Abstract Number 209

Prospective BASECAMP-1 experience in patients with gastrointestinal (GI) cancer: Identifying patients with human leukocyte antigen (HLA) loss of heterozygosity (LOH) for a future therapeutic trial exploiting LOH as a tumor vulnerability

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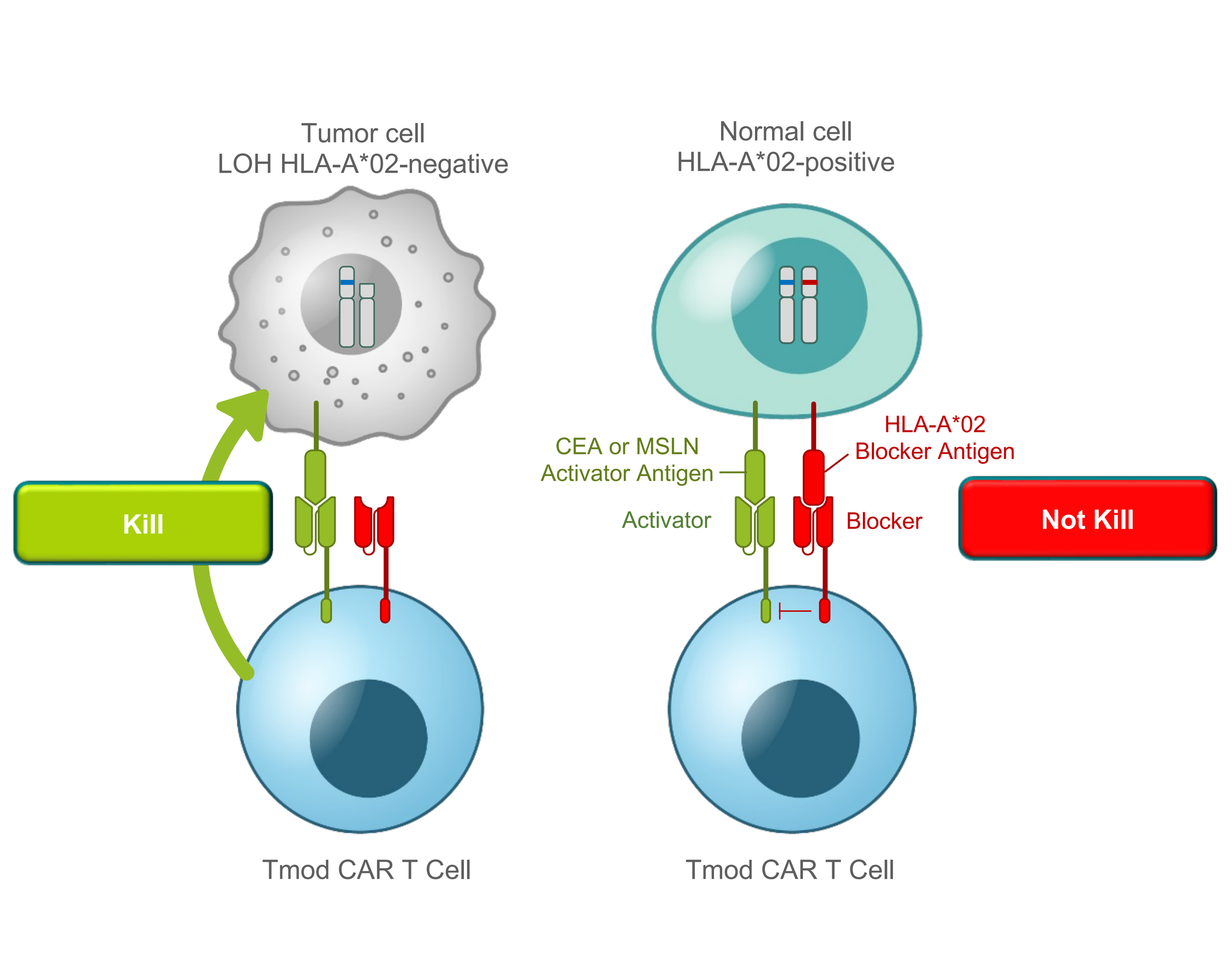
BACKGROUND AND STUDY OBJECTIVES

- Metastatic colorectal, pancreatic (PANC), gastric, and esophageal cancers are the leading causes of gastrointestinal (GI) cancer-related mortality (metastatic 5-year survival rate of 15%, 3%, 6% and 5% 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 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 which 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 a MSLN-directed Tmod construct utilizing a LIR-1-based inhibitory receptor (blocker) targeting human leukocyte antigen (HLA)-A*02
- HLA loss of heterozygosity (LOH) may provide a means to distinguish between 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]
- HLA-A*02 LOH can only be therapeutically exploited if patients are identifiable through a feasible and timely clinical workflow.
  - The Tempus xT is a next-generation sequencing (NGS) platform commonly used for patients with GI cancer which can readily identify HLA 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 A2B530 CEA Tmod or 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)

