The background is a solid teal color. It is decorated with white molecular structure patterns, consisting of interconnected circles and lines representing atoms and bonds. These patterns are most prominent on the left side, where they form a vertical column, and on the right side, where they form a large, branching structure resembling a tree or a complex network. The overall aesthetic is scientific and modern.

Preclinical Evaluation of ALLO-605, an Allogeneic BCMA TurboCAR T™ Cell Therapy for the Treatment of Multiple Myeloma

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Disclosures

- Full-time employee of Allogene Therapeutics
- Equity interest in Allogene Therapeutics

ALLO-605 (BCMA) utilizes TALEN® gene-editing technology pioneered and owned by Collectis. Allogene has an exclusive license to the Collectis technology for allogeneic products directed at this target and holds all global development and commercial rights for this investigational candidate.

Disclaimers

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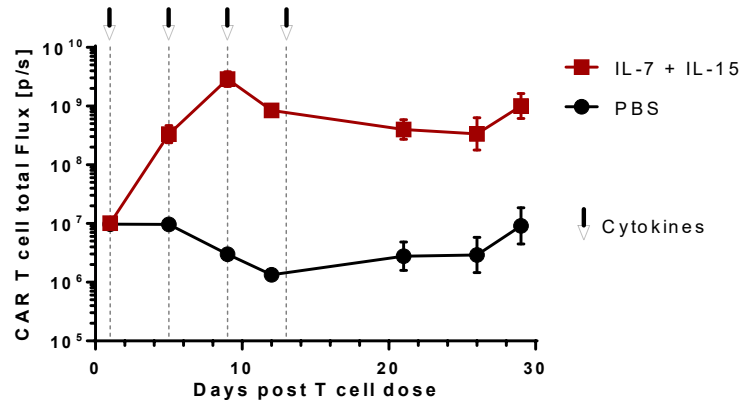
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Abstract

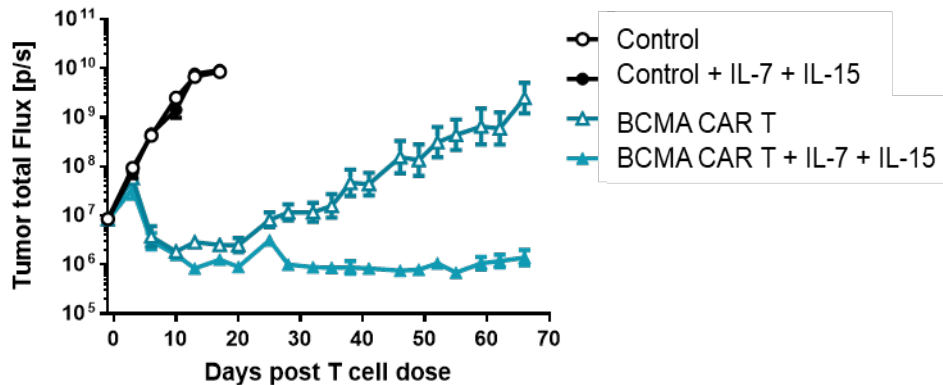
Chimeric antigen receptor (CAR) T cells have demonstrated unprecedented efficacy in heavily pretreated relapsed and/or refractory multiple myeloma (MM) patients but may require further engineering to achieve their greatest potential. Enhancing cell expansion and persistence by providing cytokine support has been shown to improve the long-term antitumor activity of adoptively transferred CAR T cells in preclinical models. Combining CAR T cells with systemically-administered cytokines or cytokine mimetics can however result in toxicities and adverse events. Alternatively, cytokine signaling can be provided in a constitutive, CAR T cell-intrinsic fashion, by exogenous expression of a Constitutively Active Chimeric Cytokine Receptor (CACCR). CACCRs can be engineered by combining a membrane-tethered dimerization and JAK-binding domain derived from the thrombopoietin receptor (TpoR) fused to an intracellular signaling domain derived from a cytokine receptor. We investigated the impact of CACCR expression on the phenotype, functionality, persistence, and safety profile of allogeneic CAR T cells targeting BCMA to produce a second generation allogeneic BCMA “TurboCAR T™” candidate (ALLO-605). BCMA CAR T cells were generated from healthy donor T cells by lentiviral transduction with a CAR construct followed by genetic inactivation of the TRAC and CD52 loci using Collectis’ TALEN® gene editing technology. Allogeneic BCMA TurboCAR™ T cells, engineered for stoichiometric expression of the CAR and a CACCR via a self-cleaving peptide, were produced similarly. Constitutive expression of the CACCR during manufacturing had no negative effects on CAR T cell phenotype or yield and resulted in a product with over 60% stem cell memory/central memory T cells. In vitro, BCMA TurboCAR T™ cells showed enhanced cytokine secretion, polyfunctionality and improved serial killing activity. In a disseminated mouse model of multiple myeloma, BCMA TurboCAR T™ cells exhibited at least a 2-fold increase in peak expansion and enhanced survival and persistence compared to BCMA CAR T cells, resulting in prolonged antitumor responses and delaying relapses. Despite this enhanced persistence, we found that exposure to target cells was absolutely required for the expansion and long-term activity of BCMA TurboCAR T™ cells and no evidence of target- and cytokine-independent proliferation was observed. Since adoptive cell therapies have the potential to elicit toxicities in some patients, two alternative approaches to modulate BCMA TurboCAR T™ cell activity were investigated. First, we tested the ability of a CD20-based off-switch incorporated within the CAR to sensitize cells to rituximab and found effective depletion of BCMA TurboCAR T™ cells by complement or effector cells in vitro and in vivo in the presence of the antibody. In addition, we confirmed rapid inhibition of BCMA TurboCAR T™ cells by the protein tyrosine kinase inhibitor dasatinib, which has been shown to interfere with LCK activity. The prolonged persistence and antitumor responses seen in preclinical models along with a favorable safety profile of BCMA TurboCAR™ T cells support clinical investigation of ALLO-605 in relapsed or refractory multiple myeloma.

