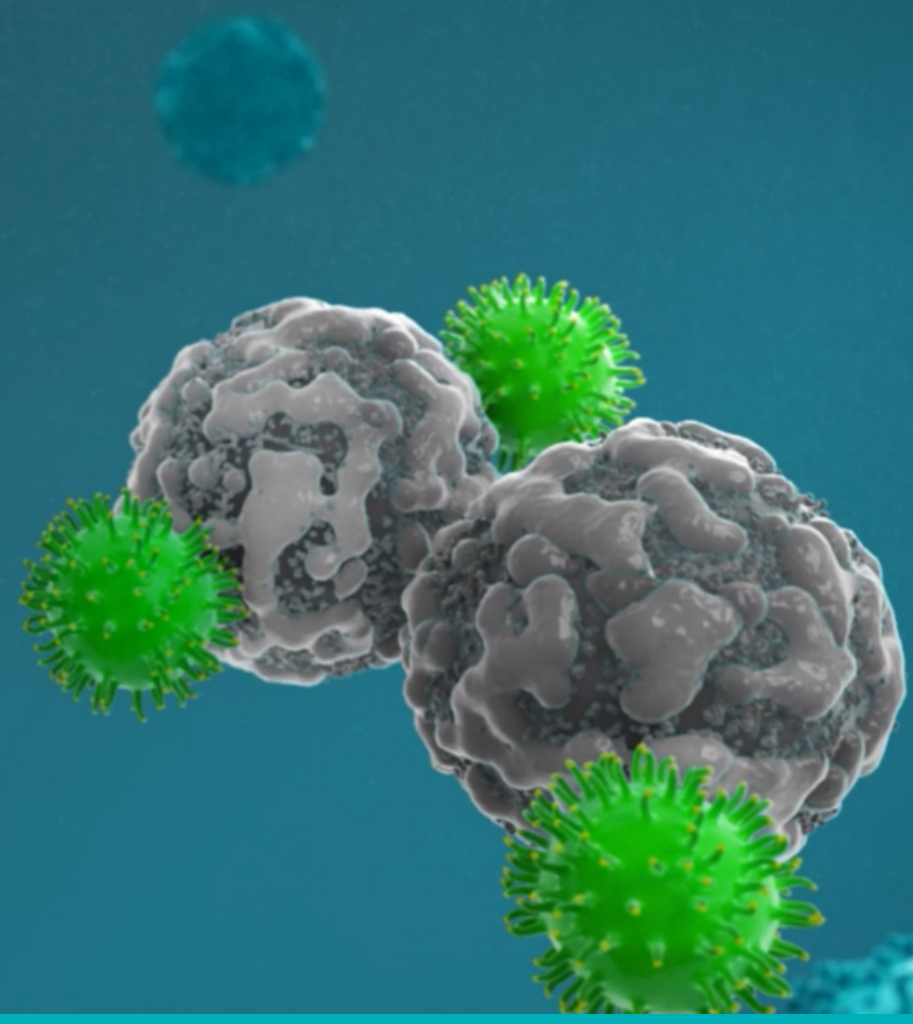


BASECAMP-1: Leveraging Human Leukocyte Antigen A (HLA-A) Loss of Heterozygosity (LOH) in Solid Tumors to Identify Patients for Carcinoembryonic Antigen (CEA) and Mesothelin (MSLN) Logic-Gated Tmod Chimeric Antigen Receptor (CAR) T-Cell Therapy



