

# Preclinical Safety Validation of a Stabilized Viral Vector Direct Injection Approach to the Cervical Spinal Cord

Thais Federici, Ph.D.<sup>1</sup>, Jonathan Riley, B.S.E.<sup>2</sup>, John Park, M.D.<sup>3</sup>, Mark Bain, M.D.<sup>3</sup>, and Nicholas M. Boulis, M.D.<sup>1</sup>

## Abstract

The current lack of a validated intraspinal delivery approach precludes translation of promising cell or viral-based therapeutics for treatment of varied spinal cord afflictions. We have developed a stabilized cervical microinjection platform with the intent of precise delivery to intraspinal sites of interest. Nine 30–40 kg female swine underwent coordinate-based microinjection AAV2-GFP at three injected volumes (10, 25, and 50  $\mu\text{L}$  ( $n = 3/\text{group}$ )) and matched infusion rates (1.0, 2.5, and 5.0  $\mu\text{L}/\text{min}$ ) over a period ( $t = 10$  minutes). Preliminary validation is provided by behavioral and targeting data demonstrating safe delivery of a viral vector carrying a fluorescent reporter gene to the cervical spinal cord ventral horn.

**Keywords:** targeted delivery, gene therapy, cervical spinal cord, adeno-associated viral vector, pig

## Introduction

Afflictions of the spinal cord, whether neurodegenerative, oncologic, or traumatic, pose unique and significant challenges towards the end of achieving effective therapeutic intervention. Specific pathologic entities, including amyotrophic lateral sclerosis (ALS)<sup>1,2</sup> or spinal muscular atrophy (SMA),<sup>3</sup> intramedullary cord tumors,<sup>4</sup> and spinal cord injury (SCI)<sup>5,6</sup> may be amenable to cell or viral-based therapeutic approaches tailored to address the fundamental dysfunction within the local neuronal microenvironment. One of the most significant barriers towards clinical translation and assessment of these molecular therapeutic strategies in Phase 1 clinical trials remains validation of a safe, accurate therapeutics delivery approach to the spinal cord gray matter. To our knowledge, the closest related studies have examined either convection enhanced delivery of macromolecules to the adjacent white matter<sup>7</sup> and of multiple substrates to the heterogeneous pontine region.<sup>8,9</sup> We have previously published preliminary data demonstrating our ability to deliver cellular grafts to the lumbar<sup>10</sup> or cervical<sup>11</sup> ventral horn utilizing a prototype stabilized microinjection platform. Herein, we extend our prior experiences to a gene therapy delivery protocol (AAV2-GFP) characterized by a ( $t = 3$  weeks) survival period.

## Methods

### Surgical procedure

We have described the surgical procedure in detail.<sup>10,11</sup> Briefly, the surgical field was prepared in the standard sterile fashion. A 22 cm midline incision was performed between the occiput and the C7 spinous process. An avascular midline dissection of the posterior spinal elements was then performed followed by a cervical laminectomy at the C3–C4 levels. The dura was incised with a No. 15 blade scalpel and a pial opening created with microbipolar coagulation followed by use of a No. 11 blade to assure hemostasis. *Figure 1A* demonstrates the microinjection platform fabricated by the Cleveland Clinic Prototype Laboratory. This was used in combination with a Narishige M0-97 hydraulic microdrive (East Meadow, NY), capable of submillimetric depth adjustment. *Figure 1B* demonstrates an *in vivo* view of the platform and microdrive. The microinjection cannula (0.015" OD, 0.011"

ID; 27.5 Gauge) was inserted to a depth of 4 mm, withdrawn to a depth of 3.5 mm, and adeno-associated viral vector serotype 2 (AAV2-GFP) delivery (4e12 vg/mL, Ceregene, Inc., San Diego, CA) commenced with aid of a Harvard p99 (Harvard Apparatus Inc., Boston, MA) programmable infusion pump. *Table 1* introduces the study design. Briefly, microinjection occurred over a range of matched volumes (10, 25, and 50  $\mu\text{L}$  ( $n = 3/\text{group}$ )) and infusion rates (1.0, 2.5, and 5.0  $\mu\text{L}/\text{minute}$ ), respectively. A constant intraspinal residence time was present between groups, including 10 minutes for delivery and an additional 5 minutes to prevent reflux along the cannula track. The dura was closed in a running watertight fashion with 4-0 Nurulon<sup>®</sup>. A four-layer closure included deep musculature (0 Vicryl<sup>®</sup>), fascia (0 Vicryl<sup>®</sup>), subdermal (2-0 Vicryl<sup>®</sup>), and skin (3-0 Ethilon<sup>®</sup>).

