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Cell-based therapy for Parkinson’s disease: A journey through decades towards the light side of the Force

Malin Parmar¹²*, Olof Torper¹² and Janelle Drouin-Ouellet¹²

¹Developmental and Regenerative Neurobiology, Department of Experimental Medical Science, Wallenberg Neuroscience Center and ²Lund Stem Cell Center, Lund University, 22184 Lund, Sweden

CORRESPONDENCE

* Malin Parmar: malin.parmar@med.lu.se

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**ABSTRACT**

This review describes the history, development and evolution of cell-based replacement therapy for Parkinson’s disease (PD), from the first pioneering trials with fetal ventral midbrain progenitors to future trials using stem cells as well as reprogrammed cells. In the spirit of Tom Isaacs, the review takes parallels to the storyline of Star Wars, including the temptations from the dark side and the continuous fight for the light side of the Force. It is subdivided into headings based on the original movies, spanning from A New Hope to the Last Jedi.

**ABBREVIATIONS LIST**

DA: Dopaminergic  
GMP: Good manufacturing practice  
hESCs: human embryonic stem cells  
HLA: human leucocyte antigen  
iNs: induced neurons  
iPSCs: induced pluripotent stem cells  
MHC: Major histocompatibility complex  
NIH: National Institutes of Health  
PD: Parkinson’s disease  
PET: Positron emission tomography  
UPDRS: Unified Parkinson’s Disease Rating Scale  
VM: Ventral midbrain

**INTRODUCTION**

It was with great sadness that we all received the news about Tom’s passing. He was a force for good in the Parkinson’s disease (PD) community and he will be greatly missed. For Tom, nothing was too serious that you could not joke about it. It is in the spirit of Tom that we write this review on cell-based therapies for PD. We take inspiration from his spectacular Star Wars themed talk at the World
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The Empire Strikes Back – NIH funded trials

The lift on the ban of federal funding for research using fetal tissue in 1993 enabled two National Institutes of Health (NIH) funded double-blind placebo-controlled trials to take place in the mid-90’s. This was seen as an expected and valuable follow-up to the open label studies. However, the design of these trials were significantly different than the earlier open-label studies (Barker et al., 2015a). The Colorado/Columbia trial transplanted 20 patients with severe PD, with another 20 receiving sham-transplantation surgeries (13 of whom received a graft one year after sham-surgery) (Freed et al., 2001). In this study, tissue was stored for up to four weeks before being transplanted and none of the patients received immunosuppressive drugs. The primary outcome of the trial was negative, despite a modest improvement observed in younger patients (Freed et al., 2001). This, combined with the development of graft-induced dyskinesias in five grafted patients, lead to the conclusion that this approach needed further refinement. In the Tampa/Mount Sinai/Rush trial, 23 patients were transplanted with either one or four donors and 11 patients randomized into sham surgery. Similar to the first NIH-founded trial (Freed et al., 2001), there was no significant effect of the transplantation on the primary and secondary endpoints. In addition, more than half the grafted patients developed graft induced dyskinesias (GID) (Olanow et al., 2003). As these two double-blind, placebo-controlled trials failed to show significant clinical benefit – likely reflective of the study design - what was initially seen as a great opportunity, took a turn for the worse. As a result, the overall view on cell-based replacement therapy for PD was then considered inefficacious, leading to the field being left dormant for the coming years.

Return of the Jedi – Transeuro

In the scientific transplantation community however, the conclusion that transplantation of fetal VM tissue was not efficacious for PD was considered premature. For one, it was clear that some patients in the early open-label trials showed remarkable clinical improvements (Lindvall et al., 1990, Piccini et al., 1999). Moreover, longer term follow-ups of the patients from the double-blind, placebo-controlled studies suggested that more time (3-5 years) was needed for the graft to provide significant clinical improvements (Ma et al., 2010). Around the same time, continuous follow-up clinical data as well as post-mortem evaluation of the grafts from the earlier open-labeled studies demonstrated that long lasting improvement (for a minimum of 10 years, and so far up to 20 years), without the need for any dopaminergic medication could be achieved in a subset of patients (Piccini et al., 1999; Politis et al., 2011; Kefalopoulou et al., 2014). Taken together a more comprehensive
evaluation from the open label, as well as from the double blind, placebo-controlled trials, indicated that this approach can work very well over long time, but that the outcome is highly variable.

