in the clinic is not a welcomed approach by regulatory and industry stakeholders. A failure in the clinic may push the field back to the drawing board, thus rigorous laboratory efforts in evaluating safety, efficacy and mechanism of action should proceed with translating cell therapy to the clinic in mind.

Detante, Jolkkonen: Currently, there are around 30 ongoing trials utilizing MNCs or MSCs as cell products and exploring the potential utility of allogenic administration. In the next few years results from the larger trials will validate the benefit of cell therapy after stroke, probably first in selected populations of stroke patients. The development of neurorestorative therapies for stroke in general should carefully take into consideration all translational and safety concerns in order to allow an efficient transfer from bench to bedside. There may be some very interesting innovations such as combinatorial approaches (e.g., co-treatment with growth factors, stem cell modification, co-transplantation of synergic cells) or the use of biomaterials (e.g., 'protective' scaffolds, hydro-gels) to enhance survival of the grafted cells after IC infusion and, thus, to prolong their beneficial effects. Much of this will depend on the availability of funding. Particularly in Europe, funding for stroke-related research has been meagre for a long time. The Horizon 2020 program now offers some hope of providing the desperately needed funding for translational and early-phase clinical studies with cell therapies. We must hope that this positive support will lead to the development of breakthrough clinical products, which in turn will fuel further cell therapy research so that in the future a patient suffering from a stroke will have as good a prognosis as one suffering from a heart attack today.

Acknowledgments

References

1. Diamandis T, Borlongan CV. One, two, three steps toward cell therapy for stroke. Stroke. 2015; 46(2):588–91. [PubMed: 25503552]

2. Savitz SI, Cramer SC, Wechsler L. Stem cells as an emerging paradigm in stroke 3: enhancing the development of clinical trials. Stroke. 2014; 45(2):634–9. [PubMed: 24368562]

3. Langhorne P, Bernhardt J, Kwakkel G. Stroke rehabilitation. Lancet. 2011; 377(9778):1693–702. [PubMed: 21571152]

4. Van Praag H, Kempermann G, Gage F. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nature Neuroscience. 1999; 2:266–270. [PubMed: 10195220]

5. Mattson MP. Neuroprotective signalling and the aging brain: take away my food and let me run. Brain Res. 2000; 886(1–2):47–53. [PubMed: 11119686]

6. Rodriguez JJ, Noristani HN, Olabarria M, et al. Voluntary running and environmental enrichment restores impaired hippocampal neurogenesis in a triple transgenic mouse model of Alzheimer's disease. Curr Alzheimer Res. 2011; 8(7):707–17. [PubMed: 21453244]

7. Zigmond MJ, Cameron JL, Leak RK, et al. Triggering endogenous neuroprotective processes through exercise in models of dopamine deficiency. Parkinsonism Relat Disord. 2009; 3:S42–S45. [PubMed: 20083005]

8. Ploughman M, Attwood Z, White N, et al. Endurance exercise facilitates relearning of forelimb motor skill after focal ischemia. Eur J Neurosci. 2007; 25(11):3453–60. [PubMed: 17553014]

9. Griesbach GS, Hovda DA, Molteni R, et al. Voluntary exercise following traumatic brain injury: brain-derived neurotrophic factor upregulation and recovery of function. Neuroscience. 2004; 125(1):129–39. [PubMed: 15051152]

10. Yasuhara T, Hara K, Matsukawa N, et al. Lack of exercise, via hindlimb suspension, impedes endogenous neurogenesis. Neuroscience. 2007; 149(1):182–91. [PubMed: 17869433]

11. Nomura S, Kami K, Kawano F, et al. Effects of hindlimb unloading on neurogenesis in the hippocampus of newly weaned rats. Neurosci Lett. 2012; 509(2):76–81. [PubMed: 22206837]

12. Veena J, Shankaranarayana Rao BS, Srikumar BN. Regulation of adult neurogenesis in the hippocampus by stress, acetylcholine and dopamine. J Nat Sci Biol Med. 2011; 2(1):26–37. [PubMed: 22470231]

13. Han TK, Lee JK, Leem YH. Chronic exercise prevents repeated restraint stress-provoked enhancement of immobility in forced swimming test in overiectomized mice. Metab Brain Dis. 2015; 30(3):711–8. [PubMed: 25344674]

