Pharmacological characterization of GR1803, a novel BCMA × CD3 bispecific antibody for multiple myeloma treatment

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Abstract

B-cell maturation antigen (BCMA) is an ideal target for the treatment of multiple myeloma (MM), and a bispecific antibody (bsAb) of BCMA × CD3 has entered clinical trials with great potential. In this study, we characterized GR1803, a novel bsAb for MM treatment. We reported that GR1803 bound to recombinant BCMA, CD3, BCMA-positive ($BCMA^+$) MM cells, and human T cells; that it induced T cell activation and cytokine release dependent on the presence of $BCMA^+$ cells; and that it effectively killed MM cells with EC_{50} values less than 10 ng/mL. We found that GR1803 induced MM cells to release various cytokines, including interleukin (IL)-2, IL-4, IL-6, IL-8, IL-10, interferon-g (IFN-γ), GM-CSF, and TNF-α. Finally, we found that GR1803 significantly and dose-dependently inhibited the growth of tumors specifically expressing human BCMA *in vivo*. Taken together, these results demonstrate that GR1803, a novel BCMA × CD3 bsAb, efficiently and selectively kills MM cells and represents a novel immunotherapy for treating MM.

Introduction

Multiple myeloma (MM) is the second most common hematologic malignancy, accounting for about 1% of cancers and 13% of hematological malignancies [1]. MM is a malignant tumor of plasma cells in bone marrow; its typical features include abnormal proliferation of plasma cells accompanied by excessive production of monoclonal immunoglobulin[2, 3]. The current therapies for MM include the use of immunomodulators, proteasome inhibitors, histone deacetylase inhibitors, and monoclonal antibodies[4, 5, 6, 7, 8, 9]. Although there has been clinical progress in these drug therapies, the 5-year survival rate of myeloma patients is only about 50%[10, 11]. Therefore, it is important to seek novel MM therapeutics.

BCMA (CD269), which is a member of the tumor necrosis factor receptor (TNFR) family, has two ligands: the proliferation-inducing ligand, APRIL, and B cell activating factor (BAFF). These ligands are mainly produced under paracrine signaling in bone marrow stroma [12, 13]. The binding of a ligand to BCMA promotes the growth and survival of MM cells through the phosphoinositide-3-kinase–protein kinase B/Akt (PI3K-PKB/Akt), rat sarcoma/mitogen-activated protein kinase (RAS/MAPK) and nuclear factor κ-light-chain enhancer of activated B cells (NF-κB) signaling pathways[12, 14, 15, 16]. BCMA is highly and selectively expressed in normal and malignant plasma cells[17, 18], including most MM cell lines (80–100%), and the level in malignant plasma cells is significantly higher than that in normal plasma cells[19, 20]. The expression level of BCMA continues to increase with the differentiation of B cells and the development of MM [20, 21, 22], and thus BCMA is an ideal target for the treatment of MM.

Novel immunotherapies for MM, such as chimeric antigen receptor T cell (CAR-T), bispecific antibody (bsAb), and antibody-drug conjugation (ADC), have recently achieved remarkable clinical responses in patients with relapsed and refractory MM [23, 24, 25, 26, 27]. Among them, bsAb represents a novel class of immunotherapy and is one of the most promising options. BCMA × CD3 bsAb simultaneously binds tumor cells and T cells; this induces T cells to release perforin and granzyme B, which kill tumor cells[28, 29].

In this study, we report that GR1803, a BCMA × CD3 bispecific antibody, effectively activates T cells and selectively kills MM cells both *in vitro* and *in vivo*, and represents a novel immunotherapy for treating MM. Currently, GR1803 has entered into phase I clinical trials in China.

Materials And Methods

Reagents and antibodies

GR1803, anti-BCMA monoclonal antibody (mAb), and anti-CD3 mAb were provided by Genrix (Shanghai) Biopharmaceutical Co., Ltd. Recombinant BCMA (human, mouse, cynomolgus), human BAFF, APRIL, and CD3E (cynomolgus, human) were purchased from Novo Protein Scientific, Inc. (Shanghai, China). Recombinant BCMA (rhesus, rat) and human CD3D were purchased from Sino Biological, Inc. (Beijing, China).

Cell Lines And Cell Culture

The MM cell lines NCI-H929, MM.1S, RPMI-8226, and U266 were obtained from the American Type Culture Collection (Manassas, VA, USA). Fresh peripheral blood mononuclear cells (PBMCs) were obtained from AllCells Co. (Shanghai, China). Natural killer (NK) cells were isolated from PBMCs using a human NK Cell Isolation Kit (Miltenyi, Germany). Cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated FBS at 37°C in a humidified 5% CO_2 atmosphere.

Binding And Blocking Assays

ELISA (enzyme-linked immunosorbent assay) plates (Greiner Bio-one, Germany) were coated overnight with BCMA or CD3s in PBS (phosphate buffered saline; 137 mmol/L NaCl, 2.7 mmol/L KCl, 10 mmol/L Na_2HPO_4, and 1.8 mmol/L KH_2PO_4, pH 7.4) at 4°C. The plates were blocked with 5% fat-free milk powder in PBS-T (PBS with 0.05% Tween 20), incubated with GR1803 for 1 h at 37℃, and then incubated with HRP-conjugated anti-human Ig kappa secondary antibody (1:1,000 dilution; EMD Millipore, Billerica, MA, USA). The plates were washed and incubated at room temperature (RT) for 2 min with substrate solution (citrate buffer containing 1 mg/mL OPD and 3‰ H_2O_2), and the reaction was stopped by sulfuric acid (H_2SO_4). Optical density 450 nm (OD_{450}) was determined using a plate reader.

The effect of GR1803 on the interaction of APRIL or BAFF with BCMA was determined by ELISA. BCMA coating, blocking, and GR1803 antibody incubation were carried out as described above. The plates were washed three times with PBS-T, loaded with 100 μL of APRIL (2 μg/mL) or BAFF (2 μg/mL), and incubated for 1 h at 37℃. Optical density was determined at 450 nm using a plate reader.

