A BCMAxCD3 bispecific T cell–engaging antibody demonstrates robust antitumor efficacy similar to that of anti-BCMA CAR T cells

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Key Points
• REGN5458 is a BCMAxCD3 bispecific T-cell–engaging antibody that potently kills BCMA+ myeloma cells.
• BCMAxCD3 bsAb shows similar antitumor activity compared with BCMA CAR T cells but with different in vivo kinetics.

CD3-engaging bispecific antibodies (bsAbs) and chimeric antigen receptor (CAR) T cells are potent therapeutic approaches for redirecting patient T cells to recognize and kill tumors. Here we describe a fully human bsAb (REGN5458) that binds to B-cell maturation antigen (BCMA) and CD3, and compare its antitumor activities vs those of anti-BCMA CAR T cells to identify differences in efficacy and mechanism of action. In vitro, BCMAxCD3 bsAb efficiently induced polyclonal T-cell killing of primary human plasma cells and multiple myeloma (MM) cell lines expressing a range of BCMA cell surface densities. In vivo, BCMAxCD3 bsAb suppressed the growth of human MM tumors in murine xenogeneic models and showed potent combinatorial efficacy with programmed cell death protein 1 blockade. BCMAxCD3 bsAb administration to cynomolgus monkeys was well tolerated, resulting in the depletion of BCMA+ cells and mild inflammatory responses characterized by transient increases in C-reactive protein and serum cytokines. The antitumor efficacy of BCMAxCD3 bsAb was compared with BCMA-specific CAR T cells containing a BCMA-binding single-chain variable fragment derived from REGN5458. Both BCMAxCD3 bsAb and anti-BCMA CAR T cells showed similar targeted cytotoxicity of MM cell lines and primary MM cells in vitro. In head-to-head in vivo studies, BCMAxCD3 bsAb rapidly cleared established systemic MM tumors, whereas CAR T cells cleared tumors with slower kinetics. Thus, using the same BCMA-binding domain, these results suggest that BCMAxCD3 bsAb rapidly exerts its therapeutic effects by engaging T cells already in place at the tumor site, whereas anti-BCMA CAR T cells require time to traffic to the tumor site, activate, and numerically expand before exerting antitumor effects.

Introduction

Multiple myeloma (MM) is the second most-common hematologic malignancy in the United States, with ~32,270 new cases and 12,830 deaths estimated in 2020. Treatment with drug combinations comprising cytotoxic chemotherapy, corticosteroids, immunomodulatory drugs, proteasome inhibitors, and monoclonal antibodies targeting CD38 have shown clinical efficacy, and consolidation therapy with autologous stem cell transplantation is available for patients who are sufficiently fit to undergo this treatment. Despite these advances, MM remains an incurable disease. Furthermore, patients have lower response rates and shorter response durations with successive lines of therapy, highlighting the unmet need.
Targeted immunotherapy approaches in MM are emerging to fill this clinical need. CD3-engaging bispecific molecules and chimeric antigen receptor (CAR) T cells are approaches that redirect T cells to recognize and kill MM cells. CD3-engaging bispecific antibodies (bsAbs) crosslink the T-cell receptor/CD3 complex when engaging a tumor antigen on cancer cells, facilitating T-cell activation and tumor cell killing through perforin and granzyme B release. This therapeutic strategy has shown antitumor effects against myeloma in multiple preclinical studies and several CD3-engaging bispecific molecules have shown activity in the clinical setting. CAR T-cell therapy involves re-infusion of a patient's T cells after ex vivo engineering to express CARs specific for tumor antigens in order to trigger T-cell signaling and tumor cell killing. CAR T-cell therapies can be highly efficacious in the clinic; CD19-specific CAR T cells have been approved for the treatment of patients with B-cell malignancies, and numerous clinical trials are ongoing in both hematologic and solid tumors. Although both technologies redirect patient T cells to recognize and kill tumor cells, differences in format, manufacturing, and in vivo properties differentiate these therapeutic modalities.

B-cell maturation antigen (BCMA, TNFRSF17) is a confirmed cell surface target for MM. BCMA is expressed on malignant plasma cells from patients with MM, with normal tissue expression limited primarily to plasma cells and a subset of activated B cells. Multiple BCMAxCD3 bsAbs and anti-BCMA CAR T cells are being tested in the clinic to treat MM, and both therapeutic modalities have shown encouraging clinical efficacy in MM and acceptable safety profiles. To date, head-to-head comparisons of antitumor efficacy and mechanism of action between bsAbs and CAR T cells have not been carefully assessed preclinically. The current report describes the generation of REGN5458 (BCMAxCD3 bsAb), a fully human bsAb that binds to BCMA and CD3, created by an established platform for the generation of full-length, fully human bsAbs amenable to production by standard antibody manufacturing techniques. Because both BCMAxCD3 bsAbs and anti-BCMA CAR T cells are under intense clinical investigation, we also performed studies to compare this BCMAxCD3 bsAb vs BCMA-targeted CAR T cells that use the same anti-BCMA binding domain.

We found that REGN5458 BCMAxCD3 bsAb has potent in vitro and in vivo antitumor activity in multiple preclinical models and is able to deplete BCMA+ cells in non-human primates. Both BCMAxCD3 bsAb and anti-BCMA CAR T cells showed similarly potent antitumor efficacy in both in vitro and in vivo models. Notably, however, BCMAxCD3 bsAb cleared tumors in vivo with more rapid kinetics than anti-BCMA CAR T cells, highlighting mechanistic differences between these 2 therapeutic platforms.

Methods

Generation of BCMAxCD3 bsAb (REGN5458)

VelocImmune mice were immunized to generate CD3- or BCMA-specific antibodies. Lead antibodies for CD3 and BCMA were assembled into a bispecific format, as previously described, with a human immunoglobulin G4 constant region containing a S228P substitution in the hinge region to minimize half-antibody formation, substitutions to minimize antibody-mediated effector functions, and a mutation on the CD3 Fc to limit protein A binding. Antibodies were produced in Chinese hamster ovary cells and purified by using protein A chromatography as previously described.

