

Submission ID #: 69778

Scriptwriter Name: Poornima G

Project Page Link: <https://review.jove.com/account/file-uploader?src=21282338>

Title: Stereotactic Intracranial Implantation of Glioblastoma Patient-Derived Xenograft Cell Lines in Mice

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Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**
- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**
- 3. Filming location:** Will the filming need to take place in multiple locations? **No**
- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 25

Number of Shots: 55

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

REQUIRED: Summarize your research focus and the main questions it aims to answer.

- 1.1. **Dani Burgenske:** Our research investigates glioblastoma using patient-derived xenograft models to evaluate therapeutic strategies and better replicate tumor heterogeneity observed in clinical settings.

- 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Dani Burgenske:** Most studies rely on established cell lines that lack tumor heterogeneity, limiting predictive value; this protocol uses patient-derived models that more accurately reflect clinical glioblastoma.

- 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Dani Burgenske:** This protocol enables reliable establishment of intracranial glioblastoma patient-derived xenograft models, supporting reproducible studies with minimal surgical complications.

- 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Dani Burgenske:** Critical considerations include careful cell preparation, appropriate animal selection, and precise intracranial cell injection to ensure consistent tumor establishment and experimental success.

- 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.5. **Dani Burgenske:** Following intracranial injection, models can be used for therapeutic evaluation, serial MRI imaging to monitor tumor growth, and histological analyses to assess tumor burden.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the Mayo Clinic

Protocol

2. Preparation of Surgical Workspace and the Animal

Demonstrators: Katie Bakken, Brett Carlson, Lauren Ott

- 2.1. To begin, spray down the work surface with a disinfectant to set up preparation station [1]. Cover the heating pad with disposable absorbent pad and set out all the equipment needed [2]. Next, set up the surgical station by covering the heating pad with a sterile drape [3]. Place all the sterilized instruments and required materials for the procedure onto the prepared sterile worksurface [4].
 - 2.1.1. WIDE: Talent standing at the preparation station and spraying disinfectant.
 - 2.1.2. Talent covering the heating pad with disposable absorbent pad and placing the tray containing all the equipment on the worksurface.
 - 2.1.3. Talent covering heating pad with a sterile drape.
 - 2.1.4. Talent placing sterilized surgical materials onto the sterile work surface
- 2.2. Ensure that the injection station is equipped with stereotaxic injection frames and autoinjector pumps [1]. Obtain 100 to 300,000 glioblastoma patient-derived xenograft cells in PBS [2].
 - 2.2.1. Talent showing the fully equipped injection station and pointing to the stereotaxic injection frames and autoinjector pumps.
 - 2.2.2. Talent placing the vial labelled “GBM PDX” with prepared cells in an ice box placed on the injection station.
- 2.3. Set up the recovery station in a separate area equipped with a heating pad [1].
 - 2.3.1. Talent positioning a heating pad in a designated recovery area.
- 2.4. Now, position the anesthetized mouse prone on the table for the procedure [1-TXT].
 - 2.4.1. Talent placing the anesthetized mouse prone on the table. **TXT: Anesthesia: 100 mg/kg Ketamine + 10 mg/kg Xylazine; IP; 10 mL/kg**
- 2.5. Confirm that the mouse has reached an adequate plane of anesthesia by verifying non-ambulatory behavior and lack of response to toe pinch or other stimuli [1]. Apply artificial tears to the eyes [2]. Using a sterile cotton-tipped applicator, scrub the incision

site with 70 percent ethanol starting at the top of the head and moving outward in a spiral pattern till the shoulder [3]. Repeat the scrubbing using povidone-iodine and perform a total of three alternating applications [4]. Administer analgesic such as carprofen subcutaneously according to institutional guidelines [5].

2.5.1. Talent assessing anesthetic depth by pinching the toe.

2.5.2. Talent applying artificial tears to the mouse eyes.

2.5.3. Talent scrubbing the head with ethanol from the head and moving outward in a spiral pattern.

2.5.4. Talent repeating scrubbing with povidone-iodine.

2.5.5. Talent administering the analgesic injection subcutaneously.

2.6. If using Radio Frequency Identification chips for identification, prepare the skin from the midline to the right flank as previously demonstrated [1] and insert the chip subcutaneously [2].

2.6.1. Talent scrubbing the right flank area using alternating ethanol and povidone-iodine.

2.6.2. Talent inserting the Radio Frequency Identification chip subcutaneously.

3. Mouse Cranial Exposure and Burr Hole Creation

3.1. Now, place the anesthetized mouse on a clean paper towel and transfer it to the surgical station [1].

3.1.1. Talent placing mouse on a paper towel and handing it to the surgical station.

3.2. Using forceps, grasp the prepared skin at the nape of the anesthetized mouse [1] and transfer the mouse from the paper towel to cotton gauze within the sterile field [2].