Cell Binding Assay

NCI-H929 cells ($BCMA^+$) were incubated with serially diluted GR1803 or anti-BCMA mAb for 1 h at 4℃, and then with FITC-conjugated anti-human IgG Fc (EMD Millipore, Billerica, MA, USA) for 1 h in the dark. The binding activity was measured by flow cytometry. The same method was used to measure the binding activity of GR1803 and anti-CD3 mAb to $CD3^+$ T cells.

T Cell Activation

NCI-H929 cells were co-incubated with various concentrations of GR1803 in the presence of PBMCs in 96-well U-bottom plates for 48 h at an effector-to-target (E:T) ratio of 1:1. The cells were stained with anti-CD107a-APC, anti-CD25-PerCP, and anti-CD69-FICT antibodies (Sino Biological, Inc., Beijing, China) and detected by flow cytometry.

The dependence of T cell activation by GR1803 on target cells was examined by luciferase assay using pGL4.30 luc2P-NFAT-RE-Hygro plasmid-transfected Jurkat (Jurkat-luc) cells. The Jurkat-luc cells were cultured alone or with NCI-H929 cells for 6 h in the presence of various concentrations of GR1803. After incubation, cells were collected and lysed, and the supernatant was assessed for luciferase activity, which reflects T cell activation.

Quantification Of Cytokines

NCI-H929 cells were co-incubated with various concentrations of GR1803 in the presence of PBMCs in 96-well U-bottom plates for 48 h at an E:T ratio of 1:1. The amounts of IL-2 and IFN-γ in T cells were quantified by HTRF-based activation assays performed using a human IL-2/IFN-γ kit (Cisbio, Codolet, USA) according to the manufacturer's protocol.

To determine the effects of GR1803 on the release of cytokines, including IL-2, IL-4, IL-6, IL-8, IL-10, GM-CSF, IFN-γ, and TNF-α, PBMCs were co-incubated with various concentrations of GR1803 in the presence of NCI-H929 cells for 6 h, 24 h, and 48 h, and the cytokines released to the supernatant were determined with a Bio-plex™ 200 system using a Bio-Plex Pro Human Cytokine 8-plex Assay (Bio-Rad, Hercules, CA, USA).

In vitro lysis assay

MM cells were co-incubated with various concentrations of GR1803 in the presence of PBMCs in 96-well U-bottom plates for 48 h at an E:T ratio of 1:1, and then stained with anti-CD138-FITC antibody (Becton Dickinson, Franklin Lakes, NJ, USA). $CD138^+$ cells were detected by flow cytometry and counted as surviving MM cells. The lysis (%) of MM cells was calculated as: 1-(absolute number of surviving $CD138^+$ cells in treated wells/absolute number of surviving $CD138^+$ cells in untreated wells) ×100%. Data from lysis assays were analyzed using a four-parameter non-linear fit model integrated, as applied using GraphPad Prism Version 8.0 (GraphPad Software, San Diego, CA, USA).

To determine the optimal E:T ratio, NCI-H929-luc cells were co-incubated with PBMCs in the presence of GR1803 (10 μg/mL) at E:T ratios of 1:1, 1:2, 1:5, and 1:10. The surviving NCI-H929-luc cells were determined by luciferase assay.

Antibody-dependent Cell-mediated Cytotoxicity (Adcc) And Complement-dependent Cytotoxicity (Cdc)

NCI-H929 cells were stained with Calcein-AM (Dojindo Laboratories, Kumamoto, Japan) for 30 min in dark and incubated with NK cells in the presence of GR1803 (100 μg/mL) at an E:T ratio of 5:1 for 4 h. The percentage of ADCC killing was calculated according to the mean fluorescence intensity compared with that of the control, which was completely lysed.

The CDC of GR1803 was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. NCI-H929 and Daudi cells were plated in 96-well plates and treated with GR1803 (100 μg/mL) or rituximab (100 μg/mL) for 4 h. MTT was added and the generated formazan crystals were solubilized with dimethyl sulfoxide. The optical density of the resulting solution was read at 490 nm using a Bio-Tek Synergy H4 (Winooski, VT, USA) [30].

Western Blotting

Western blotting
To determine the effect of GR1803 on NF-κB signaling stimulated by APRIL, NCI-H929 cells were serum starved (0.2% BSA/RPMI-1640) for 12 h and stimulated with APRIL (200 ng/mL) for 20 min, with or without GR1803. Nuclear proteins were extracted using a Subcellular Protein Fractionation Kit for Cultured Cells (Thermo Scientific, San Jose, CA, USA) according to the manufacturer's protocols. The expression of p65 was detected by Western blot analysis [30]. Histone H3 was detected as an internal control.

Flow Cytometric Assay For Cell Apoptosis

NCI-H929 cells were incubated with PBMCs in the presence of various concentrations of GR1803 for 48 h. An Annexin V-FITC/Propidium iodide (PI) Apoptosis detection kit (Dalian Meilun Biotechnology Co., Ltd., Dalian, China) was employed to examine the apoptosis of T cells, as described by the manufacturer.

In vivo **study**

6- to 8-week-old female B-hCD3EDG mice (C57BL/6-*Cd3e*$^{tm1(CD3E)}$*Cd3d*$^{tm1(CD3D)}$*Cd3g*$^{tm1(CD3G)}$/Bcgen), which are genetically modified to express human CD3E, CD3D, and CD3G, were purchased from Biocytogen Pharmaceuticals Co., Ltd (Beijing, China) and subcutaneously inoculated with MC38/BCMA cells. Tumor-bearing mice were randomized into groups and intravenously (i.v.) injected withvehicle or

GR1803 when the average tumor volume reached approximately 50–100 mm^3. Tumor volume was calculated as (length × $width^2$)/2, and body weig-ht was monitored as an indicator of general health.

