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Melanoma-reactive T cells take up residence

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Abstract

Shedding light on the mechanisms that underlie durable response to immunotherapy, a new study evaluating memory T cell responses in long-term melanoma survivors treated with immunotherapy finds that tumor-associated clonotypes are sustained over years and persist as expanded, IFNγ-expressing T_{RM} cells in the skin with T_{EM} counterparts in the blood.

Cancer immunosurveillance mediated by $CD8^+$ cytotoxic T cells (CTLs) leads to T cell receptor (TCR) recognition of tumor-associated antigens followed by killing of tumor cells, enabling favourable patient outcomes. More recently, $CD8^+$ tissue-resident memory cells (T_{RM}), a distinct subset of non-recirculating memory $CD8^+$ T cells, have been recognised as critical players in anti-tumor immune responses, suggesting that the quality rather than just the quantity of tumor-infiltrating $CD8^+$ T cells is important for clinical outcomes[1–3]. Despite the protective effect of $CD8^+$ CTLs, most tumors fail to regress as a result of the immunosuppressive milieu fostered by the tumor. In addition to suppressive populations such as $CD4^+$ regulatory T cells (T_{regs}), tumor-associated fibroblasts and macrophages, tumor immune escape is facilitated by T cell "exhaustion". Chronic antigen stimulation within tumors leads to a state of T cell dysfunction and increased expression of multiple inhibitory receptors or immune checkpoints[4]. Immunotherapy serves to counteract these inhibitory pathways, and can reinvigorate the T cells and enhance T cell infiltration, expansion and effector functions within tumors[5].

Immunotherapy achieves remarkable and often durable clinical response in a variety of advanced cancers, however such responses occur only in a minority of patients[6]. The factors that promote therapeutic response versus treatment resistance has been the subject of much investigation. A comprehensive understanding of the multifaceted mechanisms, globally as well as at the level of the individual cancer and patient, would guide choice of immunotherapy to promote robust anti-tumor immune responses required for tumor regression and improved patient outcomes. Tumor-intrinsic features such as tumor neoantigen load on the one hand, PD-L1 expression, disruption of antigen presentation and co-optation of mesenchymal transition or angiogenesis on the other, have been reported to impact response[7]. Recently, response to immunotherapy has been related to host-intrinsic

factors such as HLA genotype[8]. The immune correlates of response to immunotherapy have also been studied in different compartments during treatment. Pre-existing tumor immune infiltration and an increase in the numbers of $CD8^+$ TILs early during immunotherapy has been reported in treatment responders[9]. In peripheral blood, the ratio of reinvigorated PD-1^+CTLA-4^+ "exhausted" $CD8^+$ T cells to tumor burden has been proposed as a predictive biomarker of response[10]. In addition, several studies have found a positive correlation with signatures of T cell states, including activation, exhaustion, cytotoxicity, or the presence of a $TCF1^+$ stem-like CTL subset to clinical response[11,12]. Whilst these elegant studies provide insights into potential mechanisms activated by treatment, the features of durable anti-tumor immune responses in those patients that demonstrate long-term clinical remission remain unknown.

In this issue of Nature Cancer, Han et al. report the molecular features of $CD8^+$ memory T cell responses across tissues and over years in patients with malignant melanoma (MM) who are long-term survivors following immunotherapy (Figure 1). The authors previously showed that vitiligo, resulting from autoimmune destruction of normal host melanocytes, is required to sustain melanocyte antigen-specific T cells in mouse models, consistent with its recognition as a positive prognostic factor in melanoma patients[13]. In the current study, they evaluated matched melanoma tissue, distant vitiligo-affected skin and blood collected at least one year after initiation of a variety of immunotherapy regimens, and one month after therapy completion, from four long-term MM survivors. By application of paired single-cell RNA sequencing (scRNA-seq) and T-cell receptor (TCR) sequencing (scTCR-seq) to flow-sorted $CD8^+$ T cells, they generated an integrated map of transcriptional profiles and TCR specificities and tracked them over time.

