

Opinion

Ushering in Integrated T Cell Repertoire Profiling in Cancer

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Advances in immune profiling techniques have dramatically changed the cancer immunotherapy and monitoring landscape. High-throughput protein and gene expression technologies have paved the way for the discovery of therapeutic targets and biomarkers, and have made monitoring therapeutic response possible through the ability to independently assay the phenotype, specificity, exhaustion status, and lineage of single T cells. Although valuable insights into response profiling have been gained with current technologies, it has become evident that single-method profiling is insufficient to accurately capture an antitumor T cell response. We discuss and propose new methods that combine multiple axes of analysis to provide a comprehensive analysis of T cell repertoire in the fight against cancer.

T Cell Repertoire and Tumor Immunity

Immunotherapy plays a critical role in the modern treatment of both solid and blood tumors. As the field matures, a diverse range of therapeutics that harness T cell cytotoxicity are used in the fight against cancer, from personalized vaccines [1] and oncolytic viruses [2], to immune checkpoint blockade (ICPB) [3] and adoptive cell transfer (ACT) therapy [4]. As T cells take the center stage of cancer immunotherapy, a deep understanding of the intrinsic properties of T cells and their responses to extrinsic environments are important to new therapeutic development and treatment monitoring. Immune cell repertoire profiling that aims to evaluate immune receptor composition, antigen specificity and crossreactivity, and functional and activation status of the antigen-receptor-expressing cells is one research area that holds promise for biomarker and immune signature discovery, with the potential to aid patient stratification as well as target discovery and therapeutic development.

In this Opinion, we focus on repertoire profiling of CD8⁺ T cells, which are able to directly enact antitumor immunity, given antigen recognition through their unique T cell receptor (TCR) [5,6]. Collectively, the CD8⁺ TCR repertoire determines an individual's ability to respond to diverse antigens quickly and effectively. This endogenous immunity is the basis for therapies relying on the patient's own immune system, including vaccines and ICPB (Figure 1). Additional therapies introduce new immunity using receptor-engineered T cells to target tumor antigens. Therefore, being able to quantify the completeness and functional competence of this repertoire is important to identify patients that are most likely to benefit from a specific immunotherapy. Assessing these characteristics requires profiling highly heterogeneous T cell populations at the single-cell level in a high-throughput manner. Comprehensive and multifaceted T cell profiling is therefore critical to improve the success and safety of current T cell-based immunotherapy, as well as in developing new strategies in this area.

T Cell Profiling in Cancer Therapeutics

T cell-based immunotherapies operate upon one of two main premises: inhibition of the suppressing factors to endogenous antitumor immunity; or induction of new antitumor

Highlights

Bulk TCR and single-cell TCR repertoire analysis can provide T cell clonal expansion information.

Single-cell RNA sequencing and CyTOF can provide cell phenotype and functional status analysis of T cells.

DNA-barcoded pMHC tetramers enable high-throughput screening of T cell antigen specificity.

Integrating TCR antigen specificity, TCR sequences, and T cell phenotype and functional status at single-cell level provides comprehensive T cell profiling, which is critical for T cell-based cancer immunotherapy and monitoring.

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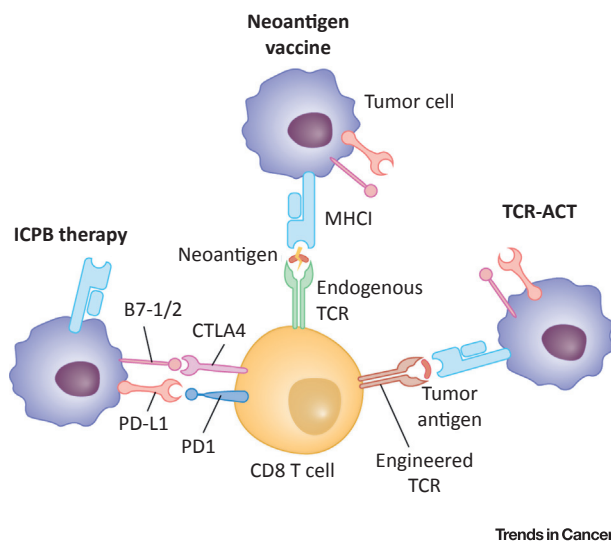


