POSTER # 3201

CB-012, an allogeneic anti-CLL-1 CAR-T cell therapy engineered with next-generation CRISPR technology to resist both the immunosuppressive tumor microenvironment and immune cell-mediated rejection, for patients with relapsed or refractory acute myeloid leukemia

Elizabeth Garner[1], Erin Kelly[1], Sai Namburi[1], Cian Colgan[1], Tristan Fowler[1], Devin Mutha[1], Art Aviles[1], Morena Stanaway[1], Raymond Guo[1], Zili An[1], Emilie Degagné [1], George Kwong[1], Leslie Edwards[1], Emma Jakes[1], McKay Shaw[1], Benjamin Schilling[1], Jeremy Huynh[2], Ricky Luu[1], Max Sidorov[1], Rhonda Mousali[1], Mikk Otsmaa[1], Justin Skoble[1], Steven Kanner[1]

[1]Caribou Biosciences, Inc., Berkeley, California, USA ; [2] Former Caribou Employee; *Disclosures*: All authors are current or former employees of Caribou Biosciences, Inc. and may have Caribou stock options and/or own Caribou stock.

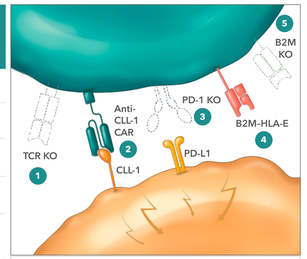
Background

CB-012 is an allogeneic anti-CLL-1 CAR-T cell therapy in development for evaluation in relapsed or refractory acute myeloid leukemia (r/r AML). CB-012 is engineered with a next-generation CRISPR genome-editing technology to leverage both checkpoint disruption and immune cloaking to improve CAR-T cell antitumor activity. CLL-1 is a compelling therapeutic target as it is highly expressed on AML tumor cells and leukemic stem cells, but not expressed on hematopoietic stem cells. It has also been established as a target in published human proof-of-concept studies. In preclinical studies, CB-012 demonstrated significant antitumor efficacy and specific CLL-1-targeted cytolytic activity. The genome-editing strategy employed to manufacture and armor CB-012 conferred a functional advantage relative to the immunosuppressive tumor microenvironment associated with r/r AML.

CB-012: anti-CLL-1 allogeneic CAR-T cell therapy with a PD-1 knockout and immune cloaking

Key attributes	CB-012	Other allogenic CAR-Ts for AML
Cas12a chRDNA editing for enhanced genomic integrity • Reduced off-target editing and enhanced insertion rates	✓	✗
1 ***TRAC* gene knockout (KO)** • Eliminates TCR expression, reduces GvHD risk	✓	Varies
2 **Human anti-CLL-1 CAR site-specifically inserted into *TRAC* gene** • Eliminates random integration, targets tumor antigen	✓	Varies
3 **PD-1 KO for enhanced antitumor activity** Potentially better therapeutic index via initial tumor debulking	✓	✗
4 **B2M-HLA-E-peptide fusion site-specifically inserted into *B2M* gene** • Blunts NK cell-mediated rejection	✓	✗
5 ***B2M* gene KO** • Reduces HLA class I presentation and T cell-mediated rejection	✓	✗

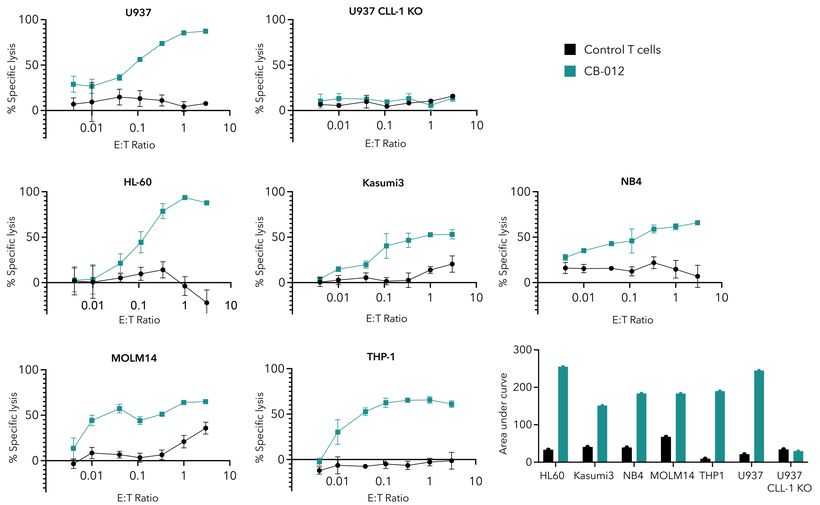
CB-012 uses a potent, fully human anti-CLL-1 scFv with a CD28 costimulatory domain



