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# Correlation of Mesothelin (MSLN) Expression Measured by RNA Sequencing (RNAseq) and Immunohistochemistry (IHC) in MSLN-Expressing Tumors


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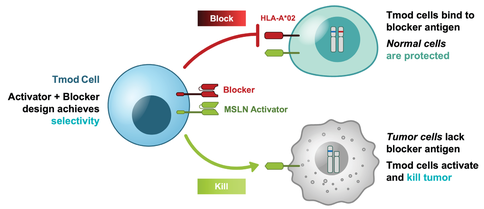
## BACKGROUND

- We are conducting clinical trials (such as EVEREST-2) of autologous logic-gated Tmod chimeric antigen receptor T-cell (CAR T) therapies
  - Tmod cells are logic-gated: the blocker component prevents CAR-mediated killing of normal cells; whereas, in tumor cells with human leukocyte antigen (HLA)-A*02 loss of heterozygosity (LOH), the blocker is no longer engaged, allowing the CAR to activate tumor cell killing (**Figure 1**)

### Figure 1. Tmod CAR Ts Discriminate Normal and Tumor Cells With HLA-A*02 LOH



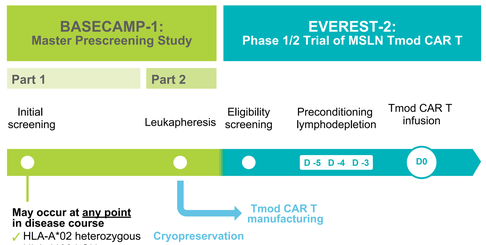

CAR T, chimeric antigen receptor T cell; HLA, human leukocyte antigen; LOH, loss of heterozygosity; MSLN, mesothelin.

- EVEREST-2 (NCT06051695) is a phase 1/2, open-label, nonrandomized study to evaluate the safety and efficacy of A2B694, a mesothelin (MSLN)-directed Tmod CAR T, in adults with MSLN-expressing solid tumors and tumor-associated HLA-A*02 LOH [1]
  - MSLN is a cell surface protein expressed in several cancer types, including mesothelioma (MESO), colorectal (CRC), non-small cell lung (NSCLC), ovarian (OVCA), and pancreatic (PANC) cancer, which can be associated with poor prognosis [2]
- To identify eligible patients for EVEREST-2, patients are screened through BASECAMP-1, a prescreening study that supports patient identification for interventional trials as well as collection of longitudinal clinical and biomarker data, including gene mutations and expression by genomic and RNA sequencing (RNAseq), for correlative analyses
  - RNAseq is an important tool for cancer research, providing expression profiles for a wide range of oncogenes and enabling research on tumor evolution and neoantigen expression [3]
  - The cost of RNAseq is approaching that of single immunohistochemistry (IHC) staining and analysis and provides more biomarker data, thus it may be a more cost-effective method for identifying patients with target protein expression [4]

## STUDY DESIGN AND OBJECTIVES

- BASECAMP-1 (NCT04981119) is an ongoing, master prescreening study (**Figure 2**) that uses a next-generation sequencing (NGS) assay (Tempus AI Inc) to identify patients with tumor-associated HLA-A*02 LOH for EVEREST-2

### Figure 2. BASECAMP-1 Study Schema




CAR T, chimeric antigen receptor T cell; D, day; HLA, human leukocyte antigen; LOH, loss of heterozygosity; MSLN, mesothelin.

- Key inclusion criteria for BASECAMP-1 include adult patients with germline HLA-A*02 heterozygosity and unresectable advanced or metastatic solid tumors and tumor-associated HLA-A*02 LOH
- Longitudinal clinical, genomic, and biomarker data were collected from participants enrolled in BASECAMP-1, providing a large dataset for translational discovery and propensity scoring
- The aim of this analysis was to determine whether MSLN expression measured by RNAseq and IHC are correlated among patients with solid tumors

## METHODS

- Tumor tissue from patients with germline heterozygous HLA-A*02 was tested for HLA-A*02 LOH using an investigational NGS device codeveloped with Tempus AI [5] that detects somatic alterations, including HLA LOH, and generates RNAseq data (eg, MSLN expression)
  - Gene-level transcripts per million (TPM) read values were determined
- Patients with HLA-A*02 LOH also submit tissue for IHC testing of MSLN expression. The IHC testing for MSLN expression was done using a laboratory-developed test (Labcorp) and the anti-MSLN clone 5B2
  - MSLN IHC expression was considered positive if the anti-MSLN clone is detected at any level
- Statistical significance was determined with ANOVA or t-tests

## MSLN EXPRESSION AS MEASURED BY IHC

- As of June 1, 2024, 64 patients had been screened for BASECAMP-1 and had MSLN IHC results; of these, 34 participants were positive for MSLN by IHC
- MSLN expression by IHC was consistently higher in participants with MESO, PANC, and OVCA vs NSCLC and CRC (**Table 1**)

### Table 1. MSLN IHC Expression by Tumor Type

| Tumor Type | Number of Samples | MSLN Positive, n (%) | MSLN Negative, n (%) |
|---|---|---|---|
| All | 64 | 34 (53) | 30 (47) |
| NSCLC | 4 | 1 (25) | 3 (75) |
| adNSCLC | 4 | 0 (0) | 4 (100) |
| sqNSCLC | 8 | 2 (25) | 6 (75) |
| MESO | 2 | 2 (100) | 0 (0) |
| CRC | 33 | 16 (48) | 17 (52) |
| PANC | 6 | 6 (100) | 0 (0) |
| OVCA | 7 | 7 (100) | 0 (0) |

ad, adenocarcinoma; CRC, colorectal cancer; IHC, immunohistochemistry; MESO, mesothelioma; MSLN, mesothelin; NSCLC, non-small cell lung cancer; OVCA, ovarian cancer; PANC, pancreatic cancer; sq, squamous cell.

