Targeting neoantigens with immunotherapy: Are we limited to pre-existing autologous neoantigen-specific T cells?

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Abstract
Several facts shape our considerations regarding the use of neoantigens as highly specific targets for immunotherapy of cancer. First, adoptive T cell therapy using TILs seems to be the most successful if the TILs include T cells specific for antigens resulting from individual mutations. Second, the success of checkpoint inhibitors is often correlated with the mutational load of tumors. However, a mutation must fulfill several criteria to be effective as a neoantigen that can be recognized by T cells. Obviously, the mutation must lead to a novel amino acid sequence (e.g. single amino acid substitution, fusion- or frameshift-sequences), and be located in a gene expressed in tumor cells. Furthermore, a peptide spanning the new sequence needs to be efficiently processed and presented by HLA molecules. Finally, a T cell response must be triggered that can specifically recognize the mutated epitope.

Targeting neoantigens as true patient-individualized epitopes requires robust processes for rational and rapid selection and validation of neoantigens as T cell targets. Currently, the most challenging step is predicting specific T cell responses. Huge efforts have been made to analyze the reactivity of patients’ T cells against mutations. However, this approach is limited to the T cell repertoire present in patients at the time of tumor resection or blood draw and might miss potential potent T cell responses that were lacking or no longer present in the patient. In our opinion, only screening the T cell repertoire of several healthy donors can answer the question if a specific mutation can trigger T cell responses. We present proof-of-concept data how we use our high-throughput T cell receptor (TCR) platform technologies and automated processes for fast and efficient screenings of T cells isolated from several partially HLA-matched/healthy donors. Promising neoantigens were predicted and T cells responses after stimulation with antigen-presenting cells either transfected with minigene constructs or loaded with peptides were compared. Peptide stimulation triggered specific T cells for most tested mutations, indicating that T cell repertoires of healthy donors can recognize neoantigens when they are forced to be presented. Also, with the clinically relevant approach using endogenously processed antigen encoded by minigenes, specific T cell responses against neoantigens presented on different HLAs were efficiently triggered, although only against some of the tested epitopes. This screening strategy has the aim to develop future TCR-based therapies and can be used for the identification of promising mutations for vaccination or as a source for TCRs for adoptive T cell therapy. Furthermore, generated data can subsequently improve algorithms predicting the immunogenicity of neoantigens.

Experimental setup

Selection of potential neoantigens

Charaterization of neoantigen-reactive TCRs

Screening for neoantigen-reactive T cell clones

Isolation of neoantigen-reactive T cell clones

Peptide processing-independent approach

Summary

We showed that T cells reactive against patient-specific mutations can be isolated from healthy, partially HLA-matched donors. This approach can be used as a screening platform for immunogenic neoantigens and as a source for patient-specific, therapeutic TCRs.