Kessous

Project 15338

Summary Comments

Overall, this is an exciting and valuable proposal. It is well-organized and easy to read. Congrats! I made many suggestions as a friendly reviewer. I hope they will help toward the success of the proposal. Please let me know if you have any questions.

**Overall Comments**

**1. Saving space and compacting the writing**. I presume that more text will be added for the final version.

a. I tried to simplify statements for clarity and space.

b. I changed the text wrap around the figures.

c. I removed spacing before and after paragraphs. I suggest if there is space, these can be restored.

d. I suggested some organizational changes to compact the proposal.

The research proposal is now about 14 pages long. After arranging the figures, there should be at least an additional partial page for new text to address revisions. The title page and abstract do not count towards the 15-page limit.

**Abstract**

**2. Significance.** At line 53, I suggest stating why significance 2 and 3 are, in fact, significant. Also, since ISF funds basic research, I suggest pondering whether to state that a clinical application is the most significant aspect of the proposal (line 55).

**Scientific Background**

**3. Subheadings.** I organized the subheadings to save space. See, for example, line 65.

**4. Figures.** I did not modify or try to move the figures, so they will now look disorganized. I felt it best to leave that to you. To save space, I suggest the following.

a. Reducing the size of figures when possible. For example, Figure 2 could be reduced in size and still be legible. Also, Table 1 can be reduced in size.

b. Place the legends for figures 1 and 2 in text boxes as with other figures. This act will permit text wrapping.

c. If necessary, slightly reduce the font size in the legend text boxes to 10 points. The text will be readable.

d. Align the figures as horizontally as possible. For example, the panels in Figure 4 could be arranged side-by-side. This strategy reduces the height of figures, which saves space.

d. I did not modify the figures or their placement. It is best if you do that.

**4. Emphasis on major points.** This point may be a matter of preference and writing style. I suggest highlighting sentences stating significance, knowledge gaps, hypotheses, and conclusions. For example, in line 127, I suggest a self-contained statement that the lack of knowledge about UBC HSCs justifies your proposal. I have noted other instances where you may want to consider such emphasis.

**5. Impact.** Along with highlighting major points, I have edited to hopefully improve the impact of important statements. These are noted in the margins at each instance. For example, at line 186, I added descriptors like “critical” and “state-of-the-art.” Reviewers will look for potential novel insights and approaches beyond the current state-of-the-art. In this regard, I suggest explicitly stating when you are presenting a new idea or approach. Likewise, I suggest stating clearly the “goal” of the proposal. Also, I suggest that “aims” reads as less impactful as there may be many aims, but generally, there are only one or proposal two goals.

**Research Objectives and Expected Significance**

**7. Objectives (line 189).**

a. I realize they may be interchangeable terms, so please accept or reject them on this basis. But I suggest stating Objectives rather than Aims. Perhaps it is a personal preference, but Objectives reads as more impactful to me. The hierarchy in my mind is goal>objective>aims>task.

b. I strongly suggest stating the Objectives explicitly as a list rather than embedded text. Reviewers will look for the list of Objectives to guide their reading. Thus, it should be distinct from the surrounding text. As an example, I created a list of six objectives. Each is numbered with a title. Each Objective should be self-explanatory or have a sentence or two to explain what will be achieved by the objective.

c. Six objectives seem a bit excessive. Reviewers may find it challenging to track so many Objectives. I suggest combining Objectives to reduce the number to three or perhaps four. For example, Objective 1 seems to stand on its own. Objectives 2, 3, and 4 might be combined as a single objective since you want to characterize cells and then use them for molecular investigations (RNASeq, mutation detection). Objectives 5 and 6 seem distinct from each other. Overall, the stated objectives depend on the relative emphasis of each and the amount of experimentation required for each to present a balanced plan.

d. Alternatively, you combine Objectives 1-3 at line 216 of the research description, indicating that the three objectives will be addressed in one section of the research plan. Combining Objectives 1-3 into a single Objective 1 seems logical, which would reduce the Objectives to four.

**8. Significance (line 201).**

a. It is refreshing that you list the significance of your proposal! Reviewers will be able to see these essential details at a glance. I suggest that you state the actual significance of several points. For example, in line 203, significance b states that you will use a multi-omics approach. The method is not inherently significant. Significance is how you use it and what new knowledge will be gained. Thus, I suggest explaining what you gain that is new. The same applies to b at line 294. Using *in vitro* and *in vivo* methods is not significant in itself, so I suggest stating what new knowledge will be gained. I suggest pondering this for the other significances as well. For d) At line 204, I suggest being more specific: What characteristics or mechanisms will you learn?

b. ISF funds basic research, so I believe you correctly excluded the clinical application from the formal significance list while still discussing it. This is a good strategy. For the other significant statements, I suggest emphasizing the basic knowledge gained. I have tried to emphasize basic knowledge here

 and throughout. While the clinical potential is exciting and a good selling point, you are presumably evaluated on the contribution of scientific knowledge.

