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INSIGHTS

Decoding TCR specificity and T cell fate at the immunological synapse

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In this issue of *JEM*, Liu et al. (https://doi.org/10.1084/jem.20251779) report PRECISE-seq, a proximity labeling platform that integrates T cell receptor specificity, functional potency, and cellular phenotype at a single-cell resolution. Using this approach, they identify an immunosuppressive Ly49$^+$ T cell state within tumors that is alleviated by PD-1 blockade.

T cell–based immunotherapies have reshaped cancer treatment, yet their clinical benefit remains constrained (Carnevale et al., 2022). This limitation likely stems from several factors, including low neoantigen burden, suboptimal T cell receptor (TCR)–antigen interaction strength, and dysfunctional or nonproductive T cell differentiation states within the tumor microenvironment (Bates et al., 2025; Cheng et al., 2026). Moreover, how TCR antigen specificity and signaling strength together instruct T cell fate decisions in tumors remains further still incomplete (Shakiba et al., 2022). A key challenge is that existing approaches capture these features in isolation (Joglekar and Li, 2021). Peptide–major histocompatibility complex (pMHC) multimer staining identifies antigen-specific T cells but largely reflects binding affinity, depends on prior epitope knowledge, and does not report functional capacity (Klenerman et al., 2002). Functional coculture assays measure reactivity, yet often alter endogenous T cell states through extended in vitro stimulation (Levy and Gros, 2022). Single-cell RNA sequencing (scRNA-seq) resolves cellular phenotype at high resolution but cannot assign antigen specificity or functional strength to individual TCR clonotypes (Tan et al., 2025). Consequently, the field has lacked an integrated framework to simultaneously determine antigen recognition, functional potency, and the resulting cellular state of T cells under physiological conditions.

PRECISE-seq enables integrated profiling of TCR specificity, potency, and phenotype

Liu et al. (2026) introduce proximity labeling for rapid screening of disease-relevant T cell repertoires (PRECISE-seq), a contact-dependent labeling strategy coupled with single-cell multi-omics. The platform is built on two key design features. First, T cells are uniformly equipped with an acceptor peptide via a two-step chemical conjugation. Second, antigen-presenting cells are engineered to express sortase A (SrtA) on their surface. Upon formation of an immunological synapse, SrtA catalyzes transpeptidation to transfer a biotinylated probe onto adjacent T cells. Because this reaction occurs strictly at sites of cell–cell contact, it minimizes background labeling and enables selective tagging independent of activation state or subset identity.

In validation experiments, PRECISE-seq detects antigen-specific T cells at frequencies as low as 0.01%. Notably, labeling intensity scales with TCR-pMHC interaction strength, as shown by graded biotin signals across altered peptide ligands with defined affinities. This provides a quantitative, activation-free readout of functional avidity. Building on this, the authors define a clonotype-level "potency score" that correlates with independently measured EC_{50} values for CMV-specific TCRs.

Applied to peripheral blood from CMV-seropositive donors, PRECISE-seq retrieves virus-specific clonotypes and reveals that high-potency clones co-express activation and exhaustion programs, consistent with the notion that repeated high-avidity stimulation drives T cell exhaustion. In tumors, however, the analysis uncovers a distinct and unexpected differentiation trajectory. Using the MC38 colon carcinoma model, the authors show that tumor-specific CD8$^+$ T cells preferentially adopt a Ly49$^+$ regulatory state (T_{Ly49}). This population expresses natural killer (NK)-associated inhibitory receptors (e.g., *Klra3*, *Klra5*), FcεRIg, and the transcription factor Helios (*Ikzf2*) and is functionally characterized by production of immunosuppressive mediators such as SPP1 (osteopontin) with minimal IFN-γ output. Adoptive transfer experiments demonstrate that T_{Ly49} cells actively promote tumor growth, establishing them as bona fide immunosuppressive effectors rather than merely dysfunctional or exhausted T cells. Trajectory analysis further indicates that T_{Ly49} diverges from effector memory T cell (T_{EM}) progenitors along a differentiation pathway distinct from the canonical T_{PEX}-to-T_{EX} exhaustion route

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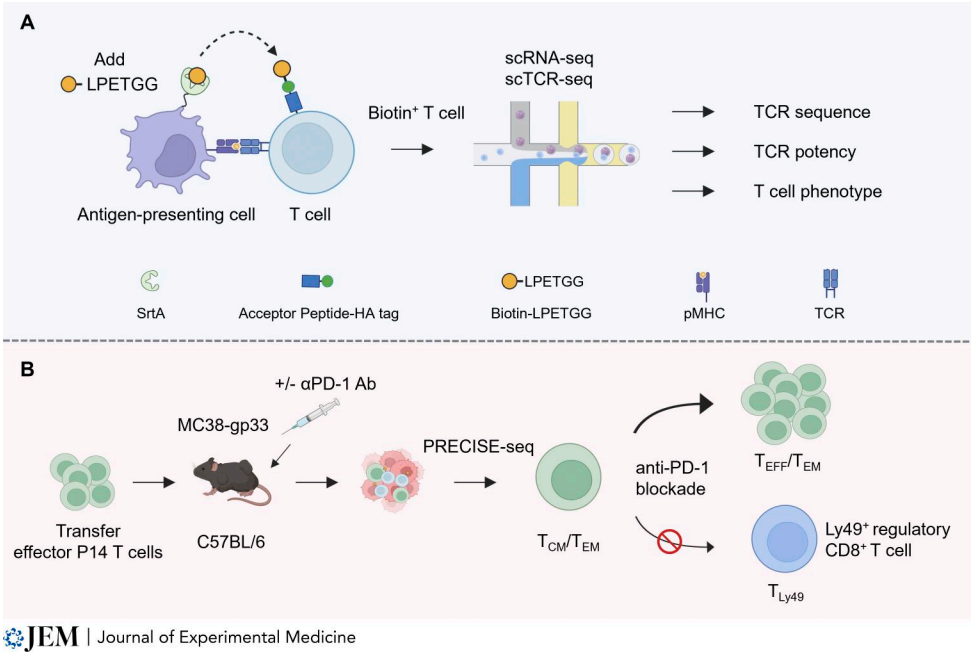

