Annals of Case Reports
Chazenbalk G, et al. Ann Case Rep: 9: 101810
www.doi.org/10.29011/2574-7754.101810
www.gavinpublishers.com

OPEN ACCESS

Case Report

GAVIN PUBLISHERS

Novel Treatment for Parkinson's Disease with Intravenous Allogeneic Pluripotent Non-Tumorigenic Adipose Stem Cell Transplantation: A Case Report

Gregorio Chazenbalk[1,2*], Lisa Riggioni Meyers[3], Maria Laura Gimeno[2], Luis Miranda Vega[4], Allison Peng[5],Freddy Henriquez Varela[6]

[1]David Geffen School of Medicine at the University of California Los Angeles, Los Angeles, CA, USA

[2]ClusterXStem Inc., Los Angeles, California, USA

[3]NeuroFT, San José, Costa Rica

[4]Provida. Laboratory de Celulas Madre, San José, Costa Rica

[5]Undergraduate Neuroscience Interdepartmental Program at the University of California Los Angeles, Los Angeles, CA, USA

[6]Hospital International La Catholica, San José, Costa Rica

***Corresponding author**: Gregorio Chazenbalk, David Geffen School of Medicine, University of California, Los Angeles, USA

Citation: Chazenbalk G, Meyers LR, Gimeno ML, Vega LM, Peng A, et al (2024) Novel Treatment for Parkinson's Disease with Intravenous Allogeneic Pluripotent Non-Tumorigenic Adipose Stem Cell Transplantation: A Case Report. Ann Case Report. 9: 1810. DOI:10.29011/2574-7754.101810

Received: 12 May 2024, **Accepted:** 16 May 2024, **Published:** 20 May 2024

Abstract

The present study evaluates the safety and efficacy of allogeneic pluripotent non-tumorigenic adipose stem cells (PASCs) intravenously (IV) injected in a patient with classical Parkinson's disease. PASCs were isolated from lipoaspirate material; pluripotency, teratogenesis, chromosomal integrity, and purity were determined accordingly. The patient was injected intravenously with 3 doses of 25 x 10^6 PASCs over time (0, 3, 10 months), and safety and symptom improvements were assessed at 0, 3, 10, and 16 months using validated clinical measurements. The patient exhibited no adverse effects from the PASC transplantation over 16 months of treatment, with significant improvements in activities of daily living, motor function, fall risk, and balance. This case report demonstrates safety and significant improvements in critical clinical outcomes of intravenous allogenic pluripotent non-tumorigenic PASC transplantation, making PASCs an excellent candidate for cell therapy in patients with PD.

Keywords: Parkinson's disease; Pluripotent Stem Cells; Non-Tumorigenic; Allogeneic; Safety; Efficacy.

Introduction

Parkinson's Disease (PD) is a progressive neurodegenerative movement disorder, characterized by degeneration of dopaminergic neurons in the substantia nigra and cardinal clinical symptoms of tremor, rigidity, and bradykinesia, alongside additional symptoms [1]. The number of PD patients has more than doubled in the past 30 years. This increasing prevalence is expected to continue, with an estimated 12 to 17 million cases worldwide by 2040. This trend is likely related to an aging population, genetic contributions, and lifestyle and environmental factors [2]. There is currently no disease-modifying therapy to prevent PD progression. Drugs, such as levodopa, dopamine agonists, or monoamine oxidase inhibitors among others, are only able to provide symptomatic relief, often with side effects, such as dyskinesia and motor fluctuations [1]. Therefore, a disease-modifying therapeutic to target PD progression is necessary.

Applications of stem cells to treat disease, aiding in tissue regeneration and functional recovery, have been demonstrated in a variety of conditions [3]. Stem cell properties, such as their ability to exert immunomodulatory effects, promote cell repair, and replace damaged cells, are the basis for their potential in PD treatment [3]. Studies have investigated mesenchymal stem cells (MSCs), human embryonic stem cells (hESCs), and induced pluripotent stem cells (iPSCs) for PD. However, these therapies are limited, due to low capacity for neuronal differentiation to neural cells in MSCs [4], and teratogenesis upon transplantation in hESCs and iPSCs [5]. Recently, the Stem-PD clinical trial began investigating the safety of dopamine progenitor cells derived from human pluripotent stem cells [6].

Pluripotent adipose-derived stem cells, PASCs (previously Muse-AT cells), exhibit non-tumorigenicity and triploblastic differentiation capability. PASCs are isolated from lipoaspirate tissue under severe cellular stress conditions, including lack of nutrients, hypoxia, low temperatures, and exposure to enzyme digestion [7,8]. They express classical pluripotency markers and HLA Class I, possess immunomodulatory properties, and have capacity for homing to damaged tissues, supporting their potential for allogeneic transplantation [8]. Safety of PASC transplantation intravenously and intracranially in naïve mice has previously been demonstrated (data not shown). Based on characteristics of PASCs, we performed a novel study in the safety and efficacy of IV injected PASCs in a patient with classical PD.

Case Presentation

We report the case of a 65-year-old Caucasian, non-Hispanic male diagnosed with PD. The patient had asymmetric extrapyramidal symptoms including bradykinesia, rigidity, and mild tremor. He did not report anosmia or REM sleep behaviour disorder, and his brain MRI was unremarkable. The patient's complete medication regimen included Carbidopa/Levodopa (Sine met) (25 mg Carbidopa/100 mg Levodopa, 4x daily) and catechol-o-methyl transferase inhibitor Opicapone (50 mg, 1x daily). Additionally, he received physical therapy for his symptoms.