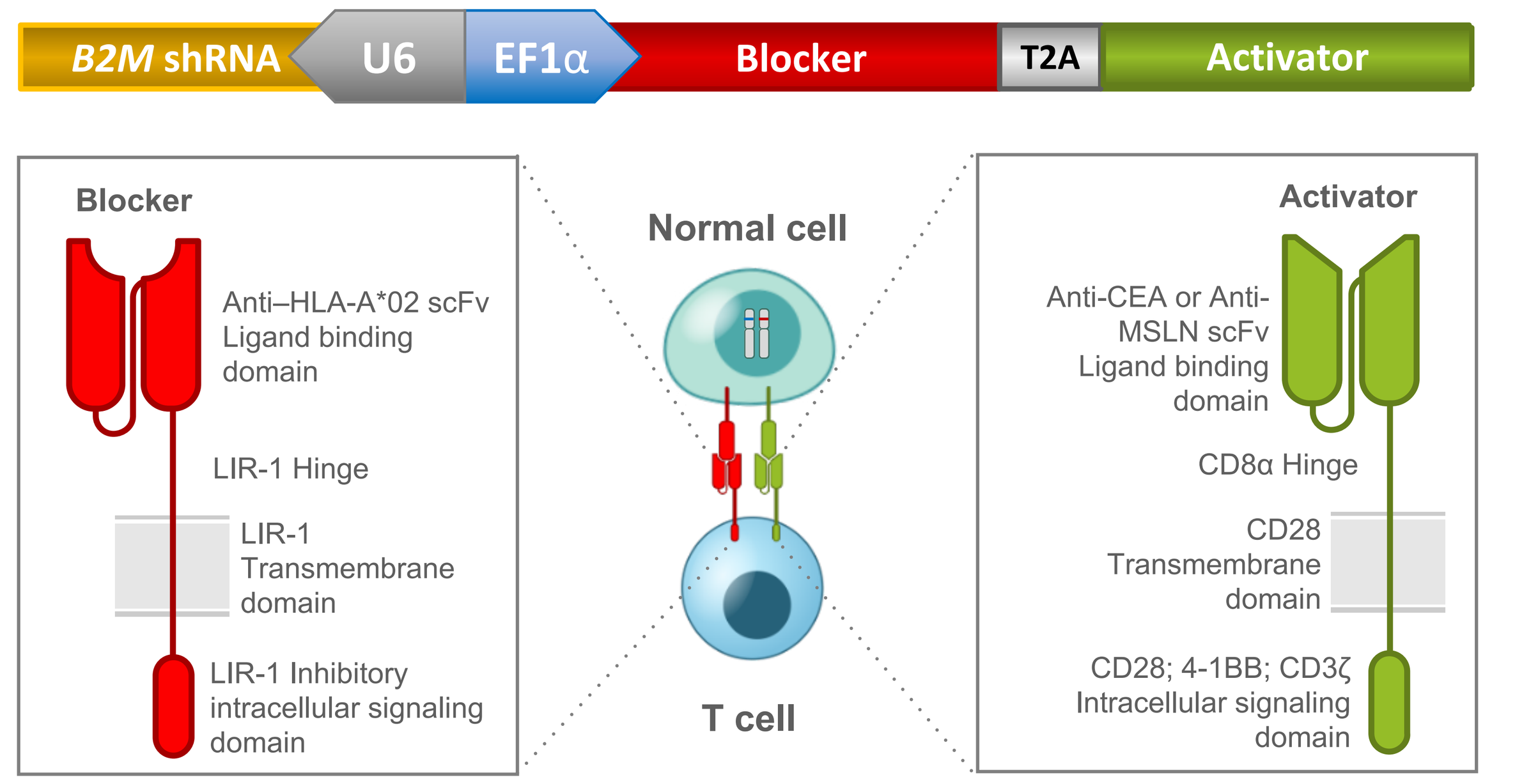


CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen 5; HLA, human leukocyte antigen; LOH, loss of heterozygosity; MSLN, mesothelin.

- 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 have dose-limiting toxicities in previous studies
- CAR T HLA-A LOH approach is independently validated by Vogelstein/Kinzler, 2021 [8]

STUDY RATIONALE (cont.)

