

Submission ID #: 72572

Scriptwriter Name: Poornima G

Project Page Link: https://review.jove.com/files_upload.php?src=22134508

Title: Focused Ultrasound Hyperthermia for Spatiotemporal Activation of Engineered Chimeric Antigen Receptor T Cells in a Prostate Cancer Mouse Model

Authors and Affiliations:

Fan Wei*, Peixiang He*, Chi Woo Yoon, Yingxiao Wang

The Alfred E. Mann Department of Biomedical Engineering, Viterbi School of Engineering,
University of Southern California

*These authors contributed equally

Corresponding Authors:

Yingxiao Wang ywang283@usc.edu

Chi Woo Yoon soulishguy@gmail.com

Email Addresses for All Authors:

Fan Wei fanwei@usc.edu

Peixiang He ph_360@usc.edu

Chi Woo Yoon soulishguy@gmail.com

Yingxiao Wang ywang283@usc.edu

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**

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? **No**

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: 20

Number of Shots: 40 (9 SC)

Interviews

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***

I have chosen 5 best answers

INTRODUCTION:

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

- 1.1. **Yingxiao Wang:** We aim to develop ultrasound-controlled cell therapies to activate engineered CAR T cells only where they are needed.
 - 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. **Peixiang He:** Solid tumor CAR T therapy lacks precise spatial control; this protocol uses focused ultrasound for localized activation.
 - 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. **Fan Wei:** This protocol allows researchers to study ultrasound-triggered CAR T activation and measure tumor response over time.
 - 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. **Fan Wei:** The key challenge is aligning the ultrasound focus, tumor, and thermocouple for stable, reproducible heating.
 - 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. **Peixiang He**: Future studies can adapt this workflow to deeper tumors, other antigens, and image-guided temperature monitoring.
 - 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.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee at the University of Southern California

Protocol

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

2. Preparation for Focused Ultrasound (FUS) Stimulation

2.1. To begin, obtain enriched and expanded Echo-Back-PSMA CAR (*P-S-M-A car*) T cells after lentiviral transduction of activated primary human T cells [1-TXT]. Then, inject the pre-activated engineered T cells into the subcutaneous xenograft tumor in an immunodeficient NSG mouse to promote tumor engagement [2].

2.1.1. Talent placing the tube with engineered cells on the work bench. **TXT: PSMA: Prostate-Specific Membrane Antigen**

CAR: Chimeric Antigen Receptor

2.1.2. Talent intratumorally injecting the mouse with the engineered cells. **TXT: See text protocol for T cell engineering and mouse injection**

Author's NOTE: We filmed the entire injection procedure, can cut right after the syringe is injected.

Authors: I have added this step to give full context for the workflow and the results as per our journal guidelines.

2.2. Three to four days after T-cell injection, prepare the focused ultrasound system for sonothermal stimulation [1]. Degas a sufficient volume of deionized water overnight using a laboratory vacuum system to reduce acoustic cavitation [1].

2.2.1. Talent turning on the main power button and showing the FUS system.

2.2.2. Talent placing the flask of deionized water in the laboratory vacuum system for degassing.

2.3. Fill the coupling cone of the focused ultrasound transducer with the degassed water [1-TXT]. Inspect the chamber and remove all the air bubbles before stimulation [2].

2.3.1. Talent filling the coupling cone with the degassed water.

2.3.2. Talent inspecting the chamber and removing the air bubbles from the coupling cone.

2.4. Mount the 1 megahertz transducer securely on a manual precision positioning stage [1].

2.4.1. Talent mounting the focused ultrasound transducer on the manual precision positioning stage.

Authors: Please provide the transducer specifications, so that the narration is complete.

Response: Custom-made 1MHz transducer

2.5. Connect the function generator to the radiofrequency power amplifier and then connect the amplifier output to the transducer [1]. Then, attach the thermometer and the needle-type thermocouple to the control computer [2].