### Behavioral assessment

A modification of the Tarlov scale, used to assess a combination of motor function and ambulation status in small animal studies, was adapted for this study. *Table 2* demonstrates the modified Tarlov scoring system. Animals were assessed preoperatively, followed by serial postoperative scoring at Days 3, 14, and prior to euthanasia at Day 21.

### Histological analysis

Following euthanasia, spinal cords were excised between the C2 and C5 levels and placed in 4% paraformaldehyde for a period of 7 days. The tissue specimens were dehydrated in a 30% sucrose solution for 1–2 days and subsequently frozen for histological analysis. Injection site identification was aided by placement of a blue 6-0 Prolene stitch in the dura overlying the microinjection site. A cryostat (Jung Frigocut 2800N, Leica Microsystems, Inc., Bannockburn, IL) was used to attain 30  $\mu\text{m}$  thickness sections for analysis. Specimens were left unstained for GFP visualization.

## Results

### Behavioral outcomes

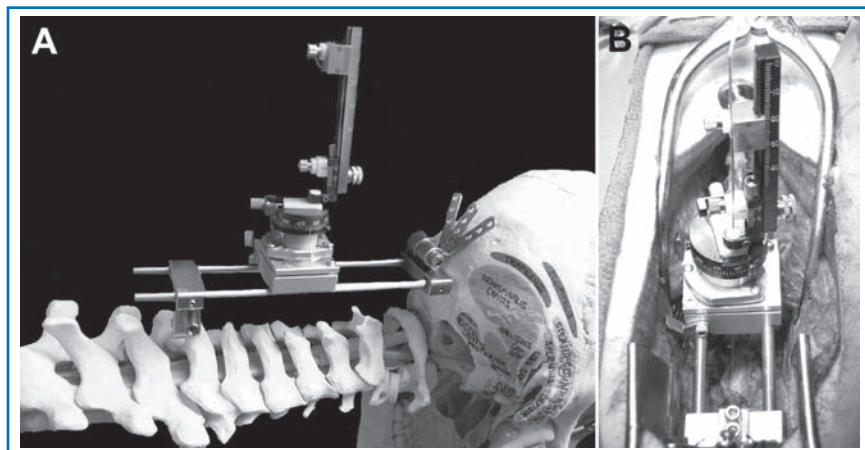
At preoperative baseline, each animal retained an intact modified Tarlov score. As demonstrated in *Figure 2*, eight of nine swine

<sup>1</sup>Emory University School of Medicine, 1365B Clifton Rd. NE, Suite B6200, Atlanta, GA 30322, USA; <sup>2</sup>Cleveland Clinic, The Lerner College of Medicine of Case Western Reserve University; <sup>3</sup>Cleveland Clinic, Neuroscience Institute, 9500 Euclid Avenue, Cleveland, OH 44195, USA.

Correspondence: Nicholas M Boulis (nboulis@emory.edu)

\*These authors contributed equally.

DOI: 10.1111/j.1752-8062.2008.00084.x



**Figure 1. Stabilized cervical intraspinal microinjection platform, *in situ* and *in vivo*.** (A) The microinjection platform is demonstrated while attached rostrally to the occiput and caudally to the spinous process of C7. (B) The platform and microdrive are demonstrated following a C3/4 laminectomy and dural opening prior to unilateral microinjection into the porcine cervical spinal cord.

recovered to baseline status by POD3 while one animal demonstrated continued improvement with return to baseline function by POD21. During necropsy, a resolving epidural hematoma was discovered. On POD 15, one Group 2 animal was found deceased in its cage following a rapid, progressive decline. A rigid, distended abdomen was observed. Necropsy revealed intestinal volvulus. Neither the observed morbidity nor the mortality was attributable to cannulation of the spinal cord or microinjection.

