Belantamab mafodotin does not induce B-cell maturation antigen loss or systemic immune dysfunction in multiple myeloma

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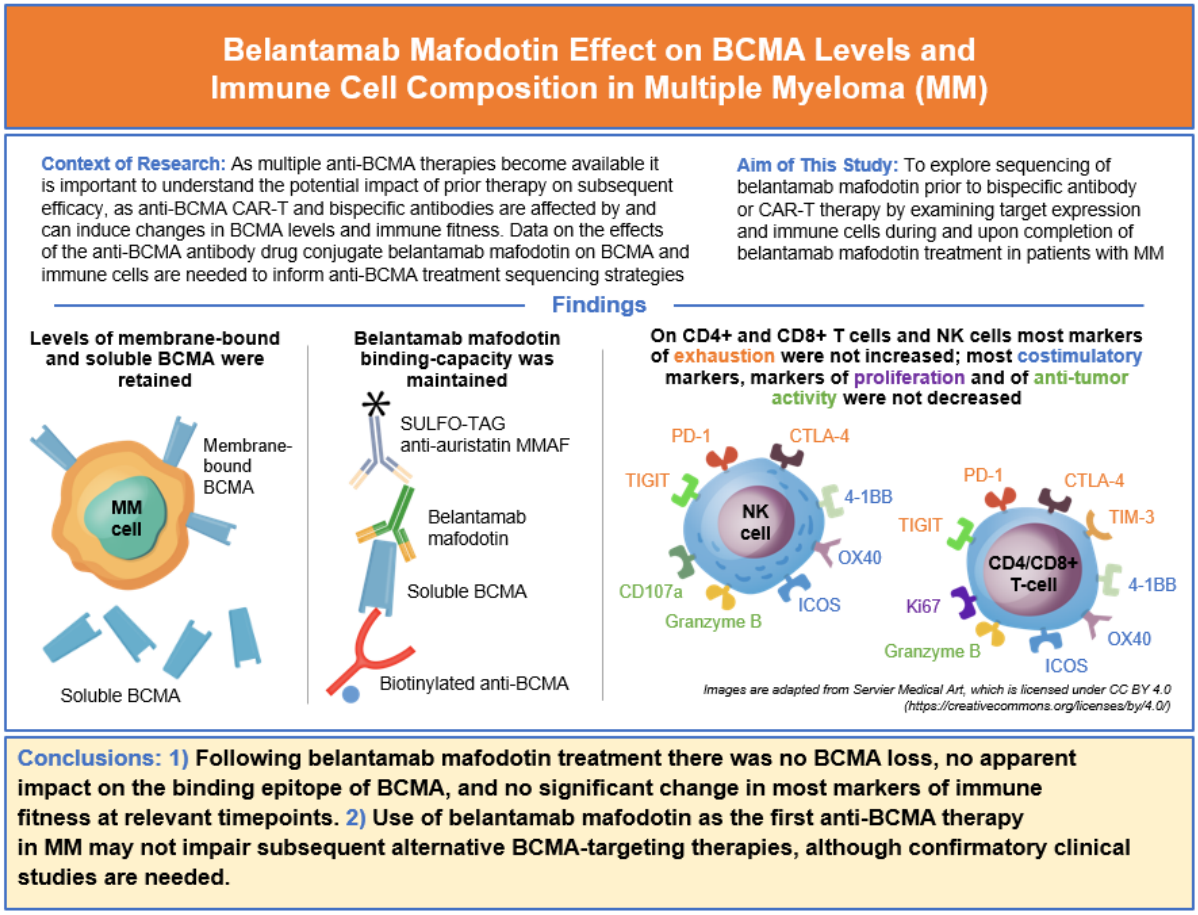
Supplemental Material



Supplemental methods

Free and complexed sBCMA

Calibration standards were prepared on the day of assay in BCMA-depleted human serum (free sBCMA: 10 µg/mL glycosylated human BCMA [huBCMA, GSK, Collegeville, PA] at 1000, 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91, 1.95, and 0.98 ng/mL; complexed sBCMA: 1 µg/mL glycosylated huBCMA and 100 µg/mL belantamab mafodotin [GSK2857916, GSK, Collegeville, PA] at 200, 66.67, 22.22, 7.41, 2.47, 0.82, 0.27, and 0.09 ng/mL). The lowest standards were used as anchor points for curve-fitting purposes only. For free sBCMA, the belantamab mafodotin antibody (GSK2857914, GSK, Collegeville, PA) was coated onto a 96-well multi-array high bind Meso Scale Discovery (MSD, Rockville, MA) plate. Calibration standards and normal and MM human serum (BioIVT, Westbury, NY) test samples containing free BCMA were added to the wells, and any free BCMA was bound by the immobilized capture antibody. Bound BCMA was detected by biotinylated anti-BCMA polyclonal goat antibody (BAF193; R&D Systems, Minneapolis, MN) and SULFO-TAG-labeled streptavidin (MSD, Rockville, MD) which generated electrochemiluminescence signals detected using a Meso Scale Discovery S600 plate reader (MSD, Rockville, MA). The electrochemiluminescence signal intensity was proportional to the amount of free BCMA in standards and serum test samples.

For complexed sBCMA, the biotinylated anti-BCMA goat polyclonal antibody (capture antibody) was coated onto each well of a streptavidin-coated 96-well MSD plate. The MSD plate was then incubated with blocking buffer. Calibration standards and normal and MM human serum test samples were added to the plate in duplicate wells, and the complexed BCMA (sBCMA-belantamab mafodotin) present in the samples was bound to the immobilized capture antibody. The complexed BCMA was further bound to ruthenylated SULFO-TAG-labeled anti-auristatin antibody (GSK, Collegeville, PA) which was used to generate electrochemiluminescence signals detected using a Meso Sector S600 plate reader (MSD, Rockville, MA). The electrochemiluminescence signal intensity was proportional to the amount of complexed BCMA in standards and human serum test samples.

Plate readers were controlled by a Dell PC workstation via MSD Discovery Workbench software, version 4.0. Data acquired were processed using Watson Laboratory Information Management System (LIMS) software, version 7.4.2 (Thermo Fisher Scientific, Waltham, MA). Calibration curves were fitted using a 4-parameter (Marquardt) logistic algorithm with $1/y^2$ weighting. Concentrations of free and complexed BCMA in the test samples were interpolated from the standard calibration curves. The mean, precision, and accuracy of individual concentrations were calculated by Watson LIMS.

Binding of belantamab mafodotin monoclonal antibody and teclistamab

Two sets of bio-layer interferometry (BLI) experiments were conducted qualitatively to address whether GSK2857914 (belantamab mafodotin without the cytotoxic payload) and teclistamab both bind to histidine tagged BCMA (His-BCMA) immobilized on a nickel (II)-nitriloacetic acid (Ni-NTA) sensor, and whether teclistamab is able to bind the GSK2857914:BCMA complex. Both experiments are described in **Supplemental Table 1** (wash steps omitted). BLI experiments were conducted using a Sartorius Octet RH16 (Sartorius, Göttingen, Germany). All samples were prepared at their stated concentrations (**Supplemental Table 1**) in 1x phosphate buffered saline at pH 7.4 with 0.05% Tween-20 (PBST) with each well on the sample plate containing 200 µL of the respective sample. Octet Streptavidin (SA) Sensors (catalogue number 18-5019; Sartorius, Göttingen, Germany) and Ni-NTA sensors (catalogue number 18-5101; Sartorius, Göttingen, Germany) were equilibrated for 600 seconds in PBST at 30°C with 600 revolution per minute (rpm) shaking prior to conducting each experiment. Biotinylated GSK2857914 (Bt-GSK'914) was generated and characterized in-house and then immobilized on SA sensors for 230 seconds at 1 µg/mL in PBST. His-BCMA (catalogue number BCA-H522y-100µg; Acro Biosystems, Newark, DE, USA) was immobilized on Ni-NTA sensors for 600 seconds at 2 µg/mL in PBST.

To address the aspect of antigen binding, Ni-NTA sensors with immobilized His-BCMA were exposed to 100 nM samples of GSK2857914 or teclistamab in PBST for 900 seconds followed by a 1200 second dissociation phase in clean PBST to diminish the effects of non-specific binding to the sensor.

Pairing experiments were conducted using both His-BCMA (Acro Biosystems, Newark, DE, USA) and recombinant human BCMA (rhBCMA) (in-house). SA sensors loaded with 1 µg/mL Bt-GSK'914 were loaded again with 100 nM of either His-BCMA or rhBCMA for 900 seconds to form a GSK2857914:BCMA complex. After a 60 second wash with PBST, each sensor was exposed to 100 nM teclistamab in PBST for 900 seconds.

Data was acquired with Octet BLI Discovery version 13.0.2.24 (Sartorius, Göttingen, Germany) and analyzed using Octet BLI Analysis version 12.2.13.4 (Sartorius, Göttingen, Germany).

Pathologist tumor cell score

The BCMA (rabbit clone E6D7B) assay was evaluated on a semi-quantitative scale, and the percentage of cancer cells staining at each of the following levels was recorded: 0 (no staining), 1+ (weak staining), 2+ (moderate staining) and 3+ (strong staining). A tumor sample was considered positive if at least 1% of tumor cells demonstrated positive expression.

H-score

An H-Score was calculated based on the summation of the product of percent of cells stained at each staining intensity using the following equation: (3 x % cells staining at "3+") + (2 x % cells staining at "2+") + (1 x % cells staining at "1+").

Additional cell types were evaluated at the maximum staining intensity and included normal adjacent tissue, endothelia, smooth muscle, fibroblast, stroma, inflammatory cells, and nerve.

Calculation of Neutrophil-to-lymphocyte ratio (NLR) and absolute lymphocyte counts (ALC)

Absolute cell counts were calculated using a single platform or dual platform method:

Single platform: $\left(\frac{Number\ of\ captured\ events}{Number\ of\ Trucount\ events}\right) \times \left(\frac{Trucount\ absolute\ number}{Sample\ volume\ [\mu L]}\right)$

Dual platform except CD14+ cells: $\%\ of\ captured\ lymphocytes \times$
$lymphocyte{:}\ leukocyte\ ratio \times white\ blood\ cells$

Dual platform, CD14+ cells: $\%\ of\ captured\ leukocytes \times white\ blood\ cells$

Flow cytometry analysis of T-cells and NK cells

Whole blood samples were drawn into 4- or 6-mL Sodium Heparin Vacutainer collection tubes (Beckton Dickinson, Franklin Lakes, NJ) from patients with MM and stored at ambient temperature. Antibodies were added to the whole blood sample, incubated, lysed, and washed per manufacturer guidelines, and data were collected using BD FACSCanto II (BD Biosciences, Franklin Lakes, NJ). Flow cytometry analysis was performed at a flow rate of ~60 µL/min, with stop gates at 10,000 lymphocyte events and 120 seconds.

Flow cytometry analysis of Granzyme B, Ki67, CD107a, PD-1, and TIGIT

Whole blood samples were drawn into sodium heparin collection tubes from patients with MM and stored at ambient temperature. A cocktail of primary stain was prepared by aliquoting multiples of each antibody plus a 10% overage into amber glass vials, then dispended into daughter tubes. Cell surface and intracellular staining were performed as described below:

- An autofluorescence (AF) extraction reference control was prepared by taking one tube of patient matrix (NaHep) through the sample preparation process without addition of antibodies. Experiments were unmixed using reference controls stored in the library
- 150 µL of bone marrow or 500 µL of whole blood patient sample was added per tube

- 3mL of the 1x Roche Lysis Buffer (Roche, Basel, Switzerland) was added, vortexed well, and incubated for 10 min at room temperature
- Tubes were spun at 500 g for 5 minutes. Supernatant was decanted without blotting
- 2 mL of Roche Lysis Buffer was used for washing, and vortexed well
- Centrifuging was performed at 500g for 5 minutes. Supernatant was decanted and tube was blotted on paper towel
- 2mL of phosphate-buffered saline (PBS) was used for washing and tubes were spun at 500 g for 5 minutes. Supernatant was decanted and blotted. The pellet was resuspended
- Fixable Viability Stain: 1 µL of 1:4 DMSO diluted Live Dead Blue was added in 999 µL of PBS solution in each tube, vortexed gently, and incubated for 30 minutes at room temperature, protected from light
- Cells were washed in BSA. To each tube, 2 mL of room temperature BD Pharmingen BSA Stain Buffer (BD Biosciences, Franklin, NJ) was added and gently vortexed. Tubes were centrifuged at 500 g for 5 minutes. Supernatant was decanted and tube blotted. The pellet was gently resuspended
- Cells were washed in 2 mL of room temperature BD Pharmingen BSA Stain Buffer (BD Biosciences, Franklin Lakes, NJ) and gently vortexed. Tubes were centrifuged at 500 g for 5 minutes, supernatant decanted, and tube blotted. The pellet was gently resuspended
- 5 µL of TruStain FC Block (BioLegend, San Diego, CA) was added and incubated for 10 min at room temperature, protected from light
- 5 µL TCR g/d antibody (BD Biosciences, Franklin Lakes, NJ) was added to each tube, vortexed well, and incubated 10 min at room temperature, protected from light.
- To each Tube 1 and Tube 2 surface antibody cocktail was added, vortexed gently, and incubated for 30 minutes at room temperature, protected from light
- Cells were washed in 2 mL of room temperature BD Pharmingen BSA Stain Buffer (BD Biosciences, Franklin, NJ) was added and gently vortexed. Tubes were centrifuged at 500 g for 5 minutes. Supernatant was decanted and tube blotted. The pellet was gently resuspended
- 2 mL FoxP3 Buffer A (BioLegend, San Diego, CA) was added and incubated for 10 min at room temperature, protected from light
- Tubes were centrifuged at 500 g for 5 minutes. Supernatant was decanted and tube blotted. The pellet was gently resuspended
- 500 µL FoxP3 Buffer C was added and incubated for 30 min at room temperature, protected from light

- Cells were washed in 2 mL of room temperature BD Pharmingen BSA Stain Buffer (BD Biosciences, Franklin, NJ) and gently vortexed. Tubes were centrifuged at 500 g for 5 minutes. Supernatant was decanted and tube blotted. The pellet was gently resuspended
- Cells were washed in 2 mL of room temperature BD Pharmingen BSA Stain Buffer (BD Biosciences, Franklin, NJ) and gently vortexed. Tubes were centrifuged at 500 g for 5 minutes. Supernatant was decanted and tube blotted. The pellet was gently resuspended
- To each Tube 1 and Tube 2 the correct volume of intracellular antibody cocktail/test was added, vortexed gently, and incubated for 30 minutes at room temperature, protected from light
- Cells were washed in 2 mL of room temperature BD Pharmingen BSA Stain Buffer (BD Biosciences, Franklin, NJ) and gently vortexed. Tubes were centrifuged at 500 g for 5 minutes. Supernatant was decanted and tube blotted. The pellet was gently resuspended
- Cells were washed in 2 mL of room temperature BD Pharmingen BSA Stain Buffer (BD Biosciences, Franklin, NJ) and gently vortexed. Tubes were centrifuged at 500 g for 5 minutes. Supernatant was decanted and tube blotted. The pellet was gently resuspended
- Cells were washed in 2 mL of room temperature BD Pharmingen BSA Stain Buffer (BD Biosciences, Franklin, NJ) and gently vortexed. Tubes were centrifuged at 500 g for 5 minutes. Supernatant was decanted and tube blotted. The pellet was gently resuspended
- Cells were resuspended in 200 µL 1X BD Stain Buffer and acquired immediately on Cytek Aurora Flow Cytometer (Cytek, Fremont, CA)

Flow cytometry analysis was performed at a medium flow rate, with stop gates at 100,000 lymphocyte events and 120 seconds, and autofluorescence extraction. Autofluorescence extraction reference control was prepared by taking one tube of patient matrix through the sample preparation process without addition of antibodies.

