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803.EMERGING TOOLS, TECHNIQUES AND ARTIFICIAL INTELLIGENCE IN HEMATOLOGY

High-Throughput Identification of Graft-Vs-Leukemia TCRs in AML Patient Samples after Allogeneic Stem Cell Transplantation

Noah J Tubo, PhD[1], Duncheng Wang, PhD[1], Qiuming He, MD PhD[1], Yibin Chen, PhD[1], Ho Ngai, PhD[1], Yanqing Huang, PhD[1], Celine Kerros, PhD[1], Xizeng Mao, PhD[1], Shoudan Liang, PhD[1], Sachet Shukla, PhD[2], Arnau Peris Cuesta[2], Pavan Bachireddy, MD[3], Julio Alcantara Montiel[2], Gheath Alatrash, PhDDO[4], Priya Koppikar, PhD[5], Jeffrey J. Molldrem, MD[6]

[1] ECLIPSE, Therapeutics Discovery and Development, The University of Texas M.D. Anderson Cancer Center, Houston
[2] Department of Hematopoietic Biology & Malignancy, The University of Texas M.D. Anderson Cancer Center, Houston
[3] Department of Hematopoietic Biology & Malignancy, The University of Texas M.D. Anderson Cancer Center, Houston, TX
[4] Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas MD Anderson Cancer Center, Pearland, TX
[5] ECLIPSE, Therapeutics Discovery and Development, The University of Texas M.D. Anderson Cancer Center, Houston, TX
[6] Department of Hematopoietic Biology and Malignancy, The University of Texas MD Anderson Cancer Center, Houston, TX

For patients with intermediate and high-risk AML/MDS, allogeneic stem cell transplantation (aSCT) is a potentially curative therapy. Efficacy is driven by the graft-versus-leukemia (GvL) response, in which donor-derived T cells recognize peptide:MHC antigens presented by leukemia cells, and subsequently kill them. The antigens that drive GvL responses are attractive therapeutic targets for engineered cell therapies and/or biologics. However, only a small number of GvL antigens have been identified, limiting the therapeutic potential of targeted immunotherapies. Deep interrogation of the T cell repertoires of patients who have responded favorably to aSCT presents an opportunity to identify *bona fide* GvL antigens, but high-throughput methods need to be developed to do this effectively.

A popular method to identify antigen-specific T cells is fluorescent peptide:MHC tetramer staining, whereby T cells whose T cell receptors (TCR) bind a particular peptide:MHC antigen become fluorescent and identifiable by flow cytometry. However, a major challenge to identifying GvL T cells using tetramers is that the prediction of immunogenic antigens - that is, those that induce a T cell response *in vivo* - is poor; studies of solid tumors suggest that only ~1% of predicted MHC-binding neoantigens are immunogenic in patients. In AML/MDS aSCT patients, there are thousands of peptides that are predicted to bind MHCI and represent potential GvL antigens, but due to practical limitations of patient blood and bone marrow sample quantities, only a small subset can be screened by flow cytometry. Other higher throughput methods using heavy-metal tagged peptide:MHC tetramers followed by mass cytometry, or DNA-barcoded tetramers followed by bulk sequencing have been employed to increase antigen screening scale, but these methods are limited in sensitivity and destroy the T cell without providing TCR sequences.

To overcome these limitations and rapidly identify GvL T cells in patient samples, we developed a single cell sequencing based method that enables the screening of thousands of peptide:MHC antigens simultaneously, using a single patient sample. We generated peptide: MHCI monomers using UV-mediated peptide exchange and generated large panels of combinatorial DNA-barcoded peptide:MHCI tetramers at microscale. We integrated our methodology into the 10X Genomics Single Cell Immune Profiling workflow to enable simultaneous recovery of TCR antigen-specificity, paired TCR alpha beta sequences, and single cell RNA-Seq data. These methods were used to screen post-transplant AML patient blood and bone marrow, and we identified expanded GvL T cell clonotypes that target novel minor histocompatibility antigens, overexpressed "aberrant self" proteins, and other leukemia associated antigens. We generated primary CD8 T cells that retrovirally express these TCRs and demonstrate that they are potent and have the expected antigen specificities.

The methods described are highly sensitive, specific, efficient, and scalable, and enable high confidence identification of antigen specific clonotypes in patient samples. They markedly expand the throughput compared to existing methods, and simultaneously yield paired alpha beta TCR sequences, facilitating a full characterization of the antigen-TCR drivers of GvL. Furthermore, these methods have broad utility beyond SCT, as they are applicable to studying T cell responses to other immune settings, including anti-cancer, autoimmune, and anti-microbial responses.

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