

Preclinical evaluation of transaxial intraputamen trajectory for enhanced distribution of grafted cells in Parkinson's disease

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OBJECTIVE The objective of this study was to develop and evaluate the feasibility and safety of a novel transaxial surgical approach for the delivery of human induced pluripotent stem cell–derived dopaminergic neuroprogenitor cells (DANPCs) into the putamen nucleus using nonhuman primates and surgical techniques and tools relevant to human clinical translation.

METHODS Nine immunosuppressed, unlesioned adult cynomolgus macaques (4 females, 5 males) received intraputamen injections of vehicle or DANPCs (0.9×10^5 to 1.1×10^5 cells/ μ L) under real-time intraoperative MRI guidance. The infusates were combined with 1-mM gadoteridol (for intraoperative MRI visualization) and delivered via two tracks per hemisphere (ventral and dorsal) using a transaxial approach. The total volumes of infusion were 25 μ L and 50 μ L for the right and left putamen, respectively (infusion rate 2.5 μ L/min). Animals were evaluated with a battery of clinical and behavioral outcome measures and euthanized 7 or 30 days postsurgery; full necropsies were performed by a board-certified veterinary pathologist. Brain tissues were collected and processed for immunohistochemistry, including against the human-specific marker STEM121.

RESULTS The optimized surgical technique and tools produced successful targeting of the putamen via the transaxial approach. Intraoperative MR images confirmed on-target intraputamen injections in all animals. All animals survived to scheduled termination without clinical evidence of neurological deficits. The first 4 animals to undergo surgery had mild brain swelling noted at the end of surgery, of which 3 had transient reduced vision; administration of mannitol therapy and reduced intravenous fluid during the surgical procedure addressed these complications. Immunostaining against STEM121 confirmed the presence of grafted cells along the injection track within the targeted putamen area of DANPC-treated animals. All adverse histological findings were limited in scope and consistent with surgical manipulation, injection procedure, and postsurgical inflammatory response to the mechanical disruption caused by the cannula insertion.

CONCLUSIONS The delivery system, injection procedure, and DANPCs were well tolerated in all animals. Prevention of mild brain swelling by mannitol dosing and reduction of intravenous fluids during surgery allowed visual effects to be avoided. The results of the study established that this novel transaxial approach can be used to correctly and safely target cell injections to the postcommissural putamen and support clinical investigation.

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KEYWORDS cell transplantation; intraoperative MRI; putamen; Parkinson; functional neurosurgery; macaque

ABBREVIATIONS AC = anterior commissure; DA = dopaminergic; DANPC = DA neuroprogenitor cell; GFAP = glial fibrillar acidic protein; H&E = hematoxylin and eosin; iMRI = intraoperative MRI; iPSC = induced pluripotent stem cell; IV = intravenous; NHP = nonhuman primate; PD = Parkinson's disease; PFA = paraformaldehyde; RT-iMRI = real-time iMRI; UW = University of Wisconsin; WNPCC = Wisconsin National Primate Research Center.

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PARKINSON's disease (PD) is characterized by neurodegeneration of nigral dopaminergic (DA) neurons that project into the striatum.¹ DA cell replacement is proposed as a PD therapy; the dorsal postcommissural putamen nucleus is a preferred grafting target due to the intense loss of DA innervation in this region and the difficulty of guiding axonal growth of nigral grafts into the putamen.

The complex neuroanatomy and behavior of nonhuman primates (NHPs) has been critical for the development and preclinical evaluation of cell replacement strategies for PD.^{2–4} A transcoronal parietal approach (from the top of the head) with multiple needle passages has typically been favored for delivering cells across the targeted putamen area. In macaques, 3–6 needle tracks are usually used.^{5–9} Because the putamen volume of humans is approximately 4–5 times larger than that of macaques,¹⁰ the clinical application of this surgical approach requires delivery of a higher number of cells via additional needle tracks to achieve equivalent target coverage. Each additional intracerebral injection carries the risk of hemorrhage or infection¹¹ and prolongs the surgical procedure, overall increasing the risk of an adverse surgical outcome.

A transaxial needle trajectory with a posterior brain entry point could be used to intersect the putamen nucleus across its anteroposterior axis. This approach would require a smaller number of intracerebral needle tracks to achieve the desired area of cell distribution, reducing the procedure risk.¹² A posterior transaxial trajectory has been widely applied for the placement of laser fibers across the hippocampus to treat epileptic seizures,^{13–16} as well as in a clinical trial for intracerebral delivery of viral vectors.¹⁷ Here, we describe the development and safety evaluation of a transaxial approach for the delivery of human induced pluripotent stem cell (iPSC)–derived midbrain DA neuroprogenitor cells (DANPCs) into the putamen nucleus of cynomolgus macaques.

Methods

Ethics Statement

Nine cynomolgus macaques (*Macaca fascicularis*) (Table 1, Fig. 1A) were used in this study. This industry-sponsored (Aspen Neuroscience, Inc.) study was performed in strict accordance with the recommendations in the National Research Council's *Guide for the Care and Use of Laboratory Animals* (8th edition, 2011) in an AAALAC International–accredited facility (Wisconsin National Primate Research Center [WNPRC], University of Wisconsin [UW]–Madison) and with the approval of the Institutional Animal Care and Use Committee. All efforts were made to minimize the number of animals utilized and to ameliorate any distress (Supplementary Methods).

The use of human stem cells was approved by the UW–Madison Stem Cell Research Oversight Committee. Aspen Neuroscience produced DANPCs from human dermal fibroblasts with the approval of the Scripps IRB. The unidentified human brain tissue used as a positive control for STEM121 immunohistochemistry was obtained from the tissue bank at the Alzheimer's Disease Research Cen-

ter at UW–Madison following documented review and approval from the IRB.

Behavioral Measures

Behaviors were assessed using video recordings and four different rating scales to score motor disability, abnormal movements, mood-related behaviors, and visual effects (Supplementary Methods). Scorers underwent reliability training and were blinded to the condition of the animals.

All behavioral training and evaluations used positive reinforcement to encourage participation of the monkeys in the tasks.⁹ Video recordings of the subjects were obtained using three cameras, each placed facing a different side of a plexiglass enclosure. After the animals were habituated to the testing environment, 15-minute video recordings were obtained twice before surgery, at 7 ± 1 days postsurgery, and at 30 ± 1 days postsurgery, as appropriate. During a recording session, an investigator familiar with the monkeys but blinded to their treatment condition presented small, desirable treats (e.g., dried fruit) to the animal, on the top and bottom of the enclosure, to assess the subject's response. The investigator then presented a treat in the center of the enclosure. The treat was slowly moved straight to the left and right, to assess the motor skills of both hands of the subject, and then in an arch, to assess visual fields.

Cell Preparation

DANPC derivation followed previously validated and published methods by Aspen Neuroscience. Dermal fibroblasts from two donors with sporadic PD were isolated¹⁸ and reprogrammed using the CytoTune-iPS Sendai Reprogramming Kit (Thermo Fisher). Multiple iPSC clones from each line were isolated, expanded, and banked.¹⁹ The iPSCs were then differentiated and analyzed for immunocytochemistry and gene expression.²⁰ Cells were shipped in cryovials to UW–Madison in a temperature-monitored liquid nitrogen shipping vessel and stored in a liquid nitrogen storage unit until formulation. Vehicle articles (Plasma-Lyte 148 10% human serum albumin, Baxter International Inc.) were shipped and stored at controlled ambient temperature until formulation.

