POSTER # 524

CB-011, a BCMA-specific allogeneic CAR-T cell therapy, engineered with next-generation CRISPR technology to knock out B2M and express a B2M–HLA-E fusion transgene to blunt immune cell-mediated rejection, for r/r multiple myeloma

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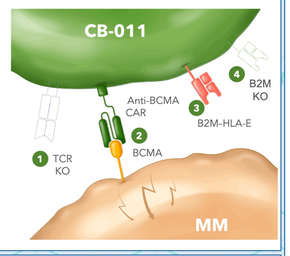
Background

The approval and commercialization of autologous chimeric antigen receptor (CAR)-T cell products have opened a path for sophisticated CAR-T cell therapies with next-generation capabilities. Healthy donor-derived allogeneic cell therapies may unlock the broad potential of engineered cells as a leading therapeutic modality. Addressing expansion, persistence, armoring, and trafficking of allogeneic immune cell therapies is critical to achieving durable patient responses. CB-011 is a genome-edited allogeneic anti-B cell maturation antigen (BCMA) CAR-T cell product candidate that will be evaluated in the CaMMouflage Phase 1 clinical trial for relapsed or refractory multiple myeloma (r/r MM). CB-011 is engineered with an "immune cloaking" genome editing strategy designed to reduce donor CAR-T cell rejection by both patient T cells and natural killer (NK) cells.

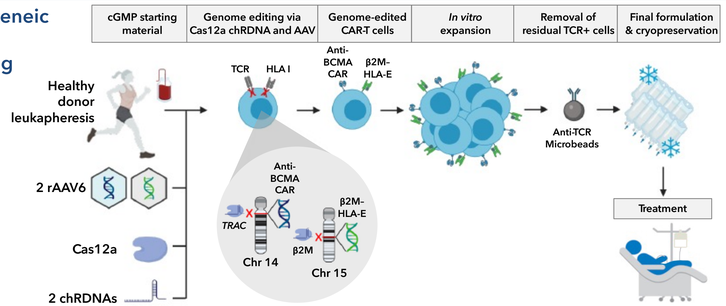
Introduction: CB-011 is an allogeneic anti-BCMA CAR-T cell therapy in clinical development for the treatment of adult patients with r/r MM

CB-011 is derived from healthy donor T cells that have been genome edited with clustered regularly interspaced short palindromic repeats (CRISPR)-hybrid RNA DNA (chRDNA) and recombinant CRISPR-associated 12a (rCas12a) endonuclease technology

1. The *T cell receptor alpha constant* (*TRAC*) gene was knocked out (KO) to prevent expression of the T cell receptor (TCR) alpha chain and assembly of cell-surface TCR α/β heterodimers to reduce potential graft versus host disease (GvHD)
2. An anti-BCMA CAR expression cassette was inserted into the *TRAC* locus by homologous recombination using a recombinant adeno-associated virus 6 (rAAV6) encoding the CAR flanked by *TRAC* homology arms to impart BCMA-specific cytotoxicity
3. A B2M-human leukocyte antigen, Class 1, E (B2M–HLA-E) fusion protein expression cassette was inserted into the *B2M* locus by using a second rAAV6 to inhibit host NK cell-mediated rejection
4. In addition, the *beta-2-microglobulin* (*B2M*) gene was knocked out with the rCas12a chRDNA technology to prevent cell-surface expression of major histocompatibility complex (MHC) Class I antigens to reduce host T cell-mediated rejection



CB-011 allogeneic CAR-T cell manufacturing process overview



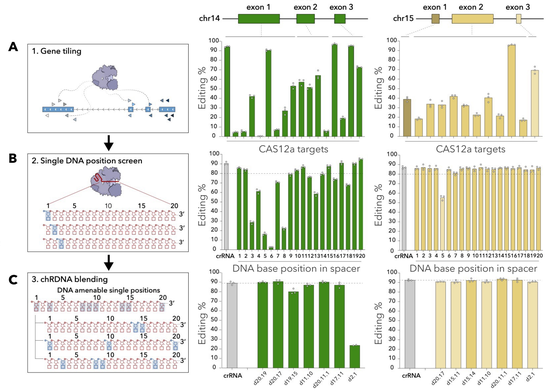
CB-011 is engineered using Cas12a chRDNA guides for precision genome editing

chRDNA guides used with Cas12a included strategically located DNA bases to confer high target specificity. Cas12a chRDNA guides were designed to target the TRAC and B2M genes for implementation in CB-011.

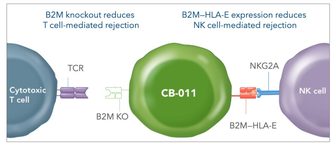
(A) The chRDNA design process begins with gene tiling to identify optimal all-RNA CRISPR guides (crRNAs). Once optimal all-RNA guides are identified, iterative DNA position screening proceeds for a given target sequence.

(B) Individual chRDNAs were tested with a single DNA base at each position in the 20-nucleotide spacer and then evaluated for on-target editing activity in T cells.