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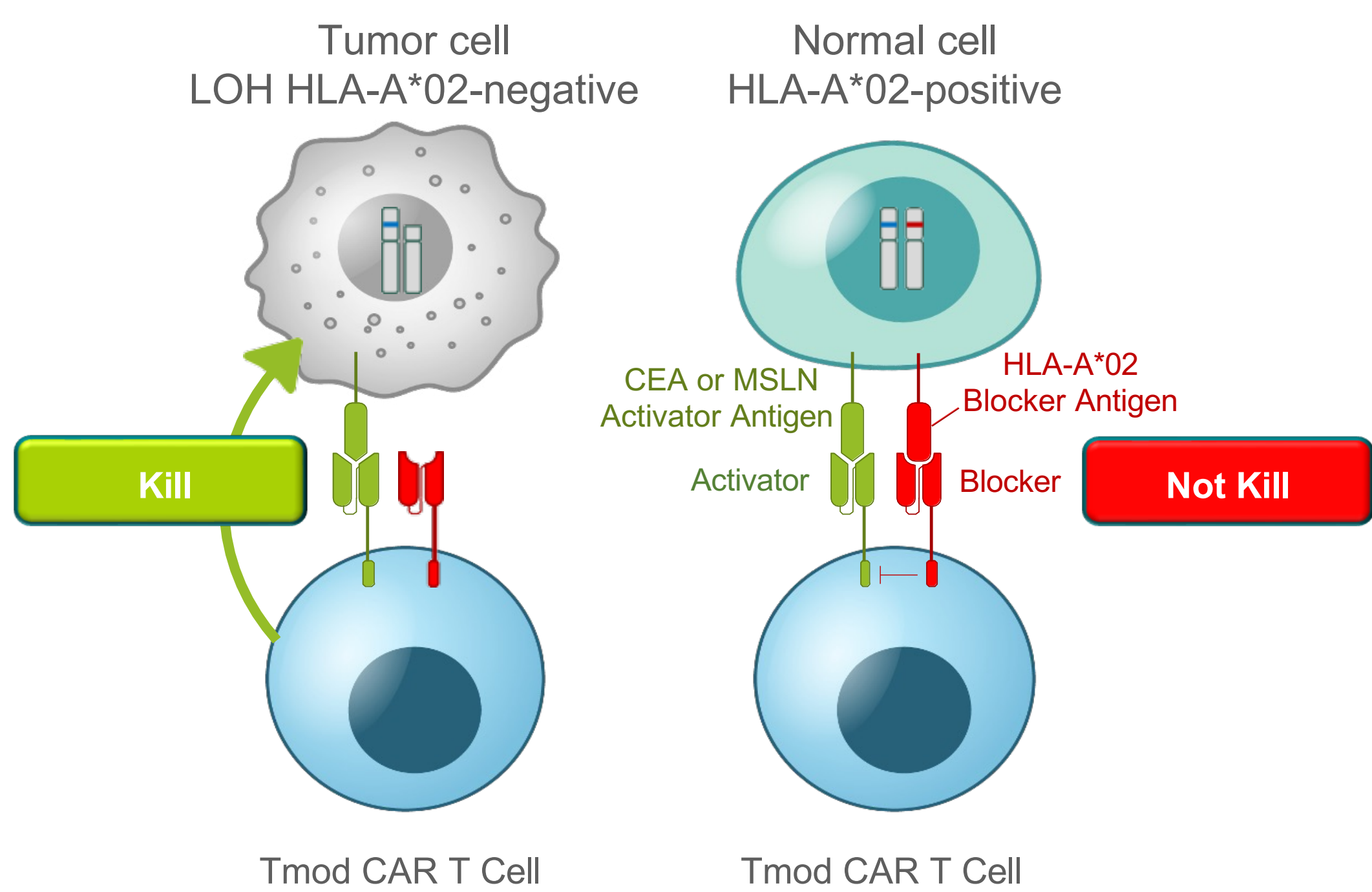
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BACKGROUND