Flow cytometry analysis of ICOS, OX40, 4-1BB, TIM-3, and CTLA-4

Whole blood samples were drawn into sodium heparin collection tubes from patients with MM and stored at ambient temperature. A cocktail of primary stain was prepared by aliquoting multiples of each antibody plus a 10% overage into Eppendorf tubes, then dispended into daughter tubes. Cell surface and intracellular staining were performed as described below:

Two polystyrene tubes were labeled per sample. 95 µL of cocktail 1 (**Supplemental Table 2**) was added to Tube 1 and 116.25 µL of cocktail 2 (**Supplemental Table 2**) to the Tube 2. By reverse pipetting, 100 µL of whole blood sample was added to each tube followed by vortexing. Tubes were transferred onto a carousel and loaded onto the Lyse/Wash Assistant (LWA). Cell wash was added to

the cell wash container, FACS Lyse in the Lyse container and PBS in the Fix container. The LWA program Duo-Lyse_30 min modified was selected. When LWA program was completed, the carousel was transferred onto the FACS Canto cytometer (BD Biosciences, Franklin Lakes, NJ). Flow cytometry analysis was performed with medium flow rate and stop gates at 50,000 events and 210 seconds.

Supplemental Table 1. Bio-layer interferometry experiments.

Experiment/Sensor	Binding/NTA Sensor	Pairing/SA Sensor	Time(s)
Load 1	PBST	Bt-GSK'914, 1 µg/mL in PBST	230
Load 2	His-BCMA, 2 µg/mL in PBST	His-BCMA or rhBCMA, 100 nM in PBST	900
Associate	Teclistamab or GSK2857914, 100 nM in PBST	Teclistamab or GSK2857914, 100 nM in PBST	900
Dissociate	PBST	PBST	1200

Bt-GSK'914, biotinylated GSK2857914; GSK2857914, belantamab mafodotin without the payload; His-BCMA, histidine tagged BCMA; NTA, nickel (II)-nitriloacetic acid; PBST, phosphate buffered saline (pH 7.4) with 0.05% Tween-20; rhBCMA, recombinant human B-cell maturation antigen; s, seconds; SA, streptavidin.

Supplemental Table 2. Flow cytometry panels.

Fluorochrome	Tube 1	Tube 2 (activation markers assays only)	Clone and catalog#	Supplier
Analysis of T cells and NK cells – Panel 1				
BV421	CD19	–	Clone: HIB19 Cat# 562440	BD Biosciences, Franklin Lakes, NJ
BV510	CD4	–	Clone: SK3 Cat# 562971	BD Biosciences, Franklin Lakes, NJ
FITC	CD3	–	Clone: SK7 Cat# 345764	BD Biosciences, Franklin Lakes, NJ
PE	CD16/CD56	–	CD16: Clone: B73.1 Cat# 332779 CD56: Clone: NCAM16.2 Cat# 345812	BD Biosciences, Franklin Lakes, NJ
PerCP-Cy5.5	CD45	–	Clone: 2D1 Cat# 332784	BD Biosciences, Franklin Lakes, NJ
PE-Cy7	–	–	–	–
APC	CD8	–	Clone: SK1 Cat# 345775	BD Biosciences, Franklin Lakes, NJ
APC-H7	CD14	–	Clone: MϕP9 Cat# 641394	BD Biosciences, Franklin Lakes, NJ
Analysis of ICOS, OX40, 4-1BB, TIM-3, and CTLA-4 – Panel 2				
FITC	CD3	CD3	Clone: SK7 Cat# 345764	BD Biosciences, Franklin Lakes, NJ
PE	-	CD336 (TIM-3)	Clone: F38-2E2 Cat# 345006	BioLegend, San Diego, CA
PerCP-Cy5.5	-	CD134 (OX40)	Clone: Ber-ACT35 Cat# 350010	BioLegend, San Diego, CA
PE-Cy7	-	CD152 (CTLA-4)	Clone: L3D10 Cat# 349914	BioLegend, San Diego, CA
APC-H7	CD8	CD8	Clone: SK1 Cat# 641400	BD Biosciences, Franklin Lakes, NJ
BV510	CD4	CD4	Clone: SK3 Cat# 562970	BD Biosciences, Franklin Lakes, NJ

APC	-	CD137 (4-1BB)	Clone: 4B4 Cat# 550890	BD Biosciences, Franklin Lakes, NJ
AF700	CD19	CD19	Clone: HIB19 Cat# 557921	BD Biosciences, Franklin Lakes, NJ
BV421	-	CD278 (ICOS)	Clone: DX29 Cat# 562901	BD Biosciences, Franklin Lakes, NJ
BV605	CD16	CD16	Clone: 3G8 Cat# 563172	BD Biosciences, Franklin Lakes, NJ
BV605	CD56	CD56	Clone: NCAM16.2 Cat# 562780	BD Biosciences, Franklin Lakes, NJ
Analysis of Granzyme B, Ki67, CD107a, PD-1, and TIGIT – Panel 3				
PE	FOXP3	FOXP3	Clone: 259D Cat# 320208	BioLegend, San Diego, CA
YG584	CD4	CD4	Clone: SK3 Cat# R7-20041	Cytek, Fremont, CA
PE-Dazzle 594	Isotype	Granzyme B	Granzyme B: Clone: QA16A02 Cat# 372216 Isotype Mouse IgG1: Clone: MOPC-21 Cat# 400176	BioLegend, San Diego, CA
PE-Fire 640	CD19	CD19	Clone: HIB19 Cat# 302274	BioLegend, San Diego, CA
PE-Cy5	–	CD107a	Clone: H4A3 Cat# 555802	BD Biosciences, Franklin Lakes, NJ
PE-Fire 700	CD25	CD25	Clone: M-A251 Cat# 356145	BioLegend, San Diego, CA
PE-Cy7	–	CD122	Clone: CF1 Cat# A53365	Beckman Coulter, Brea, CA
BUV395	CD11b	CD11b	Clone: ICRF44 Cat# 563839	BD Biosciences, Franklin Lakes, NJ
Live/Dead Blue	Fixable LD Blue	Fixable LD Blue	Viability Cat# L34962	Thermo Fisher Scientific, Waltham, MA
BUV496	CD16	CD16	Clone: 3G8 Cat# 612945	BD Biosciences, Franklin Lakes, NJ
BUV563	–	CD226	Clone: DX11 Cat# 748429	BD Biosciences, Franklin Lakes, NJ

BUV615	–	CD159a	Clone: 131411 Cat# 752302	BD Biosciences, Franklin Lakes, NJ
BUV661	CD11c	CD11c	Clone: B-ly6 Cat# 612967	BD Biosciences, Franklin Lakes, NJ
BUV737	CD56	CD56	Clone: NCAM16.2 Cat# 612766	BD Biosciences, Franklin Lakes, NJ
BUV805	CD14	CD14	Clone: M5E2 Cat# 612902	BD Biosciences, Franklin Lakes, NJ
BV421	CD27	CD27	Clone: O323 Cat# 302824	BioLegend, San Diego, CA
Pacific Blue	–	CD57	Clone: QA17A04 Cat# 393316	BioLegend, San Diego, CA
BV480	Isotype	Ki-67	Ki-67:Clone: B56 Cat# 566109 Isotype Mouse IgG1: Clone: X40 Cat# 1197793	BD Biosciences, Franklin Lakes, NJ
BV510	TCRg/d	TCRg/d	Clone: 11F2 Cat# 745026	BD Biosciences, Franklin Lakes, NJ
BV570	CD8	CD8	Clone: RPA-T8 Cat# 301038	BioLegend, San Diego, CA
BV605	–	CD336	Clone: p44-8 Cat# 744301	BD Biosciences, Franklin Lakes, NJ
BV650	–	CD279	Clone: EH12.1 Cat# 744301	BD Biosciences, Franklin Lakes, NJ
BV711	CD95	CD95	Clone: DX2 Cat# 563132	BD Biosciences, Franklin Lakes, NJ
BV750	HLA-DR	HLA-DR	Clone: L243 Cat# 307672	BioLegend, San Diego, CA
BV786	CD197	CD197	Clone: 2-L1-A Cat# 566758	BD Biosciences, Franklin Lakes, NJ
BB515	CD66b	CD66b	Clone: G10F5 Cat# 564679	BD Biosciences, Franklin Lakes, NJ
FITC	CD38	CD38	Mulitclone Cat# CYT-38F2	Cytognos, Salamanca, Spain

Alexa 532	CD45	CD45	Clone: HI30 Cat# 58-0459-42	Thermo Fisher Scientific, Waltham, MA
BB700	–	CD314	Clone: 1D11 Cat# 745863	BD Biosciences, Franklin Lakes, NJ
PerCP-eF710	–	CD69	Clone: FN50 Cat# 46-0699-42	Thermo Fisher Scientific, Waltham, MA
APC	–	TIGIT	Clone: 741182 Cat# FAB7898A	R&D Systems, Minneapolis, MN
NIR Spark 685	CD45RA	CD45RA	Clone: HI100 Cat# 304168	BioLegend, San Diego, CA
APC-Alexa 700	CD127	CD127	Clone: R34.34 Cat# A71116	Beckman Coulter, Brea, CA
APC-H7	CD3	CD3	Clone: SK7 Cat# 560176	BD Biosciences, Franklin Lakes, NJ

4-1BB, 4-immunoglobulin and cytokine receptor BB; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; ICOS, inducible T-cell co-stimulator; NK, natural killer; PD-1, programmed cell death protein 1; TIGIT, T-cell immunoreceptor with immunoglobulin and ITIM domains; TIM-3, T-cell immunoglobulin and mucin domain 3.

Supplemental Table 3. sBCMA levels across DREAMM studies.

	Baseline		Progression	
Study	n	% with detectable sBCMA expression	n	% with detectable sBCMA expression
DREAMM-1	75	100.0	51	98.0
DREAMM-2	213	99.1	183	98.9
DREAMM-7	32	100.0	32	100.0
DREAMM-8	32	100.0	32	100.0

sBCMA, soluble B-cell maturation antigen.

Supplemental Figure 1. Flow cytometry gating strategy for T-cell and NK cell identification.

Figure 1a: *FSC v SSC*
Events which display low forward and side scatter properties are identified as *Debris* and removed from subsequent analysis by creating an inverted *NOT Debris* population (see hierarchy).

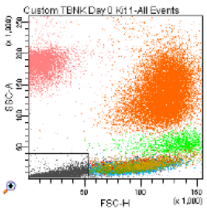


Figure 1b: *SSC v CD8_APC-H7*
From the *NOT Debris* population, TruCount *Beads* are captured. These events are used to calculate the absolute cell value for all parameters.

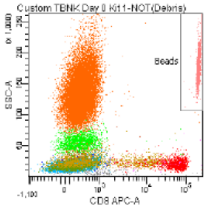


Figure 1c: *SSC v CD8 APC-H7*
From the *NOT Beads* population, monocytes are identified and gated as CD14 positive events. A *NOT Beads* gate is created to exclude the monocytes from all subsequent plots.

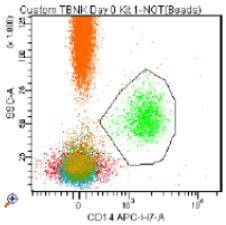


Figure 1d: *SSC v CD45_PerCPCy5.5*
From the *NOT Beads* population *Leukocytes* are identified and gated.

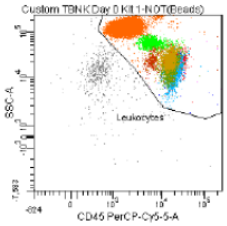


Figure 1e: *SSC v CD45_PerCPCy5.5*
From the *Leukocyte* population, $CD45^+$ lymphocytes are identified.

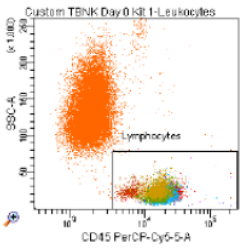


Figure 1f: *SSC v CD3_FITC*
From the *Lymphocyte* population, $CD3^+$ T cells are identified.

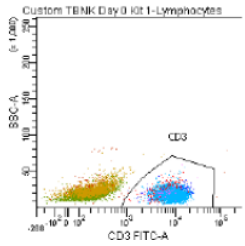


Figure 1g: *CD4_BV510 v CD8_APC*
From the *T cell* population, $CD4^+$ and $CD8^+$ cells are identified through quadrant gating.

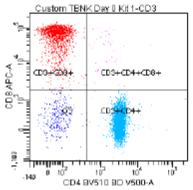


Figure 1h: *CD19_BV421 v CD56/16_APC*
From the *NOT CD3+* population, $CD3^-CD19^+$ and $CD3^-CD16^+CD56^+$ cells are identified through quadrant gating.