In retrospect, many shortcomings in the trial design of the two double-blind, placebo-controlled studies, including the immunosuppressive regime, patient selection, cell preparation, handling and storage of the fetal VM, surgical methods and site of grafting, were identified (reviewed in (Barker et al., 2013)). Stratification of the data showed that younger patients with a good response to dopaminergic medications and less severe motor scores at baseline responded well to the dopaminergic cell replacement therapy. Moreover, in the only NIH-funded study using immunosuppression, the clinical improvement seen in the transplanted patients declined shortly after the discontinuation of immunosuppression. This suggests that premature cessation of immunosuppression has an adverse effect on the clinical efficacy of the transplant – likely by causing an inflammatory response that impairs the cells’ abilities to mature, innervate and integrate into the circuitry. This is substantiated by the prominent microglial response observed around the grafts in the two post-mortem cases (Olanow et al., 2003). Thus, despite the failure of the two NIH-funded studies due to suboptimal trial designs, it was thought that a significant amount of valuable information could be assembled from those results and be useful to improve future clinical trials.

With this in mind, a task-force was created with the aim of reanalyzing all studies published using fetal VM tissue in PD patients and several criteria necessary for a positive outcome were identified (Barker et al., 2013). This in turn led in 2009 to the initiation of a new multicenter trial sponsored by the European Union, TRANSEURO, which includes:

- Selection of young patients (≤65 years old) with i) clinically less advanced PD ii) a predominant dorsal striatal dopaminergic loss and iii) without any prior disabling levodopa-induced dyskinesias
- Fetal VM tissue stored in GMP-compliant reagents and methods for a maximum of four days
- Increase in the number of fetal VM grafted (3-4 per side) with the aim to reach a minimum yield of 100,000 surviving nigral DA cells in the patient’s brain
- Optimal dissection techniques to limit the number of serotoninergic neurons to reduce the appearance of GIDs
- Tissue placed using refined neurosurgery methods in the posterior putamen using 5-7 tracts
- Triple immunosuppression therapy for 12 months
- Longer time for primary endpoint (36 months) and a longer follow-up period

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Eleven patients have been grafted in the TRANSEURO trial. The first transplantation was performed in Cambridge in May 2015 and the last patient was grafted in Lund in March 2018. Initiation of Transeuro trial led to a revitalized interest in cell-replacement therapy for PD, returning hope to the field that cell-replacement therapy could work.

THE PREQUELS

After the original Star Wars trilogy, the prequels gave an insight into what happened prior to that day on Tatooine when the droid R2-D2 delivered the holographic message from Princess Leia pleading for help. In summary, while some important information was revealed in these prequels, the story was inconsistent, it was evident that computer-generated characters couldn’t replace real actors, and Jar Jar Binks was terrible. Thus, there are striking similarities with the prequels of the fetal cell trilogy and the development of cell transplantation for PD. In an era before consensus was reached that authentic DA neurons are needed for effective repair, all sorts of catecholaminergic cells, for example from retinal pigment epithelium and adrenal medulla were used with the expectation that they would functionally replace the DA (Allen et al., 1989; Goetz et al., 1989; Olanow et al., 1990; Watts et al., 2003; Bakay et al., 2004; Stover et al., 2005). However, the use of non-neuronal cells - which did not produce appropriately regulated dopamine at high enough levels to achieve functional recovery and which survived poorly after grafting- instead of authentic VM progenitors, all ended up bearing disappointing results. Lesson learned: authenticity is crucial for a good outcome.

