activation and tdLN infiltration of cross-presenting dendritic cells (cDC1) as well as increased presentation of the SIIN-FEKL epitope of OVA specifically on this subset of dendritic cells.

Conclusions Together, our findings suggest that tethering IL-12 to tumor-specific T cells prior to adoptive transfer promotes epitope spreading through the combination of tumor cell-killing induced by the ACT therapy and IL-12-induced activation of cDC1s in the tdLN. This adjuvant activity from T cell-tethered IL-12 holds promise for overcoming antigen escape pathways that limit the efficacy of antigen-specific T cells against heterogeneous tumors

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PBMC-BASED CANCER VACCINES GENERATED WITH MICROFLUIDICS SQUEEZING DEMONSTRATE SYNERGISTIC AND DURABLE TUMOR REDUCTION IN COMBINATION WITH PD1 CHECKPOINT AND FAP TARGETED IL-2 VARIANTS

¹Matthew Booty, ¹Adam Stockmann, ¹Olivia Pryor, ¹Melissa Myint, ²Christine Trumpfheller, ²Valeria Nicolini, ²Christian Klein, ²Laura Codarri, ²Pablo Umana, ¹Armon Sharei, ¹Howard Bernstein, ¹Katherine Seidl, ¹Scott Loughhead*. ¹SQZ Biotechnologies, Watertown, MA, USA; ²Roche (pRED), Schlieren, Switzerland

Background We engineered unfractionated peripheral blood mononuclear cells (PBMCs) to function as antigen presenting cells (APCs) that generate potent CD8+ T cell responses. We investigated the combined efficacy of PBMC-based cancer vaccine with targeted interleukin 2 variants (IL2v); anti-Programmed Cell Death Protein 1 (muPD1-IL2v) and anti-Fibroblast Activation Protein (muFAP-IL2v).

Methods We generated PBMC-based cancer vaccine with microfluidic cell engineering system (Cell Squeeze®), which facilitates direct cytosolic antigen delivery and enables cell subsets within PBMCs to function as APCs. The immunocytokines used contain IL2v fused with antibody counterparts that enable targeting to tumor-associated stroma or immune cells (aFAP and aPD-1, respectively) with modified FcR binding. The IL2v moiety, compared with wild-type IL-2, has abolished binding to IL-2Ra (CD25) resulting in IL-2R β binding only, thus fully maintaining activity on NK and CD8+ T cells, while avoiding Treg activity and CD25 mediated toxicity.

Results In the murine TC-1 HPV tumor model, SQZ-PBMC-based vaccines show efficacy as monotherapy (1e6 cells administered iv on day 14 post-tumor implant), while SQZ combination therapy with targeted immunocytokines, muPD1-IL2v and muFAP-IL2v (2 mg/kg or 1 mg/kg, respectively, administered iv on days 21, 28, and 35 post-tumor implant) significantly delayed tumor growth and improved survival in murine TC-1 HPV tumor model. Median survival of combination treated groups remained undefined at day 84 post-tumor implant, while the monotherapy treated groups had calculated median survival times of 36.5, 42, and 70 days for the muFAP-IL2v, muPD1-IL2v, and SQZ monotherapy groups, respectively. Following initial tumor clearance, tumor-free mice (2/12 animals for SQZ monotherapy; 8/12 animals for SQZ with muFAP-IL2v; 11/11 animals for SQZ with muPD1-IL2v) were all re-challenged at day 84 and all remained tumor free at least 7 weeks post re-challenge, suggesting the generation of anti-tumor memory response. In a mechanistic study, SQZ-PBMCs in combination with muPD1-IL2v resulted in increased expansion of intra-tumoral, antigen-specific CD8+ T cells

compared with separate administration of either therapy (~3.6-fold over SQZ alone; ~2000-fold over muPD1-IL2v alone; per mg of tumor). Combination therapy also resulted in improved IFN γ and TNF α cytokine production by SQZ-elicited CD8+ T cells (~1.7-fold and ~9-fold, respectively, over SQZ monotherapy).

Conclusions Monotherapy with SQZ-PBMC-based cancer vaccines can drive anti-tumor responses in murine systems. These responses are enhanced by combined administration of targeted immunocytokines. Monotherapy with SQZ-PBMC-HPV is currently under clinical evaluation for HPV16+ tumor indications. These preclinical data support the combination of SQZ-PBMC with FAP-IL2v or PD1-IL2v targeted immunocytokine as promising cancer immunotherapies.

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CONTEXTUAL REPROGRAMMING OF CAR T CELLS FOR THE TREATMENT OF HER2-EXPRESSING CANCERS

¹Zhifen Yang*, ¹Lingyu Li, ¹Ahu Turkoz, ¹Pohan Chen, ¹Hana Choi, ¹Damla Inel, ²Stanley Qi, ¹Francesco Marincola. ¹Refuge Biotechnologies Inc., Menlo Park, CA, USA; ²Stanford University, Stanford, CA, USA

Background Combining checkpoint inhibition (CPI) to adoptive cell therapy (ACT) is a promising strategy to prevent chimeric antigen receptor (CAR)-engineered T cell exhaustion and improve outcomes. However, cumulative toxicities and costs limit this approach. Here, we apply a conditional, antigen-dependent non-editing CRISPR interference-(CRISPRi) modulation circuit that we originally described in yeast and eukaryotes¹⁻³ (RB-340-1) to promote CAR T resilience to checkpoint suppression extending in vivo persistence and effectiveness.

Methods RB-340-1 is an CAR T cell product engineered via synthetic biology approaches to express a combination of molecules to prevent CAR T cell exhaustion and improve solid tumor treatment outcomes. The components include two constructs. The first construct encodes an anti-HER2 (4D5) CAR single chain variable fragment (scFv), with CD28 and CD3 ζ co-stimulatory domains linked to a tobacco etch virus (TEV) protease and a programmed cell death protein 1 (PD1) promoter region-targeting single guide RNA (PD1sg). The second construct encodes a protein, linker for activation of T cells (LAT), complexed to nuclease-deactivated/dead Cas9 (dCas9)-Kruppel-associated box (Krab) via a TEV-cleavable linker. Activation of CAR brings CAR-TEV in close proximity to the LAT-dCas9-Krab complex releasing the enzyme for nuclear localization to the PD1 regulatory region to conditionally and reversibly suppress its expression. RB-340-1 was compared in vitro and in vivo against conventional and control (cRB-340-1, lacking PD1sg) HER2 CAR T cells in combination with CPI with Atezolizumab (5 mg/Kg administered intravenously twice a week).

Results RB-340-1 consistently induced higher production of homeostatic cytokines such as IL-2 resulting in significantly enhanced proliferation in vitro (figure 1a). Our in vivo data showed significantly enhanced suppression of growth of HER2 + FADU oropharyngeal cancer xenografts upon intra-tumoral (figure 1b) and systemic administration (figure 1c) and prolonged persistence of CAR T cells in vivo.