Program: CB-012

Healthy donor leukapheresis-derived T cells
Tumor antigen: CLL-1 (also known as CD371)
Indication: r/r acute myeloid leukemia (AML)
Status: IND planned for H2 2023

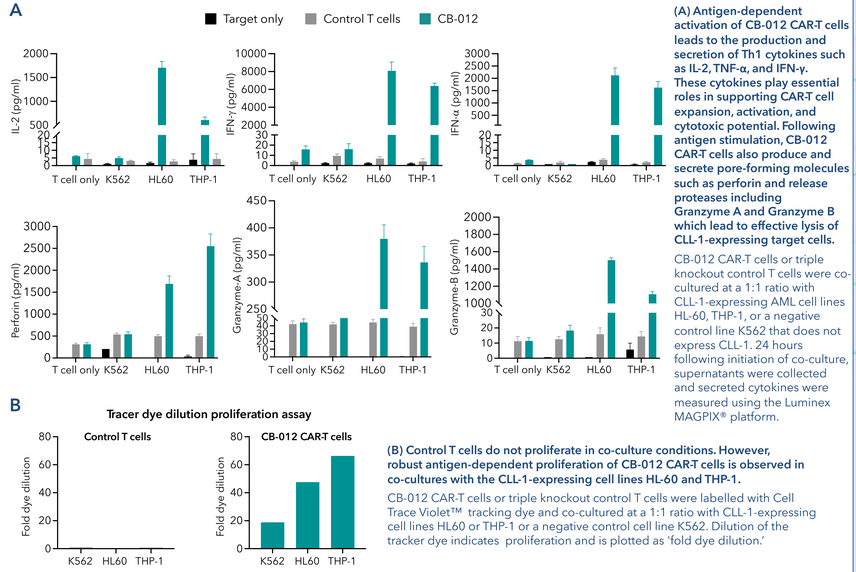
CB-012 exhibits antigen-dependent cytotoxicity *in vitro*



CB-012 CAR-T cells exhibit dose-dependent cytotoxicity at increasing effector-to-target ratios. CB-012 CAR-T cell cytotoxicity is dependent on the expression of the target antigen, CLL-1.

CB-012 CAR-T cells or triple knockout control T cells were co-cultured with CLL-1-expressing cell lines U937, MOLM-14, NB4, Kasumi3, HL-60, THP-1, or a negative control cell line engineered with a CLL-1 knockout (U937-CLL-1 KO). 48 hours following initiation of co-culture, the specific cell lysis of the CLL-1-expressing target cell population was determined by flow cytometry.

CB-012 exhibits antigen-dependent cytolytic activity *in vitro*



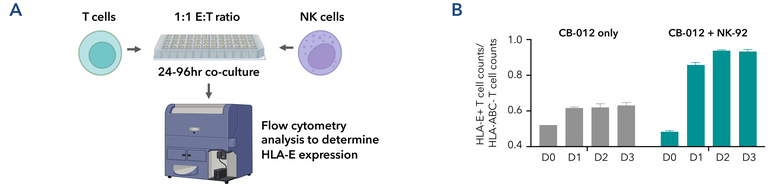
(A) Antigen-dependent activation of CB-012 CAR-T cells leads to the production and secretion of Th1 cytokines such as IL-2, TNF-α, and IFN-γ. These cytokines play essential roles in supporting CAR-T cell expansion, activation, and cytotoxic potential. Following antigen stimulation, CB-012 CAR-T cells also produce and secrete pore-forming molecules such as perforin and release proteases including Granzyme A and Granzyme B which lead to effective lysis of CLL-1-expressing target cells.

CB-012 CAR-T cells or triple knockout control T cells were co-cultured at a 1:1 ratio with CLL-1-expressing AML cell lines HL-60, THP-1, or a negative control line K562 that does not express CLL-1. 24 hours following initiation of co-culture, supernatants were collected and secreted cytokines were measured using the Luminex MAGPIX® platform.

