263 A2B694, AN AUTOLOGOUS LOGIC-GATED CELL THERAPY TARGETING MESOTHELIN

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Background Mesothelin (MSLN) is expressed on a variety of solid tumors, including mesothelioma and ovarian, uterine, gastric, pancreatic, and lung cancers.[1] However, efforts to target MSLN using cellular therapies have been hampered by severe on-target, off-tumor toxicities associated with damage to normal tissues expressing MSLN.[2] To avoid these toxicities, we have developed a logic-gated engineered cell therapy, Tmod™, which is composed of two chimeric antigen receptors (CARs): an activator that targets a tumor-associated antigen and an inhibitory receptor (blocker) gated by an antigen expressed on normal tissue but lost in tumor cells due to loss of heterozygosity (LOH). A2B694 is an MSLN-specific Tmod construct combining a third-generation MSLN CAR with an LIR-1-based inhibitory receptor specific for human leukocyte antigen A*02 (HLA-A*02).

Methods Lentivirus encoding i) the CAR, ii) the blocker, and iii) an shRNA targeting β2M was used to transduce T cells from HLA-A*02 donors and generate MSLN Tmod cells. In vitro cytotoxicity measurements were performed using fluorescence-based imaging and luciferase readouts. In vivo assessments were performed in NSG mice subcutaneously implanted with "normal" cells (MSLN[+]A*02[+]), or tumor cells (MSLN[+]A*02[-]), in the left and right flanks, respectively. Following engraftment, mice were randomized and treated intravenously with MSLN Tmod cells or controls. Grafts were measured via caliper.

Results MSLN Tmod cells preferentially killed tumor cells (MSLN[+]A*02[-]) over "normal" cells (MSLN[+]A*02[+]) in vitro, unlike clinically active comparator M5 CAR T cells, which indiscriminately killed both target cell types (figure 1A). Soluble MSLN, tested across a 0-2 µg/mL range, did not impact MSLN Tmod function. Additionally, in mixed cell cultures where T cells and tumor and "normal" cells were simultaneously cultured (1:1:1 ratio), MSLN Tmod cells selectively killed tumor targets while sparing "normal" cells. Further, MSLN Tmod cells cycled between activated and blocked states in vitro when repeatedly challenged with tumor or "normal" target cells. Finally, while MSLN CAR T cells killed both "normal" and tumor grafts in vivo, MSLN Tmod cells selectively killed tumor grafts while sparing "normal" grafts (figure 1B, C).

Conclusions A2B694 is an autologous MSLN Tmod cell product that leverages LOH at the HLA locus in cancer cells, providing a mechanism to discriminate between normal and tumor cells. BASECAMP-1 (NCT04981119), an observational study that will identify patients with somatic HLA LOH, is currently recruiting. Eligible patients with metastatic colorectal, pancreatic, or non-small cell lung cancer will be apheresed for a future A2B694 interventional study (EVEREST-2).

REFERENCES

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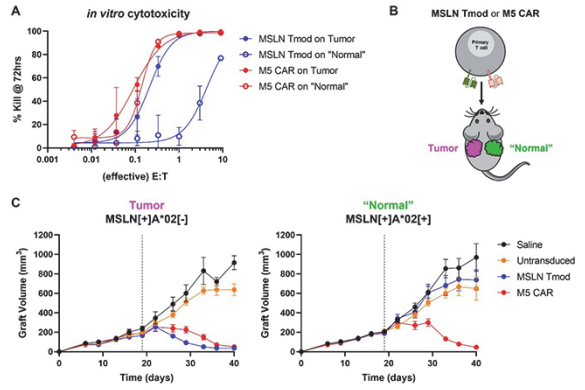
Figure 1. MSLN Tmod demonstrates selective killing of tumor vs "normal" cells in vitro and in vivo



(A) MSLN Tmod and M5 CAR killing of MSLN(+)A*02(-)luciferase(+) tumor and MSLN(+)A*02(+)luciferase(+) "normal" MS751 target cells at various E:T. Cytotoxicity was measured 72 hours after co-culture via luminescence. Data shown are for a representative donor. (B) Schematic diagram of the dual-flank tumor and "normal" MS751 xenograft model. (C) Graft sizes assessed via caliper. Left: MSLN Tmod cells kill tumor cells equivalently to M5 CAR cells. Right: M5 CAR cells kill while MSLN Tmod cells spare "normal" cells. Data shown are mean +/- SEM (n=10 animals per group).

CAR, chimeric antigen receptor; E:T, effector-to-target ratio; MSLN, mesothelin; SEM, standard error of the mean.

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