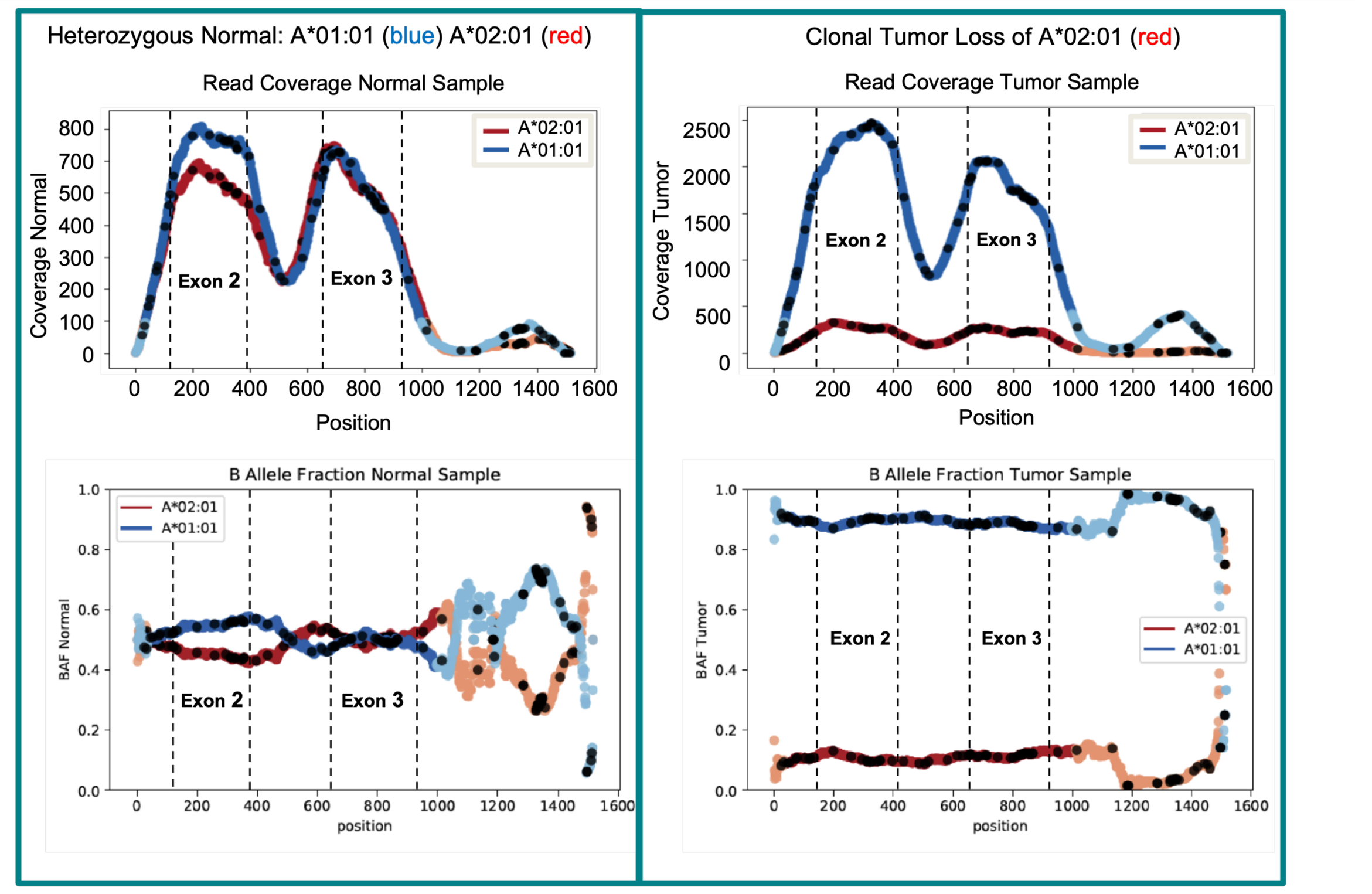
Figure 2. CEA or MSLN CAR Tmod single vector construct [10]


B2M shRNA
U6
EF1α
Blocker
T2A
Activator
Blocker
Anti–HLA-A*02 scFv Ligand binding domain
LIR-1 Hinge
LIR-1 Transmembrane domain
LIR-1 Inhibitory intracellular signaling domain
Normal cell
T cell
Activator
Anti-CEA or Anti-MSLN scFv Ligand binding domain
CD8α Hinge
CD28 Transmembrane domain
CD28; 4-1BB; CD3ζ Intracellular signaling domain

B2M shRNA, β2-microglobulin short-hairpin RNA; CEA, carcinoembryonic antigen 5; EF1α, elongation factor-1α; HLA, human leukocyte antigen; LIR, leukocyte immunoglobulin-like receptor; 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)



