**ORIGINAL ARTICLE**

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Predictive role of absolute lymphocyte count in daratumumab-treated patients with relapsed/refractory multiple myeloma

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Predictive role of absolute lymphocyte count in daratumumab-treated patients with relapsed/refractory multiple myeloma

Patients
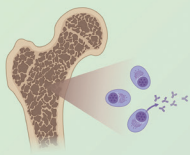
Relapsed/refractory Multiple myeloma
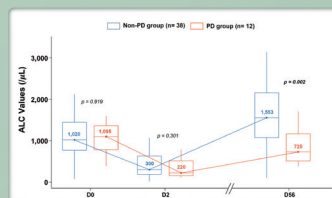
Treated with daratumumab

Method

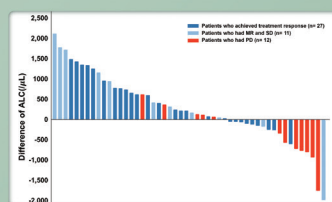Change of absolute lymphocyte count (ALC)

Aim

Survival outcomes

Result

The rapid decline and subsequent rise in ALC after daratumumab infusion was observed in better treatment responses.

ALC increase is an independent prognostic factor of the survival outcomes of patients treated with daratumumab.

Conclusion Increase in ALC during daratumumab treatment was significantly associated with prolonged survival outcomes in patients with RRMM.

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Background/Aims: Daratumumab has shown an encouraging antitumor effect in patients with multiple myeloma (MM), and was known to alter the immune properties by off-targeting immunosuppressive cells. Here, we aimed to evaluate the change in absolute lymphocyte count (ALC) as a surrogate marker for predicting survival outcomes of patients treated with daratumumab.

Methods: Between 2018 and 2021, the medical records of patients with relapsed/refractory MM (RRMM) treated with daratumumab monotherapy at 10 centers in South Korea were reviewed. We collected the ALC data at pre-infusion (D0), day 2 after the first infusion (D2), and prior to the third cycle of daratumumab therapy (D56).

Results: Fifty patients who were administered at least two cycles of daratumumab were included. Overall response rate was 54.0% after two cycles of daratumumab treatment. On D2, almost all patients experienced a marked reduction in ALC. However, an increase in ALC on D56 (ALC^{D56}) was observed in patients with non-progressive disease, whereas failure of ALC recovery was noted in those with progressive disease. Patients with $ALC^{D56} > 700/\mu L$ (n = 39, 78.0%) had prolonged progression-free survival (PFS) and overall survival (OS) than those with $ALC^{D56} \leq 700/\mu L$ (median PFS: 5.8 months vs. 2.6 months, $p = 0.025$; median OS: 24.1 months vs. 6.1 months, $p = 0.004$). In addition, $ALC^{D56} > 700/\mu L$ was a significant favorable prognostic factor for PFS (hazard ratio [HR], 0.22; $p = 0.003$) and OS (HR, 0.23; $p = 0.012$).

Conclusions: Increase in ALC during daratumumab treatment was significantly associated with prolonged survival outcomes in patients with RRMM. The ALC value can predict clinical outcomes in patients treated with daratumumab.

Keywords: Multiple myeloma; Daratumumab; Lymphocyte count; Biomarkers; Survival analysis

INTRODUCTION

The introduction of novel agents, proteasome inhibitors (PI) and immunomodulatory drugs (IMiD), and high-dose chemotherapy followed by autologous stem cell transplantation (ASCT) have contributed to a substantial improvement in the survival outcomes of patients with multiple myeloma (MM) [1]. However, the majority of patients become relapsed and/or refractory (RR) despite novel agent-based treatment regardless of receiving ASCT, and the natural course of MM is considered incurable [2]. In particular, the survival outcomes of patient refractory to PI and/or IMiD are dismal, with a median survival of 8 to 9 months and limited treatment options available for these patients [3]. Therefore, it is imperative to develop a novel therapy that has antimyeloma mechanisms different from those of PIs and IMiDs.

Daratumumab selectively binds to CD38, which is ubiquitously and highly expressed in malignant plasma cells, and mediates cell death through various mechanisms, including antibody-dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity, and antibody-dependent cellular phagocytosis [3]. In MM patients heavily pretreated with PI and/or IMiD, daratumumab treatment demonstrated promising results: an overall response rate (ORR) of 29% to 36% and a durable response duration of 6.9 to

7.6 months with a favorable safety profile [4-6]. Addition of daratumumab to backbone therapy comprising PI and/or IMiD has shown remarkable efficacy in patients with newly diagnosed MM [7,8]; thus, its earlier use as a front-line treatment appears promising and is gradually increasing. However, despite the encouraging therapeutic efficacies of daratumumab, some patients failed to respond to the treatment. Furthermore, heterogeneity was observed in the duration and quality of treatment response in patients who responded to daratumumab.

Lymphocyte count has been associated with clinical outcomes in solid cancers [9] and several hematologic malignancies [10-12]. As immunotherapy has been introduced in cancer management, the role of lymphocytes has been the hotbed of research as these cells reflect the host immune function, which determines the overall success of immune-based treatment [9]. Daratumumab was revealed to exert an immunomodulatory action by inhibiting CD38-expressing immunosuppressive cells, which might result in prolonged survival and delayed recurrence [13,14]. Given the immune-mediated action of daratumumab, it could be hypothesized that the lymphocyte count may be associated with the treatment outcomes of patients with MM treated with daratumumab. Therefore, in this study, we aimed to evaluate the changes in absolute lymphocyte count (ALC)

in patients treated with daratumumab and to identify the effect of ALC on the treatment response and survival outcomes of these patients.

METHODS

Patients

We retrospectively reviewed the medical records of patients diagnosed with MM according to the International Myeloma Working Group (IMWG) guidelines [15] at 10 medical centers in South Korea between December 2004 and June 2021. The inclusion criteria were as follows: (1) patients treated with daratumumab after more than three lines of therapy administered previously, including PI, IMiD, and/or ASCT, and (2) patients who received at least two cycles of daratumumab (total eight infusions) and underwent complete blood count (CBC) evaluation before and after daratumumab infusion. The exclusion criteria were as follows: (1) patients who received concurrent administration of other antimyeloma therapy with daratumumab and (2) patients with missing CBC values or whose treatment response was not assessed. This study was approved by the Institutional Review Board of Kyungpook National University Hospital (IRB no. 2021-05-13) and by each participating center in accordance with the Declaration of Helsinki. Written informed consent by the patients was waived due to a retrospective nature of our study.

