



ALETA-001, a CAR-T Engager protein, or CTE, optimizes anti-CD19 CAR-T cell therapies for lymphoma

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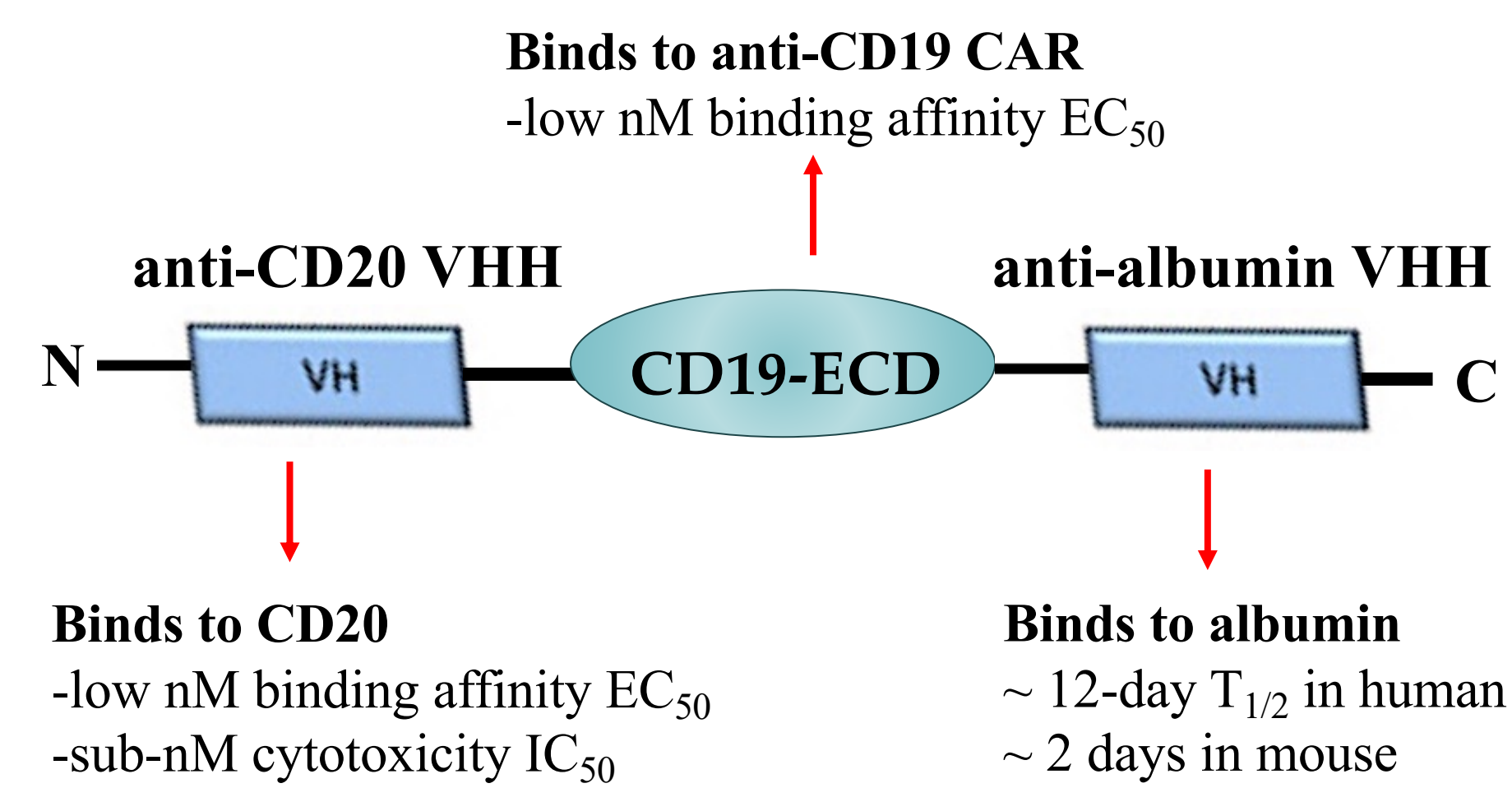
Introduction

Chimeric Antigen Receptor T cells targeting malignancies expressing CD19 (CAR19) have been widely successful, with products approved to treat B cell lymphomas (NHL) and B cell leukemia. A major limitation of CAR19 therapy is the steep relapse rate within 6 months of treatment, often due to the loss or diminution of tumor cell CD19 expression. ALETA-001 is a CAR-T Engager protein (CTE) that contains the CD19 extracellular domain (ECD), an anti-CD20 VHH to target this second antigen, and an anti-albumin VHH for half-life extension. When combined with CAR19 T cells, ALETA-001 triggers cytotoxicity through CD19 bound to CD20, thus increasing total target antigen density and preventing relapse due to loss of CD19 expression. ALETA-001 will enter Cancer Research UK-sponsored Phase 1/2 clinical trials in CAR19-treated NHL patients later this year.

