nature portfolio

Peer Review File

Phase I/II trial of iPSC-derived dopaminergic cells for Parkinson's disease

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Referee #1

(Remarks to the Author)
General Comments:

This study presents a novel approach by using iPSC-derived dopaminergic progenitors for the treatment of Parkinson's disease. The results are promising, showing both safety and efficacy over a 24-month period. The use of allogeneic transplantation without serious adverse events or tumor formation is particularly noteworthy.

The study design is generally robust, with appropriate endpoints and a thorough follow-up period. However, the open-label nature of the trial introduces potential bias, which should be acknowledged more explicitly in the limitations section. Future studies should consider a double-blind, placebo-controlled design to minimize these biases.

The sample size of seven patients is relatively small, limiting the findings' generalizability. While this is understandable given the early phase of the trial, it would be beneficial to include a larger cohort in future studies to validate these results.

Specific Comments:
1. Efficacy Outcomes:

The reported improvements in MDS-UPDRS III scores are encouraging, but the variability among patients suggests that some did not benefit as much as others. It would be helpful to explore potential factors that might explain this variability, such as differences in disease progression or patient characteristics.

The study mentions the increase in 18F-DOPA uptake, particularly in the high-dose group, as an indicator of dopaminergic activity. However, the lack of a direct correlation between this increase and motor symptom improvement raises questions. The authors should discuss potential reasons for this discrepancy and how it might influence the interpretation of the efficacy data.

Line 159~161; Authors describe that “The 18F-DOPA Ki values increased in two of the four transplanted putamen in the low-dose group and seven of the eight transplanted putamen in the high-dose group. If so, is there any case in which 18F-DOPA Ki values were not elevated in the transplanted Putamen? In that case, what are the possible causes?

2. Safety Outcomes:

While the absence of serious adverse events is a positive finding, the increase in dyskinesia in six out of seven patients warrants closer examination. The authors attribute this to maintained anti-parkinsonian medication doses, but it would be important to consider alternative explanations, such as the effect of the transplanted cells themselves, such as their survival rate and distributions.

The discussion on immunosuppression is informative, but more detail on the rationale for the chosen regimen and its long-term implications would strengthen the manuscript. It is good to know that there was no clinical difference between HLA-matched and non-matched patients (line208-209). Have the authors tested Mixed Lymphocyte Culture experiments?

3. Discussion:

The discussion could benefit from a more detailed comparison with other ongoing or previous trials using different stem cell sources (e.g., human ESCs-derived cells or human fetal ventral Mesencephalic cells). This would provide context and highlight the unique aspects of this study.

Please explain in more detail the basis for your statement that in the present study, patients who received transplants showed dyskinesia but no GIDs。Also, it would be desirable to show experimentally that the transplanted cells used in this

study do not really produce serotonergic neurons when induced to differentiate (in vitro, animal experiments).
The authors should also address the potential impact of the placebo effect and physician bias more thoroughly, given the open-label design.

Minor Comments:
1. Figures and Tables:
The figures are generally clear and informative, but adding more detailed legends, particularly for the PET imaging results, would help interpret the data.
The tables summarizing patient characteristics and outcomes are useful, but including a breakdown of individual patient responses might provide additional insights.

2. Language and Clarity:
The manuscript is well-written, but there are a few areas where the language could be more concise. For example, the explanation of the statistical methods could be streamlined.

3. Future Directions:
The authors briefly mention the need for larger, multi-center trials. More concrete suggestions for future research, such as specific patient subgroups that might benefit most from this therapy or potential modifications to the treatment protocol, would be helpful.

Referee #2

(Remarks to the Author)
In this manuscript, Sawamoto et al describe the phase 1/2 trial performed at Kyoto University Hospital, in which seven patients received bilateral putamen transplants of dopaminergic progenitors derived from iPSCs. Three patients received a low dose transplant (2.1–2.6×106 cells) and four patients received a high dose transplant (5.3–5.5×106 cells). Primary outcomes focused safety and adverse events, while secondary outcomes assessed motor symptom changes and dopamine production for 24 months. There were no serious adverse events, mild to moderate events, and no graft-induced dyskinesia. MRI showed no graft overgrowth, and this was corroborated by PET for 18F-FLT. PET also demonstrated dopamine production and suggested low microglial activation. Among six patients subjected to efficacy evaluation, four showed improvements on the UPDRS OFF score, and five improved with ON scores. The average changes of all six patients were 9.5 (20.4%) and 4.3 points (35.7%) for OFF and ON scores, respectively. Hoehn and Yahr stages improved in four patients.

While this is a very exciting area this MS does not give any new information with regard to production of the cells, any pre clinical studies that led to the trial, mechanism of graft function or any novel innovations. As such it is a clinical trial study that would be much better suited to a clinical journal. Many of the analysis need to be cleared by a clinical statistician to prove safety and more consideration needs to be given to the large placebo effect known to occur in these types of small open label trials.

Major Comments:

1. This Phase 1/2 trial is promising and is one of the first trials to describe the clinical findings from treated patients, rather than simply the preclinical findings. However, this is not entirely novel given several similar clinical trials that have been described using allogenic pluripotent embryonic stem cell-derived dopaminergic progenitors. Critically, clinical findings have been published for a compassionate use case study using autologous iPSC-derived dopaminergic neurons with a single treated patient (Schweitzer et al, 2020, NEJM). This published paper showed very similar data and findings for the treated PD patient as shown in this current study. Although a single case does not constitute a formal safety study as presented here by Sawamoto et al.
2. This trial demonstrated safety in the treated patients, and therefore met the primary endpoint for this trial. However, dopamine levels (if can assume the levels came from the transplanted cells and not a placebo effect) remained low – at 0.0054, which is about half the value of healthy individuals with 0.010-0.015. Therefore, it is likely that more cells will ultimately need to be delivered, now that these two doses have been proven safe in humans.
3. Authors should downplay the discussion section that “This first clinical trial using iPSCs confirmed that iPSC-derived DA progenitors can survive without forming tumors and produce DA in the putamen of PD patients”. This is a bit of an overstatement, given 1. the above mentioned case report with autologous iPSC-derived DA progenitors into a PD patient and given 2. the below discussed need for post-mortem data in order to make these claims.
4. Uptake of the GE180 ligand was used to assess microglial activation, and this was found to be low in patients. However, the authors should be careful not to claim that this one marker can monitor immune rejection, as further post-mortem analysis should be performed with markers for additional cell types involved in rejection. Donor-specific antibodies should also be assessed given the allogenic cell source.
5. The authors mention the placebo effect as one of the limitations of an open label trial, which is especially a concern and has been demonstrated in past cell-based open label trials for Parkinson’s disease. Given this concern, the authors should be careful about claiming that the delivered dopaminergic progenitors survived and produced dopamine. The fact that 18F-DOPA Ki values in the putamen showed higher increases in the high-dose group compared to the low dose group is promising. However, this increase did not correlate with improved motor symptoms. And to definitively claim cell survival and dopamine production would require analysis of post-mortem cases. While, the 18F-DOPA data demonstrates that dopamine is present, it cannot confirm whether this is from the grafted cells or from the patient, from endogenous dopamine due a