Six animals received infusions of DANPCs from two different cell lines (Table 1). The cell suspension to be administered was prepared up to 24 hours before each surgery (one preparation per animal), maintained on wet ice or equivalent, and used within 25 hours. Predose cell number and viability were calculated using an automated cell counter (NucleoCounter NC-200, ChemoMetec). The data were used for the preparation of the final required concentration of cells; the acceptable range was 0.9×10^5 to 1.1×10^5 cells/ μ L and $> 90\%$ viability. Gadoteridol (1 mM) was added to the cell suspension or vehicle (1:500 dilution) before loading into a 500- μ L glass syringe with an MRI-compatible brass-threaded plunger (catalog no. 81242, Hamilton Company). The loaded syringes (one per hemisphere for low and high doses) were packed in a sterile pouch and placed inside a biomedical cooler (CSafe PX1L, CSafe Global), where they were kept at 2°C – 8°C for transportation and until use.

TABLE 1. Demographics and treatment assignments

Animal ID	Age, yrs	Sex	Weight, kg	Treatment	Postop Period, days	Preinfusion Cell Viability, %	Left Syringe		Right Syringe	
							Time at Room Temperature, mins	Postinfusion Cell Viability, %	Time at Room Temperature, mins	Postinfusion Cell Viability, %
V7-1	15.8	M	9.46	Vehicle	7	NA	NA	NA	NA	NA
V7-2	12.0	F	6.10	Vehicle	7	NA	NA	NA	NA	NA
V30-1	9.9	M	7.94	Vehicle	30	NA	NA	NA	NA	NA
C7-1	15.2	M	10.00	Cell A	7	94.40	116	90.6	126	87.90
C7-2	11.6	F	6.24	Cell B	7	93.50	132	93.25	103	92.90
C7-3	11.1	F	4.90	Cell B	7	94.20	122	88.35	103	90.85
C30-1	12.6	M	8.50	Cell A	30	94.40	88	91.25	109	89.3
C30-2	12.8	M	8.77	Cell B	30	91.60	93	89.20	112	91.90
C30-3	13.0	F	4.18	Cell B	30	94.00	95	94.40	116	87.40

NA = not applicable.

Subjects were treated with either vehicle or human iPSC–derived midbrain DANPCs. Two cell lines were used: cell line 111_602 (cell A) and cell line 109_626 (cell B). Preinfusion cell viability is reported for all cells prior to being divided into syringes for left (low dose) and right (high dose) hemisphere infusions. Postinfusion cell viability was evaluated on the cells remaining in the low- and high-dose syringes after completion of all the respective infusions. On average, 110 minutes (minimum 88 minutes, maximum 132 minutes) elapsed between the removal of the cell-loaded syringe from the temperature-controlled environment and the end of the final infusion (i.e., time at room temperature).

Surgical Planning and Trajectory Guide Placement

An MRI-compatible stereotactic frame was modified to accommodate the transaxial surgical approach with the MRI-compatible Aspen Neuroscience custom SmartFlow Neuro Cannula (catalog no. NGS-NC-01, ClearPoint Neuro) (Fig. 1C–K; Supplementary Methods). The cannula was designed for human application in conjunction with the ClearPoint system. Given the smaller size of the cynomolgus skull, we used a customized version of our previously validated pivot point–based MRI-compatible external trajectory guide (Engineering Resources Group Inc.).^{9,21,22}

For imaging, we used a 3T GE SIGNA Premier MRI scanner (GE Healthcare) with a custom 3-inch-diameter receive-only surface coil (MR Instruments, Inc.). Up to 1 month before surgery, a baseline 3D T1-weighted MR image was obtained per subject under ketamine and dexmedetomidine anesthesia (Supplementary Methods). The scans were used to visualize the putamen in three planes (sagittal, axial, and coronal) and develop a roadmap of the cannula trajectory, including the cannula entry point and placement of the trajectory guide base.^{21,22}

All animals were immunosuppressed with tacrolimus, abatacept, and prednisolone following a previously validated dosing scheme²³ (Supplementary Methods). The day of the intracerebral infusions, the trajectory guide bases were placed under sterile surgical conditions in a state-of-the-art surgical suite adjacent to the imaging facility. The procedure was performed under isoflurane anesthesia and infusion of fentanyl at a continuous rate, with propofol boluses administered as needed (Supplementary Methods). For surgery, the monkey was placed in the prone sphinx position with the head secured by the modified MRI-compatible stereotactic frame at an approximately 30° downward angle (Fig. 1C and D). Lidocaine and prilocaine cream 2.5%/2.5% was applied to the ear canals for analgesia. Bilateral craniotomies were performed at the predefined entry points. A 45° angle base was mounted to the skull over each craniotomy with three small self-

tapping screws. After placement of the bases, the monkey was transported to the MRI suite and positioned in the MRI bed while remaining in the stereotactic frame.

In Vivo Real-Time Intraoperative MRI Targeting and Infusion

Real-time intraoperative MRI (RT-iMRI) guidance of the cannula used the modified pivot point–based MRI-compatible external trajectory guide^{22,24,25} (Supplementary Methods). When the trajectory angle (anteroposterior, mediolateral direction) was confirmed to be on target, the stem was locked into position. The fluid-filled cannula was removed, the remote introducer was fastened to the alignment stem, and the modified guiding insert was placed in the stem. The selected preloaded low- or high-dose syringe with cells or vehicle was extracted from the medical cooler and, in the case of cell infusions, gently rotated to resuspend cells in the media. The syringe was connected to the cannula and its infusion line, and the plunger was advanced to fill the line and cannula with vehicle or cells. The cannula was then threaded through the guidance system until the ferrule was secured to the remote introducer and advanced to position.

Two needle tracks per hemisphere (dorsal and ventral) were planned, with an intraputamenal trajectory starting at the coronal level of the anterior commissure (AC) and ending approximately 4 mm caudal (postcommissural) to the first deposit. Two deposits were made for the dorsal tracks and three for the ventral tracks; deposits were placed 2 mm apart (Fig. 1B). The right and left putamen received total volumes of 25 μ L and 50 μ L, respectively (infusion rate 2.5 μ L/min). After completion of the last deposit per needle track, a 10-minute waiting period elapsed during which a final 3D T1-weighted MR image was acquired to evaluate each trajectory and quantify the volume of the gadoteridol-positive infusion cloud (Supplementary Methods). Subsequently, the cannula was carefully extracted using the remote introducer, and the syringe was placed back in the medical cooler and transported to the

cell laboratory for analyses of postsurgical cell viability (as described above).

After completion of all infusions, the animal was transported back to the surgical room, where the bases were removed and the incision was closed in layers.⁹

Necropsy and Tissue Processing

At 7 or 30 days after surgery, animals were deeply anesthetized with sodium pentobarbital (≥ 25 mg/kg intravenous [IV]) and transcardially perfused with heparinized saline, followed by 4% paraformaldehyde (PFA).^{26,27} A full necropsy of all animals was performed by a veterinary pathologist board-certified by the American College of Veterinary Pathologists; postmortem findings in peripheral tissues and the spinal cord were considered incidental (Supplementary Methods).

Brains were collected, cut into three coronal blocks, and postfixed in 4% PFA for 24–48 hours. The brain block containing the nigrostriatal system was cryoprotected by immersion in a graded (10%–30%) sucrose/0.1 M phosphate-buffered saline (pH 7.2) solution, cut frozen (40- μ m sections) in the coronal plane on a sliding knife microtome, and sections were kept frozen in a cryoprotectant solution until use. The occipital brain block was processed for paraffin embedding, cut (8- μ m sections) in the coronal plane on a rotary microtome, and the tissue was mounted on positively charged slides.

