SQZ™ EAPCS GENERATED FROM PBMCs BY DELIVERY OF MULTIPLE MRNAs ENCODING FOR ANTIGENS, COSTIMULATORY PROTEINS, AND ENGINEERED CYTOKINES

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Background Antigen-specific CD8+ T cells are critical components of mounting an effective immune response against tumors. Generation of antigen-specific T cells require interactions with multiple signals produced by antigen presenting cells (APCs). These signals are comprised of three components: (signal 1) the peptide-MHC complex binding to the T cell receptor, (signal 2) costimulatory molecules on the surface of APCs, and (signal 3) inflammatory cytokines binding to cognate receptors on T cells.

Methods To engineer all major cell subsets of human peripheral blood mononuclear cells (PBMCs) to become enhanced APCs (eAPCs), we used Cell Squeeze® technology to deliver multiple mRNA encoding for non-self-antigens (signal 1), CD86 (signal 2), and/or membrane-bound cytokines (signal 3). The signal 3 molecules, membrane-bound IL-12 (mbIL-12) and membrane-bound IL-2 (mbIL-2), are chimeric proteins designed to increase the localized concentration of the cytokines and limit off-target effects. Flow cytometry and western blots were used to confirm the translation of each of the delivered mRNA. The increased capabilities of these enhanced APCs were assessed in vitro by culturing the APCs with antigen-specific T cells for multiple days before measuring the functionality of antigen-specific T cells via intracellular cytokine staining or ELISA.

Results We demonstrate that Cell Squeeze® processing of PBMCs with mRNA encoding for signals 1, 2, and 3 results in highly effective enhanced APCs in vitro. In a single squeeze process, efficient delivery and translation of up to five mRNA is observed in all major PBMC cell subsets including T cells, B cells, NK cells, and monocytes. Once translated, the chimeric mbIL-2 and mbIL-12 can bind to their cognate receptors and exhibit minimal shedding from the surface. We show that enhanced APCs can present antigenic peptides derived from mRNA encoding for a foreign antigen on MHC complexes in an HLA agnostic manner, which drives antigen-specific T cell responses. The addition of CD86, mbIL-2, and mbIL-12 further enhance the activation and potency of antigen-specific T cells, as measured by an increase in the secretion of inflammatory cytokines upon restimulation (i.e. IFNγ).

Conclusions Cell squeezing of human PBMCs with mRNA encoding for signals 1, 2, and 3 has the potential to generate enhanced APCs that drive robust CD8+ T cell response against multiple targets across several disease areas. The versatility of the Cell Squeeze® technology potentially enables rapid exchange of mRNA to other antigens or T cell activation signals.

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