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Advancing T cell–based cancer therapy with single-cell technologies

To accelerate the development of T cell–based immunotherapies that are effective for more patients with cancer, there is an urgent need to decipher the precise attributes of the ideal therapeutic T cell. In March 2021, the Parker Institute of Cancer Immunotherapy and 10x Genomics partnered to bring together a group of T cell immunotherapy researchers and single-cell-technology innovators for a day's workshop. Participants evaluated the current cutting edge of knowledge, identified areas for focused technology development, and put forward a call to action to the field. Insights were provided on how to best leverage single-cell technologies and key areas for future development were proposed — with the goal of facilitating a better understanding of T cell research and translation of this research into effective cancer immunotherapies. The key points of discussion that emerged from this workshop are summarized here.

In the field of oncology, autologous T cell therapies have been developed and approved for B cell malignancies, with limited success in solid cancers[1]. There has been a rapid expansion in the number of T cell therapies in clinical development for solid tumors worldwide, including autologous T cells expanded or engineered to express chimeric antigen receptors (CARs) or recombinant T cell antigen receptors (TCRs) and, in the past decade, with edited genomes[2]. But developing effective T cell therapies for patients with solid tumors brings new challenges: arming cells for durability and efficacy, surmounting suppressive tumor microenvironments, and avoiding deleterious toxicity, off-tumor activation, target antigen heterogeneity and exhaustion. Considerable effort is being focused on understanding the mechanisms underlying successful T cell–targeting therapies, such as monoclonal antibodies to the inhibitory immunoreceptor PD-1 (programmed cell death protein 1) and its ligand PD-L1 (programmed death ligand 1) in patients with solid tumors — using translational data sets as a blueprint for the attributes of T cells that drive lasting clinical benefit.

T cell biology is dynamic, heterogeneous and complex. Rare clonal populations, driven by interactions within and outside the immune compartment, are therapeutically relevant, but analyzing T cells in bulk precludes nuanced knowledge of the rare tumor-reactive T cell. Once cells are assessed at the single-cell level, rare cell populations can be identified and true understanding of cellular activity and identity can be realized.

Improvements in genomic, fluidic and computational tools have ushered in a new era of multi-omics readouts in individual cells. Today, single-cell technologies enable deep characterization of cell state through high-dimensional measurements, including at the level of the TCR sequence[3], transcriptome[4], surface proteome[5], transcriptionally accessible chromatin[6,7], or some combination of these[8]. Capabilities in tracing cell lineage, improvements in cell throughput and resolution, and incorporation of the temporal context of T cells create a rich picture of the cellular and molecular complexity of T cells in cancer. Single-cell technologies, therefore, provide a robust toolkit with which to uncover the fundamental biology of T cells during an anti-tumor immune response.

The workshop focused on the application and development of single-cell technologies for the investigation of T cell biology in immunotherapy-treated patients with cancer. Thought leaders explored how to efficiently leverage this information to inform the development of T cell–based therapies (Fig. 1). We report here on the compelling aspects of fundamental T cell biology that are being used for T cell therapeutics, the gaps that remain, and

