March 25, 2024

Dear CIRM Staff and Members of the ICOC,

We wish to thank the Grants Working Group for the thorough, carefully considered review of our CIRM DISC0 grant application "Gene-edited CD 19 CAR-T cells with superior proliferation, persistence and serial-killing activity". We were gratified to receive a highly favorable score of 83 (13th out of 43 considered applications) that left us just 2 points under of the funding cutoff.

Among some of the positive feedback we were thankful to receive were the following review comments:

> Strength: Could provide an interesting new approach to substantially modulate CAR-Ts and prevent exhaustion.
>
> This may indeed enable greater expansion and longevity for CAR-T cells.
>
> The rationale is based on a specific hypothesis and selective disruption of the <redacted> gene. The logic is very convincing. The preliminary data suggest the target as relevant and support the aims.
>
> The preliminary data are strong and derived from multiple subjects.
>
> The rationale is sound and based on extensive research in known pathways of T cell signaling.
>
> The proposed aims are straightforward and logical and backed by clear examples in the preliminary data. It is very feasible in the timeline.
>
> The DEI statement was well-considered and described several useful activities.

In examining the GWG members' critiques, we noticed that the key over-riding concerns affecting the score were issues that we have in the application, or that we can very easily address. Our hope is that clarification here could potentially satisfy the issues raised and move the application score, which was deemed at the very edge of having 'exceptional merit', into the fundable group. Our point-by-point responses are below:

1. Across three of the five review subcategories, the GWG raised concerns about the X-linked nature of our gene of interest:

   > Weakness: Since the approach involves targeting an X-linked gene, more details on the effect and feasibility across the sexes are needed. While safety is not yet relevant, suggestions on how to move this forward would be needed.

Not taking into account the sex of T-cell donors as a likely source of heterogeneity in the degree of expansion seems a significant slip. Males have a single copy of the target gene, females two copies. If only one copy is KO'ed in cells from a female, this would probably have much less effect on T-cell expansion than in cells of a male donor (consistent with X-linkage of the <redacted>). This effect could easily dwarf effects of racial/ethnic differences, etc. The project needs to address editing in males vs. females given the X-linked nature of the gene.

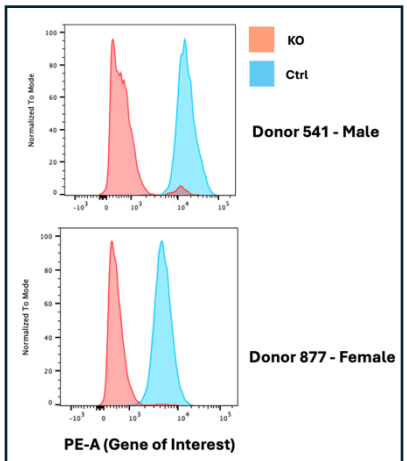
Despite claims in the DEI section that CAR T-cells from many diverse individuals will be tested, the proposal is to test most steps on cells from three patients. Information on how patients will be selected is not included. As noted, male versus female may have biggest impact, unless CRISPR KO is carried out at high multiplicity so most cells would have KO of both copies of <redacted>.

Given the potential for variation in outcome based on biological sex, the authors should address this more directly.

The targeted gene is on the X chromosome, but sex is not addressed.

Response: We appreciate the reviewers' focus on the X-linked nature of our gene of interest, and the potential impact of donor sex on our proposed studies. While we acknowledge that we should have spent more time addressing and clarifying this issue in the proposal, we believe that our own preliminary data, and published findings from others, can assuage the concerns that have been raised.

First, we wish to point out that in our preliminary studies, we have achieved identical complete <redacted> gene knockout in both male and female donor T cells. As seen in the figure, the complete knockout in donor 877 (female) is identical to that achieved in donor 541 (male), as measured by intracellular flow cytometry. We understand that it may have been an oversight to omit the 877 data from the proposal, but at the time of writing, we considered the 541 donor data to be representative. We believe this data indicates that, contrary to the reviewers' understandable concerns, we can achieve complete <redacted> knockout in male- and female-derived T cells, despite the presence of two copies of the gene in the female cells.



The gene of interest can be knocked out equally well in both male and female donor T cells under identical conditions using Cas9 electroporation.

We further note that other groups have achieved similar high CRISPR KO efficiencies of X-linked genes. For example, in (Yujuan Hou et. al., ***Challenges in Gene Therapy***

for Somatic Reverted Mosaicism in X-Linked Combined Immunodeficiency by CRISPR/Cas9 and Prime Editing, 2022, Genes (Basel), PMID: 36553615), Hou and colleagues reported in excess of 80% indel generation in the X-linked IL2RG gene in K562, a female-derived cell line which is established as having an X chromosome inactivation phenotype (PMID: 33735607). We remark that their editing was performed with a single guide RNA, whereas ours will use two (leading to greater editing efficiency). And we further point out that the Hou et al. data, along with our donor 877 data, indicates that no special efforts (i.e. an undue 'high multiplicity' of CRISPR components) will be required to achieve complete gene knockout, allowing for fair assessment of the modified CAR-T performance for both male and female donors. It's important to note, as well, that <redacted> knockout levels will always be assessed and confirmed prior to further functional characterization.

