To the Editor:

We are delighted to hear the very positive response from our reviewers and editor, and to reply to their comments below. Following Dr. Alisha DSouza’s suggestions, we have substantially edited the experimental protocol to include more detail, as is reflected in the changes tracked in the main manuscript. We have also implemented the suggested changes to the figures, and removed the video pending further editing, which, unfortunately, will not be possible within our January 3rd resubmission deadline due to the holiday closure of Yale core facilities – with the hope of being permitted to submit the edited version of the video at a later point, as soon as we possibly can. We thank the journal for the opportunity to further refine the protocol and improve its presentation, and hope that the manuscript is acceptable in its present form.

We thank you again for considering this paper for publication in JoVE.

Sincerely,

Richard L. Edelson, MD

Aaron and Marguerite Professor and Chairman

Department of Dermatology

Yale University School of Medicine

**Editorial comments:**  
Changes to be made by the author(s) regarding the manuscript:  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

*We have thoroughly reviewed the manuscript for any such issues, and hope to have eradicated them.*

2. Figure 1B: Please include a space between numbers and their corresponding units (e.g., 10 mm3). Please define s.c. in the figure legend.

*This has been done, please see the Figure 1 file.*

3. Figure 3: Please define NS in the figure legend.

*This has been done, please see the Figure 3 legend, line 588 of main manuscript.*

4. Figure 4A: Please convert it to a table, and upload the table to your Editorial Manager account as an .xls or .xlsx file.

*This has been done, please see the updated Figure 4 (with the table removed) and the accompanying Table 1 in the Excel format.*

5. Video 1: Please include a scale bar to provide context to the magnification used. Define the scale in the appropriate figure Legend.

*The video editing requires assistance from the Yale microscopy core facility, where it was first assembled. Unfortunately, the facility if closed for the holidays, only re-opening on January 2nd, which we feel is too short a time before out January 3rd resubmission deadline.*

*Therefore, with the editor’s permission, if it is possible we’d like to remove the video for now, and add it to the submission at a later point – with the scale bar added to the video and defined in the legend, and the very pertinent changes suggested by Reviewer 3 included.*

6. Please revise lines 358-360 to avoid previously published text.

*This has been done, please see the re-written lines 489-491 of main manuscript.*

7. Please define all abbreviations before use.

*We have thoroughly reviewed the manuscript for any such issues, and believe to have defined all abbreviations before first use.*

8. Please use the micro symbol µ instead of u. Please abbreviate liters to L to avoid confusion.

*This has been done, please see the tracked changes throughout the manuscript.*

9. Please revise the protocol to contain only action items that direct the reader to do something (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible. Please move the discussion about the protocol to the Discussion.

*We have revised the protocol to ensure this as best as possible, please see tracked changes throughout the manuscript. Notes have only been left where they are necessary to point out where the protocol may be modified in essential ways by the investigators following it.*

10. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. You may use the generic term followed by “(see table of materials)” to draw the readers’ attention to specific commercial names. Examples of commercial sounding language in your manuscript are: Lympholyte, Eppendorf, etc.

*All commercial language has been removed and replaced with generic product descriptions and references to the Table of Materials, as suggested. Please see thracked changes in the manuscript.*

11. Please revise the Protocol steps so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step. Use sub-steps as necessary.

*We have revised the protocol to ensure this as best as possible, please see tracked changes throughout the manuscript. Additional sub-steps have been added where necessary.*

12. Please add more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. See some examples below.  
13. 1.2: Please specify what standard conditions are.

*Standard conditions have been defined.*

14. 1.5: Please mention how animals are anesthetized (e.g., specify concentration of isoflurane) and how proper anesthetization is confirmed.

*Isoflurane concentration and method of confirming anesthesia have been included.*

15. 2.2: How many treatments are included? Please specify.

*The number of treatments has been specified more clearly.*

16. 2.3.1: Please specify the Lympholyte M protocol (centrifugation parameters). We need such details for filming.

*The Lympholyte M protocol centrifugation parameters have been specified.*

17. 2.4.1: Please provide the composition of ACK buffer. If it is purchased, please cite the Table of Materials.

*The ACK buffer is purchased, thus the Table of Materials has been cited.*

18. 5.1: What volume of blood is collected?

*The volume of blood has been specified.*

19. 6.1: Please specify incubation conditions.

*Standard incubation conditions have been defined.*

20. Table of Materials: Please sort the items in alphabetical order according to the name of material/equipment.

*The Table of Materials has been sorted in alphabetical order, please see the revised Table of Materials.*

**Reviewers' comments:**  
  
**Reviewer #1:**  
Manuscript Summary:  
The authors describe their protocol for generating physiologic immunogenic dendritic cells for use in either murine or human studies. The detailed explanations of all steps, including tumor preparation, whole blood preparation, TI plate use, and concerns for which to watch are all outlined clearly. Additionally, the figures and videos further enhance the manuscript and support the procedures being presented by the authors. I believe these protocols will be of use to those investigating dendritic cell manipulations for immunization or tolerization.  
  
Major Concerns:  
None  
  
Minor Concerns:  
Please have copy-editor check use of commas throughout manuscript (there may be too many). Also, please have all materials mentioned include company, location, and model/catalog# so that they can be easily ordered by readers interested in using the protocols described.  
  
