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Immunomodulatory Effects and Adaptive Immune Response to Daratumumab in Multiple Myeloma

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INTRODUCTION

- Daratumumab (DARA) is an IgG1κ human monoclonal antibody that binds to CD38 and inhibits the growth of CD38-expressing tumor cells by inducing the following:
  - Direct apoptosis through Fc-mediated cross-linking[1]
  - Immune-mediated tumor cell lysis through complement-dependent cytotoxicity, antibody-dependent cell-mediated cytotoxicity (ADCC), and antibody-dependent cellular phagocytosis[2,3]
  - Lysis of myeloid-derived suppressor cells (MDSCs) and a subset of regulatory T cells (T_{regs}) that express CD38[4]
  - Increased CD4$^+$ and CD8$^+$ T-cell absolute counts and total lymphocyte percentages in both peripheral blood (PB) and bone marrow (BM)[4]
- In 2 clinical studies of DARA monotherapy (16 mg/kg) in patients with relapsed and refractory multiple myeloma (GEN501 [ClinicalTrials.gov Identifier: NCT00574288] and SIRIUS [NCT01985126]), overall response rates were 36% and 29%, respectively, including complete responses (CRs) and stringent CRs.[5,6] After a median follow-up of 14.8 months, the combined estimated median overall survival (OS) was 19.9 months (95% confidence interval, 15.1-not estimable; Poster 4498)

OBJECTIVE

- To evaluate the effects of DARA on immune-cell populations and adaptive immune responses

METHODS

Patients

- In both studies, patients were ≥18 years of age, had documented myeloma requiring systemic therapy, and had an Eastern Cooperative Oncology Group performance status of ≤2
  - In GEN501, patients had relapsed from or were refractory to ≥2 prior lines of therapy, including a proteasome inhibitor (PI), an immunomodulatory drug (IMiD), chemotherapy, and autologous stem cell transplantation treatment[5]
  - In SIRIUS, patients had progressed on their most recent line of therapy and had received ≥3 prior lines of therapy, including a PI and an IMiD, or were double refractory to both a PI and an IMiD[6]

STUDY DESIGN

- GEN501 was an open-label, phase 1/2, dose-escalation and -expansion study[5]
  - In Part 1, DARA doses ranged from 0.005 mg/kg to 24 mg/kg
  - In Part 2, DARA was given as either:
    - 8 mg/kg weekly for 8 weeks, every 2 weeks for 16 weeks, and then monthly until disease progression, or
    - 16 mg/kg, with a 3-week washout after the first infusion, then weekly for 7 weeks, every 2 weeks for 14 weeks, and then monthly until disease progression
- SIRIUS was an open-label, multicenter, phase 2 study of Simon 2-stage design[6]
  - Patients received DARA 8 mg/kg every 4 weeks, or
  - DARA 16 mg/kg weekly for 8 weeks, every 2 weeks for 16 weeks, and monthly thereafter

Study Endpoints

- Best overall clinical response was determined using the International Myeloma Working Group consensus recommendations[7]
  - Non-responders included patients with a best response of minimal response, stable disease, or progressive disease
- PB and BM biopsies/aspirates were collected at screening, immediately prior to the first infusion, and at specified time points during treatment
  - Immunophenotyping was conducted by flow cytometry to enumerate various T-cell subtypes
  - T-cell clonality was measured using T-cell receptor (TCR) sequencing
  - Antiviral and alloreactive T-cell responses were assessed by enzyme-linked immunosorbent assay–based measurements of interferon γ (IFN-γ) production from PB mononuclear cells (PBMCs) stimulated with a pool of viral peptides or with allogeneic, third-party PBMCs
  - T_{reg} activity was assessed in carboxyfluorescein succinimidyl ester (CFSE)–labeled CD3$^+$CD25$^-$ autologous effector T cells, stimulated with α-CD3/CD28–coated beads

Statistical Analyses

- T-cell subpopulation counts were modeled over time with linear mixed modeling
- Two-group comparisons were performed using nonparametric Wilcoxon rank-sum tests