Figure 1. T Cell-Based Cancer Immunotherapies. Current cancer immunotherapies include (i) ICPB by blocking suppressive effects of T cell inhibitory molecules; (ii) neoantigen vaccine by stimulating T cells that recognize neoantigen-expressing tumor cells; and (iii) TCR-ACT, by engineering autologous T cells to recognize specific cancer antigens and infuse them back to patients to fight cancer cells. Abbreviations; ICPB, immune checkpoint blockade; TCR-ACT, T cell receptor redirected adoptive cell transfer.

immunity. Despite their differences, each technique discussed below relies upon the specific interaction between an effector cell and its cognate, tumor-associated antigen. Understanding and characterizing the downstream transcriptomic and epigenetic changes that these therapies have on T cells is key to improving treatment efficacy and to identifying new therapeutics.

ICPB Therapy

ICPB therapy has revolutionized the treatment of many solid [3] and blood [7] cancers, and is one of the most successful, off-the-shelf cancer therapies to date. ICPB uses antibodies to block T cell inhibitory molecules, such as PD-1/PD-L1 or CTLA-4, to reinvigorate existing T cells without regard to specificity (Figure 1). This is often seen as ‘taking the brakes off’ of the immune system and can result in polyclonal reinvigoration of cellular antitumor immunity [8]. Approximately 20–30% of patients achieve an objective response with single ICPB, although this varies between disease types, and combination therapy can achieve a higher response rate [9,10]. Regardless of patient outcome, ICPB also activates T cells that are not tumor specific, which can result in autoimmune off-tumor toxicity that ranges from skin depigmentation (vitiligo) to severe myocarditis resulting in cardiac death [11].

Toxicity resulting from ICPB has driven research into pretreatment or early treatment patient stratification methods to determine which patients are most likely to achieve an objective response [12,13]. Early stratification methods use single-molecule expression or simple metrics to predict patient response to ICPB [14]. Tumor PD-L1 expression, mutation rate, and predicted neoantigen burden were proposed and studied for this purpose, but were not robust in their prediction of patient outcome. Because ICPB relies upon a diverse set of effector cells, high-throughput profiling methods are needed to quickly and accurately assess the state of response.

Neoantigen-Based Vaccine

Like ICPB, antitumor vaccines often rely upon a diverse immune response to elicit tumor recession. Neoantigen vaccines in particular attempt to bolster the immune response by increasing the awareness of the immune system to specific, aberrant proteins found within the tumor [15]. It is hypothesized that because neoantigens are not part of the host proteome,

the thymic negative selection process preserves T cells bearing high-affinity TCRs towards neoantigens [16,17]. Administering a mixture of neoantigens as a therapeutic vaccine should stimulate functional T cells to execute cytotoxicity toward neoantigen-expressing cancer cells.

Three studies [15,18,19] independently demonstrated the feasibility and therapeutic potential of selecting personalized neoantigens and using them as therapeutic vaccines. All three studies performed extensive analysis to identify a set of personalized neoantigens, which were then injected into the respective patients. All groups demonstrated vaccine-related complete and/or partial objective responses. In two of the studies, there were patients who relapsed, but subsequently achieved complete response after anti-PD-1 was added to the treatment. The necessity of the PD-1 blockade demonstrates that presence of tumor-responsive T cells and their awareness of the antigen is not sufficient to always elicit tumor rejection. Thus, other metrics are needed to quantify the ability of the patient's immune system to respond to similar treatment.

ACT Therapy

ACT therapy physically introduces antitumor immunity by intravenous infusion of large quantities of tumor-reactive T cells [4]. The infused cells are generated in one of two ways: expansion of autologous tumor-infiltrating lymphocytes (TILs; TIL-ACT); or genetic redirection of autologous T cells by TCR transduction (TCR-ACT). In recent years, the therapeutic potential of ACT therapy has been demonstrated in colorectal cancer (CRC) [20,21], synovial cell sarcoma and melanoma [22,23] and breast cancer [24] among others.