ALLO-605 (BCMA TurboCAR T™) cells express a constitutively active chimeric cytokine receptor (Turbodomain) providing “signal 3” in a CAR T cell-intrinsic fashion

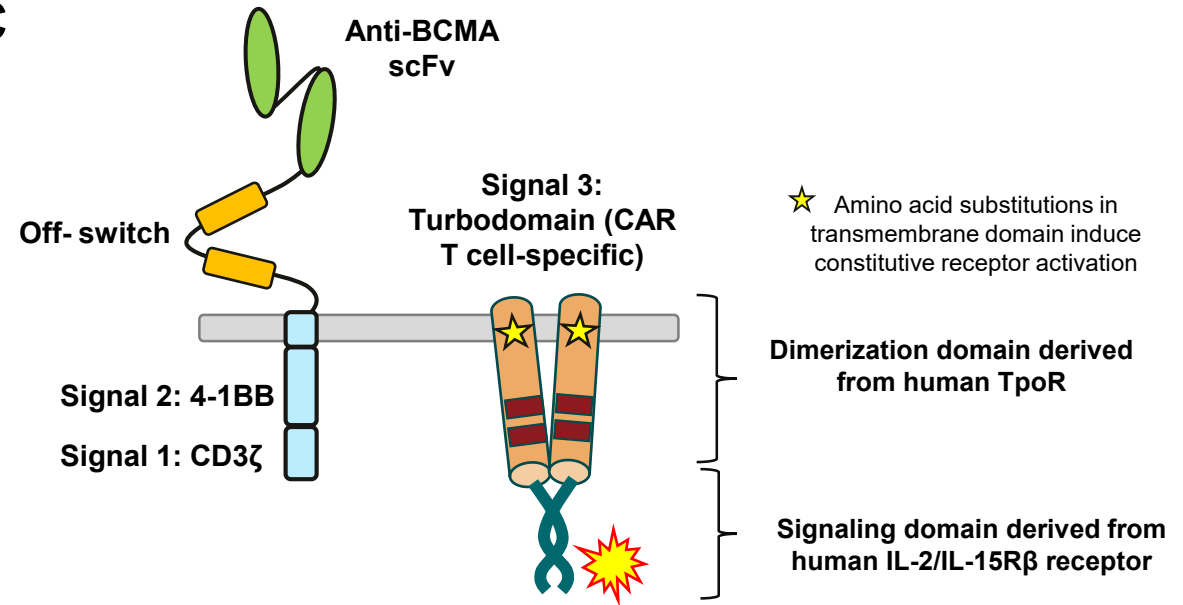
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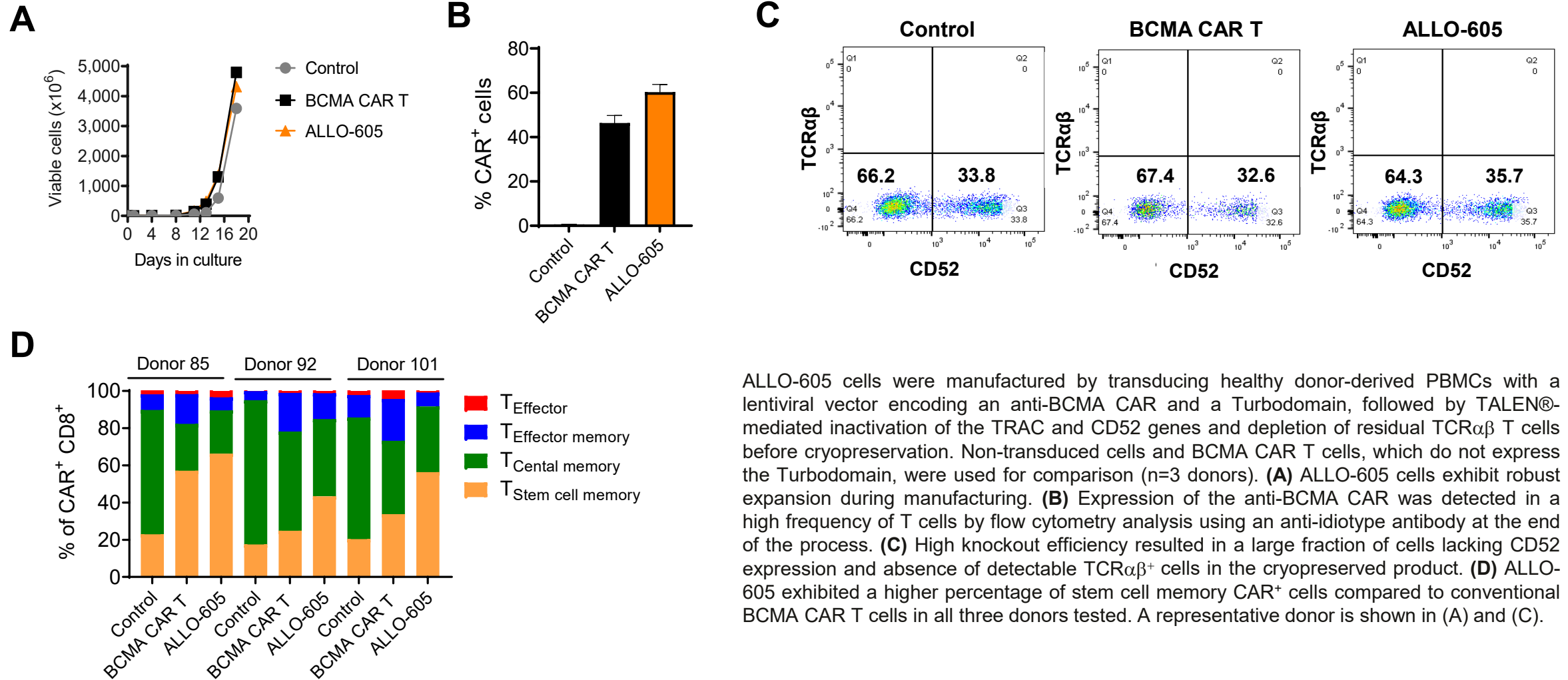


