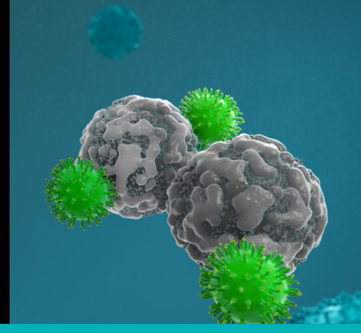
Abstract Number 27

# BASECAMP-1: Leveraging Human Leukocyte Antigen A (HLA-A) Loss of Heterozygosity (LOH) in Solid Tumors to Identify Patients for Carcinoembryonic Antigen (CEA) and Mesothelin (MSLN) Logic-Gated Tmod Chimeric Antigen Receptor (CAR) T-Cell Therapy




Caleb J. Smith[1], Sally Lau[2], Edward Garon[3], Sandip Patel[4], Yanyan Lou[5], Daniel Greenwald[6], Vamsidhar Velcheti[2], Kedar Kirtane[7], Yi Lin[1], Sattva Neelapu[8], Frederick L. Locke[7], Sarah Larson[3], Marcela V. Maus[9], Diane M. Simeone[2], Scott Kopetz[8], Ariane Lozac'hmeur[10], Ameen A. Salahudeen[11], Judy Vong[12], Kirstin B. Liechty[12], John S. Welch[12], Eric W. Ng[12], William Y. Go[12], David Maloney[13], Julian R. Molina[1]

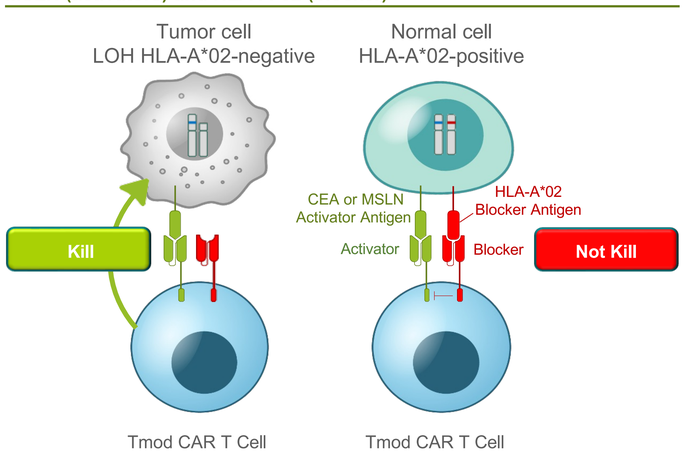
[1]Division of Medical Oncology, Mayo Clinic, Rochester, MN, USA; [2]New York University Langone Health, New York, NY, USA; [3]David Geffen School of Medicine at University of California, at Los Angeles, Los Angeles, CA, USA; [4]Department of Medical Oncology, University of California, San Diego, San Diego, CA, USA; [5]Mayo Clinic, Jacksonville, FL, USA; [6]University of California, at Los Angeles, Los Angeles, CA, USA; [7]H. Lee Moffitt Cancer Center, Tampa, FL, USA; [8]Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; [9]Massachusetts General Cancer Center, Boston, MA, USA; [10]Tempus Labs, Inc., Chicago, IL, USA; [11]Biological Modeling, Tempus Labs, Inc., Division of Hematology Oncology, University of Illinois at Chicago, Chicago, IL, USA; [12]A2 Biotherapeutics, Inc., Agoura Hills, CA, USA; [13]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA


## BACKGROUND

- Metastatic non-small cell lung cancer and mesothelioma are thoracic malignancies with poor outcomes, with 5-year survival rates of 8% [1]
- Chimeric antigen receptor (CAR) T-cell therapy has demonstrated improved 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 distinguish cancer cells from normal cells. In previous studies, the use of carcinoembryonic antigen 5 (CEA) T-cell receptors and mesothelin (MSLN) CARs both resulted in dose-limiting on-target, off-tumor toxicities [4-6]
- Tmod™ CAR T-cell is a logic-gated cell therapy that addresses these challenges by leveraging dual receptors capable of killing tumor cells while leaving healthy cells intact [7]. Tmod platform technology is a versatile system that may be applied to T cells and natural killer cells in autologous and allogeneic settings
  - A2B530 is a CEA-directed and A2B694 is an MSLN-directed Tmod construct utilizing a leukocyte immunoglobulin-like receptor 1 (LIR-1)–based inhibitory receptor (blocker) targeting human leukocyte antigen (HLA)-A*02
- HLA loss of heterozygosity (LOH) may provide a means to distinguish tumor from normal tissue in a definitive manner due to this irreversible, clonal loss within tumor cells [7,8]. 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
- In the Tempus real-world database, LOH occurs in 12.2% to 26.0% of advanced solid tumors with an average of 16.3% in 10,867 samples tested [9]
- The Tempus xT is a clinical diagnostic test commonly used for patients with lung cancer that can readily identify HLA-A*02:01 LOH
- BASECAMP-1 (NCT04981119) is an ongoing 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
  - Eligible patients identified in BASECAMP-1 will be referred to the EVEREST-1 A2B530 CEA Tmod or EVEREST-2 A2B694 MSLN Tmod interventional studies

## STUDY RATIONALE

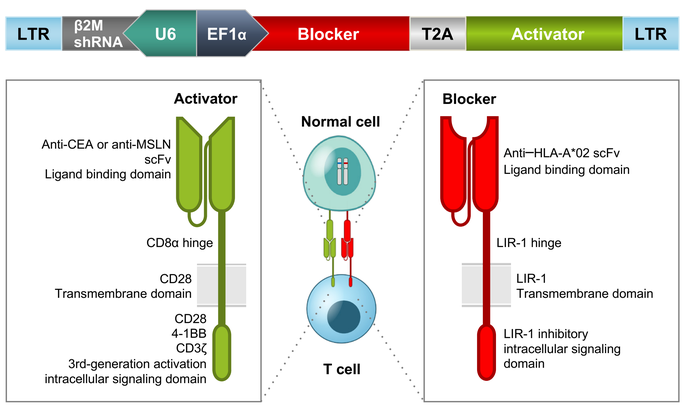
### Figure 1. Logic-gated CAR T with the goal of reducing toxicity: CEA or MSLN (activators) and HLA-A*02 (blocker)




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

- A2 Bio's Tmod CAR T HLA LOH approach has been published by Hamburger et al 2020 (**Figure 1**) [7]
  - HLA was selected as blocker target; first blocker HLA-A*02 is the most prevalent allele in the US population
  - Activators include CEA and MSLN, which are both well-studied targets but showed dose-limiting toxicities in previous studies
- CAR T HLA-A LOH approach is independently validated by Vogelstein/Kinzler, 2021 [8]

### Figure 2. CEA or MSLN CAR Tmod single vector construct

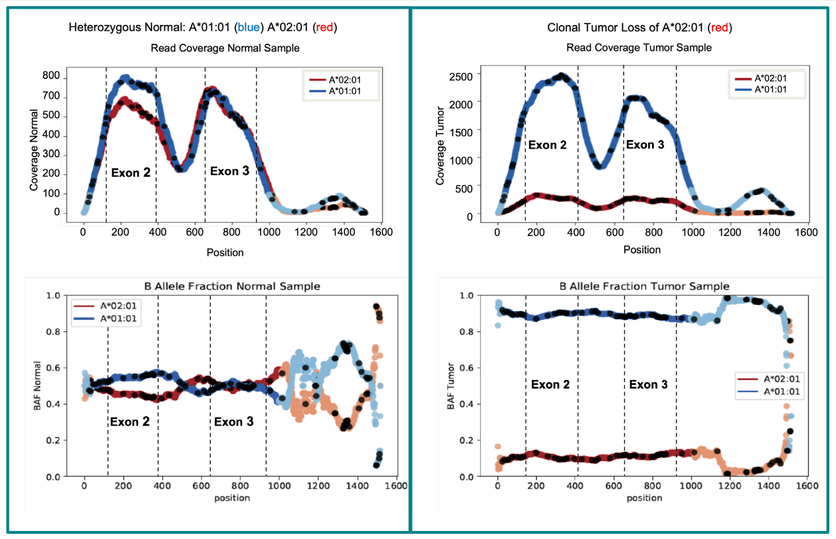



β2M shRNA, β2-microglobulin short-hairpin RNA; CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen 5; EF1a, elongation factor-1; HLA, human leukocyte antigen; LIR-1, leukocyte immunoglobulin-like receptor 1; LTR, long terminal repeat; MSLN, mesothelin; scFv, single-chain variable fragment; T2A, thosea asigna virus 2A.