## MSLN EXPRESSION MEASURED BY RNAseq

- As of June 1, 2024, 314 patients had been screened for BASECAMP-1 and had RNAseq results
- MSLN expression by RNAseq was consistently higher in participants with PANC or OVCA vs CRC or NSCLC ($P$<0.001) (**Table 2**)

### Table 2: MSLN RNAseq Expression by Tumor Type

| Tumor Type | Number of Samples | Mean TPM[a] (SD) |
|---|---|---|
| All | 314 | 5.1 (2.3) |
| adNSCLC | 25 | 3.8 (2.4) |
| sqNSCLC | 100 | 3.1 (1.8) |
| MESO | 148 | 4.5 (4.2) |
| CRC | 4 | 4.4 (2.0) |
| PANC | 4 | 6.1 (1.8) |
| OVCA | 33 | 7.2 (2.1) |

[a]MSLN log2 TPM + 1.
ad, adenocarcinoma; CRC, colorectal cancer; MESO, mesothelioma; MSLN, mesothelin; NSCLC, non-small cell lung cancer; OVCA, ovarian cancer: PANC, pancreatic cancer; RNAseq, RNA sequencing; SD, standard deviation; sq, squamous cell; TPM, transcripts per million.


### References

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  - Mark L. Sandberg, PhD, Scientific Director of Therapeutic Technology
  - Sanam Shafaattalab, PhD, Scientist
  - Wendy Langeberg, PhD, Clinical Development Associate Director

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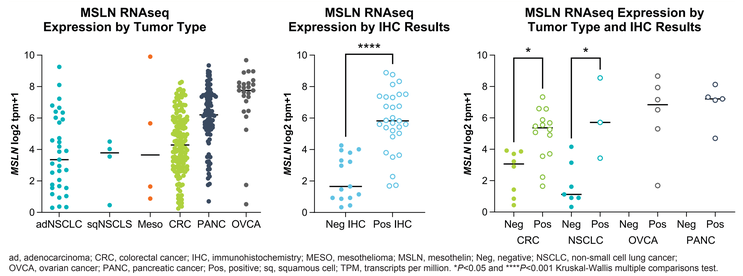
## CORRELATION OF MSLN EXPRESSION AS MEASURED BY IHC VS RNAseq

- A total of 34 participants had both RNAseq and IHC results available for correlative analyses
- MSLN expression by RNAseq and IHC were correlated overall ($P$<0.001) (**Figure 3**)

### Figure 3. MSLN Expression by IHC vs RNAseq




ad, adenocarcinoma; CRC, colorectal cancer; IHC, immunohistochemistry; MESO, mesothelioma; MSLN, mesothelin; Neg, negative; NSCLC, non-small cell lung cancer; OVCA, ovarian cancer; PANC, pancreatic cancer; Pos, positive; sq, squamous cell; TPM, transcripts per million. *$P$<0.05 and ****$P$<0.001 Kruskal-Wallis multiple comparisons test.

- Among the 8 participants with OVCA or PANC who were IHC+ for MSLN, only 1 had an RNAseq value below 6 log2 TPM+1; this participant had mesonephric-like ovarian adenocarcinoma, which may account for the low value (**Table 3**)
  - The one MESO sample with both RNAseq and IHC available was IHC+ and had a high RNAseq value

### Table 3: MSLN Expression Measured by RNAseq by IHC Status

| Tumor Type | MSLN Positive by IHC | | MSLN Negative by IHC | |
|---|---|---|---|---|
| | Number of Samples (n) | Mean TPM[a] (SD) | Number of Samples (n) | Mean TPM[a] (SD) |
| All | 20 | 6.0 (2.1) | 14 | 2.3 (1.7) |
| adNSCLC | 2 | 4.7 (1.7) | 7 | 1.5 (1.3) |
| sqNSCLC | 0 | — | 0 | — |
| MESO | 1 | 5.7 | 0 | — |
| CRC | 8 | 5.1 (2.1) | 7 | 3.1 (1.6) |
| PANC | 3 | 7.8 (0.6) | 0 | — |
| OVCA | 5 | 6.6 (2.8) | 0 | — |

[a]MSLN log2 TPM + 1.
ad, adenocarcinoma; CRC, colorectal cancer; IHC, immunohistochemistry; MESO, mesothelioma; MSLN, mesothelin; NSCLC, non-small cell lung cancer; OVCA, ovarian cancer; PANC, pancreatic cancer; RNAseq, RNA sequencing; SD, standard deviation; sq, squamous cell; TPM, transcripts per million.

## CONCLUSIONS

- MSLN expression measured by IHC is highly correlated to RNAseq expression in tumor tissue, supporting the use of RNAseq to identify patients with MSLN expression for interventional clinical trials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