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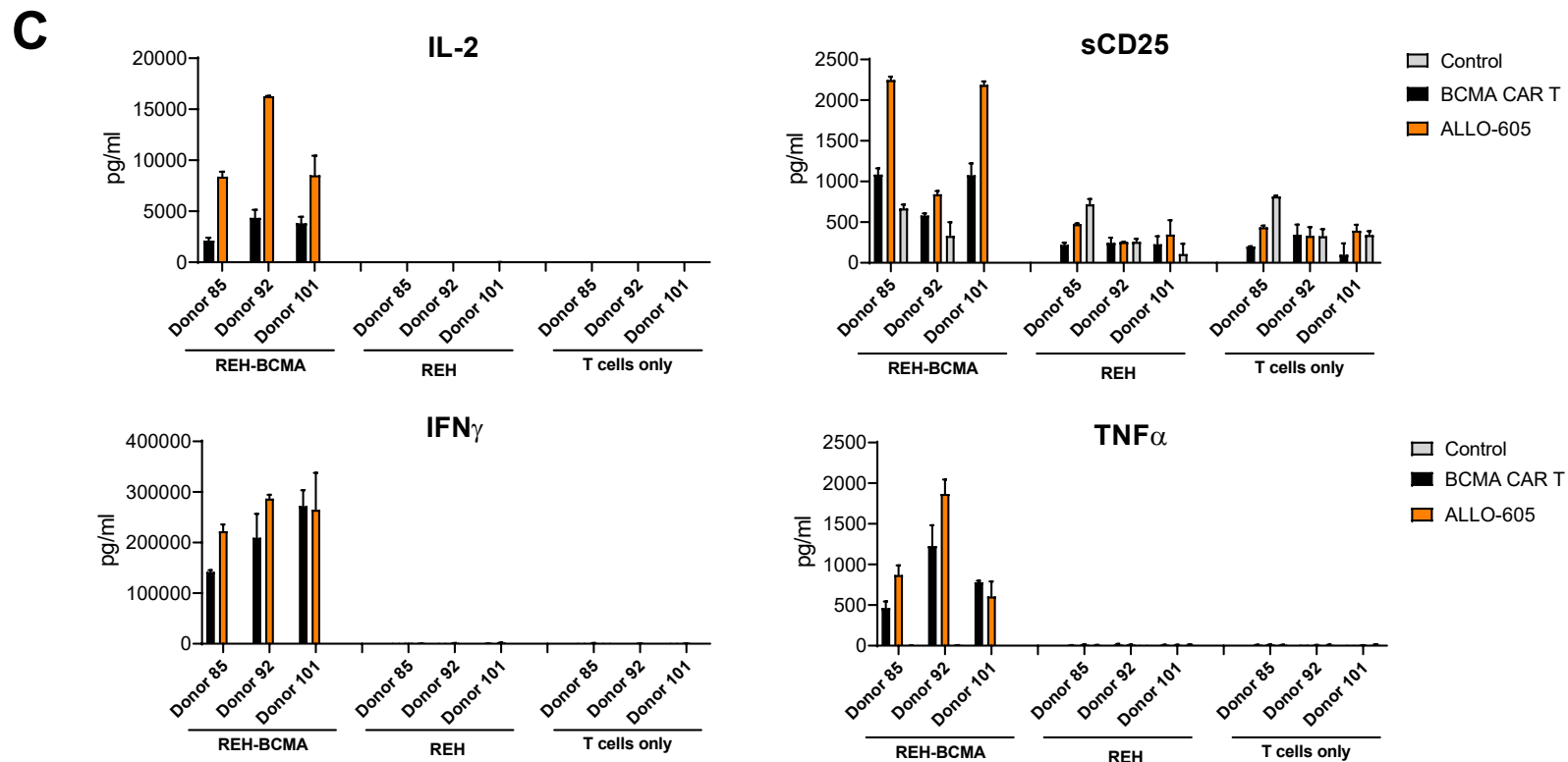
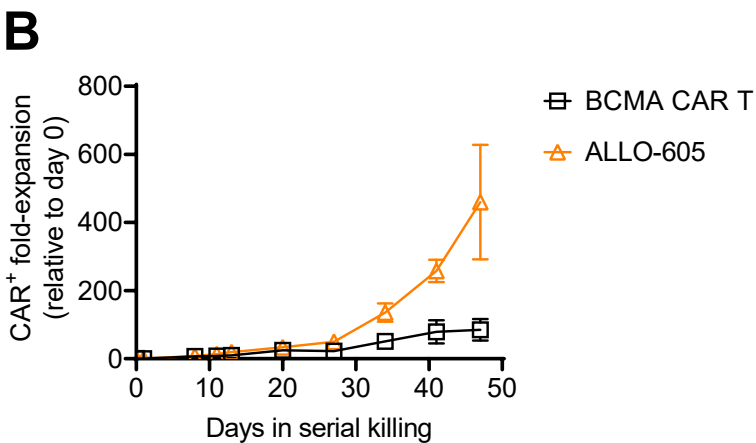
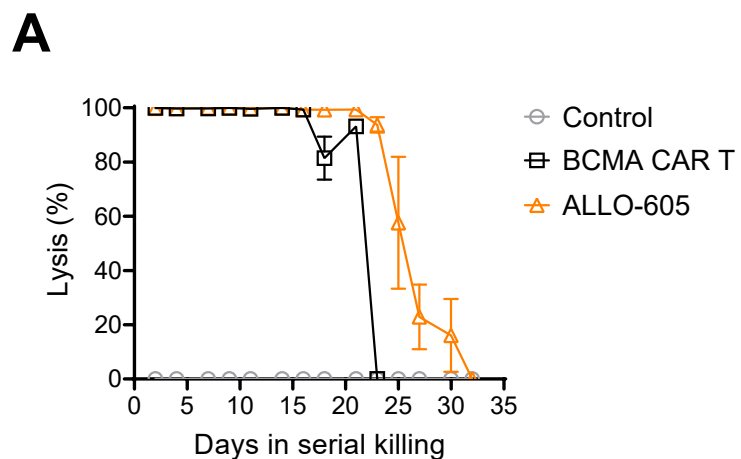
(A-B) The potential benefit of exogenous cytokine support on BCMA CAR T cell activity and persistence was investigated in NSG mice bearing Molp-8 tumors. **(A)** In vivo expansion of Click Beetle Red (CBR)-labeled CAR T cells in mice administered IL-7 and IL-15 or PBS control (n=5). **(B)** NSG mice intravenously infused with luciferase-labeled Molp-8 cells received 5×10^6 unlabeled BCMA CAR T cells 9 days later, with or without systemic cytokine support as described in (A) and tumor burden was monitored by bioluminescence (n=8-10). **(C)** ALLO-605 cells co-express an anti-BCMA CAR and a Turbodomain, which is a chimeric receptor that provides cytokine signaling in a CAR T cell-intrinsic manner. Turbodomains are comprised of two primary domains: (1) a cell membrane-bound dimerization and JAK-activating domain derived from the thrombopoietin receptor (TpoR) coupled to (2) an intracellular signaling domain containing phosphorylatable tyrosine residues derived from the human IL-2/IL-15Rβ receptor.