**Ventral horn targeting**

Ventral horn targeting and GFP transgene expression were achieved in each microinjection group. Though quantification of vector biodistribution was not the primary study endpoint, *Figure 3* demonstrates a “dose-dependent” and “targeted” GFP expression between groups. Observance of increasing GFP expression with microinjection volume and rate serves as evidence for a lack of payload reflux along the cannula track. Multilevel injection may further augment vector biodistribution along the neuraxis.

**Discussion**

The data presented above extend previous findings from our laboratory demonstrating an ability to safely cannulate a specific intraspinal site of interest for therapeutics delivery. *Figure 2* demonstrates a return to baseline function by POD3 in eight of nine animals, with all animals eventually achieving a return to neurologic baseline. Neither the episode of forelimb dysfunction nor the mortality was attributable to intraspinal cannulation, infusion, or viral delivery. *Figure 3* demonstrates accurate transgene delivery to the ventral horn, irrespective of infusion volume. Ventral horn targeting,

localized gene distribution, lack of tissue damage at the injection site, and a lack of association between behavioral outcomes and infusion parameters over a range of volumes and infusion rates were noted.

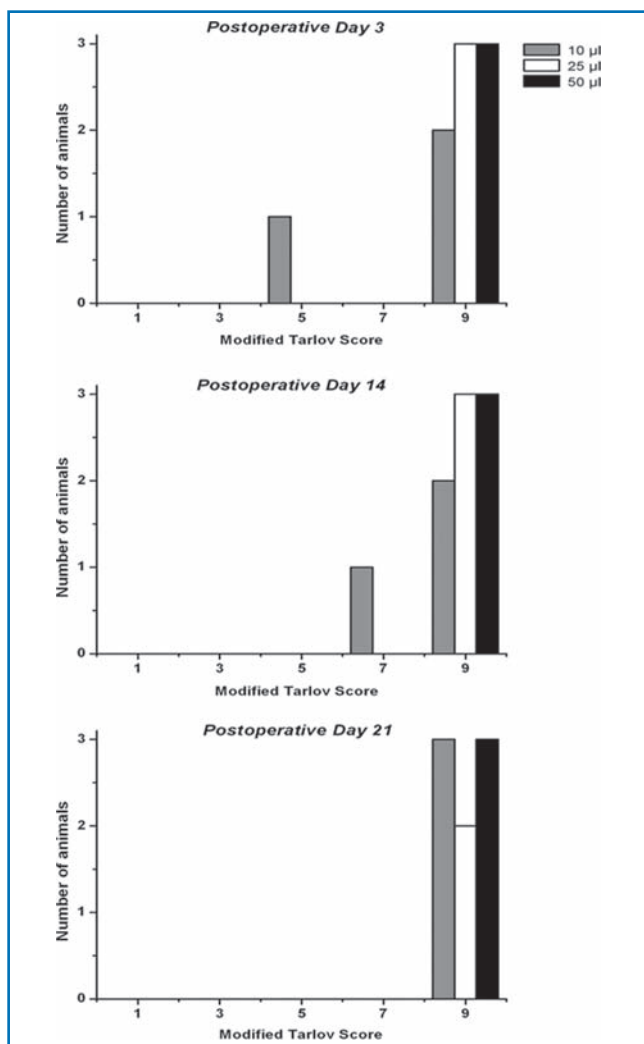
Our prior studies have utilized both stabilized lumbar<sup>10</sup> and cervical<sup>11</sup> approaches to deliver cell-based grafts to the ventral horn, utilizing microelectrode recording and coordinate-based microinjection targeting strategies, respectively. To our knowledge, these efforts represented the first reported attempts to cannulate and deliver a viral vector to a specific target within the spinal cord of a large animal, for the purpose of clinical translation. We extend the potential generalizability for the safety of stabilized microinjection to delivery of viral vectors engineered to achieve therapeutic effect.