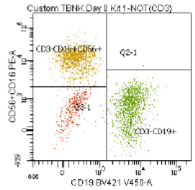
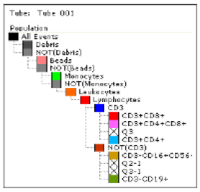


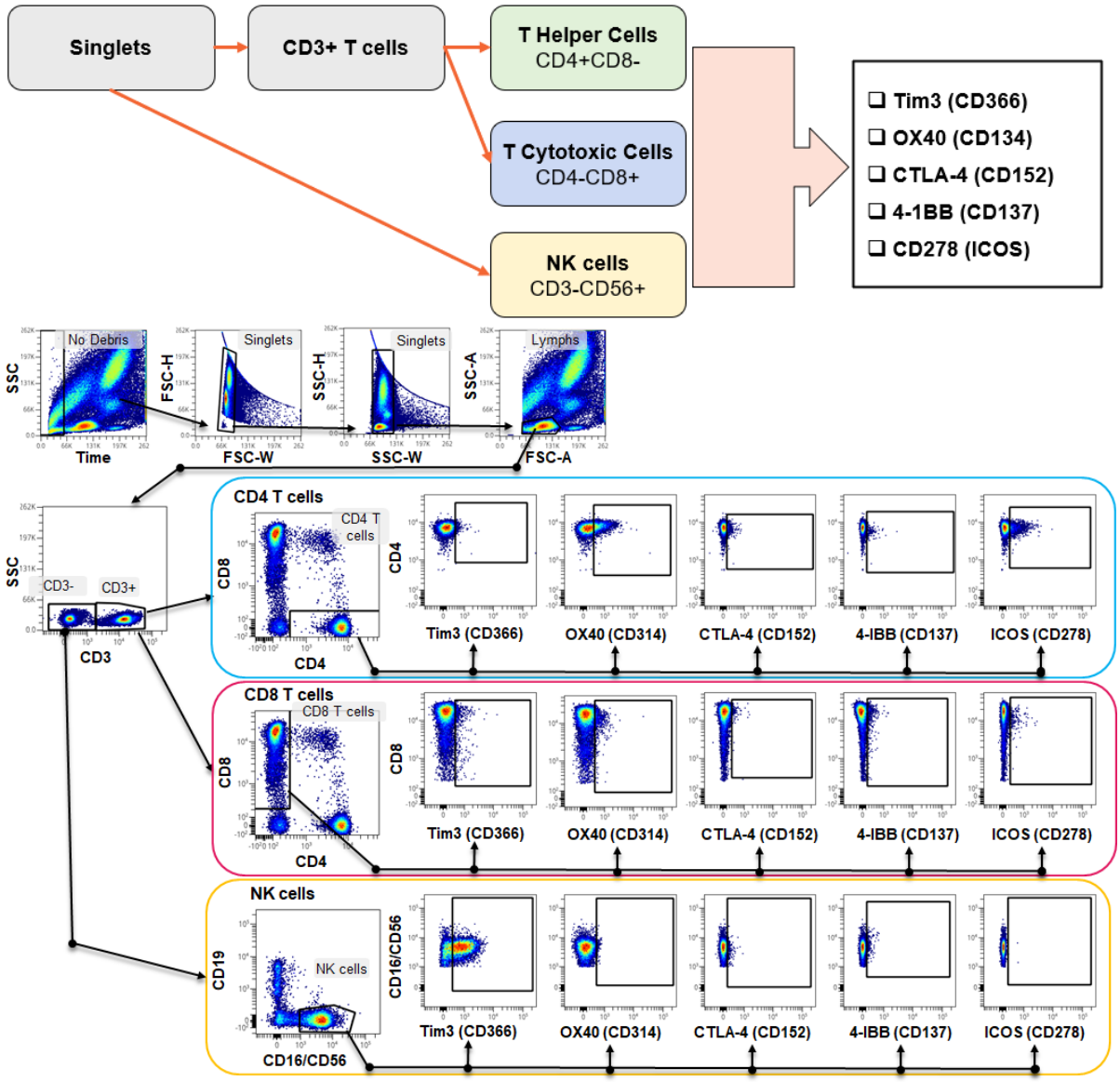
Figure 1i; Hierarchy



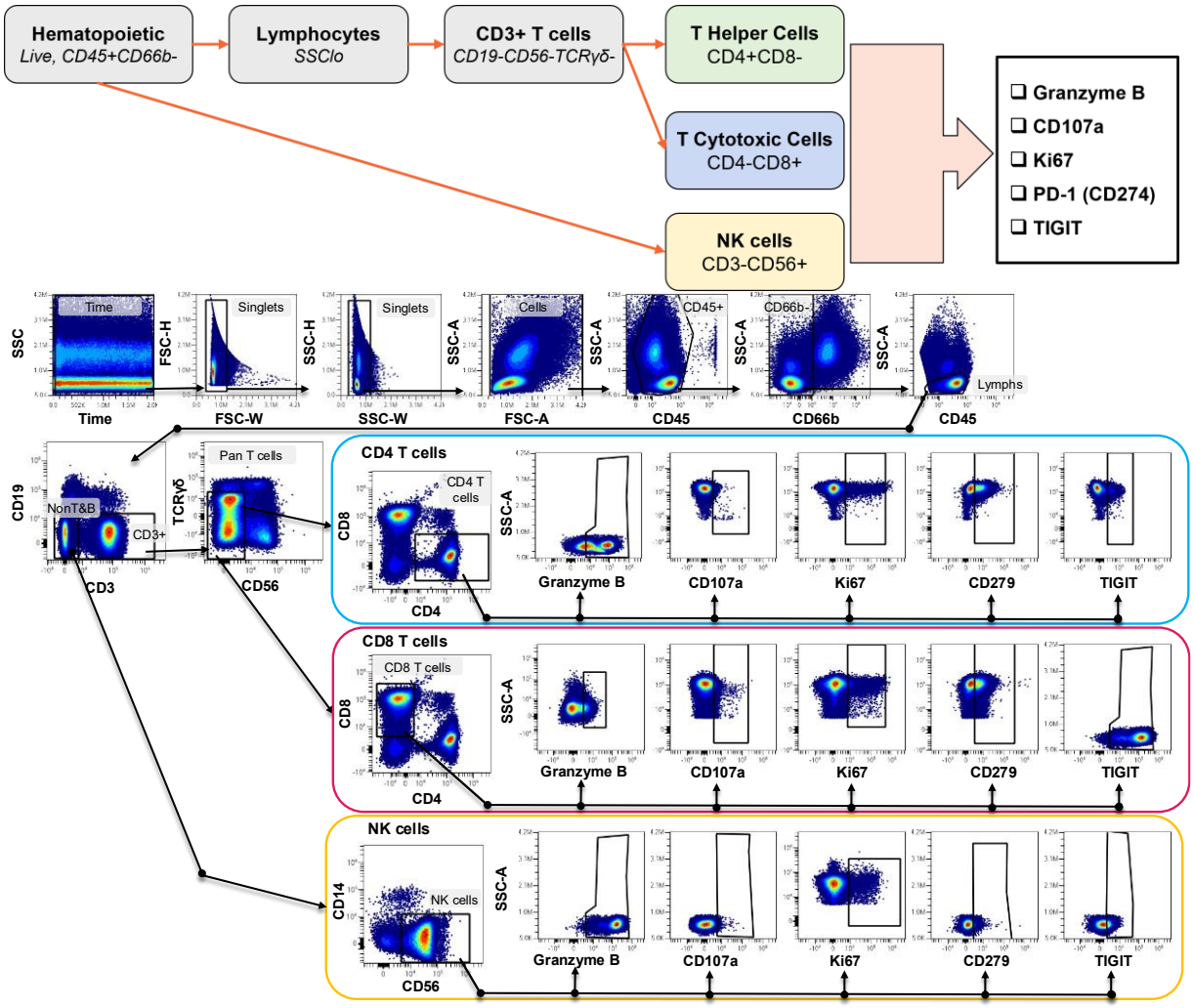
Supplemental Figure 2. Flow cytometry gating strategy for identification of subpopulations and expression of activation markers in T cells, B cells, monocytes, and NK cells.

(A) ICOS, OX40, 4-1BB, TIM-3, and CTLA-4 (panel 1) and (B) Granzyme B, Ki67, CD107a, PD-1, TIGIT (panel 2).

A



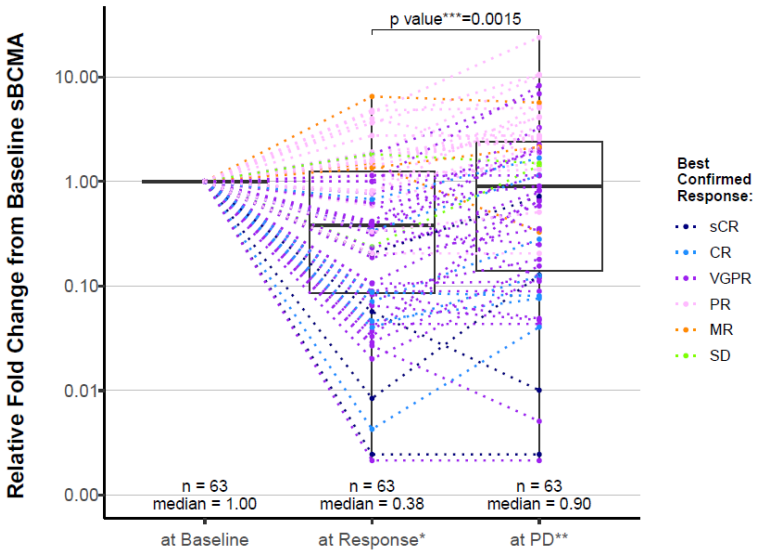
B



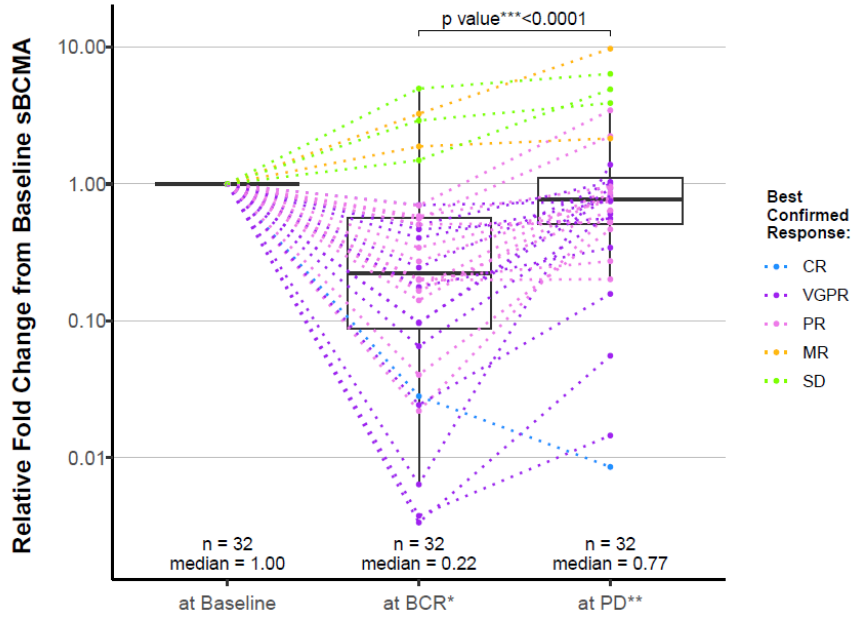
4-1BB, 4-immunoglobulin and cytokine receptor BB; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; FSC, forward scatter; NK, natural killer; PD-1, programmed cell death protein 1; SSC, side scatter; TIGIT, T-cell immunoreceptor with immunoglobulin and ITIM domains; TIM-3, T-cell immunoglobulin and mucin domain 3.

Supplemental Figure 3. Soluble B-cell maturation antigen levels at baseline, at best response, and at disease progression by best confirmed response. Monotherapy in DREAMM-2 (A); belantamab mafodotin with bortezomib and dexamethasone in DREAMM-7 (B).

A



B

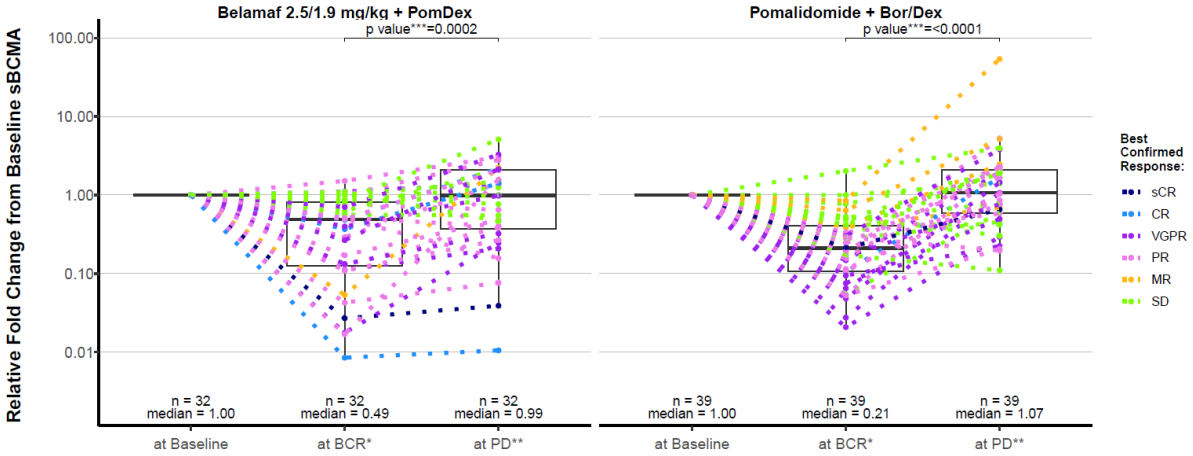


The black center line within the box represents the median value, the lower limit of the box represents the 25th percentile, the upper limit of the box represents the 75th percentile, and whiskers represent the 5th and 95th percentiles.

BCMA, B-cell maturation antigen; BCR, best confirmed response; BVd, belantamab mafodotin, bortezomib, and dexamethasone; CR, complete response; MR, minimal response; PD, progressive

disease; PR, partial response; sBCMA, soluble B-cell maturation antigen; sCR, stringent complete response; SD, stable disease; VGPR, very good partial response.

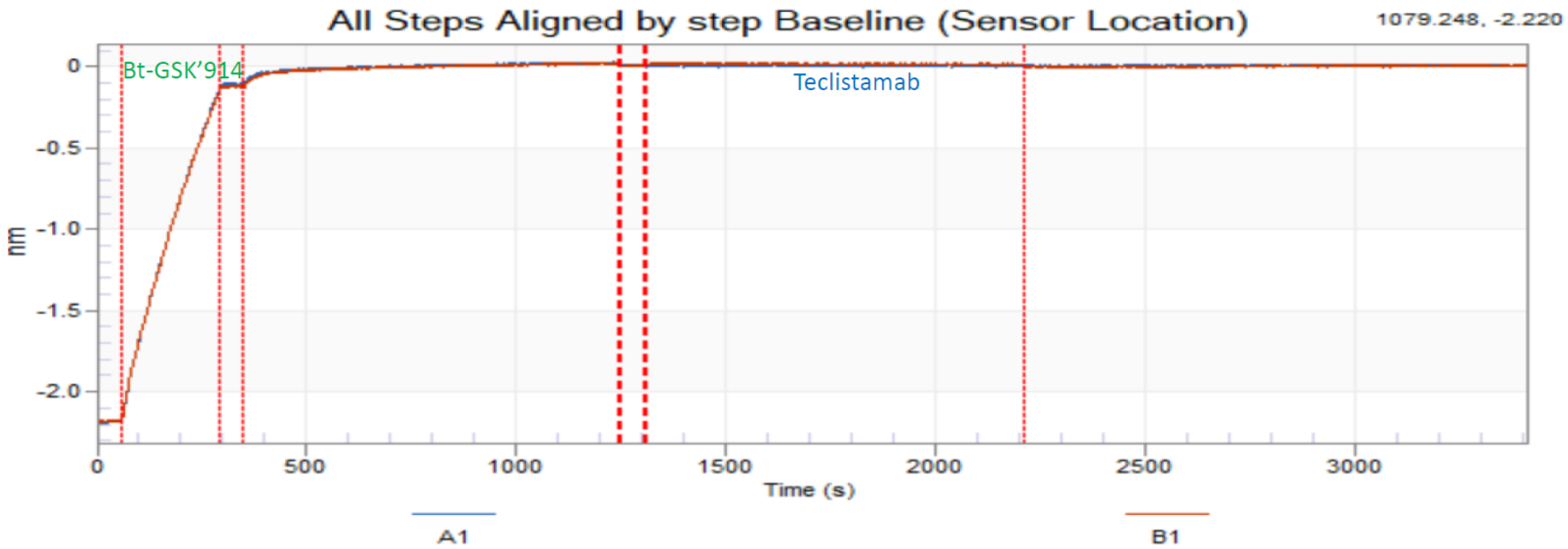
Supplemental Figure 4. Soluble B-cell maturation antigen levels at baseline, at best response, and at disease progression by best confirmed response in patients receiving belantamab mafodotin with pomalidomide and dexamethasone or pomalidomide with bortezomib and dexamethasone in DREAMM-8.



