Abstract Number 229

A2B530, an autologous CEA-directed Tmod T-cell therapy with an inhibitory receptor gated by HLA-A*02 to target colorectal, pancreatic, and lung cancer

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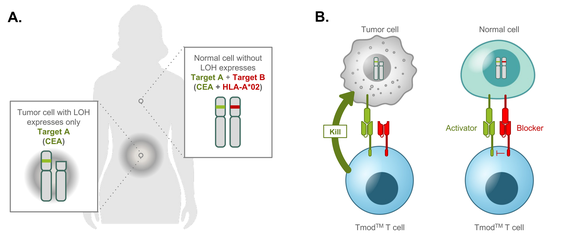
BACKGROUND

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 [1,2]. To overcome this, we have developed Tmod™, a logic-gated T-cell therapy platform (**Figure 1**). Tmod constructs are composed of an activating chimeric antigen receptor (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) [3,4]. A2B530 is a CEA-directed Tmod construct utilizing a leukocyte immunoglobulin-like receptor (LIR) 1–based inhibitory receptor (blocker) targeting human leukocyte antigen A*02 (HLA-A*02).

Figure 1. The Tmod platform utilizes tumor loss of heterozygosity to differentiate tumor from normal cells



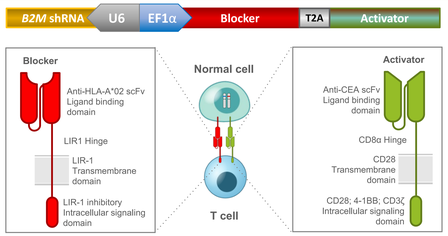
The supreme challenge in oncology: Discrimination between tumor and normal tissue. (**A**) LOH, which affects ≈20% of the tumor genome, is a large target opportunity for all-or-none tumor vs normal discrimination via the Tmod blocker. Patients with germline heterozygosity of A*02 and clonal LOH of A*02 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 typical CAR, which is directed at antigens expressed on tumor cells (eg, CEA) and a blocking receptor, which targets antigens that are expressed on normal cells but are absent on tumor cells (eg, HLA-A*02).

CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen; HLA, human leukocyte antigen; LOH, loss of heterozygosity.

METHODS

To generate CEA Tmod, T cells from HLA-A*02(+) donors were transduced with a single lentivirus construct to express i) the CAR, ii) the blocker, and iii) a short-hairpin RNA targeting B2-macroglobulin (**Figure 2**). Cytotoxicity was measured by culturing CEA(+) target cell line pairs, A*02(-) and A*02(+), expressing either green or red fluorescent protein, with engineered T cells and quantifying live target cells over time. In vivo activity was examined using NOD scid gamma (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 CAR Tmod cells or control T cells.

Figure 2. Molecular composition of CEA Tmod

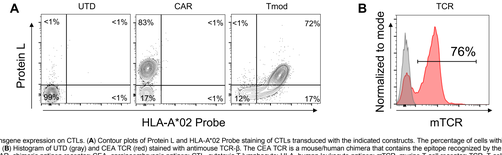


Molecular composition of CEA-targeted Tmod constructs. The activator and blocker receptors are co-expressed in a single construct and the encoded fusion protein is cleaved in the cell to generate the activator and blocker. B2M-shRNA encoded in the same vector will ensure downregulation of all class I surface expression to mitigate self HLA-A*02 and blocker cis-interaction.

B2M shRNA, β2-microglobulin short-hairpin RNA; CEA, carcinoembryonic antigen 5; EF1α, elongation factor-1; HLA, human leukocyte antigen; LIR, leukocyte immunoglobulin-like receptor; scFv, single-chain variable fragment; T2A, thosea asigna virus 2A.

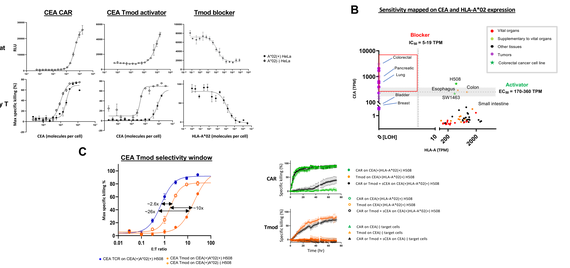
RESULTS

Figure 3. Tmod expression in primary human T cells by flow cytometry



Representative transgene expression on CTLs. (**A**) Contour plots of Protein L and HLA-A*02 Probe staining of CTLs transduced with the indicated constructs. The percentage of cells within each quadrant is shown. (**B**) Histogram of UTD (gray) and CEA TCR (red) stained with antimouse TCR-β. The CEA TCR is a mouse/human chimera that contains the epitope recognized by the antimouse TCR-β antibody. CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen; CTL, cytotoxic T-lymphocyte; HLA, human leukocyte antigen; mTCR, murine T-cell receptor; TCR, T-cell receptor; UTD, untransduced.

