Abstract Number 636

BASECAMP-1: A master prescreening study to identify patients with high-risk or metastatic solid tumors with HLA loss of heterozygosity in preparation for Tmod™ CAR T-cell therapy trials

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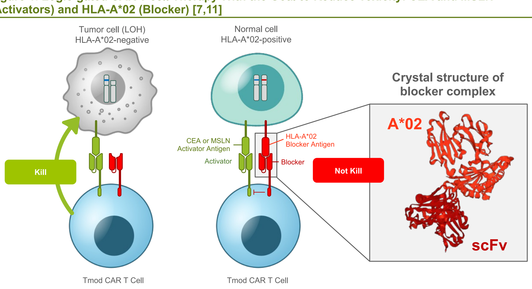
BACKGROUND AND STUDY OBJECTIVES

- Solid tumors comprise >90% of cancers. Metastatic non-small cell lung cancer (NSCLC), colorectal cancer (CRC), and pancreatic cancer (PANC) are the leading causes of cancer-related mortality in the US with 5-year relative survival rates of 9%, 14%, and 3%, respectively [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. 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]
- 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; this approach was published by Hamburger et al in 2020 and independently verified in 2021 [7,8]. HLA-A*02 LOH can only be therapeutically exploited if patients are identifiable through a feasible and timely clinical workflow
  - Among patients with advanced solid tumors, HLA-A LOH occurs in 16.3% of patients (**Table 1**) [9,10]
- Tmod, a novel logic-gated CAR T-cell therapy, utilizes a blocking receptor to discriminate tumor from normal cells, thus mitigating on-target, off-tumor toxicity (**Figure 1**) [7]. A2B530 is a CEA-directed and A2B694 is a MSLN-directed Tmod construct utilizing an LIR-1–based inhibitory receptor (blocker) targeting human leukocyte antigen (HLA)-A*02, the most prevalent allele in the United States (**Figure 2**)
  - HLA-A*02 allele prevalence differs based on race and/or national origin (**Figure 3**), but A2B530 and A2B694 blockers recognize all HLA-A*02 alleles, allowing for potential broad benefit across a diverse patient population
- BASECAMP-1 (NCT04981119) is an ongoing prescreening study to 1) identify patients with tumor-associated HLA-A*02 LOH and who are eligible for Tmod CAR T-cell therapy, and 2) obtain leukapheresis in preparation for the autologous CAR T-cell therapy trials EVEREST-1 (A2B530 targeting CEA; NCT05736731) and EVEREST-2 (A2B694 targeting MSLN; NCT06051695)

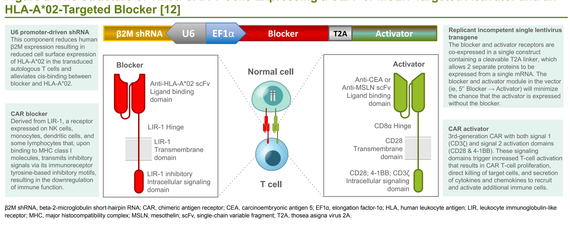
STUDY RATIONALE

Figure 1. Logic-gated CAR T-cell Therapy With the Goal to Reduce Toxicity: CEA and MSLN (Activators) and HLA-A*02 (Blocker) [7,11]



CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen 5; HLA, human leukocyte antigen; LOH, loss of heterozygosity; MSLN, mesothelin; scFv, single-chain variable fragment.