JEM | Journal of Experimental Medicine

PRECISE-seq links TCR potency to T cell fate in the tumor microenvironment. (A) Schematic of the PRECISE-seq workflow. T cells conjugated with an AP-HA tag are cocultured with antigen-presenting cells expressing SrtA. Upon formation of an immunological synapse, SrtA catalyzes the transfer of biotinylated probes onto adjacent T cells in a contact-dependent manner. The resulting labeling intensity reflects TCR-pMHC interaction strength. Biotin⁺ T cells are subsequently isolated and subjected to scRNA-seq coupled with TCR profiling, enabling simultaneous readout of antigen specificity, functional potency, and cellular phenotype. (B) Model of tumor-specific T cell fate decisions revealed by PRECISE-seq. Effector P14 T cells are adoptively transferred into C57BL/6 mice bearing MC38-gp33 tumors, with or without αPD-1 antibody treatment. PRECISE-seq analysis shows that tumor-specific T_{CM} /T_{EM} can differentiate into an immunosuppressive T_{Ly49}. PD-1 blockade reduces T_{Ly49} differentiation while promoting effector T_{EM}/T_{EFF} expansion. AP-HA tag, acceptor peptide–HA tag; T_{CM}, central memory T cells.

(Lan et al., 2024), suggesting a parallel fate decision. Importantly, PD-1 blockade markedly reduces the frequency of T_{Ly49} while expanding effector T_{EM} and T_{EFF} compartments among tumor-specific clonotypes. This shift is mirrored in human datasets across multiple cancer types, including mismatch repair–deficient colorectal cancer, hepatocellular carcinoma, and metastatic melanoma, positioning Ly49/KIR⁺ CD8⁺ T cells as a clinically relevant immunoregulatory state linked to responsiveness to checkpoint blockade.

Significance

At the technical level, PRECISE-seq unifies three dimensions that have traditionally been analyzed separately into a single-cell framework. Its ability to infer TCR functional potency directly from labeling intensity, without prior epitope knowledge or in vitro stimulation, is particularly advantageous for translational settings, where preserving endogenous T cell states is critical.

At the biological level, the identification of T_{Ly49} as a distinct immunoregulatory fate for tumor-specific CD8⁺ T cells challenges the prevailing view that exhaustion is the dominant dysfunctional endpoint in tumors (Ford et al., 2022). Rather than passively losing effector function, these cells actively support tumor progression through a defined suppressive program, reframing how T cell fate decisions are conceptualized in the tumor microenvironment. Moreover, the finding that PD-1 blockade shifts the balance away from T_{Ly49} cells toward effector populations provides a mechanistic lens on checkpoint therapy responsiveness and highlights the T_{EM}/T_{EFF}-to-T_{Ly49} ratio as a potential predictive biomarker.

Outstanding questions and future directions

Several key questions emerge. First, although T_{Ly49} cells are linked to human KIR⁺ CD8⁺ T cells using patient scRNA-seq data (Li et al., 2022), their suppressive function in human tumors remains to be directly established. Whether these cells share analogous differentiation trajectories, effector programs, and sensitivity to αPD-1 antibody requires experimental validation. Second, the relationship between TCR potency and T_{Ly49} differentiation is currently correlative. It remains unclear whether high-avidity TCR signaling actively instructs this regulatory fate, or whether extrinsic factors, such as persistent antigen exposure, inflammatory context, or metabolic stress, are the dominant drivers. This question could be addressed by transferring T cells engineered with affinity-graded TCRs against the same antigen into tumor-bearing hosts. More broadly, it raises the possibility that the T cell fate is not linearly dictated by signal strength, but instead follows an optimal "window" in which effector function is maximized while avoiding regulatory or exhausted states (Shakiba et al., 2022). Third, it will be important to determine whether PRECISE-seq labeling intensity faithfully reports functional avidity in solid tumor environments, where hypoxia, acidity, and extracellular matrix constraints may influence cell–cell contact and synapse formation (Wang et al., 2024).

From a translational perspective, applying PRECISE-seq longitudinally to patient samples collected before and during immunotherapy could enable real-time tracking of T cell state

transitions, offering mechanistic insight into response and resistance. In the context of adoptive cell therapy, the platform provides a rational framework for selecting TCRs that balance sufficient antigen reactivity with favorable differentiation potential, avoiding receptors whose excessive avidity may inadvertently bias cells toward regulatory fates. Integrating potency measurements with fate prediction may therefore enhance the design of next-generation T cell–based therapies.

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