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Running head: No BCMA loss/immune effects post belamaf

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Authorship contributions

H.M. participated in conceptualization, data acquisition, data analysis, and data interpretation; **M.M** participated in data interpretation; **S.T** participated in data interpretation; **K.W** participated in data interpretation; **T.M-D.** participated in data acquisition and data analysis; **G.F-B.** participated in data acquisition and data analysis; **Q.H.** participated in data acquisition and data analysis; **Y.M.** participated in data acquisition and data analysis; **S.P.** participated in data acquisition and data analysis; **S.S.** participated in data acquisition and data analysis; **X.Z.** participated in data acquisition, data analysis, and data interpretation; **P.G.R** participated in data interpretation; **A.D.C** participated in data interpretation; **D.E.L.** participated in conceptualization, data acquisition, data analysis, and data interpretation. All authors reviewed and revised the manuscript, approved the final version, and agreed to submit the manuscript for publication.

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Data sharing statement

Information about GSK's data sharing commitments and access requests to anonymized individual participant data and associated documents can be requested for further research from https://www.gsk-studyregister.com/en/.

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Abstract

Various drug classes target B-cell maturation antigen (BCMA) including chimeric antigen receptor T-cell (CAR-T) therapies, bispecific antibodies (bsAbs), and antibody-drug conjugates (ADCs). Outcomes with CAR-T and bsAb therapies in multiple myeloma (MM) have been affected by T-cell exhaustion, and abrogated expression/mutation of the BCMA target has been observed with anti-BCMA therapies. Optimal anti-BCMA sequencing strategies are needed to improve long-term clinical outcomes. We used data from multiple clinical studies of the ADC belantamab mafodotin (as monotherapy and combination regimens) to explore its impact on BCMA levels and binding (using electrochemiluminescence methodology) and T-cell/natural killer (NK) cell fitness (including cell counts, expression of functional markers), to determine whether belantamab mafodotin could be sequenced ahead of other BCMA-targeting therapies for MM. Levels of free soluble BCMA (sBCMA), measured at the best-confirmed response (BCR) and at progression, dropped at BCR but returned to near baseline at time of disease progression. There was no apparent impact on the binding epitope of BCMA, as indicated by the retention of belantamab mafodotin binding to sBCMA. No significant changes in cell counts or expression of T-cell exhaustion markers (PD-1, TIGIT, TIM-3 [except NK cells], or CTLA-4) and co-stimulatory markers (ICOS [except CD4+ T-cells], OX40, 4-1BB) were observed at relevant timepoints (up to 4 or 21+ months depending on the marker). No negative impact was observed on expression of proliferation (Ki67) and antitumor activity (granzyme B, CD107a) markers. Pending confirmatory studies, our results indicate potential for utilizing belantamab mafodotin ahead of other anti-BCMA therapies in MM.

Keywords: Belantamab mafodotin; B-cell maturation antigen; T-cell exhaustion; multiple myeloma; sequencing

Introduction

Multiple myeloma (MM) is a hematological cancer that follows a relapsing course.[1,2] The treatment landscape has advanced with novel therapies that improve outcomes; however, most patients continue to relapse and remissions become shorter as lines of therapy (LOTs) progress.[1,2] Moreover, with the various drug classes available for MM treatment and the heterogeneity of the patient population with MM, the choice of therapy and sequencing of LOTs has become more complex.[1,2] The use of multi-drug regimens for first-line therapy further complicates treatment decisions in the relapsed/refractory MM (RRMM) setting, with retreatment with the same drug/drug class becoming more prevalent and producing limited clinical benefit.[3] Therefore, effective treatments with novel mechanisms of action are needed after relapse. Several B-cell maturation antigen (BCMA)-targeting treatment modalities have shown high clinical benefit and/or activity in RRMM,[4] which may help address the issue of retreatment and reduced efficacy in this setting.

BCMA is overexpressed on MM plasma cells, and its activation leads to cell survival and proliferation.[4,5] When membrane-bound BCMA (mBCMA) is shed, soluble BCMA (sBCMA) is circulated (and can be used as a surrogate for mBCMA).[4,6] Higher levels of sBCMA are inversely correlated with clinical outcomes and response to future treatment in MM,[4] suggesting that sBCMA, like M-protein, is a potential prognostic factor and biomarker for MM disease burden.[4,5] Minimal BCMA expression on tissues other than plasma cells makes it an ideal target for MM treatment.[7] BCMA-targeting agents available or being evaluated for MM include chimeric antigen receptor T-cell (CAR-T) therapies, bispecific antibodies (bsAbs), and antibody-drug conjugates (ADCs).[5]

The anti-BCMA ADC belantamab mafodotin has been investigated as monotherapy, demonstrating consistent overall response rates (ORR) of 32–41% at 2.5 mg/kg in heavily pretreated RRMM, and in combination regimens for MM, showing significant progression-free survival (DREAMM-7 and DREAMM-8 studies) and overall survival (DREAMM-7) benefits in the combination regimens.[8] Belantamab mafodotin comprises a humanized, afucosylated, IgG antibody conjugated to the microtubule inhibitor monomethyl auristatin F, and its antitumor effects occur through immunogenic cell death, direct cytotoxicity, antibody-dependent cellular cytotoxicity, and antibody-dependent cellular phagocytosis.[7,9] With the emergence of BCMA-targeting CAR-T, bsAb, and ADC therapies, it is imperative to understand

factors that can impact their efficacy, including whether the use of a given anti-BCMA drug class impacts the use of subsequent alternative anti-BCMA drug classes.

All three anti-BCMA treatment modalities rely on target presence; however, outcomes with both CAR-T and bsAb therapies are also dependent on baseline immune fitness.[10-13] That is, patients with higher levels of naïve/effector T-cells and lower levels of regulatory T-cells and inhibitory receptors/T-cell exhaustion markers (programmed cell death protein-1 [PD-1], T-cell immunoreceptor with immunoglobulin and tyrosine-based inhibitory motif [TIGIT], T-cell immunoglobulin and mucin domain 3 [TIM-3], and cytotoxic T-lymphocyte-associated protein 4 [CTLA-4]) have better outcomes.[10-13] Conversely, reduced immune fitness and the emergence of T-cells with an exhausted phenotype, potentially due to chronic activation, characterize non-responders in patients treated with teclistamab[11] and are common features of patients previously treated with CAR-T or bsAbs.[10,13-15] Although infrequent, antigen escape can occur after BCMA-directed treatment, resulting in the emergence of MM clones with target-antigen loss/mutation and reduced responses to subsequent anti-BCMA therapies.[12,16-18] Studies have examined response rates of patients exposed to >1 BCMA-targeting modality. Responses have been observed with teclistamab or ciltacabtagene autoleucel treatment in patients previously exposed to BCMA-directed CAR-T or bsAb therapies, or belantamab mafodotin.[19,20] Of note, the evidence available regarding exposure to ≥1 anti-BCMA agent is limited by small patient numbers, late LOTs, and use of other LOTs between the BCMA therapies, all of which complicate interpretation.

Potential resistance to anti-BCMA therapies over time highlights a need for BCMA-directed treatments that do not impact immune fitness or the ability of subsequent anti-BCMA therapies to bind to BCMA on MM cells. Additionally, there is a need to explore sequencing to determine if there is benefit to multiple BCMA-targeting therapy lines, whether they can be used consecutively, and what the optimal sequence would be. We assessed the impact of belantamab mafodotin on the target and immune fitness, and thus its potential to influence subsequent BCMA-targeting modalities by using translational data to evaluate BCMA levels and immune cell function during and after belantamab mafodotin treatment in patients with MM.

Methods

Studies

This is a post-hoc analysis of data from studies of belantamab mafodotin as monotherapy (phase 1 DREAMM-1 [NCT02064387], phase 2 DREAMM-2 [NCT03525678], phase 3 DREAMM-3 [NCT04162210], phase 1 DREAMM-12 [NCT04398745], phase 2 DREAMM-14 [NCT05064358]) or combinations (phase 1/2 DREAMM-5 [belantamab mafodotin with nirogacestat; NCT04126200], phase 1/2 DREAMM-6 [belantamab mafodotin with lenalidomide and dexamethasone or with bortezomib and dexamethasone; NCT03544281], phase 3 DREAMM-7 [belantamab mafodotin with bortezomib and dexamethasone; NCT04246047], phase 3 DREAMM-8 [belantamab mafodotin with pomalidomide and dexamethasone; NCT04484623]) in patients with RRMM and in patients with newly-diagnosed MM (phase 1 DREAMM-9 [belantamab mafodotin with bortezomib, lenalidomide, and dexamethasone; NCT04091126]).[21-29] All studies complied with the Declaration of Helsinki and Good Clinical Practice guidelines. Trial protocols/amendments were approved by appropriate ethics bodies at participating institutions, and patients provided written informed consent.

Target antigen expression and binding

Free and complexed sBCMA

Free and complexed sBCMA were analyzed using electrochemiluminescence methodology (**Supplemental Materials**), to evaluate belantamab mafodotin binding to sBCMA and determine whether the binding epitope is altered at progression. Software/calculations used for analyses of free/complexed sBCMA are described in the **Supplemental Materials**.

Membrane-bound BCMA

Formalin-fixed and paraffin-embedded bone marrow biopsy samples were examined for BCMA and CD138 expression. Hematoxylin and eosin-stained tissue was used to identify areas with malignant plasma cells for immunohistochemistry (IHC)-based assays, at Mosaic laboratories (Lake Forest, CA) in accordance with Mosaic Laboratories' standard operating procedures. Slides were scanned using an Aperio AT Turbo system (Aperio, Vista, CA); staining was evaluated by a pathologist. Evaluation of

reactivity involved the pathologist tumor cell score indicating the level of cellular staining present and H-Score indicating intensity of staining (scoring described in **Supplemental Materials**).

Binding of belantamab mafodotin monoclonal antibody and teclistamab

Qualitative biolayer interferometry (BLI) was used to assess binding and pairing of GSK2857914 (belantamab mafodotin without the cytotoxic payload) and teclistamab with BCMA antigens (**Supplemental Methods/Supplemental Table 1**).

Immune fitness

Neutrophil-to-lymphocyte ratio (NLR) and absolute lymphocyte counts (ALC) were analyzed using complete blood count and differential counts (calculations described in **Supplemental Materials**).

T-cells and natural killer (NK) cells were analyzed using flow cytometry as described in the **Supplemental Material** (**Supplemental Table 2, Supplemental Figure 1**). T-cell, B cell, monocyte, and NK cell activation markers in whole blood were also evaluated using two separate flow cytometry assays (antibodies and fluorochromes presented in **Supplemental Table 2**). Levels (median fluorescence intensity and percent expression) of T-cell functional markers were evaluated. These included the T-cell suppression markers PD-1, TIGIT, TIM-3, and CTLA-4, the costimulatory receptors inducible T-cell co-stimulator (ICOS), OX40, and 4-immunoglobulin and cytokine receptor BB (4-1BB),[30,31] and the intracellular and intranuclear activation markers Ki67 (proliferation marker), Granzyme B (marker of T-cell and NK cell antitumor activity), and CD107a (NK cell degranulation marker).[32] Sample preparation, cell surface and intracellular staining, and flow cytometry analysis were performed as described in the **Supplemental Materials/Supplemental Figure 2**. Generalized least-squares or mixed models were used to model longitudinal data for flow cytometry. Samples were taken from patients across all studies assessed.

Results

Impact of belantamab mafodotin on target antigen expression

As BCMA expression is essential to successful treatment with anti-BCMA therapies, levels of mBCMA prior to treatment with belantamab mafodotin were assessed (**Figure 1**) and showed that mBCMA was consistently measurable independent of number of prior LOTs (n=492 across 1 to ≥7 prior LOTs

[p=0.5965]). Baseline mBCMA levels were not significantly different between LOTs. This suggests that BCMA is a good target in patients with MM, regardless of the therapy line.

In addition to evaluating BCMA levels prior to belantamab mafodotin treatment, we evaluated sBCMA levels (a surrogate for BCMA expression) during treatment and after progressive disease (PD) to determine if there was BCMA loss, which could impact subsequent anti-BCMA agents. Due to the competitive binding of belantamab mafodotin with the reagents used to assess free sBCMA, selected timepoints were prior to dosing or >4 days post-dose. Among patients who had progressed at the time of this analysis, sBCMA was detectable at baseline in nearly all monotherapy-treated patients (100% [n=75] in DREAMM-1 and 99.1% [n=213] in DREAMM-2) and in all combination therapy-treated patients in DREAMM-7 and DREAMM-8 (n=32 each); at PD, 51 (98.0%) DREAMM-1, 183 (98.9%) DREAMM-2, 32 (100%) DREAMM-7, and 32 (100%) DREAMM-8 patients had detectable sBCMA (**Supplemental Table 3**). Among patients who had progressed at the time of this analysis and had sBCMA levels available at baseline, best confirmed response, and progression, sBCMA levels dropped at the time of response to belantamab mafodotin monotherapy (n=63) and the belantamab mafodotin, bortezomib, and dexamethasone (BVd) combination (n=32) but remained measurable and returned to near baseline at the time of PD (p=0.0169 [monotherapy] and p=0.1582 [BVd] for comparison between baseline and progression levels, **Figure 2 and Supplemental Figure 3**). Similarly, sBCMA was measurable at progression in patients who had progressive disease by the time of this analysis after receiving belantamab mafodotin, pomalidomide, and dexamethasone (BPd), and the median change from baseline was similar to that of the comparator (pomalidomide, bortezomib, and dexamethasone [PVd]) (**Supplemental Figure 4**). Optional progressive disease bone marrow samples were only successfully collected from 3 patients across the studies. IHC images of BCMA/CD138 expression from these 3 patient samples showed that mBCMA levels were measurable at complete response (CR) and at PD in BPd-treated patients (n=2) and PVd-treated patients (n=1) (**Figure 3**).

Overall, the sBCMA and mBCMA results together indicate essentially no BCMA target loss after belantamab mafodotin monotherapy or combination therapy, with mBCMA expression and median change in sBCMA levels after BPd comparable to the standard of care therapy (PVd). Furthermore, sBCMA levels followed a similar trend as mBCMA, remaining measurable after belantamab mafodotin treatment.

Impact of belantamab mafodotin on target binding site

Distinct mechanisms underlying MM antigen escape post-anti-BCMA therapy are slowly emerging.[16,17] Not only is the presence of the target fundamental to BCMA-targeting therapies, but binding capacity and potential impact of mutations on the target are important to understand. We explored the impact of belantamab mafodotin on its binding epitope by generating complexed sBCMA (belantamab mafodotin bound to sBCMA) concentration data. Complexed sBCMA levels increased markedly and were maintained throughout belantamab mafodotin treatment (with and without nirogacestat [n=3–35 across timepoints]; **Figure 4A**), including up to the timepoint at which progressive disease was observed (n=20 patients who progressed; **Figure 4B**), and regardless of response status. Data suggest that there is no apparent impact on the belantamab mafodotin BCMA binding epitope upon PD.

To inform whether the apparent lack of impact on the belantamab mafodotin BCMA binding epitope translates into lack of impact on the binding epitope of the bsAb teclistamab, binding analysis was performed. Binding experiments showed that both GSK2857914 and teclistamab independently bind to histidine tagged BCMA (His-BCMA). Pairing experiments showed that a saturated biotinylated GSK2857914:BCMA complex was formed for both His-BCMA and recombinant human BCMA (rhBCMA) over the second loading period. Further exposure of these immobilized complexes to teclistamab indicated little to no interaction (**Supplemental Figure 5**). This suggests that GSK2857914 and teclistamab share similar or overlapping epitopes on the BCMA antigen. Together with the complexed sBCMA data that showed belantamab mafodotin binding across timepoints, these data support that teclistamab would likely not be impacted by prior belantamab mafodotin treatment.

Impact of belantamab mafodotin on immune fitness

Immune fitness is fundamental to the success of CAR-T and bsAb therapies[10-13]; as such, it is essential to determine what impact belantamab mafodotin has on the immune cells that drive their mechanisms of action. Initially, global immune fitness was examined. Complete blood counts with differential showed that ALC and NLR were not significantly altered throughout 73 weeks of treatment with belantamab mafodotin monotherapy in DREAMM-2 (n=2–208 across timepoints; p=0.306 for ALC and p=0.898 for NLR; **Figure 5A, B**), and when evaluated by responders (n=2–77 across timepoints) and non-responders (n=2–131), there was no difference in ALC or NLR (p=0.836 for ALC and p=0.997 for NLR for responders versus non-responders). In addition, CD8+ (p=0.284) and the ratio of CD4+ to CD8+ cells (p=0.103) was

not impacted by belantamab mafodotin treatment (n=10–139 across timepoints); CD4+ counts were significantly increased after 21+ months of therapy (p=0.031) (**Figure 5C–E**).

Next, we focused directly on factors affecting peripheral immune profiles of CD4+ T-cells, CD8+ T-cells, and NK cells, which can impact CAR-T and bsAb mechanisms of action. CD4+/CD8+ T-cell profiles were not significantly altered over the course of belantamab mafodotin treatment when evaluated using data from DREAMM-5 (combination; n=4–11 across timepoints) and DREAMM-14 (monotherapy; n=13–142 across timepoints). At relevant timepoints (i.e., the final timepoints assessed), these cells showed no increase in percent expression from baseline in the T-cell exhaustion markers PD-1, TIGIT, TIM-3, or CTLA-4, nor any decrease in costimulatory markers ICOS, OX40, or 4-1BB, with the exception of a significant increase in TIM-3 expression on NK cells ($p<0.001$; median at baseline, 44%; median at 21+ months, 71%) and a significant decrease in ICOS expression on CD4+ cells ($p<0.001$; median at baseline, 22%; median at 21+ months, 18%) (**Figures 6 and 7**, **Supplemental Figures 6–11)**. There was also no significant increase in the regulatory T-cell (T_{reg}) population (**Supplemental Figure 12**). CD4+ T-cells, CD8+ T-cells, and NK cells retained their immune cell activity during treatment with belantamab mafodotin, as evidenced by no significant changes in percent expression of Granzyme B in all three cell types and by no significant change in CD107a expression in NK cells (**Figure 8**). Both CD4+ and CD8+ T-cells retained their proliferative capacity, with the percentage of cells expressing Ki67 remaining similar to baseline throughout treatment.

Taken together, the immune fitness data demonstrate that belantamab mafodotin generally has no impact on total lymphocyte and T-cell numbers, CD4/CD8 T-cell ratio, or expression of phenotypic markers associated with poor responses to CAR-T and bsAb therapies.

Discussion

BCMA-targeting CAR-T, bsAb, and ADC treatments have fundamentally different mechanisms of action, and studies suggest that the effects of anti-BCMA therapies on target expression and immune cell composition may impact the efficacy of subsequent BCMA-targeting agents.[12,16,17] Therefore, it is important to consider sequencing of these treatments to optimize patient outcomes. Using data from multiple DREAMM studies, we examined the impact of belantamab mafodotin on the BCMA target and

immune cells during and after treatment in patients who received belantamab mafodotin, to explore factors that might impact sequencing of belantamab mafodotin prior to bsAb or CAR-T cell therapy.

Several studies have shown that treatment with BCMA-directed T cell-engaging therapies may affect BCMA expression through genomic deletion or loss of functional recognition and binding[17,33,34]; this may particularly be an issue after bsAb therapy, where loss or alteration of BCMA was noted in 43% of progressing patients in a recent study.[17] In contrast, genomic changes resulting in BCMA loss after belantamab mafodotin have been reported in only a single patient to date.[16] In the current study with a larger patient population, we evaluated the impact of belantamab mafodotin on BCMA levels using reagents that compete for the BCMA binding site with belantamab mafodotin, and found that sBCMA levels were reduced at best confirmed response, likely reflecting the reduction of malignant plasma cells, and returned to near-baseline levels at PD. sBCMA levels at PD may not have fully returned to baseline due to lower tumor burden or early detection of progression. The recovery to near-baseline sBCMA levels indicates that belantamab mafodotin treatment does not result in complete loss of target, that complete target loss is unlikely to be the primary mechanism of escape from belantamab mafodotin, and consequently, that other BCMA-directed therapies will still be able to bind BCMA on MM cells after treatment with belantamab mafodotin; however, further analyses are required to determine whether mechanisms exist that would lead to downregulation of BCMA in a subclonal population of cells in patients treated with belantamab mafodotin. In addition to no complete loss of target, we found evidence that belantamab mafodotin binds to sBCMA throughout the course of treatment, including when patients progress, suggesting that there was no apparent impact on the binding site of belantamab mafodotin. As the greatest selective pressure on MM cell clones would be expected at the belantamab mafodotin binding epitope during treatment, the apparent lack of change detected in this region suggests that the binding sites of other anti-BCMA therapies are unlikely to be impacted following belantamab mafodotin treatment.[33-36] While we also found that BPd-treated patients had measurable mBCMA levels at CR and at PD, it is important to acknowledge the potential presence of BCMA mutations that could impact belantamab mafodotin binding.[17,34] As such, the reliance on belantamab mafodotin binding to sBCMA and IHC samples from a small number of patients is a limitation of this analysis, and sequencing or structural modeling analyses of BCMA are required to confirm whether belantamab mafodotin induces genomic changes on the target that could impact binding sites of other BCMA-targeting agents. Analyses of complexed sBCMA levels of other anti-BCMA

therapies following belantamab mafodotin relapse are currently ongoing. The preliminary epitope binding data reported here showed that belantamab mafodotin monoclonal antibody and teclistamab share a similar BCMA binding epitope; therefore, no impact on teclistamab binding would be expected following belantamab mafodotin treatment.