ALETA-001

ALETA-001 is a multifunctional biologic for injection that contains an anti-CD20 llama VHH linked to an optimized CD19 protein and further linked to an anti-albumin llama VHH.

Figure 1. Design of the ALETA-001 CAR-T Engager protein.



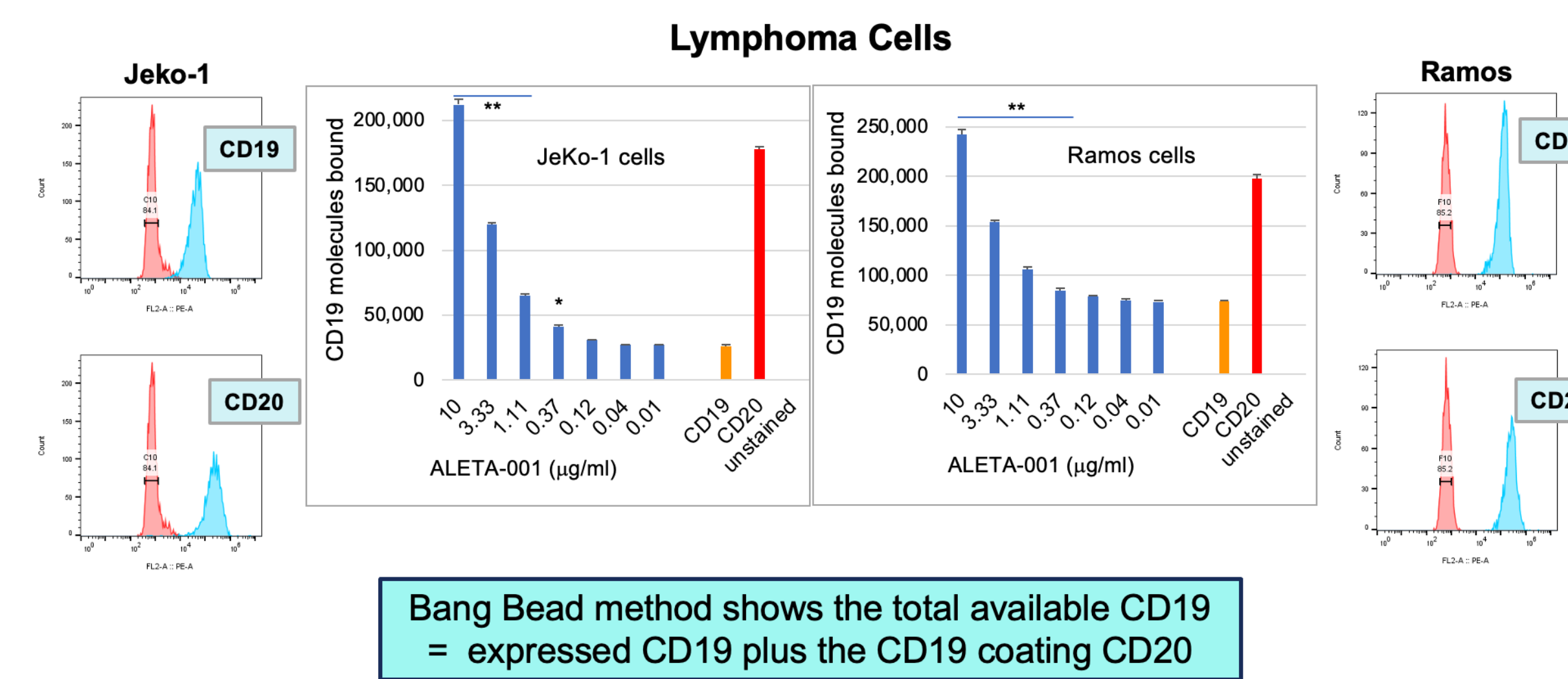
- ALETA-001 binds to CD20 on B cell tumor cells, displays CD19 on the tumor cell surface thereby activating anti-CD19 CAR T cells, and binds to albumin, providing for a long half-life upon injection.
- Aleta-001 increases CD19 antigen density and/or replaces lost CD19 expression by coating cell surface CD20 with CD19.
- Binds to any CAR19: T cell (autologous, allogeneic), NK, $\gamma\delta$, iPSC, or other.
- 55 KDa monomer utilizes standard biologic production and can be dosed “off-the-shelf”.