Based on gene expression program, the authors report enrichment of T_{RM} cells in tumor and vitiligo-affected skin, which was confirmed by flow cytometry and immunohistochemistry. Unsupervised clustering analysis demonstrated five clusters (C1–5) comprising predominantly of $CD8^+$ T cells from blood and five clusters (C6–10) consisting of those from tumor and/or skin. Blood-derived clusters (C1–5) were each enriched for $CD8^+$ T cells with different circulating phenotypes such as effector memory (T_{EM}), effector (T_{EFF}), central memory (T_{CM}), naïve (T_{NAV}) and mucosal-associated invariant T cells (MAIT). Among the tissue-derived clusters, C6 was enriched for skin $CD8^+$ T cells whilst C7 was composed of $CD8^+$ T cells with a *TCF7*+ stem cell memory phenotype (T_{SCM}) from the tumor. Three clusters (C8, C9, C10) were composed of $CD8^+$ T cells from both skin and tumor, and strikingly, each of these three clusters were enriched for T_{RM} cells with distinct functional and phenotypic features. T_{RM} cells in C8 (T_{RM}-FOS) showed features linked to TCR signaling whilst those in C10 (T_{RM}-TOX) were enriched for transcripts encoding immune checkpoints (TOX, LAG3, PDCD1 and CTLA4), cytotoxicity mediators (PRF1, GZMB), transcription factor BLIMP1 and the classical T_{RM} marker, CD103. Cluster C9 (T_{RM}-IFNG) was composed of T_{RM} cells marked by high expression of effector molecules such as cytokines (IFNG, TNF) and chemokines (CCL3, CCL4). By overlaying T_{RM} subset signatures onto data from the TCGA dataset, the authors report that T_{RM}-IFNG cells were the most significant predictor of patient survival, even after adjusting for confounders such as TIL density and other clinical variables. Interestingly, the T_{RM}-TOX^+ cells also had positive predictive value despite displaying an "exhausted" phenotype. We envision that this

subset may be important particularly in the setting of large tumors early in treatment given that the “exhausted” phenotype imposed by TOX following chronic antigenic stimulation is critical for preventing T cell overstimulation, activation-induced cell death and limiting collateral tissue damage[14]. Although the authors did not correlate the non-T_{RM} clusters with patient survival, it will be interesting and pertinent to elucidate if the tumor *TCF7*-expressing TSCM subset is linked to long-term outcome, given that expansion of these cells has been favourably associated with response to immunotherapy in prior studies[11].

Next, Han et al. leveraged paired scTCR-seq data from the same four MM patients to correlate transcriptomic profiles with T cell clonality across tissue compartments. Of all the tumor-associated clonotypes that were expanded within tumors, they found that thirty-three clonotypes contained counterparts in vitiligo-affected skin. Among the thirty-three clonotypes, fifteen were also identified in the blood (“Resident/Circulating” clonotypes) whilst eighteen were confined to tumor and skin (“Resident-Only” clonotypes). Notably, the “Resident/Circulating” clonotypes were enriched in T_{RM}-IFNG cells in tumor and skin, and existed as T_{EM} cells in the blood, suggesting a common clonal precursor for these cells. The “Resident/Circulating” clonotypes also had the highest frequencies indicating robust expansion among the T_{RM}-IFNG cells. Furthermore, the authors identified melanoma differentiation antigen (MDA)-specific responses in both skin and blood in a separate cohort of MM survivors, although the precise specificities of the “Resident/Circulating” clonotypes were not evaluated and warrants future investigation.

A unique aspect of this study is the demonstration of durable $CD8^+$ T cell memory. In seven long-term MM survivors, the authors performed bulk TCRβ DNA sequencing on skin, blood, and archival primary and metastatic tumors and showed long-term persistence of tumor-associated clonotypes in vitiligo-affected skin and blood, even up to nine years following tumor resection. The authors report that skin, relative to blood, showed greater enrichment for tumor-associated TCR repertoire and those clonotypes that were expanded in the skin were also more expanded in the blood. These findings suggest that the skin not only can act as a ‘museum’ of how immunological challenges were overcome in the past, but that the skin may also play a crucial role in the maintenance of anti-melanoma immunity. To shed light on the gene expression profile of these long-lived clonotypes, Han et al. analyzed one patient who had optimal archival tumor TCR DNA sequencing data and scTCR-seq data and found that the persistent tumor-associated clonotypes were indeed T_{RM}-IFNG cells in the skin and T_{EM} cells in the blood.

In summary, systematic analysis by Han et al. links long-term response and patient survival following immunotherapy to the persistence of a distinct T_{RM} subset with effector function and shared tumor-associated TCRs in vitiligo-affected skin. Given that the skin-resident tumor-associated clonotypes had clonally expanded counterparts in the blood as T_{EM} cells, their findings suggest that skin may serve as a niche of host-wide memory response. T_{RM} cells have been associated with prognosis in a wide variety of cancers even in treatment-naïve settings. Whether and how the phenotypic and functional properties of T_{RM} cells change in a tissue-specific and context-dependent manner, and which subsets are most critical for tumor elimination at specific stages are not known. Future studies that will construct a trajectory of $CD8^+$ T cell profiles integrated with multi-omics data in a

longitudinal fashion will provide comprehensive insights into T cell responses and enable rational choice of immunotherapy at every stage of disease to promote long-term remission.

