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# Title: Murine Model of Leukemia Relapse to Induction Chemotherapy for Acute Lymphoblastic Leukemia

**Authors and Affiliations:**
**Manuella Munuera Hoff[1,2], Juliana Ronchi Corrêa[1], Samara de Sousa Mariano[1], Amilcar Cardoso[3], Silvia Regina Brandalise[3], José Andrés Yunes[1,3,4]**

[1]Leukemia Biology Laboratory, Boldrini Children's Center
[2]Graduate Program in Genetics and Molecular Biology, University of Campinas
[3]Boldrini Children's Center
[4]Department of Translational Medicine, Faculty of Medical Sciences, University of Campinas

**Corresponding Authors:**
José Andrés Yunes andres@boldrini.org.br

**Email Addresses for All Authors:**
Manuella Munuera Hoff manumunuerahoff@gmail.com
Juliana Ronchi Corrêa juliana.ronchi@gmail.com
Samara de Sousa Mariano samarasouzama@gmail.com
Amilcar Cardoso amilcar.cardoso@boldrini.org.br
Silvia Regina Brandalise silvia@boldrini.org.br
José Andrés Yunes andres@boldrini.org.br

# 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.**

**Current Protocol Length**
Number of Steps: 25
Number of Shots: 46

# Introduction

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

1.1. **Manuella Munuera Hoff:** Our research establishes a B-ALL PDX relapse model in mice to investigate how leukemic cells acquire drug resistance. We hope to uncover mechanisms driving relapse that may guide future therapeutic strategies.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What are the current experimental challenges?

1.2. **José Andrés Yunes:** Our main challenge is the lack of an immune system in NSG mice, limiting leukemia–immune interactions. A potential solution is adapting the protocol to murine leukemia in immunocompetent hosts.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1*

What research gap are you addressing with your protocol?

1.3. **Manuella Munuera Hoff:** Our study addresses gaps by enabling exploration of chemotherapy-resistant cells while under treatment in vivo, capturing dynamic resistance evolution beyond MRD.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.1.1*

What advantage does your protocol offer compared to other techniques?

1.4. **Manuella Munuera Hoff:** Our protocol generates human pediatric ALL resistant cells in vivo, using PDX models to mimic full clinical relapse cycles, enabling access to relapse biology often unattainable through in vitro systems.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.2.1*

What new scientific questions have your results paved the way for?

1.5. **José Andrés Yunes:** Our model paves the way to explore genetic and epigenetic drivers of relapse, microenvironmental influences on resistance, patient-specific variability, and to test novel therapies, including immunotherapies, against relapsed B-ALL.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.8.1*

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

**Ethics Title Card**

This research has been approved by the Institutional Ethics Committee at Comissão de Ética no Uso de Animais from Centro Infantil Boldrini (CEUA/Boldrini 0047-2024)

# Protocol

## 2. Preparation of Leukemia Samples and Mouse Transplantation

**Demonstrator:** Manuella Munuera Hoff

2.1. To begin, thaw cryopreserved acute lymphoblastic leukemia cells that were obtained from the patient's bone marrow aspirate **[1]**.

2.1.1. WIDE: Talent holding a cryovial and placing it in a water bath to thaw.

2.2. Transfer the thawed sample into a 15-milliliter centrifuge tube **[1]** and add 14 milliliters of sterile PBS to wash the cells off the cryopreservation solution **[2]**. Place the tube into a centrifuge and spin at 300 *g* for 5 minutes **[3]**. Then, carefully remove and discard the supernatant without disturbing the pellet **[4]**.

2.2.1. Talent pipetting the thawed cell suspension into a 15 milliliter centrifuge tube.

2.2.2. Talent pipetting PBS into the tube.

2.2.3. Talent placing the tube into the centrifuge and closing the lid.

2.2.4. Talent aspirating and discarding the supernatant from the centrifuge tube.

2.3. Resuspend the cell pellet in sterile PBS to obtain around 5 to 10 million viable cells in 100 microliters for each animal **[1]**. Keep the prepared transplant solution on ice until transplantation **[2]**.

2.3.1. Talent pipetting PBS into the centrifuge tube and resuspending the pellet.

2.3.2. Close-up of the prepared transplant solution tube being placed on ice.

2.4. For transplantation, select 2-month-old male or female NSG *(N-S-G)* mice for leukemia transplantation **[1]**.

2.4.1. Talent opening a cage and selecting a healthy 2-month-old NSG mouse.

2.5. Place one mouse at a time in a secure and approved mouse restrainer, ensuring the tail remains outside and easily accessible for transplantation **[1]**.

2.5.1. Talent carefully securing a mouse into the restrainer, leaving the tail exposed.

2.6. Now, position the restrained mouse 30 centimeters from an infrared light source to ensure proper vein expansion **[1]**. Adjust the exposure time based on the power of the lamp, keeping the tail exposed to medium power light for 5 minutes **[2-TXT]**.

