Re: manuscript, JoVE59223 "Patient-derived orthotopic xenograft models enabling human urothelial cell carcinoma and colorectal cancer implant, growth, and spontaneous metastasis"

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.

Answer: We proofread and made changes were needed.

2. Please revise lines 67-75, 93-99, 253-255, 258-259, 300-302, 322-324 to avoid previously published text.

Answer We revised above listed sentences as instructed.

3. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. "This figure has been modified from [citation]."

Answer: We replaced all published figures with new data.

4. Please upload each Figure individually to your Editorial Manager account as a .png, .tiff, .pdf, .svg, .eps, .psd, or .ai file.

Answer: We uploaded each Figure individually as a tiff file as instructed.

5. Figure 3: Please include a scale bar for all images taken with a microscope to provide context to the magnification used. Define the scale in the appropriate figure Legend.

Answer: We included a scale bar in images as instructed and added the scale in the corresponding Figure Legend.

6. Please revise the title to be more concise and reflect the content of the protocol.

Answer: We revises the title as instructed.

7. Please provide an email address for each author.

Answer: Ravan Moret (rmoret@ochsner.org), Linh Hellmers (linh.hellmers@ochsner.org), Xin Zhang (xzhang@ochsner.org), Jessie Gills (jgills@ochsner.org), Nathan Hite (nathan.hite@ochsner.org), Aaron Klinger (aaron.klinger@ochsner.org), Grace A Maresh (grace.maresh@ochsner.org), Daniel Canter (daniel.canter@ochsner.org), Stephen Bardot (sbardot@ochsner.org), David A Margolin (damargolin@ochsner.org), Li Li (lli@ochsner.org)

8. Please remove the sub-headings within the Long Abstract.

Answer: All sub-heading in the Long Abstract have been removed.

9. Please define all abbreviations before use.

Answer: Abbreviations have defined prior to use as instructed.

10. Please convert centrifuge speeds to centrifugal force (x g) instead of revolutions per minute (rpm).

Answer: Centrifuge speeds are expressed as centrifugal force (x g).

11. 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: Nair, Bovie, Hamilton Microliter, IVIS Lumina, etc.

Answer: All commercial/trademark language has been removed as instructed.

12. 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.

Answer: Protocol has been revised to be in imperative tense throughout all steps.

13. 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 examples below.

Answer: We added more detail and explanation to steps that appeared vague.

14. 2.2: Please specify the reaction conditions (temperature and time) for trypsinization. Please provide the composition of media containing serum.

Answer: Reaction conditions for trypsinization and media composition has been specified in the protocol as instructed.

15. 3.2.1: What is used to mince tissues?

Answer: Surgical Scissors

16. 3.2.2: Where is the tissue implanted? And what volume of tissue is implanted?

Answer: a total volume of 8mm^3 is evenly distributed between the left and right flank.

17. 4.1: How to determine the size of the xenograft?

Answer: Tumors are measured with a digital caliper.

18. 5.1: Please specify the age, gender and species of mouse used.

Answer: The mice used are 6-8 week old female, NOD/SCID mice, describer in step 3.2. This activity is a continuation on the same mouse.

19. 5.3: Please specify the concentration of isoflurane.

Answer: 2.5% isoflurane in 100% oxygen, 1L/min.

20. 5.6: Please describe how to harvest tumor from mouse flank and specify what is imaged and how.

Answer: Tent skin directly above tumor on mouse flank. Cut away skin using surgical scissors. Place tumor in petri dish and image entire dish in imaging machine.

21. 5.7: How to remove non-bright sections?

Answer: Non-bright sections are separated from bright section using sterile surgical scissors or scalpel.

22. 6.1: What is used to mince tumor?

Answer: Surgical Scissors.

23. 7.1.4: How to confirm that mice are sedated?

Answer: To confirm sedation a toe pinch is performed. If mouse is unresponsive to the pinch then it is determined to be completely sedated. (Note added to step)

24. 10.1: Please specify the euthanasia method.

