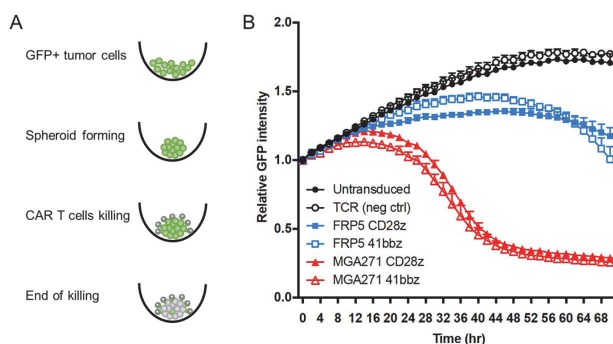
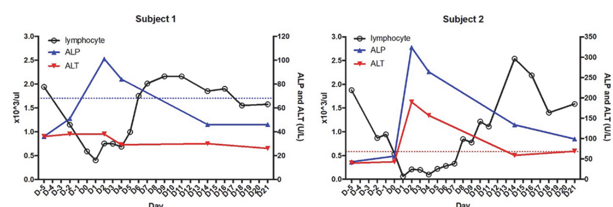
the CAR products following lymphodepletion was confirmed in two healthy dogs (figure 1B).

Results Canine solid tumors were confirmed to be B7H3 positive in almost all cases. Using the GALV-pseudotyped retrovirus system, transduction was efficient with up to 70% CAR+ cells. Post-transduction expansion was over 100 folds. B7H3 CAR transduced canine T cells were able to eliminate B7H3+ canine tumor spheroids effectively (figure 2). Safety of the CAR T cells (dose: $1 \times 10^9/m^2$) were confirmed in both healthy animals following cyclophosphamide lymphodepletion. After week 6, cetuximab was given to the subjects to deplete EGFR+ cells. Subject 2 experienced fever after CAR T cell administration. Both dogs showed elevated serum ALP and ALT levels and returned to normal (figure 3). No other treatment-related adverse events were observed. Information of the CAR T cell products can be found in table 1.



Abstract 139 Figure 2 Killing of canine OSA spheroids by canine CAR T ce
(A) Scheme of tumor cell spheroid forming and killing. The loss of GFP can be measured for cytotoxicity readout (B) FRP5 and MGA271 CAR T cells can effectively kill canine cancer spheroids. Experiments were done in triplicates and error bars indicate SD



Abstract 139 Figure 3 Dynamics of peripheral lymphocytes, serum ALP and Current treatment regimen effectively decreased peripheral lymphocytes number after cyclophosphamide and fludarabine administration (D-4 and D-3) and increased serum ALP and ALT level after CAR T cell infusion (D0). Dashed line in both graphs show the upper limit of ALP and ALT levels, which are both 68U/L

Conclusions We demonstrated that, similar to human cancers, B7H3 is a target in canine solid tumors. We successfully generated canine B7H3 specific CAR T cell products that are highly efficient at killing canine 3D tumor spheroids using a production protocol that closely models human CAR T cell production procedure and confirmed the safety in vivo. We plan to test and optimize various approaches to enhance CAR T cell efficacy for solid tumor treatment both in vitro and in canine sarcoma patients.

Ethics Approval The study was approved by Fred Hutchinson Cancer Research Center's Institutional Animal Care and Use Committee (IACUC), approval number PROTO201900860

Abstract 139 Table 1 Infused CAR T cell product information

	Subject 1	Subject 2
Weight (kg)	16	15.4
Infused cells (x10^6)	641	625
Production start (x10^6)	20	20
Production end (x10^6)	2729	6560
Transduction efficiency	32.9%	47.6%

Both subjects are adult male beagle mix

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140 ADOPTIVE TRANSFER OF T CELLS SURFACE-TETHERED WITH IL-12 PROMOTE ANTIGEN SPREADING FOR ENHANCED ANTI-TUMOR EFFICACY

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Background Acquired resistance is a major limiting factor for durable T cell therapies in solid tumors. Antigen escape pathways such as insufficient antigen coverage or loss of target antigen remain major resistance mechanisms that need to be addressed in order to expand the field of T cell therapies. Interleukin-12 (IL-12) is a potent stimulator of innate and adaptive immune cells that holds strong potential for cancer immunotherapy, but its clinical utility has been limited by high systemic toxicities. We have previously shown that tethering an IL-12 immunocytokine to the surface of T cells prior to adoptive cell transfer (ACT) safely improves anti-tumor efficacy by promoting T cell function specifically in the tumor. Here, we demonstrate that cell-tethered IL-12 delivers adjuvant activity that leads to priming and expansion of bystander, tumor-specific T cells, and thereby counteract common immune escape pathways.

Methods Adjuvant activity of IL12-tethered pmel T cells, reactive towards the gp100 antigen of B16 tumors, was evaluated in the B16-OVA syngeneic mouse model. Notably, adoptive transfer of IL12-tethered pmel T cells, but not pmel T cells alone, resulted in proliferation of endogenous tumor infiltrating lymphocytes. To assess whether this reflected tumor-specific T cell responses, we used dextramer staining against non-targeted, tumor-specific antigens and found that both abundance and activation increased following cell-tethered IL-12 treatment. Encouraged by these findings, the OT-1 model was used to track epitope spreading to tumor-specific naïve T cells. Following treatment with IL-12-tethered PMEL T cells, we tracked the proliferation and tumor engraftment of labelled, naïve OT-I T cells, which are reactive towards the non-targeted OVA antigen.

Results Cell-tethered-IL12, but neither ACT nor ACT and systemically administered IL-12, induced proliferation and engraftment OT-1 T cells in tumor-draining lymph nodes (tdLNs) and tumors of B16-OVA-bearing mice. This effect was antigen-dependent as the OT-I T cells were not primed in B16.F10 (OVA antigen-negative) tumors. Mechanistically, this priming was associated with IL-12-induced increases in

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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.

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