To study the effect of GR1803 on BCMA expression in tumor tissues and T cells percentage in the peripheral blood, tumor tissues were removed and blood samples were collected at the indicated times after tumor bearing-mice were treated with a single i.v. injection of GR1803. The expression level of BCMA was detected by Western blot analysis and the T cell percentage were counted by flow cytometry using anti-mCD45-PE and anti-hCD3-APC antibodies (Becton Dickinson, Franklin Lakes, NJ, USA). All animal experiments were carried out in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences (Shanghai, China).

Data analysis

Data were analyzed using the GraphPad Prism Version 8 software. Non-linear regression analyses were carried out to generate dose-response curves and to calculate EC_{50} values. The results of repeated experiments are presented as means ± SD. A two-tailed Student's t test was used to test for significance, as indicated. Differences were considered significant at a *P*-value < 0.05.

Results

GR1803 binds to both BCMA and CD3

GR1803 is constructed as a symmetric two-arm IgG_1-based human antibody that simultaneously binds to BCMA and CD3, it contains the same light chain structure, avoiding the occurrence of mismatch. Knob and Hole structure further enhances the accuracy of matching (Fig. 1A). We first evaluated the binding of GR1803 to BCMA and found that GR1803 dose-dependently bound to human BCMA (EC_{50} = 479.4 ± 44.1 ng/mL) and exhibited a binding activity that was about 10-fold less than that of anti-BCMA mAb (EC_{50} = 53.2 ± 2.7 ng/mL). Similar results were obtained for cynomolgus BCMA. Overall, we found that GR1803 selectively bound to recombinant human BCMA, cynomolgus BCMA, and rhesus BCMA, but not rat or mouse BCMA (Fig. 1B). GR1803 also dose-dependently bound to NCI-H929 cells, probably due to their high-level expression of BCMA (Fig. 1B).

Next, we examined the binding of GR1803 to CD3s. The anti-CD3 part of GR1803 was generated by immunizing mice with human CD3D and CD3E complex. As would be expected from this, the anti-CD3 mAb bound to both CD3E and CD3D with high affinity and a stronger binding activity than that of GR1803. The binding of both GR1803 and anti-CD3 mAb to CD3s was not species-specific. GR1803 and anti-CD3 mAb dose-dependently bound to human T cells (Fig. 1C).

We next used ELISA to assess the effect of GR1803 on the interaction of the ligands, APRIL or BAFF, with BCMA. Whereas the anti-BCMA mAb significantly blocked the binding of BAFF with BCMA, GR1803 did not block the binding of either ligand with BCMA (Fig. 1D). This was confirmed by a Western blot analysis

showing that the APRIL-induced nuclear localization of p65 in NCI-H929 cells was blocked by the anti-BCMA mAb, but not by GR1803 (Fig. 1E).

Gr1803 Induces Bcma-dependent T-cell Activation

After simultaneously binding to MM cells and T cells, BsAb BCMA × CD3 may promote cross-linking of the two cell types to form cytolytic synapses and activate T cells [31]. As expected, GR1803 upregulated the expression levels of CD25, CD69, and CD107a (Fig. 2A) and dose-dependently induced the release of the cytokines, IFN-γ and IL-2 (Fig. 2B), indicating that it triggered the activation of T cells. GR1803 significantly induced T cell activation only when $BCMA^+$ cells were present, indicating that its ability to activate T cells was dependent on $BCMA^+$ cells (Fig. 2C).

Gr1803 Induces Mm Cell Lysis

To determine the anti-MM activity of GR1803, we tested it against a panel of MM cell lines with different expression levels of BCMA.BCMA was expressed in MM and highly expressed in NCI-H929 (Fig. 3A). A significant positive correlation was observed between the PBMC:MM cell ratio (E:T ratio) and the lysis activity of GR1803 (Fig. 3B). GR1803 induced dose-dependent lysis of MM cells (EC_{50} value of 0.9 ~ 7.6 ng/mL) in the presence of PBMCs at an E:T ratio of 1:1, irrespective of the BCMA expression in the cell lines (Fig. 3C). To further elucidate the mechanism by which GR1803 kills tumor cells, we assayed the ADCC and CDC of GR1803 against MM cells. GR1803 had weak ADCC and no CDC for the tested MM cells (Fig. 4A, B).

GR1803 promotes the release of multiple cytokines from PBMCs in vitro

CRS (Cytokine release syndrome) is a systemic inflammatory response that is caused by a variety of factors and occurs as a result of high-level immune activation. It is a potentially life-threatening toxic side effect that is commonly observed in patients treated with CD3 bispecific antibodies or CAR-T[32, 33]. To address whether GR1803 induces CRS, we assessed the effect of GR1803 on the release of various cytokines to the co-culture supernatants of PBMCs and NCI-H929 cells. We found that GR1803 time- and dose-dependently promoted the releases of IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, GM-CSF, and INF-γ (Fig. 5). These results suggest that the clinical use of GR1803 might carry a risk of inducing CRS.

Gr1803 Significantly Inhibits The Growth Of Tumors Expressing Bcma

The anti-MM effects of GR1803 were further evaluated *in vivo* in MC38/BCMA tumor model mice. As shown in Fig. 6A, GR1803 significantly inhibited the growth of MC38/BCMA tumors when mice were given a single intravenous injection of GR1803 at a dose of 0.1 or 0.3 mg/kg. The tumor-bearing mice

tolerated these doses well, as evidenced by the absence of significant body weight loss during the course of the experiment in all groups. Consistent with this, GR1803 significantly down-regulated BCMA expression in tumor tissues, beginning at 4 h after injection and continuing for at least 10 days post-injection (Fig. 6B).

