Poster#LB009

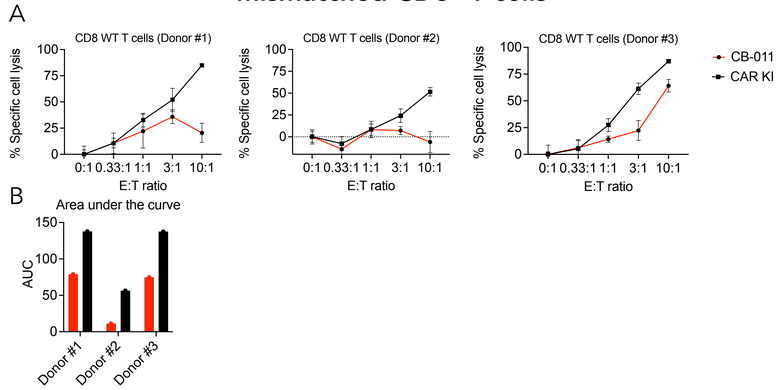
A BCMA-specific allogeneic CAR-T cell therapy (CB-011) genome-engineered to express an HLA-E fusion transgene to prevent immune cell rejection

Elizabeth Garner*[1], Emilie Degagné*[1], Suparna Roy[1], Paul Donohoue[1], Tristan Fowler[1], Morena Stanaway[1], Vanina Vicena[1], Devin Mutha[1], Benjamin Shilling[1], McKay Shaw[1], Mara Bryan[1], Leslie Edwards[1], Stephen Smith[1], Bryan Kohrs[1], Lynda Banh[1,2], Kyle McSweeney[1,3], Justin Skoble[1], and Steven Kanner**[1].

Abstract

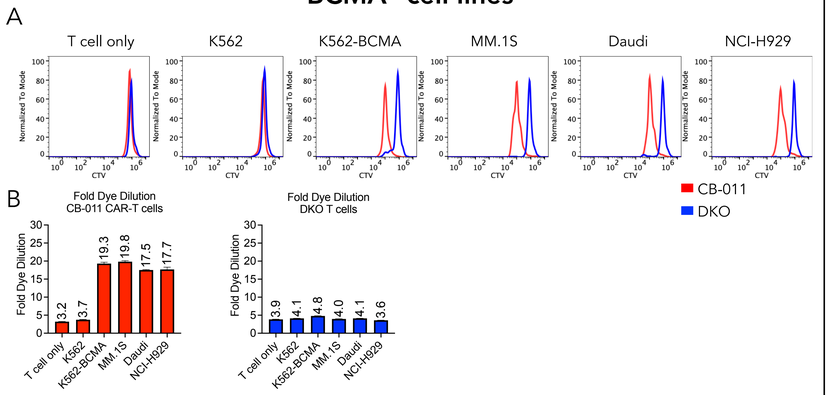
The approval and commercial launch of multiple first-generation CD19- or BCMA-directed, autologous CAR-T cell products have laid the foundation and opened a path for the development of more advanced cellular therapeutics, including CAR-T cell products with next-generation capabilities. Among these newer designs, allogeneic cell therapies are positioned to unlock the broad potential of engineered immune cells as a leading therapeutic modality. However, expansion, persistence, armoring, and trafficking of allogeneic CAR-T cells are critical to achieving long-term efficacy. Caribou Biosciences is advancing a BCMA-specific allogeneic CAR-T cell product candidate, CB-011, with an immune cloaking approach that includes both the removal of the endogenous B2M protein and the insertion of a B2M-HLA-E-peptide (B2M–HLA-E) fusion protein transgene. This strategy is designed to blunt CAR-T cell rejection by both recipient T cells and NK cells, and CB-011 is in preclinical development for relapsed or refractory multiple myeloma (r/r MM). We used Cas12a chRDNA guides in the manufacture of CB-011 to make four edits, including site-specific insertion of a proprietary humanized anti-BCMA CAR into the TRAC locus with high specificity and efficiency, thus eliminating TCR expression to prevent graft-versus-host disease (GvHD). In addition, we inserted a gene encoding a B2M–HLA-E fusion protein into the native B2M gene locus. This method simultaneously prevents the expression of the native B2M protein and expresses a minor HLA class I antigen HLA-E to blunt both T- and NK-mediated rejection of the CAR-T cells by the recipient's immune system. B2M is a protein that stabilizes all HLA class I antigens on the cell surface, therefore its knockout eliminates endogenous HLA class I presentation on the surface of the CAR-T cells. To demonstrate that the B2M–HLA-E fusion protein expression protects CB-011 from NK-mediated cell killing, we developed an *in vitro* competition assay in which CB-011 cells are co-incubated with NK cells. We observed that CAR-T cells expressing the B2M–HLA-E fusion have a survival advantage over cells that do not express the fusion in the presence of NK cells *in vitro*, indicating that they could resist killing by a recipient's NK cells and potentially circulate longer. The BCMA-specific CAR leads to long-term survival in mice bearing established orthotopically-engrafted MM tumor cells. Furthermore, mice treated with high doses of CB-011 did not experience symptoms typical of GvHD. This strategy should enable CB-011 CAR-T cells to remain in circulation longer in recipients, providing for increased potential of antitumor activity.