2.6.1. Talent positioning the mouse in front of the infrared light at the specified distance.

2.6.2. Close-up shot of the mouse tail illuminated by infrared light. **TXT: Avoid overexposure to prevent burns and stress**

2.7. Next, using an insulin syringe with an attached 12.7 by 0.33-millimeter needle, inject 100 microliters of the transplant solution into the tail vein **[1]**. After injection, place the transplanted mouse into a specific pathogen-free facility and allow it to rest for 15 days **[2]**.

2.7.1. Talent performing the injection with the insulin syringe into the tail vein of the restrained mouse.

2.7.2. Talent transferring the injected mouse into a clean cage inside the specific pathogen-free facility.

## 3. Peripheral Blood Collection and Processing for Flow Cytometry

3.1. Retrieve the animal for blood collection and restrain it securely by holding its head **[1]**. With the right hand, puncture the facial vein of the submandibular plexus using a sterile, sharp, disposable lancet **[2]**.

3.1.1. Talent demonstrating manual restraint of the mouse with the left hand, holding the head firmly.

3.1.2. Talent puncturing the facial vein with a lancet and blood emerging.

3.2. Collect 50 microliters of peripheral blood from each mouse in a 1.5 milliter centrifuge tube containing 8 microliters of 50 millimolar EDTA *(E-D-T-A)* 2 weeks after inoculation ~~and then once per week thereafter~~ **[1-TXT]**. After mixing, pipette 50 microliters of the sample into a flow cytometry tube **[2]**.
**NOTE: Shot 3.3.2 moved after 3.2.1. The VO has also been moved**

3.2.1. Shot of blood being collected in a 1.5 L centrifuge tube containing 8 µL EDTA. **TXT: Collect blood once a week; Pipette 50 µL of mixture into a flow cytometry tube**

3.3.2 : Talent transferring the homogenized blood mixture into a flow cytometry tube.

3.3. ~~Place the collected blood sample into a 1.5 milliliter centrifuge tube containing 8 microliters of 50 millimolar EDTA to prevent coagulation **[1]**. After mixing, pipette 50 microliters of the sample into a flow cytometry tube **[2]**.~~

**NOTE: Shot 3.3.2 moved after 3.2.1. The VO has also been moved**

3.3.1. ~~Talent transferring the blood into a labeled centrifuge tube preloaded with ethylenediaminetetraacetic acid~~.

3.3.2. Talent transferring the homogenized blood mixture into a flow cytometry tube.

3.4. Prepare an antibody mixture consisting of anti-mouse CD45 *(C-D-Forty-five)* antibody, anti-human CD45 antibody, anti-human CD19 *(C-D-Nineteen)* antibody, and sterile PBS as the diluent **[1]**.

3.4.1. Talent inverting a tube containing antibody cocktail.

3.5. Add 10 microliters of the antibody mixture to each blood sample **[1]** and gently tap the tube with the index finger to homogenize the sample **[2]**. Incubate the samples for 30 minutes at room temperature while shielding them from light **[3-TXT]**.

3.5.1. Talent pipetting antibody mixture into a flow cytometry tube containing blood.

3.5.2. Talent tapping the tube gently with an index finger to mix.

3.5.3. Close-up shot of tubes wrapped in foil ~~being kept in a dark chamber~~. **TXT: Lyse the RBCs**

3.6. After washing the leukemia cells with PBS, resuspend them with 200 microliters of sterile PBS by gently tapping the cytometry tube with the index finger **[1]**.

3.6.1. Talent resuspending cells by tapping the cytometry tube filled with PBS.

3.7. Employ standard flow cytometry methodologies to determine the percentage of human CD-45 positive cells in the peripheral blood relative to mouse CD45 positive cells **[1]**.

3.7.1. Talent operating the flow cytometer.

## 4. Drug Administration an Recovery of Leukemic Cells

4.1. Initiate treatment when the percentage of human CD45-positive cells in the peripheral blood reaches a median range between 0.2 percent and 1 percent **[1]**.

4.1.1. Talent picking up a mouse from its cage.

4.2. Weigh the animals designated for treatment **[1]** and calculate the arithmetic mean of the weights to determine the appropriate dose of each drug for the group **[2]**.

4.2.1. Talent placing a mice onto a digital scale and recording weights.

4.2.2. Talent making some calculations and writing down in a notebook.

4.3. Now, manually restrain the animal with the left hand, keeping the ventral portion facing upward **[1]**. Locate the lower right quadrant of the abdomen **[2]**. Using a sterile, sharp, single-use injection needle, inject 100 microliters of the drug solution into the lower right quadrant intraperitoneally **[3-TXT]**.