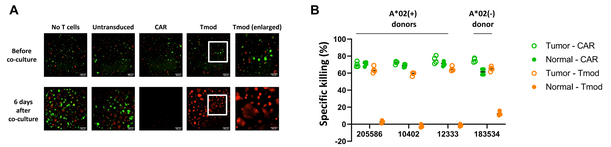
Figure 4. CEA Tmod cells exhibit sensitivity and on-target, off-tumor selectivity in vitro



(**A**) mRNA titration was used to test the sensitivities of CEA activator and HLA-A*02 blocker in CEA Tmod and CAR constructs. Cell surface expression of CEA and HLA-A*02 was determined by mRNA titration in HeLa cells. CEA Tmod-activator and -blocker sensitivities were measured as a function of molecules per cell in HeLa cells using Jurkat or primary T cells. For EC_{50} values, CEA mRNA was titrated in CEA(−)A*02(+) HeLa (filled circles) or CEA(−)HLA-A*02(−) HeLa cells (open circles). For IC_{50} values, HLA-A*02 mRNA was titrated in CEA(+)HLA-A*02(−) HeLa cells (**B**) CEA and HLA-A standard curves were generated using CEA surface expression data generated in-house, from reference 2 (for CEA) and from mRNA data from The Genotype-Tissue Expression (GTEx) database. EC_{50} and IC_{50} values were determined as in (**A**). The gray bars represent the range of EC_{50} or IC_{50} values determined in Jurkat and primary T cells. Purple points represent tumor types with HLA-A expression set at 0 TPM to account for selection of HLA-A*02(−) tumors by LOH. Tumor data are from TCGA database, normal tissue data are from the GTEx database. (**C, Left**) An example of E:T ratio comparison in primary T-cell cytotoxicity assays with one donor is shown. (**C, Right**) The effect of sCEA (10 μg/mL) on cytotoxicity in HS08 cells was measured. Data from one representative HLA-A*02(+) donor are shown here. Each data point is an average of five technical replicates. Killing was evaluated at an E:T ratio of 3:1. Data are presented as means ± SD throughout the figure.

CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen; EC_{50}, half maximal effective concentration; E:T, effector to target; GTEx, Genotype-Tissue Expression; HLA, human leukocyte antigen; IC_{50}, half maximal inhibitory concentration; LOH, loss of heterozygosity; RLU, relative luminescence unit; sCEA, soluble CEA; SD, standard deviation; TCGA, the Cancer Genome Atlas; TCR, T-cell receptor; TPM, transcripts per million.

Figure 5. CEA Tmod cells selectively kill tumor cells while sparing "normal" cells in mixed cultures

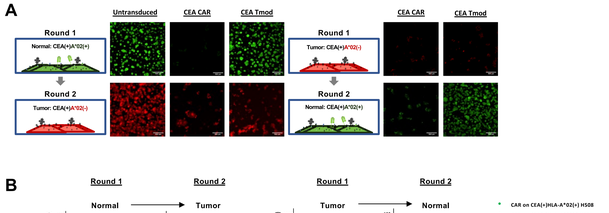


(**A**) Selective cytotoxicity of CEA Tmod was measured in 1:1 mixed HLA-A*02(−) and HLA-A*02(+) cell cultures. HS08 CEA(+)A*02(−) cells stably express GFP. HS08 CEA(+)A*02(+) cells stably express RFP. The scale bars are 500 μm. E:T: 3:1. Areas in the white boxes are enlarged on the right. The same color scales were used for images in each color channel. (**B**) Quantification of cytotoxicity imaging data in (**A**) is shown at 24 hours of coculture. Averages from four replicates are shown. Labels on the x-axis represent donor identification numbers.

CAR, chimeric antigen receptor; E:T, effector to target; GFP, green fluorescent protein; RFP, red fluorescent protein.

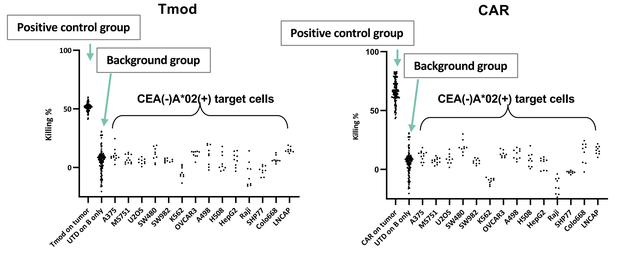
RESULTS (cont.)

