A SINGLE-CELL TRANSCRIPTOMIC ATLAS OF HUMAN NK CELLS TO GUIDE CANCER IMMUNOTHERAPY

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Background
Natural killer (NK) cell can serve as an effective anti-cancer treatment, and enhancing NK function has been shown to enhance patient outcomes. Therefore, insight into NK cell states and subtypes may lead to new treatment options. Initial characterization of NK cells relied on flow cytometry with surface markers dividing NK cells into less mature CD56bright NKs and more mature CD56dim NKs. More recently, single-cell RNAseq profiling has enabled deeper characterization and revealed three novel subtypes: cytokine-induced memory-like (CIML), adaptive, low ribosomal, and type I IFN responding NKs, which enhanced the previously known CD56bright, CD56dim, and CD56dim CD57+ subtypes. Here, we extend this single cell subtyping by building a cancer-focused NK cell Atlas that integrates 25 public datasets from multiple types of cancers and delineates both the subtypes and states of the NK cells.

Methods
Single cell sequencing datasets were downloaded from the studies listed in [table 1]. NK cells were identified using the HaiTam cell type prediction algorithm v1. Harmony v0.1.0 was used for batch correction and ACTIONet v3.0.0 was used for data integration and cell state identification. Cell state abundance comparisons were performed using 1-way ANOVA with Dunnett’s post hoc test.

Results
We generated an Atlas of 89,704 NK cells from 281 donors in 21 cancer focused studies, 3 studies using healthy donors, and 1 study of ulcerative colitis. We identified 12 unique NK cellular states in our Atlas. Some states recapitulated known NK subtypes such as CIMLs and type 1 IFN responders. By contrast, CD56dim NKs were represented by 2 cellular states and CD56bright NKs were represented by 3 states. We found that one of these CD56bright states is more abundant in blood from renal cell cancer patients than in blood from healthy donors (2.1-fold change; p < 0.02). Compared to other CD56bright states, this overrepresented state expresses higher levels of the cytotoxic gene GZMK and of migratory markers CD44, CXCR3 and SELL.

Conclusions
Our Atlas describes 12 NK cell states reflecting maturation, activation, and exhaustion in cancer. These states provide a framework for assessing co-expression of targets for NK modulators and for understanding the effects of treatments on NK cells.

REFERENCES
Abstract 1010 Figure 1  UMAP of the cellular states in the NK atlas
Each unique cellular state identified in this study has a unique color assigned to it and has been plotted in the UMAP.