T-cell activation and cytotoxicity assays

BCMAxCD3-mediated activation of reporter cell lines was performed as described; details are given in the supplemental Methods. To evaluate MM cell killing, human peripheral blood mononuclear cells (PBMCs) were plated in complete media at 1 × 10^6 cells/mL and incubated overnight at 37°C to deplete adherent cells. NCI-H929 or MOLP-8 cells were labeled with 1 μM of CellTracker Violet fluorescent tracking dye (Thermo Fisher Scientific), and 1 × 10^6 cells/well were plated in round-bottom 96-well plates (4:1 effector-to-target [E:T] ratio) with nonadherent PBMCs and serial dilutions of BCMAxCD3 for 48 hours at 37°C. To evaluate killing of primary human or cynomolgus plasma cells, enriched CD138+ cells (CD138+ Positive-Selection Kit; Stemcell Technologies) were cultured with REGN5458 and autologous PBMCs labeled with 1 μM of Vybrant CFDA SE fluorescent dye (Thermo Fisher Scientific) at 10:1 (human) or 5:1 (cyto) E:T ratios for 48 hours at 37°C. Surviving target cells and T-cell activation were analyzed by flow cytometry as previously described; details are provided in the supplemental Methods.

Murine tumor models

All animal procedures were approved by the Regeneron Pharmaceuticals Institutional Animal Care and Use Committee. For subcutaneous models, 7- to 10-week-old NOD-scidIL2Rγc null (NSG) mice (The Jackson Laboratory) were injected in the flank with a mixture of 5 × 10^6 tumor cells and 5 × 10^5 human PBMCs (ReachBio), and bsAb administered at the indicated times. Tumor volume was measured twice weekly by using calipers and calculated by the following formula: volume = (length × width^2)/2. For systemic models: On day 0, 7- to 10-week-old NSG mice were injected intravenously with 5 × 10^6 U266-Luc or 2 × 10^6 MOLP-8-Luc cells. Mice were injected intraperitoneally with 4 to 5 × 10^6 PBMCs (ReachBio) on either day –21 (U266 model) or day –10 (MOLP-8 model). bsAb was administered at the indicated times, and mice were analyzed by bioluminescence imaging (BLI) throughout the study to track tumor burden. In the OPM-2-Luc model, NSG mice were injected on day 0 with 5 × 10^6 OPM-2-Luc cells. Some animals were injected intraperitoneally with 4 to 5 × 10^6 PBMC 12 days before treatment with bsAb, while other animals were injected intravenously with CAR T cells, and tumor burden was monitored by BLI. In each experiment, the same donor's PBMCs were used to both engraft mice with effector cells and to generate CAR T cells for adoptive transfer into tumor-bearing mice.

CAR T-cell generation and in vitro assays

CAR sequences were synthesized (GenScript) and cloned into the pLX-EXP1a-ires-eGFP vector (Takara), and 293T packaging cells were transfected to generate lentivirus. T cells were purified from normal donor PBMCs (ReachBio) by using magnetic beads (Thermo Fisher Scientific) and activated with CD3/CD28 microbeads (Thermo Fisher Scientific) in the presence of recombinant human interleukin-2 (IL-2) (100 U/mL; PeproTech) in CTS OpTmizer media (Thermo Fisher Scientific) for 2 days before spin-transduction with lentivirus. T cells were expanded with CD3/CD28 microbeads and recombinant human IL-2 (100 U/mL) for 17 more days. In vitro killing assays using CAR T cells are described in the supplemental Methods.
Statistical analysis
Data are presented as mean ± standard error of the mean (SEM). The statistical tests and the associated P values are indicated in the figure legends or text.

Results
REGN5458 is a BCMAxCD3 bsAb that triggers T-cell activation, cytotoxicity, and cytokine production in the presence of BCMA+ target cells
REGN5458 (BCMAxCD3 bsAb) was generated by combining anti-BCMA and anti-CD3 antibodies (produced by immunizing VeloImmune mice) using previously described methods. This BCMAxCD3 bsAb bound specifically to CD3 on CD4+ and CD8+ T cells (50% effective concentration = 120 nM), and to the BCMAhigh NCI-H929 and BCMAlow MOLP-8 MM cell lines, which express ~110,000 and ~4,700 cell surface copies of BCMA, respectively (supplemental Figure 1A-F; supplemental Table 1). BCMAxCD3 bsAb also bound to CD2+CD45lowCD319+ cells from MM patient bone marrow (BM) samples expressing a range (918-86,777 copies; median, 3,155 copies) of cell surface densities of BCMA (supplemental Figure 1G; supplemental Table 2). These findings suggest that the MOLP-8 cell line may be a good surrogate for patient MM cells due to the cell surface density of BCMA expressed by this cell line. BCMAxCD3 bsAb activated NFAT signaling in effector cells and induced cytotoxicity against a variety of BCMA+ lines with 50% effective concentration values <1 nM, accompanied by upregulation of the activation marker CD25 on T cells (Figure 1A-D; supplemental Figure 2; supplemental Table 3) and cytokine and granzyme production (Figure 1E-G). In vitro cytotoxicity is induced at 1 bsAb dilution concentration below where granzyme/cytokines are detectable in the supernatant, potentially suggesting that cytotoxicity and cytokine production are decoupled from each other or that the signaling thresholds for cytotoxicity and cytokine production are different, or potentially reflecting the lower sensitivity of cytokine detection techniques. Furthermore, BCMAxCD3 bsAb killed CD138+ SLAM-F7+ human plasma cells (Figure 1H), but no effect was seen on mature CD20+B cells (Figure 1I). Thus, BCMAxCD3 bsAb activates T cells to kill BCMA+ human plasma cells and MM cell lines expressing various levels of BCMA. Notably, this includes lines that express BCMA levels similar to human MM cells (MOLP-8 cells).

BCMAxCD3 bsAb binding leads to cell surface BCMA accumulation on MM cell lines
BCMA is cleaved by γ-secretase in the plasma membrane, releasing soluble BCMA from the cell surface. Inhibition of γ-secretase cleavage through addition of γ-secretase inhibitors, such as N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT), leads to increased surface levels of BCMA on plasma cells and concomitant reduction of soluble BCMA. To investigate whether antibody binding to BCMA also increases surface BCMA levels on MM cell lines, we performed flow cytometry analysis of anti-BCMA antibody binding to cells incubated overnight in the presence or absence of BCMAxCD3 bsAb or DAPT. Because REGN5458 blocks the binding of other anti-BCMA antibodies, BCMA surface levels were determined by incubation with additional BCMAxCD3 bsAb followed by detection with an anti-Fc secondary antibody or by the addition of the anti-Fc secondary antibody alone. Compared with cells incubated in media alone, overnight incubation of NCI-H929 or MOLP-8 MM cells with DAPT led to increased BCMA surface levels (Figure 1J). Overnight incubation of the cell lines with 100 μg/mL BCMAxCD3 bsAb also led to an increase in cell surface BCMA expression levels. BCMA levels detected by anti-Fc alone were similar to levels seen when 100 μg/mL BCMAxCD3 bsAb was added immediately before detection. This outcome suggests that BCMAxCD3 bsAb added before overnight culture stabilized BCMA molecules and allowed cell surface accumulation. Currently established methods to measure soluble BMCA are not tolerant to REGN5458, and therefore the level of soluble BCMA in cell supernatants was not evaluated.