Reduced immune fitness and T-cell exhaustion related to prior treatment can negatively impact outcomes with CAR-T and bsAb therapies, and may even be caused by these therapies themselves,[10,11,13-15] which may limit the clinical utility of sequencing these agents as first BCMA-targeting therapy. In our study, immune system impairment was not observed with belantamab mafodotin, as evidenced by no significant changes in ALC or NLR, and at relevant timepoints, no increase in expression of exhaustion markers (PD-1, TIGIT, TIM-3 [except NK cells], CTLA-4), and no decrease in expression of costimulatory markers (ICOS [except CD4+ T-cells], OX40, 4-1BB), activation markers (Granzyme B, CD107a), or proliferation markers (Ki67) on CD4+/CD8+ T-cells and/or NK cells. This suggests that cell counts remain stable during belantamab mafodotin treatment, and cells retain their proliferative and cytolytic capacity; however, additional studies would be beneficial to demonstrate that these remain stable over longer time frames, including at PD, as would be expected for patients treated in earlier LOTs. TIM-3 was the only T-cell exhaustion marker that significantly increased at the final available assessment. While TIM-3 is thought to have an immunosuppressive role in various cancer types,[37] in patients with acute myeloid leukemia, its expression on NK cells was associated with improved effector functions and improved disease outcomes.[38] Further research is required to determine the impact of TIM-3 expression in MM. CD4+ to CD8+ T-cell ratio is an important predictor of MM prognosis, with lower ratios associated with poorer survival.[39] Higher CD4+ to CD8+ ratios are required in the leukapheresis product of CAR-T therapies to optimize CAR-T expansion and response.[40] In our analysis, the CD4+ to CD8+ T-cell ratio was not impacted by belantamab mafodotin treatment, indicating that belantamab mafodotin may not affect CAR-T treatment outcomes. Similarly, the lack of belantamab mafodotin effect on T-cell expression and exhaustion suggests that belantamab mafodotin would not impact subsequent bsAb treatment.[11]

Previous studies have examined the clinical impact of sequencing alternative BCMA-targeting agents following belantamab mafodotin or other anti-BCMA therapy, with varying outcomes reported. In these studies, CAR-T or bsAb treatment after belantamab mafodotin or other anti-BCMA therapy demonstrated potential for response in most patients, but the reported ORR, PFS, and/or duration of

responses were generally lower than that seen in patients without prior anti-BCMA therapy.[19,20,41-45] These studies were limited by small sample sizes, heterogenous and heavily pretreated patient populations, high rates of extramedullary disease in the patient populations studied, and/or multiple LOTs and variable intervals between anti-BCMA treatments, leading to difficulty drawing definitive conclusions. Shorter duration of prior anti-BCMA treatment and a longer interval between the therapies were associated with improved outcomes with CAR-T and teclistamab.[19,20,41,44,46] Specifically, an interval of ≥9 months between prior anti-BCMA therapies and teclistamab resulted in greater response rates and PFS than shorter intervals,[45,46] while patients recently treated with belantamab mafodotin (regardless of the timing of prior belantamab mafodotin exposure) had similar teclistamab response rates to patients without prior anti-BCMA therapies.[46] Due to shedding of mBCMA and the half-life of belantamab mafodotin (~14 days), it is not believed that long-term belantamab mafodotin binding to mBCMA is responsible for the lower efficacy observed with anti-BCMA treatment following belantamab mafodotin in some studies.[4,47] It is currently unknown whether there are resistance mechanisms to belantamab mafodotin that may impact outcomes with subsequent anti-BCMA therapies,[8] and future studies investigating resistance mechanisms are required. It is also important to note that CAR-T therapies can potentially be impacted by treatment status at the time of CAR-T manufacturing, which may have affected previous CAR-T sequencing reports. A study indicated that compared to newly-diagnosed patients, CAR-T treatments in patients who relapsed at the time of manufacturing had reduced proliferative and antitumor capacity.[10]

Data from the DREAMM-7 and DREAMM-8 studies indicate that belantamab mafodotin has high efficacy in combination regimens in the second-line-or-later setting.[22,24,48] DREAMM-7 showed a robust PFS benefit (23-month increase in PFS with BVd versus daratumumab plus bortezomib and dexamethasone), with maintenance of the PFS benefit in the next LOT (median PFS2 hazard ratio 0.56 [95% confidence interval 0.41–0.76]) and a subsequent OS benefit.[48] DREAMM-8 also showed a significant PFS benefit for BPd versus PVd, which was maintained into the next line of therapy (hazard ratio 0.61 [95% confidence interval 0.43–0.86]); follow-up for OS is ongoing in DREAMM-8.[22] The long-term effects demonstrated with BVd could indicate that immune-mediated effects of belantamab mafodotin are carried into the next LOT.[8]

Belantamab mafodotin represents a highly accessible anti-BCMA option due to its potential for wide availability and ease of administration.[49] Given the efficacy and manageable safety profile of belantamab

mafodotin combination regimens shown in clinical trials,[22,24,48] and the lack of changes in target expression/binding and immune cell composition demonstrated in our analysis, sequencing belantamab mafodotin as the first BCMA-targeting treatment may offer high clinical efficacy without compromising the use of subsequent BCMA-targeting therapies; however, rigorous prospective clinical studies of anti-BCMA efficacy after belantamab mafodotin treatment are required to support these findings in matched patient populations.

Limitations of our study include small sample sizes for IHC analysis of mBCMA and PD-1/TIGIT flow cytometry data and the lack of data on T-cell function. The immune fitness data may be limited by the use of data from 3 different trials, which included different doses and schedules of belantamab mafodotin, monotherapy and combination regimens, and differing follow-up periods; subgroup analyses in larger study populations and in the frontline setting would be valuable to further examine the impacts of belantamab mafodotin. PD was reached in few patients at the time of this analysis due to the long PFS and duration of response with BVd and BPd,[22,48] thereby limiting the number of PD samples collected for analysis of sBCMA levels.

Follow-up clinical data to determine the impact of belantamab mafodotin on subsequent exposure to a BCMA-directed CAR-T/bsAb therapy are limited. However, a recent study examining subsequent therapies after BVd treatment in DREAMM-7 found that the median time from the start of bsAb exposure (including teclistamab as fourth to seventh LOT) to progression or death was 11.7 months.[50] This is comparable to the median PFS seen with teclistamab in the MajesTEC-1 study,[51] which was 11.4 months at a median follow up of 30.4 months. However, as noted, this dataset is limited and further, more extensive, follow-up clinical studies of belantamab mafodotin in RRMM are required.

To our knowledge, this is the first study to analyze the effects of belantamab mafodotin monotherapy and combination treatments on sBCMA and immune cell profiles across multiple studies in MM. Belantamab mafodotin provides durable and sustained benefit to patients with MM,[22,24] without impairing BCMA expression or binding, nor immune cell composition. Collectively our data suggest that belantamab mafodotin may not directly impact subsequent alternative BCMA-targeting therapies although confirmatory clinical studies are needed.

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Figure 1. Membrane-bound B-cell maturation antigen levels at baseline across lines of therapy.*

*Includes third-line-or-later monotherapy from DREAMM-2, DREAMM-3, DREAMM-12, and DREAMM-14; second-line-or-later combinations from DREAMM-6, DREAMM-7, and DREAMM-8; and a first-line combination from DREAMM-9.

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, whiskers represent the 5th and 95th percentiles, and black circles represent outliers.
mBCMA, membrane-bound B-cell maturation antigen.

Figure 2. B-cell maturation antigen levels at baseline, at best response, and at disease progression in patients receiving belantamab mafodotin as monotherapy in DREAMM-2 (A) or in the belantamab mafodotin, bortezomib, dexamethasone regimen in DREAMM-7 (B).

*At response, minimum within best achieved response; †at progressive disease, latest recorded measure at progressive disease or later. Error bars represent 95% confidence intervals.
BCMA, B-cell maturation antigen; BCR, best confirmed response; BVd, belantamab mafodotin, bortezomib, and dexamethasone; PD, progressive disease; sBCMA, soluble B-cell maturation antigen.

Figure 3. Membrane-bound B-cell maturation antigen and CD138 levels at screening and at response or disease progression in patients receiving belantamab mafodotin with pomalidomide and dexamethasone (BPd) or pomalidomide with bortezomib and dexamethasone (PVd) in DREAMM-8. Patient receiving BPd, measured at screening and disease progression (A). Patient receiving BPd, measured at screening and complete response (B). Patient receiving PVd, measured at screening and disease progression (C).

BCMA, B-cell maturation antigen; BPd, belantamab mafodotin, pomalidomide, and dexamethasone; CR, complete response; mBCMA, membrane-bound B-cell maturation antigen; PD, progressive disease; PVd, pomalidomide, bortezomib, and dexamethasone.

Figure 4. Belantamab mafodotin soluble B-cell maturation antigen (sBCMA) binding. Geometric mean of fold-change in complex sBCMA from baseline (A). Complex sBCMA concentration among patients who experienced disease progression (B).*

*Data are from the DREAMM-5 study of belantamab mafodotin with and without nirogacestat, and included are timepoints with 3 or more patients per response status and treatment arm.
†at progressive disease, samples taken within 4 weeks of clinical confirmation of progressive disease. In panel B, baseline sBCMA levels for two patients with complex sBCMA exceeding the non-quantifiable threshold were adjusted to 0.09.
Monotherapy: C1D1 n=35; C2D1 n=30; C4D1 n=11; C6D1 n=3.
Combination: C1D1 n=29; C2D1 n=27; C4D1 n=17; C6D1 n=7.
BCMA, B-cell maturation antigen; C1D1, Cycle 1, Day 1; H, hours; PD, progressive disease; PR, partial response; PRE, pre-dose; sBCMA, soluble B-cell maturation antigen; SD, stable disease; VGPR, very good partial response.

Figure 5. Impact of belantamab mafodotin on cell counts. Neutrophil-to-lymphocyte ratio (A), absolute lymphocyte count (B), CD8+ counts (C), CD4+ counts (D), and CD4/CD8+ counts (E) over time.*

*Neutrophil-to-lymphocyte ratio and absolute lymphocyte count data from the DREAMM-2 study of belantamab mafodotin monotherapy, and CD4/CD8+ counts are from the DREAMM-14 study of belantamab mafodotin monotherapy.
Patients who were not evaluable for response were excluded from the analyses of neutrophil-to-lymphocyte ratio and absolute lymphocyte count.
C1D1, Cycle 1, Day 1.

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

*TIM-3 data are from the DREAMM-14 study of belantamab mafodotin monotherapy.
C1D1, Cycle 1, Day 1; NK, natural killer; PD, disease progression; TIM-3, T-cell immunoglobulin and mucin domain 3.

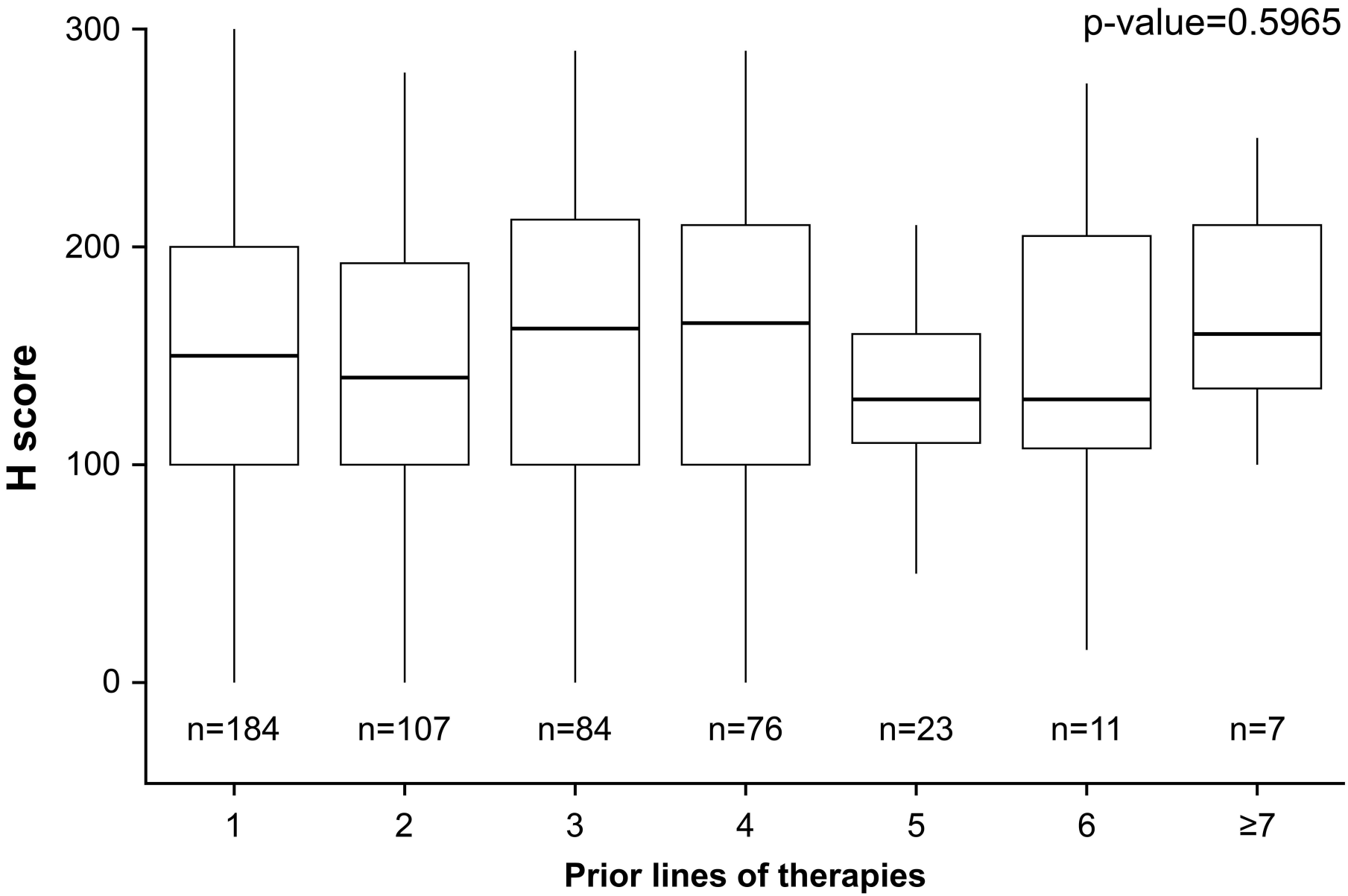
Figure 7. Impact of belantamab mafodotin on T-cell exhaustion markers PD-1 and TIGIT.*

*PD-1 and TIGIT data are from the DREAMM-5 study of belantamab mafodotin with nirogacestat.
NK, natural killer; PD-1, programmed cell death protein 1; PD, disease progression; TIGIT, T-cell immunoreceptor with immunoglobulin and ITIM domains.

Figure 8. Impact of belantamab mafodotin on immune cell activity (Granzyme B, CD107a) and proliferation (Ki67).*

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

NK, natural killer.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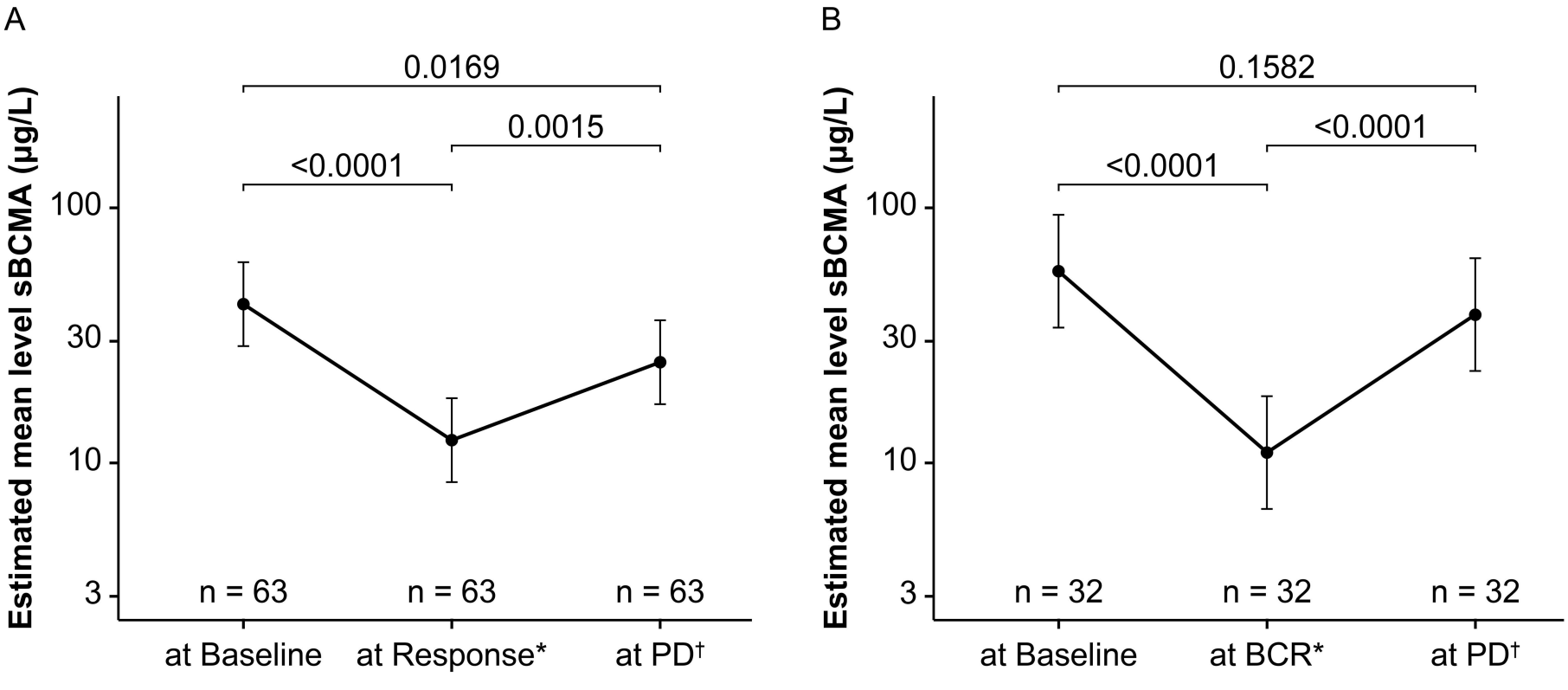
1
2
3
4
5
6
≥7
Prior lines of therapies


