

Anti-CD38 monoclonal antibody impairs CD34+ mobilization and affects clonogenic potential in multiple myeloma patients

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Background - Induction with daratumumab-based regimens followed by autologous stem cell transplantation is the current standard for newly diagnosed multiple myeloma (NDMM) patients eligible for intensive chemotherapy. However, concerns emerged regarding potential negative effects following daratumumab-based treatment on CD34+ mobilization. We here compared CD34+ mobilization and clonogenic potential between daratumumab and non-daratumumab based therapy without upfront plerixafor administration among patients affected by NDMM.

Materials and methods - Clinical, mobilization and clonogenic data from 41 consecutively enrolled NDMM patients were analyzed. Patients underwent collection of autologous CD34+ by apheresis at the ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy, from January 2021 to March 2023. Clonogenicity analysis was performed on BFU-E and CFU-GM.

Results - Seventy-five percent of daratumumab-treated patients underwent >1 apheresis, compared to 24% of non-daratumumab patients ($p=0.0017$). Daratumumab-treated patients had significantly lower CD34+ count (mean 38 vs 79/ μL , respectively; $p=0.0011$), with a median CD34+ harvest of $3.98 \times 10^6/\text{kg}$ (range 1.68-9.18) vs $6.87 \times 10^6/\text{kg}$ (range 1.63-16.85) in non-daratumumab-treated ($p=0.0006$). In multivariate analysis the likelihood of undergoing >1 apheresis was significantly higher in older patients (OR 1.2, 95% CI 1-1.4, $Z=2.10$, $p=0.03$) and daratumumab-treated patients (OR 15, 95% CI 2.8-129, $p=0.004$). Moreover, daratumumab-based induction therapy demonstrated an independent negative association with BFU-E colony formation ($p=0.0148$), even when accounting for patient age and CD34+ levels.

Discussion - Our findings underscore the impact of daratumumab-based treatment on CD34+ mobilization in a real-life, upfront plerixafor-free population of NDMM patients. Higher probability of requiring multiple apheresis occurred among daratumumab-treated patients. Interestingly, the observation that daratumumab might negatively impact BFU-E colony formation, independent of CD34+ cell count, offers novel biological perspectives. Appropriate strategies should be adopted by the Apheresis teams to mitigate these potential negative effects.

Key words: multiple myeloma, anti-CD38 monoclonal antibody, mobilization, collection, apheresis.

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INTRODUCTION

Multiple myeloma (MM) is a hematologic malignancy with a worldwide incidence of 160,000 cases and a global mortality of 106,000 patients for the year 2018¹. It is characterized by presence of abnormal clonal plasma cells in the bone marrow, with potential for uncontrolled growth causing destructive bone lesions, kidney injury, anemia and hypercalcemia². The introduction into clinical practice of daratumumab, a humanized monoclonal antibody targeting CD38, has revolutionized the therapeutic landscape for MM, particularly in the setting of transplant-eligible patients. The updated 2021 European Haematology Association (EHA) and the European Society of Medical Oncology (ESMO) guidelines recommend the triplet proteasome inhibitor bortezomib plus dexamethasone with the addition of lenalidomide (VRd) or thalidomide (VTd) plus daratumumab as first options in the treatment of newly diagnosed multiple myeloma (NDMM) followed by autologous stem cell transplant (ASCT) and lenalidomide maintenance³.

Nonetheless, as daratumumab became increasingly integrated into routine clinical practice, concerns about its potential negative impact on hematopoietic stem cell (HSC) mobilization yields emerged⁴⁻⁷.

Considering that CD38 is expressed on both MM cells and CD34+ HSCs, investigations have been conducted to elucidate the underlying biological factors contributing to these observations. Nevertheless, *in vitro* studies evaluating the effects of daratumumab on mobilized CD34+ progenitor cells from MM patients demonstrated no evidence of toxicity⁸.

In this setting, the aim of our study is to evaluate the impact of daratumumab on autologous CD34+ mobilization yields and on CD34+ clonogenicity in a real-life population of daratumumab and non-daratumumab treated patients, who did not receive pre-emptive upfront therapy with plerixafor as mobilizing agent.

MATERIALS AND METHODS

Study population

This study is a retrospective case-control comparison of daratumumab and non-daratumumab treated patients, conducted in a real-life, upfront plerixafor-free MM population. Data from 41 consecutive newly diagnosed MM (NDMM) patients, balanced in numbers between

daratumumab and non-daratumumab induction therapies, have been included. Patients underwent mobilization and collection of autologous CD34+ by apheresis at ASST Grande Ospedale Metropolitano (GOM) Niguarda from January 2021 to March 2023. This retrospective observational study protocol was approved by the Comitato Etico Milano Area 3 upon notification by the Principal Investigator (RCr) and informed consent is provided by patients for the related procedures and treatments. Inclusion criteria stipulated participants must be above 18 years of age, have a confirmed MM diagnosis at ASST GOM Niguarda, Milano/Ospedale Manzoni, Lecco. Patients who received plerixafor upfront for their first mobilization were excluded from the study. As of our local policy, plerixafor on-demand is not envisaged for NDMM patients⁹.

METHODS

The following data were retrospectively collected for each patient:

1. demographic and baseline characteristics: sex, age at mobilization, weight (expressed in kg), disease status at mobilization (as defined by International Myeloma Working Group-IMWG - Uniform Response Criteria for Multiple Myeloma¹⁰), MM characteristics;
2. treatment characteristics: induction therapy (either daratumumab-based or non daratumumab-based), mobilization strategy (cyclophosphamide monotherapy at a dose of 2-3 g/m² was administered as mobilizing regimen to all patients unless contraindicated and granulocyte colony-stimulating factor [G-CSF] at a dose of 5 µg/kg/d), previous radiotherapy (as dichotomous variable "yes/no"), number of weeks from the end of the last induction therapy cycle to CD34+ mobilization, need for plerixafor administration following mobilization failure (i.e., failing to reach the set target of 6×10⁶ CD34+ cells/kg);
3. CD34+ mobilization yields: number of CD34+ before cell harvest (i.e., CD34+ count, expressed in CD34/µL) number of harvested CD34+ ×10⁶/kg, total number of apheresis needed to reach the set target (i.e., 6×10⁶ CD34+/kg);
4. engraftment time: median time to absolute neutrophil count (ANC) recovery (defined as ANC >500/mm³

for three consecutive days without the support of G-CSF) and to platelets (PLT) recovery (defined as PLT >20,000/mm³ for three consecutive days without transfusion support).

