Abstract
Number
639

BASECAMP-1: Leveraging HLA-A loss of heterozygosity in solid tumors by NGS to identify patients with relapsed solid tumors for future CEA and MSLN logic-gated Tmod™ CAR T-cell therapy

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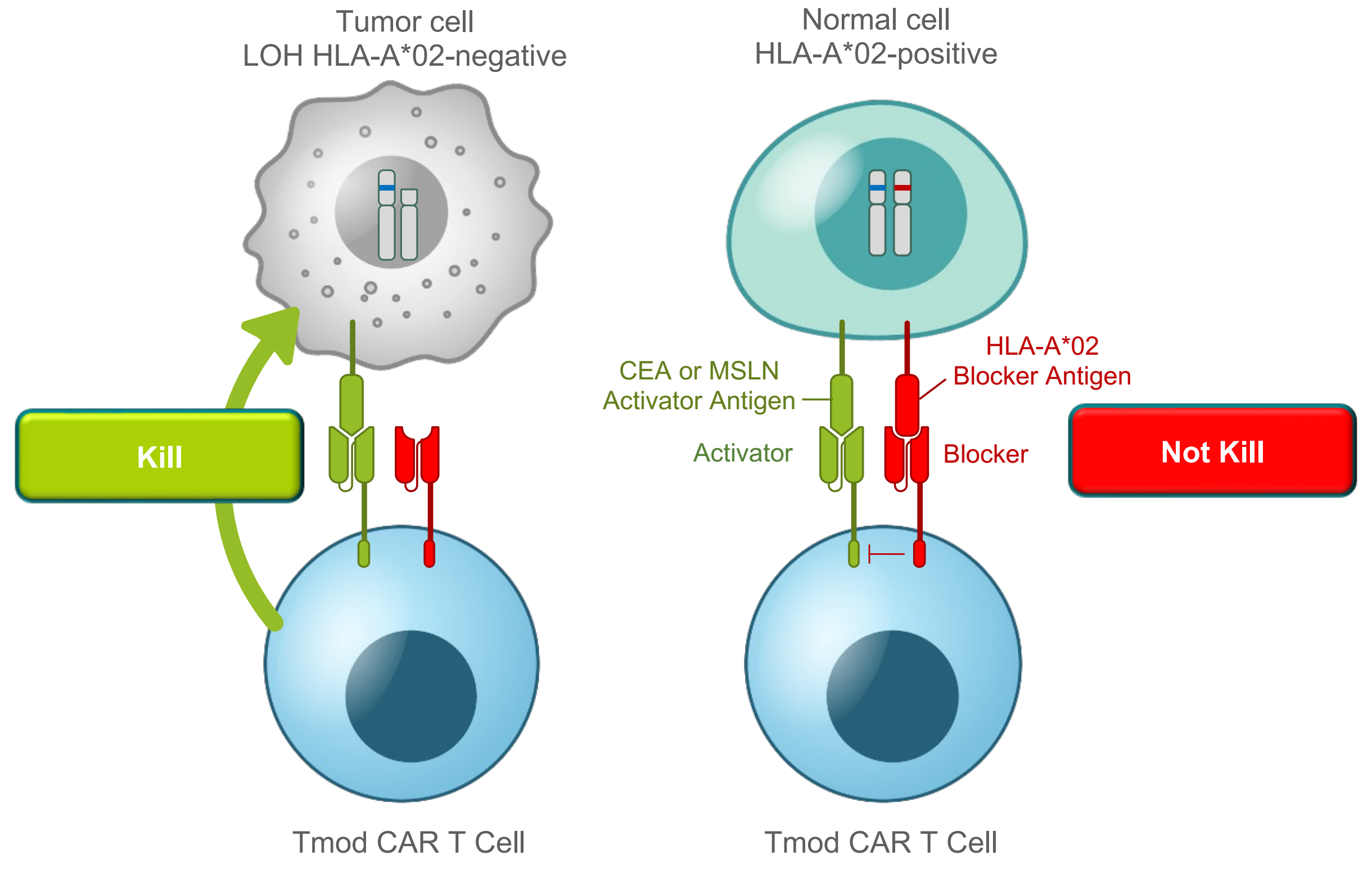
BACKGROUND AND STUDY OBJECTIVES

- Solid tumors comprise >90% of cancers. Non-small cell lung cancer (NSCLC), colorectal cancer (CRC), and pancreatic cancer (PANC) are the leading causes of cancer-related mortality (metastatic 5-year survival rate of 8%, 15%, 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 that distinguish 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 [4-6]
- 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 both utilizing a leukocyte immunoglobulin-like receptor (LIR) 1-based inhibitory receptor (blocker) targeting HLA-A*02
- Human leukocyte antigen loss of heterozygosity (HLA LOH) may provide a means to distinguish between tumor and 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-A allele lost only in tumor cells
- Based on the Tempus xT real-world database, HLA-A LOH occurs in 12.2% to 26.0% of advanced solid tumors with an average of 16.3% in 10,867 samples tested [9]
- BASECAMP-1 is an ongoing study with the following key objectives: 1) To determine and identify patients with somatic HLA-A 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 A2B530 CEA Tmod and 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)