- CAR activator: 3rd-generation CAR T with both signal 1 (CD3ζ) and signal 2 activation domains (CD28 and 4-1BB)
- CAR blocker: LIR-1 is a member of the immune inhibitory receptor family and contains 4 immunoreceptor tyrosine-based inhibition motifs in its signaling domain [10]
- Replicant incompetent single lentivirus transgene: The activator and blocker receptors are co-expressed in a single construct containing a cleavable T2A linker (**Figure 2**)

## STUDY RATIONALE (cont.)

### Figure 3. Read coverage and B allele fraction (ratio of coverage for allele 1 and allele 2)



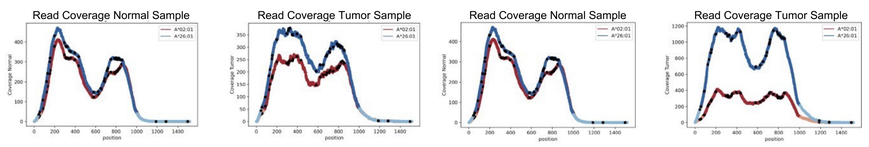

- A representative example of clonal HLA LOH (**Figure 3**), where discordance is observed in read coverage of HLA-A*02:01 between the tumor and matched-normal samples [9,11]
- HLA-A*02:01 LOH can be reliably detected using the Tempus xT clinical diagnostic test (**Table 1**)

### Table 1. Frequency of HLA-A LOH in advanced solid tumors[a]

| | Tempus HLA-A LOH advanced disease real-world data [9] | | TCGA HLA-A LOH primary tumors [12] | | Montesion et al [13] | |
|---|---|---|---|---|---|---|
| | Samples, n | HLA-A LOH frequency, % | Samples, n | HLA-A LOH frequency, % | Samples, n | HLA-A LOH frequency, % |
| NSCLC | 1,915 | 23.1 | 501 | 25.3 | 13,240 | 23 |
| Mesothelioma | 7 | 14.3 | 87 | 11.5 | 404 | 12.4 |
| Colorectal cancer | 1,854 | 15.6 | 615 | 9.6 | 10,682 | 15.3 |
| Gastroesophageal cancer | 506 | 20.8 | 625 | 16.2 | 3,174 | 22.2 |
| Pancreatic cancer | 675 | 19.6 | 184 | 33.1 | 4,049 | 23.4 |
| Prostate cancer | 998[b] | 3.4[b] | 500 | 4.5 | 2,774 | 5.8 |
| Ovarian, fallopian tube, primary peritoneal cancers | 569 | 16 | 579 | 17.1 | 4,996 | 15.7 |
| Breast cancer | 1,447 | 12.2 | 1,080 | 13.6 | 9,686 | 13.2 |
| Head and neck squamous cell carcinoma | 208 | 26 | 522 | 16.1 | 1,134 | 27.2 |

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

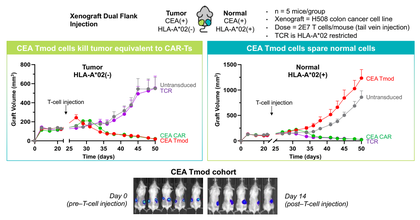
### Figure 4. Higher tumor purity allows for more accurate prediction of HLA-A*02 LOH




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

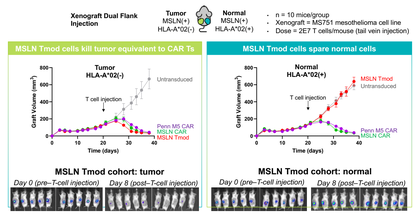
- In vivo studies show that Tmod maintains selectivity
- Tumor (HLA-A*02[-]) and "normal" (HLA-A*02[+]) cells were implanted subcutaneously in NOD scid gamma (NSG) mice
- CAR T cells or Tmod CAR T cells were administered via tail veins when tumor reached 100-150 mm$^3$
- Approximately 2 weeks following 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 or MSLN CAR T cells (shown in green) experienced regressions of both tumor and "normal" tumor grafts (**Figures 5** and **6**)

