A PSMA-Targeting CD3 Bispecific Antibody Induces Antitumor Responses that Are Enhanced by 4-1BB Costimulation

Danica Chiu, Richard Tavaré, Lauric Haber, Olulanu H. Aina, Kristin Vazzana, Priyanka Ram, Makenzie Danton, Jennifer Finney, Sumreen Jalal, Pamela Krueger, Jason T. Giulieo, Danshe Ma, Eric Smith, Gavin Thurston, Jessica R. Kirshner, and Alison Crawford

ABSTRACT

Patients with hematologic cancers have improved outcomes after treatment with bispecific antibodies that bind to CD3 on T cells and that redirect T cells toward cancer cells. However, clinical benefit against solid tumors remains to be shown. We made a bispecific antibody that targets both the common prostate tumor–specific antigen PSMA and CD3 (PSMAxCD3) and provide evidence for tumor inhibition in several preclinical solid tumor models. Mice expressing the human extracellular regions of CD3 and PSMA were generated to examine antitumor efficacy in the presence of an intact immune system and PSMA expression in normal tissues. PSMAxCD3 accumulated in PSMA-expressing tissues and tumors as detected by immuno-PET imaging.

Although PSMAxCD3 induced T-cell activation and showed antitumor efficacy in mice with low tumor burden, PSMAxCD3 lost efficacy against larger solid tumors, mirroring the difficulty of treating solid tumors in the clinic. Costimulatory receptors can enhance T-cell responses. We show here that costimulation can enhance the antitumor efficacy of PSMAxCD3. In particular, 4-1BB stimulation in combination with PSMAxCD3 enhanced T-cell activation and proliferation, boosted efficacy against larger tumors, and induced T-cell memory, leading to durable antitumor responses. The combination of CD3 bispecific antibodies and anti-4-1BB costimulation represents a therapeutic approach for the treatment of solid tumors.

Introduction

Over the last decade, the success of immune checkpoint inhibitors in patients has fueled the clinical development of diverse cancer immunotherapy drugs (1). Success in the clinic is limited to a subset of patients and varies depending on the cancer type. The success of checkpoint inhibitors has been associated with tumors with high mutation burden, that is, expressing neoantigens recognized by tumor reactive T cells (2). However, immunotherapy targeting the otherwise “nonimmunogenic” tumors remains a challenge.

CD3 bispecific antibodies activate T cells by cross-linking the CD3 complex in the presence of a tumor-associated antigen, resulting in targeted tumor cell death independent of endogenous T-cell recognition or MHC restriction (3). This suggests that CD3 bispecific antibodies can be efficacious even in tumors with low mutation burden. The success of the CD19-directed CD3–T-cell-engager blinatumomab has promoted development of agents with both hematologic and solid cancer indications. However, despite the number of CD3 bispecific antibodies targeting solid tumors in preclinical and clinical evaluation (4), clinical benefit has yet to be shown. This lack of benefit may be attributed to a lack of optimal tumor-specific targets or to barriers in the solid tumor microenvironment that may suppress T-cell entry, activation or survival in the tumor (5, 6). Furthermore, it is unclear how the size of the tumor affects CD3 bispecific function and whether the addition of costimulation can boost responses to CD3 bispecific antibodies in solid tumors.

PSMA (FOLH1) is an integral cell surface membrane protein that is frequently overexpressed in prostate cancer and is often associated with androgen-independent prostate cancer and secondary metastatic lesions (7). PSMA is also expressed on the neovasculature of some clear cell renal carcinomas, bladder, colon, and breast cancers making it an attractive cell surface target for late-stage malignancies (8). Preclinical studies have demonstrated the potential of targeting PSMA as a prostate cancer antigen with various PSMAxCD3 bispecific formats in xenogeneic mouse models (9–12). Indeed, multiple clinical trials are ongoing for the treatment of castration-resistant prostate cancer (CRPC; ClinicalTrials.gov). Further investigation into the function and mechanism of PSMAxCD3 bispecific antibodies will be informative in supporting the clinical development of such agents.

We generated an antihuman PSMAxCD3 bispecific antibody (PSMAxCD3) and evaluated its efficacy in vitro and in vivo. To examine this bispecific antibody in an immune-competent model, we genetically engineered a human target (HuT) mouse to express both human PSMA and CD3. Immuno-PET imaging of 89Zr-labeled PSMAxCD3 revealed biodistribution of PSMAxCD3 in both peripheral PSMA-expressing tissues and tumor. Treatment with PSMAxCD3 eradicated small murine tumors but when tumors were larger, efficacy was limited despite PSMAxCD3 targeting to the tumor and inducing T-cell activation. We sought to determine whether the addition of costimulatory signals could enhance the T-cell response and result in antitumor efficacy. The TNFR family receptors are upregulated on activated T cells and can induce expansion of effector T cells and subsequent formation of memory T cells (13). Clinical trials targeting OX40 were completed for prostate cancer and are ongoing for advanced malignancies (14). Here, we focused on 4-1BB (TNfrs9), another member of the TNFR family. The benefit of providing 4-1BB...
costimulation in the treatment of prostate cancer has been demonstrated in preclinical prostate cancer models (15, 16). Second generation 4-1BB chimeric antigen receptor (CAR) T cells against prostate stem cell antigen (PSCA-CAR) show superior CAR T-cell persistence and control of disease survival compared with CD28+ CAR T cells (17), highlighting the advantages 4-1BB confers.

We demonstrate that 4-1BB costimulation enhanced CD8+ T-cell infiltration, prolonged T-cell activation, and increased proliferation in larger tumors compared with PSMAxCD3 alone. This led to greater antitumor efficacy against larger established tumors and generation of T-cell memory demonstrating durable antitumor responses. Thus, therapeutic combination of CD3 bispecific antibodies and 4-1BB costimulation enhanced responses against solid tumors.

Materials and Methods

Study design

The main objective of our studies was to determine the antitumor efficacy of PSMAxCD3 (a bispecific antibody targeting PSMA and CD3) alone or in combination with anti-mouse 4-1BB. In all studies, bispecific anti-CD3 with an irrelevant targeting arm (CD3-binding control) was used as a control. In vivo efficacy was evaluated in both xenogeneic and syngeneic mouse models. In the xenogeneic models, male NSG mice were engrafted with human PBMCs and the human prostate cancer cell lines C4-2 or 22Rv1 (sample size 5 mice per group). For the syngeneic models (sample size 5–10 mice per group), male HuT mice genetically engineered to express human PSMA and human CD3 were then bred to homozygosity. For the syngeneic models, the human extracellular portion of CD3 (human region of the CD3 genes (Cd3) with 5% FBS (VWR), 5% Nu Serum IV (VWR), 5 µg/mL of insulin (Gemini Bio-Products), 10 mmol/L dehydroepiandrosterone (Sigma-Aldrich), and 2 mmol/L L-glutamine (Irvine Scientific), 500 µg/mL of penicillin, 500 µg/mL streptomycin (Thermo Fisher Scientific), and 50 µg/mL G418 (Thermo Fisher Scientific).

