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Endovascular Selective Intra-Arterial Infusion of Mesenchymal Stem Cells Loaded With Delta-24 in a Canine Model

BACKGROUND: Delta-24-RGD, an oncolytic adenovirus, shows promise against glioblastoma. To enhance virus delivery, we recently demonstrated that human bone marrow-derived mesenchymal stem cells loaded with Delta-24-RGD (hMSC-D24) can eradicate glioblastomas in mouse models. There are no studies examining the safety of endovascular selective intra-arterial (ESIA) infusions of MSC-D24 in large animals simulating human clinical situations.

OBJECTIVE: To perform canine preclinical studies testing the feasibility and safety of delivering increasing doses of hMSCs-D24 via ESIA infusions.

METHODS: ESIA infusions of hMSC-D24 were performed in the cerebral circulation of 10 normal canines in the target vessels (internal carotid artery [ICA]/P1) via transfemoral approach using commercially available microcatheters. Increasing concentrations of hMSC-D24 or particles (as a positive control) were injected into 1 hemisphere; saline (negative control) was infused contralaterally. Toxicity (particularly embolic stroke) was assessed on postinfusion angiography, diffusion-weighted magnetic resonance imaging, clinical exam, and necropsy.

RESULTS: ESIA injections were performed in the ICA (n = 7) or P1 (n = 3). In 2 animals injected with particles (positive control), strokes were detected by all assays. Of 6 canines injected with hMSC-D24 through the anterior circulation, escalating dose from 2×10^6 cells/20 mL to 1×10^8 cells/10 mL resulted in no strokes. Two animals had ischemic and hemorrhagic strokes after posterior cerebral artery catheterization. A survival experiment of 2 subjects resulted in no complications detected for 24-h before euthanization.

CONCLUSION: This novel study simulating ESIA infusion demonstrates that MSCs-D24 can be infused safely at least up to doses of 1×10^8 cells/10 mL (10^7 cells/ml) in the canine anterior circulation using commercially available microcatheters. These findings support a clinical trial of ESIA infusion of hMSCs-D24.

KEY WORDS: Endovascular, Cerebrovascular, Glioma, Glioblastoma, Microcatheter, Superselective, Intra-arterial

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Glioblastoma (GBM) is recalcitrant to conventional therapies.[1] We have developed a novel tumor-selective oncolytic adenovirus, Delta-24-RGD (Delta-24) to combat GBM.[2] In a recent clinical trial, we showed that Delta-24 is capable of replicating in human tumors after direct intratumoral injection, resulting in cell death through oncolysis, while also triggering an anti-glioma CD8 T cell immune-mediated response, all of which is capable of resulting in long term tumor eradication.[3] This trial has positioned Delta-24 as a promising novel viro-immunotherapy.[4]

Unfortunately, this trial also showed that direct intratumoral injection of Delta-24 is not an ideal method for delivery. Intratumoral injection results in limited initial

ABBREVIATIONS: ADC, apparent diffusion coefficient; **ESIA,** endovascular selective intra-arterial; **GBM,** Glioblastoma; **GFP,** green fluorescent protein; **IA,** intra-arterial; **ICA,** internal carotid artery; **MR,** magnetic resonance; **MRI,** magnetic resonance imaging; **MTD,** maximal tested dose; **PCA,** posterior cerebral artery; **SAH,** subarachnoid hemorrhage

Supplemental digital content is available for this article at www.neurosurgery-online.com.

distribution as the virus was localized primarily around the injection catheter. Alternative delivery methods that distribute large amounts of virus widely through the tumor would be of benefit and increase the potential for more robust oncolytic effects. Intravascular delivery meets these requirements, but naked adenovirus is prohibited by immune-mediated viral inactivation and by peripheral organ toxicity, particularly viral-induced liver failure.

We recently showed that human bone marrow-derived mesenchymal stem cells (BM-hMSC) are capable of homing to gliomas.[5-7] BM-hMSC loaded with Delta-24 (hMSCs-D24) maintain their ability to home to gliomas after intra-arterial (IA) injection, and are capable of delivering and releasing Delta-24 into human glioma xenografts in immune-deficient mice.[5] In several independent experiments, hMSCs-D24 treatment resulted in a significant improvement in median survival and cures in 30% to 40% of mice.[5] These data provide a rationale for using hMSCs to deliver Delta-24 to patients with GBMs in an effort to enhance viral delivery.

The clinical implementation of IA delivery of hMSCs-D24 in patients with GBM has received little attention up to now. IA delivery of "empty" hMSCs has been tested in clinical trials for other neurological disorders,[8,9] but the safety[10] and feasibility of delivering virally loaded BM-hMSC have not been assessed. To begin to address this, we recently reported in Vitro studies demonstrating that hMSCs-D24 are compatible with several clinical-grade microcatheters, and co-infusion with medications commonly used in endovascular procedures (verapamil, heparin), either alone or in combination does not alter hMSC-D24 viability.[11]

We now build upon these in Vitro studies by assessing the in Vivo feasibility of endovascular selective intra-arterial (ESIA) delivery[12] of hMSCs-D24 in a nontumor-bearing canine model. We establish the technical feasibility, safety, and maximum tolerated dose in a large animal model on a scale similar to humans.

METHODS

Preparation, Labeling, and Transduction of MSCs

hMSCs were prepared and transfected with DNX-2401 (Delta-24-RGD) as previously described,[11] with green fluorescent protein (GFP) labeling of the vector.[5] Full description is provided in Supplemental Digital Content 1. The stem cells were approximately 18 to 20 microns in size.

Dose Escalation

A stepwise dose escalation was used with each subsequent subject, starting at 2×10^6 cells/20 mL. Doses were chosen based on prior mouse studies and human studies.[3,5]

Animal Procedure

Full descriptions of the procedure are available by protocol (**Supplemental Digital Content 1**), and for each individual procedure (**Supplemental Digital Content 2**). Briefly, adult mongrel canines were anesthetized with complete general anesthesia. All procedures were approved by the institutional animal care and use committee (IACUC). In the animal angiography suite, via right femoral arterial access, the target vessel (distal internal carotid artery [ICA] for anterior circulation, P1 posterior cerebral artery (PCA) for posterior circulation) was selected with the microcatheter (Echelon 14, Medtronic, inner diameter 0.017″). Animals were systemically heparinized. Baseline angiography was performed in each hemisphere. Infusion of the experimental agent was performed in one hemisphere over 25 to 30 min. The opposite ICA or PCA was then accessed and a normal saline infusion of equal duration and volume was performed as an internal control. The experimental hemisphere was infused first followed by the control injections of normal saline to maximize the infusion to magnetic resonance imaging (MRI) time. Postinfusion angiography was performed to assess for angiographic stroke. The anesthetized animals then underwent MRI, at least 1-h postinfusion to allow complications to be detected while accounting for the logistics of maintaining anesthesia. Animals were then euthanized. Gross anatomical pathology and histological analyses were performed by independent veterinary pathologists.