placebo effect.
6. Along the same lines, authors need to be careful to not extrapolate too much for secondary outcome effects based on the UPDRS scores. Indeed, they correctly state that “these evaluations are subjective and reflect the patient’s perceptions.” This test could be swayed both by patients that feel they are doing better as they know they received a treatment as well as swayed by patients that had high treatment expectations that they then felt were not met.
7. Six of the seven patients showed some worsening of dyskinesias, which the authors contribute not to graft-induced dyskinesias, but rather due to the medications - as the doses were maintained throughout the trial. In the recent similar BlueRock trial, medication doses were decreased after cell treatment. The authors should discuss why medication doses were not reduced if the cell treatment was efficacious.
8. MRI showed a gradual increase in the graft size based on an increase in the hyperintense areas in the targeted putamen in all patients over 24 months. The authors state that there is no evidence of tumor-like abnormal enlargement, based on the absence of 18F-FLT uptake, a marker of cellular proliferation. One, these results do not appear to match if MRI shows an increase in graft size and yet the PET data does not suggest cell proliferation. Two, it could be concerning if the iPSC-derived dopaminergic progenitors continue to proliferate for up to a 24-months after engraftment. As such, the authors should further address the proliferation rate of these cells in long-term preclinical models to ensure safety, or discuss this data if it is available.
9. The authors provide a promising therapy for Parkinson’s disease, using iPSC-derived dopaminergic progenitors. While these cells have the potential for autologous transplantation, this trial used an allogenic cell source. Future trials with these cells should assess autologous transplants, as has been done in the case study by Schweizer et al, 2020 NEJM.
Minor comments:
1. Authors should describe the rationale for the selected low and high doses.
2. The generation of human iPSCs and induction of dopaminergic progenitors are both briefly mentioned. Authors should provide more details on both aspects in the Methods. What were cells sorted for on Days 11-13.

Referee #3

(Remarks to the Author)
Clinical trial of allogeneic transplantation of iPSC-derived dopaminergic progenitors for Parkinson's disease

It's a beautiful piece of work that's interesting.

In this Phase 1/2 trial, seven patients received bilateral transplantation of induced pluripotent stem cell (iPSC)-derived dopaminergic progenitors. Safety and adverse events were assessed over 24 months. There were no serious adverse events, 73 mild to moderate events. Magnetic resonance imaging did not show excessive graft growth. Ki values for 18F-DOPA in the putamen increased by 44.7%, with greater increases in the highest dose group. This clinical trial demonstrated that dopaminergic progenitors appear to survive by producing dopamine without forming tumors.

This is a partly original study, because iPSC-derived dopaminergic progenitors are original. Indeed, only one case of autologous transplantation has recently been reported (ref 10). At the end of the introduction, the authors clearly explain the scientific background and what differentiates their approach.

The methodological approach is valid for safety and imaging data. However, PET scan data alone cannot demonstrate perfect graft survival. These data are important but not definitive, as dopamine production may also be associated with a placebo effect. Moreover, in the case of organ transplantation, immunosuppressive therapy must be maintained. How do the authors explain the lack of need for tacrolimus at 15 months? Is it cellular destruction? Are there not enough neurons left? This should be clearly discussed and not only toward positive results....

Quantitative analysis of graft size showed a progressive increase in volume over 24 months. Is this related to an inflammatory response with graft-versus-host disease? The Flair MRI sequence and fluorine-18-fluorothymidine (18F-FLT) cannot rule out an inflammatory process. Imaging is not precise enough for this. We can only conclude that there is no major rejection reaction or encephalitis. The authors need to tone down their comments on these aspects. This should be clearly discussed and not only toward positive results....

I find the statement “ Neck stiffness and painful dystonia have been noted in cases of PD” a little too facile. This is clearly a known adverse effect of transplantation that should be declared as probably related! The authors should honestly declare it as a pharmacovigilance item.

More seriously, the authors state in the abstract that there was no induction of dyskinesias, yet they quote in the article “Unified Dyskinesia Rating Scale (UDysRS) total scores increased at 24 months in six patients, with the exception of those with Parkinson's disease.” They therefore need to clarify that, on the contrary, there is evidence in favor of worsening dyskinesias. It's not very precise and a little too simplistic to say that it wasn't bothersome... but what is bothersome? that the dyskinesias don't prevent the performance of activities of daily living. Dyskinesias are always unpleasant and aggravation is present. We therefore need to remove this statement from the abstract and honestly state that there is an increase in dyskinesias throughout the manuscript, and discuss the reasons why.

Furthermore, they report that of the six patients evaluated for efficacy, four showed an improvement in the Movement Disorder Society Unified Parkinson's Disease Rating Scale part III OFF score, and five an improvement in the ON score. The mean changes for the six patients were 9.5 (20.4%) and 4.3 points (35.7%) for the OFF and ON scores, respectively.

This in no way demonstrates efficacy, but at most a slight improvement of 30% or less, bearing in mind that the placebo effect averages 33% in pharmacology and can reach up to 50% in Parkinson's neurosurgery. Even more surprisingly, while the effect was small in terms of UPDRS Part III score, the authors reported an improvement in Hoehn and Yahr stages in four patients... We know that it is extremely difficult to improve these stages without having a massive symptomatic impact, so this seems inconsistent.

All in all, the authors have neither the power nor the methodology to present efficacy data. Improvement is weak for a dopaminergic treatment and remains within the placebo effect range in an open label trial. The authors put only one word in the discussion that the placebo effect is not controlled, but throughout the manuscript they report efficacy. This must absolutely be removed from the abstract and diminished in the article with a long paragraph in the discussion explaining all the limitations of this weak effect and the methodology that prevent any analysis of efficacy. At best they can talk about effects...

Version 1:

Reviewer comments:

Referee #1

(Remarks to the Author)
The revised manuscript well addressed my points. Thus, the manuscript is acceptable for publication.

Referee #2

(Remarks to the Author)
The authors have done a very good job of answering all of my previous questions - no further comments.

Referee #3

(Remarks to the Author)
The authors have responded satisfactorily.

We thank the editors and reviewers for their time and the valuable suggestions and comments they provided on our manuscript. In the original submission, we focused primarily on presenting our clinical data. In this revised manuscript, following the reviewers' comments, we have included additional interpretations, and new results from transplanting the same donor cells used in the patients into rat PD models. In the following point-by-point responses, the reviewers' comments are in black, our responses are in blue, and the revised text is in red.

Referee #1 (Remarks to the Author):

General Comments:

This study presents a novel approach by using iPSC-derived dopaminergic progenitors for the treatment of Parkinson's disease. The results are promising, showing both safety and efficacy over a 24-month period. The use of allogeneic transplantation without serious adverse events or tumor formation is particularly noteworthy.

The study design is generally robust, with appropriate endpoints and a thorough follow-up period. However, the open-label nature of the trial introduces potential bias, which should be acknowledged more explicitly in the limitations section. Future studies should consider a double-blind, placebo-controlled design to minimize these biases.