PASCs were isolated from human lipoaspirate material, and pluripotency, non-tumorigenicity, chromosomal integrity, and purity were determined as previously described [7,8] (See Supplementary Materials). The patient received 3 IV doses of highly purified PASCs (25 x 10^6 PASCs/dose) at three time points (0, 3, and 10 months). Before each injection, the patient's clinical symptoms were assessed, as well as after 16 months of treatment. Validated clinical measures, such as the 39-item Parkinson's Disease Questionnaire (PDQ-39), Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS), Five-Times Sit-to-Stand Test, Mini Balance Evaluation Systems Test (Mini-BESTest), Timed "Up & Go" Test (TUG), 360-Degree Turn Test, Clinical Test of Sensory Interaction & Balance (CTSIB), and Limits of Stability were used to assess the patient's conditions (See Supplementary Materials).

After the first dose of PASCs, the patient began taking dopamine agonist Pramipexole, but it was soon discontinued as the patient developed Pisa syndrome. After the second PASC dose, the patient became noncompliant with his medication regimen. He experienced a fall 1 month after the second dose of PASCs, and his Carbidopa/Levodopa dosage was increased to 6x daily. The patient was also prescribed monoamine oxidase-B inhibitor Rasagiline (1 mg, 1x daily) and Modafinil for fatigue.

Three months after the second injection, the patient was found to have a great reduction in symptoms, as his walking speed, gait, and balance improved. Carbidopa/Levodopa dosage was lowered to 4x daily, and Modafinil was discontinued. After the third PASC dose, the patient's clinical presentation was stable, and he continued to take Carbidopa/Levodopa, Opicapone, and Rasagiline at similar dosages.

The improvement in the patient's PDQ-39 score, which assesses ability to complete activities of daily living (ADL), surpassed the threshold of minimal clinically important difference decreasing from baseline evaluation (17.94%) to the 16-month evaluation (3.85%). The PDQ-39 was separated into eight domains, including mobility, ADL, emotional well-being, stigma, social support, cognition, communication, and bodily discomfort. During the final evaluation, seven domains show a decrease in scores compared to baseline, with exception of the "bodily discomfort" domain, which remained the same (Table 1).

The patient also showed improvements in MDS-UPDRS score, which assesses ADL (Part I & Part II), and motor function (Part III). Compared to baseline, the patient's MDS-UPDRS total, Part I, and Part III scores were greatly decreased after treatment. Additionally, his performance on the 360-Degree Turn Test, which assesses dynamic balance, also improved from baseline through the course of the study. Mini-BESTest, which assesses balance alongside dynamic movement, remained similar throughout the study course, within normal range values. The patient's TUG time, which assesses mobility and fall risk, showed improvement compared to baseline (Table 1).

The patient's CTSIB stability scores, which assess balance, showed improvement from baseline, especially in more difficult conditions (Figure 1). In Condition #1, standing on a firm surface with eyes open, his scores ranged from 88-93 without any notable changes from evaluations at 0, 3, 10, and 16 months. There was a slightly wider range of scores varying from 83-94 in Condition #2, standing on a firm surface with eyes closed. Condition #3 showed an increase in scores, from inability to complete standing on an unstable surface with eyes open at baseline, to a score of 78 at 16 months. Condition #4 showed significant improvement from baseline at 3 and 10 months, but not at 16 months (Figure 1). Improvements in Path Length & Velocity are described in Supplementary Results.

Tests	0 Months (Baseline)	3 Months	10 Months	16 Months
PDQ-39 Mobility*	3	10	9	0
PDQ-39 ADL*	3	7	6	2
PDQ-39 Emotional Well-Being*	3	2	2	0
PDQ-39 Stigma*	4	5	4	2
PDQ-39 Social Support*	6	6	6	0
PDQ-39 Recognition*	6	2	2	0
PDQ-39 Communication*	1	2	2	0
PDQ-39 Bodily Discomfort*	2	1	1	2
PDQ-39 Total Score	17.94%	22.40%	20.51%	3.85%
MDS-UPDRS Part I†	17	3	1	3
MDS-UPDRS Part II†	3	7	9	2
MDS-UPDRS Part III†	44	16	32	27
MDS-UPDRS Part IV†	0	0	0	0
MDS-UPDRS Total Score	64	26	42	32
5 Times Sit to Stand (sec)	17.02	15.62	18.18	13.75

Mini-BESTest‡	24	23	24	24
Timed Up & Go (sec)	9.22	9.53	9.2	8.37
360-Degree Turn Test Average Steps: Right	10	8	7	7
360-Degree Turn Test Time: Right (sec)	4.14	4.23	4.05	4.26
360-Degree Turn Test Average Steps: Left	8	7	7	6.5
360-Degree Turn Test Time: Left (sec)	3.69	4.46	4.35	3.23

* Lower scores in each PDQ-39 domain reflect better quality of life. Mobility corresponds to items #1-10 on the PDQ-39, with 10 total items; ADL corresponds to items #11-16 on the PDQ-39, with 6 total items; Emotional Well-Being corresponds to items #17-22 on the PDQ-39, with 6 total items; Stigma corresponds to items #23-26 on the PDQ-39, with 4 total items; Social Support corresponds to items #27-29 on the PDQ-39, with 3 total items; Cognition corresponds to items #30-33 on the PDQ-39, with 4 total items; Communication corresponds to items #34-36 on the PDQ-39, with 3 total items; Bodily Discomfort corresponds to items #37-39 on the PDQ-39, with 3 total items.