 1.2. Criteria for evaluating the research proposals: The sole criterion for evaluating the research is

scientific excellence, assessed by the following, while only outstanding proposals are funded:

1.2.1. Originality and Innovation (In the Humanities – only if applicable);

1.2.2. Project importance and contribution to scientific knowledge;

1.2.3. Suitability of methods for proving the research hypothesis;

1.2.4. Suitability of researchers’ scientific background to the project. 1.2.1. Originality and Innovation (In the Humanities – only if applicable);

1.2.2. Project importance and contribution to scientific knowledge;

1.2.3. Suitability of methods for proving the research hypothesis;

1.2.4. Suitability of researchers’ scientific background to the project.

**9. Writing in the first person.** Most of the research plan is written in the third person. While this has been the convention in the past, this section is now typically written in the first person or is a mixture of the first and third person. I have modified selected sentences to add text in the first and third person.

**10. Methods details (line 283).** Reviewers may find fault with the lack of detail about some methods.

a. As a friendly reviewer, I suggest being more specific about how many samples will be analyzed for SNPs and Nanostrong. Where will the analyses be done? For the Nanostrong, you will not have independent replicates of UBC samples. Is a single sample enough to get reliable gene expression data? What statistical analyses will be done for the DNA or RNA work? I suggest providing as much detail as possible. While these are standard techniques, it is important to demonstrate your knowledge and expertise in writing.

b. The proposal does not clearly state what expertise exists in the lab. If you need assistance with molecular or other analyses, I suggest noting any collaborators who may add expertise to the proposal, such as molecular biologists, cell biologists, bioinformaticians, or statisticians.

c. Other details include growth medium formulations and vendor city and country locations.

d. Reviewers may cite insufficient detail as a negative. At line 289, for example, what are the expected outcomes of the correlation work? It is a significant effort. Can you describe the methods for correlation and any statistics that may be involved? What will the output look like? What results do you predict? Again, I suggest details so reviewers can assess whether the methods are appropriate and sufficient for the objectives.

e. I suggest, if needed, shortening the background section to make space for more detailed methods. As long as the background is sufficiently informative, detailed methods take precedence as an evaluation criterion. I hope these tips are helpful.

**11. Figure order.** It is generally useful for the first figure to be a general overview of the project and its goals. The current Figure 3 seems to be more appropriate as Figure 1. It overviews the project, giving reviewers an overview earlier in the text. As Figure 1, it will provide a framework for placing the objectives in context. The current Figure 1 describes how you will isolate cells. Perhaps that is a better Figure 2.

**12. Stating the rationale for each task within the Objectives.**

a. There are many tasks or experiments for each of the Objectives. As a friendly reviewer, it is not always evident why the task is being performed. As a simple example, in line 236, the first sentence is, “Using previously established panels in our lab, we will analyze cell surface phenotypic markers of HSC and HSPC populations.” The sentence states what is being done but not why, which will immediately tell reviewers the intention of the task. In this case, the sentence could be, “To identify UBC-HSCs present in our UBC samples, we will use a previously established panel in our lab,” etc. The reason is stated in the following sentences, but it will be clearer and more consistent if stated in the first sentence. Other instances are at lines 227, 232, 236, 244, 252, 260, 295, and 113.

b. At line 275, I suggest stating what mechanisms you will examine by variant and transcript experiments. As a non-expert, it is not apparent. I don’t think such mechanisms are mentioned in the Scientific Background section, so it should be described on line 275.

c. At line 289, I suggest stating why you want to collect clinical and demographic information. This seems critical. For example, are you planning to use correlation as a predictive tool for choosing optimal UBC-HSCs?

d. At line 295, why do you need to measure the severity of GDM and PET?

**13. Stating the output of the proposal.** As a friendly reviewer, I strongly suggest that at the end of the experimental plan section (line 321), there be text stating clearly how the in vivo and in vitro data, including the variants and transcriptome data, will be integrated and what you expect to achieve from that combined data. Figure 3 presents a list of the tasks you propose but does not summarize what will be achieved by combining the experimental knowledge. As suggested previously, the current Figure 3 could serve as Figure 1. At line 321, I suggest a different figure that shows the inputs and outputs of the proposal. It might show the cell collection and processing in vitro and in vivo, then the output in the form of predictive models and improved HSC quality if those are your expected proposal results. Are you looking for predictive features for higher-quality HSCs? Are you hoping to discover new pathways or mechanisms that affect HSC quality due to pregnancy complications? As written, what you expect to achieve through the experimentation is vague.

**14. Statistics (line 232).** I suggest combining this section into the relevant Objective task. The concept is to preempt reviewer questions. When reading earlier sections, I wondered about statistical methods. Would the statistics fit into sections 10 (line 289) or 11 (line 295)?

