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B7-H3-targeted 4-1BB activation potentiates CD8 T cell-dependent antitumor immunity without systemic toxicity

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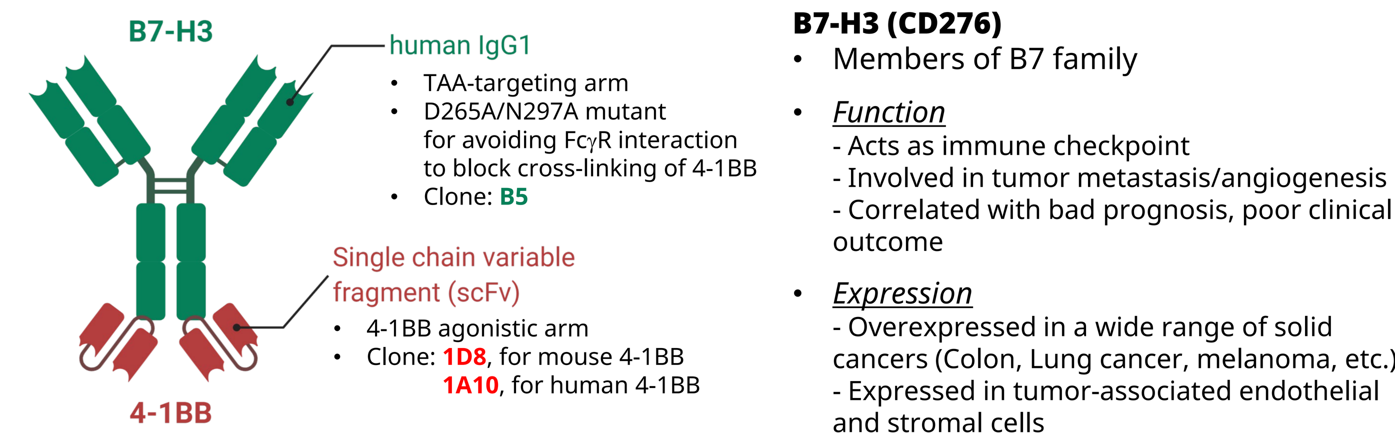
Abstract

4-1BB is a costimulatory receptor on activated T and NK cells, and the stimulation of 4-1BB by the natural ligand or agonistic mAb enhances cellular proliferation and effector functions. Immunotherapy targeting 4-1BB has been tested for cancer patients; however, dose-limiting toxicities of 4-1BB agonists restrict further clinical development. B7-H3 (CD276) is overexpressed on the cell surface of multiple cancers and tumor-associated endothelial cells, yet barely on healthy adult tissues. To restrict 4-1BB stimulation activity in tumors, we have developed an FcγR-binding null bispecific IgG1 antibody consisting of B7-H3-targeting IgG (B5) and two anti-4-1BB single-chain variable fragments. The B7-H3×4-1BB bispecific antibody (B5×1D8) shows a potent in vitro T cell costimulatory activity in the presence of B7-H3 on the tumor cells. B5×1D8 rapidly accumulates in B7-H3-overexpressing tumors compared to 4-1BB agonistic mAb, 1D8. B5×1D8 elicits a 4-1BB-dependent anti-tumor response in three different B7-H3-overexpressing murine tumor models. More importantly, in contrast to 1D8, B5×1D8 does not induce any observable immune-related adverse events (irAEs). Treatment of B5×1D8 increases the density, cytokine production, and proliferation of CD8 T cells in the tumor. Characterization of TILs indicates that B5×1D8 increases the number of PD-1⁺TIM-3⁺ "terminal effector" CD8 T cells for eliminating tumor cells. Furthermore, a combination of B5×1D8 and immune checkpoint blockade (ICB), anti-PD1, synergistically inhibits tumor growth. The human 4-1BB-targeting bispecific antibody also induces B7-H3-dependent 4-1BB costimulation and inhibits tumor growth in human 4-1BB knock-in (KI) mice. In sum, our data suggest that the B7-H3×4-1BB bispecific antibody represents an alternative form of IgG-based 4-1BB agonistic mAb for effective and safe cancer immunotherapy against B7-H3 positive cancers as monotherapy and combination therapy with other immunotherapy, like ICB.