BCMAxCD3 bsAb shows antitumor efficacy in xenogeneic MM tumor models
Antitumor efficacy of BCMAxCD3 bsAb was examined in xenograft MM models in immunodeficient NSG mice. Mice bearing BCMAhigh NCI-H929 tumors received BCMAxCD3 bsAb immediately upon tumor implantation (Figure 2A; supplemental Figure 3A) or when tumors had established for 5 days (starting tumor volume, ~65 mm3) (Figure 2B; supplemental Figure 3B). In both models, BCMAxCD3 bsAb exhibited dose-dependent antitumor activity. Similar dose-dependent effects were seen when mice with BCMAlow MOLP-8 tumors were treated with BCMAxCD3 bsAb (Figure 2C; supplemental Figure 3C). Furthermore, dose-dependent systemic inflammatory cytokine responses (interferon-γ [IFN-γ] and IL-2) were observed after BCMAxCD3 bsAb treatment but not in control-treated animals.

To examine the in vivo effects of BCMAxCD3 bsAb in a model that recapitulates the BM localization of patient MM cells, NSG mice were injected intravenously with U266 (Figure 2D) or MOLP-8 (Figure 2E) MM cell lines that localize and grow in the BM. These MM lines were engineered to express luciferase, and tumor growth was monitored by using BLI. To provide effector T cells, PBMCs were engulfed before treating mice with BCMAxCD3 bsAb. The tumor burden (BLI) of established U266-Luc tumors was reduced by BCMAxCD3 bsAb treatment, and 3 of 5 animals receiving bsAb also led to an increase in cell surface BCMA expression levels. BCMAxCD3 bsAb also delayed tumor growth (Figure 2E; supplemental Figure 5). Thus, BCMAxCD3 bsAb showed antitumor efficacy in multiple xenogeneic tumor models, including models that potentially recapitulate the BM tumor microenvironment (TME).

BCMAxCD3 bsAb shows antitumor efficacy in immunocompetent, syngeneic BCMA+ tumor models and combinatorial efficacy with PD-1 blockade
To investigate antitumor efficacy of BCMAxCD3 bsAb in immunocompetent animals, mice expressing human CD3ε,31 were used. Although no syngeneic MM models were available for testing, BCMAxCD3 bsAb caused dose-dependent inhibition of tumor growth of syngeneic B16 melanoma, MC38 colon carcinoma, or EL4 thymoma cells engineered to express human BCMA (ie, B16/hBCMA, MC38/hBCMA, EL4/hBCMA cells) (Figure 3A-B; supplemental Figure 6). The programmed cell death protein 1 (PD-1)-expressing cell death-ligand 1 (PD-L1) pathway is a clinically relevant negative regulator of T-cell activation, and PD-L1 is expressed on malignant plasma cells from patients with MM but not normal plasma.
Figure 1. BCMAxCD3 bsAb mediates human T-cell activation and redirected killing of MM cell lines and human plasma cells, but not B cells, and stabilizes cell surface BCMA expression. (A-G) Adherent cell-depleted PBMCs were incubated with NCI-H929 (A-B) or MOLP-8 (C-G) target cells, plus a range of concentrations of BCMAxCD3 bsAb (blue circles), CD3-binding control bsAb (red squares), or bivalent parental anti-BCMA mAb (yellow triangles) for 48 hours. In panels A and C, cell viability was measured by flow cytometry analysis, and values represent mean ± SEM frequencies of viable target cells from duplicate samples; in panels B and D, CD25 expression on CD8⁺ T cells was assessed by flow cytometry analysis in the same samples, and values represent mean ± SEM frequencies of CD25⁺ cells among CD8⁺ cells. Cytokine concentrations in the tissue culture supernatant of MOLP-8 cell cultures were assessed by cytometric bead array, and values represent concentrations of IFN-γ (panel E), tumor necrosis factor-α (TNF-α) (panel F) and granzyme B (panel G) at the end of the assay. (H) Enriched CD138⁺ human BM plasma cells were cultured with autologous PBMCs, with viability of CD138⁺ SLAMF7⁺ cells analyzed as in panel A. (I) Cell viability of CD20⁺ B cells present in the cultures from panel H. (J) BCMAxCD3 bsAb binding leads to the accumulation of cell surface BCMA on MM cell lines. NCI-H929 or MOLP-8 MM cells were incubated at 37°C overnight with media alone (lavender bars),
cells. We therefore assessed whether PD-1 blockade enhances the antitumor effects of BCMAxCD3 bsAb. Using an anti-mouse PD-1 antibody in CD3-humanized mice bearing established MC38/hBCMA tumors expressing murine PD-L1 (supplemental Figure 7), PD-1 blockade alone (1 of 10 mice tumor free) or suboptimal BCMAxCD3 bsAb doses of 0.04 mg/kg (0 of 10 mice tumor free) modestly affected tumor growth compared with mice given control antibodies. However, when anti-PD1 monoclonal antibody was combined with BCMAxCD3 bsAb, significant antitumor efficacy was seen, with 7 of 10 mice tumor free ($P < .0001$, $P = .003$, and $P < .0001$ at day 18 vs control mice, PD-1 antibody-treated mice, and BCMAxCD3 bsAb-treated mice, respectively, by 2-way analysis of variance) (Figure 3C). Thus, PD-1 blockade exhibited a combinatorial antitumor effect when administered with low-dose BCMAxCD3 bsAb in this model.

**BCMAxCD3 bsAb pharmacokinetic and pharmacodynamic variables in cynomolgus monkeys**

BCMAxCD3 bsAb (REGN5458) triggers cynomolgus monkey T-cell cytotoxicity and upregulation of CD25 upon coculture with cells expressing cynomolgus BCMA (supplemental Figure 8). Cynomolgus T cells also kill cynomolgus plasma cells, showing that BCMAxCD3 bsAb cross-reacts with cynomolgus BCMA and CD3. To assess tolerability and pharmacokinetic parameters of BCMAxCD3 bsAb in non-human primates, a multiple-dose safety study was conducted in cynomolgus monkeys. A total of 12 monkeys per treatment group (6 male and 6 female) received 5 weekly IV injections of BCMAxCD3 bsAb at 0.1, 1, or 10 mg/kg. In all dose groups, BCMAxCD3 bsAb was well tolerated, and all animals survived to scheduled necropsy. Toxicokinetic analysis revealed dose-proportional exposures and linear kinetics across dose groups, with no sex differences (Figure 4A). Continuous exposure to BCMAxCD3 bsAb was observed throughout the dosing phase in most animals, and exposure was maintained until the end of the recovery phase in ~10% of the animals. The elimination half-life of BCMAxCD3 bsAb was ~6.5 to 7.7 days.