The sample size of seven patients is relatively small, limiting the findings' generalizability. While this is understandable given the early phase of the trial, it would be beneficial to include a larger cohort in future studies to validate these results.

We appreciate the positive and instructive comments. We have discussed the placebo responses (please see the answer below), and added the following sentence in the discussion section (345-347):

Future studies should consider a double-blind, placebo-controlled design to minimize these biases. Lastly, the findings of this single-center, small-sample trial should be confirmed in multi-center, large-sample trials with appropriate controls.

Specific Comments:

1. Efficacy Outcomes:

¬ The reported improvements in MDS-UPDRS III scores are encouraging, but the variability among patients suggests that some did not benefit as much as others. It would

be helpful to explore potential factors that might explain this variability, such as differences in disease progression or patient characteristics.

We agree that improvements in MDS-UPDRS part III score are variable among patients. As discussed in the original manuscript, we think it can be attributed to their faster neurodegeneration, especially in the non-dopaminergic systems. In addition, we added another factor, age, to the discussion (288-290).

These two patients exhibited a higher degree of deterioration in motor symptoms than other patients during the on-time, suggesting that faster neurodegeneration, especially in the non-dopaminergic systems, diminished the beneficial effects produced by the graft during the trial period. Notably, PD05 was 69 years old at baseline, and as previous studies on fetal transplants suggest[4,5], younger patients with less severe symptoms appear to be more suitable candidates for this treatment. Considering these results, refining patient eligibility criteria may enhance the efficacy of this treatment.

¬ The study mentions the increase in 18F-DOPA uptake, particularly in the high-dose group, as an indicator of dopaminergic activity. However, the lack of a direct correlation between this increase and motor symptom improvement raises questions. The authors should discuss potential reasons for this discrepancy and how it might influence the interpretation of the efficacy data.

We discussed the relationship between ^{18}F-DOPA uptake and motor symptom improvements (300-314) by adding a new figure (Extended Data Fig. 6i,j):

Plots of pre- and post-transplant Ki values and MDS-UPDRS part III OFF scores revealed a mild overall trend but no clear correlation at the individual level (Extended Data Fig. 6i,j). This lack of correlation may be because of the complexity of cell replacement therapy: ^{18}F-DOPA uptake does not necessarily reflect the activation of postsynaptic neurons, and motor symptoms are influenced by both dopaminergic and non-dopaminergic neural circuits. The functional impact of grafted cells likely requires more than just DA delivery. Successful integration of the graft into the host brain is crucial for achieving meaningful clinical recovery in PD[38]. Another possibility is that the absolute Ki value is more important than its level of increase and that the number of surviving DA neurons is still insufficient. Previous studies have reported that Ki values of healthy individuals range from 0.010 to 0.017 (with our experience indicating a range of 0.010 to 0.015). While Parkinsonian symptoms typically emerge when Ki values decrease by 41% to 58% from the normal range[39-41], even the highest Ki value observed at 24 months fell

within the range associated with the onset of initial symptoms.

¬ Line 159~161; Authors describe that "The 18F-DOPA Ki values increased in two of the four transplanted putamen in the low-dose group and seven of the eight transplanted putamen in the high-dose group. If so, is there any case in which 18F-DOPA Ki values were not elevated in the transplanted Putamen? In that case, what are the possible causes?

As shown in Extended Data Fig. 6c-h, ^{18}F-DOPA uptake increased in the injection sites in all cases. We have added the following discussion (314-324):

In addition, three out of 12 putamen samples (PD02R, PD02L, PD06L) did not show an increase in ^{18}F-DOPA uptake (Extended Data Fig. 6a,b). This may be due to the technical limitation of measuring uptake in the whole putamen. However, PET images revealed distinct uptake at the injection sites (Extended Data Fig. 6c-h), which may have contributed to symptom improvement. Importantly, there was no difference in adverse events between low- and high-dose groups, and neither graft overgrowth nor GIDs were observed, even in the high-dose group. Considering these results, implanting more cells across a broader area may be necessary to achieve more substantial therapeutic effects. The favorable safety profile observed in this trial provides an opportunity to explore whether a higher dose across a wider region can offer greater clinical efficacy.

2. Safety Outcomes:

¬ While the absence of serious adverse events is a positive finding, the increase in dyskinesia in six out of seven patients warrants closer examination. The authors attribute this to maintained anti-parkinsonian medication doses, but it would be important to consider alternative explanations, such as the effect of the transplanted cells themselves, such as their survival rate and distributions.

Following the reviewer's suggestion, we have added the discussion (230-235):

(Original)

This clinical presentation is typical for drug-induced dyskinesia and not for GIDs, which occur during the off-time and are often observed in the lower extremities. It also indicates DA production by the grafted cells in the patient's brain.

(Revised)

This clinical presentation is typical for drug-induced dyskinesia and not for GIDs, which occur during the off-time and are often observed predominantly in the lower extremities[4,5,24]. This suggests that grafted cells replicate the effects of levodopa,

including the tendency to induce dyskinesias in susceptible patients. Alternatively, a focal rather than diffuse distribution of DA stimulation from the graft may exacerbate dyskinesia[25].

¬ The discussion on immunosuppression is informative, but more detail on the rationale for the chosen regimen and its long-term implications would strengthen the manuscript. It is good to know that there was no clinical difference between HLA-matched and non-matched patients (line208-209). Have the authors tested Mixed Lymphocyte Culture experiments?

The optimal immunosuppression regimen for allogeneic neural cell transplantation is currently unknown. To minimize the risk of side effects from immunosuppressant drugs, we discontinued tacrolimus treatment at 15 months, following the approach used in previous studies.
We conducted several experiments to investigate the differences between HLA-matched and non-matched patients. The results of these experiments will be reported elsewhere in a separate publication.
We have added the following discussion (244-254):
Our previous non-human primate studies demonstrated no acute immune response following allogeneic transplantation of monkey iPSC-derived DA progenitors without immunosuppression[30]. Furthermore, tacrolimus alone effectively suppressed immune responses during both allo- (monkey to monkey)[31] and xeno-transplantation (human to monkey)[12]. Based on these findings, we used tacrolimus as the sole immunosuppressant in our clinical trial. Histological analyses from previous fetal cell transplantation studies have shown that grafted DA neurons can survive for 9 to 16 years, even when immunosuppression is discontinued 6 to 18 months post-transplantation[32,33]. Additionally, our PET study at 3, 6, and 12 months showed no ^{18}F-GE180 uptake, suggesting the absence of significant inflammation. As a result, we discontinued tacrolimus treatment at 15 months.

3. Discussion:
¬ The discussion could benefit from a more detailed comparison with other ongoing or previous trials using different stem cell sources (e.g., human ESCs-derived cells or human fetal ventral Mesencephalic cells). This would provide context and highlight the unique aspects of this study.

