Background
Nearly all colorectal and most pancreatic and lung cancers express carcinoembryonic antigen (CEA). However, due to its expression in normal gut epithelial cells, CEA-targeted therapies have resulted in on-target, off-tumor toxicity. To overcome this, we have developed Tmod™, a logic-gated T-cell therapy platform. Tmod constructs are composed of an activating CAR or T-cell receptor that targets a tumor antigen and an inhibitory receptor recognizing an antigen expressed on normal healthy tissues, but not on tumor cells due to loss of heterozygosity (LOH). A2B530 is a CEA-directed Tmod construct utilizing an LIR-1-based inhibitory receptor (blocker) targeting human leukocyte antigen A*02 (HLA-A*02).

Methods
To generate CEA Tmod, T cells from HLA-A*02(+) donors were transduced with a single lentivirus to express i) the CAR, ii) the blocker, and iii) an shRNA targeting β2M. Cytotoxicity was measured by culturing CEA(+) target cell line pairs (A*02(-) and A*02(+)), expressing either GFP or RFP, with engineered T cells and quantifying live target cells over time. In vivo activity was examined using NSG mice subcutaneously implanted with “normal” (CEA(+)A*02(+)) and tumor cells (CEA(+)A*02(-)), in the right and left flanks. Mice were treated intravenously with CEA Tmod cells or control T cells.

Results
Control CEA CAR T cells killed CEA(+) target cell lines in vitro irrespective of HLA-A*02 expression. In contrast, CEA Tmod cells selectively killed tumor cells (CEA(+)A*02(-)) while sparing “normal” cells (CEA(+)A*02(+)). In mixed target cell cultures, CEA Tmod cells killed only the A*02(-) target cells, whereas the CEA CAR T cells killed both the A*02(-) and A*02(+) cell lines. Further, CEA Tmod cells exhibited bidirectional control between the activated and blocked states. While mice treated with control CEA CAR T cells experienced a reduction in volume and bioluminescence of both normal and tumor grafts, CEA Tmod cells specifically cleared A*02(-) tumors in mice (table 1). Finally, although expansion of Tmod cells in peripheral blood trended lower than CAR and TCR controls, anti-tumor activity was comparable in these groups.

Conclusions
A2B530 is an autologous CEA Tmod cell product that exploits common LOH at the HLA locus in cancer cells, enabling these engineered T cells to discriminate between normal and tumor cells. BASECAMP-1 (NCT04981119), an observational study identifying patients with somatic HLA LOH, is recruiting. Eligible patients with metastatic colorectal, pancreatic, or non-small cell lung cancer will be apheresed for a future A2B530 EVEREST-1 interventional study.

REFERENCES