Here we present results from ALETA-001 preclinical work that further elucidate the mechanisms of action.

Disclosure: all authors are employees and/or stockholders of Aleta Biotherapeutics Inc.

MOA – 1. ALETA-001 increases apparent CD19 density on CD19-positive lymphoma cells.

Figure 2. Incubation with ALETA-001 binds the CD19 ECD onto CD20, and this increases the amount of detectable CD19 by FACs analysis. Left: JeKo-1, Mantle Cell Lymphoma. Right: Ramos, Hodgkin Lymphoma

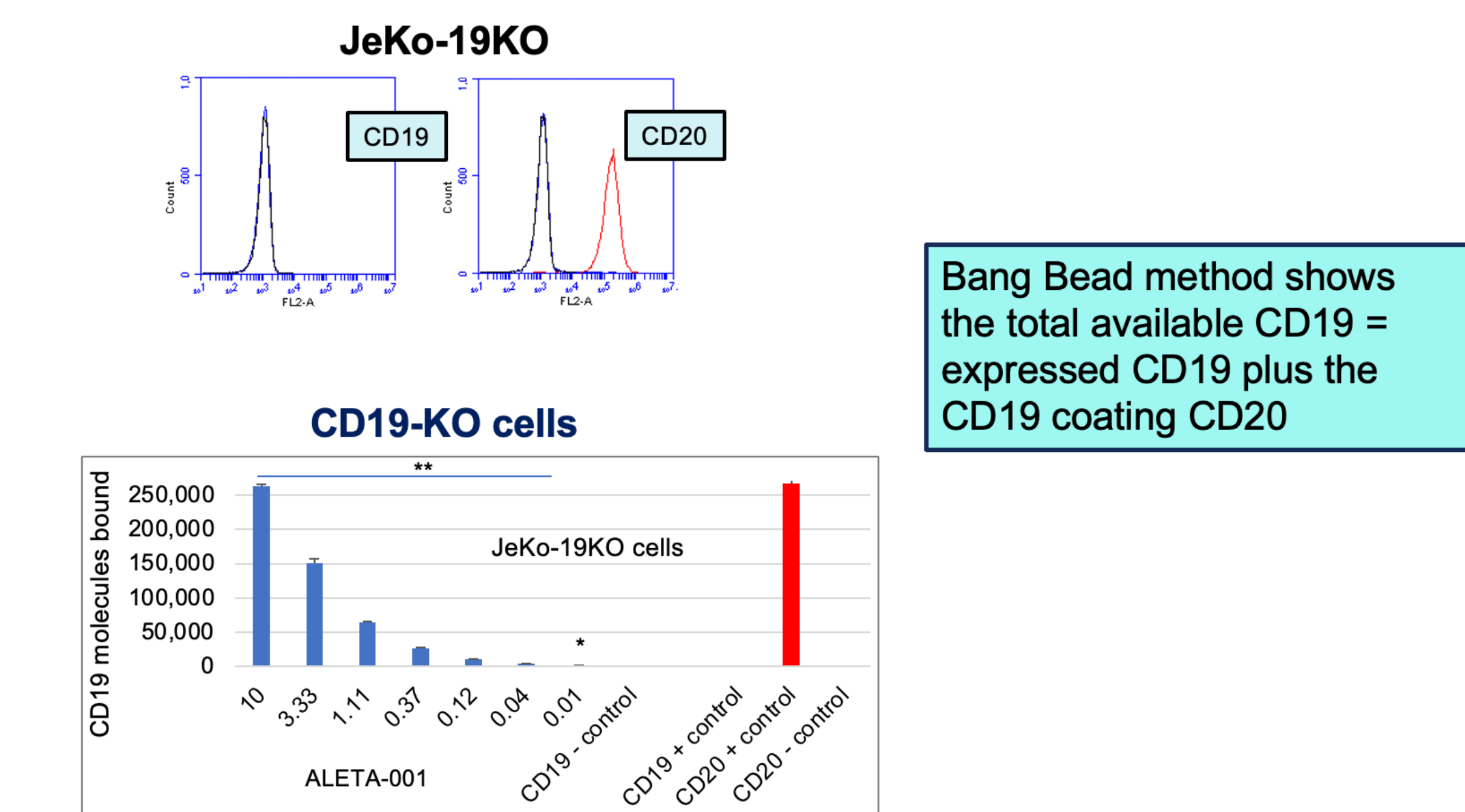


Bang Bead method shows the total available CD19 = expressed CD19 plus the CD19 coating CD20