2.5.1. Talent connecting the function generator to the amplifier and the amplifier output to the transducer.

2.5.2. Talent connecting the thermometer and the needle-type thermocouple to the control computer.

2.6. Before preparing the animal, turn on the function generator, amplifier, thermometer, and computer. Verify that all electrical connections are secure [1].

2.6.1. Talent switching on the instruments and checking that the connections are secure.

3. Mouse Preparation

3.1. Position the anesthetized tumor-bearing mouse laterally on a heating pad maintained at 37 degrees Celsius, with the subcutaneous flank tumor facing upward [1-TXT]. Secure the mouse gently with laboratory tape [2].

3.1.1. Talent positioning the anesthetized mouse laterally on the heating pad with the tumor facing upward. **TXT: Anesthesia: 1.5% Isoflurane**

3.1.2. Talent securing the animal with tape.

Authors: Please provide the isoflurane concentration for anesthesia, as our veterinary reviewers will ask for this information in the video.

Response: 1.5%

3.2. Shave the hair surrounding the tumor [1]. If necessary, apply a depilatory cream to remove any residual hair and wipe the skin thoroughly [2]. Clean the exposed skin with alcohol [3], and mark the tumor center with a fine-tip skin marker [4].

- 3.2.1. Talent shaving the hair surrounding the tumor.
- 3.2.2. Talent applying depilatory cream and then wiping the area down thoroughly.
- 3.2.3. Talent cleaning the area with an alcohol wipe.
- 3.2.4. Close-up of Talent marking the center of the tumor on the skin.

4. Thermocouple Placement

- 4.1. Using a sterile small-gauge needle, create a skin opening approximately 2 to 3 millimeters from the tumor edge [1].
 - 4.1.1. Close-up of Talent creating the skin opening with a sterile needle beside the tumor edge.
- 4.2. Insert a 29-gauge needle-type thermocouple through the opening at a shallow angle, approximately parallel to the skin surface [1]. Advance the active tip toward the marked tumor center and position it near the tumor mid-depth [2].
 - 4.2.1. Close-up of Talent inserting the needle-type thermocouple through the skin opening at a shallow angle.
 - 4.2.2. Close-up of Talent advancing the thermocouple tip toward the center of the tumor.

Authors: Please give some specifications of the needle-thermocouple. Example: Gauge, type, etc. **Response: 29 gauge**

Authors: During filming, please point out the marked tumor center and the insertion site before inserting the thermocouple.

- 4.3. Secure the thermocouple with laboratory tape to prevent displacement during stimulation [1]. Then, apply a generous layer of degassed ultrasound gel over the tumor and around the insertion site [2].
 - 4.3.1. Talent securing the thermocouple with laboratory tape.
 - 4.3.2. Talent applying the degassed ultrasound gel over the tumor.

5. Focused Ultrasound Transducer Positioning

- 5.1. Using the positioning stage, slowly lower the transducer toward the tumor and center the acoustic coupling cone over the tumor mark [1].

5.1.1. Close-up of lowering the transducer toward the tumor and aligning it over the marked tumor using the positioning stage.

5.2. Gently bring the cone membrane into contact with the ultrasound gel and confirm continuous acoustic coupling. Check the interface for air bubbles [1]. Adjust the transducer height and angle to stabilize the cone over the target [2].

5.2.1. Close-up of bringing the cone membrane into contact with the ultrasound gel.

5.2.2. Close-up of talent inspecting and then adjusting the transducer height and angle over the target.

6. Proportional-Integral-Derivative (PID)-Controlled Stimulation and Animal Recovery

6.1. Launch the dedicated PID controller software on the control computer. Ensure that the function generator and the thermometer are recognized [1]. ~~Select or enter the communication ports assigned to the function generator and the thermometer. Verify that both devices are recognized [2].~~

6.1.1. SCREEN: The PID controller software being launched on the control computer.

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.