Positive Controls

In 2 animals, a "positive control" was performed, with the intention to induce a stroke by endovascular means that would be detectable on MRI and pathology. In these controls, LC Bead Lumi™ 70-micron particles (BTG, London, United Kingdom) were used to embolize 1 hemisphere to induce an ischemic stroke. Otherwise, the procedure was performed as above.

Survival Experiments

Two animals were infused at the maximum dose as a survival experiment. Immediately after the procedure, the animals were recovered from anesthesia and extubated. They were monitored for 24 h with neurological[13] and systemic assessments. After 24 h postprocedure, an MRI was performed. The animals were then euthanized and assessed as above.

Postprocedure Assessments

Imaging protocol

The MRI studies were performed on a 3-Tesla MRI system (Trio Tim, Siemens Healthcare, Erlangen, Germany). T1, T2, T2 FLAIR, and diffusion weighted imaging with apparent diffusion coefficient (ADC) maps were generated. Please refer to Supplemental Digital Content 1 for further MRI acquisition detail.

Magnetic resonance image processing

The magnetic resonance (MR) images were transferred to Horos (The Horos Project, Horosproject.org). Two regions of interest were drawn on the ADC maps in the centrum semiovale and cerebellum in each dog. In addition, a region of interest was drawn on the ADC map in focal areas of abnormally low signal intensity. The ADC mean values and minimum values were recorded. A board-certified diagnostic radiologist (MC) read all the MRIs and was blinded to the clinical protocol and pathologic findings.

Tissue Analyses

Organs (eyes, brain, lungs, heart, liver, spleen, and kidneys) were harvested and representative sections were taken. Gross inspection was

TABLE 1. Angiography and Infusion Summary

Subject	Age	Weight	Infusion concentration/volume	Cell infusion duration; Location	Control injection duration; Location	Angiographic result	Procedure outcomes
Canine 1	10 mo 13 d	35 kg (77 lbs)	2×10^6 cells/20 mL	32 min; L ICA	17 min; R ICA	No angiographic occlusions	No complications
Canine 2	8 mo 6 d	27.5 kg (60.5 lbs)	1×10^7 cells/20 mL	40 min 50 s; L ICA	15 min 28 s; R ICA	No angiographic occlusions	No complications
Canine 3	9 mo 22 d	28.3 kg (61.8 lbs)	2×10^7 cells/20 mL	22 min 21 s; L ICA	20 min 34 s; R ICA	No angiographic occlusions	No complications
Canine 4	10 mo 26 d	28.8 kg (63.5 lbs)	1×10^8 cells/20 mL	21 min; R ICA	17 min; L ICA	No angiographic occlusions	No complications
Canine 5	8 mo 22 d	33.1 kg (72.9 lbs)	1×10^8 cells/20 mL	21 min 34 s; L PCA	18 min; R PCA	No angiographic occlusions	Technical difficulty in PCA access
Canine 6	9 mo 14 d	32.7 kg (72.1 lbs)	1×10^8 cells/10 mL	11 min 26 s; L PCA	10 min 12 s; R PCA	No angiographic occlusions	Basal cistern/perivascular SAH, no stroke or other complications
Canine 7	8 mo 15 d	27.6 kg (60.8 lbs)	Lumi Particles; Positive control #1	R PCA	L PCA no injection	Slowed flow in R PCA without occlusion	Basal cistern/perivascular SAH, no stroke or other complications
Canine 8	9 mo 27 d	33.6 kg (74.1 lbs)	Lumi Particles; Positive control #2	R ICA	No injection	Complete occlusion of R ICA, stagnant LICA flow	Numerous particles found in meningeal vessels and brain on both hemispheres.
Canine 9	8 mo 20 d	26.0 kg (57.3 lbs)	1×10^8 cells/10 mL Survival #1	R ICA	L ICA	No angiographic occlusions	No complications
Canine 10	9 mo 2 d	29.3 kg (64.6 lbs)	1×10^8 cells/10 mL Survival #2	L ICA	R ICA	No angiographic occlusions	No complications

Angiographic result is summarized in comparison to baseline angiography prior to infusion. Technical notes or complications are also described. Full procedural reports are available in Supplemental data. For all ICA injections, a Synchro2 microwire (0.014", Stryker Neurovascular) over an Echelon 14 microcatheter was used. PCA injections were more technically challenging. When inaccessible with the Synchro2/Echelon 14 system, a Marathon microcatheter (Medtronic) over a Mirage microwire (.008", Medtronic) was used.

performed to assess for large vessel injury or organ injury. Histological analysis was performed for any signs of end-organ damage. A board-certified veterinary pathologist performed all necropsy and histological analyses. GFP was used to localize the GFP-labeled BM-hMSCs in the tissues and brain. Histological sections (N = 10/organ) were analyzed by fluorescent microscopy to identify hMSCs-D24 in brain and peripheral organs.

Statistical Analysis

Statistical analysis was performed with GraphPad Prism (GraphPad Software, San Diego, California). Standard deviation is given for means where appropriate.

RESULTS

Subjects and Procedural Outcome

Ten adult canines were included in this study. Infusion was achieved in all 10 (**Supplemental Digital Content 1**). The ICAs were catheterized in 7 canines and the PCAs were accessed in three. The procedural results and animal subject details are summarized in Table 1. Microcatheter access and selective angiography of the anterior and posterior circulation are shown in Figure 1.

Infusion Doses and Angiographic Outcomes After Infusions

In 8 animals, hMSCs-D24 was infused in one hemisphere and saline (negative control) was infused into the opposite hemisphere (Table 1). Specifically, in each animal hMSC-D24 was infused over 20 to 30 min using a handheld technique. The cell dose was increased with each subsequent animal starting at 2×10^6 cells in 20 mL up to 1×10^8 cells in 20 mL (Table 1). This was then concentrated to 1×10^8 cells in 10 mL. No strokes were found on baseline angiography. Post-infusion angiography showed no evidence of embolic strokes after infusion of hMSC-D24 at all dose levels or after infusion of saline into the ICA (N = 5) or PCA (N = 3).