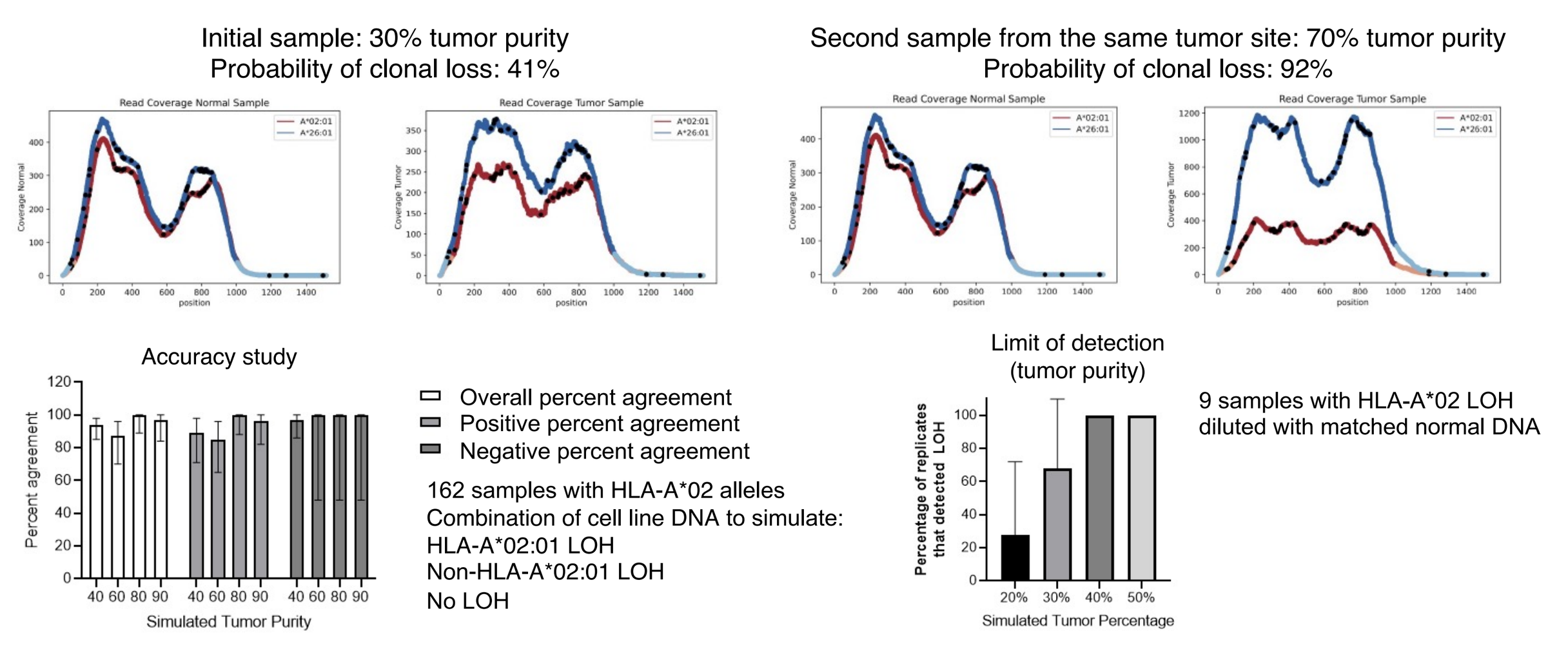
- A representative example of clonal HLA LOH (**Figure 3**), where a discordance is observed in read coverage of HLA-A*02:01 between the tumor and matched-normal samples [9, 11]
- HLA-A*02 LOH can be reliably detected using the Tempus xT next-generation sequencing (NGS) assay (**Table 1**)

Table 1. Frequency of HLA-A LOH in advanced GI 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)
Range in GI cancers	15.6%-20.8% (n=3,035)	9.6%-33.1% (n=1,424)
Colorectal	15.6% (n=1,854)	9.6% (n=615)
Pancreatic	19.6% (n=675)	33.1% (n=184)
Gastroesophageal	20.8% (n=506)	16.2% (n=625)

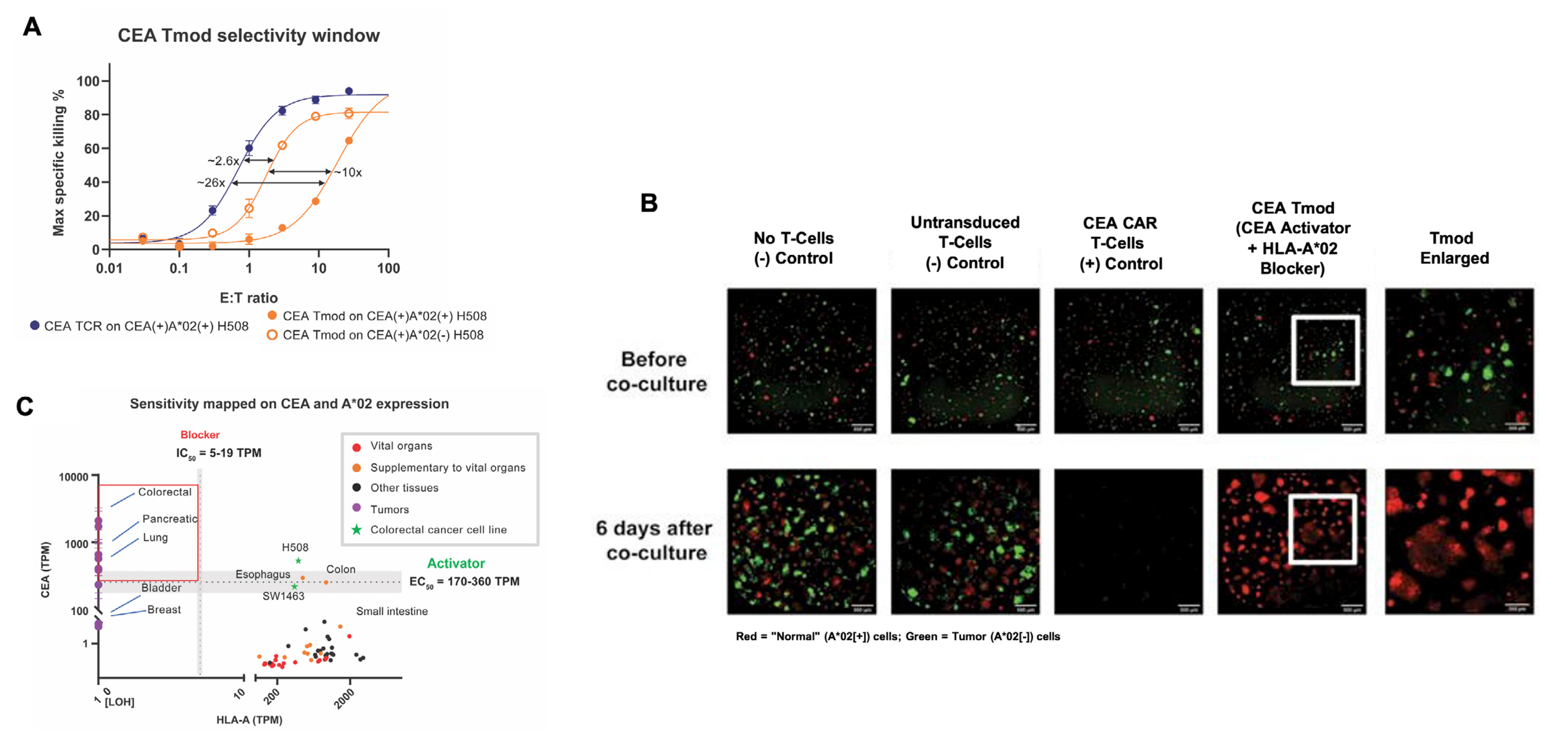
HLA, human leukocyte antigen; LOH, loss of heterozygosity; TCGA, The Cancer Genome Atlas.
[a]Tempus data contain more advanced disease, and TCGA data have more primary tumors.