ALLO-605 can be successfully manufactured from healthy-donor PBMCs



ALLO-605 cells were manufactured by transducing healthy donor-derived PBMCs with a lentiviral vector encoding an anti-BCMA CAR and a Turbodomains, followed by TALEN-mediated inactivation of the TRAC and CD52 genes and depletion of residual TCRαβ T cells before cryopreservation. Non-transduced cells and BCMA CAR T cells, which do not express the Turbodomains, were used for comparison (n=3 donors). **(A)** ALLO-605 cells exhibit robust expansion during manufacturing. **(B)** Expression of the anti-BCMA CAR was detected in a high frequency of T cells by flow cytometry analysis using an anti-idiotypic antibody at the end of the process. **(C)** High knockout efficiency resulted in a large fraction of cells lacking CD52 expression and absence of detectable TCRαβ⁺ cells in the cryopreserved product. **(D)** ALLO-605 exhibited a higher percentage of stem cell memory CAR⁺ cells compared to conventional BCMA CAR T cells in all three donors tested. A representative donor is shown in (A) and (C).

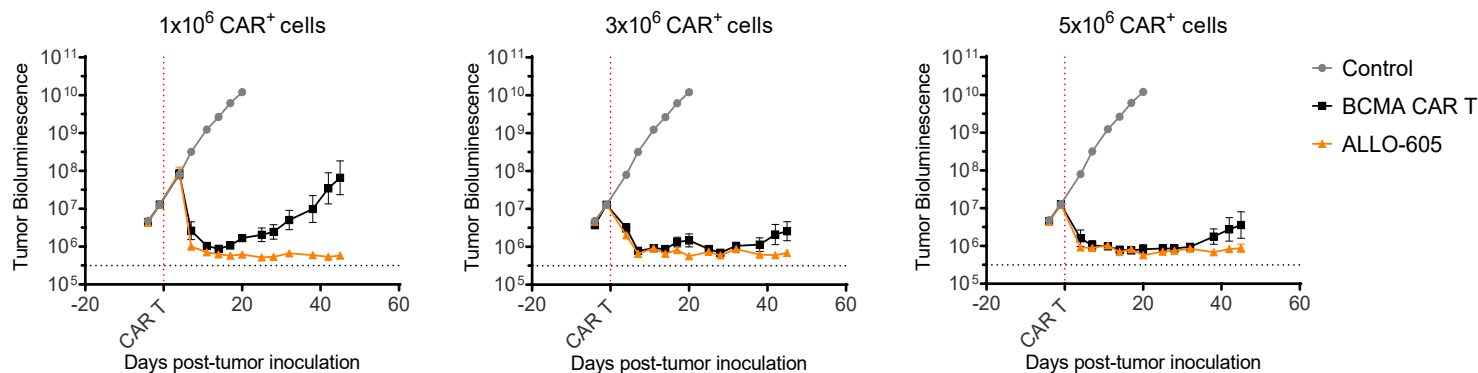
ALLO-605 displayed enhanced long-term cytotoxic activity and expansion upon repeated stimulation with target cells



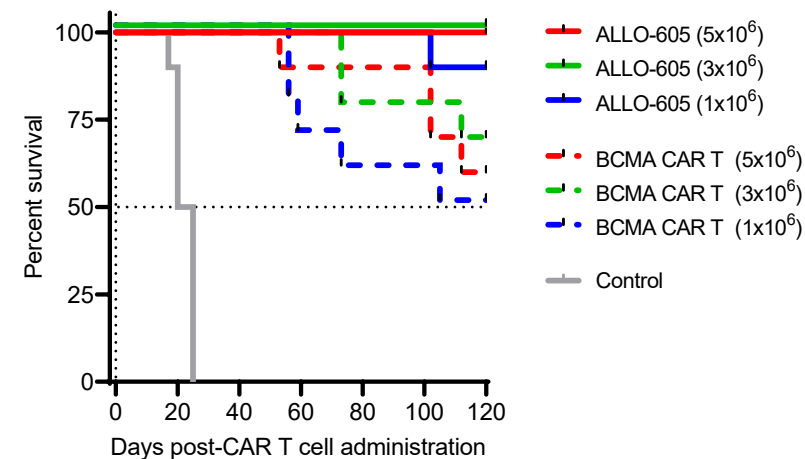
Expression of the Turbodomains in ALLO-605 cells results in improved killing, robust target-dependent expansion and enhanced cytokine release (n=3 donors). **(A)** Long-term cytotoxic activity and **(B)** target-dependent expansion of ALLO-605 following repeated stimulation with MM.1S-Luc-GFP target cells. A representative donor (donor 92) is shown. **(C)** CAR T cells were evaluated for effector function by measuring secreted cytokines in the presence of target cells (REH-BCMA). BCMA-negative REH cells and T cells only were used as controls.

ALLO-605 showed longer persistence and delayed tumor relapses in an orthotopic mouse model of multiple myeloma