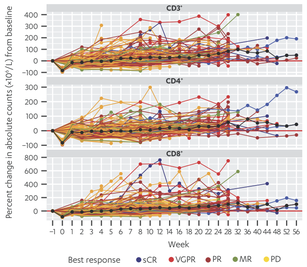
RESULTS

Patients

- Data from 148 patients who received DARA 16 mg/kg (42 in GEN501 and 106 in SIRIUS) were analyzed for changes in immune response
  - Median (range) age was 64 (31-84) years
  - Median time (range) from diagnosis was 5.12 (0.77-23.77) years
  - 53% were male
  - 91% were refractory to their last line of treatment, 86% were refractory to both a PI and an IMiD, and 76% received ≥3 prior lines of therapy

Effect of DARA on T-Cell Expansion

- In PB (n = 58), significant mean increases in CD3$^+$ (44%), CD4$^+$ (32%), and CD8$^+$ (62%) T-cell counts per 100 days were seen with DARA treatment (**Figure 1**)
- Similar expansion was observed in BM (n = 58), with median maximum percent increases of 20%, 6%, and 27% for CD3$^+$, CD4$^+$, and CD8$^+$ T-cell counts, respectively (**Table 1**)



PB, peripheral blood; DARA, daratumumab; sCR, stringent complete response; VGPR, very good partial response; PR, partial response; MR, minimal response; PD, progressive disease.
Longitudinal data representation of the percent change from baseline of absolute CD3$^+$, CD4$^+$, and CD8$^+$ T-cell counts over time in PB.
Lines represent connected data points of individual patients that are colored by best overall clinical response.
The black line shows the overall median percent change over time.
Only visits with more than 2 data points are shown.

Figure 1. CD3$^+$, CD4$^+$, and CD8$^+$ T-cell counts increase in PB with DARA treatment.

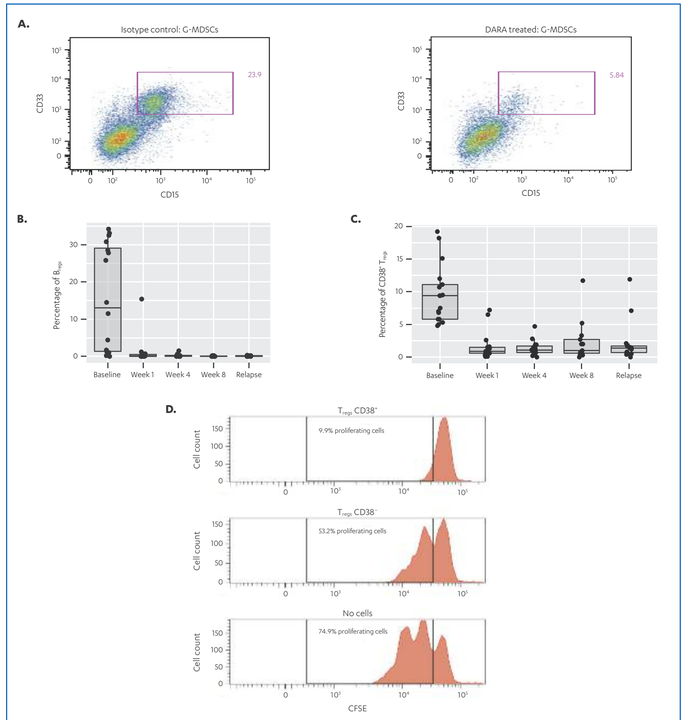
Table 1. CD3$^+$, CD4$^+$, and CD8$^+$ T-Cell Counts Increase in BM With DARA Treatment

	Percent change from baseline at on-treatment visit (% of lymphocytes) n = 58		
	CD45$^+$CD3$^+$	CD45$^+$CD3$^+$ CD4$^+$	CD45$^+$CD3$^+$ CD8$^+$
Minimum	−40.40	−60.7	−10.89
1st quartile	12.13	−8.67	14.58
Median	19.95	5.66	26.99
Mean	29.28	13.42	39.09
3rd quartile	47.65	25.34	53.71
Maximum	121.6	125.5	187.9

BM, bone marrow; DARA, daratumumab.