TIL-ACT is cumbersome; it requires substantial tumor resection prior to therapy, an intensive search for tumor-antigen-specific TILs, and a long outgrowth period [4]. However, by using immune profiling of the infusion product and direct TIL isolates, much can be learned about the endogenous antitumor response in CD4⁺ and CD8⁺ T cells. In contrast, TCR-ACT is less constrained by the patients' own T cells, because it can use tumor-antigen-specific TCRs identified from other sources [25]. These TCRs can be introduced to T cells isolated from patients' peripheral blood. Two main challenges have prevented the extensive use of TCR-ACT: discovery of robust, immunogenic tumor antigens, and rapid identification of therapeutic TCRs. These represent areas where integrated T cell profiling technologies could impact patient care (see below).

Single-Axis T Cell Repertoire Profiling

Studies on T cell repertoire profiling often focus upon single axes to parse out information about TILs and peripheral T cells. Key technologies here use immune repertoire sequencing technologies, cytometry by time of flight (CyTOF) or single-cell RNA sequencing (scRNA-seq) to examine the T cell clonal expansion, activation, and phenotype.

TCR Repertoire Sequencing

Recently, we [26,27] and others [28] developed several second-generation immune repertoire sequencing technologies that use molecular identifiers to reduce sequencing and PCR errors that are common to the first generation of immune repertoire sequencing techniques [29–31]. This approach reduces error from more than one nucleotide in every TCR sequence to one nucleotide in 150 TCRs. Immune repertoire sequencing has been used to show that the clonal diversity index of mucosal TCR repertoire predicts prognosis in gastric cancer [32]. Others [33] have examined the TCR intratumor heterogeneity in lung adenocarcinomas and established the correlation between high degrees of TCR intratumor heterogeneity and increased risk of postsurgical relapse and shorter disease-free survival. Another study showed that a less diverse and more clonally expanded TCR repertoire in pretreatment tissue significantly correlated with clinical response to anti-PD-1 treatment in advanced melanoma patients [34].

Development in informatics to piece out TCR and B cell receptor (BCR) sequences from bulk RNA sequencing data enables TCR repertoire analysis from existing large cohorts of sequencing data, which could potentially be used for prognostic prediction. This analysis showed that public TCRs are common in tumors [35], which is consistent with another study using HLA-A2⁺ patient samples [36] that suggested that, aside from neoantigens, T cells recognizing shared antigens are also prevalent. Other studies went a step further and identified potential immunogenic somatic mutations on the basis of their co-occurrence with CDR3 sequences [37,38]. More recently, an improved algorithm was developed to extract TCR and BCR sequence information from bulk RNA sequencing data [39], revealing that high intratumoral immunoglobulin heavy chain expression levels were associated with longer survival.

Single-Cell Gene Expression and Phenotypic Profiling of T Cells

Using scRNA-seq, several groups have documented heterogeneity of TILs in mice [40,41] and humans [42–44] for different tumor types. Dysfunction is a common feature of TILs and the gene modules for TIL dysfunction and activation are enriched in different TIL subpopulations. CyTOF [45] analysis echoes scRNA-seq results on heterogeneity of TIL gene expression. In-depth studies in both humans and mice showed that anti-CTLA-4 and anti-PD-1 treatments target different subsets of TILs and that they induce immune responses by distinct cellular mechanisms [46].

Two studies have used CyTOF in combination with other methods to examine possible biomarkers in the peripheral blood of stage IV melanoma patients before anti-PD-1 treatment that could be used to predict responders from non-responders. These two studies, however, generated two different sets of biomarkers. Huang *et al.* [47] showed that, despite most patients demonstrating an immunological response to anti-PD-1 treatment, the magnitude of circulating exhausted CD8⁺ T cell reinvigoration and tumor burden correlated with clinical response. Krieg *et al.* [48] also examined peripheral blood samples and found that the frequency of CD14⁺CD16⁻HLA-DR^{hi} monocytes is a strong predictor of progression-free and overall survival in response to anti-PD-1 therapy. Two recent studies used scRNA-seq and examined 12 346 and 6311 TILs, respectively. Guo *et al.* [49], found that while the ratio of pre-exhausted to exhausted T cells was positively correlated with better prognosis, enrichment of the gene signature of activated tumor regulatory T cells correlated with poor prognosis in lung adenocarcinoma. However, Sava *et al.* [50] showed that the gene signature of CD8⁺ tissue-resident memory T cells was associated with improved survival. These different prognostic factors reflect the complexity of the antitumor T cell response as well as the difference between sampling blood and TILs.