Cells and cell lines

TRAMP-C2-hPSMA cells were generated by transducing human PSMA into TRAMP-C2 (ATCC) cells and passed in vivo. TRAMP-C2-hPSMA cells were maintained in DMEM high glucose (Irvine Scientific) with 5% FBS (VWR), 5% Nu Serum IV (VWR), 5 µg/mL of insulin (Gemini Bio-Products), 10 mmol/L dehydroepiandrosterone (Sigma-Aldrich), and 2 mmol/L L-glutamine (Irvine Scientific), 500 µg/mL of penicillin, 500 µg/mL streptomycin (Thermo Fisher Scientific), and 50 µg/mL G418 (Thermo Fisher Scientific).

C4-2 cells (ATCC) were maintained in DMEIT12 media (Irvine Scientific) with 10% FBS (VWR), 500 µg/mL of l-glutamine/penicillin/streptomycin (Thermo Fisher Scientific), 12.5 µg/mL Adenine (Sigma Aldrich), 13.65 µg/mL Triiodo Thyronine/T3 (Sigma Aldrich), 0.244 µg/mL d-Biotin (Sigma Aldrich), 4.4 µg/mL Apo-Transferrin (Sigma Aldrich), and 5 µg/mL Insulin (Gemini Bio-Products).

22Rv1 cells (ATCC) are maintained in RPMI (Irvine Scientific) supplemented with 10% FBS (VWR), 500 µg/mL of l-glutamine/penicillin/streptomycin (Thermo Fisher Scientific).

PC-3 cells (ATCC) are maintained in Kaighn’s Nutrient Mixture F12 (Irvine Scientific) supplemented with 10% FBS (VWR), 500 µg/mL of glutamine/penicillin/streptomycin (Thermo Fisher Scientific).

Flow cytometry of PSMAxCD3 binding

Flow cytometric analysis was utilized to determine binding of PSMAxCD3 or parental antibodies to preactivated human T cells, followed by detection with a PE-anti-human IgG antibody. Human T cells were preactivated with anti-CD3/CD28 for 6 days. Post activation, activated human T cells were incubated for 30 minutes at 4°C with 10 µg/mL of antibody. After incubation, the cells were washed twice with cold PBS (1% FBS) then PE-anti-human secondary antibody was added to the cells and incubated for an additional 30 minutes. CD3-binding antibody, no antibody, or secondary only staining were included as a controls. After incubation, cells were analyzed by flow cytometry on a BD FACS Canto II. For PSMA binding to tumor cells, C4-2, 22Rv1, and PC-3 cell lines were tested for Mycoplasma by real-time PCR assay in 2013, 2019, 2015, 2018, and 2012 respectively, and authenticated by short tandem repeat profiling (IDEXX BioResearch). All experiments were conducted with low-passage cell cultures (< passage 5). PBMCs were obtained from ReachBio (WA) for in vivo studies or leukopaks from the New York Blood Center for in vitro studies.
Flow cytometry–based cytotoxicity and activation assay using naïve human effector cells

22Rv1, C4-2, or PC-3 cells were labeled with 1 μmol/L of Violet Cell Tracker and plated overnight at 37°C. Human PBMCs were plated in supplemented RPMI media at 1 × 10^6 cells/mL and incubated overnight at 37°C. Target cells were coincubated with adherent cell-depleted naïve PBMC (effector/target cell 4:1) and serial dilutions of either PSMAxCD3 or the CD3-binding control for 48 hours at 37°C. Cells were analyzed by flow cytometry. Cells were stained with a dead/live far red cell tracker (Invitrogen). For the assessment of specificity of killing, cells were gated on Violet cell tracker labeled populations. Percentage of live target cells was reported as follows: adjusted killing, cells were gated on Violet cell tracker labeled populations. naïve human effector cells were injected with 5 × 10^5 ID8-VEGF/huMUC16 tumor cells were injected intraperitoneally. Mice were dosed with 1 mg/kg of MUC16xCD3 twice a week for a total of three doses. These mice were weighed twice weekly and checked for the clinical sign of swollen bellies indicative of ascites formation. Following institutional guidelines, mice were euthanized when ascites has developed and mice weighed >20% of starting weight (22). For studies with combination studies with anti-mouse 4-1BB (LOB12.3; BioXcell), CD3-binding control groups were treated with a Rat-IgG isotype control (BE0088; BioXcell) and anti-mouse 4-1BB groups were treated with CD3-binding control. For the tumor memory studies, mice that cleared the tumor in response to treatment were rechallenged with 1 × 10^6 TRAMP-C2-hPSMA or TRAMP-C2-WT cells at least 30 days post tumor injection on the opposite flank.

Small-animal PET

A precalibrated Sofie Biosciences G8 PET/CT instrument (Sofie Biosciences and PerkinElmer) was used to acquire PET and CT images. The energy window ranged from 150 to 650 keV with a reconstructed resolution of 1.4 mm at the center of the field of view. On day 6 after dosing, mice were anesthetized using isoflurane and kept under continuous flow of isoflurane during a 10-minute static PET acquisition followed by CT acquisition. Decay-corrected PET data and CT data were processed using VivoQuant software (inviCRO Imaging Services) into false-colored coregistered PET-CT maximum intensity projections on a color scale calibrated to indicate a signal range of 0% to 15% of injected dose per volume, expressed as %ID/g. For ex vivo biodistribution analysis, mice were euthanized following PET/CT acquisition. Blood, normal tissues, and tumor were then harvested and placed into counting tubes. The γ-emission radioactivity for all samples were counted on an automatic γ counter (AMG, Hiden) and results reported in normalized counts per minute (cpm). The %ID for each sample was the determined using samples counts relative to dose-standards counts prepared from the original injected material. Subsequently, the individual %ID/g values were derived by dividing the %ID value by the respective weight of the appropriate blood, tissues, or tumor sample.

