9 A PAN-CANCER MULTI-OMIC IMMUNE SINGLE-CELL ATLAS FOR CANCER IMMUNOTHERAPY: FOCUS ON CD4 + T CELLS

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Background Despite the success of immunotherapy, clinical responses remain difficult to predict, likely due to diverging tumor immune cell composition and function. Advances in single-cell analysis have revealed heterogeneous immune cell activity within and across individuals with cancer. While CD8 + ?tumor-infiltrating lymphocytes (TILs) have been extensively studied,[1–4] a pan-cancer consensus annotation of CD4+ TIL in immunotherapy is lacking. Robust identification of CD4+ T-cells from admixed single-cell transcriptomes is challenging due to low CD4 transcript expression and CD4+CD8+ T cells. Poor harmonization of CD4+ T-cell annotations across datasets compromises reproducibility and generalization. Here, we present the Cancer Immunotherapy T-cell Atlas (CITA), a harmonized, metadata-rich, pan-cancer, single-cell omics resource, spanning over 1.3M T cells, aimed at discovering CD4+ T-cell related features impacting immunotherapy response.

Methods Publicly available single-cell RNA sequencing (scRNA-seq) data were used to generate the CD4+ T-cell consensus re-annotation and the CITA. Raw count data and metadata were obtained from the Gene Expression Omnibus (GEO) or manuscript supplementary data. Individual datasets were processed using standardized bioinformatics workflow for quality control, integration, normalization, and batch correction.

Results We collected scRNAseq data and clinical metadata from 23 published datasets from 320 donors, across 30 different cancers, 20 immunotherapies, and from diverse tissue types and sequencing platforms[3,5–25] (figure 1). Existing immune cell annotations were harmonized by mapping to our reference cell identity labels, and T cells were subsetted for the CITA. To enable consensus-driven annotation, we resolved precise CD4+ T-cell transcriptional profiles from publicly available, FACS-sorted CD4+ T-cell scRNAseq datasets from liver, lung, and colorectal cancers.[21,22,26] We found CD4+ T cells homogeneously distributed in 12 main clusters across cancer types (figure 2). Foxp3+ regulatory T cells (Tregs) segregated into circulating/naive, tissue-resident, and effector Tregs, consistent with prior studies.[27] Moreover, we resolved naive, central, effector, tissue-resident, activated, and highly proliferating CD4+Foxp3- T cells, as well as Tbet+ Th1, and T follicular helper (Tfh) cells, co-expressing cytotoxic or canonical Tfh genes respectively (figure 2).

Conclusions The CITA provides the foundation for pan-cancer, harmonized, metadata-rich compendium of single-cell omics T-cell data from treatment-naive and immunotherapy-treated patients. Our CD4+ T-cell consensus re-annotation in conjunction with existing and new machine-learning-based classification methods automates annotation of new and existing CD4 +T-cell datasets. CITA will be a publicly available software and data resource at http://cita.cells.ucsc.edu and will include new datasets as they are released.

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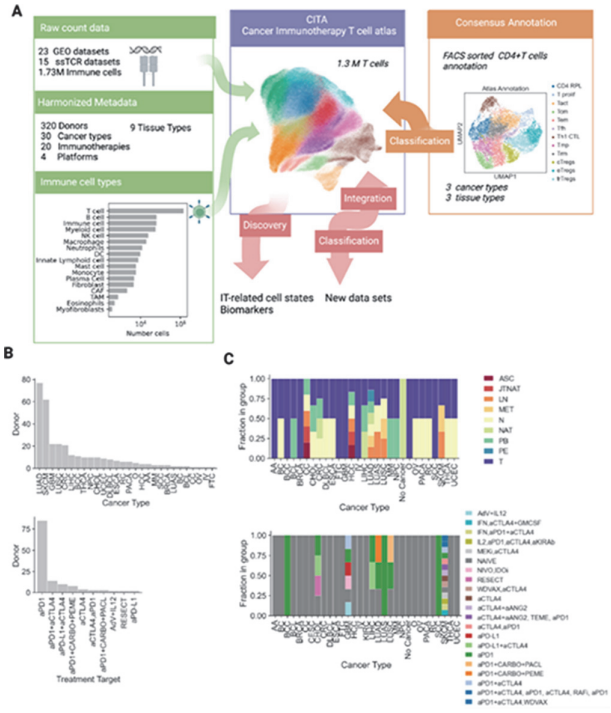
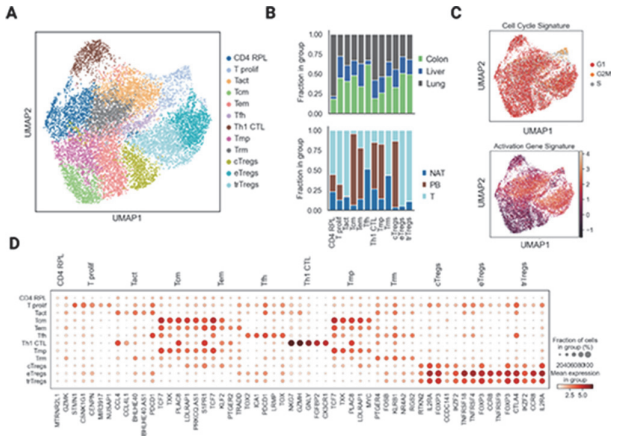