The black center line within the box represents the median value, the lower limit of the box represents the 25th percentile, the upper limit of the box represents the 75th percentile, and whiskers represent the 5th and 95th percentiles.

BCR, best confirmed response; BPd, belantamab mafodotin, pomalidomide, and dexamethasone; CR, complete response; MR, minimal response; PD, progressive disease; PR, partial response; PVd, pomalidomide, bortezomib, and dexamethasone; sBCMA, soluble B-cell maturation antigen; sCR, stringent complete response; SD, stable disease; VGPR, very good partial response.

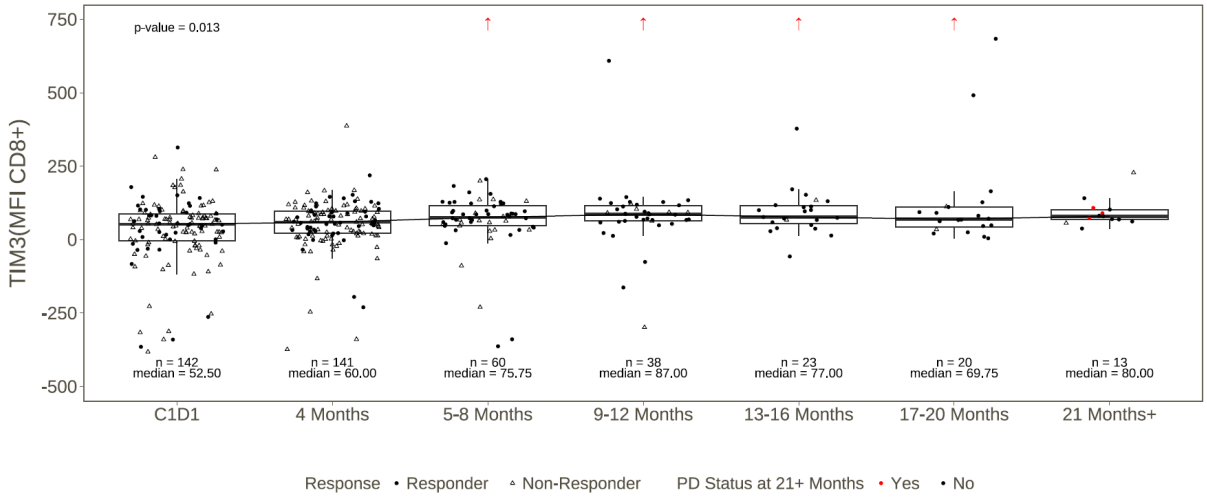
Supplemental Figure 5. Binding of belantamab mafodotin monoclonal antibody and teclistamab to B-cell maturation antigen



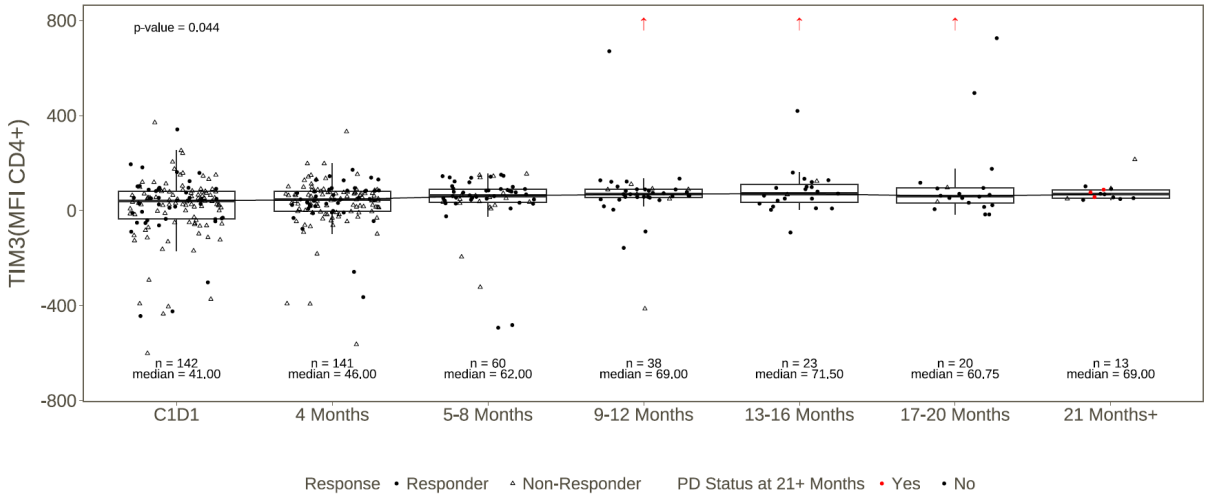
Bt-GSK'914; biotinylated GSK2857914; GSK2857914, belantamab mafodotin without the payload.

Supplemental Figure 6. Impact of belantamab mafodotin on median fluorescence intensity of T-cell exhaustion marker TIM-3.*

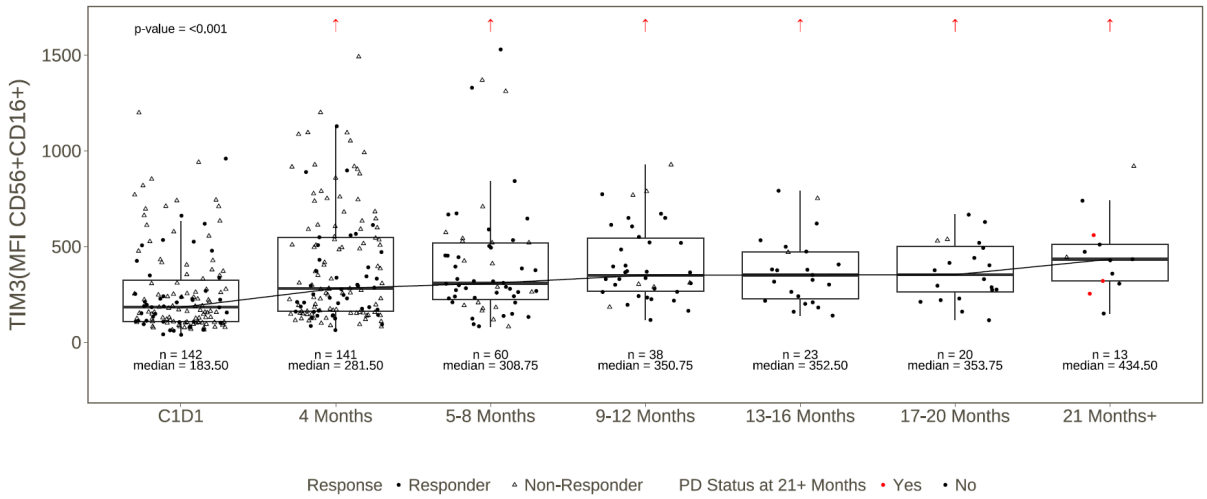
CD8+ T cells



CD4+ T cells



NK cells



*Data are from the DREAMM-14 study of belantamab mafodotin monotherapy.

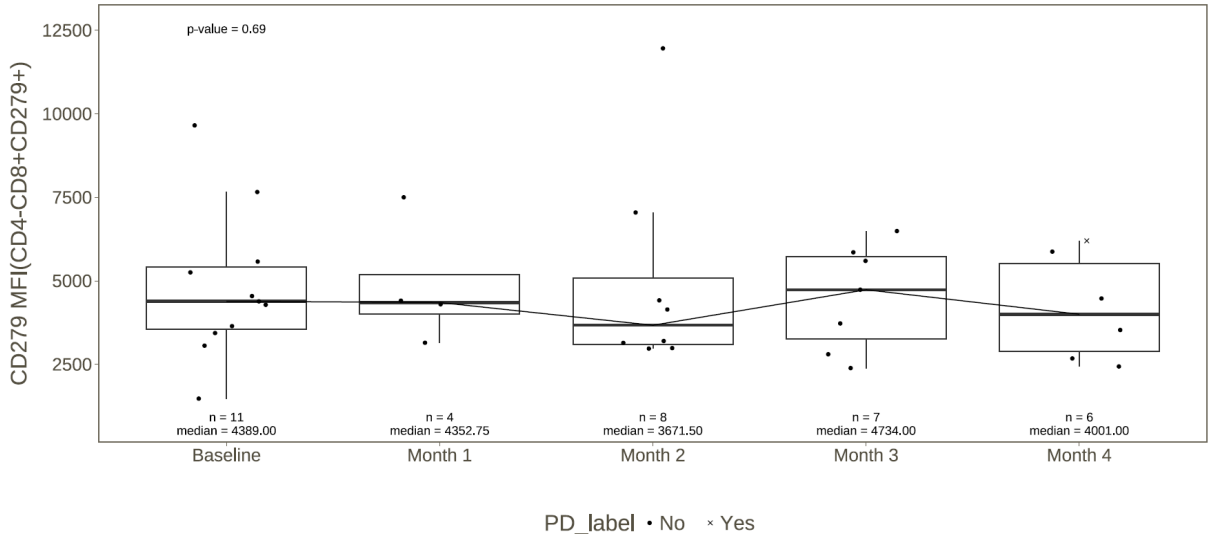
The median of the timepoints within each time category for each patient was utilized. The direction of the red arrows indicates significant increase or decrease from C1D1 for each timepoint.

C1D1, cycle 1 day 1; MFI, median fluorescence intensity; NK, natural killer; TIM-3, T-cell immunoglobulin and mucin domain 3.

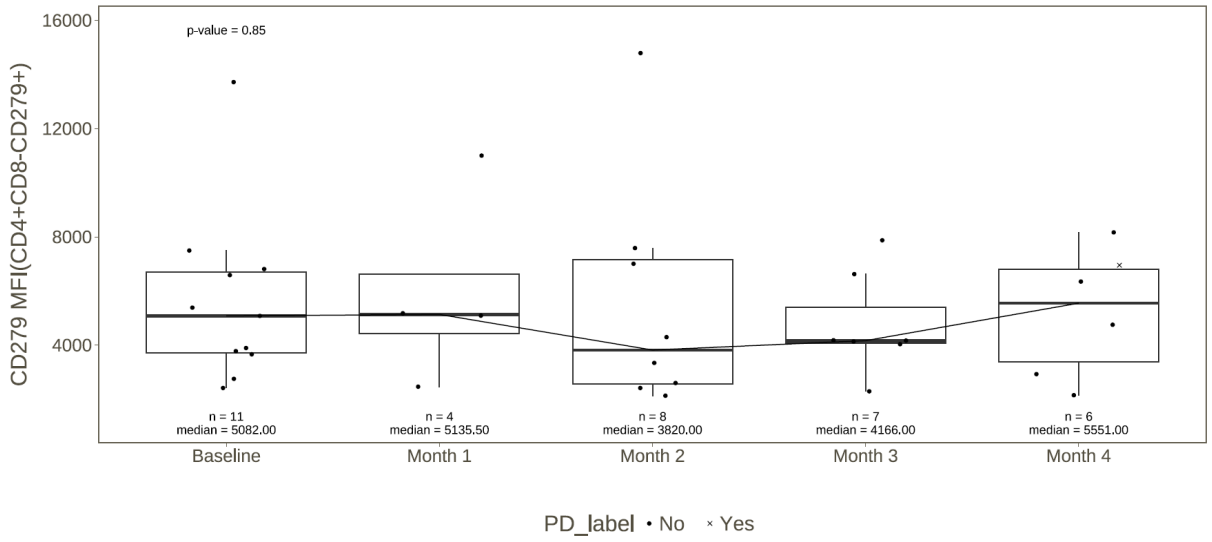
Supplemental Figure 7. Impact of belantamab mafodotin on median fluorescence intensity of T-cell exhaustion markers. PD-1 (A) and TIGIT (B).*