**15. Pitfalls (line 342).** Are there any pitfalls with the variant or Nanostring expression analyses?

**Preliminary Results**

**16. Less emphasis on peripheral results.** Starting at line 416, significant data (Fig. 9) is shown for a related but different project. The intention is to show that the humanized mice methodology used previously applies to the methods proposed here. I understand why this is important, but it seems like a point of potential confusion because it shows a preliminary result not directly related to the proposal. I suggest some alternatives to consider.

a. Is the previous work published, so can you cite that work as an example? This tact would permit omitting the extra figure to save space.

b. Alternatively, perhaps a description of the work and the key points are sufficient to highlight your applicable experience.

c. Another possibility is to describe the work in the project Feasibility section.

d. Regardless, Fig. 9 has many small panels that reviewers will glance over, I suspect. If included, can the figure be made simpler with four panels, for example?

e. If the figure and text are included, perhaps a subheading like “Prior expertise with a humanized mouse model” would clarify for reviewers that you are pointing to expertise from another project.

**17. Placement of Preliminary Results.** I suggest relocating Preliminary Results immediately after the research plan at line 322. The ethical approval, feasibility, and pitfalls sections can then follow.

**18. Summary statements.** As noted in comment 13, the proposal should have strong summary statements indicating what you will achieve from the proposal and the significance of the results. The last information about the project details will be at the end of the Preliminary Results. I suggest another short summary statement about what will be achieved and its significance to the field, science, and society, if appropriate. The statement will leave reviewers with a positive vision of the proposal.

**19. DNA/RNA work**.

a. Do you have any preliminary data concerning the variant and transcriptome work? You have collected samples. Have any been sent out for an initial experiment?

b. I raise this question because, as an overall comment, the proposal is easy to understand and integrates the objectives except for the DNA/RNA work. It seems unclear what the expectation is for this objective or how it will be integrated into the final project output. As I understand the proposal, the objective seems expendable without affecting the overall proposal. To better integrate Objective 4, task 9, I suggest a stronger justification for the work in the Objectives section (line 275) and a clearer idea of how it integrates into the overall project at the end of the experimental plan (line 321). This includes an explanation of what mechanisms you plan to detect or discover. I suggest that we want to avoid making these experiments read as extraneous.

Edit 2

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**1. Abstract.** The abstract reads well and is succinct.

**2. Text boxes.** I suggest placing all figure legends in text boxes rather than within the main text for easier adjustment and text wrapping. At line 112, I placed the legend in a box.

**3. The research plan length is over the 15-page limit.** The research plan has a 15-page limit (A4 size). Including the title page and abstract (one page each), the total length is 18 pages as written. Thus, the research plan is 16 paragraphs as written.

a. I formatted it to the correct A4 size from the letter. Maybe my computer did this.

b. ISF specifies 2cm margins (0.8 inches). I have set the margins at 0.8 inches. The length is now below 15 pages.

c. If you need more space to address final edits, I suggest removing spaces between sections. While neater in appearance, they are not essential.

**4. DNA/RNA work.** This section is much improved, with details equivalent to the other methods. Line 309 states that samples will be tested (sequenced) in duplicate. As a friendly reviewer, can you explain the duplicates? Are these technical replicates from RNA extracted once, or are they replicates from two separate RNA extractions from each UCB sample? In other words, will you extract RNA from cells independently and then sequence it, or will you sequence the same RNA samples twice? If I understand correctly, you will examine expression for specific genes across your samples, providing gene expression and variation. Thus, your 2x expression threshold will be applied between your patient UBC groups. If this is correct, what is the value of the duplicate sequencing?

**5. Figure shifting.** Editing starting at Line 428 resulted in changes to figure positioning. I tried to address this unsuccessfully, so I left these for you to address. I suggest that all figure legends be borderless, like Figure 1. Grouping each figure and legend into individual PDFs will make repositioning easier.

**6. Summary statement (line 494).** This ending statement is nice. I suggest merging it with the existing text as a final paragraph rather than a separate heading. It will be apparent that it is a summary paragraph.

**7. Merging the RNA/DNA work.** I mentioned this in Comment 19 for the first edit. The methods are much better, and some details make what will be done more explicit. My only thought as a friendly reviewer is to return gently to the earlier point. It is not apparent how the RNA work will be integrated into the whole to uncover molecular mechanisms. It is clear from the DNA work that you will get markers. What will it mean to have differences in transcription of stem cell-related genes between UBC groups? How will this work identify molecular mechanisms (Objective 2)? What criteria determine if gene expression changes point to a mechanism? Will you look for clusters of related genes that point to a particular pathway? What might those pathways be? Known stress pathways? As a non-stem cell expert, this seems vague. I suggest text explaining how you will use the data for reviewers.

**8. References.** The limit for references is five pages. Your list is six pages. However, the references may be single-spaced. While your list is single-spaced, there are extra spaces between references. Removing those spaces should get you close to or below the page limit. Alternatively, there may be a more compact citation format.

Overall, the proposal was well organized before and is even better now, with a balanced level of detail throughout. It is an exciting and relevant proposal, and I wish you all the best with the submission. Please let me know if you have any questions!