Thank you for the valuable suggestion. To date, no papers have been published on clinical trials using human ESCs. We have added a discussion on clinical trials involving human fetal ventral mesencephalon (325-337):

Previous open-label trials using human hfVM demonstrated that cells engrafted into the host striatum synthesized dopamine and improved motor symptoms. In favorable cases, symptom improvement persisted for over ten years without severe adverse events[33,42]. While two double-blind, placebo-controlled trials did not find significant differences between graft and control groups, they did show significant motor symptom improvement in specific subgroups. These findings suggest cell replacement therapy may be beneficial if appropriate patients are selected. One beneficial subgroup may be patients 60 years old or younger, while another included those with less severe stages (below 50 points as evaluated by the original UPDRS part III OFF score[43]). Although our results did not fully align with age-related findings, it is notable that the worst case (PD05) was the oldest patient, and the best case (PD08) was the youngest. In terms of MDS-UPDRS part III OFF scores, patients PD02 and PD08, both with scores under 50, demonstrated symptom improvement.

¬ Please explain in more detail the basis for your statement that in the present study, patients who received transplants showed dyskinesia but no GIDs. Also, it would be desirable to show experimentally that the transplanted cells used in this study do not really produce serotonergic neurons when induced to differentiate (in vitro, animal experiments).

As mentioned above, we have revised the discussion (230-235) on dyskinesia accordingly:

(Original)

This clinical presentation is typical for drug-induced dyskinesia and not for GIDs, which occur during the off-time and are often observed in the lower extremities. It also indicates DA production by the grafted cells in the patient's brain.

(Revised)

This clinical presentation is typical for drug-induced dyskinesia and not for GIDs, which occur during the off-time and are often observed predominantly in the lower extremities[4,5,24]. This suggests that grafted cells replicate the effects of levodopa, including the tendency to induce dyskinesias in susceptible patients. Alternatively, a focal rather than diffuse distribution of DA stimulation from the graft may exacerbate dyskinesia[25].

We have included new results from transplanting the same donor cells used in patients into PD model rats (Extended Data Fig. 2), as well as single-cell RT-qPCR analysis of donor cells (Extended Data Fig. 1). The results showed no expression of TPH2 in the donor cells and no 5HT-positive cells in grafts. We have added the following results and discussion (119-120, 127-128, 238-239):

Importantly, no TPH2-expressing (a marker for serotonergic neurons) cells were detected.

Furthermore, no 5-hydroxytryptamine (5-HT)-positive cells (a marker for serotonergic neurons) were detected.

As a result, we detected no 5-HT-positive cells in donor cell-derived grafts in rat PD models.

¬ The authors should also address the potential impact of the placebo effect and physician bias more thoroughly, given the open-label design.

As the reviewer pointed out, the placebo response is one of the concerns of an open-label trial. We have added the following discussion (266-278):

As this is an open-label trial without a control group, it is important to consider the potential influence of the placebo effect and observer bias. A systematic analysis of placebo responses (placebo effect plus observer bias) in nine double-blind, randomized trials reported an average improvement of 4.3 points in MDS-UPDRS part III OFF scores, with a 95% confidence interval of 3.1 to 5.6 by a mean observation time of 11.3 months[34]. Additionally, a PET study conducted in four of nine trials showed no significant increase in ^{18}F-DOPA uptake in sham-operated groups. Furthermore, the placebo effect in PD patients is thought to be mediated by the release, rather than synthesis, of endogenous DA in the striatum[35]. Based on these findings, at least three patients (PD02, PD04, PD08) in this trial exhibited motor symptom improvements exceeding what could be attributed to placebo responses, potentially due to DA synthesized by the graft. This interpretation should be further validated through post-mortem histological examinations in the future.

Minor Comments:

1. Figures and Tables:

¬ The figures are generally clear and informative, but adding more detailed legends, particularly for the PET imaging results, would help interpret the data.

Thank you for the helpful suggestion. We made the figure legends more understandable

for readers as below.

Figure 1. Enrollment and follow-up

a, Patients were recruited and evaluated at Kyoto University Hospital between August 2018 and January 2019. The first three patients were categorized into the low-dose subgroup, while the remaining four were classified into the high-dose subgroup. One registered patient was excluded before surgery due to a COVID-19 infection. The first patient received unilateral surgeries with an eight-month interval between procedures and was included only in the safety assessment. Efficacy analysis was conducted on the remaining six patients. **b,** After providing informed consent, patients were enrolled in this clinical trial and underwent neurological evaluation for more than six months. If no significant symptomatic changes were observed during this period, patients were re-enrolled for surgery and underwent further neurological evaluation (including MDS-UPDRS) and an ^{18}F-DOPA PET study. Following cell transplantation, brain imaging (MRI and PET) and neurological assessments (including MDS-UPDRS) were performed at 3, 6, 12, 18, and 24 months. PET studies included ^{18}F-DOPA (to assess dopamine synthesis), ^{18}F-GE180 (to detect inflammation), and ^{18}F-FLT (to assess cell proliferation). For immunosuppression, tacrolimus (0.06 mg/kg twice daily) was administered, with dosage adjusted to maintain target trough levels of 5–10 ng/mL. The dose was reduced by half at 12 months and discontinued at 15 months.

Figure 2. Chronological changes in clinical endpoints

a,b, Chronological changes in the Unified Dyskinesia Rating Scale (UDysRS) scores for each patient from registration (0M) to the end of the observation period (24M) for safety assessment. **b,** Score changes from baseline, with the mean represented by the black line. Absolute and relative (percentage) changes at 24 months are shown on the right. **c-e,** Chronological changes in Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part III scores during the medication-off (c, d) and medication-on (e, f) periods for each patient from registration (0M) to the end of observation (24M) for efficacy assessment. **d,f,** Score changes from baseline, with the mean represented by the black line. Absolute and relative (percentage) changes at 24 months are shown on the right.

Figure 3. Dopamine synthesis detected by ^{18}F-DOPA PET

a,b, Chronological changes in fluorine-18-L-dihydroxyphenylalanine (^{18}F-DOPA) Ki values (average of both sides) in the putamen for each patient from registration (0M) to the end of the observation period (24M). **b,** Ki value changes from baseline, with the

mean represented by the black line. Absolute and relative (percentage) changes at 24 months are shown on the right. **c,** ^{18}F-DOPA Ki value (average of both sides) changes from baseline in the caudate nucleus for each patient, indicating pathological deterioration in PD. **d**, Ratio of Ki values between the putamen and the caudate nucleus, highlighting the impact of cell transplantation on pathological deterioration. The percentage changes at 24 months are shown on the right. **e,** Semiquantitative ^{18}F-DOPA images generated at 80–90 minutes post-injection by subtracting the occipital background signal and normalizing the result to the occipital activity in patient PD08 (Pre-Op: pre-operation, 12M: 12 months, 24M: 24 months). The color change from dark green to red in the bilateral putamen indicates increased ^{18}F-DOPA uptake, reflecting dopamine synthesis by grafted cells.

¬ The tables summarizing patient characteristics and outcomes are useful, but including a breakdown of individual patient responses might provide additional insights.

