The impact of one: single cell analysis of T cell states in human cancer

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Chapter 3

An atlas of intratumoral T cells

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Intratumoral T cell composition is relevant for disease outcome across tumor types

The T cells that are present in human tumors display a diversity of cell states. Because not all T cells have an equal capacity to contribute to anti-tumor responses, understanding this diversity is critical to define their role in natural tumor control and cancer immunotherapy. On page 1462 of this issue, Zheng *et al.* (*1*) describe a "T cell atlas" containing transcriptional profiles of T cells across 21 cancer types, addressing aspects such as recurring T cell states, cell differentiation trajectories, and prognostic value. Although there are many ways to slice these data, aspects of particular interest are the pan-cancer identification of T cell subsets that may play an active role in tumor control, and the observation that the relative abundance of T cells with distinct states has prognostic value that transcends tumor type, which takes a step toward immune type-based patient stratification.

The capacity of T cells to recognize and eliminate tumor cells forms the mechanistic basis for the activity of immune checkpoint blocking therapies that have revolutionized cancer care. There is compelling evidence though that the contribution of individual T cells to tumor control varies strongly, and appears associated with their cell state. Specifically, analysis of T cell infiltrates in human tumors has demonstrated that only a small fraction of T cells at such sites is tumor-reactive (*2*). In at least some tumor types, the expression of hallmarks of dysfunction (or "exhaustion"), including expression of inhibitory receptors such as programmed cell death protein 1 (PD-1), can be used to distinguish these tumor-reactive T cells from neighboring "bystander" cells (*3, 4*). Also within the tumor-reactive T cell compartment, T cells differ in their capacity to convey anti-tumor effects. Studies in mouse models have for example shown that cells with an early dysfunctional cell state, characterized by the expression of transcription factor 7 (*TCF7*) and longer-term renewal potential, are particularly important for a durable response to immune checkpoint therapy (*5, 6*).

A central question that is addressed by Zheng *et al*. is how the properties of the T cell pool with presumed tumor-reactivity compare across cancer types. $CD8^+$ T cells that are enriched at the tumor site and that show clonal expansion, as inferred from T cell receptor (TCR) sequencing, are predominantly observed in the cell pool with a (terminal) exhaustion (Tex) phenotype, consistent with chronic antigen exposure driving T cell dysfunction. Notably, the very same T cell states showed enhanced gene expression signatures associated with both cell division and TCR signaling, tempting one to conclude that tumor-reactive T cells in many human cancers are actively responding to tumor cells even in the absence of therapy. Similarly, use of the latter criteria on the $CD4^+$ T cell compartment identified a regulatory T cell (Tregs) population that expresses TNF receptor superfamily member 9 (*TNFRSF9*), which encodes the activation marker 4-1BB, as most highly enriched for potential tumor-reactivity. This demonstrates that both cytotoxic and suppressive tumor-reactive T cell populations may potentially be identified across cancer types based on their transcriptional characteristics, holding promise for both diagnostic and therapeutic applications.

Continuous antigen exposure forms a major driver of T cell dysfunction, but the varied presence of antigen as well as additional cell-bound and soluble factors, such as immune checkpoint ligands and transforming growth factor-β (TGFβ), at the tumor site, provide ample

opportunity for potential diversification in this process. Based on their pan-cancer dataset, Zheng *et al*. propose a model in which two distinct differentiation paths lead to a state of terminal dysfunction, an observation that extends recent work in non-small cell lung cancer (NSCLC)(*7*). Notably, both Tex cell differentiation paths, characterized by the presence of either a granzyme K (*GZMK*$^{+}$) or zinc finger protein 683 (*ZNF683*$^{+}$) intermediate CD8^{+} T cell state, were shown to co-exist in a substantial part of tumors. This may be explained by intratumoral heterogeneity in the signals that drive dysfunction but could also reflect the developmental origin of these T cell populations outside of the tumor - for instance due to differential imprinting of naive T cells during priming, a matter that deserves further attention. Notably, the preferential connection of the *TCF7*$^{+}$ Tex population with T cell states from the *GZMK*$^{+}$ differentiation path raises the question whether there are additional T cell populations with a comparable level of stemness, or whether the capacity for self-renewal may differ between these trajectories.