Effect of DARA on Regulatory Cells Expressing CD38

- CD38$^+$ granulocytic myeloid-derived suppressor cells (MDSCs), generated in vitro, were highly sensitive to DARA-mediated ADCC (**Figure 2A**)
- CD38$^+$ regulatory B cells (B_{regs}) from DARA-treated patients (n = 16) were depleted compared to baseline ($P = 0.0018$) at Week 1 of DARA treatment and remained depleted during treatment (**Figure 2B**)
- A novel subpopulation of T_{regs} (CD4$^+$CD25$^+$CD127^{dim}) was identified that expressed high levels of CD38 prior to activation
  - These T_{regs} comprised approximately 10% of peripheral T_{regs}
  - These cells were highly sensitive to DARA and exhibited a significant, rapid decline following the first dose (n = 17; 8.88×10^{-16} at Week 1 vs baseline) and remained depleted throughout treatment (**Figure 2C**)
  - Changes in CD38$^+$ T_{regs} were similar in responders and non-responders, but the CD8$^+$ T-cell:T_{reg} ratio was significantly higher at Week 8 in patients who showed a response to DARA ($P = 0.0095$)
  - In ex vivo analyses, CD38$^+$ T_{regs} suppressed T-cell proliferation more robustly than CD38$^-$ T_{regs} or controls
    - Histograms showing the dilution of CFSE dye due to cell division are shown in **Figure 2D**



DARA, daratumumab; B_{reg}, regulatory B cell; T_{reg}, regulatory T cell; CFSE, carboxyfluorescein succinimidyl ester.

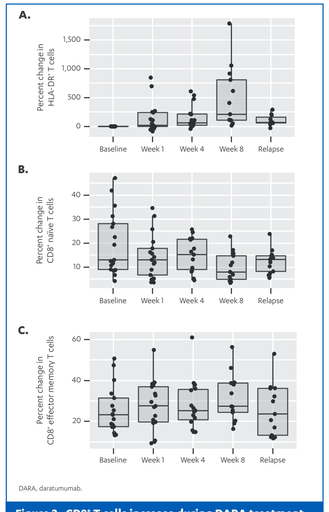
Figure 2. CD38-expressing regulatory cells decline with DARA treatment.

Changes in CD4$^+$ and CD8$^+$ T Cells

- The CD8$^+$:CD4$^+$ and CD8$^+$:T_{reg} ratios increased significantly over time in PB and BM with DARA treatment
  - In PB
    - The median (range) ratio of CD8$^+$:CD4$^+$ T cells increased from 1.21 (0.16-18.35) at baseline to 1.99 (0.26-9.51; $P = 5.1\times10^{-5}$) and 1.92 (0.26-8.17; $P = 0.00017$) at Weeks 8 and 16, respectively
    - CD8$^+$:T_{reg} ratio increased from 15.23 (2.33-185.5) at baseline to 29.21 (3.5-230.67; $P = 1.7857\times10^{-7}$) and 25.05 (4.33-98.91; $P = 4.1245\times10^{-7}$) at Weeks 8 and 16, respectively
  - In BM
    - The median (range) ratio of CD8$^+$:CD4$^+$ ratio increased from 1.46 (0.33-9.97) at baseline to 2.19 (0.30-8.56; $P = 0.00016$) at Week 12 (±1 cycle)
    - CD8$^+$:T_{reg} ratio increased from 13.72 (3.06-157) at baseline to 26.57 (4.51-208.25; $P = 2.7758\times10^{-7}$) at Week 12 (±1 cycle)
- Patients who responded to treatment (n = 45) showed significantly greater maximum increases from baseline than non-responders (n = 93) in CD3$^+$ ($P = 0.00012$), CD4$^+$ ($P = 0.00031$), and CD8$^+$ ($P = 0.00018$) T cells
- In a subset of 17 patients from the GEN501 study, peripheral HLA-DR$^+$ CD8$^+$ T cells were significantly increased during DARA treatment compared with baseline ($P = 1.25\times10^{-5}$ at Week 8) and declined when patients relapsed (**Figure 3A**)
  - This change was accompanied by a decrease in naïve CD8$^+$ T cells (**Figure 3B**) and a concomitant increase in CD8$^+$ effector memory T cells (**Figure 3C**)