Xenogeneic tumor model

Xenogeneic studies were carried out in 20 to 24 weeks old male NSG (NOD SCID γ-chain knockout) mice (Jackson Laboratory). 22Rv1 tumor cells were subcutaneously injected with 5 × 10^6 tumor cells and 1.66 × 10^6 human PBMCs (ReachBio). C4-2 tumor cells were subcutaneously injected with 5 × 10^6 tumor cells and 1 × 10^6 human PBMCs (ReachBio). Mice were treated on day 0 with PSMAxCD3 or CD3-binding control twice a week for a total of three doses to examine antitumor efficacy. Tumor growth was measured using calipers. Tumor volume based on caliper measurements were calculated by the formula: volume = (length × width)^2/2. For the MUC16 model, 5 × 10^6 ID8-VEGF/huMUC16 tumor cells were injected intraperitoneally. Mice were dosed with 1 mg/kg of MUC16xCD3 twice a week for a total of three doses. These mice were weighed twice weekly and checked for the clinical sign of swollen bellies indicative of ascites formation. Following institutional guidelines, mice were euthanized when ascites has developed and mice weighed >20% of starting weight (22). For studies with combination studies with anti-mouse 4-1BB (LOB12.3; BioXcell), CD3-binding control groups were treated with a Rat-IgG isotype control (BE0088; BioXcell) and anti-mouse 4-1BB groups were treated with CD3-binding control. For the tumor memory studies, mice that cleared the tumor in response to treatment were rechallenged with 1 × 10^6 TRAMP-C2-hPSMA or TRAMP-C2-WT cells at least 30 days post tumor injection on the opposite flank.

Determination of antibody-binding capacity on PSMA-expressing cells

The antibody binding capacity (ABC) of PSMAxCD3 on endogenous cell lines expressing various levels of PSMA was assessed by FACS using a Quantum Simply Cellular antihuman IgG Kit and following the manufacturer’s instructions (Bangs Laboratories). Ten μg/mL of Alexa Fluor-488-labeled-PSMAxCD3 was added to the cells and incubated for 30 minutes at 4°C. Ten μg/mL of Alexa Fluor-488-labeled-PSMAxCD3 was added to the cells and incubated for 30 minutes at 4°C. The same concentration of PSMAxCD3-Alexa Fluor-488 was added to the provided anti-human standard beads, and incubated 30 minutes at 4°C. After incubation, cells and beads were washed, re-suspended in 200 μL cold PBS containing 1% filtered PBS and analyzed by flow cytometry on a BD FACS Canto II. The ABC of PSMAxCD3 for each cell line was interpolated from the standards’ maximum fluorescence Intensity.

Antibodies for flow cytometry

To detect T cells, we used a combination of antibodies to CD45 (30-F11; Biologend), CD90.2 (30-H12; Biologend), CD8 (53-6.7; BD Pharmigen), CD4 (GK1.5; BD Pharmigen), and FOXP3 (FJK-165; E Biosciences). T cells were identified as CD45^, CD90.2^, CD8^, CD4^, or CD4^ FOXP3^+. T-cell activation was examined using antibodies to Granzyme B (GB11; BD Pharmigen), K67 (16A8; Biologend), and 4-1BB (IAH2; BD Pharmigen). Staining was carried out using the Ebioscience FOXP3 staining buffer set. Tumor cells were identified by CD45^, PSMA^+ (LNI-17; Biologend). A mouse Mice TCR VB screening panel (BD Pharmigen) was used to determine VB usage. Samples were analyzed on a BD LSRFortessa (BD Biosciences).

Measuring total hIgG concentrations for pharmacokinetic study

Total antibody concentrations of PSMAxCD3 were determined by an immunoassay specific for detecting human IgG using a sandwich ELISA. A goat anti-hFcy polyclonal antibody (Jackson ImmunoResearch Laboratory) was passively adsorbed to a microtiter plate overnight at 4°C followed by a nonspecific binding block. Serum samples were allowed to bind to plates for 1 hour at room temperature. After washing, plate captured human IgG antibodies were detected using a horseradish peroxidase (HRP)–conjugated goat anti-hFcy polyclonal antibody (Jackson ImmunoResearch) followed by addition of the chromogenic HRP-substrate, 3,3’,5,5’-tetramethylbenzidine (TMB; BD Pharmingen). TMB was used to detect HRP activity, and the resultant OD450 was read on a PerkinElmer Victor X4 Multimode Plate Reader.
Antibody conjugation for PET imaging

In preparation of $^{89}$Zr-radiolabeling and subsequent immuno-PET imaging studies, deferoxamine (DFO) conjugates of the PSMAxCD3, anti-PSMA, and anti-CD3-binding control antibodies were generated via an amine reactive, heterobifunctional chelator, p-isothiocyanatobenzyl-deferoxamine (p-SCN-Bn-DFO; Macroscopy). In short, each antibody was prepared to 10 mg/mL in 150 mMol/L NaCl 50 mMol/L carbonate, pH 9.0 buffer, followed by dropwise addition and gentle agitation of p-SCN-Bn-DFO to NEAT DMSO (Sigma-Aldrich) to a final reaction ratio of four-fold excess over antibody (mol/mol) in 2% DMSO (v/v). The reaction proceeded at 37°C for 0.5 hours, quiescent. The reaction mixture was buffered exchanged by a PD-10 column (GE Healthcare) into 10 mMol/L L listidine, pH 5.5 (Sigma-Aldrich), as per manufacturer’s instructions. The elution, the DFO-antibody conjugate, was sterile filtered and stored frozen for future use. The monomeric protein purity for all DFO-antibody conjugates was ≥98% by size-exclusion high performance liquid chromatography (SE-HPLC) monitoring the absorption at 280 nm and was corroborated SDS-PAGE. The DFO-to-antibody ratios were determined to be 1.6, 1.8, and 1.4-to-1 for PSMAxCD3, anti-PSMA, and anti-CD3-binding control, respectively, by UV absorption spectroscopy and using the extinction coefficients of the DFO moiety.

DFO-antibody conjugate radiolabeling

To prepare DFO-antibody conjugates for radiolabeling, 250 μg of each was brought to a total volume of 200 μL with 1 M HEPES, pH 7.4 (Teknova). In a separate vial, 3 μCi $^{89}$Zr-oxalic acid (3D Imaging) was buffered to pH 7.4 with approximately 1000 μL of 1 M HEPES, pH 7.4. The DFO-antibody conjugate and buffered $^{89}$Zr solutions were combined, gently mixed by pipet and incubated at room temperature for 45 minutes, quiescent. The reaction mixture was promptly buffer exchanged by a PD-10 column, preconditioned with 250 mMol/L NaAcN, pH 5.5 (Sigma-Aldrich), as per manufacturer’s instructions. The elution, the $^{89}$Zr-antibody, was sterile filtered and assayed for radiochemical purity by SE-HPLC with inline gamma emission detector. All $^{89}$Zr-antibodies had a purity ≥95%, with ≤1.1% unincorporated $^{89}$Zr present. The specific activity for $^{89}$Zr-antibodies ranged between 12.7 and 15.8 μCi/μg and was determined via the protein concentration (measured by UV absorption spectroscopy) and a dose calibrator (Capinctect) set for $^{89}$Zr. The target specific binding, that is, immuno-reactivity (IR), of the $^{89}$Zr-antibodies was measured by a cell-based assay and was ≥80% for target antibodies and ≤5% for the non-target antibody.

