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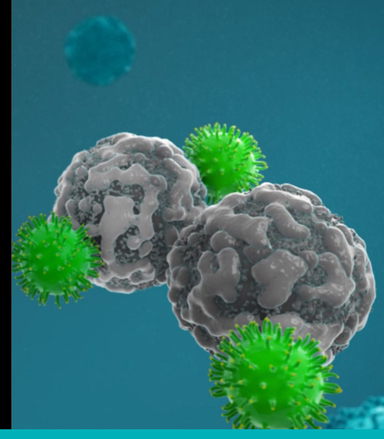
EVEREST-2: A seamless phase 1/2 study of A2B694, a mesothelin (MSLN) logic-gated Tmod CAR T-cell therapy, in patients with solid tumors that show MSLN expression and human leukocyte antigen (HLA)-A*02 loss of heterozygosity (LOH)



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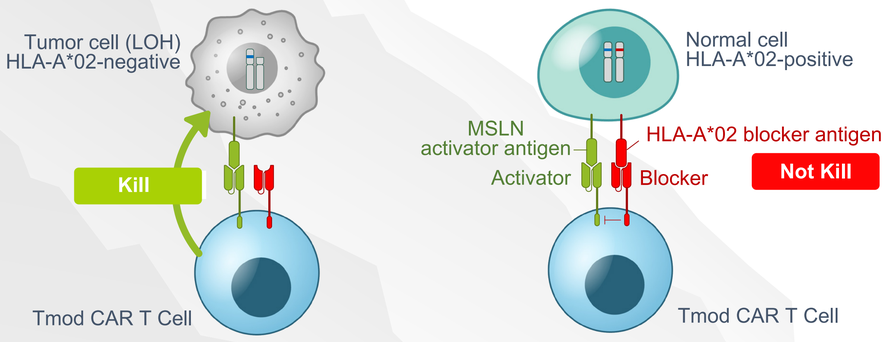
BACKGROUND AND STUDY OBJECTIVES

- Implementation of chimeric antigen receptor T-cell (CAR T) therapies in solid tumors has been challenging due to a lack of tumor-specific targets that discriminate cancer from normal cells; for example, CAR T and T-cell receptor fusion therapies targeting mesothelin (MSLN), which is normally expressed in mesothelial cells, but can be upregulated in colorectal, ovarian, lung, and pancreatic cancers, have been hampered by on-target, off-tumor toxicity, including fatal events [1-3]
- A2B694 is an MSLN-directed Tmod™ CAR T therapy construct that combines a CAR-activating receptor with a leukocyte immunoglobulin-like receptor-1–based inhibitory receptor (LIR-1; blocker) targeting HLA-A*02 to discriminate tumor from normal cells (**Figures 1 and 2**) [4,5]
  - The activator receptor recognizes MSLN on the surface of both tumor and normal cells
  - The blocker receptor recognizes an HLA-A*02 allele: for patients who are germline HLA-A*02 heterozygous, loss of the allele may occur in tumor cells, known as loss of heterozygosity (LOH) [6], which can be detected using the Tempus next-generation sequencing (NGS; **Table 1**)
- EVEREST-2 (NCT06051695), the 2nd A2 Bio interventional clinical trial, is a seamless, phase 1/2, open-label, nonrandomized study to evaluate the safety and efficacy of A2B694 in adult patients with solid tumors

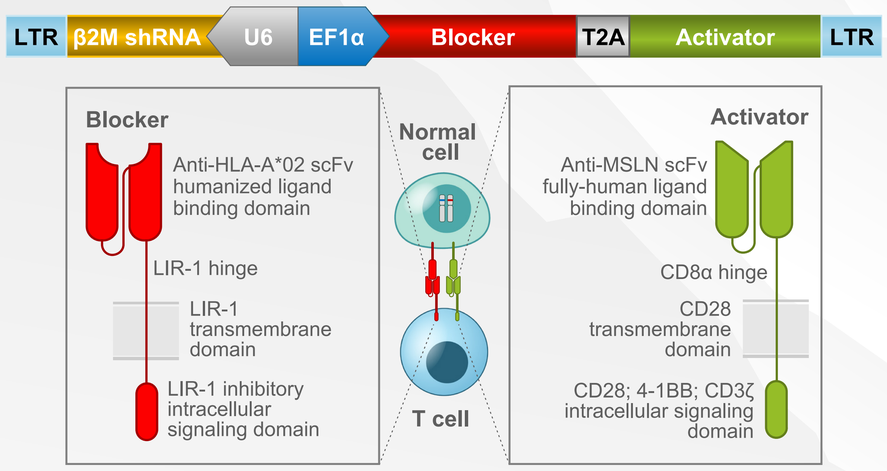
STUDY RATIONALE

Figure 1. Logic-Gated CAR T With the Goal to Reduce Toxicity: MSLN (Activator) and HLA-A*02 (Blocker) [4]



