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A2B694, an autologous logic-gated cell therapy targeting mesothelin

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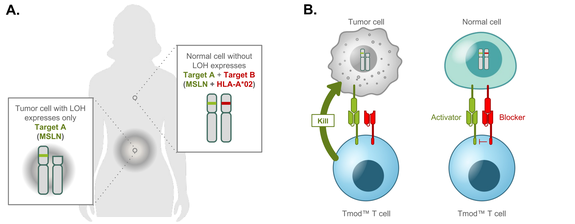
BACKGROUND

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™ (**Figure 1**), which is composed of 2 chimeric antigen receptors: an activator that targets a tumor-associated antigen and an inhibitory 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 blocker specific for human leukocyte antigen A*02 (HLA-A*02).

Figure 1. Tmod platform uses tumor LOH to differentiate tumor from normal cells

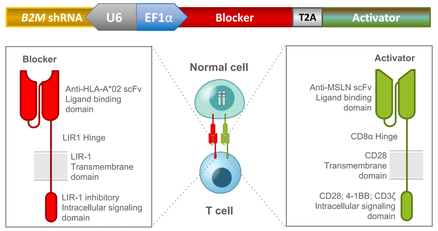


The supreme challenge in oncology: Discrimination between tumor and normal tissue. (**A**) LOH, which affects ~20% of the tumor genome, is a large target opportunity for all-or-none tumor vs. normal discrimination via the Tmod blocker. Patients with germline heterozygosity of A*02 and clonal LOH of A*02 in their tumors (red bar on chromosome on the right) are selected. (**B**) Tmod consists of a T cell modified with 2 receptors: an activator, which can be a CAR or TCR that is directed at antigens expressed on tumor cells (eg, MSLN) and a blocker, which recognizes antigens that are expressed on normal cells but are absent on tumor cells (eg, HLA-A*02).
CAR, chimeric antigen receptor; HLA, human leukocyte antigen; LOH, loss of heterozygosity; MSLN, mesothelin.

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 (**Figure 2**). In vitro cytotoxicity measurements were performed using fluorescence-based imaging and luciferase readouts. In vivo assessments were performed in NOD scid gamma (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 A2B694 T cells or controls. Grafts were measured via caliper.

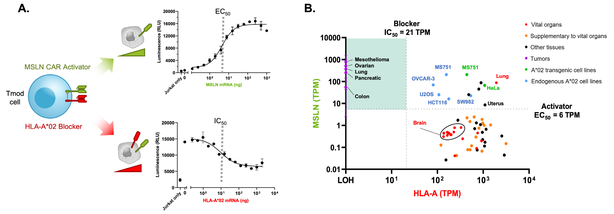
Figure 2. Molecular composition of MSLN Tmod



Molecular composition of MSLN-targeted Tmod constructs. The activator and blocker receptors are co-expressed in a single construct and the encoded fusion protein is cleaved in the cell to generate the activator and blocker. B2M-shRNA encoded in the same vector will ensure downregulation of HLA class I surface expression to mitigate self HLA-A*02 and blocker cis-interaction.
B2M shRNA, β2-microglobulin short-hairpin RNA; EF1a, elongation factor-1; HLA, human leukocyte antigen; LIR, leukocyte immunoglobulin-like receptor; MSLN, mesothelin; scFv, single-chain variable fragment; T2A, thosea asigna virus 2A.

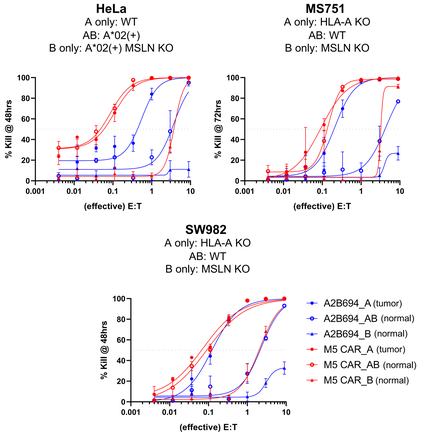
RESULTS

Figure 3. On-target safety and efficacy: MSLN Tmod sensitivity mapped on MSLN and A*02 expression suggests an effective therapeutic



On-target safety and efficacy. (**A**) MSLN mRNA transfected HEK293T and A*02 mRNA transfected HeLa cell targets co-cultured with MSLN CAR or MSLN Tmod expressing Jurkat cells are used to quantify CAR and blocker sensitivities, respectively. (**B**) MSLN Tmod are predicted to kill tumors while protecting normal tissues. Data representation requires standard curves (not shown) that relate surface protein level to RNA-Seq value for cell lines and tissues. Standards are also used to convert CAR and blocker sensitivities. Purple points represent tumor types with HLA-A expression set at 0 TPM to account for selection of A*02(-) tumors by LOH. Tumor data from TCGA database; normal tissue data from the GTEx database; cell line data from DepMap.
CAR, chimeric antigen receptor; DepMap, the Dependency Map portal; EC_{50}, half maximal effective concentration; GTEx, Genotype-Tissue Expression; HLA, human leukocyte antigen; IC_{50}, half maximal inhibitory concentration; MSLN, mesothelin; TCGA, the Cancer Genome Atlas; TPM, transcripts per million.