IR assay

Each $^{89}$Zr-antibody required two 500 μL aliquots of hPSMA-transfected HEK293 cells (generated in house) at 5 × 10⁶ cells/mL. The $^{89}$Zr-antibody (20 ng) was added to an aliquot and allowed to incubate at 37°C, 5% CO₂ for 45 minutes. Cells were centrifuged at 1,500 rpm for 5 minutes and the supernatant was collected. The second 500 μL aliquot of cells was pelleted and the supernatant of the first pellet was introduced, followed by incubation and separation as above. Each cell pellet was washed twice with 1 mL fresh cell culture media, centrifuging at 1,500 rpm for 5 minutes between each wash. Wash supernatants were collected. The final activities for all components (each cell pellet resuspended in 500 μL of cell culture media, reaction supernatants, and wash supernatants) were measured with a gamma counter (Hidex). IR was determined by sum of both pellets’ activity divided by sum of the activity of all components, times 100%.

Tissue preparation

Tumors were mechanically disrupted and digested for 9 minutes at 42°C in the presence of collagenase II (175 units/mL; Worthington), collagenase IV (200 units/mL; Gibco), and DNase I (400 units/mL; Sigma). The digested material was then passed through a cell strainer (BD Biosciences). Spleens were directly passed through a cell strainer (BD Biosciences).

In vitro and in vivo measurement of serum cytokine concentrations

Supernatant of in vitro assays or sera from in vivo studies were analyzed using V-plex Human ProInflammatory-10 Plex Kit or the Mouse V-plex ProInflammatory-10 Plex Kit following the manufacturer’s instructions (Meso Scale Diagnostics).

Histology on mouse tissues

Tissues were fixed in 10% neutral buffered formalin for 36 hours before replacing with 70% ethanol, embedded, and sectioned for IHC staining. Five-micron-thick paraffin sections of tissues or tumors were stained with anti-PSMA (ERP6253; Abcam), anti-CD3 (A045229; DAKO), anti-CD4 (Ab183685; Abcam), anti-CD8 (AS151; eBiosciences) or anti-FOX3 (12653; Cell Signaling Technology) on the Ventana Discovery XT (Ventana) Automated IHC staining system using the Ventana DAB Map Detection Kit. Slides were manually counterstained with hematoxylin, dehydrated, and coverslipped. Images were acquired on the Aperio AT 2 slide scanner (Leica Biosystems) and analyzed using Indica HALO software (Indica Labs). Tissue embedding and H&E stains were performed by Histoserv, Inc.

Statistical analysis

Data are presented as means ± SD and means ± SEM as stated in the figure legends. Statistically significant differences were tested using specific tests in Prism (Graphpad Software, Inc.) as indicated in the figure legends. P < 0.05 was considered statistically significant.

Results

PSMAxCD3 induces target-dependent T-cell activation and tumor cell cytotoxicity in vitro

PSMAxCD3 was generated by immunizing VelocImmune mice separately for human PSMA and CD3 antibodies, and combining the resultant antibodies into a bispecific format (23, 24). The resulting PSMAxCD3 bispecific antibody is a hinge-stabilized, effector minimized, IgG4 isotype (25). Flow cytometry analysis demonstrated that PSMAxCD3 specifically bound to PSMA on human CD4⁺ and CD8⁺ T cells (Fig. 1A). Furthermore, PSMAxCD3 specifically bound to 22Rv1 and C4-2, human prostate tumor cell lines that endogenously express PSMA, but not to PC-3 that lack PSMA. The ABC for 22Rv1 and C4-2 was 12.200 and 283.200, respectively, indicating that PSMAxCD3 can specifically bind to both low and high antigen expressing cell lines (Fig. 1B). Binding of PSMAxCD3 was specific to cells expressing human PSMA (Fig. 1C).

To evaluate the cytotoxic potential of PSMAxCD3, an in vitro flow cytometry-based cell-killing assay was performed. PSMAxCD3 induced the killing of 22Rv1 (EC₅₀ = 1.23 × 10⁻¹¹) and C4-2 (EC₅₀ = 9.6 × 10⁻¹⁵) cells, whereas the CD3-binding control did not, showing that cross-linking via the tumor targeting arm was required for the killing (Fig. 1D). In response to PSMAxCD3, the early activation marker CD69, late activation marker CD25, and the inhibitory receptor PD-1 were elevated on T cells when incubated with 22Rv1 and C4-2 cells. Neither tumor cell lysis nor T-cell activation markers were observed when assays were performed with PC-3 cells demonstrating PSMA-dependent activity (Fig. 1D; Supplementary Fig. S1A). PSMAxCD3 also induced cytokine release (IFNγ and
when T cells were incubated with PSMA expressing C4-2 or 22Rv1 tumor cells (Supplementary Fig. S1B). These results demonstrated that PSMAxCD3 is able to induce target dependent, CD3-mediated T-cell activation resulting in killing of PSMA expressing tumor cells in vitro.

**PSMAxCD3 inhibits growth of human prostate tumor xenografts in mice**

The promising in vitro data led us to investigate the activity of PSMAxCD3 in two subcutaneous tumor xenograft mouse models using 22Rv1 and C4-2 cells. Human PBMCs were delivered as a source of human T cells at the time of tumor implantation and mice were treated immediately with CD3-binding control or PSMAxCD3 antibodies. Compared with the CD3-binding control, mice treated with 0.1 or 1 mg/kg of PSMAxCD3 resulted in a dose-dependent tumor growth inhibition (Fig. 1E). Mice implanted with C4-2 tumor cells showed significant tumor growth inhibition with as low as 0.01 mg/kg of PSMAxCD3 (Fig. 1F). These studies demonstrate that low doses of PSMAxCD3 can control the growth of both low and high PSMA-expressing human prostate tumor xenografts in vivo.