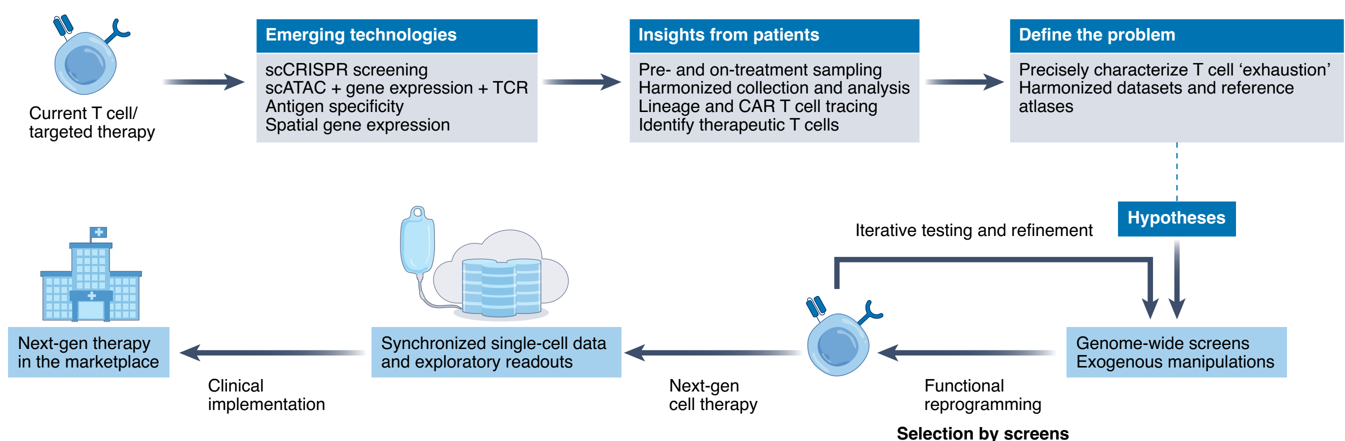

Fig. 1 | Model for the design of effective T cell-based cancer therapies. This roadmap leverages single-cell technologies for patient-centric research and the development of next-generation cancer therapies. sc, single-cell; Next-gen, next-generation.

Box 1 | Types of clinical T cell analyses that will inform improvements in T cell therapeutic research and development

T cell state: molecular and functional phenotype. Single-cell resolution of the epigenome, transcriptome, and protein expression allow the correlation of cell state with clinical attributes such as response and resistance to immunotherapy and relapse after immunotherapy.

T cell fate: future identity of daughter cells. TCR sequencing of single cells leverages the TCR CDR3 sequence to trace mother–daughter relationships, characterizing the fate of T cells in response to and/or as an immunotherapy. Useful when combined with T cell state analysis.

TCR specificity: what antigen the T cell recognizes. Knowledge of whether the patient's T cells being analyzed recognize the tumor or not will be revolutionary, not only for therapeutic choices for patients (for example, anti-PD-1 if the patient has their own tumor-reactive T cells, or CAR T cells if none are detected) but also for adding relevant context to T cell state and fate studies as described above.

Spatial context: position of T cells in the tumor microenvironment and their interplay with other cell types. T cell function within a three-dimensional tissue microenvironment, and cellular relationships are lost when tissue is dissociated for some single-cell profiling applications. High-dimensional profiling of cells, from multicellular resolution to subcellular resolution, is providing novel insights into interdependent cellular interactions that widen the lens for appreciating mechanisms of successful T cell–based therapies.

Tracking gene-edited T cell therapies in patients: methods for detecting and analyzing autologous T cell therapy products over time in patients with cancer bring additional technical complexity to single-cell approaches as methods are needed to (1) identify the therapy in the background of endogenous T cells ('needle in a haystack'); (2) track gene edits with fidelity; and (3) ensure the technology is compatible with sample preparation in the clinical setting (for example, formalin-fixed tissue).

approaches to filling these gaps. Finally, we provide a call to action to improve and accelerate the analysis of clinical samples and the development of single-cell technologies.

Defining the therapeutically relevant T cell

Building a reference data set. John Wherry (University of Pennsylvania) opened his keynote address with the challenge that CD8$^+$ T cell molecular and functional phenotypes (cell state) and the future identity of the daughter cells (cell fate) remain poorly defined. This can be attributed, at least in part, to the absence of a common reference data set that could be used by the field as a benchmark for analyzing research samples. To address this, Josephine Giles in the Wherry group has generated a healthy human T cell epigenome and transcriptome data set using bulk ATAC (assay for transposase-accessible chromatin) sequencing (ATAC-seq) and RNA sequencing (RNA-seq), respectively, from canonical CD8$^+$ (and some CD4$^+$) T cell subsets[9]. Investigation of these data demonstrated that chromatin structure provided a separation of functional T cell states superior to that achieved by gene transcription; moreover, accessible chromatin regions that are distal to the gene promoter provide resolution of CD8$^+$ T cell subsets superior to that provided by promoter-proximal accessible chromatin regions or RNA-seq. The Wherry group is using this data set as a reference map to put RNA-seq and ATAC-seq data from PD-1 inhibitor trials into context using canonical cell-type definitions.