A

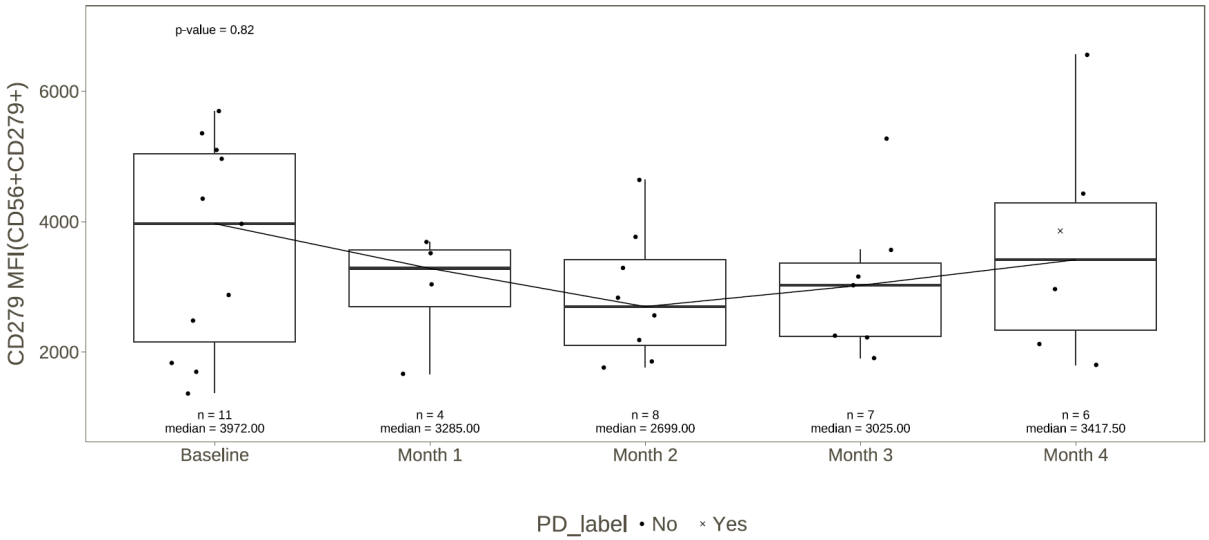
CD8+ T cells



CD4+ T cells

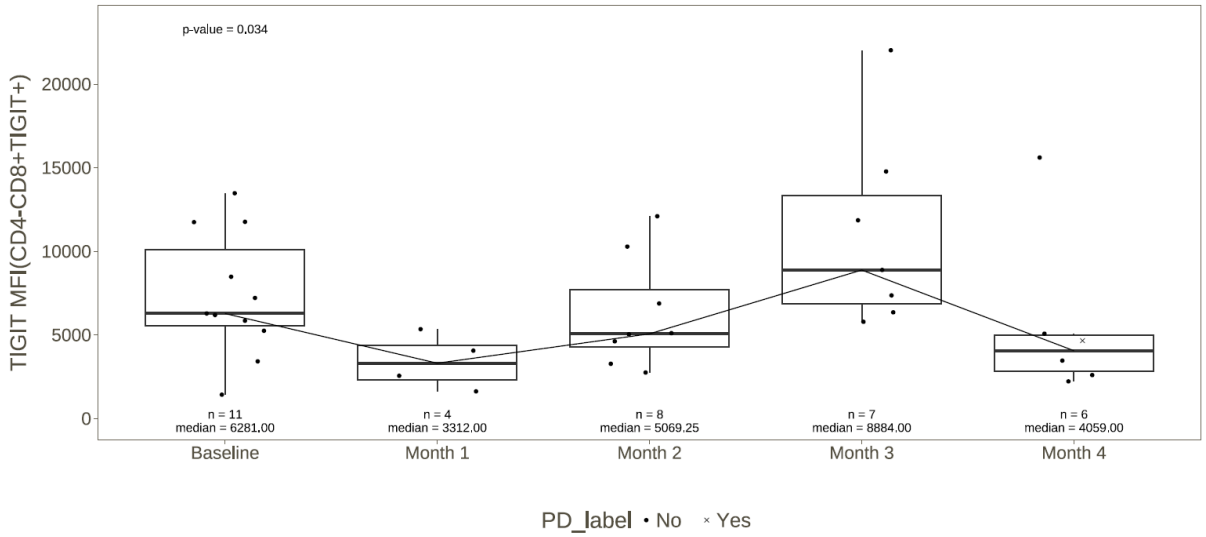


NK cells

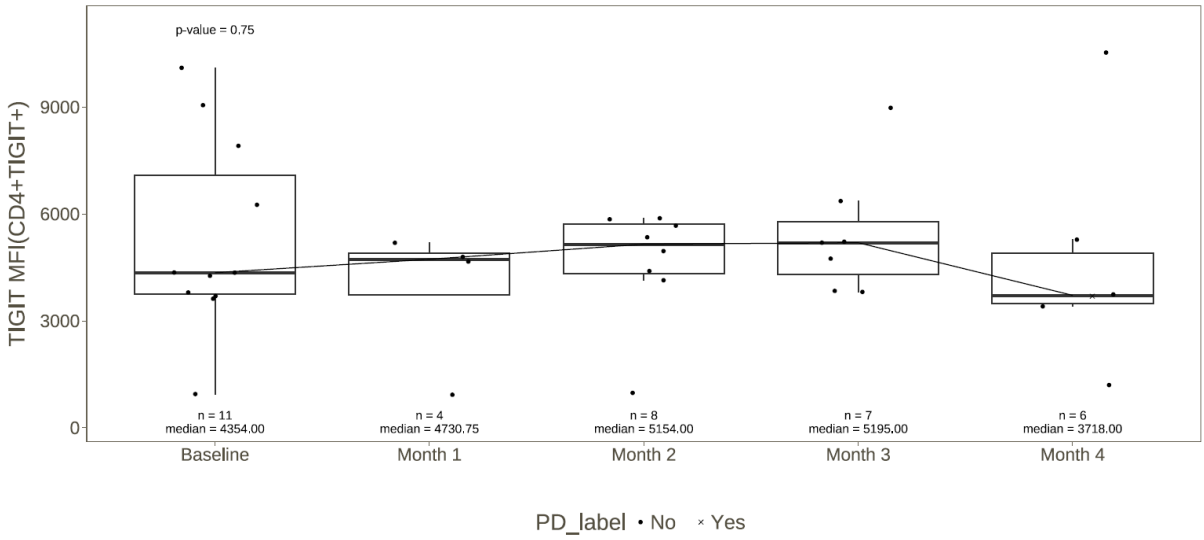


B

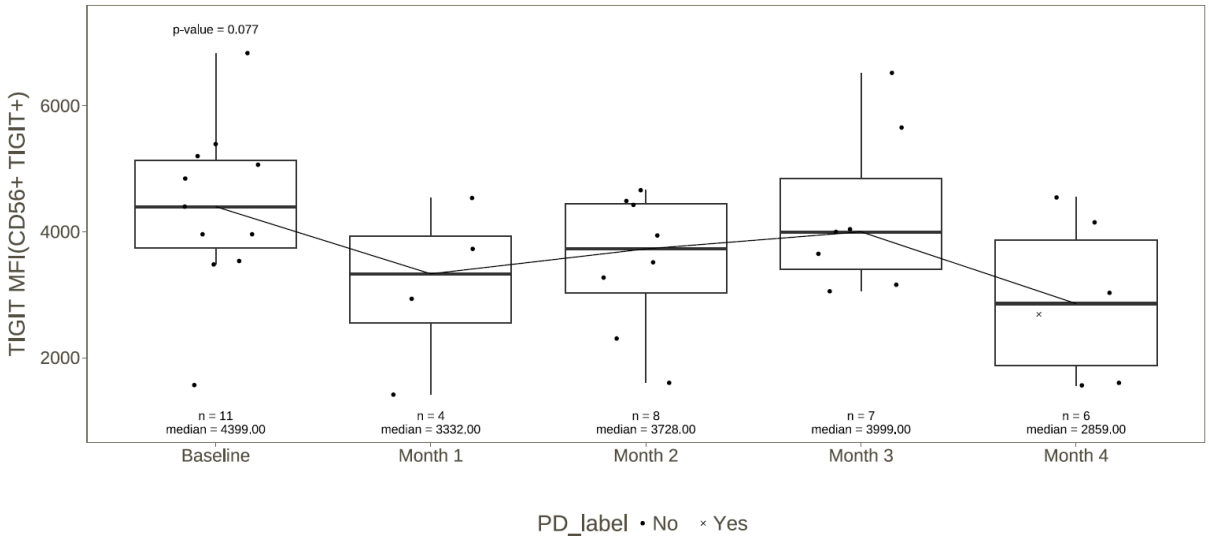
CD8+ T cells



CD4+ T cells



NK cells



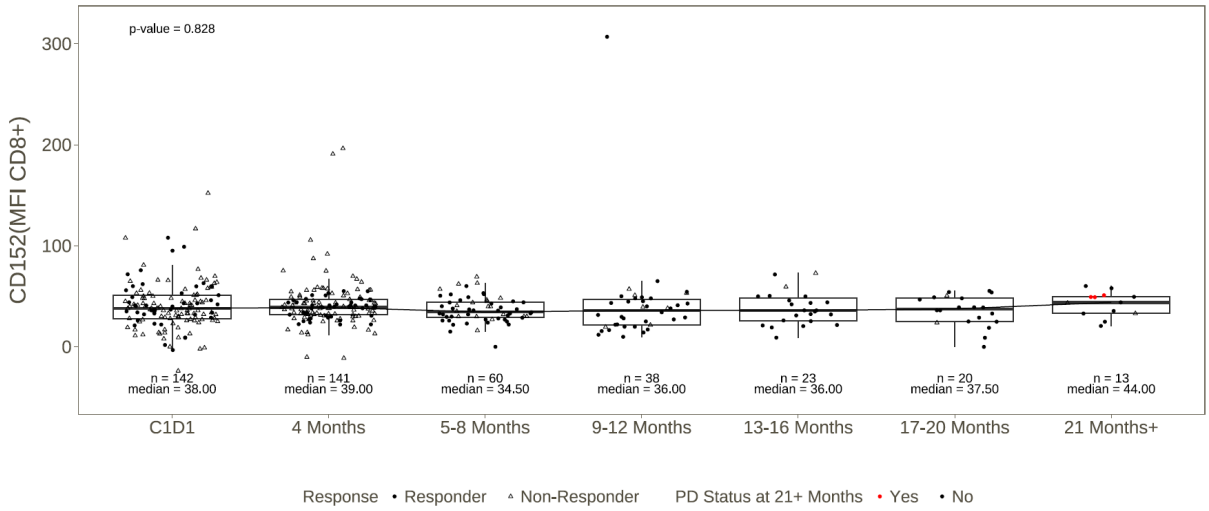
*Data are from the DREAMM-5 study of belantamab mafodotin with nirogacestat.

MFI, median fluorescence intensity; NK, natural killer; PD-1, programmed cell death protein 1; TIM-3, T-cell immunoglobulin and mucin domain 3; TIGIT, T-cell immunoreceptor with immunoglobulin and ITIM domains.

Supplemental Figure 8. Impact of belantamab mafodotin on T-cell exhaustion marker CTLA-4.*

CD8+ T cells.

Median fluorescence intensity



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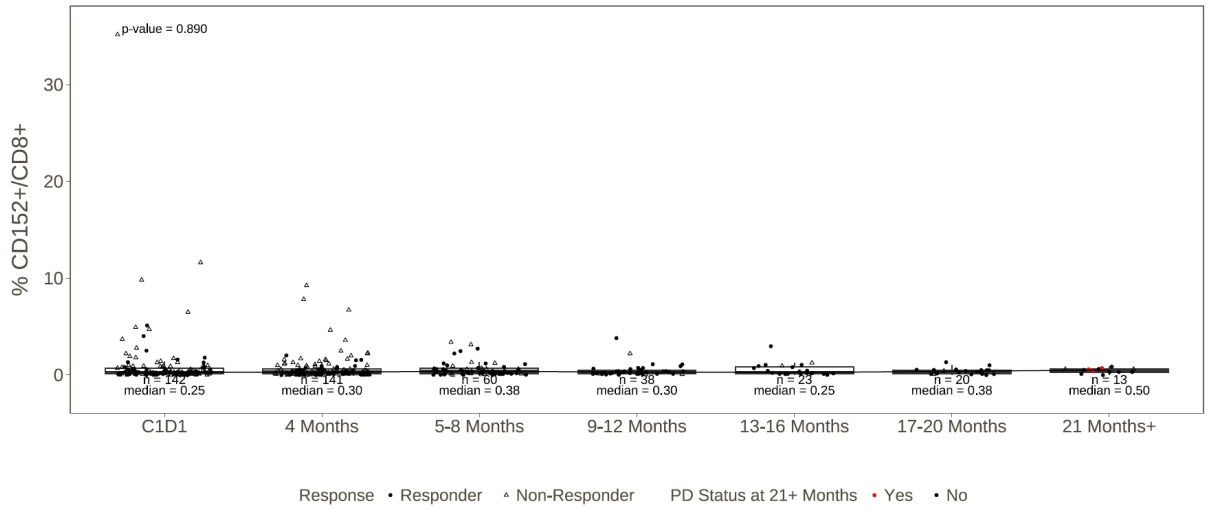
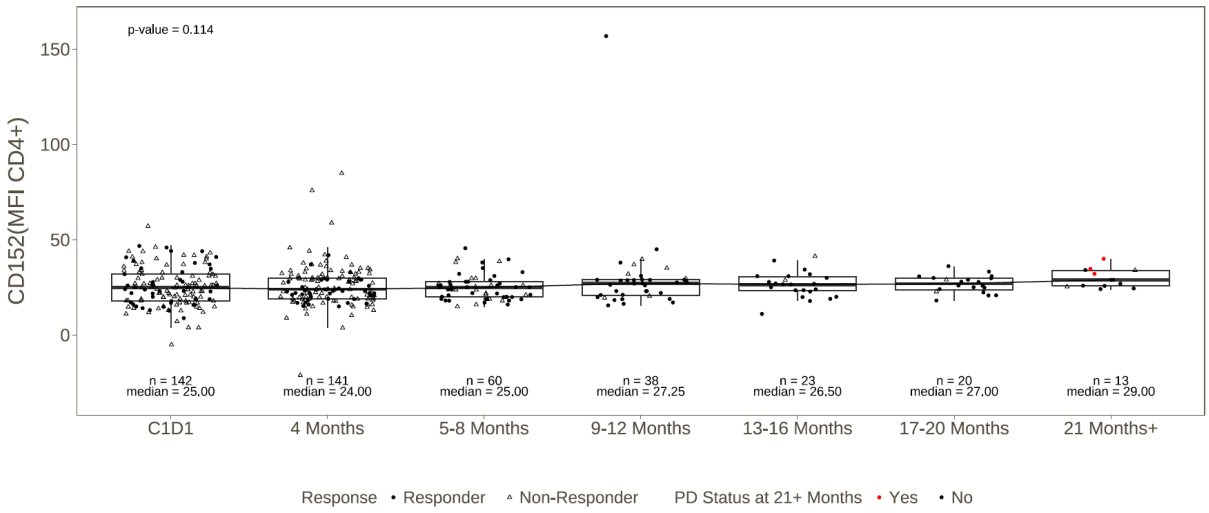


