*Written responses to supplemental questions for the "**CD4+ T cell immunity is dependent on an intrinsic stem-like program**" webinar (7/18/2024).*

1) Will some transferred TCF+ (stem like) cells differentiate into effector cells in recipients?

- Dr. Li: Yes, then can, but some still retain stem features.

2) Beautiful work Wenhao - you are bringing in new insights into the transcriptional mechanisms underlying observations nearly 30 years ago that effector cytokine-producing T cells arise only after multiple cell divisions! Well done

- Dr. Chen: Thanks for bringing this point up. [The] work from about 30 years ago using CFSE labeling to track T cell fate in vivo is truly striking.

3) Beautiful work! Do you have any insight into the respective contribution of the stem/effector populations to TFH and allo antibody formation?

- Dr. Chen: This is a critical but unaddressed question. I believe that TFH cells represent one of the differentiation fates of stem-like CD4 T cells, thereby contributing to allo-antibody production. Moreover, since TFH cells express TCF1, they themselves can further differentiate into effector cells if they leave the GC environment. Dr. Chen Dong's group published this point in Nature Immunology a few weeks ago.

4) Can the TEa cells transduced to have forced expression of Tcf1 reject or are they stuck in temness like the Irf4-KO T cells?

- Dr. Chen: Thank you for your great question. Since we haven't performed the skin graft survival experiment with TCF1 overexpression, I don't have a conclusive answer at this time. But we have extensive data that TCF1+ cells fail to mediate rejection w/o downregulating TCF1.

5) Wenhao, a similar bifurcation to stem memory vs. effector function has been observed in tumor-specific T cell responses - how do the aspects and mechanisms you show for alloreactive T cells compare to anti-tumor T cell effector vs. memory?

- Dr. Li: In the tumor setting, stem-like T cells represent a subset of exhausted T cells which can be invigorated upon checkpoint blockade treatment, this is a critical aspect of tumor immunology. By contrast, stem-like T cells in the transplant setting mainly develop into effector cells, and preventing this process is crucial for transplant success.

6) Beautiful work and presentation. Do all effector cells have to go through a TCF+ state or can they bypass and differentiate directly into effectors. Any idea how Tregs might work on these two cell states?

- Dr. Li: Terminal Teff cells die quickly, and if the pool size is kept small enough, the graft can withstand them and survive. However, if they are present in large numbers (like many TCF1 knockout cells that bypass the stem-like state), the graft gets rejected. Stem-like T cells, without differentiating into Teff cells, fail to reject the graft despite their self-renewal potential. The Treg question is an interesting one, how do Tregs fit in these dynamics? Self-renewal or differentiation or both? Hopefully, we will have some answers soon…