~~6.1.2. SCREEN: The communication ports being entered and both devices being recognized.~~ **Author's NOTE:** Many steps are default settings and we cant show it

6.2. After entering the experiment name, verify the ultrasound operating parameters [1]. ~~the target temperature, the total stimulation duration, and the maximum input voltage limit [1-TXT]. Then, enter the ultrasound operating parameters, including the acoustic frequency, the pulse repetition period, and the duty factor [2].~~

6.2.1. LAB MEDIA: Figure 2 **Author's NOTE:** The screen captures are not needed, display figure 2

6.2.2. **SCREEN:** ~~The experiment name, the target temperature, the duration, and the voltage limit are being entered.~~

6.2.3. **SCREEN:** ~~The acoustic frequency, the pulse repetition period, and the duty factor being entered.~~

Authors: Should we display the parameters for the above 2 steps as text overlay? Or would you provide the parameters used during the shoot after the filming is over?

Either is fine. We will use the software's default settings.

Authors: Please provide the details in the screen recording

6.3. Verify that the baseline temperature is stable and click **Start** to initiate the closed-loop stimulation [1]. Monitor the real-time temperature, elapsed time, output voltage, and system status throughout the procedure [2].

6.3.1. SCREEN: The Start button being clicked to initiate the closed-loop stimulation.

6.3.2. SCREEN: The real-time temperature, elapsed time, and output voltage being monitored.

6.4. The software will automatically stop when the programmed stimulation period ends, or when the temperature reading is higher than the set safety limit of 45 degrees Celsius [1]. **Author's NOTE:** Authors gave a revised narration for this step

6.4.1. **SCREEN:** The stimulation software stopping automatically upon noting an increase in temperature.

Authors: Please give us the predefined temperature safety limit, so that we can state the value on screen.

Response: <45 Celsius (the software can auto-shut down)

7. Completion of the Stimulation and Animal Recovery

7.1. At the end of the programmed period, confirm that the software has terminated the voltage output. If necessary, stop the program manually [1]. Then, raise the transducer away from the animal using the positioning stage [2].

7.1.1. SCREEN: The voltage output being confirmed as terminated at the end of the run (hover the cursor over the required area).

7.1.2. Talent raising the transducer away from the animal using the positioning stage.

7.2. Remove the thermocouple carefully from the tumor [1]. Wipe away the ultrasound gel using warm water and paper towels [2].

7.2.1. Close-up of Talent removing the thermocouple from the tumor.

7.2.2. Talent wiping away the ultrasound gel with warm water and paper towels.

7.3. Finally, remove the securing tape [1] and place the mouse on a warm, clean recovery surface [2]. Monitor the mouse continuously until normal movement resumes, and return it to its home cage only after full recovery [3]. Repeat the localized focused ultrasound stimulation four days after the first treatment [4]. Continue monitoring

tumor progression by in vivo bioluminescence imaging every two to three days throughout the treatment period [5]. **Authors:** Please check the **changed** narration for the last 2 sentences

7.3.1. Talent removing the securing tape.

7.3.2. Talent placing the mouse on the warm recovery surface.

7.3.3. Talent monitoring the mouse until normal movement resumes and placing the animal in cage.

7.3.4. Reuse 5.1.1 and 5.2.2

7.3.5. Talent placing the animal in the bioluminescence imaging setup or operating the bioluminescence system. **Authors:** This is a representative shot to provide full context to the results.