Neoantigen-Based Immune Profiling

Early studies in mice [51] and humans [52] showed that tumor mutations are important targets for ICPB, which led to the hypothesis that it may work best in tumors with high mutation burden [16]. Studies in melanoma [53] and non-small cell lung cancer [54] supported this hypothesis. In addition, it was found that patients with mismatch-repair-deficient CRC are more responsive to ICPB therapy than patients with mismatch-repair-proficient CRC [55]. Recently, this observation was extended to 11 other solid tumor types [56]. These studies demonstrate that mutational load is associated with the degree of clinical benefit but is not sufficient to predict benefit alone.

Integrated Repertoire Analysis Technologies

Technological development has made it possible to pair information on two different axes in single cells, such as linking TCR affinity with TCR sequences, and pairing TCR sequences with antigen specificity and crossreactivity.

Antigen-Specific TCR Affinity and Receptor Sequence Profiling

MHC multimers [6,57] have long enabled detection of antigen-specific T cells from patient tissues and peripheral blood. However, tetramer staining intensity is not well-correlated with T cell function [58], while TCR affinity is [59]. Traditionally, TCR affinity has been quantified in solution (3D affinity) by the surface plasmon resonance method, such as Biacore, which involves labor-intensive generation of soluble TCRs and pMHC. More recently, we [58,60,61] and others [62] have shown that TCR affinity measured at the interface of T cells and antigen-presenting cells, which is referred to as 2D affinity, correlates better with T cell function compared with TCR affinity obtained in solution. Thus, 2D TCR affinity could be used to identify therapeutic TCRs. However, the traditional 2D TCR affinity measurement technologies are low-throughput and are optimum for therapeutic TCR discovery. We developed an *in situ* TCR affinity and sequence test (iTAST) [58] that can measure 2D TCR affinity on individual human primary T cells and at the same time obtain its TCR sequences. With a throughput of 75 cells per day, iTAST can be used to find TCRs of any specified 2D affinity and potentially increase the speed of the discovery of therapeutic TCRs.

TCR Sequence and Antigen-Specificity Pairing

Until recently, large-scale assessment of TCR antigen specificity and crossreactivity in TILs and other T cells has been experimentally challenging. We recently made contributions to expanding knowledge in this particular area through the development of a new technology to link simultaneously antigen specificity to TCR sequences on thousands of single T cells for hundreds of peptides [63]. We named it TetTCR-Seq for tetramer-associated TCR sequencing. We achieved this by using DNAs to label pMHC tetramer specificities [64] instead of fluorophore [57,65] or heavy metal ion [66] labels; thus drastically expanding the number of antigen species that can be interrogated at once, as well as the number of bound antigen species on single T cells. The ability to detect multiple species of pMHC binding onto the same T cell enables the detection of TCR crossreactivity.

We have demonstrated the advantage of linking antigen specificity with TCR sequences in studying the potential of neoantigen-recognizing TCRs to crossreact with wild-type antigen counterparts. Using published and experimentally validated neoantigens, we showed that neoantigen-recognizing TCRs that crossreact with wild-type antigen counterparts are prevalent. TetTCR-Seq therefore enables the identification of neoantigens that are capable of inducing wild-type crossreactive T cells as well as the identification of neoantigen-specific TCRs that are not crossreactive and are of potential therapeutic value.