Treatment and response evaluation

Daratumumab was intravenously infused at a dose of 16 mg/kg weekly (days 1, 8, 15, and 22) for the first two cycles, every 2 weeks (days 1 and 15) for the subsequent four cycles, and every 4 weeks thereafter. According to the IMWG consensus criteria, treatment responses were classified as follows: complete response (CR), very good partial response (VGPR), partial response (PR), minimal response, stable disease (SD), and progressive disease (PD) [16]. The ORRs were CR, VGPR, and PR, respectively. Achieving SD or a better response was defined as non-progressive disease (non-PD). Response was evaluated after completing two cycles (eight infusions) of daratumumab.

Collecting complete blood count results

The CBC assessment included evaluation of hemoglobin, white blood cell count, absolute neutrophil count (ANC),

ALC, and platelet (PLT) count. To monitor the serial changes in CBC during daratumumab therapy, we collected the CBC results performed on the following days: D0, the day or the previous day of the first daratumumab infusion; D2, from the second to the fourth day after the first infusion; and D56, the day prior to starting the third cycle of daratumumab therapy (Supplementary Fig. 1). Unless the patients proceeded with the third cycle of daratumumab for any reason, the CBC for D56 was replaced with the results obtained within 7 days after completing the second cycle (eighth infusion) of daratumumab.

Statistical analysis

Data related to patient characteristics are presented as proportions and medians. Continuous variables were compared using a two-sample t test or analysis of variance, and categorical data were compared using the chi-square test. Progression-free survival (PFS) was defined as the time from diagnosis to disease progression. Overall survival (OS) was defined as the time from diagnosis to the time of the last follow-up or all-cause death. PFS and OS curves were plotted using the Kaplan-Meier method, and group comparisons were performed using a log-rank test. Prognostic factors affecting OS and PFS were evaluated using a Cox regression model. Factors with $p < 0.1$ in the univariate analysis were included in the multivariate analysis, and $p < 0.05$ were considered significant. A receiver operating characteristic (ROC) curve analysis was conducted to determine the optimal cut-off value of variables that predict treatment outcomes. R statistical software 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria; available at http://www.r-project.org) and SPSS version 20.0 (IBM Co., Armonk, NY, USA) were used for all statistical analyses.

RESULTS

Patient characteristics and treatment outcomes

Fifty patients with relapsed/refractory MM (RRMM) who received daratumumab monotherapy were included in the final analysis (Supplementary Fig. 2). The median age at the initiation of daratumumab therapy was 64.0 years (range, 40 to 86), and 24 patients (48.0%) were aged 65 years or older. Twenty-seven patients (54.0%) were male. The Eastern Cooperative Oncology Group (ECOG) performance

Table 1. Baseline characteristics of patients and clinical outcomes after daratumumab

Characteristic	Overall (n = 50)	ALC at day 56 after daratumumab		p value
		> 700/μL (n = 39)	≤ 700/μL (n = 11)	
Age, yr	64.0 (40–86)	63.8 (40–86)	67.0 (53–82)	0.623
Age ≥ 65	24 (48.0)	18 (46.2)	6 (54.5)	
Sex				0.520
Male	27 (54.0)	22 (56.4)	5 (45.5)	
Female	23 (46.0)	17 (44.6)	6 (54.5)	
ECOG PS				0.411
0–1	40 (80.0)	32 (82.1)	8 (72.7)	
2–3	8 (16.0)	5 (12.8)	3 (27.3)	
Unknown	2 (4.0)	2 (5.1)	0 (0.0)	
Subtype				0.080
IgG	36 (72.0)	28 (71.8)	8 (72.7)	
IgA	5 (10.0)	2 (5.1)	3 (27.3)	
Light chain type	8 (16.0)	8 (20.5)	0	
Unknown	1 (2.0)	1 (2.6)	0	
ISS				0.056
I, II	25 (50.0)	22 (56.4)	3 (27.3)	
III	21 (42.0)	13 (33.3)	8 (72.7)	
Unknown	4 (8.0)	4 (10.3)	0	
R-ISS				0.348
I, II	34 (68.0)	26 (66.7)	8 (72.7)	
III	10 (20.0)	7 (17.9)	3 (27.3)	
Unknown	6 (12.0)	6 (15.4)	0	
Previous lines of therapy	4 (3–7)	4 (3–7)	4 (3–5)	1.000
3 lines	18 (36.0)	14 (35.9)	4 (36.4)	
4–7 lines	32 (64.0)	25 (64.1)	7 (63.6)	
Prior use of PI	50 (100.0)	39 (100)	11 (100)	
Bortezomib	48 (96.0)	38 (97.4)	10 (90.9)	0.395
Carfilzomib	26 (52.0)	16 (41.0)	10 (90.9)	0.003
Prior use of IMiD	50 (100.0)	39 (100)	11 (100)	
Thalidomide	34 (86.0)	27 (69.2)	7 (63.6)	0.728
Lenalidomide	49 (98.0)	38 (97.4)	11 (100.0)	1.000
Pomalidomide	30 (60.0)	25 (64.1)	5 (45.5)	0.331
Previous ASCT	30 (60.0)	25 (64.1)	5 (45.5)	0.311
Cycles of daratumumab	4.5 (2–25)	5 (2–25)	3 (2–11)	0.004
Response[a]				0.093
CR	2 (4.0)	2 (5.1)	0	
VGPR	6 (12.0)	5 (12.8)	1 (9.1)	
PR	19 (38.0)	18 (20.5)	1 (9.1)	
MR	4 (8.0)	3 (7.7)	1 (9.1)	
SD	7 (14.0)	5 (12.8)	2 (18.2)	

Table 1. Continued

Characteristic	Overall (n = 50)	ALC at day 56 after daratumumab		p value
		> 700/μL (n = 39)	≤ 700/μL (n = 11)	
PD	12 (24.0)	6 (15.4)	6 (54.5)	
Overall response rate	27 (54.0)	25 (64.1)	2 (18.2)	0.018
ALC at D0, /μL	1,070 (70–3,430)	1,120 (330–3,430)	800 (70–1,590)	0.024
ALC at D2, /μL	295 (20–3,100)	300 (40–3,100)	240 (20–780)	0.198
ALC at D56, /μL	1,388 (100–3,140)	1,600 (720–3,140)	510 (100–700)	< 0.001