CD4+ T cells

Median fluorescence intensity



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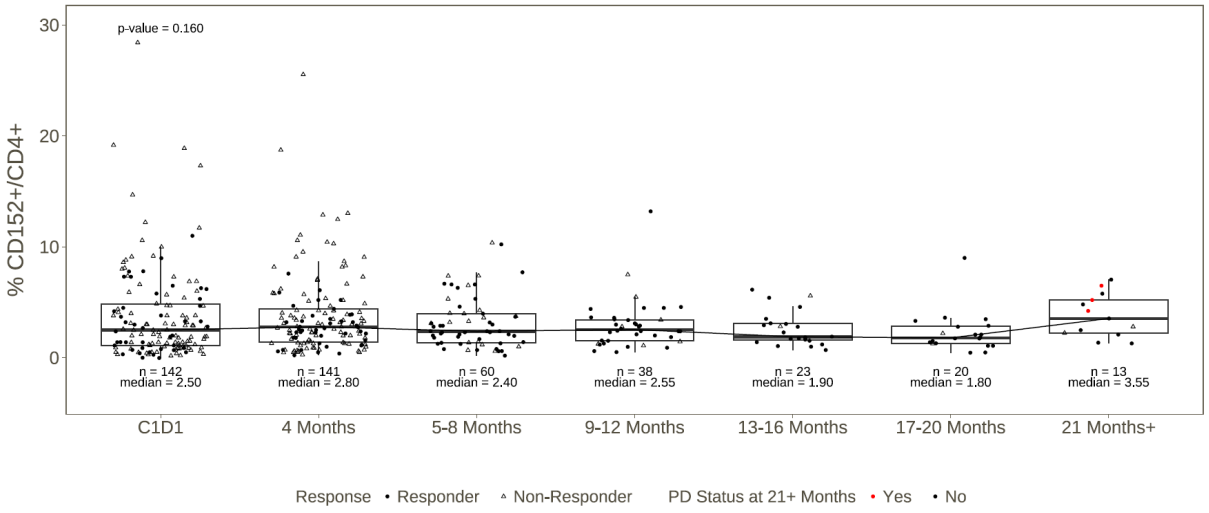
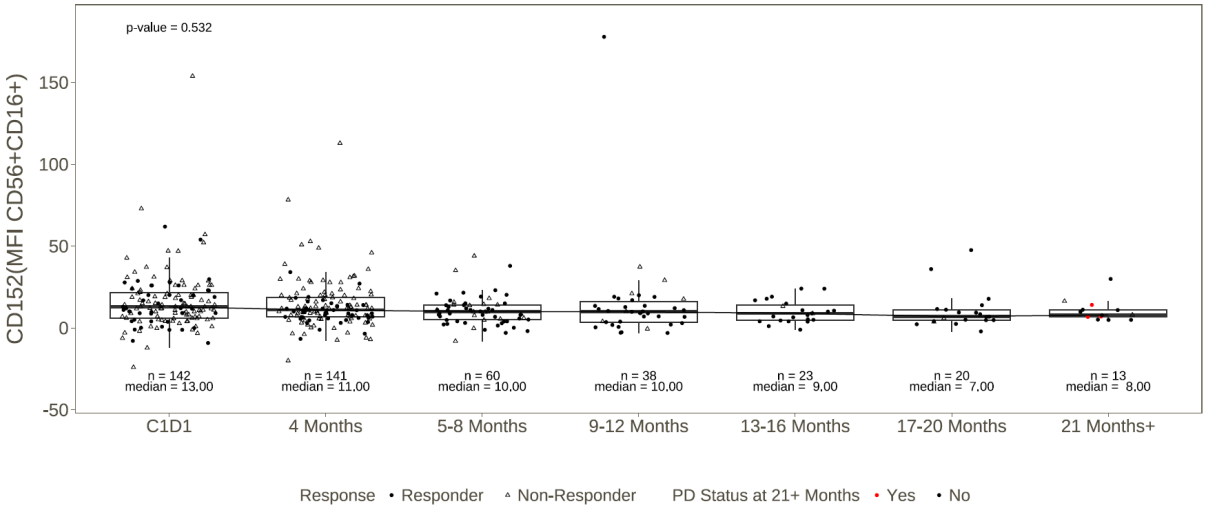


NK cells

Median fluorescence intensity



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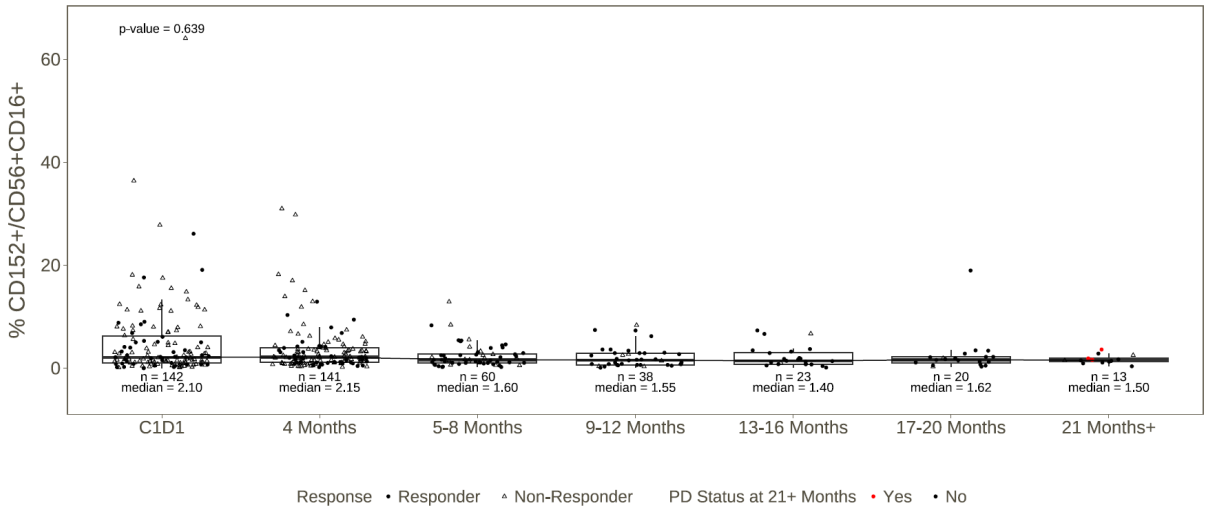


*Data are from the DREAMM-14 study of belantamab mafodotin monotherapy.

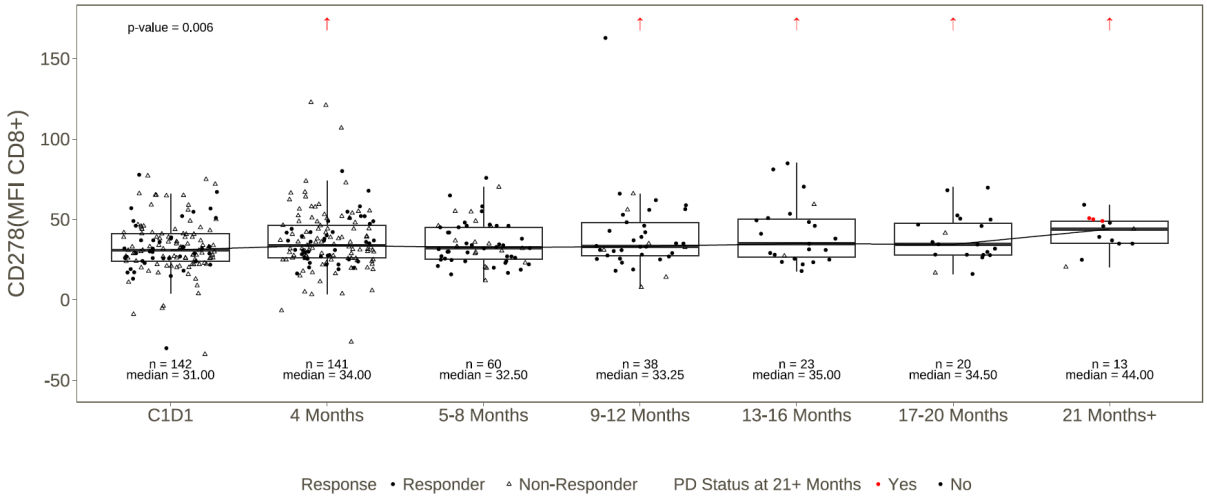
The median of the timepoints within each time category for each patient was utilized.

C1D1, Cycle 1, Day 1; CTLA-4, cytotoxic T-lymphocyte-associated protein; MFI, median fluorescence intensity; NK, natural killer.

Supplemental Figure 9. Impact of belantamab mafodotin on costimulatory marker ICOS*

CD8+ T-cells

Median fluorescence intensity



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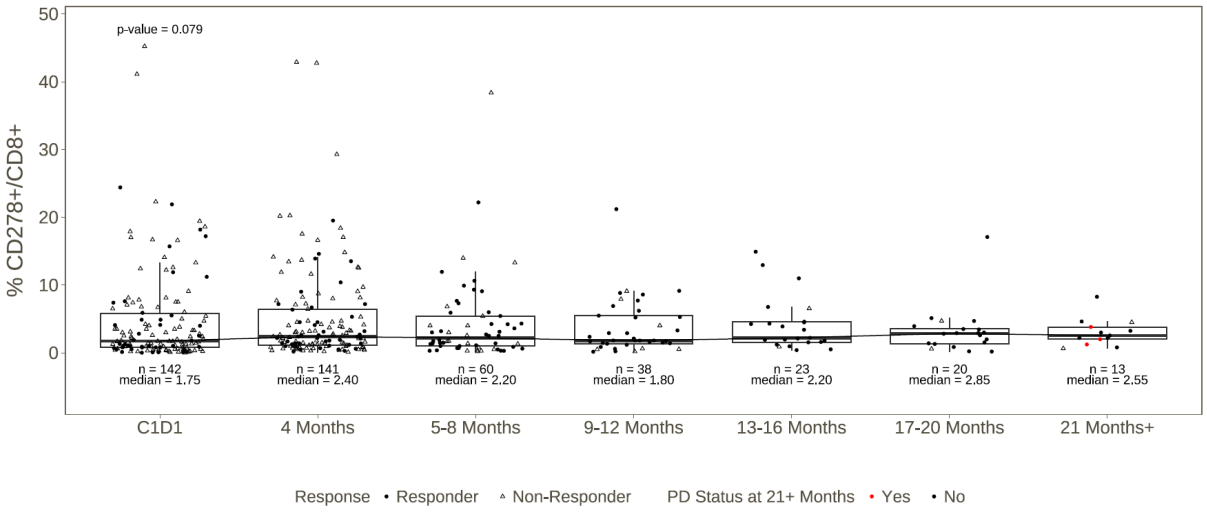
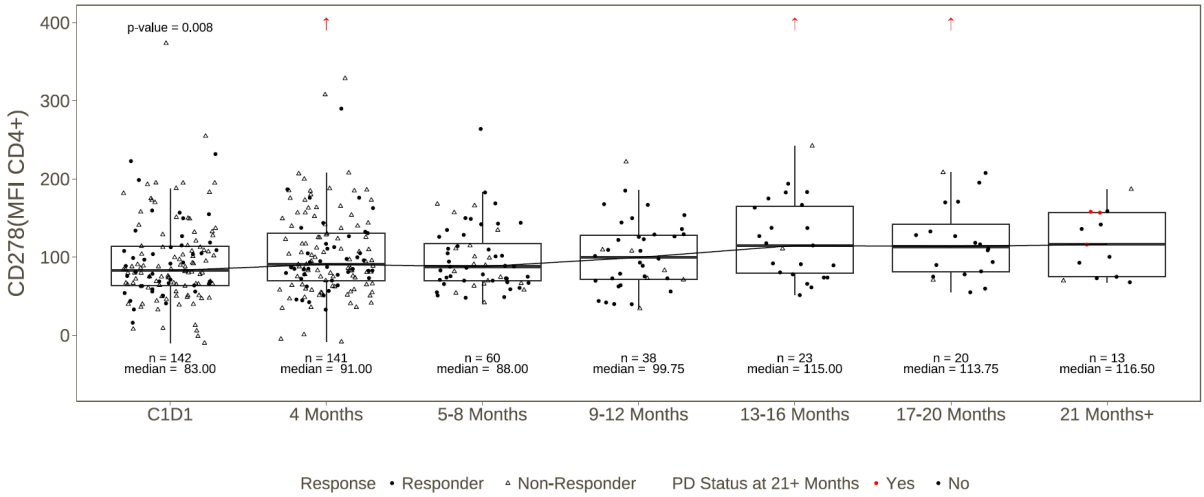


CD4+ T cells

Median fluorescence intensity



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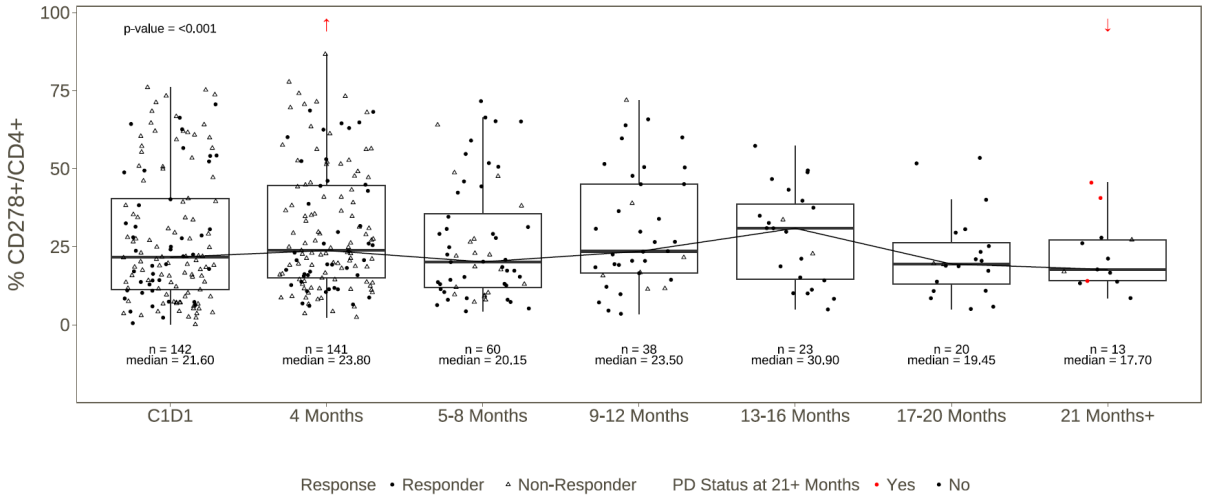
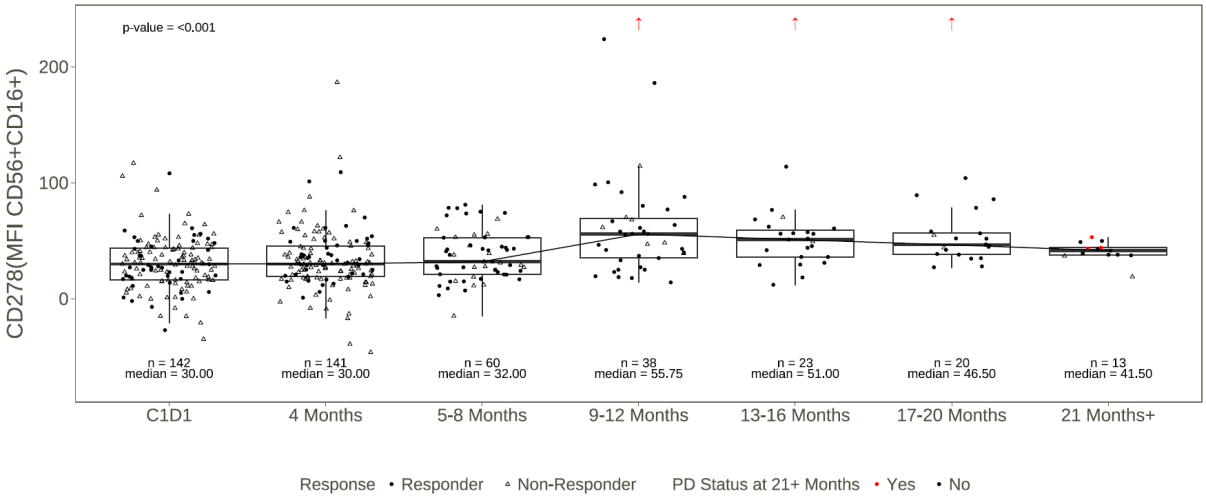


NK cells

Median fluorescence intensity



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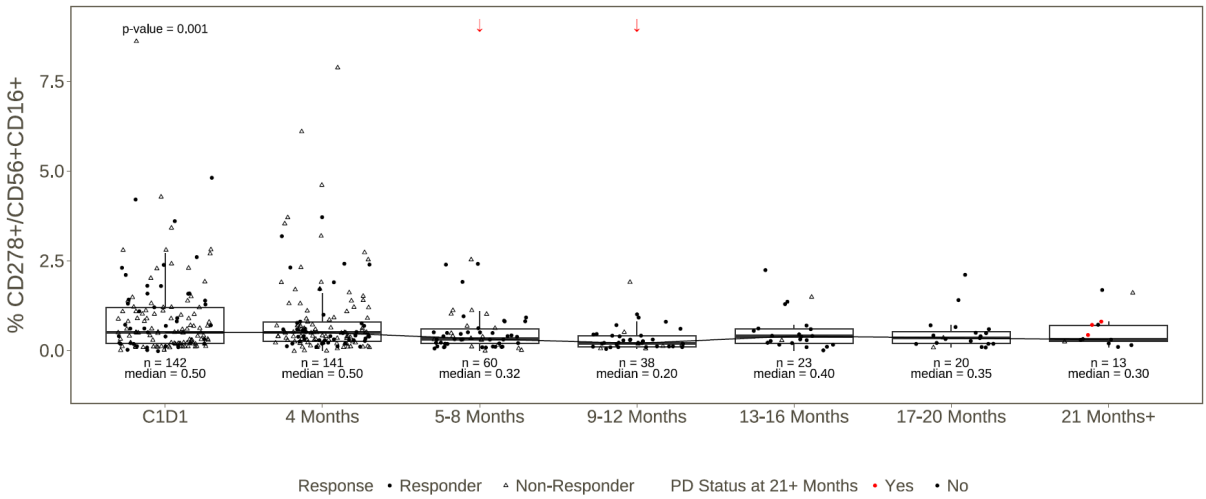


*Data are from the DREAMM-14 study of belantamab mafodotin monotherapy.