High-Throughput Integrated Single T Cell Profiling Is the Future

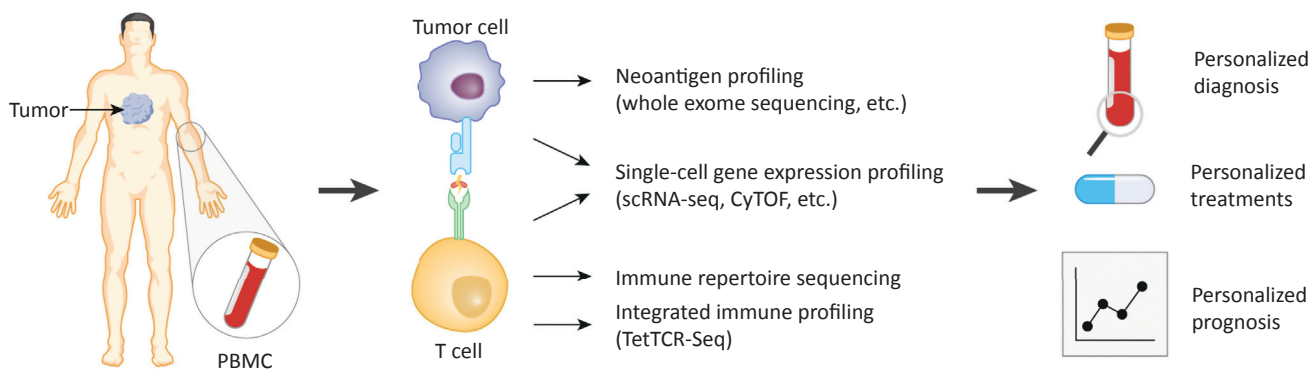
A recent case study [24] demonstrated drastic changes in TCR repertoire when comparing between *in vitro* expanded, neoantigen-recognizing TILs and TCRs that can be detected in peripheral blood 6 weeks after infusion. Antigen specificities that were nearly undetectable at infusion were represented by dominant clonotypes after 6 weeks. Furthermore, some of these same clonotypes were those present in high quantities before *in vitro* expansion, suggesting a degree of reversion to the original TIL repertoire. These dynamic composition changes of TCR repertoire are likely to be shaped by the antigen-expression pattern in cancer cells and the suppressive tumor microenvironment, thus highlighting the importance of a comprehensive understanding of these interactions in directing therapy.

High-throughput gene expression analysis of tumors enables the use of computational models to address major scientific questions about tumor immunogenicity. For example, a recent study predicted the immunogenicity of neoantigens by combining the likelihood of neoantigen

presentation by MHC and the probability of recognition by T cells [12]. In that study, the authors developed a neoantigen fitness model that predicted tumor response to ICPB. Part of this model relies upon the assumption that a neoantigen is more likely to be recognized by T cells if it is similar to known peptides in Immune Epitope Database (IEDB) Appendix A. The positive correlation observed under this assumption could have several implications: responding TCRs could be crossreactive to a foreign antigen, or that certain peptide characteristics result in more robust immune responses. Thus, we identified a need for a comprehensive understanding of how tumor-associated epitopes and their expression impacts recognition by TILs. TIL repertoire profiling in a high-throughput manner, such as TetTCR-Seq, will provide critical knowledge about the relationship between antigens and TCR repertoire in cancer immunology, and will be a useful approach to select therapeutic candidates in cancer immunotherapy.

As discussed above, the suppressive tumor microenvironment also impacts the function and differentiation state of T cells. The clinical benefit of CTLA-4 and PD-1 or PD-L1 in some cancers has motivated the search for new ICPB targets and the testing of ICPB combinations. Despite many positive outcomes, the wide range of detrimental side effects associated with ICPB demands new metrics that can be used to correlate and eventually predict patients' response before therapy. Studies using scRNA-seq or CyTOF on TILs found that independent sets of immune parameters on different T cell subsets could be used to correlate patient benefit with ICPB [46,48,50]. Despite these improvements, many patients cannot be accurately stratified and most metrics are not widely applicable to all cancer types.