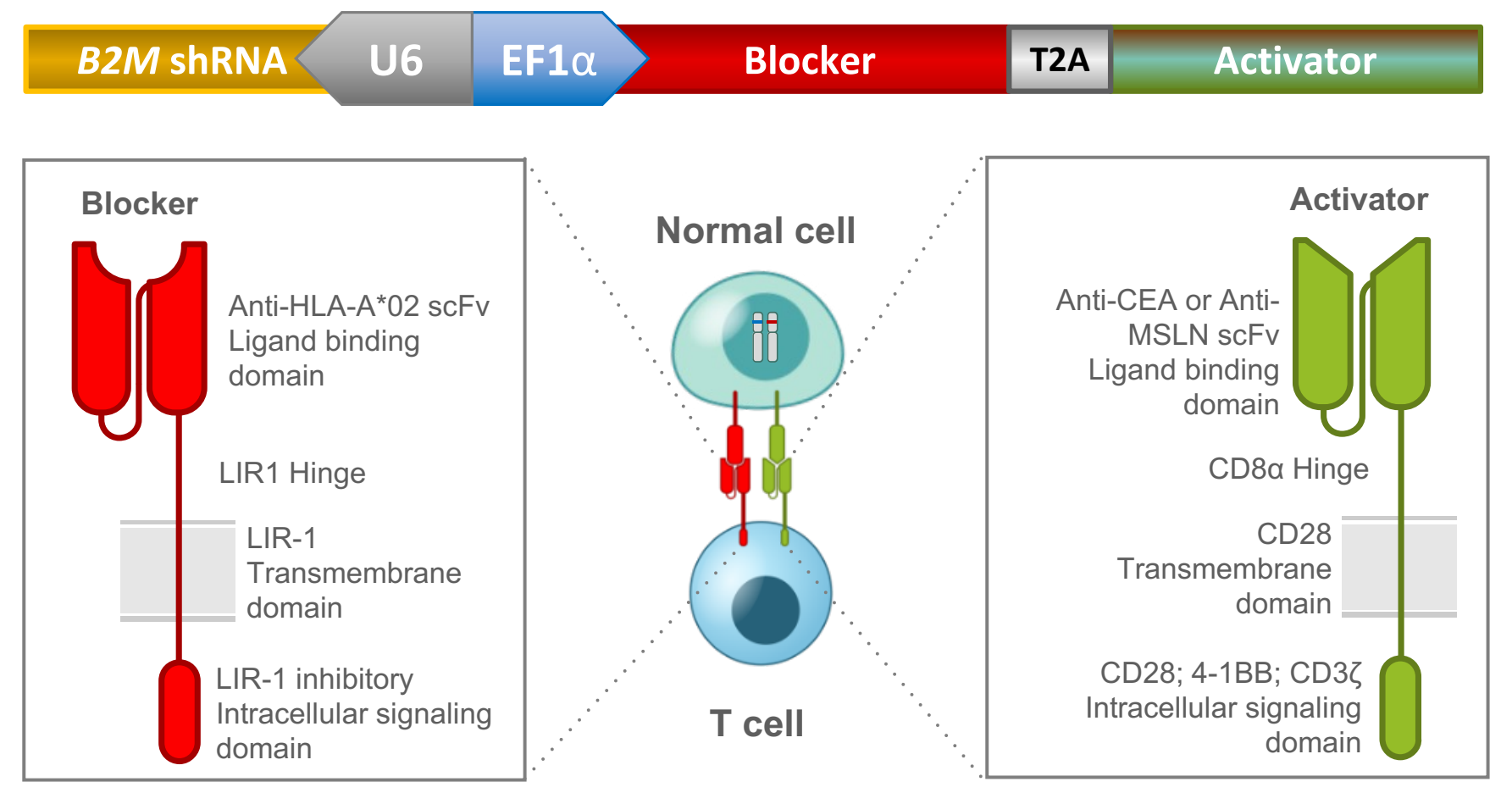


CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen; HLA, human leukocyte antigen; LOH, loss of heterozygosity; MSLN, mesothelin.

- A2 Bio's Tmod CAR T HLA-A 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 have dose-limiting toxicities in previous studies
- CAR T HLA-A LOH approach is independently validated by Vogelstein/Kinzler, 2021 [8]

STUDY RATIONALE (cont.)

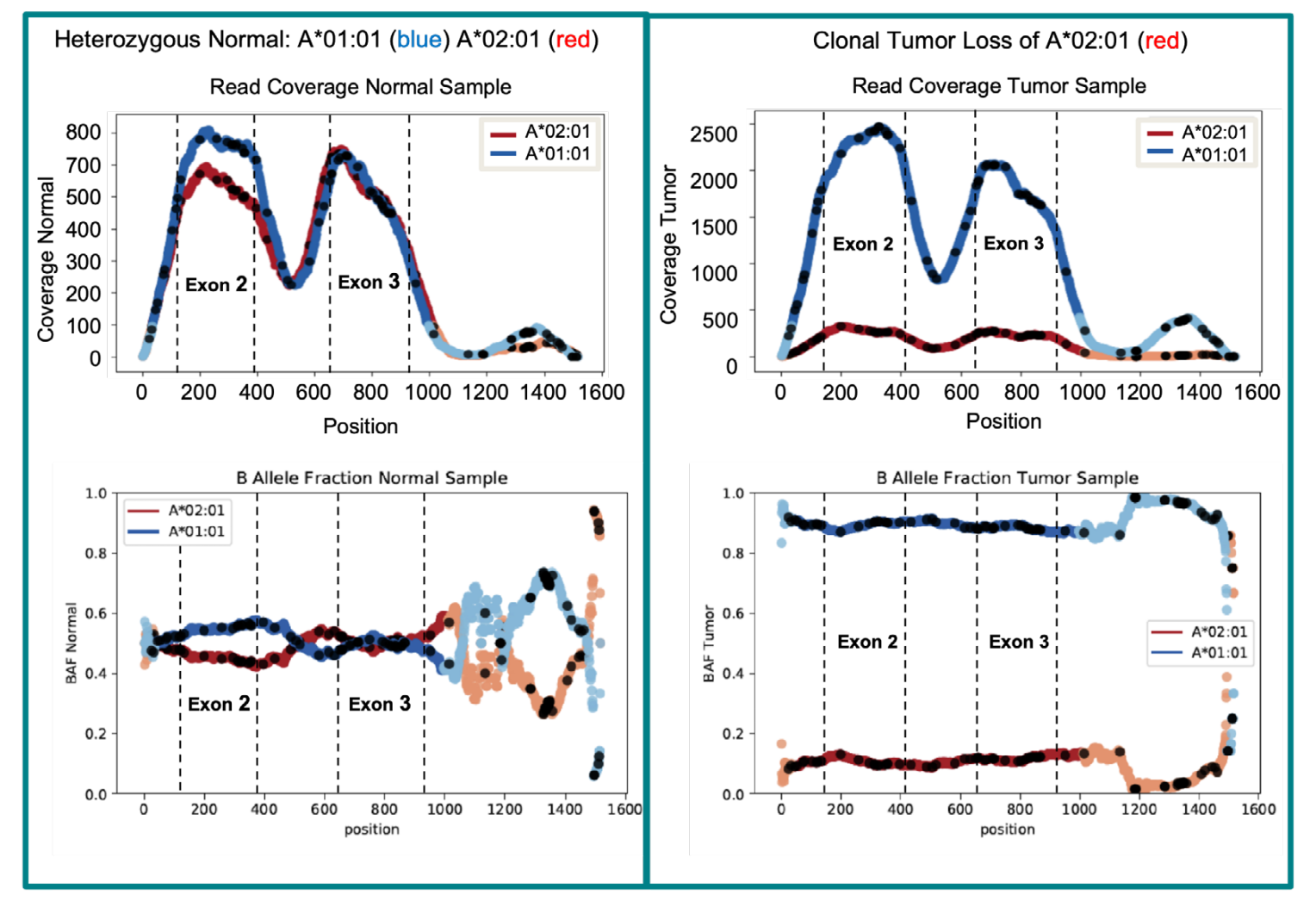
Figure 2. CEA or MSLN CAR Tmod single vector construct [10]



