

HUNTR™: A HYPERPLEX PLATFORM FOR THE DISCOVERY OF NEOANTIGEN-REACTIVE T-CELL RECEPTORS

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Background Solid tumors harbor mutations that can give rise to neoantigens recognized by T-cell receptors (TCRs) expressed on tumor infiltrating lymphocytes (TILs). We have developed a library of TCR-T cells targeting hotspot mutations based on the non-viral *Sleeping Beauty* transposon/transposase system, which is presently being evaluated in a first-in-human phase 1/2 study in patients with non-small cell lung, colorectal, endometrial, pancreatic, ovarian, and bile duct cancers. Current methods of TCR discovery require large volume blood draws or immortalization of primary cells with live viruses to make antigen presenting cells (APCs), limiting the ability to efficiently discover new TCRs and is only applicable to some patients. We developed hunTR™ (human neoantigen T-cell Receptor), a rapid, hyperplex platform for the discovery of neoantigen-reactive TCRs from limited starting material.

Methods TILs sorted from dissociated tumor specimens were processed to obtain TCR sequences and gene expression profiles on a single cell basis. These data were fed into a novel bioinformatics pipeline that identifies TCRs with predicted neoantigen reactivity. TCRs were reconstructed in *Sleeping Beauty* transposon plasmids and expressed in TCR cells, an engineered cell line capable of detecting TCR reactivity to both class I and class II HLA-restricted neoepitopes. Somatic single nucleotide variants, short insertions/deletions, and germline class I and II HLA alleles were called for each patient. neoAPCs were engineered to express relevant patient-derived neoantigens and HLA molecules. TCR cells were cocultured with a matrix of neoAPCs, and conditions yielding a neoantigen reactivity were identified.

Results A total of 3.1×10^5 TCR+HLA+neoantigen combinations were evaluated in seven patients with a mean plexity of 4.4×10^4 per patient. All specimens screened (colorectal n=3, endometrial n=2, breast n=2) yielded at least one neoantigen-reactive TCR. The 57 neoantigen-reactive TCRs (19% of 304 total TCRs screened) identified targeted 20 mutations, including one shared *KRAS* and 19 personal mutations. Of these, 81% were restricted by HLA class II while 19% were restricted by class I. A median reactive hit rate of 14% was achieved per patient (range 5-31%) with an average of three unique neoantigen specificities (range 1-6).

Conclusions In conclusion, hunTR is a hyperplex screening platform that identifies neoantigen-reactive TCRs. hunTR allows for the expansion of our hotspot mutation-targeted TCR library, increasing the addressable population of solid tumor patients (with matching hotspot mutation and HLA allele) eligible for TCR-T cell treatment. In addition, hunTR is applicable for personalized TCR-T therapy such that most solid tumor patients could be eligible for mutation-targeted cell therapy.

Ethics Approval Human-derived specimens were obtained through a commercial source that adheres to all applicable regulations and guidelines of the relevant countries including the US.

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