MRI Outcomes

Postprocedure MRI was performed in all subjects to assess for intracerebral complications, particularly ischemic stroke (Table 2, **Supplemental Digital Content 3**). Quantitative

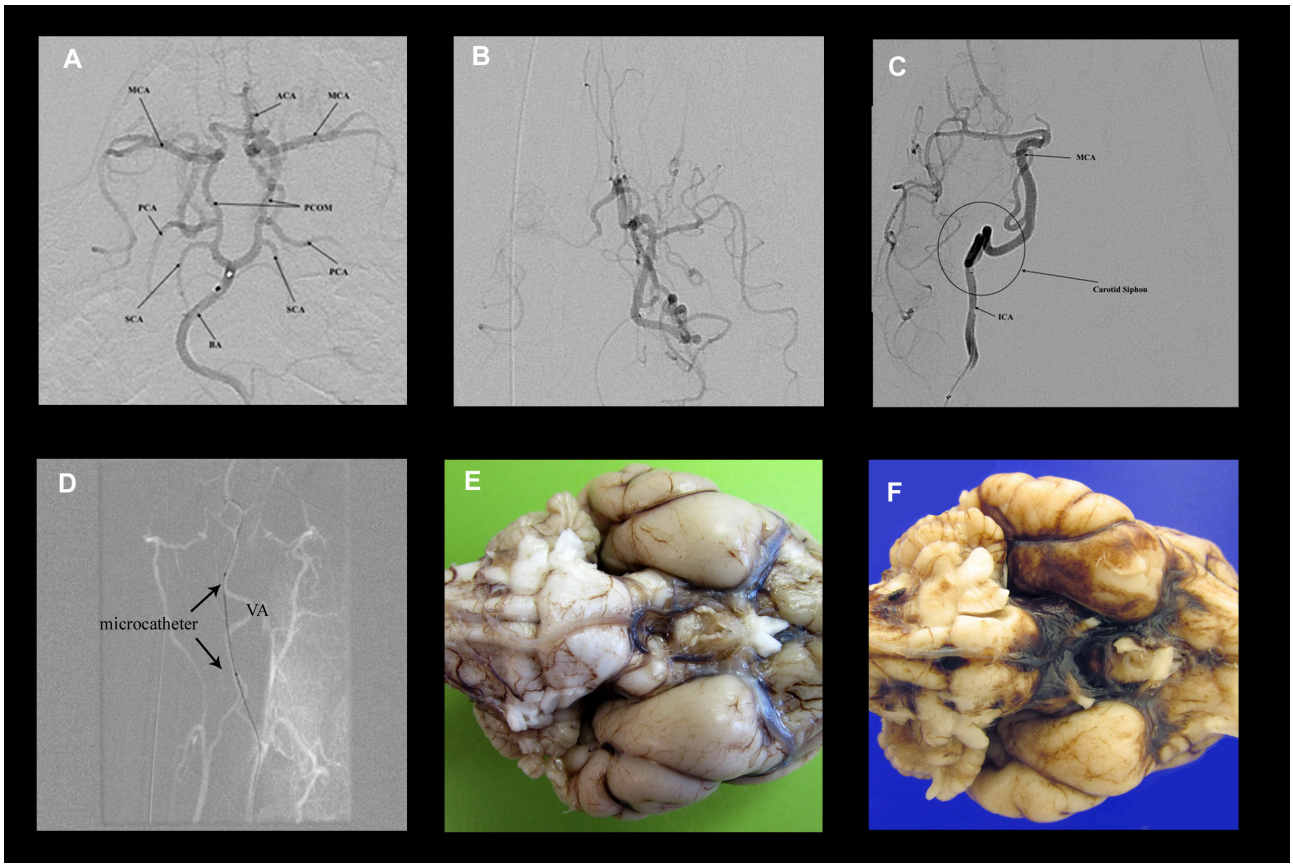

FIGURE 1. *Angiography in canine subjects.* **A**, *Diagnostic cerebral angiogram (DSA) of the circle of Willis in the canine. The microcatheter is positioned near the basilar apex (BA). Both the anterior circulation (anterior cerebral artery (ACA), middle cerebral artery (MCA)) and posterior circulation (posterior communicating artery (PCOM), posterior cerebral artery (PCA), and superior cerebellar arteries (SCAs)) are visible.* **B**, *DSA of the posterior circulation with the microcatheter positioned in the P1 PCA. Via the PCOM, the anterior circulation is partially opacified along with the PCA. This demonstrates that the canine has a posterior circulation dominance compared the anterior circulation.* **C**, *DSA of the anterior circulation with the microcatheter positioned in the proximal ICA at the bottom of the panel. The carotid siphon is very tortuous, precluding further selective access.* **D**, *microcatheter access via the vertebral artery, roadmap view. The microcatheter can be seen outside of the roadmap of the artery. This demonstrates that the vessel is highly mobile and being easily straightened by a flexible, soft microcatheter. It is likely this manipulation that led to vessel injury in posterior circulation subjects.* **E**, *Gross pathology looking at the ventral brainstem and cerebrum in subject 1, with no procedural complications.* **F**, *Same view in subject 6 demonstrating diffuse basal cistern SAH.*

analysis was based on previous canine MRI studies.[14,15] The normal mean ADC values have been determined to be above 700 $\mu m^2/s$ (Table 3).

Embolic Particle-Infusion (Positive Control)

To test the sensitivity of our assays for their ability to detect stroke, Lumi radio-opaque embolic particles were infused (subject 7 in the PCA and subject 8 in the ICA) and demonstrated angiographic occlusion, radiographic stroke, and pathologic occlusion (Figure 2).

MSC-D24-Infusion

No strokes were observed in the anterior circulation infusions with hMSCs-D24 (n = 6). MRI was normal at doses up to 1×10^8 cells/10 mL (Figure 3), including in both survival experiments (Table 2, Figure 4). However, in 2 canines with infusions through the posterior circulation, ischemia in and out of the territory of MSC infusion was noted.