4.3.1. Talent restraining the mouse securely with its ventral portion facing up.

4.3.2. Close-up shot of the talent identifying the lower right quadrant of the abdomen.

4.3.3. Talent injecting the drug administration solution intraperitoneally with a sterile needle. **TXT: Allow mice to rest for 4 weeks**

4.4. To recover the leukemic cells, use sterile surgical scissors and tweezers to make a small incision in the animal's abdominal skin and remove it **[1]**.

4.4.1. Talent making a precise incision with surgical scissors and retracting the skin using tweezers to reveal the peritoneum.

4.5. Make a small incision in the peritoneum **[1]** and extend the incision until there is full access to the abdominal organs **[2]**.

4.5.1. Talent cutting the peritoneum with scissors.

4.5.2. Shot of the exposed the abdominal cavity.

4.6. Locate the spleen beneath the stomach **[1]** and remove it carefully using tweezers **[2].** Place the extracted spleen in a container with sterile PBS for further processing **[3]**.

4.6.1. Talent pointing to the spleen in the abdominal cavity.

4.6.2. Talent extracting the spleen with tweezers.

4.6.3. Talent placing the harvested spleen into a sterile container with PBS.

4.7. For femur harvesting, open the peritoneal cavity to gain access to the hind legs of the animal **[1]** and locate the femur bone **[2].**

4.7.1. Talent making a careful incision and retracting tissue to expose the hind legs.

4.7.2. Talent pointing to the femur inside the hind leg.

4.8. Using scissors, cut at the joints that connect the femur with the pelvis and with the tibia and fibula to remove it **[1-TXT]**.

4.8.1. Talent cutting at the joints to release the femur. **TXT: Macerate the organs on a cell strainer to harvest cells**

4.9. Place the harvested cells in a 15-milliliter centrifuge tube **[1]** and spin at 420 *g* for 30 minutes using a swing bucket rotor without acceleration and without brake to preserve the separation of phases **[2]**.

4.9.1. Talent loading the 15 milliliter tube with cell suspension.

4.9.2. Talent placing the tube into the centrifuge.

4.10. Using a sterile disposable Pasteur pipette, carefully remove and discard the top aqueous phase **[1]**. With a new sterile disposable Pasteur pipette, collect the leukemic cell layer that has formed **[2]** into a fresh 15 milliliter centrifuge tube **[3]**.

4.10.1. Talent pipetting and discarding the top aqueous phase into a waste container.

4.10.2. Talent using a pipette to collect the distinct leukemic cell layer.

4.10.3. Talent adding the collected leukemic cells into a clean 15 milliliter centrifuge tube.

4.11. Finally, cryopreserve the isolated leukemic cells following standard cell culture cryopreservation guidelines **[1]**.

4.11.1. Talent placing the cell tube in a freezer or liquid nitrogen container.

# Results

## 5. Results

5.1. In the mouse model transplanted with leukemic cells from good responder patients, VXLD *(V-X-L-D)* treatment induced remission **[1-TXT]**, but with repeated cycles the interval between remission and relapse became progressively shorter, indicating drug resistance **[2]**.

5.1.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the blue treatment line between 50 and 100 on X-axis.* **TXT: VXLD: Vincristine, Dexamethasone, L-Asparaginase, and Daunorubicin**

5.1.2. LAB MEDIA: Figure 2A. *Video editor: Highlight the blue treatment line that rises between 100 and 150 on X-axis*.

5.2. In the mouse model transplanted with leukemic cells from poor responder patients, VXLD treatment failed to induce remission and only slowed disease progression for 3 weeks before leukemic load exceeded tolerable limits, demonstrating refractoriness **[1]**.

5.2.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the blue treatment line*

5.3. In another poorly responding leukemia, VXLD treatment temporarily induced remission **[1]**, but relapse occurred rapidly after a single rest week without treatment, indicating short-lived remission **[2]**.

5.3.1. LAB MEDIA: Figure 2C. *Video editor: Highlight the blue treatment line in the left graph*.

5.3.2. LAB MEDIA: Figure 2C. *Video editor: Highlight the blue treatment line in the right graph.*

PRONUNCIATION GUIDE:

**1. Murine**
Pronunciation link:
https://www.merriam-webster.com/dictionary/murine
IPA: /ˈmjʊraɪn/
Phonetic Spelling: myoo-rine

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**2. Leukemia**
Pronunciation link:
https://www.merriam-webster.com/dictionary/leukemia
IPA: /luˈkiːmiə/
Phonetic Spelling: loo-kee-mee-uh

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**3. Lymphoblastic**
Pronunciation link:
https://www.merriam-webster.com/dictionary/lymphoblast
IPA: /ˌlɪmfəˈblæstɪk/
Phonetic Spelling: lim-foh-blas-tik