14. Barha CK, Brummelte S, Lieblich Se, et al. Chronic restraint stress in adolescence differentially influences hypothalamic–pituitary–adrenal axis function and adult hippocampal neurogenesis in male and female rats. Hippocampus. 2011; 21(11):1216–27. [PubMed: 20665592]

15. Watson N, Ji X, Yasuhara T. No pain, no gain: lack of exercise obstructs neurogenesis. Cell Transplant. 2015; 24(4):591–7. [PubMed: 25806858]

16. Detante O, Jaillard A, Moisan A, et al. Biotherapies in stroke. Rev Neurol (Paris). 2014; 170(12): 779–798. [PubMed: 25459115]

17. Kucia M, Reca R, Campbell FR, et al. A population of very small embryonic-like (VSEL) CXCR4(+)SSEA-1(+)Oct-4+ stem cells identified in adult bone marrow. Leukemia. 2006; 20(5): 857–69. [PubMed: 16498386]

18. Wakao S, Kitada M, Kuroda Y, et al. Multilineage- differentiating stress- enduring (MUSE) cells are a primary source of induced pluripotent stem cells in human fibroblasts. PNAS. 2011; 108(24): 9875–9880. [PubMed: 21628574]

19. Wu J, Okamura D, Li M, et al. An alternative pluripotent state confers interspecies chimaeric competency. Nature. 2015; 521(7552):316–21. [PubMed: 25945737]

20. Onteniente B. The multiple aspects of stroke and stem cell therapy. Curr Mol Med. 2013; 13(5): 821–831. [PubMed: 23642063]

21. Reyes S, Tajiri N, Borlongan CV. Development in intracerebral stem cell grafts. Exp Rev Neurother. 2015; 15(4):381–93.

22. Borlongan CV, Hadman M, Sanberg CD, et al. Central nervous system entry of peripherally injected umbilical cord blood cells is not required for neuroprotection in stroke. Stroke. 2004; 35(10):2385–9. [PubMed: 15345799]

23. Tajiri N, Quach DM, Kaneko Y, et al. Behavioral and histopathological assessment of adult ischemic rat brains after intracerebral transplantation of NSI-556RSC cell lines. PLoS One. 2014; 9(3):e9408.

24. Mitkari, B.; Kerkelä, E.; Nystedt, J., et al. Advances in the Treatment of Ischemic Stroke. Maurizio Balestrino (Ed.); 2012. Toward a more effective intravascular cell therapy in stroke; p. 141-160.

25. Shindo A, Liang AC, Maki T, et al. Subcortical ischemic vascular disease: roles of oligodendrocyte function in experimental models of subcortical white-matter injury. J Cereb Blood Flow Metab. 2015

26. Seo JH, Miyamoto N, Hayakawa K, et al. Oligodendrocyte precursors induce early blood- brain barrier opening after white mater injury. J Clin Invest. 2013; 123(2):782–786. [PubMed: 23281396]

27. Lipsanen A, Jolkkonen J. Experimental approaches to study functional recovery following cerebral ischemia. Cell Mol Life Sci. 2011; 68(18):3007–3017. [PubMed: 21626271]

28. Hicks A, Schallert T, Jolkkonen J. Cell-based therapies and functional outcome in experimental stroke. Cell Stem Cell. 2009; 5(2):139–140. [PubMed: 19664986]

29. Boltze J, Lukomska B, Jolkkonen J. Mesenchymal stromal cells in stroke: improvement of motor recovery or functional compensation? J Cereb Blood Flow Metab. 2014; 34(8):1420–1421. [PubMed: 24849662]

30. Stroke Therapy Academic Industry Roundtable. Recommendations for standards regarding preclinical neuroprotective and restorative drugs. Stroke. 1999; 30(12):2752–2758. [PubMed: 10583007]

31. Stem Cell Therapies as an Emerging Paradigm in Stroke Participants. Stem Cell Therapies as an Emerging Paradigm in Stroke (STEPS): bridging basic and clinical science for cellular and neurogenic factor therapy in treating stroke. Stroke J Cereb Circ. 2009; 40(2):510–515.