B2M shRNA, β2-microglobulin short-hairpin RNA; CEA, carcinoembryonic antigen; EF1a, elongation factor-1; HLA, human leukocyte antigen; LIR, leukocyte immunoglobulin-like receptor; LTR, long terminal repeat; MSLN, mesothelin; scFv, single-chain variable fragment; T2A, thosea asigna virus 2A

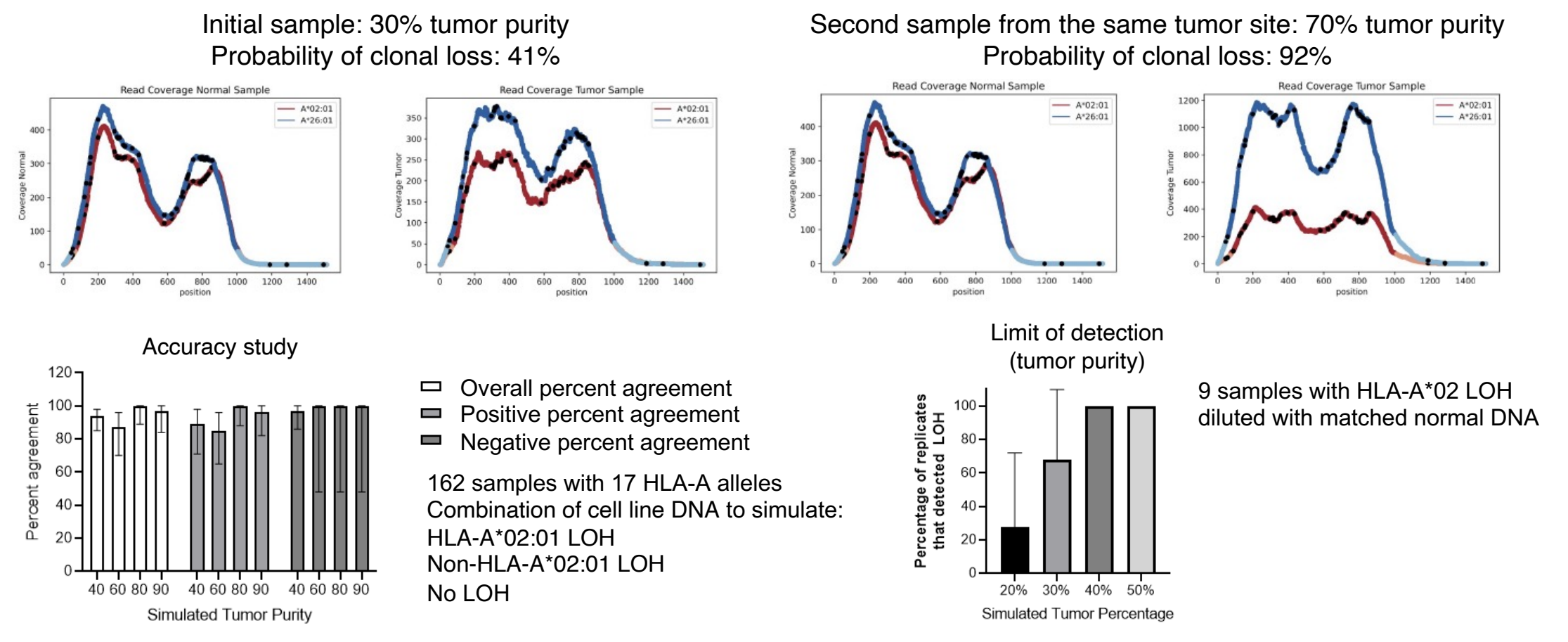
- CAR activator: 3rd-generation CAR T with both signal 1 (CD3ζ) and signal 2 activation domains (CD28 & 4-1BB)
- CAR blocker: LIR-1 is a member of the immune inhibitory receptor family and contains 4 immunoreceptor tyrosine-based inhibition motifs in its signaling domain [10]
- Replicant incompetent single lentivirus transgene: The activator and blocker receptors are co-expressed in a single construct containing a cleavable T2A linker (**Figure 2**)

Figure 3. Read coverage and B allele fraction (ratio of coverage for allele 1 and allele 2)



- A representative example of clonal HLA-A LOH (**Figure 3**), where a discordance is observed in read coverage of HLA-A*02:01 between the tumor and matched-normal samples [9, 11]
- HLA-A LOH can be reliably detected using the Tempus xT next-generation sequencing (NGS) assay (**Table 1**)