Introduction of B7-H3×4-1BB bsAb



Functional Characterization of B7-H3×4-1BB bsAb

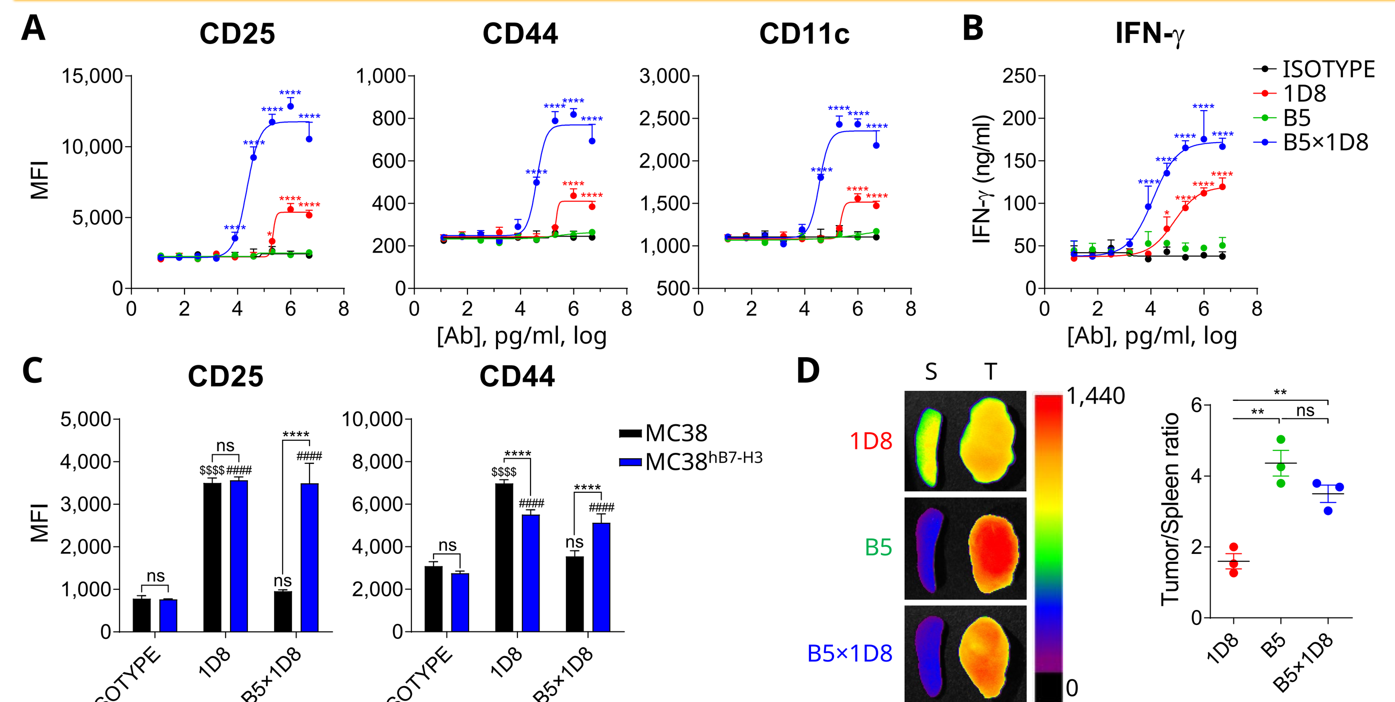


Figure 1. (**A** and **B**) Dose-dependent costimulatory activity of human IgG1 isotype, 1D8, B5, and B5×1D8 on CD8 T cells stimulated with anti-CD3ε (1 μg/ml) and irradiated MC38^{hB7-H3} cells. Flow cytometric analysis of surface expressions on CD8 T cells (A) and IFN-γ secretion by ELISA (B) 72 hours after stimulation. (**C**) Flow cytometric analysis of surface expressions on CD8 T cells stimulated with anti-CD3ε (1 μg/ml) and irradiated MC38 or MC38^{hB7-H3} cells with indicated antibodies (1 μg/ml) 72 hours after stimulation. (**D**) Representative *ex vivo* fluorescence images of spleen (S) and tumor (T) (left), and tumor-to-spleen ratio (right) from MC38^{hB7-H3} tumor-bearing mice 24 hours after intravenous injection of 37.5 μg of 680XL-labeled mAb (1D8 and B5) or 50.0 μg of 680XL-labeled B5×1D8 (*n* = 3/group). */\$/#*P* < 0.05; **/\$\$/##*P* < 0.01; ***/\$\$\$/###*P* < 0.001; and ****/\$\$\$\$/####*P* < 0.0001, two-way ANOVA with Bonferroni posttests compared with hIgG1 isotype group (A and B); two-way ANOVA with Bonferroni posttests (C); and one-way ANOVA with Bonferroni's multiple comparison test (D). ns, not significant. For (D), * compares two cell lines, \$ (for MC38) and # (for MC38^{hB7-H3}) compare each treatment in one cell line. Data presented as mean ± SD.