† MDS-UPDRS Part I is scored out of 52 points, with a higher score corresponding to more severe nonmotor symptoms regarding ADL; MDS-UPDRS Part II is scored out of 52 points, with a higher score corresponding to more severe motor symptoms regarding ADL; MDS-UPDRS Part III is scored out of 132 points, with a higher score corresponding to more severe symptoms upon motor examination; MDS-UPDRS Part IV is scored out of 24 points, with a higher score corresponding to more severe symptoms of motor complications

‡ The Mini-BESTest is scored out of 28 points, with a lower score corresponding to greater balance deficits.

Table 1: PD Assessments.

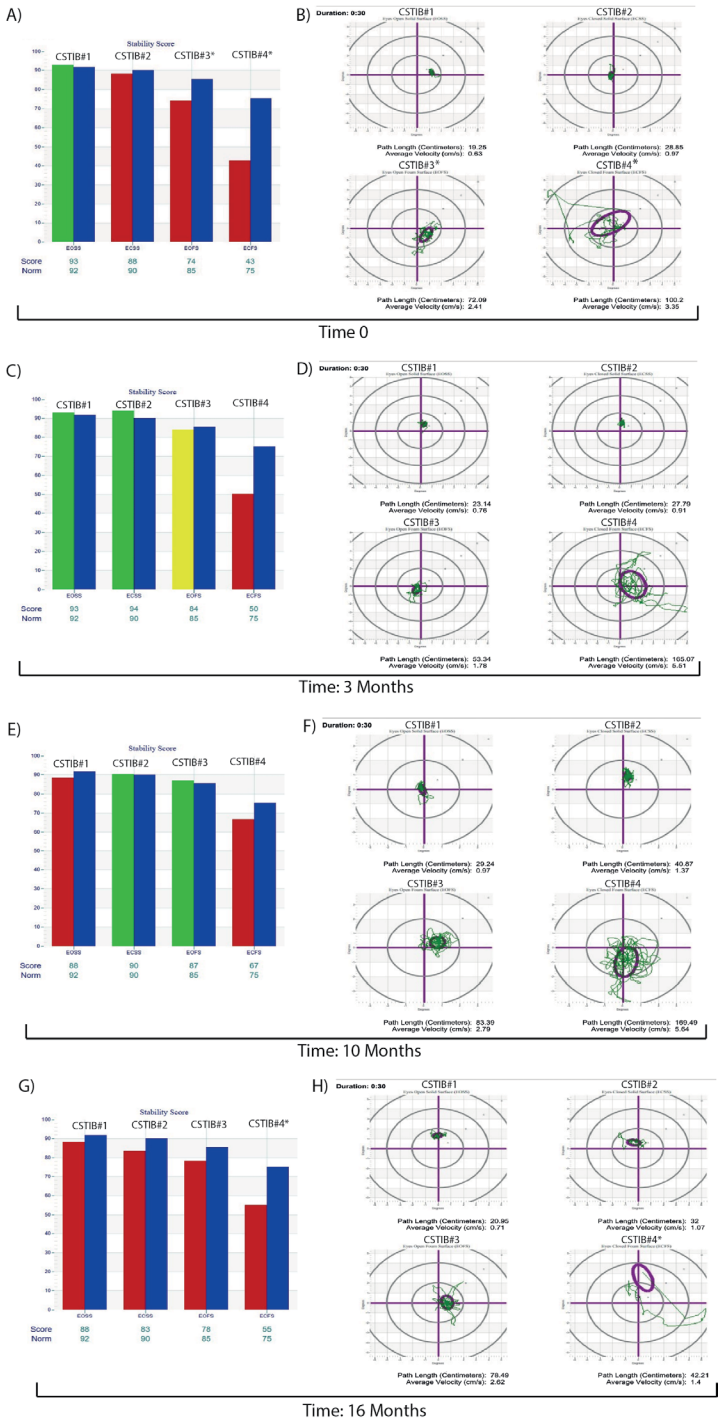


Figure 1: Clinical Test of Sensory Interaction and Balance (CTSIB). (A, C, E, G) Stability scores at baseline, 3 months, 10 months, and 16 months, compared to reference scores. Higher scores reflect increased balance and stability. (B, D, F, H) Path length, average velocity, and representation of 95% confidence ellipse of mass acceleration in the horizontal plane to characterize postural sway. Lower path length reflects better balance. Lower velocity reflects a greater ability to control directional displacement of the center of gravity. *Indicates loss of balance before the end of the test (30 seconds).

Discussion

PASCs express pluripotent stem cell markers and immunomodulatory properties [7,8], making them suitable for allogeneic transplantation. The present study confirms that PASCs are pluripotent, non-tumorigenic, and can be obtained efficiently with a high degree of purity in one single step [7,8]. Injection of 3 doses in a PD patient did not produce adverse effects over a span of 16 months. The patient showed significant improvements in PD symptoms, including motor function, balance, stability, and ADL. Additionally, his medication dosage at the conclusion of the study was stable and similar to his dosage before treatment.

Previous case reports have reported MSCs [9,10] and iPSC-derived progenitor cells [11] to treat PD patients. These reports showed clinical improvement assessed primarily via MDS-UPDRS and PDQ-39 scores, alongside neuroimaging. MSCs have lower differentiation capabilities and cannot differentiate into dopaminergic neurons, limiting their ability to replace degenerating tissues. iPSCs possess this differentiation capability but are teratogenic; therefore, only progenitors derived from iPSCs could be used to treat PD patients.