### Figure 5. CEA Tmod (A2B530) in vivo study demonstrates potency comparable to NCI benchmark CEA TCR-T [4, 14]




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

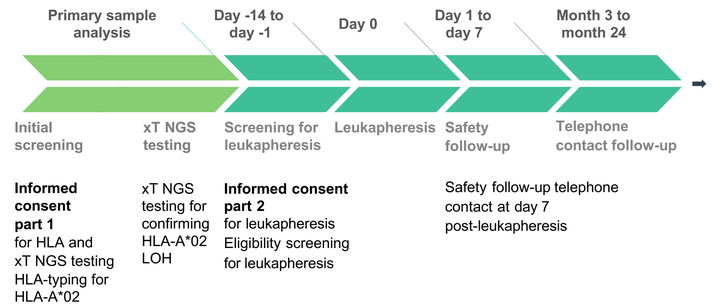
### Figure 6. MSLN Tmod (A2B694) in vivo study demonstrates potency comparable to M5 benchmark MSLN CAR T [15]




CAR, chimeric antigen receptor; HLA, human leukocyte antigen; MSLN, mesothelin.

## STUDY DESIGN AND METHODS

### Figure 7. Study schema for BASECAMP-1 (NCT04981119)



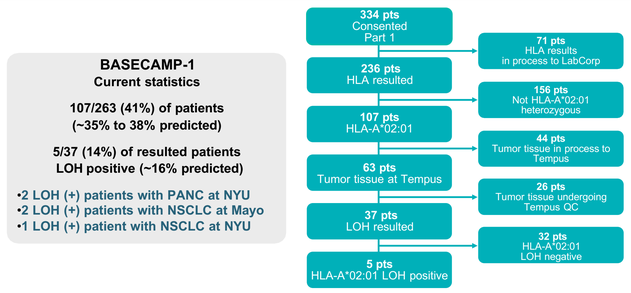

CEA, carcinoembryonic antigen 5; CRC, colorectal cancer; HLA, human leukocyte antigen; LOH, loss of heterozygosity; MSLN, mesothelin; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; PANC, pancreatic cancer.

- Participants will be initially screened to identify germline HLA-A*02 heterozygosity by central NGS. If HLA-A*02 heterozygosity is confirmed, primary tumor tissue will be analyzed by xT NGS testing to determine if somatic tumor HLA-A*02 LOH is present. 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 the autologous EVEREST-1 A2B530 CEA Tmod and EVEREST-2 A2B694 MSLN Tmod interventional studies when clinically appropriate

### Figure 8. Tempus clinical workflow


NGS, next-generation sequencing; PI, principal investigator.

### Figure 9. BASECAMP-1 progress to date and screening process details: 5 HLA LOH patients identified (Updated data cut on February 6, 2023)




HLA, human leukocyte antigen; LOH, loss of heterozygosity; NSCLC, non-small cell lung cancer; PANC, pancreatic cancer; QC, quality control.

## SITE LIST

- **NYU Langone Medical Center**
  - Principal Investigator: Diane Simeone, MD
  - Sub-Investigator: Theodore Welling, MD
  - Sub-Investigator: Sally Lau, MD
- **Mayo Clinic, Rochester**
  - Principal Investigator: Julian Molina, MD, PhD
  - Sub-Investigator: Yi Lin, MD, PhD
  - Sub-Investigator: Caleb Smith, MD
- **MD Anderson Cancer Center**
  - Principal Investigator: Maria Pia Morelli, MD, PhD
  - Sub-Investigator: Scott Kopetz, MD, PhD
- **University of California, Los Angeles**
  - Principal Investigator: J. Randolph Hecht, MD
  - Sub-Investigator: Edward Garon, MD
  - Sub-Investigator: Daniel Greenwald, MD
- **Moffitt Cancer Center**
  - Principal Investigator: Kedar Kirtane, MD
  - Sub-Investigator: Frederick Locke, MD
  - Sub-Investigator: Tiago Biachi, MD
- **City of Hope**
  - Principal Investigator: Marwan Fakih, MD
- **University of California, San Diego**
  - Principal Investigator: Sandip Patel, MD
  - Sub-Investigator: Andrew Lowy, MD

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