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


Initial sample: 30% tumor purity
Probability of clonal loss: 41%
Second sample from the same tumor site: 70% tumor purity
Probability of clonal loss: 92%
Accuracy study
Overall percent agreement
Positive percent agreement
Negative percent agreement
162 samples with HLA-A*02 alleles
Combination of cell line DNA to simulate:
HLA-A*02:01 LOH
Non-HLA-A*02:01 LOH
No LOH
Limit of detection (tumor purity)
9 samples with HLA-A*02 LOH diluted with matched normal DNA

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

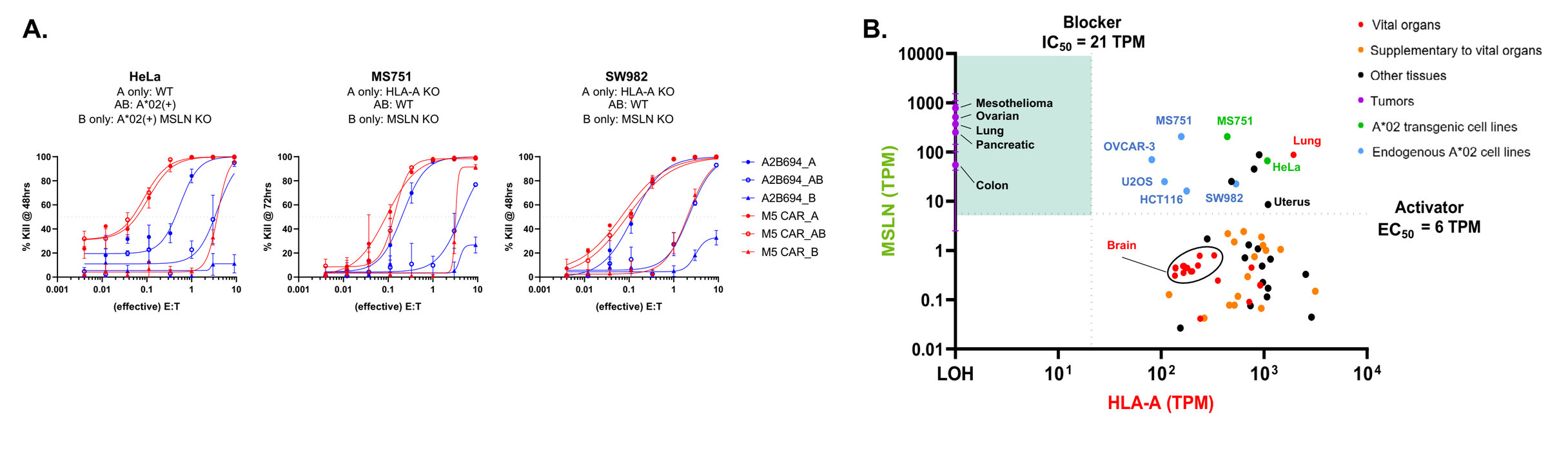
Figure 5. CEA Tmod (A2B530) in vitro study provides a therapeutic safety window comparable to NCI benchmark CEA TCR-T [4,13]



CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen 5; EC_{50}, half maximal effective concentration; E:T, effector-to-target; HLA, human leukocyte antigen; IC_{50}, half maximal inhibitory concentration; NCI, National Cancer Institute; 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*02 standard plots were generated using CEA surface expression data from mRNA data (**Figure 5C**)
  - CEA Tmod Jurkat or T-cell effective concentration and inhibitory concentration were graphed with the tumor and normal expression values for the CEA and A*02 antigens, along with multiple cell lines

