activation and tdLN infiltration of cross-presenting dendritic cells (cDC1) as well as increased presentation of the SIINFEKL 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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141 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

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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-2Rgb 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 IFNγ and TNFα 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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142 CONTEXTUAL REPROGRAMMING OF CAR T CELLS FOR THE TREATMENT OF HER2-EXPRESSING CANCERS

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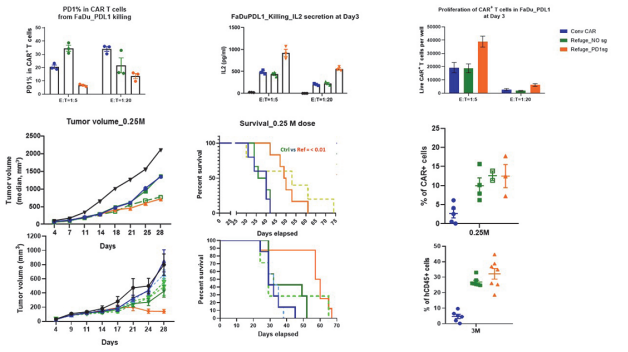
Background Combining checkpoint inhibition (CPI) to adoptive cell therapy (ACT) is a promising strategy to prevent chimeric antigen receptor (CAR)-engineered T cell exhaustion and improve outcomes. However, cumulative toxicities and costs limit this approach. Here, we apply a conditional, antigen-dependent non-editing CRISPR interference-(CRISPRi) modulation circuit that we originally described in yeast and eukaryotes[1–3] (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 CD3ζ 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.



Abstract 142 Figure 1 Rb-340-1 performance in vitro and in vivo RB-340-1 (orange) decreased PD-1 expression resulting in enhanced cytokine production and proliferation in vitro (figure 1a) and superior tumor suppression in vivo after intra-tumoral (figure 1b) or intravenous (figure 1c) administration compared to conventional CAR T cells (blue) or cRB-340-1 (green). Conventional CAR T cells or cRB-340-1 CAR T combination treatment with PDL1 blockade (Atezolizumab) is shown as dashed line. Colonization of tumors by human CD45+ cells is shown at the bottom of figure 1b & 1c

Conclusions Intrinsic conditional regulation of checkpoint expression in CAR T cells provides a simplified approach to combination therapies that limits systemic toxicities and reduces costs. Since the expression of multiple genes can be simultaneously controlled by CRISPRi, broader applications can be envisioned in the future.

Ethics Approval Not Applicable

Consent Not applicable

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144 RTX-240, AN ALLOGENEIC ENGINEERED RED BLOOD CELL EXPRESSING 4–1BBL AND IL-15TP, PROMOTES NK CELL FUNCTIONALITY IN VITRO AND IN VIVO

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Background Agonist antibodies and recombinant cytokines have had limited success in the clinic due to three factors: severe toxicity leading to a narrow therapeutic index, the diminished activity of an agonistic antibody compared with natural ligand, and the lack of multiple signals needed to effectively activate most cell types. To address these limitations, Rubius Therapeutics has developed RTX-240, an allogeneic cellular therapy using red blood cells genetically engineered to express 4-1BBL and IL-15/IL-15Ra fusion (IL-15TP) in their natural conformation on the cell surface. RTX-240 is designed to recapitulate human biology by broadly stimulating adaptive and innate immunity to generate an anti-tumor response and provide improved safety due to the restricted biodistribution of red blood cells to the vasculature. Here we demonstrate that RTX-240 is highly active in preclinical models.

Methods PBMCs or NK cells were treated with RTX-240 in vitro. mRBC-240 was used for in vivo studies.

Results Treatment of either PBMCs or isolated NK cells with RTX-240 induced a dose-dependent increase in NK cell activation, proliferation and functionality. These effects were further enhanced with increased 4-1BBL and IL-15TP expression on the surface of RTX-240. NK cell counts, NKp44 and Trail expression were increased 150, 4.6 and 6-fold over media control, respectively. Activation of NK cells with RTX-240, followed by incubation with K562 targets enhanced NK cell cytotoxicity (1.3-2.8 over control), that was accompanied by increased NK cell activation (CD69) and degranulation (CD107a) (3.1-fold and 1.9-fold, respectively). RTX-240-activated NK cells showed higher frequency of CD56dim/CD16+ NK cells, which have been reported to induce natural and ADCC-dependent cytotoxicity. Correspondingly, RTX-240 promoted enhanced ADCC-induced killing of Raji cells when combined with anti-CD20 mAb (1.4-fold over control). Intravenous administration of mRBC-240 to a B16F10 intravenous lung metastases model led to NK cell expansion on Day 4 (3.8-fold over control). These NK cells were cytotoxic (Granzyme B+) and highly proliferative (Ki67+) (1.4-fold and 18.8-fold over control, respectively). Treatment with mRBC-240 increased the frequency of terminally differentiated NK cells (NK1.1+/CD11b+/CD27-/KLRG1+) in the tumor (2.1-fold increase over control). Terminally differentiated NK cells are highly cytotoxic and their frequency in the tumor was strongly correlated with efficacy in this model ($p=0.0001$).

Conclusions Taken together, these data indicate that RTX-240 promotes NK cell activity and functionality in preclinical models. RTX-240 has now entered a first-in-human Phase 1 trial for the treatment of patients with relapsed/refractory or locally advanced solid tumors, with a planned arm evaluating RTX-240 in relapsed/refractory acute myeloid leukemia.

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145 PRECLINICAL DEVELOPMENT OF EDIT-201, A MULTIPLEXED CRISPR-CAS12A GENE EDITED HEALTHY DONOR DERIVED NK CELLS DEMONSTRATING IMPROVED PERSISTENCE AND RESISTANCE TO THE TUMOR MICROENVIRONMENT

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Background Natural killer (NK) cells distinguish tumor from healthy tissue via multiple mechanisms, including recognition of stress ligands and loss of MHC class I expression. However, effector function of allogeneic NK cells can be diminished by the lack of functional persistence, as well as tumor-intrinsic immunosuppressive mechanisms, such as production of TGF-β. We developed a next-generation allogeneic NK cell therapy using CRISPR-Cas12a gene editing to enhance NK cell function through knockout of the CISH and TGFBR2 genes. We hypothesized that knockout of CISH, a negative regulator of IL-2/IL-15 signaling, would improve NK cell effector function, while knockout of the TGF-β receptor gene,