

Supplementary methods

Bispecific antibodies characterization and purification

The IgG BsAbs were purified from CHO supernatants using a protein A column with a PBS running buffer and a 0.1 M Glycine at pH 3.2 elution buffer using the AKTA Pure system (GE Healthcare). The eluants were neutralized using a 1 M Tris-HCl solution at pH 8.5. Eluants were passed through a 120 mL Superdex-200 column in PBS buffer and concentrated using VIVASPIND15 concentrators with at 10K cutoff. Protein concentrations were measured (UV280nm) using a NanoDrop.

Proteins were initially characterized by SDS-PAGE and analytical SEC with in-line static light scattering. Analytical SEC with in-line light scattering (SEC/LC) was performed by injecting 40-80 µg of each sample directly from affinity purification (prior to preparative SEC) onto a Phenomenex Yarra G3000 SEC analytical HPLC (7.7x300mm) column equilibrated in buffer containing 100mM Na-phosphate, 150mM NaCl, 0.02% NaN₃ at pH 6.8, using an Agilent 1100 HPLC system. Static light scattering data for material eluted from the SEC column were collected using a miniDAWN TREOS static light scattering detector coupled to an Optilab T-rEX in-line refractive index meter (Wyatt Technologies). UV data were analyzed using HPCHEM (Agilent). Molecular weights of the proteins were determined by their static light scattering profiles using the ASTRA V software (Wyatt Technologies). Endotoxin level was tested using EndoSafe PTS from Charles River Laboratories.

Pharmacokinetics of HER-2/CD3 BsAbs

Pharmacokinetic studies of the HER-2/CD3 IgG BsAbs, pertuzumab IgG1, and chimeric SP34 IgG1_N297Q were performed by single dose intravenous injection of 10 mg/kg per test article

into male Balb/c mice as described previously¹. ELISA serological quantification of the parental mAbs were performed by coating a High Bind U-well 96-well plate (Greiner Microolon) with 2 µg/ml antigen (hCD3 delta-Fc or hHer2-Fc) in 50 mM sodium carbonate, pH 9.3 overnight at 4 °C. The plates were washed with PBS+0.1%, Tween20 (PBST), blocked for 1 hour with BlockerTM Casein in PBS (Thermo Scientific) at room temperature (RT), and washed with PBST. Mouse plasma dilutions starting at 1:50 with 1:2 serial dilutions in 1% BSA in PBST were added for 1 hour at RT. The plates were washed with PBST and either a 1:1000 diluted Goat anti-human lambda-AP (Southern Biotech) for anti-CD3 IgG or Goat anti-human kappa-AP (Southern Biotech) for anti-HER-2 IgG1 in 1% BSA in PBST were added for 1 hour at RT. The detection reagent was washed off followed by the addition of PNPP Substrate (Thermo Scientific). Colorimetric readout measured the absorbance at 450 nm on a SpectraMax 190 plate reader. For the BsAbs, the hHER-2-Fc was coated at 2 µg/mL on the plate and bound BsAb was detected using a biotinylated hCD3 epsilon/delta-Fc to ensure bispecificity. The biotinylated detection antigen was then detected with Streptavidin-AP (Jackson ImmunoResearch).

Flow cytometry

Flow cytometric evaluation of cell surface proteins on T cells 48h after mixing SKOV3 cells and biologic test articles. The human T cells (ALLCELLS frozen normal human peripheral blood CD3+ Pan T cells, Cat# PB009-1F, Lot # A5715/Donor 1 and A5647/Donor 2) were co-cultured for 48 hrs in RPMI 1640 /10% FBS Corning/gentamicin (Gibco) at 37 °C, 5% CO₂ were removed from redirected lysis assay plates after exposure to SKOV3 tumor cells and various IgGs and IgG BsAbs (E:T 10:1). The BsAb and mAb triplicates were transferred into 96

well plate, spun at 170g for 7 minutes, aspirated, resuspended in PBS and pooled. Next the human T cells were counted. All subsequent steps were performed on ice. The cells were resuspended in Blocking buffer for 15 min, pelleted, washed 2x, and resuspended in Wash buffer before adding 50 μ L of the cells ($\sim 0.15 \times 10^6$ cells/well) to 96-well plates (Corning 3799.) Alexa-488 Mouse Anti-Human CD4 (BD Biosciences Cat# 557695) and Pacific Blue Mouse Anti-Human CD8 (BD Biosciences Cat#558207) were added to the human T cells. Labeled T cell activation markers: APC-Cy7 Mouse Anti-Human CD69 (BD Biosciences cat# 557756) and APC-Mouse Anti-Human CD25 (BD Biosciences Cat# 555434) were added to plate with Donor 1 and incubated for 45 minutes. Labeled checkpoint inhibitors: PerCP-eFluor 710 Mouse Anti-Human CD279 [PD-1] (Invitrogen cat# 46-2799-42), APC- Mouse Anti-Human CD152 [CTLA-4] (Biolegend Cat# 349908) and PE-Mouse anti-Human CD137 [4-1BB] (BD Biosciences Cat#561701) were added to plate with Donor 2 and incubated for 45 minutes. The cells were pelleted and washed 3x again. Finally, the cells were resuspended in Wash buffer with 1:1000 PI (Molecular Probes Cat#P3566) and covered with foil. The cells along with OneComp eBeads (eBioscience Cat# 01-1111-42) were then analyzed on a Becton Dickinson LSRFortessa flow cytometer acquiring with BD FACSDiva software and data were analyzed using FlowJo version 10.

Quantitation of HER-2 and PD-L1 receptors per cell by flow cytometry. The SKOV3 tumor cells (ATCC Cat#HTB-77) and OVCAR3 (ATCC Cat#HTB-161) in RPMI 1640 /10% FBS (Corning), gentamicin (Gibco) at 37 °C, 5% CO₂ were lifted from the flasks using Accutase (Innovative Technologies Cat#AT104). Cells were transferred to centrifuge tubes and pelleted by centrifugation at 170g for 7 minutes. Cells were washed once with PBS buffer. All subsequent steps were performed on ice. Henceforth, “Wash buffer” was PBS/2% FBS/0.05% NaN₃ /10% Normal Goat Serum with extra 10% FBS. “Blocking buffer” was Wash buffer supplemented with Human BD Fc Block (BD Biosciences

Cat#564220.) The cells were resuspended in Blocking buffer for 15 min, pelleted, washed 2x, and resuspended in Wash buffer before adding 50 μ L of the cells (0.5×10^6 cells/well) to 96-well plates (Corning 3799). The mAbs were added to the wells at 30 mg/ml and incubated for 45 minutes. The cells were pelleted, washed 2x, and the supernatants were aspirated again before adding R-Phycoerythrin-conjugated Goat anti-Human IgG (H+L) (Jackson Cat#109-116-088) in Wash buffer to tumor cells. Both cell lines were incubated with the secondary detection reagent for 45 minutes. The cells were pelleted and washed 2x again. Finally, the cells were resuspended in LIVE/DEAD stain PI (Molecular Probes Cat#P3566) to assess cell viability and covered with foil. Flow cytometry with the cells was then acquired on a Becton Dickinson LSRFortessa flow cytometer acquiring with BD FACSDiva software. The data was analyzed using FlowJo version 10. Quantibrite™ PE beads (BD Bioscience Cat# #340495) were used to determine number of cell surface HER-2 receptor/tumor cell according to the manufacturer's instructions.

