An efficient platform for antigen-specific T cell discovery in AML after allogeneic hematopoietic cell transplantation

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Background: Donor-derived T cells mediate graft-versus-leukemia (GvL) effects through recognition of leukemia-associated antigens. Identification and characterization of antigen-specific T cells may improve understanding of leukemia-directed immune response and enable development of adoptive T-cell transfer for post-transplant relapse. Purpose: We aimed to establish a platform for antigen-specific T-cell identification, T cell receptor (TCR) discovery and functional TCR reactivity profiling. As proof-of-principle, we investigated T cell specificity for the Epstein-Barr virus (EBV)-derived HLA-A*02:01-restricted antigen CLGGLLTMV (CLG).

Methods: Mononuclear cells from stem cell grafts are incubated overnight with 1 µg/mL CLG peptide and further expanded for seven days with 500 IU/ml IL-2. Antigen-specific CD8+ T cells are identified using HLA-A*02:01/CLG tetramers and single-cell sorted for TCRαβ Oxford Nanopore sequencing. Reconstructed TCRs containing murine constant regions are synthesized, cloned into a MP71 vector and expressed in primary T cells. TCR transduced T cells are co-cultured with peptide-loaded antigen-presenting cells (APCs) and activation was assessed by CD137 expression.

Results: We implemented a published protocol for expansion of antigen-specific T cells from stem cell grafts followed by TCR long-read sequencing of single-sorted tetramer-positive CD8+ T cells. CLG-specific CD8+ T cells were enriched from 0.82% to 5.31% following peptide stimulation and expansion. TCR sequencing identified a dominant clonotype characterized by the complementarity-determining region (CDR3) amino acid sequences CAVLMDSNYQLIW and CASSDDGMNTEAFF, which have previously been reported as CLG-reactive. To establish a functional TCR validation assay, we re-expressed CLG-specific TCR and assessed its response to CLG-loaded APCs. The TCR was efficiently expressed (70% transduction efficiency), and antigen exposure induced CD137 upregulation in 44.6% of CD8+ T cells, confirming specific recognition of CLG.

Conclusion: We have established a platform for antigen-specific T cell identification, and functional TCR validation. We will use this platform to identify GvL-mediating TCRs to improve leukemic control after stem cell transplantation.