Optimize cell and gene therapies with single cell multiomics

Introduction

One of the more promising types of cancer immunotherapy involves engineering immune cells to recognize and attack cancer. While immune cell therapies like chimeric antigen receptor (CAR) T-cell therapy have been effective for treating some cancers, untangling the mechanisms behind patient response, resistance, and development of toxicities after infusion continues to be a critical focus of ongoing research. Whether characterizing changes in the functional state of engineered cells, discovering on-target off-tissue toxicities, or tracking clonal diversity over time, researchers need methodological approaches that offer the scale and resolution required to address their questions of interest. Now, researchers studying cell and gene therapies are applying single cell sequencing and immune profiling tools from 10x Genomics to fundamentally alter our understanding of cancer immunotherapies like CAR T-cell therapy and accelerate novel translational applications in therapeutic development and treatment.

Browse the collection of publications below to see how researchers are using 10x Genomics technology to study the complexities of cell and gene therapies for treating cancer.

Featured publication	Experiment snapshot	Research highlights
Pooled Knockin Targeting for Genome Engineering of Cellular Immunotherapies TL Roth, et al. *Cell.* (2020).	**Research area**: Cancer, immuno-oncology, assay method **10x Genomics products**: Chromium Single Cell 3' Gene Expression **Sample type**: Human blood cells	Developed a new method for functional screening called "pooled knockin sequencing," or PoKI-seq, which combines single cell transcriptome analysis and pooled CRISPR knockin screening Created a library of 36 genetic constructs, each believed to modulate T-cell function in some way, and tested which constructs enhance T-cell function in vitro and in vivo Discovered that knocking in exogenous TGF-βR2–derived receptors most potently impacts T-cell function in mouse models by converting a suppressive signal in the tumor microenvironment into a proliferation and effector state–inducing signal PoKI-seq is a powerful approach to measure how genetic edits impact cell abundance and cell state, in both in vitro and in vivo models

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Characteristics of Anti-CD19 CAR T Cell Infusion Products Associated with Efficacy and Toxicity in Patients with Large B Cell Lymphomas Q Deng, et al. *Nat Med.* (2020).	**Research area:** Cancer, immuno-oncology **10x Genomics products:** Chromium Single Cell 5′ Gene Expression, Immune Profiling **Sample type:** Human blood cells and core needle biopsies of tumor **Clinical timepoints:** Infusion product, CAR T-naive tumor biopsy, tumor biopsy at progression	Performed scRNA-seq of 137,326 residual cells after infusion of CAR T cells into 24 patients with large B-cell lymphoma (LBCL) Exhausted CD8 and CD4 T cells were enriched in patients with partial response/progressive disease while memory CD8 T cells were 3x more enriched in patients with complete response at 3-month follow-up PET/CT Used immune profiling to show that CD8 T cells with the exhaustion signature are polyclonal and characterized by increased proportion of receptor genes *LAG3* and *TIM3* Study shows that variation in single cell transcriptional profiles of CD19 CAR T-cell infusion product contributes to differences in efficacy and toxicity of the therapy in LBCL patients
Single-Cell Analyses Identify Brain Mural Cells Expressing CD19 as Potential Off-Tumor Targets for CAR-T Immunotherapies KR Parker, et al. *Cell.* (2020).	**Research area:** Cancer, immuno-oncology **10x Genomics products:** Chromium Single Cell 5′ Gene Expression **Sample type:** Human brain cells, mouse models of CD19 CAR T-cell therapy	Used scRNA-seq data from 2,364 human prefrontal cortex cells to identify CD19 expression in brain mural cells CD19 expression is present across multiple independent datasets and in pericytes as well as vSMCs Mouse mural cells show lower levels of CD19 expression, suggesting importance of using human tissues Findings suggest mechanism for neurotoxicity in CD19 CAR T-cell therapies with implications for designing immunotherapies
Clonal Kinetics and Single-Cell Transcriptional Profiling of CAR-T Cells in Patients Undergoing CD19 CAR-T Immunotherapy A Sheih, et al. *Nat Commun.* (2020).	**Research area:** Cancer, immuno-oncology **10x Genomics products:** Chromium Single Cell 5′ Gene Expression, Immune Profiling **Sample type:** Human blood cells **Clinical timepoints:** Infusion product, longitudinal blood samples post-treatment (up to ~4 months)	Performed scRNA-seq on 62,167 $CD8^+$ CAR T cells from the infusion product (IP) and blood, finding that the transcriptional profile of CAR T cells in the blood progressively progressively diverges over time in vivo from that of the IP Found four transcriptionally distinct clusters of $CD8^+$ CAR T cells from the IP Pairing gene expression and T-cell receptor (TCR) sequence analysis showed clones that expanded after infusion are primarily derived from clusters with high expression of cytotoxicity and proliferation genes Study demonstrates the value of characterizing CAR T-cell kinetics and fate after infusion, helping to improve therapy design