**Immuno-PET imaging demonstrates in vivo biodistribution of PSMAxCD3 in HuT mice**

Xenogeneic models use immunodeficient mice which lack mature B, T, and natural killer (NK) cells. To examine PSMAxCD3 efficacy in a model with endogenous immune cells and expressing human PSMA, we genetically engineered immunocompetent HuT mice expressing human PSMA and CD3. In these mice, the mouse genomic loci were

---

**Figure 1.**

PSMAxCD3 induces target-dependent tumor cell cytotoxicity in vitro and in vivo. **A,** Specific binding to CD3 on human CD4⁺ and CD8⁺ T cells by flow cytometry. **B,** Specific binding to human PSMA on human prostate cancer cell lines 22Rv1, C4-2, and PC-3 by flow cytometry. **C,** Specific binding to human PSMA on HEK 293, PC-3, B16F10, and TRAMP-C2 cell lines without transfection or after transfection with human PSMA. **D,** Specific cell killing assay of 22Rv1, C4-2, or PC-3 tumor cells incubated with serial dilutions of PSMAxCD3 or CD3-binding control in the presence of human PBMCs. Mean values are shown as SEM (duplicate wells, three replicates). **E,** NSG mice were coimplanted with 22Rv1 cells and human PBMCs subcutaneously. Mice were dosed on days 0, 3, and 7 (arrows) with 0.1 or 1 mg/kg of PSMAxCD3 or 1 mg/kg of CD3-binding control. **F,** NSG mice were coimplanted with C4-2 cells and human PBMCs subcutaneously. Mice were dosed on days 0, 3, and 7 (arrows) with 0.01 or 0.1 mg/kg of PSMAxCD3 or 0.1 mg/kg of CD3-binding control. Mean tumor volumes are shown as SEM (n = 5, three replicates; **,** P < 0.0001). Statistical significance is measured by two-way ANOVA compared with CD3-binding control.
deleted and replaced with the orthologous regions of human sequences. Similar to published reports of endogenous PSMA expression in mice, the knock-in human PSMA transcript was detected in spinal cord, brain, liver, kidney, testes, and salivary glands of these mice, whereas minimal expression was found in prostate (Fig. 2A; refs. 26, 27). In addition, PSMA protein expression was confirmed by IHC and showed a similar expression pattern in normal tissues of HuT mice (Supplementary Fig. S2A; ref. 28).

To determine the in vivo bioavailability of PSMA antigen and the distribution of PSMAxCD3 in HuT mice, we used immuno-PET imaging. Mice were injected with 89Zr-anti-PSMA (the bivalent antibody used to generate PSMAxCD3), 89Zr-PSMAxCD3 bispecific antibody, or 89Zr-CD3-bispecific binding control (Fig. 2B). Mice injected with bivalent 89Zr-anti-PSMA showed specific uptake in the liver, kidneys, epididymis, lacrimal glands, and salivary glands, whereas there was no specific targeting in the same tissues of the mice that...
were injected with \(^{89}\)Zr-CD3-binding bispecific control since uptake was below that found for blood (Fig. 2C and D). Although the brain and testes were identified as PSMA expressing tissues in our studies, mice injected with \(^{89}\)Zr-anti-PSMA showed no targeting to these tissues, possibly due to the blood–brain barrier that precludes antigen access (Fig. 2B). Mice injected with \(^{89}\)Zr-PSMAxCD3 bispecific showed a similar distribution profile as the bivalent \(^{89}\)Zr-anti-PSMA, except for reduced uptake in the kidney and increased uptake in the spleen (Fig. 2C). Some difference in uptake is expected given that \(^{89}\)Zr-anti-PSMA has higher PSMA binding avidity compared with \(^{89}\)Zr-PSMAxCD3, which can drive greater uptake in some PSMA-expressing tissues. Uptake in the liver showed minor kinetic differences: the highest uptake of \(^{89}\)Zr-PSMA is observed on Day 1 whereas the highest uptake of \(^{89}\)Zr-PSMAxCD3 is observed on Day 4 after injection. As PSMAxCD3 has low affinity to CD3, it does not greatly redistribute to the lymphoid tissues. To further confirm that tissue distribution is mainly driven by the PSMA binding and not by CD3, we examined the clearance of PSMAxCD3 bispecific from the serum in mice humanized for either CD3 alone, or both CD3 and PSMA. Although serum drug concentrations of the HuT (CD3) mice were similar to WT mice, HuT (PSMA and CD3) mice showed faster drug clearance from the serum (Fig. 2E). Thus, PSMAxCD3 distribution in the HuT mice is driven mainly by the PSMA-binding arm, resulting in uptake in select PSMA-expressing tissues.

**HuT mice have normal T-cell development and respond to anti-human CD3 stimulation**

The genetic humanization of PSMA and CD3 in mice did not alter the thymic development of PSMA T cells, as determined by T-cell receptor (TCR) VB usage (Supplementary Fig. S2B; ref. 22). HuT mice also have similar total T-cell numbers and proportions of CD4\(^{+}\), CD8\(^{+}\), and regulatory T cells (Treg) compared with WT mice (Supplementary Fig. S2C). To assess whether PSMAxCD3 can induce systemic activation of T cells, HuT mice were injected with PBS, CD3-binding control bispecific, PSMAxCD3 bispecific, or a positive control of anti-human CD3 (OKT3). Transient reduction of T cells from the blood has been previously described after CD3 stimulation (29) and was observed after PSMAxCD3 or OKT3 administration in the HuT mice, indicating both OKT3 and PSMAxCD3 induced T-cell activation (Supplementary Fig. S2D). The transient T-cell reduction was accompanied by an increase in systemic cytokines (Supplementary Fig. S2E). Mice treated with CD3-binding control and PBS did not show these effects. Furthermore, T-cell reduction and cytokine increase were prevented if PSMA target availability was blocked by pre-dosing mice with anti-PSMA, demonstrating that activity of PSMAxCD3 is dependent on clustering through PSMA binding (Supplementary Fig. S2F and S2G).

**PSMAxCD3 is efficacious in HuT mice with low tumor burden**

HuT mice were implanted with a mouse prostate adenocarcinoma cell line expressing human PSMA (TRAMP-C2-hPSMA). PSMAxCD3 antibody treatment initiated on the day of tumor implantation prevented tumor growth compared with the mice that received the control CD3-binding antibody (Fig. 3A). When PSMAxCD3 treatment was given once tumors were approximately 50 mm\(^3\), PSMAxCD3 still induced a significant tumor growth delay compared with the CD3-binding control (Fig. 3B). Furthermore, mice that cleared the established tumor completely after PSMAxCD3 treatment remained tumor-free long term (Fig. 3C) and were able to reject a secondary tumor challenge, demonstrating that PSMAxCD3 antitumor activity can result in rejection of a secondary challenge with the same tumor (Fig. 3D). Despite the efficacy induced with these early treatment regimes, when treatment start was delayed until tumors were approximately 200 mm\(^3\), only a transient antitumor response was observed, and antitumor efficacy was significantly diminished (Fig. 3E). Flow cytometry confirmed that PSMA target expression was still maintained on TRAMP-C2-hPSMA tumors, indicating that the lack of efficacy was not due to the loss of target (Fig. 3F). Furthermore, a higher dose of PSMAxCD3 at 20 mg/kg was insufficient to control 200 mm\(^3\) tumors (Supplementary Fig. S3A), even when PSMA target expression was maintained (Supplementary Fig. S3B).

