FINAL SCRIPT: APPROVED FOR FILMING

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Title: Enhancing Chimeric Antigen Receptor-Extracellular Vesicles (CAR-EV) Technology: The Future of Cancer Therapy

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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**
   SCREEN: 2.6.2,2.9.1, 2.11.1, 2.12.1, 2.13.1

   Videographer: Please capture the screen of the instrument for all shots labeled SCREEN

3. **Filming location:** Will the filming need to take place in multiple locations? **No.**

Current Protocol Length
Number of Steps: 12
Number of Shots: 24

Introduction

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

1.1. **Gregory Rice** : Our research focuses on developing exosome-based diagnostics and therapeutics. Our objective is the engineering of Chimeric Antigen Receptor - exosome therapeutics that are safer, and more effective and accessible than cell-based therapies in treating solid tumors.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.3*

What research gap are you addressing with your protocol?

1.2. **Kartini Asari:** Our EXO-NET and EXO-ACE platforms enable scalable, reproducible, and GMP-compliant exosome isolation with high yield and purity, addressing limitations of traditional methods for research and therapeutics.

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

What advantage does your protocol offer compared to other techniques?

1.3. **Sadman Bhuiyan:** EXO-NET and EXO-ACE enable rapid, scalable, and GMP-compliant EV isolation with superior purity and reproducibility, outperforming centrifugation and precipitation for research, diagnostics, and therapeutic applications.

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

How will your findings advance research in your field?

1.4. **Ramin Khanabdali:** We have developed a rapid, scalable and validate method to engineer CAR-EVs and deliver next-generation cell-free targeted therapeutics, providing a robust workflow that can be applied to other disease conditions where there is clinical need.

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

What research questions will your laboratory focus on in the future?

1.5. **Gregory Rice:** Our next goal is to use the methods that we have developed to generate CAR-EV-based, targeted RNA delivery for the treatment of cancer and other inflammatory diseases

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

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

2. Extracellular Vesicle Isolation and Enrichment Using Ion Exchange Chromatography and High-Throughput Pan-Exosome Capture

Demonstrator: Sadman Bhuiyan and Carlos Palma

2.1. To begin, bring the ion exchange column to room temperature by allowing it to stand upright for 15 minutes **[1]**. Using a pipette, add 10 column volumes of regeneration buffer **[2]**, followed by 10 column volumes of equilibration buffer **[3]**.

2.1.1. WIDE: Talent placing the column on the benchtop.

2.1.2. Talent pipetting 10 column volumes of regeneration buffer into the column.

2.1.3. Talent pipetting 10 column volumes of equilibration buffer into the column.

2.2. Pass the thawed conditioned medium through a 0.22-micrometer polyethersulfone filter **[1]**.

2.2.1. Talent passing the thawed conditioned medium through a 0.22 micrometer PES filter into a clean container.

2.3. Now, add up to 31.2 column volumes of the filtered sample into the equilibrated column **[1]**. Then add 10 column volumes of wash buffer **[2]**, followed by 2.5 column volumes of elution buffer **[3].** Collect the flow through containing the enriched extracellular vesicles **[4]**.

2.3.1. Talent loading the filtered sample into the ion exchange column using a pipette.

2.3.2. Talent adding 10 column volumes of wash buffer to the column.

2.3.3. Talent adding 2.5 column volumes of elution buffer.

2.3.4. Talent collecting the flow-through in a collection tube.

2.4. Using 30 kilodalton ultrafiltration centrifugal filters and a diluent, perform buffer exchange on the extracellular vesicle eluate, ensuring a minimum 100-fold dilution **[1]**. Adjust the final volume of the exchanged solution based on experimental needs **[2]**.

2.4.1. Talent pipetting extracellular vesicle eluate into the 30 kilodalton ultrafiltration device and adding diluent solution.

2.4.2. Talent adjusting the final volume after buffer exchange according to experimental requirements.

2.5. Pass the enriched extracellular vesicle sample through a 0.22-micrometer filter to sterilize it for *in vitro* assays **[1]**. Determine the size distribution, concentration, and yield of CAR *(car)* extracellular vesicles using nanoparticle tracking analysis **[2]**.

2.5.1. Talent filtering the extracellular vesicle sample through a 0.22 micrometer filter into a sterile container.

2.5.2. Talent loading the sample into the nanoparticle tracking instrument and initiating the analysis.

2.6. For high-throughput pan-exosome extracellular vesicle isolation, switch on and log into the instrument **[1].** Configure the settings to establish the **HT EV** *(H-T E-V)* **Protein Isolation Protocol [2-TXT]**.

2.6.1. Talent switching on and logging into the instrument.

2.6.2. SCREEN: Shot of the HT EV Protein Isolation Protocol being selected. **TXT: The system will request plate loading in reverse order**

Videographer: Please capture the screen of the instrument for this shot

2.7. Prepare a Tip Comb Plate by placing a tip comb into a 96-well deep well plate **[1]**. Add 1 milliliter of PBS into each well to prepare 3 Wash Plates **[2]**.