PD-L1 molecules/tumor cell was determined after a 24-hour exposure to IgG BsAbs or control mAbs and human T cells in SKOV3 and OVCAR3 tumor cells using a similar method as described for HER-2 using an anti-PD-L1 (BioLegend clone 29E.2A3, Cat#329706) (**Supplementary Fig. S7**). The 24-hour supernatant cultures (with no T cells) were transferred to a 2nd plate of new SKOV3 and OVCAR3 tumor cells. 24 hours later, upregulation of PD-L1 expression on the tumor cells was determine from the 2nd 6-well plate (with cytokines from the original supernatant only cultures) by FACS Analysis.

ELISA Binding activity of in-house IgG4PAA versions of Nivolumab and Urelumab

Binding activity for in-house Nivolumab and Urelumab hIgG4PAA, was measured by Capture ELISAs. High Bind U-well 96-well plate (Greiner Microton) were coated overnight at 4 °C with 2 μ g/ml of Goat anti-Human kappa-unlabeled (Southern Biotech Cat#2060-01) in 50 mM sodium carbonate, pH 9.3. The plates were washed with PBST, blocked for 1 hour with

95 Blocker™ Casein in PBS [blocking buffer] (Thermo Scientific Cat#37528) at room
96 temperature (RT), and washed with PBST. Serial 1:3 serial dilutions of antibody (starting at
97 20 µg/ml) in blocking buffer were added and incubated 1 hour at RT. The plates were washed
98 with PBST. Both human extracellular domains of hPD-1 and h4-1BB were generated in-house
99 by standard molecular biology, transient transfection in CHO and purification using C-terminal
100 Histidine tags followed by a preparative SEC polish. hPD-1 ECD-His-biotin at 1 µg/ml in
101 blocking buffer was added to Nivolumab plate. h4-1BB ECD-biotin at 2 µg/ml in blocking
102 buffer was added to the Urelumab plate. The plates were washed with PBST and Streptavidin-
103 AP (Jackson ImmunoResearch Laboratories Cat#016-050-084) in blocking buffer was added for
104 1 hour at RT. The detection reagent was washed off followed by the addition of PNPP Substrate
105 (Thermo Scientific cat# 37621). Colorimetric readout was performed by reading the
106 absorbance at 405 nm on a SpectraMax 190 plate reader.

107

Supplementary Data

Supplementary Table S1. BsAb exposure data in humanized mice. Serum samples were taken 2 hrs after intravenous administration (5 mg/kg IgGs). Concentrations were determined using a CD3/HER-2 extracellular domain protein sandwich ELISA and purified test articles to generate standard curves. The determined concentrations matched what was expected based on the BsAb pharmacokinetic study in Balb-c mice (**Fig. 1D**).

Sample Source	Bispecific Concentration (µg/mL)
HER-2/CD3 IgG1AAQ BsAb	290±110
Pert+anti-CD3 IgGs†	0
Pertuzumab + SP34 IgGs†	0
Vehicle	0
HER-2/CD3 IgG1AAQ IgG BsAb†	105±8

†Serum collected 24 hours after an additional dose on day 30.

‡No signal observed (or expected) as bispecific binding is required to produce a signal within the ELISA.

Supplementary Table S2. IFN γ levels (pg/mL) in the serum of NSG-His mice on Day 2 after treatment with specific affinity modified BsAbs and at the end of the study (EOS). Statistical significance was not reached for any of the BsAb groups versus the vehicle primarily due to inconsistent high versus low cytokine values in the BsAb groups. It is clear, though, that certain groups had mice with high IFN γ either at Day 2 or at the EOS while the vehicle and the low affinity anti-CD3 BsAb group did not.

Test articles	Day 2	StdDev	p-value	EOS	StdDev	p-value
Vehicle	29	19	-	50	61	
SP34/Pertuz IgG BsAb (46nM/8nM)	239	276	0.07	38	24	0.65
SP34/Pertuz IgG BsAb (600nM/8nM)	26	22	0.75	25	20	0.36
SP34/Pertuz IgG BsAb (46nM/70nM)	39	35	0.52	192	304	0.27

127

128 **Supplementary Table S3.** Cytokine levels (pg/mL) in cell culture media 48 hours after mixing
 129 primary T cells and various test articles (0.1 nM) with SKOV3 cells (10:1 T cell:Tumor cell).

130 Each value is the mean of three separate reactions with independently mixed T cells, SKOV3

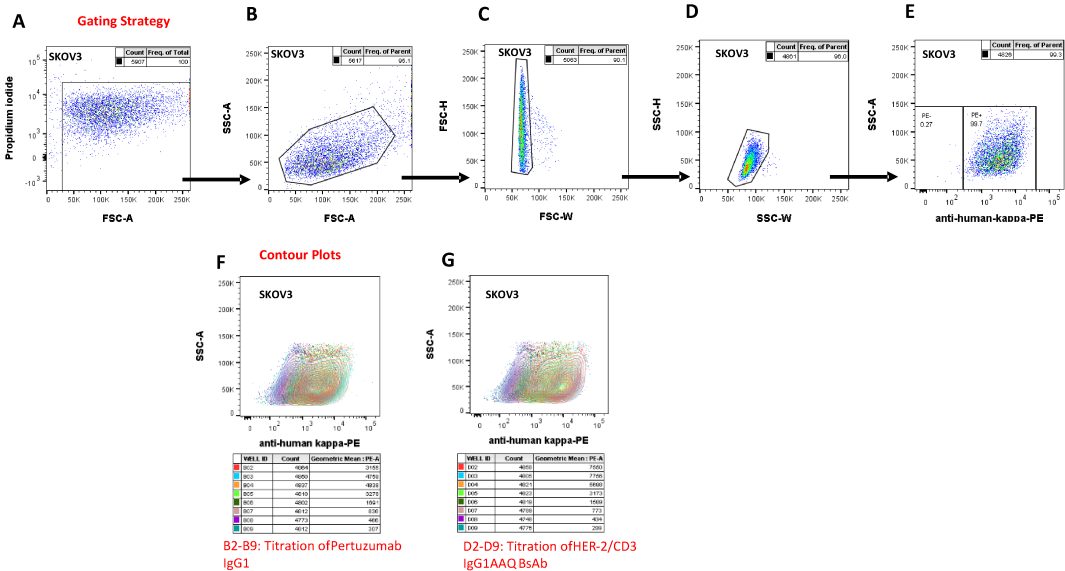
131 cells, and/or test article and the error is the standard deviation.