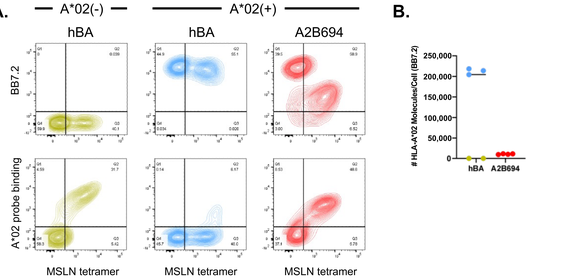
Figure 4. A2B694 T cells demonstrate selective killing of tumor cells in vitro



Cytotoxicity data of A2B694 from a representative HLA-A*02(+) donor against 3 endogenous MSLN(+) cell lines with native or engineered (eg, A*02(+)) expression of HLA-A*02. Killing was measured either 48 or 72 hours after co-culture via luminescence. Clinically active M5 CAR expressing cells are shown for comparison.
CAR, chimeric antigen receptor; E:T, effector to target; HLA, human leukocyte antigen; KO, knock-out; MSLN, mesothelin; WT, wild-type.

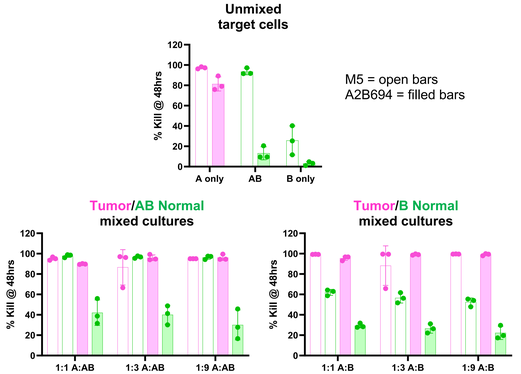
RESULTS (cont.)

Figure 5. A2B694 is an autologous T-cell product: B2M-shRNA downregulates HLA-A*02 surface expression and restores blocker availability in A*02(+) donors



B2M-shRNA in A2B694 T cells downregulates HLA-A*02 surface expression. (**A**) In the absence of B2M-shRNA (ie, hBA = blocker and activator construct without B2M-shRNA) in A*02(+) donors, surface HLA-A*02 expression limits blocker availability due to cis-interaction of the blocker with HLA-A*02. In the presence of B2M-shRNA, HLA-A*02 is downregulated (as shown with anti-HLA-A*02 staining using antibody clone BB7.2), restoring blocker binding to A*02 tetramer. (**B**) Quantification of surface HLA-A*02 molecules. Colors refer to construct and cell type as described in part A. Each symbol represents a different donor (n=2-4).
B2M shRNA, β2-microglobulin short-hairpin RNA; HLA, human leukocyte antigen; MSLN, mesothelin.

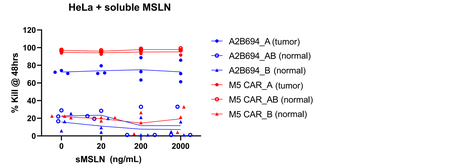
Figure 6. A2B694 T cells selectively kill tumor cells in mixed cultures with normal cells



A2B694 T cells selectively kill tumor cells in mixed culture with AB or B only normal HeLa target cells. Tumor cells are MSLN(+)A*02(-) cells that stably express firefly luciferase. AB normal target cells are MSLN(+)A*02(+) cells and B only normal target cells are MSLN(-)A*02(+) that stably express renilla luciferase. Killing was measured 48 hours after co-culture via luminescence. Each symbol represents a different A*02(+) donor (n=3).
MSLN, mesothelin.

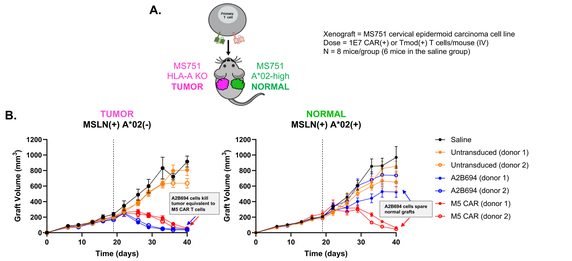
RESULTS (cont.)

Figure 7. Soluble MSLN does not impact A2B694 sensitivity or selectivity



Soluble, full-length mesothelin (sMSLN) does not impact A2B694 sensitivity or selectivity across a range of concentrations. M5 CAR is shown for comparison. Killing was measured 48 hours after co-culture via luminescence. Each symbol represents a different A*02(+) donor (n=3).
CAR, chimeric antigen receptor; MSLN, mesothelin; sMSLN, soluble mesothelin.

Figure 8. A2B694 T cells demonstrate selective killing of tumors in vivo



A2B694 T cells selectively kill tumors in vivo. (**A**) Schematic diagram of the dual-flank tumor and normal MS751 xenograft model. (**B**) Graft sizes assessed via caliper. Left: A2B694 cells kill tumor grafts equivalently to M5 CAR cells. Right: M5 CAR cells kill while A2B694 cells spare normal grafts. Data shown are mean ± SEM (n=8 animals per group).
CAR, chimeric antigen receptor; HLA, human leukocyte antigen; KO, knock-out; MSLN, mesothelin; SEM, standard error of the mean.

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, ovarian, mesothelioma or non-small cell lung cancer will be apheresed for a future A2B694 interventional study (EVEREST-2).

References

1. Hassan R, et al. J Clin Oncol. 2016;34(34):4171-4179.
2. Tanyi JL, et al. Presented at: Cellicon Valley '21: The Future of Cell and Gene Therapies; May 6-7, 2021; virtual symposium.

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