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



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

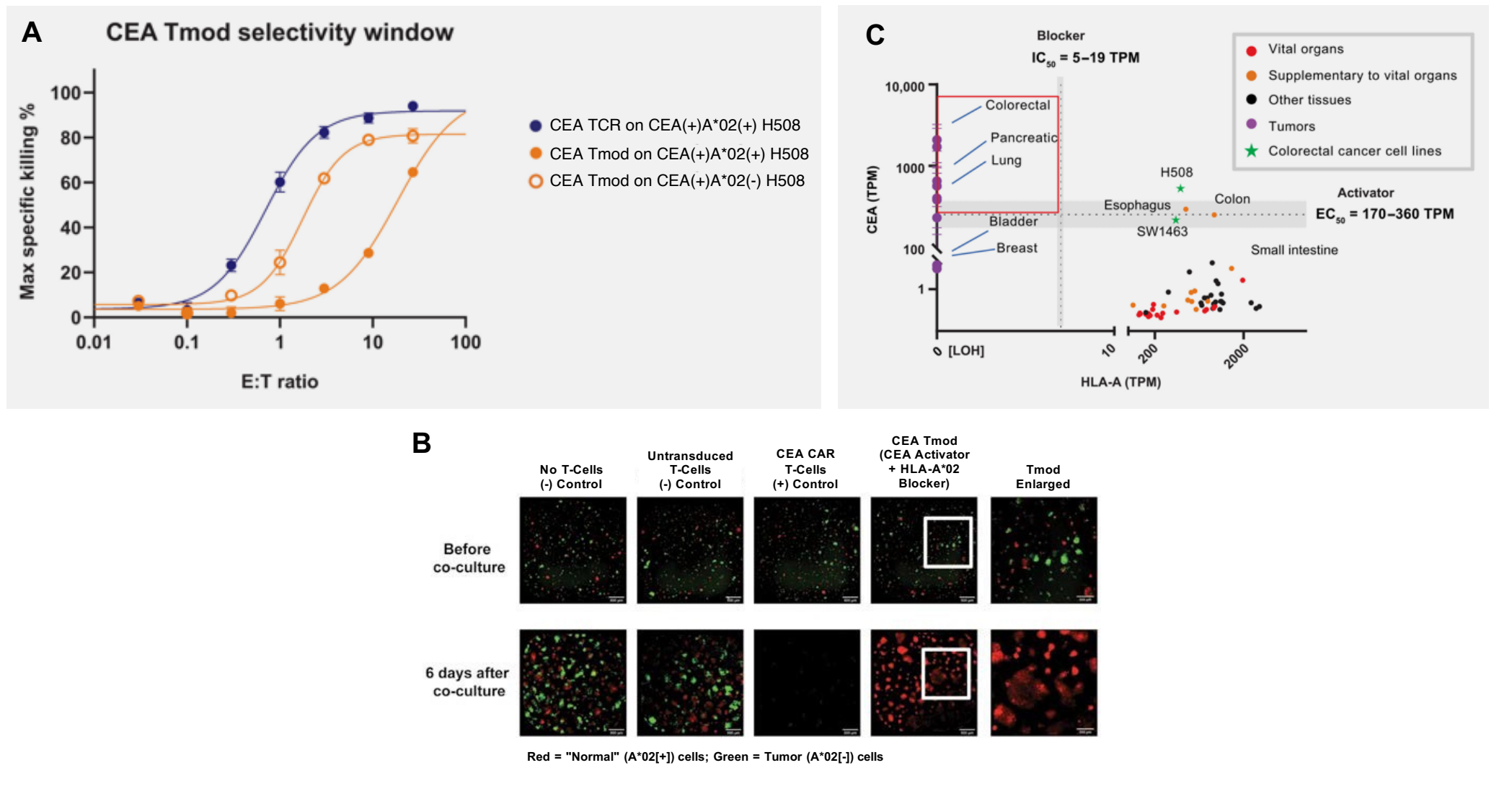
STUDY RATIONALE (cont.)

Table 1. Frequency of HLA-A LOH in advanced tumors[a]

	Tempus HLA-A LOH advanced disease real-world [9]	TCGA HLA-A LOH primary tumors [12]
Average	16.3% (n=10,867)	12.6% (n=10,844)
Non-small cell lung cancer	23.1% (n=1,915)	25.3% (n=501)
Head and neck cancer	26.0% (n=208)	16.1% (n=522)
Breast cancer	12.2% (n=1,447)	13.6% (n=1,080)
Ovarian cancer	16.0% (n=569)	17.1% (n=579)
Colorectal cancer	15.6% (n=1,854)	9.6% (n=615)
Pancreatic cancer	19.6% (n=675)	33.1% (n=184)
Gastroesophageal cancer	20.8% (n=506)	16.2% (n=625)
Mesothelioma	14.3% (n=7)	11.5% (n=87)

HLA, human leukocyte antigen; LOH, loss of heterozygosity; TCGA, The Cancer Genome Atlas.
[a]Tempus data contain more advanced disease, and TCGA data have more primary tumors.