Test Article	IFN γ	IL-2	IL-6	TNF α	IL-8	IL-10	IL-13	IL-12	IL-1 β	IL-4
SKOV3 cells	0.3 \pm 0.1	<1	10 \pm 0.3	0.4 \pm 0.3	400 \pm 30	0.1 \pm 0.04	3 \pm 2	<0.1	<0.2	<0.1
SKOV3 cells + Tcells	19 \pm 6	8 \pm 5	10 \pm 1	0.8 \pm 0.3	280 \pm 20	0.1 \pm 0.05	3 \pm 0.4	<0.1	<0.2	<0.1
IgG1 control	0.1 nM	3 \pm 1	4 \pm 5	8 \pm 1	0.4 \pm 0.1	260 \pm 30	0.1 \pm 0.02	1 \pm 0.5	<0.1	<0.2
	0.01 nM	6 \pm 5	4 \pm 3	9 \pm 1	0.5 \pm 0.2	270 \pm 50	0.1 \pm 0.02	3 \pm 0.6	<0.1	<0.2
	0.001 nM	10 \pm 10	5 \pm 3	8 \pm 2	0.5 \pm 0.2	260 \pm 40	0.1 \pm 0.03	2 \pm 1	<0.1	<0.2
SP34 IgG1 N297Q	0.1 nM	68 \pm 9	11 \pm 6	28 \pm 2	6.7 \pm 0.4	500 \pm 40	0.9 \pm 0.2	2 \pm 1	<0.1	<0.2
	0.01 nM	13 \pm 2	8 \pm 7	11 \pm 1	0.9 \pm 0.4	280 \pm 20	0.2 \pm 0.03	3 \pm 0.4	<0.1	<0.2
	0.001 nM	12 \pm 8	5 \pm 4	9 \pm 2	0.6 \pm 0.4	280 \pm 50	0.1 \pm 0.02	3 \pm 0.6	<0.1	<0.2
Pertuzumab IgG1	0.1 nM	5 \pm 4	2 \pm 3	8 \pm 1	0.3 \pm 0.2	250 \pm 20	<0.1	1 \pm 1	<0.1	<0.2
	0.01 nM	7 \pm 6	0.7 \pm 0.2	8 \pm 1	0.4 \pm 0.1	240 \pm 20	0.14 \pm 0.03	3 \pm 0.6	<0.1	<0.2
	0.001 nM	11 \pm 7	8 \pm 2	10 \pm 2	0.8 \pm 0.2	270 \pm 10	<0.1	3 \pm 0.5	<0.1	<0.2
SP34+Pertuz IgG	0.1 nM	63 \pm 16	8 \pm 2	26 \pm 1	5.2 \pm 0.2	490 \pm 20	0.4 \pm 0.2	2 \pm 1	<0.1	<0.2
	0.01 nM	12 \pm 5	4 \pm 3	9 \pm 2	0.6 \pm 0.2	240 \pm 30	0.1 \pm 0.02	1 \pm 1	<0.1	<0.2
	0.001 nM	13 \pm 20	3 \pm 3	8 \pm 2	0.6 \pm 0.1	270 \pm 10	0.1 \pm 0.03	3 \pm 0.4	<0.1	<0.2
SP34/Pertuz IgGBsAb	0.1 nM	15000 \pm 1000	2000 \pm 200	110 \pm 10	1100 \pm 40	1910 \pm 20	350 \pm 40	60 \pm 3	0.3 \pm 0.3	2.4 \pm 0.1
	0.01 nM	10000 \pm 200	190 \pm 20	240 \pm 30	350 \pm 30	2060 \pm 30	170 \pm 10	40 \pm 4	0.5 \pm 0.1	4.2 \pm 0.5
	0.001 nM	110 \pm 25	17 \pm 1	210 \pm 10	21 \pm 1	1920 \pm 60	4 \pm 1	16 \pm 1	1.0 \pm 0.7	1.7 \pm 0.3
SP34_F100fH/Pertuz IgGBsAb	0.1 nM	13000 \pm 900	410 \pm 30	150 \pm 5	580 \pm 50	1880 \pm 30	240 \pm 10	47 \pm 4	0.4 \pm 0.1	2.9 \pm 0.2
	0.01 nM	94 \pm 16	12 \pm 1	230 \pm 20	20 \pm 3	1900 \pm 40	7 \pm 1	16 \pm 9	0.6 \pm 0.2	2.0 \pm 0.2
	0.001 nM	11 \pm 9	6 \pm 4	8 \pm 1	0.6 \pm 0.1	260 \pm 30	0.13 \pm 0.03	3 \pm 0.2	<0.1	<0.2