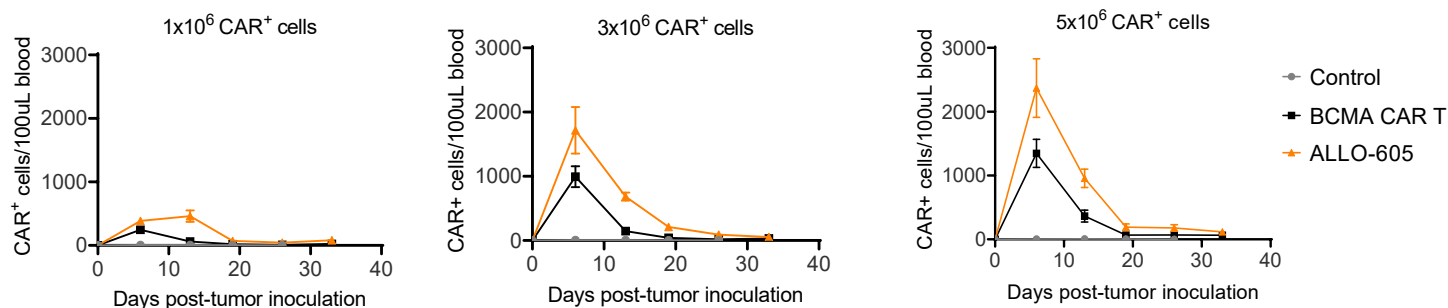
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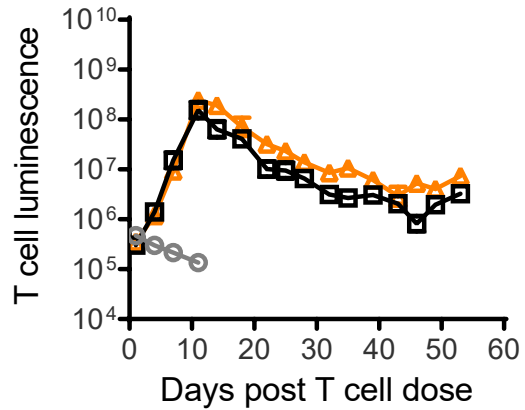
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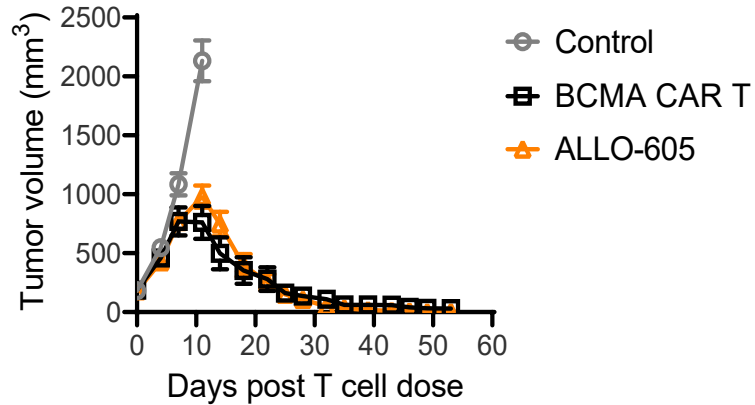
The antitumor efficacy of ALLO-605 was evaluated in the MM.1S model of multiple myeloma. NSG mice engrafted with MM.1S-Luc-GFP tumor cells were treated with 1×10^6 , 3×10^6 , or 5×10^6 CAR⁺ cells and tumor burden was monitored by bioluminescence. Treatment with ALLO-605 resulted in rapid and durable antitumor responses (A) that prolonged the survival of mice compared to mice receiving control cells or BCMA CAR T cells (B). The improved antitumor efficacy of ALLO-605 was associated with higher peak expansion and longer persistence of CAR⁺ cells (C).

