ABSTRACT

Background
CLL-1 is a compelling therapeutic target for AML as it is highly expressed on AML tumor cells and leukemic stem cells but is not expressed on hematopoietic stem cells. CB-012 was engineered with a next-generation Cas12a CRISPR hybrid RNAi-DNA (cRNA) genome-editing technology and leverages both checkpoint disruption and immune cloaking arming strategies to potentially improve antitumor activity.

The CB-012 anti-CLL-1 CAR was developed with a fully human sCD137 and the CD28 costimulatory domain and is currently in development for the treatment of relapsed or refractory AML (t/n AML). Here we describe preclinical studies that supported the CB-012 IND clearance by the FDA in October 2023.

Methods
Cas12a-cRNA guides were implemented to generate five gene edits in the manufacture of CB-012. A multiplex genome-editing strategy was designed to enhance the antitumor activity of CB-012 through prevention of G4D, PD-1 checkpoint disruption, and suppression of allograft rejection. In vitro and in vivo studies established the specificity of antigen binding, antigen-dependent activity, and toxicologic potential.

Results
CB-012 demonstrated potent antigen-dependent expansion and cytotoxic activity against CLL-1- human AML, T cells and patient-derived cells in co-cultures. In AML xenograft models, a single dose of CB-012 CAR T cells resulted in robust tumor control, leading to significant prolongation of survival. CB-012 co-culture with multiple CLL-1-negative cell types representing vital tissues demonstrated that the anti-CLL-1 sCD137 does not exhibit tissue cross-reactivity. In an unbiased cell surface protein microarray, the anti-CLL-1 sCD137 demonstrated highly specific interaction with human CLL-1, with no detectable non-specific interactions. CB-012 CAR T cells exhibited limited tissue infiltration and expansion in treatment naive, immunocompromised murine models.

Conclusion
CB-012, the first allophenic anti-CLL-1 CAR-T cell therapy using both checkpoint disruption and immune cloaking arming, demonstrated specific and potent CLL-1-targeted cytotoxic activity in vitro and in vivo. Specificity of the anti-CLL-1 sCD137 was demonstrated in an unbiased protein binding study and no adverse safety signals were observed from CB-012 in murine toxicology models. These preclinical studies supported the IND clearance of CB-012, which is being evaluated in the AMpLify trial, a Phase 1 clinical trial in patients with AML (NCT01438044).

CONCLUSIONS
• CLL-1 is a compelling therapeutic target for AML as it is highly expressed on AML tumor cells and leukemic stem cells but is not expressed on hematopoietic stem cells.
• CB-012 is the first allophenic anti-CLL-1 CAR-T cell therapy using checkpoint disruption and immune cloaking arming strategies, engineered with a next-generation Cas12a CRISPR technology.
• Specificity of the anti-CLL-1 fully human sCD137 was demonstrated in an unbiased protein-binding study and no adverse safety signals were observed in murine toxicology models.
• A single dose of CB-012 resulted in robust tumor control, leading to significant prolongation of survival in AML xenograft models.
• Data from these preclinical studies supported the IND clearance of CB-012 by the FDA in October 2023.