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Title: A Neonatal Intravenous Transplant Model for Assessment of Early-Life Immune Activities Against Cancer Cells

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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? **Yes**

If **Yes**, we will need you to record using screen recording software.

We recommend using the screen capture program [OBS](https://review.jove.com/v/5848/screen-capture-instructions-for-authors?status=a7854k). JoVE's tutorial for using OBS Studio is provided at this link: <https://review.jove.com/v/5848/screen-capture-instructions-for-authors?status=a7854k>

As these files are necessary for finalizing your script, please upload all screen-captured video files to your project page as soon as possible.

Videographer: Please record the computer screen for the shots labeled as SCREEN. The screen should be in focus and the fonts should be clearly visible.

- 3. Filming location:** Will the filming need to take place in multiple locations? **Yes**

- 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: 17

Number of Shots: 30

Introduction

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

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

Answers are slightly edited in accordance with journal guidelines and we have retained 5 best answers.

INTRODUCTION:

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

- 1.1. **Maryam Aletaha** : We study how early-life immunity influences pediatric cancer progression, and whether neonatal immune responses can suppress or promote cancer progression.
 - 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. **Maryam Aletaha** : Current models lack precise neonatal intravenous delivery; this protocol ensures efficient delivery and consistent modeling of early-life immune responses.
 - 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. **Nakaw Young**: This protocol enables longitudinal tracking of cancer progression and evaluation of early-life immune modulation using bioluminescence imaging.
 - 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. **Nakaw Young**: The main challenge is accurate temporal vein injection within a narrow neonatal window, requiring careful technique and proper visualization.

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

How can future studies build on this work? Describe the questions or research directions that can be investigated next.

- 1.5. ~~Zahra Savoji~~~~Nakaw-Young~~: Future studies can explore different pediatric cancers and immune stimuli, vaccines, or therapies to define mechanisms of immune-mediated cancer control in early life.

- 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera **NOTE: Use the author provided 1.5 statement.**

Zahra-1.5.1.MOV

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

Ethics Title Card

This research has been approved by the Animal Care Committee of the University of British Columbia

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

2. Temporal Vein Injection of Cancer Cells into P1-P2 Mouse Pups

Demonstrators: Nakaw Young, Ali Farrokhi

2.1. To begin, place freshly prepared suspensions of luciferase-tagged leukemic cells derived from E μ -Ret (*E-myoo-ret*) mice [1] or neuroblastoma cells derived from TH-MYCN (*T-H-Mik-en*) mice on ice before neonatal transplantation [2].

2.1.1. WIDE: Talent standing at the workstation with tubes and ice box. Placing tubes with cell suspension on ice.

2.1.2. Close-up of the labelled tubes placed on ice.

2.2. Load a 100-microliter glass syringe with 20 microliters of the cell suspension containing 1×10^4 leukemic cells or 5×10^4 neuroblastoma cells [1]. Then, attach a 30-gauge needle to the syringe [2]. Prime the needle by expelling a small amount of suspension back into the tube [3].

2.2.2, Talent loading cell suspension into a glass syringe with a needle, without air bubble formation.

2.2.1, Talent attaching a 30-gauge needle. Added action: Expelling the suspension back into the tube to fill the dead volume of the syringe and needle.

NOTE: Authors modified the shot order and combined this step. So filmed in this sequence: Loading the syringe, attaching needle and pushing the liquid.

2.3. Prepare a transilluminated stage by placing a cold light source beneath a plexiglass platform [1].

2.3.1. Talent positioning a cold light source under a plexiglass platform.

2.4. Next, place a sterile napkin with a central hole on the stage [1] and then place a small piece of modeling clay to stabilize the needle during injection [2]. Position the hypothermia-immobilized postnatal day 1 or 2 pup on the napkin [3-TXT] and adjust its position until the temporal vein is clearly visible [4-TXT].

2.4.1. Talent placing a sterile napkin with a central hole onto the stage.

2.6.1, Talent positioning modeling clay next to the pup's head

NOTE: Show step 2.6.1 here after 2.4.1. Show 2.4.1, 2.6.1 and then 2.4.2

2.4.2. Talent positioning the immobilized pup on the napkin. **TXT: Follow institutionally approved guidelines for immobilizing the pup**

2.4.3. Talent adjusting the pup's position and pointing to the exposed temporal vein. **TXT: Use BALB-c pup for leukemic cells and TH-MYCN pup for neuroblastoma cells**

2.5. Wear magnifying eyewear to visualize the injection site [1].

2.5.1. Talent wearing and adjusting magnifying eyewear.

~~2.6. Place a small piece of modeling clay adjacent to the pup's head to stabilize the needle during injection [1].~~

~~2.6.1. Talent positioning modeling clay next to the pup's head.~~

NOTE: Moved after 2.4.1

2.7. Then, insert the needle bevel-up into the visible temporal vein [1] and push the plunger to slowly inject the cell suspension. Observe vascular filling to confirm successful intravenous delivery [2]. Apply gentle pressure to the injection site using a sterile cotton swab [3].