Collection by apheresis

Daily monitoring of peripheral CD34+ levels was performed from day +10 following the initiation of the mobilization regimen. Apheresis procedures were initiated as soon as the CD34+ count exceeded 20/μL and they continued until the target cell count was reached or as dictated by the CD34+ kinetics if a downward trend was observed. Collection was realized employing Spectra Optia™ (Terumo BCT, Lakewood CO, USA) by connecting the patient through peripheral or central venous access, the latter whenever peripheral veins were not appropriate for the procedure. ACD-A was used as anticoagulant in the apheresis kit and calcium gluconate was administered to patient as intravenous infusion during collection, to prevent ACD-induced hypocalcemia. The overall volume of blood processed depended upon the initial CD34+ value before collection and the locally established collection efficiency. Local policy allows for processing a maximum of three total blood volumes per day.

Statistical analysis

The primary objective of this analysis was to ascertain whether patients undergoing daratumumab-based induction therapy had an increased probability of requiring more apheresis procedures compared to those not receiving daratumumab. Secondary objectives, pre-specified before data analysis, included the evaluation of any differences in CD34+ count levels at initial mobilization, the association of CD34+ levels greater than 20/μL as a predictor for target achievement, any differences in median CD34+ harvest and in clonogenic tests. The predefined target was set at 6×10⁶ CD34+/kg and was assessed as a nominal dichotomous variable. Comparative analyses were performed using the Mann-Whitney U test for continuous variables and the Chi-square or Fisher's exact test for categorical variables. Sensitivity and specificity of CD34+/μL >20/μL as a predictor were analysed using Receiver Operating Characteristic (ROC) analysis. The impact of daratumumab-based therapy on the primary objective, adjusted for other potential confounding factors, was evaluated through multiple logistic regression.

The need for one or more apheresis sessions was regarded as a nominal dichotomous variable. The influence of independent variables on continuous outcomes was assessed using multiple linear regression. Failure to achieve the target was defined when the predefined set target of 6×10⁶ CD34+/kg was not obtained.

Clonogenicity essays

Clonogenicity assessment was conducted on stored harvested CD34+ cells from both groups, wherein quantification of colony forming unit-granulocyte-macrophage (CFU-GM) and burst forming unit-erythroid (BFU-E) at a rate of ×10⁴/kg was performed. The impact of daratumumab on clonogenicity was evaluated in a multivariate analysis considering both patient age and CD34+ values. CFU-GM, BFU-E and CD34+ were evaluated as continuous variables in multiple linear regression.

RESULTS

Patient cohort description

A total of 41 consecutive patients with NDMM, who underwent collection of autologous CD34+ by apheresis between January 2021 and March 2023 at ASST GOM Niguarda, were analyzed. Median age was 62 years (range, 39 to 72), and 71% (No.=29) of the cohort was male. Twenty patients received daratumumab-based induction therapy (48.8%), while 21 (52.5%) patients belonged to the non-daratumumab group. Patients received a median of 4 cycles (range, 4 to 11 cycles) of induction chemotherapy, with a median of 4 cycles (range, 4 to 6) in the daratumumab group and 5 cycles (range, 4 to 11) in the non-daratumumab group respectively. Median number of weeks from the last daratumumab administration to mobilization was 6 (range, 4 to 10), while a median of 7 weeks (range, 2 to 13) passed from the last non daratumumab-based chemotherapy cycle to mobilization. Baseline patient and disease characteristics were well balanced between the two induction therapy groups, **Table I**. No statistically significant differences were reported between the two groups (daratumumab and non-daratumumab based induction therapy) when considering patients who did and did not receive radiotherapy before mobilization (p=0.40) and when considering platelet level before mobilization (i.e., below or above the value of 170,000/mm³, p=0.20), *Online supplementary Figure S1*.

Moreover, no statistically significant difference emerged in the baseline response rates at the time of mobilization ($p=0.34$), *Online supplementary Figure S2*.

Autologous CD34+ mobilization yields

Seventy-five percent (No.=15) of the daratumumab-treated patients had to undergo >1 apheresis, compared to only

Table I - Baseline patients and disease characteristics as divided in the two induction therapy groups: daratumumab-based and non daratumumab-based therapy

Characteristics	Daratumumab group (No.=20)	Non-daratumumab group (No.=21)
Age, yr, median (range)	62 (44-72)	60 (39-71)
Male sex, No. (%)	15 (75)	14 (66)
Weight, kg, No. (%)	74 (49-110)	73 (54-106)
Subtype, No. (%)		
IgG	15 (75)	13 (62)
IgA	3 (15)	5 (24)
IgE	0	0
Light chain	2 (10)	2 (9)
Non secretory	0	1 (5)
Previous RT, No. (%)	1 (5)	4 (19)
Induction therapy type, No. (%)		
Dara-VTD	17 (85)	0
Dara-VCD	2 (10)	0
Dara-RD	1 (5)	0
VTD	0	16 (76)
Other	0	5 (24)
Number of full CHT cycles, median (range)	4 (4-6)	5 (4-11)
Number of weeks from the end of the last CHT cycle to mobilization, median (range)	6 (4-10)	7 (2-13)
BM infiltration before mobilization		
absent, No. (%)	5 (25)	6 (28)
<30%, No. (%)	15 (75)	13 (62)
>30%, No. (%)	0	2 (9)
Hb level before mobilization		
8-13 g/dL, No. (%)	20 (100)	21 (100)
<8 g/dL, No. (%)	0	0
WBC level before mobilization		
<5.000/mm ³ , No. (%)	2 (10)	4 (19)
>5.000/mm ³ , No. (%)	18 (90)	17 (81)
PLT level before mobilization		
<170.000/mm ³ , No. (%)	12 (60)	17 (81)
>170.000/mm ³ , No. (%)	8 (40)	4 (19)

RT: radiotherapy; CHT: chemotherapy; BM: bone marrow; Hb: hemoglobin; WBC: white blood cells; PLT: platelets.