Values are presented as median (range) or number (%).
ALC, absolute lymphocyte count; ECOG PS, Eastern Cooperative Oncology Group performance status; Ig, immunoglobulin; ISS, International Staging System; R-ISS, Revised International Staging System; PI, proteasome inhibitor; IMiD, immunomodulatory drug; ASCT, autologous stem cell transplantation; CR, complete response; VGPR, very good partial response; PR, partial response; MR, minimal response; SD, stable disease; PD, progressive disease.
[a]Response was evaluated after two cycles of daratumumab treatment.

status score was 0 or 1 in 40 patients (80.0%) and 2 or 3 in eight patients (16.0%). Immunoglobulin G (IgG) was detected in a majority of the patients (n = 36, 72.0%). IgA, light chain type, and unknown were found in five (10.0%), eight (16.0%), and one patient (2.0%), respectively. Of the total patients, 34 (68.0%) were classified as Revised International Staging System (R-ISS) I or II, and 10 (20.0%) were classified as the R-ISS III. Patients received a median of four (range, 3 to 7) previous lines of treatment before the initiation of daratumumab therapy. All patients were refractory to PI and/or IMiD and/or relapsed after receiving these regimens, 60% patients (n = 30) underwent ASCT. The median number of daratumumab treatment cycles was 4.5 (range, 2 to 25). Sixteen patients (32.0%) were still receiving daratumumab at the time of data analysis (Supplementary Table 1). In terms of the treatment response after completing the second cycle of daratumumab, the observed responses were as follows: PR or better in 27 patients (54 %), CR in two (4.0%), VGPR in six (12.0%), and PR in 19 patients (38.0%). Meanwhile, 12 patients (24.0%) experienced progression after two cycles of daratumumab. The detailed patient characteristics and treatment outcomes are summarized in Table 1.

CBC trend after daratumumab treatment

The median ALC value was 1,070/μL (range, 70 to 3,430) on D0 but sharply dropped to 295/μL (range, 20 to 3,100) on D2. After two cycles of daratumumab therapy, the ALC value recovered to 1,388/μL (range, 100 to 3,140) (Table 1). Meanwhile, the hemoglobin value, ANC, and PLT counts followed a trend different from that of ALC during the course of treatment, although the PLT count slightly decreased on D2 (Supplementary Table 2).

An ROC plot was generated to determine the optimal ALC value based on the overall response of the patients (PR or better), and the cut-off value for ALC on D56 (ALC^{D56}) was determined as 700/μL (area under the curve = 0.668; p = 0.023) (Supplementary Fig. 3). No association was found between the ALC value on D0 or D2 and treatment response. Overall, 39 (78.0%) and 11 (22.0%) patients had an ALC^{D56} > 700/μL (ALC^{D56} > 700) and ALC^{D56} ≤ 700/μL (ALC^{D56} ≤ 700), respectively. The distribution of each characteristic was not significantly different according to the ALC^{D56} value (Table 1); however, patients with ALC^{D56} > 700 received more cycles of daratumumab therapy (Supplementary Fig. 4) and showed a higher baseline ALC value than those with ALC^{D56} ≤ 700 (Table 1). Interestingly, the ALC value increased from 1,120 to 1,600/μL in patients with ALC^{D56} > 700 during daratumumab treatment but decreased in those with ALC^{D56} ≤ 700 (Table 1). Among the other components of CBC, the PLT count showed a trend similar to that of the ALC value during daratumumab treatment (Supplementary Table 2).

Change in ALC according to treatment response: non-PD vs. PD

We evaluated the changes in the ALC values of patients according to treatment response: non-PD or PD. In 38 patients (76.0%) who had SD or better (i.e., non-PD) ALC values changed dynamically: 1,020, 300, and 1,553/μL on D0, D2, and D56, respectively (Fig. 1A). In contrast, for patients with PD (n = 12, 24%), the median ALC values did not completely recover after two cycles of daratumumab treatment: 1,095,

220, and 725/μL on D0, D2, and D56, respectively (Fig. 1A). Between the patients with non-PD and PD, no difference was observed in the baseline ALC values ($p = 0.919$). However, a noticeable difference in ALC values was identified on D56 ($p = 0.002$) (Fig. 1A). A waterfall plot showed a difference in ALC values from D56 to D0 in all patients; most patients with non-PD (n = 27, 71.1% [27/38]) showed an increase in ALC values after receiving daratumumab therapy, whereas those with PD showed a decrease in ALC values (n = 7, 58% [7/12]) as shown in Fig. 1B. The changes in ALC values of each patient on D0, D2, and D56 are shown in Supplementary Fig. 5. Meanwhile, no significant changes in ANC and PLT values during daratumumab therapy were observed (Supplementary Fig. 6).
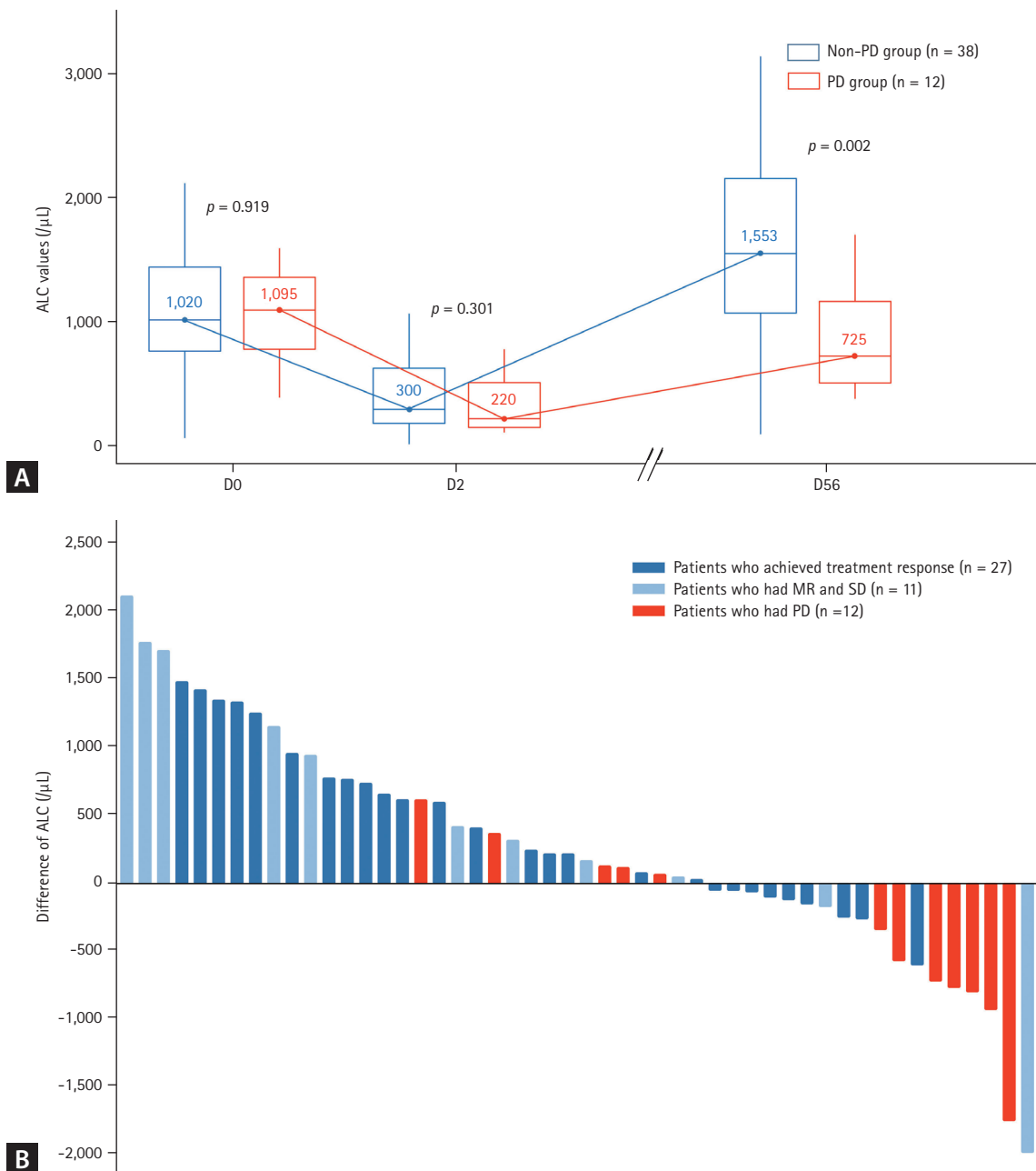