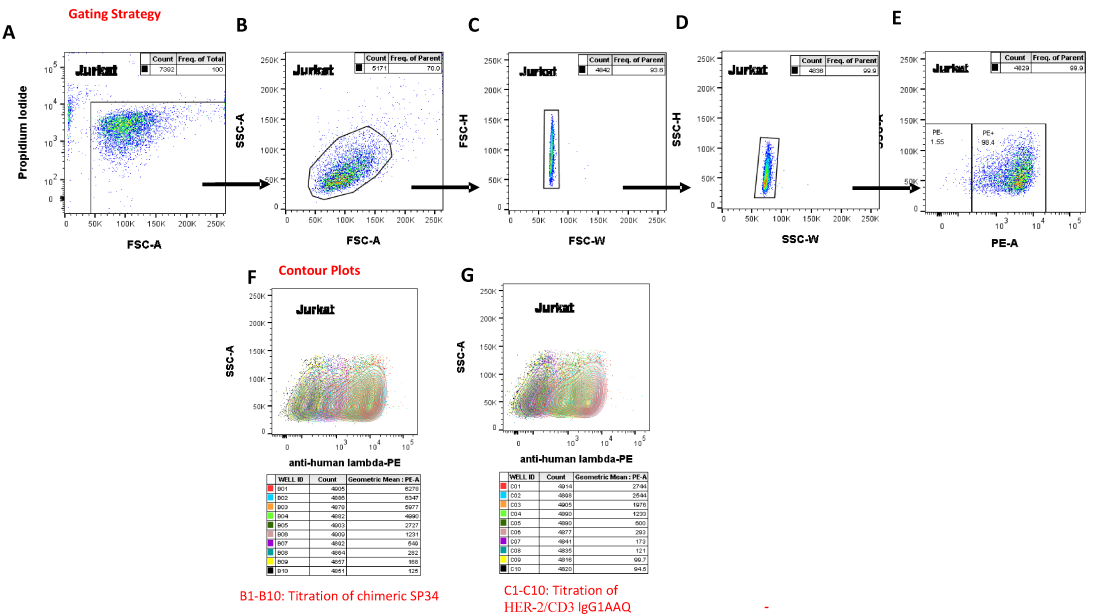
132

133

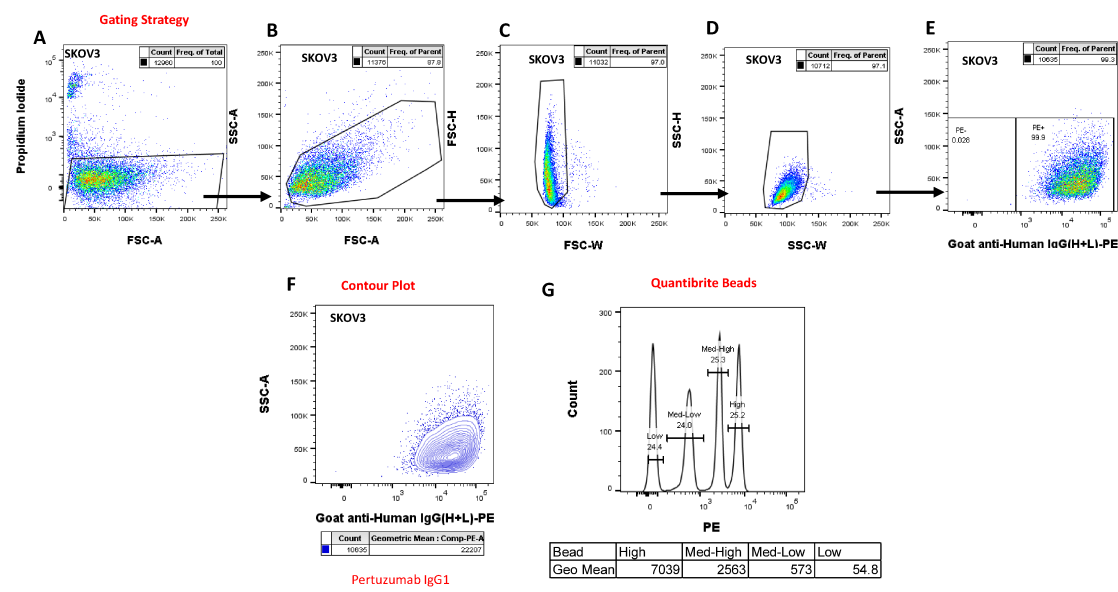
134



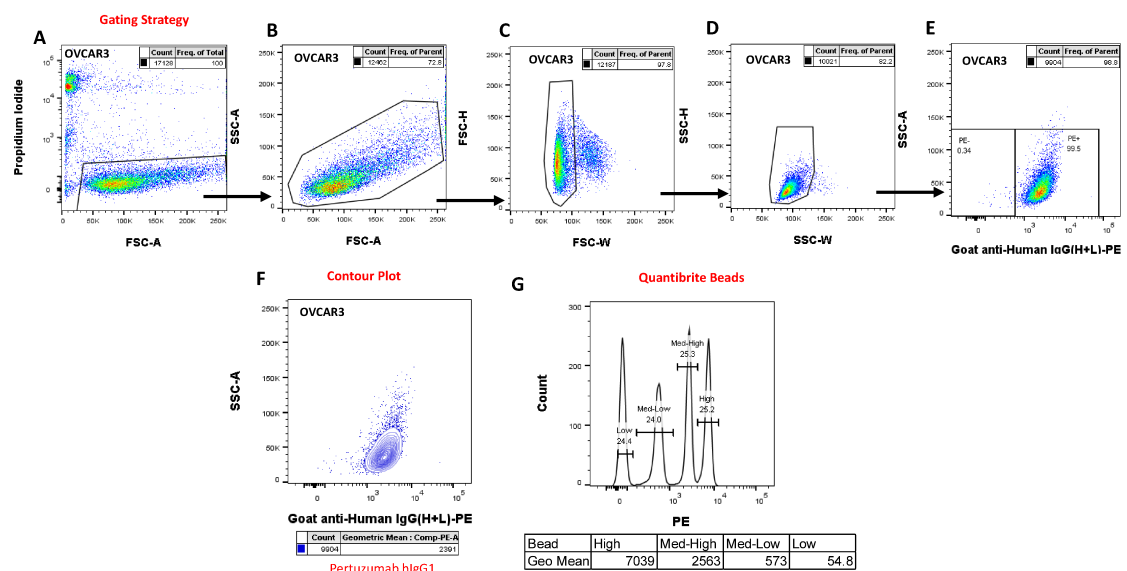
Supplementary Figure S1A. Flow cytometry gating strategy and Contour Plots for Fig. 2A. SKOV3 cells were centrifuged and incubated for 45 minutes with HER-2/CD3 BsAbs and Pertuzumab (anti-HER-2). After washing, cells were stained with anti-human kappa-PE and incubated for 45 minutes. Propidium iodide was used as the live/dead discriminator. Acquisition was performed on a Becton Dickinson LSRFortessa using BD FACSDiva software. Analysis was performed using FlowJo v10.7.1. Cells were initially gated based on A: FSC-A vs Propidium Iodide to exclude dead cells. B: FSC-A vs SSC-A to identify the distribution of cells based on light scatter. C and D: FSC-H vs FSC-W and SSC-H vs SSC-W for doublet discrimination. E: anti-human kappa-PE vs SSC-A for determination of positivity. Contour Plots F: B2-B9 titration of Pertuzumab IgG1. G: D2-D9 titration of HER-2/CD3 IgG1AAQ.