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

Figure 2. The Structure of Tmod CAR Ts Expressing an MSLN-Targeted Activator and an HLA-A*02-Targeted Blocker



The Tmod CAR construct is designed for safety with the LIR-1 inhibitory blocker [7] transcribing before the anti-MSLN activator

β2M shRNA, beta-2-microglobulin short-hairpin RNA; CAR, chimeric antigen receptor; CD, cluster of differentiation; EF1α, elongation factor 1 alpha; HLA, human leukocyte antigen; LIR, leukocyte immunoglobulin-like receptor; MSLN, mesothelin; scFv, single-chain variable fragment; T2A, thosea asigna virus 2A.

Table 1. Frequency of HLA-A LOH in Advanced Tumors [8]

	Tempus HLA-A LOH advanced disease real-world
Average, % (n)	16.3 (10,867)
Colorectal cancer, % (n)	15.6 (1854)
Mesothelioma, % (n)	14.3 (7)
NSCLC, % (n)	23.1 (1915)
Ovarian, fallopian tube, primary peritoneal cancer, % (n)	16.0 (569)
Pancreatic cancer, % (n)	19.6 (675)

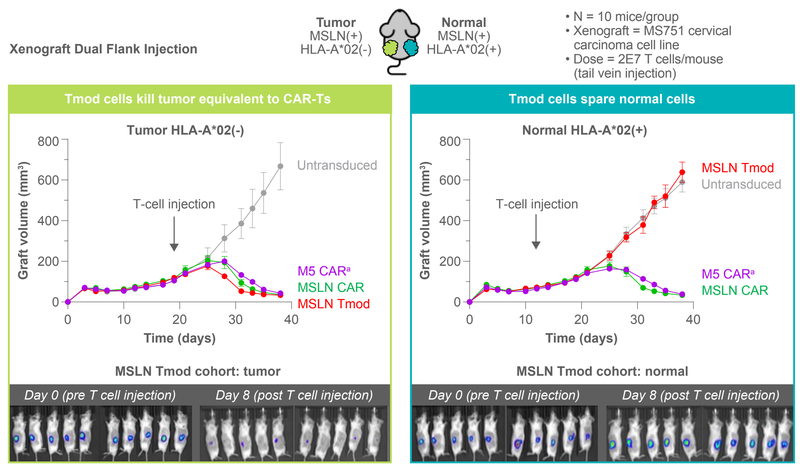
HLA, human leukocyte antigen; LOH, loss of heterozygosity; NSCLC, non-small cell lung cancer.

STUDY RATIONALE (CONTINUED)

Nonclinical Studies

- Nonclinical studies of A2B694 demonstrated improved selectivity and a therapeutic safety window with comparable efficacy to the MSLN-directed M5 CAR T [2,9]
  - Approximately 2 weeks following cell infusion, A2B694 treated NOD scid gamma mice experienced selective regression of tumor grafts (HLA-A*02-), while "normal" grafts (HLA-A*02+) continued to grow. Mice treated with MSLN-targeted M5 CAR Ts experienced regressions of both tumor and "normal" grafts (**Figure 3**)

Figure 3. MSLN Tmod (A2B694) In Vivo Study Demonstrates Efficacy Comparable to M5 CAR T Benchmark

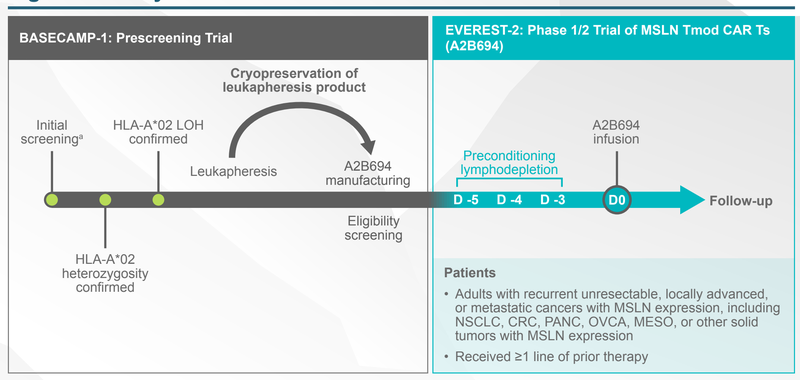


[a]M5 CAR is a MSLN-directed CAR T therapy that was clinically tested in a phase I trial [2].
CAR, chimeric antigen receptor; HLA, human leukocyte antigen; MSLN, mesothelin; UTD, untransduced T-cells.