Figure 1. Changes in absolute lymphocyte count (ALC) values during daratumumab therapy. (A) Box plot depicting changes in ALC values according to treatment response. Each pair of boxes shows the ALC^{D0}, ALC^{D2}, and ALC^{D56} values of patients treated with daratumumab. (B) Waterfall plot of differences in ALC values in all patients. The values on the y-axis indicate the differences in the ALC values (from ALC^{D56} to ALC^{D0}). PD, progressive disease; MR, minimal response; SD, stable disease.

Survival outcomes per ALC value

With a median follow-up of 7.2 months (range, 38.3 to 1.7), the survival outcomes were distinctively different based on the ALC^{D56} value. The median PFS was 5.8 months (range, 4.4 to 13.7) and 2.6 months (range, 1.6 to 7.8) for patients with $ALC^{D56} > 700$ and $ALC^{D56} \leq 700$, respectively ($p = 0.025$). Meanwhile, the median OS was 24.1 months (range, 12.2 to not applicable [NA]) and 6.1 months (range, 2.9 to NA) for patients with $ALC^{D56} > 700$ and ≤ 700, respectively ($p = 0.004$) (Fig. 2). Among patients with $ALC^{D56} \leq 700$, two were still receiving daratumumab at the time of data analysis; however, half of them died without subsequent treatment (Supplementary Table 1). In the univariate analysis, the following factors contributed to survival outcomes: sex, age, ECOG, R-ISS, number of previous lines of treatment, prior use of pomalidomide, previous ASCT, and ALC^{D56} value. In the multivariate analysis, $ALC^{D56} > 700$ was significantly associated with prolonged PFS (hazard ratio [HR], 0.22; 95% confidence interval [CI], 0.08 to 0.59; $p = 0.003$) and OS (HR, 0.23; 95% CI, 0.08 to 0.73; $p = 0.012$), as shown in Fig. 3. The other factors that contributed to the reduction in PFS were R-ISS stage III (HR, 10.46; 95% CI, 2.52 to 43.34; $p = 0.001$) and prior use of pomalido-

mide (HR, 3.23; 95% CI, 1.07 to 9.76; $p = 0.038$) (Fig. 3A). Patients who underwent ASCT prior to daratumumab treatment showed a lower risk of death (HR, 0.28; 95% CI, 0.08 to 0.98; $p = 0.046$) (Fig. 3B).