(B) Control T cells do not proliferate in co-culture conditions. However, robust antigen-dependent proliferation of CB-012 CAR-T cells is observed in co-cultures with the CLL-1-expressing cell lines HL-60 and THP-1.

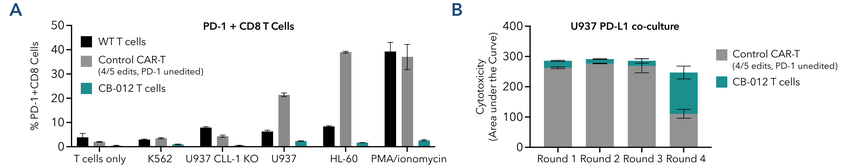
CB-012 CAR-T cells or triple knockout control T cells were labelled with Cell Trace Violet™ tracking dye and co-cultured at a 1:1 ratio with CLL-1-expressing cell lines HL60 or THP-1 or a negative control cell line K562. Dilution of the tracker dye indicates proliferation and is plotted as 'fold dye dilution.'

Immune cloaking armoring protects CB-012 from NK cell-mediated cytotoxicity *in vitro*



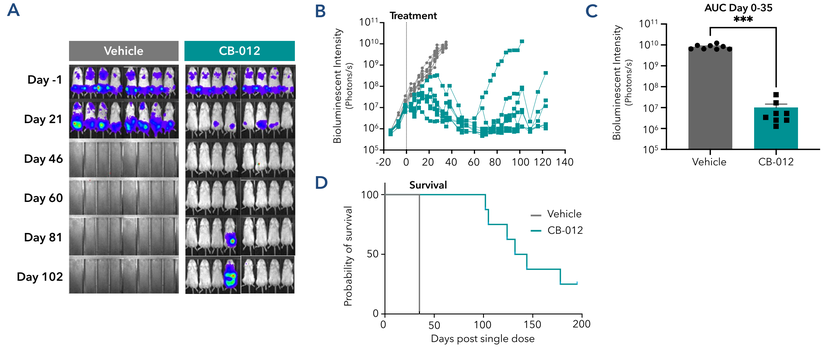
In the presence of NK cells, the CB-012 HLA-E-expressing, ABC-negative population increases demonstrating protection of the HLA-E fusion-expressing population from NK cell-mediated cytotoxicity. In the absence of NK cells, the HLA-E-expressing population is stable, indicating no intrinsic survival advantage. (A) CB-012 cells were co-cultured with NK-92 cells at a 1:1 ratio in the presence of IL-2. The percentage of HLA-E-positive and HLA-E-negative CB-012 cells were monitored by flow cytometry over time. The HLA-ABC-negative population represents a surrogate marker to identify the B2M knockout population. **(B)** The ratio of HLA-E-expressing CB-012 CAR-T cells within this population over three days of culture in the presence or absence of NK-92 cells is shown.

Checkpoint disruption armoring prevents PD-1 expression and maintains CB-012 cytotoxic potential *in vitro*



(A) PD-1 is a receptor expressed on activated T cells that can contribute to the inhibition of effective antitumor immunity. CB-012 CAR-T cells are engineered to abrogate PD-1 expression to enhance CAR-T cell activation in the presence of antigen. To validate the loss of PD-1 expression, cells unedited at *PDCD1* or CB-012 (which includes a *PDCD1* knockout) were cultured at a 1:1 ratio with target cells or stimulated with phorbol myristate acetate (PMA) / ionomycin for 24 hours. PD-1 expression was evaluated by flow cytometry and expressed as a percentage of the CD8+ population. When cultured alone or with K562 target cells that do not express CLL-1, a small fraction of CB-012 CAR-T cells express PD-1 on the cell surface. In co-cultures with the CLL-1-expressing target line HL-60, PD-1 expression is upregulated in control CAR-T cells unedited at *PDCD1*, but not in CB-012 CAR-T cells. Similarly, when stimulated with a T cell activation cocktail containing PMA and ionomycin, PD-1 expression is induced in control T cells and in control CAR-T cells unedited at *PDCD1*, but not in CB-012 CAR-T cells. This demonstrates that the knockout of *PDCD1* functions to eliminate expression of PD-1 upon CB-012 CAR-T cell activation. **(B) CB-012 CAR-T cells maintain cytotoxic potential following multiple rounds of challenge.** CB-012 CAR-T cells or control CAR-T cells unedited at *PDCD1* were co-cultured with CLL-1- and PD-L1-expressing target cells. Cytotoxicity was summarized as area under the curve and plotted over 4 rounds of re-challenge with target cells.