Gr1803 Induces T Cell Apoptosis In A Bcma-dependent Manner

In an *in vivo* efficacy study of GR1803, we observed a clear and rapid decrease of the percentage of peripheral blood $CD3^+$ T cells in all GR1803-treated mice. The percentage of $hCD3^+/mCD45^+$ cells significantly decreased at 5 min after administration and peaked on day 7 post-treatment (Fig. 7A). To elucidate the mechanism responsible for this decrease in T cells, we assessed the effect of GR1803 on T cell apoptosis *in vitro*. T cells exhibited apoptosis when treated with GR1803 at concentrations above 10 ng/mL in the presence of $BCMA^+$ cells (Fig. 7B), indicating that the decrease of T cell percentage caused by GR1803 in the peripheral blood of tumor-bearing mice *in vivo* is probably due to GR1803-induced T cell apoptosis. Considering that the EC_{50} values of GR1803 for killing MM cells were less than 10 ng/mL, we postulate that the apoptotic actions of GR1803 within the effective dose range will be tolerated by patients.

Discussion

BCMA is an ideal target for treating MM based on its selective expression in MM cells. Indeed, bsAbs of BCMA × CD3 have made great progress in clinical trials. For example, the BCMA × CD3 bsAb, JNJ-64007957 (teclistamab), achieved an overall response rate (ORR) of 65% at 1.5 mg/kg, while the BCMA × CD3 bsAb, PF-06863135 (elranatamab), achieved an ORR of 70% at 215 μg/kg in patients with MM[34, 35]. However, 30～40% of MM patients do not respond to these two bsAbs, prompting researchers to continue seeking novel, more potent BCMA × CD3 bsAbs. In this report, we demonstrate that GR1803, a novel BCMA × CD3 bispecific antibody, efficiently killed MM cells both *in vitro* and *in vivo* with stronger activity than the previously reported BCMA × CD3 bsAbs [29, 36], suggesting that GR1803 may have stronger clinical efficacy.

The EC_{50} values of GR1803 in killing MM cells *in vitro* were less than 10 ng/mL at an E:T ratio of 1:1, whereas PF-06863135 and JNJ-64007957 were reported to require higher E:T and doses for their killing activities[29, 36]. The stronger activity of GR1803 may be associated with its ability to simultaneously bind CD3D and CD3E (Fig. 1C). This could facilitate the activation of T cells and the killing of MM cells.

BCMA activates the NF-κB signaling pathways when bound by its ligand, APRIL, to promote MM growth and survival [12]. Therefore, blocking the binding of APRIL to BCMA and inhibiting the activation of NF-κB pathways has been thought to be an ideal strategy for BCMA × CD3 bsAb design. Interestingly, GR1803 shows potent MM cell-killing activity even though it does not block the binding of APRIL to BCMA or

inhibit NF-κB signaling. This suggests that blocking the binding of BCMA with its ligands and inhibiting BCMA-NF-κB pathways is not necessary for the anti-MM function of a BCMA × CD3 bsAb.

Although GR1803 was found to induce MM cell lysis in a BCMA-dependent manner, the killing activity of GR1803 toward MM cells was not affected by the expression level of BCMA (Fig. 3). For example, the expression of BCMA is low in U266 cells and high in NCI-H929 cells, but both cell lines showed a similar sensitivity to GR1803. Our findings are consistent with previous reports[28, 29]. This phenomenon is also observed during anti-BCMA CAR T-cell treatment, where the killing activity of anti-BCMA CAR T-cell requires the presence of BCMA but is not affected by its expression level [37]. Here, we observed that the killing activity of GR1803 was mainly associated with the E:T ratio.

CRS is observed in patients treated with BCMA × CD3 bsAb therapies[34, 35]. We found that GR1803 significantly and dose-dependently promoted the releases of TNF-α, IFN-γ, GM-CSF, IL-2, IL-4, IL-6, IL-8, and IL-10. This indicates that CRS should be closely monitored when patients with MM are treated with GR1803 in the clinic.

GR1803 significantly inhibited the growth of $BCMA^+$ tumors, but was also observed to significantly decrease the percentage of T cells in peripheral blood of tumor-bearing mice as soon as 5 min post-dosing. Further studies showed that GR1803 induced T cell apoptosis *in vitro*. We believe that GR1803 decreases T cells in tumor-bearing mice through inducing T cell apoptosis, and speculate that this apoptosis-inducing activity may be linked to its stronger T cell activation ability. The GR1803-induced decrease in the T cell count peaked on day 7 after administration and gradually returned to normal status thereafter, indicating that this effect is reversible. This suggests that although GR1803 carries a risk for decreasing T cells in the clinic, this side effect should be manageable. Nevertheless, both CRS and T cell counts should be closely monitored in clinical trials.

In conclusion, we herein report that GR1803 is a novel BCMA × CD3 bispecific antibody with stronger anti-MM activity than the previously reported versions, and therefore warrants further clinical study.

Statements And Declarations

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Author contributions Liguang Lou designed the study, guided the research, and revised the manuscript. Lili Li performed the experiments, analyzed the data, and wrote the draft manuscript. Xiangling Chen and Yongpeng Li helped collect some data. All authors read and approved the final manuscript.

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Data availability All data are available on reasonable request.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

Ethical approval All animal experiments were carried out in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences (Shanghai, China).

Informed consent Not applicable.