STUDY DESIGN

- EVEREST-2 (NCT06051695) is a first-in-human, phase 1/2, multicenter, open-label, nonrandomized study to evaluate the safety and efficacy of a single-dose of A2B694 Tmod CAR Ts in adults with recurrent unresectable, locally advanced, or metastatic cancers with MSLN expression
- Patients are enrolled to EVEREST-2 through BASECAMP-1 (NCT04981119), a master prescreening study that identifies patients with HLA LOH at any time in the course of their disease; enrolled patients undergo leukapheresis and, when clinically appropriate, CAR Ts are manufactured for the EVEREST-2 study (**Figure 4**)

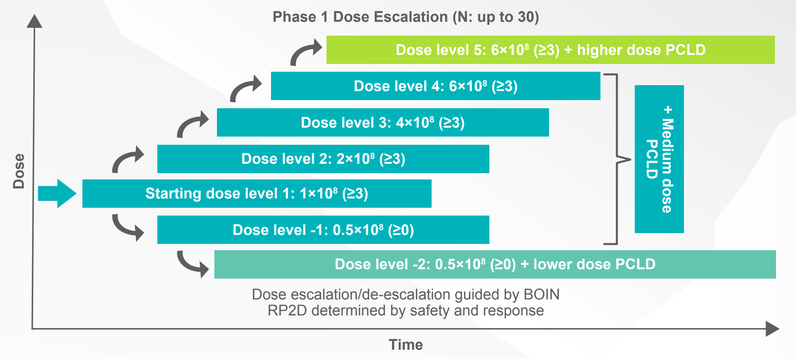
Figure 4. Study Schema: BASECAMP-1 to EVEREST-2



[a] May occur at any point in disease course.
CAR, chimeric antigen receptor; CRC, colorectal cancer; HLA, human leukocyte antigen; LOH, loss of heterozygosity; MESO, mesothelioma; MSLN, mesothelin; NSCLC, non-small cell lung cancer; OVCA, ovarian cancer; PANC, pancreatic cancer.

- The phase 1 dose escalation portion of the study employs a Bayesian optimal interval design (BOIN) to assess the safety and tolerability of A2B694 and to determine a recommended phase 2 dose (RP2D; **Figure 5**); up to 30 patients will be included in the dose escalation

Figure 5. EVEREST-2 Phase 1 Dose Escalation/Expansion and Phase 2 Study Design



BOIN, Bayesian optimal interval design; PCLD, preconditioning lymphodepletion; RP2D, recommended phase 2 dose.

STUDY DESIGN (CONTINUED)

Inclusion Criteria

- Appropriately enrolled in the BASECAMP-1 study, with tissue demonstrating LOH of HLA-A*02 by NGS (whenever possible from the primary site), successful apheresis and peripheral blood mononuclear cell processing, and with sufficient stored cells available for Tmod therapy
- Histologically confirmed recurrent unresectable, locally advanced, or metastatic CRC, NSCLC, PANC, OVCA, MESO, or other solid tumors with MSLN expression; measurable disease is required with lesions of >1.0 cm by computed tomography
- Received previous required therapy for the appropriate solid tumor disease as described in the protocol
- Has adequate organ function as described in the protocol
- ECOG performance status of 0 to 1
- Life expectancy of ≥3 months
- Willing to comply with study schedule of assessments including long-term safety follow-up

Figure 6. EVEREST-2 Study Objectives and Endpoints

Objectives

- **Phase 1:** Determine the safety and the optimal dose of A2B694 (after PCLD) in participants with solid tumor disease
- **Phase 2:** Determine the further safety and efficacy of A2B694

Primary Endpoints

- **Phase 1:** Rate of adverse events and dose-limiting toxicities by dose levels; recommended phase 2 dose
- **Phase 2:** Overall response rate

Secondary Endpoints

- Persistence of A2B694
- Serum cytokine analysis

AE, adverse event; DLT, dose-limiting toxicity; ICR, independent central review; RECIST, Response Evaluation Criteria in Solid Tumors; RP2D, recommended phase 2 dose.

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