2.7.1. Talent placing a tip comb into a 96-well plate.

2.7.2. Talent pipetting 1 milliliter of phosphate-buffered saline into each well of three deep-well 'Wash plates.

2.8. Now prepare an Elution plate by pipetting 35 microliters of 1 percent SDS buffer into each well of a 96-well plate **[1]**. Then prepare a Binding plate by adding 1 milliliter of the conditioned medium **[2]**.

2.8.1. Talent pipetting 35 microliters of 1 percent SDS buffer into each well of a separate plate labeled "Elution".

2.8.2. Talent pipetting 1 milliliter of conditioned medium.

2.9. On the automated isolation system, begin the protocol by selecting the program **HT EV Protein Isolation Protocol** and select the **Play** button **[1]**. Open the instrument to begin loading the plates **[2]**. Follow the instrument prompts and load the prepared Elution plates, followed by the three Wash deep-well plates into the instrument **[3]**.

2.9.1. SCREEN: Shot of the HT EV Protein Isolation Protocol being clicked and the Play button being pressed.

Videographer: Please capture the screen of the instrument for this shot

2.9.2. Talent opening the instrument door.

2.9.3. Talent loading the plates into the high-throughput automated instrument.

2.10. Just prior to loading the Binding plate, add 30 microliters of pan-exosome capture beads into each well **[1]**. Load the Binding plate into the instrument **[2]**. Then, load the Tip Comb Plate **[3],** and close the instrument to initiate Binding **[4].** The system will mix the conditioned medium sample and magnetic beads continuously at slow speed for 30 minutes, facilitating the efficient capture of extracellular vesicles **[5].**

2.10.1. Talent adding 30 microliters of pan-exosome capture beads into each well of the 'Binding' plate.

2.10.2. Shot of the binding plate being loaded into the instrument.

2.10.3. Talent loading the 'tip well comb' plate into the instrument.

2.10.4. Talent closing the instrument door.

Added shot: Shot of system mixing the medium sample and magnetic beads.

2.11. Upon completion of the binding step, allow the system to perform three wash steps, mixing at a slow speed for 30 seconds to remove non-specific contaminants **[1-TXT].**

2.11.1. SCREEN: Shot of the instrument interface displaying "Wash 1", "wash 2", "Wash 3". **TXT" The system will execute 5 capture cycles of 30 s between each wash**
Videographer: Please capture the screen of the instrument for this shot

2.12. Allow the automated system to initiate extracellular vesicle lysis for protein recovery by performing a 30-second bottom mix **[1]**. During incubation, the instrument will pause for 7 minutes and 30 seconds while keeping the pipette tips positioned above each well **[2]**.

2.12.1. SCREEN: Shot of the system interface displaying 'EV Protein lysate', initiating 30-second bottom mixing.
Videographer: Please capture the screen of the instrument for this shot

2.12.2. Shot of the pipette tips positioned above the wells.

2.13. Initiate another 30-second mix followed by another 7-minute and 30-second pause, **[1-TXT]**. When the protocol concludes, the system will lift the used tip comb, leaving the solution in the 96-well plate and the magnetic beads attached to the tips **[2]**.

2.13.1. SCREEN: Shot of the mixing being initiated on the system. **TXT: The system will perform 3 cycles of capture and release, prior to bead removal from the final EV lysate**
Videographer: Please capture the screen of the instrument for this shot

2.13.2. Shot of the tip comb being lifted off the plate.

2.14. Finally, the instrument will proceed to the Leave step, returning the Tip Comb into the Tip Comb Plate **[1]**. Open the instrument and remove the plates **[2]**.

2.14.1. Shot of the tip comb being returned into the tip comb plate.

2.14.2. Talent opening the instrument cover and removing the used tip comb, binding, wash and elution plates.

Results

3. Results

3.1. GFP *(G-F-P)* fluorescence confirmed CAR *(car)* expression in transduced T cells, with localization evident in cell clusters **[1]**, and co-localization verified by overlaying brightfield and GFP channels **[2]**.

3.1.1. LAB MEDIA: Figure 2. *Video editor: Highlight the GFP panel.*

3.1.2. LAB MEDIA: Figure 2. *Video editor: Highlight the merged image*

3.2. Nanoparticle tracking analysis revealed a significantly higher concentration of extracellular vesicles in the enriched EV fraction compared to conditioned media for both EGFR *(E-G-F-R)* and HER2 *(Her-Two)* CAR constructs **[1]**.

3.2.1. LAB MEDIA: Figure 3A. *Video editor: Highlight the blue EV columns for EGFR and HER2*

3.3. Western blotting confirmed the presence of granzyme B in CAR-expressing cells and in the isolated extracellular vesicles **[1]**, with calnexin absent in the extracellular vesicles, indicating lack of cellular contamination **[2].**

3.3.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the top row "Granzyme B" EGFR CAR-T and HER2//GFP CAR-T lanes.*

3.3.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the calnexin row under the "EVs" column*

3.4. EGFR-targeting CAR extracellular vesicles significantly reduced MCF-7 *(M-C-F-Seven)* breast cancer cell viability by 70% **[1]** and K562 *(K-Five-Six-Two)* blood cancer cell viability by 40% **[2]**.

