CAR, which can actively target the GD2 antigen overexpressed on GBM (Figure 1A). For E-NK cell radiolabeling, zirconium-89 (89Zr, ½ life = 78 Hr) radiotracer was attached covalently to the E-NK cell surface via conjugation with DFO-Bz-NCS in a range of doses from 50–600 μCi.

**Results**

An optimal balance between labeling efficiency and cell viability was attained at 120 μCi 89Zr resulting in 39% labeling efficiency and 46% cell viability over for 48 hours. After labeling, the NK cells maintained their in vitro killing activity against GBM cells (figure 1B). The 89Zr labeled E-NK cells were administered intravenously in mice containing intracranial GBM10 tumors at week 5 post-implant. PET imaging was performed at 1 and 2 days later and gamma imaging ex vivo at 4 days. Free 89Zr was visible diffusely throughout the body with low levels in the brain. The majority of 89Zr labeled E-NK cell groups localized to the lungs with detectable activity elsewhere in various organs (figure 1C and 1D).

**Abstract 138 Figure 1** PET imaging and gamma counting of the engineered NK cells

Figure 1 (A) Multifunctional, responsive CAR constructs; (B) In vitro killing activity against GBM43 cells after co-incubation with 89Zr labeled NK cells at an E:T ratio of 10 for 4 h with LDH assay (N=3); (C) & (D) In vivo PET imaging and ex vivo gamma counting with 89Zr at week 5 in 10 mice during 4 days, GBM intracranial implantation to NSG male mouse, 89Zr, 89Zr + NK cell, or 89Zr + E NK cell (7 × 106 cells with 500 μCi). Administration involved through intravenous injection, Qimage was used for the PET/MRI co-registration and analysis.

**Conclusions**

We generated multifunctional E-NK cells which showed the improved killing of GBM cells using novel targeting approaches, including the blockade of CD73-mediated adenosinergic signaling. We also optimized E-NK cell radiolabeling with 89Zr for GB10 therapy in vitro and in vivo fate mapping against a xenograft of patient-derived GBM.

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**References**


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**Abstract 139**

**Establishment of Canine CAR T Cells Treatment Model for Solid Tumor Immunotherapy Development**

1. Shihong Zhang, 1 Karan Kohli, 1 Rachel Black, 1 Brian Hayes, 1 Cassandra Miller, 2 Mari Maeda-Whitaker, 3 Brett Schroeder, 4 Craig Abrams, 5 Bernard Seguin, 4 Stephen Gottschalk, 4 Peter Moore, 4 Beverly Tork-Stoth, 5 Seth Pollack. 6 Fred Hutchinson Cancer Research Center, Seattle, WA, USA; 1 Canine Cancer Alliance, Seattle, WA, USA; 2 Colorado State University, Fort Collins, CO, USA; 3 St. Jude Children’s Research Hospital, Memphis, TN, USA; 4 University of California, Davis, CA, USA

**Background**

Chimeric antigen receptor (CAR) T cell therapy has transformed therapy for hematological malignancies but has not yet been established as standard of care for any solid tumors. One obstacle for human solid tumor immunotherapy research is the lack of clinically relevant, immunocompetent animal models. In this study, we sought to establish CAR T cells for naturally occurring canine sarcomas in client owned animals as a model for human CAR T cell therapy.

**Methods**

Archived FFPE, freshly isolated canine solid tumor samples as well as tumor lines were tested for B7H3 expression by immunohistochemistry (IHC) and flow cytometry analysis. We designed CARs using the scFv from the human B7H3-specific antibody MGA271 and confirmed the cross-reactivity to canine B7H3 (construct information see figure 1A). A truncated EGFR (tEGFR) was included in the construct to allow for IHC and flow cytometry testing for the presence of CAR T cells. Killing efficiency was evaluated using 3D tumor spheroid killing assays to monitor dynamics. Safety of...
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 × 10^6/m²) 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.

**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

<table>
<thead>
<tr>
<th>Subject</th>
<th>Weight (kg)</th>
<th>Infused cells (x10^6)</th>
<th>Production start (x10^6)</th>
<th>Production end (x10^6)</th>
<th>Transduction efficiency</th>
</tr>
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<tbody>
<tr>
<td>Subject 1</td>
<td>16</td>
<td>641</td>
<td>20</td>
<td>2729</td>
<td>32.9%</td>
</tr>
<tr>
<td>Subject 2</td>
<td>15.4</td>
<td>625</td>
<td>20</td>
<td>6560</td>
<td>47.6%</td>
</tr>
</tbody>
</table>

Both subjects are adult male beagle mix

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**Abstract 139**

**Figure 2** Killing of canine OSA spheroids by canine CAR T cell

(A) Scheme of tumor cell spheroid forming and killing. The loss of GFP can be measured for cytotoxicity readout (B) FRPS 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

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 engulfment 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 engulfment OT-1 T cells in tumor-draining lymph nodes (dLNs) 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...