Author's NOTE: We had multiple shots for this step. Use the TAKE 8, the one before the last one, as best shot.

2.7.1. Talent inserting the needle bevel-up into the temporal vein and performing the injection.

2.7.2. Close-up of pushing the plunger to inject and pointing to the vascular filling .
Videographer: Zoom in through the magnifier and capture the minute details for this step

2.7.3. Talent applying gentle pressure to the injection site with a cotton swab.

2.8. Then, place the pup on a warmed recovery surface at approximately 37 degrees Celsius with a towel barrier [1]. Allow recovery until normal movement and pink coloration return [2].

2.8.1. Talent placing the pup onto a warmed recovery surface with a towel barrier.

2.8.2. Shot of pup regaining normal movement and pink coloration. **NOTE: 2.8.2 and 2.9.1 are combined as 2.9 monitoring step**

2.9. Monitor the pup for 5 to 10 minutes after recovery [1] and return the pup to the dam [2].

2.9.1. Talent observing the pup during the monitoring period.

2.9.2. Talent placing the pup back with the dam.

2.10. Rinse the syringe with PBS followed by a syringe cleaning solution [1], and finally with 70 percent ethanol [2].

2.10.1. Talent rinsing the syringe sequentially with phosphate-buffered saline and cleaning solution.

2.10.2. Talent rinsing the syringe with 70 percent ethanol. **Author's NOTE: We took three shots for three washes; PBS, Cleaning Solution, and ethanol (2.10.1, 2.10.2 and 2.10.3)**

3. Bioluminescence Imaging (BLI) of Injected Pup

Demonstrator: Maryam Aletha

3.1. Prepare the pup for Bioluminescence Imaging six days after the temporal vein injection [1].

3.1.1. Talent placing the pup on the work desk.

3.2. Retrieve an aliquot of filter-sterilized D-Luciferin from the freezer [1-TXT].

3.2.1. Talent taking out the tube with D-Luciferin from the freezer. **TXT: D-Luciferin: 10 µg/µL in PBS**

3.3. Using a 30-gauge needle, inject the pup with 50 microliters of D-Luciferin intraperitoneally [1].

3.3.1. Talent performing intraperitoneal D-Luciferin injection on the pup with a syringe.

- 3.4. Next, place the anesthetized pup on a warmed imaging stage at approximately 37 degrees Celsius [1-TXT].
 - 3.4.1. Talent positioning anesthetized pup on a warmed imaging stage. **TXT: Anesthesia: Isoflurane; Induction: 3 - 4%; Maintenance: 1.5 - 2%**
- 3.5. Ten minutes after the injection, acquire bioluminescence and X-ray images using consistent exposure settings [1]. Set the exposure time to 120 seconds, use medium binning, and set the X-ray power level to High [2].
 - 3.5.1. Talent opening the imaging software.
 - 3.5.2. SCREEN: SCREEN.mp4 00:00-00:01 *Video editor: Highlight 'Exposure = 120', 'Binning – Medium (4)' and 'X ray power – High' at the bottom of the software when the Vo mentions the respective terms*
- 3.6. Intraperitoneally inject postnatal day 7 pups with murine cytomegalovirus or mCMV (M-C-M-V) six days after temporal vein transplantation of cancer cells [1-TXT].
 - 3.6.1. Representative shot of IP injecting virus into the P7 pup. **TXT: mCMV: 1×10^4 PFU in 50 μ L PBS; Control: PBS** *Video editor: Show a long cut here to indicate the next step is performed later*
- 3.7. Repeat the Bioluminescence Imaging procedure 10 days post-viral injection to evaluate the effect of infection-driven immune activation on tumor progression [1]. Then, define whole-body regions of interest for each mouse using bioluminescence imaging software [2] and quantify the emitted light [3].
 - 3.7.1. SCREEN: SCREEN.mp4 00:02-00:06.
 - 3.7.2. SCREEN: SCREEN.mp4 00:06-00:16.
 - 3.7.3. SCREEN: SCREEN.mp4 00:17-00:20. *Video editor: Highlight the 'Mean Rad' column in the wide box popup*
- 3.8. Finally, export the quantified region of interest values for downstream statistical analysis [1].
 - 3.8.1. SCREEN: SCREEN.mp4 00:21-00:23.

