FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 68013
Scriptwriter Name: Debopriya Sadhukhan
Project Page Link: https://review.jove.com/account/file-uploader?src=20744013

Title: Studying Interactions Between Myeloid Cells and CAR T Cells In Vitro and In Vivo

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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**

   Can you record movies/images using your own microscope camera?

   No

   OLYMPUS CKX53

   If a dissection or stereo microscope is required for your protocol, please list all shots from the script that will be visualized using the microscope (shots are indicated with the 3-digit numbers, like 2.1.1, 2.1.2, etc.).

   2.3.2

   NOTE: Videographer has filmed the scope shots

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? **Yes**

   How far apart are the locations? **From Floor 13 to floor 20**.

Current Protocol Length
Number of Steps: 14
Number of Shots: 33

Introduction

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

1.1. **Saad S. Kenderian:** Our study provides simplified *in vitro* and *in vivo* models to understand the impacts of human immunosuppressive macrophages on CART19 antitumor activities in the context of mantle cell lymphoma.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.2., 3.3.1.*

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

1.2. **R. Leo Sakemura:** The commonly used technology to study human macrophages or monocytes in mice is to engraft immunodeficient mice with human hematopoietic stem cells after irradiation.

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

What are the current experimental challenges?

1.3. **Kun Yun:** Engrafting immunodeficient mice with human hematopoietic stem cells to study human myeloid cells in mice can be time-consuming and expensive. The engraftment efficiency can be limited as well.

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

What research gap are you addressing with your protocol?

1.4. **Brooke Kimball:** We established more simplified in vitro and in vivo models to study interactions between human macrophages, CART, and tumor cells. It can be used to test other macrophage-targeted immunotherapies as well.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 2.*

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

Ethics Title Card

This research follows the guidelines of Mayo Clinic's Institutional Review Board (IRB), Institutional Biosafety Committee (IBC), and was approved by the Department of Comparative Medicine's Institutional Animal Care and Use Committee

2. Establishment of Xenograft Model with Both Human Macrophages and Tumor in NSG Mice

Demonstrators: Truc N. Huynh and Kun Yun

2.1. To begin, centrifuge the isolated 10 million human classical monocytes at 300 *g* for 5 minutes at 4 degrees Celsius **[1-TXT]**. Aspirate the supernatant **[2]** and resuspend the cells in culture media to a final concentration of 1 million cells per milliliter **[3]**.

2.1.1. WIDE: Talent placing tubes with monocytes into a centrifuge. **TXT: Confirm the purity of monocytes by flow cytometry before this step**

2.1.2. Talent aspirating supernatant.

2.1.3. Talent resuspending cells in culture media.

2.2. Add human recombinant GM-CSF *(G-M C-S-F)* to the suspension to reach a final concentration of 10 nanograms per milliliter and mix thoroughly **[1]**. Transfer the monocytes into a T25 *(T-twenty-five)* tissue culture flask **[2]** and incubate them at 37 degrees Celsius for 7 days **[3]**.

2.2.1. Talent pipetting GM-CSF into the cell suspension and mixing.

2.2.2. Talent transferring the mixture to a T25 flask.

2.2.3. Talent placing the flask into a 37 degrees Celsius incubator.

2.3. On day 7, pipette the cells vigorously on ice every 10 minutes **[1]**, checking under a microscope until most macrophages detach **[2]**. Transfer the detached cells into a 15 milliliter conical tube on ice **[3]**. Then, wash the cells with ice-cold PBS to loosen the remaining cells **[4]** and combine them in the conical tube **[5]**.

2.3.1. Talent pipetting the cell suspension on ice.

2.3.2. SCOPE: A microscope shot of the detached macrophages.

2.3.3. alent transferring detached macrophages into a conical tube placed on ice.

2.3.4. Talent rinsing the cells with ice-cold PBS.

2.3.5. Talent mixing the contents in the conical tube.

2.4. Count the cells using an automated cell counter **[1]** and centrifuge them at 300 *g* for 5 minutes at 4 degrees Celsius **[2]**.