Saline-Infusion

Normal saline infusion was performed in each of the 8 MSC-D24-infused subjects in the contralateral hemisphere as a control for the baseline risk of ischemic stroke from the access and infusion. ADC analysis confirmed ischemic stroke in 1 of 8 hemispheres infused (12.5%) with saline (Figure 4). In this subject, the MSC-infused hemisphere did not demonstrate any complications.

TABLE 2. MRI Results

Subject	Cell concentration	Cell infusion location/duration	Saline infusion location/duration	MR findings	MRI notes
Canine 1	2×10^6 cells/20 mL	32 min 0 s L ICA	17 min 0 s; R ICA	normal	
Canine 2	1×10^7 cells/20 mL	40 min 50 s; L ICA	15 min 28 s; R ICA	left thalamic/left brainstem infarct	Infarct uncorrelated with cell infusion; technical issue, related to access
Canine 3	2×10^7 cells/20 mL	22 min 21 s; L ICA	20 min 34 s; R ICA	normal	
Canine 4	1×10^8 cells/20 mL	21 min 0 s; R ICA	17 min 0 s; L ICA	normal	
Canine 5	1×10^8 cells/20 mL	21 min 34 s; L PCA	18 min 0 s; R PCA	left cerebellar infarct, IVH	Left PCA cell infusion, likely access-related stroke
Canine 6	1×10^8 cells/10 mL	11 min 26 s; L PCA	10 min 12 s; R PCA	diffuse stroke, IVH	Left PCA cell infusion, likely access-related stroke
Canine 7	Positive stroke control	R PCA particles Positive control #1	no injection	diffuse stroke, IVH, SAH	Right PCA PVA particle embolization, but likely access-related stroke
Canine 8	Positive stroke control	R ICA particles Positive control #2	no injection	diffuse stroke	Right ICA Lumi particle embolization; penetrated bilaterally
Canine 9	1×10^8 cells/10 mL	R ICA	L ICA	normal	Survival
Canine 10	1×10^8 cells/10 mL	L ICA	R ICA	normal	Survival

The stroke in subject 2 was in the contralateral posterior circulation territory relative to the cell infusion; thus it was determined (independently by the reading radiologist) that it was likely related to access rather than cell infusion. The remaining MRI-detected complications occurred in posterior circulation access cases. Quantitative analysis of MRI results is shown in Table 3. MRI was found to be sensitive to hemorrhagic and ischemic complications in the 2 positive control subjects (7 and 8), which were accessed via the right PCA and ICA, respectively. The PCA infusion subject had embolic particles in the vessel infused and had diffuse ischemic stroke, but also intraventricular hemorrhage (IVH) and SAH, which would be attributable to microcatheterization of the posterior circulation rather than the particles themselves. Subject 8 had a more characteristic global ischemic caused from right ICA infusion of embolic particles. This confirmed the sensitivity of MRI to detect hemorrhagic as well as ischemic complications.

Pathology–Gross, Histology, and Fluorescence

Gross pathology and histological analyses of representative sections were performed on the brain, eyes, liver, kidneys, lungs, and liver of all cell infusion subjects (**Supplemental Digital Content 4**).

Brain

Cerebral complications were noted by pathology in subjects 6 and 7, which were both accessed via the posterior circulation. Subarachnoid hemorrhage (SAH) around the basilar artery and caudal brain stem was seen, likely as a result of traumatic microcatheterization (Figure 1). In the remaining 6 animals that received hMSC-D24 into the anterior circulation, there was no gross or histological evidence of strokes on necropsy (Table 4, Figure 5). GFP-labeled hMSC-D24 cells were not identified in histological analysis in any cerebral sections (in either hemisphere).

In the positive control animals (subjects 7 and 8), H&E histology demonstrated the expected intravascular sequestration and occlusion by the embolic particles. No histological changes associated with ischemia were seen, which would not be expected in the short time window following stroke (1-2 h). This confirms MRI/ADC analysis to be the more sensitive assay.

Peripheral Organs

Peripheral organs were removed in all animals; gross inspection revealed no abnormalities. Analyses by standard light microscopy in the eight animals infused with hMSC-D24 did not detect any pathological findings (Figure 6). Importantly, all sections were analyzed by fluorescent microscopy to detect GFP-labeled MSC-D24, and none were found.

Survival Experiments

Two dogs were recovered after the procedure with reversal of anesthesia and were assessed by clinical exams for 24 h. There were no immediate or delayed postprocedural clinical signs in both animals. Both subjects had normal postprocedural vital signs, neurological exam (assessed by the canine stroke scale), and general systemic assessment.

DISCUSSION

We present here a translational study of ESIA infusion of hMSC-D24 in a nontumor-bearing canines. All procedural aspects of the study were designed to recapitulate the clinical setting of a human Phase 1 dose-escalation trial. We show for the first time that GMP-grade hMSC-D24 can be safely infused in the anterior circulation up to doses of 10^7 cells/ml with

TABLE 3. Quantitative MRI Analysis

Subject	Supratentorial		Infratentorial		Lesions (Location, ADC mean/min)
	ADC mean	ADC min	ADC mean	ADC min	
Canine 1	768.9	628	816	718	
Canine 2	804	630	894	684	L Thalamus (596/564) L Brainstem (555/339)
Canine 3	764	710	821	789	
Canine 4	848	685	746	614	
Canine 5	868	694	878	709	L Cerebellum (485/276) L Medial Temporal (593/364) R Parietal (853/186) L Parietal (710/403)
Canine 6	519	472	452	371	Global ischemia
Canine 7	492	360	503	342	Global ischemia
Canine 8	469	397	533	375	Global ischemia
Canine 9	756	682	702	591	
Canine 10	823	676	827	691	

ADC map was used to assess for ischemia as described in the Methods section. Based on previous canine MRI studies the normal mean ADC values have been determined to be above 700 $\mu m^2/s$.