- 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

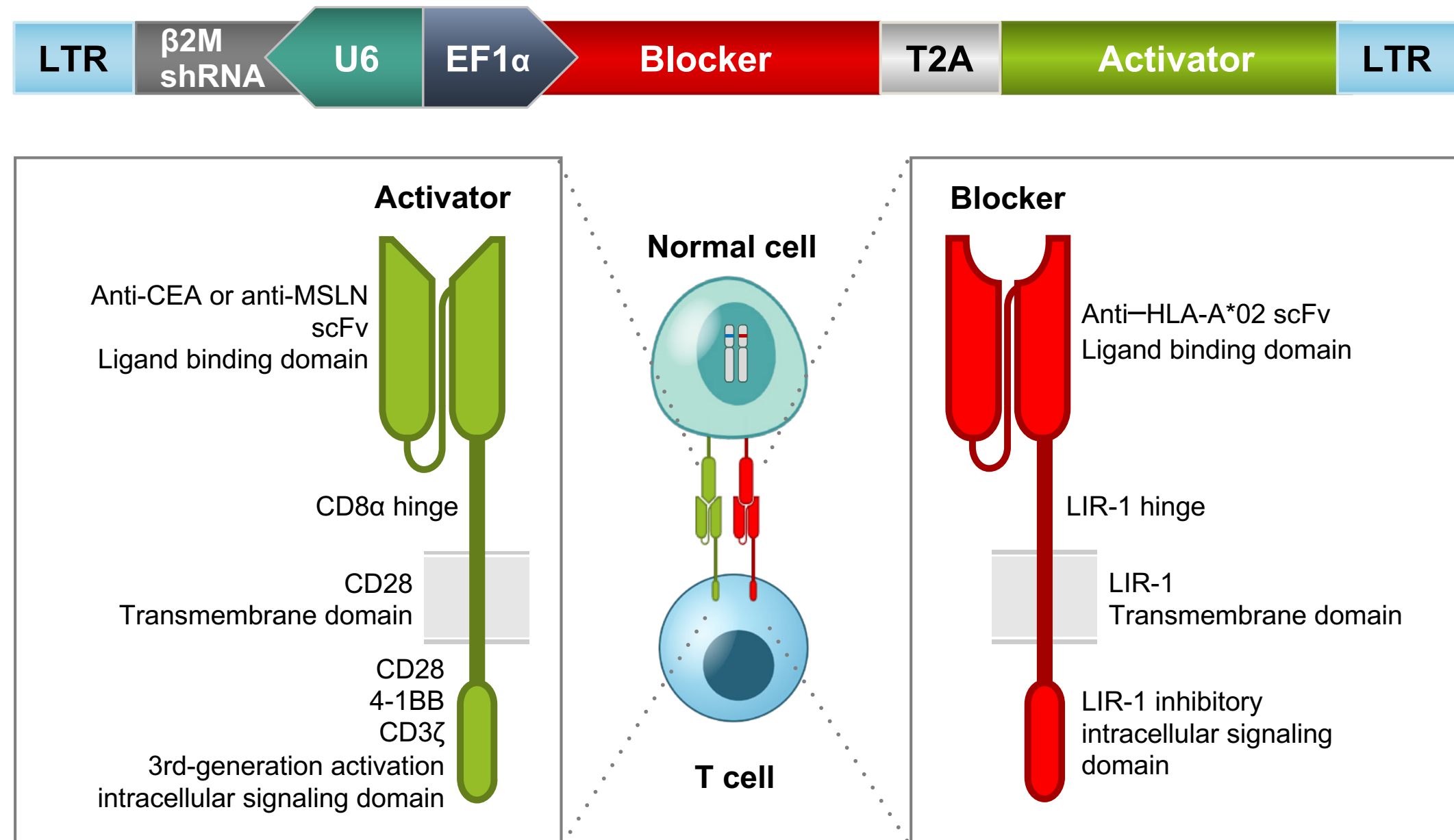
Figure 1. Logic-gated CAR T with the goal of reducing toxicity: CEA or MSLN (activators) and HLA-A*02 (blocker)