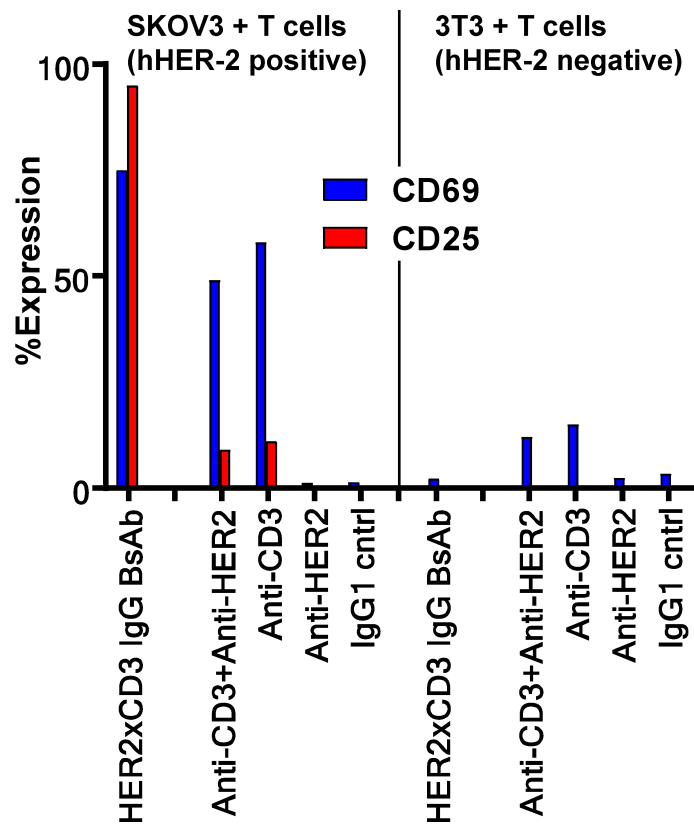
Supplementary Figure S1B. Flow cytometry gating strategy and Contour Plots for Fig. 2A. Jurkat cells were centrifuged and incubated for 45 minutes with HER-2/CD3 BsAbs and chimeric SP34 (anti-CD3). After washing, cells were stained with anti-human lambda-PE and incubated for 45 minutes. Acquisition was performed on a Becton Dickinson LSRFortessa using BD FACSDiva software. Analysis was performed using FlowJo v10.7.1. A-D: Live cell discrimination, doublet elimination using scattering patterns was performed as in **Supplementary Fig. S1A**. E: anti-human lambda-PE vs SSC-A for determination of positivity. Contour Plots: F: B1-B10 titration of chimeric SP34. G: C1-C10 titration of HER-2/CD3 IgG1AAQ



Supplementary Figure S2A. QuantiBrite™ bead quantitation of HER-2 levels on SKOV3 cells. SKOV3 were centrifuged and incubated for 45 minutes with Pertuzumab (30 µg/ml). After washing, cells were stained with anti-human IgG (H+L)-PE and incubated for 45 minutes. Acquisition was performed on a Becton Dickinson LSRFortessa using BD FACSDiva software. Analysis was performed using FlowJo v10.7.1. A-D: Live cell discrimination, doublet elimination using scattering patterns was performed as in **Supplementary Fig. S1A**. E: anti-human IgG(H+L)-PE vs SSC-A for determination of positivity. Contour Plots F: Pertuzumab IgG1. G: Analysis of Quantibrite Beads (BD Cat#340495, Lot#22755)

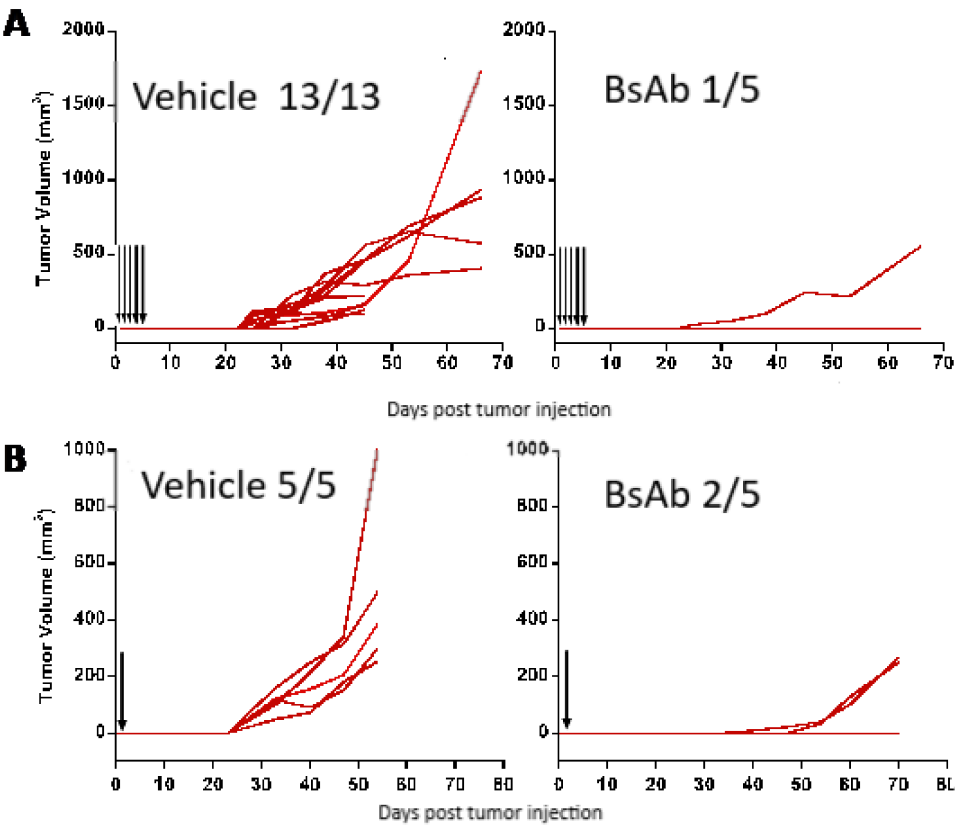


Supplementary Figure S2B. QuantiBrite™ bead quantitation of HER-2 levels on OVCAR3 cells. Quantitation of HER-2 on OVCAR3 cells was performed using a procedure identical to that described in the caption of **Supplementary Fig. S2A**.