Absence of irAEs following B7-H3×4-1BB bsAb Treatment

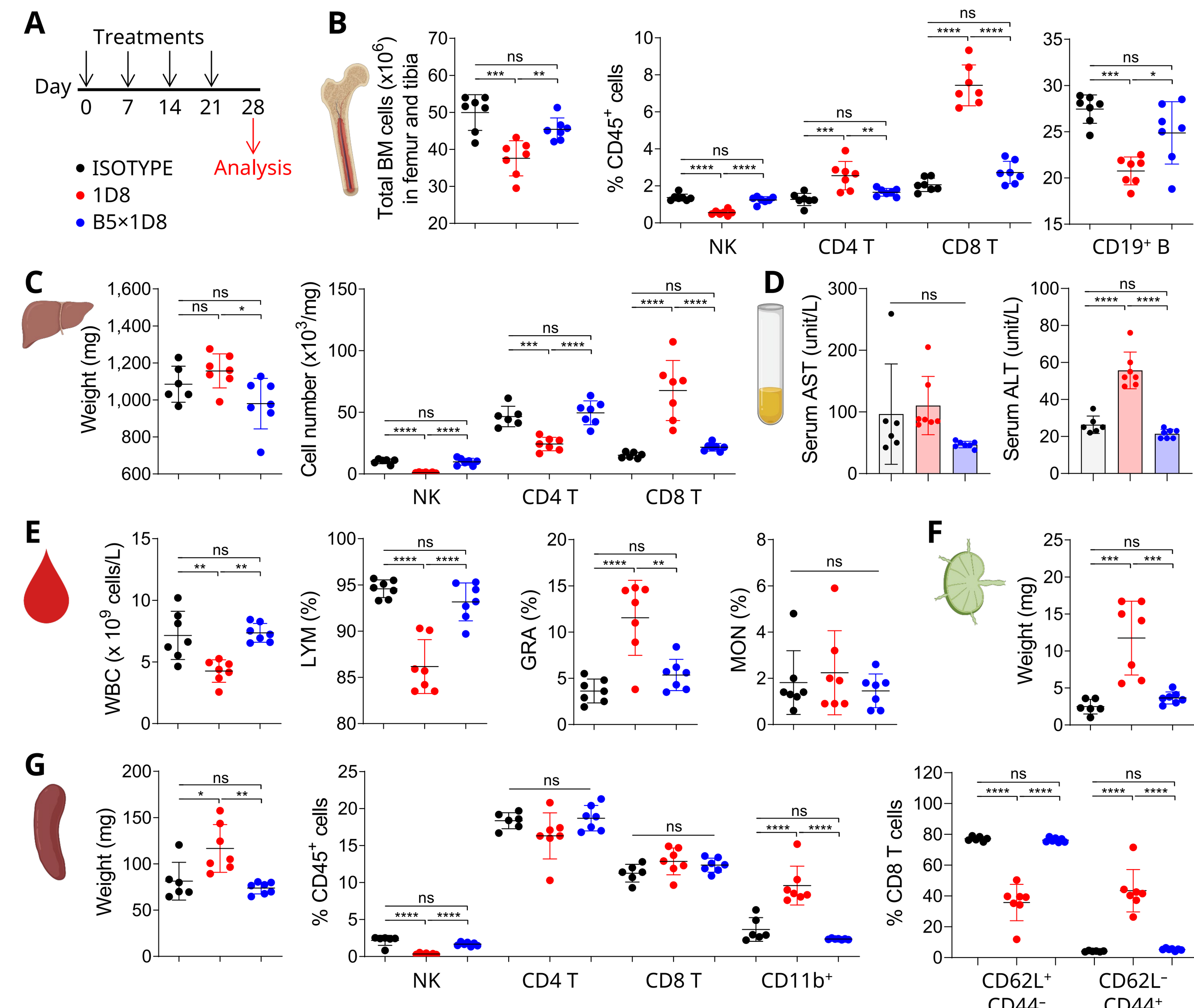


Figure 2. (**A**) Experimental scheme. C57BL/6 naïve mice (*n* = 6-7 per group) were treated with indicated antibodies once a week. Systemic alterations in each organ of antibody-treated mice were addressed 7 days after the last treatment. (**B**) Number of bone marrow (BM) cells (left), NK-, CD4 T-, and CD8 T cell frequency (middle), B cell frequency (right) from femur and tibia. (**C**) Serum AST (left) and ALT (right). (**D**) Liver weight (left), liver-infiltrated NK-, CD4 T-, and CD8 T cell number (right). (**E**) Peripheral blood cell population analyzed by the CBC counter. WBC; white blood cells, LYM; lymphocytes, GRA; granulocytes, MON; monocytes. (**F**) Weight of inguinal lymph node. (**G**) Spleen weight (left), NK-, CD4 T-, CD8 T-, and CD11b⁺ myeloid cell population (middle), and subtypes of CD8 T cell (right). The immune population in BM, liver, and spleen, was analyzed by flow cytometry. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; and *****P* < 0.0001, one-way ANOVA with Bonferroni's multiple comparison test (for B to G). ns, not significant. Data presented as mean ± SD. All icons were "Created with BioRender.com."