3.2.1. Talent holding the mouse at the nape with forceps.

3.2.2. Talent placing the mouse onto sterile gauze in the surgical field.

3.3. Apply pressure to the skin over the skull [1]. Using a scalpel, make an approximately 1-centimeter midline incision from the back of the skull to hind side of the eyes [2].

3.3.1. Talent stabilizing the skull by applying pressure.

3.3.2. Talent making a midline incision with a scalpel.

- 3.4. Then, locate the bregma [1]. Move the drill bit to the injection site by approximating 1 millimeter forward and 2 millimeters lateral to the right using the width of the bit [2].
 - 3.4.1. Talent identifying/pointing to the bregma on the skull.
 - 3.4.2. Talent positioning the drill bit relative to bregma.
- 3.5. Turn on the drill and adjust the speed between 15 to 20 thousand Revolutions Per Minute [1]. Apply slight pressure with the drill bit to the skull until penetration occurs and immediately remove pressure [2]. After drilling, transfer the mouse back to the paper towel for handoff to the injection station [3].
 - 3.5.1. Talent activating the drill and adjusting the speed.
 - 3.5.2. Talent drilling into the skull and releasing pressure upon penetration.
 - 3.5.3. Talent moving mouse back to the paper towel for transfer.

4. Intracranial Injection of Glioblastoma Patient-Derived Xenograft Cells

- 4.1. Next, remove the cells from the ice bucket and flick the tube to ensure the cells form a homogeneous suspension [1].
 - 4.1.1. Talent taking the cell tube from ice and flicking it.
- 4.2. Using the pump, load between 3.1 and 3.5 microliters of cells into the syringe [1]. Confirm that the suspension appears cloudy [2]. If required, troubleshoot clogs by flushing with sterile PBS or replacing the needle if necessary [3].
 - 4.2.1. Talent loading cell suspension into the syringe using the pump.
 - 4.2.2. Shot of syringe contents showing cloudy suspension.
 - 4.2.3. Talent flushing PBS into the needle-syringe setup and replacing the needle.
- 4.3. Recap the cells and return them to wet ice [1]. Clean the needle tip with a sterile alcohol wipe to remove excess cells and prevent seeding in the meninges [2].
 - 4.3.1. Talent recapping the cell tube and placing it on ice.
 - 4.3.2. Talent wiping the needle tip with a sterile alcohol wipe.
- 4.4. Load the mouse onto the bite blocks and secure them with ear pins [1]. Ensure that the

skull is stable and level in both planes and adjust as necessary [2]. Cover the animal from the shoulder to the haunch with a plastic wrap to preserve body heat during injection [3].

4.4.1. Talent positioning a mouse onto the bite block and securing ear pins.

4.4.2. Talent adjusting the head position to level the skull.

4.4.3. Talent covering the mouse with plastic wrap.

4.5. Using a sterile cotton swab, move the skin aside to expose both the bregma and burr hole [1].

4.5.1. Talent retracting the skin with a sterile cotton swab to reveal the skull landmarks.

4.6. Next, place the needle tip over the bregma just above the skull [1] and dial in coordinates to 1 millimeter forward and 2 millimeters lateral to the right [2].

4.6.1. Talent positioning the needle tip above the bregma.

4.6.2. Talent adjusting the injector coordinates.

4.7. Then, lower the needle tip until the bevel reaches the top of the skull [1]. Set a depth of 3 millimeters from the skull surface and start the pump [2]. Remove the needle from the brain 1 minute after the injection [3] and then relocate the mouse from the bite block to a paper towel [4].

4.7.1. Talent lowering the needle to the skull surface.

4.7.2. Talent setting depth and initiating the injection pump.

4.7.3. Talent holding position after injection completion.

4.7.4. Talent removing the mouse from the apparatus and placing it on a paper towel.

5. Wound Closure and Post-Operative Care

5.1. Break off a pea-sized amount of bone wax and roll it between fingers to soften [1]. Using sterile forceps, transfer the mouse back to the surgical field as shown previously [2]. With the non-dominant hand, separate the skin to expose the burr hole and gently swipe the wax over the burr hole once to seal [3].

