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 VH21 to target this second antigen, and an anti-albumin VH2 for half-life extension. When combined with CAR19 T cells, ALETA-001 restricts cytokinetics 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 VH21 linked to an optimized CAR19 protein and further linked to an anti-albumin llama VH21.**

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, γδ, 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.

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: JeCo-1, Mantle Cell Lymphoma; Right: Ramos, Hodgkin Lymphoma

ALETA-001 binding increases the detectable CD19 on JeCo-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 JeCo-1 experimental knockout cells, while exceeding normal CD19 expression.

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

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

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

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

ALETA-001 eliminates CD19-negative lymphoma in CAR19 treated animals: CD19-deficient JeCo-1 Mantle Cell Lymphoma (MCL) were implanted into NRG mice and allowed to establish for 4 days before CAR19 T cells and ex vivo 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 5b. JeCo-Ko cells

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

Figure 6. In vivo CD19-negative lymphoma model

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 CAR19s who fail to reach a complete response at month 1 or relapse from a CR by month 6. The trial will begin in the H2/23.