Microbiology 204
Discussion Session #6 December 5, 2018
1-2:30PM CL220
Faculty leader: Cliff Lowell

Kamphorst et al. PD-1 targeting is CD28 dependent. Science 355, 1423 (2017)
Hui et al. CD28 is the target for PD-1 inhibition. Science 355, 1428 (2017)

Everyone should read these papers before coming to class.

LEARNING OBJECTIVES:
To understand mechanisms of PD-1 blockade in tumor immunotherapy. How does PD-1 inhibit T cell function?

STUDENT ASSIGNMENTS
1. Discussion leader (see instruction page for advice on leading the discussion).

2. Remind the group of CD28 costimulation. What are the ligands? What molecule on T-cells competes for the ligands? How can manipulation of CD28 co-stimulatory molecules be used to amplify or repress T cell function? Show the usual picture of a T cell interacting with an APC along with all the stimulatory/inhibitory molecules.

3. **FIRST PAPER:** Explain Fig. 1, panels A-C. It may help to draw out what molecules are being blocked on the APC/T-cell interacting picture. The observation that PD-1 blockade works to re-stimulate exhausted T cells following chronic LCMV infection, suggests that we should use this antibody in chronic human viral infections. Would it work?

4. Explain Fig. 1, panels D – J. How does this differ from panels A – C?

5. Explain Fig. 2, panels A- C. Make sure to define what P14 T cells are.

6. Explain Fig. 2, panels D – H. How does this approach differ from panels A – C. What is the really conclusive way the authors demonstrate that cell-intrinsic CD28 signaling is required for PD-1 blockade to work?

7. Explain Fig. 3. What do you think is missing in this figure? (answer: characterization of the immune infiltrate into the tumor, I would think. What should it look like in the different groups?).

8. Figure 4 is simply awful. Explain it and say why it stinks. (they show the responding cells are CD28+, but don't show the non-responding cells!). One of the issues brought up at the end is the cell localization of PD-1 versus CD28 ligands. Most tumors don't express B7 molecules, but we think PD-L1 expression on tumors is driving T cell suppression. Is that correct? What cells express both PD-L1 and B7 molecules?

9. **SECOND PAPER:** Explain the background to the paper. How is it thought that engagement of PD-1 or CTLA-4 suppresses T-cell activation? What is the biochemical mechanism?

10. Explain the assay set up in Fig. 1. Go through the panels, but don't worry too much about the supplemental data for this figure. While there is a good effort to determine binding partners for phospho-PD-1, there is less of an effort to determine kinases. Do you think looking at Lck and Csk alone is adequate?

11. Go over Fig. 2. This is the most critical figure in the paper.

12. Go over Fig. 3 briefly. This is a short/supportive figure, although I am confused why this seemed to work without PD-L1 in the bilayer. Maybe the answer is Fig. S13.

13. Explain Fig. 4. This is the cell based equivalent to Fig 2. Does the effect on CD28 phosphorylation look subtle to you on the western blot (but a bit better on the quantitation – panel D)? Do you think 50% decrease in CD28 phosphorylation is sufficient to decrease its signaling function? In general would you believe this paper if it weren't proceeded by the Kamphorst paper?

TO THE GROUP: What is the really big implication of these papers? What is the REAL target for anti-tumor therapy we should be going after that will be much cheaper and easier than using a blocking mAb against PD-1?

Student assignment #s

1. Jennifer Umhoefer
2. Adam Wade-Vallance
3. Benjamin Wheeler
4. Darwin Kwok
5. Suraj Makhija
6. Nick Mroz
7. Rachel DeBarge
8. Julie Cole
9. Ki Hyun Kim
10. Brian J Woo
11. Iowis Zhu
12. Marissa Chou
13. Cody Mowery
14.

(NOTE : if you will miss a discussion session, inform Dr. Lowell in advance; if assignments have already been made, you should additionally make a trade with one of your classmates who does not have an assignment that week so that your assignment is covered).