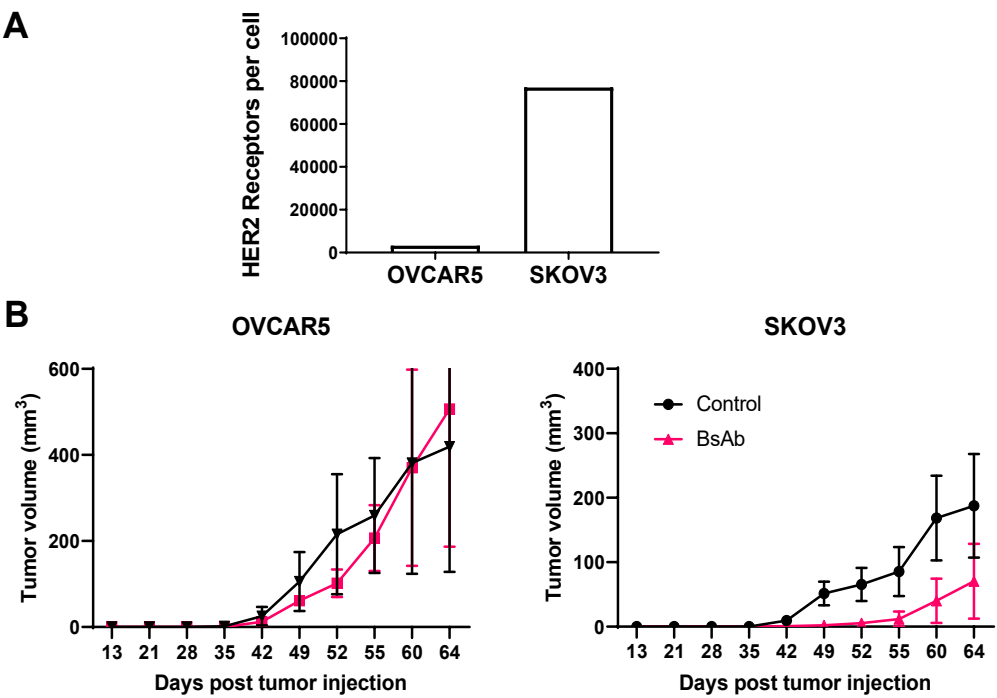
Supplementary Figure S3. CD69 and CD25 activation marker upregulation on primary T cells by various test articles. Upregulation was measured after a 48 h incubation with human HER-2^HI SKOV3 cells (left side) or human HER-2 negative mouse 3T3 cells (right side) in the presence of the HER-2/CD3 IgG BsAbs, the parental mAbs as well as a combination of the parental mAbs (all at 1 nM) using flow cytometry.

188

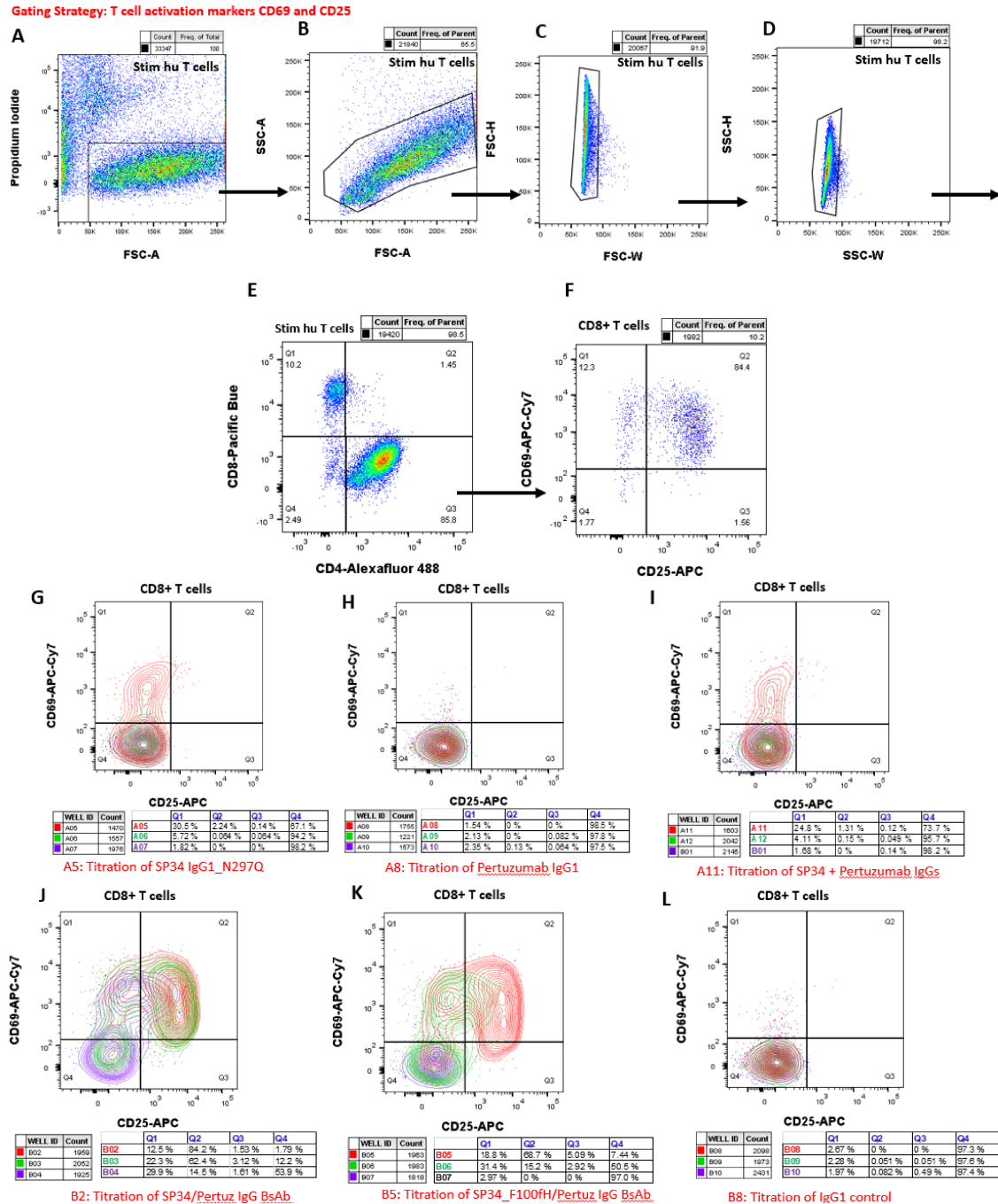


189
190
191
192
193
194
195
196
197
198
199
200

Supplementary Figure S4. Individual caliper-based tumor outgrowth curves of SKOV3 tumors in NSG-His mice administered with either 5 consecutive daily doses (**A**) or a single dose (**B**) starting 1 day after tumor cell implantation. Doses were performed with saline (left) or 5 mg/kg HER-2/CD3 IgG BsAb (right). The imaging data for this study is shown in **Fig. 2C**. Numbers in the upper righthand corners indicate the number of mice where measurable tumors were observed (numerator) per the number in the entire group (denominator).