Antitumor Efficacy of B7-H3×4-1BB bsAb

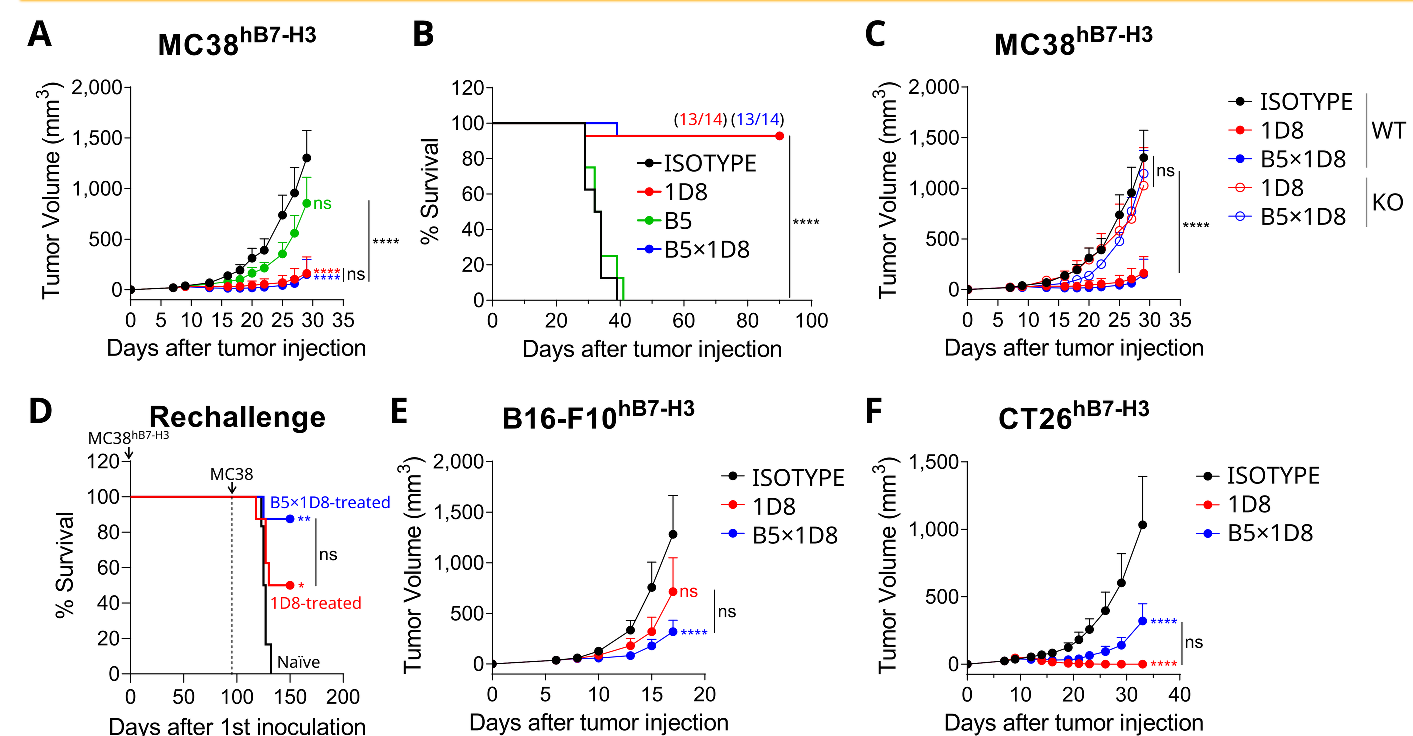


Figure 3. (**A** to **C**) MC38^{hB7-H3} tumor-bearing C57BL/6 or 4-1BB KO mice (*n* = 7-14 per group) were treated with indicated antibodies on day 7 and 10 after tumor injection and analyzed for tumor growth (A), survival (B) for C57BL/6 mice, and tumor growth for C57BL/6 and 4-1BB KO mice (C). Numbers in survival curves indicate tumor-free mice/total mice at the end of the experiment. (**D**) Long-term survivors (*n* = 6-8 per group) from 4-1BB agonist treatments (A) were rechallenged with MC38 and analyzed for survival. (**E**) B16-F10^{hB7-H3} tumor-bearing C57BL/6 mice (*n* = 10 per group) were treated with indicated antibodies on day 6, 12, and 15 after tumor injection and analyzed for tumor growth. (**F**) CT26^{hB7-H3} tumor-bearing BALB/c mice (*n* = 11 per group) were treated with indicated antibodies on day 7 and 10 after tumor injection and analyzed for tumor growth. 10.0 μg for mAb and 13.3 μg for bsAb were intraperitoneally administered in all experiments. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; and *****P* < 0.0001, two-way ANOVA with Bonferroni posttests for (A, E, and F); and Log-rank (Mantel-Cox) test for (B and D). ns, not significant. Data presented as mean ± SEM.