| Swine number               |     | Injection volume (µl) |    |    |
|----------------------------|-----|-----------------------|----|----|
|                            |     | 10                    | 25 | 50 |
| Injection Rate (µL/minute) | 1   | Group 1 (n = 3)       |    |    |
|                            | 2.5 | Group 2 (n = 3)       |    |    |
|                            | 5   | Group 3 (n = 3)       |    |    |

**Table 1.** Microinjection group study design.

|   |  |
|---|--|
| 0 | No voluntary movements   |
| 1 | Perceptible movements at joints                                  |
| 2 | Good movements at joints but inability to stand                  |
| 3 | Ability to get up and stand with assistance <1 minute            |
| 4 | Ability to get up with assistance and stand unassisted <1 minute |
| 5 | Ability to get up with assistance and stand unassisted >1 minute |
| 6 | Ability to get up and stand unassisted >1 minute                 |
| 7 | Ability to walk <1 minute  |
| 8 | Ability to walk >1 minute  |
| 9 | Full recovery and normal walking                                 |

**Table 2.** Modified Tarlov scoring system.



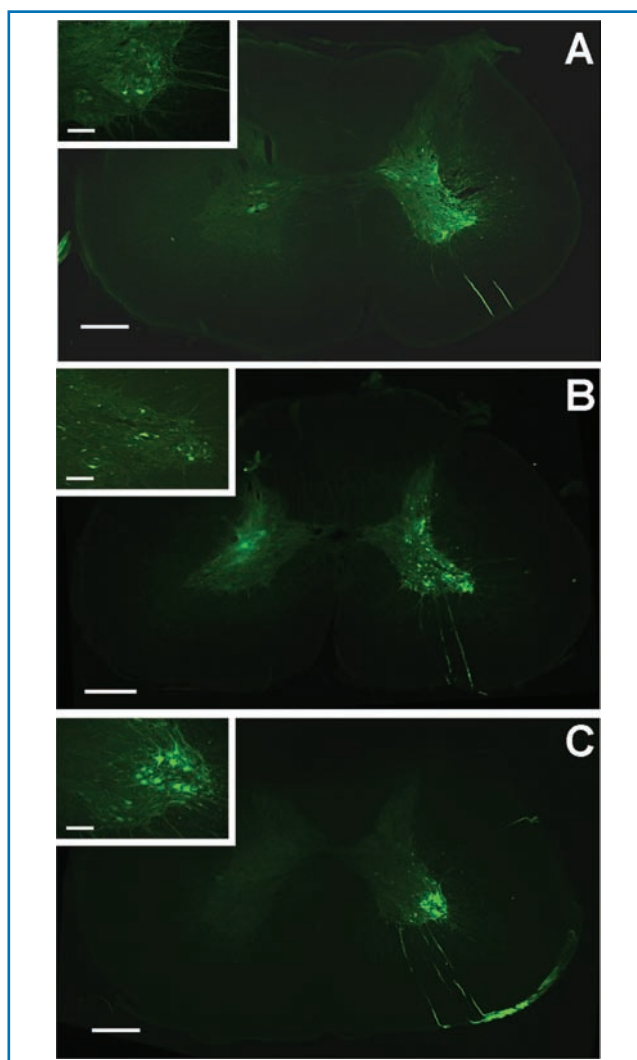
**Figure 2. Postmicroinjection behavioral outcomes.** **Top panel:** A maximal Tarlov score was achieved in all but one animal at postoperative Day 3. The affected swine was in Group 1 (10 µL injection group). **Middle panel:** By Day 14, behavioral improvement was noted in this animal. **Bottom panel:** At euthanasia, the affected swine from Group 1 had recovered to preoperative baseline. On Day 15, one animal from Group 2 was found deceased in its cage following a rapidly deteriorating course. Necropsy demonstrated the presence of intestinal volvulus.

## Acknowledgments

We would like to thank Shujun Zhou for her contribution to this work and Ceregene, Inc. for providing the viral vector. This research was supported by the ALS Association.

