

## Higher cyclophosphamide dose grants optimal stem-cell collection after daratumumab-based induction in multiple myeloma

by Carmine Liberatore, Tommaso Perini, Cecilia Passeri, Valeria Ferla, Francesca Fioritoni, Virginia Girlando, Ornella Iuliani, Alessia Orsini, Guido Montanaro, Francesca Farina, Alessandro Di Nicola, Rosamaria Nitti, Stefano Pulini, Sara Mastaglio, Stella Santarone, Milena Coppola, Sarah Markt, Patrizia Accorsi, Fabio Ciceri, Magda Marcatti, and Mauro Di Ianni

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## LETTER TO THE EDITOR

### *Higher cyclophosphamide dose grants optimal stem-cell collection after daratumumab-based induction in multiple myeloma*

#### Short title

Stem-cell yield with HD-CTX after Daratumumab use

#### Authors

Carmine Liberatore<sup>1,\*</sup>, Tommaso Perini<sup>2,3,\*</sup>, Cecilia Passeri<sup>4</sup>, Valeria Ferla<sup>2</sup>, Francesca Fioritoni<sup>1</sup>, Virginia Girlando<sup>2,5</sup>, Ornella Iuliani<sup>4</sup>, Alessia Orsini<sup>6</sup>, Guido Montanaro<sup>1</sup>, Francesca Farina<sup>2</sup>, Alessandro Di Nicola<sup>7</sup>, Rosamaria Nitti<sup>2,5,6</sup>, Stefano Pulini<sup>1</sup>, Sara Mastaglio<sup>2</sup>, Stella Santarone<sup>1</sup>, Milena Coppola<sup>6</sup>, Sarah Markt<sup>2</sup>, Patrizia Accorsi<sup>4</sup>, Fabio Ciceri<sup>2,5</sup>, Magda Marcatti<sup>2, +</sup>, Mauro Di Ianni<sup>1,7, +</sup>.

#### Affiliations:

<sup>1</sup>Hematology Unit, Department of Oncology and Hematology, Ospedale Santo Spirito, Pescara, Italy; <sup>2</sup>Hematology and Bone Marrow Transplant Unit, IRCCS Ospedale San Raffaele, Milan, Italy; <sup>3</sup>Age Related Diseases Laboratory, Division of Genetics and Cell Biology, IRCCS Ospedale San Raffaele, Milan, Italy; <sup>4</sup>Blood Bank Unit, Department of Oncology and Hematology, Ospedale Santo Spirito, Pescara, Italy; <sup>5</sup>Vita-Salute San Raffaele University, Milan, Italy; <sup>6</sup>Immunohematology and

Transfusion Medicine Unit, IRCCS Ospedale San Raffaele, Milan, Italy; <sup>7</sup>Department of Medicine and Sciences of Aging, University of Chieti-Pescara, Chieti, Italy

\* first co-authors

+ last co-authors

• corresponding authors

### **Authorship Contributions**

All co-authors (C.L.; T.P., C.P., V.F., F.F., V.G., O.I., A.O., G.M., F.F., R.N., S.P., S.M., S.S., M.C., S.M., P.A., F.C., M.M., M.D.I.) contributed to patients' clinical care. C.L. and T.P wrote the manuscript. M.M and M.D.I. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest.

### **Data sharing statement**

Original data are available in anonymous form upon request by contacting corresponding author.

## **Correspondence**

Carmine Liberatore

Hematology Unit

Ospedale Santo Spirito,

via Fonte Romana 8, 65124, Pescara

Phone +39 0854252853

Fax +39 0854252904

e-mail: [carmine.liberatore@asl.pe.it](mailto:carmine.liberatore@asl.pe.it)

Tommaso Perini

Hematology and Bone Marrow Transplant Unit

IRCCS Ospedale San Raffaele

Via Olgettina 60, 20132 Milano

Phone +39 0226433903

Fax +39 0226434769

e-mail: [perini.tommaso@hsr.it](mailto:perini.tommaso@hsr.it)