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Figures

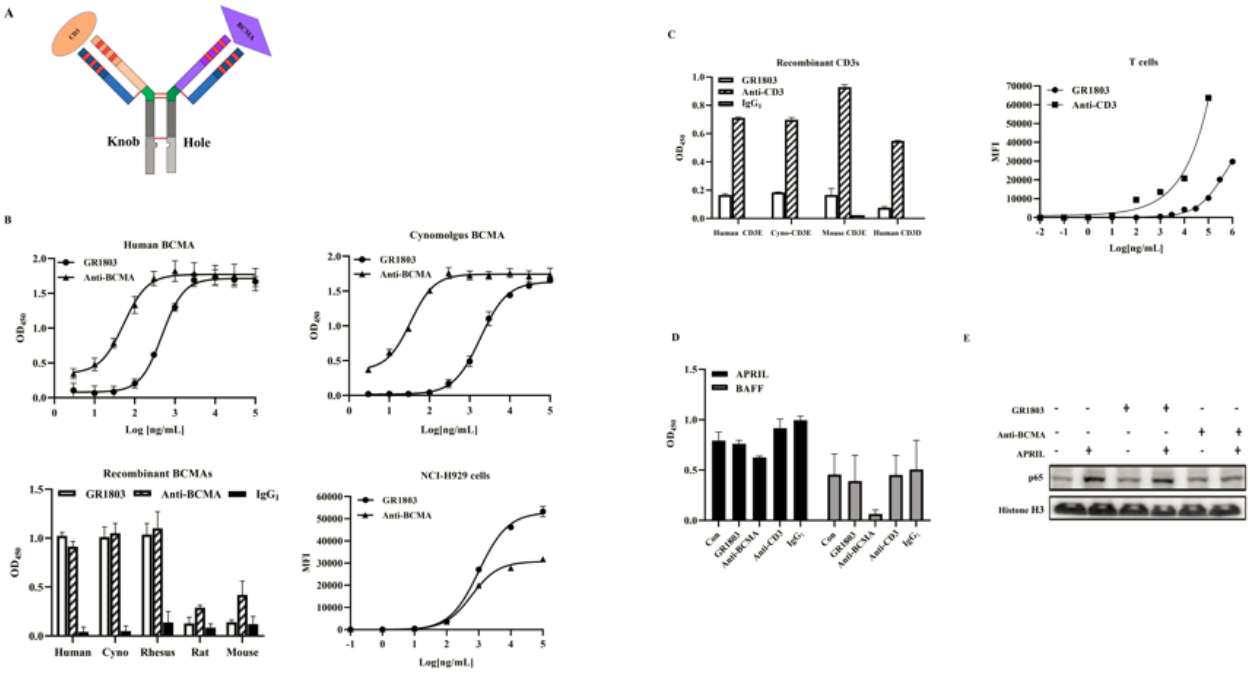

Figure 1

Binding of GR1803 to both BCMA and CD3.

A. The structure of GR1803. B. Binding of GR1803 to recombinant BCMA derived from different species or the MM cell line, NCI-H929. The binding of GR1803 to BCMA was determined by ELISA (enzyme linked immunosorbent assay) and that to NCI-H929 cells was assessed by flow cytometry. C. Binding of GR1803 to recombinant CD3s derived from different species or human T cells. The binding of GR1803 to CD3s was determined by ELISA and that to T cells was assessed by flow cytometry. D. The effect of GR1803 on the interaction of APRIL or BAFF with BCMA, as determined by ELISA. Data are presented as the means ± SD (error bars; n=2). E. The effect of GR1803 on the APRIL-induced nuclear localization of p65. Serum-starved NCI-H929 cells were pretreated with GR1803, and then stimulated with APRIL (200 ng/mL) for 20 min. Nuclear protein was extracted and analyzed by Western blotting using the indicated antibodies.

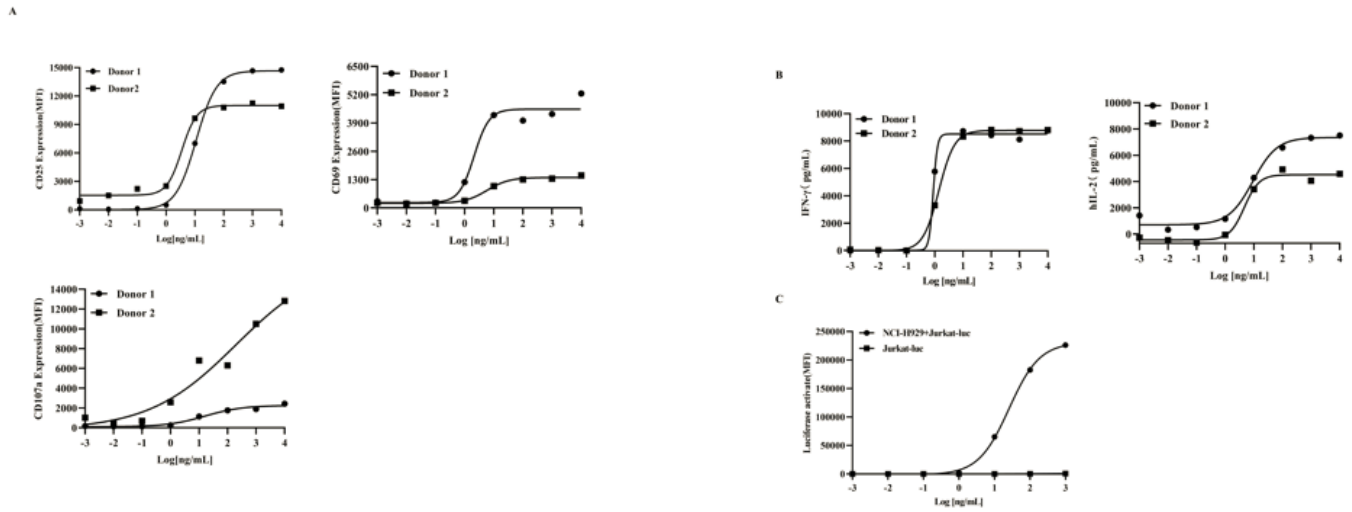

Figure 2

GR1803 activates T cells and promotes cytokine release in a BCMA-dependent manner.

A, B. NCI-H929 cells were incubated with PBMCs from healthy donors in the presence of various concentrations of GR1803 for 48 h. The expression levels mean fluorescence intensity (MFI) of CD25, CD69, and CD107a in T cells were determined by flow cytometry using anti-CD25-PerCP, anti-CD69-FITC, anti-CD107a-APC antibodies (A). The cytokines (IL-2, IFN-γ) released to cell culture supernatants were determined by HTRF (B). C. Target cell-dependent T cell activation. Jurkat-luc cells were incubated with various concentrations of GR1803 for 6 h in the presence or absence of NCI-H929 cells. Jurkat-luc cell activation was detected by luciferase assay.

