Regulatory T cells hamper the efficacy of T-cell-engaging bispecific antibody therapy

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Supplementary information

Key materials and resources

REAGENT or RESOURCE	Source/ References
Experimental models: Mouse strains	
C57BL/6J wild type	WEHI, maintained in house.
Foxp3DTR	Guillerey et al.[1]
Antibodies	
Anti-TCRβ (H57-597)	Biolegend
Anti-mouse CD4 Antibody (RM4-5)	Biolegend
Anti-Foxp3 (FJK-16s)	Thermo Fisher Scientific
Anti-CD8α (53-6.7)	Biolegend
Anti-mouse CD69 (H1.2F3)	Biolegend
Anti-mouse PD-1 (29F.IA12)	Biolegend
Anti-mouse Tigit (1G9)	Biolegend
Anti-mouse IFNγ (XMG1.2)	Thermo Fisher Scientific
ActinRed™ 555 ReadyProbes™ Reagent (Rhodamine phalloidin)	Thermo Fisher Scientific
Anti-Human CD11a (LFA-1alpha)	Thermo Fisher Scientific
Anti-mouse CD16/32 (2.4G2)	Prepared in house
7-AAD Viability Staining Solution	Biolegend
Zombie Acqua™ Fixable Viability Kit	Biolegend
Anti-Human FoxP3 (236A/E7)	BD Biosciences
Anti-Human CD25 (M-A251)	Biolegend
Anti-Human CD4 (SK3)	BD Biosciences
Anti-Human CD152 (CTLA-4) (L3D10)	Biolegend
Anti-Human CD8a (HIT8a)	Biolegend
Anti-Human CD3 (SK7)	BD Biosciences
Anti-Human/Mouse/Rat CD278 (ICOS) (C398.4A)	Biolegend
Therapeutic antibodies	
Rat IgG$_{2a}$ isotype control (1-1)	Leinco Technologies, Inc.
anti-mouse CD3/BCMA	Bristol Myers Squibb, Casey *et al.*[2]
Human anti-BCMA-antiCD3 bispecific antibody	BPS Bioscience
Human anti-HER2-antiCD3 bispecific antibody	Absolute Antibody
Reagents, Recombinant Proteins	
Lymphoprep™	STEMCELL Technologies
Diphtheria Toxin (DT)	Sigma-Aldrich
Red Blood Cell Lysis Solution (10x)	Miltenyi Biotec
Recombinant hIL-2	Tecin Teceleukin
Assay kits	
CellTrace™ Violet Cell Proliferation kit	Thermo Fisher Scientific

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PKH26 Red Fluorescent Cell Linker Kit	Sigma-Aldrich
Image-iT™ Fixative Solutions	Thermo Fisher Scientific
EasySep™ Human CD8+ T cell Isolation Kit	STEMCELL Technologies
EasySep™ Human CD4+ T cell Isolation Kit	STEMCELL Technologies
EasySep™ Human CD138 Positive Selection Kit II	STEMCELL Technologies
EasySep™ Human CD4+CD127lowCD25+ Regulatory T cellI Isolation Kit	STEMCELL Technologies
ImmunoCult™ Human CD3/CD28 T Cell Activator	STEMCELL Technologies
Cell Stimulation Cocktail plus protein transport inhibitor	Thermo Fisher Scientific
Foxp3 Transcription Factor Staining Buffer set	Thermo Fisher Scientific
BD Liquid Counting beads	BD Biosciences
Mouse Granzyme B ELISA kit	Thermo Fisher Scientific
Mouse Interferon-γ ELISA kit	Thermo Fisher Scientific
Human IL-10 Quantikine ELISA Kit	R&D Systems
Human Interferon-γ ELISA kit	Thermo Fisher Scientific
Sebia Hydragel 30 B1-B2	Abacus dx
Multiple myeloma cell lines	
Vk14551-EGFP cells	Casey *et al*. [2]
JJN-3	From Slavica Vuckovic
JJN-3-GFP	In this paper
RPMI8226-GFP-luciferase	From Slavica Vuckovic
Software	
Graphpad Prism 8.0 software	GraphPad Software, Inc.
MetaMorph, Microscope Imaging, Microscopy Analysis Software	Molecular Devices, Inc.
FlowJo™ v10 software	BD Biosciences

References

1.   Guillerey C, Nakamura K, Pichler AC, et al. Chemotherapy followed by anti-CD137 mAb immunotherapy improves disease control in a mouse myeloma model. JCI Insight. 2019;5(14).