ALETA-001 binding increases the detectable CD19 on JeKo-1 cells from ~25,000 molecules to more than 200,000 molecules, an amount equivalent to the sum of CD19 expressed (orange bar) + CD20 expressed. Similarly, ALETA-001 binding increases the detectable CD19 on Ramos cells from ~75,000 to nearly 250,000, closely resembling the sum of CD19 (orange) + CD20 (red).

MOA – 2. ALETA-001 replaces CD19 that is otherwise lost from JeKo-1-CD19 knockout cells, while exceeding normal CD19 expression

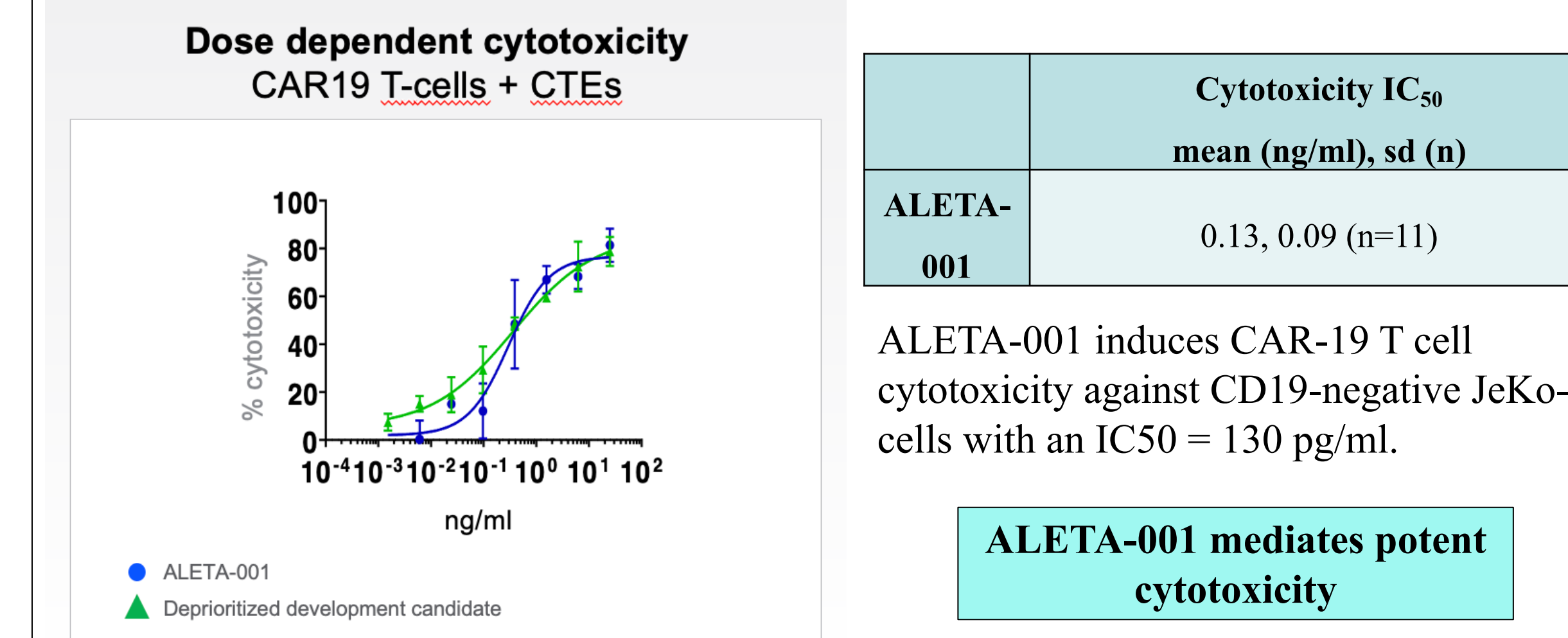
Figure 2. Incubation with ALETA-001 replaces CD19 lost from JeKo-1-CD19 Knockout cells at a level higher than native CD19 expression and matching CD20 expression.