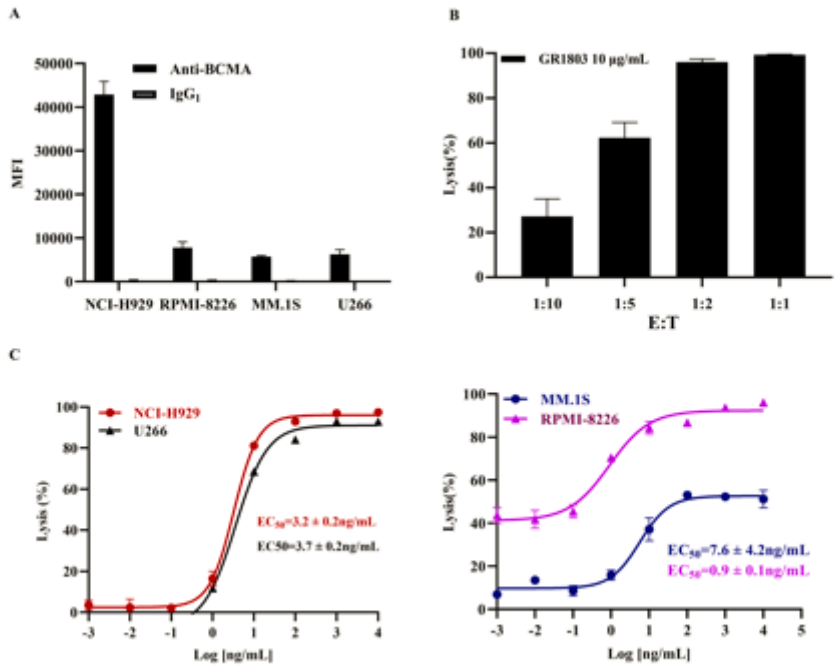

Figure 3

GR1803 induces MM cell lysis.

A. BCMA expression of MM cell lines, as determined by flow cytometry. B. GR1803-mediated cell lysis is associated with the effector: target cell ratio. NCI-H929-luc cells were incubated with PBMCs from healthy donors in the presence of 10 μg/mL of GR1803 for 48 h, and cell lysis was determined by luciferase assay. C. GR1803-mediated MM cells lysis. MM cells (NCI-H929, U266, MM.1S or RPMI-8226) were incubated with PBMCs from healthy donors in the presence of various concentrations of GR1803 for 48 h, and cell lysis was calculated from the surviving $CD138^+$ cells (MM cells) by flow cytometry using anti-CD138-FITC antibody.

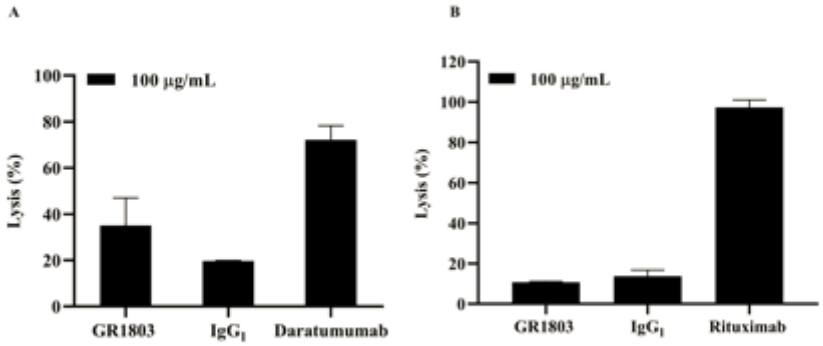

Figure 4

ADCC and CDC of GR1803.

A. The ADCC of GR1803. Calcein-AM-labeled NCI-H929 cells were incubated with GR1803 or daratumumab (100 μg/mL) in the presence of natural killer cells for 4 h, and cells lysis was monitored by assessing the release of calcein from NCI-H929 cells. B. The CDC of GR1803. NCI-H929 cells were incubated with GR1803 in the presence of human serum for 4 h, cell viability was determined by MTT assay, and cell lysis was calculated. The CDC of rituximab against Daudi cells was used as a positive control.