Answer: Mice are euthanized via CO_2 inhalation.

25. 10.4.3: Please specify for how long and at what temperature are the organs and tumor fixed in formalin.

Answer: Tumor and organs are fixed in 10% neutral buffered saline for 48 hours at ambient temperature.

26. Please combine some of the shorter Protocol steps so that individual steps contain 2-3 actions and maximum of 4 sentences per step.

Answer: Some short steps combined as instructed.

27. Please include single-line spaces between all paragraphs, headings, steps, etc.

Answer: Single line spacing used throughout manuscript as instructed.

28. After you have made all the recommended changes to your protocol (listed above), please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

Answer: Essential steps were highlighted (less than 2.75) pages as instructed.

29. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense. Please do not highlight any steps describing anesthetization and euthanasia.

Answer: Only complete sentences were highlighted. Highlighted steps describing anesthetization and euthanasia were removed as instructed.

30. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.

Answer: All sub-steps and relevant details are highlighted as instructed.

31. Discussion: As we are a methods journal, please also discuss critical steps within the protocol, any modifications and troubleshooting of the technique, and any limitations of the technique.

Answer: We discussed these issues in lines 326-336 of the discussion section.

32. References: Please do not abbreviate journal titles.

Answer: Full names of all journals are listed as instructed.

33. Table of Equipment and Materials: Please sort the items in alphabetical order according to the Name of Materials.

Reviewers' comments:

Reviewer #1:
Manuscript Summary:
This manuscript describes methods to generate patient derived tumor xenografts in mice that retain their metastatic ability and are similar to the original tumor.
The methodology is generally clear except for the few points listed below. The model will be very useful for the deeper analysis of tumor metastasis. The authors have generated models with good survival of mice for up to 100 days.

Major Concerns:
None it is a well written manuscript. The manuscript details the development of metastatic orthotopic cancer models.

Minor Concerns:
Line 226, 10.4.2 It is stated "Weigh tumor and image separately.' I am not sure what this means. Are the authors referring to organs perhaps rather than images?

Answer: Step was re-written to be clearer.

Line 190, 7.6.1 It appears that the cells were instilled in the bladder for a few seconds. Do the tumor cells remain in the bladder after the catheter removal? Could the authors clarify this point or clarify the duration that the tumor cells remain in the bladder.

Answer: After installation the catheter remains in the bladder for several seconds allowing for cells to adhere to the bladder wall. Cells remain in the bladder and develop into a primary tumor.

Line 179, How certain are the authors that they are not accidently puncturing the bladder wall with the electro cautery wire?

Answer: The wire has been previously measured to determine the appropriate length. The wire is taped at 0.025" limiting its reach to only 1mm past the edge of the catheter. After removal mice are monitored for signs of bleeding to assure the bladder wall or urethra hasn't be punctured.

Line 120, The source of HK cells are not mentioned anywhere.

Answer: As stated in line 132: "HK cells are normal human follicular dendritic cells and can be grown and expanded for ≤ 15 passages *in vitro*." A reference of Kim et al Journal of Immunology 1995, 155 (3), 1101 was included for more details.

Table 1, The tumor implantation rate of 83% is good but it is lower than the tumor implantation rate via the rectal route. Could this be related to issues with the electro cautery technique or timing of cell instillation in the bladder?

Answer: Implantation of colon cancer tumor cells are using intra-rectal route, while bladder cancer cells are using intra-bladder instillation. No direct comparison was done with same patient tumor cells in both models. In addition, within one model, we have seen different implantation rates with different individual patient tumors. It is difficult to comment on the reason for the difference in tumor implantation rates.