Bang Bead method shows the total available CD19 = expressed CD19 plus the CD19 coating CD20

ALETA-001 binding increases the detectable CD19 on JeKo-1-CD19 knockout cells from ~0 molecules to more than 250,000 molecules, an amount equivalent to CD20 expression (red bar)

Figure 3. Incubation with ALETA-001 mediates CAR-19 cytotoxicity against JeKo-CD19-knockout cells, at pg/ml concentrations.

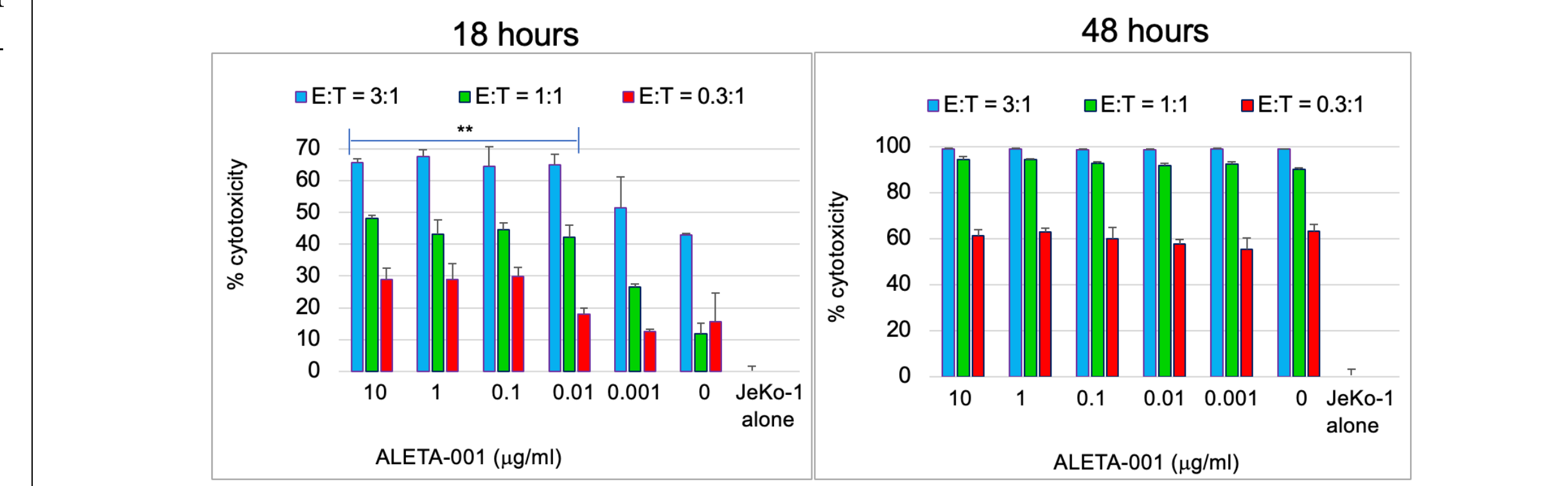


ALETA-001 induces CAR-19 T cell cytotoxicity against CD19-negative JeKo-1 cells with an IC50 = 130 pg/ml.

ALETA-001 mediates potent cytotoxicity

MOA – 3. ALETA-001 increases the effectiveness of the CAR-T cell pool and supports more effective killing of wildtype JeKo-1 cells that express both CD19 and CD20.

Figure 4. Incubation with ALETA-001 increases CAR-19 cytotoxicity against wildtype JeKo-1 lymphoma cells, with more rapid killing at lower E:T ratios measured at 18 hours.