Analysis of the relative abundance of different T cell states across cancers made it possible to distinguish eight 'immune types' of cancer, as based on their immune composition, characterized by (amongst others) greater or lesser abundance of Tex, *TNFRSF9*$^{+}$ Tregs, and various memory T cell states with a lower level of dysfunction. Two of these immune types share a sizable Tex cell population, but differ in the abundance of *TNFRSF9*$^{+}$ Tregs, a difference that is likely to impact the activity of different immunotherapeutic strategies. Furthermore, Zheng *et al*. observed that patients with either of these "Tex high" immune type cancers had reduced survival compared with "Tex low" immune types that show less T cell dysfunction.

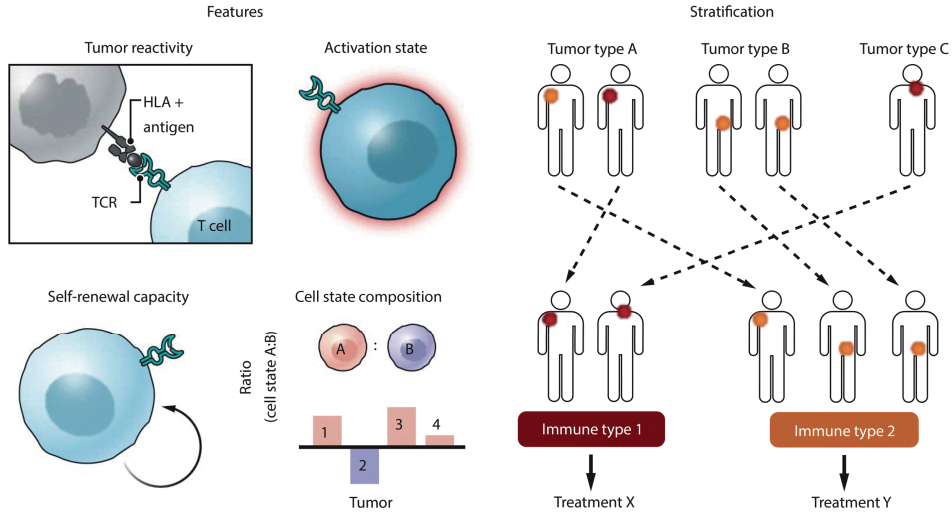

Figure 1. T cell immune types of cancer

Various characteristics of T cells that infiltrate tumors are relevant to therapeutic response, such as the presence of tumor-reactive T cells, their activation state, self-renewal capacity, and the balance between suppressive and effector T cell states. These characteristics may define cancer immune types that can stratify patients to optimize therapy.

Similarly, Tex low melanomas showed an improved response to immune checkpoint blockade compared to Tex high tumors (*8*). Given the enrichment of tumor-reactive T cells in the dysfunctional T cell pool (*9*), these data argue for a model in which patient outcome is less determined by the presence of a sizable tumor-reactive T cell pool but more so by the capacity to maintain T cell reactivity over time. It will be important to translate the concept of immune types, reflecting aspects such as capacity for T cell renewal and tumor recognition, into assays that can be incorporated into routine diagnostics (see the figure). As with any atlas, the inclusion of additional layers of information should further increase its value in the coming years. For example, information on epigenetic state may help to understand to what extent T cell populations can (durably) be reactivated by therapy. Furthermore, a combined analysis of T cells and other immune cell types appears attractive, because crosstalk between immune cell subsets likely explains part of the diversity in T cell states observed. Arguably, the most valuable addition touches on a much more central aspect of any map: its spatial resolution. T cells that reside in human tumors may be present either in stromal or parenchymal areas, or in tertiary lymphoid structures or other immune cell niches (*10*), thereby determining the signals to which these cells are exposed. A future map that couples T cell state to their presence in defined tumor areas will help to dissect how specific T cell pools shape their local environment, and how the functional state of T cells is influenced by the cellular neighborhood in which they grow up.

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