Effect of daratumumab on normal plasma cells, polyclonal immunoglobulin levels, and vaccination responses in extensively pre-treated multiple myeloma patients

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SUPPLEMENTAL MATERIALS

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Patient material

Bone marrow (BM) samples were obtained from 25 relapsed/refractory multiple myeloma (RRMM) patients, who were treated with daratumumab monotherapy in the GEN501 study, at baseline, after 3 months of treatment, and to confirm complete response or disease progression. MM cells and normal plasma cells (PCs) in these samples were quantified and analyzed for cell surface marker expression using flow cytometry. Additionally, normal PCs were analyzed in BM samples from 5 healthy controls. All samples were analyzed within 24 hours after BM aspiration.

Peripheral blood serum samples were obtained from 30 RRMM patients, who were treated with daratumumab monotherapy in the first part of the DARA/ATRA study, at baseline, at start of each new cycle of daratumumab treatment (Q4 weeks) and at the time of disease progression. Serum samples were obtained directly before the initiation of the daratumumab infusion. Uninvolved, polyclonal immunoglobulins (IgA, IgE and IgM for IgG myeloma; IgG, IgE and IgM for IgA myeloma; and IgG, IgA, IgE, and IgM for light-chain only myeloma) were assessed in these samples using nephelometry. There were no cases of IgE or IgM myeloma. IgA, IgM, and IgE levels below the detection limit were set on the lower limit of detection (LLOD; IgA 0.22 g/L; IgM 0.16 g/L and IgE 2.0 kU/L). Patients receiving intravenous immunoglobulin suppletion (IVIG, n=3) as prophylaxis against infection, were excluded from

polyclonal IgG analysis, and patients were excluded if baseline values were missing (n=5 for IgE evaluation).

Both the GEN501 (NCT00574288) and DARA/ATRA (NCT02751255) studies included adult MM patients with measurable disease, who were relapsed from or refractory to ≥2 prior lines of treatment, and had an Eastern Cooperative Oncology Group performance status 0-2.[1] Patients described in this report received daratumumab monotherapy in both studies, according to the approved dose and schedule (16 mg/kg weekly for 8 weeks, biweekly for 16 weeks, followed by monthly infusions).[1]

The study site ethics committee approved the protocols, which were conducted according to the principles of the Declaration of Helsinki, the International Conference on Harmonization, and the Guidelines for Good Clinical Practice. All patients gave written informed consent.

Bone marrow mononuclear cells

BM mononuclear cells were isolated from BM aspirates by Ficoll-Hypaque density-gradient centrifugation.

Flow cytometric analysis

MM cells and normal PCs in BM samples from MM patients and healthy donors were identified and analyzed for cell surface marker expression by staining cells with appropriate dilutions of CD38 Pacific blue, CD138 PerCP-Cy5.5, CD56 Pe-Cy7 (all Becton Dickinson), CD45 Pacific Orange (Invitrogen), CD19 APC-A750 (Beckman Coulter), combined with cytoplasmic staining for immunoglobulin light chains using monoclonal anti-kappa APC (Becton Dickinson) and polyclonal anti-lambda PE (DAKO). To assess the expression of CD38 in these samples irrespective of daratumumab treatment, cells were also stained with Humax-003 FITC (Janssen

Pharmaceuticals), which binds to a CD38 epitope distinct from the epitope bound by daratumumab. Red blood cells were lysed (BD Pharm Lyse, Becton Dickinson) directly before flow cytometric analysis. Malignant PCs were distinguished from normal PCs based on CD56, CD19 and CD45 expression. The frequency of normal PCs was calculated based on the total number of cells, with a detection limit of 10^{-5}. Flow cytometry was performed using a Canto II flow cytometer (Becton Dickinson). Fluorescent labeled beads (CS&T beads, Becton Dickinson) were used daily to monitor the performance of the flow cytometer and verify optical path and stream flow. This procedure enables controlled standardized results and allows the determination of long-term drifts and incidental changes within the flow cytometer. No changes were observed which could affect the results. Compensation beads were used to determine spectral overlap, compensation was automatically calculated using Diva software. Flow cytometry data were analyzed using FACS Diva software.

Antibody response to vaccination

RRMM patients were vaccinated against *Streptococcus (S.)pneumoniae*, *Haemophilus (H.)influenzae* type B, and seasonal influenza. Patients with a history of prior vaccination against *S.pneumoniae* or *H.influenzae* type B were excluded. Vaccination response was assessed in RRMM patients receiving daratumumab monotherapy. As a control group, we vaccinated RRMM patients treated with a non-daratumumab containing regimen, who otherwise had a comparable treatment history (Supplementary Table 3). Importantly, 8 of these 10 patients daratumumab-naïve RRMM patients were treated with an immunomodulatory drug (IMiD)-containing regimen, which may augment vaccine responses in MM.[2] Patients were evaluable for antibody response to vaccination if baseline and post vaccination titers were available.