Reviewer #2:

Manuscript Summary:
I reviewed the manuscript titled "Patient-derived orthotopic xenograft models enabling human urothelial cell carcinoma and colorectal cancer implant, growth, and spontaneous metastasis" by Moret and colleagues. The authors reported the development of patient-derived orthotopic xenograft (PDOX) models of urothelial cancer (UCC) and colorectal cancer (CRC). The authors isolated cancer cells from patients' and described technique using dissociated cancer cells from surgically excised patients' tumor samples injected into the bladder and rectum of NOD/SCID mice. Fluorescent/luminescent tumor cells were injected with HK cells (follicular NK cells). Tumor growth and metastasis were monitored by BLI and IR imaging. Tumor metastases were also reported. The authors compared the PDX tissues with the primary tumors. They showed H&E as well as ki67 immunostaining. The manuscript is clearly written. The rational and surgical technical approaches are clearly explained.

Major Concerns:
1. Viral transduction of dissociated cancer cells is not clearly described. The authors should indicate the technique of viral transduction, the source of viruses, multiplicity of infection, method of detection of the transduction efficiency and selection of transduced cells. These details should be described.

Answer: On line 159 it states: "Tumor is directly injected with single dose of Luc/RFP-lentivirus (50ul/tumor, 1:30 dilution from concentrated high tier lentivirus stock)." Revised section 4 and 5 added more details on monitoring and selection of transduced cells.

2. The source of HK cells was not specified. The ratio of the numbers of cancer cells to HK cells was not specified.

Answer: As stated in line 132: "HK cells are normal human follicular dendritic cells and can be grown and expanded for ≤ 15 passages *in vitro*." A reference of Kim et al Journal of Immunology 1995, 155 (3), 1101 was included for more details. 0.3 million HK cells were used for each mouse in both CRC and UCC models, various numbers of cancel cells were used based on disease stage and tumor cell aggressiveness.

3. The authors should show immunostaining of the HK cells (NK cell markers) and inflammatory and immune cell infiltration in the PDX and compare that with the profile of the primary tumors.

Answer: HK cells are a follicular dendritic cell line isolated from human tonsils. They are similar to lymph node stromal cells are reported in our previous publication (*FASEB Journal*. 2015, 29 (8), 3571). HK cells are not NK cells. We have data shown that HK cells will last up to 2 weeks post co-injection with tumor cells using GFP tagged HK cells and detected by live mouse GFP imaging (unpublished). Therefore, at the end point (at least 6 weeks post co-injection of cancer cells and HK cells, HK cells may not be detectable. In addition, NO/SCOD mice are immunodeficient mice that do not have immune cells such as T cells and B cells as well as with reduced NK cells. It will be difficult to compare PDX with patient primary tumor for immune cell infiltration.

4. Since the authors used traumatic cauterization of the bladder, they should show the extent of the inflammatory reactions instigated by the cauterization in the bladders of non-tumor bearing mice.

Answer: As shown in Figure 1, H&E staining of mouse bladders after electro-stimulation with or without tumor cell instillation. Inflammatory cell (small and dense) infiltration is observed between bladder lumen mucosa and tumor growth (left), however, inflammatory reaction is not as obvious in non-tumor bearing mice (right).

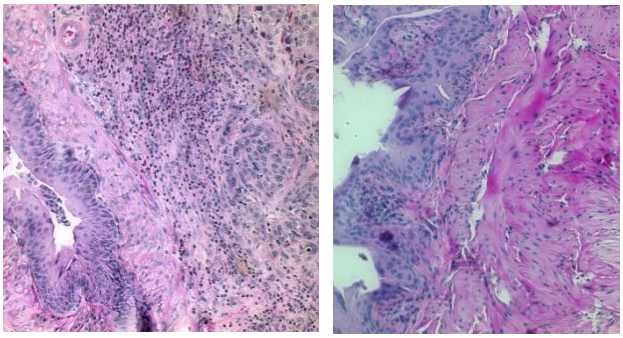

Figure 1. H&E staining of mouse bladder after electro-stimulation. Inflammatory cell (small and dense) infiltration is observed between bladder lumen mucosa and tumor growth (left), however, inflammatory reaction is not as obvious in non-tumor bearing mice (right).