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POSTER ABSTRACTS

506.BONE MARROW MICROENVIRONMENT

Tumor Reactive T Cells Reside in the Bone Marrow and Are Associated with Clinical Response to Hematological Malignancies

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T cell receptors (TCRs) play a crucial role in orchestrating cellular immunity in both health and disease, particularly in the context of cancer. Although immunotherapy and stem cell transplantation have shown promising clinical responses in hematological malignancies, these have not been linked to the presence of endogenous tumor reactive T cells. Moreover, the specificities and phenotypes of such tumor recognizing TCRs in these largely low immunogenic entities are unknown. In this study, we addressed this gap by developing an approach to map TCR specificities and transcriptional phenotypes of human bone marrow-resident T cells directly *ex vivo*, focusing on patients who were treatment-naïve at the time of biopsy. This allowed us to generalize our findings beyond immune checkpoint inhibitor-treated patients, a limitation of previous studies in solid cancers.

To discover T cells that specifically recognize cancer cells or viral epitopes, we developed a multiplexed profiling approach using microfluidic reaction chambers, optical cytokine detection and TCR sequencing of patient-derived primary T cells. We were able to confidently map TCR recognition of 25,957 bone marrow-resident T cells from n=20 hematological cancer patients. Based on the acquired functional data and reverse phenotyping of screened TCRs to their original cell state *in situ*, we built a bone marrow T cell atlas charting >300,000 expression profiles in human bone marrow-resident T cells, their TCR specificities as well as their longitudinal clonal dynamics upon therapeutic perturbations in n=40 patients with newly diagnosed multiple myeloma (MM) and acute myeloid leukemia (AML).

Our approach detected tumor-reactive T cell clones in the bone marrow of all screened individuals with varying frequency (1-5% of T cells), which were largely found to display a CD8+ effector-memory or progenitor-exhausted phenotype. In contrast, non-tumor reactive clones were either CD4+ or enriched for viral specificities. We identified a conserved signature of anti-tumor reactivity across patients. This signature included the *ITGB1* gene, which encodes CD29 and was uniformly expressed in tumor reactive T cells. Importantly, *ITGB1* expression was specifically restricted to tumor reactive, compared to virus-specific or bystander T cells. In two independent mouse tumor models, we found that CD29 protein was significantly upregulated on homed T cells carrying tumor reactive TCRs compared to non-reactive TCRs.

A causal link between tumor reactive T cells and clinical response to hematological cancers would be strengthened by finding that these cells specifically recognize autologous cancer epitopes and clonally expand as part of a clinical anti-tumor response. We therefore cloned patient-individualized TCR libraries and performed immunoprecipitation of MHC class I : peptide complexes from cancer cells. We validated TCR binding against ~1-20 epitopes per patient, which were mostly derived from cancer/testis antigens (CTAs) and novel open reading frames (nORFs). Finally, we classified tumor reactive TCRs in three clinical cohorts: Notably, we observed selective expansion of tumor reactive TCRs upon autologous stem cell transplantation, which was associated with deep clinical response in newly diagnosed MM patients. Conversely, the failure of tumor

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reactive TCRs to clonally expand on-treatment underlined clinical non-response to bispecific BCMAxCD3 antibody therapy (MM) or combination therapy with immune checkpoint inhibitors and Azacytidine (AML).
We show here that, unexpectedly, clinically relevant endogenous anti-tumor reactivity lies in a rare subset of bone marrow-resident T cells. The recurrent detection of these immunotherapy-responsive TCRs in patients with MM or AML revealed the selective clonal expansion of tumor-reactive TCRs following autologous stem cell transplantation. We describe a conserved gene signature of tumor reactivity that diverges from lymphocytes infiltrating solid tumors and includes CD29 as an indicator of antigen-specific T cell homing. Our findings facilitate the antigen-agnostic identification of tumor-reactive TCRs with potential for future patient-individualized cell therapies and suggest the relevance of immune responses against alternative epitopes, such as cancer/testis antigens, in hematological malignancies with low mutational loads.

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