ALLO-605 expansion and persistence in vivo was target-dependent

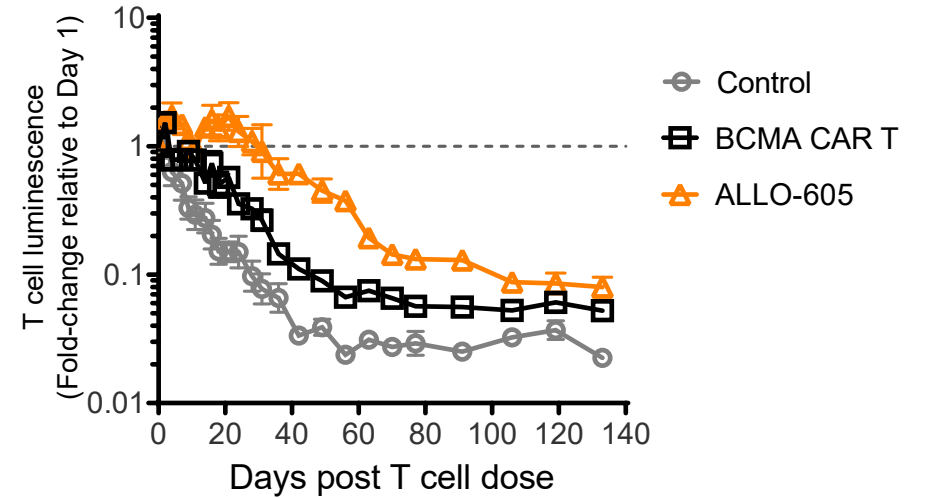
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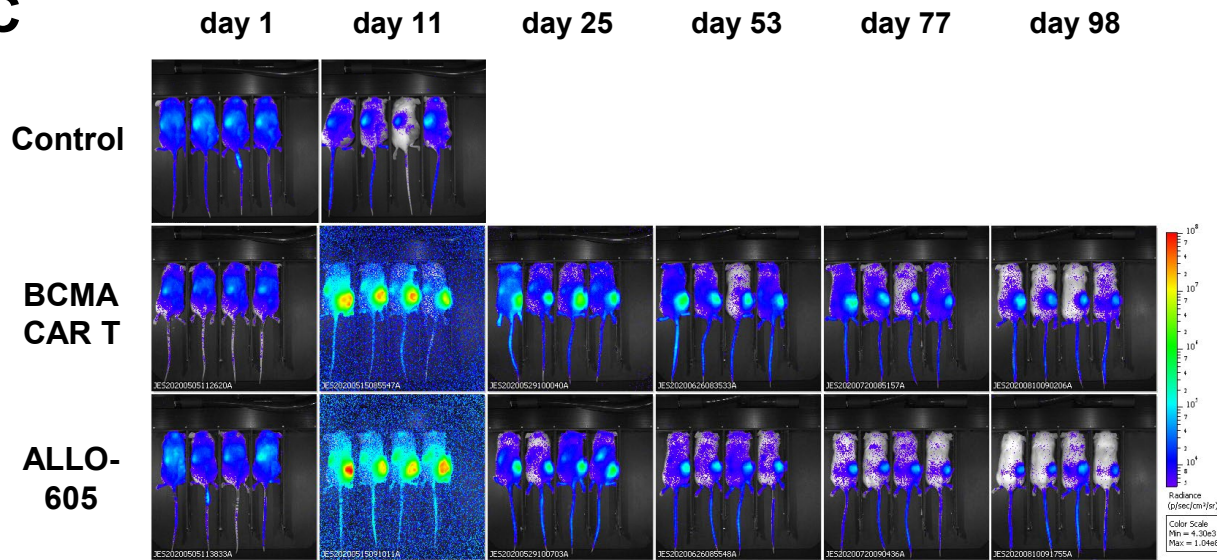
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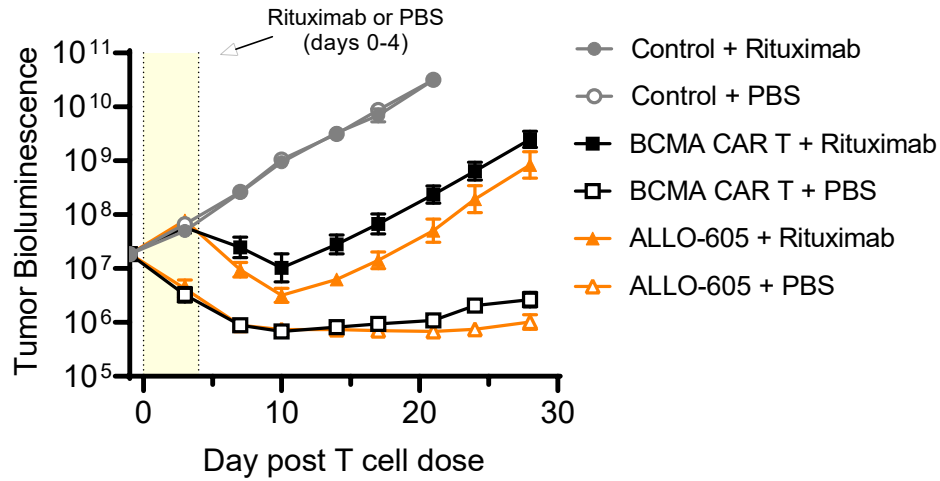
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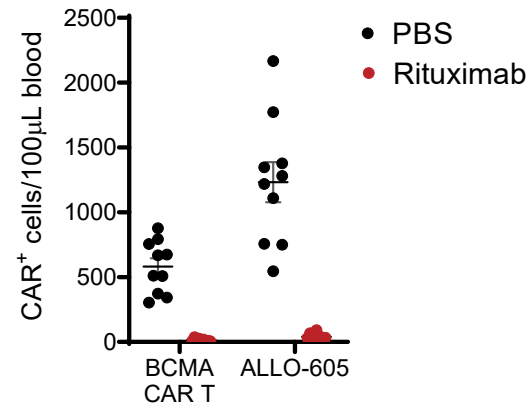
CBR-labeled ALLO-605 cells were administered to NSG mice bearing subcutaneous tumors (A-C) or to non-tumor-bearing mice (D) and their persistence was measured using bioluminescence. Other mice received CBR-labeled non-transduced cells (control) or CBR-labeled BCMA CAR T cells. Following infusion, ALLO-605 and BCMA CAR T cells expanded (A) and cleared the tumors (B), and this was followed by a gradual decline in luminescence to similar levels in both groups (A). CAR⁺ cells were progressively localized to the tumor site (C). (D) In the absence of target (tumor cells), ALLO-605 cells persisted for ~30 days and then gradually declined, reaching nadir levels comparable to BCMA CAR T cells and control cells around day 100.

ALLO-605 cells can be selectively depleted with rituximab and were sensitive to inactivation with dasatinib