Changes in CD8 TILs following B7-H3×4-1BB bsAb Treatment

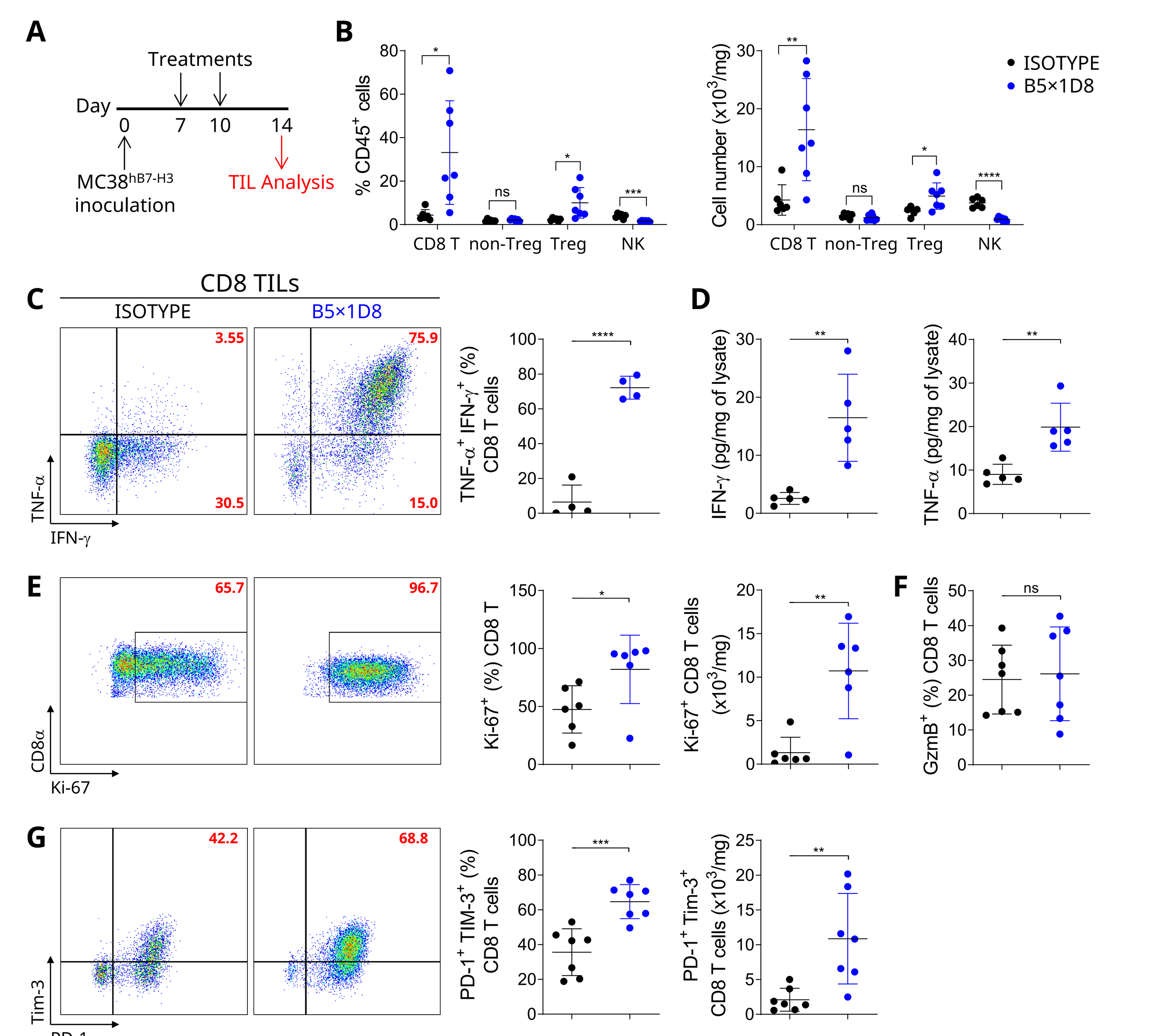


Figure 4. (**A**) MC38^{hB7-H3} tumor-bearing C57BL/6 mice (*n* = 4-7 per group) were intraperitoneally treated with 10.0 μg of hIgG1 isotype or 13.3 μg of B5×1D8. and tumor tissues were analyzed 4 days after last treatment. (**B** and **C**) Flow cytometric analysis of TIL composition (B, left), cell count per mg of tumor (B, right), and TNF-α and IFN-γ in restimulated CD8 TILs (B). (**D**) The protein level of TNF-α and IFN-γ in the tumor lysate by ELISA. (**E** to **G**) Flow cytometric analysis of Ki-67 (E), GzmB (F), and PD-1/Tim-3 (G) expression in CD8 TILs. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; and *****P* < 0.0001, unpaired Student's *t*-test for (B to G). ns, not significant. Data presented as mean ± SD.

