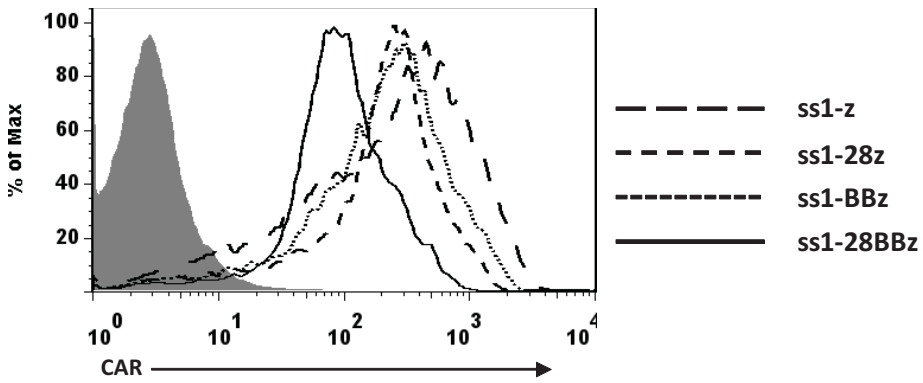
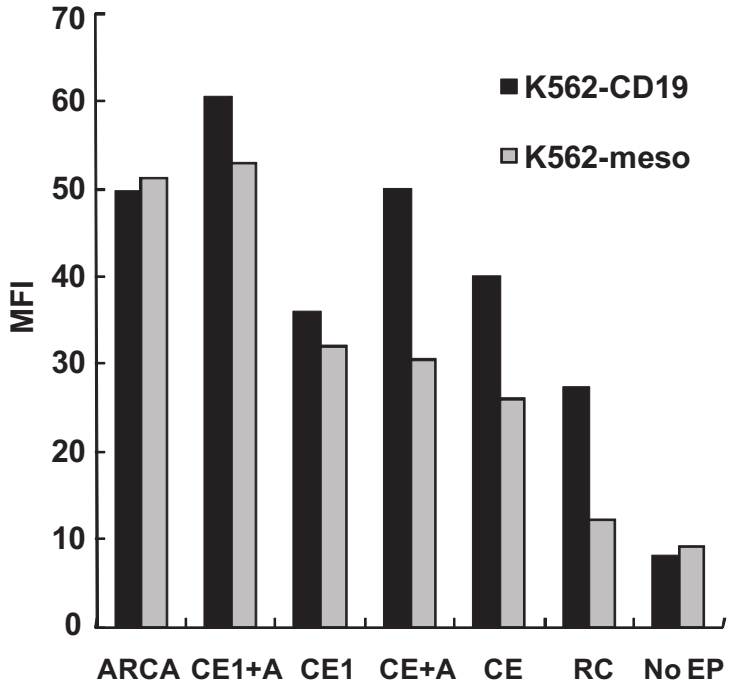
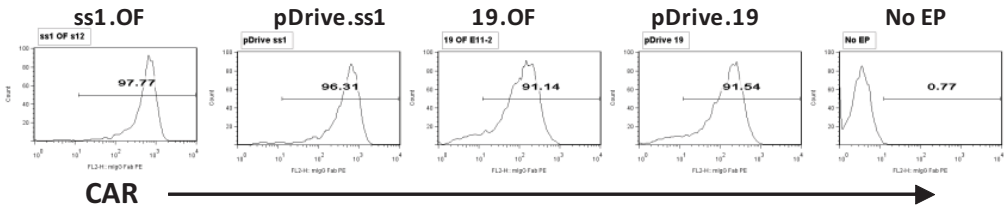


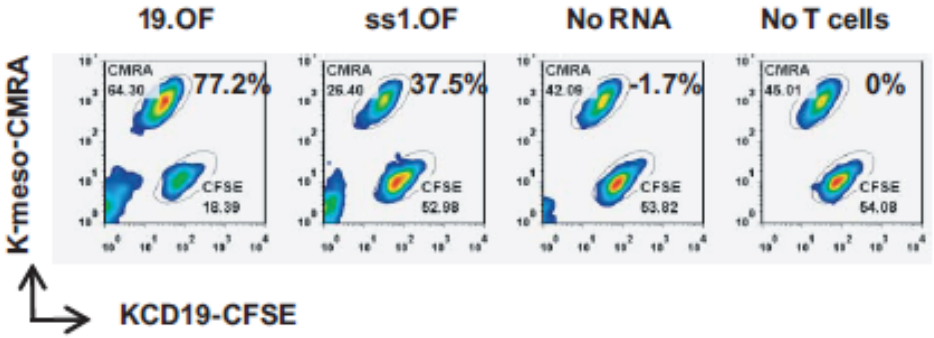
Supplemental Figure 1. Varying levels of CAR transgene expression on T cells. Activated T cells were electroporated with RNA encoding anti-mesothelin scFv ss1 CARs with the indicated signaling moieties, and flow cytometry used to measure surface expression 19 hours after electroporation. T cells electroporated without RNA were used as negative control. Experiments are representative of at least 2 independent experiments.