Even though the prequels were not successful in their own right, they were certainly necessary to better shape new developments. The message from the prequels is today perhaps more relevant than ever as the sequel trilogy is unravelling - with new therapies being developed based on stem cells and reprogrammed cells (see below). Entering this new and exciting era, we need to remember the valuable lessons learnt from the prequels: you need the real thing to succeed. In the transplantation field, this means that the cells that we make from stem cells or via reprogramming need to be authentic and functional midbrain (A9) DA neurons, acting in a similar manner and potency as fetal DA neurons.
THE SEQUELS

The Force Awakens – Stem Cells

Despite the encouraging results, work with human fetal tissue presents a number of ethical and logistical problems as well as issues of standardization, and thus does not represent a realistic therapeutic option in the future. For large-scale applications, readily available, renewable, and bankable cells are therefore absolutely necessary, and this is why the field has turned to stem cells. Among the different stem cell sources available (Figure 2), human embryonic stem cells (hESCs) seem the most likely to go to clinical trials next in PD (Barker et al., 2017). Since the generation of hESCs were first reported (Thomson et al., 1998), a number of protocols on how to differentiate them into neurons, and further specify them into DA neurons have been reported (reviewed in (Barker et al., 2015a)). Newer protocols where DA neurons are formed via a floor plate intermediate were the first to show satisfactory functionality after transplantation (Kriks et al., 2011; Kirkeby et al., 2012), and since then, a number of studies have documented their authenticity, function and potency (Grealish et al., 2014; Grealish et al., 2015; Steinbeck et al., 2015; Chen et al., 2016). These cells can now be obtained under GMP compliant conditions (Kirkeby et al., 2017a; Nolbrant et al., 2017), and are on the verge of entering clinical trials (Barker et al., 2017; Kirkeby et al., 2017b; Studer, 2017). These rapid developments in the field have greatly renewed enthusiasm and created new hope for cell based repair in PD. However, while working together on a global level to bring stem cell based therapies to the clinic in an accelerated manner (Barker et al., 2015b), we are also fighting a battle against the Dark Side of unproven stem cell therapies (Box 2).

The Last Jedi – Cellular Reprogramming

Stem cells certainly have great potential to generate cells for transplantation, but there is no good story without an unexpected twist. In the latest Star Wars movie, new and previously unknown Jedi(s) suddenly appear and give the impression that the future looks more promising than ever. In a similar way, cellular reprogramming has revolutionized medical sciences by allowing large scale studies of patient-specific cells including those of the nervous system, and with that, a new and previously unconsidered source of cells for therapy are emerging.
Cell reprogramming was first reported by Sir John Gurdon in the early 60’s and revealed the multipotency of somatic cells by generating tadpoles from nuclei isolated from the frog intestine (GURDON, 1962). Decades later, this became a hot topic when it was linked to the cloning of Dolly the sheep, followed by the revolutionary findings by Shinya Yamanaka who discovered that human skin cells can be reprogrammed into induced pluripotent stem cells (iPSCs), simply by delivering a small number of transcription factors (Takahashi et al., 2007). Of high relevance and important for the stem cell transplantation field is that iPSCs seem to carry the same differentiation potential as ESCs, and therefore can be used in a similar manner to generate authentic and functional DA neurons for transplantation (Kriks et al., 2011; Kikuchi et al., 2017). In addition, iPSCs have the benefit that they can be generated from any individual, which opens up the possibility of developing patient-specific or HLA-matched personalized medicine (Taylor et al., 2012). Emerging studies shows that in fact MHC matching improves cell viability and function of iPSC-derived DA neurons in non-human primates (Morizane et al., 2017).