DISCUSSION

This study evaluated the dynamic changes in ALC values and the prognostic impact of ALC increase on the survival outcomes of patients treated with daratumumab. Almost all the patients experienced a rapid decline in ALC immediately after the first infusion of daratumumab, regardless of treatment response. However, the subsequent changes in the ALC values in response to further infusions of daratumumab were significantly different according to the treatment response; failure of ALC recovery was noted in patients with PD after daratumumab treatment, whereas the ALC value increased in those without PD. This immediate decline in ALC may be associated with a marked reduction in CD38 expression in plasma cells and non-plasma immune cells [17] or could be transiently influenced by the pre-infusion of steroids to manage infusion-related reactions to daratumumab
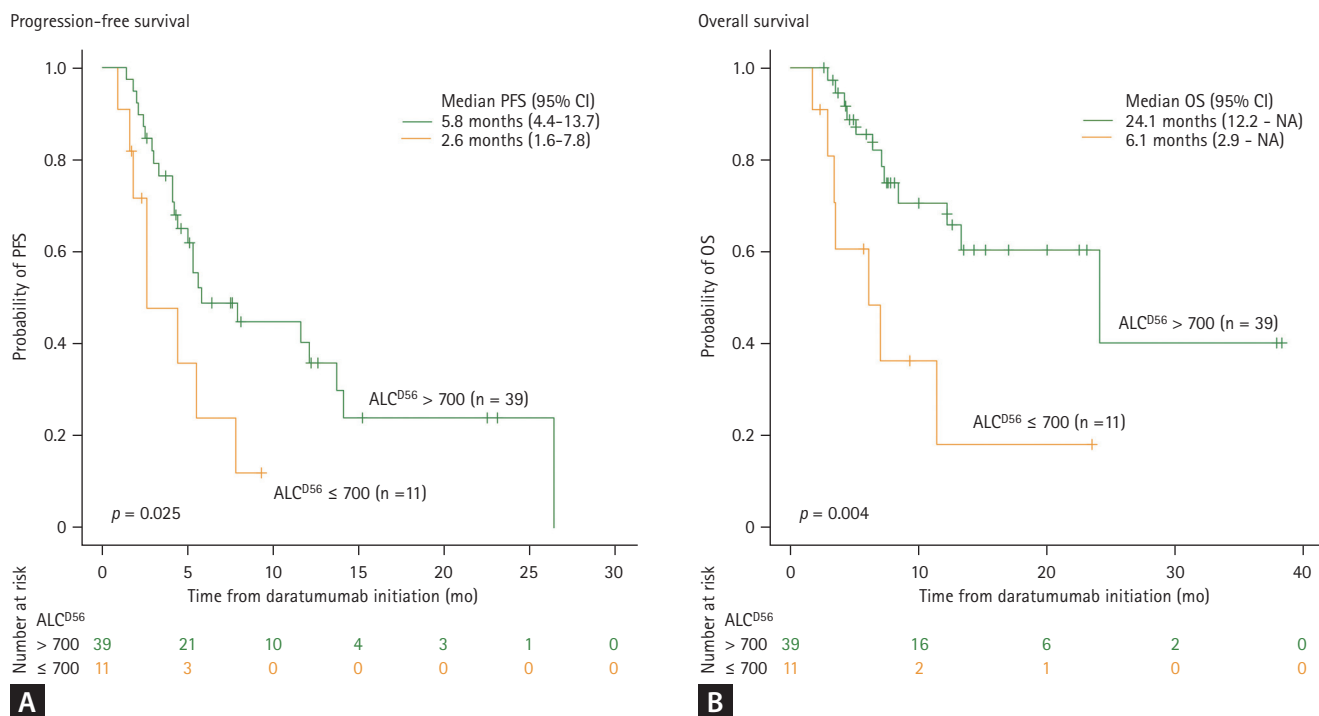

Figure 2. Survival analysis of each absolute lymphocyte counts at the day prior to the third cycle of daratumumab (ALC^{D56}) value after daratumumab treatment. (A) Progression-free survival (PFS), (B) overall survival (OS). NA, not applicable.

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[18]. However, the difference in ALC change according to treatment response may be involved in the immunomodulatory role of daratumumab. In a study evaluating regulatory T cells (Tregs) in patients treated with daratumumab, the absolute number of CD38-expressing Tregs before treatment was higher in patients who responded to daratumumab compared to those who did not responded to the therapy [19]. Indeed, an increase in the absolute number of T cells such as CD3+, CD4+, and CD8+ from baseline, which may be a consequence of Treg suppression by daratumumab treatment, was observed in daratumumab responders [13]. Taken together, the difference in ALC values between patients with and without PD could be attributed to the collective immune response modulated by daratumumab.

The host immune status determines treatment outcomes and has become more significant in the era of immune-based therapy. Therefore, an easily measurable and reliable immune marker is required to predict the clinical outcomes of patients undergoing immunotherapy. In patients with cancer, ALC is an established immune marker for predicting survival outcomes [9]. Several studies have reported its prognostic role in patients with MM. A retrospective analysis of 537 patients with MM demonstrated that an ALC > 1.4×10^9/L at diagnosis was associated with better

OS [20]. After induction therapy, patients with an ALC > 0.8 $\times 10^9$/L also had longer OS [21]. Additionally, early recovery of ALC after ASCT is an independent prognostic factor for OS [22]. In this study, the role of ALC as a surrogate marker for survival in patients treated with daratumumab was confirmed. Our study followed the serial changes in ALC values and showed an increasing trend of ALC in patients without PD. Furthermore, ALC^{D56} > 700/µL after the second cycle of daratumumab was a significant prognostic factor for patient survival outcomes.

In this study, patients who previously received pomalidomide before the initiation of daratumumab therapy had a shorter PFS than those who did not. However, the underlying mechanisms remain unknown. Because therapeutic efficacy of daratumumab has been observed in IMiD-refractory or -relapsed patients in clinical trials [4,5]. Considering that the addition of IMiD to daratumumab-refractory MM patients can act synergistically to overcome drug resistance [23], the expected alteration in immune properties by treatment with a single IMiD might be insufficient to prime an immunological environment to fight against malignant plasma cells. In another explanation, the sequence of therapy may be important in heavily treated patients with MM. A previous single-center retrospective observational study of
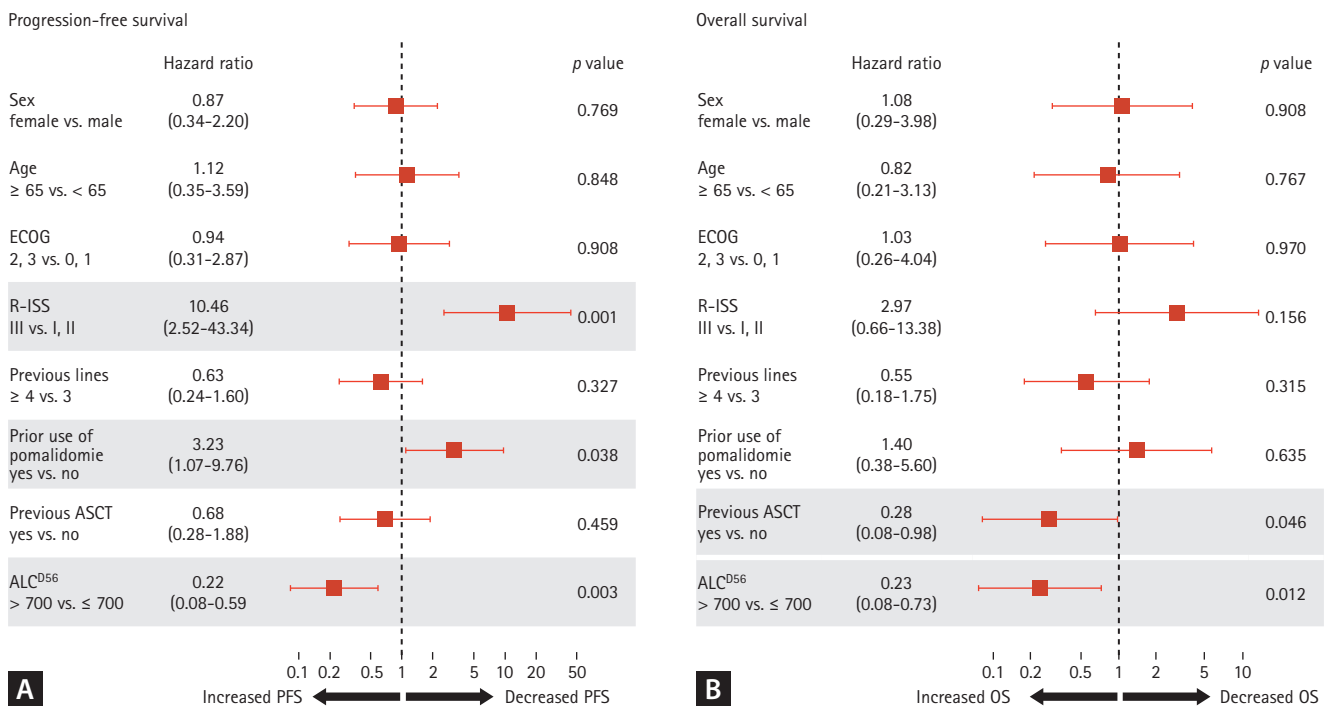