2.   Casey M, Tu C, Harrison SJ, Nakamura K. Invariant NKT cells dictate antitumor immunity elicited by a bispecific antibody cotargeting CD3 and BCMA. Blood Adv. 2022;6(17):5165-5170.

Supplementary Figure 1
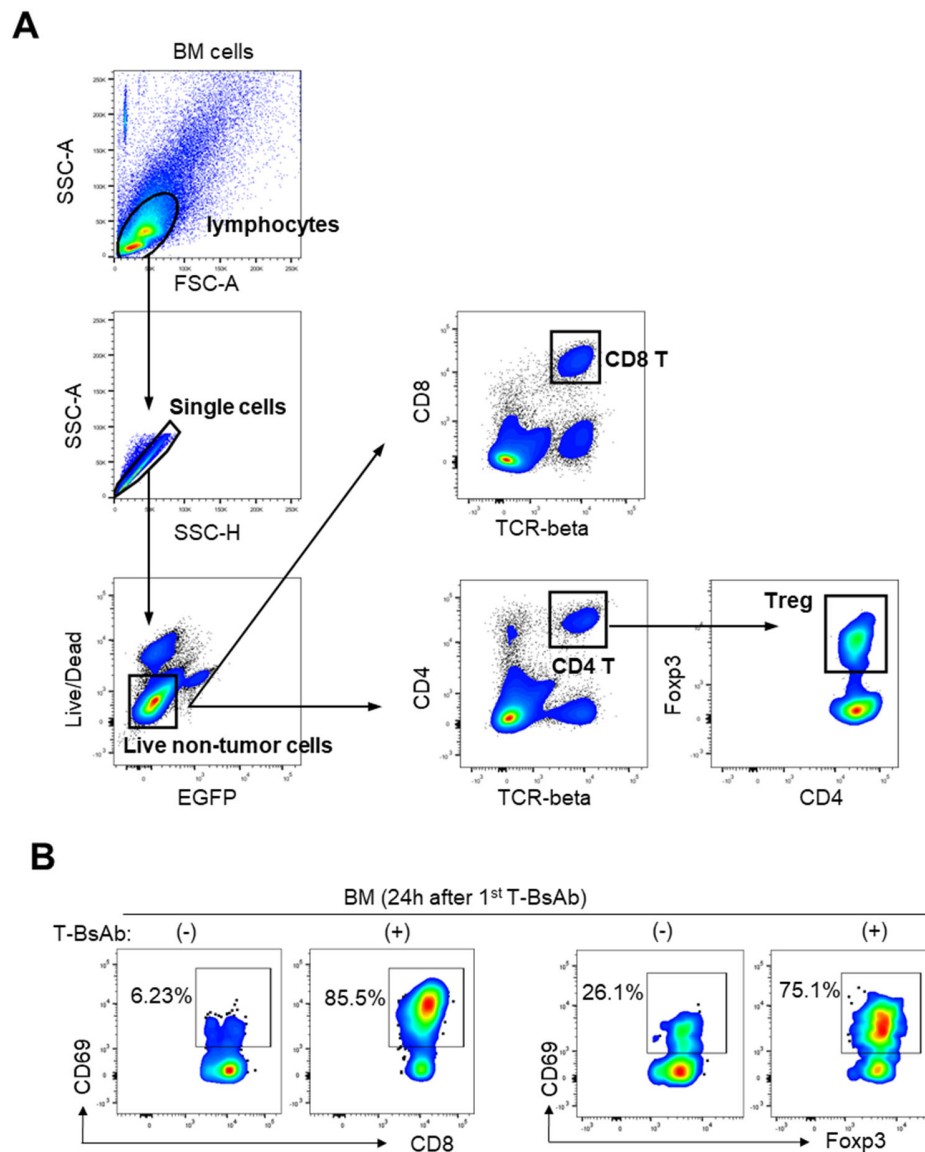
Supplementary Figure 1. T-BsAb-induced early activation of BM CD8 T cells and regulatory T (Treg) cells.