Figure 2. The Structure of Tmod CAR T Cells Expressing a CEA- or MSLN-Targeted Activator and an HLA-A*02-Targeted Blocker [12]



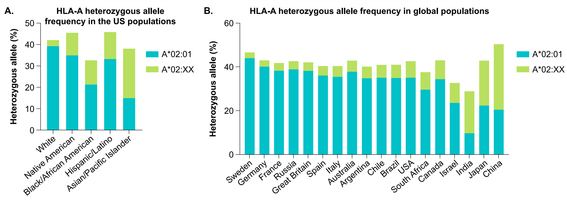
β2M shRNA, beta-2-microglobulin short-hairpin RNA; CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen 5; EF1α, elongation factor-1α; HLA, human leukocyte antigen; LIR, leukocyte immunoglobulin-like receptor; MHC, major histocompatibility complex; MSLN, mesothelin; scFv, single-chain variable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

Table 1. Frequency of HLA-A LOH in Advanced Tumors [9,10,13]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)
NSCLC, % (n)	23.1 (1,915)	25.3 (501)
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)
Ovarian cancer, % (n)	16.0 (569)	17.1 (579)
Mesothelioma, % (n)	14.3 (7)	11.5 (87)

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.

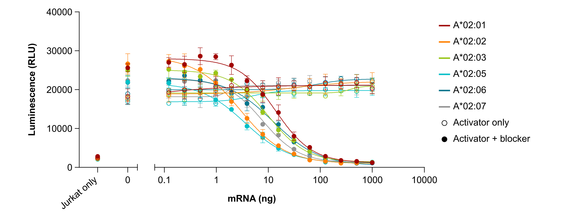
Figure 3. Frequencies of HLA-A*02 Alleles in the US and Global Populations (NMDP)



HLA, human leukocyte antigen; NMDP, National Marrow Donor Program; US, United States.

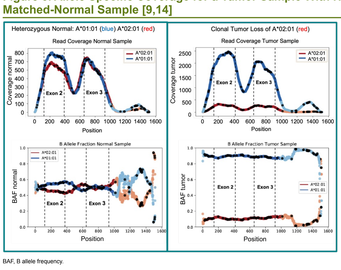
- While initial trial design focused on HLA-A*02:01, inclusion of additional allele subvariants to the Tempus NGS assay can expand the patient population by 7% and increase patient diversity (**Figure 3**)
  - 42.8% of North American patients with diverse national origins are expected to be HLA-A*02 heterozygous vs 35% HLA-A*02:01 heterozygous (**Figure 3A**)
  - HLA-A*02 global distribution reflects the racial and ethnic distributions in the US (**Figure 3B**)
- Importantly, the HLA-A*02-targeted blocker can recognize additional HLA-A*02 alleles (**Figure 4**)

Figure 4. The LIR-1–Based Inhibitory Receptor (Blocker) Recognizes Additional HLA-A*02 Alleles [11]



LIR, leukocyte immunoglobulin-like receptor; mRNA, messenger RNA; RLU, relative luminescence unit.

Figure 5. Allele Specific Coverage for a Tumor Sample With HLA-A*02:01 LOH and Its Matched-Normal Sample [9,14]



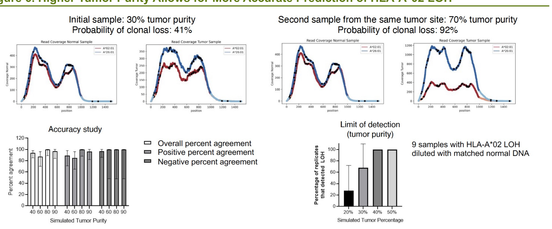
BAF, B allele frequency.

HLA LOH Detection

- HLA-A LOH can be reliably detected using the Tempus xT next-generation sequencing (NGS) assay (**Table 1**)
- **Figure 5** shows a representative example of clonal HLA-A LOH, in which a discordance is observed in read coverage of HLA-A*02:01 between the tumor and matched-normal samples [9,14]

STUDY RATIONALE (CONTINUED)

Figure 6. Higher Tumor Purity Allows for More Accurate Prediction of HLA-A*02 LOH



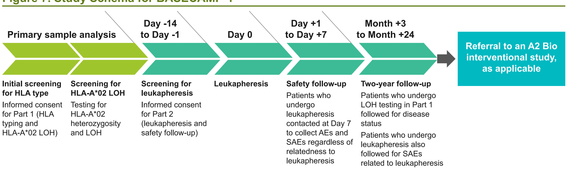
HLA, human leukocyte antigen; LOH, loss of heterozygosity.

