Submission ID #: 68694
Scriptwriter Name: Poornima G
Project Page Link: https://review.jove.com/account/file-uploader?src=20951718

Title: An Orthotopic Sciatic Nerve Xenograft for Neurofibromatosis Type 1 Neurofibromas

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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? **YES**

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: 08
Number of Shots: 18

Introduction

NOTE to VO: Please record the interview statements.

1.1. This research focuses on neurofibromas, a type of benign nerve sheath tumor, to develop a preclinical model suitable for pharmacological studies.

1.1.1. *2.3.1.*

What are the current experimental challenges?

1.2. The challenge of this model was to establish an orthotopic intraneural graft of human immortalized cells.

1.2.1. *2.5.1.*

How will these findings advance research in the field?

1.3. Only one treatment is currently approved for neurofibroma. While this represents a significant advance, there are still limitations, including toxicity and the development of resistance. The preclinical model will enable the screening of new compounds.

1.3.1. *2.6.1*

Ethics Title Card

This research has been approved by the Ethics Committee at the Institut Curie CEEA-IC

Protocol

2. Sciatic Nerve Injection Procedure

Demonstrator: Mikael Hivelin

Videographer: Please do not film or cover the talent's face throughout the shoot. They do not wish to appear on the video. Please cover all the procedures in close-ups

2.1. To begin, place the anesthetized and analgesia-treated mouse on its right side and gently spread its hind legs apart **[1-TXT].** Use povidone-iodine to prepare a sterile surgical field **[2]** and cover the surgical area with a sterile drape before making the incision **[3]**. NOTE: The VO for the moved shot is added here from 2.2

2.1.1. Talent positioning the anesthetized mouse on its right side and gently spreading its hind legs. **TXT: Anesthesia: Ketamine (80 mg/kg); Xylazine (15 mg/kg) i.p; Buprenorphine (0.1 mg/kg, s.c)**

2.2.1 Talent applying povidone-iodine to the surgical area.

2.1.2. Talent placing a sterile drape over the surgical area.

~~2.2. Use povidone-iodine to prepare a sterile surgical field **[1].**~~

2.2.1. ~~Talent applying povidone-iodine to the surgical area.~~ NOTE: This step is moved before 2.1.2

2.3. Then, make a 5-millimeter skin incision parallel to the femur using fine scissors **[1]**. Use fine forceps and micro scissors to gently separate the underlying muscle layers while avoiding damage to blood vessels **[2]**. Identify the sciatic nerve as a white, cord-like structure located medial to the femur **[3]**.

2.3.1. Talent using a fine scissors to make a small skin incision along the femur. NOTE: slated 2.1.2 but check the name file)

2.3.2. Talent using fine forceps and micro scissors to dissect muscle layers carefully.

2.3.3. Close-up shot of the exposed sciatic nerve, showing its distinct white cord-like appearance.

2.4. Next, take the cell suspension to be injected in a 1.5-milliliter tube **[1]** and gently flick the tube to resuspend any settled cells **[2]**. Load 4 microliters of the suspension into a 10 microliter Hamilton syringe fitted with a 33-gauge needle **[3]**.

2.4.1. Talent picking up the tube with suspension from storage rack. NOTE: Use 2sd one

2.4.2. Talent flicking a 1.5 milliliter tube to resuspend cells. NOTE: This shot is filmed with 2.4.1)

2.4.3. Talent loading 4 microliters of cell suspension into a 10 microliter Hamilton

syringe with a 33 gauge needle.

2.5. Now, insert the needle parallel to the sciatic nerve axis to a depth of approximately 2 millimeters **[1]**. Slowly start injecting to create pressure and a gap between the nerve and the peripheral sheath, and advance the needle for approximately 0.8 millimeters **[2]**. Then, slowly inject the suspension while withdrawing the needle from the nerve **[3-TXT]**.

2.5.1. Talent inserting the needle alongside the sciatic nerve. NOTE: tc 21/00)

2.5.2. Talent advancing the needle. NOTE: Filmed with 2.5.1 , tc 1/19/00)

2.5.3. Talent pushing the plunger of the syringe. **TXT: Avoid any tension or movement of the nerve** NOTE: MED, and SCOPE is filmed with 2.5.1 , tc 1/25/00)

2.6. Perform the injection without stretching the sciatic nerve and do not use clamps **[1]**. Wait for 10 to 15 seconds before slowly withdrawing the needle to minimize reflux **[2]**.