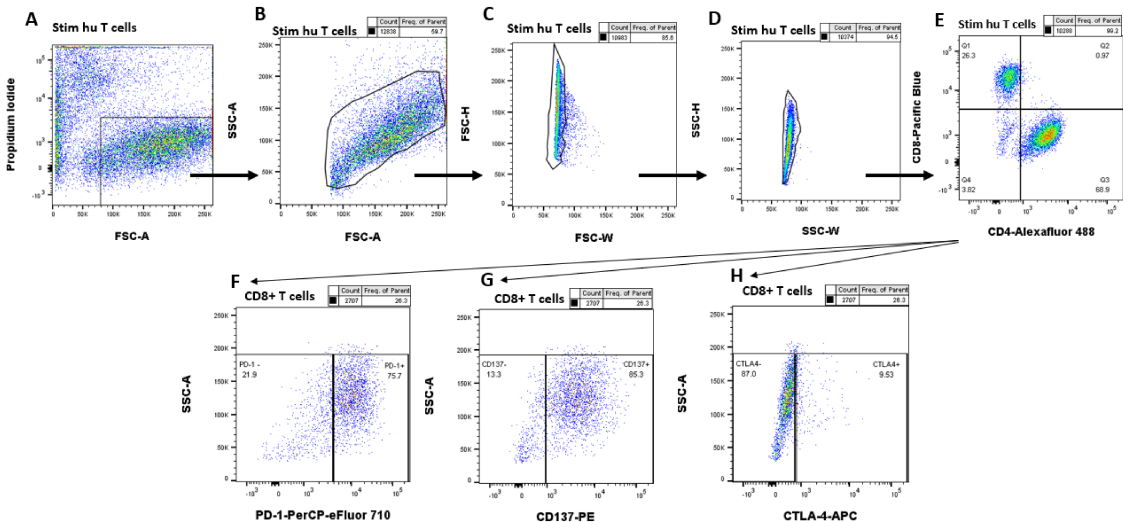


Supplementary Figure S5. Effect of HER level on target tumor cell. **A** HER2 expression was measured on OVCAR5 and SKOV3 tumor cells before injection in mice. **B** Caliper-based tumor outgrowth curves of Her2^{HI} SKOV3 and HER2^{LO} OVCAR5 tumors in NSG-His mice administered a single dose 1 day after tumor cell implantation. Doses were performed with saline (control) or 5 mg/kg HER-2/CD3 IgG BsAb.

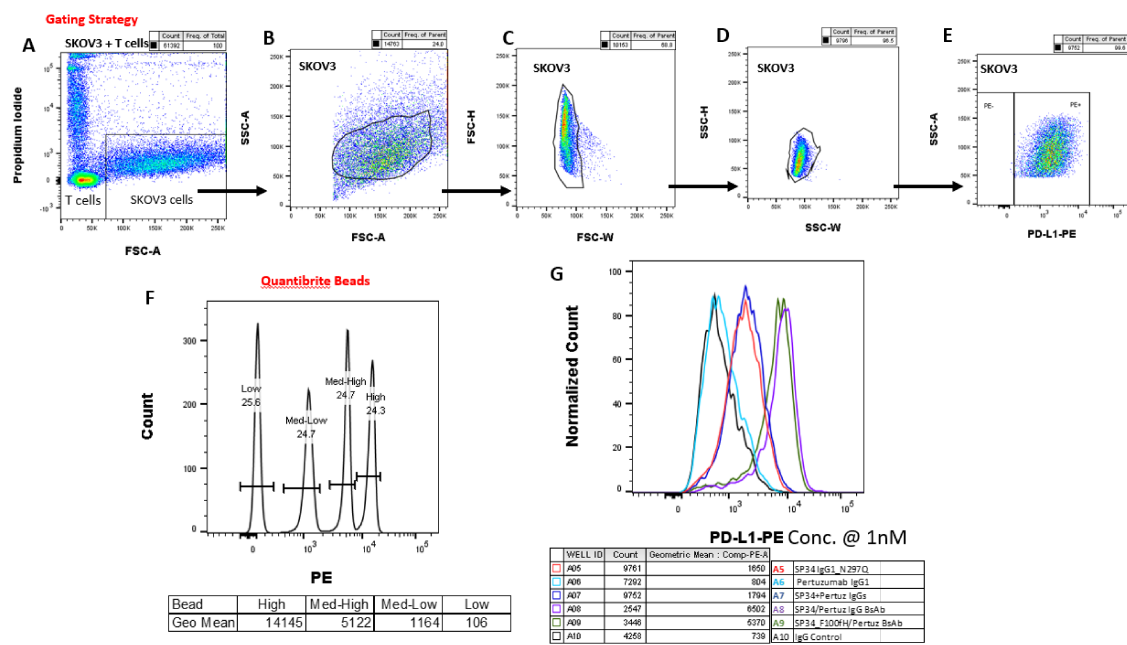


Supplementary Figure S6. Gating strategy and FACS contour plots evaluating CD69 and CD25 on human T cells incubated with test articles and tumor cells. After 48 hours exposure to SKOV3 tumor cells expressing HER-2 and titrating various IgGs and IgG BsAbs (0.1, 0.01 and 0.001nM.), human T cells were centrifuged and incubated for 45 minutes with Alexa-488 Mouse Anti-Human CD4, Pacific Blue Mouse Anti-Human CD8, APC-Cy7 Mouse Anti-Human

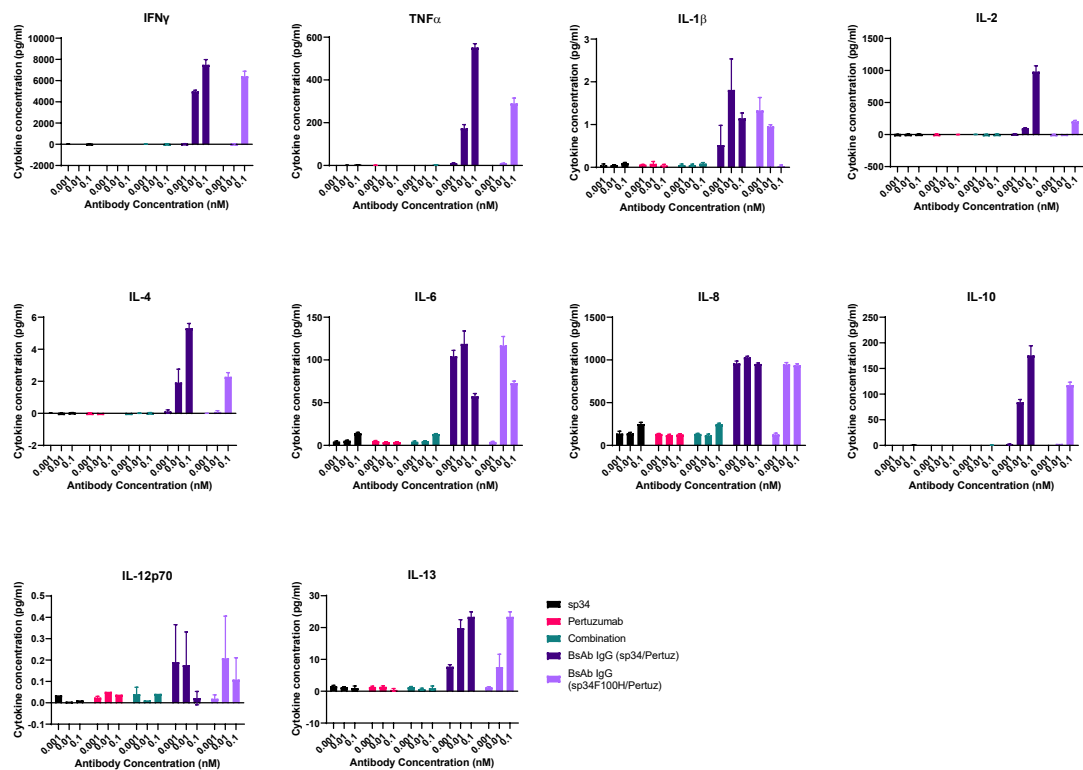
CD69 and APC- Mouse Anti-Human CD25. Acquisition was performed on a Becton Dickinson LSRFortessa using BD FACSDiva software. Analysis was performed using FlowJo v10.7.1. A-D: Live cell discrimination, doublet elimination using scattering patterns was performed as in **Supplementary Fig. S1A**. E: Alexa-488 Mouse Anti-Human CD4 and Pacific Blue Mouse Anti-Human CD8 for determination of CD8 and CD4 population frequency. F: APC-Cy7 Mouse Anti-Human CD69 and APC-Mouse Anti-Human CD25 for determination of CD69 and CD25 population frequency. Contour plots are provided for the 0.001, 0.01, and 0.1 nM tirations of G: A5-A7 titration of SP34 IgG1_N297Q, H: A8-A10: Titration of Pertuzumab IgG1, I: A11-12, B1: Titration of SP34+Pertuzumab IgGs. J: titration of B2-B4: Titration of SP34/Pertuz IgG BsAb. K: B5-B7: Titration of SP34_F100fH/Pertuz IgG BsAb, L: B8-B10: Titration of IgG1.