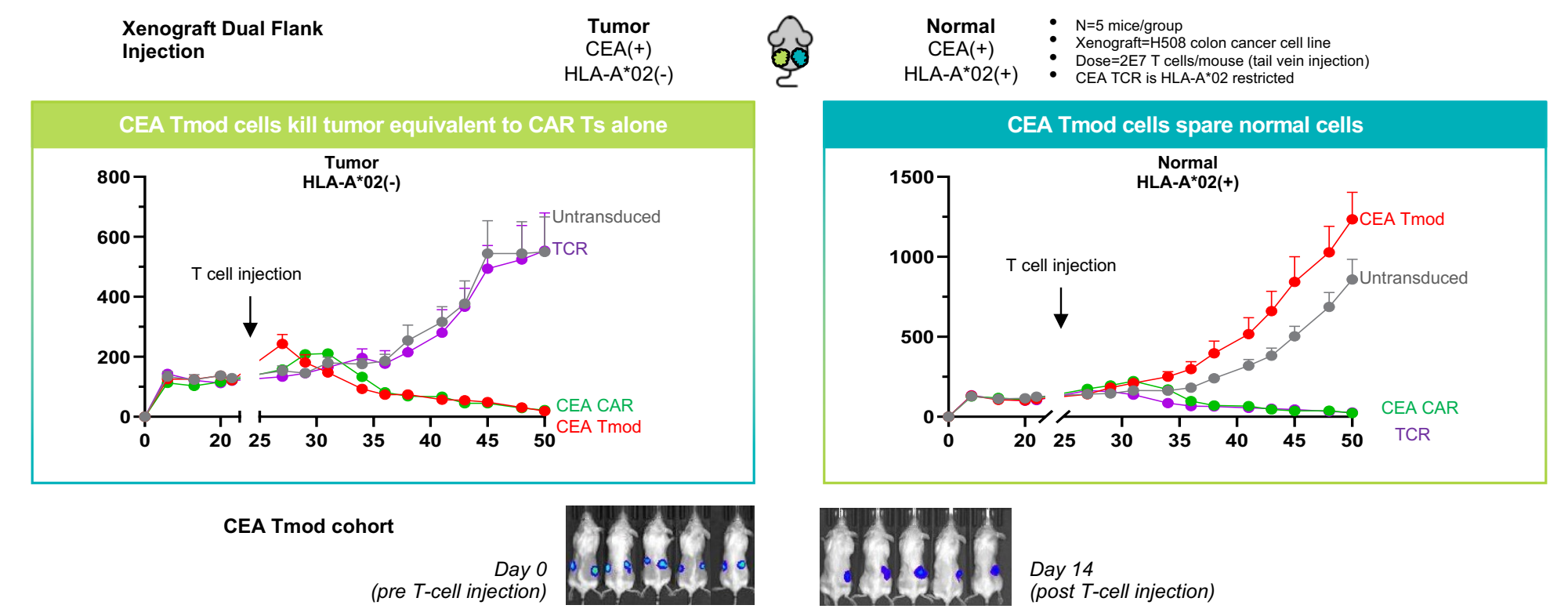
Figure 5. CEA Tmod (A2B530) in vitro study provides a therapeutic safety window comparable to NCI benchmark CEA TCR-T [4,13]



CEA, carcinoembryonic antigen; EC_{50}, half maximal effective concentration; E:T, effector to target; HLA, human leukocyte antigen; IC_{50}, half maximal inhibitory concentration; TCR, T-cell receptor; TPM, total particulate matter.

- Tmod provided selectivity at varying effector-to-target (E:T) ratios with "normal" CEA(+)A*02(+) cells and tumor CEA(+)A*02(-) colon cancer cell lines (**Figure 5A**)
- Mixed A*02(+) and A*02(-) cell cultures show Tmod's ability to discriminate between "normal" (A*02[+]) and tumor (A*02[-]) cells (**Figure 5B**)
- CEA and HLA-A standard plots were generated using CEA surface expression data from mRNA data (**Figure 5C**)
  - CEA Tmod Jurkat or T-cell EC and IC were graphed with the tumor and normal expression values for the CEA and A*02 antigens, along with multiple cell lines

Figure 6. CEA Tmod (A2B530) in vivo study demonstrates potency comparable to NCI benchmark CEA TCR-T [4,13]

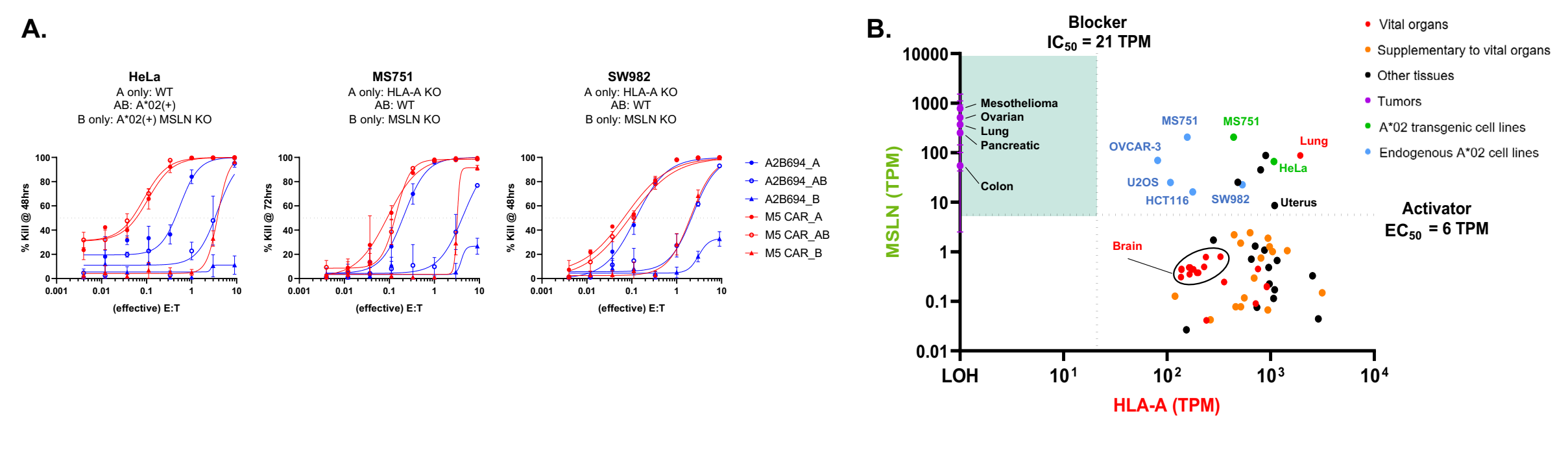


CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen; HLA, human leukocyte antigen; TCR, T-cell receptor.

- In vivo studies show that Tmod maintains selectivity
- In order to allow for adequate tumor (HLA-A*02[-]) and "normal" (HLA-A*02[+]) cells to be established, tumor and "normal" 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 mm^3 (Day 0)
- 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 6 and 8**)

STUDY RATIONALE (cont.)