We realize that the phenotype that we are trying to emulate is primarily described in males with X-linked lymphoproliferative disease and donor sex could play a role in the effectiveness of our proposed CAR-T modification. Indeed, our proposal includes donor sex as a variable we will account for in our study. However, we hope that we have allayed the unease of the committee regarding the danger to our study if we could not achieve full knockout for all donor phenotypes.

2. Also, in three of the categories, the reviewers cited that our application was lacking in attention to details about safety:

   While safety is not yet relevant, suggestions on how to move this forward would be needed.

   Whether this would significantly improve clinical outcomes is not yet clear. It also may increase risks associated with CAR T-cell therapy, such as the development of leukemias arising from the engineered CAR T-cells. This already occurs with sufficient frequency so that the FDA recently imposed a "black box" warning label on multiple approved CAR T-cell products. Potential risks should have been discussed in the application.

   The "black box" labeling from the FDA appears to be the only discussion of safety.

   Response: The reviewers' wise focus on safety, especially given the specific nature of our proposed gene modification, is something we applaud and share. We could have more clearly delineated, in a specific subsection, the efforts we plan to take to address safety concerns. However, we would like to mention that rather than being listed in a single section, our risk mitigation plans and observations regarding safety were distributed throughout the document.

   For example, CRISPR-mediated gene editing is well-known to cause off-target mutagenic effects. That is why we stated in Milestone 2, "*Additionally, we will utilize*

the UC Davis Genomics Shared Resource to perform whole exome sequencing of the lead candidate edited T-cells to look for off-target genic alterations by Cas9."

As another example, we mentioned in the 'Rationale' section that we have paid specific attention to a known adverse consequence of our proposed gene knockout in humans, and have seen no evidence of this: "*we have seen in preliminary studies that CAR-mediated cytotoxicity of CD19+ target cells proceeds normally, and the modified cells eliminate the tumor burden, which prevents the <redacted> seen in <redacted> patients.*"

Along the same lines, we specifically mentioned in the Milestone 3 'Pitfalls, preventive measures and alternative approaches' section the following: "*An additional concern is that the <redacted> KO CAR-Ts will unleash a graft-versus-host effect on the mice, or that <redacted> KO CAR-Ts may become transformed. We have examined this latter possibility in preliminary studies, and have seen no evidence that <redacted> KO CAR-Ts can continue dividing once antigenic stimulation is removed. However, if we notice evidence of transformation or GVHD (via weight loss, early death not attributable to tumor burden, or results from hematoxylin and eosin [H&E] staining), we may still obtain useful data by incorporating a suicide cassette into the CAR-Ts*."

We wish to stress that our mention of the inclusion of a suicide cassette (for example through a dimerizable caspase, or an epitope marker such as CD20 that would allow for antibody-mediated depletion), is a common safety enhancement that is proposed in the CAR-T literature.

We hope that the committee recognizes that we are not questioning the reviewers' strong focus on safety, but rather that we share their concerns. We regret that we did not include a specific section addressing safety, but we feel that the proposal, as originally written, already includes checkpoints to specifically address these issues.

3. Concerns about our proposal related to diversity, equity and inclusion (DEI) were raised in the two review categories (including DEI):

   The promise in the DEI section to systematically explore effects of <redacted> KO on cells from many individuals of diverse ethnicity, etc., unfortunately seems rather empty. The numbers required may have been impractical.

   The potential DEI-oriented impact depends on the applicant's collection of broadly representative samples; it is not clear that this will be actively pursued.

   Despite claims in the DEI section that CAR T-cells from many diverse individuals will be tested, the proposal is to test most steps on cells from three patients. Information on how patients will be selected is not included.

Response: We thank the reviewers for their comments regarding our efforts in this very important topic. We understand that it is easy to overlook this aspect of the application, and we wish to respond to those doubts.

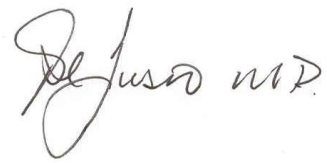
We have already verified with our donor blood vendor, Vitalant, that we can specifically ask for blood provided by donors of specific sex and ethnicity. In this way, we have and will 'actively pursue' the goal of obtaining blood samples from a donor pool that matches the ethnic diversity of California's population.

The proposal to perform most analyses using cells from >= 3 donors will admittedly make it difficult to achieve full representation for individual components of our study. However, as noted in our DEI statement, "*As we expect to utilize donor blood from perhaps upwards of 50 or more donors during the course of this project, we anticipate that we can intentionally include a great diversity of backgrounds in our study*."

We understand and appreciate the reviewers' desire to hold us accountable for statements made regarding DEI, and look forward to following through on our plans, should we be recommended for funding. DEI is a central, daily commitment at UC Davis.

In closing, we wish to again thank the GWG for their insightful and appropriate comments. Finally, we thank the Independent Citizens' Oversight Committee for considering our rebuttal and hope that it will inspire a re-examination of our score, particularly in light of how close we were to the level required to achieve funding and the importance of our future investigational product.

Sincerely,



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