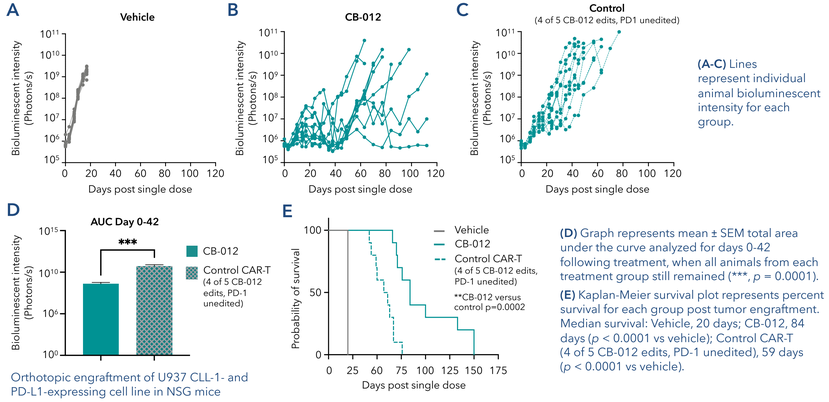
CB-012 demonstrates potent antitumor activity in a xenograft model of AML



CB-012 demonstrates significant antitumor efficacy and prolonged survival in a CLL1-positive human tumor xenograft model of AML. Human acute myeloid leukemia HL-60-GFP-ffLuc+ tumor cells were engrafted intravenously in NSG mice on day 0 and a single bolus dose of vehicle or CB-012 was given intravenously on day 21. **(A)** IVIS images show tumor burden for each animal on different days following treatment. **(B)** Lines represent individual animal bioluminescent intensity for each group. **(C)** Graph represents mean ± SEM total area under the curve analyzed for days 0-35 following treatment, when all animals from each group remained alive (***, $p = 0.0002$). **(D)** Kaplan-Meier survival plot represents percent survival for each group post tumor engraftment. Median survival: Vehicle, 35 days; CB-012, 138 days ($p = 0.0001$ vs vehicle).

CB-012 checkpoint disruption armoring enhances antitumor activity in PD-L1-expressing xenograft model

CB-012 demonstrated significant antitumor efficacy and prolonged survival when compared to vehicle and control CAR-T cells in a CLL-1-positive PD-L1-overexpressing human tumor xenograft. Human CLL-1-expressing U937-PD-L1+-GFP-ffLuc+ tumor cells were engrafted intravenously in NSG mice on day 0, and a single bolus dose of vehicle, or CB-012, or control CAR-T (4 of 5 CB-012 edits, PD-1 unedited) was given intravenously on day 3.



(A-C) Lines represent individual animal bioluminescent intensity for each group.

Orthotopic engraftment of U937 CLL-1- and PD-L1-expressing cell line in NSG mice

(D) Graph represents mean ± SEM total area under the curve analyzed for days 0-42 following treatment, when all animals from each treatment group still remained (***, $p = 0.0001$).

(E) Kaplan-Meier survival plot represents percent survival for each group post tumor engraftment. Median survival: Vehicle, 20 days; CB-012, 84 days ($p < 0.0001$ vs vehicle); Control CAR-T (4 of 5 CB-012 edits, PD-1 unedited), 59 days ($p < 0.0001$ vs vehicle).

Summary

- CB-012 is a next-generation CRISPR-edited allogeneic anti-CLL-1 CAR-T cell therapy in preclinical development for the treatment of adult patients with r/r AML
- Cas12a chRDNA genome-editing technology was used to engineer 5 edits in the manufacture of CB-012 and has been shown to provide insertion efficiency, reduced off-target editing, and enhanced genomic integrity
- CB-012 is engineered with immune cloaking and checkpoint disruption strategies designed to enhance antitumor activity
- CB-012 CAR-T cells demonstrate potent antitumor activity *in vitro* and enhanced survival in AML xenograft models
- CB-012 IND application submission planned for H2 2023

CORRESPONDING AUTHOR: Steven Kanner, PhD (skanner@cariboubio.com), Caribou Biosciences, Inc.

American Association for Cancer Research Annual Meeting
April 14-19, 2023 | Orange County Convention Center | Orlando, Florida