Immunohistochemistry and Immunofluorescence

Serial coronal brain sections containing the nigrostriatal system from all monkeys were immunostained using the primary antibodies human-specific cell marker (STEM121) (1:4000; Y404410, Takara), microglia/macrophages (CD68) (1:250; M08914, Dako), or mononuclear cells (CD45) (1:250; M0701, Dako) and counterstained with Nissl, as previously described.⁹ Negative (no primary antibody) and positive controls were run in parallel. Brain tissue from vehicle-treated animals was used as experimental negative controls; human brain tissue was used as STEM121+ controls. Occipital brain sections were similarly stained for STEM121.

Triple immunofluorescence staining was performed to evaluate colocalization of STEM121, β III-tubulin (neurofilament marker; 1:500; PA5-86069, Invitrogen), and glial fibrillar acidic protein (GFAP) (astrocytic marker; 1:500; 13-0300, Invitrogen) using previously validated methods.⁹ Confocal images were obtained using a Nikon A1R confocal microscope with 405-, 488-, 561-, and 640-nm wavelength lasers using NIS-Elements version 5.20.02.

Graft Evaluation

Coronal brain sections (approximately 10 sections per animal), 240 μ m apart, immunostained against STEM121 and counterstained with Nissl, were used to map canula trajectory and DANPC distribution. All brightfield imaging was performed using a Nikon Eclipse E800 equipped with a Nikon Digital Sight 1000 camera. The distance of the coronal sections from the AC was determined using a rhesus macaque stereotactic atlas.²⁸ Coronal brain sections across the cerebrum from all subjects were also

stained with hematoxylin and eosin (H&E) and evaluated by a board-certified veterinary pathologist for evidence of hemorrhage, edema, and other adverse events.

Statistics

Data collection and analysis were performed by investigators blinded to the treatment groups. Prism (version 9.0, GraphPad Software) was used for calculating descriptive statistics and statistical analysis.

Results

In Vivo Outcomes

The animals were generally healthy during the study, with no specific evidence of effects by the tested article as supported by blood chemistry and complete blood count findings (Supplementary Tables 1 and 2). Hyperglycemia and hypertriglyceridemia were occasional findings, most likely secondary to the immunosuppression regimen, and did not correlate with the tacrolimus levels (Supplementary Table 3). One animal (V7-1) presented with spontaneous hyperglycemia before surgery and required insulin therapy.

The 9 macaques in the study successfully received bilateral injections into the putamen nucleus (Table 1). The 36 infusions (9 subjects, 2 hemispheres, 2 needle tracks per hemisphere) followed transaxial trajectory plans based on the baseline MR images. Intraoperative MRI confirmed appropriate injection tracks in all animals and every canula track (Fig. 2). Backflow of gadoteridol-positive infusate outside the putamen target was observed during some infusions of vehicle- and DANPC-treated subjects (e.g., V7-2 and C7-2, left 20- μ L infusion) (Fig. 2). High cell viability of the DANPCs was confirmed after infusions (Table 1).

After V30-1 and V7-1 (first 2 surgical subjects) were noted to have mild brain swelling at the surgical site during wound closure, the remaining 7 animals received mannitol infusions during closure of the incision (Supplementary Table 4). As brain swelling was still noted in C30-3 and C7-2 (the following 2 surgical subjects), the volume of IV fluids administered during surgery was reduced (Supplementary Table 4). Three of the 4 subjects with brain swelling had some difficulties performing the visual field tests at the 7-day evaluation (Supplementary Table 5). V7-1 presented with minimal reduced tracking on the right visual field, and V30-1 had reduced tracking on the right and left visual fields. C7-2 displayed more reduced tracking of treats and had some difficulties grasping treats on both fields, probably compounded by postoperative periocular edema, which was limited to this subject and considered to be due to head placement in the stereotactic frame. Of the remaining 5 subjects, only C7-3 presented minimally reduced tracking and grasping on the right visual field, which veterinarians noted to improve prior to euthanasia at 7 days. At the 30-day evaluation, assigned animals, including V30-1, performed visual tasks within the normal range.

Signs of motor disability or abnormal movements were not observed in any subject with the exception of C30-2, which presented mild bradykinesia at the 30-day evaluation (Supplementary Table 5), likely associated with the