5.1.1. Talent softening bone wax between fingers by rolling.

5.1.2. Talent transferring the mouse to the sterile field with forceps.

- 5.1.3. Talent applying bone wax over the burr hole.
- 5.2. Close the head incision using 1 to 2 wound closure clips [1]. Ensure the closure is secure without excessive tightness, allowing normal eye closure and avoiding skin tension [2]. Then, transfer the mouse to a paper towel for recovery. Inject an anesthesia reversal agent if using a sensitive animal strain [3].
 - 5.2.1. Talent closing the incision with 1-2 wound close clips.
 - 5.2.2. Shot showing properly aligned and tension-free closure.
 - 5.2.3. Talent placing the mouse on a paper towel for recovery.
- 5.3. Return the animal to the home cage placed partially on a heat source with one-third of the cage off heat [1].
 - 5.3.1. Talent placing the mouse into a cage positioned partially on a heating surface.
- 5.4. Actively monitor the mouse until full recovery of consciousness, approximately 1 hour [1]. Every 20 minutes, perform manual stimulation including picking up, rubbing, and gentle toe pinching until the animal is ambulatory and return the mouse to the housing room [2]. ~~Then, return the recovered mouse to the animal housing room [3].~~ NOTE: For removed shot, VO is added in concise format in previous step.
 - 5.4.1. Talent observing the mouse in the recovery cage.
 - 5.4.2. Talent handling and stimulating mouse.
 - 5.4.3. ~~Talent transferring recovered mouse to the housing area.~~ NOTE: Not filmed.
- 5.5. Monitor the mouse daily for at least 10 to 14 days following surgery and note the survival days [1].
 - 5.5.1. Talent looking at the mouse placed in the cage and noting something in a book.
- 5.6. Once moribund criteria are met, remove the animal for euthanasia following the institutional guidelines [1]. Harvest the brain and fix it in formalin for downstream analysis [2]. Perform microscopy to assess the tumor after hematoxylin and eosin staining or immunofluorescence staining [3].
 - 5.6.1. Talent removing the animal from the cage.
 - 5.6.2. Talent placing brain tissue it a jar labelled "Formalin".

- 5.6.3. Wide shot of placing the sample under a microscope or the talent viewing something under a microscope or talent placing the slide in a container labeled “H&E Stain”.

Results

6. Results

6.1. Intracranial tumor was confirmed in the harvested brain by hematoxylin and eosin staining [1]. Immunofluorescence staining and imaging confirmed the presence of tumors as unilateral, well-demarcated masses [2].

6.1.1. LAB MEDIA: Figure 3A.

6.1.2. LAB MEDIA: Figure 3B.

6.2. Peak body weight loss occurred within 24 hours after surgery [1] and fully resolved within 72 to 96 hours when no post-operative complications arose [2].

6.2.1. LAB MEDIA: Figure 4. *Video editor: Highlight the lowest point of the weight curve for day 1 on X-axis.*

6.2.2. LAB MEDIA: Figure 4. *Video editor: Highlight data points for day 3 and 4 on X-axis.*

6.3. The time of animal decline was consistent across experiments and cell densities when survival was tracked after intracranial implantation [1].

6.3.1. LAB MEDIA: Figure 5. *Video editor: Show the survival curves in both the graphs.*

Pronunciation guides:

1. Disinfectant

Pronunciation link: <https://www.merriam-webster.com/dictionary/disinfectant>

IPA: /ˌdɪs.ɪnˈfɛk.tənt/

Phonetic Spelling: dis-in-fek-tuhnt

2. Absorbent

Pronunciation link: <https://www.merriam-webster.com/dictionary/absorbent>

IPA: /əbˈzɔːr.bənt/

Phonetic Spelling: uhb-zor-buhnt

3. Stereotaxic

Pronunciation link: <https://www.merriam-webster.com/medical/stereotaxic>

IPA: /ˌstɛr.i.ooˈtæks.ɪk/

Phonetic Spelling: steh-ree-oh-tak-sik

4. Autoinjector

Pronunciation link: <https://www.merriam-webster.com/medical/autoinjector>

IPA: /ˌɔː.təʊ.ɪnˈdʒɛk.tər/

Phonetic Spelling: aw-toh-in-jek-ter

5. Glioblastoma

Pronunciation link: <https://www.merriam-webster.com/medical/glioblastoma>

IPA: /ˌɡliː.əʊ.blæˈstəʊ.mə/

Phonetic Spelling: glee-oh-blas-toh-muh

6. Xenograft

Pronunciation link: <https://www.merriam-webster.com/medical/xenograft>

IPA: /ˈziː.nəʊ.græft/

Phonetic Spelling: zee-noh-graft

7. Anesthetized

Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetized>

IPA: /əˈnɛs.θə.taɪzd/

Phonetic Spelling: uh-nes-thuh-tyzd

8. Ketamine

Pronunciation link: <https://www.merriam-webster.com/medical/ketamine>

IPA: /ˈkɛt.ə.miːn/

Phonetic Spelling: ket-uh-meen

9. Xylazine

Pronunciation link: <https://www.merriam-webster.com/medical/xylazine>

IPA: /ˈzaɪ.lə.ziːn/

Phonetic Spelling: zy-luh-zeen

10. Ambulatory

Pronunciation link: <https://www.merriam-webster.com/dictionary/ambulatory>

IPA: /ˈæm.bjə.ləˈtɔːr.i/

Phonetic Spelling: am-byuh-luh-tor-ee

11. Povidone-iodine

Pronunciation link: <https://www.merriam-webster.com/medical/povidone-iodine>

IPA: /ˌpɑː.vɪ.doʊn ˈaɪ.əˌdaɪn/

Phonetic Spelling: pah-vih-dohn eye-uh-dyne

12. Analgesic

Pronunciation link: <https://www.merriam-webster.com/dictionary/analgesic>

IPA: /ˌæn.əlˈdʒiː.zɪk/

Phonetic Spelling: an-uhl-jee-zik

13. Carprofen

Pronunciation link: <https://www.merriam-webster.com/medical/carprofen>

IPA: /ˈkɑːr.prəʊ.fɛn/

Phonetic Spelling: kar-proh-fen

14. Subcutaneously
Pronunciation link: <https://www.merriam-webster.com/dictionary/subcutaneously>
IPA: /ˌsʌb.kjuːˈteɪ.ni.əs.li/
Phonetic Spelling: sub·kyoo·tay·nee·uhs·lee
15. Cranial
Pronunciation link: <https://www.merriam-webster.com/dictionary/cranial>
IPA: /ˈkreɪ.ni.əl/
Phonetic Spelling: kray·nee·uhl
16. Bregma
Pronunciation link: <https://www.merriam-webster.com/medical/bregma>
IPA: /ˈbrɛɡ.mə/
Phonetic Spelling: breg·muh
17. Revolutions
Pronunciation link: <https://www.merriam-webster.com/dictionary/revolution>
IPA: /ˌrɛv.əˈluːʃənz/
Phonetic Spelling: rev·uh·loo·shunz
18. Intracranial
Pronunciation link: <https://www.merriam-webster.com/medical/intracranial>
IPA: /ˌɪn.trəˈkreɪ.ni.əl/
Phonetic Spelling: in·truh·kray·nee·uhl
19. Homogeneous
Pronunciation link: <https://www.merriam-webster.com/dictionary/homogeneous>
IPA: /ˌhoʊ.məˈdʒiː.ni.əs/
Phonetic Spelling: hoh·muh·jee·nee·uhs
20. Microliters
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
IPA: /ˈmaɪ.kroʊ.liː.tərz/
Phonetic Spelling: my·kroh·lee·terz
21. Meninges
Pronunciation link: <https://www.merriam-webster.com/medical/meninges>
IPA: /məˈnɪn.dʒiːz/
Phonetic Spelling: muh·nin·jeez
22. Bevel
Pronunciation link: <https://www.merriam-webster.com/dictionary/bevel>
IPA: /ˈbev.əl/
Phonetic Spelling: bev·uhl
23. Moribund
Pronunciation link: <https://www.merriam-webster.com/dictionary/moribund>
IPA: /ˈmɔːr.ə.bʌnd/

Phonetic Spelling: mor·uh·bund

24. Euthanasia

Pronunciation link: <https://www.merriam-webster.com/dictionary/euthanasia>

IPA: /ju:.θə'nei.zə/

Phonetic Spelling: yoo·thuh·nay·zhuh

25. Formalin

Pronunciation link: <https://www.merriam-webster.com/medical/formalin>

IPA: /'fɔ:r.mə.lɪn/

Phonetic Spelling: ফর্মালিন·muh·lin

26. Microscopy

Pronunciation link: <https://www.merriam-webster.com/dictionary/microscopy>

IPA: /maɪ'krɑ:skə.pi/

Phonetic Spelling: my·krah·skuh·pee

27. Hematoxylin

Pronunciation link: <https://www.merriam-webster.com/medical/hematoxylin>

IPA: /hi:mə'tɔ:k.sɪ.lɪn/

Phonetic Spelling: hee·muh·tok·suh·lin

28. Eosin

Pronunciation link: <https://www.merriam-webster.com/medical/eosin>

IPA: /'i:ɒs.ɪn/

Phonetic Spelling: ee·oh·sin

29. Immunofluorescence

Pronunciation link: <https://www.merriam-webster.com/medical/immunofluorescence>

IPA: /ɪmju:.nɒʊ.flɔ'res.əns/

Phonetic Spelling: ih·myoo·noh·floor·es·uhns

30. Unilateral

Pronunciation link: <https://www.merriam-webster.com/dictionary/unilateral>

IPA: /ju:.nə'læt.ər.əl/

Phonetic Spelling: yoo·nuh·lat·er·uhl

31. Demarcated

Pronunciation link: <https://www.merriam-webster.com/dictionary/demarcate>

IPA: /di:'mɑ:r.keɪ.tɪd/

Phonetic Spelling: dee·mar·kay·tid