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EVEREST-1: A seamless phase 1/2 study of CEA-directed logic-gated Tmod™ CAR T-cell therapy (A2B530) in adults with solid tumors associated with CEA expression also exhibiting HLA loss of heterozygosity (LOH)

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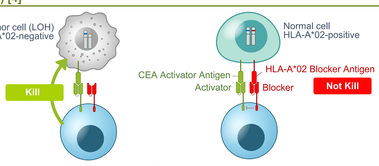
BACKGROUND AND STUDY OBJECTIVES

- Chimeric antigen receptor (CAR) T-cell therapy has demonstrated clinical efficacy in hematologic malignancies [1]; however, implementation of these therapies in solid tumors has been challenging due to a lack of tumor-specific targets that discriminate cancer from normal cells
  - Previous studies using carcinoembryonic antigen 5 (CEA) T-cell receptors and T-cell engagers have resulted in dose-limiting, on-target, off-tumor toxicities [2,3]
- Tmod CAR T-cell therapy addresses challenges of on-target, off-tumor toxicity by combining a CAR-activating receptor with a blocking receptor to discriminate tumor from normal cells (**Figures 1** and **2**) [4,5]
- A2B530 is a CEA-directed Tmod CAR T-cell construct utilizing a leukocyte immunoglobulin-like receptor-1-based inhibitory receptor (blocker) targeting human leukocyte antigen (HLA)-A*02 (**Figure 2**)
- The activator receptor recognizes CEA on the surface of both tumor and normal cells; CEA is normally widely expressed in epithelial cells, particularly of the gastrointestinal (GI) system and can be upregulated in GI and lung tumors (**Figure 3**)
- The blocker receptor recognizes an HLA-A*02 allele that is present in normal cells and often lost in tumor cells [6]
  - For patients who are germline HLA-A*02 heterozygous for the allele, loss of the allele in tumor cells is called LOH
  - LOH for HLA-A*02 is observed in solid tumor malignancies and can be detected using the Tempus next-generation sequencing (NGS) testing
- Tmod cells are logic-gated: the blocker component prevents CAR-mediated killing of normal cells; whereas, in tumor cells with LOH, the blocker is no longer engaged, allowing the CAR to activate tumor cell killing (**Table 1**)
- EVEREST-1 (NCT05736731) is a seamless, phase 1/2, open-label, nonrandomized study to evaluate the safety and efficacy of A2B530, a logic-gated CEA-targeting Tmod CAR T-cell therapy, in adult patients

STUDY RATIONALE

Figure 1. Logic-gated CAR T-cell Therapy With the Goal to Reduce Toxicity: CEA (Activator) and HLA-A*02 (Blocker) [4]

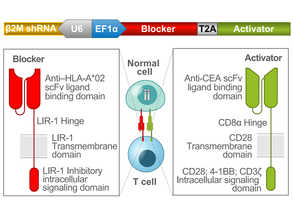


CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen 5; HLA, human leukocyte antigen; LOH, loss of heterozygosity.

Figure 2. The Structure of Tmod CAR T Cells Expressing a CEA-Targeted Activator and an HLA-A*02-Targeted Blocker [7]

U6 promoter-driven shRNA
This component reduces human β2M expression resulting in reduced cell surface expression of HLA-A*02 in the transduced autologous T cells and alleviates cis-binding between blocker and HLA-A*02.

CAR blocker
Derived from LIR-1, a receptor expressed on NK cells, monocytes, dendritic cells, and some lymphocytes that, upon binding to MHC class I molecules, transmits inhibitory signals via its immunoreceptor tyrosine-based inhibitory motifs, resulting in the downregulation of immune function.

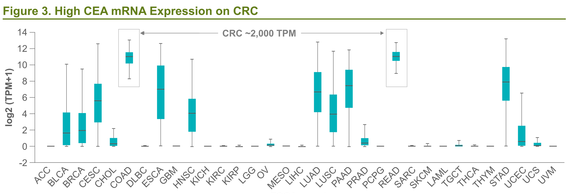


Replicant incompetent single lentivirus transgene
The blocker and activator receptors are co-expressed in a single construct containing a cleavable T2A linker, which allows 2 separate proteins to be expressed from a single mRNA. The blocker and activator module in the vector (ie, 5′ Blocker → Activator) will minimize the chance that the activator is expressed without the blocker.

CAR activator
3rd-generation CAR with both signal 1 (CD3ζ) and signal 2 activation domains (CD28 & 4-1BB). These signaling domains trigger increased T-cell activation that results in CAR T-cell proliferation, direct killing of target cells, and secretion of cytokines and chemokines to recruit and activate additional immune cells.

β2M shRNA, beta-2-microglobulin short-hairpin RNA; CAR, chimeric antigen receptor; CD, cluster of differentiation; CEA, carcinoembryonic antigen 5; EF1α, elongation factor-1α; HLA, human leukocyte antigen; LIR, leukocyte immunoglobulin-like receptor; MHC, major histocompatibility complex; scFv, single-chain variable fragment; T2A, thosea asigna virus 2A.