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Quadruplet induction with Daratumumab, Bortezomib, Thalidomide and Dexamethasone (Dara-VTd) followed by autologous stem-cell transplantation (ASCT) and Dara-VTd consolidation has become the standard treatment for transplant-eligible newly diagnosed multiple myeloma patients (NDMM) in Europe.<sup>1</sup> Both improved response rates and progression-free survival (PFS) advantage were reported in Dara-VTd arm of CASSIOPEIA trial.<sup>2</sup> Nonetheless, concerns emerged with stem-cell mobilization and collection after Daratumumab.<sup>3</sup> Following cyclophosphamide 2 to 3 g/m<sup>2</sup> and granulocyte colony-stimulating factor (G-CSF) 10 µg/kg/day, patients treated with Daratumumab experienced greater use of plerixafor, longer leukapheresis and lower total number of CD34+cells/Kg collected per patient. Although the proportion of patients undergoing ASCT and obtaining hematopoietic reconstitution was similar between arms, longer intervals to platelet and neutrophil engraftment were observed in those receiving Daratumumab.<sup>3</sup> Similar results after Daratumumab exposure were reported in both GRIFFIN and MASTER study with chemo-free mobilization approach based on G-CSF and plerixafor as well as in recent real-life reports with cyclophosphamide and G-CSF.<sup>4-6</sup>

We report a collaborative, retrospective analysis of NDMM that consecutively underwent stem-cell mobilization and collection after Dara-VTd induction at two Italian centers (Ospedale Santo Spirito, Pescara, and IRCCS Ospedale San Raffaele, Milano). Inclusion criteria were NDMM and eligibility to ASCT. After Dara-VTd induction, patients received high-dose cyclophosphamide (HD-CTX) 4 g/m<sup>2</sup> as per institutional practice and were monitored in outpatient setting. G-CSF was administered at a dose of 5 µg/kg/day starting on day 3 to 5 after HD-CTX, with optional increase to 10 µg/kg/day at 48 hours before leukapheresis. Plerixafor 0.24 mg/kg was administered on demand on the day of planned leukapheresis either in patients with <20 CD34+cells/µl or in those predicted to be poor mobilizer according to subsequent criteria: 1) white blood cells count > 10x10<sup>9</sup>/L together with CD34+ count <15/µL; 2) ratio of patient body weight (kg) and CD34+/µL >

2; 3) yield < 25% of total CD34+ target dose on first day of leukapheresis. Pre-planned total target dose was  $10 \times 10^6$  CD34+cells/Kg to allow for multiple ASCT. Aim of the study was to evaluate safety and efficacy of mobilization with HD-CTX  $4 \text{ g/m}^2$  in terms of stem-cell yield and transplantation outcomes. Patients were treated according to current institutional programs upon written informed consent for transplantation procedures and use of medical records for research.

From 1<sup>st</sup> December 2021 to 28<sup>th</sup> February 2023, 64 NDMM consecutively received Dara-VTd at our institutions. At data cut-off, 47 patients completed induction and were included in this analysis whereas 17 patients were still receiving induction (Figure 1). At diagnosis median age was 62 years (range: 38-71), 13 patients (28%) were ISS III, 5 patients (11%) were R-ISS III and 26 patients (55%) were R2-ISS III-IV. Seventeen patients (36%) had high-risk cytogenetic abnormalities as del17p13, t(4;14) and t(14;16). Overall, a median of 4 Dara-VTd induction cycle (range: 4-6) was administered. Notably, 36 patients (76%) required Thalidomide dose reduction, 12 patients (25%) needed Bortezomib dose reduction and in 2 patients (4%) a single Daratumumab administration was omitted due to adverse events and/or intolerance. Grade III-IV adverse events occurred in 10 patients (21%) (Figure 1). After Dara-VTd induction, overall response rate was 98%, with 22 patients (47%) in very good partial remission, 3 patients (6%) in complete remission and 11 patients (23%) in stringent complete remission. One patient progressed soon after fourth Dara-VTd cycle. After a median of 133 days (range: 113-232) from start of induction and 32 days (range: 16-93) from last Daratumumab administration, 46 patients received HD-CTX  $4 \text{ g/m}^2$ . Most frequent grade III-IV adverse events were anemia (n=8; 17%), neutropenia (n=40; 87%), thrombocytopenia (n=21; 45%), and febrile neutropenia (n=4; 8%) (Figure 1). Notably, only 2 patient (4%) required platelets transfusion and 2 patients (4%) discontinued mobilization due to febrile neutropenia. Fourteen patients (30%) doubled G-CSF dose at 48 hours before leukapheresis. After a median of 11 days (range: 9-16) from HD-CTX, 43 out of 46 patients (93%)