**PSMAxCD3 targets tumors independent of size, but efficacy is restricted to smaller tumors**

To investigate whether antitumor efficacy is determined by the total tumor burden or by the local tumor environment, a bilateral tumor model was established with each mouse harboring a small ( \(<50\) mm\(^3\) ) and large tumors ( \(<150\) mm\(^3\) ) on opposite flanks (Fig. 4A). PSMAxCD3 was still able to delay tumor progression of the smaller tumors in mice containing a larger tumor on the opposite flank (Fig. 4B) but had limited effect on the larger tumor in the same animal (Fig. 4C). These findings show that PSMAxCD3 efficacy is not determined by the total tumor burden or systemic T-cell dysfunction but by tumor-intrinsic factors. To determine whether PSMAxCD3 can penetrate larger tumors as well as smaller tumors, we utilized PET imaging in the double tumor model. Once the tumors on opposite flanks were at 50 and 150 mm\(^3\) (Fig. 4D), \(^{89}\)Zr-PSMAxCD3 or \(^{89}\)Zr-CD3-binding control antibodies were injected into HuT mice bearing small and large tumors on opposite flanks (Fig. 4E). Mice showed specific uptake of \(^{89}\)Zr-PSMAxCD3 in peripheral PSMA expressing tissues and tumors (Fig. 4F). In contrast, mice treated with the \(^{89}\)Zr-CD3-binding control showed no specific uptake in tissues. Any localization to the tumor region could be explained by vascularization of the tumor and not specific targeting given that the amount of uptake is below the activity in the blood (Fig. 4G and F). Furthermore, ex vivo biodistribution analysis confirmed that PSMAxCD3 similarly targets small and large tumors (Fig. 4G), therefore the lack of antitumor response is not due to the inability of PSMAxCD3 to target to larger tumors.

**PSMAxCD3 induces T-cell infiltration and activation in both small and large tumors**

To assess the density and spatial distribution of intratumoral T cells, we analyzed tumors by IHC. Both small and large tumors were infiltrated with CD8\(^{+}\) and CD4\(^{+}\) T cells (Fig. 5A). Tumors were then examined 3 days after treating with PSMAxCD3 or CD3-binding control. PSMAxCD3 induced no significant effect on the density of CD4\(^{+}\) T cells (Fig. 5B). In contrast, PSMAxCD3 treatment resulted in increased CD8\(^{+}\) T-cell density in both 50 and 200 mm\(^3\) tumors (Fig. 5C). In addition, FOXP3\(^{+}\) Treg cell density were similar across all groups (Fig. 5D). Because T cells were present in similar densities in the small and large tumors, we used RNAseq to determine if T-cell activation markers were increased similarly after PSMAxCD3 treatment. PSMAxCD3 induced the expression of activation markers (IFN\(\gamma\), TNF\(\alpha\), granzymes, and perforin) and inhibitory receptors (PD-1, LAG-3, Tim-3, and CD160) in both small and large tumors (Supplementary Fig. S4A).

To examine T-cell activation more thoroughly, flow cytometry was carried out on intratumoral CD8\(^{+}\) and CD4\(^{+}\) T cells from both small and large tumors. CD4\(^{+}\) and CD8\(^{+}\) T cells upregulated the cytolytic marker granzyeme B and proliferation marker Ki67 after PSMAxCD3 treatment (Fig. 5E and F). Cytokine such as IFN\(\gamma\), IL2, and TNF\(\alpha\) were increased in tumor-bearing HuT mice at 4 hours posttreatment with PSMAxCD3 and returned to baseline concentrations by 72 hours.
indicating a strong but transient T-cell response (Fig. 5G). Although there is a systemic T-cell response and activation of intratumoral T cells in both small and large tumor bearing mice, only mice bearing the small tumors showed a significant reduction in tumor size by 48 hours after dosing (Fig. 5H), suggesting that the initial response appears to be sufficient to eliminate small tumors, however it may not be sufficient to overcome rapidly proliferating larger tumors. Because we demonstrated that T cells are still functionally active, providing additional costimulation to increase the existing T-cell response might result in an antitumor response to larger tumors.

**PSMAxCD3 treatment with 4-1BB costimulation is efficacious against larger tumors**

Because 4-1BB is an activation-induced marker for tumor-reactive T cells (30), we examined 4-1BB expression on tumor infiltrating T cells of the large tumor after PSMAxCD3 treatment. Similar to the CD8⁺ T cells of the small tumor (Supplementary Fig. 4B), PSMAxCD3 induced activation-dependent 4-1BB surface expression on intratumoral CD4⁺ (Fig. 6A) and CD8⁺ (Fig. 6B) T cells, but not on splenic T cells in mice. We determined whether costimulation of the 4-1BB pathway could increase tumor specific T-cell activity and antitumor efficacy against larger tumors. Both PSMAxCD3 and anti-murine 4-1BB treatment as single agents showed some delay in tumor growth but little tumor clearance. In contrast, a single treatment of PSMAxCD3 with anti-4-1BB resulted in significant antitumor efficacy (Fig. 6C; Supplementary Fig. 5A) and complete clearance of over 50% of the tumors by day 60 (Fig. 6D). Mice that received the higher dose of PSMAxCD3 in combination with anti-4-1BB experienced transient weight loss. This transient weight loss was not as severe in mice that received a lower dose of PSMAxCD3 with anti-4-1BB even though efficacy was maintained (Fig. 6D; Supplementary Fig. S5B). To confirm that 4-1BB signaling occurred in the mice treated with anti-4-1BB, we examined whether the downstream signaling pathways of 4-1BB were elevated. Tumors from mice treated with PSMAxCD3 in combination with anti-4-1BB showed elevated mRNA expression of TRAF1 adaptor protein, which is essential to 4-1BB-induced activation pathways as well as upregulation of survival genes Bcl2, Bcl-XL (Bcl2l1), and Bfl-1 (Bcl2a1a; Fig. 6E; ref. 30).