32. Kordower JH, Freeman TB, Vingerhoets FJ, et al. Neuropathological evidence of graft survival and striatal reinnervation after the transplantation of fetal mesencephalic tissue in a patient with Parkinson's disease. N Engl J Med. 1995; 332(17):1118–24. [PubMed: 7700284]

33. Peschanski M, Defer G, N'Guyen JP, et al. Bilateral motor improvement and alteration of L-dopa effect in two patients with Parkinson's disease following intrastriatal transplantation of foetal ventral mesencephalon. Brain. 1994; 117(pt 3):487–99. [PubMed: 8032859]

34. Spencer DD, Robbins RJ, Naftolin F, et al. Unilateral transplantation of human fetal mesencephalic tissue into the caudate nucleus of patients with Parkinson's disease. N Engl J Med. 1992; 327(22): 1541–8. [PubMed: 1435880]

35. Lindvall O, Backlund EO, Farde L, et al. Transplantation in Parkinson's disease: two cases of adrenal medullary grafts to the putamen. Ann Neurol. 1987; 22(4):457–68. [PubMed: 3435067]

36. Backlund EO, Granberg PO, Hamberger B, et al. Transplantation of adrenal medullary tissue to striatum in parkinsonism. J Neurosurgery. 1985; 62(2):169–173.

37. Watson DJ, Longhi L, Lee EB, et al. Genetically modified NT2N human neuronal cells mediate long-term gene expression as CNS grafts *in vivo* and improve functional cognitive outcome following experimental traumatic brain injury. J Neuropathol Exp Neurol. 2003; 62(4):368–80. [PubMed: 12722829]

38. Nelson PT, Kondziolka D, Wechsler L, et al. Clonal human (hNT) neuron grafts for stroke therapy: neuropathology in a patient 27 months after implantation. Am J Pathol. 2002; 160(4):1201–6. [PubMed: 11943704]

39. Mack GS. ReNeuron and StemCells get green light for neural stem cell trials. Nat Biotechnol. 2011; 29(2):95–7. [PubMed: 21301419]

40. Savitz SI, Misra V, Kasam M, et al. Intravenous autologous bone marrow mononuclear cells for ischemic stroke. Ann Neurol. 2011; 70(1):59–69. [PubMed: 21786299]

41. Jeong H, Yim HW, Cho YS, et al. Efficacy and safety of stem cell therapies for patients with stroke: a systematic review and single arm meta-analysis. Int J Stem Cells. 2014; 7(2):63–69. [PubMed: 25473443]

42. Lee JS, Hong JM, Moon GJ, et al. A long-term follow-up study of intravenous autologous mesenchymal stem cell transplantation in patients with ischemic stroke. Stem Cells. 2010; 28(6): 1099–1106. [PubMed: 20506226]

43. Kondziolka D, Steinberg GK, Wechsler L, et al. Neurotransplantation for patients with subcortical motor stroke: a Phase 2 randomized trial. J Neurosurg. 2005; 103(1):38–45. [PubMed: 16121971]

Biographies

Cesar V Borlongan is a distinguished professor and vice chairman for research at the University of South Florida Morsani College of Medicine in the Department of Neurosurgery and Brain Repair. He is a world leader in stem cell research for stroke therapy, with his highly innovative translational bench-to-bed approach facilitating the initiation of US FDA-approved clinical trials of cell transplantation in neurological disorders, including the world's first cell therapy in stroke patients.

Jukka Jolkkonen is an Adjunct Professor at the University of Eastern Finland. His main interest has been on translational stroke recovery and particularly on how to enhance brain plasticity and how best to assess the functional outcome in experimental animals. He is the co-editor with Piotr Walczak of the book Cell-Based Therapies in Stroke.

Olivier Detante is a neurologist at the Grenoble University Hospital (France) and Associate Professor at the Grenoble Alpes University. His main interest is stroke care and recovery, and clinical development of cell therapy for stroke. As visiting associate professor at the Institute of Regenerative Medicine of Kyoto University, he studied reparative biomaterials combined with cell therapy for stroke. He is the principal investigator of the French clinical trial about autologous cell therapy after stroke.