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Cytokine response and heme cell depletion and recovery after treatment with Tmod logic-gated chimeric antigen receptor T cells (CAR Ts) in patients with solid tumors

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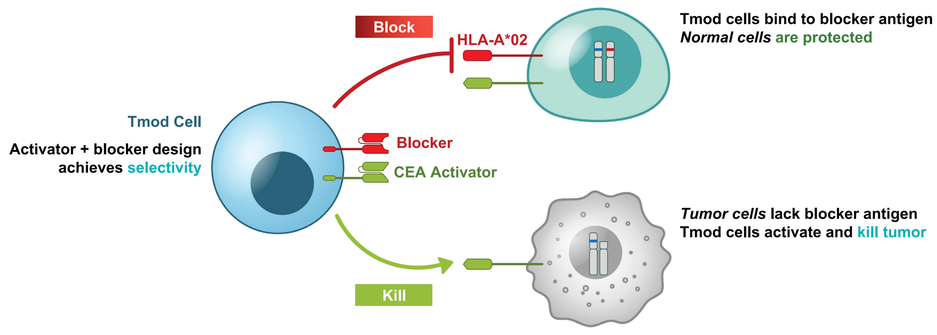
BACKGROUND:

- The response to lymphodepleting chemotherapy (LD) is critical for effective treatment of hematologic malignancies using CD19-directed CAR Ts [1, 2]
  - However, response to LD in patients with solid tumors is less understood
- Therefore, to better elucidate responses to LD, we characterized the cytokine response and kinetics of peripheral blood hematopoietic cell depletion and recovery in participants enrolled in EVEREST-1

EVEREST-1 TRIAL:

- EVEREST-1 (NCT05736731) is a first-in-human, phase 1/2, multicenter, open-label, nonrandomized study to evaluate the safety and efficacy of a single dose of A2B530, a carcinoembryonic antigen (CEA)-targeted, logic-gated, Tmod CAR T (**Figure 1**), in adults with recurrent unresectable, locally advanced, or metastatic non-small cell lung cancer (NSCLC), colorectal cancer (CRC), pancreatic cancer (PANC), or other solid tumor associated with CEA expression

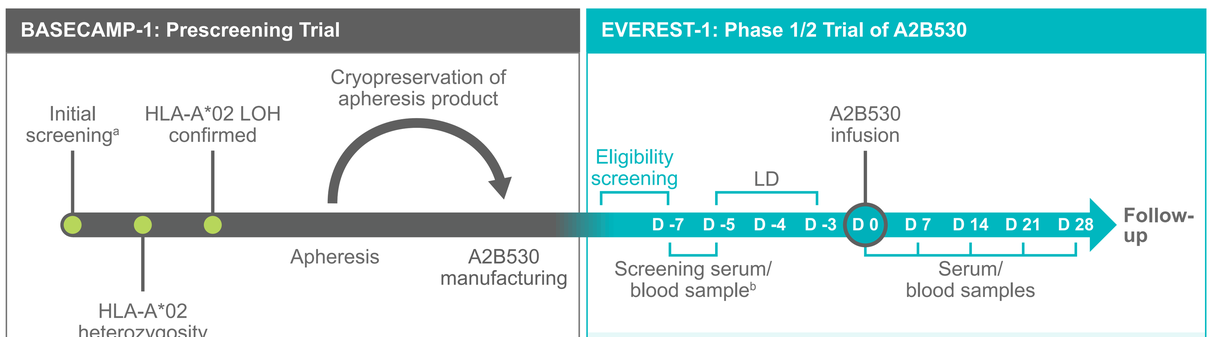
Figure 1: Logic-Gated CAR T Therapy With the Goal to Reduce Toxicity: CEA (Activator) and HLA-A*02 (Blocker) [3]



CEA, carcinoembryonic antigen; HLA, human leukocyte antigen.