2.4.1. Talent loading cells into an automated cell counter.

2.4.2. Talent placing the tube in a centrifuge.

2.5. Aspirate the supernatant **[1]** and then wash and resuspend the pellet with 5 milliliters of ice-cold PBS **[2-TXT]**.

2.5.1. Talent aspirating the supernatant.

2.5.2. Talent adding PBS to the tube and resuspending the pellet. **TXT: Repeat centrifugation and aspirate the supernatant**

2.6. After centrifuging the tube again and aspirating the supernatant, resuspend the final cell pellet in ice-cold PBS to reach a concentration of 20 million cells per milliliter **[1]**. Transfer the solution to a 1.5 milliliter microcentrifuge tube **[2]** and keep it on ice **[3]**.

2.6.1. Talent resuspending the cell pellet to achieve the desired concentration.

2.6.2. Talent transferring cell suspension into a microcentrifuge tube.

2.6.3. Talent placing the tube on ice.

2.7. Next, transfer the prepared 15 million luciferase-positive JeKo *(Jee-koh)* cells into a 15-milliliter conical tube **[1]**.

2.7.1. Talent pipetting JeKo-1 cells into a conical tube from a culture flask.

2.8. After centrifuging the JeKo cells, aspirate the supernatant **[1-TXT]**. Resuspend the pellet in ice-cold PBS to a concentration of 40 million cells per milliliter **[2]**.

2.8.1. Talent removing supernatant from the JeKo-1 cells tube (after centrifugation). **TXT: 5 min, 300 x *g*, 4 ^{o}C**

2.8.2. Talent resuspending the cells in PBS.

2.9. Now, mix the same volumes of the macrophage suspension and tumor cell suspension in a fresh 1.5-milliliter microcentrifuge tube **[1]**. Add the same volume of solubilized basement membrane matrix and mix thoroughly on ice **[2-TXT]**.

2.9.1. Talent combining macrophage and tumor cell suspensions in a tube.

2.9.2. Talent adding basement membrane matrix and mixing on ice. **TXT: Thaw the basement membrane matrix on ice overnight**

2.10. As a control, mix the remaining JeKo cells with the same volume of PBS in a new microcentrifuge tube **[1]**. Add the same volume of solubilized basement membrane matrix and mix on ice **[2]**.

2.10.1. Talent preparing the control tube with JeKo-1 and PBS.

2.10.2. Talent adding solubilized basement membrane matrix to the control and mixing while keeping the tube on ice.

3. Subcutaneous Cell Injection in Anesthetized NSG Mice

Demonstrator: Brooke Kimball

3.1. After anesthetizing the mice, place them on a heated stage with nose cones **[1-TXT]**. Apply an ophthalmic ointment to both eyes of the mice to prevent corneal damage **[2]**.

3.1.1. Talent placing an anesthetized mouse on a heated stage with nose cones. **TXT: Prepare ten 6 - 8-week-old NSG mice (~20 g); Only 1 mouse is used for demonstration; Anesthesia: 2.5% Isoflurane**

3.1.2. Talent applying ointment to the mouse's eyes.

3.2. Shave the right flank of each mouse to expose the skin **[1-TXT]**. Load 100 microliters of the JeKo-macrophage mixture into a 0.5 milliliter syringe and remove air bubbles **[2]**.

3.2.1. A shot of the shaved flank of the anesthetized mouse. **TXT: Confirm anesthesia by verifying the loss of postural and pedal reflexes**

3.2.2. Talent loading the cell mixture into syringes and tapping out bubbles.

3.3. Gently lift the exposed skin and insert the needle into the raised skin area while applying finger pressure to the site **[1-TXT]**.