A
0.0169
0.0015
<0.0001
Estimated mean level sBCMA (µg/L)
100
30
10
3
n = 63
n = 63
n = 63
at Baseline
at Response*
at PD†
B
0.1582
<0.0001
<0.0001
Estimated mean level sBCMA (µg/L)
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30
10
3
n = 32
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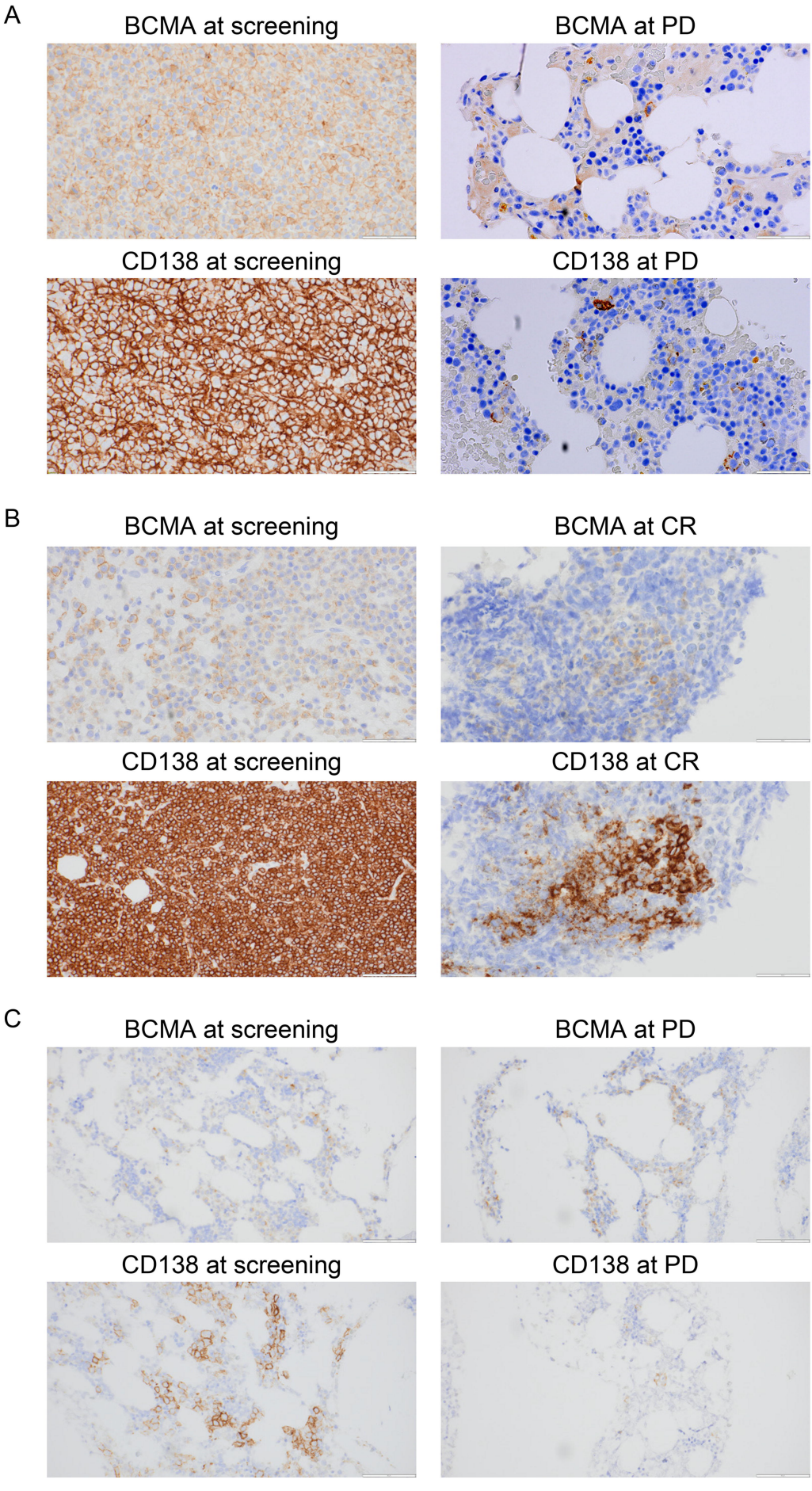
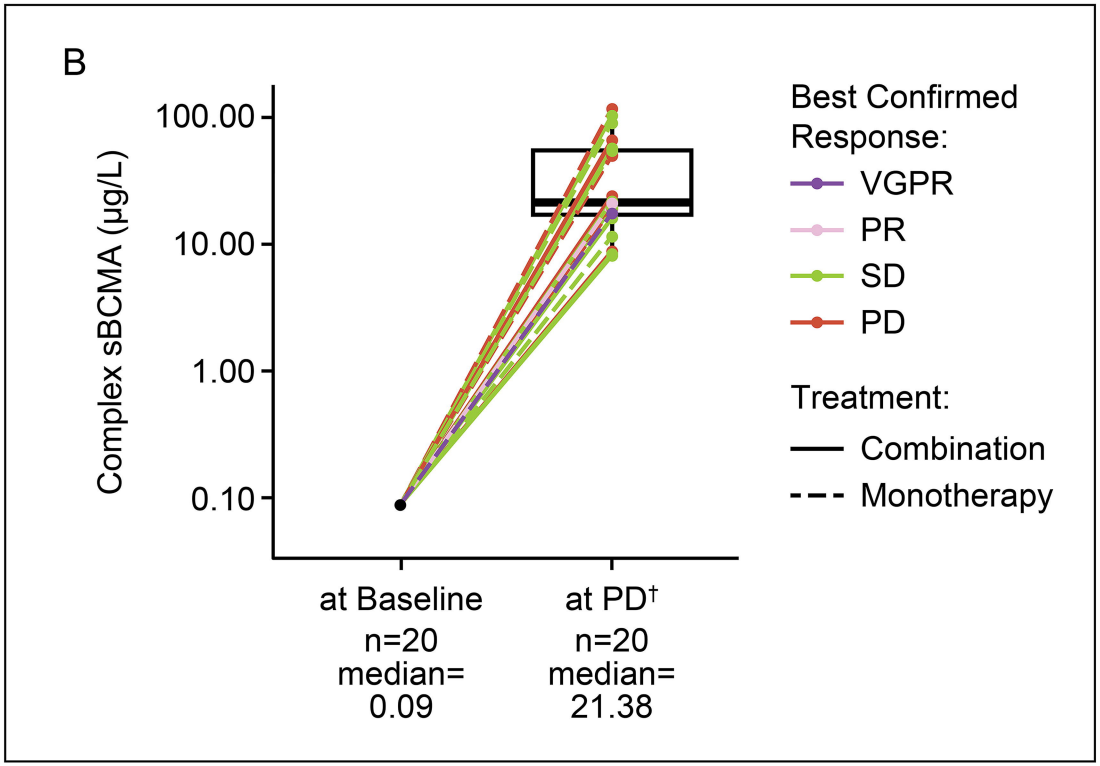
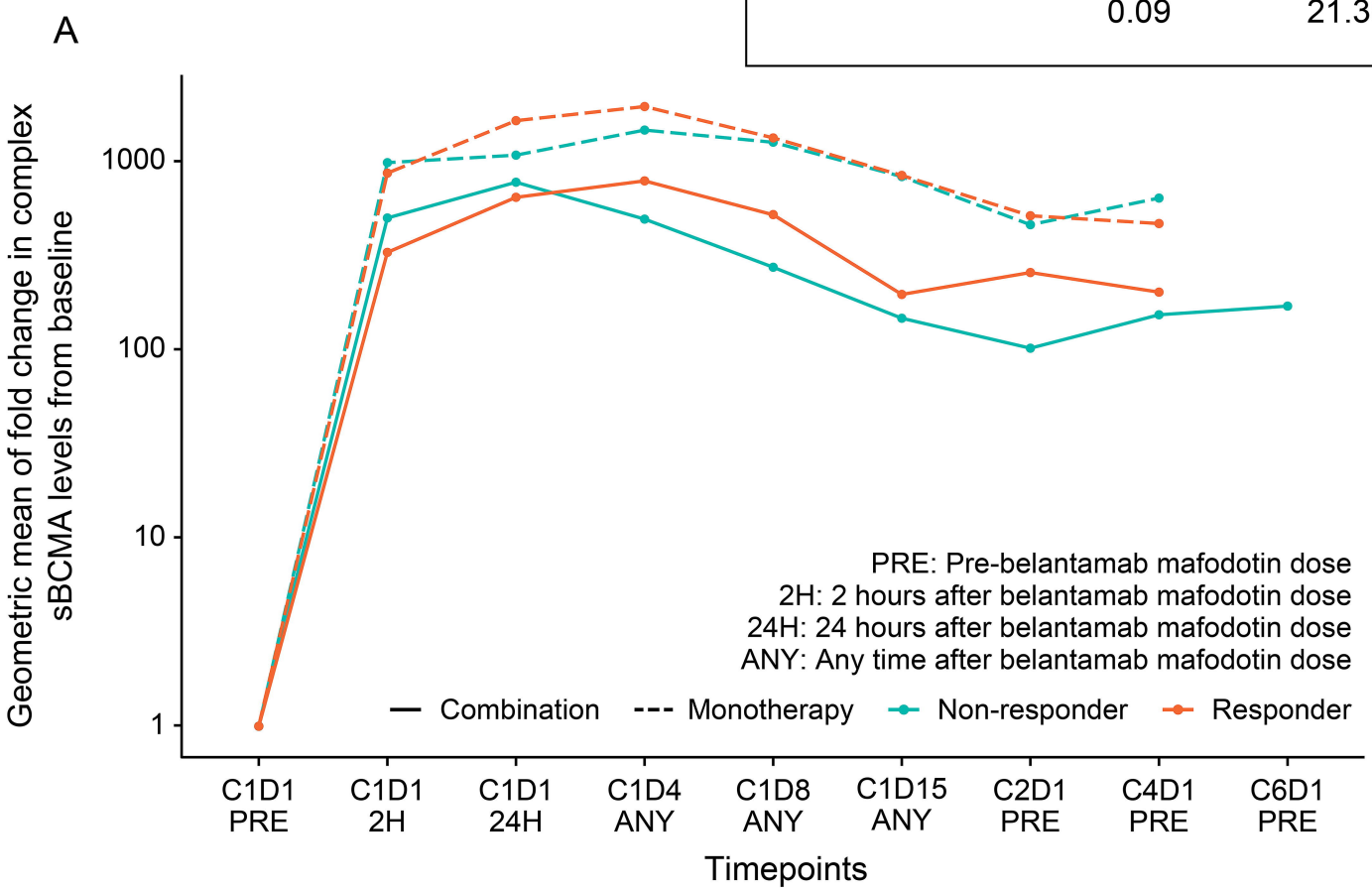
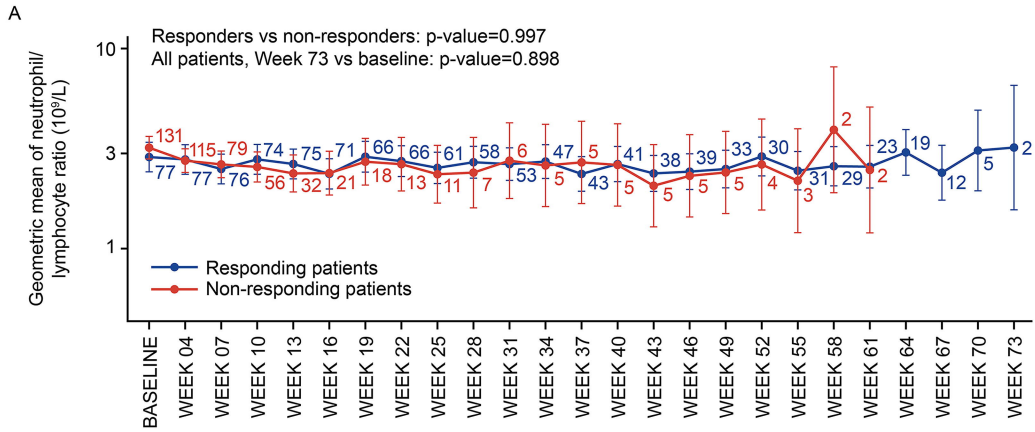
n = 32
at Baseline
at BCR*
at PD†


A
BCMA at screening
BCMA at PD
CD138 at screening
CD138 at PD
B
BCMA at screening
BCMA at CR
CD138 at screening
CD138 at CR
C
BCMA at screening
BCMA at PD
CD138 at screening
CD138 at PD


B
Complex sBCMA (µg/L)
100.00
10.00
1.00
0.10
at Baseline
n=20
median=
0.09
at PD†
n=20
median=
21.38
Best Confirmed Response:
VGPR
PR
SD
PD
Treatment:
Combination
Monotherapy


A
Geometric mean of fold change in complex sBCMA levels from baseline
1000
100
10
1
C1D1 PRE
C1D1 2H
C1D1 24H
C1D4 ANY
C1D8 ANY
C1D15 ANY
C2D1 PRE
C4D1 PRE
C6D1 PRE
Timepoints
PRE: Pre-belantamab mafodotin dose
2H: 2 hours after belantamab mafodotin dose
24H: 24 hours after belantamab mafodotin dose
ANY: Any time after belantamab mafodotin dose
Combination
Monotherapy
Non-responder
Responder


A
Responders vs non-responders: p-value=0.997
All patients, Week 73 vs baseline: p-value=0.898
Geometric mean of neutrophil/ lymphocyte ratio (10⁹/L)
Responding patients
Non-responding patients
BASELINE
WEEK 04
WEEK 07
WEEK 10
WEEK 13
WEEK 16
WEEK 19
WEEK 22
WEEK 25
WEEK 28
WEEK 31
WEEK 34
WEEK 37
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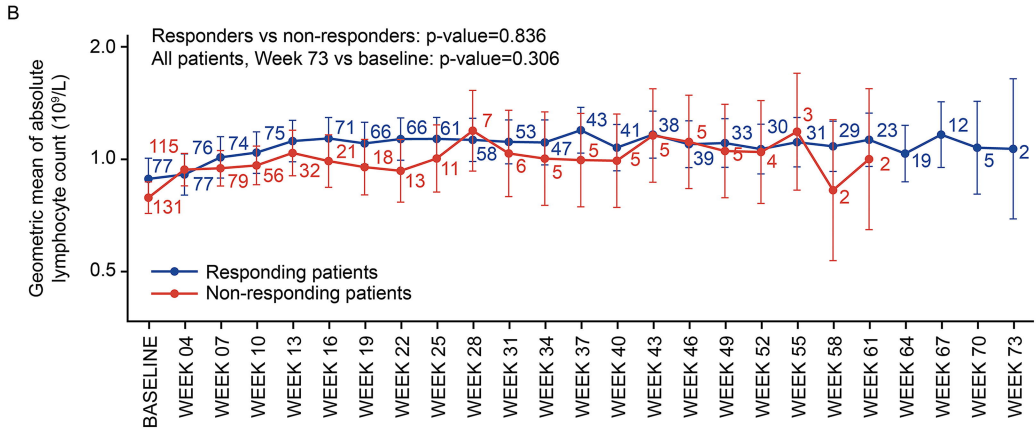
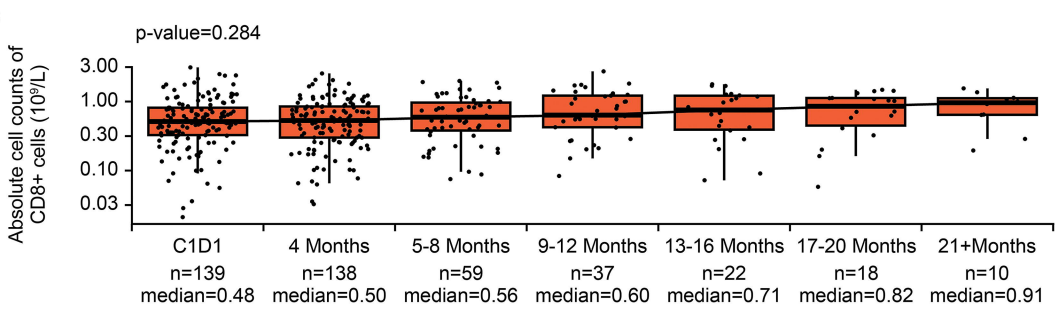
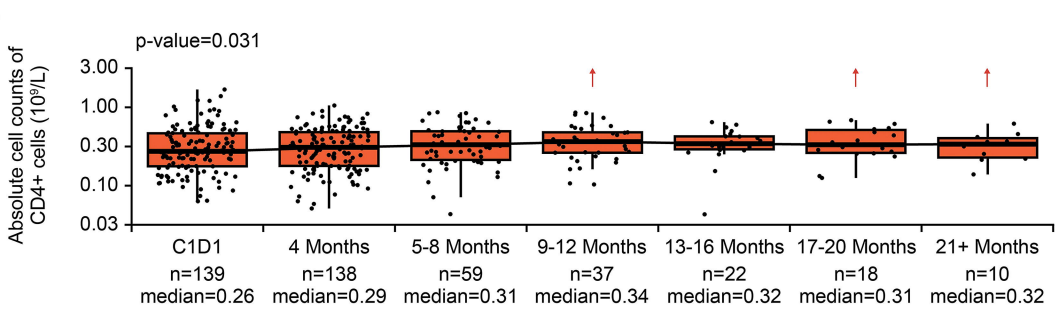
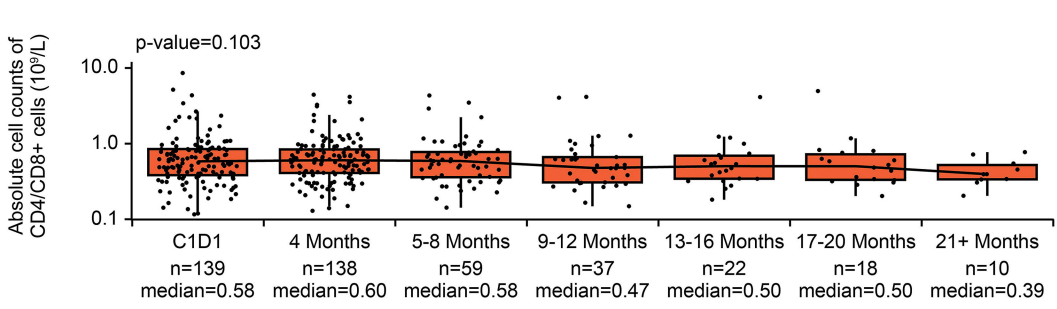
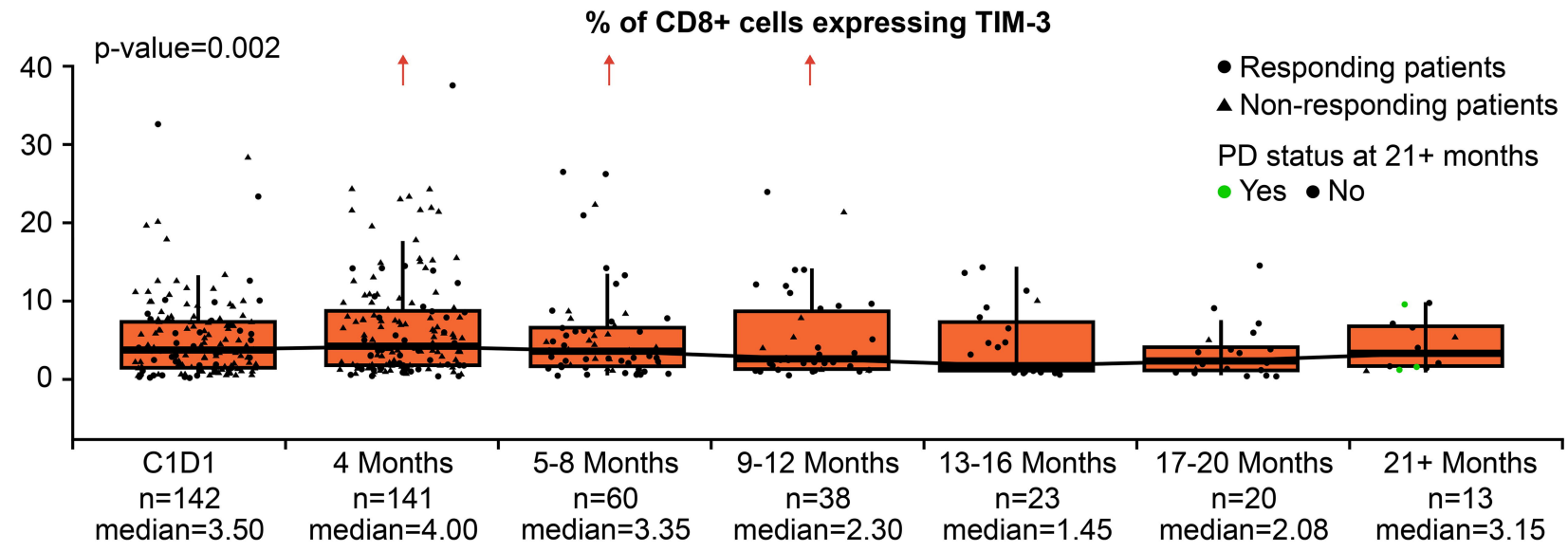
WEEK 58
WEEK 61
WEEK 64
WEEK 67
WEEK 70
WEEK 73

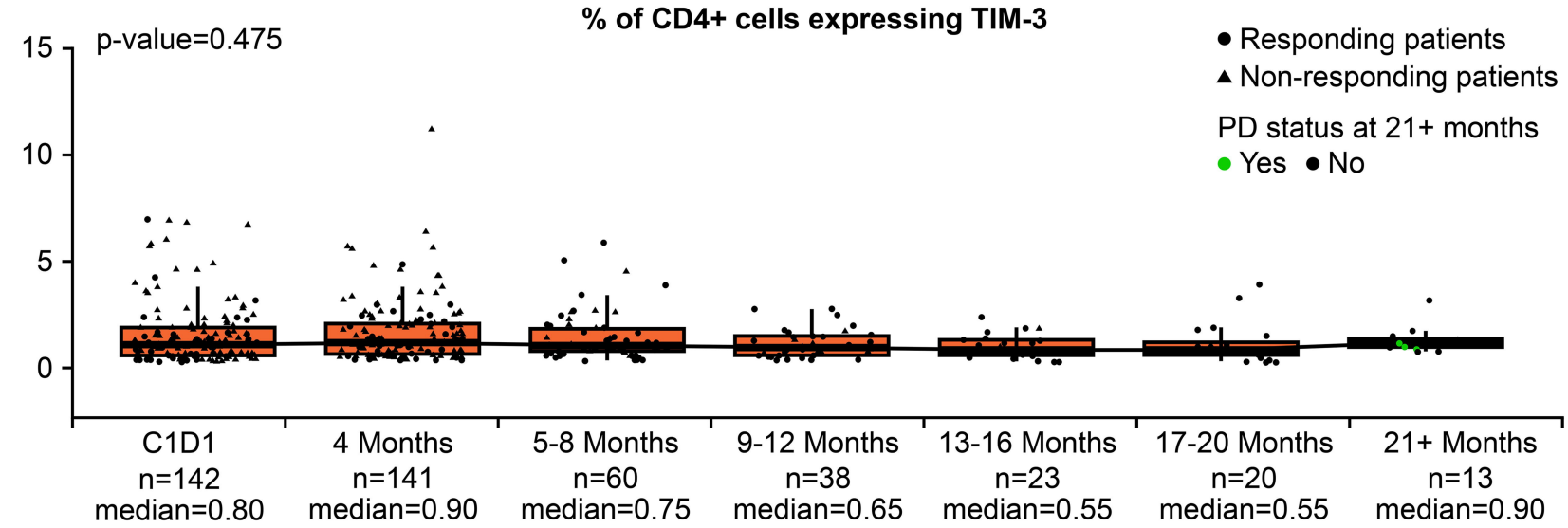

B
Responders vs non-responders: p-value=0.836
All patients, Week 73 vs baseline: p-value=0.306
Geometric mean of absolute lymphocyte count (10⁹/L)
Responding patients
Non-responding patients
BASELINE
WEEK 04
WEEK 07
WEEK 10
WEEK 13
WEEK 16
WEEK 19
WEEK 22
WEEK 25
WEEK 28
WEEK 31
WEEK 34
WEEK 37
WEEK 40
WEEK 43
WEEK 46
WEEK 49
WEEK 52
WEEK 55
WEEK 58
WEEK 61
WEEK 64
WEEK 67
WEEK 70
WEEK 73

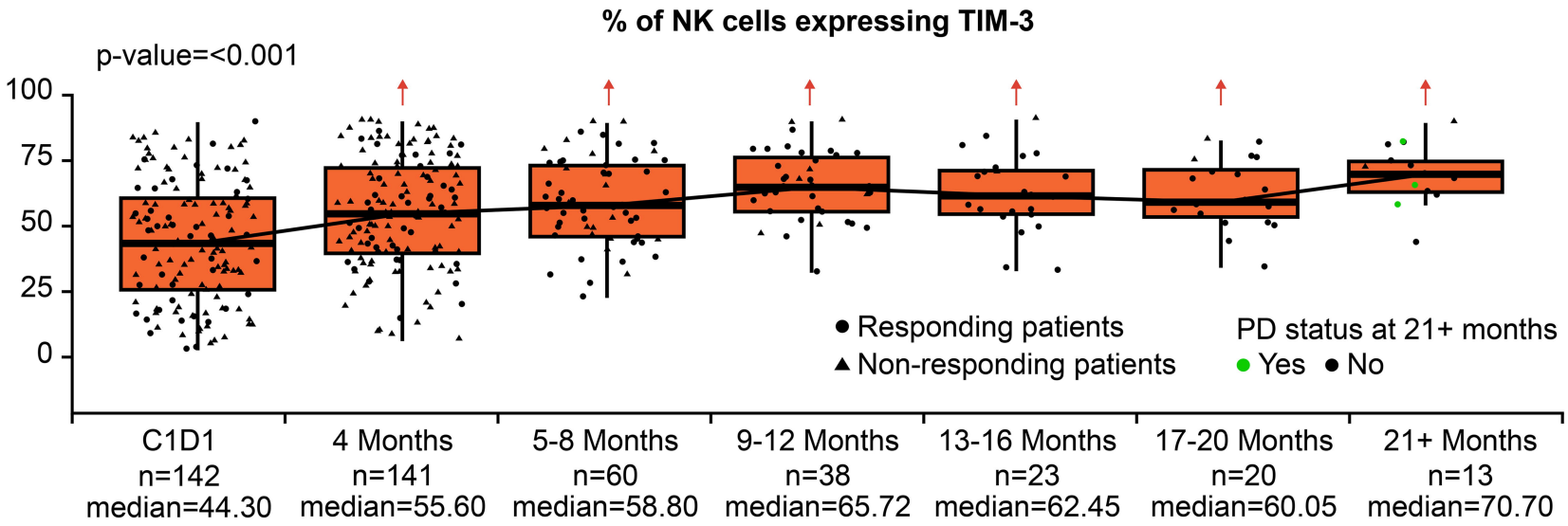

C
p-value=0.284
Absolute cell counts of CD8+ cells (10⁹/L)
C1D1 n=139 median=0.48
4 Months n=138 median=0.50
5-8 Months n=59 median=0.56
9-12 Months n=37 median=0.60
13-16 Months n=22 median=0.71
17-20 Months n=18 median=0.82
21+Months n=10 median=0.91