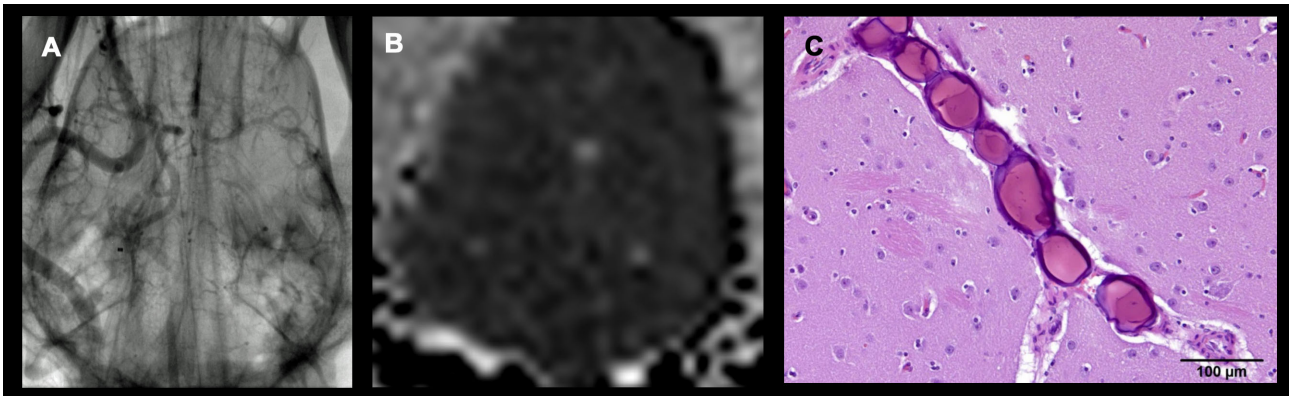

FIGURE 2. *Multimodality assessment of toxicity. Toxicity of the cell infusion (stroke) was assessed by angiography, MRI, and necropsy. These are shown in the positive control subjects 7 and 8.* **A**, *Angiography demonstrating contrast stagnation and subtracted vascular cast of the brain caused by radio-opaque embolic particle injection. This demonstrates the expected appearance and detectability of large vessel stroke by angiography. The result was most dramatic in subject 8, shown here, with complete occlusion of the right, demonstrating our ability to identify angiographic occlusion in this animal model.* **B**, *MRI Brain, FLAIR sequence demonstrating diffuse sulcal effacement and gray-white matter blurring. The paired ADC sequence shows diffusely low ADC signal.* **C**, *In all sections of the cerebrum and midbrain examined there were numerous intravascular, spherical, laminated foreign bodies approximately 40-100 microns in diameter; these were seen especially along the middle cerebral artery and in small arterioles. While the vessels were occluded, histological development of stroke was not yet observed.*

commercially available microcatheters. These data support translation of this strategy in a human clinical trial.

Maximal Tested Dose

The initial dose started at 2×10^6 cells/20 mL and was escalated in each subsequent animal to 1×10^8 cells/20 mL (5×10^6 cells/ml). Once this dose was tolerated in two subjects, the cell solution was concentrated to 1×10^8 cells/10 mL (1×10^7/ml). Qualitatively, the character of the cell solution changed from clear/non-viscous at the low dose to viscous and cloudy, necessitating continuous agitation at 1×10^8 cells/10 mL. Despite this difference in composition, no dose-related ischemic

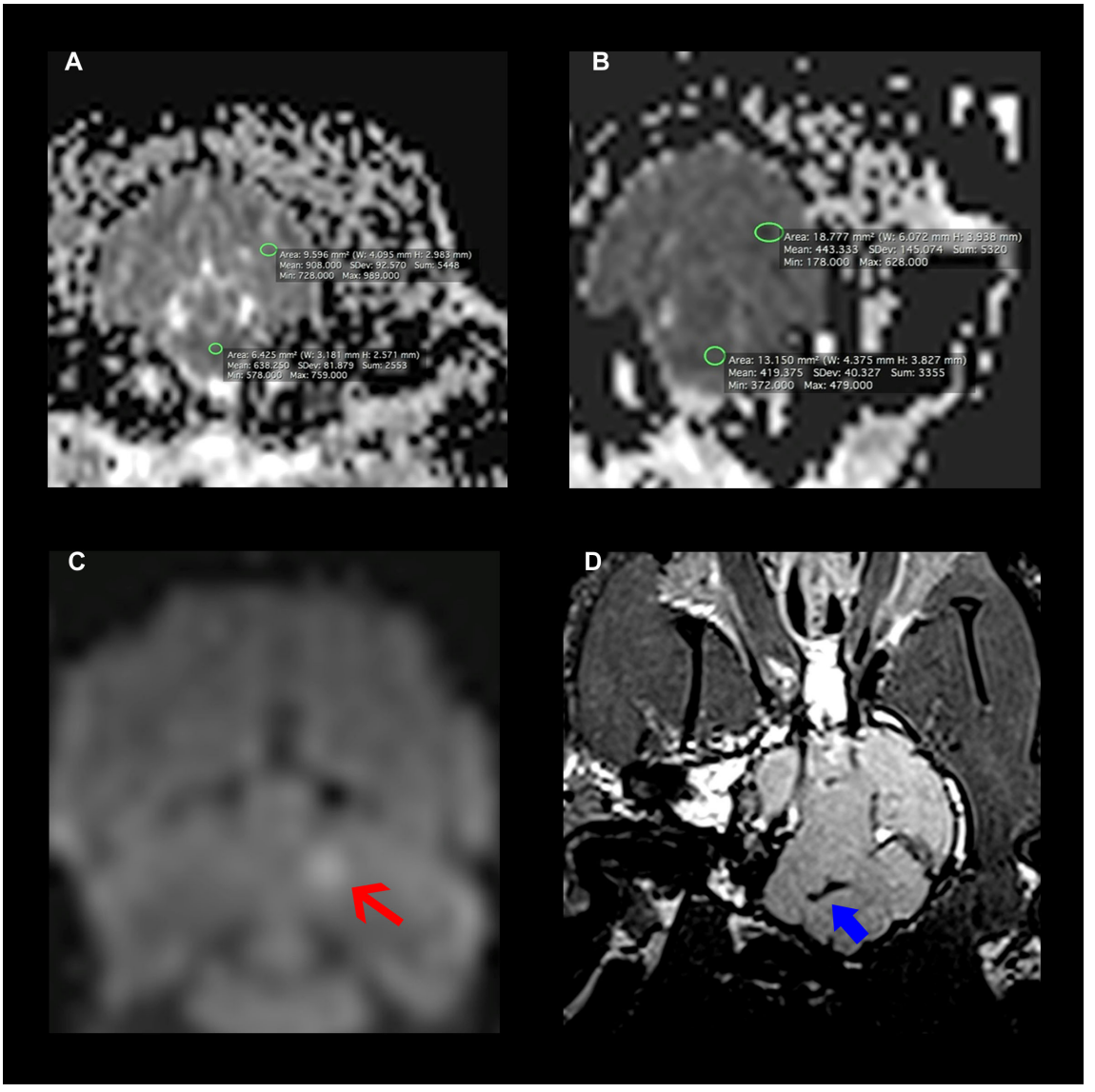

FIGURE 3. *Selected postprocedure brain MRIs.* **A**, *Subject 1, normal MRI ADC map. Circled are selected regions showing supratentorial and infratentorial minimum ADC values of 908 and 578 respectively.* **B**, *Subject 6, MRI ADC map demonstrating global ischemia. Circled are selected regions showing supratentorial and infratentorial minimum ADC values of 443 and 372 respectively.* **C**, *Subject 5, MRI diffusion weighted imaging (DWI) sequence demonstrating focal signal abnormality that had a corresponding low ADC signal, consistent with acute infarct in the left cerebellum (red arrow).* **D**, *Subject 6, MRI FLAIR sequence demonstrating low signal consistent with layering of blood (SAH) in the basal cisterns (blue thick arrow).*