Results

8. Results

- 8.1. Following focused ultrasound stimulation of the tumor, the closed-loop feedback system [1] maintained localized hyperthermia at approximately 43 degrees Celsius with limited temperature fluctuation [2].
 - 8.1.1. LAB MEDIA: Figure 3A
 - 8.1.2. LAB MEDIA: Figure 3A. *Video editor: Please emphasize the flat plateau of the temperature trace at 43 degrees Celsius between 0 to 600 marks on X axis*
- 8.2. To validate sonothermal activation independently, the same focused ultrasound stimulation workflow was applied to tumors expressing a heat shock promoter-driven firefly luciferase reporter [1]. Focused ultrasound significantly increased the reporter activity [2]. Bioluminescence at the stimulated site increased markedly six hours after the treatment [3].
 - 8.2.1. LAB MEDIA: Figure 3B *Video editor: Please emphasize the HSP-Fluc box on top*
 - 8.2.2. LAB MEDIA: Figure 3B. *Video editor: Please emphasize the FUS-positive bar and the P value above it*
 - 8.2.3. LAB MEDIA: Figure 3C. *Video editor: Please emphasize the FUS-positive mouse in the After panel*
- 8.3. In the PSMA-positive prostate tumor model, ultrasound-activated EchoBack CAR T cells sustained tumor suppression [1], whereas the tumor burden progressively increased in mice receiving unmodified T cells [2].
 - 8.3.1. LAB MEDIA: Figure 4C. *Video editor: Please emphasize the flat EchoBack block on the right of the plot, then the rising plain T cell block on the left*
 - 8.3.2. LAB MEDIA: Figure 4D. *Video editor: Please sequentially emphasize the Day 11 to the Day 22 rows of the Plain T column*

1. Focused ultrasound

Pronunciation link: <https://www.merriam-webster.com/dictionary/ultrasound>

IPA: /'foukəst 'ʌltrəˌsəʊnd/

Phonetic Spelling: FOH·kuhst UHL·truh·sound

2. PSMA

Pronunciation link: No confirmed link found

IPA: /ˌpiː.ɛs.ɛm'eɪ/

Phonetic Spelling: pee·ess·em·AY

3. Prostate-Specific Membrane Antigen

Pronunciation link: No confirmed link found

IPA: /'prɑːsteɪt spə'sɪfɪk 'mɛmˌbreɪn 'æntɪdʒən/

Phonetic Spelling: PRAH·stayt spuh·SIF·ik MEM·brayn AN·tih·juhn

4. Chimeric Antigen Receptor

Pronunciation link: No confirmed link found

IPA: /kaɪ'mɛrɪk 'æntɪdʒən rɪ'sɛptə/

Phonetic Spelling: ky·MAIR·ik AN·tih·juhn rih·SEP·ter

5. Lentiviral

Pronunciation link: <https://www.merriam-webster.com/dictionary/lentivirus>

IPA: /ˌlɛntɪ'vaɪrəl/

Phonetic Spelling: len·tih·VY·ruhl

6. Transduction

Pronunciation link: <https://www.merriam-webster.com/dictionary/transduction>

IPA: /trænz'dʌkʃən/

Phonetic Spelling: tranz·DUK·shuhn

7. Subcutaneous

Pronunciation link: <https://www.merriam-webster.com/dictionary/subcutaneous>

IPA: /ˌsʌbkjʊ'teɪniəs/

Phonetic Spelling: sub·kyoo·TAY·nee·uhs

8. Xenograft

Pronunciation link: <https://www.merriam-webster.com/dictionary/xenograft>
IPA: /'zi:nəˌgræft/
Phonetic Spelling: ZEE-nuh-graft

9. Immunodeficient

Pronunciation link: <https://www.merriam-webster.com/dictionary/immunodeficient>
IPA: /ˌɪmjənoʊdɪˈfɪənt/
Phonetic Spelling: im-yuh-noh-dih-FISH-uhnt

10. Intratumorally

Pronunciation link: No confirmed link found
IPA: /ˌɪntrəˈtu:mərəli/
Phonetic Spelling: in-truh-TOO-mer-uh-lee

11. Sonothermal

Pronunciation link: No confirmed link found
IPA: /ˌsɔ:nəʊˈθɜ:məl/
Phonetic Spelling: sah-noh-THER-muhl

12. Deionized

Pronunciation link: <https://www.merriam-webster.com/dictionary/deionize>
IPA: /diːˈaɪəˌnaɪzd/
Phonetic Spelling: dee-EYE-uh-nyzd