3.4.1. LAB MEDIA: Figure 4A. *Video editor: Please highlight the red points on the left graph (MCF-7 Breast Cancer cells)*

3.4.2. LAB MEDIA: Figure 4A. *Video editor: Please highlight the red points on the right graph (K-562 Blood Cancer cells)*

3.5. HER2-targeting CAR extracellular vesicles about 10% reduction in MCF-7 breast cancer cell viability **[1]**, while having no observable effect on K562 blood cancer cells **[2]**.

3.5.1. LAB MEDIA: Figure 4B. *Video editor: Please highlight the blue points on the left graph (MCF-7 Breast Cancer cells)*

3.5.2. LAB MEDIA: Figure 4B. *Video editor: Please highlight the right graph (K-562 Blood Cancer cells)*

Pronunciation Guide:

1. Chimeric Antigen Receptor

- **Pronunciation link: HowToPronounce.com provides audio for *Chimeric Antigen Receptor* How To Pronounce**
- **IPA (American): /kɪˈmɛrɪk ˈæn(t)ɪdʒən rɪˈsɛptər/**
- **Phonetic Spelling: kih-MAIR-ik AN-ti-jun ri-SEP-ter**

2. Extracellular Vesicles

- **Pronunciation link: HowToPronounce.com offers audio examples How To Pronounce**
- **IPA: /ˌɛkstrəˈsɛljələr ˈvɛsɪkəlz/**
- **Phonetic Spelling: ek-stru-SELL-yuh-lur VES-ih-kulz**

3. CAR-EV (Chimeric Antigen Receptor-Extracellular Vesicles)

(A compound term—each part separately.)

- **Pronunciation link: See #1 and #2 above.**
- **IPA: /si eɪ ɑr-i vi/**
- **Phonetic Spelling: see-A-R E-V**

Additional Terms

4. Exosome

- **Pronunciation link: Not explicitly found in your sources, but typically listed in HowToPronounce.**
  (No confirmed link found in provided search results.)
- **IPA: /ˈɛksoʊsoʊm/**
- **Phonetic Spelling: EK-soh-sohm**

5. Ion Exchange Chromatography

- **Pronunciation link: Not located in the current results.**
  (No confirmed link found.)
- **IPA: /ˌaɪən ɪksˈtʃeɪndʒ ˌkroʊmətəˈgræfi/**
- **Phonetic Spelling: EYE-on iks-CHAYNJ kroh-mah-TOG-ruh-fee**

6. Ultrafiltration

- **Pronunciation link: (Not in search results.)**
  (No confirmed link found.)
- **IPA: /ˌʌltrəˌfɪlˈtreɪʃən/**

- **Phonetic Spelling: UL-truh-fil-TRAY-shun**

7. Nanoparticle Tracking Analysis

- **Pronunciation link: (Not captured.)**
  (No confirmed link found.)
- **IPA: /ˌnænoʊˈpɑrtɪkəl ˈtrækɪŋ əˈnæləsɪs/**
- **Phonetic Spelling: NAN-oh-PAR-tih-kul TRACK-ing uh-NAL-ih-sis**

8. GMP-compliant *(Good Manufacturing Practice-compliant)*

- **Pronunciation link: (Not located.)**
  (No confirmed link found.)
- **IPA: /ˌdʒiː ɛm pi kəmˈplaɪənt/**
- **Phonetic Spelling: G-M-P kum-PLY-ent**

9. Phosphate-Buffered Saline (PBS) *(for context)*

- **Pronunciation link: (Not included in results.)**
  (No confirmed link found.)
- **IPA: /ˈfɒsfeɪt ˈbʌfərd ˈseɪlɪn/**
- **Phonetic Spelling: FOS-fate BUH-ferd SAY-lin**

10. SDS (Sodium Dodecyl Sulfate) *(for context)*

- **Pronunciation link: (Not found.)**
  (No confirmed link found.)
- **IPA: /ˌsoʊdiəm doʊˈdɛsəl ˈsʌlfeɪt/ (abbrev. /ɛs-di-ˈɛs/)**
- **Phonetic Spelling: SO-dee-um doh-DES-il SUL-fate; S-D-S**

11. Nanovesicle *(a variation of EV context)*

- **Pronunciation link: (Not found.)**
  (No confirmed link found.)
- **IPA: /ˈnænoʊˌvɛsɪkəl/**
- **Phonetic Spelling: NAN-oh-VES-ih-kul**

12. Centrifugation *(implied in isolation technique)*

- **Pronunciation link: (Not in search results.)**
  (No confirmed link found.)
- **IPA: /ˌsɛntrɪfjuˈgeɪʃən/**
- **Phonetic Spelling: sen-tri-fyoo-GAY-shun**