Synergistic Effect of B7-H3×4-1BB bsAb with PD-1 Blockade

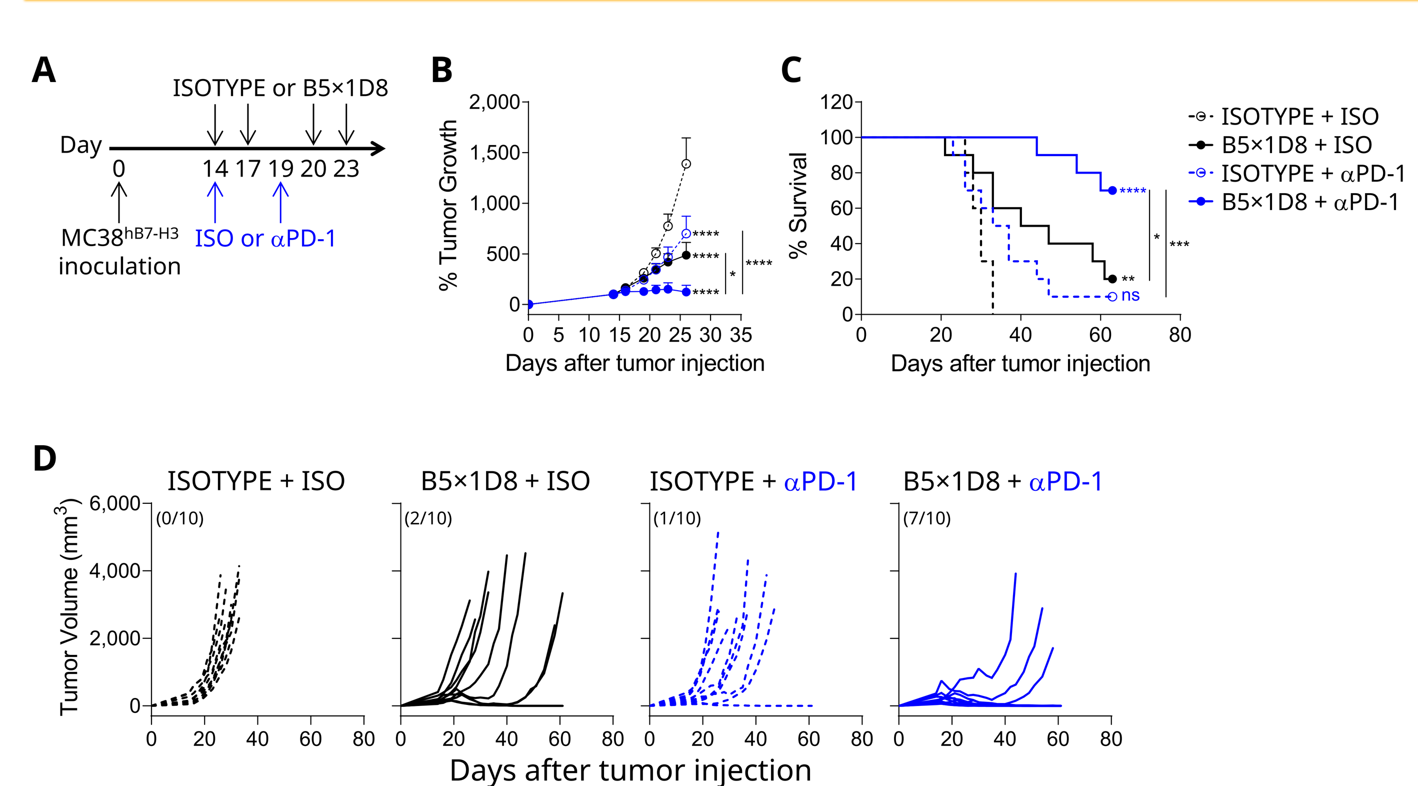


Figure 5. (**A**) Experimental scheme of combination therapy of B7-H3×4-1BB bsAb and anti PD-1 in MC38^{hB7-H3} tumor-bearing C57BL/6 mice (*n* = 10/group). 37.5 μg of hIgG1 ISOTYPE or 50.0 μg or bsAb were administered intraperitoneally with 200 μg of rat IgG2a isotype (ISO) or anti-PD-1 (αPD-1) from day 14 after tumor injection (when the tumor reached an average volume of 100-200 mm³). (**B** to **D**) Tumor growth (basal tumor volume at day 14) curves (B), survival curves (C), and tumor growth curves for individual mice (D). Numbers in (D) indicate tumor-free/total mice ratios. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; and *****P* < 0.0001, two-way ANOVA with Bonferroni posttests for (B); and Log-rank (Mantel-Cox) test for (C). ns, not significant. Data presented as mean ± SEM.

