Abstract Number 491

BASECAMP-1: An observational study to identify relapsed solid tumor patients with human leukocyte antigen (HLA) loss of heterozygosity (LOH) and leukapheresis for future CAR T-cell therapy

Julian R. Molina[1], William Y. Go[2], Scott Kopetz[3], Diane M. Simeone[4], Sandip P. Patel[5], Yi Lin[1], Kirstin B. Liechty[2], Michelle Fan-Port[2], Jason Perera[6], Armen Mardiros[2], Karl Beutner[6], Ariane Lozac'hmeur[6], Eric W. Ng[2], David G. Maloney[7], and J. Randolph Hecht[8]

[1]Mayo Clinic, Rochester, MN, USA; [2]A2 Biotherapeutics, Inc., Agoura Hills, CA USA; [3]The University of Texas MD Anderson Cancer Center, Houston, TX, USA; [4]New York University Langone Health, New York, NY, USA; [5]University of California San Diego, San Diego, CA, USA; [6]Tempus, Chicago, IL, USA; [7]Fred Hutchinson Cancer Research Center, Seattle, WA, USA; [8]University of California Los Angeles, Los Angeles, CA, USA

BACKGROUND AND BASECAMP-1 STUDY OBJECTIVES

- Solid tumors comprise >90% of cancers. Metastatic colorectal cancer (CRC), non-small cell lung cancer (NSCLC), and pancreatic cancer (PANC) are among the leading causes of cancer-related mortality[1]
- Chimeric antigen receptor (CAR) T-cell therapy has demonstrated clinical outcomes in hematologic malignancies.[2,3] However, translating engineered T-cell therapies to solid tumors proves difficult due to a lack of tumor-specific targets that discriminate cancer cells from normal cells. In previous studies, the use of a carcinoembryonic antigen (CEA) T-cell receptors and mesothelin (MSLN) CARs both resulted in dose-limiting on-target, off-tumor toxicities[4,5]
- Tmod™ CAR T-cell therapy addresses these challenges by leveraging dual receptors to create a robust AND-NOT signal integrator capable of killing tumor cells, while leaving healthy cells intact.[6] Tmod platform technology is a versatile system that may be applied to T cells and natural killer cells in autologous and allogeneic settings
- Human leukocyte antigen loss of heterozygosity (HLA LOH) offers a definitive tumor versus normal discriminator target for CAR T-cell therapy.[6,7] The 2 receptors of the Tmod CAR T-cell platform comprise an activator that recognizes an antigen present on the surface of normal and tumor cells and a blocker that recognizes a second surface antigen from an HLA allele lost only in tumor cells
- HLA LOH has been observed in ~13% across all solid tumors and up to 33% of primary PANCs.[8] New technologies have shown higher HLA LOH rates; however, it is unclear whether patients with HLA LOH in their primary tumor tissues are at higher risk for recurrence
- BASECAMP-1 is an observational study with key objectives: 1) To determine and identify patients with somatic HLA LOH eligible for Tmod CAR T-cell therapy, and 2) Subsequent leukapheresis and manufacturing feasibility for future Tmod CAR T-cell trials

METHODS

- BASECAMP-1 (NCT04981119)
  - Eligibility criteria: Pathologically confirmed solid tumors, eg, CRC, NSCLC, PANC, gastric and esophageal cancer, ovarian cancer, mesothelioma, small cell lung cancer, breast cancer, or head and neck cancer that are metastatic, unresectable locally advanced, or in the Investigator's opinion, the patient is high risk for incurable relapse within 2 years
  - BASECAMP-1 is a noninterventional, observational study to evaluate patients with solid tumors with a high risk of relapse for incurable disease. No interventional therapy will be administered on this study. Participants will be screened for HLA type, and based on results, participants will have archived tumor tissue tested by next-generation sequencing (NGS) and be followed for up to 2 years. Based on the tumor NGS results, participants will be leukapheresed for peripheral blood mononuclear cell collection to store their T cells for a future interventional study upon relapse

STUDY RATIONALE

- BASECAMP-1 will include solid tumor patients with a high unmet medical need (**Table 1**). Eligible patients with CRC, NSCLC and PANC with HLA-A*02 LOH will be leukapheresed for future EVEREST interventional studies
- The Tmod platform creates a therapeutic window for greater efficacy and safety by providing new access to clinically validated targets (**Figure 1A**)
- Tmod CAR T-cell therapy addresses the challenge of discriminating between tumor and normal cells by leveraging dual receptors to create a robust AND-NOT signal integrator capable of killing tumor cells, while leaving healthy cells intact (**Figure 1B**)
- Tmod CAR T-cells detect a normal cell presenting the blocker and activator antigen; Tmod CAR T-cells remain quiescent
- However, when Tmod CAR T-cells detect a tumor cell which lacks the blocker antigen and presents an activator antigen, Tmod CAR T-cells activate cytolytic activity to kill the tumor cell