underwent leukapheresis; 21 of them (49%) received plerixafor. All patients undergoing leukapheresis completed stem-cell collection, harvesting a mean total amount of  $10.68 \times 10^6$  CD34+cells/kg (SD 2.54) (range: 4.92-18.8). Notably, at first day of leukapheresis the mean amount of CD34+cells/kg collected was  $6.98 \times 10^6$  (SD 3.60) (range: 1,4-17,6) and plerixafor was given in 6 patients (14%). Mean collection efficiency (CE) was high in our cohort and the majority of patients (n=28/43; 65%) required 2 days of apheresis to reach the collection target of  $10 \times 10^6$  CD34+cells/kg. Details on stem-cell mobilization and harvesting are shown in Table 1. Overall, 3 out of 46 patients (7%) who received HD-CTX  $4 \text{ g/m}^2$  and G-CSF did not undergo stem-cell collection. One patient discontinued mobilization due to concomitant Sars-Cov2 infection. Unfortunately, both subsequent rescue attempts with chemo-free G-CSF  $10 \text{ }\mu\text{g/kg/day}$  + plerixafor and then CTX  $2 \text{ g/m}^2$  + G-CSF  $10 \text{ }\mu\text{g/kg/day}$  + plerixafor failed. Two patients discontinued mobilization due to febrile neutropenia but were later able to collect enough CD34+cells/Kg for single ASCT with a second procedure (a chemo-free regimen and a bone marrow harvest, respectively). After a median of 195 days from start of induction (range: 164-283) and 49 days (range: 33-96) from stem-cell collection, 35/43 (81%) patients who completed leukapheresis already underwent ASCT at time of data cut-off. Mean number of infused CD34+cells per patient was  $4.84 \times 10^6/\text{kg}$  (SD 1.20) (range: 2.96-9.86). All patient obtained stable neutrophils and platelets engraftments after a median of 12 days (range: 9-14) and 16 days (range: 10-25), respectively. No unexpected toxicities of transplant procedure were reported. At last follow up, all patients are alive.

Recent years have seen the introduction of several novel agents for the treatment of NDMM, with improved response rates and prolonged survival. Nonetheless, ASCT still remains a standard of care for younger fit patients.<sup>1,7</sup> A long-standing debate exists about the value of double ASCT in MM: the phase 3 STaMINA trial showed a PFS benefit in high-risk patients receiving tandem ASCT,

whereas in the EMN02/HO95 trial both PFS and overall survival advantage were reported.<sup>8-10</sup> To date both EHA-ESMO and NCCN guidelines still recommend double ASCT as an option for patients who do not achieve at least a VGPR after the first ASCT and those with high-risk features (defined by cytogenetics or clinical characteristics as extramedullary disease).<sup>1,7</sup> Notably, ongoing randomized trials still incorporate a double ASCT arm for MRD-defined high risk patients (MIDAS study; NCT04934475). A second transplantation can be also considered at the time of disease relapse in carefully selected patients.<sup>1,7</sup> More recently, cryopreserved autologous CD34+ cells has been used as rescue of prolonged cytopenias after CAR-T, further increasing the need for high collection numbers considering the expected increase in CAR-T use in the next future.<sup>11,12</sup> Although a minimum dose of  $2 \times 10^6$  CD34+cells/Kg is sufficient for a single ASCT, the optimal dose for a timely and stable hematopoietic reconstitution usually ranges between 3 and  $5 \times 10^6$  CD34+cells/Kg. Therefore, whenever feasible, the collection target should reach 8-10  $\times 10^6$  CD34+cells/Kg to allow for multiple ASCT.<sup>13,14</sup> Many centers are now implementing chemo-free mobilization protocols, although these are associated with an increased risk of mobilization failure or low stem cell yield among patients exposed to Daratumumab.<sup>5,6</sup> Cyclophosphamide is the most common chemotherapeutic agent used for mobilization in NDMM, with dosage ranging from 1.5 g/m<sup>2</sup> to 4 g/m<sup>2</sup>. Despite greater toxicities, higher doses are associated with improved stem-cell mobilization and harvesting, lower rate of collection failure and improved post-transplantation engraftment.<sup>14,15</sup> The present analysis represents, to the best of our knowledge, the first report of a mobilization strategy based on HD-CTX 4 g/m<sup>2</sup> after Daratumumab-based quadruplet induction in NDMM. In our population, HD-CTX 4 g/m<sup>2</sup> was administered early after the end of induction and was well tolerated with limited toxicities in an outpatient setting. Notably, the majority of patients successfully underwent leukapheresis after a median of 11 days. In our cohort, we observed a high proportion of poor mobilizers after Daratumumab exposure and frequent need for plerixafor to



