Abstract 6323

Preclinical evaluation of CB-012, an allogeneic anti-CLL-1 CAR-T cell therapy, that exhibits specific and potent cytotoxicity in acute myeloid leukemia (AML) xenograft models

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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 RNA-DNA (chRDNA) genome-editing technology and leverages both checkpoint disruption and immune cloaking armoring strategies to potentially improve antitumor activity. The CB-012 anti-CLL-1 CAR was developed with a fully human scFv and the CD28 costimulatory domain and is currently in development for the treatment of relapsed or refractory AML (r/r AML). Here we describe preclinical studies that supported the CB-012 IND clearance by the FDA in October 2023.

Methods

Cas12a chRDNA guides were implemented to generate five genome 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 GvHD, PD-1 checkpoint disruption, and suppression of allograft rejection. In vitro and in vivo studies evaluated the specificity of antigen binding, antigen-dependent activity, and toxicologic potential.

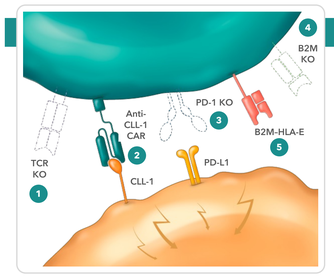
Results

CB-012 demonstrated potent antigen-dependent expansion and cytotoxic activity against CLL-1$^+$ human AML cell lines 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 scFv does not exhibit tissue cross-reactivity. In an unbiased cell surface protein microarray, the anti-CLL-1 scFv 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 naïve, immunocompromised murine models.

Conclusion

CB-012, the first allogeneic anti-CLL-1 CAR-T cell therapy using both checkpoint disruption and immune cloaking armoring, demonstrated specific and potent CLL-1-targeted cytolytic activity in vitro and in vivo. Specificity of the anti-CLL-1 scFv 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, first-in-human clinical trial for patients with r/r AML (NCT06128044).

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



Armored with 5 genome edits

1. ***TRAC* gene knockout (KO)**
   - Eliminates TCR expression, reduces GvHD risk
2. **Human anti-CLL-1 CAR site-specifically inserted into *TRAC* gene**
   - Eliminates random integration, targets tumor antigen
3. **PD-1 KO for enhanced antitumor activity**
   - Potentially better therapeutic index via initial tumor debulking
4. ***B2M* gene KO**
   - Reduces HLA class I presentation and T cell-mediated rejection
5. **B2M-HLA-E-peptide fusion site-specifically inserted into *B2M* gene**
   - Blunts NK cell-mediated rejection

- 1st CAR-T with **checkpoint inhibition and immune cloaking** (PD-1 KO, B2M KO + B2M-HLA-E-peptide fusion) to enter the clinic[1]
- Cas12a chRDNA editing for reduced off-target editing and **enhanced insertion rates**
- Potent, fully human **anti-CLL-1** scFv [2] with a CD28 costimulatory domain

[1] To Caribou's knowledge

[2] Anti-CLL-1-specific scFv exclusively licensed from Memorial Sloan Kettering Cancer Center for allogeneic cell therapies

CB-012 demonstrates antigen-specific cytotoxicity, proliferation, and cytokine secretion against CLL-1-expressing AML cell lines

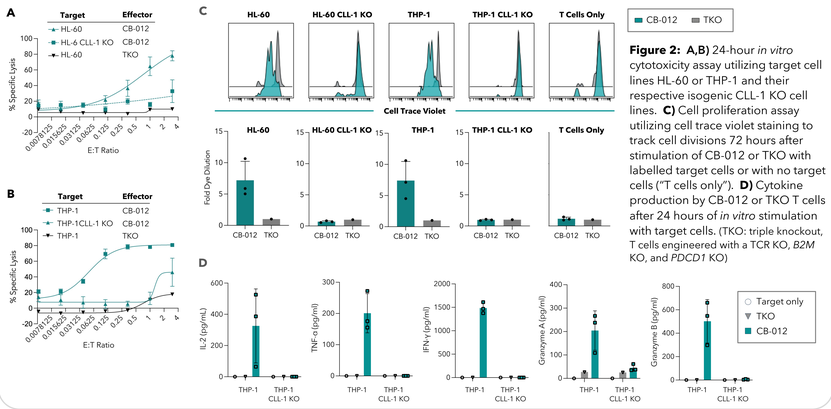


Figure 2: A,B) 24-hour *in vitro* cytotoxicity assay utilizing target cell lines HL-60 or THP-1 and their respective isogenic CLL-1 KO cell lines. **C)** Cell proliferation assay utilizing cell trace violet staining to track cell divisions 72 hours after stimulation of CB-012 or TKO with labelled target cells or with no target cells ("T cells only"). **D)** Cytokine production by CB-012 or TKO T cells after 24 hours of *in vitro* stimulation with target cells. (TKO: triple knockout, T cells engineered with a TCR KO, *B2M* KO, and *PDCD1* KO)