- LOH results can be obtained within a clinically feasible workflow and timeframe; however, the assay sensitivity declines below a tumor purity of 40% [9]
- The impact of tumor purity on LOH sensitivity was highlighted in a patient with a low initial sample tumor purity (30%) that resulted in a 41% probability of HLA-A*02:01 LOH (below positive threshold). A second sample with a higher tumor purity (70%), obtained from formalin-fixed, paraffin-embedded sections, resulted in a 92% probability of HLA-A*02:01 LOH (positive; **Figure 6**)

STUDY DESIGN AND METHODS

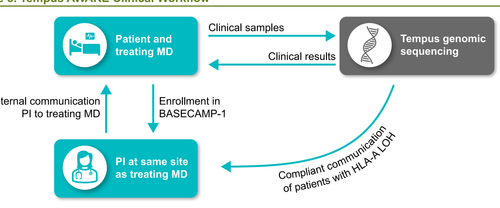
- Patients with metastatic solid tumors or at high risk of relapse will be screened for baseline HLA-A*02. Tumor tissue from patients with germline HLA-A*02 heterozygosity will be analyzed for somatic tumor HLA-A*02 LOH via Tempus NGS (**Figures 7 and 8**)
- In addition, patients may be identified via the Tempus AWARE program (**Figure 8**). AWARE analyzes tissue from patients submitted to Tempus as part of the patient's routine clinical workup. Institutional investigators are then informed of molecular results and can communicate with treating physicians regarding enrollment opportunities
- Patients with tumors demonstrating HLA-A*02 LOH may be screened for subsequent leukapheresis. Leukapheresis can be done at any time during the disease course resulting in the collection of T cells in better condition, prior to additional lines of anti-cancer therapy
- Banked T cells will be available to be manufactured for the EVEREST-1 and EVEREST-2 studies

Figure 7. Study Schema for BASECAMP-1



AE, adverse event; HLA, human leukocyte antigen; LOH, loss of heterozygosity; SAE, serious adverse event.

Figure 8. Tempus AWARE Clinical Workflow



HLA, human leukocyte antigen; LOH, loss of heterozygosity; PI, principal investigator.

RESULTS

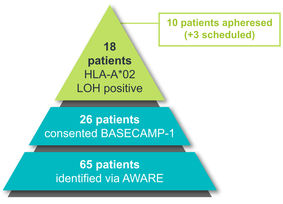
- As of October 2, 2023, 880 patients have been consented at 11 institutions through standard screening and AWARE matching. HLA-A*02 LOH status is available for 191 patients. A total of 28 patients have been confirmed HLA-A*02 LOH positive and 16 have been apheresed (**Figure 9**)
- Through the AWARE program, deployed since January 2022, at programs that send tumor tissue to Tempus, 65 patients with study-specific disease types with HLA-A*02 LOH have been identified
  - This demonstrates the feasibility of leveraging a diagnostic during routine clinical workup to identify patients with rare, molecularly defined disease for personalized clinical studies

Figure 9. BASECAMP-1 Progress to Date and Screening Process Details (October 2, 2023)

BASECAMP-1 Current Statistics

43% (376/880)	of patients **HLA-A*02 heterozygous** (≈42-45% predicted)
15% (28/191)	of resulted patients **HLA-A*02 LOH positive** (≈8% predicted)
16	patients apheresed