## References

- Dodge JC, Haidet AM, Yang W, Passini MA, Hester M, Clarke J, Roskelley EM, Treleaven CM, Rizo L, Martin H, Kim SH, Kaspar R, Taksir TV, Griffiths DA, Cheng SH, Shihabuddin LS, Kaspar BK. Delivery of AAV-IGF-1 to the CNS extends survival in ALS mice through modification of aberrant glial cell activity. *Mol Ther*. 2008; 16(6): 1056–1064.
- Kaspar BK, Llado J, Sherkat N, Rothstein JD, Gage FH. Retrograde viral delivery of IGF-1 prolongs survival in a mouse ALS model. *Science*. 2003; 301(5634): 839–842.
- Corti S, Nizzardo M, Nardini M, Donadoni C, Salani S, Ronchi D, Saladino F, Bordoni A, Fortunato F, Del Bo R, Papadimitriou D, Locatelli F, Menozzi G, Strazzer S, Bresolin N, Comi GP. Neural stem cell transplantation can ameliorate the phenotype of a mouse model of spinal muscular atrophy. *J Clin Invest*. 2008; 118(10): 3316–3330.
- Colak A, Goodman JC, Chen SH, Woo SL, Grossman RG, Shine HD. Adenovirus-mediated gene therapy for experimental spinal cord tumors: Tumoricidal efficacy and functional outcome. *Brain Res*. 1995; 691(1-2): 76–82.



**Figure 3. AAV-GFP ventral horn expression.** Specimens from each group demonstrate GFP expression 3 weeks after injections. Accuracy of the injection and a dose-dependent increase in fluorescence can be observed. **(A)** Group 1–10 µL, 1.0 µL/minute; **(B)** Group 2–25 µL, 2.5 µL/minute; **(C)** Group 3–50 µL, 5.0 µL/minute. Scale bars: 500 µm **(A)–(C)**; 200 µm **(A)–(C)** inset.

- Pallini R, Vitiani LR, Bez A, Casalbore P, Facchiano F, Di Giorgi Gerevini V, Falchetti ML, Fernandez E, Maira G, Peschle C, Parati E. Homologous transplantation of neural stem cells to the injured spinal cord of mice. *Neurosurgery*. 2005; 57(5): 1014–1025.
- Deshpande DM, Kim YS, Martinez T, Carmen J, Dike S, Shats I, Rubin LL, Drummond J, Krishnan C, Hoke A, Maragakis N, Shefner J, Rothstein JD, Kerr DA. Recovery from paralysis in adult rats using embryonic stem cells. *Ann Neurol*. 2006; 60(1): 32–44.
- Lonser RR, Gogate N, Morrison PF, Wood JD, Oldfield EH. Direct convective delivery of macromolecules to the spinal cord. *J Neurosurg*. 1998; 89(4): 616–622.
- Lonser RR, Walbridge S, Garmestani K, Butman JA, Walters HA, Vortmeyer AO, Morrison PF, Brechtel MW, Oldfield EH. Successful and safe perfusion of the primate brainstem: In vivo magnetic resonance imaging of macromolecular distribution during infusion. *J Neurosurg*. 2002; 97(4): 905–913.
- Lonser RR, Warren KE, Butman JA, Quezado Z, Robison RA, Walbridge S, Schiffman R, Merrill M, Walker ML, Park DM, Croteau D, Brady RO, Oldfield EH. Real-time image-guided direct convective perfusion of intrinsic brainstem lesions. Technical note. *J Neurosurg*. 2007; 107(1): 190–197.
- Riley J, Butler J, Baker KB, McClelland S, III, Teng Q, Yang J, Garrity-Moses M, Federici T, Boulis NM. Targeted spinal cord therapeutics delivery: stabilized platform and microelectrode recording guidance validation. *Stereotact Funct Neurosurg*. 2008; 86(2): 67–74.
- Riley J, Federici T, Park J, Suzuki M, Franz C K, Tork C, McHugh J, Teng Q, Svendsen S, Boulis N. Cervical spinal cord therapeutic delivery: Preclinical safety validation of a stabilized microinjection platform. *Neurosurgery*. In press, 2009.