Figure 7. MSLN Tmod (A2B694) in vitro study provides a therapeutic safety window comparable to M5 benchmark MSLN CAR-T [14]

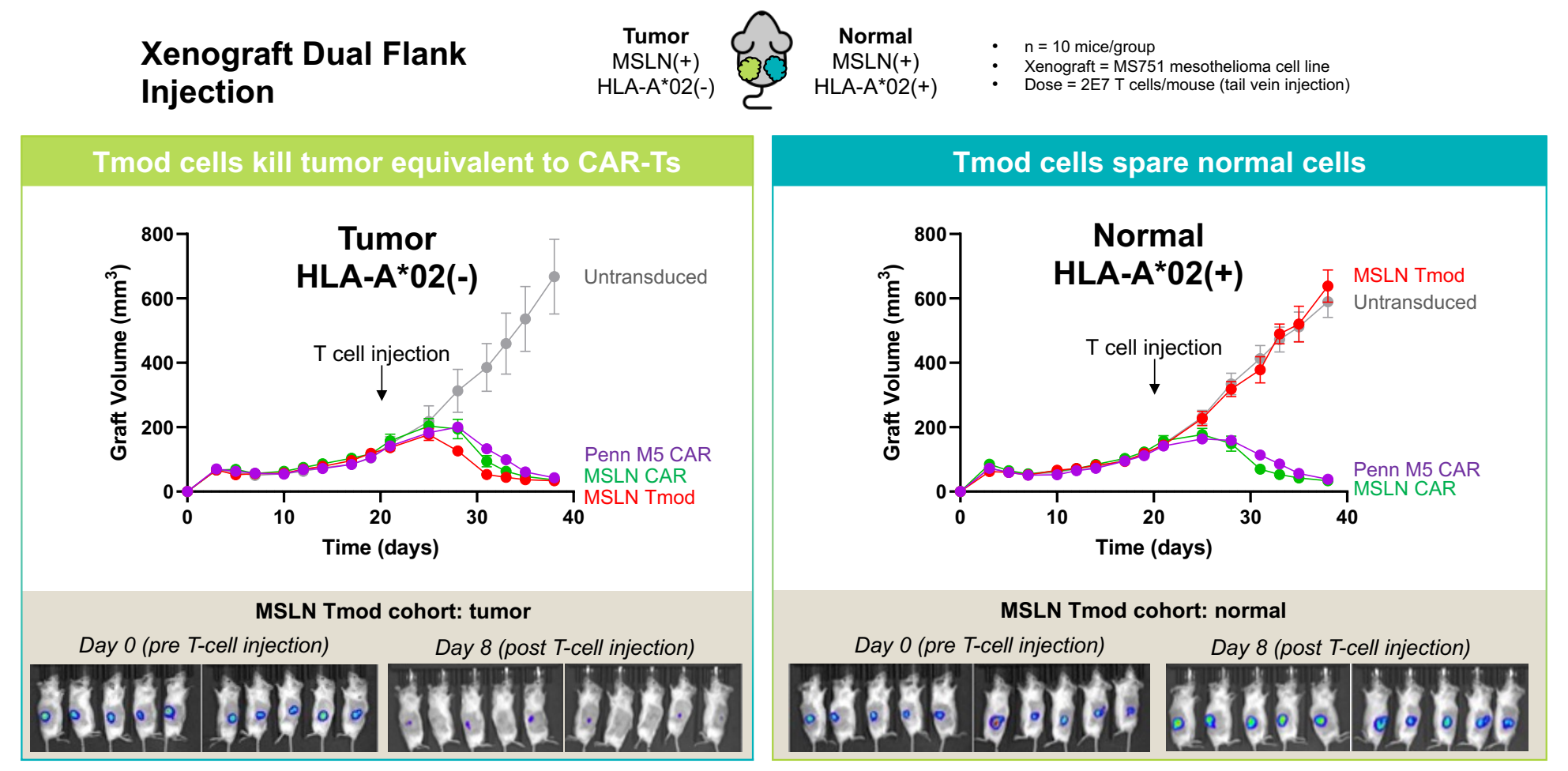


(**A**) Cytotoxicity data of A2B694 constructs from a representative HLA-A*02(+) donor against three endogenous MSLN(+) cell lines with native or engineered expression of HLA-A*02. M5 CAR expressing cells are shown for comparison.

(**B**) MSLN Tmod are predicted to kill tumors while protecting normal tissues. Data representation requires standard curves (not shown) that relate surface protein level to RNA-Seq value for cell lines and tissues. Purple points represent tumor types with HLA-A expression set at 0 TPM to account for selection of A*02(-) tumors by LOH. Tumor data from TCGA database; normal tissue data from the GTEx database.

CAR, chimeric antigen receptor; EC_{50}, half maximal effective concentration; GTEx, Genotype-Tissue Expression; HLA, human leukocyte antigen; IC_{50}, half maximal inhibitory concentration; MSLN, mesothelin; TCGA, the Cancer Genome Atlas; TPM, total particulate matter.

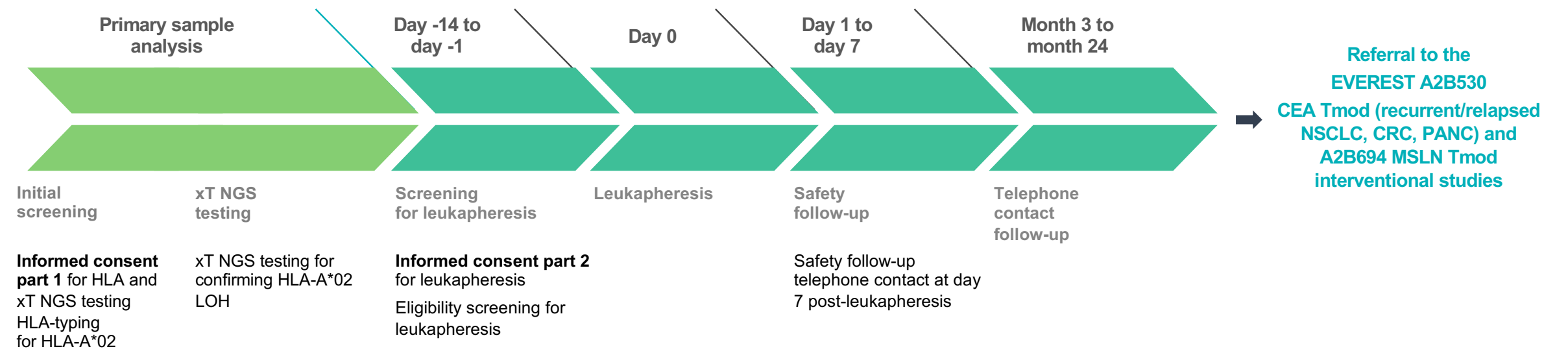
Figure 8. MSLN Tmod (A2B694) in vivo study demonstrates potency comparable to M5 benchmark MSLN CAR-T [14]