Figure 3. Multivariate Cox regression analyses. (A) Progression-free survival (PFS), (B) overall survival (OS). ECOG, Eastern Cooperative Oncology Group; R-ISS, Revised International Staging System; ASCT, autologous stem cell transplantation; ALC, absolute lymphocyte count.

patients with RRMM showed that patients who were re-treated with IMiDs and PIs after daratumumab monotherapy had impressive ORRs of 52% and 67%, respectively [24]. Additionally, an increase in ALC, which reflects the immune status of the patients, would warrant the use of IMiDs or cellular therapies after daratumumab treatment to improve long-term outcomes.

Despite presenting some credible findings, this study had several limitations. First, we did not perform flow cytometry analysis to identify the type and proportion of subsets consisting of ALC, although we postulated that an increase in ALC during daratumumab treatment might be associated with a favorable immune outcome, such as T cell expansion. Second, changes in ALC values were not identified in patients who received combination therapy. As the use of daratumumab in combination with IMiD and/or PI is increasing, the clinical relevance of ALC in survival outcomes should also be verified in patients treated with three or four regimens. Lastly, medical conditions affecting the CBC value may concurrently exist during daratumumab treatment. Owing to the retrospective nature of this study, we could not control such conditions that may alter the ALC values. However, patients with medical conditions, such as uncontrolled infections that could change the immune profiles, were excluded.

Nevertheless, our findings suggest that ALC is a potential surrogate marker for predicting survival outcomes in patients undergoing daratumumab-based therapy. ALC increase was an independent prognostic factor for survival outcomes in patients treated with daratumumab. Moreover, persistent decrease in ALC values after daratumumab therapy significantly correlated with unfavorable treatment outcomes.

KEY MESSAGE

1. The rapid decline and subsequent rise in absolute lymphocyte count (ALC) after daratumumab infusion was observed in multiple myeloma patients with better treatment responses.
2. ALC increase is an independent prognostic factor of the survival outcomes of patients treated with daratumumab.
3. ALC could be a surrogate marker for predicting the survival outcomes of patients treated with daratumumab.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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Supplementary Table 1. Subsequent treatments after daratumumab therapy

Subsequent treatments	Overall (n = 50)	ALC^{D56} > 700/μL (n = 39)	ALC^{D56} ≤ 700/μL (n = 11)
IMiDs[a]	7 (14.0)	6 (15.4)	1 (9.1)
PIs[a]	7 (14.0)	7 (17.9)	0
Cytotoxic chemotherapy	7 (14.0)	5 (12.8)	2 (18.2)
No treatment	2 (4.0)	2 (5.1)	0 (0.0)
Death	10 (20.0)	4 (10.3)	6 (54.5)
Others	1 (2.0)	1[b] (2.6)	0
Daratumumab[c]	16 (32.0)	14 (35.9)	2 (18.2)

Values are presented as number (%).

ALC^{D56}, absolute lymphocyte counts at the day prior to the third cycle of daratumumab; IMiD, immunomodulatory drug; PI, proteasome inhibitor.

[a]No patient was treated with combination therapy with IMiDs and PIs.

[b]One patient was diagnosed with myelodysplastic syndrome during daratumumab therapy.
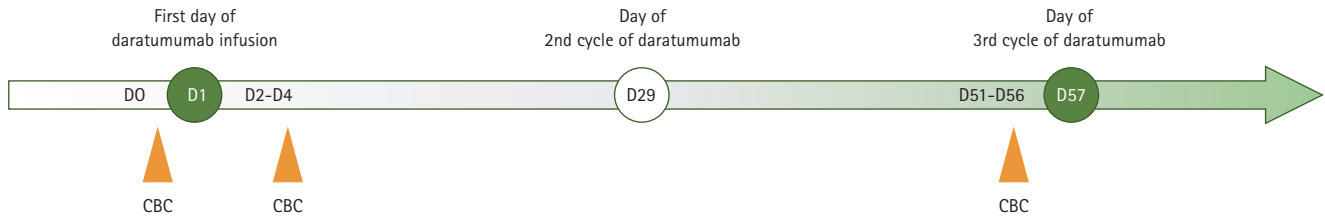
[c]Patients who responded to daratumumab continued the therapy.

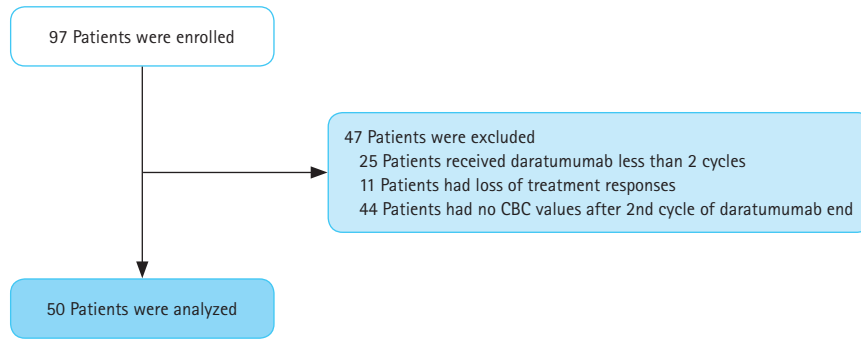**Supplementary Table 2. CBC during the first two cycles of daratumumab therapy**