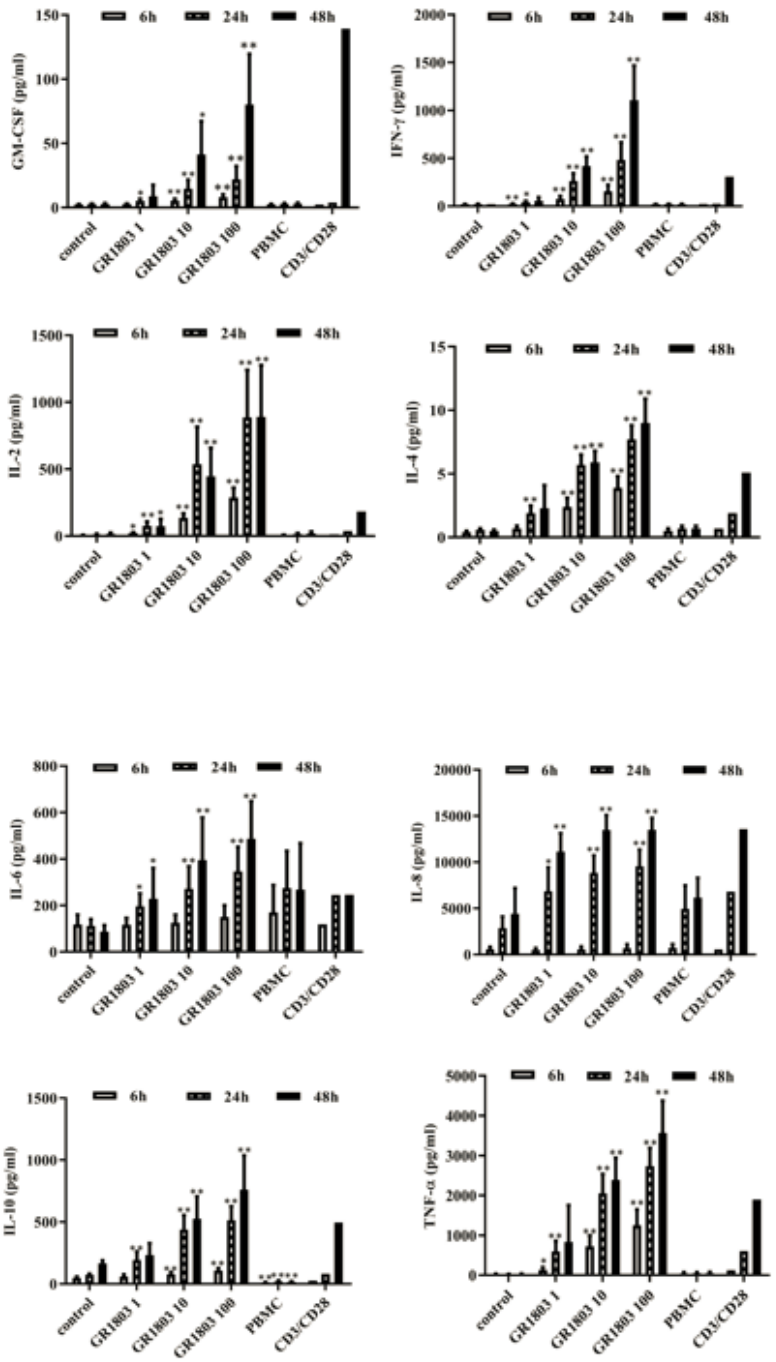

Figure 5

GR1803 promotes the release of various cytokines from PBMCs.

PBMCs from six healthy donors were incubated with various concentrations of GR1803 for 6 h, 24 h, and 48 h in the presence of NCI-H929 cells (E:T=1:1). The levels of eight cytokines (GM-CSF, TNF-α, IFN-γ, IL-2,

IL-4, IL-6, IL-8, IL-10) were detected with a Bio-Plex Pro Human Cytokine 8-plex Assay. *P□0.05, **P□0.01 vs. control; n=6.

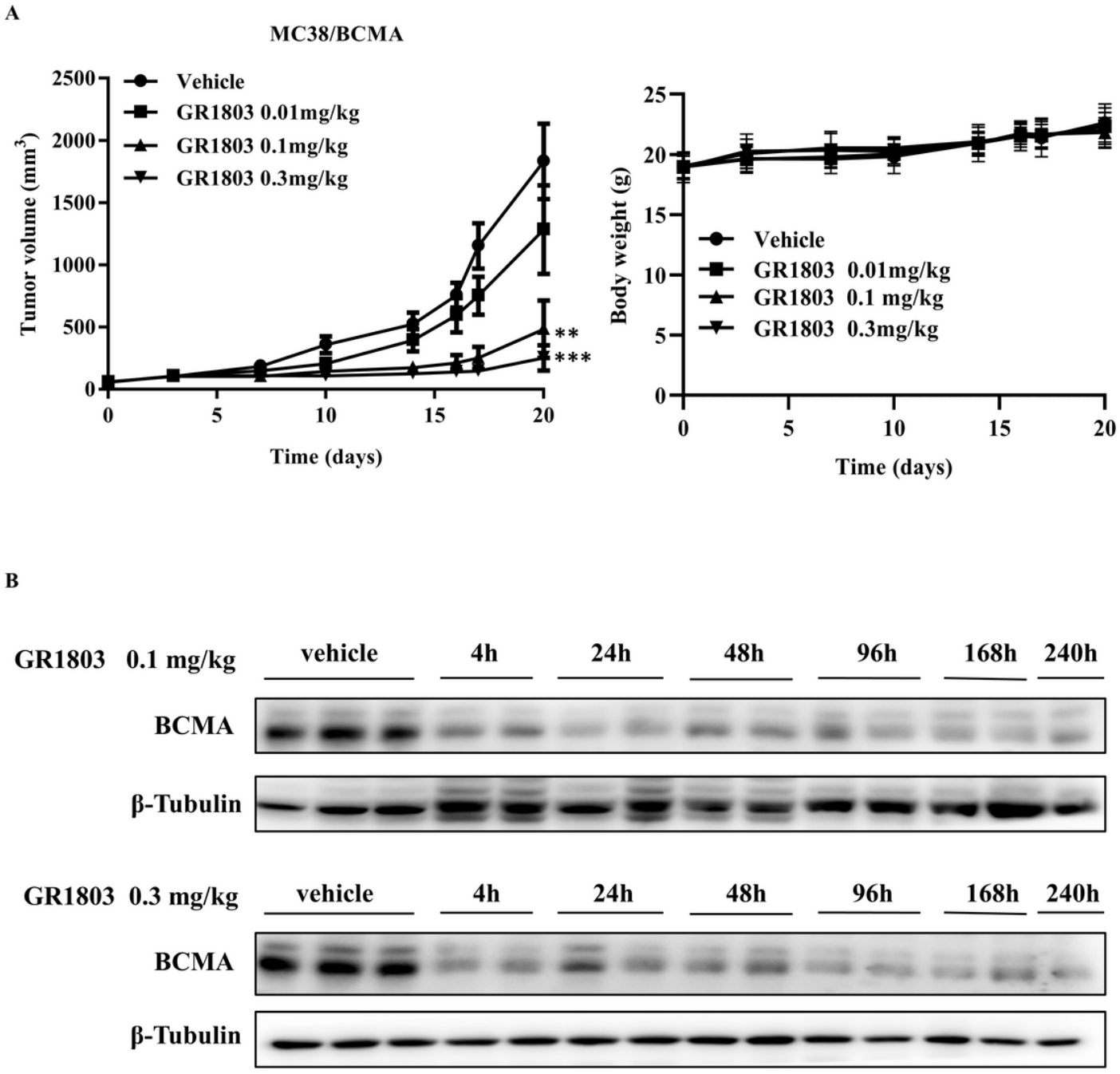

Figure 6

GR1803 inhibits the growth of BCMA$^+$ tumors *in vivo*.