- Participants are enrolled to EVEREST-1 through BASECAMP-1 (NCT04981119), a master prescreening study that identifies patients with human leukocyte antigen loss of heterozygosity (HLA LOH) at any time in the course of their disease; enrolled participants undergo leukapheresis and, when clinically appropriate, CAR Ts are manufactured for the EVEREST-1 study (**Figure 2**)

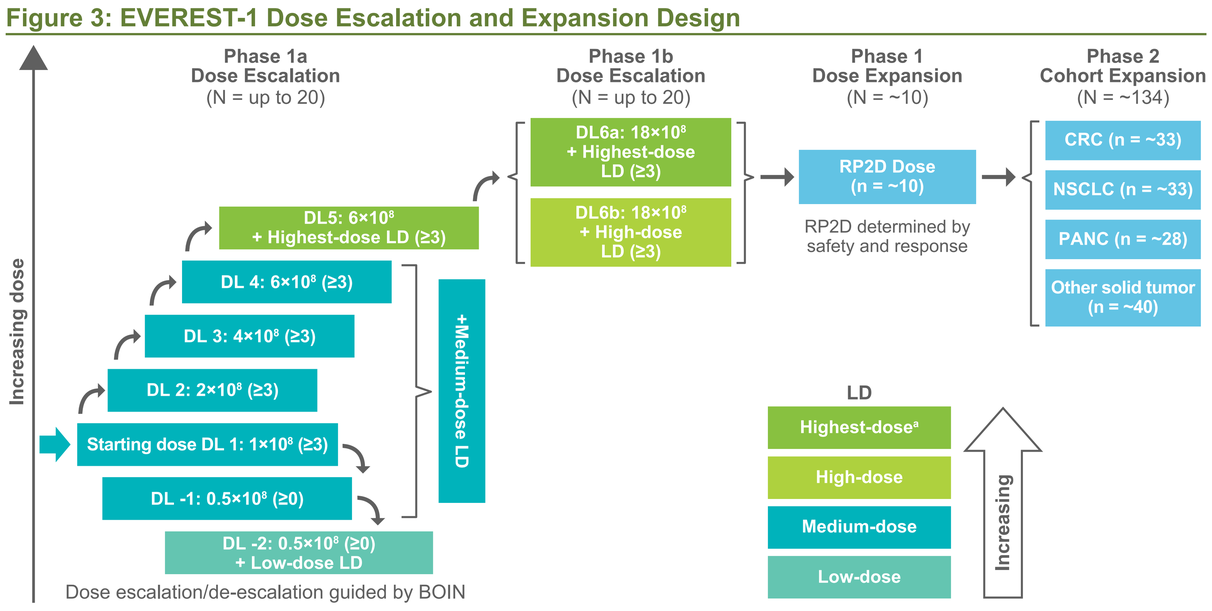
Figure 2: EVEREST-1 Study Design



[a] May occur at any time in disease course. [b] Screening serum and blood samples taken at D -7 for participants who receive the highest dose LD and at D -5 for all other DLs. [c] For patients with CRC or PANC, CEA assessment will be performed retrospectively, and the result is not needed for enrollment.
CEA, carcinoembryonic antigen; CRC, colorectal cancer; D, day; DL, dose level; DLT, dose-limiting toxicity; HLA, human leucocyte antigen; LD, lymphodepleting chemotherapy; NSCLC, non-small cell lung cancer; PANC, pancreatic cancer.

- The phase 1 dose escalation portion of the study employs a Bayesian optimal interval design to assess the safety and tolerability of A2B530 and to determine a recommended phase 2 dose (**Figure 3**); 9 to 40 participants will be included in the dose escalation
  - Serum and blood samples were collected at prespecified timepoints per the study protocol (**Figure 2**)
  - T, B, and natural killer (NK) cells were enumerated using a quantitative flow cytometry assay (Labcorp). Cytokines (other than C-reactive protein [CRP]) were quantified using the Ella automated ELISA platform (ProteinSimple) and measured in duplicate. CRP was measured using an immunoturbidometric assay (Labcorp).

Figure 3: EVEREST-1 Dose Escalation and Expansion Design



[a]Highest dose LD is similar to the LD used in prior cellular therapy trials [4, 5]
BOIN, Bayesian optimal interval design; CRC, colorectal cancer; DL, dose level; DLT, dose-limiting toxicity; IL, interleukin; LD, lymphodepleting chemotherapy; NSCLC, non-small cell lung cancer; PANC, pancreatic cancer; RP2D, recommended phase 2 dose.

- The first participant was dosed in EVEREST-1 in May 2023 and, as of January 15, 2025, 14 participants have been enrolled (**Table 1**)
- Dose levels (DLs) 1-5 have been administered, with 3 participants through DL 4 and 2 participants at DL 5

Table 1: Participant Demographics and Baseline Characteristics

Characteristic	Participants (N = 14)
Gender, n (%)	
Female	6 (43)
Male	8 (57)
Median age (range), years	
Female	61 (46–76)
Male	58 (34–70)
Cancer type, n (%)	
PANC	4 (29)
CRC	10 (71)
Median prior lines of anti-cancer therapy (range)	
PANC	2 (1–3)
CRC	2 (1–6)
Race, n (%)	
White	11 (79)
Asian	1 (7)
Other	2 (14)
Ethnicity, n (%)	
Hispanic	2 (14)
Not Hispanic	12 (86)

CRC, colorectal cancer; PANC, pancreatic cancer.

