FINAL SCRIPT: APPROVED FOR FILMING

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Title: Establishment of Orthotopic Patient-Derived Xenograft Models for Brain Tumors Using a Stereotaxic Device

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **MM/DD/YYYY**

When you are ready to submit your video files, please contact our Content Manager, Utkarsh Khare.

Current Protocol Length

Number of Steps: 16
Number of Shots: 40

Introduction

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

1.1. **Maria Tsoli:** In my research, we focus on developing new treatments for aggressive childhood brain cancers like Diffuse Midline Gliomas, high-grade gliomas, ependymomas, and medulloblastomas. We aim to find new drug targets and develop therapies that can make a difference in patients while also studying how these tumours affect their surrounding brain environment.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.6*

What technologies are currently used to advance research in your field?

1.2. **Maria Tsoli:** In pediatric brain tumor research, we develop patient-derived xenograft models using intracranial injections, advanced imaging, and high-throughput drug screening. Techniques like single-cell and spatial transcriptomics help us understand tumor impacts and treatment effects, enabling accurate models for exploring new therapies

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.7*

What significant findings have you established in your field?

1.3. **Maria Tsoli:** Our Brain Tumor Group has developed pediatric brain tumor PDX models through the ZERO trial and Hotrods autopsy program. We found Diffuse Midline Gliomas resist drugs due to the intact blood-brain barrier. Promising therapies targeting epigenetic pathways and polyamine metabolism improved PDX survival and are now in clinical development.

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

What research questions will your laboratory focus on in the future?

1.4. **Maria Tsoli:** Using orthotopic brain tumour models, we aim to understand how tumours affect brain vasculature. By applying advanced techniques like spatial transcriptomics, we want to identify pathways impacted by tumours. This knowledge will help us identify BBB modulators that can loosen the barrier, allowing drug treatments to penetrate more effectively.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.11*

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 University of New South Wales

Protocol

2. Preparation of Brain Tumor Cells for Intracranial Injection for Brain Tumor Models

Demonstrator: Aaminah Khan

Protocol

2.1. To begin, thaw cultured neurosphere-forming brain tumor cells **[1].** In a biosafety cabinet, carefully pipette out the cultured cells and culture medium from the tissue culture flask **[2]** into a 50-milliliter conical tube with a serological pipette **[3].**

2.1.1. WIDE: Talent places frozen culture of brain tumor cells on the workspace.

2.1.2. Talent carefully pipetting out the culture with a serological pipette.

2.1.3. Shot of the culture being transferred into a 50 mL conical tube.

2.2. Centrifuge the conical tube at 300 *g* for up to 5 minutes at room temperature **[1].** With a 25-milliliter pipette, aspirate the medium above the pellet, leaving approximately 0.5 milliliters in the tube **[2].**

2.2.1. Talent placing the conical tube into the centrifuge and closing the lid.

2.2.2. Shot of the supernatant being removed with a 25 mL pipette carefully, leaving 0.5 milliliters above the cell pellet.

2.3. Gently pipette the cells up and down 10 times with a 1-milliliter pipette to dissociate the cell pellet **[1].** Add trypan blue to the cell suspension **[2]** and count with a hemocytometer **[3].**

2.3.1. Shot of the cell suspension being pipetted up and down with a 1 mL pipette.

2.3.2. Talent adds trypan blue to the cell suspension.

2.3.3. Shot of the stained cell suspension being pipetted onto a hemocytometer.

2.4. Aliquot cell suspension containing 2 million cells into a fresh tube **[1].** Add 0.5 milliliter PBS to the cell suspension **[2],** then centrifuge at 300 *g* for 5 minutes at 4 degrees Celsius **[3].** Discard the supernatant and wash again **[4-TXT].**

2.4.1. Talent aliquoting the appropriate cell volume into a fresh tube.

2.4.2. Shot of 0.5 mL PBS being added to the cell suspension.

2.4.3. Shot of tubes being placed in the centrifuge.

2.4.4. Shot of supernatant being discarded from the tubes. **TXT: Perform PBS wash 2x**

2.5. After the final wash, aspirate as much PBS as possible **[1].** Then place the cell pellet and 50 microliters of extracellular matrix hydrogel on ice **[2].**

2.5.1. Shot of the final PBS wash being pipetted out, ensuring minimal volume remains.

2.5.2. Talent placing the tube containing the cell pellet and ECM hydrogel on ice.

2.6. Mix the cell pellet with the extracellular matrix hydrogel promptly to prepare for intracranial injection **[1-TXT].**

2.6.1. Talent mixing the cell pellet with ECM hydrogel on ice. **TXT: Cell suspension: 1 x 10^5 cells/ µL**

3. Intracranial Injection Protocol for Establishing Brain Tumor Models in Mice

Demonstrator: Maria Tsoli & Caitlin Ung

3.1. To begin, mount an anesthetized mouse onto a stereotaxic device **[1-TXT]**. Ensure the front teeth are fixed in the incision bar and the nose cone secures the animal in place **[2].**