Insights from therapeutic targeting of PD-1. Therapies directed against PD-1 and PD-L1 have resulted in cancer regression and have extended survival for ~15% of patients with metastatic cancer, which has revolutionized research and clinical development strategies in oncology[10]. In 2021, 5,761 oncology clinical trials involving monoclonal antibodies that target PD-1–PD-L1 pathways were reported in the United States[11], which provided an unprecedented opportunity for learning about mechanisms of clinical response and resistance to these therapies. We are certain that CD8$^+$ T cells mediate anti-tumor immunity in this context and provide long-lasting protection through memory generation. However, questions remain, such as how tumor-specific T cells can be identified and what determines the fate trajectories of these T cells. Deep analysis of T cells provides not only insights into T cell–intrinsic mechanisms of response and resistance and potential biomarkers for therapies directed against PD-1, but also broader insights into how T cells act in patients with cancer. This approach is being used to inform research and development of T cell–based therapeutics, as current preclinical models are limited in their ability to be predictive of the efficacy, potency, differentiation and durability of these therapies in patients (discussed further below).

Ansu Satpathy (Stanford University) develops and uses single-cell, multi-modal analysis for insights into T cell responses during cancer immunotherapy. He presented an ongoing interpretation of the Yost et al. study that sought to trace the fate of T cells in patients with basal cell carcinoma treated with antibody to PD-1 (anti-PD-1)[12]. Emerging data suggest that tumor-infiltrating lymphocytes (TILs) with the same TCR clonotype have similar transcriptional programs. This suggests that the state of TILs can be imprinted by the TCR; understanding the mechanism of TCR-directed fate decisions will therefore be crucial for the therapeutic application of TILs and TCR- or CAR-engineered T cells. Ansu Satpathy, Mathew Hellman (Memorial Sloan Kettering Cancer Center) and collaborators addressed the question of the resistance of solid tumors to anti-PD-1 by analyzing TILs in distinct regions of non–small-cell lung cancer tumors, before and after therapy, in patients who had mixed responses across sites (including pathological responses and resistance)[13]. This study design controlled for the challenge of sampling heterogeneity, which will be discussed later in this report. Single-cell TCR sequencing and single-cell RNA-seq (scRNA-seq) analyses revealed that dominant clonotypes with memory effector and stem-cell-like transcriptomes expanded and were present in both treatment-responsive sites and treatment-resistant sites. Thus, in this study, there was no evidence that dysfunction or lack of T cells, per se, was associated with regional resistance to PD-1-blockade therapy, which has been suggested as a predictive biomarker for anti-PD-1-naive patients with melanoma[14]. However, the challenge of comparison across studies was emphasized in the discussions. The considerable challenge of data harmonization across translational data sets was a common theme throughout the workshop.

Improving adoptive T cell therapy

Tracing the fate of adoptive cell therapy in patients with cancer not only provides insights into mechanisms of response and resistance, but also provides patient-relevant data that can inform the design of the next generation of cell therapies. Ansu Satpathy discussed the use of multi-modal single-cell analysis in longitudinal sampling of a recent first in-human study of a T cell therapy product with multiple CRISPR–Cas9 gene edits: physiological expression of a TCR with editing of the locus encoding its TCR

Table 1 | Challenges being solved by technology development that were discussed at the workshop

Critical research needs	Technology development to address needs
Reduction of high sequencing costs	Sample multiplexing[8] and targeted panels
Increase in cell throughput in single-cell studies to observe rare cell types of interest	Chromium X (microfluidic instrument for high-throughput single-cell analysis)
Immunoreceptor specificity	BEAM (barcode-enabled antigen mapping: high-throughput mapping of antigen recognition by B cells or T cells), TCRex[22], TCRGP[23], NetTCR[24] and TcellMatch (all are computational approaches to predicting ligands on the basis TCR sequence)
Multiomics analysis of tissue sections	TCR plus RNA[25], XYZeq[26], Visium[27] (spatial co-detection of whole transcriptome and protein markers), and Xenium in situ (instrument for subcellular targeted spatial profiling)
Higher resolution spatial analysis (on tissue sections)	MIBI-TOF[28] (protein detection with heavy-metal-tagged antibodies) and Xenium in situ
Multi-modal data-set integration	Various algorithms[29–33]
Analysis of archival clinical samples	Fixed: chromium-fixed RNA profiling (single-cell analysis of dissociated PFA-fixed cells and FFPE tissue). Visium for FFPE tissue, Visium CytAssist (instrument for spatial analysis of pre-mounted tissue slides), Xenium in situ
Temporal cell tracing	Single-cell division tracking plus antigen receptor mtDNA-seq for non-lymphocytes[7]
Gene integration tracking	PoKi-seq[21], CAR T cell integration site analysis[34]
Gene regulation and network interactions; need for data science and/or machine learning methods	Single-cell ChIP-seq[35]