RESULTS:

- T, B, and NK cells decreased from Day -7/-5 (T cell median: 789 cells/μL) to Day 0 (T cell median: 26 cells/μL) after LD administration (**Table 2**)

Table 2: Immune Cell Counts in Patients Who Received LD in EVEREST-1

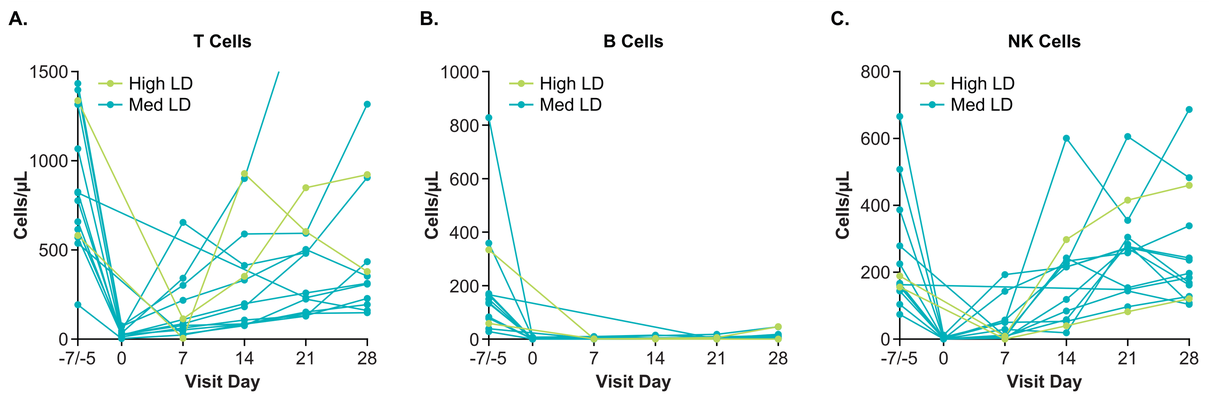
	Day -7/-5	Day 0	Day 28
T cells			
N	14	10	14
Median (/μL)	789	26	366
Range (/μL)	193-1433	3-75	148-2758
B cells			
N	14	10	14
Median (/μL)	144	1	10
Range (/μL)	28-828	0-7	0-47
NK cells			
N	14	10	14
Median (/μL)	165	2	191
Range (/μL)	74-666	0-12	104-687

	Day -7/-5	Day 0	Day 28
Hemoglobin			
N	14	14	14
Median (g/dL)	12	10.5	11.1
Range (g/dL)	8.7-15.3	9.2-13.6	8.5-14.1
Neutrophils			
N	14	14	14
Median (×10⁹/L)	3.7	1.7	2.9
Range (×10⁹/L)	2.2-7.3	0.1-18.0	0.4-4.5
Platelets			
N	14	14	14
Median (×10⁹/L)	198	142	195
Range (×10⁹/L)	111-440	73-512	34-314

LD, lymphodepleting chemotherapy; NK, natural killer.

- T and NK cells recovered slowly starting on Day 7, and, by Day 28, increased in all 14 participants (T cell median: 366 cells/μL; NK cell median: 191 cells/μL; **Figure 4A, 4C**)
- B cells remained low through Day 28 (**Figure 4B**)