24% (No.=5) in the non-daratumumab group ($p=0.0017$), **Figure 1A**. A total of three blood volumes for each apheresis were processed for most patients, unless lower volumes were sufficient to reach the target, upon pre-apheresis calculation according to the local known collection efficiency. The total blood volumes processed during each apheresis and the CD34+ cells collected are shown in the Supplemental **Table SI**. In the same table are detailed the patients receiving apheresis through central venous catheter vs peripheral access (No.=6 vs No.=35 respectively). A correlation emerged between induction therapy (daratumumab-based and non daratumumab-based) and

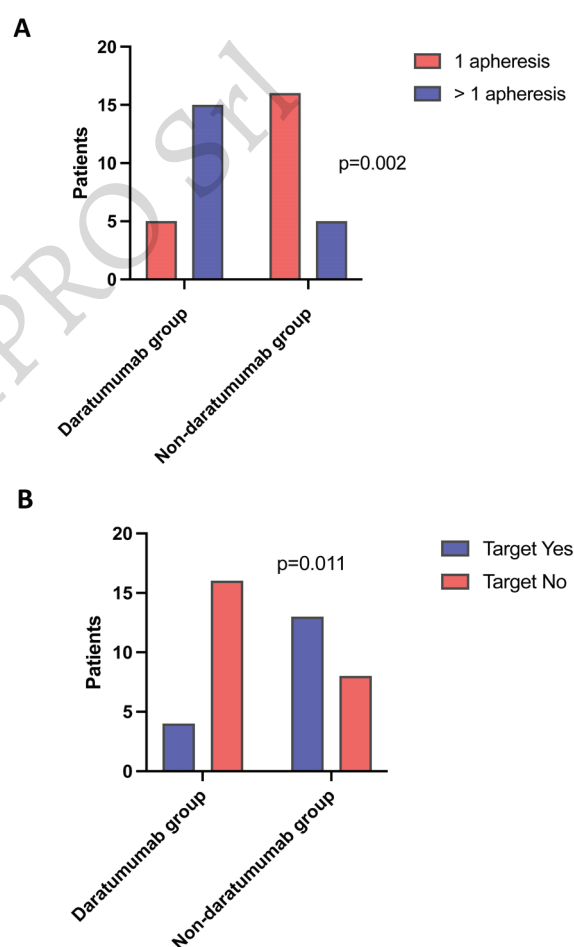


Figure 1 - Number of apheresis and CD34+ target according to induction therapy

A) Number of patients who had to undergo 1 (red) vs >1 (blue) apheresis sessions in order to reach the set CD34+ target, divided into the two induction therapy groups (daratumumab-based and non daratumumab-based). **B)** Number of patients who reached the set CD34+ target (i.e., $6 \times 10^6/\text{kg}$) divided into the two induction therapy groups (daratumumab-based and non daratumumab-based).

the number of apheresis needed to reach the set target (i.e., 6×10^6 CD34+/kg). Sixty-two percent (No.=13) of patients in the non-daratumumab group reached the set CD34+ target at first collection, compared to only 20% (No.=4) in the daratumumab group. The differences between the two groups were statistically significant ($p=0.01$), **Figure 1B**. Moreover, patients who received induction therapy containing daratumumab had significantly lower CD34+ count than non-daratumumab patients (mean 38 vs 79/ μ L, difference between means 40.4; 95% confidence interval (CI) 17-63 respectively; $p=0.0011$), **Figure 2A**. Interestingly, according to a ROC analysis, the CD34+ count value of 54.49/ μ L appeared to be the most effective predictor of target achievement probability within our cohort, with a sensibility of 82% and specificity of 76%, **Figure 2B**. Median CD34+ harvest was 3.98×10^6 /kg (range 1.68-9.18) in patients who received induction therapy with daratumumab, while 6.87×10^6 /kg (range 1.63-16.85) in non-daratumumab treated patients. Plerixafor at a dose of 0.24 mg/kg was administered as a rescue strategy to 25% (No.=5) of patients who underwent >1 apheresis, 80% of them (i.e., 4/5) being daratumumab patients.

Previous radiotherapy and low number of platelets before mobilization did not have an impact on the number of apheresis needed to reach the set CD34+ target ($p=0.34$ and $p=0.17$, respectively), *Online supplementary Figure S3*. Furthermore, also the grade of response (i.e., VGPR or better) before CD34+ mobilization resulted not to have any statistically significant impact both on the number of apheresis needed to reach the set CD34+ target and on the collected CD34+/ μ L ($p=0.20$ and $p=0.06$, respectively), *Online supplementary Figure S4*.

A multiple logistic regression analysis was performed considering age, sex, weight, induction therapy type (daratumumab and non daratumumab-based therapy) and disease status at mobilization. According to this analysis the probability of undergoing >1 apheresis was significantly increased in older patients (odds ratio [OR] 1.2, 95% CI 1.03-1.40, $Z=2.10$, $p=0.03$) –**Figure 3**– and in patients who had received daratumumab-based induction therapy (OR 15, 95% CI 2.8-129, $p=0.004$). In multivariate analysis, daratumumab induction therapy was significantly associated with a lower CD34+ count ($p=0.0056$).