Results

4. Results

- 4.1. Bioluminescence imaging combined with X-ray imaging confirmed successful engraftment of leukemic cells [1] and neuroblastoma cells at postnatal day 7, revealing that temporal vein injection achieved 100% procedural success [2].
 - 4.1.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the blue/green bioluminescent regions in all mice*
 - 4.1.2. LAB MEDIA: Figure 3A. *Video editor: Highlight the blue/green bioluminescent regions in all mice*
- 4.2. mCMV-infected mice showed a significant reduction in leukemia burden at postnatal day 17 [1] compared with control mice [2].
 - 4.2.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the blue/green bioluminescent regions in all mice labeled mCMV*
 - 4.2.2. LAB MEDIA: Figure 2B. *Video editor: Highlight the blue/green/yellow/red coloured signals in Cnt mice*
- 4.3. Quantification of bioluminescence signal demonstrated a significant reduction in leukemic burden in mCMV-infected mice [1] compared with controls at postnatal day 17 [2].
 - 4.3.1. LAB MEDIA: Figure 2C. *Video editor: Highlight the red squares at P 17 for mCMV*
 - 4.3.2. LAB MEDIA: Figure 2C. *Video editor: Highlight the blue circles at P 17*
- 4.4. Flow cytometric quantification showed significantly fewer leukemic cells in the spleen and bone marrow of mCMV-treated mice [1] compared with controls [2].
 - 4.4.1. LAB MEDIA: Figure 2E *Video editor: Highlight the red points*
 - 4.4.2. LAB MEDIA: Figure 2E. *Video editor: Highlight the blue points*
- 4.5. In contrast, the neuroblastoma burden at postnatal day 17 was comparable between mCMV-infected mice and control mice [1].
 - 4.5.1. LAB MEDIA: Figure 3B and C. *Video editor: Highlight the mCMV and control groups.*

1. Luciferase

Pronunciation link: https://www.merriam-webster.com/dictionary/luciferase

IPA: /'lu:səfəˌreɪs/

Phonetic Spelling: loo·suh·fuh·rays

2. Leukemic

Pronunciation link: https://www.merriam-webster.com/dictionary/leukemic

IPA: /lu'ki:mɪk/

Phonetic Spelling: loo·kee·mik

3. Neuroblastoma

Pronunciation link: https://www.merriam-webster.com/dictionary/neuroblastoma

IPA: /ˌnʊrəʊblæ'stoʊmə/, /ˌnjʊrəʊblæ'stoʊmə/

Phonetic Spelling: noo·roh·blas·toh·muh

4. Microliter

Pronunciation link: https://www.merriam-webster.com/dictionary/microliter

IPA: /'maɪkroʊˌli:tər/

Phonetic Spelling: my·kroh·lee·ter

5. Plexiglass

Pronunciation link: https://www.merriam-webster.com/dictionary/plexiglass

IPA: /'pleksi,glæs/

Phonetic Spelling: plek·see·glas

6. Hypothermia

Pronunciation link: https://www.merriam-webster.com/dictionary/hypothermia

IPA: /,haɪpə'θɜːmiə/

Phonetic Spelling: hy·puh·thur·mee·uh

7. Temporal

Pronunciation link: https://www.merriam-webster.com/dictionary/temporal

IPA: /'tempərəl/

Phonetic Spelling: tem·pruhl

8. Intravenous

Pronunciation link: https://www.merriam-webster.com/dictionary/intravenous

IPA: /,ɪntrə'viːnəs/

Phonetic Spelling: in·truh·vee·nuhs

9. Bioluminescence

Pronunciation link: https://www.merriam-webster.com/dictionary/bioluminescence

IPA: /,baɪoʊˌluːmə'nesəns/

Phonetic Spelling: by·oh·loo·muh·nes·uhns

10. Aliquot

Pronunciation link: [https://www.merriam-

webster.com/dictionary/aliquot](https://www.merriam-webster.com/dictionary/aliquot)

IPA: /'æli,kwɑ:t/

Phonetic Spelling: al·ih·kwaht

11. D-Luciferin

Pronunciation link: No confirmed link found

IPA: /di: lu: 'sɪfərɪn/

Phonetic Spelling: dee loo·sif·uh·rin

12. Intraperitoneally

Pronunciation link: https://www.merriam-webster.com/dictionary/intraperitoneal

IPA: /,ɪnrəˌperɪtə'ni:əli/

Phonetic Spelling: in·truh·peh·ruh·tuh·nee·uh·lee

13. Isoflurane

Pronunciation link: https://www.merriam-webster.com/medical/isoflurane

IPA: /,aɪsoʊ'flʊreɪn/

Phonetic Spelling: eye·soh·floor·ayn

14. Cytomegalovirus

Pronunciation link: https://www.merriam-webster.com/medical/cytomegalovirus

IPA: /,saɪtoʊˌmegələ'vaɪrəs/

Phonetic Spelling: sy·toh·meg·uh·luh·vy·ruhs

15. Engraftment

Pronunciation link: https://www.merriam-webster.com/medical/engraftment

IPA: /ɪnˈgræftmənt/

Phonetic Spelling: in-graft-muhnt

16. Quantification

Pronunciation link: https://www.merriam-webster.com/dictionary/quantification

IPA: /ˌkwɑːntəfəˈkeɪʃən/

Phonetic Spelling: kwon-tuh-fuh-kay-shuhn

17. Flow Cytometric

Pronunciation link: https://www.merriam-webster.com/medical/cytometry

IPA: /ˌfloʊ ˌsaɪtəˈmetrɪk/

Phonetic Spelling: floh sy-tuh-me-trik

18. Phosphate-buffered

Pronunciation link: https://www.merriam-webster.com/dictionary/phosphate

IPA: /ˈfɑːsfet ˈbʌfərd/

Phonetic Spelling: fos-fayt buf-erd