3.3.1. Talent lifting mouse skin and inserting the needle carefully. **TXT: Avoid moving the needle once inserted to prevent punctures and cell leakage**

3.3.2. Talent maintaining needle position and stabilizing injection site.

3.4. Slowly inject the cells into the subcutaneous layer **[1]** and gently withdraw the needle **[2-TXT]**.

3.4.1. Talent slowly injecting the cell solution into the subcutaneous layer.

3.4.2. Talent withdrawing the needle. **TXT: Repeat the injection for all 10 mice; 5 mice receive both JeKo-1 and macrophages; 5 mice receive only JeKo-1**

Results

4. Results

4.1. To evaluate the in vivo impact of immunosuppressive macrophages, tumor burden progression was tracked by bioluminescence imaging in NSG mice engrafted with **[1]** JeKo cells alone **[2]** or in combination with differentiated macrophages **[3]**.

4.1.1. LAB MEDIA: Figure 6.

4.1.2. LAB MEDIA: Figure 6. *Video Editor: Highlight the blue curve.*

4.1.3. LAB MEDIA: Figure 6. *Video Editor: Highlight the red curve.*

4.2. The presence of differentiated macrophages markedly accelerated tumor progression in vivo, as indicated by a significantly greater tumor burden in co-engrafted mice by day 17 **[1]**, with a highly significant difference compared to JeKo alone **[2]**.

4.2.1. LAB MEDIA: Figure 6. *Video Editor: Highlight the red curve*.

4.2.2. LAB MEDIA: Figure 6. *Video Editor: Highlight the blue curve*.

Pronunciation guide:

1. Centrifuge

- **Pronunciation link**: (merriam-webster.com)
- **IPA**: /ˈsɛn.trəˌfjuːdʒ/
- **Phonetic Spelling**: SEN-truh-fyooj

2. Monocyte

- **Pronunciation link**: (merriam-webster.com)
- **IPA**: /ˈmɒn.əˌsaɪt/
- **Phonetic Spelling**: MON-uh-syte

3. Supernatant

- **Pronunciation link**: (merriam-webster.com)
- **IPA**: /ˌsuː.pərˈneɪ.tənt/
- **Phonetic Spelling**: SOO-pur-NAY-tuhnt

4. Resuspend

- **Pronunciation link**: (merriam-webster.com)
- **IPA**: /ˌriː.səˈspɛnd/
- **Phonetic Spelling**: REE-suh-SPEND

5. Recombinant

- **Pronunciation link**: (merriam-webster.com)
- **IPA**: /rɪˈkɒm.bɪ.nənt/
- **Phonetic Spelling**: ri-KOM-bih-nuhnt

6. GM-CSF

- **Pronunciation link**: (merriam-webster.com)
- **IPA**: /ˌdʒiːˌɛm ˌsiːˌɛsˈɛf/
- **Phonetic Spelling**: GEE-EM SEE-ES-EF

7. Macrophage

- **Pronunciation link**: (merriam-webster.com)
- **IPA**: /ˈmæk.rəˌfeɪdʒ/
- **Phonetic Spelling**: MAK-ruh-fayj

8. PBS

- **Pronunciation link**: (merriam-webster.com)
- **IPA**: /ˌpiː.biːˈɛs/
- **Phonetic Spelling**: PEE-BEE-ESS

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9. Subcutaneous

- **Pronunciation link**: (merriam-webster.com)
- **IPA**: /ˌsʌb.kjuːˈteɪ.ni.əs/
- **Phonetic Spelling**: SUB-kyoo-TAY-nee-uhs

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10. Ophthalmic

- **Pronunciation link**: (merriam-webster.com)
- **IPA**: /ɑːfˈθæl.mɪk/
- **Phonetic Spelling**: ahf-THAL-mik

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11. Bioluminescence

- **Pronunciation link**: (merriam-webster.com)
- **IPA**: /ˌbaɪ.oʊ.luː.mɪˈnɛs.əns/
- **Phonetic Spelling**: BY-oh-loo-mih-NESS-uhns