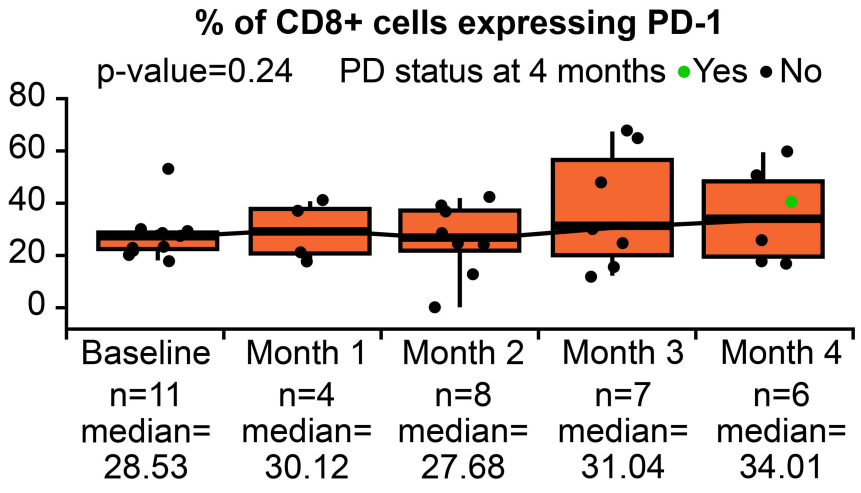

D
p-value=0.031
Absolute cell counts of CD4+ cells (10⁹/L)
C1D1 n=139 median=0.26
4 Months n=138 median=0.29
5-8 Months n=59 median=0.31
9-12 Months n=37 median=0.34
13-16 Months n=22 median=0.32
17-20 Months n=18 median=0.31
21+ Months n=10 median=0.32

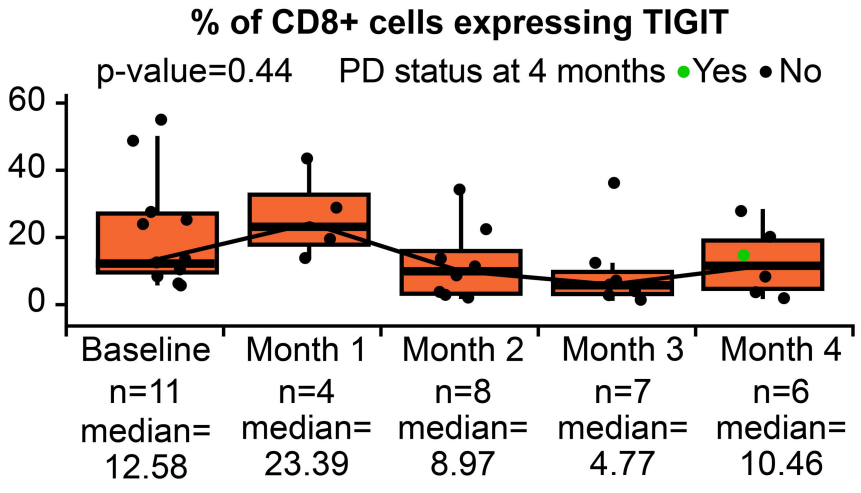

E
p-value=0.103
Absolute cell counts of CD4/CD8+ cells (10⁹/L)
C1D1 n=139 median=0.58
4 Months n=138 median=0.60
5-8 Months n=59 median=0.58
9-12 Months n=37 median=0.47
13-16 Months n=22 median=0.50
17-20 Months n=18 median=0.50
21+ Months n=10 median=0.39

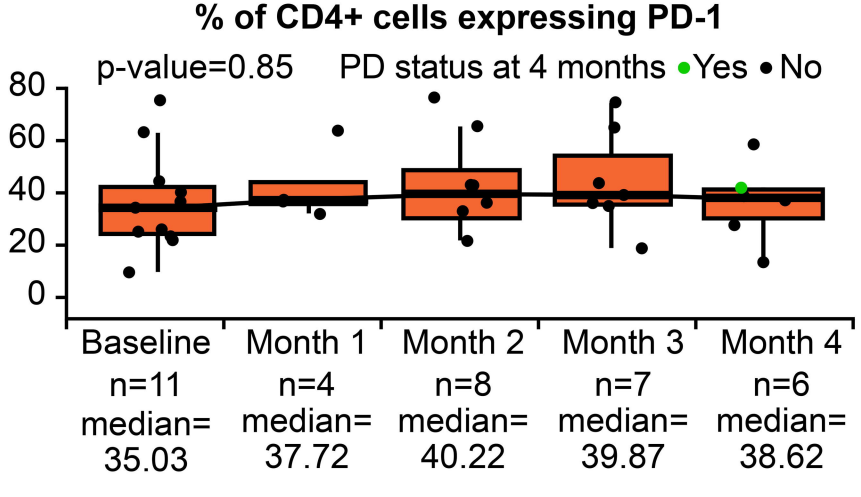

% of CD8+ cells expressing TIM-3
p-value=0.002
40
30
20
10
0
● Responding patients
▲ Non-responding patients
PD status at 21+ months
● Yes ● No
C1D1
n=142
median=3.50
4 Months
n=141
median=4.00
5-8 Months
n=60
median=3.35
9-12 Months
n=38
median=2.30
13-16 Months
n=23
median=1.45
17-20 Months
n=20
median=2.08
21+ Months
n=13
median=3.15

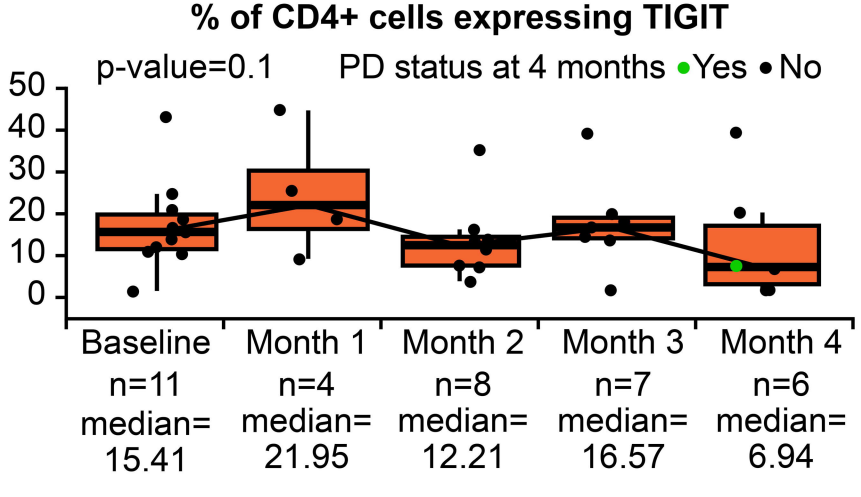

% of CD4+ cells expressing TIM-3
p-value=0.475
15
10
5
0
● Responding patients
▲ Non-responding patients
PD status at 21+ months
● Yes ● No
C1D1
n=142
median=0.80
4 Months
n=141
median=0.90
5-8 Months
n=60
median=0.75
9-12 Months
n=38
median=0.65
13-16 Months
n=23
median=0.55
17-20 Months
n=20
median=0.55
21+ Months
n=13
median=0.90

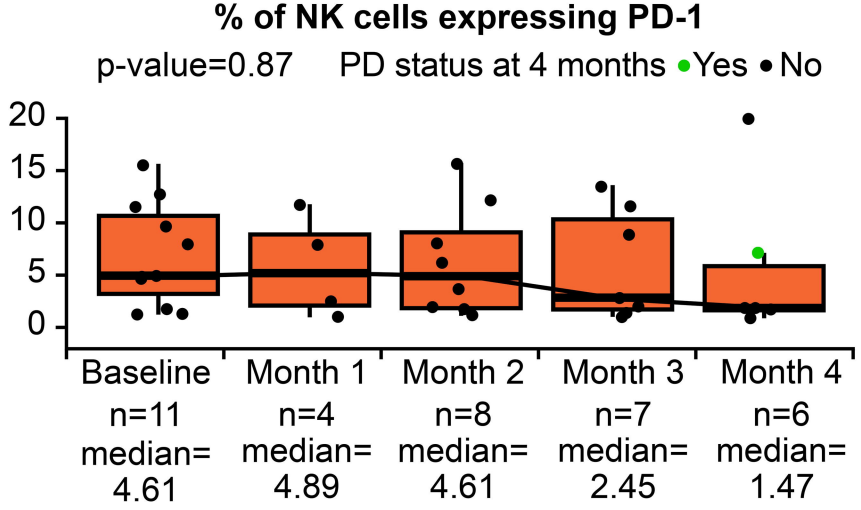

% of NK cells expressing TIM-3
p-value=<0.001
100
75
50
25
0
● Responding patients
▲ Non-responding patients
PD status at 21+ months
● Yes ● No
C1D1
n=142
median=44.30
4 Months
n=141
median=55.60
5-8 Months
n=60
median=58.80
9-12 Months
n=38
median=65.72
13-16 Months
n=23
median=62.45
17-20 Months
n=20
median=60.05
21+ Months
n=13
median=70.70

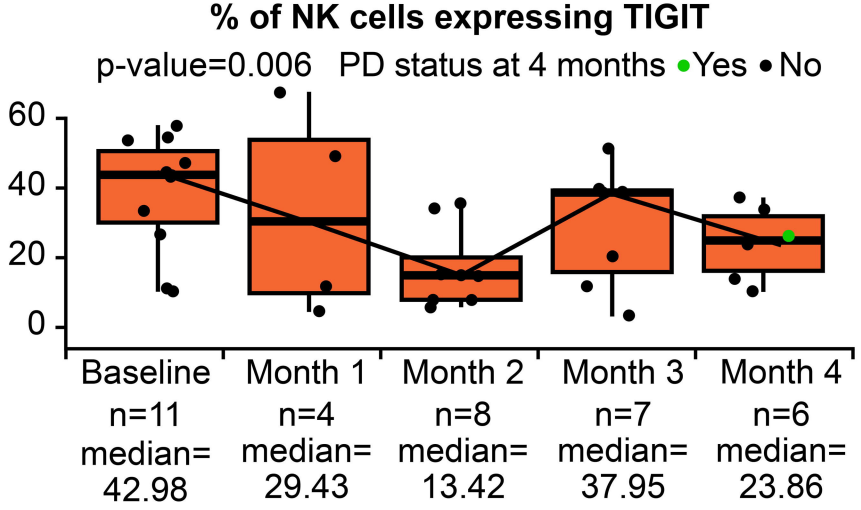

% of CD8+ cells expressing PD-1
p-value=0.24
PD status at 4 months
Yes
No
80
60
40
20
0
Baseline n=11 median= 28.53
Month 1 n=4 median= 30.12
Month 2 n=8 median= 27.68
Month 3 n=7 median= 31.04
Month 4 n=6 median= 34.01

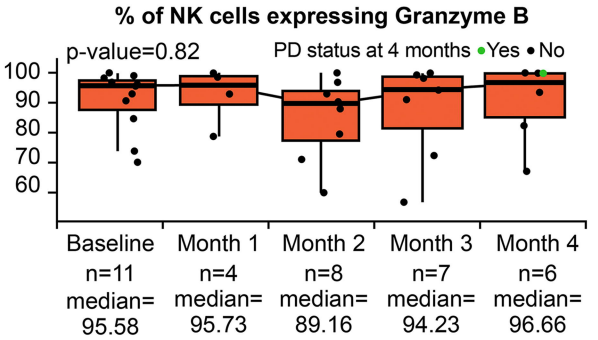

% of CD8+ cells expressing TIGIT
p-value=0.44
PD status at 4 months
Yes
No
60
40
20
0
Baseline n=11 median= 12.58
Month 1 n=4 median= 23.39
Month 2 n=8 median= 8.97
Month 3 n=7 median= 4.77
Month 4 n=6 median= 10.46

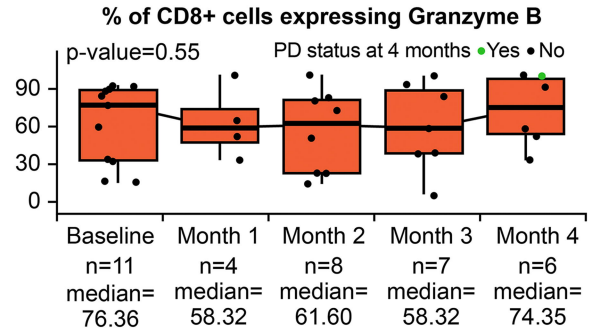

% of CD4+ cells expressing PD-1
p-value=0.85
PD status at 4 months
Yes
No
80
60
40
20
0
Baseline n=11 median= 35.03
Month 1 n=4 median= 37.72
Month 2 n=8 median= 40.22
Month 3 n=7 median= 39.87
Month 4 n=6 median= 38.62

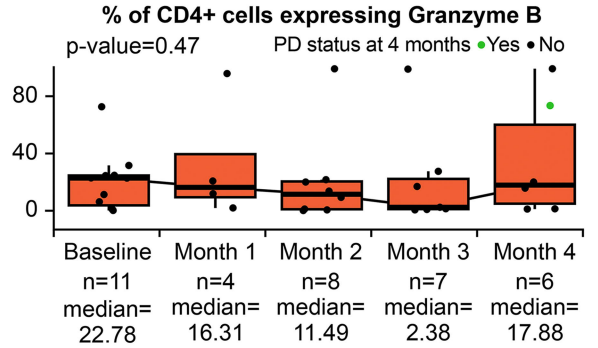

% of CD4+ cells expressing TIGIT
p-value=0.1
PD status at 4 months
Yes
No
50
40
30
20
10
0
Baseline n=11 median= 15.41
Month 1 n=4 median= 21.95
Month 2 n=8 median= 12.21
Month 3 n=7 median= 16.57
Month 4 n=6 median= 6.94

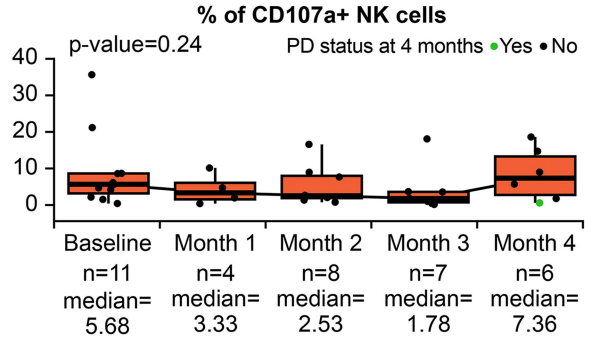

% of NK cells expressing PD-1
p-value=0.87
PD status at 4 months
Yes
No
20
15
10
5
0
Baseline n=11 median= 4.61
Month 1 n=4 median= 4.89
Month 2 n=8 median= 4.61
Month 3 n=7 median= 2.45
Month 4 n=6 median= 1.47

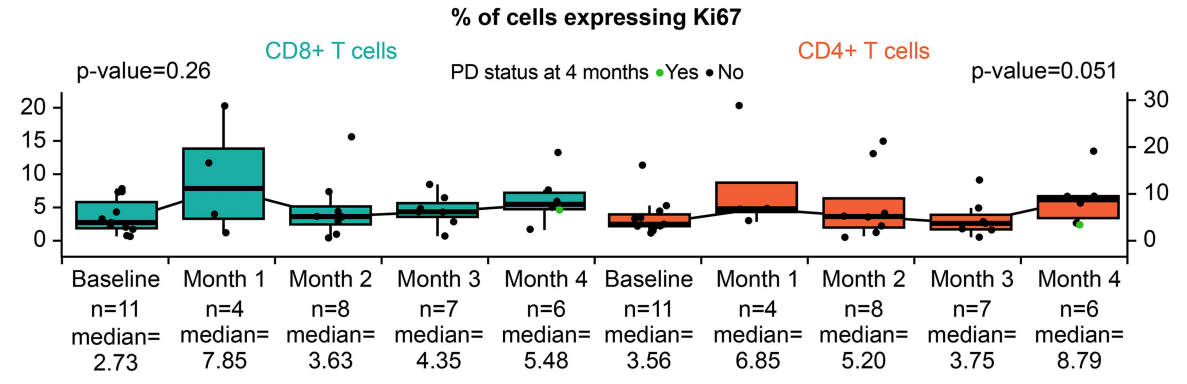

% of NK cells expressing TIGIT
p-value=0.006
PD status at 4 months
Yes
No
60
40
20
0
Baseline n=11 median= 42.98
Month 1 n=4 median= 29.43
Month 2 n=8 median= 13.42
Month 3 n=7 median= 37.95
Month 4 n=6 median= 23.86


% of NK cells expressing Granzyme B
p-value=0.82
PD status at 4 months •Yes •No
100
90
80
70
60
Baseline n=11 median= 95.58
Month 1 n=4 median= 95.73
Month 2 n=8 median= 89.16
Month 3 n=7 median= 94.23
Month 4 n=6 median= 96.66


% of CD8+ cells expressing Granzyme B
p-value=0.55
PD status at 4 months •Yes •No
90
60
30
0
Baseline n=11 median= 76.36
Month 1 n=4 median= 58.32
Month 2 n=8 median= 61.60
Month 3 n=7 median= 58.32
Month 4 n=6 median= 74.35


% of CD4+ cells expressing Granzyme B
p-value=0.47
PD status at 4 months •Yes •No
80
40
0
Baseline n=11 median= 22.78
Month 1 n=4 median= 16.31
Month 2 n=8 median= 11.49
Month 3 n=7 median= 2.38
Month 4 n=6 median= 17.88


% of CD107a+ NK cells
p-value=0.24
PD status at 4 months •Yes •No
40
30
20
10
0
Baseline n=11 median= 5.68
Month 1 n=4 median= 3.33
Month 2 n=8 median= 2.53
Month 3 n=7 median= 1.78
Month 4 n=6 median= 7.36


% of cells expressing Ki67
CD8+ T cells
CD4+ T cells
p-value=0.26
PD status at 4 months •Yes •No
p-value=0.051
20
15
10
5
0
30
20
10
0
Baseline n=11 median= 2.73
Month 1 n=4 median= 7.85
Month 2 n=8 median= 3.63
Month 3 n=7 median= 4.35
Month 4 n=6 median= 5.48
Baseline n=11 median= 3.56
Month 1 n=4 median= 6.85
Month 2 n=8 median= 5.20
Month 3 n=7 median= 3.75
Month 4 n=6 median= 8.79

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

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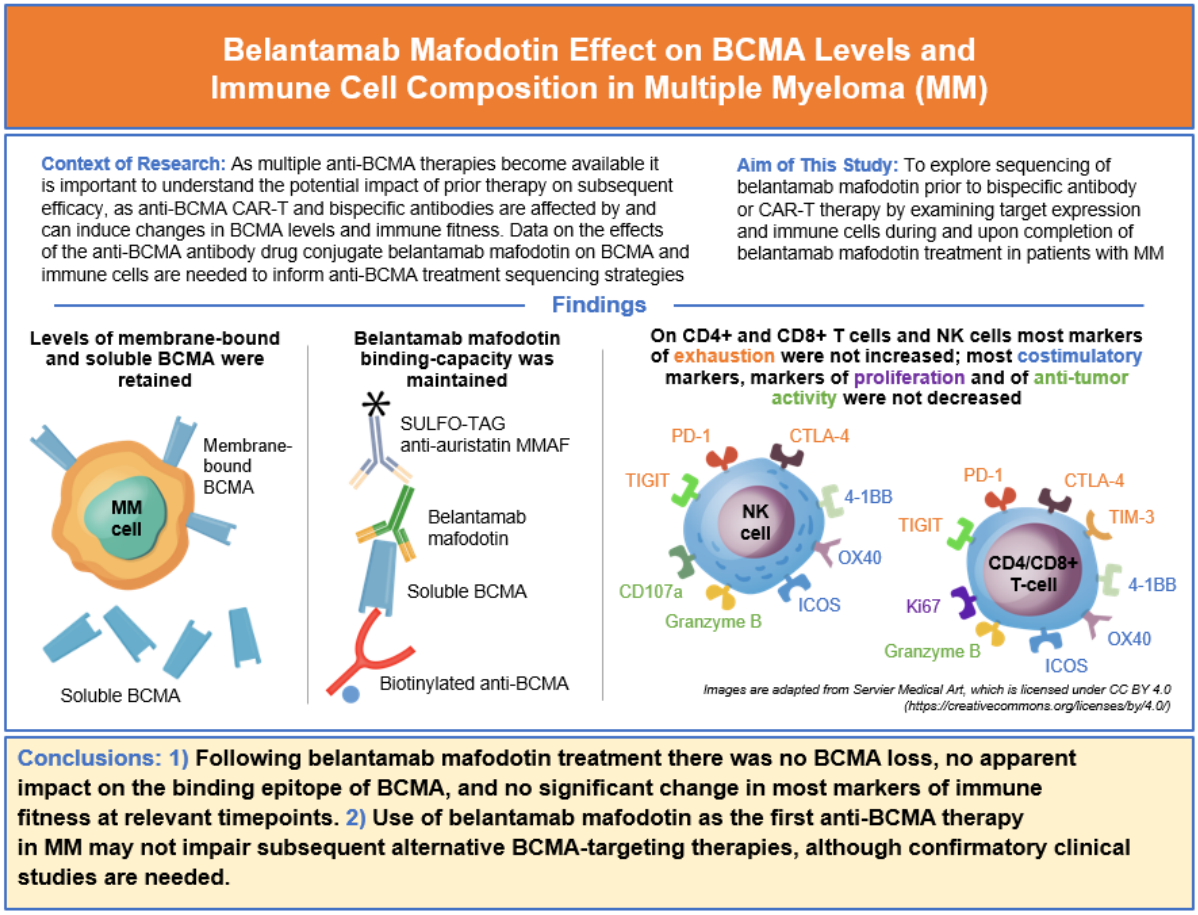
Supplemental Material



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).

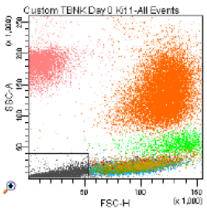


Figure 1b: *SSC v CD8_APC-H7*
From the *NOT Debris* population, TruCount *Beads* are captured. These events are used to calculate the absolute cell value for all parameters.