Currently, clinical trials investigating the safety and dosage of MSCs in PD are underway [12,13]. A case report showed that a patient who received 26 doses of MSCs showed improvement of symptoms with no adverse effects over 2 years [10]. A recent Phase I dose-escalation trial at the University of Texas in Houston demonstrated no serious adverse reactions to infusions of doses of 1, 3, 6, or 10×10^6 stem cells/kg in PD patients [14]. Only the highest dose had the most significant effects in the reduction of MDS-UPDRS scores and inflammatory markers. Following its completion, a Phase IIa clinical trial investigated allogeneic MSCs in 45 patients, who received 3 doses of either MSCs every 3 months (10×10^6 stem cells/kg/dose) or placebo [12].

Notably, the dosage of pluripotent PASCs in our present study is 100 times lower than the dosage of multipotent MSCs used in the Phase IIa study [12] (2.5 x 10^7 PASCs versus 2.4 x 10^9 MSCs every 3 months in an 80 kg patient), indicating a significant advantage for use of PASCs in PD patients. This is in agreement with previous studies using pluripotent stem cells similar to PASCs, named Muse cells, which comprise a small percentage of the MSC population (~1%) [15]. Therefore, isolation and transplantation of PASCs has the potential to be more efficient and lower cost than MSC transplantation.

MDS-UPDRS scores are considered the "gold standard" test to evaluate motor and non-motor functions in PD patients. In the present study we incorporated additional quantitative measures of clinical improvements, supplementing MDS-UPDRS results such as PDQ-39, 5 Times Sit to Stand, Mini-BESTest, TUG, 360-Degree Turn, and CTSIB tests, strengthening our conclusions and allowing additional information regarding the improvements of PD symptoms.

Since the present study is a case report, it is difficult to directly draw definitive conclusions. A randomized, placebo-controlled, double-blinded clinical trial is currently underway to evaluate 40 patients treated with PASCs or a placebo control to confirm the potential therapeutic benefits of PASCs transplantation in PD patients [16].

Conclusions

In summary, this case report shows that allogeneic, IV injected PASCs in a PD patient are safe and produce significant improvements in motor function, balance, stability, and ADL, making PASCs a promising candidate to treat PD as well as other neurodegenerative disorders.

Supplementary Materials

Detailed Methods:

PASC Isolation

Liposuction of human abdominal subcutaneous lipoaspirate material was performed following standard protocol [17] in selected donor patients who were non-obese healthy individuals, 30-50 years old, without infectious diseases (Human Immunodeficiency Virus, Hepatitis A, B and C Virus, Covid-19, Epstein-Barr Virus, T-lymphotropic virus type 1, Citomegalovirus, Epstein-Barr Virus, and Zika Virus).

PASCs were isolated as previously described in the literature [7,8]. Briefly, the lipoaspirate material was washed with phosphate buffer (PBS) until blood was completely removed, and tissue was incubated with 0.1% (wt/vol) collagenase in Alpha-MEM for 30 minutes at 37°C with agitation, followed O/N incubation at 4°C under hypoxic conditions. After centrifugation, cell pellets were depleted from erythrocytes using red blood cell lysis buffer, and after centrifugation, the remaining cell pellet containing PASCs was washed with PBS and then cryopreserved and stored in liquid nitrogen. PASCs cells were thawed, cultured in suspension using 60-mm non-adherent plastic dishes in the presence of alpha MEM + 20% KnockOut serum replacement for 24 hours at 37°C, 5 % CO_2 for further cell characteristic studies. Pool of PASCs from 5 different donors with similar number of cells was used for the study.

Pluripotent characteristics of PACS

PASCs in suspension were spun onto glass slides, and fixed by the addition of cold methanol. The cells were then incubated with the following primary antibodies: anti-stage-specific embryonic antigen (SSEA-4; Abcam) anti-human embryonic cell marker OCT-4; Abcam). Secondary antibodies, used at 1

to 500 dilution, were either mouse Alexa Fluor 647 conjugated dye (Thermo Fisher), and rabbit Alexa Fluor 488 conjugated dye (Thermo Fisher). The slides were mounted with Mowiol (Sigma-Aldrich). Images were acquired on an inverted Zeiss LSM710 (Carl Zeiss MicroscopyGmbH, Jena, Germany, http://www.zeiss.com). Data acquisition was performed using ZEN Black 2011 software (Carl Zeiss Microscopy).

Analysis of viability and quantification automatic cell counter

Resuspended PASCs were mixed with equal volume of 0.4% trypan blue (ThermoFisher Scientific, Waltham, Massachusetts), and 10 µl was transferred to a chamber slide. Number of living cells vs dead cells were determined using a cell counter (Countess 3 Automated Cell Counter, ThermoFisher, Waltham, Massachusetts).

Flow Cytometry Analysis of viability and quantification

Resuspended PASCs in 100 µl in PBS containing 0.5% BSA, 1% Sodium Azide were incubated with 10 µl of propidium Iodide (ThermoFisher Scientific, Waltham, Massachusetts) at 4°C for 30 minutes in the darkness, after 2 times washes with PBS, PASCs were resuspended with 300 µl with PBS transferred to 5 ml round-bottom tubes (Trucount tubes (BD Bioscience, Franklin Lakes, New Jersey). Analysis was performed using the Cytoflex LX (Beckman Coulter, Brea, California).

Analysis of teratogenesis

Teratogenesis of PASCs was determined using ALDEFLUORTM for Aldehyde dehydrogenase (ALDH) detection following standard protocol (STEM CELL Technologies, Vancouver).