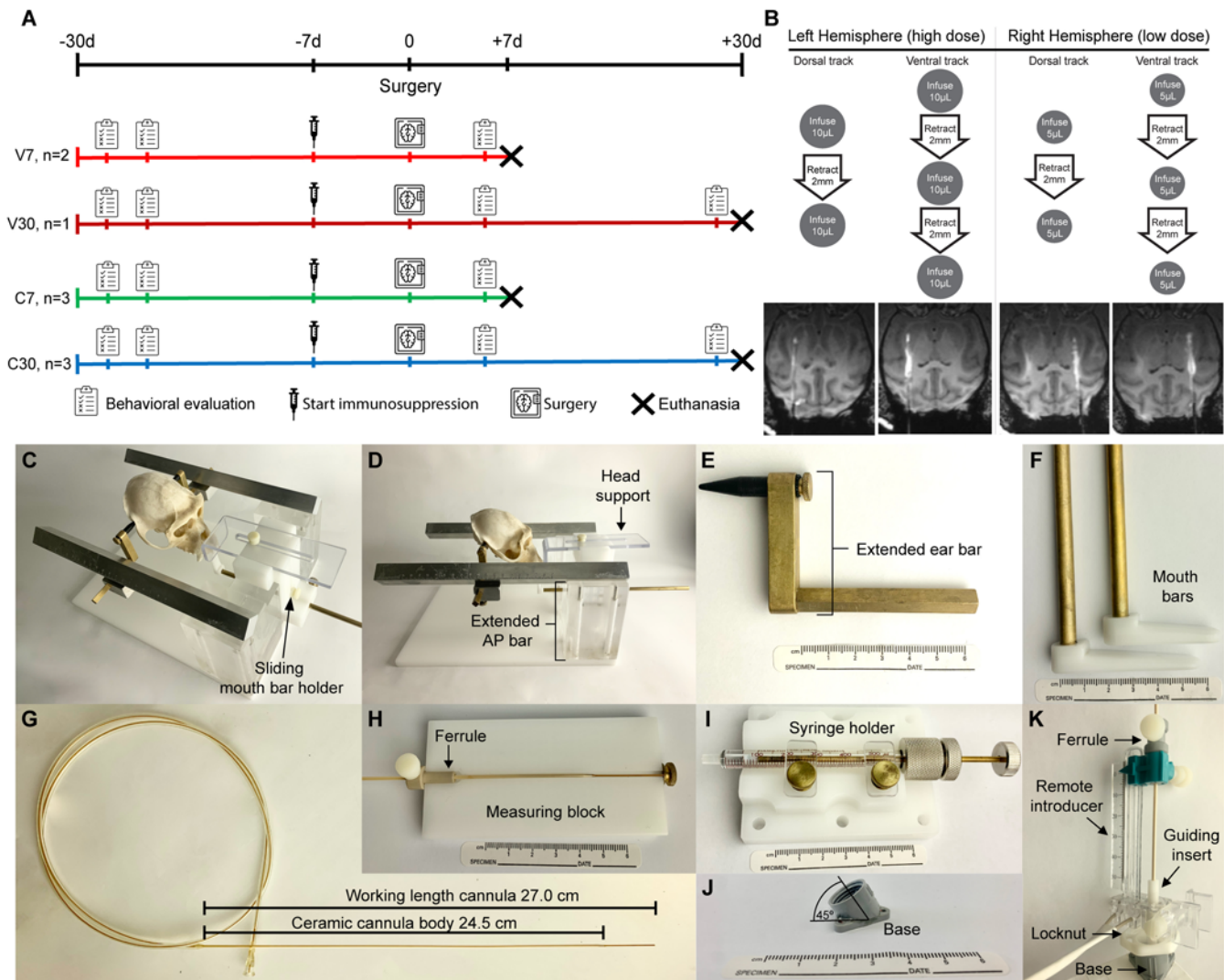


FIG. 1. Experimental design, dosing plan, and MRI-compatible infusion system. **A:** Timeline and study design. Nine cynomolgus macaques received intraputamen injections of vehicle (V) or cells (C). The animals were evaluated with a battery of clinical and behavioral outcome measures and euthanized 7 (V7, C7) or 30 days (V30, C30) postsurgery. **B:** Schematic of infusion volumes dosed per hemisphere (images obtained in C30-3). **C and D:** An MRI-compatible stereotactic frame was modified into a modular design, allowing for adaptation to differently sized subjects and surgical approach. The height of the anteroposterior (AP) frame was raised to fit the head of the monkey when it was placed in the prone to modified sphinx position; a contoured plexiglass piece was used to secure the head in the optimal angle. **E:** The vertical portion of the brass ear bars was extended to 50 mm to accommodate the coil and compatible infusion system during the MRI procedures. **F:** Sliding long or short palate bars were attached to the frame to further secure the subject's head in the modified sphinx position. **G:** MRI-compatible Aspen Neuro Cannula (ClearPoint Neuro). **H:** A ferrule was fastened to the ceramic body of the cannula at 10 mm from the tip using a custom-designed measurement block. **I:** MRI-compatible syringe (500 μ L) in syringe holder. **J:** Surgical base at a 45° angle. **K:** MRI-compatible infusion system with cannula and ferrule threaded through the alignment stem and remote introducer, secured by a locknut to the base.

development of a hematological neoplasia confirmed at necropsy. No changes in mood behaviors were detected; slightly anxious or submissive behaviors were observed at baseline, usually persisting in the following testing sessions (Supplementary Table 5).

Postmortem Outcomes

Postmortem brain analyses of vehicle- and cell-treated animals identified the two transaxial needle trajectories per hemisphere, consistent with iMR images. Evaluation

of H&E-stained coronal brain sections showed findings consistent with surgical manipulation, cannulation, and cell administration. Along the cannula track in the putamen, foci of hypercellularity consistent with cell grafts were present (Supplementary Fig. 1). Mononuclear cells and very scant hemorrhage were regularly observed within foci of hypercellularity; scant hemorrhage was occasionally noted adjacent to cannula tracks. No evidence of acute inflammation was detected in any subject (Supplementary Fig. 1). In the occipital lobe, there was mechanical dis-

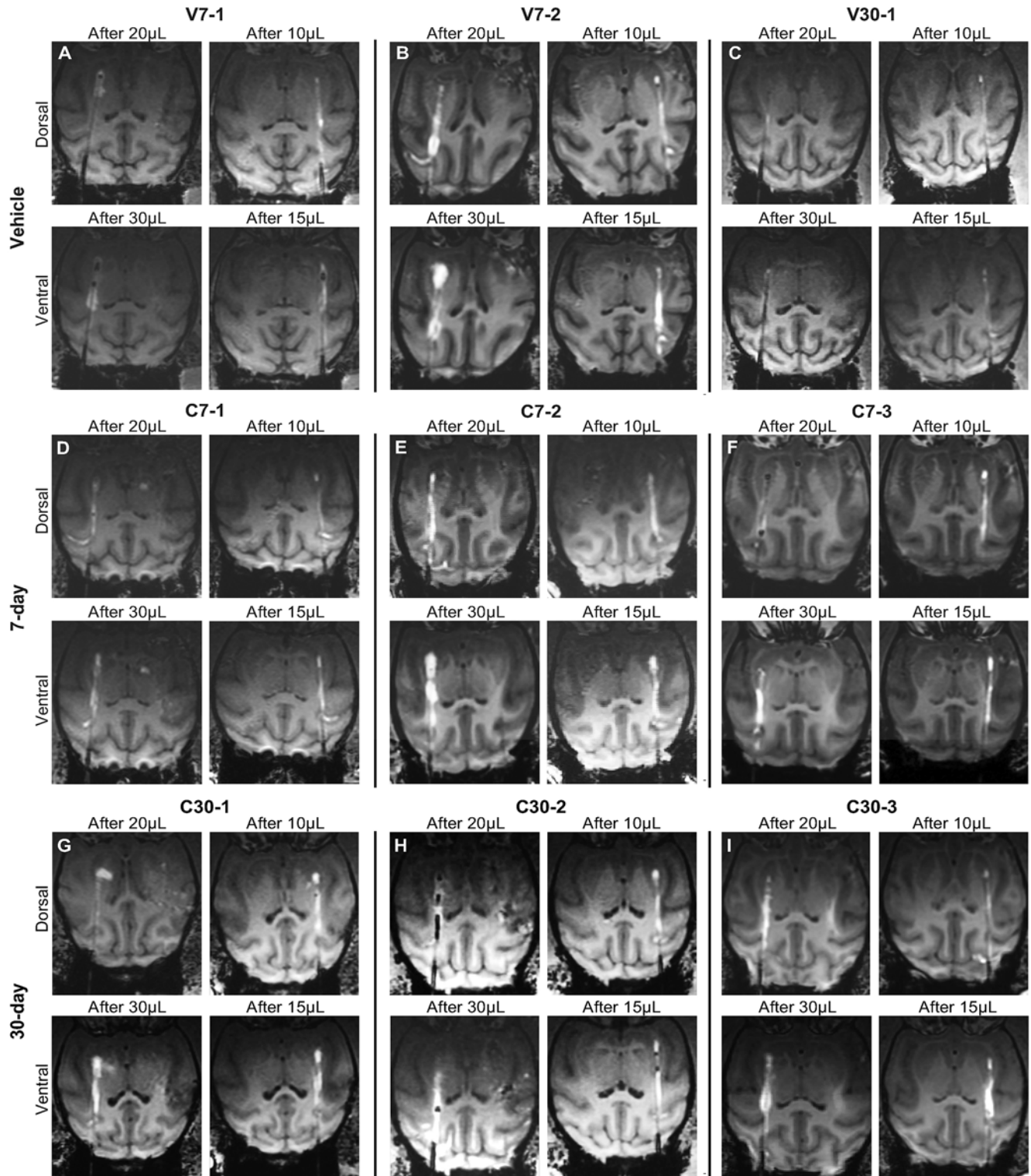


FIG. 2. Transverse T1-weighted BRAVO intraoperative MR images across each cannula trajectory for all subjects. Intracerebral injections consisted of two needle tracks per hemisphere (1 dorsal, 1 ventral), with a high (*left*; total volume 50 μ L) and a low (*right*; total volume 25 μ L) dose of 1-mM gadoteridol in Plasma-Lyte 148 with up to 10% human serum albumin (**A–C**) or human DANPCs (1×10^5 cells/ μ L) (**D–I**). All infusions were done at a rate of 2.5 μ L/min, with two deposits for the dorsal tracks and three deposits for the ventral tracks. The first intraputamenal deposit was placed at the coronal level of the AC; deposits were separated by 2 mm. After completing the last deposit per needle track, a 10-minute waiting period elapsed before carefully extracting the cannula using the remote introducer. During the waiting period, a final MR image was acquired (showcased here). Note the imaging artifact generated by the gadoteridol-filled cannula present in the brain at the time of MRI acquisition.