(C) The positions where DNA bases did not reduce editing efficiencies were combined in subsequent chRDNA guide designs to identify optimal chRDNAs with multiple DNA bases in the optimized sequence.

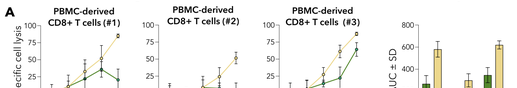


CB-011 immune cloaking armoring strategy suppresses cytotoxic T and NK cell-mediated allograft rejection

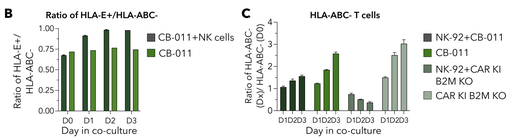


(A) HLA class I mismatched CD8+ T cells from 3 different donors were co-cultured with CB-011 CAR-T cells (green) or CAR-T cells without the immune cloaking engineering (sand). Immune cloaking engineering reduces mismatched T cell mediated cytotoxicity. **(B)** CB-011 cells were co-cultured with NK-92 cells at a 1:1 ratio in the absence of IL-2. The percentage of HLA-E positive and HLA-E negative CB-011 cells were monitored over time. The HLA-ABC negative population represents a surrogate marker to identify the B2M knock-out population. The ratio of HLA-E expressing CB-011 CAR-T cells within this population over three days of culture in the presence or absence of NK-92 cells is shown. In the absence of NK cells, the HLA-E expressing population is stable indicating no intrinsic survival advantage. In the presence of NK cells, the HLA-E expressing ABC negative population increases demonstrating protection of the HLA-E fusion expressing population from NK cell mediated cytotoxicity. **(C)** CB-011 CAR-T cells or CAR-T cells with a B2M knock out without HLA-E fusion engineering (CAR KI B2M KO) were cultured in media supplemented with IL-2 in the presence or absence of NK cells. In the absence of NK cells, IL-2 supplemented T cells increase. In the presence of NK cells, T cells engineered without the HLA-E fusion are susceptible to NK mediated lysis and numbers decline while CB-011 CAR-T cells are resistant.

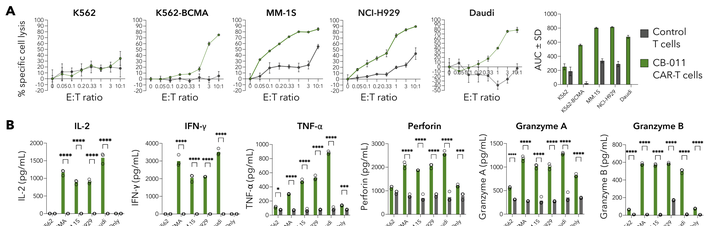
B2M KO removes all endogenous HLA class I presentation to **reduce T cell-mediated rejection**



B2M–HLA-E-peptide fusion insertion **blunts NK cell-mediated rejection**

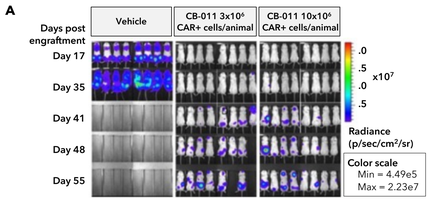


CB-011 CAR-T cells demonstrate potent BCMA targeted cytotoxicity and cytokine secretion in vitro



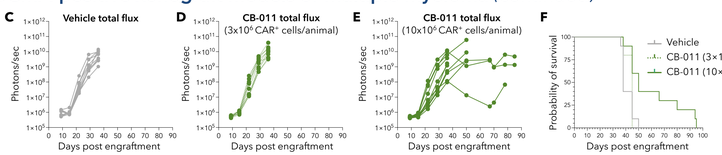
(A) CB-011 CAR-T cells or double knock-out control T cells were co-cultured with BCMA-expressing cell lines MM.1S, NCI-H929, Daudi or the engineered BCMA-expressing cell line K562. 48 hours following initiation of co-culture the specific cell lysis of the BCMA expressing target cell population was determined by flow cytometry. CB-011 CAR-T cells exhibit dose-dependent cytotoxicity at increasing effector to target ratios. CB-011 CAR-T cell cytotoxicity is dependent on the expression of the target antigen, BCMA. **(B)** CB-011 CAR-T cells or double knock-out control T cells were co-cultured with BCMA-expressing cell lines MM.1S, NCI-H929, Daudi, or the engineered BCMA-expressing cell line K562. 24 hours following initiation of co-culture, supernatants were collected and secreted cytokines were measured using the Luminex MAGPIX® platform. Antigen-dependent activation of CB-011 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 stimulation, CB-011 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 BCMA expressing target cells.

