Abstract Number 190

Next-Generation Sequencing (NGS) to Identify Relapsed Gastrointestinal (GI) Solid Tumor Patients With Human Leukocyte Antigen (HLA) Loss of Heterozygosity (LOH) for Future Logic-Gated CAR T Therapy to Reduce On-Target Off-Tumor Toxicity

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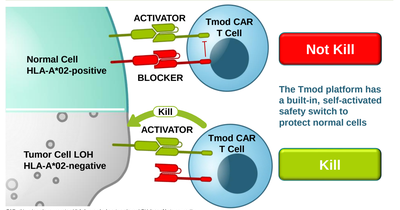
BACKGROUND AND RATIONALE: TARGETING HLA LOH

- Solid tumors comprise >90% of cancers. Metastatic colorectal (CRC), pancreatic (PANC), and gastroesophageal (GE) cancers are the leading causes of gastrointestinal (GI) cancer-related mortality (5-year survival rate, 14%, 3% and ~5%-6%, 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 that discriminate cancer cells from normal cells. In previous studies, the use of carcinoembryonic antigen (CEA) T-cell receptors and mesothelin (MSLN) CARs both resulted in dose-limiting on-target, off-tumor toxicities[4,14]
- 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.[7] 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.[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
- HLA LOH has been observed in ~13% across solid tumors and up to 33% of primary pancreatic cancers.[9] 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 of recurrence
- Prevalence of HLA LOH across advanced GI tumors is unknown in the real-world setting
- The Tempus xT-Onco next-generation sequencing (NGS) database of patients with multiple GI tumors can help determine HLA-A LOH in advanced disease in the real-world setting
- With the Tempus xT-Onco standard-of-care NGS assay, patients with GI cancer can be readily identified for HLA-A LOH and for future treatment with Tmod logic-gated CAR T therapy to reduce on-target off-tumor toxicity
- We are currently screening advanced CRC, PANC, and non-small cell lung cancer patients for HLA-A*02 LOH within the BASECAMP-1 study (NCT04981119) to then identify and bank T cells for potential eligible subjects for future autologous Tmod CAR T cell therapy in EVEREST interventional studies

LOGIC-GATED CAR T WITH GOAL TO REDUCE TOXICITY: CEA AND MSLN (ACTIVATORS) AND HLA-A*02 (BLOCKER)

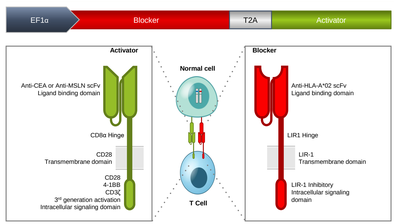
Figure 1A. Tmod CAR T Platform Activator (eg, CEA, MSLN) and HLA-A*02 Blocker: Discrimination Between Tumor and Normal Cells



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

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

Figure 1B. CEA or MSLN CAR Tmod Single Vector Construct



CEA, carcinoembryonic antigen; LIR, leukocyte immunoglobulin-like receptor; MSLN, mesothelin; scFv, single-chain variable fragment.

- CAR Activator: 3rd Generation CAR T with both signal 1 (CD3ζ) and signal 2 activation domains (CD28 & 4-1BB)
- CAR Blocker: LIR-1 is a member of the immune inhibitory receptor family and contains 4 ITIMs 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 1B**)

METHODS

- 21,053 cancer patients in the Tempus database, as of December 1, 2021, were evaluated (**Figure 2A**)
- CRC, PANC, and GE cancer patients with ≥ stage 3 were then extracted from the database, and frequencies of HLA LOH were identified (ie, whether loss occurred across high-frequency HLA-A alleles)
- The occurrence of HLA LOH in GI tumors of 3035 patients was assessed based on paired germline and somatic DNA sequencing using a research assay[7]
- Mutations in *KRAS*, *EGFR*, and *BRAF*, as well as microsatellite instability (MSI) status were examined to determine any association with HLA-A LOH
- Resection or biopsy of the 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 samples are compared with tumor samples to determine loss of heterozygosity based on exons 2 and 3 of HLA (**Figure 2B**)

Figure 2A. GI Cancers in the Tempus xT-Onco Real-World Database

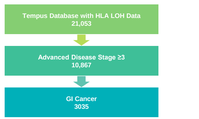
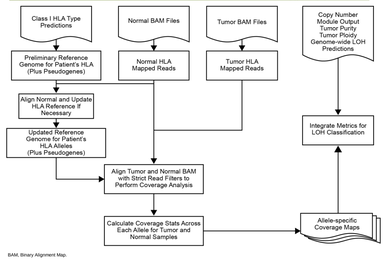


Figure 2B. Tempus HLA Typing and Analysis of Loss of Heterozygosity (LOH)



BAM, Binary Alignment Map.