PFA, paraformaldehyde; FFPE, formaldehyde-fixed paraffin-embedded; mtDNA-seq, mitochondrial DNA sequencing; ChIP-seq, chromatin immunoprecipitation followed by deep sequencing.

α-chain and β-chain so the TCR recognizes the intracellular tumor antigen NY-ESO-1, and deletion of PD-1 to overcome exhaustion[15] (as exhausted CD8$^+$ T cells are associated with ineffective anti-tumor responses in solid tumors[16]). In this trial, led by Carl June and collaborators at the University of Pennsylvania, the cell-therapy product was administered to three patients with advanced, refractory solid cancers[15]. Over time (by day 113), the infused therapeutic cells took on an exhausted phenotype. Strikingly, cells in which PD-1 was deleted did not persist, regardless of other gene modifications, whereas TCR transgene–positive cells with deletions in the TCR α-chain had improved durability. These findings agreed with previously published work on the importance of PD-1 in controlling activation-induced death in early T cell activation[17] and improved TCR cell therapy when recombinant genes are targeted to the locus encoding the TCR α-chain[18]. These results also emphasized the actionable insights that can arise from temporal, single-cell analysis of adoptive T cell products in patients.

Discussions in a breakout-session on single-cell profiling for T cell therapy development, led by Phil Greenberg (University of Washington and Fred Hutchinson Cancer Center), coalesced around the central challenge that there are inadequate insights into the type of CD8$^+$ T cell that drives successful immunotherapy. For example, the presence of PD-1$^+$TCF-1$^+$ self-renewing stem-like T cells in the tumor microenvironment is associated with a response to anti-PD-1 in patients with kidney, prostate or bladder cancer;[19] however, questions remain: is this broadly applicable across cancer types and stages, are these the defining or only properties that drive the success of adoptive T cell therapy, and how can these beneficial properties in T cells be engineered in such a way that they both persist in patients and provide the necessary anti-tumor activity?

Current strategies aimed at generating desirable therapeutic T cell phenotypes include signaling modifiers (for example, switch receptors that convert inhibitory signals to stimulatory ones), targeting metabolic pathways, and epigenetic and genetic modifiers. Orthogonal approaches of gene engineering and synthetic biology were discussed in depth. Alex Marson (Gladstone-UCSF Institute for Genomic Immunology in San Francisco) discussed several recent studies that made use of pooled CRISPR screens of genetic manipulations to elucidate and improve T cell functionality for adoptive cell therapies, such as improvements in proliferative capacity and antigen sensitivity, even in suppressive environments. As an example, he presented data from colleagues Eric Shifrut and Julia Carnevaele demonstrating that deletion of RASA2 — a regulator of RAS activity that controls proliferation and differentiation in non-oncogenic cells — improved T cell activation potential in challenging environments associated with solid tumors. The Marson lab has also adapted several pooled CRISPR screening methods used in primary human immune cells to be compatible with droplet-based scRNA-seq phenotyping (also known as 'Perturb-seq'[20]). For example, pooled CRISPR knock-ins at a defined site can be coupled with scRNA-seq ('Poki-seq') to parallelize discovery of synthetic knock-in sequences that improve T cell function[21]. In addition to complementing selectable phenotypic readouts such as cytokine production and proliferation, these high-dimensional single-cell readouts will help to uncover signaling mechanisms and identify potential trade-offs (for example, between strong effector function and maintenance of stemness) in the use of synthetic biology to generate better T cells for cancer therapy.