B7-H3×h4-1BB bsAb in Human 4-1BB System

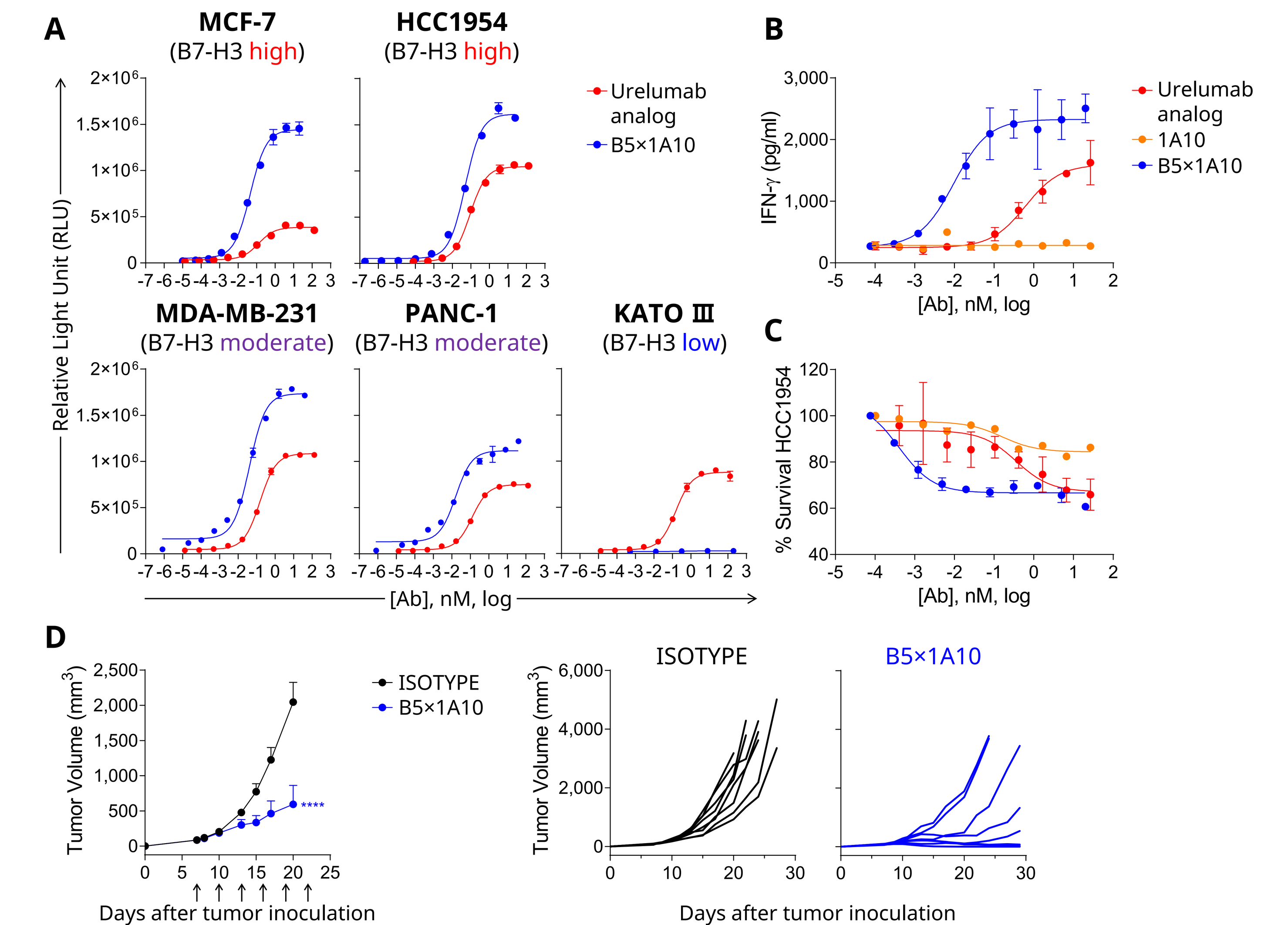
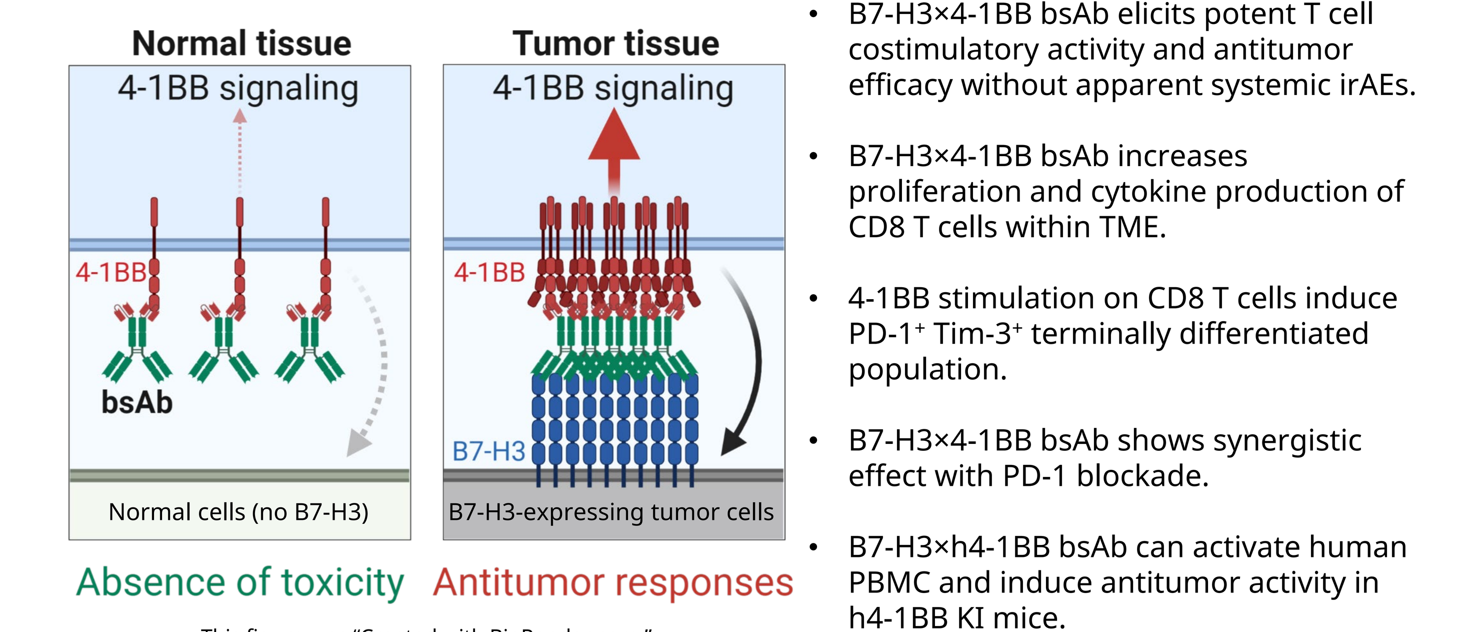