**PSMAxCD3 and 4-1BB costimulation increases expansion and activation of CD8⁺ T cells**

Mice treated with PSMAxCD3 in combination with anti-4-1BB showed enhanced and sustained cytokine induction up to 96 hours after treatment, whereas cytokine concentrations returned to baseline in mice treated with PSMAxCD3 alone (Fig. 7A). At 48 hours after dosing, PSMAxCD3 alone or in combination with anti-4-1BB...
increased the percentage of granzyme B and Ki67-expressing CD8\(^+\) (Fig. 7B) and CD4\(^+\) (Supplementary Fig. S6A) T cells in the large tumor. By 96 hours, an increase in activated T-cell numbers was restricted to the group treated with the combination of PSMAxCD3 with 4-1BB costimulation and this effect was stronger in the CD8\(^+\) T cells (Fig. 7C) than in the CD4\(^+\) T cells (Supplementary Fig. S6B). These effects were due to an increase in percentage of activated T cells as well as an increase in total CD8\(^+\) T-cell numbers (Fig. 7D–F). Given that the role of 4-1BB has also been debated on regulatory T cells, tumors were also examined total numbers of tumor infiltrating FOXP3\(^+\) CD4\(^+\) T cells. Although total numbers of Treg cells were similar across treatment groups, the CD8\(^+\)-to-Treg ratio was significantly increased due to the expansion of CD8\(^+\) T cells of mice that received the combination treatment (Fig. 7G).

To determine whether the mice that had cleared the tumors after combination treatment can generate a protective memory response, we re-challenged these mice with either the same tumor line (TRAMP-C2-PSMA) or the original TRAMP-C2 tumor cells lacking human PSMA expression. Mice were protected from both tumors indicating the existence of a PSMA-independent immunologic memory (Fig. 7H and I). These data demonstrate that PSMAxCD3 induces activation, cytokine production, and proliferation of T cells immediately following treatment, however, combination with anti-mouse 4-1BB can prolong and enhance these effects to achieve antitumor efficacy against larger tumors.

To determine if this combinatorial effect with anti-mouse 4-1BB was unique to PSMAxCD3 and/or this model or whether it could be translated across multiple different cancer indications, we examined...
the combinatorial effect of anti-41BB with a CD3 bispecific targeting MUC16 (an antigen upregulated in ovarian cancer as well as other cancer types) in a murine ovarian tumor model (22). The addition of anti-mouse 4-1BB significantly improved the survival of mice compared with those that received MUC16xCD3 alone. These mice experienced no body weight loss (Supplementary Fig. S7A and S7B). This demonstrates that 4-1BB can combine with several CD3 bispecific antibodies targeting different antigens and this combination could be beneficial across various cancer indications.

Discussion

Clinical trials with CD3 bispecific antibodies against solid tumors are still in the early stages and it remains to be seen if CD3 bispecific antibodies will show as much benefit here. We illustrate that a PSMAxCD3 bispecific induces antitumor efficacy against smaller tumors but is less efficient at combating larger tumors. Reduced efficacy with higher tumor burden has previously been demonstrated with checkpoint blockade therapies in humans: anti-PD-1-induced immunologic responses are less effective if the starting tumor burden is high (31, 32). These clinical lessons suggest that a combinatorial approach may be required to eradicate larger solid tumor lesions. We showed that the size of the individual tumor can impact therapeutic efficacy, suggesting that smaller tumor lesions may be easier to clear than larger lesions in the same patient.

Our results demonstrated that PSMAxCD3 induced intratumoral CD8⁺ T-cell infiltration, activation, and proliferation in both the small and large tumors but the addition of 4-1BB signaling was required to achieve efficacy against larger tumors. Alternative approaches that use CAR-T cells have demonstrated the benefit of including costimulatory signaling to enhance their activity (33). The 4-1BB pathway enhances the magnitude and duration of T-cell responses by promoting cell

Figure 5.
PSMAxCD3 induces T-cell infiltration and activation in both small and larger tumors. A, IHC analysis and quantification of positively stained CD3, CD4⁺, and CD8⁺ T cells in 50 and 200 mm² TRAMP-C2-hPSMA tumor sections, 40× (n = 5; two replicates). Tumor-bearing HuT mice were treated with 5 mg/kg of CD3-binding control or PSMAxCD3, and T cells were examined by IHC at 72 hours after dose. B, Representative images (40×) of CD4 stained tumor sections and quantification of positively stained cells. C, Representative images (40×) of CD8 stained tumor sections and quantification of positively stained cells. Statistical significance measured by one-way ANOVA compared with CD3-binding controls (***, P < 0.0001; ***, P < 0.01; studies conducted n = 5, two replicates). D, Representative images (40×) of FOXP3 stained tumor sections and quantification of positively stained cells. Tumor-infiltrating T cells were examined for activation and proliferation markers 48 hours after treatment. E, Representative flow plots and percentages of granzyme B⁺ CD8⁺ and CD4⁺ T cells (**, P < 0.01; studies conducted n = 5–15). F, Representative flow plots and percentages of Ki67⁺ CD8⁺ and CD4⁺ T cells. Statistical significance is measured by Mann-Whitney test compared with CD3-binding control (***, P < 0.0001; studies conducted n = 5–15; multiple 50 mm² tumors under 0.1 g were combined, two replicates). G, Serum cytokine concentrations were measured by MSD at 4 and 72 hours after dosing. H, Individual tumor weights at 48 hours after treatment. Statistical significance is measured by Mann-Whitney test compared with CD3-binding control (***, P < 0.0001). NS, not significant.
survival, limiting treatment-induced antigen-induced cell death (AICD), reversing anergy, and subsequently generating memory T cells to promote antitumor activity (34, 35). Activated CD4+ and CD8+ T cells can upregulate many costimulatory molecules but 4-1BB is upregulated primarily on CD8+ T cells, indicating that 4-1BB may have a role in expanding cytotoxic CD8+ T cells (36, 37). Indeed, we observed greater antitumor efficacy and intratumoral CD8+ T-cell activation in mice that received PSMAxCD3 with 4-1BB costimulation.

In addition to T cells, 4-1BB is also expressed on NK and Treg cells (30). The role of 4-1BB on Treg cells remains unclear, and we did not observe any effects on the numbers of Treg cells (37, 38). Stimulation of activation-induced 4-1BB on NK cells can increase their cytolytic activity, providing a secondary mechanism to boost antitumor efficacy. Therefore, although combining PSMAxCD3 with anti-4-1BB increased T-cell function and numbers in our studies, anti-4-1BB may play additional roles on NK cells or Treg cells, recruiting additional immune cells against the tumor.