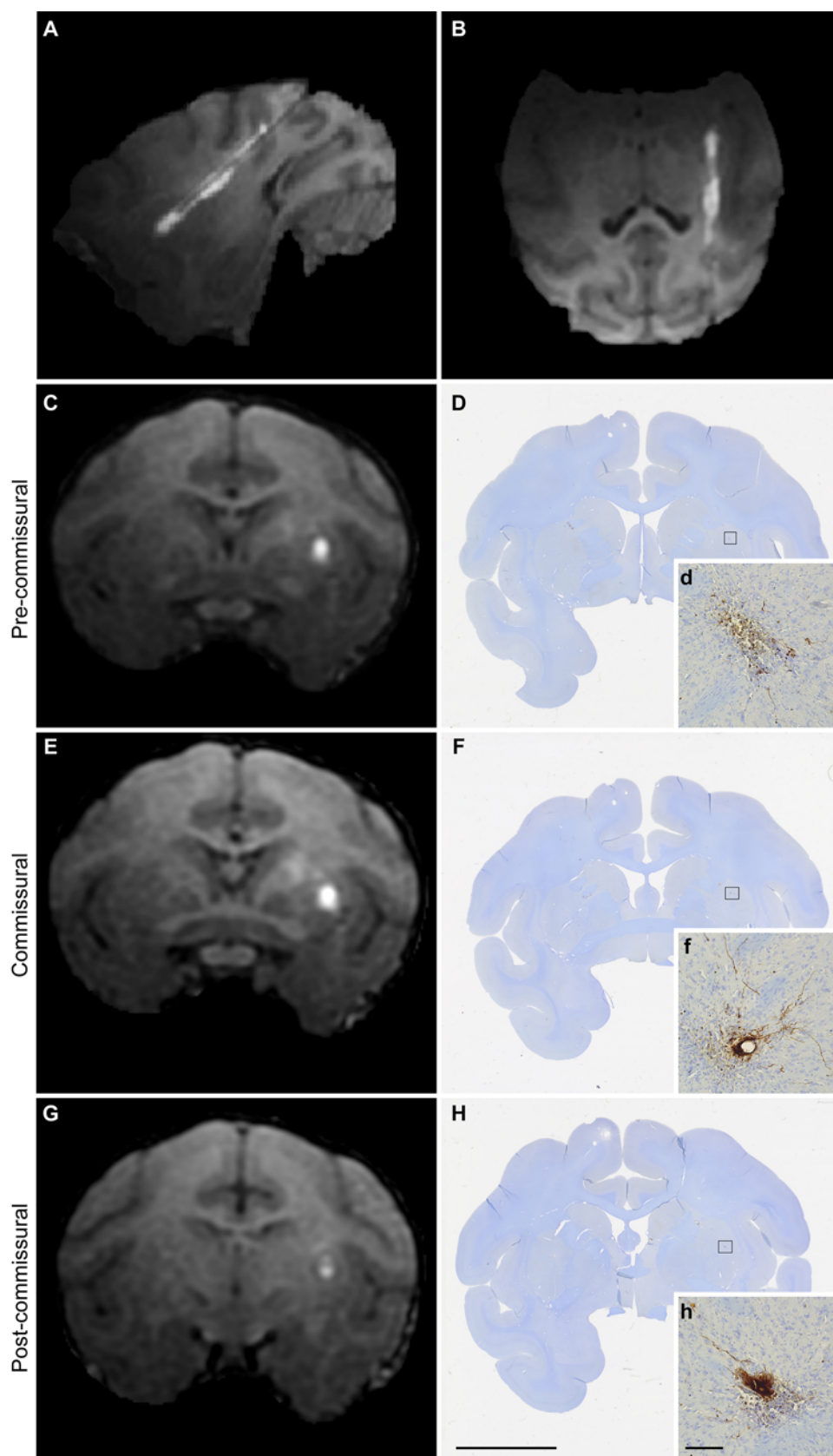


FIG. 3. Intraoperative MRI cannula trajectory and corresponding distribution of expression of the human cell marker STEM121. Representative sagittal (A) and transverse (B) intraoperative T1-weighted BRAVO MR images of the right ventral needle track after a 15- μ L infusion of cells with 1-mM gadoteridol (obtained in C30-1). **FIG. 3. (continued)**→

FIG. 3. Coronal MR brain images of the same infusion at the precommissural (C), commissural (E), and postcommissural (G) levels of the gadoteridol-positive cloud. At 30 days postinfusion, STEM121+ human cells were detected in the areas of infusion at the precommissural (D), commissural (F), and postcommissural (H) coronal levels. **Insets** (d, f, h) are higher-magnification images of the boxed areas. Scale bars = 2 cm (D, F, H) and 100 μ m (d, f, h).

ruption of the meninges and neuropil, variable amounts of hemorrhage, and hypercellularity of the neuropil along the cannula track, which was surrounded by normal neuropil. All these findings were expected and consistent with surgery and immunological response 7 and 30 days postsurgery (Supplementary Fig. 2).

Immunostaining using the human-specific antibody STEM121 identified grafted cells within the foci of hypercellularity present within the transaxial intraputamenal needle tracks (Figs. 3 and 4), but not across occipital regions, including in areas where gadoteridol-positive backflow was detected using iMRI (Supplementary Fig. 3). At 7 and 30 days postinfusion, STEM121 expression was detected in 5 of the 6 DANPC-injected animals, pri-

marily in structures with neuronal fiber-like morphology surrounding the needle tracks across the putamenal targets (Fig. 4). Triple-label immunofluorescence confirmed the neuronal (STEM121+/ β III-tubulin+) nature of the grafted cells, which were adjacent to STEM121-/GFAP+ macaque astrocytes (Fig. 5). STEM121+ fibers were detected up to approximately 2.88 mm rostral and 12.48 mm caudal to the AC; they were more abundant and easier to identify at 30 days than at 7 days (Fig. 6).

Consistent with H&E findings, CD68+ microglia (Fig. 7A–H) and CD45+ mononuclear cells (Fig. 7I–P) were detected in the putamen of all vehicle- and cell-treated subjects following the transaxial trajectory of the cannula. Across treatment groups and time points, CD68+ cells