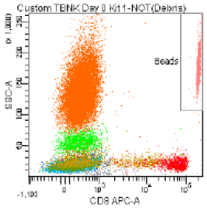


Figure 1c: *SSC v CD8 APC-H7*
From the *NOT Beads* population, monocytes are identified and gated as CD14 positive events. A *NOT Beads* gate is created to exclude the monocytes from all subsequent plots.

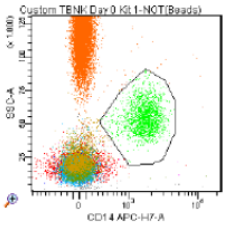


Figure 1d: *SSC v CD45_PerCPCy5.5*
From the *NOT Beads* population *Leukocytes* are identified and gated.

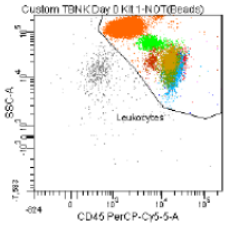


Figure 1e: *SSC v CD45_PerCPCy5.5*
From the *Leukocyte* population, $CD45^+$ lymphocytes are identified.

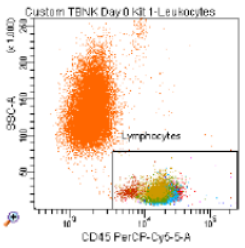


Figure 1f: *SSC v CD3_FITC*
From the *Lymphocyte* population, $CD3^+$ T cells are identified.

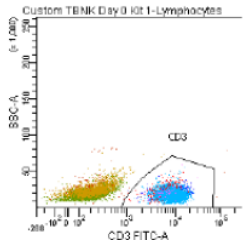


Figure 1g: *CD4_BV510 v CD8_APC*
From the *T cell* population, $CD4^+$ and $CD8^+$ cells are identified through quadrant gating.

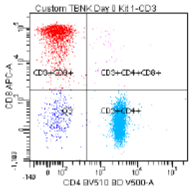


Figure 1h: *CD19_BV421 v CD56/16_APC*
From the *NOT CD3+* population, $CD3^-CD19^+$ and $CD3^-CD16^+CD56^+$ cells are identified through quadrant gating.

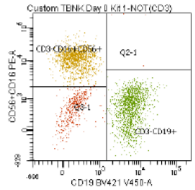
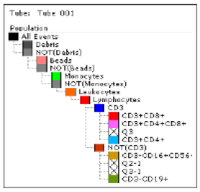


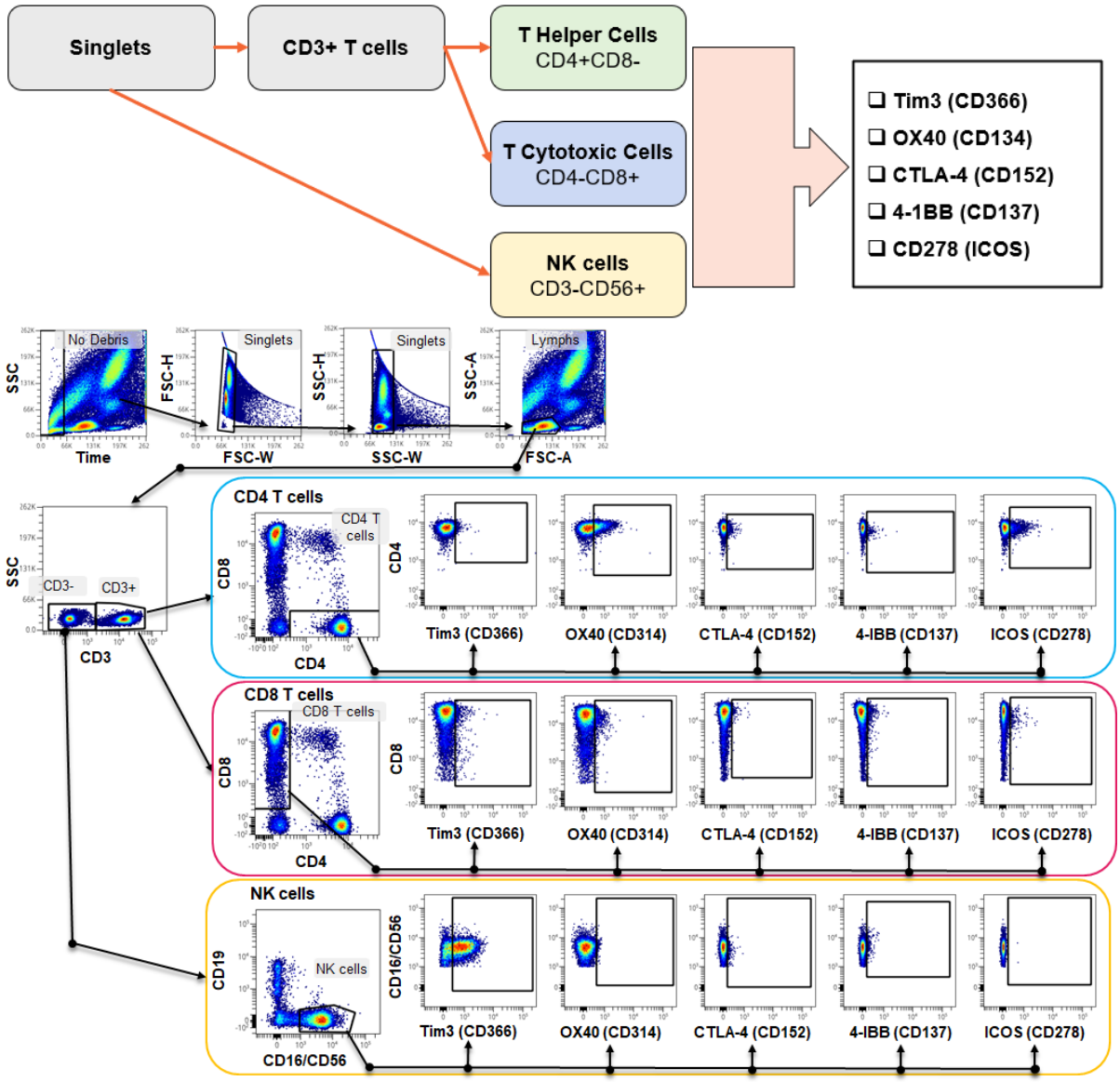
Figure 1i; Hierarchy



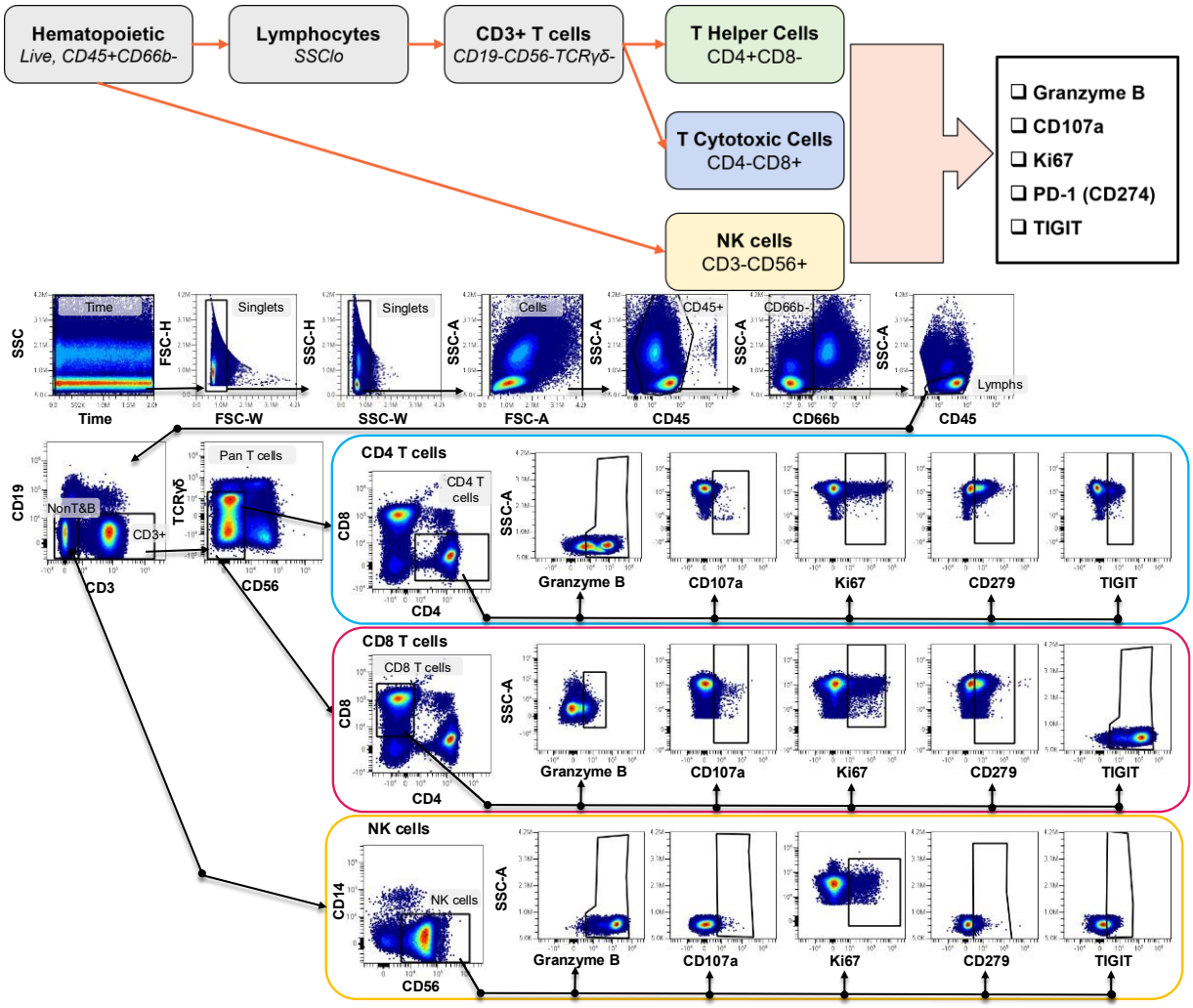
Supplemental Figure 2. Flow cytometry gating strategy for identification of subpopulations and expression of activation markers in T cells, B cells, monocytes, and NK cells.

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

A



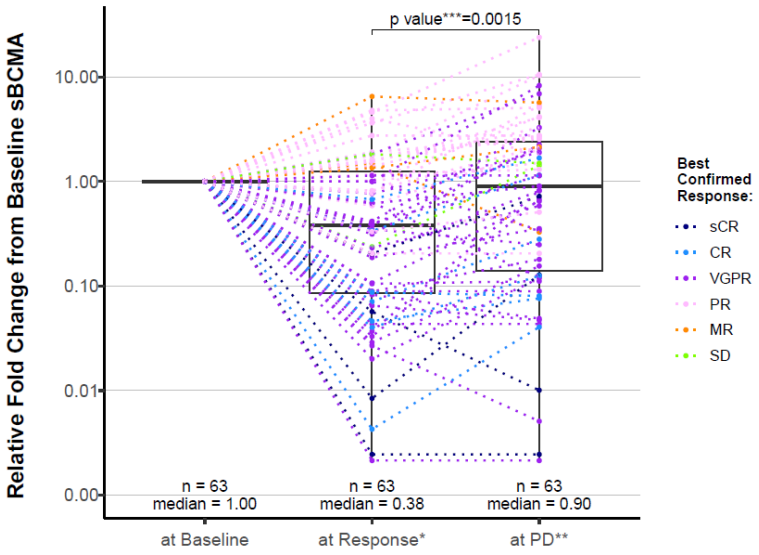
B



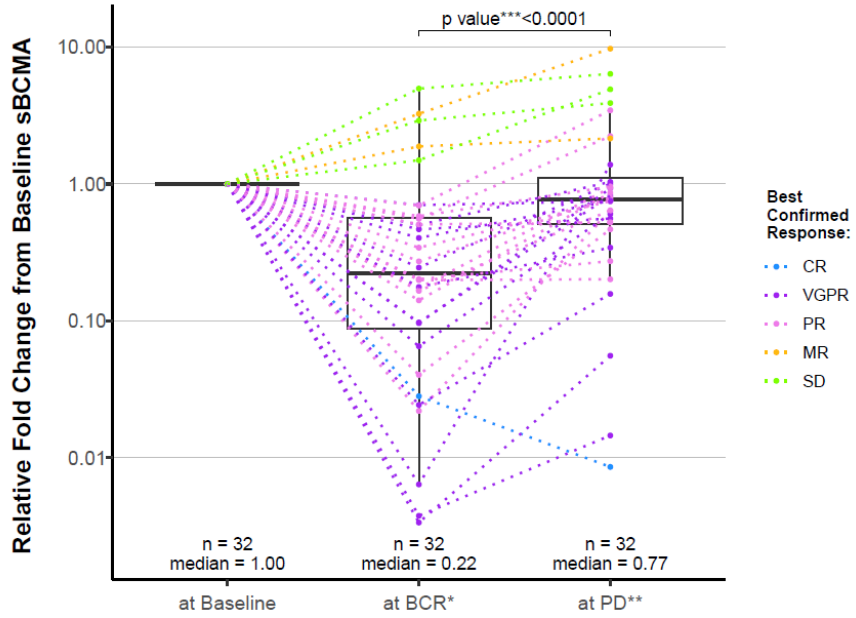
4-1BB, 4-immunoglobulin and cytokine receptor BB; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; FSC, forward scatter; NK, natural killer; PD-1, programmed cell death protein 1; SSC, side scatter; TIGIT, T-cell immunoreceptor with immunoglobulin and ITIM domains; TIM-3, T-cell immunoglobulin and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

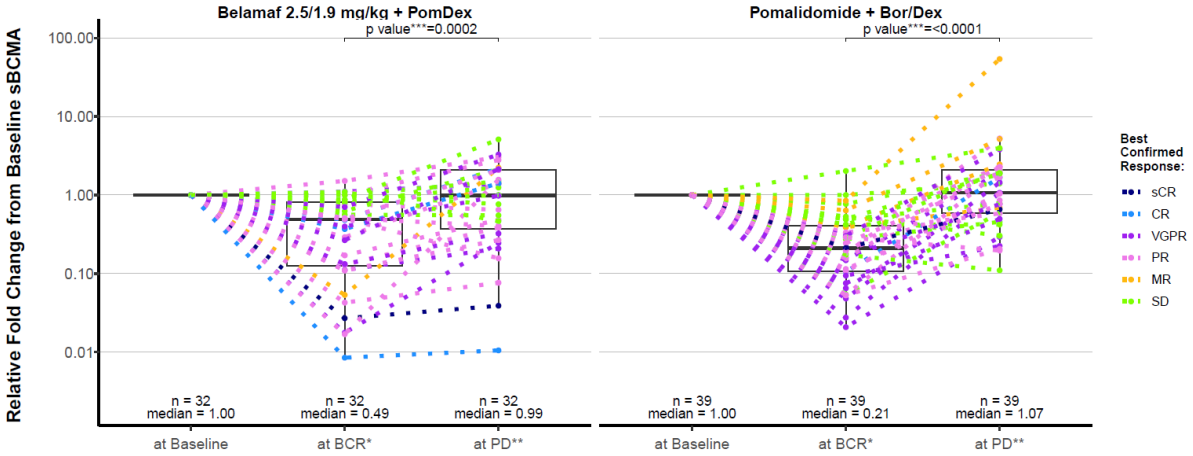


The black center line within the box represents the median value, the lower limit of the box represents the 25th percentile, the upper limit of the box represents the 75th percentile, and whiskers represent the 5th and 95th percentiles.

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.

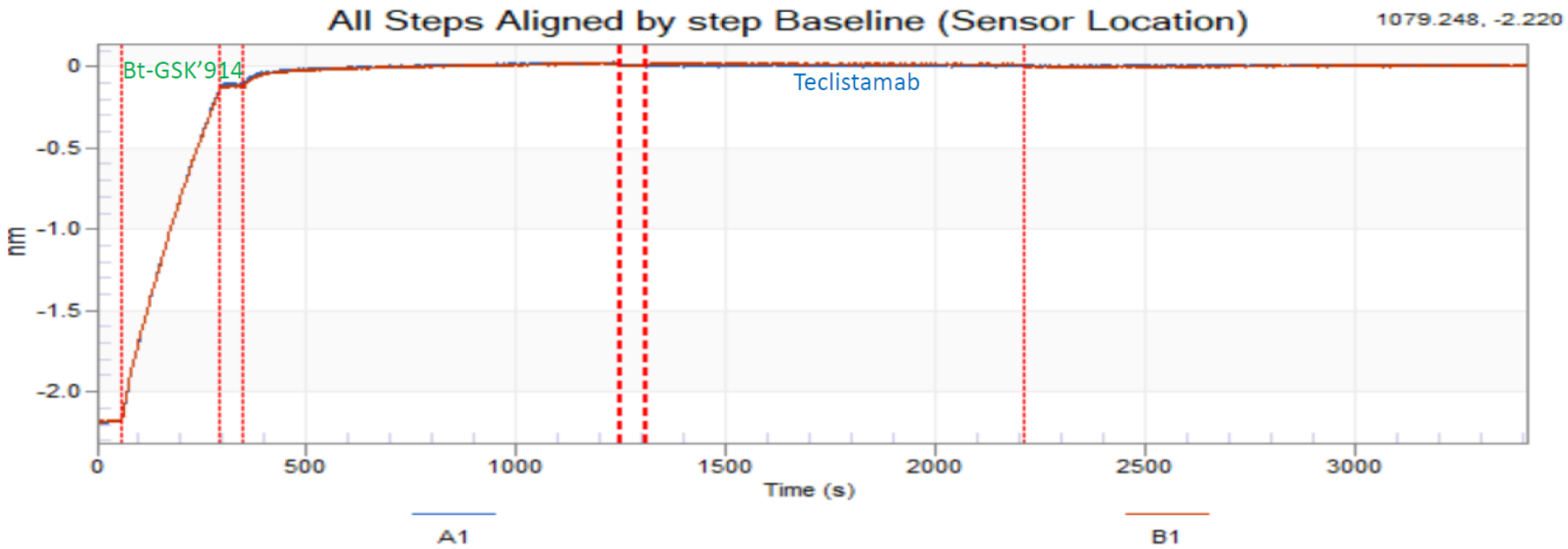
Supplemental Figure 4. Soluble B-cell maturation antigen levels at baseline, at best response, and at disease progression by best confirmed response in patients receiving belantamab mafodotin with pomalidomide and dexamethasone or pomalidomide with bortezomib and dexamethasone in DREAMM-8.



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.

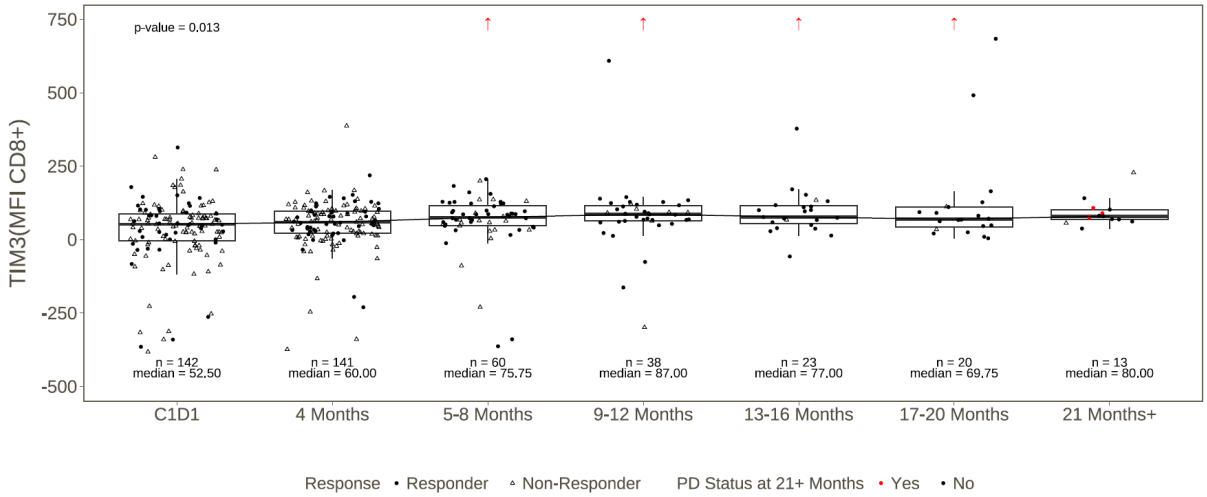
Supplemental Figure 5. Binding of belantamab mafodotin monoclonal antibody and teclistamab to B-cell maturation antigen



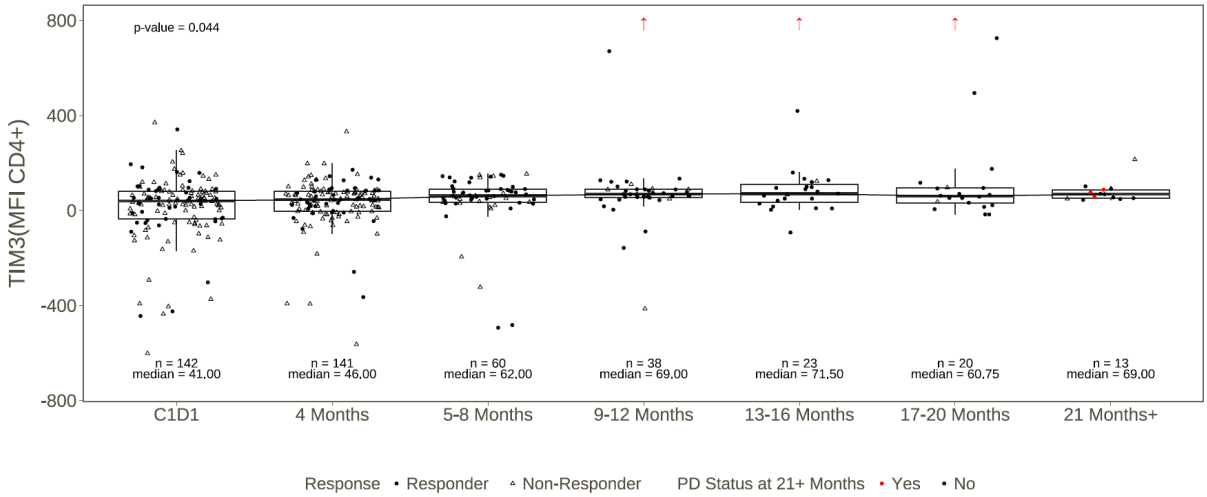
Bt-GSK'914; biotinylated GSK2857914; GSK2857914, belantamab mafodotin without the payload.