Figure 6. MSLN Tmod (A2B694) in vitro study provides a therapeutic safety window comparable to M5 benchmark MSLN CAR-T [14]

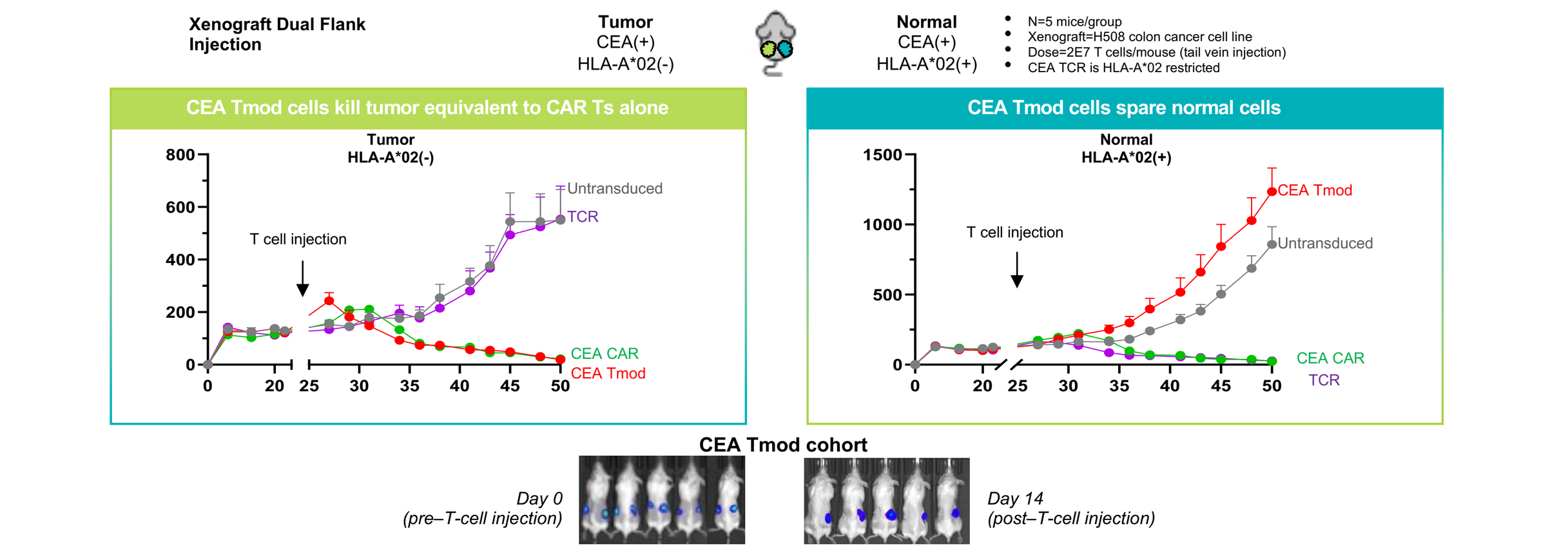


(**A**) Cytotoxicity data of A2B694 constructs from a representative HLA-A*02(+) donor against three endogenous MSLN(+) cell lines with native or engineered expression of HLA-A*02. M5 CAR expressing cells are shown for comparison.

(**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.

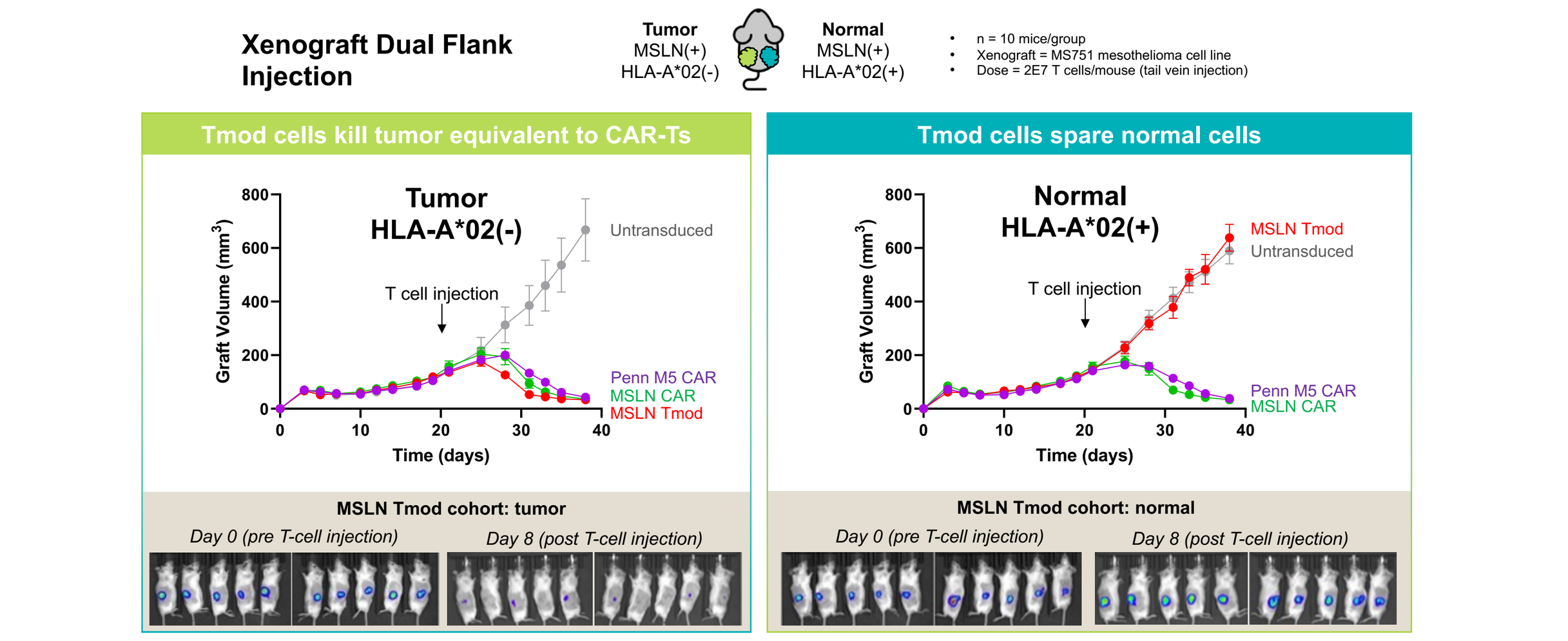
CAR, chimeric antigen receptor; EC_{50}, half maximal effective concentration; GTEx, Genotype-Tissue Expression; HLA, human leukocyte antigen; IC_{50}, half maximal inhibitory concentration; MSLN, mesothelin; TCGA, the Cancer Genome Atlas; TPM, total particulate matter.