complications were identified at any level based on post-infusion angiography, MRI or necropsy. All complications were due to endovascular access (discussed below). We did not reach an maximal tested dose (MTD), suggesting that higher doses may be possible, though due to the tendency for cells to precipitate out, further escalation was not performed. Based on mouse studies, this is expected to in the high therapeutic range.

Systemic Toxicity

A second aim of the experiment was to identify any systemic toxicity associated with ESIA infusion. The distribution of

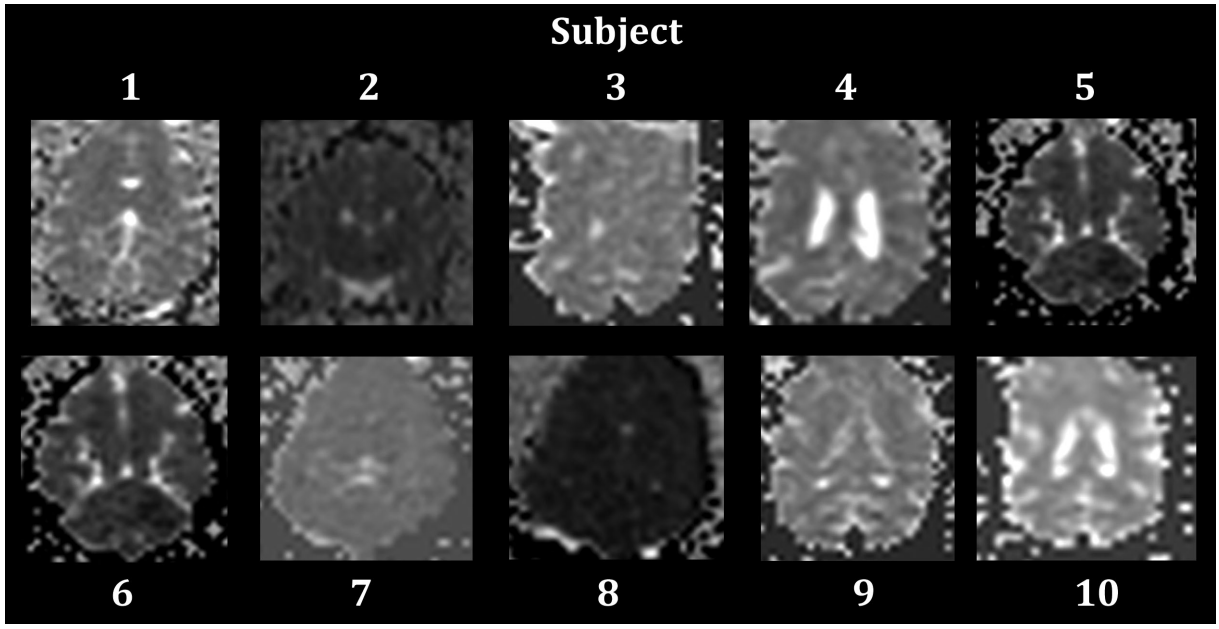

FIGURE 4. *Selected ADC slices from all 10 subjects. MRI results are summarized for all 10 in Table 2. Subject 2 had a left thalamic stroke thought to be a result of the control saline infusion through the left ICA, given the large posterior communicating arteries in the canine (Figure 1). Subject 5 had a left cerebellar stroke. Subject 6 had a diffuse stroke. Subjects 7 and 8, positive stroke controls in the right PCA and right ICA respectively, also had diffuse strokes.*

the agent in this experiment does not perfectly recapitulate the human scenario, as the nontumor bearing canine model does not have any tumor to attract the hMSCs-D24, and thus it would be expected for the cells to circulate through the cerebrovasculature and enter the heart to be systemically distributed. However, no systemic toxicities were identified in any of the eight canines who received infusions with hMSC-D24, including 2 survival subjects with clinical examinations. In previous experiments with intravenous infusions, MSCs have been found primarily in the lungs and secondarily in the liver and other organs.[16] Intraarterial infusions have been noted to avoid pulmonary entrapment compared with intravenous infusion.[17] Our findings are consistent with these reports. Specifically, fluorescent microscopy assessments of histological sections from systemic organs for distribution of ESIA-injected GFP-labeled MSCs did not identify GFP-labeled cells in any peripheral organs. In addition, GFP-labeled cells were not identified in the brains of any subjects. These results suggest that without a tumor to attract them,[7,18] hMSCs-D24 pass through the normal cerebrovasculature and are not sequestered into peripheral organs, including the lungs at the concentrations tested in these studies. How the cells are eliminated from the body and will require further studies using continuous, whole body, real-time tracking methods.

Technical Feasibility

One of the goals of the experiment was to determine technical feasibility of the procedure, including hMSC-D24 production, procedural logistics, endovascular access, and infusion. Cells were successfully produced by a GMP laboratory, transported in a sterile container, and temporarily stored on ice into the procedure room. They were thawed during the access phase and then drawn up at room temperature once the target vessel was catheterized. The solution was mixed frequently during the infusion phase to avoid precipitation of the cells.

Selective catheterizations of the distal ICA were feasible and safe. Although catheterization of the P2 was feasible, the access resulted in stroke and SAH. Compared with humans, the small caliber and laxity of the canine basilar artery made catheterization technically difficult. We suspect that the SAH observed was from avulsion of small perforators due to the arterial hypermobility during catheterization.

