

BIOCHEMISTRY SEMINAR

Mapping the molecular mechanisms of CAR T cell activation at single cell and single molecule resolution

Kevin Scrudgers

Ph.D Candidate (Low-Nam Group), Purdue University



Abstract: Chimeric Antigen Receptor (CAR) T cells are engineered to repurpose the lethal capacity of cytotoxic T cells toward elimination of target cells via secretion of lytic molecules. While there have been some remarkable successes in applying CAR T cell technology toward elimination of blood cancers, the overall realization of the therapeutic potential of this cell-based immunotherapy remains limited. Second generation CARs are comprised of domains from the native T cell receptor (TCR), its coreceptor CD28 or 4-1BB, and an extracellular binding domain based on a single chain antibody that engages a target surface cell marker, typically one that is overexpressed. The extent to which these modular CARs successfully engage the T cell signaling machinery and activate remains poorly understood. We hypothesized that a molecular understanding of the mechanisms governing CAR T cell activation will improve efficacy of therapies and overcome some on-target off-tumor toxicities that can be lethal. Direct observation of potent elimination of tumor targets emphasized the need to observe how individual CAR T cells generate robust cytotoxic responses. We developed an in vitro reconstitution system based on biomimetic supported lipid bilayers and high-resolution Total Internal Reflection Fluorescence (TIRF) microscopy to map the CAR T cell impulse-response function. Individual ligand binding events collected by living CAR T cells were imaged along with the lytic granule polarization outcome. Surprisingly, we found that CAR T cells were capable of rapid polarization after small collecting numbers of binding events, challenging the established notion that extensive CAR engagement is necessary for activation. This low molecular threshold may underscore systemic toxicities from spurious CAR T cell activation. Also unexpected was the identification of a significant fraction of CAR T cells with negligible binding, even at high agonist densities. Taken together, these data emphasize that a small subset of the CAR T cell population is dominating the response. Unexpectedly, this single molecule, single cell sensitivity bears a strong resemblance to the activity of the TCR. Perturbation of the native TCR strongly dampened CAR T cell activation, suggesting that the receptors may undergo a previously unknown crosstalk. I will discuss these insights that prompt new directions for engineering safer and more precise CAR T cell therapies.



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3:30 pm



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