BCMAxCD3 bsAb-related clinical observations were limited to emesis (predominantly within 4 hours of the first dose) and liquid feces (occurring transiently throughout the dosing phase). There were no adverse effects of BCMAxCD3 bsAb treatment on toxicity end points (ie, body weight, food consumption, physical examinations, vital signs, safety pharmacology [ie, electrocardiography, neurologic examination, respiratory examination], peripheral blood immunophenotyping, clinical pathology [ie, urinalysis, hematology, serum chemistry], macroscopic or microscopic pathology) at either terminal or recovery necropsy. Pharmacologically anticipated, dose-related, mild, and reversible elevations of circulating inflammatory markers (C-reactive protein and IL-6) were observed within 1 day after the initial dose. However, these elevations were not apparent after subsequent doses, except for C-reactive protein elevations at the 10 mg/kg dose (Figure 4B-D). Similar results were observed with other serum cytokines (IFN-γ, IL-2, IL-15, and IL-10) (supplemental Figure 9). In accordance with the increase in serum cytokine values, T-cell redistribution was detected after BCMAxCD3 bsAb administration, as absolute T-cell numbers transiently decreased in the circulation during the dosing period and returned to baseline levels during recovery (supplemental Figure 10); this outcome has been described for several other CD3 bispecific molecules reactive with hematologic targets. CD20^{-} B-cell absolute numbers in the circulation were also decreased during the dosing period and subsequently recovered during the recovery phase, consistent with previous reports that low levels of BCMA are expressed on mature cynomolgus B cells. Consistently, immunohistochemistry analysis of spleens and mesenteric lymph nodes revealed the absence of CD138^{+} plasma cells 1 week after the final BCMAxCD3 bsAb dose, with a concurrent modest decrease in the number of CD20^{-} B cells, while CD3^{+} T cells slightly increased (supplemental Figures 11 and 12). After a 12-week dose-free recovery phase, CD138 staining was once again detected, indicating that plasma cells had populated the lymphoid organs.

A separate pharmacology study was performed in cynomolgus monkeys to assess the short-term effect of a single dose of BCMAxCD3 bsAb on BCMA^{+} BM plasma cell, B-cell, and T-cell frequencies. As expected, BCMAxCD3 bsAb depleted the majority of BM plasma cells 7 days after infusion at all doses tested (Figure 4D). BM B cells were depleted in a dose-dependent manner, consistent with the peripheral blood depletion noted earlier. BM T-cell frequencies were not affected by BCMAxCD3 bsAb. Thus, these 2 studies show BCMAxCD3 bsAb-directed depletion of BCMA^{+} cells in lymphoid tissues of cynomolgus monkeys, thereby indicating on-target activity in nonhuman primates.

**BCMAxCD3 bsAb and anti-BCMA CAR T cells exhibit similar in vitro and in vivo antitumor activities, with distinct in vivo antitumor kinetics**

Because both BCMAxCD3 bsAbs and anti-BCMA CAR T cells are under clinical investigation, we compared BCMAxCD3 bsAb vs BCMA-targeted CAR T cells containing identical tumor antigen (BCMA)—binding domains. To generate anti-BCMA CAR T cells, the variable heavy chain and variable light chain of the anti-BCMA arm of REGN5458 were reformatted as a single-chain variable fragment–based CAR construct containing CD8 hinge/transmembrane, 4-1BB costimulatory, and CD3z signaling domains, plus an internal ribosome entry site followed by a green fluorescent protein reporter (supplemental Figure 13A). This CAR architecture and the 4-1BB signaling domain were chosen because the same domain is used in clinical-stage BCMA CAR T cells from commercial entities, although CD28 costimulatory domains have been explored clinically by academic laboratories. This CAR expressed efficiently in primary T cells (supplemental Figure 13B). Similar in vitro cytotoxicity was noted for both BCMA CAR T cells and BCMAxCD3 bsAb against BCMA^{+} NCI-H929 cells or BCMA-targeted MOLP-8 cells (Figure 5A) using effector cells derived from the same donor. Similarly, both
Figure 2. BCMAxCD3 bsAb shows antitumor efficacy in a dose-dependent manner in xenogeneic MM tumor models. (A-C) NSG mice were coimplanted subcutaneously with a mixture of NCI-H929 MM cells and human PBMCs (A-B) or MOLP-8 MM cells and human PBMCs (C). The mice were either immediately treated with the indicated dose of BCMAxCD3 bsAb, CD3-binding control bsAb, or phosphate-buffered saline (PBS) and continued to be dosed twice weekly, for a total of 7 doses (panels A and C), or tumors were allowed to establish for 5 days before twice weekly dosing was initiated (panel B, 7 total doses). In panels A-C, values in the top graphs represent mean ± SEM tumor volumes (n = 7 per group) for the indicated treatments; values in the middle and bottom graphs represent serum IFN-γ and IL-2 concentrations, respectively, from individual animals 4 hours after the first injection of the indicated bsAb. Bars indicate mean ± SEM values. Significant differences between the indicated sample and mice given CD3-binding control bsAb, as measured by 2-way analysis of variance, are indicated. **P < .01. (D) NSG mice that had been engrafted intraperitoneally with PBMCs were injected intravenously with U266-Luc MM cells on day 0. After 31 days, the mice bearing established tumors were treated with 4 mg/kg
BCMAxCD3 bsAb and anti-BCMA CAR T cells mediated killing >90% of CD138⁺ SLAMF7⁺ MM patient blasts (Figure 5B).