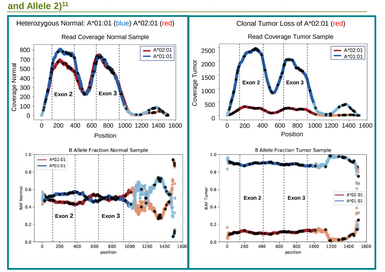
LOH can be reliably detected using the Tempus xT-Onco NGS assay

- A representative example of clinical HLA LOH (**Figure 3**) where a discordance is observed in read coverage of HLA-A*02:01 between the tumor and matched-normal samples

Clonal LOH is determined based on B Allele Fraction (BAF), LogR difference, and tumor purity

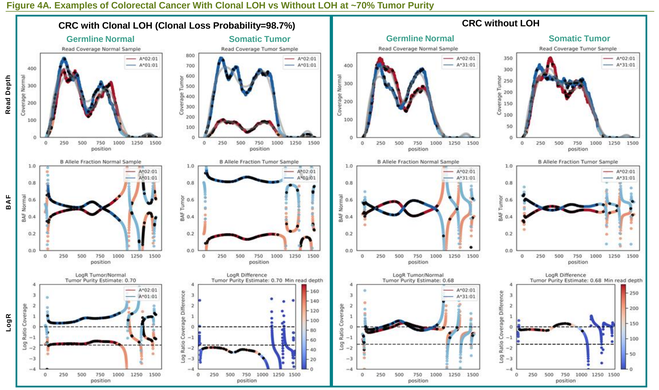
- Tumor purity of the tumor sample is determined by the pathologist
- Ratio of BAF between tumor and matched normal captures the magnitude of the LOH signal between tumor and matched normal samples. The BAF in the normal sample without LOH will be ~0.5; however, if the tumor purity increases, the BAF will decrease from 0.5 proportionally to the tumor purity in the tumor sample with LOH
- Ratio of observed to expected LogR differences represent the magnitude of the loss event. For a clonal loss in a tumor sample with a given tumor purity, the expected LogR difference is $\log_2(1-\text{Tumor Purity})$. The ratio describes whether the loss meets or exceeds the expected loss for a clonal LOH event (**Figures 4A and 4B**)

Figure 3. Read Coverage and B Allele Fraction (Ratio of coverage for Allele 1 and Allele 2)[11]



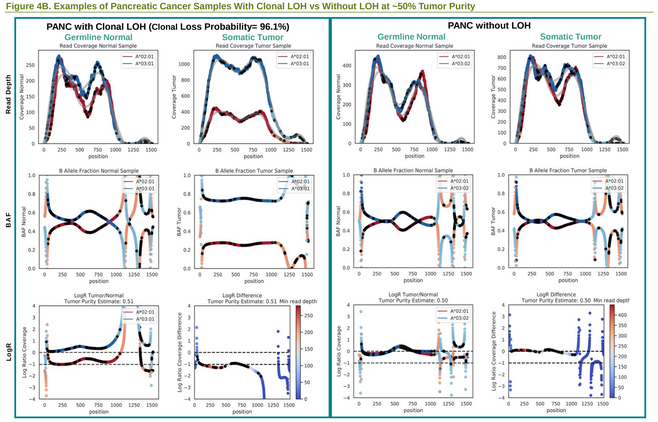
RESULTS: Tempus xT-Onco Can Demonstrate Clonal LOH at Various Levels of Tumor Purity

Figure 4A. Examples of Colorectal Cancer With Clonal LOH vs Without LOH at ~70% Tumor Purity



BAF, B allele frequency; CRC, colorectal; LOH, loss of heterozygosity.

Figure 4B. Examples of Pancreatic Cancer Samples With Clonal LOH vs Without LOH at ~50% Tumor Purity



BAF, B allele frequency; LOH, loss of heterozygosity; PANC pancreatic cancer

Table 1. Frequency of HLA-A LOH in Advanced GI Tumors[a]

	Tempus HLA-A LOH Advanced Disease Real-World	TCGA HLA-A LOH Primary Tumors
Average	16.3% (n=10,867)	12.6% (n=10,844)
Range in GI cancers	15.6%-20.8% (n=3035)	9.6%-33.1% (n=1424)
Colorectal	15.6% (n=1854)	9.6% (n=615)
Pancreatic	19.6% (n=675)	33.1% (n=184)
Gastroesophageal	20.8% (n=506)	16.2% (n=625)

[a]Tempus data contain more advanced disease, and TCGA data have more primary tumors.

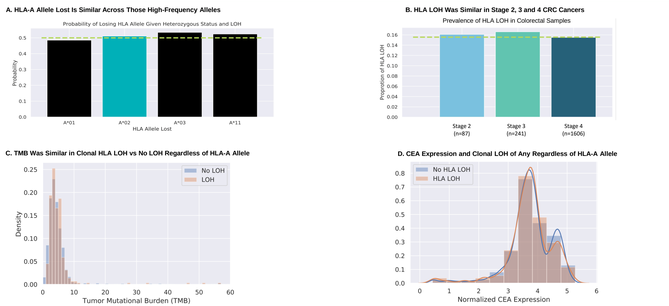
Table 2. Clinical Biomarkers Were Similar in No HLA LOH and HLA LOH Cohorts[a]

HLA LOH Status, n (%)[b]	*BRAF*	*KRAS*	*NRAS*	*ERBB2*	*NTRK1*	*NTRK2*	*NTRK3*	*EGFR*	MSI High	TMB High[c]
No LOH (N=1148)	116 (10)	498 (43)	52 (5)	39 (3)	16 (1)	17 (1)	37 (3)	11 (1)	36 (3)	51 (4)
LOH (N=289)	18 (6)	134 (46)	15 (5)	13 (4)	2 (1)	3 (1)	8 (3)	6 (2)	9 (3)	13 (4)

[a]All reported mutations were included (pathogenic or unknown significance). No difference was significant using Fisher exact test. [b]LOH and no LOH cohorts are stage- and age-matched. [c]TMB≥10.
HLA, human leukocyte antigen; LOH, loss of heterozygosity; MSI, microsatellite instability; TMB, tumor mutational burden.

