Abstract Number 634

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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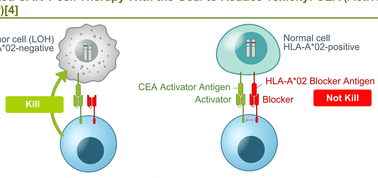
BACKGROUND AND STUDY OBJECTIVES

- 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 construct utilizing a leukocyte immunoglobulin-like receptor-1-based inhibitory receptor (blocker) targeting 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 a human leukocyte antigen (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]

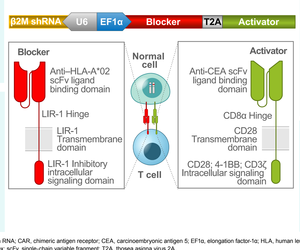


CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen 5; HLA, human leukocyte antigen; LOH, loss of heterozygosity.

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.

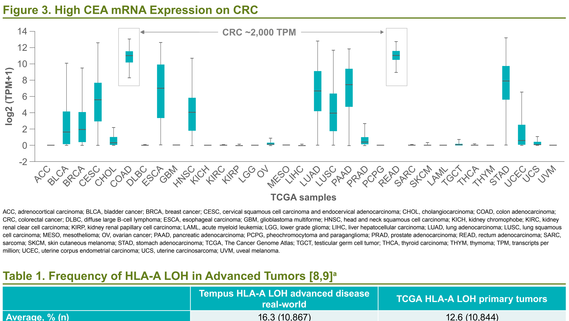


Replicant incompetent single lentivirus transgene
The blocker and activator receptors are co-expressed in a single construct containing a cleavable T2A linker, which allows 2 separate proteins to be expressed from a single mRNA. The blocker and activator module in the vector (ie, 5′ Blocker → Activator) 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; 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

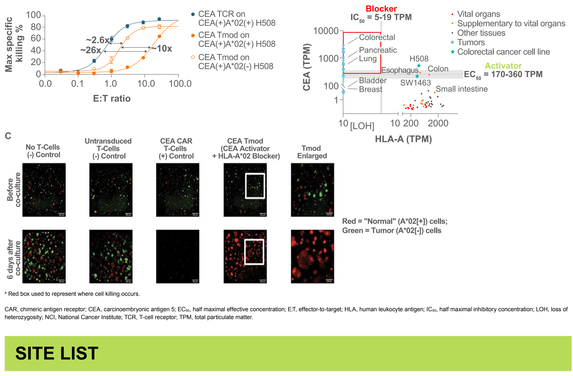
	Tempus HLA-A LOH advanced disease real-world	TCGA HLA-A LOH primary tumors
Average, % (n)	16.3 (10,867)	12.6 (10,844)
Colorectal cancer, % (n)	15.6 (1,854)	9.6 (615)
Gastroesophageal cancer, % (n)	20.8 (506)	16.2 (625)
Pancreatic cancer, % (n)	19.6 (675)	33.1 (184)
NSCLC, % (n)	23.1 (1,915)	25.3 (501)

ªTempus 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 selectivity, efficacy, and a therapeutic safety window comparable to National Cancer Institute (NCI) benchmark CEA T-cell receptor T-cell (TCR-T) (**Figures 4** and **5**)
- Tmod provided selectivity at varying effector-to-target (E:T) 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 (A2B530) In Vitro Study Provides a Therapeutic Safety Window Comparable to NCI Benchmark CEA TCR-T [2,6]

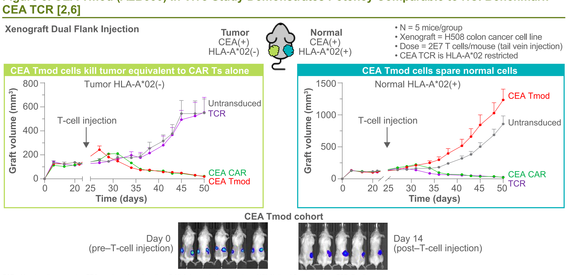


Red = "Normal" (A*02[+]) cells; Green = Tumor (A*02[-]) cells

* 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.

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

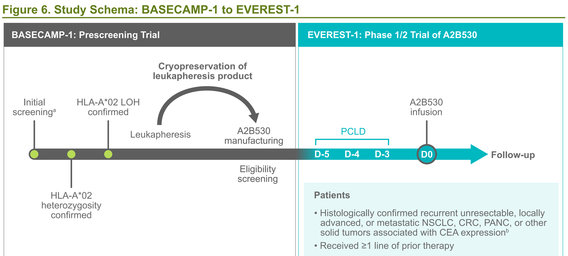


CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen 5; HLA, human leukocyte antigen; NCI, National Cancer Institute; TCR, T-cell receptor.

- 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

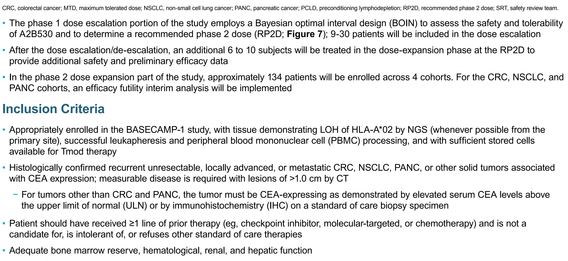
Figure 6. Study Schema: BASECAMP-1 to EVEREST-1



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; HLA, human leukocyte antigen; LOH, loss of heterozygosity; 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 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 are used to manufacture A2B530 for the EVEREST-1 study (**Figure 6**)

Figure 7. EVEREST-1 Phase 1 Dose Escalation/Expansion and Phase 2 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 1-5 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 subject ≥50 kg, any subject <50 kg would receive the previous dose level that was deemed safe in subjects ≥50 kg with the exception of dose level -1, where the subject would receive half the dose.