Figure 7. 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; NCI, National Cancer Institute; TCR, T-cell receptor.

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

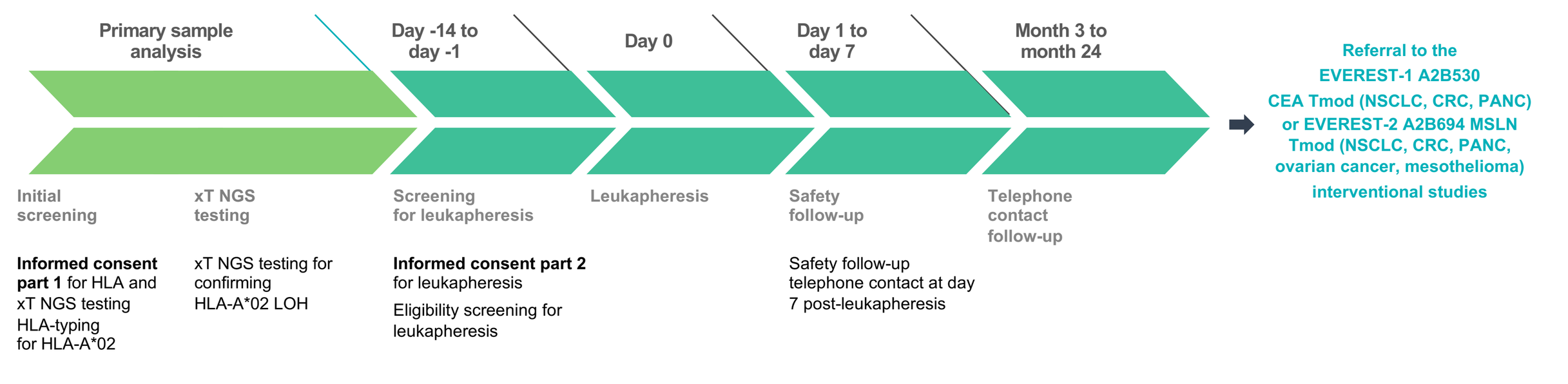

Xenograft Dual Flank Injection
Tmod cells kill tumor equivalent to CAR-Ts
Tmod cells spare normal cells
Tumor HLA-A*02(-)
Normal HLA-A*02(+)
Time (days)
MSLN Tmod cohort: tumor
MSLN Tmod cohort: normal
Day 0 (pre T-cell injection)
Day 8 (post T-cell injection)

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

- 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 mm^3
- 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 7 and 8**)