Determination of Karyotypes

PASCs were grown in Amnio Max culture medium (ThermoFisher Scientific, Waltham, Massachusetts) for 10 days. Cells were then fixed in 4% formaldehyde, stained with 5 µl/ml Quinacrine-Hoechst (ThermoFisher Scientific, Waltham, Massachusetts),micro photographed using optic microscope (Olympus BX53, Life Science, St. Petersburg, Florida), and then analysed by Digital Karyotyping Automated Scanning HiBAND (Applied Spectral Image, Carslbad, California).

PASC implantation

Patient was IV injected with highly purified PASCs (25 x 10^6 PASCs/dose) at three time points (0, 3, and 10 months), Clinical and non-neuromotor/motor characteristics were assessed a few days before each injection as well as 16 months after first injection.

PDQ-39

The 39-item Parkinson's Disease Questionnaire (PDQ-39) consists of 39 questions used to assess quality of life answered by the patient. Items assessed in the PDQ-39 include ADL, attention and working memory, cognition, communication, depression, functional mobility, quality of life, social relationships, and social support [18]. The final score is transformed on a linear scale from 0% to 100%, with a higher score reflecting lower quality of life.

MDS-UPDRS

The Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) is widely used to quantify clinical characteristics of PD in adults. The scale assesses aspects of PD, and it is divided into 4 domains: nonmotor experiences of daily living (Part I), motor experiences of daily living (Part II), motor examination (Part III), and motor complications (Part IV). The MDS-UPDRS has a total of 65 items, and the assessment takes approximately 30 minutes to complete. Assessments using the MDS-UPDRS were conducted during the "ON" state.

Mini-BESTest

The Mini Balance Evaluation Systems Test (Mini-BESTest) aims to measure dynamic balance and associated movement during translational motion. The test consists of 14 items, with a maximum score of 28 points, with a higher test result, the greater the patient's functionality.

Five-Times Sit-to-Stand Test

The Five-times sit-to-stand Test is a tool that quantifies the ability of patients suffering from diseases associated with balance problems, as in the case of PD, to perform transitional motions. In addition, it has been used as a measure of lower limb strength and to assess fall risk. Participants begin the test seated, and they are instructed to cross their arms and come to a full standing position before sitting again. The time for the patient to stand and sit five times is recorded.

Timed "Up & Go" Test

The Timed "Up & Go" (TUG) test consists of observing and timing the patient as he gets up from an armchair, walking for 3 meters, turns, and returns and sits down again. It has been used to measure balance and fall risk in PD patients [19].

360-degree Turn Test

This test measures dynamic balance in the bipedal. In this test, the number of steps and the time that it takes the patient to make a complete 360-degree turn [20].

Balance and Postural Stability (CTSIB)

The Clinical Test of Sensory Interaction & Balance (CTSIB) systematically measures the influence of visual, vestibular, and somatosensory input on bipedal balance. Specifically, this test measures the bipedal balance for 30 seconds in four different

conditions that are classified as firm surface with eyes open (Condition #1), firm surface with eyes closed (Condition #2), unstable surface with eyes open (Condition #3), and unstable surface with eyes closed (Condition #4) [20]. Path length reflects balance, with lower path length indicating greater balance. Velocity indicates the ability to control directional displacement of the center of gravity, with lower velocity indicating greater ability. The Limits of Stability test uses the force platform to accurately measure the individual's ability to reach their limits of stability [20]. The Limits of Stability test assesses the displacement of center of gravity in the forward, backward, left, and right directions. This test also measures the time it takes to reach each target and the percentage of targets attained. Lower times reflect quicker and more efficient attainment of targets. Higher percentages in each of the eight stability domains and the total stability score indicate greater stability, nearing the normative percentage.

Supplementary Results

PASC characterization isolated from lipoaspirate material of 5 different donors

As we specified in the PASC preparation, we utilized a pool of PASCs obtained from 5 different donors with a similar number of cells per donor. After 5 days in culture, PASCs formed tightly packed clusters with an approximate diameter of 50–150 μm, expressing the pluripotent stem cell markers SSEA-4 (red) and OCT4 (green) detected by immunofluorescence (Supplemental Figure 1A). FACS analysis using PI showed that the percentage of death in the 5 PASC donors were between 2-5% after preparation (Supplemental Figure 1B). PASCs cultured for 10 days demonstrated integrity of 23 pairs of chromosomes indicating normal karyotype from each patient donor (Supplemental Figure 1C). FACS in PASCs in culture did not show teratogenic activity determined by ALDH production (Supplemental Figure 1D) in contrast to the negative and positive control of the assay, Supplemental Figure 1D-i) and 1D-ii), respectively.

