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703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

CLL-1-Targeted Allogeneic CAR-T Cells Exhibit High on-Target Specificity and Potent Cytotoxicity in Preclinical Models of Acute Myeloid Leukemia

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Background: CLL-1 is a compelling therapeutic target for acute myeloid leukemia (AML) as it is highly expressed on AML tumor cells and leukemic stem cells, but is not expressed on hematopoietic stem cells. An allogeneic anti-CLL-1 CAR-T cell therapy (CB-012) is in development for relapsed or refractory (r/r) AML. The CAR was generated with a fully human scFv targeting CLL-1 that was selected from a panel of scFvs. CB-012 was engineered with a next-generation Cas12a CRISPR hybrid RNA-DNA (chRDNA) genome-editing technology to leverage both checkpoint disruption and immune cloaking for potentially improved antitumor activity.
Methods: Cas12a chRDNA guides were implemented to generate five genome edits in the manufacture of CB-012. A fully human anti-CLL-1 CAR transgene was site-specifically inserted into the *TRAC* gene, thereby eliminating TCR expression to reduce graft-versus-host disease. A B2M-HLA-E fusion transgene was inserted into the native *B2M* gene, preventing expression of all HLA class I antigens except HLA-E, to blunt both T and NK cell-mediated allograft rejection of the CAR-T cells. A knockout of the *PDCD1* gene prevented PD-1 receptor expression and thus PD-L1 ligand binding to prolong antitumor activity. This multiplex genome-editing strategy was designed to enhance the antitumor activity of CB-012. *In vitro* and *in vivo* studies evaluated specificity for antigen binding, antigen-dependent activity, and preclinical safety assessments.
Results: CB-012 CAR-T cells express a fully human anti-CLL-1 scFv-containing CAR construct and demonstrate potent antigen-dependent cytotoxic activity in human AML cell line co-cultures. In AML xenograft models, a single-dose administration of CB-012 CAR-T cells exerted robust tumor control, leading to significant prolongation of survival. Cell binding studies suggested that the anti-CLL-1 scFv does not exhibit tissue cross-reactivity when examined in the context of a collection of cell types representing vital tissues. In an unbiased cell surface protein microarray, the anti-CLL-1 scFv demonstrated specific interaction with human CLL-1, without detectable non-specific interactions. CB-012 CAR-T cells exhibited limited tissue infiltration and expansion in treatment naïve, immunocompromised mouse models.
Conclusion: CB-012 demonstrated highly specific and potent CLL-1-targeted cytolytic activity *in vitro* and *in vivo*. Specificity of the anti-CLL-1 scFv was further demonstrated by screening in an unbiased protein-binding study and no adverse safety signals were observed in murine models. These data support advancing the development of CB-012 into a first-in-human clinical trial for patients with r/r AML.

Disclosures Francica: *Caribou Biosciences:* Current Employment. **Garner:** *Caribou Biosciences:* Current Employment, Current equity holder in publicly-traded company. **Namburi:** *Caribou Biosciences:* Current Employment. **Colgan:** *Caribou Biosciences:* Current Employment. **Fowler:** *Caribou Biosciences:* Current Employment. **Mutha:** *Caribou Biosciences:* Ended employment in the past 24 months. **Aviles:** *Caribou Biosciences:* Current Employment. **Stanaway:** *Caribou Biosciences:* Current Employment. **Guo:** *Caribou Biosciences:* Current Employment. **An:** *Caribou Biosciences:* Current Employment. **Kelly:** *Caribou Biosciences:* Ended employment in the past 24 months. **Degagne:** *Caribou Biosciences:* Current Employment. **Kwong:** *Caribou Biosciences:* Current Employment. **Edwards:** *Caribou Biosciences:* Current Employment. **Jakes:** *Caribou Biosciences:* Current Employment. **Shaw:** *Caribou Biosciences:* Current Employment. **Schilling:** *Caribou Biosciences:* Current Employment. **Huynh:** *Caribou Biosciences:* Ended employment in the past 24 months. **Luu:** *Caribou Biosciences:* Current Employment. **Sidorov:** *Caribou Biosciences:* Current Employment. **Mousali:** *Caribou Biosciences:* Current Employment. **Otsmaa:** *Caribou Biosciences:* Current Employment. **Lauer:** *Caribou Biosciences:* Current Employment. **Skoble:** *Caribou Biosciences:*

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