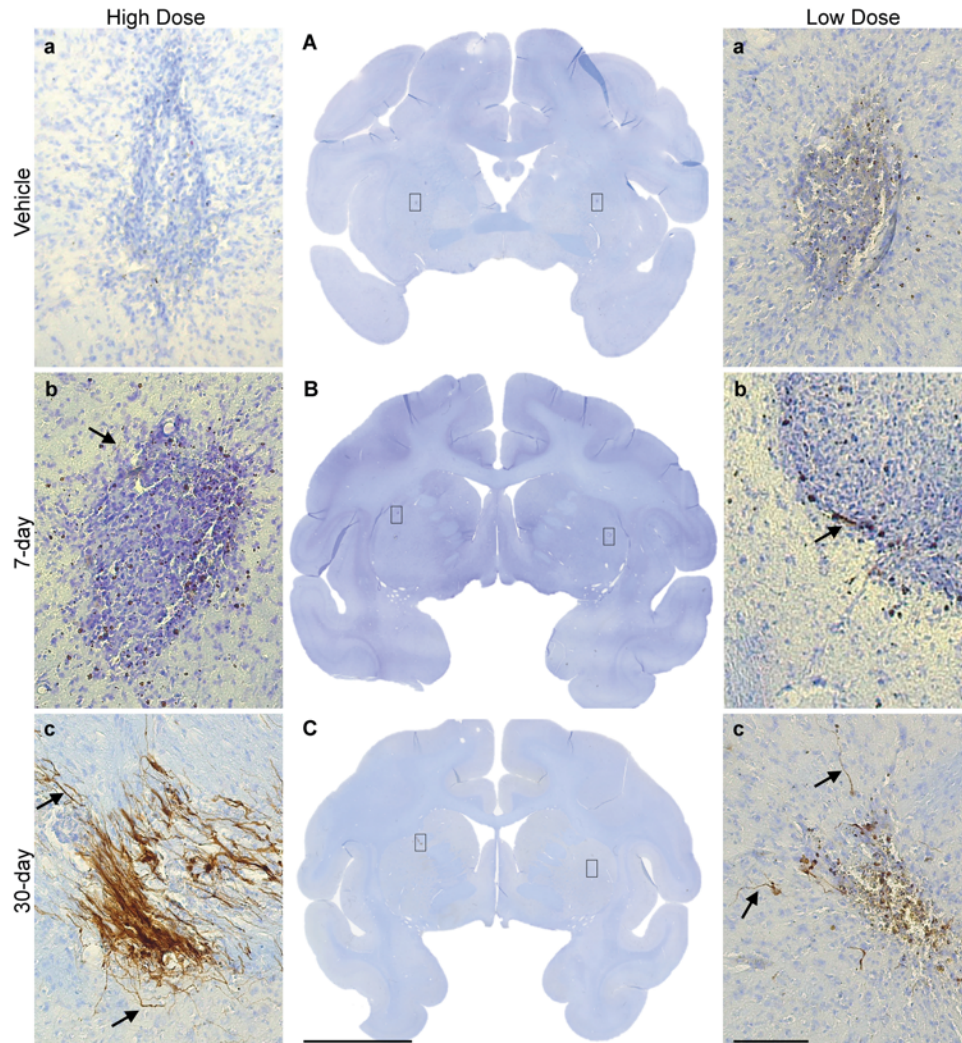


FIG. 4. Putamenal STEM121 expression across treatment groups. Coronal brain sections obtained in vehicle-treated 7-day (V7-1) (A), cell-treated 7-day (C7-2) (B), and cell-treated 30-day (C30-1) (C) subjects immunostained against STEM121 with Nissl counterstaining. Higher-magnification images (a–c) of corresponding boxed areas. Black arrows point to STEM121+ fibers. Scale bars = 2 cm (A–C) and 100 μ m (a–c).

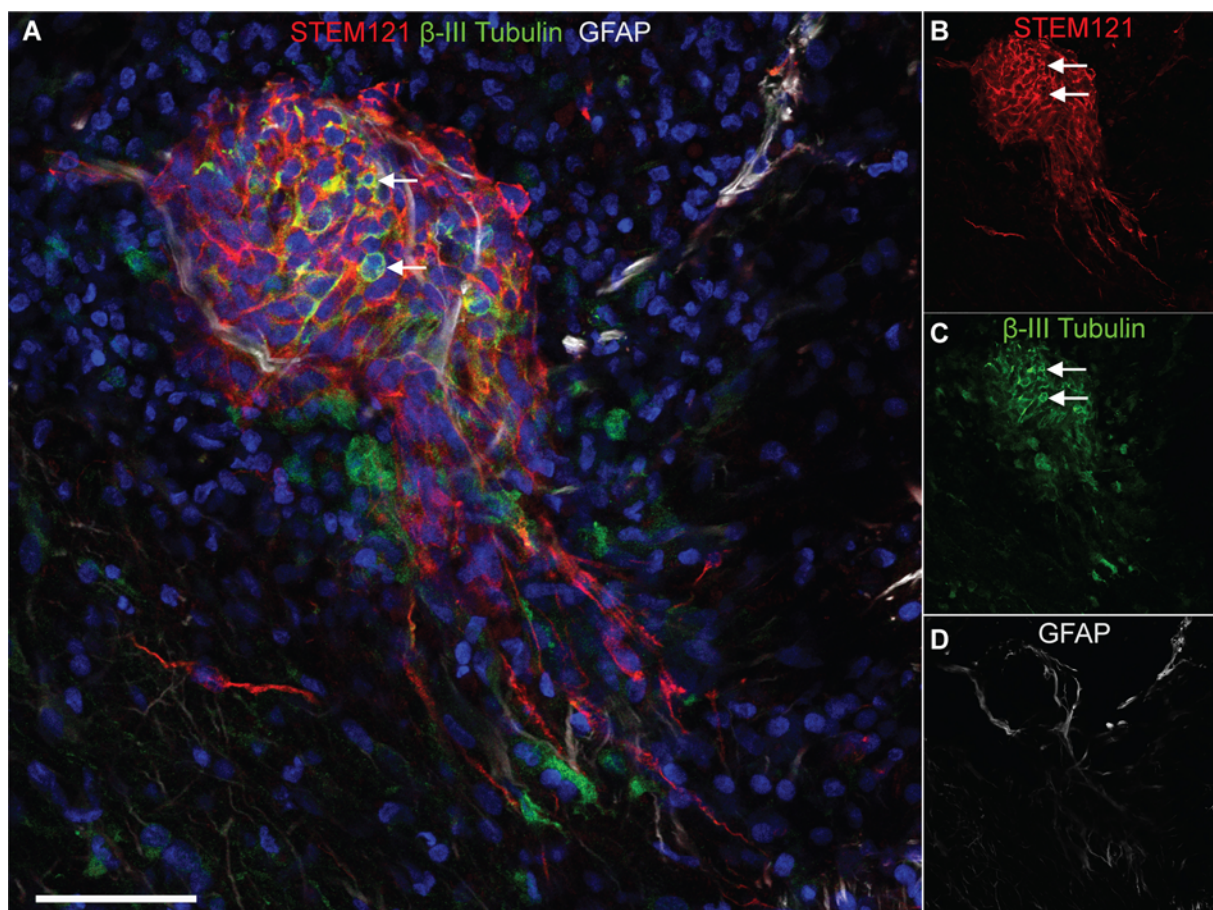


FIG. 5. Phenotype of grafted STEM121+ DANPCs. Confocal image (A) of triple-label immunofluorescence for STEM121 (B; red), β III-tubulin (C; green), and GFAP (D; white); cell nuclei are visualized with 4',6-diamidino-2-phenylindole (blue). The human cells (STEM121+) colocalized with the neuronal marker β III-tubulin (white arrows). A few GFAP+ astrocytes were detected in the grafted area. Scale bar = 50 μ m.

were more abundant than CD45+ cells; no differences were detected in the DANPC-injected animal without discernable STEM121+ cells (C7-3) (Supplementary Fig. 4).

Discussion

The results of this study in cynomolgus macaques demonstrate the feasibility and safety of a novel transaxial approach for cell delivery into the putamen nucleus. The 36 transaxial infusions of vehicle or DANPCs completed in 9 monkeys were accurately placed across the dorsal and ventral axis of the commissural to postcommissural putamen nucleus using RT-iMRI guidance and an MRI-compatible delivery system.

DANPCs from two different human cell lines were successfully infused using the MRI-compatible Aspen Neuroscience custom SmartFlow Neuro Cannula and syringe delivery system designed for human use. To accommodate for the monkey anatomy, the pivot-point base was optimized for use with the human cannula and entry angle, in combination with modifications to the NHP stereotactic frame for appropriate head-holding. The custom Navigus base plate and stereotactic frame were created solely for this NHP study, as the ClearPoint Neuro SmartFrame will

be used in the planned first-in-human trial. Aiming to inform this clinical translation, we injected one NHP brain hemisphere with the planned maximum clinical dose, and the opposite hemisphere received twice the planned maximum clinical dose, both scaled by putaminal volume.