Thank you for the helpful suggestion. We have added the amount and relative (percentage) change to the graph representing chronological changes in clinical endpoints. (Fig. 2b,d,f, and Fig. 3b,d)

2. Language and Clarity:

¬ The manuscript is well-written, but there are a few areas where the language could be more concise. For example, the explanation of the statistical methods could be streamlined.

We revised the manuscript to make it more concise.

3. Future Directions:

The authors briefly mention the need for larger, multi-center trials. More concrete suggestions for future research, such as specific patient subgroups that might benefit most from this therapy or potential modifications to the treatment protocol, would be helpful.

We have added the following discussion (288-290, 322-324, 354-356):

Notably, PD05 was 69 years old at baseline, and as previous studies on fetal transplants suggest[4,5], younger patients with less severe symptoms appear to be more suitable candidates for this treatment.

The favorable safety profile observed in this trial provides an opportunity to explore whether a higher dose across a wider region can offer greater clinical efficacy.

Future strategies may combine cell transplantation with gene therapy, medication, and rehabilitation to enhance efficacy[44]. Additionally, as demonstrated in a single case study[8], autologous transplantation using iPSCs may also be a promising option.

Referee #2 (Remarks to the Author):

In this manuscript, Sawamoto et al describe the phase 1/2 trial performed at Kyoto University Hospital, in which seven patients received bilateral putamen transplants of dopaminergic progenitors derived from iPSCs. Three patients received a low dose transplant (2.1–2.6×106 cells) and four patients received a high dose transplant (5.3–5.5×106 cells). Primary outcomes focused safety and adverse events, while secondary outcomes assessed motor symptom changes and dopamine production for 24 months. There were no serious adverse events, mild to moderate events, and no graft-induced dyskinesia. MRI showed no graft overgrowth, and this was corroborated by PET for 18F-FLT. PET also demonstrated dopamine production and suggested low microglial activation. Among six patients subjected to efficacy evaluation, four showed improvements on the UPDRS OFF score, and five improved with ON scores. The average changes of all six patients were 9.5 (20.4%) and 4.3 points (35.7%) for OFF and ON scores, respectively. Hoehn and Yahr stages improved in four patients.

While this is a very exciting area this MS does not give any new information with regard to production of the cells, any pre clinical studies that led to the trial, mechanism of graft function or any novel innovations. As such it is a clinical trial study that would be much better suited to a clinical journal. Many of the analysis need to be cleared by a clinical statistician to prove safety and more consideration needs to be given to the large placebo effect known to occur in these types of small open label trials.

We appreciate the valuable comments. We have previously reported a novel method for sorting DA progenitors (Doi et al., Stem Cell Rep 2: 337-350, 2014), conducted non-human primate studies (Morizane et al., Stem Cell Rep 1: 283-292, 2013; Kikuchi et al., Nature 548: 592-596, 2017; Morizane et al., Nat Commun 8: 385, 2017), and completed a preclinical study involving genetic and epigenetic analyses as well as a 52-week

tumorigenicity assessment (Doi et al., Nat Commun 11: 3369, 2020). These studies provided important insights, and accordingly, this first multi-case clinical trial using iPSCs introduced novel findings based on these previous studies. In response to the reviewer's suggestion, we have included new results from transplanting the same donor cells used in the patients into PD model rats, as well as single-cell RT-qPCR analysis of the donor cells.

Major Comments:

1. This Phase 1/2 trial is promising and is one of the first trials to describe the clinical findings from treated patients, rather than simply the preclinical findings. However, this is not entirely novel given several similar clinical trials that have been described using allogenic pluripotent embryonic stem cell-derived dopaminergic progenitors. Critically, clinical findings have been published for a compassionate use case study using autologous iPSC-derived dopaminergic neurons with a single treated patient (Schweitzer et al, 2020, NEJM). This published paper showed very similar data and findings for the treated PD patient as shown in this current study. Although a single case does not constitute a formal safety study as presented here by Sawamoto et al.

To date, no papers have been published on clinical trials using human ESCs. As the reviewer mentioned, there is one report involving human iPSCs. While it is a groundbreaking study, a compassionate use case differs significantly from a clinical trial, even with a small sample size. To emphasize the iPSC clinical study, we have revised a part of the introduction section as below (70-72):

(Original)

Clinical trials using human embryonic stem cells (ESCs) are also ongoing (NCT04802733, NCT05635409). Regarding induced pluripotent stem cells (iPSCs), a single case of autologous transplantation was recently reported.

(Revised)

Recently, a single compassionate use case of autologous transplantation suggested the potential of induced pluripotent stem cells (iPSCs)[8]. Moreover, clinical trials using human embryonic stem cells (ESCs) are also ongoing (NCT04802733[9], NCT05635409[10]).

We also have added a discussion on clinical trials involving human fetal ventral mesencephalon (325-337):

Previous open-label trials using human hfVM demonstrated that cells engrafted into the

host striatum synthesized dopamine and improved motor symptoms. In favorable cases, symptom improvement persisted for over ten years without severe adverse events[33,42]. While two double-blind, placebo-controlled trials did not find significant differences between graft and control groups, they did show significant motor symptom improvement in specific subgroups. These findings suggest cell replacement therapy may be beneficial if appropriate patients are selected. One beneficial subgroup may be patients 60 years old or younger, while another included those with less severe stages (below 50 points as evaluated by the original UPDRS part III OFF score[43]). Although our results did not fully align with age-related findings, it is notable that the worst case (PD05) was the oldest patient, and the best case (PD08) was the youngest. In terms of MDS-UPDRS part III OFF scores, patients PD02 and PD08, both with scores under 50, demonstrated symptom improvement.

2. This trial demonstrated safety in the treated patients, and therefore met the primary endpoint for this trial. However, dopamine levels (if can assume the levels came from the transplanted cells and not a placebo effect) remained low – at 0.0054, which is about half the value of healthy individuals with 0.010-0.015. Therefore, it is likely that more cells will ultimately need to be delivered, now that these two doses have been proven safe in humans.

We agree with the reviewer's comment. We have added a figure illustrating the relationship between ^{18}F-DOPA uptake and symptom improvement (Extended Data Fig. 6i,j) and have included a discussion on the possibility of increasing the donor cell dose (300-324):

Plots of pre- and post-transplant Ki values and MDS-UPDRS part III OFF scores revealed a mild overall trend but no clear correlation at the individual level (Extended Data Fig. 6i,j). This lack of correlation may be because of the complexity of cell replacement therapy: ^{18}F-DOPA uptake does not necessarily reflect the activation of postsynaptic neurons, and motor symptoms are influenced by both dopaminergic and non-dopaminergic neural circuits. The functional impact of grafted cells likely requires more than just DA delivery. Successful integration of the graft into the host brain is crucial for achieving meaningful clinical recovery in PD[38]. Another possibility is that the absolute Ki value is more important than its level of increase and that the number of surviving DA neurons is still insufficient. Previous studies have reported that Ki values of healthy individuals range from 0.010 to 0.017 (with our experience indicating a range of 0.010 to 0.015). While Parkinsonian symptoms typically emerge when Ki values decrease by 41%

to 58% from the normal range[39-41], even the highest Ki value observed at 24 months fell within the range associated with the onset of initial symptoms. In addition, three out of 12 putamen samples (PD02R, PD02L, PD06L) did not show an increase in ^{18}F-DOPA uptake (Extended Data Fig. 6a,b). This may be due to the technical limitation of measuring uptake in the whole putamen. However, PET images revealed distinct uptake at the injection sites (Extended Data Fig. 6c-h), which may have contributed to symptom improvement. Importantly, there was no difference in adverse events between low- and high-dose groups, and neither graft overgrowth nor GIDs were observed, even in the high-dose group. Considering these results, implanting more cells across a broader area may be necessary to achieve more substantial therapeutic effects. The favorable safety profile observed in this trial provides an opportunity to explore whether a higher dose across a wider region can offer greater clinical efficacy.

