**Supplemental Figure Legends**

*Supplemental Figure 1. Schematic representations of viral-engineered constructs for transductions of T cells.*

Our basic CAR constructs were constructed from a human CD8 leader sequence followed by a murine single chain monoclonal antibody fragment targeting either human mesothelin (meso-scFv) or murine fibroblast activation protein (FAP-scFv) followed by a human CD8 hinge sequence fused to the cytoplasmic domain of human 41BB which was fused to the cytoplasmic domain of CD3z. The RISR-RIAD construct into which myc and ddk (FLAG) tags were incorporated, was synthesized by Integrated DNA Technologies in the pIDT.SMART cloning plasmid. The insert was subcloned downstream of CAR constructs after in IRES sequence in retroviral vectors (MigR1) that were used to transduce mouse T cells. Primary murine T cells were isolated and transduced with these retroviral particles as previously described (2).

This included:

1. The mesoCAR targeting human mesothelin, named MigR1.mesoCAR-RIAD
2. The murine FAPCAR targeting mouse FAP (1), named MigR1.FAPCAR-RIAD
3. For transgene-tracking purposes, we cloned in a GFP cassette in the MigR1.mesoCAR construct
4. We also made a CAR-mCherry fusion protein that was inserted in front of the RIAD cassette in the MigR1 vector (MigR1.mesoCAR-mCherry-RIAD).

For use in human T cells, the insert was also cloned into CAR plasmids used to make lentiviral vectors (pTRPE).

1. Our control vector expressed GFP followed by a T2A sequence and then mesoCAR (pTRPE-mesoCAR).
2. The pTRPE.mesoCAR-RIAD construct was made by excising the GFP cassette and inserting the RISR-RIAD construct.
3. For tracking purposes, we made a third construct expressing RISR-RIAD followed by a fusion protein of mesoCAR and mCherry. The isolation, bead activation, transduction using pTRPE.mesoCAR and pTRPE.mesoCAR-RIAD, and subsequent expansion of primary human T cells were carried out as previously described (3).

*Supplemental Figure 2. Successful transduction of primary T cells and detection of transgenes,*

CAR transduction efficiency in lentiviral-transduced primary human T cells (left panel), and murine T cells (right panel) was determined using a primary biotinylated goat anti-mouse IgG recognizing the F(ab’)2 fragment of the CAR (followed by a secondary APC-Cy7-conjugated streptavidin antibody). RIAD expression was measured in the same cells using an anti-myc or anti-ddk antibody.

*Supplemental Figure 3. Phenotypic characterization of human CAR T cells at baseline.*

Human T cells before (donor T cells) and after transduction (CAR+) with mesoCAR or mesoCAR-RIAD and expansion were examined by flow cytometry. A representative example is illustrated; cells were gated on CAR+CD8+ T cells.

1. CD4 to CD8 ratio: The ratio of CD4:CD8 cells after transduction varied among donors, but after CAR transduction, there was almost always a skewing toward more CD8 T cells. However, the RIAD transgene did not appear to alter this ratio
2. T cell differentiation: After T cell expansion with CD3/CD28 beads, almost all of the transduced T cells were of the memory (CD45RO+) phenotype with a predominance of central memory (CD62Lhi) cells. Again, there was no difference between the CAR and CAR-RIAD cells.
3. Activation/Inhibition phenotype: We saw no differences in expression levels of CD69, CD25, HLA-DR, 4-1BB, LAG3, PD1, TIGIT, or TIM3.

*Supplemental Figure 4. Phenotypic characterization of murine CAR T cells at baseline.*

Expanded, but non-transduced murine T cells and murine T cells after transduction with mesoCAR or mesoCAR-RIAD and expansion were examined by flow cytometry. A representative example is illustrated; cells were gated on CAR+CD8+ T cells.

1. CD4 to CD8 ratio: We saw relatively equal numbers of CD4 and CD8 T cells, with no clear effect of the RIAD transgene.
2. T cell differentiation: Both the CD8 CAR and CAR-RIAD T cells were mostly of the effector memory (CD62Llo/CD44hi) phenotype, while the CD4 CARs had more of a central memory phenotype (CD62Lhi/CD44intermediate), with no differences in the CAR-RIAD group.
3. Activation/Inhibition phenotype: We saw no differences in expression levels of CD69, CD25, HLA-DR, 4-1BB, LAG3, PD1, TIGIT, or TIM3.

*Supplemental Figure 5. Heightened cytokine production by CAR-RIAD T cells upon in vitro restimulation with mesothelin-coated beads.*

Human T cells expressing mesoCAR and mesoCAR-RIAD were examined at baseline or were stimulated with mesothelin-coated beads or with PMA/ionomycin overnight in the presence of GolgiStop, and stained for the indicated cytokines using flow cytometry. The percent of T cells expressing both IL2 and IFNγ are shown. RIADCAR T cells showed increased cytokine production at baseline and after meso-bead stimulation with similar production of IL-2 and IFNγ after PMA/ionomycin stimulation.

*Supplemental Figure 6. Densitometry analyses of signaling proteins.*

The phosphorylation status of key signaling proteins – pERK, pLckY394, and pAkt – in human mesoCAR and mesoCAR-RIAD T cells was assessed by western blotting as shown in Figure 2A, and quantified using densitometry analysis.

*Supplemental Figure 7. Expression of PGE2 in our tumor cells and the effect of mesoCAR-RIAD T cells on tumors that do not express the antigen mesothelin.*

1. Our tumor cell lines, AE17meso (murine mesothelioma cell line) and EMmeso (human mesothelioma cell line) express high levels of PGE2 as assessed by ELISA. Note the levels seen in normal tissues (lung and liver) is much lower. Data shown are means ± SEM, n = 3 replicates per group.
2. Adoptively transferred mesoCAR-RIAD T cells were injected into mice bearing AE17ova (non-antigen-expressing) tumor cells. The increased activity seen in the mesoCAR-RIAD cells does not non-specifically boost endogenous anti-tumor immunity in this model of data shown are means ± SEM, n = 8 mice per group.

*Supplemental Figure 8. Greater influx of CD4+ CAR T cells in human and murine tumors.*

A. EMmeso tumors harvested at Day 32 post T cell-transfer were digested, pooled, and analyzed using flow cytometry analysis. Panel A is a blowup of the human CD4 TIL data shown in Figure 4A. There was a significant increase in the CD4 T cells in the CAR-RIAD tumors, although the number of CD4 T cells was low.

B. To examine a model where T cells can be measured at an earlier time point, we used the murine AE17meso model. CAR T cells were made from CD45.1 mice and infused into congenic CD45.2 mice. At Day 3 post-adoptive transfer, we performed flow cytometry analysis of the murine tumor-infiltrating lymphocyte (TIL) population. The left panel shows two examples of tracings where we used an anti-CD45.1 antibody to identify the transferred T cells. As with the human model, we also observed a greater influx of murine mesoCAR-RIAD CD8+ T cells into the tumors by flow cytometry. The right hand panel shows the CD4 and CD8 T cells in the CD45.1 population. Again, CD8+ TILs outnumbered CD4+ TILs at an approximate 10:1 ratio.

**References:**

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