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

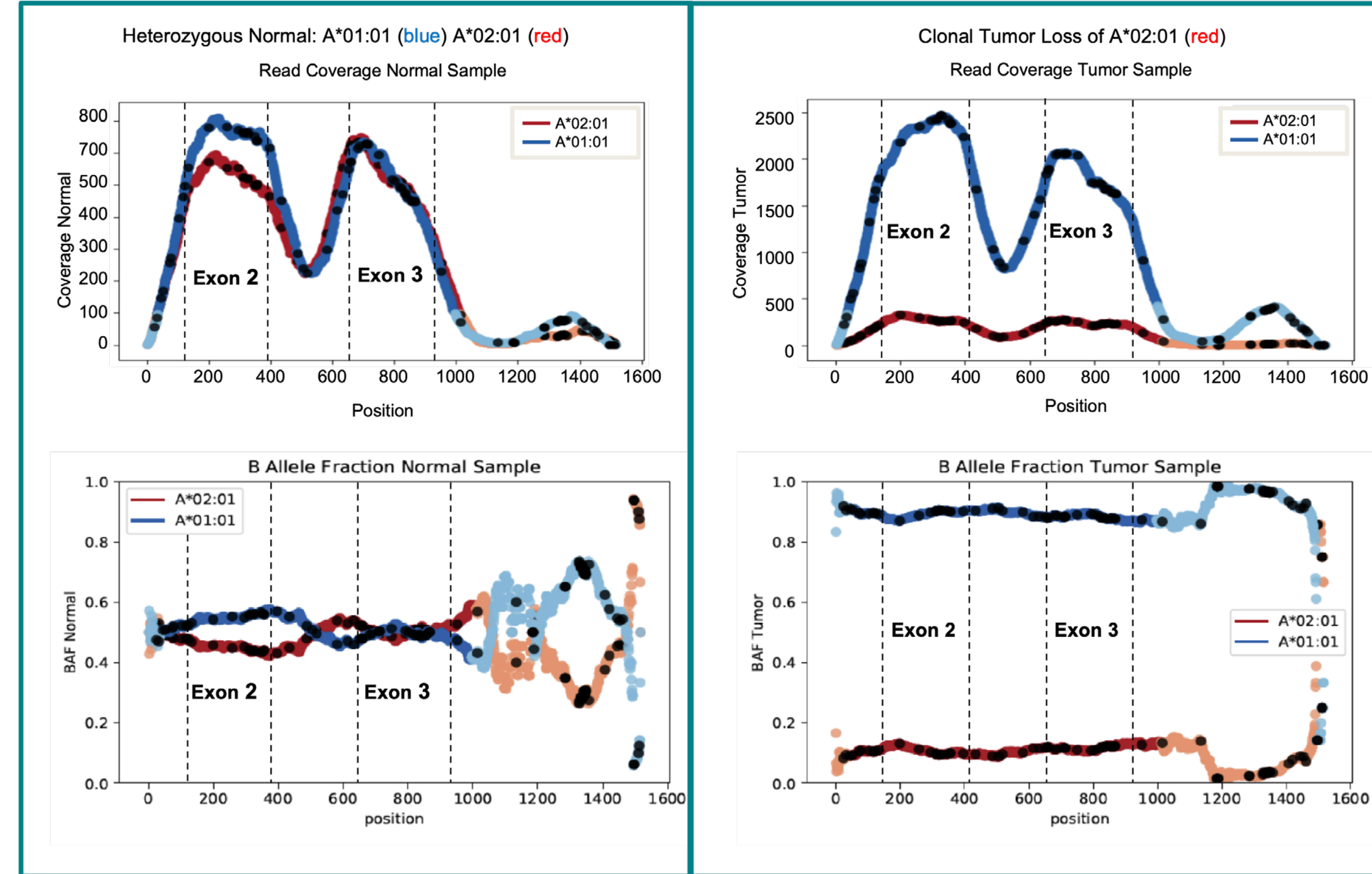


β2M shRNA, β2-microglobulin short-hairpin RNA; CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen 5; EF1α, elongation factor-1; HLA, human leukocyte antigen; LIR-1, leukocyte immunoglobulin-like receptor 1; LTR, long terminal repeat; MSLN, mesothelin; scFv, single chain variable fragment; T2A, thioesterase active site 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)

