RBC-DERIVED, ACTIVATING ANTIGEN CARRIERS (SQZ AACs) PRIME POTENT T CELL RESPONSES AND DRIVE TUMOR REGRESSION IN VIVO

Katarina Blagovic*, Carolyn Smith, Lindsay Moore, Emrah Ilker Ozay, Armon Sharei, Howard Bernstein, Scott Loughhead. *SQZ Biotechnologies, Watertown, MA, USA*

Background T cell responses are at the core of checkpoint inhibitor success in treating cancer; however, generating targeted antigen presentation to stimulate T cell responses remains challenging. Here, we take advantage of the natural process of eryptosis by professional antigen presenting cells (APCs) to drive antigen presentation and T cell activation in human and mouse models. Through the delivery of tumor antigen and adjuvant to red blood cells (RBCs) using the Cell Squeeze® platform, we generate activating antigen carriers (AACs) for use in tumor-specific cancer vaccines.

Methods Following intravenous AAC administration, we measured clearance kinetics of AACs and characterized the site and cell type of AAC uptake. We investigated upregulation of activation markers on phagocytes that engulf AACs, and the effect of priming and boosting on endogenous T cell responses. To determine the ability of AACs to control implanted tumors, we measured tumor growth rates in mice therapeutically treated with AACs. Tumor growth of AAC-treated mice in combination with a chemotherapy treatment was also assessed. Additionally, the in vitro uptake of adjuvant loaded human AACs and resultant maturation of monocyte-derived dendritic cells (MoDCs) was measured to qualify adjuvant delivery. Peptide antigen delivery to human AACs was measured with flow cytometry and fluorescence microscopy.

Results Squeezing effectively loads AACs with antigen and adjuvant and leads to exposure of phosphatidylserine on the AAC membrane. When administered into a mouse, mouse AACs were cleared from circulation within one hour and were engulfed by professional phagocytes in both the spleen and liver. In vivo, AACs induced upregulation of maturation markers on endogenous dendritic cells (DCs) and macrophages. Therapeutic AACs administration significantly slowed growth of the HPV-associated tumor, TC-1, and extended survival of treated animals. These anti-tumor responses correlated with >500-fold increase in antigen-specific CD8+ tumor-infiltrating lymphocytes compared to untreated mice. Boosting enhanced endogenous T cell responses and enhanced the efficacy of low dose vaccinations in a tumor model. Combination with early (days 7 and 9) or late (days 17 and 24) treatment with a chemotherapeutic agent cleared tumors in treated animals. In an in vitro human system, the intracellular delivery of peptide antigen and adjuvant to human AACs induced MoDC maturation and stimulated E7-specific CD8+ T cell responses.

Conclusions AACs loaded with antigen and adjuvant can effectively drive antigen presentation and prime a potent anti-tumor response in mice. These preclinical data support the further study of SQZ AACs as an immunotherapy for cancer treatment.

Ethics Approval All methods were performed in accordance with the relevant guidelines and regulations. Animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) at SQZ Biotechnologies, using the recommendations from the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the Office of Laboratory Animal Welfare. All activities were also

conducted in accordance with Public Health Service (PHS) Policy on Humane Use and Care of Laboratory Animals.

<http://dx.doi.org/10.1136/jitc-2021-SITC2021.156>