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CRISPR-Engineered T Cells in Patients with Refractory Cancer EA Stadtmauer, et al. *Science*. (2020).	**Research area**: Immuno-oncology, cancer **10x Genomics products**: Chromium Single Cell 5′ Gene Expression, Immune Profiling **Sample type**: Human blood cells **Clinical timepoints:** Longitudinal blood samples post-treatment (up to ~4 months)	First human phase I clinical trial to test the safety and feasibility of multiple CRISPR-Cas9 gene edits to T cells Introduced cancer-specific TCR transgene (NY-ESO-1); deleted *TRAC* and *TRBC* genes to reduce TCR mispairing; deleted *PDCD1* to limit T-cell exhaustion Infusions of engineered T cells were well tolerated in three patients and showed persistence 9 months after infusion scRNA-seq analysis of longitudinal blood samples from one patient revealed heterogeneous transcriptomic phenotypes in the engineered product as cells evolved over time in vivo
c-Jun Overexpression in CAR T Cells Induces Exhaustion Resistance RC Lynn, et al. *Nature*. (2019).	**Research area**: Immuno-oncology, cancer **10x Genomics products**: Chromium Single Cell Gene Expression **Sample type**: Human, mouse tumor cells	Performed scRNA-seq on 804 CD19-CAR and 726 GD2-CAR T cells to reveal an exhaustion signature of GD2-CAR T cells in differentially expressed activation-associated genes, inhibitory receptors and inflammatory chemokines or cytokines, and genes associated with naive and memory T cells scRNA-seq validated that the bZIP family members JUN, JUNB, JUND, and ATF4 were some of the most differentially expressed in exhausted GD2-CAR T cells scRNA-seq performed on 6,946 Her2-BBz and 10,985 JUNHer2-BBz CAR T cells (JUN-overexpressing) revealed a more activated transcriptional program and down-regulation of many exhaustion-associated genes in JUN-overexpressing cells Study shows that CAR T cells can be engineered for enhanced antitumor activity, in this case, engineered to overexpress c-Jun, which improved antitumor potency in five different mouse tumor models in vivo

Additional references:

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3. JA Rath et al., Single-Cell Transcriptomics Identifies Multiple Pathways Underlying Antitumor Function of TCR- and CD8αβ-engineered Human CD4^{+} T Cells. *Sci Adv*. 6, eaaz7809 (2020).
4. RI Klein Geltink et al., Metabolic Conditioning of CD8^{+} Effector T Cells for Adoptive Cell Therapy. *Nat Metab*. 2, 703–716 (2020).
5. N Singh et al., Impaired Death Receptor Signaling in Leukemia Causes Antigen-Independent Resistance by Inducing CAR T-cell Dysfunction. *Cancer Discov*. (2020). DOI: 10.1158/2159-8290.CD-19-0813
6. YC Lu et al., Single-Cell Transcriptome Analysis Reveals Gene Signatures Associated with T-Cell Persistence Following Adoptive Cell Therapy. *Cancer Immunol Res*. (2019). DOI: 10.1158/2326-6066.CIR-19-0299
7. AG Chapuis et al., T Cell Receptor Gene Therapy Targeting WT1 Prevents Acute Myeloid Leukemia Relapse Post-Transplant. *Nat Med*. 25, 1064–1072 (2019).
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