Background Immunotherapies represent promising approaches to treat metastatic solid tumors, yet their response rates remain low.\(^1\)\(^2\) Identifying antitumor T cells, their antigens, and their cognate T cell receptors (TCRs) can provide crucial insights into the design of next-generation cellular immunotherapies. Circulating T cells from the peripheral blood (PBL) can provide a rich and non-invasive source for identifying and studying antitumor T cells as an alternative to tumor-infiltrating lymphocytes (TIL). Yet, pre-surgery antitumor T cell frequencies in PBL of patients with metastatic cancer are often low,\(^3\)\(^4\) limiting the accurate definition of their phenotypic states.

Methods We employed single-cell phenotypic profiling of 36 vetted neoantigen-specific T cell clones from 6 metastatic epithelial cancer patients to derive the transcriptional and cell surface protein signatures of PBL-resident antitumor CD8\(^+\) T cells (NeoTCRPBL). In 4 samples, we compared T cell gene signatures of 24 paired neoantigen TCR clonotypes between the PBL and TIL compartments. We developed a NeoTCRPBL gene signature to assess its sensitivity and specificity in discovering new antitumor TCRs from PBL of prospective patients with different tumor types. Finally, we compared the frequency and avidity of antitumor TCR clonotypes between the TIL and PBL compartments in all patients.

Results Blood-resident NeoTCRPBL T cells were clonally expanded, but low in frequency (<0.001–0.002% per clone) necessitating their enrichment for studies. NeoTCRPBL T cells exhibited phenotypes distinct from common T cell subsets and bystander viral-reactive T cells displaying transcriptional programs of both dysfunctional and tissue-resident memory T cells (figure 1A,B). Within the same patient, intra-clonotype comparison of 24 TIL-and PBL-neoantigen-specific T cell clones suggested that relative to their TIL counterparts, circulating NeoTCRPBL T cells displayed less-dysfunctional immunotherapy-response associated progenitor phenotypic states (figure 1C).\(^5\)\(^–\)\(^7\) Combined analysis of 100 antitumor T cell clones revealed that circulating NeoTCRPBL T cells largely targeted the same clonal, subclonal neoantigens with comparable avidity as TIL (>79% shared), but their TCR-repertoire was only partially shared with TIL (47% shared) (figure 1F-G). Finally, prediction and testing of 64 clonally expanded NeoTCRPBL signature-enriched TCRs from prospective samples discovered 20 neoantigen-TCRs demonstrating that NeoTCRPBL signature can successfully identify antitumor TCRs from very low circulating PBL frequencies (<0.002%) (figure 1D,E).

Conclusions Circulating antitumor T cells are low in frequency exhibiting distinct clonotypic repertoire and phenotypic states in patients with metastatic solid tumors. The NeoTCRPBL signature provides an alternative source for identifying antitumor T cells and their TCRs non-invasively from pre-surgery blood samples enabling immune monitoring and designing cancer immunotherapies.

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REFERENCES
Abstract 1216 Figure 1  (A) Transcriptional states of circulating CD8+ cells from six metastatic colon cancer patients’ blood samples and projection of neoantigen- and viral-reactive clones on the UMAP. (B) Schematic representing the combined TIL+ PBL neoantigen T cell phenotypic states analysis within each patient. (C) Average gene signature scores (scGSEA) scores of immunotherapy response and non-response associated gene signatures within each individual neoantigen-reactive clone compared between TIL and PBL compartment. ****P < 0.0001 by Paired T-test per each neoantigen T cell clonotype. (D) FACS-sorting enrichment gating of circulating CD8+ T cells from patient for scRNA. (E) Projection of predicted antitumor T cells from PBL based on NeoTCRPBL gene signature (left) and results of testing, 5 antitumor TCRs were discovered (right). (F) Summary of the landscape of neoantigen-reactive TCR clonotypes and their cognate neoantigens shared between TIL and PBL. (G) Functional avidity of 44 NeoTCR clones that were either found only in the PBL compartment (Blood), TIL compartment (Tumor), or shared between PBL and TIL (Shared).

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