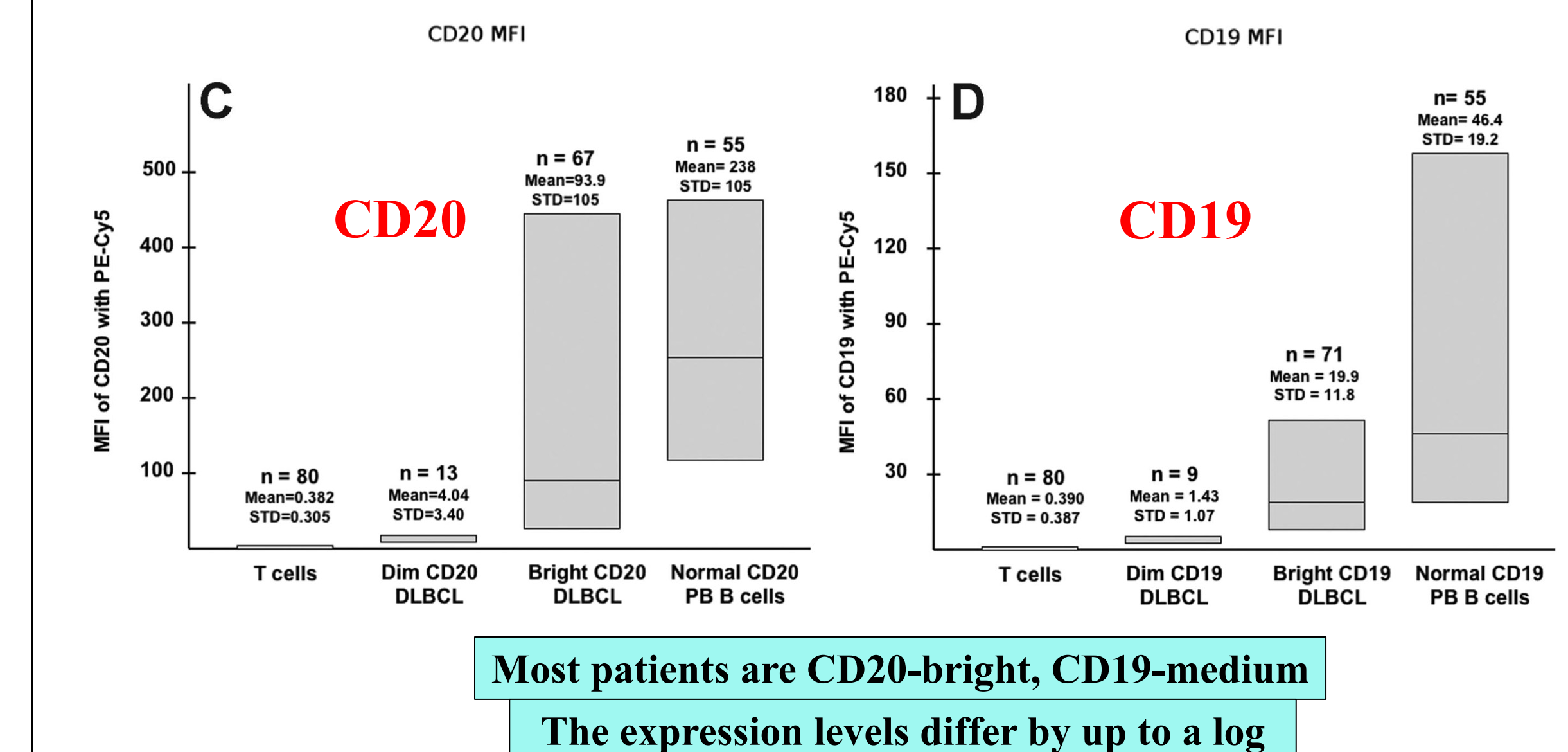


Higher antigen density = More rapid CAR19 activation and cytotoxicity, and at lower E:T ratios

Using E:T ratios of 0.3:1 to 3:1 we observed more rapid lymphoma cell killing at 18 hours, indicating that CAR-T activation and functionality were enhanced by the presence of ALETA-001. The effect was discernable with as little as 1 ng/ml ALETA-001 at E:T ratios of 3:1 and 1:1 and was robust with 10 ng/ml ALETA-001 at all tested E:T ratios. By 48 hours all incubation conditions had “caught up” and the effect was obscured.