reach the pre-planned target dose of  $10 \times 10^6$  CD34+cells/Kg. Nonetheless, HD-CTX  $4 \text{ g/m}^2$  together with patient-tailored plerixafor allowed high CD34+ cells collection in the majority of patients, with a mean total amount of  $10,68 \times 10^6$  CD34+ cells/kg. Notably, high collection numbers with limited plerixafor administration were already achieved in the first day of leukapheresis. These results compare favorably to those reported in the Dara-VTd arm of CASSIOPEIA trial, where lower CTX doses ( $2\text{-}3 \text{ g/m}^2$ ) led to collection of a mean of  $6.7 \times 10^6$  CD34+cells/kg.<sup>3</sup> We also observed a low incidence of mobilization failure in our cohort. Patients could safely undergo ASCT, with timely hematological engraftment. While limitations of this study include retrospective non-randomized nature and patients number, multicentricity limits single-center effects and bias in patient management.

In conclusion, HD-CTX  $4 \text{ g/m}^2$  and G-CSF after Dara-VTd induction in NDMM proved feasible in the outpatient setting and effective in terms of stem-cells mobilization. Increased dose of CTX together with on-demand administration of plerixafor allowed high collection numbers of CD34+cells per patient, thus limiting the potential detrimental effects of daratumumab on leukapheresis procedures and ensuring sufficient stem-cells for multiple ASCT and rescue of CART-related cytopenia.

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## Table

<b>Table 1. Characteristics of stem-cell mobilization and harvesting</b>	
Days from start of induction to HD-CTX: median (range)	133 (113-232)
Days from last Daratumumab to HD-CTX: median (range)	32 (16-93)
Days from HD-CTX to first day of leukapheresis: mean [SD] (range)	11.6 [1.65] (9-16),
Total G-CSF ( $\mu\text{g}/\text{kg}$ ) administered per patient: mean [SD] (range)	440 [128] (240-768)
Peripheral white blood cells/ $\mu\text{l}$ on first day of leukapheresis: median (range)	13.400 (1.600 -67.000)
Peripheral CD34+cells/ $\mu\text{l}$ on first day of leukapheresis: median (range)	57 (20-226)
Number of days of leukapheresis:	
- 1	15/43 (35%)
- 2	28/43 (65%)
Total number of leukapheresis days: mean [SD] (range)	1.7 [0.48] (1-2)
Plerixafor use: number (%)	21/43 (49%)
- Indication $<20$ CD34+cells/ $\mu\text{l}$	14/21 (66%)
- Other	7/21 (34%)
Amount of CD34+cells $\times 10^6/\text{kg}$ collected per patient: mean [SD] (range)	
- Day 1	6,98 (1,4-17,6)
- Day 2	5,77 (3,2-12,6)
- Total	10,68 [2.54] (4,94-18,8)
Collection efficiency: mean [SD] (range)	
- Day 1	64% [17] (18-99)
- Day 2	71% [19] (26-100)
Total blood volume processed (liter): mean [SD] (range)	4.58 [2] (1,6-9,6)

Table 1 shows characteristics of stem-cell mobilization and harvesting. *HD-CTX: high-dose cyclophosphamide. SD: standard deviation. G-CSF: granulocyte colony-stimulating factor.*

## Figure Legends

**Profile of analyzed population.** Figure 1 shows distribution of analyzed patients. *NDMM*: newly-diagnosed multiple myeloma patients. *HD-CTX*: high-dose cyclophosphamide. *ASCT*: autologous stem-cell transplantation. *PNP*: peripheral neuropathy.