3.1.1. WIDE: Talent places an anesthetized mouse onto a stereotaxic device. **TXT: Anesthesia: Isoflurane inhalation**

3.1.2. Talent fixing the teeth and positioning the nose cone securely.

3.2. Disinfect the mouse's head with an iodine-soaked cotton tip followed by an isopropanol wipe **[1].** Then apply corneal eye ointment to both eyes to prevent drying **[2].**

3.2.1. Talent disinfecting the mouse's head with iodine and isopropanol wipes.

3.2.2. CU: Talent applying corneal eye ointment to both eyes.

3.3. Make an incision at the base of the cerebellum **[1]** and extend it across the cranium to the midpoint, to create a 1-centimeter-long cut along the superior aspect of the skull **[2].**

3.3.1. Shot of an incision being made at the base of the cerebellum.

3.3.2. Shot of the incision being extended across the cranium to the midpoint.

3.4. Tighten the skin between the ears to expose the skull **[1]** and then tighten the ear bards to secure the head **[2]**. Clean the skull surface and dry it thoroughly with a cotton bud **[3].**

3.4.1. Shot of the skin between the ears being tightened and the skull being seen.

3.4.2. Talent tightening the ear bars to secure the mouse's head in place.

3.4.3. Talent cleaning and drying the skull surface with a cotton bud.

3.5. Secure the drill onto the stereotaxic frame and locate the bregma or lambdoid structure using the drill **[1].** Once the coordinates are established, carefully drill a small burr hole in the bone at the designated site **[2].**

3.5.1. Close-up of the drill being aligned with bregma or lambdoid structure on the stereotaxic frame.

3.5.2. CU: Shot of the drill creating a small burr hole at the designated coordinates.
AND
TEXT ON PLAIN BACKGROUND:
Diffuse midline glioma (DMG): X = +0.5, Y = -5.5, Z = -3.1 from bregma

Supratentorial brain tumor (GBM or ependymoma): X = +1.5, Y = +1, Z = -3 from bregma

Infratentorial brain tumor (Medulloblastoma, ependymoma): X = +2, Y = - 2, Z = -2 from lambdoid structure.
Video Editor: Please play both shots side by side

3.6. Resuspend the prepared brain tumor injection cell suspension multiple times using a pipette, ensuring air bubbles are avoided **[1].** Draw 2 microliters of the cell mixture into a prewashed, cold glass microsyringe **[2]** and attach the syringe to the stereotaxic frame **[3].** Adjust the needle to the skull tip to prepare for injection **[4].**
3.6.1. Shot of the injection cell suspension being resuspended using a pipette.
3.6.2. Talent loading 2 µL of the cell mixture into a glass microsyringe.
3.6.3. Shot of the loaded syringe being attached to a stereotaxic frame.
3.6.4. Talent adjusting the needle to the skull tip.

3.7. Within 30 seconds, inject the cells into the drilled region **[1].** Wipe away any refluxed cell suspension with an isopropanol wipe, during the injection **[2].**
3.7.1. Close-up of the syringe injecting the cells into the burr hole over 30 seconds.
3.7.2. Talent drying any refluxed cell suspension with an isopropanol wipe during the injection.

3.8. Leave the syringe in place for 1 minute to prevent backflow and allow the extracellular matrix hydrogel to settle **[1].** Then remove the syringe **[2]** and clean the wound with an isopropanol wipe **[3]**. Seal the incision with skin glue or wound clips if necessary **[4].**
3.8.1. Close-up of the syringe remaining in place after injection.
3.8.2. Talent removing the syringe.
3.8.3. Talent wiping the wound with an isopropanol wipe.
3.8.4. Shot of the wound being sealed with skin glue or wound clips.

3.9. Place the mouse in a recumbent position in a clean recovery cage with a heat lamp or heating pad to maintain warmth **[1].** Monitor the animal continuously until it begins moving independently and normally **[2].**
3.9.1. Talent placing the animal in a recovery cage under a heat lamp.