A xenogeneic BCMA⁺ OPM-2-Luc disseminated MM model was developed to simultaneously compare the antitumor efficacies of BCMAxCD3 bsAb and anti-BCMA CAR T cells in vivo. OPM-2 tumors localize to and grow in the BM, thereby creating a TME within the BM. To investigate bsAb effects, mice with established tumors were engrafted with normal human PBMCs and treated with BCMAxCD3 bsAb (REGN5458) at 15 or 21 days after tumor injection, to treat animals with low or high tumor burdens, respectively (Figure 5C). Alternatively, the mice were injected with anti-BCMA CAR⁺ T cells 15 or 21 days after tumor injection (Figure 5D). Similar differentiation states (mixture of ∼25% CD45RO⁺CCR7⁺ central-memory and ∼75% CD45RO⁺CCR7⁺ effector-memory) were seen between T cells engrafted in the BM of NSG mice and BCMA CAR⁺ T cells at the end of ex vivo culture before infusion into mice (supplemental Figure 16); this finding suggests similar degrees of fitness between engrafted T cells and CAR T cells before adoptive transfer. Engrafted T cells exhibited a modestly increased activation profile and were PD-1⁺ and LAG-3-low, whereas BCMA CAR⁺ T cells were PD-1-low and LAG-3⁻. T cells engrafted in NSG BM may also resemble the phenotypic characteristics of BM T cells from patients with MM; a majority are the effector-memory phenotype that expresses PD-1.39,40

After treatment with BCMAxCD3 bsAb on day 15, tumor burden (BLI level) was rapidly reduced to background levels by day 18 (Figure 5C, left). By contrast, after treatment with anti-BCMA CAR T cells on day 15, tumor burden decrease was delayed until days 21 to 25 (Figure 5D, left). After BCMAxCD3 bsAb treatment of larger tumors on day 21, tumor burden was rapidly reduced to background levels in 3 of 5 animals by day 26, and BLI levels remained at background levels in these 3 mice for the duration of the study (Figure 5C, right). In contrast, CAR T cells showed no effect on BLI levels through day 34, as tumor burden continued to increase similarly to control CAR T-cell–treated mice (Figure 5D, right). Starting on day 38, tumor burden in BCMA CAR T-cell–treated mice began to decrease and reached background levels by day 47, with 3 of 5 animals surviving and remaining tumor free. In addition, CAR T cells with a CD28 signaling domain (CD28/CD3z) exhibited more rapid antitumor kinetics (more closely resembling BCMAxCD3 bsAb efficacy) than 4-1BB/CD3z BCMA CAR T cells with a 4-1BB signaling domain (supplemental Figure 17), consistent with the enhanced signaling properties of the CD28 signaling domain.41 Thus, BCMAxCD3 bsAb (REGN5458) treatment showed potent antitumor activity, with tumor burden reduced to background levels within several days. In comparison, anti-BCMA CAR T cells with the 4-1BB/CD3z signaling domains being used in the clinic also exhibited potent antitumor efficacy and reduced tumor burden to background levels but with considerably slower kinetics.

BCMAxCD3 bsAB induces rapid T-cell activation and cytokine production in the TME, while BCMA CAR T cells expand more slowly

To understand how BCMAxCD3 bsAb and BCMA CAR T cells can exhibit similar antitumor efficacies but with different kinetics, the serum cytokines and the BM TME from mice bearing BM-localized OPM-2 tumors (Figure 5C-D) were analyzed at various time points after bsAb or CAR T cell (4-1BB/CD3z CAR) dosing in a second experiment. Kinetics of tumor clearance in this study were similar to those of the previous study (supplemental Figure 18).

Serum IFN-γ induced by BCMAxCD3 bsAb peaked at 4 hours postdose and declined over a 7-day period (Figure 6A). By contrast, serum IFN-γ levels remained low 4 hours after BCMA CAR T-cell administration, and steadily increased by day 7 to levels >20-fold higher than those induced after BCMAxCD3 bsAb dosing. Thus, IFN-γ production kinetics mirrored the kinetics of antitumor efficacy.

One day after BCMAxCD3 bsAb dosing, absolute T-cell numbers in the BM TME were increased compared with controls (P = .07, day 1), and they tripled between days 1 and 7 compared with control bsAb-treated mice (P < .01) (Figure 6B). By contrast, BCMA CAR T cells were scarcely detectable in the BM at 1 day post infusion but expanded ~12-fold by day 7 compared with control CAR T cells (P ≤ .01). Thus, T-cell expansion within the TME was observed with both modalities but with different kinetics and magnitudes that mirrored antitumor efficacy and serum cytokine induction.

Phenotypic analysis of BM TME T cells from mice treated with BCMAxCD3 bsAb revealed increases in the activation markers CD25 and 4-1BB, and increased intracellular granzyme B, at day 1 (P < .01 vs control bsAb for each parameter) (Figure 6C-E). By day 7, when tumors had been cleared (supplemental Figure 18), BM T cells no longer expressed these activation markers. In BCMA CAR T-cell–treated animals, too few T cells were found in the BM TME for reliable flow cytometry analysis at day 1, but BCMA CAR T cells were activated (upregulated CD25, 4-1BB, and granzyme B expression compared with CAR⁺ cells) on day 7. Thus, the kinetics of T-cell activation also mirrored those of antitumor efficacy, T-cell expansion, and serum cytokine levels.

Discussion

In these studies, we developed models to directly compare antitumor activities of a novel BCMAxCD3 bsAb vs anti-BCMA CAR T cells. Both BCMAxCD3 bsAb and anti-BCMA CAR T cells eliminated established systemic MM tumors in vivo but with different kinetics: BCMAxCD3 bsAb treatment resulted in rapid tumor clearance, whereas anti-BCMA CAR T-cell treatment was not potent early but ultimately induced tumor clearance. Mechanistically, we noted inflammatory cytokine production, and T-cell activation and expansion in the BM TME that peaked between 4 and 24 hours after BCMAxCD3

Figure 2. (continued) BCMAxCD3 bsAb, CD3-binding control bsAb, or PBS and continued to be dosed twice weekly, for a total of 6 doses. (E) NSG mice that had been engrafted intraperitoneally with PBMCs were injected intravenously with MOLP-8-Luc MM cells and immediately treated with 0.4 mg/kg BCMAxCD3 bsAb, CD3-binding control bsAb, or PBS and continued to be dosed twice weekly, for a total of 3 doses. In panels D-E, values in the left graphs represent mean ± SEM radiancemeasurements of tumor burden as determined by BLI imaging using an IVIS Spectrum device (n = 5 mice per group), with individual BLI data shown in the right graphs. Mice without tumors (open black diamonds) are included in each panel to indicate background BLI. BLI images corresponding to data in panels D and E are shown in supplemental Figures 5 and 6, respectively. Blue arrows indicate the time at which mice were given their first dose of antibody.
bsAb injection before decreasing over 7 days. By contrast, elevation in inflammatory cytokines was not observed, and CAR T cells were barely detectable in the BM TME 24 hours after BCMA CAR T-cell infusion. Noticeably, within 1 week of infusion, activated CAR T cells had expanded in the BM TME, and a sizeable serum inflammatory cytokine response had developed. Thus, BCMAxCD3