One challenge of targeting solid tumor malignances is that target expression is rarely tumor-cell restricted. Nontumor expression of antigen may cause “on-target off-tumor” activity that can limit treatment safety. Our studies demonstrated that even in the presence of PSMA-expressing normal tissues, PSMAxCD3 could target to the PSMA-expressing tumor. This targeting resulted in antitumor efficacy with no evident toxicity. However, weight loss occurred when high doses of PSMAxCD3 were given in combination with anti-mouse 4-1BB, suggesting that care must be taken to avoid limiting toxicities when combining these immunotherapeutic agents. Others have shown that high concentrations of anti-4-1BB monotherapy can result in polyclonal activation of CD8+ T cells and systemic cytokine release as well as splenomegaly and hepatomegaly in mice (39, 40). Whether other receptors from the TNFR superfamily such as OX40 may provide costimulation and efficacy with lower toxicities remains unknown. A possibility is to target the “Signal 2” to the tumor, potentially limiting systemic toxicity. One could target “Signal 1” with one antigen and “Signal 2” with a second antigen to increase T-cell killing to tumor cells. Combination with checkpoint blockade may also be beneficial. Indeed, our work demonstrated that PD-1 blockade could enhance the efficacy of a MUC16xCD3 bispecific antibody in pre-clinical models, supporting testing of this combination in the clinic (22).

Our studies illustrate the ability of PSMAxCD3 bispecific antibody to activate intratumoral T cells and highlight how 4-1BB

Figure 6. PSMAxCD3 treatment combined with 4-1BB costimulation is efficacious against larger tumors. A, Representative flow plots and MFI of 4-1BB expression on splenic and tumor CD4+ T cells in HuT mice (n = 5) with large 200 mm3 tumors treated with 5 mg/kg of CD3-binding control or PSMAxCD3 48 hours after dose. B, Representative flow plots and MFI of 4-1BB expression on splenic and tumor CD8+ T cells in HuT mice (n = 5) with large 200 mm3 tumors treated with 5 mg/kg of CD3-binding control or PSMAxCD3 48 hours after dose. Established 200 mm3 TRAMP-C2-hPMSA tumors were treated once on day 9 (arrow) with 5 mg/kg CD3-binding control, 2.5 mg/kg anti-4-1BB, 1 mg/kg PSMAxCD3, 5 mg/kg PSMAxCD3, 1 mg/kg PSMA with 2.5 mg/kg anti-4-1BB, or 5 mg/kg PSMAxCD3 with 2.5 mg/kg anti-4-1BB. C, Mean tumor volume is shown as SEM (n = 10, 3 replicates). Average tumor growth curve is plotted until first mouse of each group was euthanized. D, Survival curve represents tumor-free mice. Significance is measured by Gehan-Breslow-Wilcoxon test compared with CD3-binding control (**, P < 0.0001). E, Relative expression of 4-1BB pathway genes in tumors 72 hours after dose was measured by TaqMan. Data are shown as SEM (n = 6; ****, P < 0.0001; ***, P < 0.001; **, P < 0.01; *, P < 0.05). Statistical significance measured by one-way ANOVA compared with CD3-binding control. MFI, mean fluorescence intensity; NS, not significant.
costimulation can enhance the magnitude and duration of the T-cell response, leading to antitumor efficacy against larger tumors. We provide a rationale for combining CD3 bispecific antibodies with 4-1BB costimulation as a strategy when treating solid tumors to achieve better overall antitumor activity.

Disclosure of Potential Conflicts of Interest
D. Chiu is a full-time employee at Regeneron Pharmaceuticals. R. Tavaré is a full-time employee at Regeneron Pharmaceuticals. L. Haber is a full-time employee at Regeneron Pharmaceuticals. K. Vazzana is a full-time employee at Regeneron Pharmaceuticals. P. Ram is a full-time employee at Regeneron Pharmaceuticals. M. Danton is a full-time employee at Regeneron Pharmaceuticals. J. Finney is a full-time employee at Regeneron Pharmaceuticals. J. Kirshner is a full-time employee at Regeneron Pharmaceuticals. A. Crawford is a full-time employee at Regeneron Pharmaceuticals. J. Finney, J.T. Giurleo, A. Crawford Disclosure of Potential Conflicts of Interest
D. Chiu is a full-time employee at Regeneron Pharmaceuticals. R. Tavaré is a full-time employee at Regeneron Pharmaceuticals. L. Haber is a full-time employee at Regeneron Pharmaceuticals. K. Vazzana is a full-time employee at Regeneron Pharmaceuticals. P. Ram is a full-time employee at Regeneron Pharmaceuticals. M. Danton is a full-time employee at Regeneron Pharmaceuticals. J. Finney is a full-time employee at Regeneron Pharmaceuticals. J. Kirshner is a full-time employee at Regeneron Pharmaceuticals. A. Crawford is a full-time employee at Regeneron Pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions
Conception and design: D. Chiu, L. Haber, G. Thurston, J.R. Kirshner, A. Crawford Development of methodology: D. Chiu, R. Tavaré, P. Krueger, D. Ma Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): D. Chiu, R. Tavaré, O.H. Aina, K. Vazzana, P. Ram, M. Danton, J. Finney, J.T. Giurleo, A. Crawford Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): D. Chiu, R. Tavaré, L. Haber, O.H. Aina, J. Finney, P. Krueger, D. Ma, A. Crawford Writing, review, and/or revision of the manuscript: D. Chiu, R. Tavaré, L. Haber, O.H. Aina, P. Krueger, E. Smith, G. Thurston, J.R. Kirshner, A. Crawford Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D. Chiu, S. Jalal Study supervision: L. Haber, E. Smith, A. Crawford

Acknowledgments
The authors thank all Regeneron employees who contributed to the generation and characterization of PSMAxCD3, including Ashok Badehe, Ashique Rafique, Drew Dudgen, Frank DelFino, Douglas MacDonald, William C. Olson, Jeannette Fairhurst, Tammy Huang, Joel Martin, John C. Lin, Chia Jen Siao, William Pousayimirou, Alejo Mujica, Harry Polites, Travis Gorenc, Andria Skinner, and Nicholas Papadopoulos.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received July 8, 2019, revised November 27, 2019; accepted March 10, 2020, published first March 17, 2020.
References


A PSMA-Targeting CD3 Bispecific Antibody Induces Antitumor Responses that Are Enhanced by 4-1BB Costimulation

Danica Chiu, Richard Tavaré, Lauric Haber, et al.


Access the most recent version of this article at:
doi:10.1158/2326-6066.CIR-19-0518

Access the most recent supplemental material at:
http://cancerimmunolres.aacrjournals.org/content/suppl/2020/03/17/2326-6066.CIR-19-0518.DC1

This article cites 40 articles, 17 of which you can access for free at:
http://cancerimmunolres.aacrjournals.org/content/8/5/596.full#ref-list-1

This article has been cited by 1 HighWire-hosted articles. Access the articles at:
http://cancerimmunolres.aacrjournals.org/content/8/5/596.full#related-urls

Sign up to receive free email-alerts related to this article or journal.

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

To request permission to re-use all or part of this article, use this link
http://cancerimmunolres.aacrjournals.org/content/8/5/596.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.