We propose that a high-throughput technology that provides a comprehensive evaluation of a patient's T cell repertoire in terms of antigen specificity, T cell phenotype, activation/exhaustion status, and T cell clonal expansion could provide new knowledge for patient stratification and be directly used for ICPB patients. This technology would extensively profile the T cell repertoire in patients receiving ICPB therapy to learn which metrics constitute a good response first, then to predict ICPB efficacy (Figure 2). As previously discussed, neoantigen vaccines have been shown to have therapeutic value in melanoma, and mutational load has been correlated with clinical benefit of ICPB in melanoma and other types of cancer [17]. Thus, neo-antigen specific T cells are an attractive effector in the fight against cancer. A recent study however, showed that TILs recognizing non-tumor antigens are much more prevalent compared to TILs recognizing



Trends in Cancer

Figure 2. Benefits of T Cell Repertoire Profiling in Cancer Immunology and Immunotherapy. Current methods of T cell repertoire profiling from tumor-infiltrating lymphocytes and PBMCs include neoantigen profiling, single cell gene expression profiling, and immune repertoire profiling. An integrated immune profiling that combines above all aspects can help to guide a personalized diagnosis, treatment plans, and prognosis. Abbreviations: CyTOF, cytometry by time of flight; PBMC, peripheral blood mononuclear cell; scRNA-seq, single-cell RNA sequencing; TetTCR-seq, tetramer-associated T cell receptor sequencing.

neoantigens or tumor-associated antigens in both lung cancer and CRC [67]. It is possible that the lack of tumor-specific T cells found in that study resulted from the unique characteristics of lung cancer and CRC, although the former is also believed to harbor high numbers of mutations [17]. It is also possible that the neoantigen search space is limited by the number of pMHCs that can be tested (Box 1).

The proposed integrated T cell profiling technology would advance our understanding of the interaction between cancer and the immune system. The high-throughput pMHC screening capacity of the integrated technology could offer a near exhaustive screening of all personalized neoantigens from individual patients, while the scRNA-seq aspect of the technology could provide an in-depth analysis of TIL functional and activation/exhaustion state. scRNA-seq is

Box 1. Immune Repertoire Characteristics

Immune Receptor Repertoire

T cells and B cells are able to recognize a large and diverse pool of antigens through their immune receptors. During cellular development, these receptors go through variable, diversity, and joining (VDJ) region recombination that enables a potential 10^{18} unique receptors for T cells and 10^{13} for B cells [69]. Despite the theoretical possibilities, current estimates place the peripheral diversity in an adult human to be approximately 2×10^7 unique TCRs at a given time [70]. The immune repertoire refers to the identities and distribution of these immune receptors present in an individual at a given time.

Immune Cell Repertoire

The cellular repertoire differs from its receptor counterpart by including cellular characteristics. These may include phenotype, activation status, transcriptomic profiling or more recently, epigenetic analysis at the single cell level.

Clonality

When T cells proliferate, their unique TCR sequence is passed to both daughter cells. Thus, the number of T cells bearing the same TCR or their relative fraction can be used to make assumptions about how much that unique T cell or clone has proliferated. This can give insight into infection status and the presence of a given antigen.

Antigen Specificity and Crossreactivity

The individual molecular characteristics of a TCR dictate which antigens it will bind well to and those it will not. Traditionally, only one antigen is believed to associate with a particular TCR; known as TCR antigen specificity. High-throughput screening enables the detection of multiple antigens that can be bound to the same TCR. These antigens are referred to as TCR crossreactivity.

Repertoire Completeness

The collection of an individual's TCR repertoire that is able to cover all possible antigens is referred to as repertoire completeness. Repertoire completeness is important for an individual's ability to fight infection and other diseases but is difficult to evaluate due to the lack of high-throughput technologies.

Receptor Affinity

The strength of the interaction between a TCR and its ligand can be referred to as TCR:pMHC affinity. This can be measured using 2D (on cell membrane), 3D (in solution), or indirect affinity measurement methods. We and others have demonstrated correlations between affinity and *in vitro* functional capacity, which could have an impact on ACT therapies.

T Cell Exhaustion

Repeated antigen exposure combined with inhibitory signaling results in loss of T cell functionality. This loss begins as a reversible quality, but over time becomes a cellular state, featured by certain epigenetic modifications, which cannot be overcome using inhibitory molecule blockade such as anti-PD-1 antibodies. Exhausted T cells can be identified by their lack of functionality and expression of inhibitory molecules including Tim-3, LAG3, TIGIT, and PD-1.

