1208 A MULTIMODAL CYTOKINE EFFECTS ATLAS FROM MULTIPLEXED CELL BARCODING REVEALS PRINCIPLES OF T CELL PROGRAMMING

Elisa C Clark*, Sriram Pendyala, Kathleen Abadie, Matthew Wither, Arjun Kumar, Douglas Fowler, Hao Yuan Kueh. *University of Washington, Seattle, WA, USA*

Background To defend against pathogens and tumors, T cells must sense and integrate myriad extracellular signals. Cytokines mediate immune cell-cell communication and their concentrations in the external milieu conveys information about an encountered threat, its cellular location, and the state of the ongoing immune response. Approximately 30 unique cytokines are sensed by $CD8^+$ T cells, but we lack a systemic understanding for how they impact cell state and function. A comprehensive atlas of cytokine effects on T cell state and function, evaluated over well-defined signaling contexts, is needed to reveal the gene expression and functional programs accessible to T cells and provide a blueprint for T cell engineering.

Methods To measure the effect of cytokines on a massive scale we developed CellCode. CellCode leverages viral DNA barcoding to track single T cell clones subject to many treatments (~500) through subsequent pooled assays, thereby connecting signaling history to cell growth, fate, and function. Here we used CellCode to measure the impact of 28 cytokines at multiple concentrations across 4 distinct *ex vivo* stimulation contexts with varied T cell receptor (TCR, Signal 1) stimulation, CD28 costimulation (Signal 2), and IL-12 driven inflammation (Signal 3) on $CD8^+$ T cells by single cell transcriptomics, *Tcf7* (TCF1) reporter expression, and pooled competition assays of overall persistence and clonal expansion.

Results The complete set of *ex vivo* cytokine treatments recapitulated a broad spectrum of cell states including effector cell states, circulatory and resident-like memory states, and exhausted states. Despite the diversity of cell states generated, many different cytokines yielded convergent gene expression programs which led to similar growth and *Tcf7* regulation, including cytokines from distinct receptor families. Remarkably, cytokine effects were highly dependent on stimulation context, such that individual cytokines had distinct or even opposing effects on cell state depending on signaling context. For example, the immunosuppressive cytokine TGF-β up-regulated *Tcf7* expression, consistent with its known role in preserving stemness in chronic viral infections, but did so only when combined with TCR stimulation and inflammation. Moreover, many cytokines had long-lasting effects on cell viability and proliferation in pooled assays days after cytokine removal.

Conclusions Together, our results show that cytokines have lasting, signaling context-dependent effects on gene expression and cell fate, and that distinct cytokines can produce convergent cell states and fate outcomes. CellCode enables systematic profiling of cell responses to treatments at scale, with important implications for rational T cell programming for immunotherapies.

Ethics Approval All animals were used in accordance with the Institutional Animal Care and Use Committee guidelines for the University of Washington, registered protocol number 4397–01.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.1208