| CBC	Overall (n = 50)	ALC^{D56} > 700/μL (n = 39)	ALC^{D56} ≤ 700/μL (n = 11)	p value
Hemoglobin, g/dL				
D0	9.5 (6.4–14.1)	9.5 (6.4–14.1)	9.3 (6.7–11.5)	0.076
D2	9.7 (7.0–14.2)	10.0 (7.0–14.2)	9.1 (7.1–11.3)	0.156
D56	10.0 (5.1–15.4)	10.5 (7.1–15.4)	8.8 (5.1–11.9)	0.001
ANC, /μL				
D0	1,494 (231–1,420)	1,600 (231–1,420)	950 (610–1,760)	0.142
D2	2,155 (220–15,040)	2,700 (220–15,040)	1,420 (600–8,250)	0.249
D56	2,060 (50–4,634)	2,200 (169–4,634)	1,430 (50–3,560)	0.024
PLT, ×10^3/μL				
D0	110 (10–286)	124 (10–286)	64 (21–222)	0.063
D2	90 (14–245)	93 (14–245)	38 (18–121)	0.002
D56	125 (7–358)	147 (7–358)	31 (8–158)	< 0.001

Values are presented as median (range).

CBC, complete blood counts; ALC^{D56}, absolute lymphocyte counts at the day prior to the third cycle of daratumumab; ANC, absolute neutrophil count; PLT, platelet.

First day of
daratumumab infusion

Day of
2nd cycle of daratumumab

Day of
3rd cycle of daratumumab

| D0 | D1 | D2–D4 | | D29 | | D51–D56 | D57 | |

CBC   CBC   CBC

Supplementary Figure 1. The day of collecting complete blood count (CBC).

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┌──────────────────────────────┐
│ 97 Patients were enrolled     │
└──────────────────────────────┘
              │
              │          ┌────────────────────────────────────────────────────────────────┐
              ├─────────▶│ 47 Patients were excluded                                       │
              │          │   25 Patients received daratumumab less than 2 cycles           │
              │          │   11 Patients had loss of treatment responses                   │
              │          │   44 Patients had no CBC values after 2nd cycle of daratumumab end│
              ▼          └────────────────────────────────────────────────────────────────┘
┌──────────────────────────────┐
│ 50 Patients were analyzed     │
└──────────────────────────────┘
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Supplementary Figure 2. Consort diagram of the patient selection process. CBC, complete blood count.