**Figure 3.** BCMAxCD3 bsAb shows antitumor efficacy in syngeneic BCMA⁺ tumor models and synergizes with PD-1 blockade in vivo. (A-B) C57BL/6 mice that express human CD3 in place of murine CD3 (CD3-humanized mice) were implanted subcutaneously with either B16 melanoma (A) or MC38 colon carcinoma cells (B) that stably express human BCMA (B16/hBCMA and MC38/hBCMA, respectively). The mice were immediately treated with either CD3-binding control bsAb (4 mg/kg) or BCMAxCD3 bsAb (4 or 0.4 mg/kg), followed by twice weekly dosing for a total of 3 doses. Values in the left graphs indicate mean ± SEM tumor volumes (n = 6 per group) for the indicated treatments, with individual tumor growth curves shown in the right graphs. Significant differences between the indicated sample and mice receiving CD3-binding control bsAb, as measured by 2-way analysis of variance, are indicated: **P < .01. (C) CD3-humanized mice were implanted subcutaneously with MC38/hBCMA cells, and the tumors were allowed to establish for 3 days, at which time the mice were administered a CD3-binding control bsAb or BCMAxCD3 bsAb at 0.04 mg/kg, along with either anti-mouse PD-1 antibody or an isotype-matched control antibody at 4 mg/kg. Mice were given subsequent antibody doses on days 7 and 10. Values in the top graph represent mean ± SEM tumor volumes (n = 10 per group) for the indicated treatments, with individual tumor growth curves shown in the graphs below. Blue arrows indicate the time at which mice received their first dose of antibody. Significant differences between the indicated sample and mice receiving CTL bsAb + CTL Ab, as measured by 2-way analysis of variance, are indicated: *P < .05, **P < .01. Significant differences between the indicated sample and mice receiving BCMAxCD3 bsAb + CTL Ab, as measured by 2-way analysis of variance, are indicated: **P < .01.
bsAb exerts its therapeutic effect in the TME rapidly after administration, whereas BCMA CAR T cells require time to expand in the TME and to become fully efficacious.

CD3-engaging bsAbs and CAR T cells are both at the forefront of T-cell–mediated immunotherapies for cancer and both are clinically efficacious against hematologic malignancies. However, these T-cell–redirecting therapies differ in several key mechanistic aspects and have clinical advantages/disadvantages. Our studies show that these therapies targeting human BCMA both potently kill BCMA+ MM cells in vitro, regardless of whether the expression levels of BCMA are high or low. Both therapies also achieve potent

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**Figure 4.** BCMAxCD3 pharmacokinetic parameters and pharmacology in cynomolgus monkeys. (A) Cynomolgus monkeys were administered 5 weekly doses of BCMAxCD3 bsAb at dose levels of 0.1, 1, and 10 mg/kg. Blood was harvested at the indicated time points and processed to serum for analysis of BCMAxCD3 bsAb concentrations. Values represent mean ± SEM BCMAxCD3 bsAb levels at the indicated time points (n = 6-12 animals per group). Arrows indicate when BCMAxCD3 bsAb was dosed in the animals. (B-C) Serum from the same animals was also analyzed for C-reactive protein (B) and IL-6 (C) levels at the indicated time points, with values representing concentrations of these molecules from individual animals at the indicated time points. (D) In a separate study, cynomolgus monkeys were administered a single dose of BCMAxCD3 bsAb at 0.1, 1, and 5 mg/kg or vehicle control, with BM aspirates assessed by flow cytometry analysis 7 days later. Values represent the frequency of CD138+ plasma cells, CD20+ B cells, and CD2+ T cells among CD45+ leukocytes in the BM from individual animals (n = 3 per group). Horizontal lines indicate means, and error bars indicate SEM.
antitumor efficacy in vivo but with distinct kinetics: BCMAxCD3 bsAb rapidly cleared established tumors, whereas BCMA CAR T cells cleared tumors at a slower rate. These findings suggest that adoptively transferred CAR T cells require time to traffic to the tumor site and then expand to sufficient numbers to exert meaningful antitumor activity. In contrast, BCMAxCD3 bsAb may use T cells that are already present within the TME as effectors to rapidly kill tumor cells.

Although the rapid kinetics of bsAb therapy may be an advantage in the clinic, there are other attributes of the bsAb platform compared with CAR T cells. bsAbs are available immediately to any patient and do not require multi-week manufacturing times necessary to generate currently approved autologous CAR T cells. Furthermore, some patients are ineligible for CAR T-cell therapy, either due to the patient’s fitness, an inability to harvest sufficient T cells, or due to manufacturing failures.43 Such patients with too few or poorly fit peripheral T cells for CAR T-cell manufacturing may still be eligible to receive bsAb therapy, as they would likely maintain effector T cells that could respond to the bsAb. Toxicities, either due to cytokine-release syndrome or on-target/off-tumor effects, can be
more quickly mitigated for bsAb-treated patients by withdrawing treatment or implementing staged dosing strategies, such as step-up dosing. In contrast, currently approved CAR T cells activate and expand in patients and do not include “safety switches” that can rapidly turn off or kill the CAR T cells in the case of an adverse event; these types of mitigation strategies are in development, however. In the current study, peak serum IFN-γ levels induced by BCMA CAR T were >20-fold higher than those induced by BCMAxCD3 bsAb. This may be due to CAR T-cell signaling through costimulatory domain in addition to CD3z, or because tumor burdens were higher at the time of peak CAR T-cell activity due to the delayed kinetics of the CAR T-cell expansion in the TME.

CAR T cells do have several unique advantages compared with bsAbs. CAR T cells contain engineered costimulation domains (eg, 4-1BB cytodomains), which drive the memory-like phenotype and expansion/persistence of adoptively transferred cells to enhance efficacy and potentially create a reservoir of memory CAR T cells.
In contrast, bsAb efficacy is likely determined solely by the persistence/exposure of the drug, and this modality is unlikely to provide long-term antitumor T-cell memory once treatment is withdrawn.44 In solid tumors or the BM of patients with MM, the TME may contain insufficient numbers of T cells, nonfunctional T cells, or poorly fit effector T cells, potentially limiting the bsAb efficacy. However, next-generation bsAb approaches aim to harness costimulatory pathways when applied in combination with CD3-engaging bsAbs,45 and they could potentially generate more potent and lasting clinical responses. Future generations of CAR T-cell therapies may build on these characteristics and incorporate allogeneic platforms to more rapidly treat a wider array of patients, engineered safety switches to prevent toxicities, and gene edits to enhance T-cell function. Furthermore, CAR T cells can be engineered with different costimulatory signaling domains to achieve different in vivo functionalities. For example, the current study showed that BCMA CAR T-cell therapy with CD28 signaling domains exhibited more rapid tumor clearance kinetics that were similar to BCMAxCD3 bsAb treatment, compared with the slower antitumor kinetics of CAR T-cell therapy with 4-1BB domains. This result is consistent with CD28 signaling domains triggering more rapid and more potent T-cell signaling compared with 4-1BB domains.41 It is possible that BCMAxCD3 bsAb may function with kinetics similar to CD28 CAR T cells in patients. However, it is unclear how the lack of long-term persistence of CD28z CAR T cells15,16 would affect the durability CD28 CAR T cells compared with BCMAxCD3 bsAbs, which can be re-dosed multiple times.