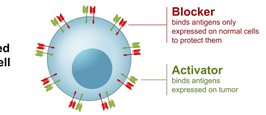
Table 1. BASECAMP-1 Will Enable EVEREST Studies to Address Recurrent and Metastatic Colorectal, Lung, and Pancreatic Cancer With Future Tmod CAR T-Cell Therapy

	High Unmet Medical Need	
	Metastatic Cancer	5-Year Survival*
Low Bar for Efficacy	Colorectal	~14%
	Lung	~7%
	Pancreatic	~3%

BASECAMP-1 Will Enable EVEREST Studies: BASECAMP-1 Observational study → EVEREST Screening → EVEREST Interventional studies

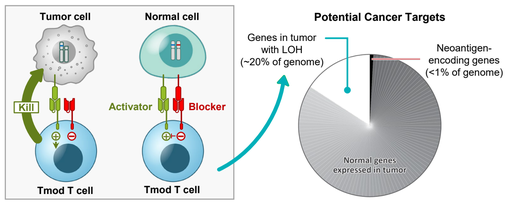
Adapted from The American Cancer Society,[1] Tas F, et al.,[9] Hamers P, et al.,[10] and Peng, et al.[11]

Figure 1A. Tmod CAR T-Cell Therapy



STUDY RATIONALE (cont.)

Figure 1B. Tmod CAR T-Cells Can Discriminate Between Tumor and Normal Cells

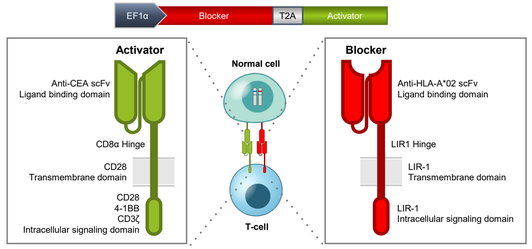


Independent validation of the HLA LOH approach[7]

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

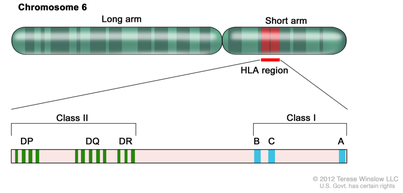
- At the molecular level, the A2B530 CEA CAR Tmod construct consists of a single lentiviral vector that encodes a cleavable fusion protein (**Figure 2**)
- The CAR activator contains a single-chain variable fragment (scFv) and a third-generation intracellular domain
  - Additional results on both the CEA and MSLN Tmod CAR T-cells are reported in Hamburger et al, SITC Abstract #122
- The blocker domain is derived from leukocyte immunoglobulin-like receptor, which is a class I MHC receptor related to natural killer inhibitory receptors
- HLA antigens are ideal blocker targets for a Tmod T-cell therapy that protects normal cells (**Figure 3**)
- Approximately 13% of all cancer patients have tumors with permanent clonal heterozygous loss of HLA, enabling access to different tumor types (up to 33% in PANCs)
- HLA-A*02 is selected as the first blocker because it is the most prevalent allele in the US population

Figure 2. A2B530 CEA CAR Tmod Single Vector Construct



CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen; HLA, human leukocyte antigen; LIR, leukocyte immunoglobulin-like receptor; MHC, major histocompatibility complex; scFv, single-chain variable fragment.

Figure 3. HLA Complex



- HLA-A LOH ranges from 10%-33% in primary solid tumors with significant unmet medical need (**Table 2**)
- Resection of the primary tumor is sent to Tempus as a standard-of-care diagnostic, which is used to identify patients with clonal loss of HLA-A*02
- Matched normal blood samples are compared with primary tumor samples to determine loss of heterozygosity based on exons 2 and 3 of HLA-A[12] (**Figure 4**)

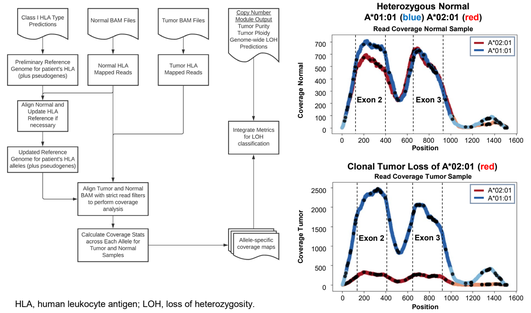
STUDY RATIONALE (cont.)