The intracerebral distribution of the gadoteridol-positive infusate observed using iMRI confirmed the transaxial trajectory and the accuracy of the targeting. Although the gadoteridol-positive cloud extended past the boundaries of the targeted putamen, no STEM121+ cells were identified beyond the target, probably because cells have greater size and weight than gadoteridol molecules, which limit their mobility within the brain parenchyma during the infusion. Backflow is frequently observed after intracerebral injection of fluids, especially when using convection-enhanced delivery methods; a relatively slow rate of infusion can be used to minimize backflow occurrence.²⁹ The originally planned infusion rate was 5 μ L/min, yet based on our pilot trials, we ultimately used 2.5 μ L/min to decrease the risk of backflow. This injection flow rate also matched the flow rates of 2–3 μ L/min previously used by our group for successful administration of neuronal precursors to the NHP putamen.^{9,22}

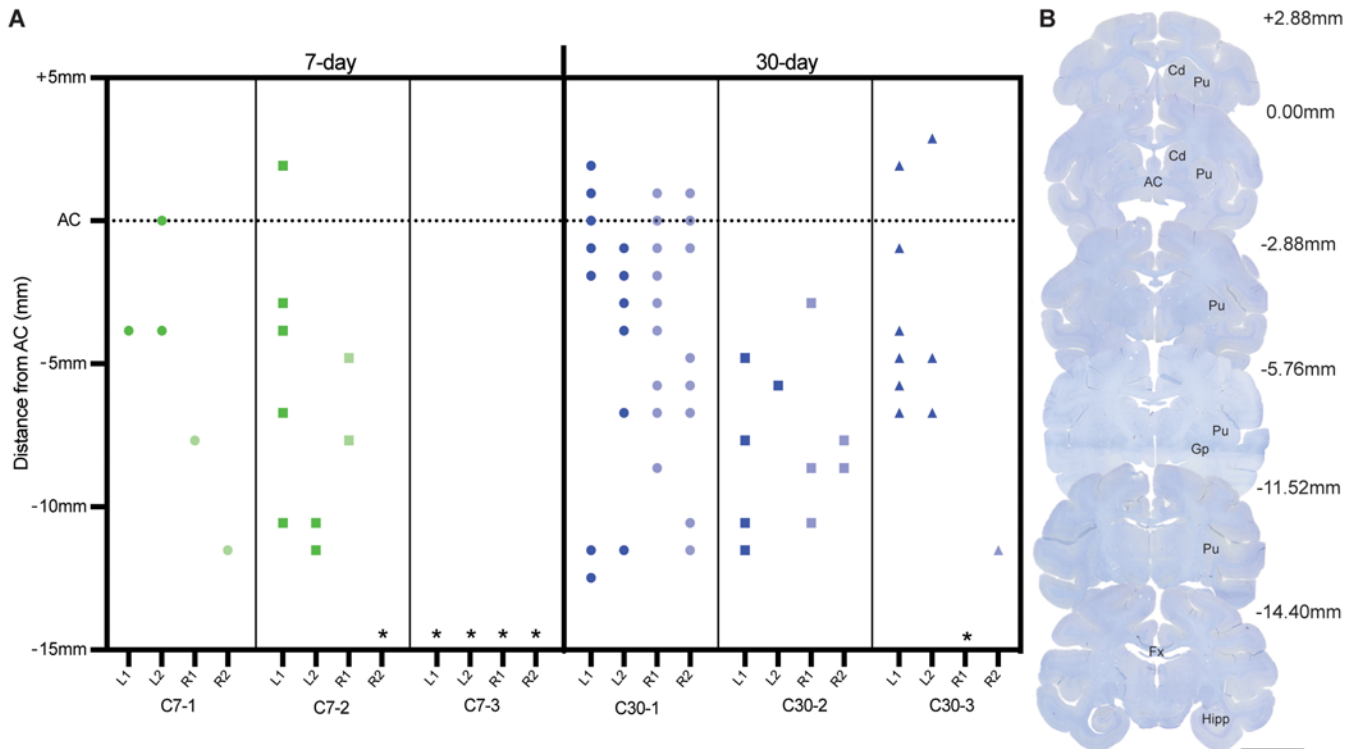


FIG. 6. Intracerebral distribution of STEM121+ fibers relative to the AC (dotted line). **A:** Serial coronal brain sections immunostained against STEM121 and counterstained with Nissl were used to detect human grafts in both brain hemispheres (left [L], right [R]), following the dorsal (R1, L1) and ventral (R2, L2) infusion trajectories in the transaxial plane. *No fibers were observed along the needle track. Symbol color corresponds to treatment group (green, 7 days; blue, 30 days), and a unique symbol shape is used for each subject. **B:** Representative images of coronal brain sections (obtained in C30-1). Scale bar = 2 cm. Cd = caudate; Fx = fornix; Gp = globus pallidus; Hipp = hippocampus; Pu = putamen.

Mild brain swelling at the surgical site was noted during wound closure of the first 4 subjects, and 3 of these animals (V30-1, V7-1, and C7-2) presented with reduced vision 7 days postsurgery. As this was a serious concern for the study animals' health and the clinical translation, we carefully reviewed our surgical methods and perioperative care. Following WNPC standard operating procedures (SOPs), we administered IV fluids at approximately 5 mL/kg/hr to these subjects during the procedure, and additional fluid boluses were given per veterinarian discretion. On consideration of the greater procedure length and minimal blood loss compared with other surgeries for which the SOPs were developed, reduction in IV fluid administration was initiated after dosing the fourth animal. Afterward, only 1 additional animal (C7-3) showed very mild reduced vision that veterinarians reported improved by the time of euthanasia. It should also be noted that the first 2 animals did not receive mannitol compared with the following 7 animals. In human patients, excessive IV dosing of Plasma-Lyte A and Plasma-Lyte 148 is known to lead to edema and is described as a warning in the drug insert.³⁰ Maintaining a systolic blood pressure < 140 mm Hg is recommended for patients undergoing intracerebral delivery of gene therapies to minimize the chance of brain bleeding.³¹ Excess IV fluids increase blood pressure, leading to increased intracranial pressure and symptoms such as blurred vision, headache, and feeling less alert. There-

fore, it is likely that increased intracranial pressure during surgery and the early postoperative period contributed to the transient reduced vision. This finding underscores the importance of monitoring blood pressure and fluid delivery during brain surgery.

Other factors may have contributed to the visual effects. The cannula penetrated and traveled through the periphery of the occipital lobe. Visual field deficits were reported in 7 of 68 patients with refractory mesial temporal lobe epilepsy treated by thermal ablation via the transaxial approach.¹⁴ Yet in these cases, issues in laser fiber placement leading to heat spread along ventricular spaces were considered the cause of the visual field cuts. In an open-label trial, temporary visual disturbances were reported in 1 of 8 PD patients who received intraputamenal delivery of viral vectors through a transaxial approach with the ClearPoint system and the subjects placed in the prone position.¹⁷ The affected patient presented with intraoperative hemorrhage; the visual deficits resolved over time. Intraoperative hemorrhage was not detected during our monkeys' surgical procedures visually or using MRI. Moreover, postmortem analyses at 7 days did not find striking differences between subjects with or without reduced vision. The finding of brain swelling in vehicle- and DANPC-treated animals nullifies a treatment-related effect. Interestingly, the right visual field was preferentially affected. The infusate volume was larger in the left (50 μ L total) than the right (25