CB-011 CAR-T cells demonstrate significant antitumor activity and enhanced survival in BCMA-positive xenograft models of multiple myeloma



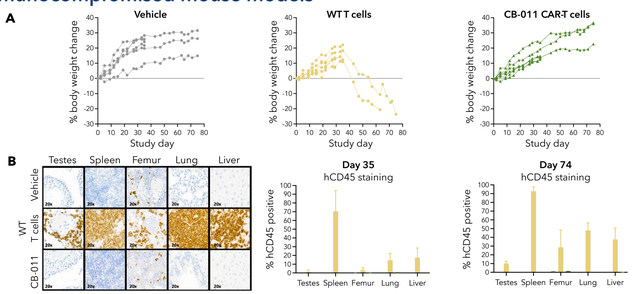
(A) Human MM.1S-GFP-Luc + tumor cells were injected intravenously in NSG mice on Day 0 and a single bolus dose of CB-011 CAR-T cells was given intravenously on Day 3 at the cell dose indicated. Bioluminescence imaging was performed using an IVIS® Spectrum system. IVIS images show tumor burden for each animal on different days following engraftment. No animals in the vehicle control survived beyond day 35. Missing animals in CAR-T cell treated groups indicate deaths. **(B)** Kaplan-Meier survival plot represents percent survival for each group post tumor engraftment. Median survival: Vehicle, 37 days; CB-011 (3x10⁶ CAR⁺ T cells/animal), 59 days (p < 0.0001 vs vehicle); CB-011 (10x10⁶ CAR⁺ T cells/animal), 64 days (p < 0.0001 vs vehicle).

CB-011 CAR-T cells demonstrate significant antitumor activity and enhanced survival in BCMA-positive xenograft models of multiple myeloma *(continued)*



(C-E) Human MM NCI-H929-GFP-Luc+ tumor cells were injected intravenously in NSG mice on Day 0 and a single bolus dose of CB-011 CAR-T cells was given intravenously on Day 3 at the cell dose indicated. Bioluminescence imaging was performed using an IVIS® Spectrum system. Lines represent individual animal bioluminescent intensity for each group. **(F)** Kaplan-Meier survival plot represents percent survival for each group post tumor engraftment. Median survival: Vehicle, 38 days; CB-011 (3x10⁶ CAR⁺ T cells/animal), 45 days (p = 0.2354 vs vehicle); CB-011 (10x10⁶ CAR⁺ T cells/animal), 50 days (p = 0.004 vs vehicle).

No GvHD or CAR-T cell tissue infiltration was detected following administration of CB-011 in immunocompromised mouse models



(A) NSG mice were treated on Day 0 with a single bolus of vehicle, wild-type unmodified T cells, or CB-011 CAR-T cells. Animal body weight was obtained by an electronic balance. Lines represent individual animal body weight percent change post treatment. Scheduled takedown at Day 35 eliminated some of the mice from body weight plots afterward. Signs of acute GvHD were observed in WT T cell dosed animals but not vehicle or CB-011 CAR-T cell dosed cohorts. % body weight decline in the WT T cell dosed animals was accompanied by clinical signs of GvHD including hunching, skin lesions and dull fur. **(B)** Example images of hCD45 staining (shown at 20x) from day 74 samples in vehicle (top row), wild-type unmodified T cells (middle row), and CB-011 CAR-T cells (bottom row) groups. Quantification of percent hCD45 staining from samples collected at Day 35 and 74, respectively are shown.

CB-011 will be evaluated in the CaMMouflage Phase 1 trial

Patients with r/r MM

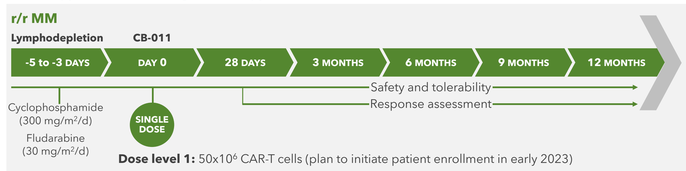
- ≥3 prior lines of therapy, including a PI, an IMiD, and an anti-CD38 antibody
- Exclusions: prior CAR-T cell therapy and/or BCMA-targeted therapy within last 3 months

Part A: 3+3 dose escalation

- Objective: safety, determine MTD, RP2D

Part B: dose expansion

- Objective: antitumor response



Dose level 1: 50x10⁶ CAR-T cells (plan to initiate patient enrollment in early 2023)

Summary

- CB-011 is a next-generation CRISPR-engineered allogeneic anti-BCMA CAR-T cell therapy in clinical development for the treatment of adult patients with r/r MM
- Cas12a chRDNA genome-editing technology was used to engineer CB-011 and provides enhanced genomic integrity and insertion efficiency, as well as reduced off-target editing
- CB-011 is engineered with an immune cloaking strategy that reduced cytotoxic T cell and NK cell-mediated allograft rejection
- CB-011 CAR-T cells demonstrate potent antitumor activity *in vitro* and enhanced survival in MM xenograft models
- No adverse safety signals were observed *in vivo*
- CB-011 will be evaluated in the CaMMouflage Phase 1 clinical trial for patients with r/r MM

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