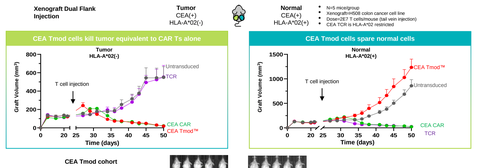
- The probability of HLA-A allele loss is similar across high-frequency alleles in LOH samples (**Figure 5A**)
- The frequency of HLA LOH was similar in stage 2, 3 and stage 4 CRC cancer samples (**Figure 5B**)
- HLA-LOH and no LOH cohorts were stage- and age-matched for TMB analysis (**Figure 5C**)
  - Difference in biomarkers was not significant using Fisher exact test
- CEA expression does not vary significantly between patients with HLA LOH or no LOH (**Figure 5D**)

Figure 5. Probability and Frequency of Clonal HLA LOH Is Similar Across a Variety of Clinical Biomarkers



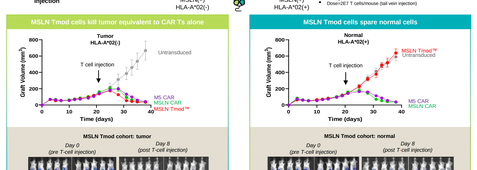
CEA, carcinoembryonic antigen; CRC, colorectal cancer; HLA, human leukocyte antigen; TMB, tumor mutational burden; LOH, loss of heterozygosity.

Figure 6A. CEA Tmod (A2B530) In Vivo Study Demonstrates Potency Comparable to NCI Benchmark CEA TCR-T[4]



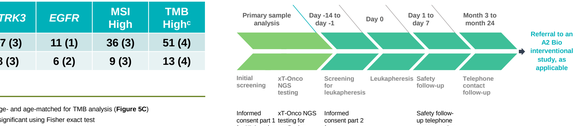
CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen; HLA, human leukocyte antigen; NCI, National Cancer Institute; TCR, T-cell receptor.

Figure 6B. MSLN Tmod (A2B694) Display High Tumor-Specific Killing Compared With Penn M5 MSLN CAR T[6]



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

Figure 7. Study Schema for BASECAMP-1 (NCT04981119)



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

CONCLUSIONS

- **Clonal HLA LOH is a clear foundational discriminator between tumor vs normal cells and can be exploited for AND-NOT logic-gated Tmod CAR T to reduce on-target off-tumor toxicity[7,8]**
- **The frequency of HLA LOH among advanced GI solid tumor cancers in Tempus real-world dataset is 16.3%, with a range of 15.6%-20.8% between colorectal, pancreatic, and gastroesophageal tumors**
- **The HLA LOH frequency observed in these late-stage advanced GI tumors is similar to that in primary tumors from TCGA, which also used germline-matched and tumor samples**
- **Patterns or distributions for canonical biomarkers and clinical factors were similar in HLA-stable and HLA LOH cohorts**
- **HLA LOH is a clonal event as identified by Tempus xT-Onco and its frequency is similar regardless of stage in GI malignancies**
- **Tempus xT-Onco NGS assay was able to identify real-world late-stage patients with clonal HLA LOH. This may be utilized for Tmod CAR T therapy to potentially enhance the therapeutic window.**
- **BASECAMP-1 (NCT04981119) study is currently enrolling to identify HLA-A*02 LOH patients with colorectal, pancreatic and non-small cell lung cancers and then to bank their T cells for future autologous EVEREST novel Tmod logic-gated CAR T therapy interventional trials. Additional HLA blockers are being developed for future clinical studies**

List of BASECAMP-1 Sites on clinicaltrials.gov as of Nov 2021

- NYU Langone Medical Center (Recruiting)
  - Principal Investigator: Diane Simeone, MD
  - Sub-Investigator: Theodore Welling, MD
- Mayo Clinic Rochester
  - Principal Investigator: Julian Molina, MD, PhD
  - Sub-Investigator: Yi Lin, MD, PhD
- MD Anderson Cancer Center
  - Principal Investigator: Scott Kopetz, MD, PhD
  - Sub-Investigator: Maria Pia Morelli, MD, PhD
- University of California San Diego
  - Principal Investigator: Sandip Patel, MD
- UCLA Medical Center
  - Principal Investigator: J. Randolph Hecht, MD
  - Sub-Investigator: Edward Garon, MD

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  - Alain Silk, PhD – Director of Regulatory Affairs at Tempus
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