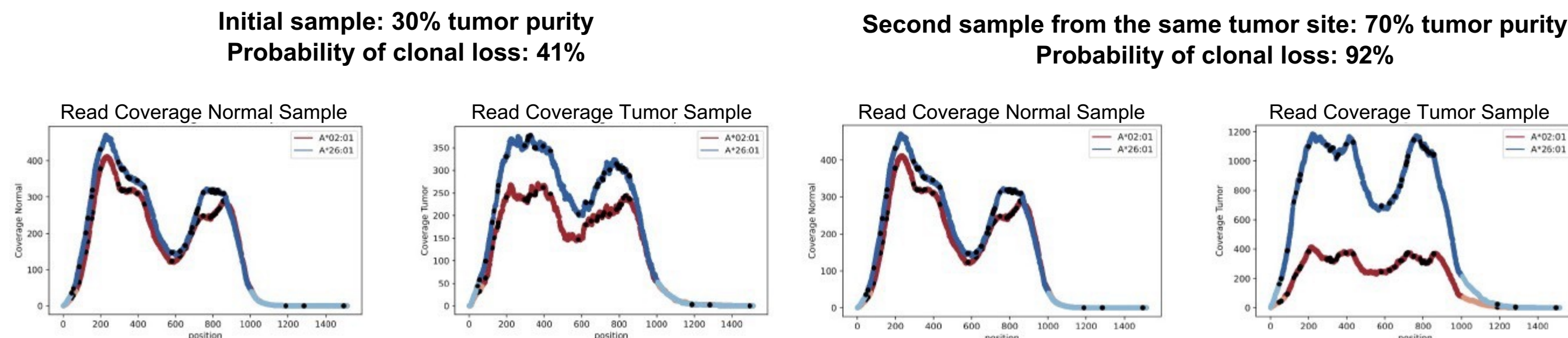
Table 1. Frequency of HLA-A LOH in advanced solid tumors^a

	Tempus HLA-A LOH advanced disease real-world data [9]		TCGA HLA-A LOH primary tumors [12]		Montesin 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.

^aTempus data contain more advanced disease, and TCGA data have more primary tumors. ^bUnpublished data.

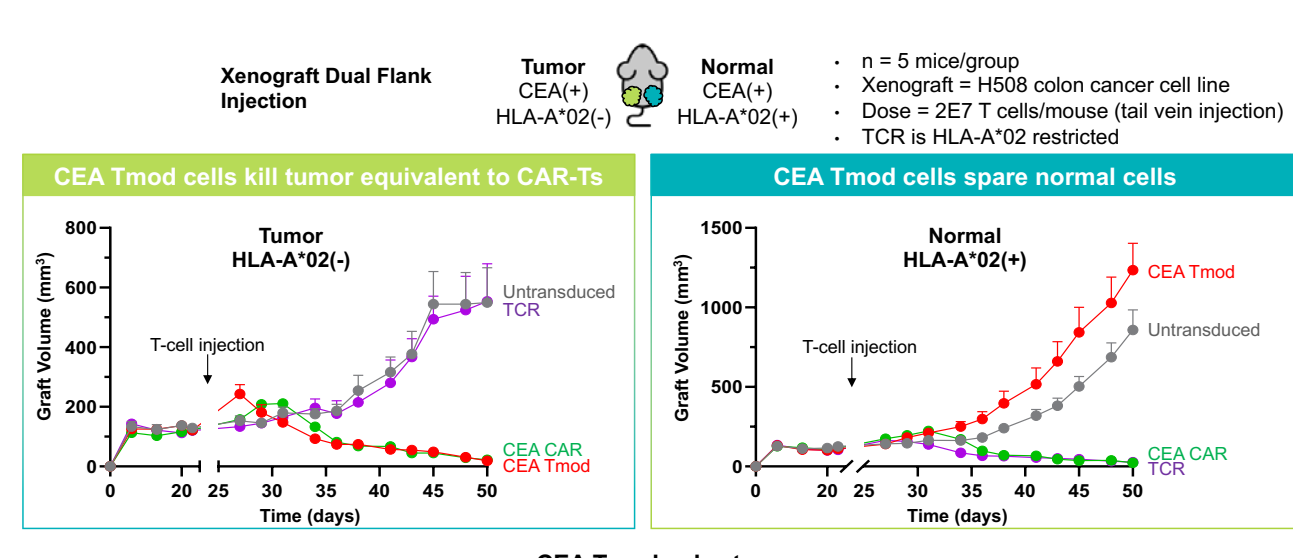
Figure 4. Higher tumor purity allows for more accurate prediction of HLA-A*02 LOH