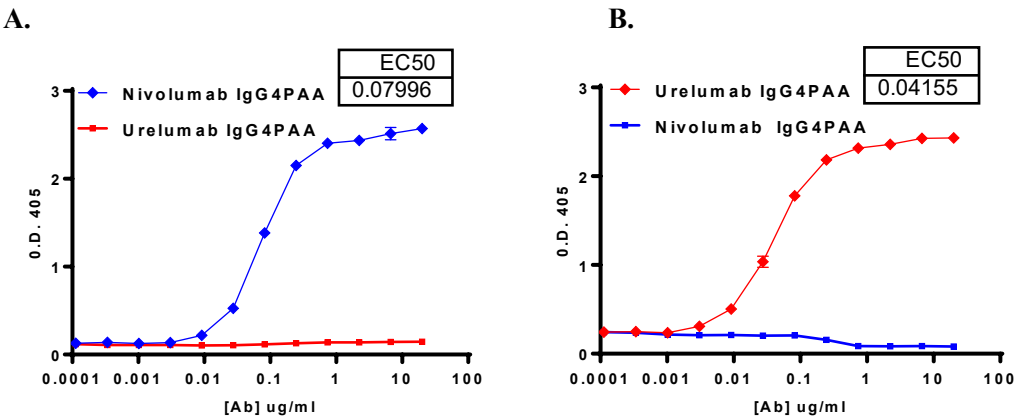
Supplementary Figure S7. Gating strategy and FACS contour plots evaluating PD-1, 4-1BB, and CTLA-4 on human T cells incubated with test articles and tumor cells. After 48 hours exposure to HER-2+ SKOV3 cells and titrating various IgGs and IgG BsAbs (0.1,0.01 and 0.001nM), human T cells were centrifuged and incubated for 45 minutes with Alexa-488 Mouse Anti-Human CD4, Pacific Blue Mouse Anti-Human CD8, PerCP-eFluor 710 Mouse Anti-Human PD-1, PE-Mouse anti-Human 4-1BB, and APC- Mouse Anti-Human CTLA-4. Acquisition was performed on a Becton Dickinson LSRFortessa using BD FACSDiva software. Analysis was performed using FlowJo v10.7.1. A-D: Live cell discrimination, doublet elimination using scattering patterns was performed as in **Supplementary Fig. S1A**. E: Alexa-488 Mouse Anti-Human CD4 and Pacific Blue Mouse Anti-Human CD8 for determination of CD8 and CD4 population frequency. Examples of PD-1, 4-1BB, and CTLA-4 detection on T cells using the wild-type HER-2/CD3 IgG BsAb at 0.1 nM: F: PerCP-eFluor 710-Mouse Anti-Human PD-1 for determination of the PD-1 population frequency. G: PE-Mouse anti-human 4-1BB for determination of the 4-1BB population. H: APC-Mouse anti-human CTLA-4 for determination of the CTLA-44 population.



Supplementary Figure S8. Flow Cytometry Quantitation of PD-L1 on SKOV3 cells after exposure to T cells and the BsAbs or mAb controls as shown in Table 2. After 24 hours exposure to primary human T cells and titrating various IgGs and HER-2/CD3 IgG BsAbs (1.0 nM), SKOV3 cells were rinsed, lifted, centrifuged and incubated for 45 minutes with PE-Mouse anti-human PD-L1. Acquisition was performed on a Becton Dickinson LSRFortessa using BD FACSDiva software. Analysis was performed using FlowJo v10.7.1. A-D: Live cell discrimination, doublet elimination using scattering patterns was performed as in **Supplementary Fig. S1A**. E: Example of PE-Mouse anti-human PD-L1 vs SSC-A for determination of PD-L1 positivity using SKOV3 cells treated with 1 nM anti-CD3 SP34 mAb. F: Analysis of Quantibrite Beads (BD Cat#340495, Lot#72299). G. Histograms of SKOV3 cells stained with PE-Mouse anti-human PD-L1 after 24 h incubation with test articles/T cells.



Supplementary Figure S9. Cytokine levels (pg/mL) in cell culture media 48 hours after mixing primary T cells and various test articles (0.1 nM) with SKOV3 cells (10:1 T cell:Tumor cell). Each value is the mean of three separate reactions with independently mixed T cells, SKOV3 cells, and/or test article and the error is the standard deviation.



272 **Supplementary Figure S10.** ELISA binding activity of in-house IgG4PAA versions of
273 Nivolumab (anti-PD-1) and Urelumab (anti-4-1BB/CD137) tested using extracellular domains of
274 human PD-1 (**A**) and human 4-1BB/CD137 (**B**). Both extracellular domains were generated in-
275 house using molecular biology and mammalian expression methods described in the *Materials*
276 *and Methods*.

277
278
279
280
281
282
283 1. Lewis SM, Wu X, Pustilnik A, et al. Generation of bispecific IgG antibodies by structure-based design of
284 an orthogonal Fab interface. *Nat Biotechnol* 2014;32(2):191-8. doi: 10.1038/nbt.2797 [published
285 Online First: 2014/01/28]
286