CB-011 CAR-T cells are protected against HLA mismatched CD8⁺ T cells



CB-011 or control HLA-I expressing CAR-T cells (CAR-KI) were co-cultured for 7 days with HLA mismatched CD8+ T cells from three donors at a range of effector to target (E:T) ratios. Specific lysis (A) and area under the curve (B) were calculated. % of specific lysis was calculated using the formula: % specific lysis = 100*(1- (count of live Target cells in wells with Effector cells/count of live cells in Target cells in Target only wells)).

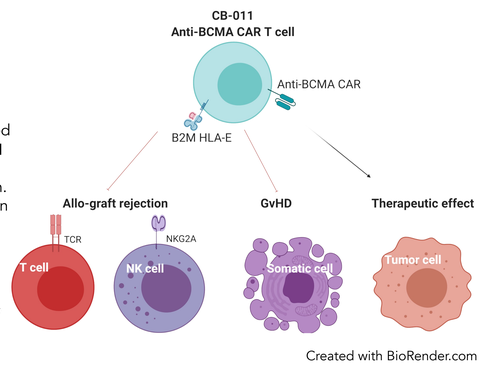
CB-011 CAR-T cells proliferate in co-cultures with BCMA⁺ cell lines



CB-011 CAR-T cells and target cell lines were incubated at a 1:1 effector to target ratio and proliferation was monitored by CTV dye dilution at 72 h. (A) Histograms of CTV staining for representative samples. (B) Fold dye dilution representing proliferation of CB-011 CAR-T cells or DKO T cells at 72 h. (Fold Dye Dilution = CTV geometric mean (0 h)/ CTV geometric mean (72 h). n = 3/group. DKO = *TRAC* KO and *B2M* KO.

Introduction

- Immune rejection of allogeneic CAR-T cells may reduce the persistence and therapeutic effect of a therapy. These concerns may be alleviated by engineering the CAR-T cell product with specific immune cloaking strategies
- CB-011 CAR-T cells are genome edited to remove B2M, which prevents class I HLA expression thus enhancing protection against host T cell rejection. However, this modification can result in the activation of host NK cells
- To prevent host NK cells from mediating rejection, CB-011 CAR-T cells are engineered to express a B2M–HLA-E fusion protein specifically designed to inhibit NK cell cytotoxic activity
- This engineering approach is designed to increase persistence and thus therapeutic benefit of CB-011 CAR-T cells



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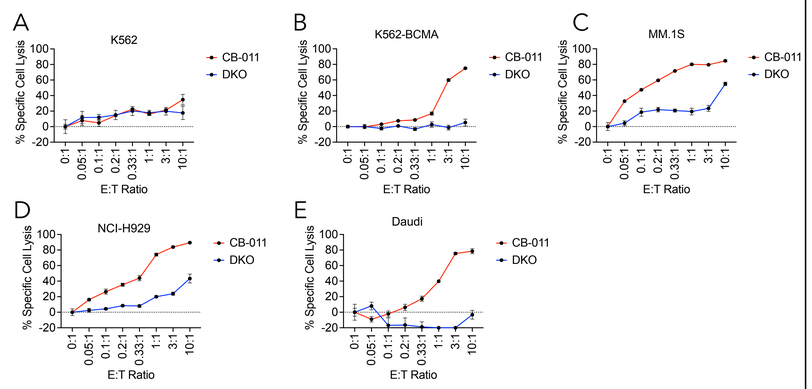
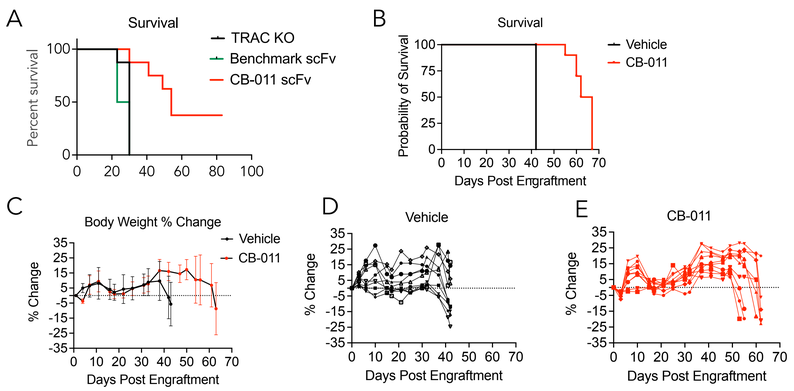
CB-011 CAR-T cells demonstrate *in vitro* cytotoxicity against a panel of BCMA⁺ cell lines



In vitro T cell cytotoxicity assay at a range of effector to target (E:T) ratios showing efficacy following 48-hour co-culture with BCMA negative cell line K562 (A) and BCMA⁺ cell lines K562-BCMA (B), MM.1S (C), NCI-H929 (D) and Daudi (E). % Specific lysis = 100*(1- (count of live Target cells in wells with Effector cells/count of live cells in Target cells in Target only wells)). DKO = *TRAC* KO and *B2M* KO.