Call to action

The workshop identified five types of analysis (Box 1) that provide crucial insights to accelerate the rational development of T cell therapies — by identifying the tumor-reactive T cells to prioritize for further study in patients treated with anti-PD-1, highlighting T cell–extrinsic factors that affect how T cells act in the tumor microenvironment, and elucidating T cell functional fidelity and activity after infusion. Additional high-priority areas in which advancements would accelerate the rational development of T cell therapies included the following:

1. **Improvement of preclinical models for T cell therapy development**. Current workflows include a phased approach from in vitro testing to in vivo xenograft models in immunodeficient mice, immune-competent tumor models and humanized mouse models and then to phase 1 clinical testing. A panel led by Lisa Butterfield (Parker Institute of Cancer Immunotherapy) investigated the value and challenges of translating preclinical data; disparate views remain

on the value of models for clinical utility. Key considerations are, first, the goal of the study and, second, whether the limitations of the model are fully appreciated within the context of that goal. For example, 'humanized' mouse models remain hybrids of mouse and human systems with both functional networks and dysfunctional networks. These attributes must be considered in the design and interpretation of preclinical proof-of-concept and mechanistic studies. Eric Shifrut (University of California, San Francisco) revealed that scRNA-seq analysis of CAR T cells in different in vitro and in vivo systems had widely disparate readouts of cell state, durability and anti-tumor activity. There remains insufficient information from engineered T cells in patients with cancer for appropriate correlation of preclinical data sets with clinical activity.

2. **Development of human single-immune-cell reference maps**. Tissue-specific atlases that define canonical immune cell types and subsets spanning all organs are needed for cataloging of normal immune cell states. Defining 'normal' states poses an inherent challenge, due to variables such as environment, race, sex and age; therefore, large sample sizes are needed to encompass divergence. Consortia-wide efforts, such as those at the Human Cell Atlas and the Foundation for the National Institutes of Health Biomarkers Consortium, are solid foundations for reference data sets for the public domain. Theresa LaValle (Parker Institute of Cancer Immunotherapy) hosted a panel discussion on the practicalities of generating reference maps for putting data sets from cancer immunotherapy trials into context. The group coalesced around the challenge of the number of immune cells needed to capture rare cell subsets, and tissue residency that affects cell state. Spleens from trauma patients and neoadjuvant surgical tumor tissues emerged as tangible efforts, some of which are ongoing.

   Finally, James Lee (University of California, San Francisco) called for standard transcriptomic data sets that can be used as controls for multivariate analysis of trial data, for which numbers are always a challenge.
3. **Harmonization of translational data sets**. The variability of trial data sets directly affects the reliability of immune-cell reference maps and cross-study comparisons. Standardization of sample collection and processing, data generation, analysis platforms and biostatistical methods are critical issues to address. Ansu Satpathy emphasized that there is a need for specialized cross-functional teams of technologists, biologists, software and machine learning engineers, and computational biologists to execute rigorous single-cell analysis of trial samples. A key call to action discussed in a breakout session led by Nick Banovich (Translational Genomics Research Institute) included the development of multi-omics single-cell and spatial atlases of longitudinal, multi-tissue samples collected from patients in cell-therapy clinical trials.
4. **Technology development**. Technology development to increase throughput, resolution, fidelity and cost is ongoing, and the workshop focused on advancements that are in the public domain and underway at 10x Genomics (Table 1). The data reviewed at this meeting were generated from single-cell technology development and application, and workshop participants acknowledged that the discussions were not inclusive of all current technologies in development in the field. Going forward, other workshops with a similar theme should be conducted with annual cadence to capture the rapid developments in both single-cell analysis and spatial analysis.

Conclusion

A diverse group of technology innovators, clinical and basic scientists, and drug developers came together to discuss the utility of single-cell technologies to inform the development of T cell therapies for solid tumors. Such cross-functional teams are crucial for success in high dimensional cell analysis that addresses complex biology.

Action on many of the points raised in this meeting report is already underway and is expected to progress rapidly. The Parker Institute of Cancer Immunotherapy, 10x Genomics and partners will continue to collaborate to generate data and technologies to provide roadmaps for defining, generating and using therapeutically relevant T cells for successful cancer therapies. ❐

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Competing interests

The authors declare no competing interests.