3. Authors should downplay the discussion section that "This first clinical trial using iPSCs confirmed that iPSC-derived DA progenitors can survive without forming tumors and produce DA in the putamen of PD patients". This is a bit of an overstatement, given 1. the above mentioned case report with autologous iPSC-derived DA progenitors into a PD patient and given 2. the below discussed need for post-mortem data in order to make these claims.

As the reviewer mentioned, there was a report involving human iPS cells. While it is a groundbreaking study, a compassionate use case differs significantly from a clinical trial, even with a small sample size.

We agree with the reviewer that post-mortem data are necessary to make definitive claims about the survival of mature DA neurons and tumorigenicity. We have added the following discussion (258-260, 277-278, 338-340):
However, further confirmation through long-term follow-up and post-mortem histological examinations is necessary for definitive conclusions.

This interpretation should be further validated through post-mortem histological examinations in the future.

First, for definitive conclusions regarding the survival of mature DA neurons, inflammation around the graft, and tumorigenicity, post-mortem histological analyses are required.

4. Uptake of the GE180 ligand was used to assess microglial activation, and this was found to be low in patients. However, the authors should be careful not to claim that this one marker can monitor immune rejection, as further post-mortem analysis should be performed with markers for additional cell types involved in rejection. Donor-specific antibodies should also be assessed given the allogenic cell source.

We also agree with the reviewer that post-mortem data are necessary to make definitive claims about inflammation around the graft.
We conducted several experiments to investigate the differences between HLA-matched and non-matched patients. The results of these experiments will be reported elsewhere in a separate publication.
We have added the following discussion (258-260, 338-340):
However, further confirmation through long-term follow-up and post-mortem histological examinations is necessary for definitive conclusions.

First, for definitive conclusions regarding the survival of mature DA neurons, inflammation around the graft, and tumorigenicity, post-mortem histological analyses are required.

5. The authors mention the placebo effect as one of the limitations of an open label trial, which is especially a concern and has been demonstrated in past cell-based open label trials for Parkinson's disease. Given this concern, the authors should be careful about claiming that the delivered dopaminergic progenitors survived and produced dopamine. The fact that 18F-DOPA Ki values in the putamen showed higher increases in the high-dose group compared to the low dose group is promising. However, this increase did not correlate with improved motor symptoms. And to definitively claim cell survival and dopamine production would require analysis of post-mortem cases. While, the 18F-DOPA data demonstrates that dopamine is present, it cannot confirm whether this is from the grafted cells or from the patient, from endogenous dopamine due a placebo effect.

As the reviewer pointed out, the placebo response is one of the concerns of an open-label trial. We have added the following discussion (266-278). Please also refer to our response to question #2 you posed.
As this is an open-label trial without a control group, it is important to consider the potential influence of the placebo effect and observer bias. A systematic analysis of

placebo responses (placebo effect plus observer bias) in nine double-blind, randomized trials reported an average improvement of 4.3 points in MDS-UPDRS part III OFF scores, with a 95% confidence interval of 3.1 to 5.6 by a mean observation time of 11.3 months[34]. Additionally, a PET study conducted in four of nine trials showed no significant increase in ^{18}F-DOPA uptake in sham-operated groups. Furthermore, the placebo effect in PD patients is thought to be mediated by the release, rather than synthesis, of endogenous DA in the striatum[35]. Based on these findings, at least three patients (PD02, PD04, PD08) in this trial exhibited motor symptom improvements exceeding what could be attributed to placebo responses, potentially due to DA synthesized by the graft. This interpretation should be further validated through post-mortem histological examinations in the future.

6. Along the same lines, authors need to be careful to not extrapolate too much for secondary outcome effects based on the UPDRS scores. Indeed, they correctly state that "these evaluations are subjective and reflect the patient's perceptions." This test could be swayed both by patients that feel they are doing better as they know they received a treatment as well as swayed by patients that had high treatment expectations that they then felt were not met.

Thank you for the insightful comment. We toned down our claims about efficacy appropriately.

7. Six of the seven patients showed some worsening of dyskinesias, which the authors contribute not to graft-induced dyskinesias, but rather due to the medications - as the doses were maintained throughout the trial. In the recent similar BlueRock trial, medication doses were decreased after cell treatment. The authors should discuss why medication doses were not reduced if the cell treatment was efficacious.

We discussed the medication dose when designing the protocol and concluded that it should be maintained at the level recorded at registration to minimize any effects from medication changes. The dose was only adjusted in cases of therapeutic necessity. We have revised the results and discussion sections (184-186, 227-229):

This approach was taken to avoid confounding the assessment of transplantation outcomes, as changes in individualized anti-parkinsonian medication could affect the results.

In this trial, six out of seven patients showed a mild worsening of dyskinesia, resulting in an average increase in the UDysRS total score of 12.3 points (116.4%) from baseline at 24 months, likely because anti-parkinsonian medication doses were maintained throughout the trial, except when therapeutic adjustments were necessary. The protocol was designed to minimize the impact of medication changes.

8. MRI showed a gradual increase in the graft size based on an increase in the hyperintense areas in the targeted putamen in all patients over 24 months. The authors state that there is no evidence of tumor-like abnormal enlargement, based on the absence of 18F-FLT uptake, a marker of cellular proliferation. One, these results do not appear to match if MRI shows an increase in graft size and yet the PET data does not suggest cell proliferation. Two, it could be concerning if the iPSC-derived dopaminergic progenitors continue to proliferate for up to a 24-months after engraftment. As such, the authors should further address the proliferation rate of these cells in long-term preclinical models to ensure safety, or discuss this data if it is available.

We believe that the progressive increase in graft size is due to the migration of the grafted cells, rather than proliferation, as discussed in the original manuscript (176-180). This conclusion was indirectly supported by the results from grafting the same donor cells into PD model rats (Extended Data Fig. 2). We agree that a post-mortem examination will be necessary for a definitive conclusion and thus we have added the following discussion to address this point (212-215, 217-218, 338-340).