STUDY DESIGN AND METHODS

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

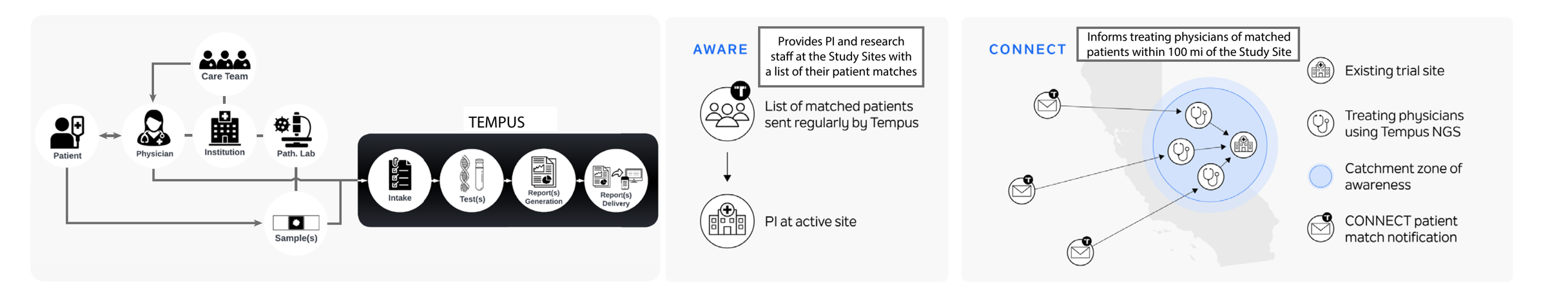

Primary sample analysis
Day -14 to day -1
Day 0
Day 1 to day 7
Month 3 to month 24
Referral to the EVEREST-1 A2B530 CEA Tmod (NSCLC, CRC, PANC) or EVEREST-2 A2B694 MSLN Tmod (NSCLC, CRC, PANC, ovarian cancer, mesothelioma) interventional studies
Initial screening
xT NGS testing
Screening for leukapheresis
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

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, tumor tissue will be analyzed by xT NGS testing to determine if somatic tumor HLA-A*02 LOH is present (**Figures 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

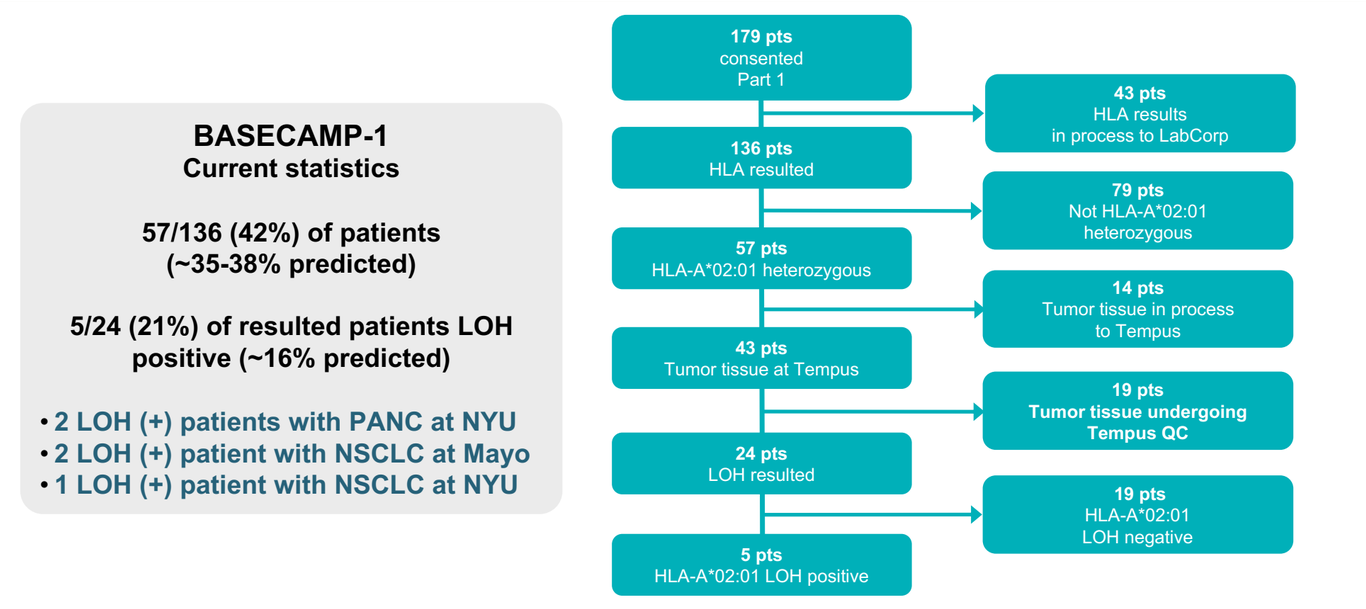
STUDY DESIGN AND METHODS (cont.)

Figure 10. Tempus clinical workflow



NGS, next-generation sequencing; PI, principal investigator.

Figure 11. BASECAMP-1 progress to date and screening process details: 5 HLA LOH patients identified (Updated data cut on December 4, 2022)



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

CONCLUSIONS

- BASECAMP-1 prospective identification of HLA-A*02 LOH is feasible in the real-world setting
- Clonal HLA LOH is an irreversible discriminator between tumor vs normal cells that 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 [9]
- Tempus xT platform is able to identify patients with clonal HLA LOH
  - LOH results can be obtained within a clinically feasible workflow and timeframe, although samples with a <40% tumor purity have a reduced sensitivity for LOH detection, an issue recurrently observed in patients with PANC
- BASECAMP-1 (NCT04981119) study is currently enrolling patients to identify HLA-A*02 LOH subjects with colorectal, pancreatic, non-small cell lung cancer, mesothelioma or ovarian cancer and then to bank their T cells for future EVEREST A2B530 CEA Tmod or A2B694 MSLN Tmod interventional studies

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