Unintentional hemorrhagic and ischemic stroke was observed in 2 animal subjects with PCA catheterization while none of the 6 animals with anterior circulation infusions had strokes in the infusion distribution. The complication rate of posterior circulation access in these canines is higher than would be expected or tolerated in humans. All neurointerventionalists were very experienced; all found the access to be quite challenging due to the

TABLE 4. Necropsy and Histology Results

Subject	Cell concentration	Cell infusion location	Saline infusion location	Gross pathology	Histology and GFP
Canine 1	2×10^6 cells/20 mL	L ICA	R ICA	No significant lesions observed	No significant lesions observed.
Canine 2	1×10^7 cells/20 mL	L ICA	R ICA	No significant lesions observed	Brain edema, meningeal, moderate, multifocal, acute. No difference observed between hemispheres. No significant lesions observed in peripheral organs.
Canine 3	2×10^7 cells/20 mL	L ICA	R ICA	No significant lesions observed	No significant lesions observed.
Canine 4	1×10^8 cells/20 mL	R ICA	L ICA	No significant lesions observed	No significant lesions observed.
Canine 5	1×10^8 cells/20 mL	L PCA	R PCA	No significant lesions observed	No evidence of stroke or vascular obstruction was observed in the brain. No significant lesions observed in peripheral organs.
Canine 6	1×10^8 cells/10 mL	L PCA	R PCA	Perivascular hemorrhage around distal BA	Hemorrhage, leptomeningeal, focally extensive and moderate, multifocal, acute. No evidence of stroke or vascular obstruction was observed in the brain. No significant lesions observed in peripheral organs.
Canine 7	Positive stroke control	R PCA particles	no infusion	Cisterna magna SAH; peri-medullary hemorrhage	Hemorrhage, leptomeningeal, regional (caudal brain stem), moderate, multifocal coalescing, acute. Intravascular foreign bodies, bilateral small arterioles – moderate number, acute. No evidence of stroke other than particle obstruction.
Canine 8	Positive stroke control	R ICA particles	no infusion	No significant lesions observed	Intracerebral intravascular beads, marked, diffuse, acute, also in the eye (choroid and optic nerve), and meninges. No morphologic evidence of ischemia was observed in the brain or eye.
Canine 9	1×10^8 cells/10 mL	R ICA	L ICA	No significant lesions observed	No morphologic indications of ischemia in the brain or other tissues are observed
Canine 10	1×10^8 cells/10 mL	L ICA	R ICA	No significant lesions observed	No morphologic indications of ischemia in the brain or other tissues are observed. Clump of large mononuclear round cells seen in a large meningeal artery.

Incidental findings (eg, hepatocellular lipidosis) that were independently determined to be unrelated are available in supplemental data reports. BA = basilar artery.

reasons above. Therefore, the dog basilar artery is not representative of the human clinical setting.

Limitations

This study is meant to be a feasibility/safety study to help define the MTD for ESIA infusion. The study has not directly assessed the efficacy of this therapy in a large animal model. Another limitation is the small sample size (limited due to cost and ethical concerns). The sensitivity of MRI in the canine may be limited by the small size of the canine cerebrum. Nevertheless, the data provide valuable information about the feasibility and safety of infusing hMSC-D24 into the cerebrovasculature of an animal whose circulation approaches that of humans in terms of size and pattern. Future study will be required in a tumor-bearing large animal model.

CONCLUSION

This first-of-its-kind study, simulating ESIA infusion of hMSCs-D24, demonstrates that hMSCs-D24 can be infused safely at least up to doses of 1×10^8 cells/10 mL. ESIA infusion is feasible in a large animal model with potential for translation to clinical practice. High-dose infusion does not result in dose-related toxicity. Complications, when encountered, were due to technical challenges inherent to the posterior circulation canine model. These findings support a clinical trial of ESIA infusion of hMSCs-D24.

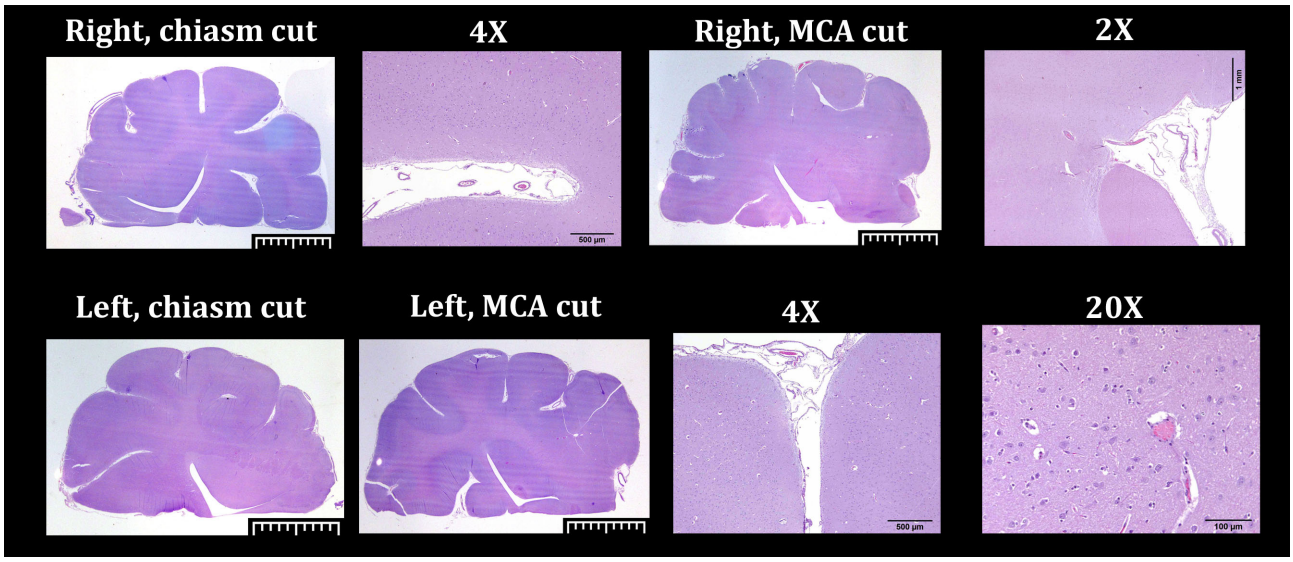

FIGURE 5. *Cerebral pathology. H&E slides of the canine cerebrum following Delta-24-MSC infusion. No histological evidence of stroke was noticed in subjects with anterior circulation infusions. Assessment of GFP-labeled cells was also performed (not shown).*