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

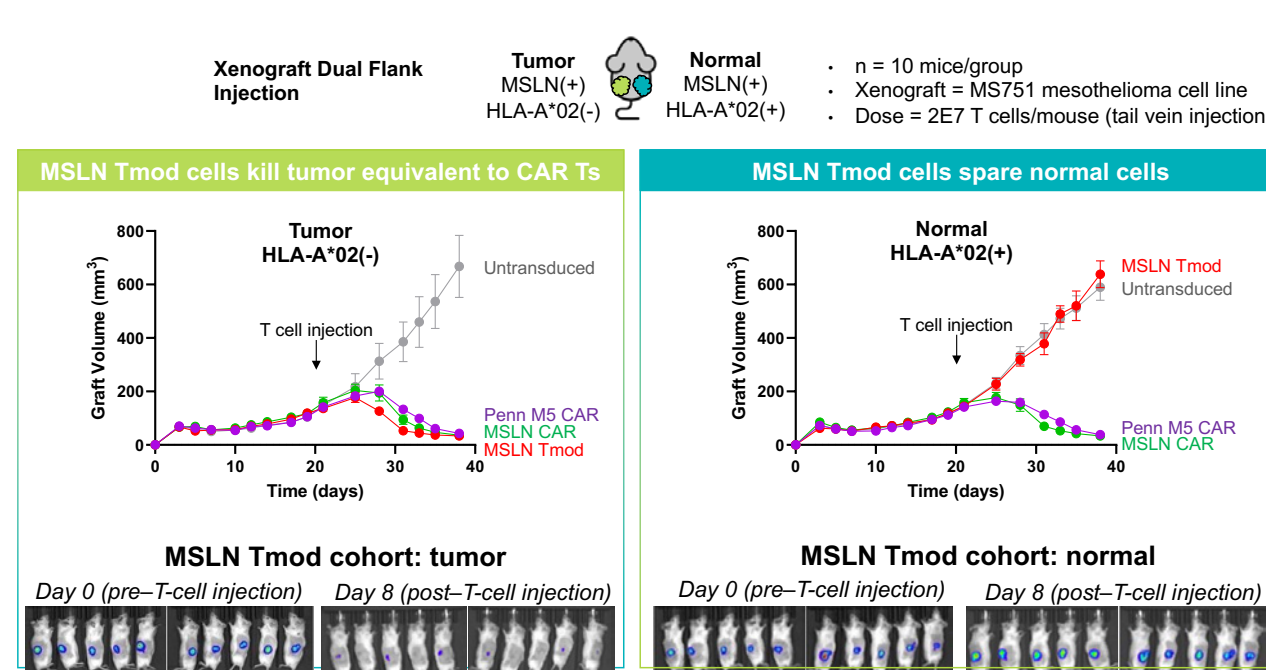
- In vivo studies show that Tmod maintains selectivity
- Tumor (HLA-A*02-) and "normal" (HLA-A*02+) cells were implanted subcutaneously in NOD scid gamma (NSG) mice
- CAR T cells or Tmod CAR T cells were administered via tail veins when tumor reached 100-150 mm³
- Approximately 2 weeks following cell 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.