  
**Reviewer #2:**  
Manuscript Summary:  
Extracorporeal photopheresis (ECP) is a FDA-approved therapy for cutaneous T cell lymphoma (CTCL), and is also widely used for treatment for many T-cell mediated diseases. Recently, the researches of applications of ECP for cancer therapy draw many attentions. Nevertheless, the mechanisms of ECP action under different diseases are not fully understood. One of obstacles is lacking a good experimental system/device to study and assess different effects in vitro and in vivo. The authors in this manuscript introduce the Transimmunization (TI) chamber, a scaled-down version of the clinical ECP leukocyte-processing device, suitable for work with both mouse models, and small human blood samples. Overall, the methods and protocols are appropriate for the methods Journal, and researchers/scientists in this field will be interested in this system.  
  
Major Concerns:  
1. Because this is a methods article, only one video for the microscopic observation is not enough to introduce a new system/device. Additional videos for some key steps are needed for readers to understand the TI, for examples, collection of peripheral blood mononuclear cells (PBMC); 8-MOP/UVA treatment of tumor cells; TI plate passage of cells; preparation of autologous mouse serum; co-incubation of PBMC with antigen; re-injection of TI-treated cells to mice.

*We thank the reviewer very much for their interest in this protocol, and for their desire to see it filmed in as much detail as possible. We wish the same, and have tried our best to select for filming these parts that are not described elsewhere, or have unique features that are essential to the TI method. These sections of the protocol, as the reviewer points out, include the collection of PBMC, the 8-MOP/UVA treatment of tumor cells, the TI plate passage of cells, the co-incubation of PBMC with antigen overnight, and the preparation of cells for re-injection.*

*We did not select the preparation of autologous mouse serum for filming, because that is a fairly common technique and is not unique to our protocol. For the same reason we did not select for filming the actual re-injection of the cells into mice, only cell preparation for injection, again feeling that retro-orbital cell injection itself is not unique to the TI method, and is a well-described veterinary protocol. However, we will be happy to include these sections for filming, should the editor grant us additional filming time.*

2. For the classic ECP therapy for patients, the whole process will be done within the same day, instead of overnight co-incubation as the TI. Is there any data/justification for overnight incubation?

*We thank the reviewer for the opportunity to clarify this key point.*

*As described in the Introduction, the TI protocol differs in several features from standard clinical ECP, the addition of overnight incubation being a very important such feature (lines 125-129). The reason for these alterations is that the Transimmunization protocol aims to not only replicate ECP in the laboratory, but to rationally optimize it. Since it has been demonstrated that antigen-loaded dendritic cell production is the key mechanism of action in ECP, it was next hypothesized that extending the time of contact between the dendritic cells newly produced during cell passage through the ECP device, and the apoptotic tumor cells also produced in the device, will allow for better tumor cell internalization and processing by dendritic cells, and thus improve the clinical impact.*

*This hypothesis was tested clinically in Cutaneous T Cell Lymphoma (CTCL), where the addition of overnight incubation alone, with no other changes to the ECP protocol, led to clinical response in 60% of patients previously refractory to standard ECP (reference 13 in the manuscript, Girardi et al, 2006). Furthermore, in the TI mouse model of ECP, the inclusion of the overnight incubation step led to tumor growth reduction with the TI protocol.*

*We therefore believe that at least in the immunogenic anti-tumor modality of ECP, the co-incubation step is an important improvement. However, it remains to be investigated whether it is equally an improvement in the tolerogenic aspect of ECP, and what length of incubation is optimal in the immunizing and the tolerizing modalities. We hope these questions with be among these studied, as the scientific exploration of ECP continues.*  
  
  
**Reviewer #3:**  
Manuscript Summary:  
I think that Edelson and col. present in this article a very important approach to mechanism of action of extracorporeal photopheresis and a derivation of it , that is the treatment of solid neoplasma based in the same mechanism of action of it.  
The article shows the knowledge of the author on this topic, and states that the continuous research in the same field may lead to important advances.  
  
Major Concerns:  
No.  
  
Minor Concerns:  
I think that watching the video does not provide important information. I suggest looking for some way to point out different type of cells that are observed or to make it more instructive.

*We thank the reviewer for their observation, and the opportunity to improve on the video. Following the reviewer’s and the editor’s suggestions, we would like to add to the video a scale bar, to give a better idea of the relative cell sizes in the device, and to also include arrows and labels pointing out the leukocytes, the gradual coating of the plate with platelets, and leukocyte rolling along the plate-bound platelets. We hope that will add to the quality and the instructive value of the video.*

*However, the video editing requires assistance from the Yale microscopy core facility, where it was first filmed and assembled. Unfortunately, the facility if closed for the holidays, only re-opening on January 2nd, which we feel is too short a time before out January 3rd resubmission deadline.*

*Therefore, with the editor’s permission, if it is possible we’d like to remove the video for now, and add it to the submission at a later point – with the scale bar added to the video and defined in the legend, and the very pertinent changes suggested by Reviewer 3 included. We still feel there is a value to the community visualizing for themselves what happens within the device during this protocol.*