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**4. Relapse**
Pronunciation link:
https://www.merriam-webster.com/dictionary/relapse
IPA: /ˈriːˌlæps/ (noun), /rɪˈlæps/ (verb)
Phonetic Spelling: ree-laps (noun), ri-laps (verb)

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**5. Induction (Chemotherapy context)**
Pronunciation link:
https://www.merriam-webster.com/dictionary/induction
IPA: /ɪnˈdʌkʃən/
Phonetic Spelling: in-duhk-shun

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**6. Immunocompetent**
Pronunciation link:
https://www.merriam-webster.com/dictionary/immunocompetent
IPA: /ˌɪm.jə.noʊˈkɑːmpɪtənt/
Phonetic Spelling: im-yoo-noh-kom-pi-tuhnt

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**7. Immunotherapies**
Pronunciation link:
https://www.merriam-webster.com/dictionary/immunotherapy
IPA: /ˌɪm.jə.noʊˈθɛrəpi/
Phonetic Spelling: im-yoo-noh-theh-ruh-pee

**8. Cryopreserved**
Pronunciation link:
https://www.merriam-webster.com/dictionary/cryopreserve
IPA: /ˌkraɪoʊprɪˈzɝːvd/
Phonetic Spelling: cry-oh-pre-zervd

**9. Centrifuge**
Pronunciation link:
https://www.merriam-webster.com/dictionary/centrifuge
IPA: /ˈsɛntrəˌfjuːdʒ/
Phonetic Spelling: sen-truh-fyooj

**10. Supernatant**
Pronunciation link:
https://www.merriam-webster.com/dictionary/supernatant
IPA: /ˈsuːpərˌneɪtənt/
Phonetic Spelling: soo-per-nay-tuhnt

**11. Pellet (biological context)**
Pronunciation link:
https://www.merriam-webster.com/dictionary/pellet
IPA: /ˈpɛlɪt/
Phonetic Spelling: peh-lit

**12. NSG (NOD scid gamma) mice**
Pronunciation link:
No confirmed link found
IPA: /ɛn ɛs ˈdʒiː maɪs/
Phonetic Spelling: N-S-G mice

**13. Submandibular**
Pronunciation link:
https://www.merriam-webster.com/dictionary/submandibular
IPA: /ˌsʌbˈmændjələr/
Phonetic Spelling: sub-man-juh-lur

**14. Plexus**
Pronunciation link:
https://www.merriam-webster.com/dictionary/plexus
IPA: /ˈplɛksəs/
Phonetic Spelling: plek-suhs

**15. Ethylenediaminetetraacetic acid (EDTA)**
Pronunciation link:
https://www.merriam-webster.com/dictionary/EDTA
IPA: /ˌɛθəˌliːndiˈæmɪnˌtɛtrəoʊˈsiːtɪk ˈæsɪd/
Phonetic Spelling: eth-uh-leen-dye-am-in-tet-ruh-oh-see-tik ass-id

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**16. Flow Cytometry**
Pronunciation link:
https://www.merriam-webster.com/dictionary/cytometry
IPA: /floʊ saɪˈtɑːmətri/
Phonetic Spelling: floh sigh-tom-uh-tree

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**17. Intraperitoneally**
Pronunciation link:
https://www.merriam-webster.com/dictionary/peritoneal
IPA: /ˌɪntrəˌpɛrɪtoʊˈniəli/
Phonetic Spelling: in-truh-peh-ri-toh-nee-uh-lee

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**18. Peritoneum**
Pronunciation link:
https://www.merriam-webster.com/dictionary/peritoneum
IPA: /ˌpɛrətnˈiəm/
Phonetic Spelling: peh-rih-tee-um

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**19. Spleen**
Pronunciation link:
https://www.merriam-webster.com/dictionary/spleen
IPA: /spliːn/
Phonetic Spelling: spleen

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**20. Femur**
Pronunciation link:
https://www.merriam-webster.com/dictionary/femur
IPA: /ˈfiːmər/
Phonetic Spelling: fee-mer

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**21. Tibia**
Pronunciation link:
https://www.merriam-webster.com/dictionary/tibia
IPA: /ˈtɪbiə/
Phonetic Spelling: tib-ee-uh

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**22. Fibula**

Pronunciation link:
https://www.merriam-webster.com/dictionary/fibula
IPA: /ˈfɪbjələ/
Phonetic Spelling: fib-yuh-luh

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**23. Pasteur pipette**
Pronunciation link:
https://www.merriam-webster.com/dictionary/Pasteur%20pipette
IPA: /ˈpæstər paɪˈpɛt/
Phonetic Spelling: pass-ter pie-pet

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**24. Refractoriness (medical context)**
Pronunciation link:
https://www.merriam-webster.com/dictionary/refractory
IPA: /rɪˈfræktərinəs/
Phonetic Spelling: ri-frak-tuh-ree-ness