Figure 3. High CEA mRNA Expression on CRC



ACC, adrenocortical carcinoma; BLCA, bladder cancer; BRCA, breast cancer; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; CRC, colorectal cancer; DLBC, diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian cancer; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TCGA, The Cancer Genome Atlas; TGCT, testicular germ cell tumor; THCA, thyroid carcinoma; THYM, thymoma; TPM, transcripts per million; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

Table 1. Frequency of HLA-A LOH in Advanced Tumors [8,9][a]

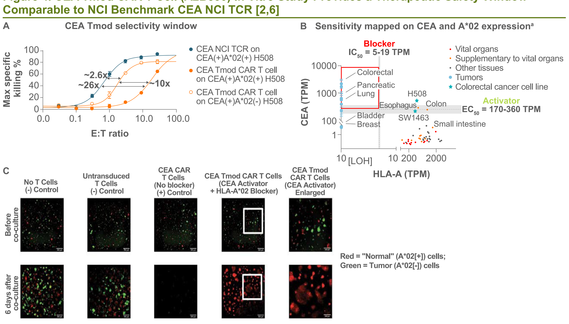
	Tempus HLA-A LOH advanced disease real-world	TCGA HLA-A LOH primary tumors
Average, % (n)	16.3 (10,867)	12.6 (10,844)
Colorectal cancer, % (n)	15.6 (1854)	9.6 (615)
Gastroesophageal cancer, % (n)	20.8 (506)	16.2 (625)
Pancreatic cancer, % (n)	19.6 (675)	33.1 (184)
NSCLC, % (n)	23.1 (1915)	25.3 (501)

[a]Tempus data contain more advanced disease and TCGA data have more primary tumors.
HLA, human leukocyte antigen; LOH, loss of heterozygosity; NSCLC, non-small cell lung cancer; TCGA, The Cancer Genome Atlas.

Nonclinical Data

- In vitro and in vivo nonclinical studies of A2B530 demonstrated improved selectivity and a therapeutic safety window with comparable efficacy to National Cancer Institute (NCI) benchmark CEA T-cell receptor T Cell (**Figures 4** and **5**)
- Tmod provided selectivity at varying effector-to-target ratios with "normal" CEA(+)A*02(+) cells and tumor CEA(+)A*02(-) colon cancer cell lines (**Figure 4A**)
- Mixed A*02(+) and A*02(-) cell cultures show the ability of Tmod to discriminate between "normal" (A*02[+]) and tumor (A*02[-]) cells (**Figure 4B**)
- CEA and HLA-A*02 standard plots were generated using CEA expression data from mRNA data (**Figure 4C**)
  - CEA Tmod Jurkat or T-cell effective concentration and inhibitory concentration were graphed with the tumor and normal expression values for the CEA and A*02 antigens, along with multiple cell lines

Figure 4. CEA Tmod CAR T Cell (A2B530) In Vitro Study Provides a Therapeutic Safety Window Comparable to NCI Benchmark CEA NCI TCR [2,6]



[a]Red box used to represent where cell killing occurs.

CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen 5; EC_{50}, half maximal effective concentration; E:T, effector-to-target; HLA, human leukocyte antigen; IC_{50}, half maximal inhibitory concentration; LOH, loss of heterozygosity; NCI, National Cancer Institute; TCR, T-cell receptor; TPM, total particulate matter.

SITE LIST

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STUDY RATIONALE (CONTINUED)

Figure 5. CEA Tmod CAR T Cell (A2B530) In Vivo Study Demonstrates Potency Comparable to NCI Benchmark CEA TCR [2,6]

CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen 5; HLA, human leukocyte antigen; NCI, National Cancer Institute; TCR, T-cell receptor.

- In vivo studies show that Tmod maintains selectivity
- Tumor (HLA-A*02[-]) and "normal" (HLA-A*02[+]) cells were implanted subcutaneously in NOD scid gamma mice
- CAR T cells or Tmod CAR T cells were administered via tail veins when tumor reached 100-150mm³
- Approximately 2 weeks after cell infusion, A2B530 treated mice experienced selective regression of tumor grafts, while "normal" tumor grafts continued to grow. Mice treated with CEA-targeted CAR T cells experienced regressions of both tumor and "normal" tumor grafts (**Figure 5**)