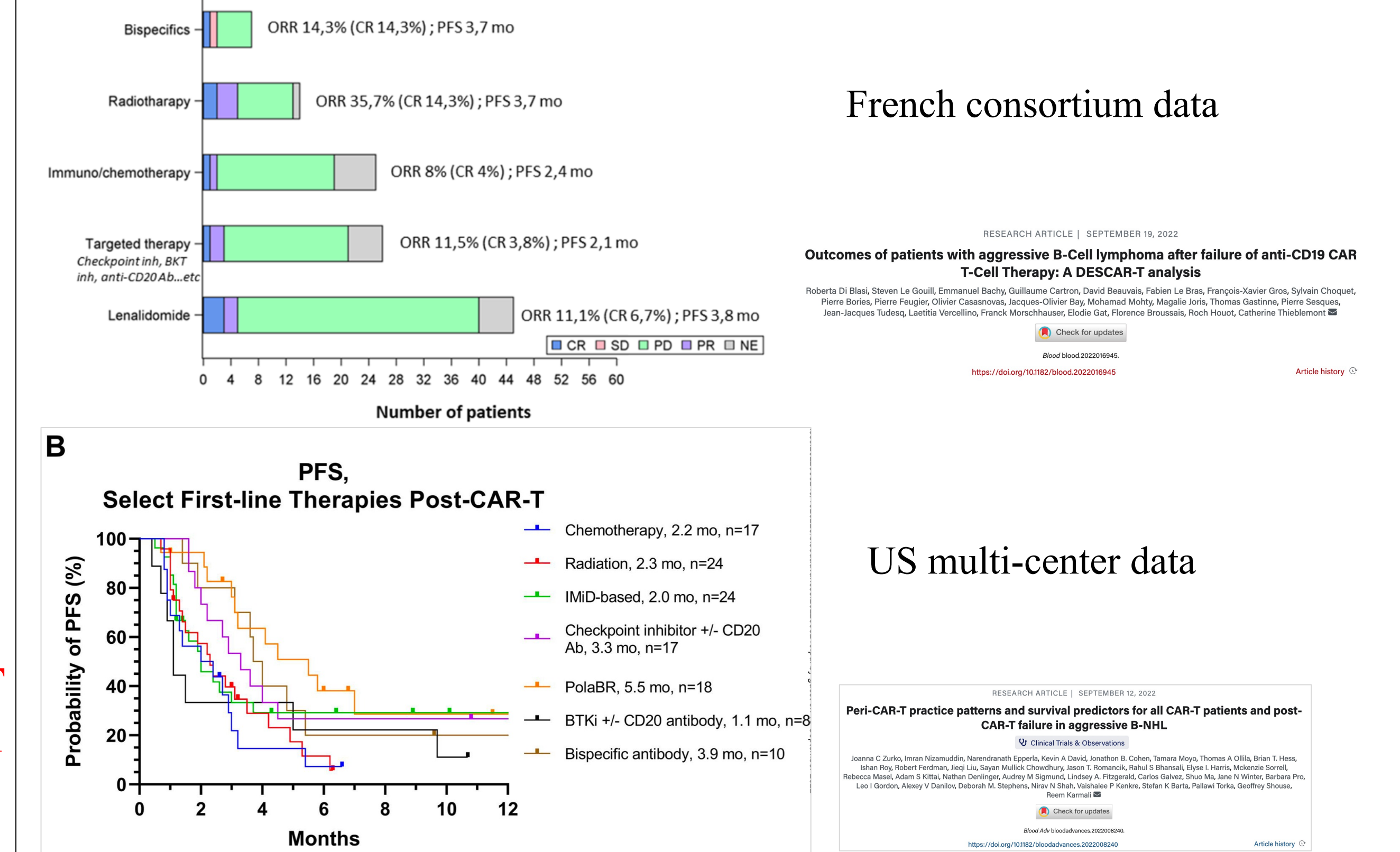
Most patient diffuse large B cell lymphoma cells (DLBCL) express much higher levels of CD20 than CD19

Figure 5a. from N.A.Johnson et al. 2009. Blood. doi:10.1182/blood-2008-09-177469



Most patients are CD20-bright, CD19-medium
The expression levels differ by up to a log