After autologous stem cell transplantation, the median

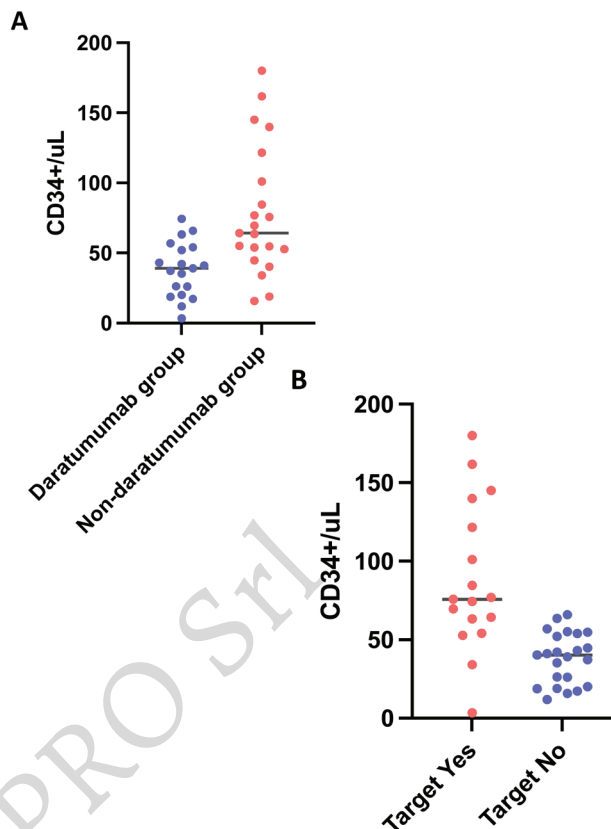


Figure 2 - Pre-apheresis CD34+ count, induction therapy group and CD34+ target

Count of CD34+/ μ L in the two induction therapy groups: daratumumab-based (blue) and non daratumumab-based (red). **A)** Results from a ROC analysis considering the count of CD34+/ μ L and the ability to reach the set CD34+ target (target reached red, target not reached blue). **B)** The CD34+ count value of 54.49/ μ L appeared to be the most effective predictor of target achievement probability, with a sensibility of 82% and specificity of 76%.

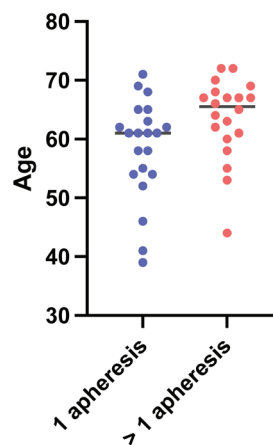


Figure 3 - Probability of undergoing 1 apheresis (blue) vs >1 apheresis (red) with respect to age

time for neutrophils engraftment was 9.5 days in the daratumumab group and 10 days in the non-daratumumab group ($p=0.16$). The median time for platelet engraftment was 10.5 days in the daratumumab group and 11 days in the non-daratumumab group ($p=0.73$), **Figure 4**.

Clonogenicity data

In order to evaluate the clonogenicity of the collected HSCs, CFU-GM and BFU-E were performed to all apheresis products. Overall, the median CFU-GM was 63.6 (95% CI $48.7-95.8$) $\times 10^4$ /kg, the median BFU-E was

60.7 (95% CI $53.1-77.40$) $\times 10^4$ /kg. Considering the single apheresis products separated in the two groups, median CFU-GM in the daratumumab-treated group was 48.01 (95% CI $29.4-79.4$) $\times 10^4$ /kg, while BFU-E was 49.8 (95% CI $33.1-60.7$) $\times 10^4$ /kg; they were 112.01 (95% CI $61.6-137.1$) $\times 10^4$ /kg and 107.17 (95% CI $76.3-135.4$) $\times 10^4$ /kg, respectively, in the non daratumumab-treated group, **Figure 5**.

Considering the established associations between age, CD34+ content, and the outcomes of clonogenic tests, our analysis assessed the influence of daratumumab

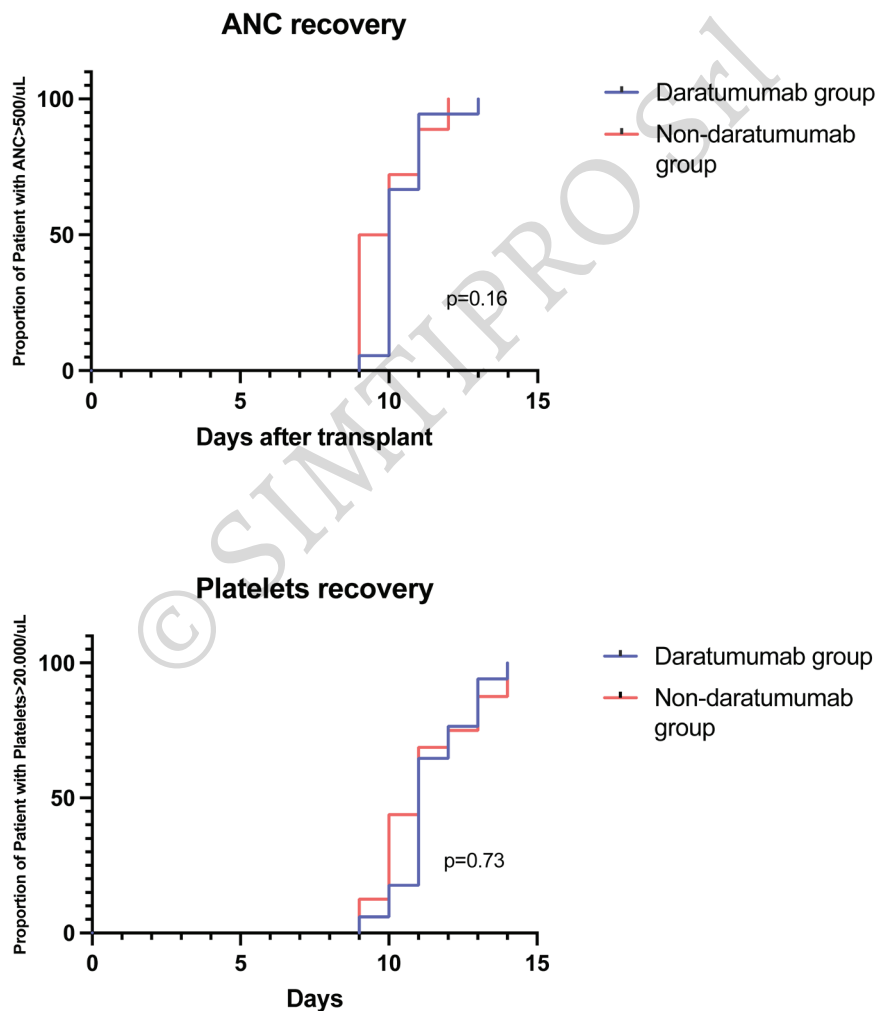


Figure 4 - Median time for neutrophils and platelets engraftment divided into the two induction therapy groups

Daratumumab-based (orange) and non-daratumumab based (light blue).
ANC: absolute neutrophil count.