PDCD1 KO in CB-012 confers significant increase in activity

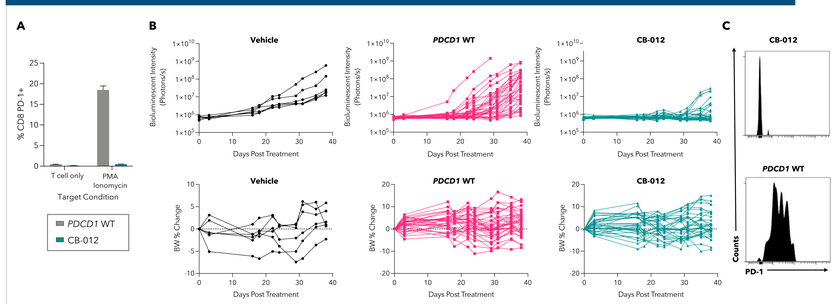


Figure 3: A) PD-1 expression was analyzed by flow cytometry on fully edited CB-012 ("CB-012") or CB-012 that was not edited at the *PDCD1* locus ("*PDCD1* WT") after 24 hours in culture +/- PMA/ionomycin stimulation. **B)** *In vivo* tumor burden as measured by luminescent intensity (top) and corresponding mouse body weight (BW%, bottom). 5 x 10^5 HL-60 cells edited to express GFP and luciferase as well as to overexpress PD-L1 were engrafted by intravenous injection and allowed to establish for 7 days before injection of 1 x 10^7 CB-012 or *PDCD1* WT CAR-T cells. **C)** *Ex vivo* expression of PD-1 on CB-012 or *PDCD1* WT T cells extracted from a terminal take down of bone marrow from surviving mice 42 days after treatment with each cell type. Data represents the concatenation of transferred edited T cells from 5 mice.

CB-012 displays minimal off-target, off-tissue activity

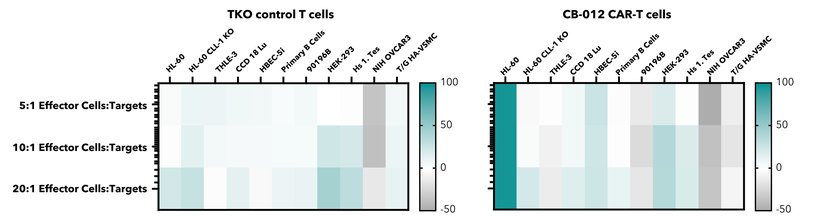
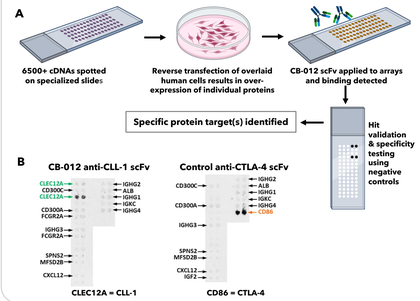


Figure 4: 9 cell lines representative of vital human tissues as well as positive and negative control cell lines (HL-60 and HL-60 CLL-1 KO, respectively) were incubated *in vitro* for 48 hours with 3 effector-to-target ratios to define potential off-tumor activity of CB-012. Cytotoxicity profiles or experimental cell lines were similar when cell lines were incubated with CB-012 as compared to cultures incubated with TKO. (TKO: triple knockout, T cells engineered with a TCR KO, *B2M* KO, and *PDCD1* KO)

Unbiased screen for off-target binding reveals high specificity of CB-012 scFv



C

Test	Specific Interactions Found
PBS (-control)	No
CTLA4 (+control)	Yes
CLEC12a (CLL-1, +Experimental control)	Yes
6,105 human plasma membrane proteins	No
400 heterodimers	No

Figure 5: A) Schematic of workflow for the Retrogenix unbiased screen for off-target scFv interactions. A library screen was performed testing the CB-012 anti-CLL-1 scFv interactions across a cell array over-expressing 6,105 individual full-length human plasma membrane proteins and cell surface-tethered human secreted proteins as well as a further 400 human heterodimers. **B)** Spot array results from the confirmation screen. All identified library interactions were re-expressed and probed with CB-012 anti-CLL-1 scFv to determine which interactions may be specific to the CB-012 scFv. All positive results were also positive in the anti-CTLA-4 control group except for CLL-1. **C)** Summary of results from screen.

CB-012 pharmacokinetics in tumor naïve murine models demonstrates limited antigen-independent expansion

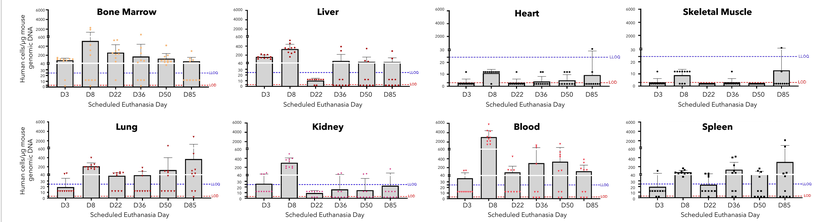


Figure 6: Non-tumor bearing NSG mice were treated with 30 x 10^6 CB-012 T cells. Persistence of cells in labelled organs was measured by sacrificing animals, processing organs, and detecting CB-012-specific sequences by ddPCR over an 85-day time course.

CB-012 AMpLify Phase 1 trial design

Figure 7:

Patients with r/r AML

- Relapsed or refractory AML patients should have received at least 1 but not more 3 prior lines of therapy
- Patients with prior allo or auto SCT are allowed
- Exclusions: prior CAR-T cell therapy and/or CLL-1-targeted therapy

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 allogeneic anti-CLL-1 CAR-T cell therapy using checkpoint disruption and immune cloaking armoring strategies, engineered with a next generation Cas12a chRDNA technology
- Specificity of the anti-CLL-1 fully human scFv 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
- A Phase 1 first-in-human clinical trial (AMpLify) in relapsed/refractory AML patients is ongoing and currently enrolling patients in the United States (NCT06128044).

ABBREVIATIONS:

AML: acute myeloid leukemia
MTD: maximum tolerated dose
RDE: recommended dose or doses for expansion
RP2D: recommended phase 2 dose
SCT: stem cell transplant
TKO: triple knockout, T cells engineered with a TCR KO, *B2M* KO, and *PDCD1* KO

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