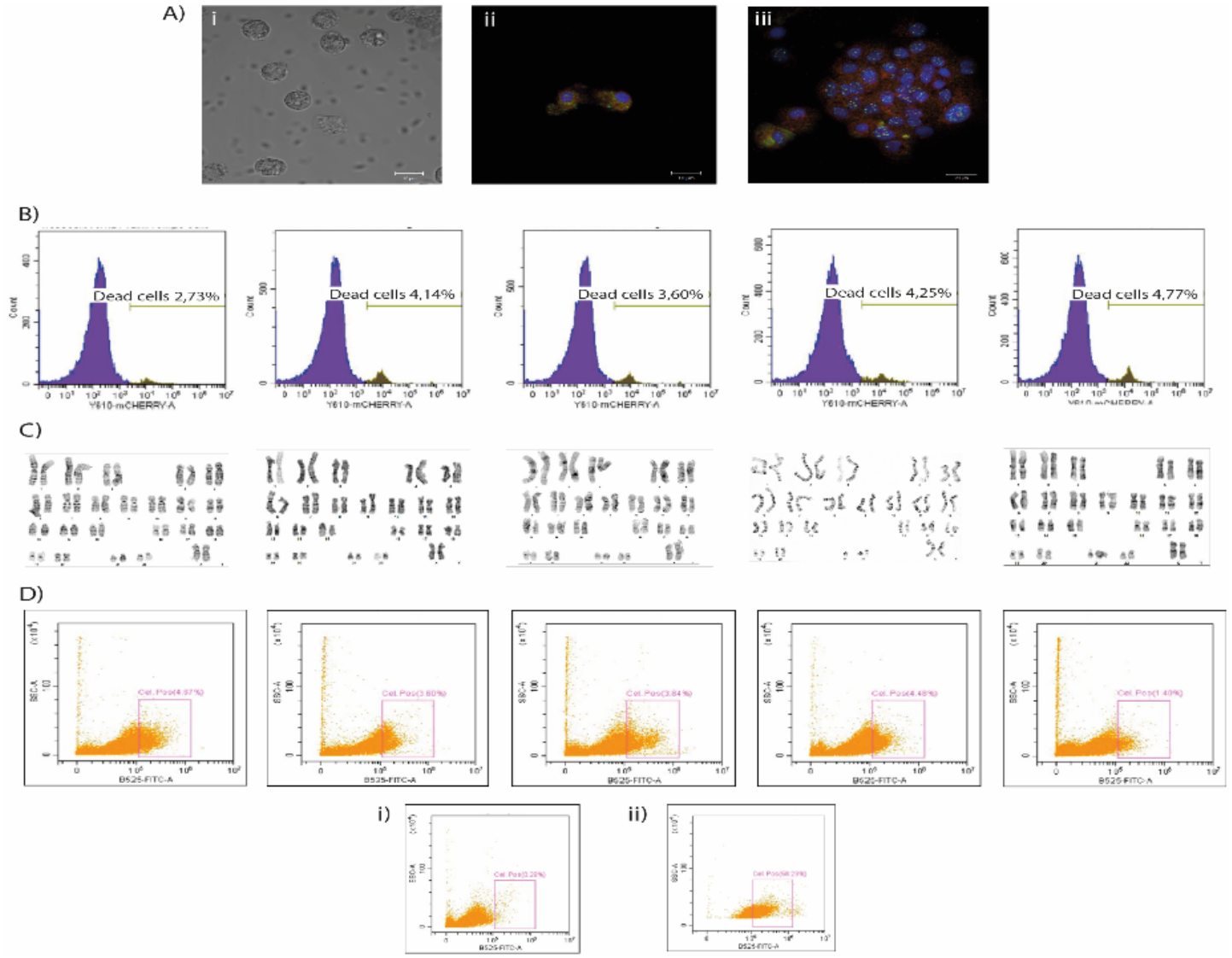
CTSIB

Path Length & Velocity

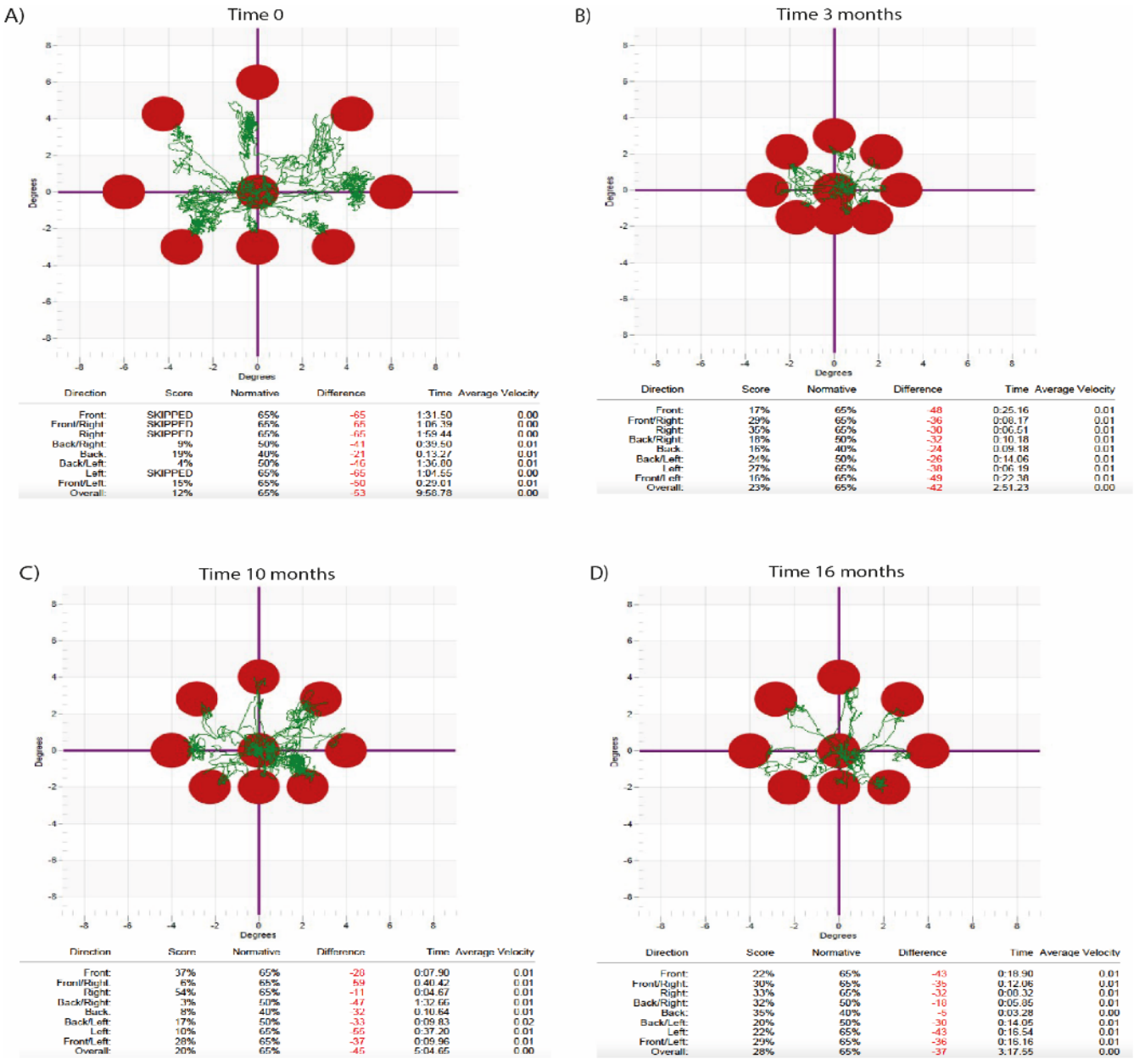
Lower path length reflects better balance. Condition #1 path length increased minimally from 19.25 cm at baseline to 20.95 cm at 16 months. Condition #2 path length demonstrated a slight increase in scores, from 28.85 cm at baseline and 32 cm at 16 months. Path length in Conditions #3 and #4 at baseline and #4 at the 16 months could not be obtained, since the subject was unable to complete the test, requiring assistance to not fall. Evaluation at 3 months demonstrated the lowest path length for Condition #3 (53.34 cm), which measured 83.39 cm at 10 months, and 78.49 cm at 16 months. Condition #4 could only be compared at 3 months (165.07 cm) and 16 months (169.64 cm), with no significant changes between these two evaluations (See Figure 1 B, D, F, H). Lower velocity reflects a greater ability to control directional displacement of the Center of gravity. As conditions increase in difficulty from #1 to #4, average velocity typically increases, reflecting the increased challenge to maintain balance with eyes closed and/or on unstable surfaces. Average velocity scores for Condition #1 slightly increased from baseline (0.63 cm/s) to 16 months (0.71 cm/s). A similar increase in time occurred in the velocity scores for Condition #2 (0.91 cm/s at baseline, 1.07cm/s at 16 months) (See Figure 1 B, D, F, H).