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

STUDY DESIGN

Figure 9. Study Schema for BASECAMP-1 (NCT04981119)


Primary sample analysis
Day -14 to day -1
Day 0
Day 1 to day 7
Month 3 to month 24

Initial screening
xT NGS testing
Screening for leukapheresis
Leukapheresis
Safety follow-up
Telephone contact follow-up

Informed consent part 1 for HLA and xT NGS testing HLA-typing for HLA-A*02
xT NGS testing for confirming HLA-A*02 LOH
Informed consent part 2 for leukapheresis
Eligibility screening for leukapheresis
Safety follow-up telephone contact at day 7 post-leukapheresis

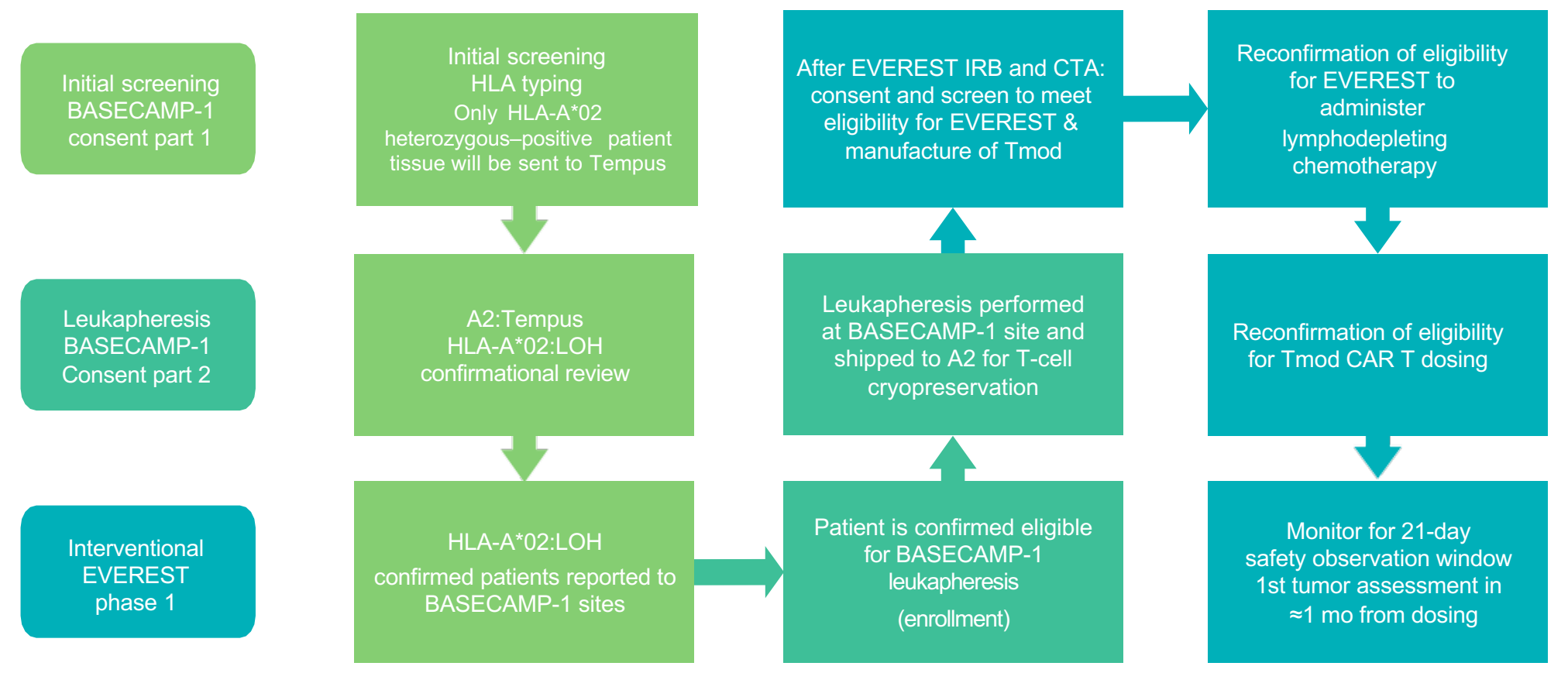
Referral to the EVEREST A2B530 CEA Tmod (recurrent/relapsed NSCLC, CRC, PANC) and A2B694 MSLN Tmod interventional studies

CEA, carcinoembryonic antigen; 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 archival tumor tissue will be analyzed by xT NGS testing to determine if somatic tumor HLA-A*02 LOH is present (**Figure 9 and 10**)
- 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 subsequent autologous Tmod CAR T-cell therapy at the time of relapse

STUDY DESIGN (cont.)