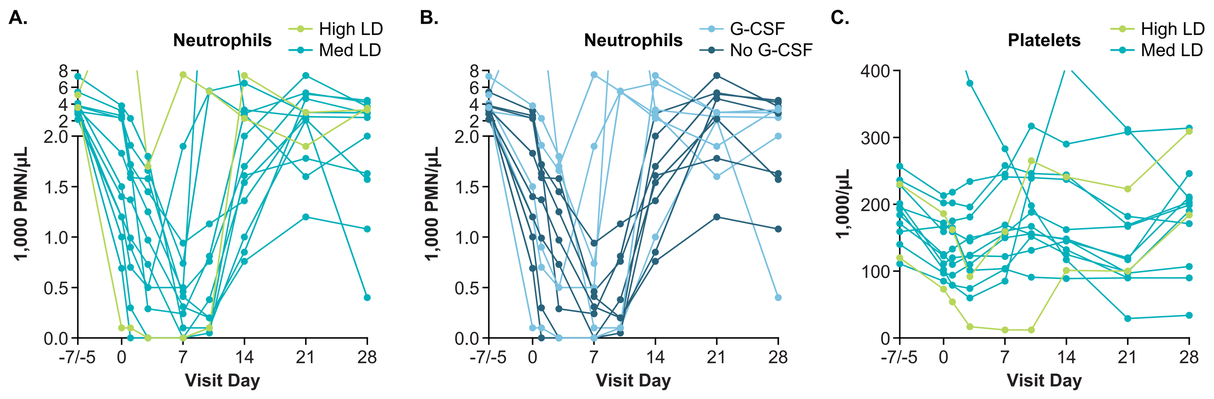
Figure 4: T-, B-, and NK-Cell Counts Over Time



LD, lymphodepleting chemotherapy; Med, medium; NK, natural killer.

- Neutrophil nadir was noted between Days 7 and 14, with more rapid recovery in participants who received prophylactic granulocyte colony-stimulating factor (**Figure 5**)
  - One participant with DL 5 experienced significant thrombocytopenia (nadir 12×10³/μl on Day 10) with recovery to 184×10³/μl on Day 28

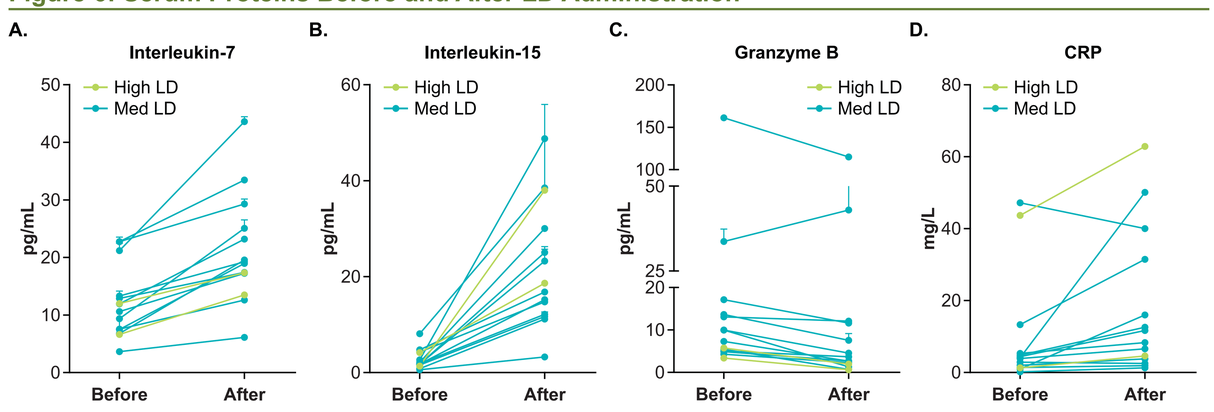
Figure 5: Neutrophil and Platelet Counts Over Time



G-CSF, granulocyte colony-stimulating factor; LD, lymphodepleting chemotherapy; Med, medium; PMN, polymorphonuclear neutrophils.

- No dose-limiting toxicities were observed in the 14 participants
- Serum concentrations of interleukin (IL)-7 and IL-15 increased in all participants after LD administration (**Figure 6**)
- In most cases, serum concentrations of Granzyme B typically decreased and CRP typically increased after LD administration

Figure 6: Serum Proteins Before and After LD Administration[a]



[a] Before sample collected on day -7 or -5 and After sample collected on day of CAR T infusion. Plotted values represent the mean value and standard deviation from duplicate wells.
CRP, C-reactive protein; LD, lymphodepleting chemotherapy; Med, medium.

CONCLUSIONS:

- In participants with solid tumors, the LD regimens used in EVEREST-1 were well tolerated, depleted patient lymphocytes, and resulted in increased serum concentrations of IL-7 and IL-15
- Future directions will include examining the comparison of medium- and high-dose LD regimens with additional participants and the effects of LD in participants enrolled in EVEREST-2 (NCT06051695), which includes participants with ovarian cancer who have previously been exposed to more platinum-based, myelosuppressive chemotherapies than patients with CRC, PANC, and NSCLC

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