SINGLE MRNA CONSTRUCTS ENCODING MULTIPLE LINKED ANTIGENS ALLOW FOR A MULTIANTEGEN-SPECIFIC CD8⁺ T CELL RESPONSE DRIVEN BY SQZ® EAPCS

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Background During cancer progression, genomic mutations develop that can generate immunogenic neoepitopes, which are potential targets for immunotherapies. In contrast to microbial and other exogenous antigens where most of the protein is immunogenic, neoepitopes are immunogenic in a narrow region of the protein due to self-tolerance for the flanking regions. Previously, we demonstrated that SQZ® eAPCs, produced from human peripheral blood mononuclear cells (PBMCs) with mRNA encoding for full-length viral antigens (98-561 aa in length), can elicit robust CD8⁺ T cell responses. To understand whether the smaller immunogenic regions of multiple neoepitopes (~25 aa) could be linked together in a single mRNA construct, we delivered various mRNA constructs encoding 5-10 model antigen fragments (e.g., HPV16 E6, HPV16 E7, mutant KRAS, and Influenza M1) linked together into PBMCs to generate SQZ® eAPCs.

Methods Model antigen fragments with known CD8⁺ T cell epitopes were linked together to form a single mRNA. Cell Squeeze® technology was used to deliver the linked antigen mRNAs directly into the cytosol of all major cell subsets of PBMCs. Western blots were used to confirm the delivery and translation of the linked antigen mRNA constructs. The MHC-I presentation of epitopes on the SQZ® eAPCs was assessed in vitro by culturing SQZ® eAPCs with antigen-specific responder T cells or TCR-transduced Jurkat-Lucia NFAT reporter cells overnight before measuring the activation response of these T cells via luciferase or IFNγ ELISA.

Results We demonstrate simultaneous antigen presentation on MHC-I by SQZ® eAPCs with a single mRNA encoding for 5 or 10 linked antigen fragments. Multiple antigen-specific responder cells are activated after co-culture with PBMCs squeezed with linked antigen mRNA, as measured by an increase in the secretion of luciferase or IFNγ.

Conclusions SQZ® eAPCs with mRNA encoding for up to 10 linked antigen fragments allows for simultaneous antigen presentation and robust CD8⁺ T cell responses. These findings further enhance the versatility of the Cell Squeeze® technology to potentially target multiple tumor-associated neoantigens (e.g., mutated KRAS) in a single mRNA construct.