The *S.pneumoniae* vaccination schedule consisted of the conjugated PCV-13 vaccine (Prevenar; Pfizer) followed by the polysaccharide PPV-23 vaccine (Pneumovax; Merck Sharp & Dohme), which were administered by intramuscular injection with an 8-week interval.[3] Specific antibody (IgG) titers were measured using enzyme-linked immunoabsorbent assay (ELISA) at baseline, 4 and 8 weeks after PCV-13 vaccination, as well as 4 and 8 weeks after PPV-23 vaccination. Response was defined as an absolute titer ≥2 μg/mL or ≥2-fold increase in 6 out of 9 analyzed pneumococcal subtypes (6B, 8, 9, 14, 15B, 19F, 20, 23F and 33F) according to the criteria proposed by Palazzo.[4] To asses fold increase in antibody titer, titers below the LLOD (0.04 μg/mL) were set to 50% of the LLOD, as described before.[5] One daratumumab-treated patient was lost to follow up and excluded from response evaluation. One daratumumab-naïve patient was not evaluable for response due to disease progression requiring a new treatment regimen, which included daratumumab.

H.influenzae type B vaccination consisted of a single intramuscular injection of the Act-Hib vaccine (Sanofi). Measurement of specific IgG was performed using ELISA at baseline, as well as 4 and 8 weeks after vaccination. Response was defined as an absolute titer ≥1 μg/mL, or ≥4-fold increase if maximum titer was between 0.15-0.99 μg/mL.[4] To assess fold increase in antibody titer, titers below the LLOD (0.11 μg/mL) were set to 50% of the LLOD, as described before.[6] Patients with protective titers at baseline were excluded from response evaluation (1 daratumumab-naïve and 2 daratumumab-treated patients).

Seasonal influenza vaccines of the 2016/2017 and 2017/2018 seasons were used, which contained components of an influenza A(H1N1)pdm09 virus, an A(H3N2) virus and a B virus (B/Brisbane/60/2008-like), according to the World Health Organization recommendations.[7, 8] Serum samples were collected at baseline, as well as 3 and 12 weeks after vaccination. Hemagglutinin inhibition assays (HAI) were performed according to standard procedures using

four hemagglutinating units of the respective vaccine strains and turkey erythrocytes.[9, 10] Data are presented as geometric mean HAI titer (GMT). Seroprotection was defined as an absolute titer ≥1:40, and seroconversion as a ≥4-fold increase in titer.[9, 11] To assess fold increase in antibody titer, titers below the LLOD (1:10) were set to 5, as described before.[12] All patients were evaluable for antibody response.

Infectious complications and microbiological evaluation following vaccination

During follow-up of the 27 vaccinated patients in this single-center study (median duration of follow-up 17 months (range 6 – 21 months; 16 months in daratumumab-naïve and 19 months in daratumumab-treated patients)), 49 infectious episodes (upper respiratory infection n=35, pneumonia n=7, flu-like symptoms n=5, and sinusitis n=2) were reported in 14 out of 17 daratumumab-treated patients (82%) and 6 out of 10 daratumumab-naïve patients (60%). Four patients (15%) had an infectious episode grade ≥3, the other infections were all grade ≤2.[13] If patients presented to hospital with fever, microbiological evaluations were performed according to local guidelines. However, in 26 episodes (53%; all grade ≤2) no microbiological evaluations were performed, since patients presented to their general physician. All patients also received antibiotic prophylaxis.[14]

Statistics

Comparisons between variables were performed using two-tailed (paired) Student's *t*-test, and Mann-Whitney *U* test or Wilcoxon matched-pairs signed-rank test in case data do not follow normal distribution. Response rates were compared using Pearson Chi-Square test. *P*-values below 0.05 were considered significant. Data were analyzed in SPSS version 22.

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SUPPLEMENTAL TABLES

Supplemental Table 1. Baseline characteristics of patients included in analysis of normal plasma cells and MM cells in bone marrow samples.

	RRMM patients n = 25	
Age, median (range)	60 (43 - 75)	
Male sex, n (%)	16 (64)	
M-protein, n (%)		
- IgG	8 (32)	
- IgA	6 (24)	
- IgD	2 (8)	
- Light chain only	7 (28)	
- Unknown	2 (8)	
Previous lines of treatment, median (range)	4 (1 - 11)	
Prior myeloma treatment, n (%)	**Exposed**	**Refractory***
- Lenalidomide	18 (72)	17 (68)
- Bortezomib	23 (92)	17 (68)
- Pomalidomide	3 (12)	3 (12)

*Refractory disease is defined as progressive disease during therapy, no response (less than PR), or progressive disease within 60 days of stopping treatment, according to the International Uniform Response Criteria for Multiple Myeloma.

Abbreviations: RRMM, relapsed/refractory multiple myeloma; n, number; IgG, immunoglobulin G; IgA, immunoglobulin A; IgD, immunoglobulin D.

Supplemental Table 2. Baseline characteristics of patients included in analysis of polyclonal immunoglobulin levels.