Figure 6. CEA Tmod cells exhibit bidirectional control between the activated and blocked states



(**A**) CEA Tmod cells were cocultured sequentially with either A*02(+) or A*02(−) target cells at an E:T ratio of 3:1 as shown. For cytotoxicity assays, T cells were transduced, enriched for blocker antigen, and transferred from one type of target cell to the next. Both A*02(+) and A*02(−) cells were labeled with GFP, but red color was used to visualize A*02(−) cells and green color was used to visualize A*02(+) cells. The same color scale was used for all images. (**B**) A summary of imaging data in (**A**) is shown. Averages from four replicates are presented. Horizontal arrows indicate the transfers between target cell types. CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen; E:T, effector to target; GFP, green fluorescent protein.

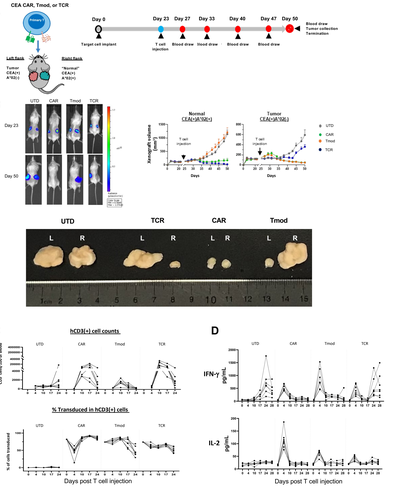
Figure 7. CEA Tmod cells exhibit minimal off-target effects in vitro



A panel of cell lines was selected to cover greater than 90% of protein-coding genes expressed at least 0.5 TPM in the GTEx database (gene expression in cell lines is derived from the Cancer Cell Line Encyclopedia database). Cytotoxicity was measured for CEA Tmod off-target selectivity using T cells from three A*02(+) donors against the indicated cell lines at an E:T ratio of 3:1. One additional A*02(−) donor was tested on A375 and MSTO1 cells. The time at which Tmod cells reached IK50 was chosen to compare percentage of killing by the CEA CAR, CEA Tmod, and UTD T cells on CEA–target cells. Positive control: CAR T or Tmod cells on CEA(+)A*02(+) target cells. Background: UTD on CEA(−)A*02(+) target cells. CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen; E:T, effector to target; GTEx, Genotype-Tissue Expression; NSG, NOD scid gamma; IK50, 50% killing on tumor cells; TPM, transcripts per million; UTD, untransduced.

RESULTS (cont.)

Figure 8. CEA Tmod cells selectively target xenografts that lack HLA-A*02 in NSG mice



(**A**) Xenograft experimental design and timeline. NSG mice were inoculated with HLA-A*02(−) tumors on the left flank and HLA-A*02(+) tumors on the right flank. The tumors were derived from the HS08 colon cancer cell line that expresses firefly luciferase. UTD T cells, CEA Tmod cells, CAR T cells, or TCR T cells were injected at 7 × 10^6 per mouse through tail vein on day 23, when tumors were 100 to 200 mm^3. Xenograft volumes were measured twice weekly before randomization and T-cell injection and thrice weekly thereafter. Blood was also drawn after cell infusion. (**B**) The average xenograft volume was measured by caliper and BLI change. Left, representative BLI images are shown. BLI images were taken before T-cell injection (day 23) and every 7 days afterward until day 50. Middle, longitudinal caliper measurements. Data are presented as means ± SEM (mm^2 mice per group for volume). One-direction error bars are used for some curves to avoid crowding. Right, representative images of tumor and "normal" grafts excised at the end of the study (day 50). (**C**) Top, quantification of human T cells in mouse blood were detected with a hCD3 mAb. Bottom, the percentage of CEA-specific receptor-expressing T cells of total hCD3(+) T cells was determined using a protein-L or an antimouse TCR antibody ($n=7$ mice per group). (**D**) Longitudinal serum IFN-γ and IL-2 measurements were examined starting on day 0 (day of T-cell injection) and at the indicated time points through day 28 ($n=7$ mice per group).

BLI, bioluminescence intensity; CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen; hCD3, human CD3; IFN, interferon; IL, interleukin; NSG, NOD scid gamma; TCR, T-cell receptor; UTD, untransduced.

CONCLUSION

- A2B530 is an autologous CEA Tmod cell product that exploits common loss of heterozygosity (LOH) at the HLA locus in cancer cells, enabling these engineered T cells to distinguish 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

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