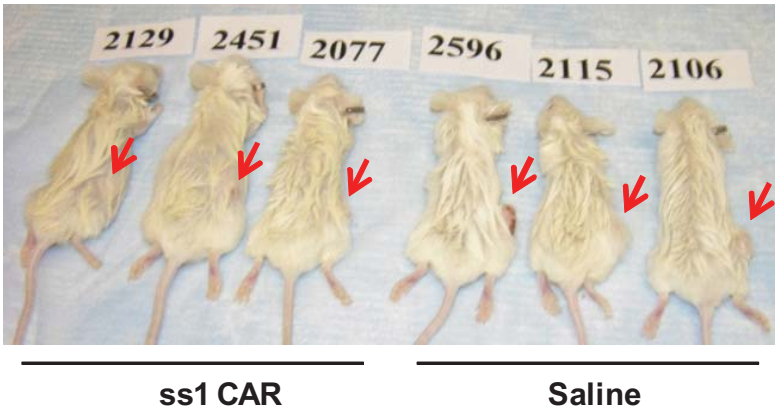
Supplemental Fig 2. T cells electroporated with ss1 RNA CARs generated by different methods were co-cultured 1:1 with targets expressing mesothelin (K562-meso) or control targets (K562-CD19) at day 1 post electroporation and cultured for 48 hours before the surface transgene expression (MFI) was measured by flow cytometry. Abbreviations: ARCA, anti-reverse capping analog; CE1+A, capping enzyme 1 plus long poly(A); CE1, capping enzyme 1 with 64 poly(A); CE+A, capping enzyme plus long poly(A) 150; RC, regular capping analog; NoEP, mock electroporated. Experiments are representative of 2 independent experiments.



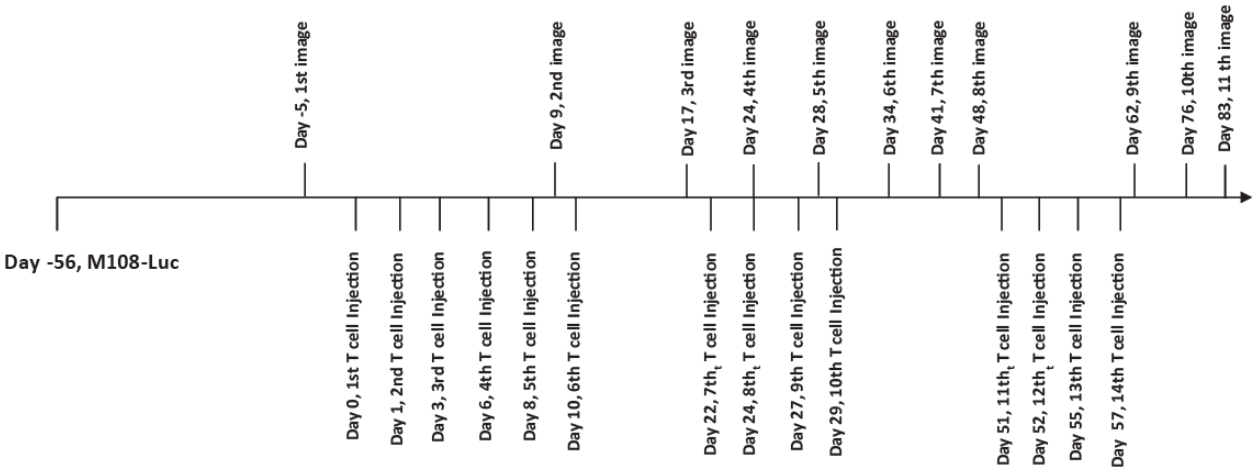
Supplemental Figure 3. Transgene expression of T cells electroporated with RNAs generated from clinical grade IVT vector pD-A.ss1.OF (ss1.OF) and pD-A.19.OF (19.OF), compared to their parental vectors pDrive-ss1.2bgUTR.150A (pDrive.ss1) and pDrive-19.2bgUTR.150A (pDrive.19) 20 hours post electroporation. Experiments are representative of at least 2 independent experiments.



Supplemental Figure 4. Specific lytic activity of T cells electroporated with ss1-bbz or 19-bbz CAR RNA. 20 hours after electroporation, a 4 hr flow-based CTL assay containing a mixture of labeled K562-CD19 or K562-meso targets at an effector:target ratio of 10:1 was used. Percentage values listed on upper right quadrant are calculated specific killing for the relevant target. Experiments are representative of at least 2 independent experiments.



Supplemental Figure 5. Mice treated as shown in figure 4A were sacrificed on day 98 after tumor inoculation and photographed. Red arrows point to the tumors.



Supplemental Figure 6. Schedule of BLI and T cell injections for experiment testing multiple injections of RNA CAR T cells that were autologous to the tumor. 30 mice were injected with $8x10^6$ M108-Luc tumor cells (IP) and the mice were randomized into 3 groups before beginning therapy with autologous T cells electroporated with the indicated CAR on day 56. T cell injections ($1x10^7$ T cells per injection) and the times of BLI are indicated. BLI commenced 5 days prior to T cell injections , to provide a baseline measurement of tumor burden.