These studies use xenograft tumors grown in immunodeficient animals, a standard but artificial model in bsAb and CAR T-cell preclinical evaluation. In our studies, phenotyping of engrafted T cells in NSG mice and ex vivo expanded CAR T cells revealed similar functional states. However, unappreciated differences that could affect the outcomes of in vivo studies may exist, such as T-cell metabolic states. Accumulating clinical data with bsAbs and CAR T cells may shed light on the differential mechanisms underpinning efficacy or lack thereof. Complex assessments of immunotherapy combinations in xenograft models are limited by the lack of an intact TME. Thus, our results strengthen the notion that PD-1 blockade and BCMAxCD3 and PD-1 blockade are likely twofold: enhanced bsAb-mediated tumor killing by effector T cells due to augmented T-cell activation in the TME, as well as boosting of endogenous immune responses against MC38/hBCMA tumor antigens released upon tumor cell killing. Thus, our results strengthen the notion that PD-1 (and other checkpoint) blockade has promise as combinatorial agents with CD3-binding bsAbs47 and CAR T-cell48 therapies, and it is possible that combining BCMAxCD3 bsAb with BCMA CAR T-cell therapy may provide even further enhancement of early and durable disease control.

Collectively, these data show that the potent preclinical antitumor activity of BCMAxCD3 bsAb is comparable to that of CAR T cells, and they provide strong rationale for clinical testing of BCMAxCD3 bsAb in patients with MM. Thus, a phase 1 clinical trial to evaluate the safety, pharmacokinetic variables, and early efficacy of REGN5458 is underway in patients with relapsed/refractory multiple MM (#NCT03761108).

Acknowledgments

The authors thank all Regeneron employees who contributed to the generation and characterization of REGN5458. This study was funded and supported by Regeneron Pharmaceuticals. Regeneron Pharmaceuticals is developing REGN5458 as a clinical compound and has filed patent applications related to this work.

Authorship

Contribution: D.J.D., K.O., T.C.M., K.B., M.W.R., S.G., F.D., J.L., E.S., G.T., and J.R.K. designed the studies; D.J.D., K.O., K.M., T.C.M., K.B., O.S., T.S., and J.K. conducted the experiments; D.J.D., K.O., K.M., T.C.M., K.B., O.S., M.W.R., S.G., P.F., F.D., E.S., and J.R.K. analyzed the data; and D.J.D., K.O., T.C.M., M.W.R., S.G., F.D., E.S., G.T., and J.R.K. wrote the manuscript.

Conflict-of-interest disclosure: All authors are employees and equity-shareholders of Regeneron Pharmaceuticals.