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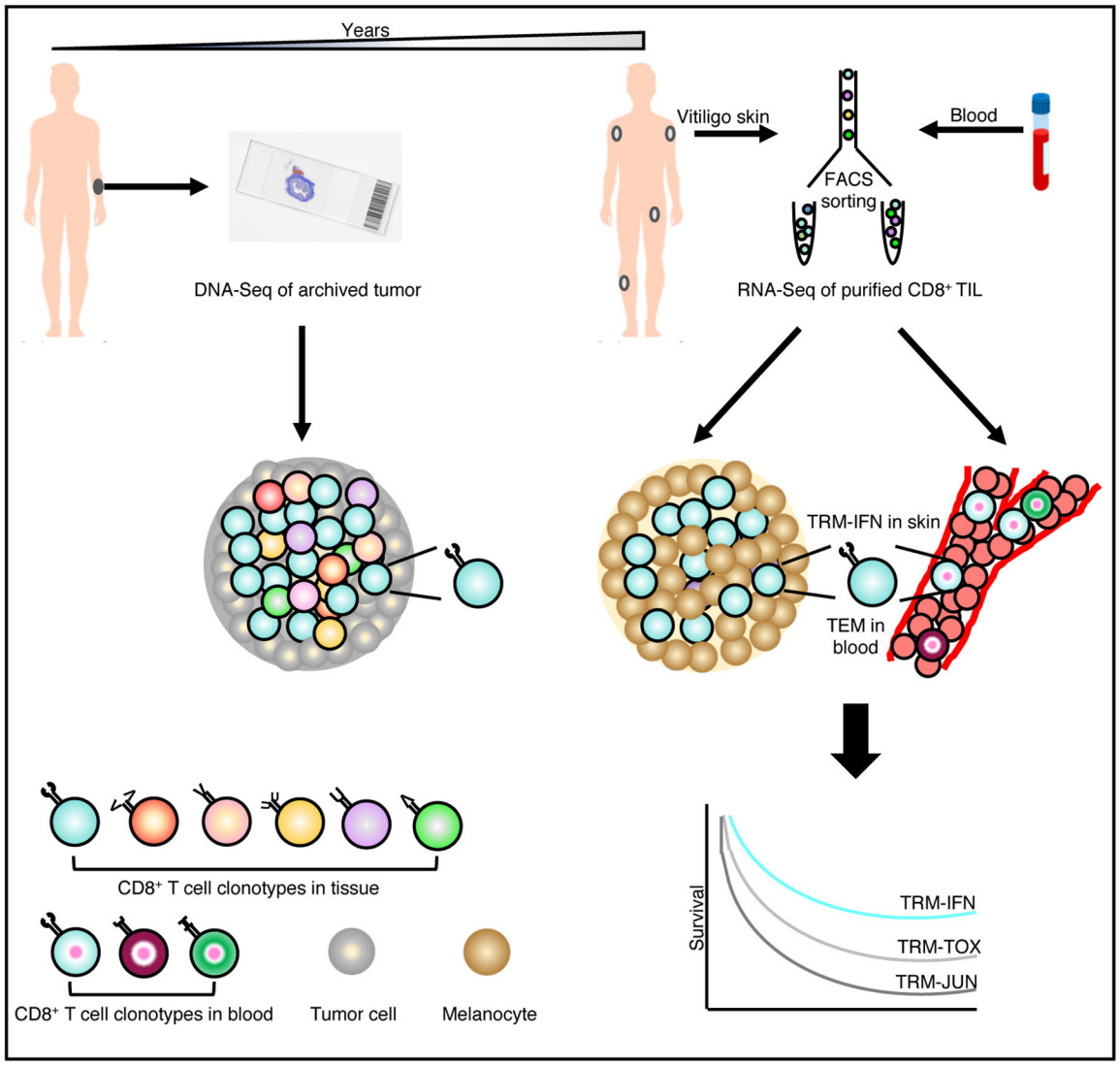

Figure 1:

In long-term melanoma survivors, tracking of tumor-infiltrating $CD8^+$ T cell clonotypes over years reveals their persistence as tissue-resident memory cells with effector capabilities (TRM-IFN) in vitiligo-affected skin and as effector memory T cells (T_{EM}) circulating in the blood. TRM-IFN subset was the most significant positive prognostic indicator relative to the other TRM subsets, TRM-TOX and TRM-JUN, with features of exhaustion, and TCR activation, respectively.