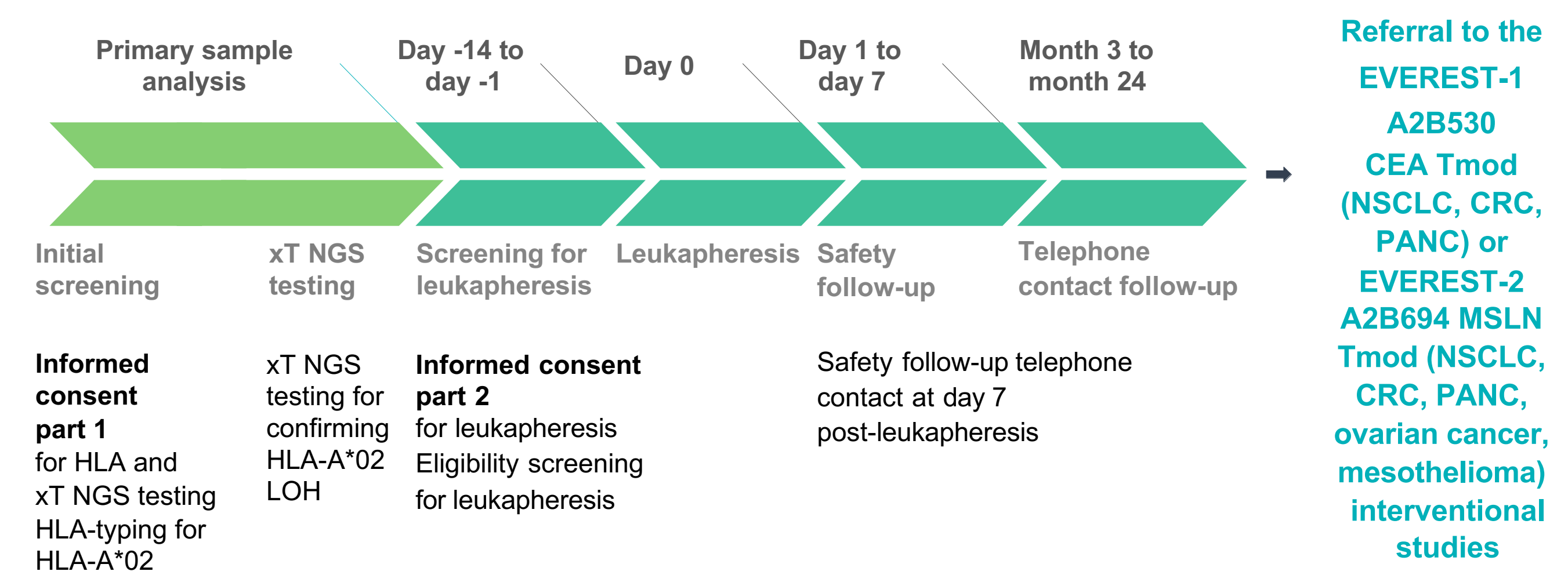
Figure 6. MSLN Tmod (A2B694) in vivo study demonstrates potency comparable to M5 benchmark MSLN CAR T [15]



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

STUDY DESIGN AND METHODS

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

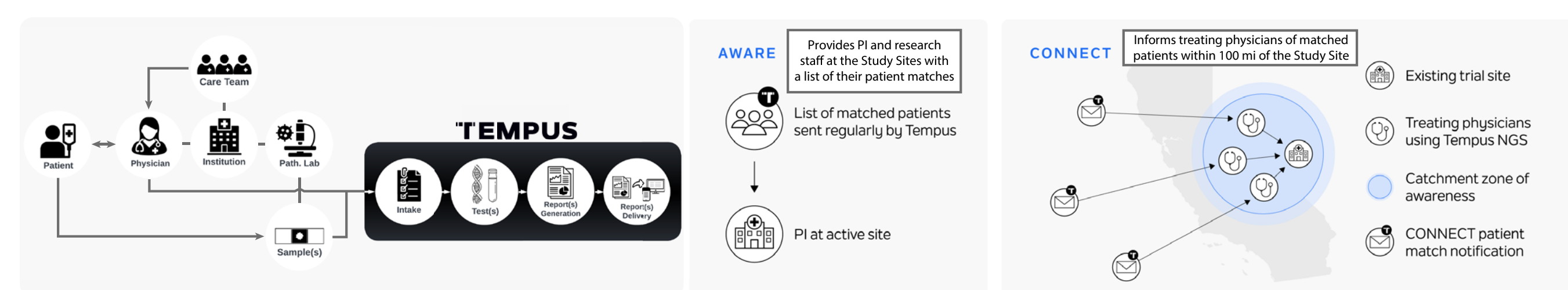


CEA, carcinoembryonic antigen 5; CRC, colorectal cancer; HLA, human leukocyte antigen; LOH, loss of heterozygosity; MSLN, mesothelin; NGS, next-generation sequencing;

NSCLC, non-small cell lung cancer; PANC, pancreatic cancer.