Neoantigen

Somatic mutations within a tumor can produce altered protein segments that are tumor restricted. Missense, nonsense, and stop-loss mutations that alter the amino acid sequence can produce neoantigens. Recent studies have also pointed to chromosomal fusions and intron retention as potential sources of neoantigens.

Box 2. High-Throughput Immune Profiling Methods

Immune Repertoire Sequencing

This refers to using various next-generation sequencing platforms to quantify the composition of different kinds of B cell receptor (BCR) or TCR sequences in a sample. The measurement can be done on either single or paired chains [71]. During B cell clonal expansion, BCRs gradually alter their sequences through somatic hypermutation and change the relative abundance of each B cell clone by selective advantage. TCR sequences remain unchanged but their abundance through clonal selection may change according to proliferation. Thus, analyzing these diversity and abundance changes of BCRs and TCRs using immune repertoire sequencing can help to evaluate clonal alteration and lineage development of B and T cells in the context of infection, vaccination, autoimmune disease, and cancer.

CytoTOF

CytoTOF is a method that uses antibodies tagged with rare heavy metal ions instead of fluorescent molecules to assess protein marker expression at the single-cell level. After antibody staining, the cells are individually atomized and ionized. The masses corresponding to the heavy metal tags are counted in discrete time-separated detector channels. The minimal overlapping of mass among these heavy metal tags, enables CyTOF to analyze more than 40 different protein targets on single cells in a sample [72].

scRNA-seq

Characterization of transcribed genes at a single-cell level through sequencing of total poly-A⁺ mRNAs. scRNA-seq has evolved from in-tube methods [73–77] to high-throughput methods including microfluidic chip-based and droplet-based single cell transcriptome amplification. These allow for processing the sample in bulk, but result in reads that can be attributed back to single cells. scRNA-seq has been useful in identifying rare cell populations, studying the development of tissues and organisms, and comparing phenotypes and functions between cell populations among others.

Outstanding Questions

What parameters are essential to distinguish individual T cells?

How would comprehensive T cell profiling help cancer diagnosis and treatment?

How can we develop high-throughput, comprehensive profiling techniques for use in research and in the clinic?

especially advantageous in its quantification of transcription factors, which may have more predictive power on cell state compared to surface markers, as suggested by the recent Tabula Muris project [68]. For example, it is possible that neoantigens recognizing T cells constitute the majority of TILs, and that they are the cells that proliferate extensively and are most exhausted, as hypothesized from other scRNA-seq only studies. However, it is also possible that these T cells that recognize cancer antigens or foreign antigens are crossreactive with cancer neoantigens that cannot be evaluated by current technologies (Box 2).

This integrated T cell profiling approach could also be used to identify therapeutic neoantigens and TCRs, as the function and activation status of neoantigen-recognizing T cells from patients would provide clues as to whether the predicted antigens were indeed expressed by cancer cells, which is an area lacking high-throughput technology development. Finally, this integrated T cell profiling approach could also provide a way to monitor cancer immunotherapy and direct personalized therapeutic regimens. For example, in ACT therapy, it could be used to monitor the expression of immune checkpoint molecules and then single or multiple ICPBs could be used in addition to the original ACT therapy. In other settings, patients resistant to ICPB therapy could be tested for the existence of T cells targeting dominant cancer antigens using this integrated therapy. If the T cell frequency to these dominant cancer antigens is low, *in vitro* expanding them or engineering patients' T cells to express therapeutic TCRs could be applied to the personalized regimen.

Concluding Remarks

By combining TetTCR-Seq with scRNA-seq and other technologies, we could accurately and comprehensively profile antigen specificity, activation/exhaustion, and clonal expansion of TILs, which are parameters essential to distinguish individual T cells. A comprehensive understanding these characteristics of T cells could, in theory, be applied to predict responders before patients receive ICPB therapy, or it could yield simple markers for patient stratification that are more clinically friendly. As tumors continue to evolve under the pressure from the immune

system, it is essential to profile both the TILs and the tumor microenvironment to comprehensively understand the interaction between tumor and T cells, in order to develop new immunotherapies and diagnostic tools.

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Resources

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