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

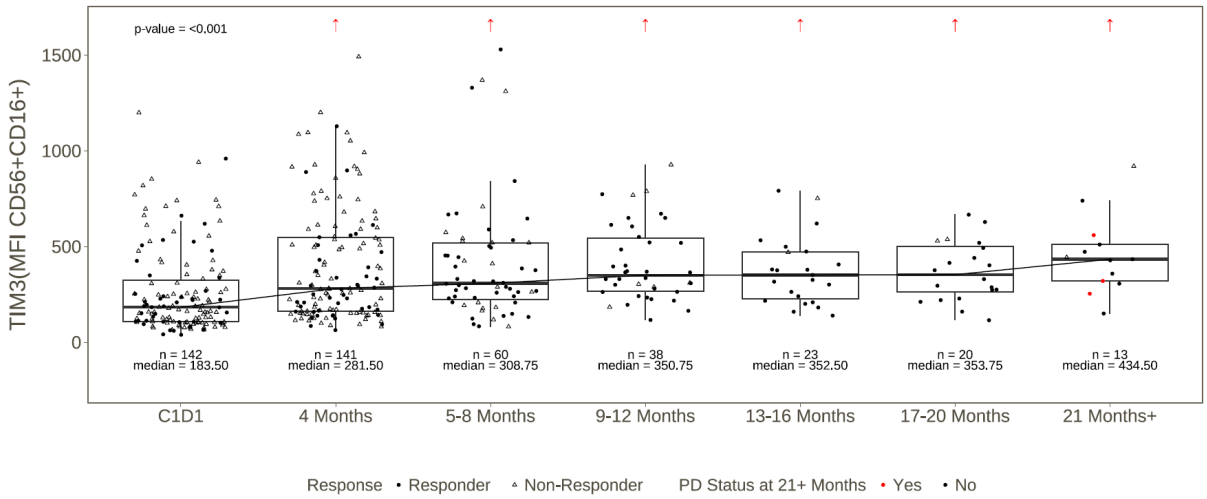
CD8+ T cells



CD4+ T cells



NK cells



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

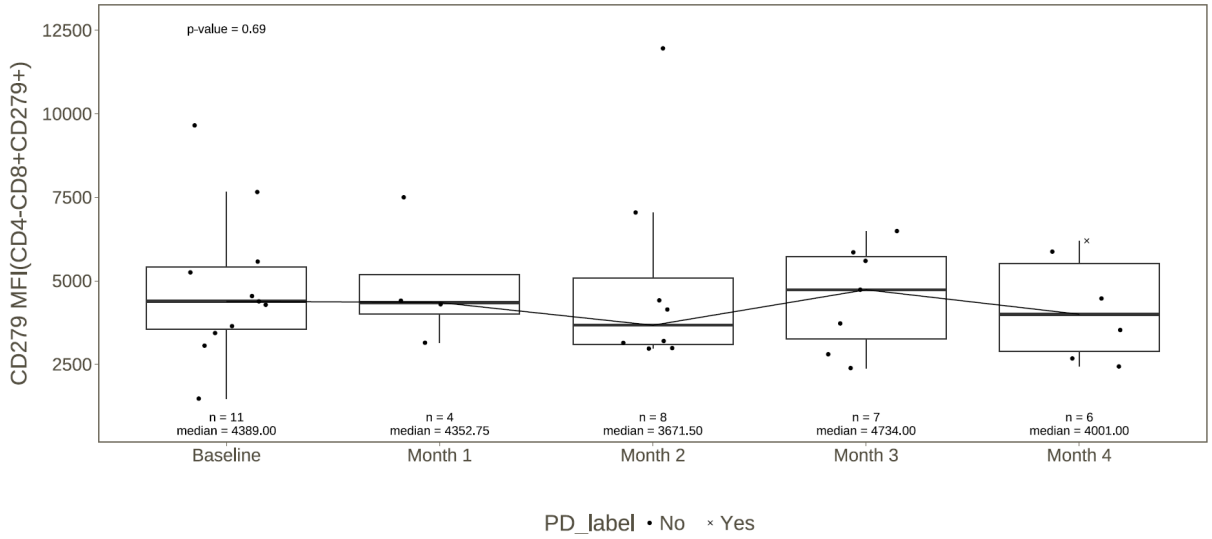
The median of the timepoints within each time category for each patient was utilized. The direction of the red arrows indicates significant increase or decrease from C1D1 for each timepoint.

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

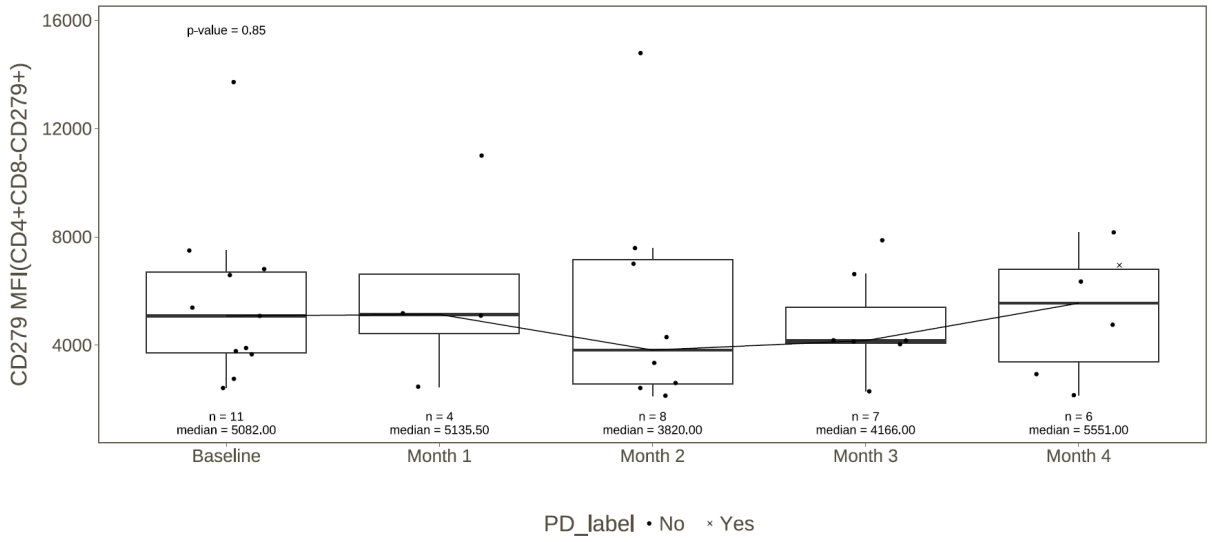
Supplemental Figure 7. Impact of belantamab mafodotin on median fluorescence intensity of T-cell exhaustion markers. PD-1 (A) and TIGIT (B).*

A

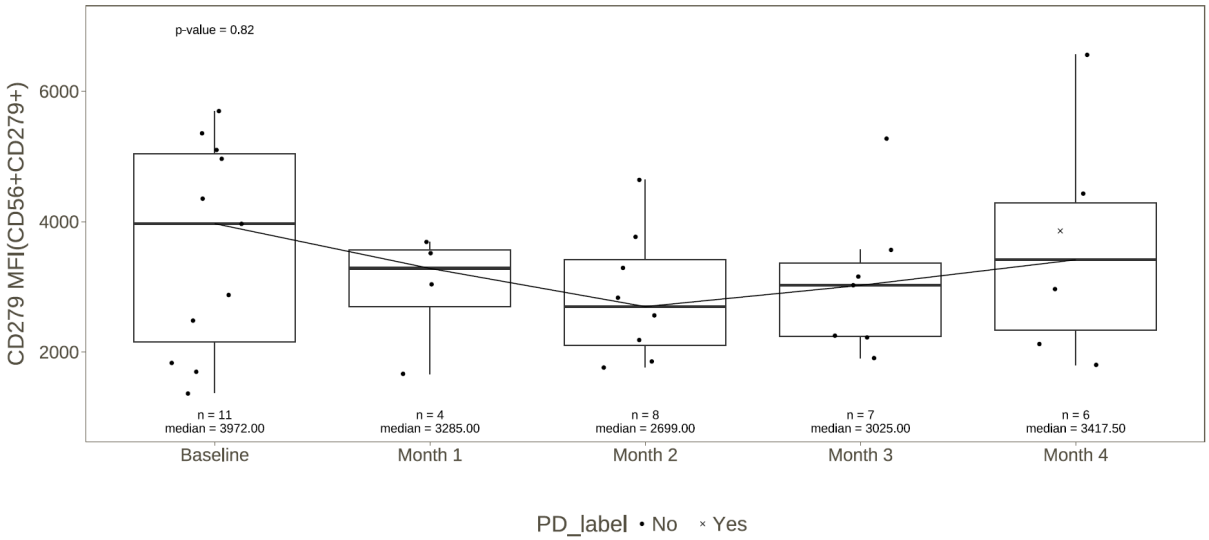
CD8+ T cells



CD4+ T cells

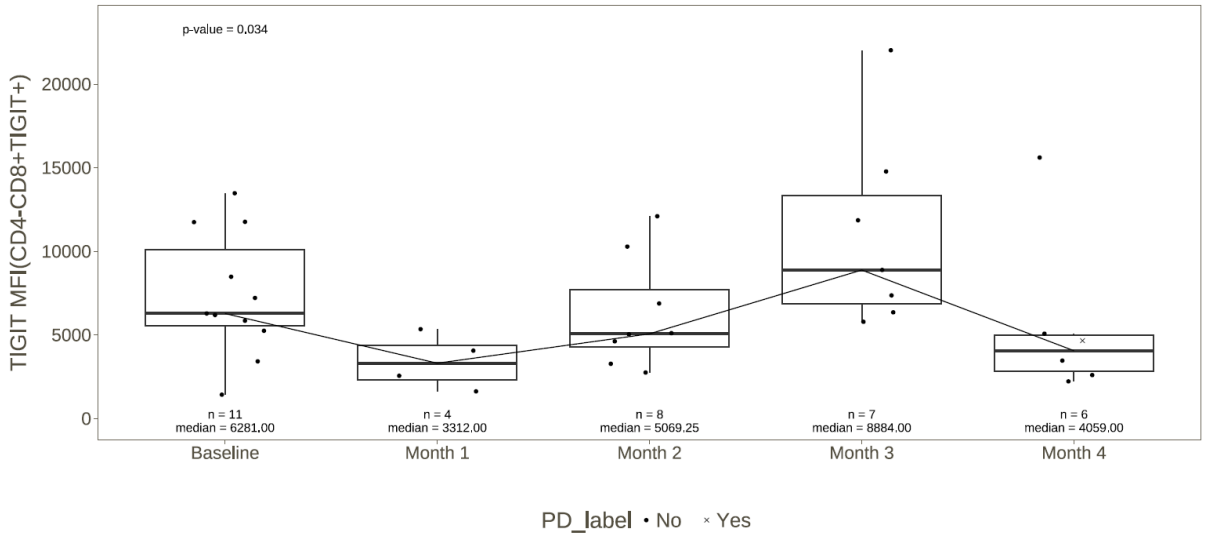


NK cells

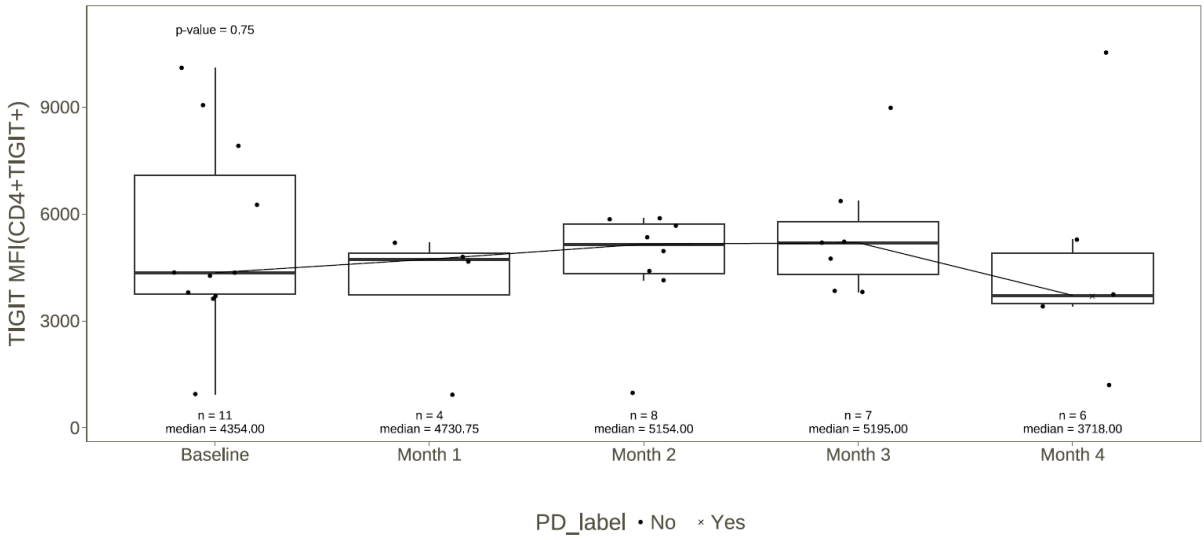


B

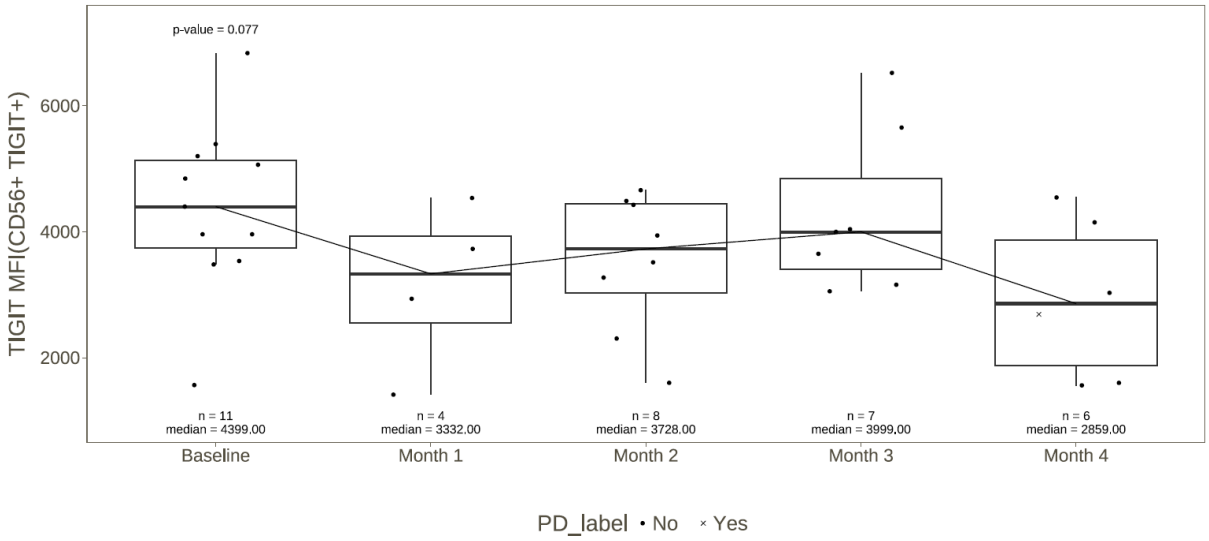
CD8+ T cells



CD4+ T cells



NK cells



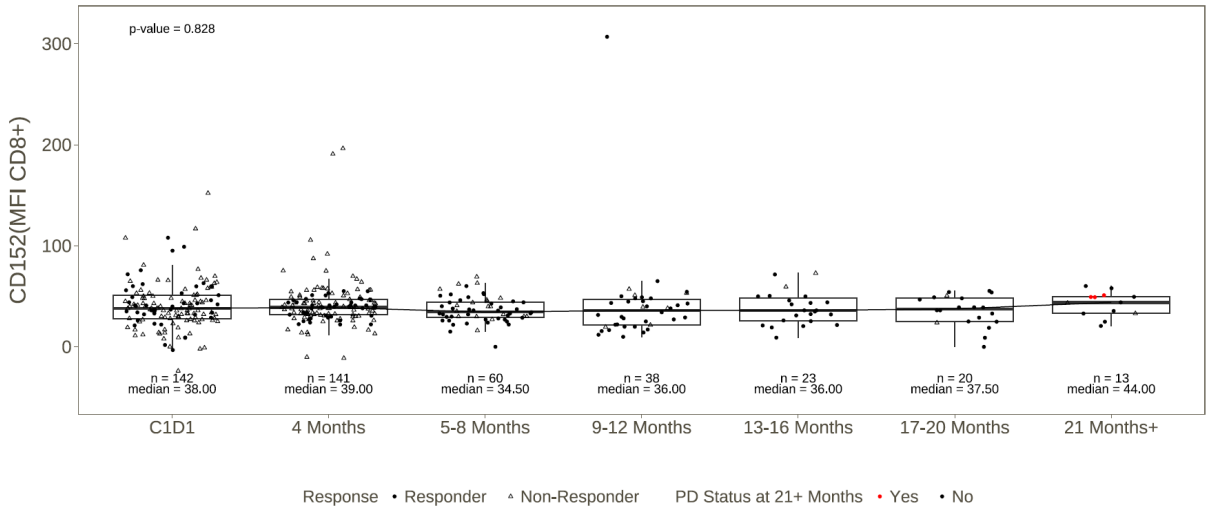
*Data are from the DREAMM-5 study of belantamab mafodotin with nirogacestat.

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.

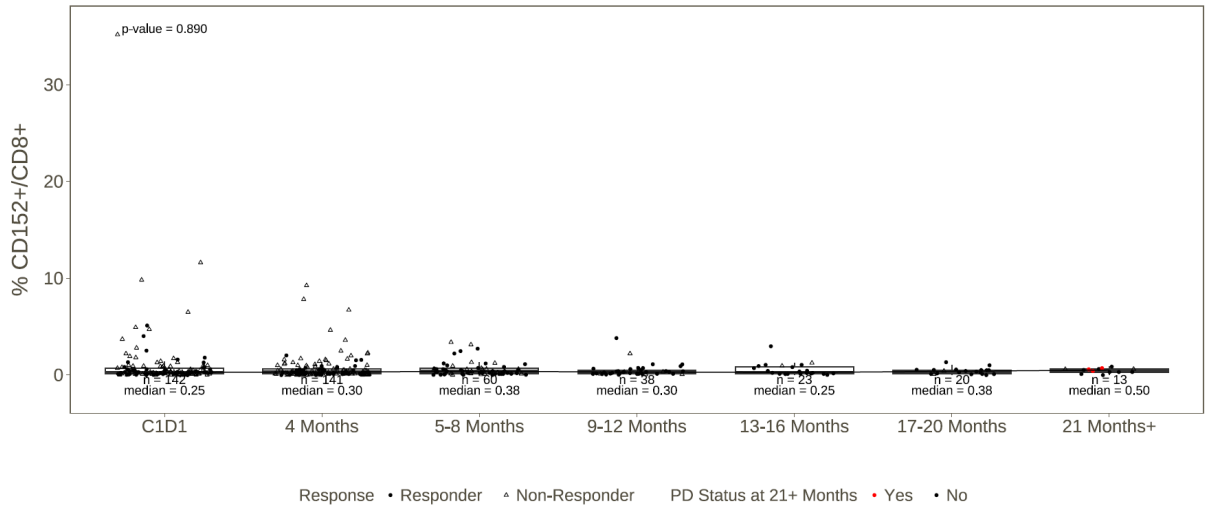
Supplemental Figure 8. Impact of belantamab mafodotin on T-cell exhaustion marker CTLA-4.*

CD8+ T cells.