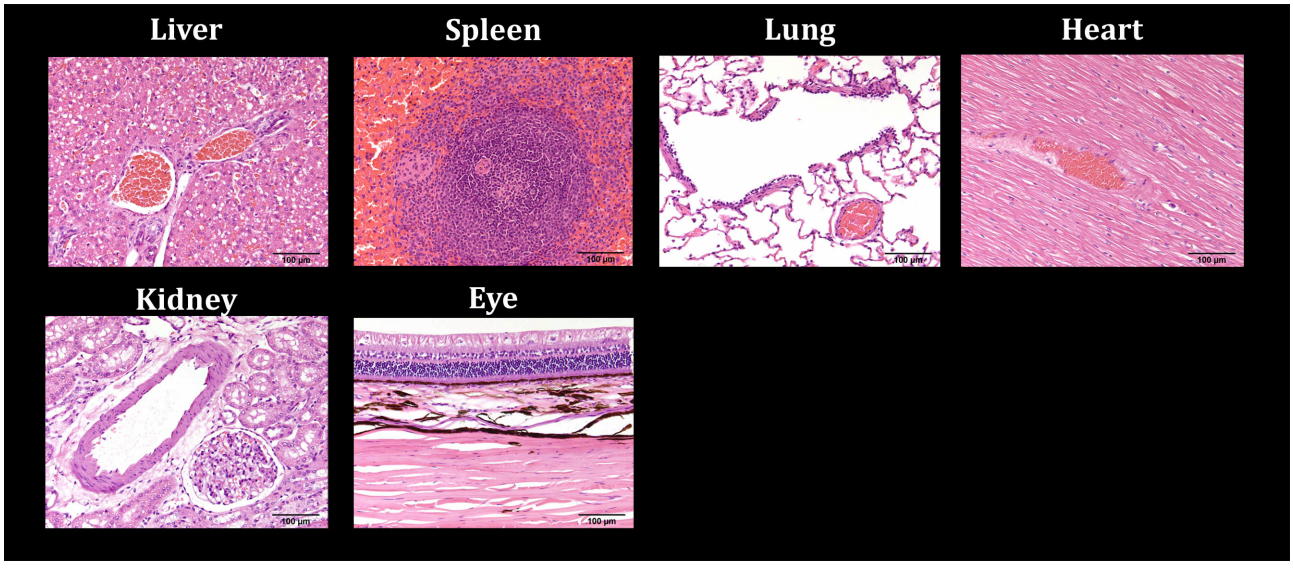

FIGURE 6. *Peripheral pathology. Representative sections were taken from each organ and stained with H&E. Delta-24-MSCs were not identified in any of the peripheral organs supplied. Further, histologic and gross damage was not seen in these organs. Specifically, there was including no evidence of inflammation, ischemia, vessel occlusion or hemorrhages in any of the eight animals infused with hMSC-D24. Embolic particles were identified in the eye in subject 8 (positive control). This confirms the angiographic observation of the ophthalmic artery supply from the ICA and represents a potential complication detectable by pathology.*

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Disclosures

Dr Lang reports being a patent holder with DNAtrix, who manufactures Delta-24-RGD. Dr Kan reports being a consultant for Stryker Neurovascular, Medtronic (manufacturer of microcatheters used here), and Cerenovus. Dr Kan is a stockholder in Vena Medical, Inc. The other authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article.

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Supplemental digital content *is available for this article at www.neurosurgery-online.com.*

Supplemental Digital Content 1. Methods. Details of animal procedure, MSC production, and MRI protocol.
Supplemental Digital Content 2. Procedure Reports. Detailed procedure report of each of the 10 surgical/endovascular procedures.
Supplemental Digital Content 3. Final MRI Reports. Detailed MRI reports read by board-certified neuroradiologist.
Supplemental Digital Content 4. Canine Path Reports. Gross and histologic pathology reports for the 10 animal subjects, including brain and peripheral organ assessment.

COMMENTS

The use of BM-hMSC loaded with Delta-24 oncolytic adenovirus has been previously shown to be efficacious in murine studies, able to infiltrate gliomas following IA delivery, eradicate GBM, and prolong survival. The authors present a novel, proof-of-concept safety and feasibility study assessing the use of endovascular selective intra-arterial (ESIA) delivery of mesenchymal stem cells loaded with Delta-24 in a canine model. While IA delivery of hMSCs has been shown in both canines and human clinical trials previously, this study is the first of its kind to assess the safety and feasibility of ESIA delivery of hMSCs loaded with an oncolytic virus that specifically targets GBM. Given the significant morbidity and poor improvement in median survival in GBM to date, this presents a promising emerging adjunctive treatment modality for this disease process.

Despite low numbers in the present study, the authors effectively demonstrates the safety and feasibility of ESIA in a large animal model. Recent advancements in IA delivery of chemotherapy and endovascular delivery of neuromodulation leads and strent-electrodes have identified new minimally invasive mechanisms for the treatment of a variety of neuropathologies. ESIA delivery of hMSCs provides an exciting new treatment modality, able to deliver high concentrations of therapeutics directly to the target tissue, thereby limiting systemic side effects and improving local efficacy. This initial translational study shows promise in anticipation of later clinical applications of this treatment paradigm.

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The authors present a study investigating the safety and feasibility of delivering human bone-marrow derived mesenchymal stem cells loaded with the oncolytic adenovirus, Delta-24-RGD intraarterially by selective endovascular catheterization. Delta-24's oncolytic potential via intratumoral injection has been previously shown, and the authors are seeking a delivery method that better covers the extent of the tumor while limiting systemic adverse effects. They were able to demonstrate the technical feasibility of intraarterial delivery of hMSC-D24 without systemic effect evidenced by the lack of hMSC in other tissues or histological signs of organ toxicity. In combination with prior referenced

studies demonstrating the ability of BM-hMSC loaded with Delta-24 to deliver the virus into human glioma xenografts along with a therapeutic benefit, this study provides sufficient evidence to support a clinical trial.

While they did experience complications with posterior circulation catheterization, they attribute this to the canine anatomy, and it is reasobable to expect that the technical aspects of the endovascular procedure can be performed safely in humans. Given the lack of effective treatments for glioblastoma, short expected survival and lack of a better model to confirm these views, proceeding with future study is warranted as this represents a potentially exciting avenue for glioblastoma treatment.

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