Abstract 9 Figure 1 Composition of the CITA
A. Pipeline for the Cancer Immunotherapy T-cell Atlas (CITA) integration including the use of consensus annotated CD4+ T-cell states based on FACS-sorted CD4 single-cell datasets. UMAP of 1.3M T cells from 23 single cell datasets from individuals with cancer, for 30 different cancer types and 9 tissue types (center). B,C. CITA harmonized metadata overview, with sampled tissue type per cancer type and treatment type by cancer type. LUAD: lung adenocarcinoma; SKCM, skin cancer melanoma; GBM, glioblastoma; LUSC, lung squamous cell carcinoma; CC/CRC, colorectal carcinoma; LIHC, liver hepatocellular carcinoma; BC/BCC, basal cell carcinoma; THCA, thyroid carcinoma; NPC, nasopharyngeal cancer; CHOL, cholangiocarcinoma; UCEC, uterine corpus endometrial carcinoma; DLBCL, diffuse large B cell lymphoma; ESCA, esophageal cancer; RC, renal cancer; PACA, pancreatic adenocarcinoma; O, oligodendroglioma; HCC, hepatocellular carcinoma; AA, anaplastic astrocytoma; MM, multiple myeloma; SCC, squamous cell carcinoma; BRCA, breast cancer; LUAS, lung adeno/squamous carcinoma; BC, basal cell carcinoma; BCL, B cell lymphoma; OV, ovarian serous cystadenocarcinoma; IV, glioma stage 4; FTC, fallopian tube carcinoma; ASC, ascite; JTNAT, joint tumor normal tissue; LN, lymph node; MET, metastasis; N, normal; NAT, normal adjacent tissue; PB, peripheral blood; PE, pleural effusion; T, tumor; CARBO, carboplatin; PEME, Pemetrexed; PACL, paclitaxel; Adv, adenovirus; RESECT, resection



Abstract 9 Figure 2 Pan-cancer CD4+ T-cell consensus annotation
A. UMAP of consensus annotation of CD4+ sorted cells from peripheral blood (PB), tumor (T) and normal adjacent tissue (NAT) from individuals with lung, colorectal or liver cancer.[21,22,26] CD4 RPL: high ribosomal protein, T prolif: proliferating, Tmp: memory precursors; Tcm: central memory, Tact: activated, Tem: effector memory, Tfh: follicular helper, Th1 CTL: T helper 1 cytolytic lymphocytes, Trm: tissue resident memory, cTregs: circulating regulatory T cells, eTregs: effector regulatory T cells, trTregs: tissue resident regulatory T cells. B. Barplot of cancer type and tissue type fractions for each cell annotation. C. UMAP of gene signature scores for cell cycle and a curated T-cell activation/terminal differentiation gene signature (n=26 genes) consisting of terminal differentiation transcription factors (e.g. ID2, RUNX3, PRDM1, TOX), cytolytic markers (e.g. GZMA, GZMB, GZMH, PRF1), co-stimulatory receptors (e.g. ICOS, TNFRSF18, TNFRSF4), and chemokines/chemokine receptors (e.g. CXCR3, CX3CR1, CXCL13) for dataset described in (A). D. Dotplot of the five most significant differentially expressed genes for each cell annotation contrasted against each other cell annotation

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