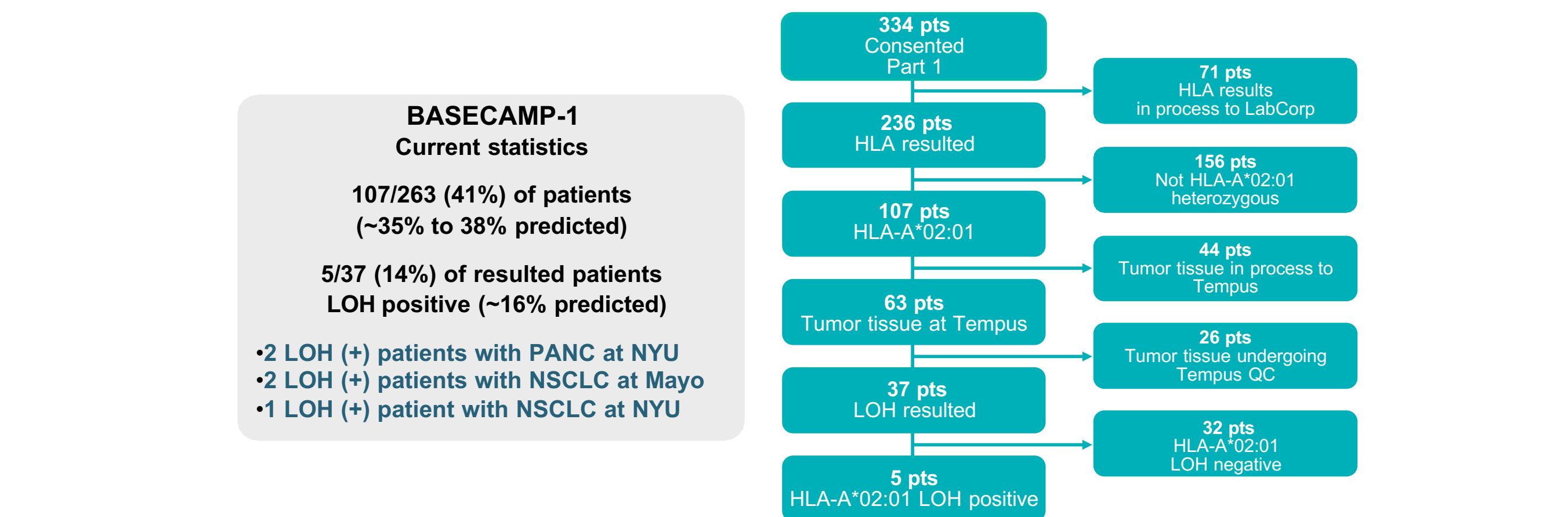
- Participants will be initially screened to identify germline HLA-A*02 heterozygosity by central NGS. If HLA-A*02 heterozygosity is confirmed, primary tumor tissue will be analyzed by xT NGS testing to determine if somatic tumor HLA-A*02 LOH is present. If the tumor demonstrates HLA-A*02 LOH and the 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.

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Acknowledgements

The authors would like to thank:

- Alexander Kamb, PhD – Founder and Chief Scientific Officer of A2 Bio
- Han Xu, PhD – Vice President of Therapeutic Technology at A2 Bio
- Agnes E. Hamburger, PhD – Vice President of Drug Discovery at A2 Bio
- Armen Mardiros, PhD – Scientific Director of Therapeutic Technology at A2 Bio
- Mark L. Sandberg, PhD – Scientific Director of Drug Discovery at A2 Bio
- Talar Tokalian, PhD – Principal Scientist at A2 Bio
- Bio Connections, LLC

This study was supported by A2 Bio