CB-011 treatment prolongs survival in mice engrafted with a multiple myeloma tumor model



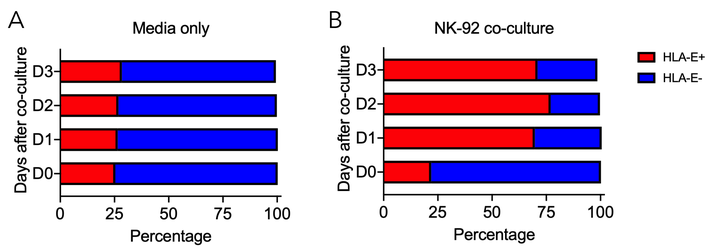
(A) Kaplan-Meier survival plots represent percent survival for each group post subcutaneous tumor engraftment. CB-011 vs Benchmark BCMA-CAR p<0.0004

(B) Kaplan-Meier survival plots represent percent survival for each group post orthotopic tumor engraftment. Vehicle vs. CB-011 (1×10^7 viable CAR+ cells/animal), p<0.0001

(C) Lines represent averages ± SD for group body weight percent change post orthotopic tumor engraftment

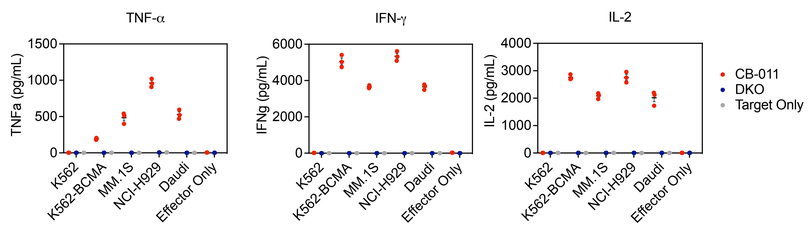
(D & E) Lines represent individual animal body weight percent change post orthotopic tumor engraftment. For all graphs, day 0 represents the day of tumor engraftment

CB-011 CAR-T cells expressing a B2M—HLA-E fusion are protected against NK-mediated cytotoxicity



CB-011 cells were co-cultured with NK-92 cells at a 1:1 ratio, and the percentage of HLA-E positive and negative CB-011 cells was monitored daily (D). Percentage of HLA-E positive and negative CB-011 cells from Day 0 though Day 3 is shown following culture in media only (A) or co-culture with NK-92 cells (B).

CB-011 CAR-T cells secrete TNF-α, IFN-γ, and IL-2 in co-cultures with BCMA⁺ cell lines



Secretion of TNF-α (left panel), IFN-γ (middle panel), and IL-2 (right panel) by effector cells was quantified by Luminex multiplex immunoassay. Effector cells were plated at a 1:1 E: T ratio with BCMA negative cell line K562 and BCMA⁺ cell lines K562-BCMA, MM.1S, Daudi, and NCI-H929. Supernatants were collected after 24 hours. DKO = *TRAC* KO and *B2M* KO.

Conclusions

- Abrogation of B2M expression and insertion of a B2M–HLA-E fusion protein protect CB-011 CAR-T cells against cytolysis by both NK and HLA mismatched CD8+ T cells as shown *in vitro* in co-culture assays
- CB-011 CAR-T cells are cytotoxic against BCMA-expressing tumor cells as demonstrated *in vitro* in cytotoxicity assays, cytokine secretion and antigen-dependent proliferation assays
- *In vivo*, CB-011 CAR-T cells prolong survival compared to a benchmark BCMA CAR or vehicle control in the MM.1S tumor xenograft mouse model of multiple myeloma
- Further *in vivo* studies will examine this immune cloaking strategy and the potential increase in therapeutic effect that it confers

* – First Authors
** – Correspondence
Questions: Please contact Steve Kanner (skanner@cariboubio.com)
www.cariboubio.com

1 – Caribou Biosciences, Inc., 2929 Seventh St #105, Berkeley, CA 94710
2 – Current affiliation: OneSignal, Inc., 2850 S Delaware St #201, San Mateo, CA 94403
3 – Current affiliation: Adicet Bio, Inc., 200 Constitution Drive, Menlo Park, CA 94025