Table 2. HLA-A Deletion Frequency in Primary Solid Tumors (TCGA)

Primary Tumor Type	HLA-A Deletion Frequency TCGA (%)
Pancreas	33
Lung squamous cell carcinoma	25
Esophagus	23
Kidney cancer	18
Bladder urothelial	18
Ovary	17
Head and neck squamous cell carcinoma	16
Glioblastoma	15
Cervical squamous cell carcinoma	14
Breast	14
Stomach	13
Lung adenocarcinoma	12
Mesothelioma	11
Cholangiocarcinoma	11
Colorectal	10

HLA, human leukocyte antigen; TCGA, The Cancer Genome Atlas.

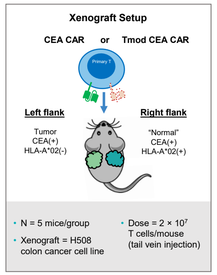
Figure 4. Tempus HLA Typing and Analysis of Loss of Heterozygosity (LOH)

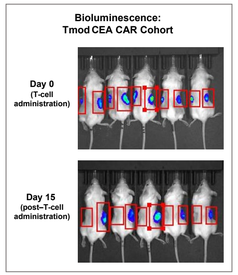


HLA, human leukocyte antigen; LOH, loss of heterozygosity.

- In vivo studies show that Tmod maintains selectivity (**Figure 5A** and **B**)
- In order to allow for adequate tumor and "normal" cell to be established, tumor and "normal" cells were implanted subcutaneously in NSG mice
- CAR T cells or Tmod CAR T-cells were administered via tail veins when tumor reached 100-150 mm^3 (Day 0)
- Approximately 2 weeks following cell infusion, Tmod CAR T-cell treated mice (shown in red) experienced selective regression of tumor grafts, while "normal" tumor grafts continued to grow. Mice treated with CEA CAR T cells (shown in green) experienced regressions of both tumor and "normal" tumor grafts

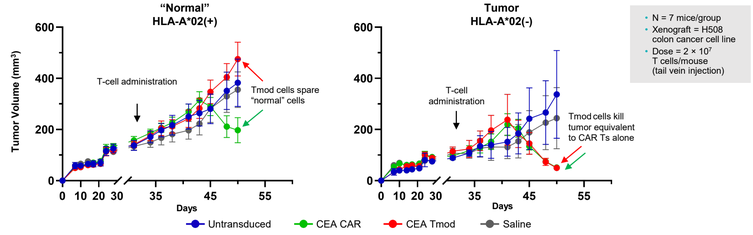
Figure 5A. A2B530 CEA Tmod CAR T-Cells Display High Tumor-Specific Killing Compared With CEA CAR T-Cells





STUDY RATIONALE (cont.)

Figure 5B. CEA Tmod Cells Display High Tumor-Specific Killing Compared With CEA CAR T-Cell Therapies



CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen; HLA, human leukocyte antigen.
Data provided by Mark L. Sandberg, PhD, A2 Bio.

STUDY DESIGN

- Participants will be initially screened to identify germline HLA-A*02 heterozygosity by central NGS. If HLA-A*02 heterozygosity is confirmed, primary archival tumor tissue will be analyzed by xT-Onco NGS testing to determine if somatic tumor HLA-A*02 LOH is present[12] (**Figure 6A** and **B**)
- If the tumor demonstrates HLA-A*02 LOH and the participant screens eligible, the participant will undergo leukapheresis
- Participants enrolled in the study who undergo leukapheresis will be evaluated for safety 7 days after leukapheresis and followed for relapsed status
- Banked T cells will be available for subsequent autologous Tmod CAR T-cell therapy at the time of relapse

Figure 6A. Patient and Tissue Flow From the BASECAMP-1 to EVEREST Studies

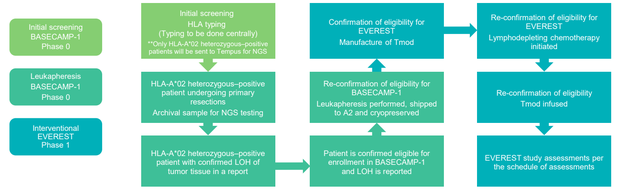
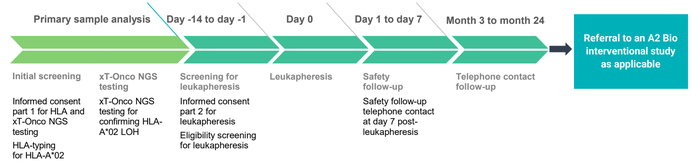


Figure 6B. Study Schema



HLA, human leukocyte antigen; LOH, loss of heterozygosity; NGS, next-generation sequencing.

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