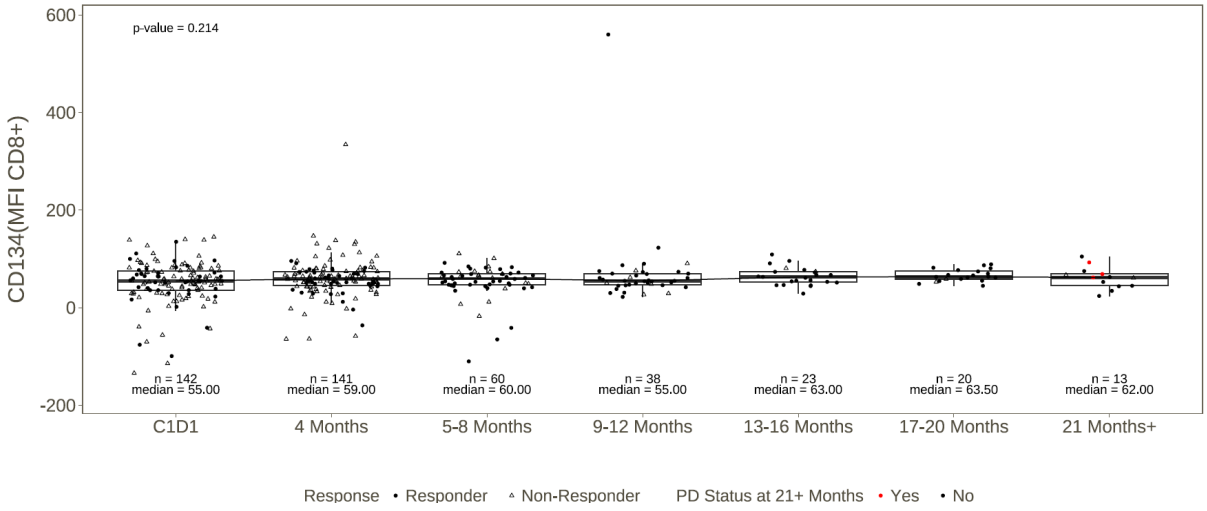
The median of the timepoints within each time category for each patient was utilized. The direction of the red arrows indicates significant increase or decrease from C1D1 for each timepoint.

C1D1, cycle 1 day 1; ICOS, inducible T-cell costimulator; MFI, median fluorescence intensity; NK natural killer.

Supplemental Figure 10. Impact of belantamab mafodotin on costimulatory marker OX40*

CD8+ T cells

Median fluorescence intensity



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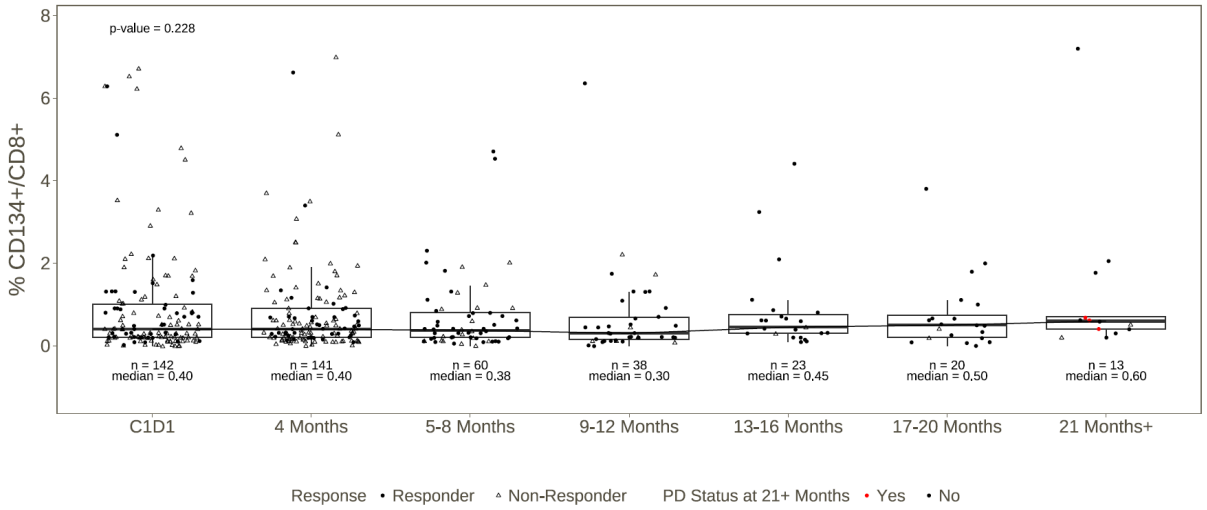
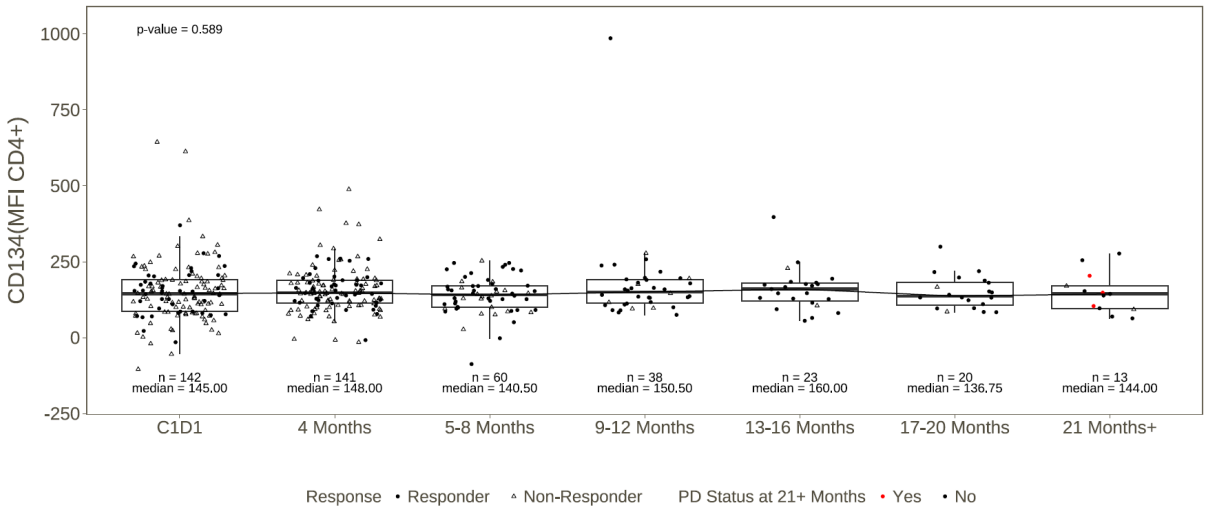


CD4+ T cells

Median fluorescence intensity



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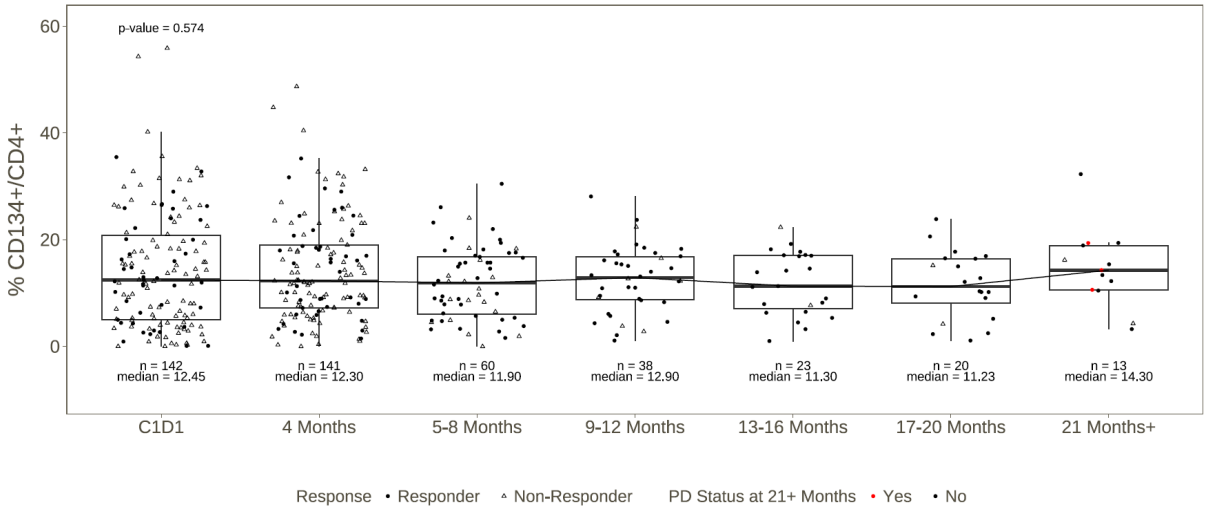
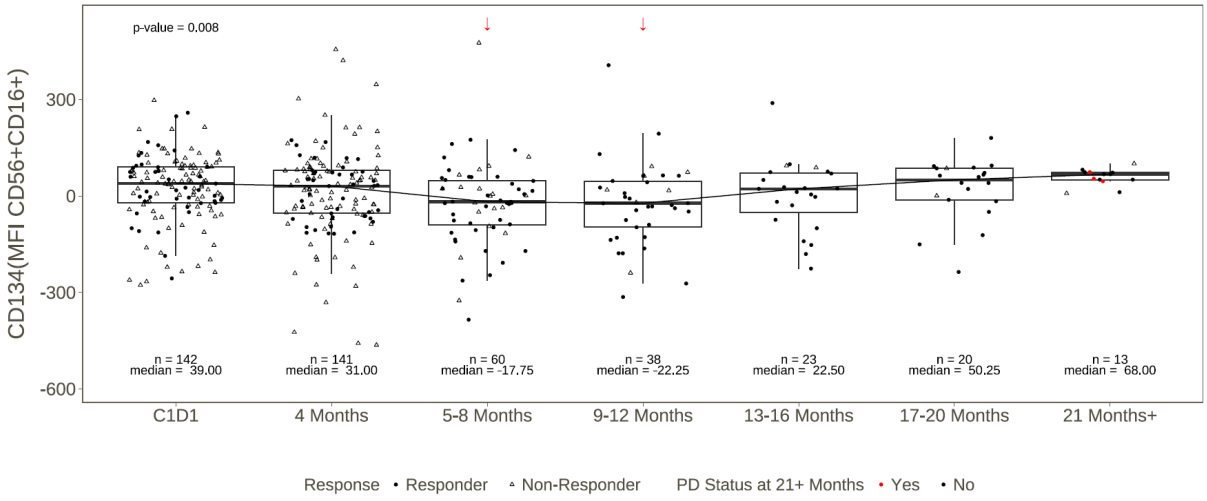


NK cells

Median fluorescence intensity



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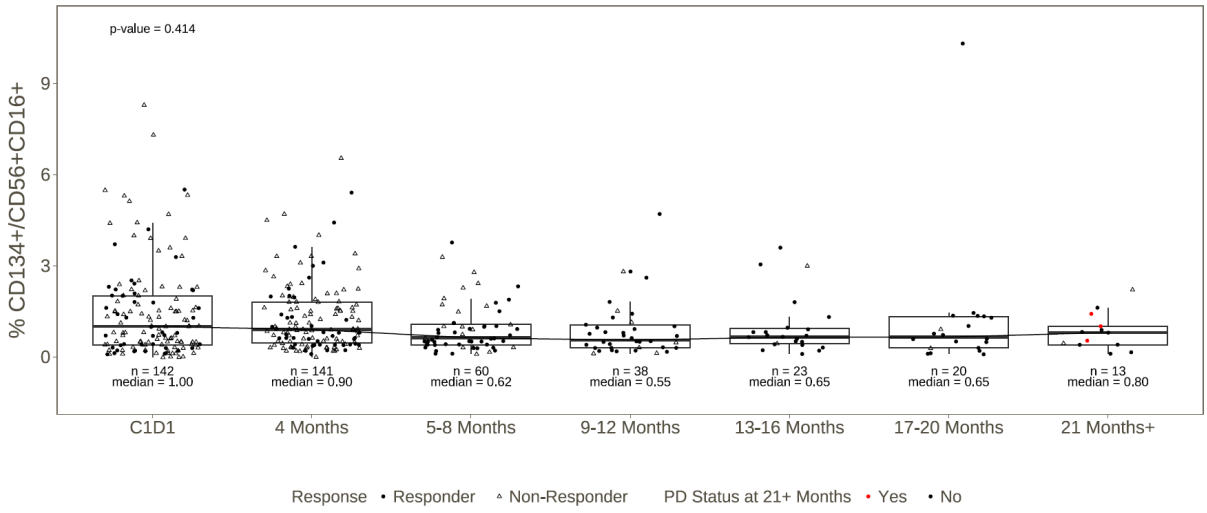


*Data are from the DREAMM-14 study of belantamab mafodotin monotherapy.