Median fluorescence intensity

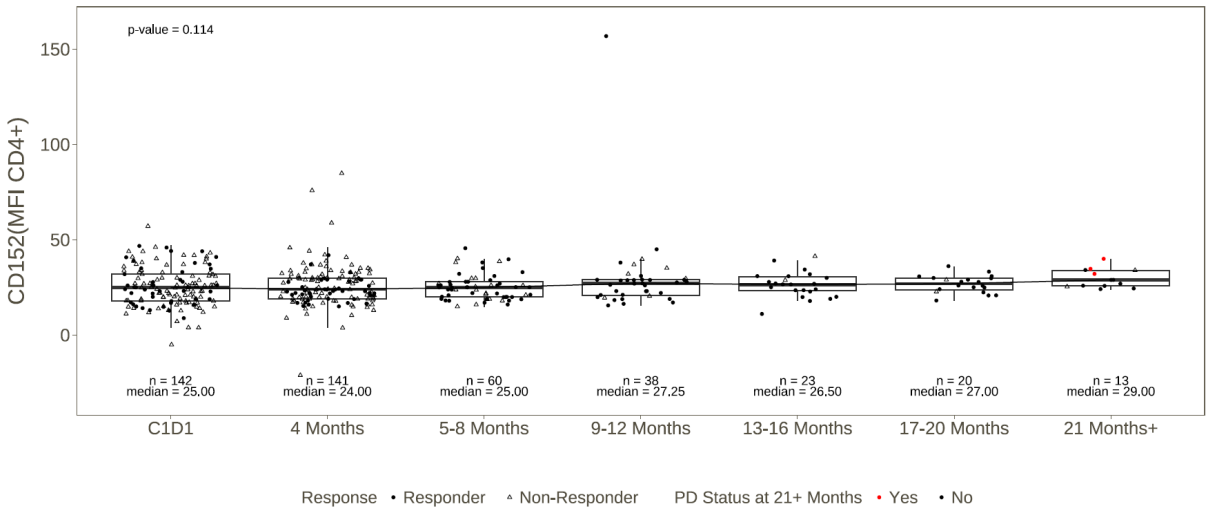


% expression

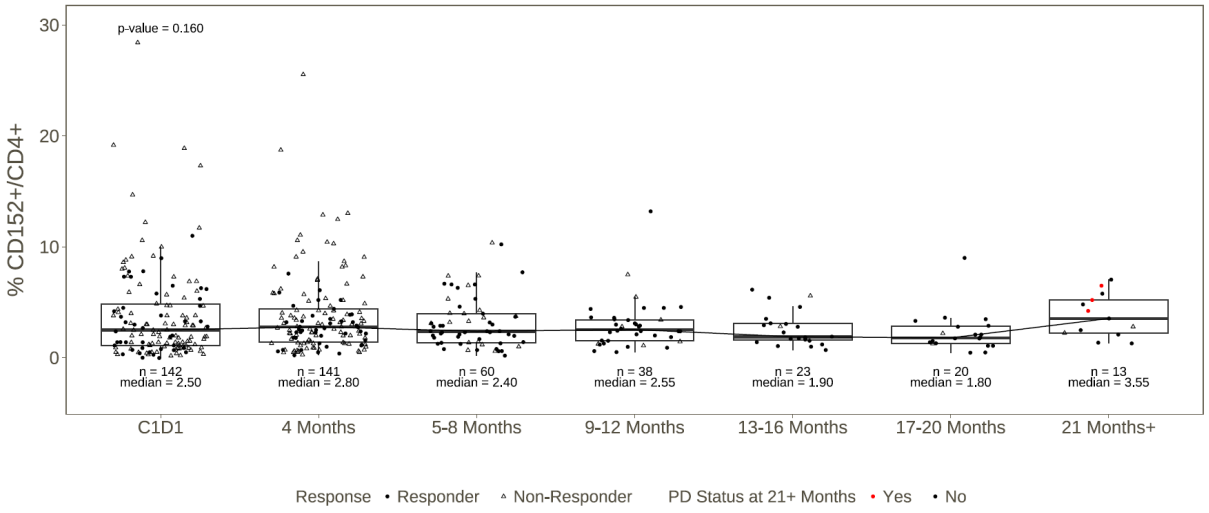


CD4+ T cells

Median fluorescence intensity

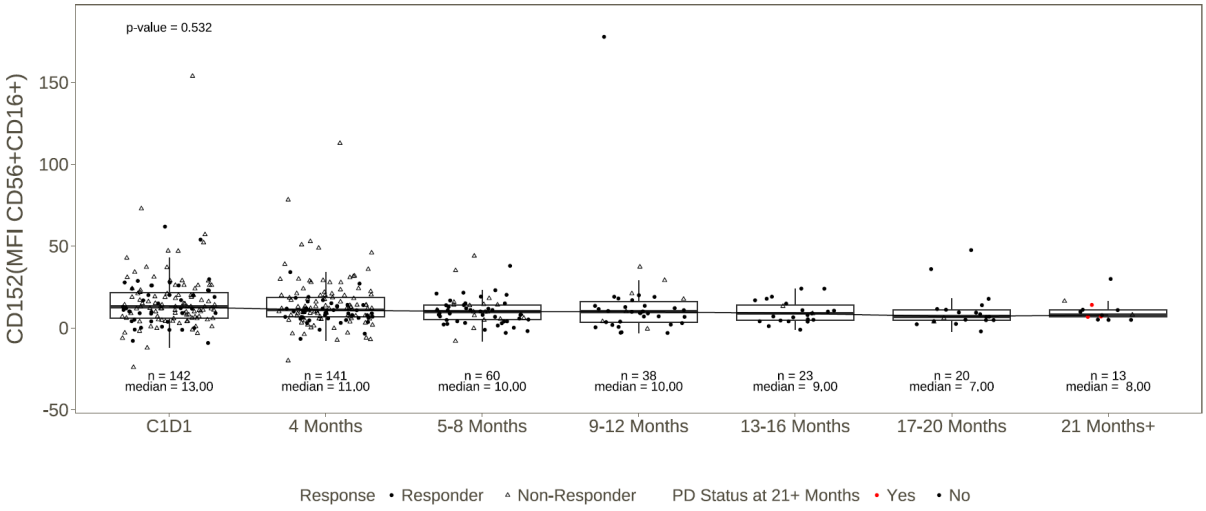


% expression

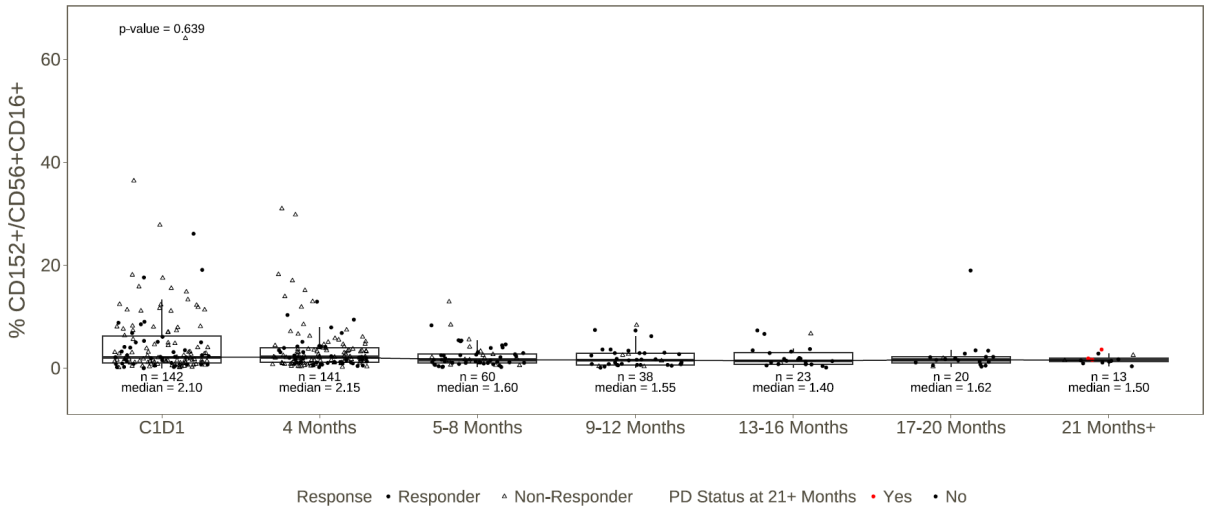


NK cells

Median fluorescence intensity



% expression



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

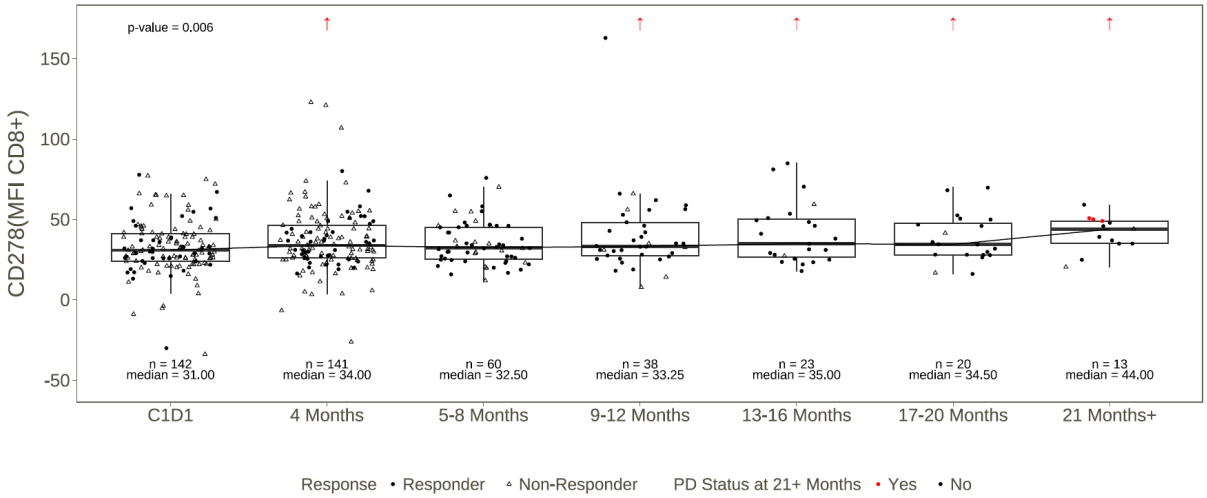
The median of the timepoints within each time category for each patient was utilized.

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

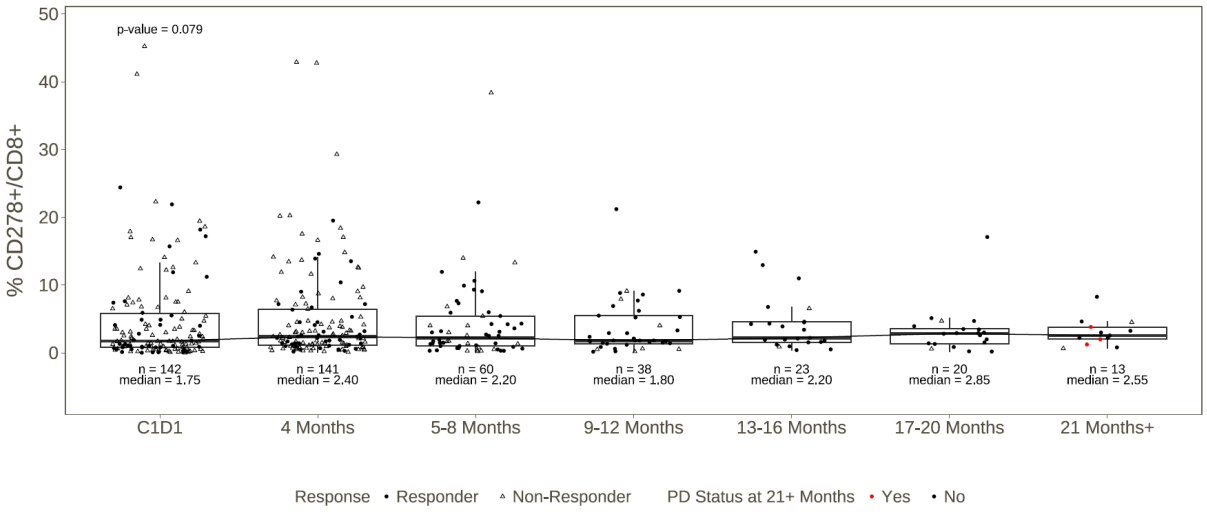
Supplemental Figure 9. Impact of belantamab mafodotin on costimulatory marker ICOS*

CD8+ T-cells

Median fluorescence intensity

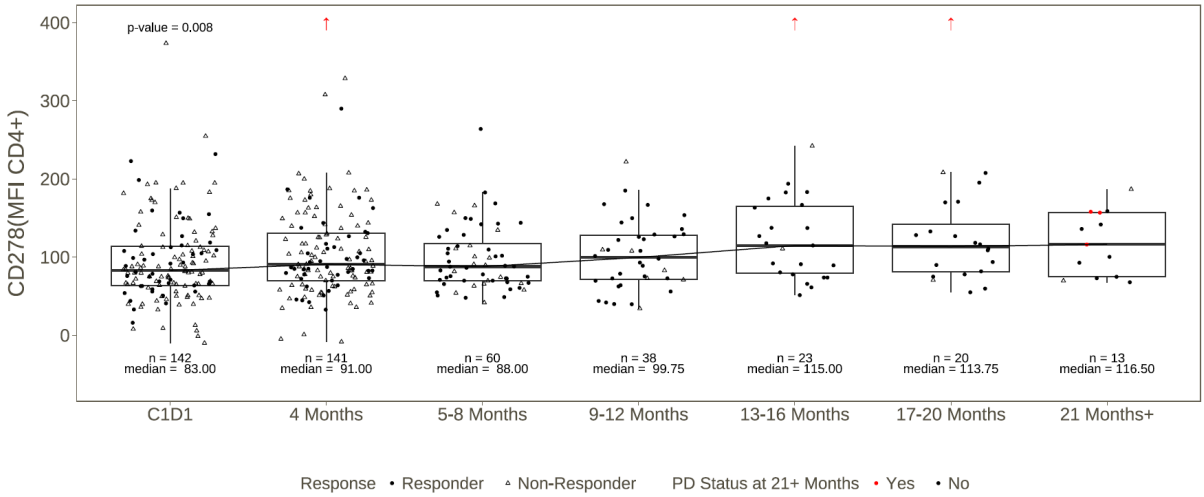


% expression

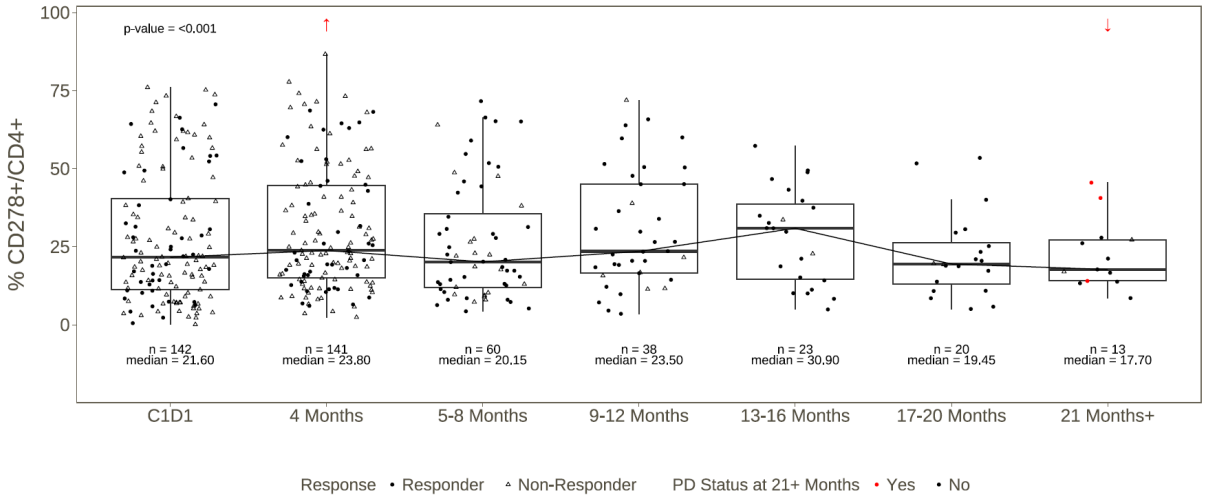


CD4+ T cells

Median fluorescence intensity

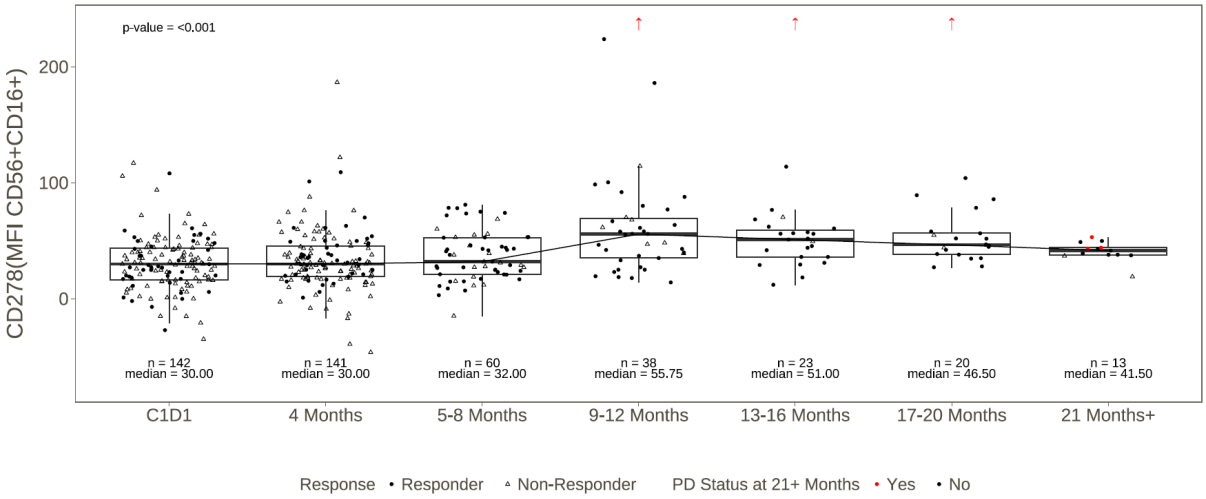


% expression

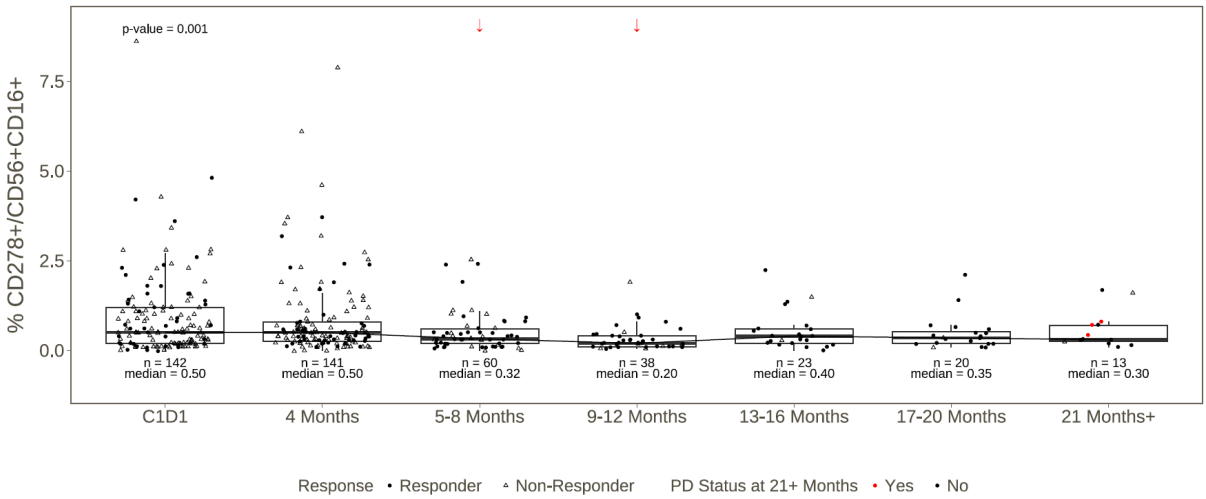


NK cells

Median fluorescence intensity



% expression



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

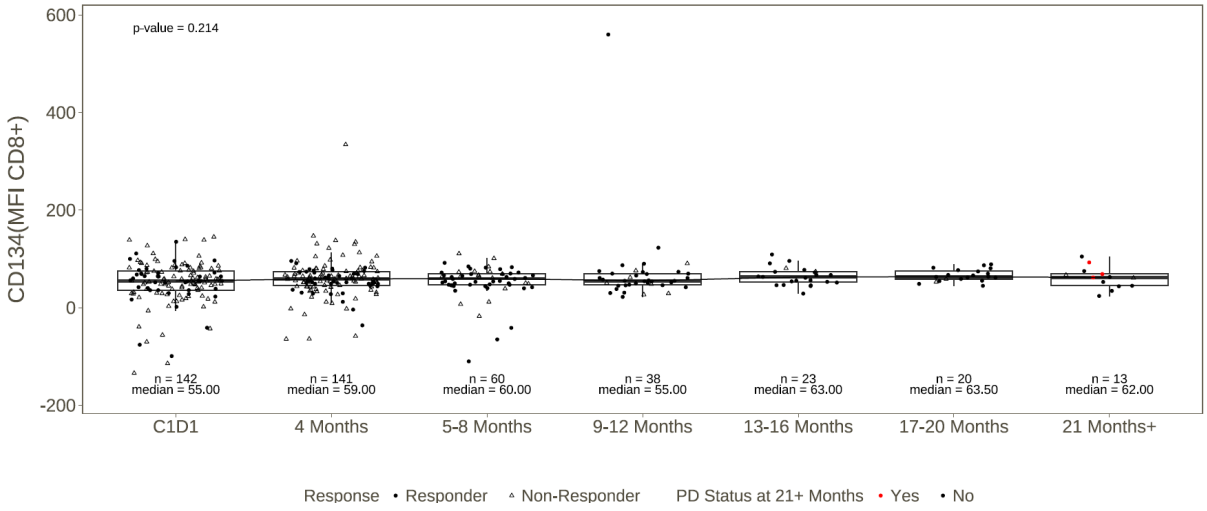
The median of the timepoints within each time category for each patient was utilized. The direction of the red arrows indicates significant increase or decrease from C1D1 for each timepoint.