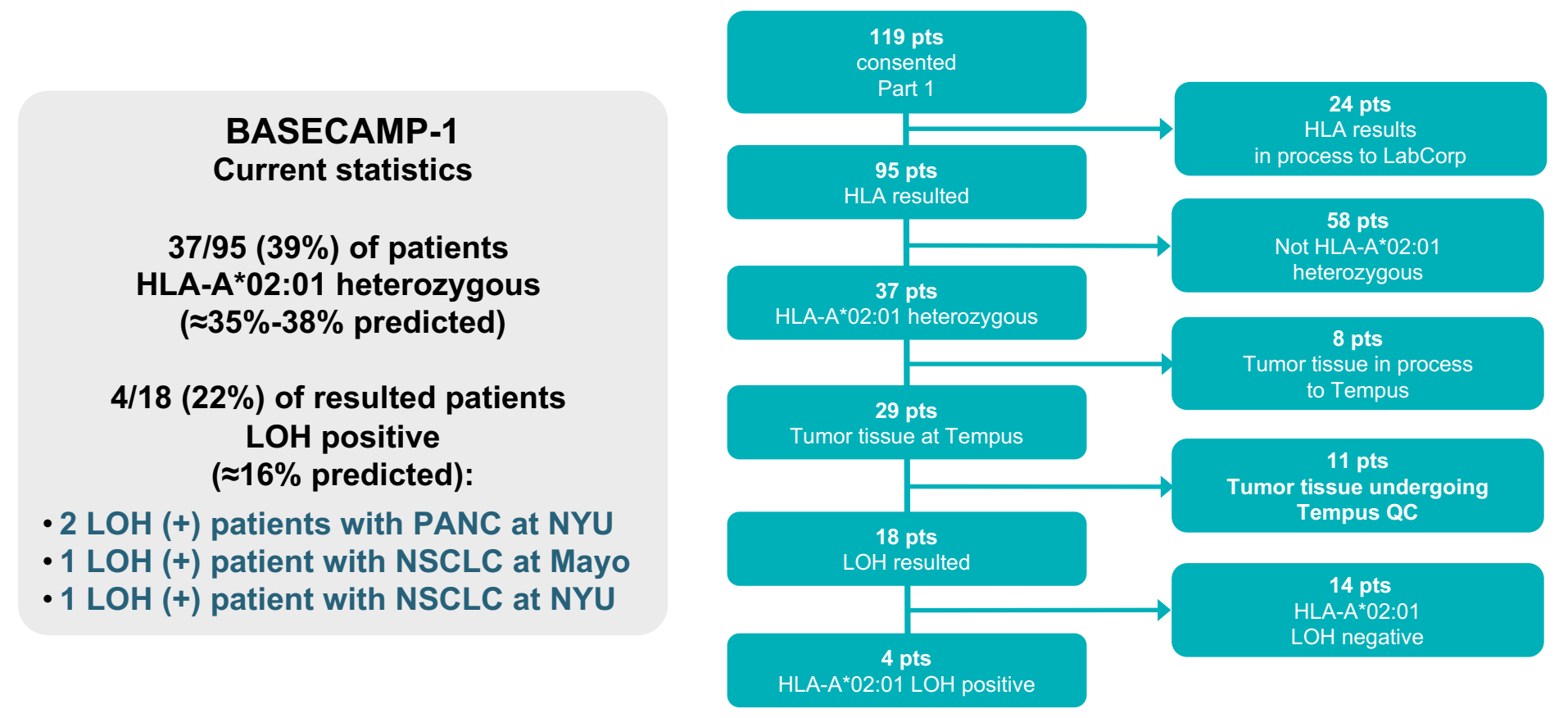
Figure 10. Patient and tissue flow from the BASECAMP-1 to EVEREST studies


Initial screening BASECAMP-1 consent part 1
Leukapheresis BASECAMP-1 Consent part 2
Interventional EVEREST phase 1

Initial screening HLA typing Only HLA-A*02 heterozygous–positive patient tissue will be sent to Tempus
A2:Tempus HLA-A*02:LOH confirmational review
HLA-A*02:LOH confirmed patients reported to BASECAMP-1 sites
Patient is confirmed eligible for BASECAMP-1 leukapheresis (enrollment)
Leukapheresis performed at BASECAMP-1 site and shipped to A2 for T-cell cryopreservation
After EVEREST IRB and CTA: consent and screen to meet eligibility for EVEREST & manufacture of Tmod
Reconfirmation of eligibility for EVEREST to administer lymphodepleting chemotherapy
Reconfirmation of eligibility for Tmod CAR T dosing
Monitor for 21-day safety observation window 1st tumor assessment in ≈1 mo from dosing

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

Figure 11. BASECAMP-1 progress to date and screening process details: 4 HLA-A LOH patients identified (Updated data cut on Oct 10, 2022)


BASECAMP-1
Current statistics

37/95 (39%) of patients
HLA-A*02:01 heterozygous
(≈35%-38% predicted)

4/18 (22%) of resulted patients
LOH positive
(≈16% predicted):

- **2 LOH (+) patients with PANC at NYU**
- **1 LOH (+) patient with NSCLC at Mayo**
- **1 LOH (+) patient with NSCLC at NYU**

119 pts consented Part 1
24 pts HLA results in process to LabCorp
95 pts HLA resulted
58 pts Not HLA-A*02:01 heterozygous
37 pts HLA-A*02:01 heterozygous
8 pts Tumor tissue in process to Tempus
29 pts Tumor tissue at Tempus
11 pts **Tumor tissue undergoing Tempus QC**
18 pts LOH resulted
14 pts HLA-A*02:01 LOH negative
4 pts HLA-A*02:01 LOH positive

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

SITE LIST

- NYU Langone Medical Center
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  - Sub-Investigator: Theodore Welling, MD
- Mayo Clinic, Rochester
  - Principal Investigator: Julian Molina, MD, PhD
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- University of California San Diego
  - Principal Investigator: Sandip Patel, MD
- City of Hope
  - Principal Investigator: Marwan Fakih, MD
- University of California Los Angeles
  - Principal Investigator: J. Randolph Hecht, MD
  - Sub-Investigator: Edward Garon, MD
- Moffitt Cancer Center
  - Principal Investigator: Kedar Kirtane, MD
  - Sub-Investigator: Frederick Locke, MD

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14. Tokatlian T, et al. *J Immunother Cancer.* 2022;10:e003826.

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