Limits of Stability

In the baseline evaluation (0 months), the subject was unable to attain 4 of the 8 total conditions. The test was administered using 6 degrees of variation from the Center, which made this test harder to achieve making the targets two degrees longer from the Center of gravity compared to four degrees used in the following three evaluation times. The subject's total stability score was 12%, and the total time to reach the targets was 9:58.78. At 3 months, the subject was able to complete all 8 conditions with a total stability score of 23% in a time of 2:51.53. At 10 months, the subject needed a total of 5:04.65 to achieve an overall score of 20%. The evaluation at 16 months was completed in a total time of 3:17.55, and the subject achieved his highest overall stability score, with a score of 28%. This score was the highest score of all four evaluations, increasing from a baseline score of 12% to the current 28%. His time at 16 months also improved from baseline by 6 minutes and 41 seconds (Supplemental Figure 2).



Supplemental Figure 1: PASC characterization isolated from lipoaspirate material of 5 different donors. (A) Floating of Individual and Cluster of PASCs in culture, i) Bright-field image of individual PASCs (1 day in culture), ii) Immunostaining of individual PASCs recognized by the pluripotent stem markers SSEA-4 (red) and OCT4 (green) (1 day in culture), and iii) immunostaining of PASC forming clusters recognized by the pluripotent stem markers SSEA-4 (red) and OCT4 (green) (10 days in culture); (B) Quantification and viability of PASCs using PI by FASCs, C) Normal karyotype assay in PASCs obtained from 5 different donors indicate by the integrity of 23 pairs of chromosomes, (D) Non-teratogenesis of PASCs in the 5 different donors indicated by low ALDH levels using the teratogenesis assay ALDEFLUOR kit, (i) Negative control using specific inhibitor for ALDH production (diethylaminobenzaldehyde, DEAB), and ii) Positive control, A549 human lung carcinoma cells. Abbreviation: PASCs pluripotent adipose stem cells; SSEA, stage-specific embryonic antigen; FACS flow cytometry; PI propidium iodide.



Supplemental Figure 2: Limits of Stability. This image depicts lines originating from the center, moving towards the outer circles, and back to the center, reflecting the patient's micro-movements to achieve the targets. An increase in the zig-zag shape of the lines represents a less efficient movement strategy used to reach the targets. Limits of stability at (A) baseline (0 months), (B) 3 months, (C) 10 months, and (D) 16 months.

Disclosure

Author Contributions: conceptualization, GC, LRM, MLG, and FHV; methodology, GC, LRM, MLG, LMV; data curation, GC, LRM, FHV; formal analysis, GC, LRM, LMV; investigation GC, MLG, FHV; writing original draft preparation, GC, AP, FHV; writing review and editing, GC, AP, FHV; supervision, GC.

All authors read and approved the final manuscript.

Study Funding: This work was supported by ClusterXStem Inc., Los Angeles, California, USA. The funder played no role in study design, data collection, analysis and interpretation of data, or the writing of this manuscript.

Ethical Standard: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards, IRB #CEC-ICIC 123-2021. Informed consent was obtained from individual participants involved in the study.