2.6.1. Shot of the injection site with the needle held still. NOTE: Filmed with 2.5.1 , tc 1/37/00

2.6.2. Talent slowly withdrawing the needle from the site. NOTE: filmed with 2.5.1 , tc 1/47/00, or MED end of 2.5.3)

2.7. Ensure that the sciatic nerve appears slightly swollen at the injection site with no leakage or discoloration **[1]**. A stable nerve appearance without extravasation confirms a successful injection **[2]**.

2.7.1. Close-up shot of the sciatic nerve showing slight swelling and no visible leakage. NOTE: filmed with 2.5.1 , tc 2/10/00)

2.7.2. Talent pointing to the nerve without extravasation. NOTE: filmed with 2.5.1 , tc 2/29/00)

2.8. Finally, close the surgical incision using 3 to 4 simple interrupted sutures with non-absorbable material to avoid inflammation **[1]**. If the surgery lasts longer than 10 minutes, administer 1 milliliter of Ringer's lactate subcutaneously **[2]**.

2.8.1. Talent suturing the incision site using the specified suture technique.

2.8.2. Talent administering a subcutaneous injection of Ringer Lactate to the mouse. **TXT: Anti-inflammatory: Meloxicam (5 mg/kg); s.c at the end of the day**

Results

3. Results

3.1. Bioluminescence imaging showed localized signal at the sciatic nerve as early as day 5 post-injection, indicating successful tumor engraftment **[1]**, with exponential signal increase by day 33, reflecting progressive tumor growth **[2]**.

3.1.1. LAB MEDIA: Figure 1A. *Video editor: Highlight the bright bioluminescent region in the left leg of both mice at day "5"*

3.1.2. LAB MEDIA: Figure 1A. *Video editor: Highlight the expanded, more intense bioluminescent region in the left leg of the mice at day "33"*

3.2. Dissection of the sciatic nerve at day 33 revealed a clearly visible, well-integrated tumor mass without signs of abscess formation **[1]**, consistent with the bioluminescence imaging **[2]**.

3.2.1. LAB MEDIA: Figure 1B. *Video editor: Highlight the large, bulging tumor at the sciatic nerve pointed by the red arrow*

3.2.2. LAB MEDIA: Figure 1B. *Video editor: Highlight the excised tumor mass on the right-hand image*

3.3. Hematoxylin and eosin staining confirmed the presence of tumor tissue within the sciatic nerve, showing dense cellularity and focal invasion in all three representative samples **[1]**.

3.3.1. LAB MEDIA: Figure 2.

Pronunciation Guide

1. **analgesia**
   Pronunciation link: https://www.merriam-webster.com/dictionary/analgesia
   IPA: /ˌænəlˈdʒiːʒə/
   Phonetic spelling: an-uhl-JEE-zhuh
2. **povidone-iodine**
   Pronunciation link: https://www.merriam-webster.com/dictionary/povidone
   (for "iodine" see https://www.merriam-webster.com/dictionary/iodine)
   IPA (approx): /ˌpoʊvɪˈdoʊn ˈaɪəˌdaɪn/
   Phonetic spelling: poh-vih-DOHN EYE-oh-dine
3. **femur**
   Pronunciation link: https://www.merriam-webster.com/dictionary/femur
   IPA: /ˈfiːmər/
   Phonetic spelling: FEE-muhr

4. **micro scissors**
   "micro" — Pronunciation link: https://www.merriam-webster.com/dictionary/micro
   IPA: /ˈmaɪkroʊ/
   Phonetic spelling: MY-kroh
   "scissors" — Pronunciation link: https://www.merriam-webster.com/dictionary/scissors
   IPA: /ˈsɪzərz/
   Phonetic spelling: SIH-zurz
5. **sciatic**
   Pronunciation link: https://www.merriam-webster.com/dictionary/sciatic
   IPA: /saɪˈætɪk/
   Phonetic spelling: sigh-AT-ik
6. **extravasation**
   Pronunciation link: https://www.merriam-webster.com/dictionary/extravasation
   IPA: /ˌɛkstrəvəˈzeɪʃən/
   Phonetic spelling: ek-struh-vuh-ZAY-shun
7. **bioluminescence**
   Pronunciation link: https://www.merriam-webster.com/dictionary/bioluminescence
   IPA: /ˌbaɪoʊˌluːməˈnɛsəns/
   Phonetic spelling: bye-oh-LOO-muh-NES-ens
8. **hematoxylin**
   Pronunciation link: https://www.merriam-webster.com/dictionary/hematoxylin
   IPA: /ˌhiːmətoʊˈzɪlɪn/
   Phonetic spelling: HEE-mah-toh-ZIL-in
9. **eosin**
   Pronunciation link: https://www.merriam-webster.com/dictionary/eosin
   IPA: /ˈiːəsɪn/
   Phonetic spelling: EE-oh-sin