CRC, colorectal cancer; MTD, maximum tolerated dose; NSCLC, non-small cell lung cancer; PANC, pancreatic cancer; PCLD, preconditioning lymphodepletion; RP2D, recommended phase 2 dose; 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**); 9-30 patients will be included in the dose escalation
- After the dose escalation/de-escalation, an additional 6 to 10 subjects will be treated in the dose-expansion phase at the RP2D to provide additional safety and preliminary efficacy data
- In the phase 2 dose expansion part of the study, approximately 134 patients will be enrolled across 4 cohorts. For the CRC, NSCLC, and PANC cohorts, an efficacy futility interim analysis will be implemented

Inclusion Criteria

- Appropriately enrolled in the BASECAMP-1 study, with tissue demonstrating LOH of HLA-A*02 by NGS (whenever possible from the primary site), successful leukapheresis and peripheral blood mononuclear cell (PBMC) processing, and with sufficient stored cells available for Tmod 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 CT
  - For tumors other than CRC and PANC, the tumor must be CEA-expressing as demonstrated by elevated serum CEA levels above the upper limit of normal (ULN) or by immunohistochemistry (IHC) on a standard of care biopsy specimen
- Patient should have received ≥1 line of prior therapy (eg, checkpoint inhibitor, molecular-targeted, or chemotherapy) and is not a candidate for, is intolerant of, or refuses other standard of care therapies
- Adequate bone marrow reserve, hematological, renal, and hepatic function
- Eastern Cooperative Oncology Group (ECOG) performance status 0-1
- Patients with previously treated stable brain metastases may participate upon sponsor agreement

Figure 8. EVEREST-1 Study Objectives and Endpoints

Primary

Phase 1

Objectives
- To evaluate the safety and tolerability of A2B530
- To determine RP2D of A2B530

Endpoints
- Incidence of DLTs and AEs by dose level

Phase 2

Objectives
- To evaluate the efficacy of A2B530

Endpoints
- ORR including confirmed CR or PR per RECIST v1.1 by ICR

Secondary

Phase 1 and 2

Objectives
- To evaluate the efficacy of A2B530 across all cohorts and within disease cohorts
- To evaluate the safety of A2B530 for each tumor type
- To evaluate the manufacturing feasibility of A2B530
- To evaluate biomarker data to correlate with clinical outcomes and guide RP2D selection (phase 1 only)
- To evaluate systemic cytokine release after A2B530 administration (phase 1 only)
- To evaluate biomarkers such as PK, PD, and immunogenicity (phase 2 only)
- To evaluate RCL detected in blood samples

Endpoints
- ORR, including confirmed CR or PR per RECIST v1.1 assessed by investigator
- For Phase 1 only: ORR by ICR, if independent review is deemed necessary by A2 Bio
- The following endpoints based on the investigator and ICR assessments:
  - DuR: PR or better lasting ≥6 months, ≥12 months, and ≥18 months
  - DOR: the time from date of initial confirmed response to the date of the first documented progressive disease or death, whichever occurs earlier
  - DCR: SD or better
  - BOR: the best response per RECIST v1.1
  - PFS: time from dosing of A2B530 to the date of disease progression or death from any cause, whichever occurs earlier
- OS: time from dosing of A2B530 to the date of death from any cause
- TEAEs (All AEs, Grade ≥3 AEs, SAEs, fatal AEs)
- TEAEs (All AEs, Grade ≥3 AEs, SAEs, fatal AEs) that are deemed related to A2B530
- DILI
- Abnormal laboratory results
- Proportion of participants with a successful manufacture of A2B530
- PK: levels and persistence of CAR T cells in blood samples
- PD: levels of cytokines in serum (eg, IL-6, TNF-α, IFN-γ, IL-15)
- Immunogenicity: incidence of anti-A2B530 antibodies
- Incidence of RCL detected in blood samples

BOR, best overall response; CR, complete response; DCR, disease control rate; DILI, drug-induced liver injury; DLT, dose-limiting toxicity; DOR, duration of response; DuR, durability of response; ICR, independent central review; IFN-γ, interferon gamma; ORR, overall response rate; OS, overall survival; PBMC, peripheral blood mononuclear cell; PCLD, preconditioning lymphodepletion; PFS, progression-free survival; PK/PD, pharmacokinetics/pharmacodynamics; PR, partial response; RECIST, response evaluation criteria in solid tumors; RCL, replication competent lentivirus; TEAE, treatment-emergent adverse event; TNF-α, tumor necrosis factor alpha.

SITE LIST

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Acknowledgements

The authors would like to thank: Alexander Kamb, PhD, Founder and Chief Scientific Officer of A2 Bio; Agnes E. Hamburger, PhD, Vice President of Drug Discovery at A2 Bio; Mark L. Sandberg, PhD, Scientific Director of Therapeutic Technology at A2 Bio; Sanam Shafaattalab, PhD, Scientist at A2 Bio; Wendy Langeberg, PhD, MPH, Clinical Development Associate Director at A2 Bio.

Medical writing support was provided by Bio Connections, LLC and funded by A2 Bio.

This study was supported by A2 Bio.

Presented at the Society for Immunotherapy of Cancer's (SITC) 38th Annual Meeting, November 1-5, 2023