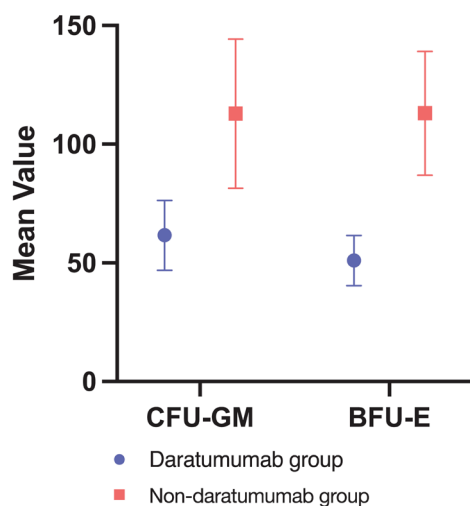


Figure 5 - CFU-GM and BFU-E mean value with 95% CI per the two induction therapy groups

Daratumumab-based (blue) and non daratumumab-based (red). CFU-GM: colony forming unit – granulocyte macrophage; BFU-E: burst forming unit – erythroid; CI: confidence interval.

on clonogenicity through a multivariate analysis that simultaneously considered patient age and $CD34+ \times 10^6/kg$. This approach aimed at evaluating the specific impact of daratumumab while accounting for the interrelationship between these two variables.

Notably, a lower incidence of BFU-E colony formation was observed in samples collected following the daratumumab-based regimen compared to those from the non-daratumumab group; indeed, a statistically significant difference between induction regimens has been observed ($p=0.0148$, no multicollinearity indexes were found among variables: variance inflation factor range 1.1-1.3). Conversely, no statistically significant difference emerged concerning CFU-GM colonies ($p=0.7071$).

DISCUSSION

To the best of our knowledge, our study represents the first investigation assessing the impact of the anti-CD38 monoclonal antibody daratumumab-based therapy on $CD34+$ mobilization yields and clonogenic potential in NDMM patients who did not receive upfront plerixafor administration, nor on-demand. The results of our study reveal a statistically significant difference in autologous HSC mobilization yields between the two examined groups, with the daratumumab-treated group

experiencing significant impairment in the mobilization process. Moreover, we report a negative effect of daratumumab-based therapy on BFU-E colony formation. While existing data do not consistently align regarding the adverse impact of anti-CD38 monoclonal antibody on $CD34+$ mobilization yields, it is crucial to acknowledge that most of these studies employed upfront plerixafor administration in conjunction with G-CSF. A relevant example can be drawn from the randomized phase 3 CASSIOPEIA study, designed to compare the effects of Dara-VTd and VTd regimens on HSC mobilization and collection outcomes. Notably, 21.7% of the Dara-VTd-treated patients and 7.9% of those receiving VTd underwent upfront plerixafor administration as a preemptive mobilization strategy. Within this context, the Dara-VTd arm exhibited distinctive characteristics, including a diminished mean count of harvested HSCs and a heightened reliance on plerixafor for mobilization purposes^{4,11}. Indeed, although certain investigations did not demonstrate a statistically significant impact of daratumumab on HSC mobilization yields, they reported a higher need of plerixafor rescue within the daratumumab-treated group⁶. Findings from the GRIFFIN¹² and MASTER¹³ trials indicated that daratumumab-treated patients receiving upfront plerixafor displayed numerically greater HSC yields compared to those who received plerixafor in an on-demand manner for rescue purposes⁷.

In line with our study, a recently published Swedish retrospective study, which did not employ upfront plerixafor administration, observed that the addition of daratumumab to the treatment regimen resulted in a substantially lower HSC yield, hence necessitating an increased mean and median duration of apheresis procedures, as compared to non daratumumab-based therapies. The study also reported an increased need for the rescue use of plerixafor to effectively facilitate HSC mobilization in these patients⁵. However, no analysis on clonogenicity potential was performed.

These findings emphasize the need for investigations encompassing larger patient cohorts to validate the observed results and, in the long run, enhance the mobilization strategy for individuals subjected to daratumumab-based therapy¹⁴.

Our study demonstrates a statistically significant variation in HSC mobilization yields within the two

analyzed groups in an upfront plerixafor-free setting, hence allowing for a more untainted analysis of outcomes. The daratumumab-administered group required an increased number of apheresis sessions to attain the predefined target, along with reduced pre-apheresis CD34+ count and CD34+ harvest levels. Importantly, despite the encountered challenges in the mobilization process, no statistically significant disparities were detected in neutrophil or platelet engraftment between the two groups after autologous stem cell transplantation. Conversely to some findings in the literature^{15,16}, our study did not reveal any statistically significant effects associated with the administration of radiotherapy before stem cell mobilization or having a low platelet count (i.e., PLT count <170,000/mm³) at the time of apheresis. This might be due to the low number of patients in our cohorts, not allowing these two variables to emerge as significant.

We believe that strategies to mitigate the risk of failure to achieve the set CD34+ target would be worthy of use. Increasing the G-CSF dosage, i.e., to 10 µg/kg/day, or optimizing the mobilization strategy by administering higher doses of cyclophosphamide might be both feasible and effective in enhancing the HSC mobilization. Recently, Liberatore *et al.*¹⁴ reported optimal HSC collection after daratumumab-based induction with cyclophosphamide 4 g/m² as mobilization strategy. Another viable option could be the “on-demand” administration of plerixafor to all patients undergoing daratumumab-based therapy. Despite its specific indications, on-demand plerixafor has been successfully employed, also in other clinical contexts⁹. Implementing these strategies alongside daratumumab-based treatment may help overcome mobilization challenges by trying to avoid multiple collections as well as reducing the mobilization failures. The achievement of the CD34+ target is indeed crucial to allow for high-dose therapy followed by ASCT, thus for the completion of the first-line therapeutic program in NDMM patients.