AWARE matches at 9 sites



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

CONCLUSIONS

- Clonal HLA LOH is an irreversible discriminator between tumor vs normal cells that can be exploited for logic-gated Tmod CAR T to reduce on-target, off-tumor toxicity [7,8]
- BASECAMP-1 (NCT04981119) study is currently enrolling patients to identify HLA-A*02 LOH patients with CRC, NSCLC, PANC, mesothelioma, or ovarian cancer and then to bank their T cells for EVEREST-1 (A2B530 CEA Tmod) or EVEREST-2 (A2B694 MSLN Tmod) studies
- As of October 2, 2023, 191 consented patients had LOH results available and 28 had LOH confirmed. Importantly, 65 patients with study-specific disease types with HLA-A*02 LOH were identified across sites through the AWARE program, compared to 10 in standard screening
- BASECAMP-1 prospective identification of HLA-A*02 LOH is feasible in the real-world setting and has led to enhanced enrollment in the EVEREST-1 study

SITE LIST

- **Banner Health, Gilbert, AZ**
  - Principal Investigator: Tomislav Dragovich, MD
- **City of Hope, Duarte, CA**
  - Principal Investigator: Marwan Fakih, MD
- **University of California San Diego, La Jolla, CA**
  - Principal Investigator: Sandip Patel, MD
- **Stanford University, Palo Alto, CA**
  - Principal Investigator: Saurabh Dahiya, MD
- **UCLA Medical Center, Santa Monica, CA**
  - Principal Investigator: J. Randolph Hecht, MD
  - Sub-Investigator: Edward Garon, MD
- **Mayo Clinic, Jacksonville, FL**
  - Principal Investigator: Hemant Murthy, MD
- **Moffitt Cancer Center, Tampa, FL**
  - Principal Investigator: Kedar Kirtane, MD
  - Sub-Investigator: Frederick Locke, MD
- **Massachusetts General Hospital/Dana-Farber Cancer Institute, Boston, MA**
  - Principal Investigator: Jong Chul Park, MD
- **Mayo Clinic, Rochester, MN**
  - Principal Investigator: Julian Molina, MD, PhD
  - Sub-Investigator: Yi Lin, MD, PhD
- **Washington University, St. Louis, MO**
  - Principal Investigator: Patrick Grierson, MD, PhD
- **NYU Langone Medical Center, New York, NY**
  - Principal Investigator: Diane Simeone, MD
  - Sub-Investigator: Theodore Welling, MD
- **The University of Texas MD Anderson Cancer Center, Houston, TX**
  - Principal Investigator: Maria Pia Morelli, MD, PhD
  - Sub-Investigator: Scott Kopetz, MD, PhD
- **Fred Hutchinson Cancer Center, Seattle, WA**
  - Principal Investigator: Jennifer Specht, MD

References

1. American Cancer Society. Cancer Facts & Figures 2023. Atlanta: American Cancer Society; 2023.
2. Locke F, et al. *N Engl J Med*. 2022;386(7):640-654.
3. Maude S, et al. *N Engl J Med*. 2018;378(5):439-448.
4. Parkhurst M, et al. *Mol Ther*. 2011;19(3):620-626.
5. Haas A, et al. *Mol Ther*. 2019;27(11):1919-1929.
6. Tanyi J, et al. Presented at: Cellicon Valley '21: The Future of Cell and Gene Therapies; May 6-7, 2021; virtual symposium.
7. Hamburger A, et al. *Mol Immunol*. 2020;128:298-310.
8. Hwang M, et al. *Proc Natl Acad Sci U S A*. 2021;118(12):e2022410118.
9. Hecht J, et al. *J Clin Oncol*. 2022;40(suppl 4):190.
10. Simeone D, et al. *J Immunother Cancer*. 2022;10(suppl 2):A670.
11. Mock J-Y, et al. *Mol Ther Oncolytics*. 2022;27:157-166.
12. Borges L, et al. *J Immunol*. 1997;159(11):5192-5196.
13. The Cancer Genome Atlas (TCGA) Research Network. Accessed June 2021. https://www.cancer.gov/tcga
14. Perera J, et al. *J Immunother Cancer*. 2019;7(suppl 1):P103.

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