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

### **Authors and Affiliations:**

**Maryam Aletaha<sup>1</sup>, Zahra Savoji<sup>1</sup>, Nakaw Young<sup>1</sup>, Gregor S. D. Reid<sup>1,2</sup>, Ali Farrokhi<sup>1,2</sup>**

<sup>1</sup>Michael Cuccione Childhood Cancer Research Program, BC Children's Hospital Research Institute

<sup>2</sup>Department of Pediatrics, University of British Columbia

### **Corresponding Authors:**

Ali Farrokhi                      farrokhi@mail.ubc.ca;afarrokhi@bcchr.ca

### **Email Addresses for All Authors:**

|                   |                                         |
|-------------------|-----------------------------------------|
| Maryam Aletaha    | maletaha@student.ubc.ca                 |
| Zahra Savoji      | zsavoji@student.ubc.ca                  |
| Nakaw Young       | nakawyoung@gmail.com                    |
| Gregor S. D. Reid | gregor.reid@ubc.ca                      |
| Ali Farrokhi      | farrokhi@mail.ubc.ca;afarrokhi@bcchr.ca |

## **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

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*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

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*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 $\mu$ -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  $1 \times 10^4$  leukemic cells or  $5 \times 10^4$  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:  $1 \times 10^4$  PFU in 50  $\mu$ 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-TXT]. 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. **TXT: Set the exposure time to 30 s**
- 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

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

IPA: /,haɪpə'tθɜːmiə/

Phonetic Spelling: hy·puh·thur·mee·uh

#### 7. Temporal

Pronunciation link: [https://www.merriam-webster.com/dictionary/temporal](https://www.merriam-webster.com/dictionary/temporal)

IPA: /'tempərəl/

Phonetic Spelling: tem·pruhl

#### 8. Intravenous

Pronunciation link: [https://www.merriam-webster.com/dictionary/intravenous](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](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](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](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](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](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](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](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](https://www.merriam-webster.com/dictionary/phosphate)

IPA: /ˈfɑːsfet ˈbʌfərd/

Phonetic Spelling: fos-fayt buf-erd