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References

1. Röllig C, Knop S, Bornhäuser M. Multiple myeloma. Lancet. 2015;385(9983):2197-2208.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7-30.
3. Gozzetti A, Candi V, Papini G, Bocchia M. Therapeutic advancements in multiple myeloma. Front Oncol. 2014;4:241.
4. Kumar SK, Dimopoulos MA, Kastritis E, et al. Natural history of relapsed myeloma, refractory to immunomodulatory drugs and proteasome inhibitors: a multicenter IMWG study. Leukemia. 2017;31(11):2443-2448.
5. Batlevi CL, Matsuki E, Brentjens RJ, Younes A. Novel immunotherapies in lymphoid malignancies. Nat Rev Clin Oncol. 2016;13(1):25-40.
6. Haas C, Knirrer E, Brischwein K, et al. Mode of cytotoxic action of T cell-engaging BiTE antibody MT110. Immunobiology. 2009;214(6):441-453.
7. Labrijn AF, Janmaat ML, Reichert JM, Parren PWHL. Bispecific antibodies: a mechanistic review of the pipeline. Nat Rev Drug Discov. 2019;18(8):585-608.
8. Smith EJ, Olson K, Haber LJ, et al. A novel, native-format bispecific antibody triggering T-cell killing of B-cells is robustly active in mouse tumor models and cynomolgus monkeys. Sci Rep. 2015;5(1):17943.
9. Freichs KA, Broekmans MEC, Marin Soto JA, et al. Preclinical activity of JNJ-7957, a novel BCMA×CD3 bispecific antibody for the treatment of multiple myeloma, is potentiated by daratumumab. Clin Cancer Res. 2020;26(9):2203-2215.
10. Hopp S, Tai YT, Blanset D, et al. A novel BCMA/CD3 bispecific T-cell engager for the treatment of multiple myeloma induces selective lysis in vitro and in vivo. Leukemia. 2017;31(10):2278.
11. Seckinger A, Delgado JA, Moser S, et al. Target expression, generation, preclinical activity, and pharmacokinetics of the BCMA-T cell bispecific antibody EM801 for multiple myeloma treatment. Cancer Cell. 2017;31(3):396-410.
12. Nagorsen D, Kucer P, Baueerle PA, Bargou R. Blinatumomab: a historical perspective. Pharmacol Ther. 2012;136(3):334-342.
13. Sasu B, Chaparro-Riggers J. T cell redirecting therapies for cancer treatment. Curr Cancer Drug Targets. 2016;16(1):22-33.
14. Topp MS, Kucer P, Gokbuget N, et al. Targeted therapy with the T-cell-engaging antibody blinatumomab of chemotherapy-refractory minimal residual disease in B-lineage acute lymphoblastic leukemia patients results in high response rate and prolonged leukemia-free survival. J Clin Oncol. 2011;29(18):2493-2498.
15. June CH, Sadelain M. Chimeric antigen receptor therapy. N Engl J Med. 2018;379(1):64-73.
16. Majzner RG, Mackall CL. Clinical lessons learned from the first leg of the CAR T cell journey. Nat Med. 2019;25(9):1341-1355.
17. Mohy M, Gautier J, Malard F, et al. CD19 chimeric antigen receptor-T cells in B-cell leukemia and lymphoma: current status and perspectives. Leukemia. 2019;33(12):2767-2778.
18. Carpenter RO, Esvbuomwan MO, Pittaluga S, et al. B-cell maturation antigen is a promising target for adoptive T-cell therapy of multiple myeloma. Clin Cancer Res. 2013;19(8):2048-2060.
19. Cho SF, Anderson KC, Tai YT, Targeting B. Targeting B cell maturation antigen (BCMA) in multiple myeloma: potential uses of BCMA-based immunotherapy. Front Immunol. 2018;9:1821.
20. Caraccio C, Krishna S, Phillips DJ, Schurck CM. Bispecific antibodies for multiple myeloma: a review of targets, drugs, clinical trials, and future directions. Front Immunol. 2020;11:501.
21. Cohen AD, Raje N, Fowler JA, Mezzi K, Scott EC, Dhodapkar MV. How to train your T cells: overcoming immune dysfunction in multiple myeloma. Clin Cancer Res. 2020;26(7):1541-1554.
22. Tustian AD, Endicott C, Adams B, Mattila J, Bak H. Development of purification processes for fully human bispecific antibodies based upon modification of protein A binding avidity. MAbs. 2016;8(4):828-838.
23. Raje N, Bendeja J, Lin Y, et al. Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma. N Engl J Med. 2019;380(18):1726-1737.
24. Topp MS, Duell J, Zugmaier G, et al. Anti-B-cell maturation antigen BiTE molecule AMG 420 induces responses in multiple myeloma. J Clin Oncol. 2020;38(8):775-783.
25. Murphy AJ, Macdonald LE, Stevens S, et al. Mice with megabase humanization of their immunoglobulin genes generate antibodies as efficiently as normal mice. Proc Natl Acad Sci U S A. 2014;111(14):5153-5158.
26. Crawford A, Haber L, Kelly MP, et al. A mucin 16 bispecific T cell-engaging antibody for the treatment of ovarian cancer. Sci Transl Med. 2019;11(497):eaau7534.
27. Li J, Piskul R, Ybarra R, et al. CD3 bispecific antibody-induced cytokine release is dispensable for cytotoxic T cell activity. Sci Transl Med. 2019;11(508):eaax861.
28. Trinklein ND, Pham D, Schellenberger U, et al. Efficient tumor killing and minimal cytokine release with novel T-cell agonist bispecific antibodies. MAbs. 2019;11(4):639-652.
29. Laurent SA, Hoffmann FS, Kuhn PH, et al. γ-Secretase directly sheds the survival receptor BCMA from plasma cells. Nat Commun. 2015;6(1):7333.
30. Struhl G, Adachi A. Requirements for presenilin-dependent cleavage of notch and other transmembrane proteins. Mol Cell. 2000;6(3):625-636.
31. Valenzuela DM, Murphy AJ, Frendewey D, et al. High-throughput engineering of the mouse genome coupled with high-resolution expression analysis. Nat Biotechnol. 2003;21(6):652-659.
32. Sun C, Mezzadra R, Schumacher TN. Regulation and function of the PD-L1 checkpoint. Immunity. 2018;48(3):434-452.
33. Rosenblatt J, Avigan D. Targeting the PD-1/PD-L1 axis in multiple myeloma: a dream or a reality? Blood. 2017;129(3):275-279.
34. Tremblay-LeMay R, Rastgou N, Chang H. Modulating PD-L1 expression in multiple myeloma: an alternative strategy to target the PD-1/PD-L1 pathway. J Hematol Oncol. 2018;11(1):46.
35. Campagne O, Delmas A, Foulard S, et al. Integrated pharmacokinetic/pharmacodynamic model of a bispecific CD3×CD123 DART molecule in nonhuman primates: evaluation of activity and impact of immunogenicity. Clin Cancer Res. 2018;24(11):2631-2641.
36. Friedman KM, Garrett TE, Evans JW, et al. Effective targeting of multiple B-cell maturation antigen-expressing hematological malignancies by anti-B-cell maturation antigen chimeric antigen receptor T cells. Hum Gene Ther. 2018;29(5):585-601.
37. Xu J, Chen L, Yang SS, et al. Exploratory trial of a biepitopic CAR T-targeting B cell maturation antigen in relapsed/refractory multiple myeloma. Proc Natl Acad Sci U S A. 2019;116(19):9543-9551.
38. Brudno JN, Maric I, Hartman SD, et al. T cells genetically modified to express an anti-B-cell maturation antigen chimeric antigen receptor cause remissions of poor-prognosis relapsed multiple myeloma. J Clin Oncol. 2018;36(22):2267-2280.
39. Sponaas AM, Yang R, Rustad EH, et al. PD1 is expressed on exhausted T cells as well as virus specific memory CD8+ T cells in the bone marrow of myeloma patients. Oncotarget. 2018;9(62):32024-32035.

40. Zhang X, Dong H, Lin W, et al. Human bone marrow: a reservoir for “enhanced effector memory” CD8+ T cells with potent recall function. J Immunol. 2006;177(10):6730-6737.

41. Salter AI, Ivey RG, Kennedy JJ, et al. Phosphoproteomic analysis of chimeric antigen receptor signaling reveals kinetic and quantitative differences that affect cell function. Sci Signal. 2018;11(544):eaat6753.

42. Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. N Engl J Med. 2018;378(5):439-448.

43. Graham C, Jozwik A, Pepper A, Benjamin R. Allogeneic CAR-T cells: more than ease of access? Cells. 2018;7(10):E155.

44. Benonisson H, Altıntaş I, Sluijter M, et al. CD3-bispecific antibody therapy turns solid tumors into inflammatory sites but does not install protective memory. Mol Cancer Ther. 2019;18(2):312-322.

45. Skokos D, Waite JC, Haber L, et al. A class of costimulatory CD28-bispecific antibodies that enhance the antitumor activity of CD3-bispecific antibodies. Sci Transl Med. 2020;12(525):eaaaw7888.

46. Olson B, Li Y, Lin Y, Liu ET, Patnaik A. Mouse models for cancer immunotherapy research. Cancer Discov. 2018;8(11):1358-1365.

47. Clynes RA, Desjarlais JR, Redirected T. Redirected T cell cytotoxicity in cancer therapy. Annu Rev Med. 2019;70(1):437-450.

48. Grosser R, Cherkassky L, Chintala N, Adusumilli PS. Combination immunotherapy with CAR T cells and checkpoint blockade for the treatment of solid tumors. Cancer Cell. 2019;36(5):471-482.