Data availability: The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Acknowledgements: Javier Mora Rodriguez, Ph.D., Norman Rojas Campos, Ph.D., Raul Rios Ritter, M.D., Carlos Zuniga Loaiza, M.D., Natalia Mora Gambo

Conflicts of Interest: Authors LRM, MLG, LMV, AP, and FHV declare no financial or non-financial competing interests. GC holds shares in ClusterXStem Inc. but declares no non-financial competing interests.

References

1. DeMaagd G, Philip A. (2015) Parkinson's Disease and Its Management. Pharmacy and Therapeutics. 40:504–532.
2. Chaudhuri KR, Azulay J-P, Odin P, Lindvall S, Domingos J, et al. (2024) Economic Burden of Parkinson's Disease: A Multinational, Real-World, Cost-of-Illness Study. Drugs - Real World Outcomes. 11:1–11.
3. Hoang DM, Pham PT, Bach TQ, Ngo ATL, Nguyen QT, et al. (2022) Stem cell-based therapy for human diseases. Signal Transduction and Targeted Therapy. 7:272.
4. Hernández R, Jiménez-Luna C, Perales-Adán J, Perazzoli G, Melguizo C, et al (2020)Differentiation of Human Mesenchymal Stem Cells towards Neuronal Lineage: Clinical Trials in Nervous System Disorders. Biomolecules & Therapeutics. 28:34–44.
5. Lee AS, Tang C, Rao MS, Weissman IL, Wu JC (2013) Tumorigenicity as a clinical hurdle for pluripotent stem cell therapies. Nat Med 19:998–1004.
6. Region S. (2024) STEM-PD Trial: A Multicentre, Single Arm, First in Human, Dose-escalation Trial, Investigating the Safety and Tolerability of Intraputamenal Transplantation of Human Embryonic Stem Cell Derived Dopaminergic Cells for Parkinson's Disease (STEM-PD Product). clinicaltrials.gov; 2024. Report No.: NCT05635409.
7. Heneidi S, Simerman AA, Keller E, Singh P, Li X, Dumesic DA, et al (2013) Awakened by Cellular Stress: Isolation and Characterization of a Novel Population of Pluripotent Stem Cells Derived from Human Adipose Tissue. PLoS ONE. 8:e64752.
8. Gimeno ML, Fuertes F, Barcala Tabarrozzi AE, Attorressi AI, Cucchiani R, et al. (2017) Pluripotent Nontumorigenic Adipose Tissue-Derived Muse Cells have Immunomodulatory Capacity Mediated by Transforming Growth Factor-β1. Stem Cells Translational Medicine. 6:161–173.
9. Carstens M, Haq I, Martinez-Cerrato J, Dos-Anjos S, Bertram K, Correa D. (2020) Sustained clinical improvement of Parkinson's disease in two patients with facially-transplanted adipose-derived stromal vascular fraction cells. Journal of Clinical Neuroscience. 81:47–51.
10. Vij R, Prossin A, Tripathy M, Kim H, Park H, Cheng T, et al (2023) Long-term, repeated doses of intravenous autologous mesenchymal stem cells for a patient with Parkinson's disease: a case report. Frontiers in Neurology. 14:1257080.
11. Schweitzer JS, Song B, Herrington TM, Park T-Y, Lee N, et al. (2020) Personalized iPSC-Derived Dopamine Progenitor Cells for Parkinson's Disease. New England Journal of Medicine. 382:1926–1932.
12. Schiess M. (2023) A Randomized, Double-blind, Placebo-controlled Trial of Allogeneic Bone Marrow-derived Mesenchymal Stem Cells as a Disease-modifying Therapy for Idiopathic Parkinson's Disease. clinicaltrials.gov; 2023. Report No: NCT04506073.
13. Hope Biosciences Stem Cell Research Foundation. A Randomized, Double-Blind, Single Center, Phase 2, Efficacy and Safety Study of Allogeneic HB-adMSCs vs Placebo for the Treatment of Patients With Parkinson's Disease. clinicaltrials.gov; 2023. Report No.: NCT04995081.
14. Schiess M, Suescun J, Doursout M, Adams C, Green C, et al (2021) Allogeneic Bone Marrow–Derived Mesenchymal Stem Cell Safety in Idiopathic Parkinson's Disease. Movement Disorders. 36:1825–1834.
15. Dezawa M. (2016) Muse Cells Provide the Pluripotency of Mesenchymal Stem Cells: Direct Contribution of Muse Cells to Tissue Regeneration. Cell Transplantation. 25:849–861.
16. ClusterXStem-Costa Rica. Tissue Regeneration and Function Recovery in Patients With Parkinson's Disease Using Allogenic Pluripotent Stem Cells Isolated From Adipose Tissue (PASCs): A Randomized Phase I/IIa Clinical Trial (REPOSE). clinicaltrials.gov; 2023. Report No.: NCT06141317.
17. Bartow MJ, Raggio BS. (2024) Liposuction. In: StatPearls. Treasure Island (FL): StatPearls Publishing.
18. Peto V, Jenkinson C, Fitzpatrick R, Greenhall R. (1995) The development and validation of a short measure of functioning and well being for individuals with Parkinson's disease. Quality of Life Research. 4:241–248.
19. Çekok K, Kahraman T, Duran G, Dönmez Çolakoğlu B, Yener G, et al (2020) Timed Up and Go Test With a Cognitive Task: Correlations With Neuropsychological Measures in People With Parkinson's Disease. Cureus. 12:e10604.
20. Dawson N, Dzurino D, Karleskint M, Tucker J. (2018) Examining the reliability, correlation, and validity of commonly used assessment tools to measure balance. Health Science Reports. 1:e98.