Figure 6. Rescue attempts for CAR19-relapsed patients yield poor outcomes

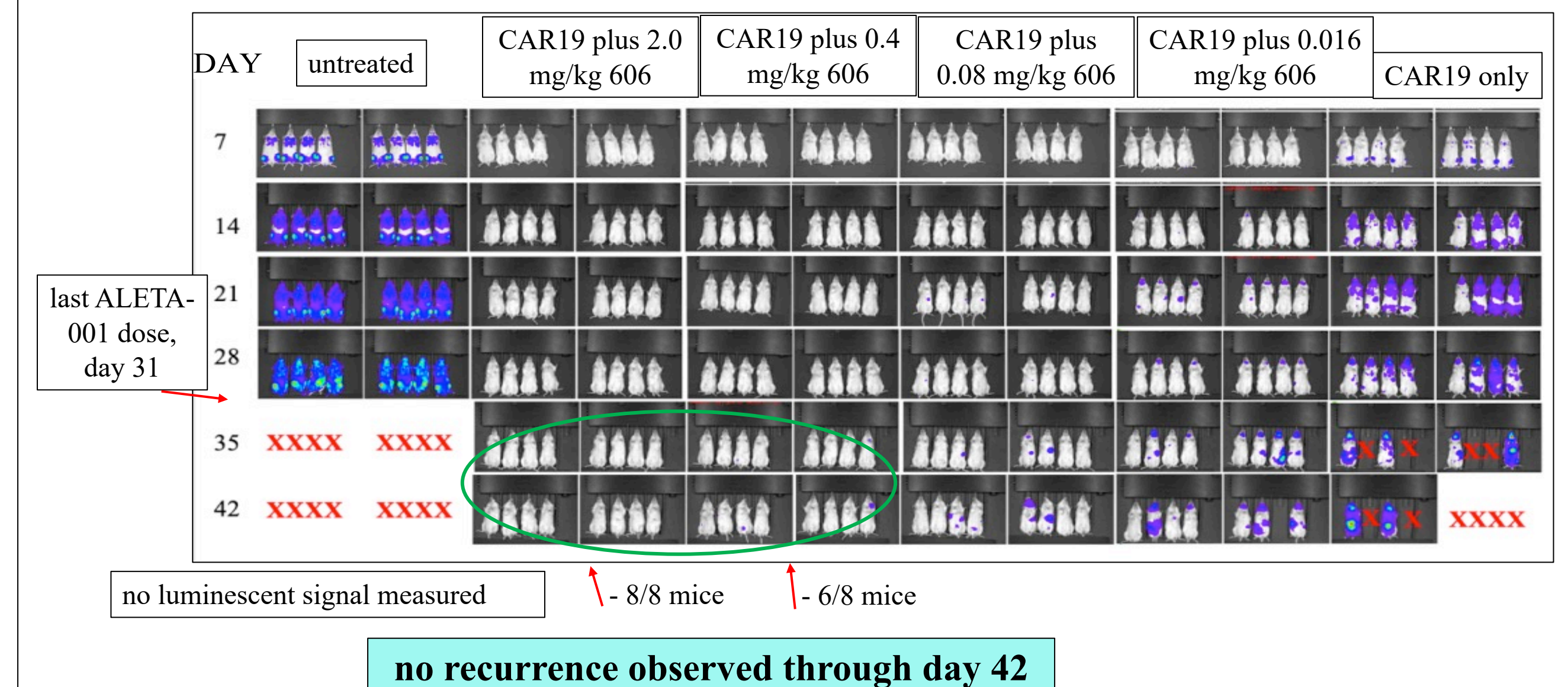


French consortium data

US multi-center data

ALETA-001 eliminates CD19-negative lymphoma in CAR-19 treated animals: CD19-deficient JeKo-1 Mantle Cell Lymphoma cells (MCL) were implanted into NSG mice and allowed to establish for 4 days before CAR-19 T cells and research-grade ALETA-001 protein was injected. The CARs were given once, and the protein dosed 3x weekly until day 31, at which point dosing stopped and animals were monitored for cancer recurrence.

Figure 6. In vivo CD19-negative lymphoma model



Animals were untreated, given CAR-19 alone, or given CAR-19 + increasing amounts of ALETA-001, dosed IV on the half-life (thus, every 3 days). Imaging was performed through day 42 post treatment initiation (4 days after CD19-negative lymphoma infusion). In the highest dose groups of 2 mg/kg there were no relapses (8/8 cured) and in the next dose group (0.4 mg/kg) 6 of 8 animals were cured.

These results support our clinical hypothesis that ALETA-001 will improve the effectiveness of existing anti-CD19 CAR-T cell therapeutics. The Phase 1/2 supported by Cancer Research UK will test this hypothesis in patients receiving CAR-19s who fail to reach a complete response at month 1 or relapse from a CR by month 6. The trial will begin in the 1H'23.