Our observation of impaired mobilization and collection of autologous HSCs following daratumumab-based therapy could potentially be attributed to the inherently low expression of CD38 on CD34+ HSCs¹⁷. The precise mechanism by which the anti-CD38 monoclonal antibody daratumumab influences HSCs mobilization yields has

not been fully elucidated yet. However, several plausible factors warrant consideration. Firstly, daratumumab interaction with the CD38 molecule on HSCs may potentially impede their mobilization process. Notably, the disruption of interactions between myeloma cells and the bone marrow microenvironment, induced by daratumumab, may result in interference with adhesion molecules within the bone marrow milieu. This phenomenon could consequently impact HSC capacity to effectively mobilize from the bone marrow and enter circulation^{18,19}. Furthermore, daratumumab immune-mediated properties, exemplified by processes like antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), possess the capacity to trigger the elimination of CD38-expressing cells²⁰, which include HSCs, influencing their mobilization dynamics.

To gain insights into the biological factors contributing to our observed findings we analyzed clonogenicity data of BFU-E and CFU-GM. Interestingly, statistically significant disparities between induction regimens were observed in the context of BFU-E colony formation, with a negative impact of daratumumab. A previous study investigating the influence of daratumumab on CD34+ mobilization and the composition of erythroid and myeloid progenitor stem cell products, as measured by the number of BFU-E, CFU-GM, and colony-forming unit cell (CFU-C), revealed comparable colony formation regardless of daratumumab use²¹. However, it is important to note that in this study 88% of daratumumab-treated patients received plerixafor prior to mobilization. This could potentially offset the effects of daratumumab and lenalidomide on graft and stem cell collection, as stated by the authors. Also, published *in vitro* studies evaluating the effects of daratumumab on mobilized CD34+ progenitor cells from myeloma patients demonstrated no evidence of toxicity and no effects on progenitor cell assays⁸.

Our results stand as the first report of a possible impact of CD38+ monoclonal antibody daratumumab on HSC clonogenic potential. One plausible elucidation might come from a previous work wherein heightened influences of CD34+CD38++ cell subpopulation on BFU-E formation were reported. Specifically, a subset of CD34+ cells characterized by elevated CD38 expression (CD34+CD38++ cells) exhibited a pronounced enrichment

in BFU-E quantities while concurrently manifesting a relative depletion in CFU-GM counts, in comparison to a control CD34+ cell population¹⁷. This observation may suggest that HSCs that have engaged with anti-CD38 monoclonal antibody, presumably leading to a reduced CD38 expression, may potentially exhibit diminished capacity to generate BFU-E colonies.

Our study has some limitations, firstly being a retrospective single-center observational study, hence involving a small number of patients, despite well balanced between the two groups. Secondly, the impact of anti-CD38 monoclonal antibodies other than daratumumab was not assessed. Although Isatuximab has recently been approved in combination therapies for the treatment of relapsed-refractory MM^{22,23} and many clinical trials have been conducted to evaluate its activity in NDMM²⁴, no analysis on mobilization with this drug has been performed so far.

Nonetheless, our study is among the first efforts to explore autologous CD34+ cell mobilization subsequent to daratumumab and non-daratumumab-based induction therapies, employing a mobilization strategy devoid of upfront plerixafor administration, nor on-demand. Our findings confirm previous observation on impaired CD34+ mobilization among daratumumab-treated patients and detect for the first time a significant impact on the generation of BFU-E colonies, standing as the first documentation of a negative impact attributed to the anti-CD38 monoclonal antibody daratumumab on the clonogenic potential of HSCs *in vitro*. Reassuringly, despite these observed effects, there were no adverse consequences on neutrophil and platelet engraftment after ASCT.

CONCLUSIONS

The current study is among the first efforts to explore autologous CD34+ cell mobilization subsequent to daratumumab and non daratumumab-based induction therapies, employing a mobilization strategy devoid of upfront plerixafor administration. In our investigation, patients subjected to induction treatment involving the anti-CD38 monoclonal antibody demonstrated an increased likelihood of multiple apheresis for successful collection, with subsequent potential discomfort for the patients. Furthermore, to the best of our knowledge,

present results stand as the first documentation of a negative impact attributed to the CD38-targeting monoclonal antibody daratumumab on the clonogenic potential of HSCs.

In light of these observations, while anti-CD38 monoclonal antibody therapy does indeed elicit effects on both mobilization yields and clonogenic potential, neutrophil and platelet engraftment after ASCT was not affected. Nevertheless, our study emphasizes that in a setting that does not employ upfront plerixafor administration as a mobilization strategy, as defined in Europe by the EMA label, patients treated with daratumumab have a higher probability of requiring multiple apheresis compared to non daratumumab-treated patients. Apheresis teams should adopt appropriate measures to mitigate this risk, trying to avoid the associated adverse effects and discomfort endured throughout the mobilization process.

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AUTHORS' CONTRIBUTIONS

AZ and RCr designed the study, collected data and wrote the manuscript. IC supervised methodology and performed the statistical analyses. LP performed the clonogenicity assays. SP collected data. AMC, MA, LB, PB, GG, PM, MLP, ER, MS, CVV, EBV, CGP, SR, RCa managed the patients or performed the apheresis procedures. All Authors revised the manuscript and approved the final version.

The Authors declare no conflicts of interest.