However, no tumorigenic overgrowth was identified, as evidenced by the absence of ^{18}F-FLT uptake, a marker of cellular proliferation, which was indirectly supported by results from the transplantation experiment using the same donor cells into PD model rats. Histological analyses at 24 or 32 weeks showed no tumor-like overgrowth, with less than 1.0% of cells being KI67-positive.

While such effects may also apply to our trial, further confirmation through long-term follow-up and post-mortem histological examinations is necessary.

First, for definitive conclusions regarding the survival of mature DA neurons, inflammation around the graft, and tumorigenicity, post-mortem histological analyses are required.

9. The authors provide a promising therapy for Parkinson's disease, using iPSC-derived dopaminergic progenitors. While these cells have the potential for autologous transplantation, this trial used an allogenic cell source. Future trials with these cells should assess autologous transplants, as has been done in the case study by Schweizer et al, 2020 NEJM.

We have added the following discussion (355-356):
Additionally, as demonstrated in a single case study[8], autologous transplantation using iPSCs may also be a promising option.

Minor comments:
1. Authors should describe the rationale for the selected low and high doses.

We have added the following explanation in the methods section (page 22).
The low dose of 5 million cells was selected based on our monkey study[12], which demonstrated both safety and efficacy. After confirming the safety of this dose over one year, the cell count was increased to 10 million.

2. The generation of human iPSCs and induction of dopaminergic progenitors are both briefly mentioned. Authors should provide more details on both aspects in the Methods. What were cells sorted for on Days 11-13.

In the results section, we described iPSCs and DA progenitors so that readers could follow the workflow of this clinical trial (104-128).
Generation of human iPSCs
The clinical-grade human iPSC line (QHJI01s04) was established from peripheral blood from a healthy individual homozygous for the most frequent haplotype in the Japanese population (HLA-A 24:02, HLA-B 52:01, HLA-DRB1 15:02, HLA-C 12:02, HLA-DQB1 06:01, HLA-DPB1 09:01), which matches 17% of the Japanese population[15,16].

Induction and transplantation of DA progenitors
DA progenitors were induced as previously described[13]. To enrich DA progenitors and eliminate non-target cells, CORIN-positive (a floor plate marker) cells were sorted on days 11–13, with sorted cells cultured in neural differentiation media to form aggregate spheres. The fresh final product containing DA progenitors meeting quality control criteria (Extended Data Table 1) was bilaterally transplanted into the putamen using a

neurosurgical navigation system. Single-cell RT-qPCR analysis confirmed the stable production of DA progenitors. iPSCs differentiated into DA neurons, with sorting for CORIN-positive cells, resulting in a final product comprising approximately 60% DA progenitors and 40% DA neurons (Extended Data Fig. 1). Importantly, no TPH2-expressing (a marker for serotonergic neurons) cells were detected. The same donor cells used for patients PD04–PD08 were transplanted into rat PD models to evaluate cell survival, proliferation, and differentiation potential (Extended Data Fig. 2). Donor cells expressed NURR1 and FOXA2 and, when grafted, differentiated into TH-positive DA neurons, improving the rotational behavior of PD model rats. At 24 or 32 weeks post-transplantation, no tumor-like overgrowth was observed, and KI67-positive (a maker for proliferating cells) cells were less than 1.0% and sparsely distributed in the grafts. Furthermore, no 5-hydroxytryptamine (5-HT)-positive cells (a marker for serotonergic neurons) were detected.

Referee #3 (Remarks to the Author):

Clinical trial of allogeneic transplantation of iPSC-derived dopaminergic progenitors for Parkinson's disease

It's a beautiful piece of work that's interesting.

In this Phase 1/2 trial, seven patients received bilateral transplantation of induced pluripotent stem cell (iPSC)-derived dopaminergic progenitors. Safety and adverse events were assessed over 24 months. There were no serious adverse events, 73 mild to moderate events. Magnetic resonance imaging did not show excessive graft growth. Ki values for 18F-DOPA in the putamen increased by 44.7%, with greater increases in the highest dose group. This clinical trial demonstrated that dopaminergic progenitors appear to survive by producing dopamine without forming tumors.

This is a partly original study, because iPSC-derived dopaminergic progenitors are original. Indeed, only one case of autologous transplantation has recently been reported (ref 10). At the end of the introduction, the authors clearly explain the scientific background and what differentiates their approach.

The methodological approach is valid for safety and imaging data. However, PET scan data alone cannot demonstrate perfect graft survival. These data are important but not definitive, as dopamine production may also be associated with a placebo effect. Moreover, in the case of organ transplantation, immunosuppressive therapy must be maintained. How do the authors explain the lack of need for tacrolimus at 15 months? Is it cellular destruction? Are there not enough neurons left?
This should be clearly discussed and not only toward positive results....

We appreciate the insightful comments. We agree with the reviewer that post-mortem data are necessary to make definitive claims about the survival of mature DA neurons. We have added the following discussion (258-260, 277-278, 338-340):
However, further confirmation through long-term follow-up and post-mortem histological examinations is necessary for definitive conclusions.

This interpretation should be further validated through post-mortem histological examinations in the future.

First, for definitive conclusions regarding the survival of mature DA neurons, inflammation around the graft, and tumorigenicity, post-mortem histological analyses are required.

The optimal immunosuppression regimen for allogeneic neural cell transplantation is currently unknown. To minimize the risk of side effects from immunosuppressant drugs, we discontinued tacrolimus treatment at 15 months, following the approach used in previous studies. We have added the following discussion (244-254):
Our previous non-human primate studies demonstrated no acute immune response following allogeneic transplantation of monkey iPSC-derived DA progenitors without immunosuppression[30]. Furthermore, tacrolimus alone effectively suppressed immune responses during both allo- (monkey to monkey)[31] and xeno-transplantation (human to monkey)[12]. Based on these findings, we used tacrolimus as the sole immunosuppressant in our clinical trial. Histological analyses from previous fetal cell transplantation studies have shown that grafted DA neurons can survive for 9 to 16 years, even when immunosuppression is discontinued 6 to 18 months post-transplantation[32,33]. Additionally, our PET study at 3, 6, and 12 months showed no ^{18}F-GE180 uptake, suggesting the absence of significant inflammation. As a result, we discontinued tacrolimus treatment at 15 months.

Quantitative analysis of graft size showed a progressive increase in volume over 24 months. Is this related to an inflammatory response with graft-versus-host disease? The Flair MRI sequence and fluorine-18-fluorothymidine (18F-FLT) cannot rule out an inflammatory process. Imaging is not precise enough for this. We can only conclude that there is no major rejection reaction or encephalitis. The authors need to tone down their comments on these aspects. This should be clearly discussed and not only toward positive results....

We believe the progressive increase in graft size is due to the spread of grafted cells rather than their proliferation, as discussed in the original manuscript (176-180). This conclusion was indirectly supported by the results from grafting the same donor cells into PD model rats (Extended Data Fig. 2). We agree with the reviewer that post-mortem data are necessary to make definitive claims about tumorigenicity and the inflammation around the grafts, so we have added the following discussion to address this point. (212-215, 217-218, 338-340).