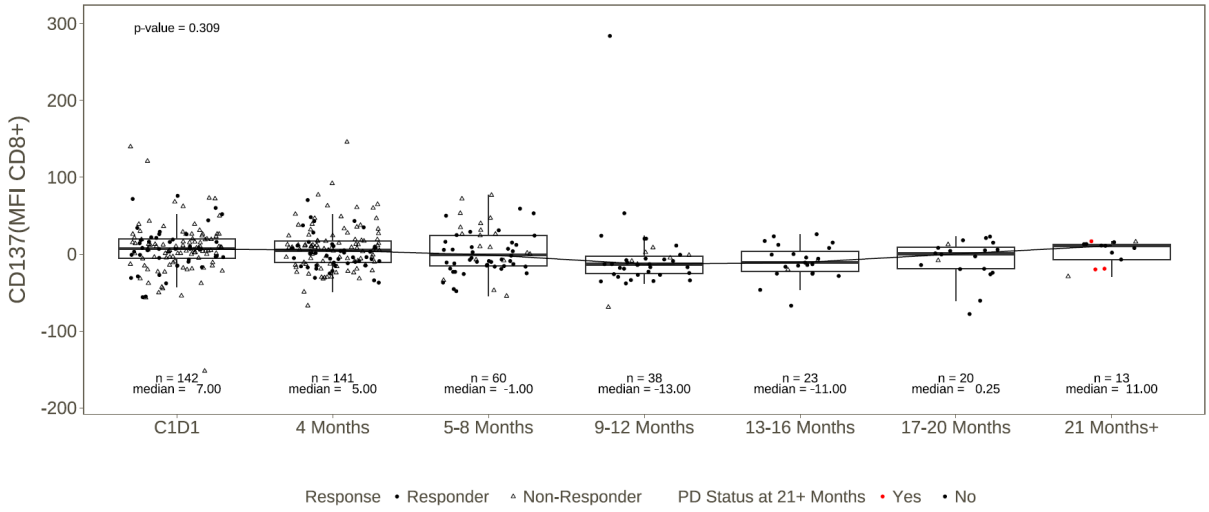
The median of the timepoints within each time category for each patient was utilized. The direction of the red arrows indicates significant increase or decrease from C1D1 for each timepoint.

C1D1, Cycle 1, Day 1; MFI, median fluorescence intensity; NK, natural killer.

Supplemental Figure 11. Impact of belantamab mafodotin on costimulatory marker 4-1BB*

CD8+ T cells

Median fluorescence intensity



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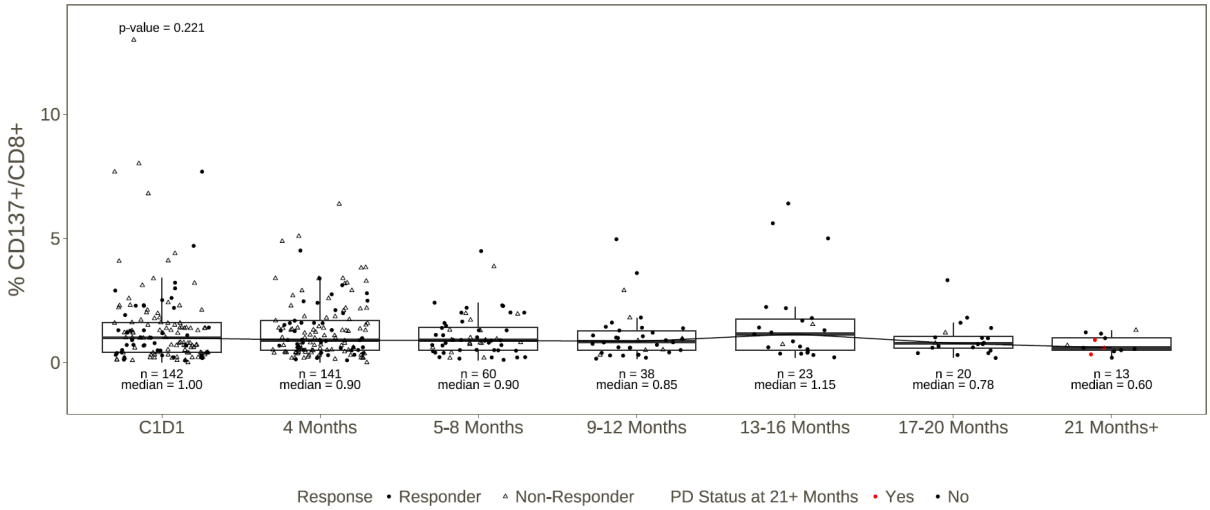
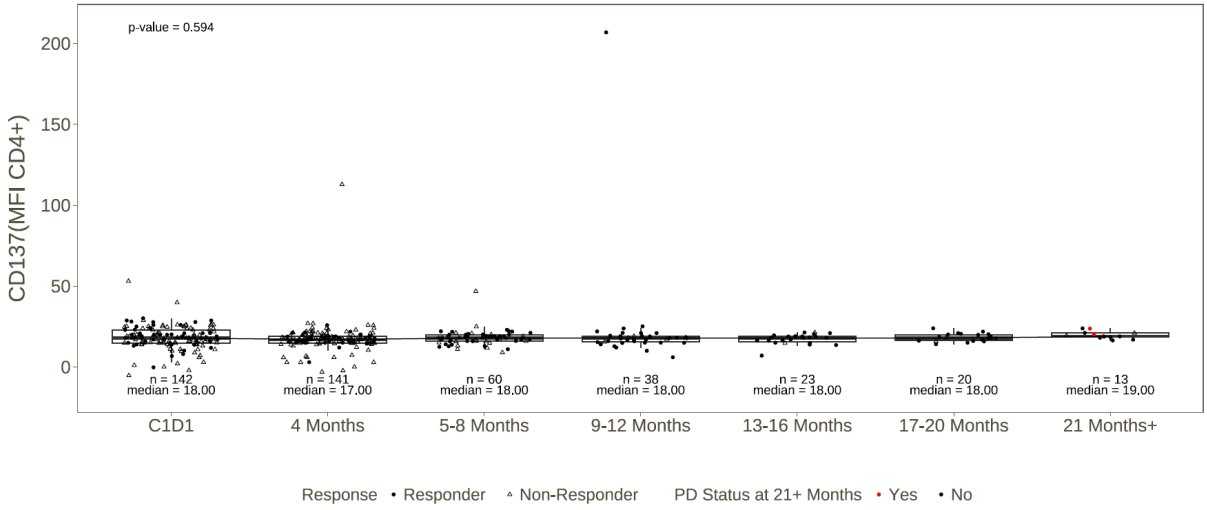


CD4+ T cells

Median fluorescence intensity



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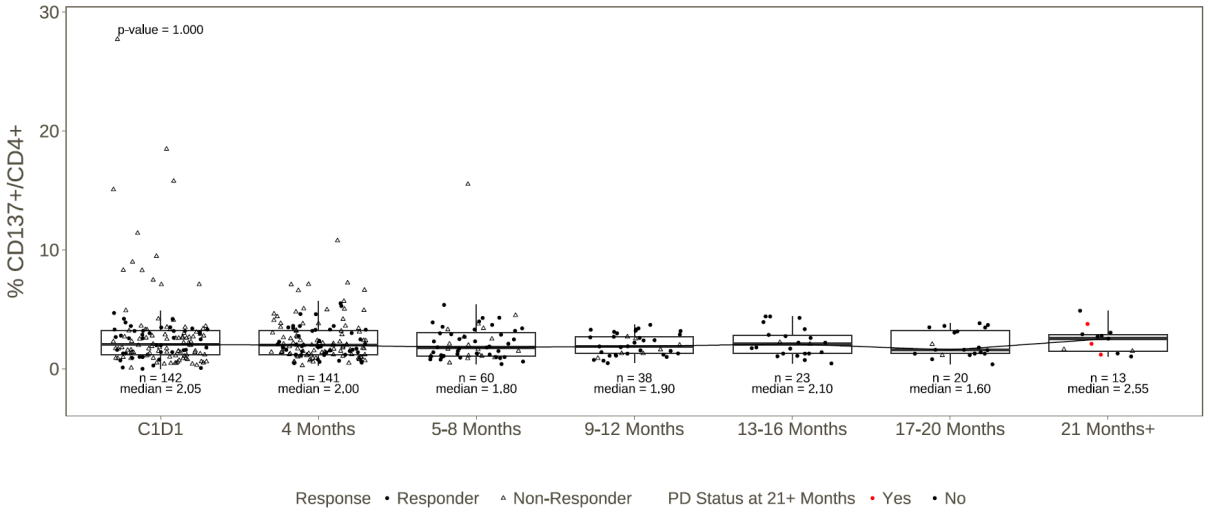
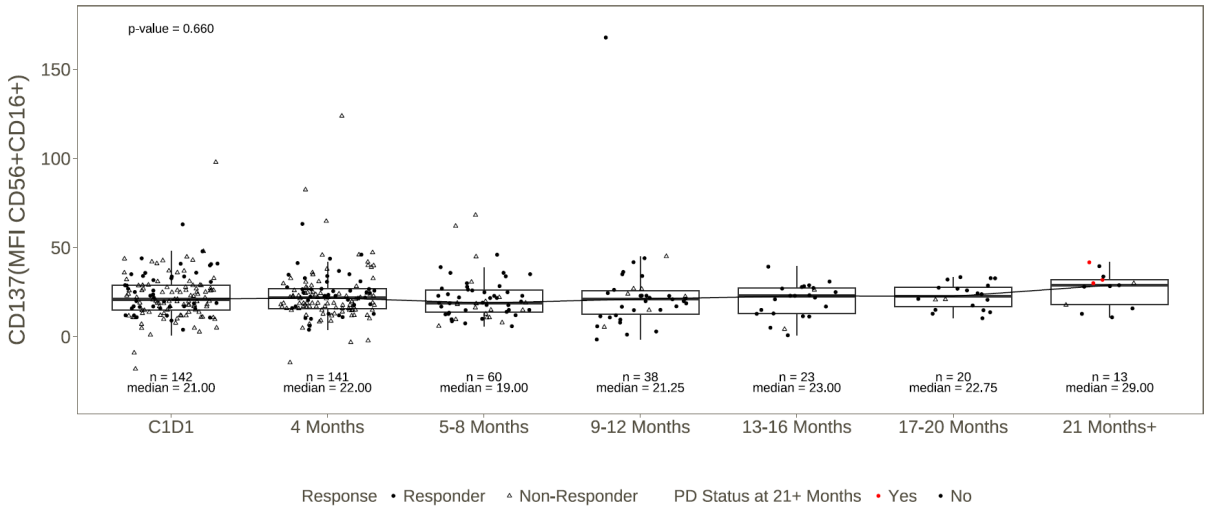


NK cells

Median fluorescence intensity



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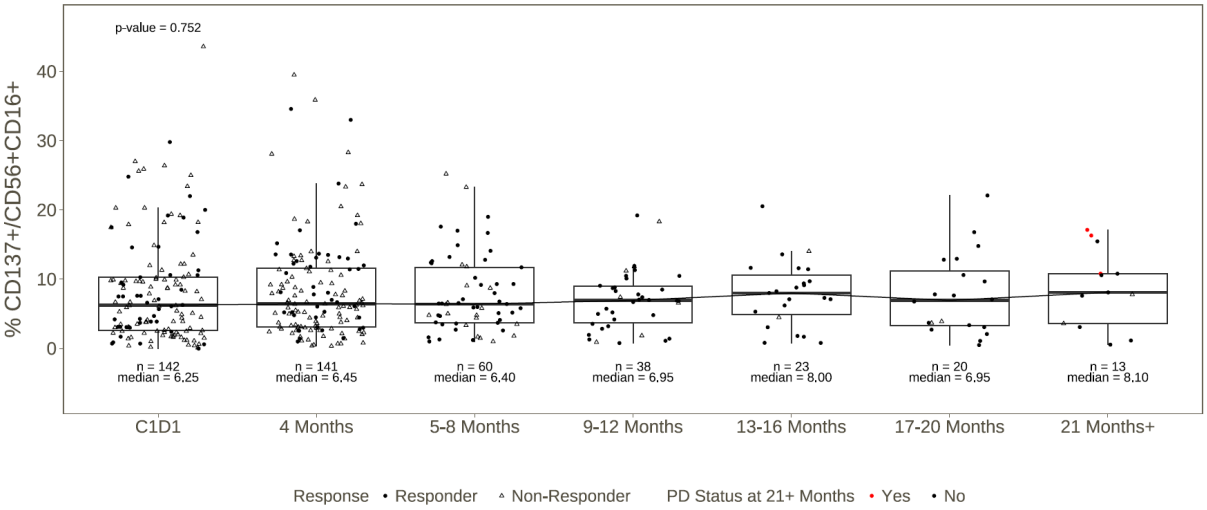
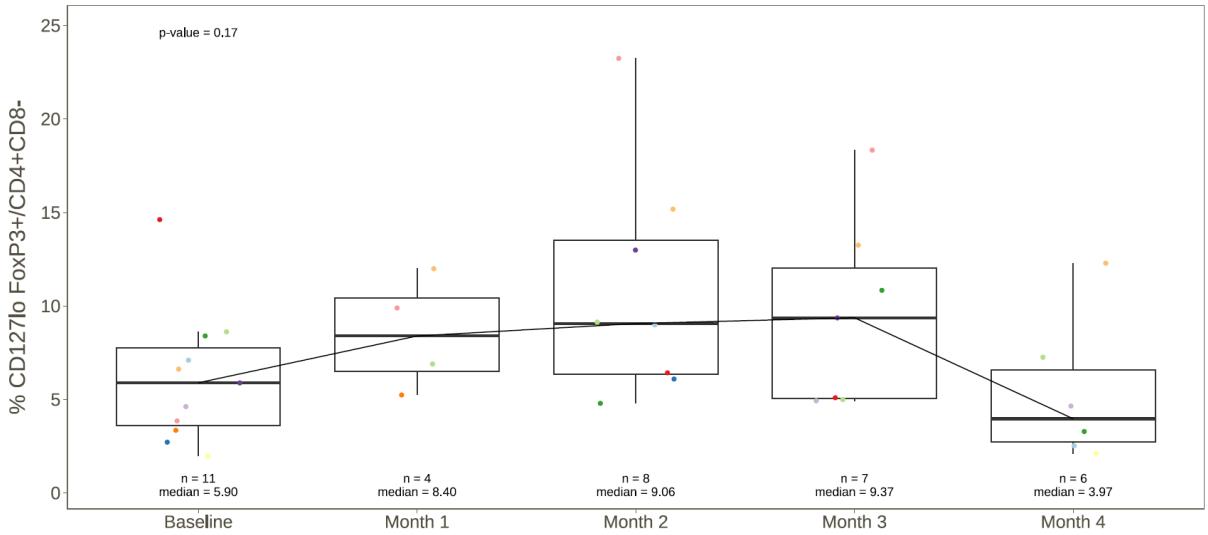


*Data are from the DREAMM-14 study of belantamab mafodotin monotherapy.

The median of the timepoints within each time category for each patient was utilized.

4-1BB, 4-immunoglobulin and cytokine receptor BB; MFI, median fluorescence intensity NK, natural killer.

Supplemental Figure 12. Impact of belantamab mafodotin on the regulatory T-cell population*



*Data are from the DREAMM-5 study of belantamab mafodotin with nirogacestat.