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ONLINE SUPPLEMENTARY CONTENT**Table SI - Characteristics of the first two apheresis procedures for the total No.=41 patients**

Patient	Daratumumab	Vascular access	CD34+ count at 1 st harvest	TBV processed during the 1 st harvest	CD34+ ×10 ⁶ /kg collected at 1 st harvest	CD34+ count at 2 nd harvest	TBV processed during the 2 nd harvest	CD34+ ×10 ⁶ /kg collected at 2 nd harvest	Tot No. of aphereses	Plerixafor
#1	Y	Peripheral	3.35	3	9.184	N/A	N/A	N/A	1	Y
#2	Y	Peripheral	54.00	3	8.628	N/A	N/A	N/A	1	N
#3	Y	Peripheral	63.29	3	6.900	N/A	N/A	N/A	1	N
#4	Y	Central	74.44	3	6.367	N/A	N/A	N/A	1	N
#5	Y	Peripheral	17.21	3	1.680	32.93	3	3.610	2	Y
#6	Y	Peripheral	26.12	3	1.941	12.48	3	1.026	4	N
#7	Y	Peripheral	11.85	3	1.728	14.38	3	1.457	4	Y
#8	Y	Peripheral	43.00	3	3.808	48.00	3	4.136	2	N
#9	Y	Peripheral	39.15	3	3.235	39.81	3	3.826	2	N
#10	Y	Peripheral	65.90	3	5.468	N/A	N/A	N/A	1	N
#11	Y	Peripheral	20.00	3	2.699	26.00	3	3.867	2	Y
#12	Y	Central	39.10	3	5.290	55.90	1	1.240	2	N
#13	Y	Peripheral	56.94	3	5.474	22.79	1.5	3.181	2	N
#14	Y	Peripheral	52.09	3	4.279	33.19	3	4.669	2	N
#15	Y	Central	37.19	3	3.771	23.81	3	2.801	2	N
#16	Y	Peripheral	42.12	3	4.151	42.70	2.5	2.635	2	N
#17	Y	Peripheral	35.23	3	3.160	65.75	2.5	4.936	2	N
#18	Y	Peripheral	41.04	3	4.434	94.57	1.5	5.737	2	N
#19	Y	Peripheral	26.25	3	2.889	21.25	3	2.388	2	N
#20	Y	Peripheral	18.81	3	1.722	16.16	3	1.336	4	N
#21	N	Peripheral	139.86	2,5	16.846	N/A	N/A	N/A	1	N
#22	N	Peripheral	145.00	2	7.693	N/A	N/A	N/A	1	N
#23	N	Peripheral	101.12	2,5	9.107	N/A	N/A	N/A	1	N
#24	N	Peripheral	180.00	2	14.201	N/A	N/A	N/A	1	N
#25	N	Peripheral	52.79	3	6.432	N/A	N/A	N/A	1	N
#26	N	Peripheral	84.53	3	7.358	N/A	N/A	N/A	1	N
#27	N	Central	121.54	2,5	10.960	N/A	N/A	N/A	1	N
#28	N	Peripheral	64.21	3	6.872	N/A	N/A	N/A	1	N
#29	N	Peripheral	69.50	3	8.635	N/A	N/A	N/A	1	N
#30	N	Peripheral	161.75	1,5	8.109	N/A	N/A	N/A	1	N
#31	N	Peripheral	75.82	3	8.302	N/A	N/A	N/A	1	N
#32	N	Central	76.90	3	8.247	N/A	N/A	N/A	1	N
#33	N	Peripheral	34.04	3	6.080	N/A	N/A	N/A	1	N
#34	N	Peripheral	18.89	3	1.631	9.40	3	0.954	2	N
#35	N	Peripheral	54.73	3	4.460	40.60	3	3.406	2	N
#36	N	Peripheral	44.73	3	5.300	N/A	N/A	N/A	1	Y
#37	N	Peripheral	15.70	3	1.712	10.92	3	1.027	2	N
#38	N	Peripheral	63.62	3	5.262	N/A	N/A	N/A	1	N
#39	N	Peripheral	40.18	3	5.051	48.58	3	2.837	2	N
#40	N	Peripheral	55.05	3	3.823	67.50	2.5	3.920	2	N
#41	N	Central	53.88	3	5.301	N/A	N/A	N/A	1	N

N: no; Y: yes; N/A: not applicable.

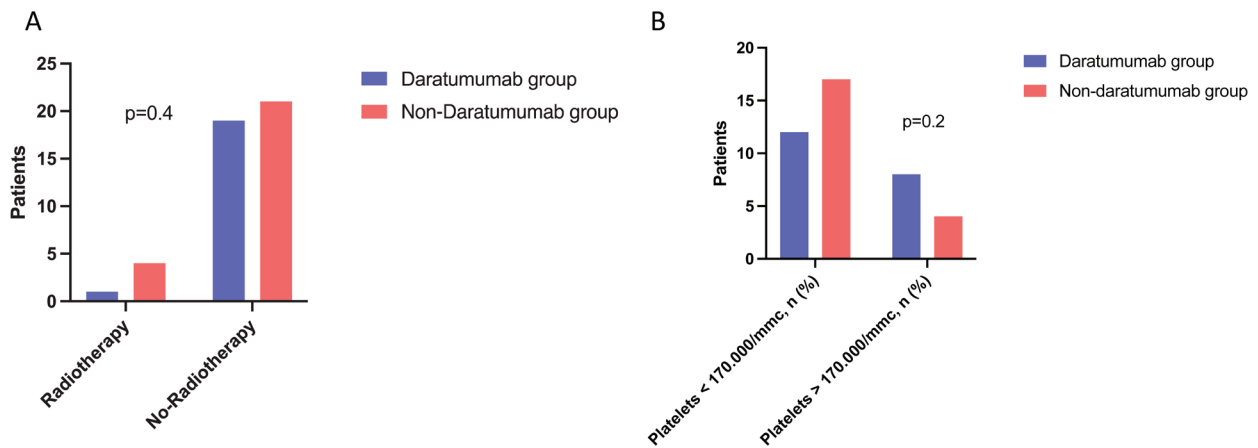


Figure S1 - Induction therapy, radiotherapy and platelets number

A) Number of patients who did or did not undergo radiotherapy before CD34+ mobilization in the two induction therapy groups: daratumumab (blue) and non-daratumumab (red). **B)** Number of patients who had less than or more than 170.000/mm³ platelets in the two induction therapy groups: daratumumab (blue) and non-daratumumab (red).