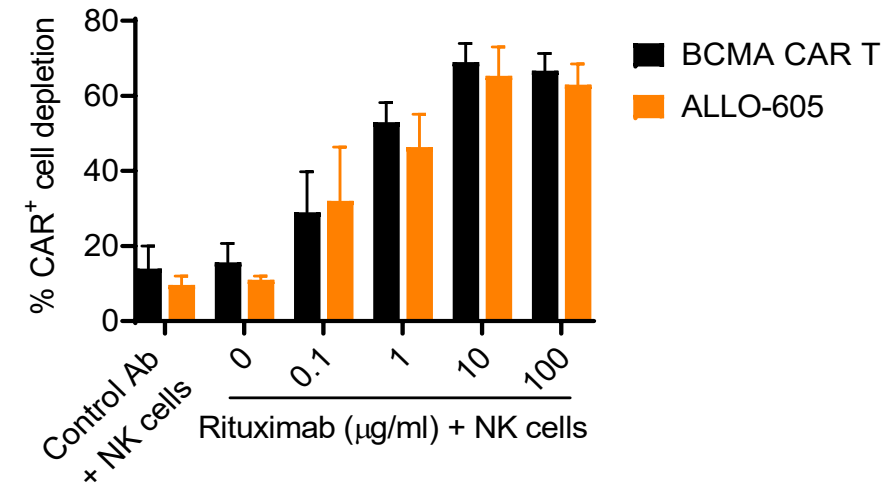
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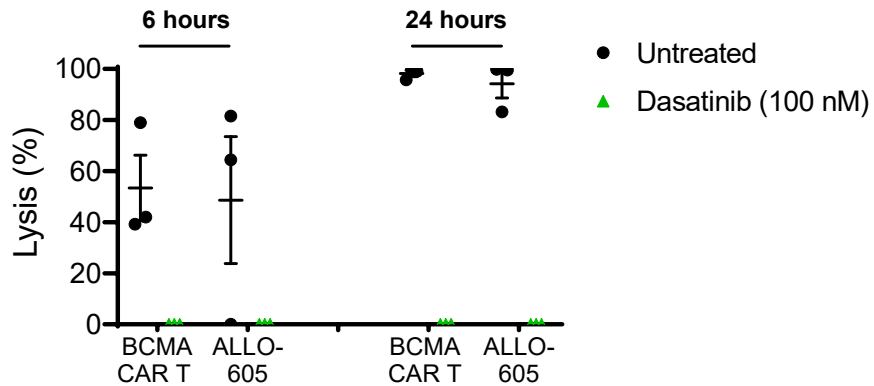
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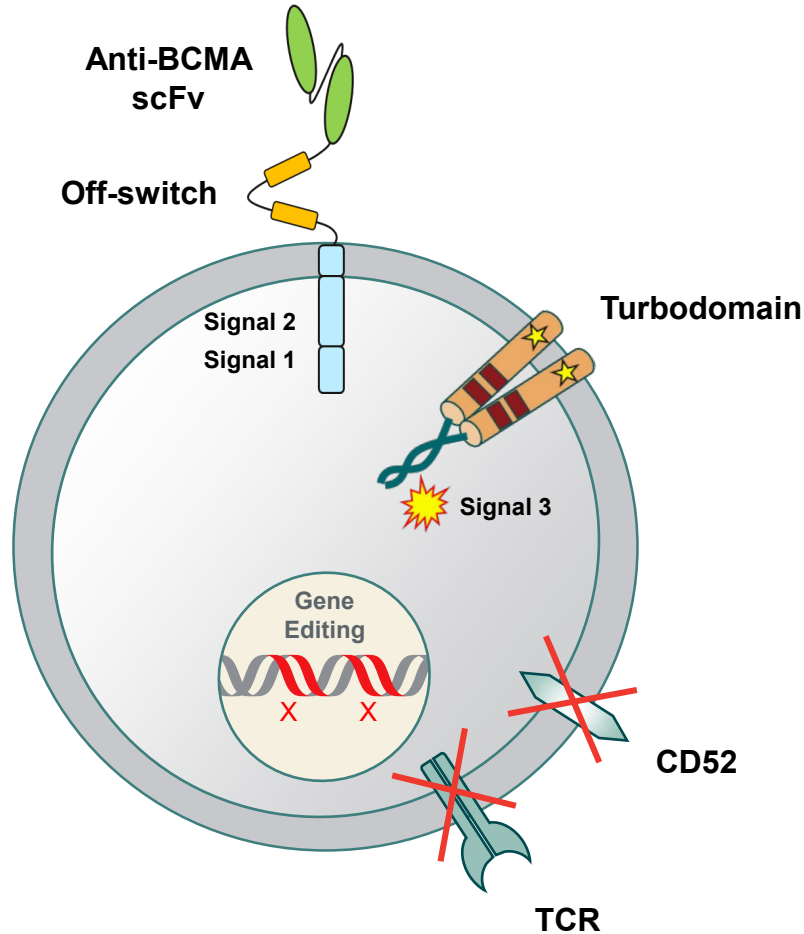


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(A) NSG mice engrafted with MM.1S-Luc-GFP cells received 3×10^6 CAR⁺ cells along with either rituximab (10 mg/kg/day, 5 days) or vehicle control. Treatment with ALLO-605 or BCMA CAR T cells significantly reduced tumor burden. Administration of rituximab blunted CAR T cell activity **(A)** and was associated with depletion of CAR⁺ cells in peripheral blood **(B)**. **(C)** The functionality of the off-switch contained in the BCMA CAR was also demonstrated through antibody-dependent cellular cytotoxicity assays by culturing ALLO-605 cells in the presence of rituximab and effector cells and enumerating residual cells by flow cytometry analysis after short-term culture. **(D)** ALLO-605 cells were effectively inactivated with a tyrosine kinase inhibitor, as shown by measuring their cytotoxic activity in the presence of dasatinib using a luminescence-based assay.

ALLO-605: Allogeneic BCMA TurboCAR T™ cells for the treatment of multiple myeloma



- Manufactured from healthy donor PBMCs for use in an allogeneic setting
- Expression of a Turbodomain conferred constitutive, CAR T cell-intrinsic cytokine signaling
- Demonstrated high potency and persistence, which was dependent on target exposure
- Effectively depleted with rituximab and sensitive to the small molecule inhibitor dasatinib
- These pre-clinical results support clinical investigation of ALLO-605 for the treatment of multiple myeloma