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

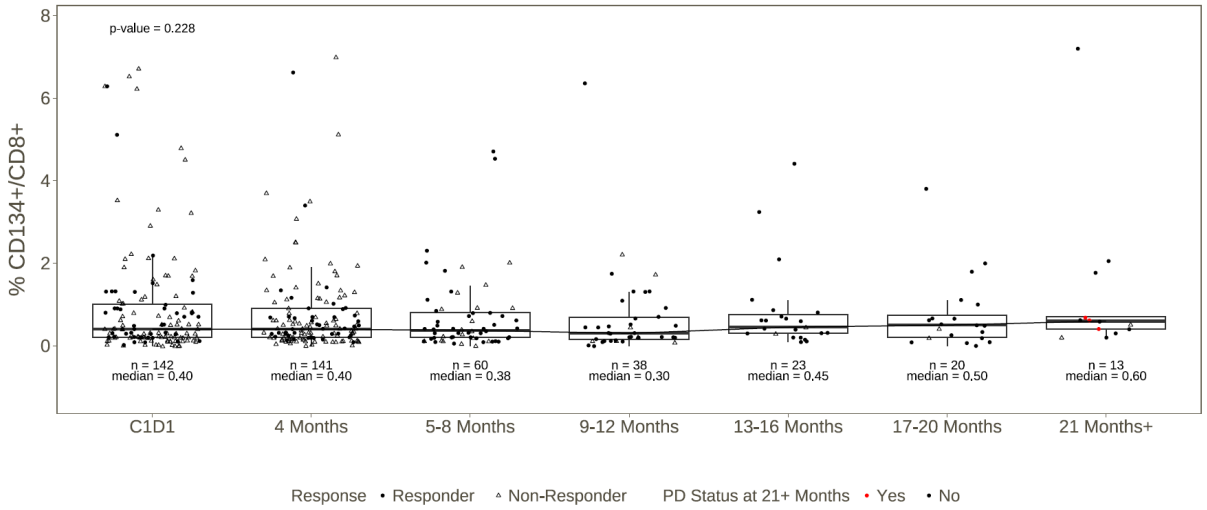
Supplemental Figure 10. Impact of belantamab mafodotin on costimulatory marker OX40*

CD8+ T cells

Median fluorescence intensity

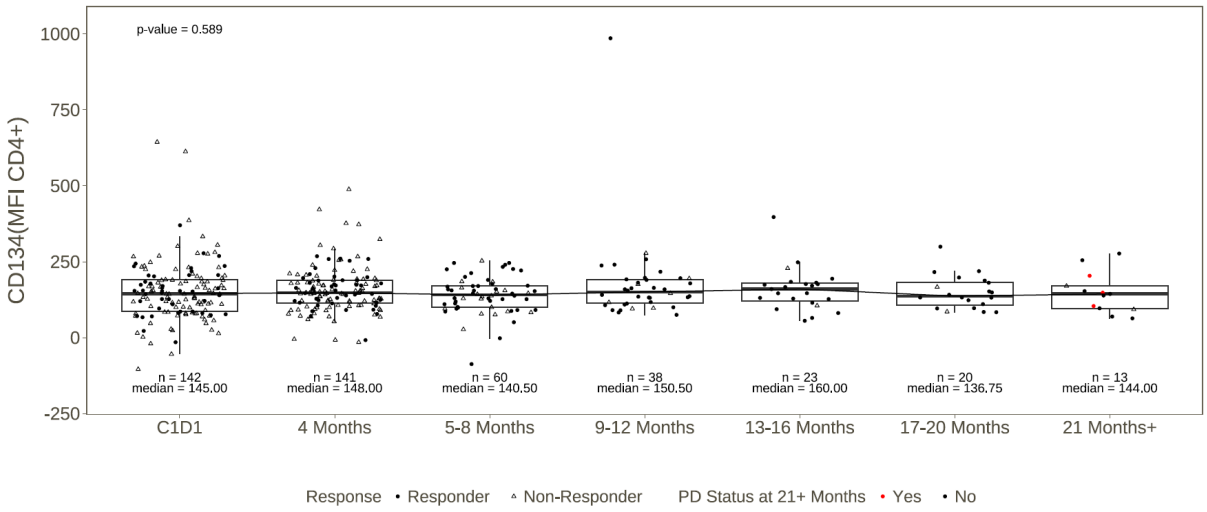


% expression

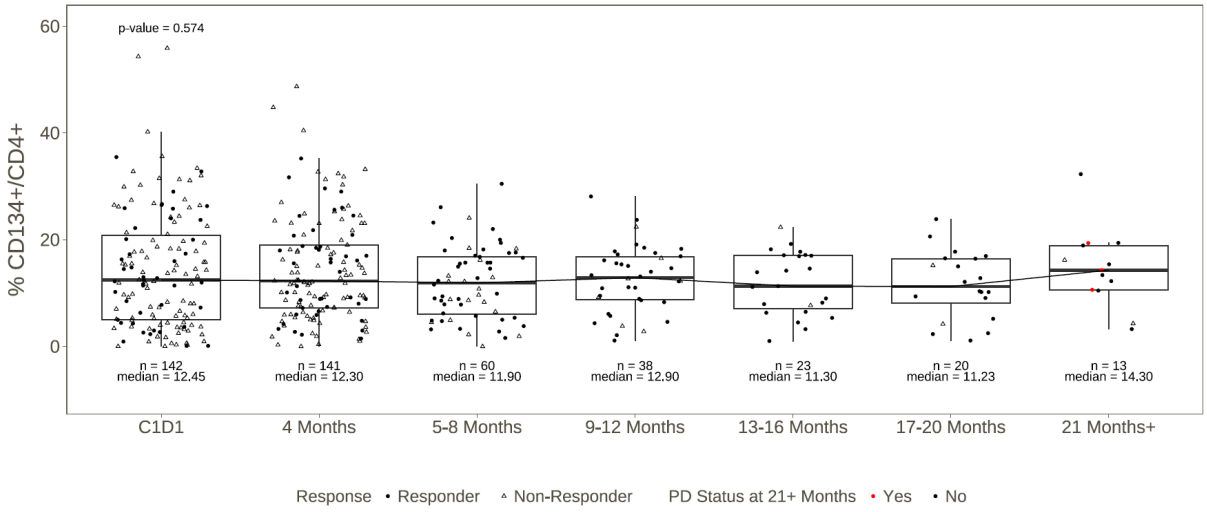


CD4+ T cells

Median fluorescence intensity

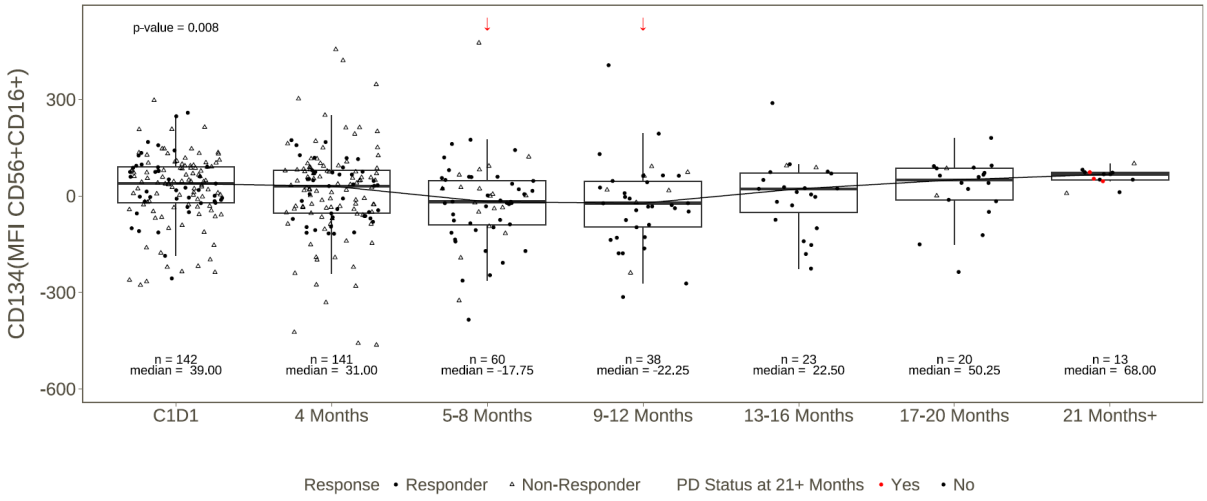


% expression

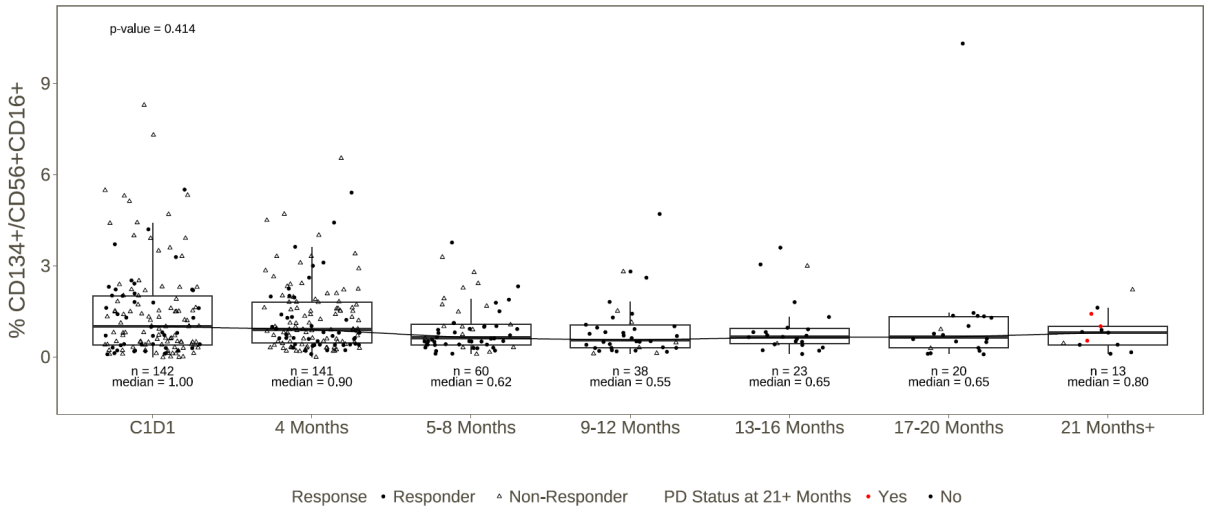


NK cells

Median fluorescence intensity



% expression



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

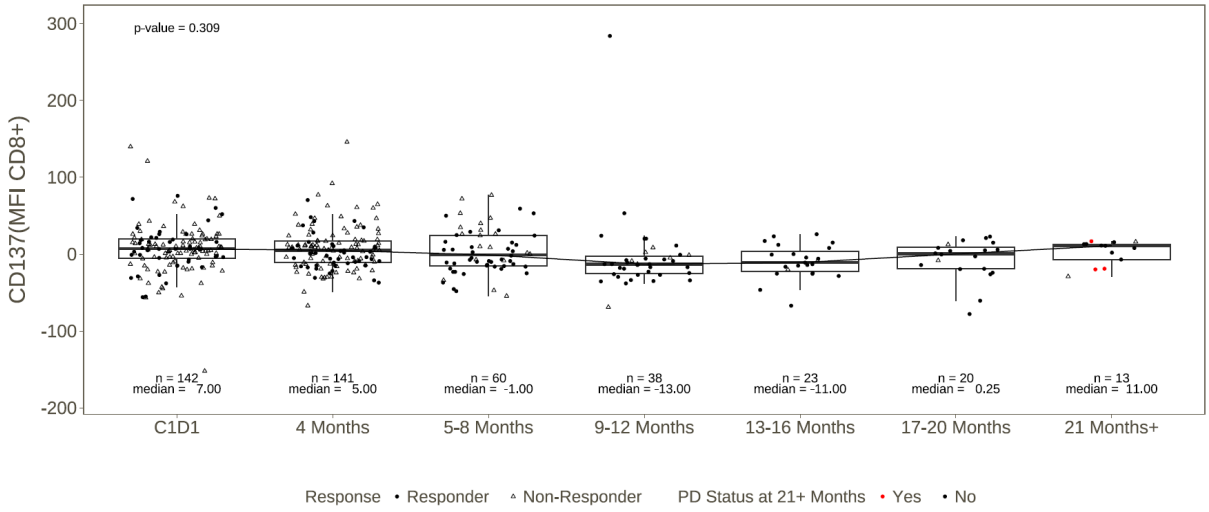
The median of the timepoints within each time category for each patient was utilized. The direction of the red arrows indicates significant increase or decrease from C1D1 for each timepoint.

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

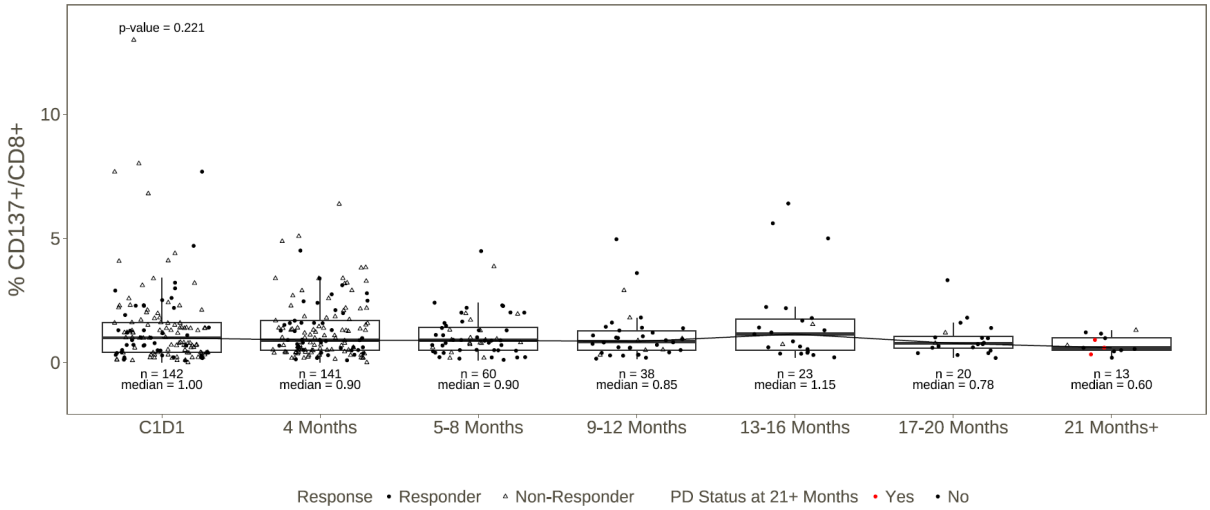
Supplemental Figure 11. Impact of belantamab mafodotin on costimulatory marker 4-1BB*

CD8+ T cells

Median fluorescence intensity

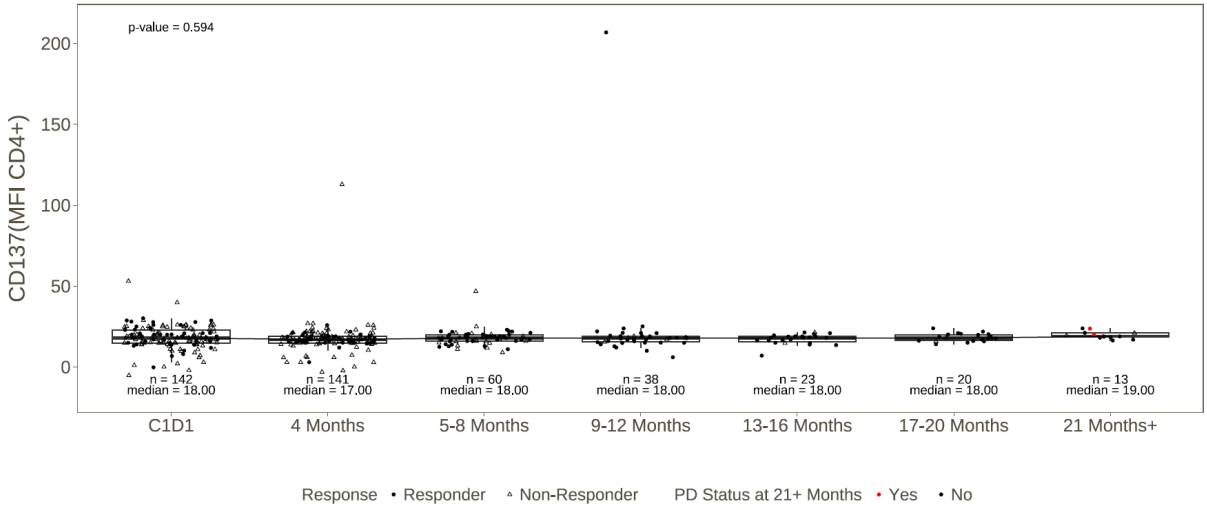


% expression

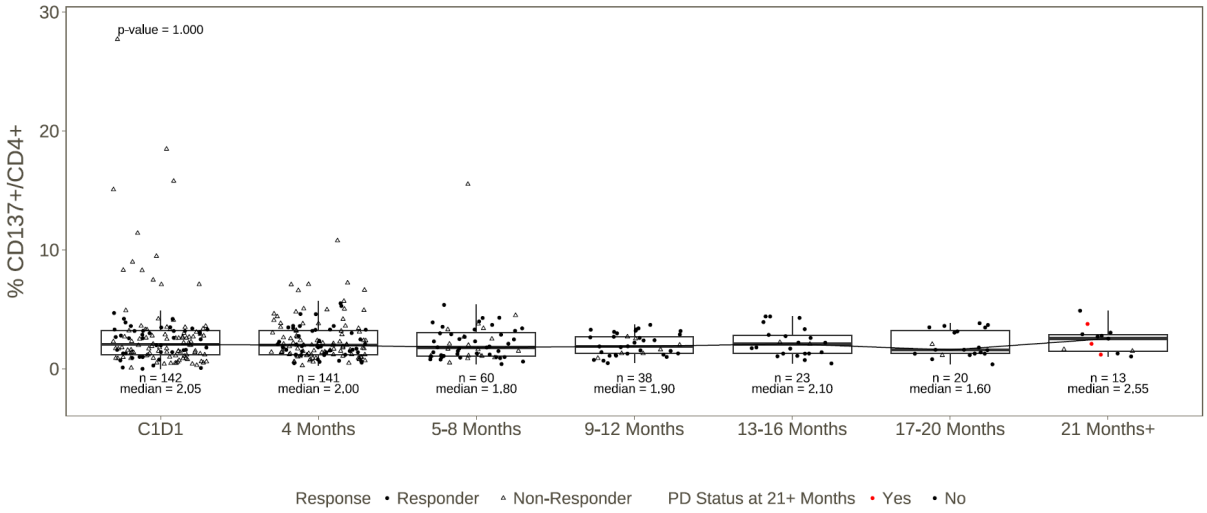


CD4+ T cells

Median fluorescence intensity

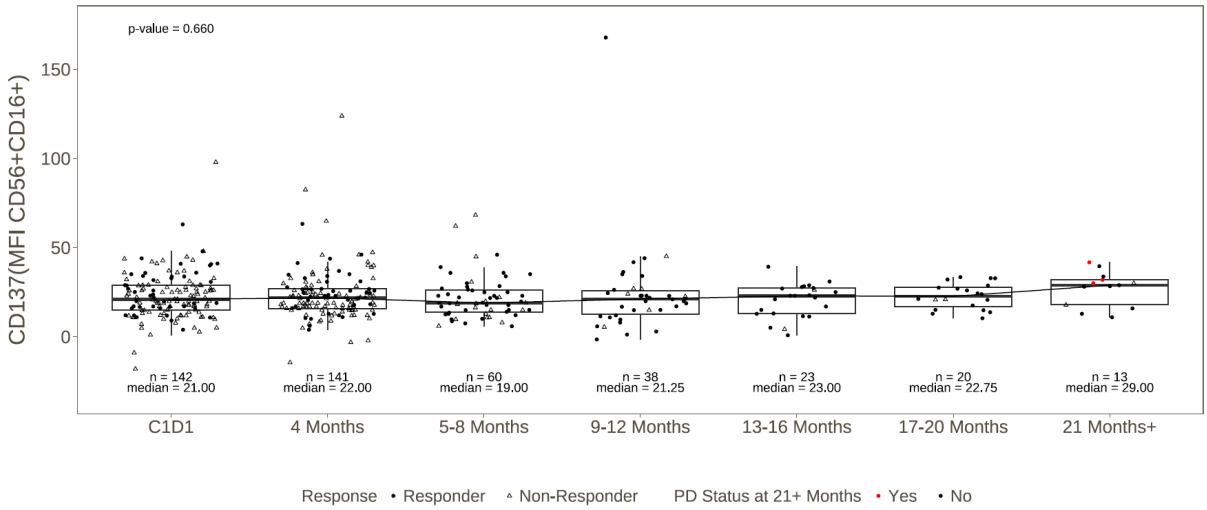


% expression

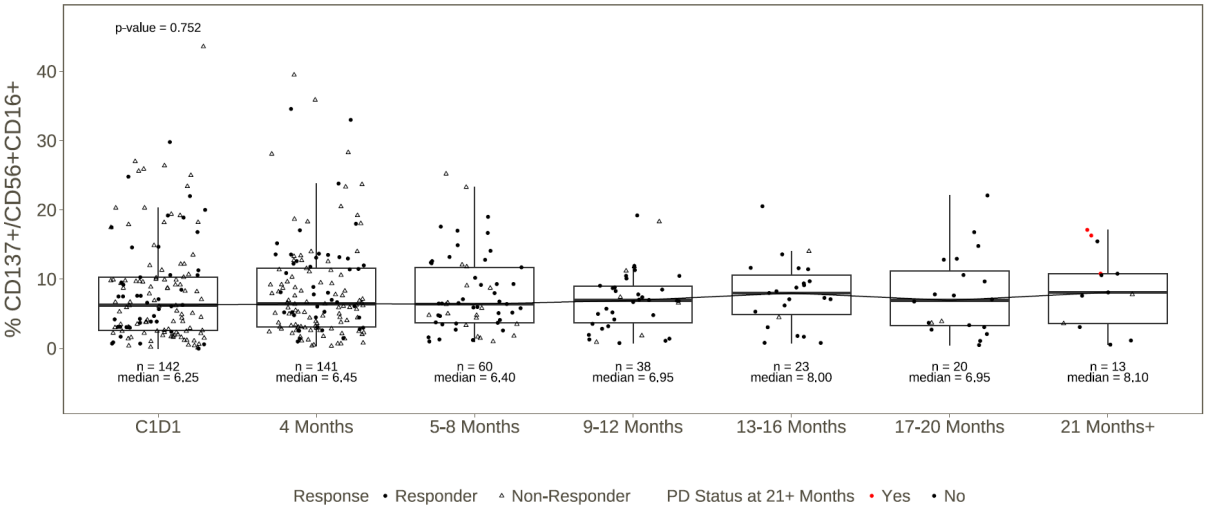


NK cells

Median fluorescence intensity



% expression

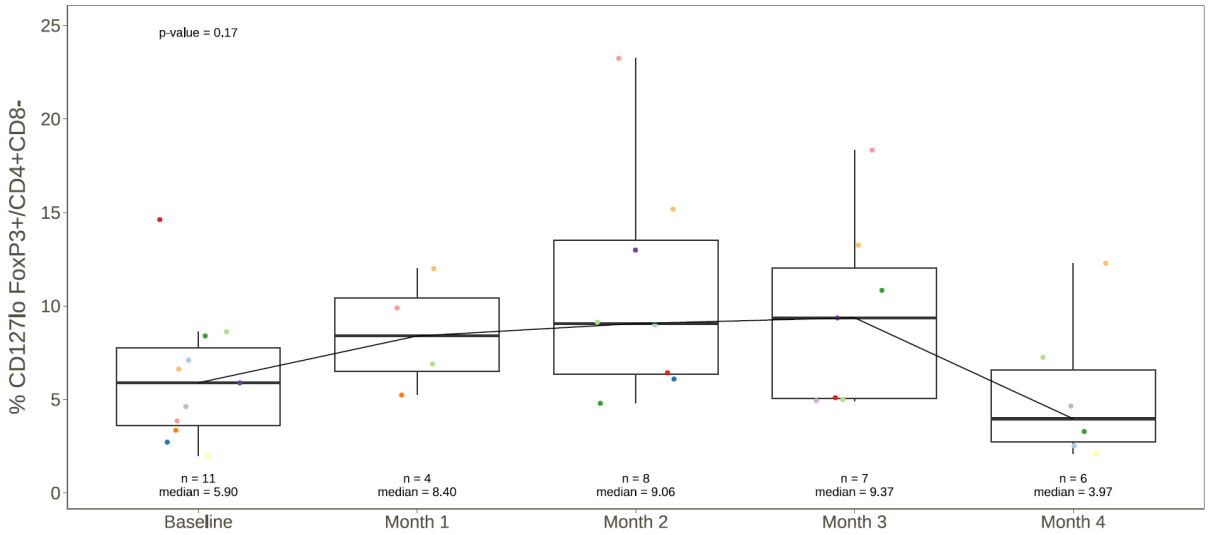


*Data are from the DREAMM-14 study of belantamab mafodotin monotherapy.

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.