A. GR1803 inhibited the growth of BCMA$^+$ MC38 tumors inoculated subcutaneously to B-hCD3EDG mice that were genetically engineered to express humanCD3E, CD3D, and CD3G. Tumor-bearing mice were

administrated with a single intravenous injection of vehicle or the indicated doses of GR1803, and tumor volumes and body weights were measured on the indicated days. Data are presented as means ± SEM for tumor volumes or means ± SD for body weight. ***P*<0.01 vs. vehicle, ****P*<0.001 vs. vehicle; n=8. B. GR1803 induced down-regulation of BCMA in tumor tissues. B-hCD3EDG mice were subcutaneously inoculated with MC38/BCMA cells. Tumor-bearing mice were administrated with a single intravenous injection of vehicle or GR1803 and tumor tissues were removed at the indicated times. The expression levels of BCMA in tumor tissues were detected by Western blot analysis.

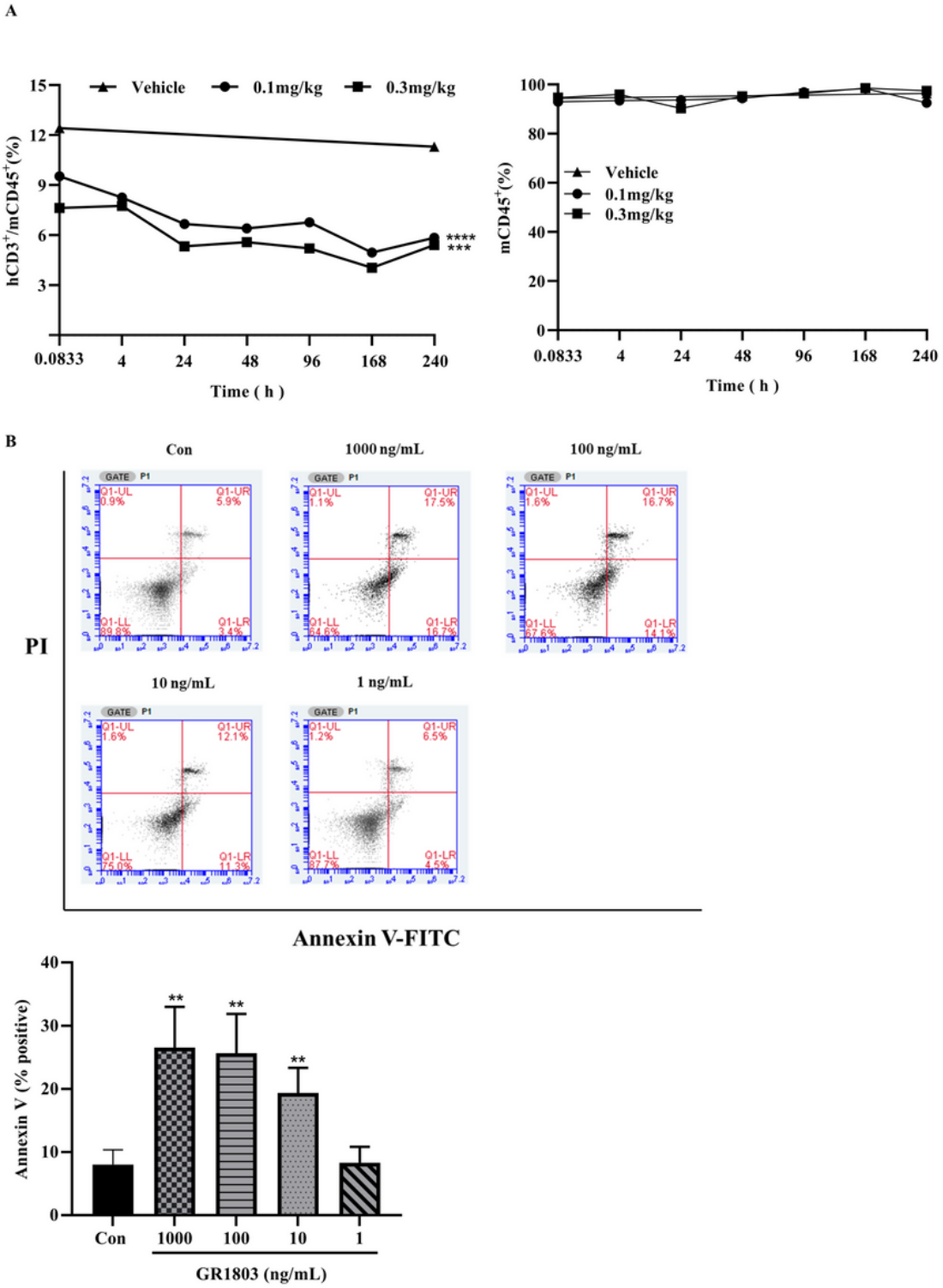

Figure 7

GR1803 induces T cell apoptosis.

A. GR1803 decreased the percentage of T cells in peripheral blood of tumor-bearing mice. B-hCD3EDG mice were subcutaneously inoculated with MC38/BCMA cells. Tumor-bearing mice were administrated with a single intravenous injection of vehicle or GR1803 and the change of $mCD45^+$(%) cells and $hCD3^+/mCD45^+$(%) cells in the peripheral blood of mice were determined by flow cytometry using anti-mCD45-PE and anti-hCD3-APC antibodies. ***P<0.001 vs. vehicle, ****P<0.0001 vs. vehicle; n=4. B. GR1803 induced the apoptosis of T cells *in vitro*. NCI-H929 cells were incubated with PBMCs in the presence of various concentrations of GR1803 for 48 h. The apoptosis of T cells was determined by flow cytometry using an Annexin V-FITC/PI apoptosis detection kit. **P<0.01 vs. control; n=4.