Reprogramming to pluripotency is based on changing the existing transcriptional program of a cell to match that of a pluripotent cells that can subsequently be differentiated into specialized cells. It is now clear that cells do not need to transition via a pluripotent stage but that one somatic cell type can be directly reprogrammed into another specialized cell, including neurons called induced neurons (iNs) (Vierbuchen et al., 2010; Pfisterer et al., 2011; Masserdotti et al., 2016). Both iPSCs and iNs have been used to generate cells from fetal and adult individuals, including individuals with neurodegenerative disease. The cells generated display characteristics of bonafide mesencephalic, DA neurons such as shared marker protein expression, matching gene expression profiles and ability for DA release and uptake (Soldner et al., 2009; Caiazzo et al., 2011; Pfisterer et al., 2011; Kikuchi et al., 2017). While currently, convincing evidence for functional recovery in transplantation studies of PD models using iNs are scarce (Torper et al., 2013; Dell’Anno et al., 2014; Pereira et al., 2014), the benefits of using directly reprogrammed cells over fetal or pluripotent cell sources in the future are numerous. First, fibroblasts for generating iNs can easily be acquired from skin biopsies, thereby avoiding any ethical concerns in terms of fetal and embryonic tissue (López-León et al., 2017). Secondly, as the fibroblasts can be obtained from individual patients, it is also possible to generate cells with matching immune profiles thereby reducing the risk of graft rejection. These first two benefits are shared with iPSCs, but a major additional benefit exclusive to iN cells is that direct reprogramming avoids the pluripotent stage and directly generates post-mitotic cells (Marro et al., 2011), thereby the risk of transplants containing proliferating cells which could develop tumors is
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**DATA ACCESSIBILITY STATEMENT:**

This review does not contain any primary data

**AUTHOR CONTRIBUTIONS STATEMENT:**

MP conceptualized the theme of the review and wrote the manuscript. OT participated to the concept and the writing. JDO participated to the concept, the writing and made the figures.

**REFERENCES:**


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pigment epithelial (hRPE) cells attached to gelatin microcarriers: a potential new cell therapy for Parkinson’s disease. J Neural Transm Suppl 215–227


**FIGURE LEGENDS:**

**Figure 1.** Pictures from Tom Isaacs’ Star Wars themed talk at the World Parkinson Congress, Portland, OR, USA, 2016 entitled “Stem cells, what they mean to people with Parkinson’s disease”. Tom, depicted as Darth Vader, used these images to illustrate “a kind of Stem Cell Wars in which stem cells can come from either a dodgy source usually combined with far-fetched and false claims about their efficacy. Or they come from a reliable source like those being studied by the previous speakers here today. So, we need to stand by these scientists, who, hopefully, in the long run will be able to offer us a source of stem cells and therapies which are evidence based.”

**Figure 2.** Schematic drawing illustrating the evolution of the different sources of cells used for replacement therapy in PD, starting with dopaminergic progenitors originating from fetal ventral midbrain (fVM). Moving on to human pluripotent stem cells (ESCs/iPSCs), and eventually to the development of dopaminergic progenitor directly reprogrammed from somatic cells such as skin fibroblasts.
**Figure 3.** Schematic representation reflecting the struggle of developing safe and efficient protocols that prevent the reprogrammed cell product to revert back to its original state. An additional concern with autologous therapy is to exclude the expression of disease-related pathology in the converted neuron.

**Box 1.**

Thirty years ago, in a galaxy near, near by...

In an attempt to replace the lost dopaminergic cells in Parkinson’s disease (PD), two Jedi Knights and their Padawans led a desperate mission to develop cell-replacement therapy using fetal ventral mesencephalic tissue. With the first small clinical trials yielding positive results, two bigger clinical trials were then conducted. Little did they know that these new trials would result in a negative outcome. It was a dark time for the rebellion. Evading the negative aura surrounding cell-based therapy for PD at the time, and building on lessons learned from these failed trials - a group of freedom fighters established a new and improved clinical trial in Europe, Transeuro. Meanwhile in the same galaxy, a small band of rebels took upon themselves to generate authentic dopaminergic neurons from pluripotent stem cells, with the hope to develop a cell therapy, even more powerful than the first. When completed, this stem cell rebellion will pave the way to a new era of scientific prosperity and reprogramming. Restoring balance to the Force, and bestowing freedom of movement for the PD patients of the galaxy.