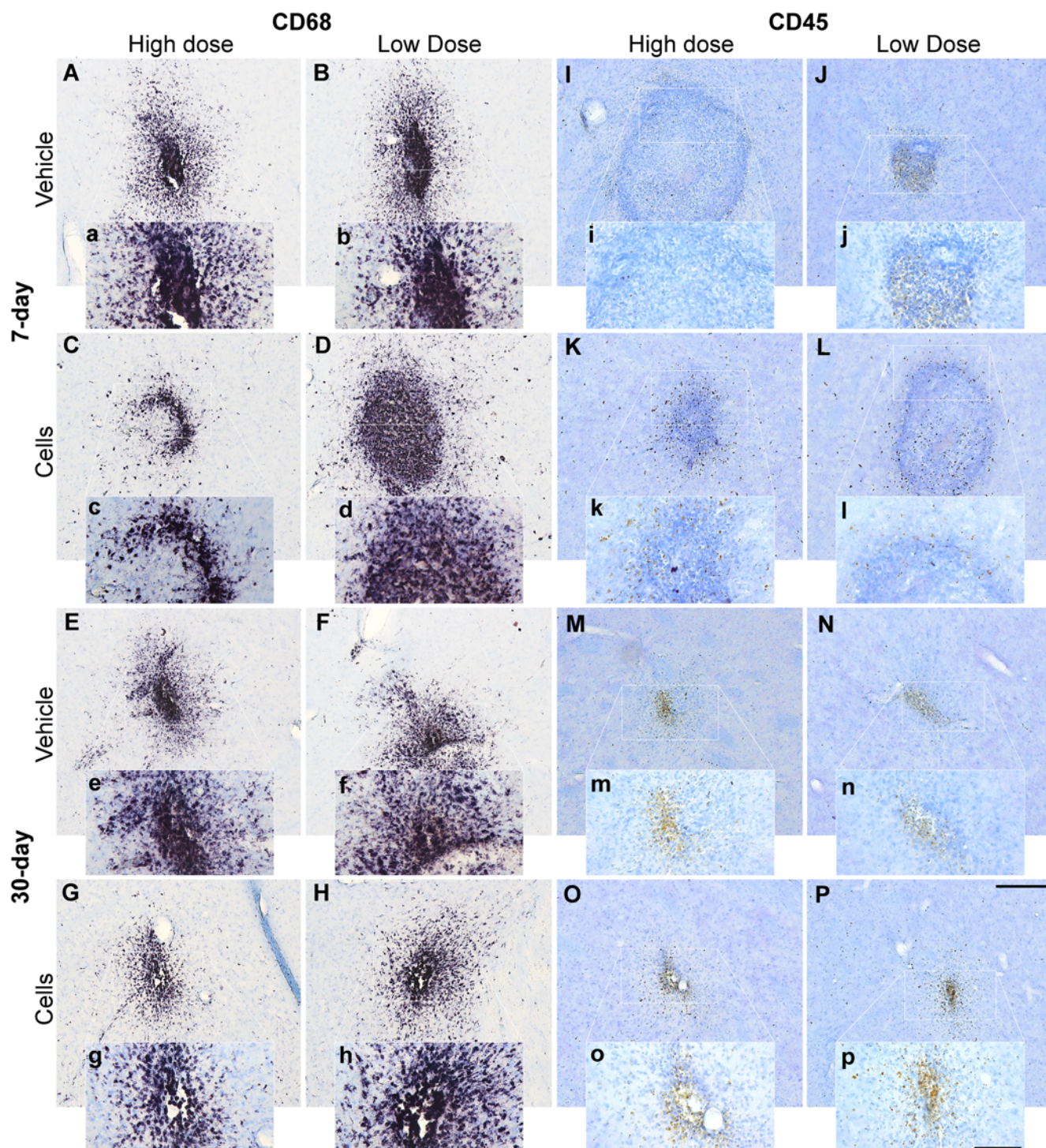


FIG. 7. Intracerebral host immune response. Representative images of putaminal infused areas immunostained against the macrophage/microglia marker CD68 (A–H) and the peripheral blood mononuclear cell marker CD45 (I–P) with Nissl counterstaining. Immune cell recruitment was limited, and it was observed in the high- and low-dose treated brain hemispheres of 7-day (V7-1) and 30-day (V30-1) vehicle-treated subjects and 7-day (C7-1) and 30-day (C30-2) cell-treated subjects. Evidence of scant/mild hemorrhage is visible as partially intact extravasated erythrocytes and orange/brown hemosiderin, a product of the breakdown of hemoglobin. Note the increased hemosiderin at 30 days relative to 7 days, consistent with resolution of the hemorrhage along the needle track. Scale bars = 250 μm (A–H) and 100 μm (a–h).

μL total) putamen, further suggesting that increased intracranial pressure played a role in the visual signs.

One of the 6 subjects injected with DANPCs (C7-3) did not have detectable STEM121+ cells. High cell viability was verified before and after surgery, and cells from the same donor and lot were successfully used in other monkeys (C7-2, C30-2, and C30-3). All brain infusions were performed by the same skilled surgeons, using the same methods and delivery system. To avoid host rejection of the xenografts, animals underwent a previously validated, intense immunosuppression paradigm, confirmed to be effective during the pilot phase of this study. Tacrolimus measures revealed consistent therapeutic levels throughout the study. The levels of methylprednisolone and abatacept were not measured (standard clinical approach); therefore, the possibility of subtherapeutic levels of these drugs cannot be negated. CD68+ microglia and CD45+ mononuclear cells were detected around the needle track of all subjects, including vehicle animals, and their appearance in C7-3 was similar to that in other subjects. These findings at 7 and 30 days postsurgery suggest that recruitment of immune cells was occurring, associated with the surgical procedure itself. Additional studies with later time points postsurgery may help us discern between immunoreaction to the xenograft versus glial reaction to repair the brain after the injection. The planned clinical translation of this approach will use autologous sources for generating DANPCs. Although autologous cell therapy development will be more labor intensive, the need for immunosuppression will be avoided, as the risk of rejection will be minimized. In that regard, our use of two different cell lines supports the feasibility of generating multiple cell lines from autologous sources.

It could be argued that two limitations of the present study are the assessment of the surgical approach in normal, nonparkinsonian NHPs and the short postsurgical period, as these choices preclude our ability to evaluate graft efficacy. A previous report by our group has shown the antiparkinsonian benefits of similarly produced DANPCs in a rodent model of PD.²⁰ In contrast, evaluation of DANPC efficacy was not a goal of this study. Rather, the aim was to assess the feasibility and safety of the approach. Future investigations in NHP models of PD with an extended postsurgical period (e.g., 12 months) would provide the disease-like state and time for transplanted cells to mature and integrate into the host brain, conditions needed to assess graft efficacy. A tantalizing possibility of the transaxial cell deployment is to extend the injected area to the precommissural putamen to treat PD nonmotor symptoms. Our previous cell transplantation studies in monkeys with PD have shown that a combination of DA-producing grafts in the pre- to postcommissural putamen may provide relief of PD motor symptoms as well as depressive and anxious behaviors.⁹ Follow-up studies to assess the impacts of brain area of cell delivery, grafted cell phenotype, and grafted cell gene expression signatures on PD motor and nonmotor symptoms are warranted.

Conclusions

The iMRI-guided transaxial approach, MRI-compati-

ble delivery system, and DANPCs were well tolerated by all subjects. Prevention of mild brain swelling by mannitol dosing and reduction of IV fluids during surgery allowed us to avoid visual effects. The transaxial surgical methods produced accurate, replicable, and safe delivery of cells across the putamen nucleus of cynomolgus macaques and supported their clinical translation.

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Supplemental Information

Online-Only Content

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