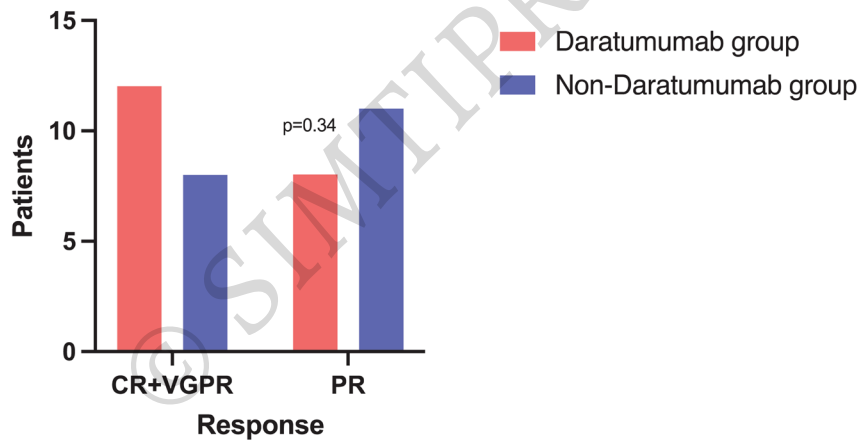


Figure S2 - Induction therapy and clinical response

Number of daratumumab-treated (red) and non-daratumumab treated patients (blue) who had achieved complete response (CR) or very good partial response (VGPR) at the time of CD34+ mobilization as opposed to patients who were reported to have achieved only a partial response (PR).

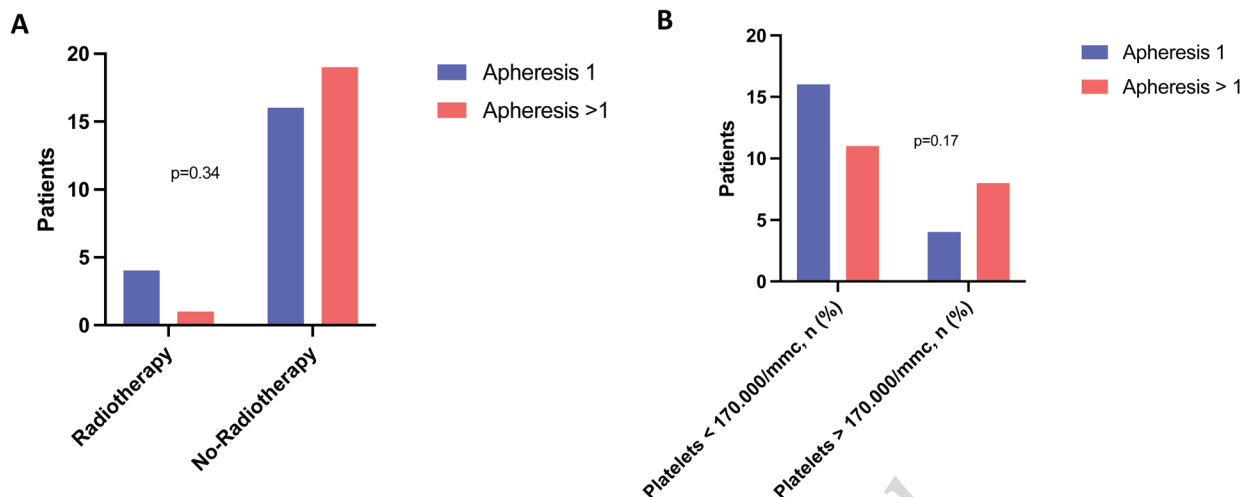


Figure S3 - Radiotherapy, platelets number and number of apheresis

Number of patients who did or did not undergo radiotherapy before CD34+ mobilization and needed to undergo one (blue) or more than one (red) apheresis sessions in order to reach the set CD34+ target
 Number of patients who had less than or more than 170.000/mm³ platelets and number of apheresis needed to reach the set CD34+ target: one (blue) and more than one (red).

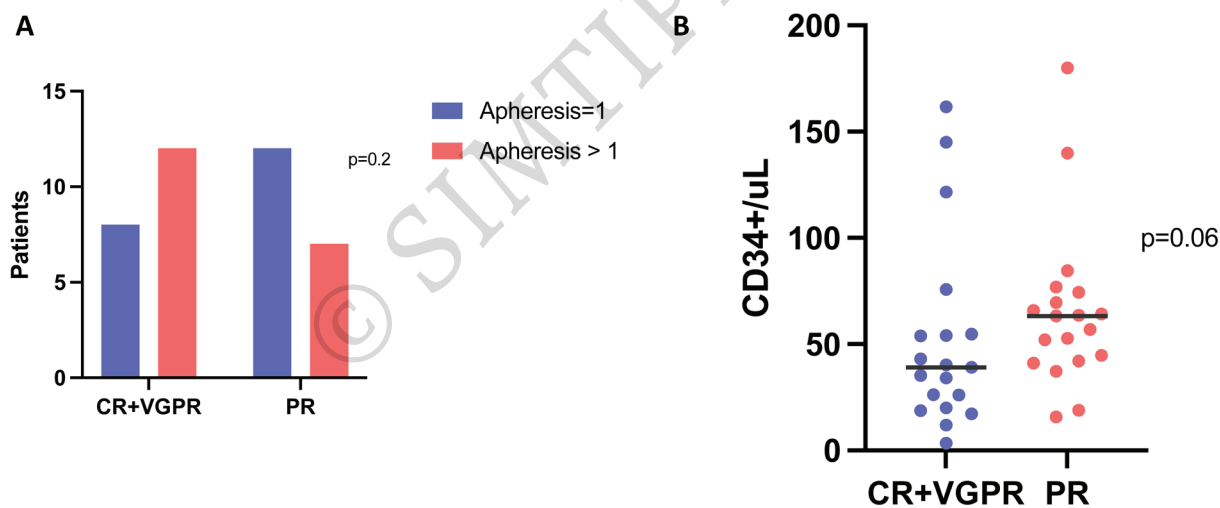


Figure S4 - Clinical response, number of apheresis and pre-apheresis CD34+ count

Number of patients who had to undergo one (blue) or more than one (red) apheresis sessions divided into two groups based on the achieved response at time of mobilization (i.e., complete response [CR] or very good partial response [VGPR] and partial response [PR]).
 Pre-apheresis CD34+ count in patients who had achieved a CR or VGPR as opposed to patients in PR.