STUDY DESIGN

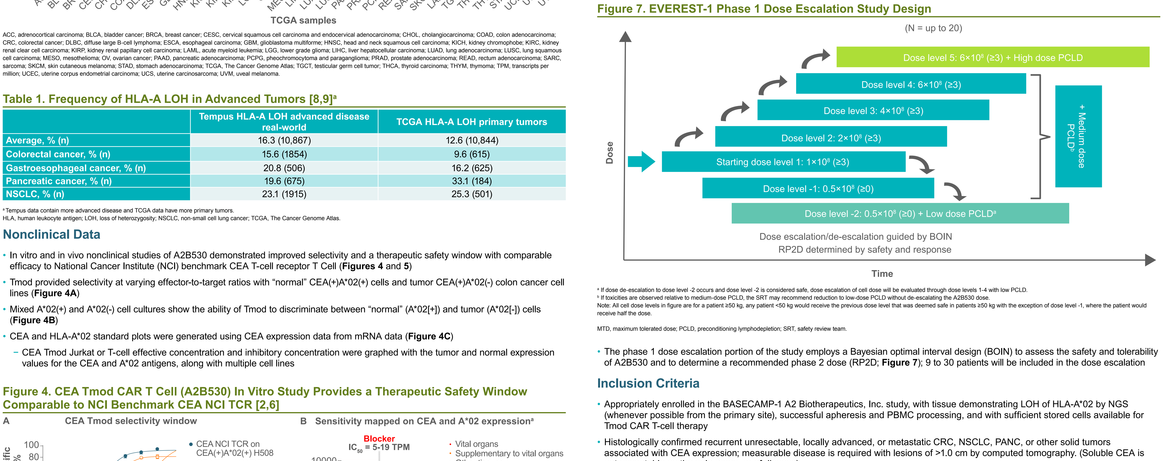
Figure 6. Study Schema: BASECAMP-1 to EVEREST-1

[a] May occur at any point in disease course. [b] For patients with CRC or PANC, CEA assessment will be performed retrospectively, and the result is not needed for enrollment.

CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen 5; CRC, colorectal cancer; HLA, human leukocyte antigen; LOH, loss of heterozygosity; NSCLC, non-small cell lung cancer; PANC, pancreatic cancer; PCLD, preconditioning lymphodepletion.

- EVEREST-1 (NCT05736731) is a first-in-human, phase 1/2, multicenter, open-label, nonrandomized study to evaluate the safety and efficacy of a single-dose of A2B530 Tmod CAR T cells in adult patients with metastatic colorectal cancer (CRC), non-small cell lung cancer (NSCLC), pancreatic cancer (PANC), or other solid tumors associated with CEA expression
- Patients are enrolled to EVEREST-1 through BASECAMP-1 (NCT04981119), a master prescreening study that identifies patients with HLA LOH at any time in the course of their disease
  - BASECAMP-1 eligible patients undergo leukapheresis and, when clinically appropriate, their banked T cells are used to manufacture A2B530 for the EVEREST-1 study (**Figure 6**)

Figure 7. EVEREST-1 Phase 1 Dose Escalation Study Design



[a] If dose de-escalation to dose level -2 occurs and dose level -2 is considered safe, dose escalation of cell dose will be evaluated through dose levels 1-4 with low PCLD.
[b] If toxicities are observed relative to medium-dose PCLD, the SRT may recommend reduction to low-dose PCLD without de-escalating the A2B530 dose.
Note: All cell dose levels in figure are for a patient ≥50 kg, any patient <50 kg would receive the previous dose level that was deemed safe in patients ≥50 kg with the exception of dose level -1, where the patient would receive half the dose.

MTD, maximum tolerated dose; PCLD, preconditioning lymphodepletion; SRT, safety review team.

- The phase 1 dose escalation portion of the study employs a Bayesian optimal interval design (BOIN) to assess the safety and tolerability of A2B530 and to determine a recommended phase 2 dose (RP2D; **Figure 7**); 9 to 30 patients will be included in the dose escalation

Inclusion Criteria

- Appropriately enrolled in the BASECAMP-1 A2 Biotherapeutics, Inc. study, with tissue demonstrating LOH of HLA-A*02 by NGS (whenever possible from the primary site), successful apheresis and PBMC processing, and with sufficient stored cells available for Tmod CAR T-cell therapy
- Histologically confirmed recurrent unresectable, locally advanced, or metastatic CRC, NSCLC, PANC, or other solid tumors associated with CEA expression; measurable disease is required with lesions of >1.0 cm by computed tomography. (Soluble CEA is not acceptable as the sole measure of disease).
- Received previous required therapy for the appropriate solid tumor disease as described in the protocol
- Has adequate organ function as described in the protocol
- Eastern Cooperative Oncology Group performance status 0 to 1
- Life expectancy of ≥3 months
- Willing to comply with study schedule of assessments including long-term safety follow-up

Figure 8. EVEREST-1 Study Objectives and Endpoints

Objectives
- **Phase 1:** Determine the safety and the optimal dose of A2B530 (after PCLD) in participants with solid tumor disease
- **Phase 2:** Determine the further safety and efficacy of A2B530

Primary Endpoints
- **Phase 1:** Rate of adverse events and dose-limiting toxicities by dose levels; recommended phase 2 dose
- **Phase 2:** Overall response rate

Secondary Endpoints
- Persistence of A2B530
- Cytokine analysis

PCLD, preconditioning lymphodepletion.

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