**Box 2. Rogue One – unproven therapies**

Just like any good story, there are a number of spin-offs. The most important one to mention here is unproven stem cells therapies. Patients are desperate for new treatments and there are a number of negligent actors that are more than happy to prey on that hope and sell “stem cell” based treatments. For a hefty sum of money, you can buy health, beauty and youth. In fact, a stem cell therapy for PD can be as cheap as 10,000-30,000 USD dollars. The snag of course is that what is for sale is not a proven stem cell therapy, but ineffective cells and potentially dangerous treatments. The stem cell field is fighting hard against unproven therapies (Barker et al., 2017; Murdoch et al., 2018). While patients are anguished by the long time it takes to bring new therapies to the clinic – this must be done in a considered way in order to bring safe and efficacious therapies to patients. The long, evidence-based road is the only way to win this battle. Tom was very active in this war against unproven therapies and now the rest of us have to join forces and carry on that important work.

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**Table 1**: Advantages and disadvantages of the different cellular approaches for transplantation in PD

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<th>Fetal VM progenitors</th>
<th>ES cells</th>
<th>iPS cells</th>
<th>Induced neurons</th>
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<td><strong>Advantages</strong></td>
<td>Grafts can survive, integrate, re-innervate the striatum and release dopamine</td>
<td>Unlimited availability of the cell product</td>
<td>Easy access of the cell source</td>
<td>Easy access of the cell source</td>
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<td>Promote functional recovery in pre-clinical PD models</td>
<td>Have the potential to form authentic DA progenitors that survive well, integrate, re-innervate the striatum, release dopamine and mature after transplantation</td>
<td>Unlimited availability of the cell product</td>
<td>Lower tumorogenic potential due to avoidance of pluripotent stage</td>
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<td>Proven efficacious in some patients</td>
<td>Promote functional recovery in pre-clinical PD models</td>
<td>Have the potential to form authentic DA progenitors that survive well, integrate, re-innervate the striatum, release dopamine and mature after transplantation</td>
<td>Fast and efficient generation that makes the cells suitable to adapt for autologousgrafting</td>
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<td>Production can be standardized and cells cryopreserved and stored</td>
<td>Promote functional recovery in pre-clinical PD models</td>
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<td>GMP manufacturing protocols already established</td>
<td>Production can be standardized and cells cryopreserved and stored</td>
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<td><strong>Disadvantages</strong></td>
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<td>Very limited availability of the tissue</td>
<td>Risk of tumor formation after grafting if not properly differentiated</td>
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<td>Current lack of studies showing the long-term survival, integration, re-innervation of the striatum and the release of dopamine</td>
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<td>Variable results from clinical trials</td>
<td>Allografting that requires immunosuppression</td>
<td>High costs</td>
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<td>Allografting that requires immunosuppression</td>
<td>Ethical issues</td>
<td>Additional risks associated with the reprogramming process</td>
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<td>Ethical issues</td>
<td><strong>The graft may contain unwanted</strong></td>
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<th>The graft may contain unwanted cell types</th>
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<td>High variability in cell product and thus more challenging to standardize</td>
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I can feel the conflict inside you...... there is still some good left in you!

you can do it, you can make a functional neuron without pathology
Thirty years ago, in a galaxy near, near by…

CELL THERAPY FOR PD

In an attempt to rescue the lost dopaminergic cells in Parkinson’s disease (PD), two Jedi Knights and their Padawans led a desperate mission to develop cell-replacement therapy using fetal ventral mesencephalic tissue. With the first small clinical trials yielding positive results, two bigger clinical trials were then conducted. Little did they know that these new trials would result in a negative outcome. It was a dark time for the rebellion. Evading the negative aura surrounding cell-based therapy for PD at the time, and building on lessons learned from these failed trials - a group of freedom fighters established a new and improved clinical trial in Europe, Transeuro. Meanwhile in the same galaxy, a small band of rebels took upon themselves to generate authentic dopaminergic neurons from pluripotent stem cells, with the hope to develop a cell therapy, even more powerful than the first. When completed, this stem cell rebellion will pave the way to a new era of scientific prosperity and reprogramming. Restoring balance to the Force, and bestowing freedom of movement for the PD patients of the galaxy.
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