Cho HJ, et al. Absolute lymphocyte count in daratumumab

KJIM
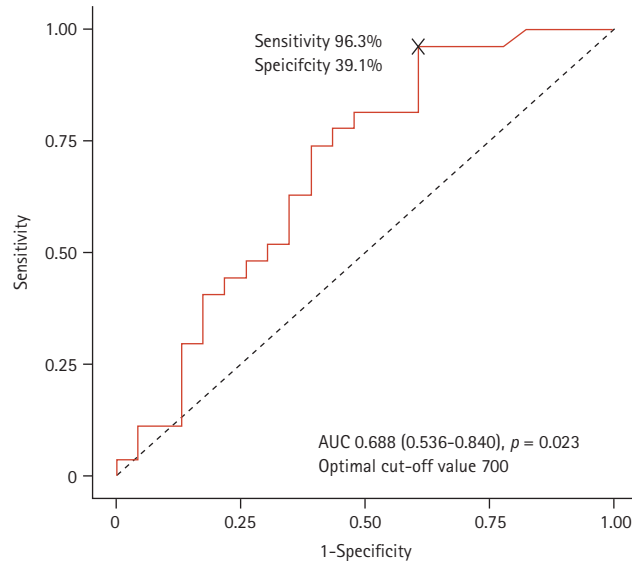
Supplementary Figure 3. Receiver operating characteristic (ROC) analysis to determine the optimal value of absolute lymphocyte counts at the day prior to the third cycle of daratumumab (ALC^{D56}) based on the treatment response. The area under the curve (AUC) is 0.668 and the cut-off value is 700.
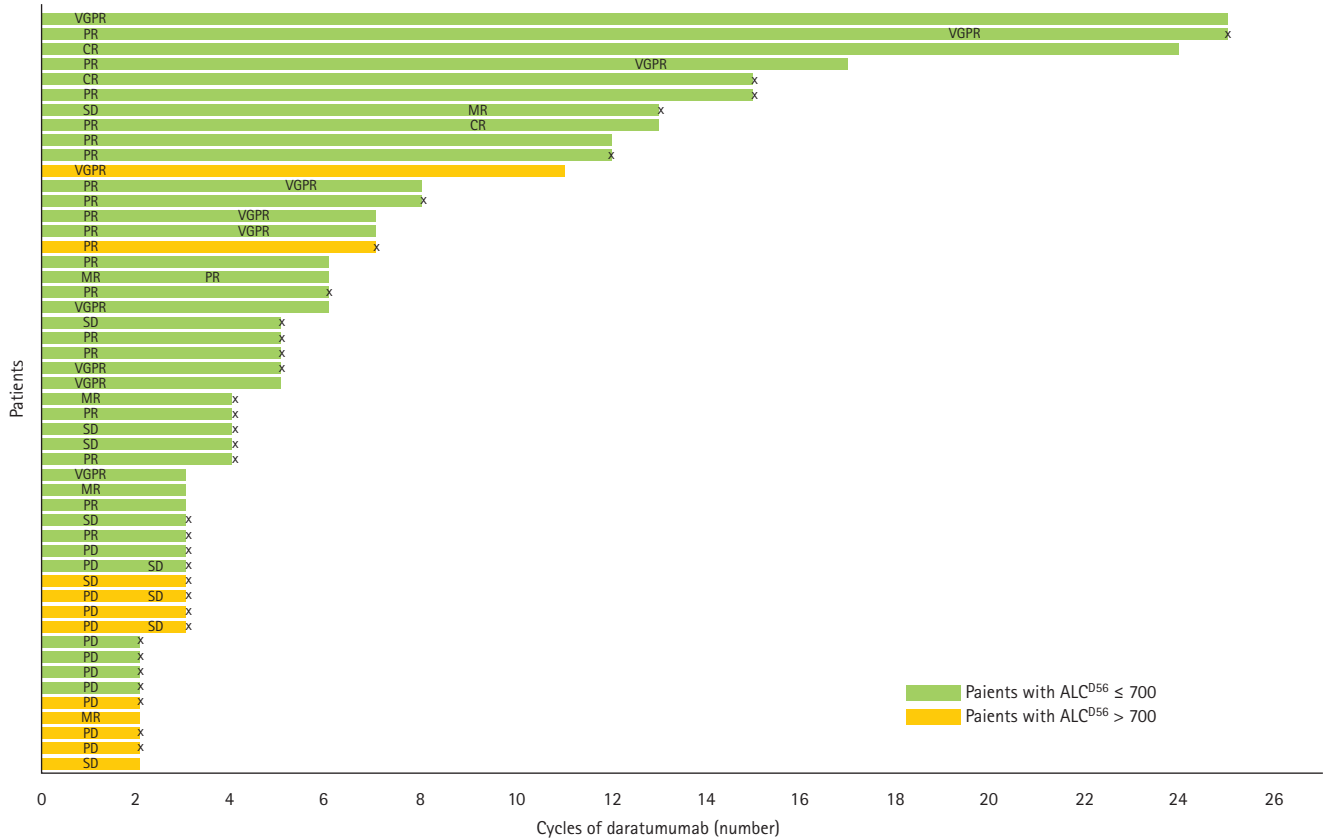
Supplementary Figure 4. Swimmer plot of all patients with treatment response. Responses shown on the left side indicate the initial responses after the second cycle of daratumumab treatment. Responses shown on the right side indicate the best response during the course of daratumumab treatment. X indicates disease progression, which is a result of treatment withdrawal. ALC^{D56}, absolute lymphocyte counts at the day prior to the third cycle of daratumumab; VGPR, very good partial response; PR, partial response; CR, complete response; SD, stable disease; MR, minimal response; PD, progressive disease.
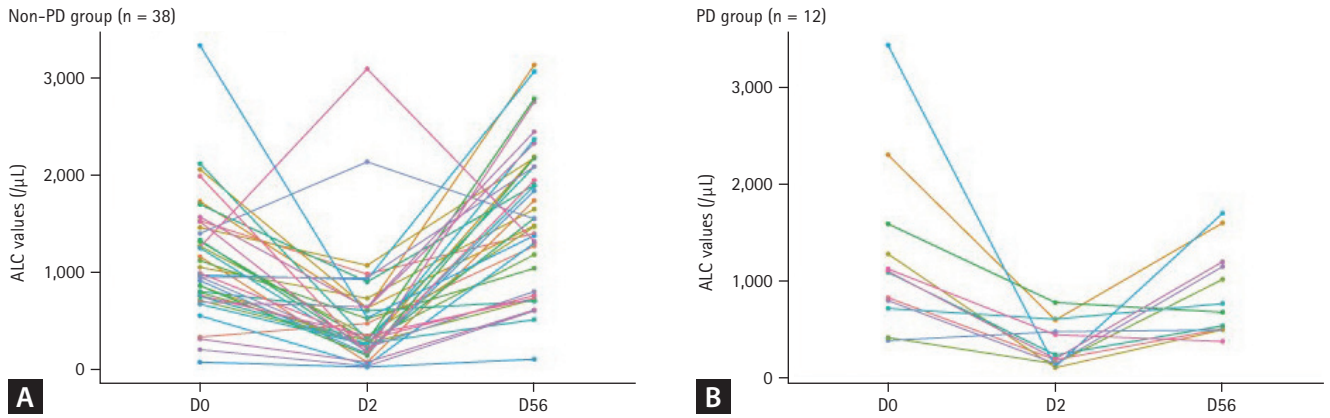
Supplementary Figure 5. Paired plots depicting the change in absolute lymphocyte count (ALC) values during the first two cycles of daratumumab therapy in each patient based on their treatment response. (A) Non-progressive disease (PD) group, (B) PD group.
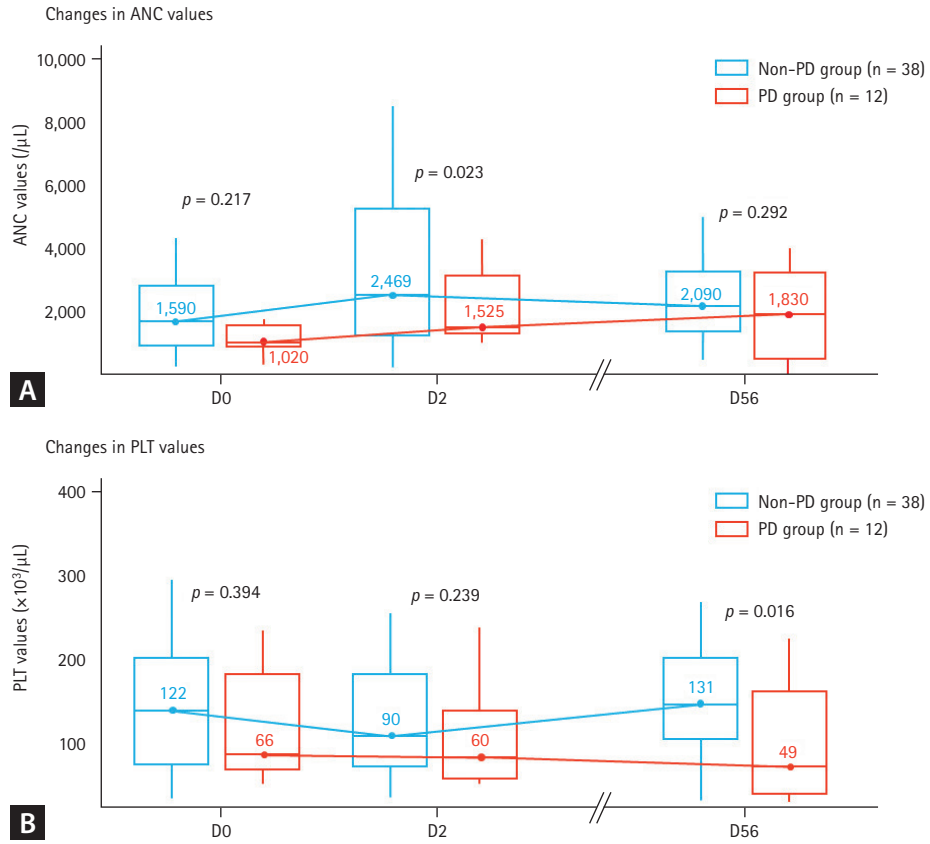
Supplementary Figure 6. Changes in (A) absolute neutrophil count (ANC) and (B) platelet (PLT) values based on treatment response during daratumumab therapy. PD, progressive disease.