13. Acoustic

Pronunciation link: <https://www.merriam-webster.com/dictionary/acoustic>
IPA: /əˈkuːstɪk/
Phonetic Spelling: uh-KOO-stik

14. Cavitation

Pronunciation link: <https://www.merriam-webster.com/dictionary/cavitation>
IPA: /ˌkævəˈteɪʃən/
Phonetic Spelling: kav-uh-TAY-shuhn

15. Transducer

Pronunciation link: <https://www.merriam-webster.com/dictionary/transducer>

IPA: /trænz' du:sə/

Phonetic Spelling: tranz·DOO·ser

16. Megahertz

Pronunciation link: <https://www.merriam-webster.com/dictionary/megahertz>

IPA: /'mægə,hɜ:ts/

Phonetic Spelling: MEG·uh·hurts

17. Radiofrequency

Pronunciation link: <https://www.merriam-webster.com/dictionary/radiofrequency>

IPA: /,reɪdiəʊ'fri:kwənsi/

Phonetic Spelling: ray·dee·oh·FREE·kwuhn·see

18. Thermometer

Pronunciation link: <https://www.merriam-webster.com/dictionary/thermometer>

IPA: /θə'mɑ:mətə/

Phonetic Spelling: ther·MAH·muh·ter

19. Thermocouple

Pronunciation link: <https://www.merriam-webster.com/dictionary/thermocouple>

IPA: /'θɜ:məʊ,kʌpəl/

Phonetic Spelling: THER·moh·kuh·puhl

20. Anesthetized

Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetize>

IPA: /ə'nesθə,tɑɪzd/

Phonetic Spelling: uh·NES·thuh·tyzd

21. Isoflurane

Pronunciation link:

<https://www.howtopronounce.com/isoflurane>

IPA: /ˌaɪsoʊˈflʊr, eɪn/

Phonetic Spelling: eye·soh·FLOOR·ayn

22. Depilatory

Pronunciation link: <https://www.merriam-webster.com/dictionary/depilatory>

IPA: /dɪˈpɪləˌtoːri/

Phonetic Spelling: dih·PIL·uh·tor·ee

23. Proportional-Integral-Derivative

Pronunciation link: No confirmed link found

IPA: /prəˈpɔːrʃənəl ˈɪntəgrəl dɪˈrɪvətɪv/

Phonetic Spelling: pruh·POR·shuh·nuhl IN·tuh·gruhl dih·RIV·uh·tiv

24. Acoustic frequency

Pronunciation link: No confirmed link found

IPA: /əˈkuːstɪk ˈfriːkwənsi/

Phonetic Spelling: uh·KOO·stik FREE·kwuhn·see

25. Bioluminescence

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

IPA: /ˌbaɪoʊˌluːməˈnɛsəns/

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

26. Hyperthermia

Pronunciation link: <https://www.merriam-webster.com/dictionary/hyperthermia>

IPA: /ˌhaɪpəˈθɜːmiə/

Phonetic Spelling: hy·per·THER·mee·uh

27. Sonothermal activation

Pronunciation link: No confirmed link found

IPA: /ˌsɔːnoʊˈθɜːməl ˌæktəˈveɪʃən/

Phonetic Spelling: sah·noh·THER·muhl ak·tuh·VAY·shuhn

28. Luciferase

Pronunciation link:

<https://www.howtopronounce.com/luciferase>
)

IPA: /lu:'sɪfəˌeɪs/

Phonetic Spelling: loo·SIF·er·ays

29. EchoBack

Pronunciation link: No confirmed link found

IPA: /'ekoʊˌbæk/

Phonetic Spelling: EK·oh·bak

30. Bioluminescence imaging

Pronunciation link: No confirmed link found

IPA: /ˌbaɪoʊˌluːməˈnesəns ˈɪmɪdʒɪŋ/

Phonetic Spelling: by·oh·loo·muh·NESS·uhns IM·ih·jing