3.9.2. Close-up of the mouse showing normal behavior.

3.10. Following the intracranial injection, monitor the mice five days per week to assess their general well-being **[1].** Record parameters such as weight loss, activity level, posture, signs of dehydration, and fur condition on a designated monitoring sheet **[2-TXT].**

3.10.1. Talent observing mice in their cages during routine monitoring.

3.10.2. Close-up of the monitoring sheet with recorded parameters such as weight, activity, and fur condition. **TXT: Document any intermittent, mild, or severe neurological symptoms**

Results

Representative Results

3.11. Animals exhibited distinct symptoms based on tumor type and injection site such as head tilting in brainstem tumors **[1]** and forebrain enlargement for cortical gliomas or ependymomas **[2]**.
3.11.1. LAB MEDIA: Figure 3A.
3.11.2. LAB MEDIA: Figure 3B

3.12. Intracranial injections of medulloblastoma D425 *(D-four-two-five)* cells at different densities revealed a direct correlation between cell density and tumor engraftment speed **[1]**, with 100,000 cells leading to the shortest median survival of 15 days **[2]**.
3.12.1. LAB MEDIA: Figure 4.
3.12.2. LAB MEDIA: Figure 4. *Video editor: Please highlight the black curve, corresponding to 100,000 cells*

3.13. High-grade glioma cells injected into the cortex resulted in a median survival of approximately 25.5 days **[1]**, with immunohistochemical analysis revealing a large, highly nucleated tumor mass and increased vascularization **[2]**, as well as abundant Ki67*(K-eye-sixty-seven)* -positive proliferative cells **[3]**.
3.13.1. LAB MEDIA: Figure 5A
3.13.2. LAB MEDIA: Figure 5B
3.13.3. LAB MEDIA: Figure 5B

3.14. Brainstem injections of high-grade glioma cells resulted in a median survival of approximately 26 days **[1]**, with immunohistochemical analysis indicating a tumor mass in the upper pons and leptomeningeal infiltration **[2]**, as well as proliferative Ki67-positive cells in infiltrated areas **[3]**.
3.14.1. LAB MEDIA: Figure 5D
3.14.2. LAB MEDIA: Figure 5E
3.14.3. LAB MEDIA: Figure 5F

Pronunciation Guide:

1. Orthotopic
Pronunciation link:
https://www.merriam-webster.com/dictionary/orthotopic
IPA: /ˌɔːr.θəˈtoʊ.pɪk/
Phonetic Spelling: or-thuh-toh-pik

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2. Xenograft
Pronunciation link:
https://www.merriam-webster.com/dictionary/xenograft
IPA: /ˈzɛn.əˌgræft/
Phonetic Spelling: zeh-nuh-graft

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3. Stereotaxic
Pronunciation link:
https://www.merriam-webster.com/dictionary/stereotaxic
IPA: /ˌstɛr.i.oʊˈtæk.sɪk/
Phonetic Spelling: stair-ee-oh-tak-sik

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4. Medulloblastoma
Pronunciation link:
https://www.merriam-webster.com/dictionary/medulloblastoma
IPA: /ˌmɛd.jə.loʊ.blæˈstoʊ.mə/
Phonetic Spelling: med-yuh-loh-blas-toh-muh

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5. Ependymoma
Pronunciation link:
https://www.howtopronounce.com/ependymoma
IPA: /ɛˌpɛn.dɪˈmoʊ.mə/
Phonetic Spelling: eh-pen-dih-moh-muh

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6. Glioma
Pronunciation link:
https://www.merriam-webster.com/dictionary/glioma
IPA: /gliˈoʊ.mə/
Phonetic Spelling: glee-oh-muh

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7. Bregma
Pronunciation link:
https://www.howtopronounce.com/bregma
IPA: /ˈbrɛg.mə/
Phonetic Spelling: breg-muh

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8. Lambdoid
Pronunciation link:
https://www.howtopronounce.com/lambdoid

IPA: /ˈlæm.dɔɪd/
Phonetic Spelling: lam-doyd

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9. Neurosphere
Pronunciation link:
https://www.howtopronounce.com/neurosphere
IPA: /ˈnjʊr.oʊ.sfɪr/
Phonetic Spelling: nyoo-roh-sfeer

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10. Trypan Blue
Pronunciation link:
https://www.howtopronounce.com/trypan-blue
IPA: /ˈtrɪp.æn bluː/
Phonetic Spelling: trip-an bloo

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11. Hemocytometer
Pronunciation link:
https://www.howtopronounce.com/hemocytometer
IPA: /ˌhiː.moʊ.saɪˈtɑː.mɪ.tɚ/
Phonetic Spelling: hee-moh-sigh-tah-mi-ter

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12. Isoflurane
Pronunciation link:
https://www.howtopronounce.com/isoflurane
IPA: /ˈaɪ.sə.flʊˌreɪn/
Phonetic Spelling: eye-suh-floo-rayn

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13. Leptomeningeal
Pronunciation link:
https://www.howtopronounce.com/leptomeningeal
IPA: /ˌlɛp.toʊ.məˈnɪn.dʒi.əl/
Phonetic Spelling: lep-toh-muh-nin-jee-uhl

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14. Ki67
Pronunciation link:
https://www.howtopronounce.com/ki67
IPA: /ˌkaɪ ˈsɪk.sti ˈsɛv.ən/
Phonetic Spelling: kai sixty-seven