Figure 6. (**A**) Dose-dependent costimulatory activity of Urelumab analog and B5×1A10 on Jurkat-NFκB-luc2/h4-1BB reporter cells. Luminescence was measured 6 hours after stimulation with indicated cancer cells. (**B** and **C**) Dose-dependent costimulatory activity of Urelumab analog, 1A10, and B5×1A10 on PBMCs stimulated with anti-human CD3 (5 μg/ml) and HCC1954 cells. IFN-γ secretion by ELISA (B) and optical cellular density by cell counting kit (C) were analyzed 72 hours after stimulation. (**D**) MC38^{hB7-H3} tumor-bearing h4-1BB KI mice (*n* = 8/group) were treated with 2.25 mg/kg of hIgG1 isotype of 3 mg/kg of B5×1A10. Black arrows (↑) indicate treatment points. Tumor growth curves of individual mice are shown on the right. *****P* < 0.0001, two-way ANOVA with Bonferroni posttests for (D). ns, not significant. Data presented as mean ± SD for (A to C) and mean ± SEM for (D).

Conclusion



- B7-H3×4-1BB bsAb elicits potent T cell costimulatory activity and antitumor efficacy without apparent systemic irAEs.
- B7-H3×4-1BB bsAb increases proliferation and cytokine production of CD8 T cells within TME.
- 4-1BB stimulation on CD8 T cells induce PD-1⁺ Tim-3⁺ terminally differentiated population.
- B7-H3×4-1BB bsAb shows synergistic effect with PD-1 blockade.
- B7-H3×h4-1BB bsAb can activate human PBMC and induce antitumor activity in h4-1BB KI mice.

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Disclosures

Yangsoon Lee, Kyeongsu Park, and Jaeho Jung are employees of ABL Bio, Inc., which develops and supports the supply of recombinant antibodies presented in this manuscript. The other authors declare no potential conflicts of interest.

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