However, no tumorigenic overgrowth was identified, as evidenced by the absence of ^{18}F-FLT uptake, a marker of cellular proliferation, which was indirectly supported by results from the transplantation experiment using the same donor cells into PD model rats. Histological analyses at 24 or 32 weeks showed no tumor-like overgrowth, with less than 1.0% of cells being KI67-positive.

While such effects may also apply to our trial, further confirmation through long-term follow-up and post-mortem histological examinations is necessary.

First, for definitive conclusions regarding the survival of mature DA neurons, inflammation around the graft, and tumorigenicity, post-mortem histological analyses are required.

In the results section, we added the word “apparent” to tone down a sentence (154):
Additionally, no patients displayed T2-weighted and fluid-attenuated inversion recovery (FLAIR) hyperintense regions nor appreciable uptake of the translocator protein-ligand, fluorine-18-flutriciclamide (^{18}F-GE180: a marker of microglial activation)[17], indicative of apparent inflammation in the putamen and surrounding areas.

I find the statement “Neck stiffness and painful dystonia have been noted in cases of PD” a little too facile. This is clearly a known adverse effect of transplantation that should be declared as probably related! The authors should honestly declare it as a pharmacovigilance item.

In the results section and Extended Data Table 1 (Extended Data Table 2 now) of the original manuscript, we considered the possibility that neck stiffness and painful dystonia are related to cell transplantation. Following the reviewer’s suggestion, we have revised the discussion (220-222):

(Original)

Neck stiffness and painful dystonia in the right upper limb were noted in PD01 during the drug-on state, a phenomenon possibly related to the grafts but sometimes appearing in the course of levodopa treatment as well.

(Revised)

Neck stiffness and painful dystonia in the right upper limb were noted in PD01 during the drug-on state, a phenomenon possibly related to the grafts.

More seriously, the authors state in the abstract that there was no induction of dyskinesias, yet they quote in the article “Unified Dyskinesia Rating Scale (UDysRS) total scores increased at 24 months in six patients, with the exception of those with Parkinson's disease.” They therefore need to clarify that, on the contrary, there is evidence in favor of worsening dyskinesias. It's not very precise and a little too simplistic to say that it wasn't bothersome... but what is bothersome? that the dyskinesias don't prevent the performance of activities of daily living. Dyskinesias are always unpleasant and aggravation is present. We therefore need to remove this statement from the abstract and honestly state that there is an increase in dyskinesias throughout the manuscript, and discuss the reasons why.

Following the reviewer’s suggestion, we have revised the abstract (42-44):

(Original)

There were no serious adverse events, 73 mild to moderate events, and no graft-induced dyskinesia.

(Revised)

There were no serious adverse events, with 73 mild to moderate events. Patients' anti-parkinsonian medication doses were maintained unless therapeutic adjustments were required, resulting in increased dyskinesia.

We have also added the following discussion (227-235):

In this trial, six out of seven patients showed a mild worsening of dyskinesia, resulting in an average increase in the UDysRS total score of 12.3 points (116.4%) from baseline at 24 months, likely because anti-parkinsonian medication doses were maintained throughout the trial, except when therapeutic adjustments were necessary. The protocol was designed to minimize the impact of medication changes. Consistently, patients recorded dyskinesia in both upper and lower extremities during the on-time period. This clinical presentation is typical for drug-induced dyskinesia and not for GIDs, which occur during the off-time and are often observed predominantly in the lower extremities[4,5,24]. This suggests that grafted cells replicate the effects of levodopa, including the tendency to induce dyskinesias in susceptible patients. Alternatively, a focal rather than diffuse distribution of DA stimulation from the graft may exacerbate dyskinesia[25].

Furthermore, they report that of the six patients evaluated for efficacy, four showed an improvement in the Movement Disorder Society Unified Parkinson's Disease Rating Scale part III OFF score, and five an improvement in the ON score. The mean changes for the six patients were 9.5 (20.4%) and 4.3 points (35.7%) for the OFF and ON scores, respectively. This in no way demonstrates efficacy, but at most a slight improvement of 30% or less, bearing in mind that the placebo effect averages 33% in pharmacology and can reach up to 50% in Parkinson's neurosurgery. Even more surprisingly, while the effect was small in terms of UPDRS Part III score, the authors reported an improvement in Hoehn and Yahr stages in four patients... We know that it is extremely difficult to improve these stages without having a massive symptomatic impact, so this seems inconsistent.

As the author pointed out, the placebo response is one of the concerns of an open-label trial. As such, we have added the following discussion (266-278):

As this is an open-label trial without a control group, it is important to consider the potential influence of the placebo effect and observer bias. A systematic analysis of placebo responses (placebo effect plus observer bias) in nine double-blind, randomized trials reported an average improvement of 4.3 points in MDS-UPDRS part III OFF scores, with a 95% confidence interval of 3.1 to 5.6 by a mean observation time of 11.3 months[34]. Additionally, a PET study conducted in four of nine trials showed no significant increase in ^{18}F-DOPA uptake in sham-operated groups. Furthermore, the placebo effect in PD patients is thought to be mediated by the release, rather than synthesis, of endogenous DA in the striatum[35]. Based on these findings, at least three patients (PD02, PD04, PD08) in this trial exhibited motor symptom improvements exceeding what could be attributed to

placebo responses, potentially due to DA synthesized by the graft. This interpretation should be further validated through post-mortem histological examinations in the future.

We have also added the following discussion about discrepancies were noted between the MDS-UPDRS part III scores and the Hoehn-Yahr stage (292-298):
In some cases, especially PD03 and PD06, discrepancies were noted between the MDS-UPDRS part III scores and the Hoehn-Yahr stage. The Hoehn-Yahr stage emphasizes postural instability and mobility issues, while the MDS-UPDRS part III offers a more comprehensive evaluation of major motor symptoms in PD. Consequently, improved postural stability and mobility may account for the greater improvement in the Hoehn-Yahr stage compared to changes observed in MDS-UPDRS part III scores of this study.

All in all, the authors have neither the power nor the methodology to present efficacy data. Improvement is weak for a dopaminergic treatment and remains within the placebo effect range in an open label trial. The authors put only one word in the discussion that the placebo effect is not controlled, but throughout the manuscript they report efficacy. This must absolutely be removed from the abstract and diminished in the article with a long paragraph in the discussion explaining all the limitations of this weak effect and the methodology that prevent any analysis of efficacy. At best they can talk about effects...

Following the reviewer's suggestion, we revised the final sentence of the abstract. We also toned down our claims about efficacy appropriately throughout the manuscript (51-53):
(Original)
This iPSC clinical trial (UMIN000033564) demonstrated that dopaminergic progenitors survived, produced dopamine without forming tumors, and improved motor symptoms, suggesting a safe and effective regenerative therapy for Parkinson's disease.
(Revised)
This clinical trial (UMIN000033564) demonstrated that allogeneic iPSC-derived dopaminergic progenitors survived, produced dopamine, and did not form tumors, thus suggesting safety and potential clinical benefits for Parkinson's disease.