(A and B) Gating strategy (A) and representative plots (B) showing frequencies of CD69+ CD8 T cells and Treg cells in the BM 24 hours after the first T-BsAb treatment. Representative results from three experiments are shown.

Supplementary Figure 2.
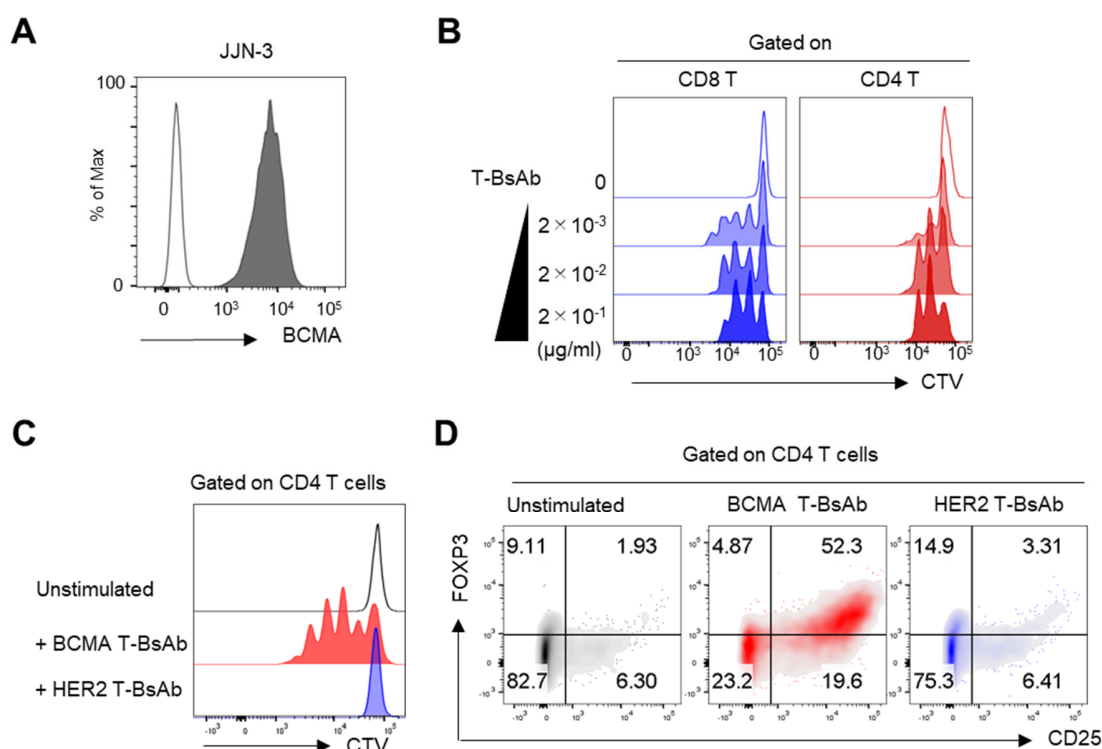
Supplementary Figure 2. The target antigen-specific activation of CD4 T cells.

(A) Representative histograms showing the expression level of BCMA in JJN-3 myeloma cells.

(B) PBMCs (4×10^5) were labeled with CellTrace Violet (CTV), and co-cultured with JJN-3 cells with indicated concentrations of T-BsAb at an effector-to-target ratio of 4:1. Representative histograms showing T-cell proliferation 4 days after co-culture.

(C and D) CD4 T cell proliferation 4 days after co-culture with JJN-3 myeloma cells in the presence of anti-BCMA or anti-HER2 T-BsAb (0.1 µg/ml) (C). FACS plots showing frequencies of CD4 T cells expressing CD25 and FOXP3 after stimulation with indicated T-BsAb (D). Representative results from 3 different donors are shown.