	RRMM patients n = 30	
Age, median (range)	70 (47 - 80)	
Male sex, n (%)	17 (57)	
M-protein, n (%)		
- IgG	21 (70)	
- IgA	1 (3)	
- Bence-Jones	1 (3)	
- Light-chain only	7 (23)	
Previous lines of treatment, median (range)	5 (2 – 11)	
Prior myeloma treatment, n of patients (%)	**Exposed**	**Refractory***
- Lenalidomide	30 (100)	27 (90)
- Bortezomib	29 (97)	20 (67)
- Pomalidomide	15 (50)	14 (47)
- Carfilzomib	3 (10)	3 (10)
- Durvalumab	6 (20)	6 (20)
- Elotuzumab	2 (7)	2 (7)
- Ixazomib	1 (3)	1 (3)
- Daratumumab	2# (7)	2 (7)

*Refractory disease is defined as progressive disease during therapy, no response (less than PR), or progressive disease within 60 days of stopping treatment, according to the International Uniform Response Criteria for Multiple Myeloma. #2 patients received daratumumab retreatment after 2 and 5 intervening lines of therapy not containing daratumumab.

Abbreviations: RRMM, relapsed and/or refractory multiple myeloma; n, number; IgG, immunoglobulin G; IgA, immunoglobulin A.

Supplemental Table 3. Baseline characteristics of vaccinated relapsed/refractory MM patients.

	RRMM Daratumumab-naïve n = 10		**RRMM Daratumumab-treated** n = 17	
Age, median (range)	67 (60 – 85)		72 (55 – 81)	
Male sex, n (%)	6 (60)		10 (59)	
M-protein, n (%)				
- IgG	4 (40)		13 (77)	
- IgA	3 (30)		1 (6)	
- Light-chain only	3 (30)		2 (12)	
- Non-secreting	0		1 (6)	
Treatment at time of vaccination, n (%)				
- IMiD	1 (10)		0	
- IMiD + dexamethasone	2 (20)		0	
- IMiD + PI + dexamethasone	2 (20)		0	
- IMiD + Cyclophosphamide + dexamethasone	3 (30)		0	
- PI + Cyclophosphamide + dexamethasone	1 (10)		0	
- Cyclophosphamide + dexamethasone	1 (10)		0	
- Daratumumab monotherapy	0		17 (100)	
Duration of treatment at time of vaccination (current regimen), median months (range)	11 (0 – 57)		2 (0.5 – 17)	
Response at time of vaccination, n (%)				
- ≥PR	7 (70)		9 (53)	
- <PR	3 (30)		8 (47)	
Previous lines of treatment, median (range)	3 (1 – 8)		4 (2 – 11)	
Prior myeloma treatment, n (%)	**Exposed**	**Refractory***	**Exposed**	**Refractory***
- Lenalidomide	10 (100)	7 (70)	17 (100)	17 (100)
- Pomalidomide	4 (40)	4 (40)	8 (47)	8 (47)
- Bortezomib	8 (80)	7 (70)	16 (94)	10 (59)
- Carfilzomib	0	0	2 (12)	2 (12)
- Ixazomib	0	0	1 (6)	1 (6)
- Daratumumab	0	0	2# (12)	2 (12)
- Durvalumab	1 (10)	1 (10)	4 (24)	4 (24)
- Elotuzumab	0	0	1 (6)	1 (6)

*Refractory disease is defined as progressive disease during therapy, no response (less than PR), or progressive disease within 60 days of stopping treatment, according to the International Uniform Response Criteria for Multiple Myeloma. #2 patients received daratumumab retreatment after 2 and 5 intervening lines of therapy not containing daratumumab.

Abbreviations: RRMM, relapsed/refractory multiple myeloma; n, number of patients; IgG, immunoglobulin G; IgA, immunoglobulin A; IMiD, immunomodulatory drug; PI, proteasome inhibitor; PR, partial response according to the IMWG criteria

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. The effect of daratumumab treatment on polyclonal IgG levels in RRMM patients persists when the maximum potential serum concentration of daratumumab is subtracted from total IgG level. Serum levels of uninvolved, polyclonal IgG were assessed using nephelometry, at baseline and after 4 weeks of daratumumab monotherapy in 7 RRMM patients. Patients with an IgG M-protein, or receiving IgG substitution therapy, were excluded from polyclonal IgG analysis. Samples were obtained directly before the first (baseline, black bar) and fifth (4 week-treatment) daratumumab infusion. A limitation of this analysis is that the evaluation of total IgG also includes daratumumab, which is an IgG-kappa antibody. This results in a relatively small absolute overestimation of the concentration of polyclonal IgG. During weekly daratumumab infusions (the first 8 weeks of treatment), the serum trough concentration reached a maximum of 0.5 g/L.[15] Therefore, we performed a separate analysis whereby this value of 0.5 g/L was subtracted from the measured polyclonal IgG values at 4 weeks (grey bar). Data represent mean ± SEM. The difference between indicated groups was calculated using Wilcoxon matched ranks test.

Abbreviations: IgG, immunoglobulin G; RRMM, relapsed/refractory multiple myeloma; SEM, standard error of mean; ns, not significant.

SUPPLEMENTAL FIGURES

Supplemental Figure 1.