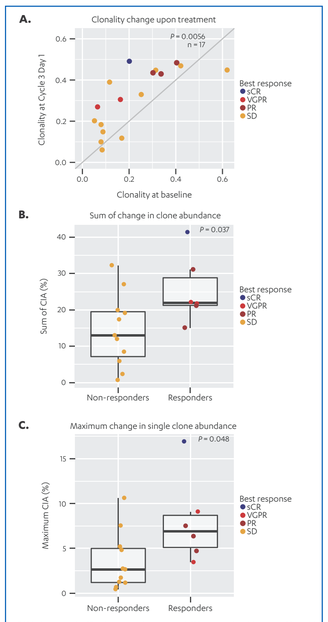
T-cell Clonality Increased With DARA Treatment

- TCR clonality significantly increased during DARA treatment compared with pre-treatment ($P = 0.0056$; **Figure 4A**) and was positively correlated with increases in CD8$^+$ T cells (Pearson's $r = 0.76$)
  - Patients who responded to treatment had greater increases in the sum of absolute change in abundance (CIA) for each expanded T-cell clone in the repertoire ($P = 0.037$; **Figure 4B**), as well as greater maximum CIA of a single clone ($P = 0.048$; **Figure 4C**) compared to non-responders
- Increased T-cell responses (IFN-γ secretion) to viral- and alloantigens were observed after DARA treatment compared to baseline, particularly in responders



DARA, daratumumab.

Figure 3. CD8$^+$ T cells increase during DARA treatment.

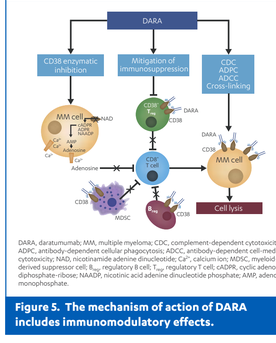


DARA, daratumumab; sCR, stringent complete response; VGPR, very good partial response; PR, partial response; SD, stable disease; CIA, change in abundance.

Figure 4. T-cell clonality increases with DARA treatment, particularly in responders.

CONCLUSIONS

- **DARA treatment in a heavily pretreated population was associated with the following immunomodulatory effects:**
  - **An expansion of CD4$^+$ T-helper cells and CD8$^+$ cytotoxic T cells**
  - **An increase in clonality of the TCR repertoire and enhanced cytotoxic T-cell responses**
    - **Increased TCR clonality and its correlation with increased CD8$^+$ T cells suggest that T-cell expansion is antigen-driven**
  - **Responders had increased T-cell responses to viral- and alloantigens, suggesting a revival of an antitumor immune response**
  - **These changes in T-cell expansion and activity were more pronounced in responders than non-responders and were often reversed with relapse**
- **CD38$^+$ B_{regs} from patients and CD38$^+$ MDSCs created in vitro were sensitive to DARA**
- **These data suggest an immunomodulatory role of DARA, in addition to its previously described mechanisms of action, which may contribute to deep clinical responses and prolonged OS (Figure 5)**
- **The revival of an antitumor immune response in some patients with heavily pretreated myeloma suggest that these immunomodulatory effects may have a broader role in cancer treatment**



DARA, daratumumab; MM, multiple myeloma; CDC, complement-dependent cytotoxicity; ADPC, antibody-dependent cellular phagocytosis; ADCC, antibody-dependent cell-mediated cytotoxicity; NAD, nicotinamide adenine dinucleotide; Ca^{2+}, calcium ion; MDSC, myeloid-derived suppressor cell; B_{reg}, regulatory B cell; T_{reg}, regulatory T cell; cADPR, cyclic adenosine diphosphate–ribose; NAADP, nicotinic acid adenine dinucleotide phosphate; AMP, adenosine monophosphate.

Figure 5. The mechanism of action of DARA includes immunomodulatory effects.

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DISCLOSURES

T. Casneuf, B. Verbist, J. Bald, K. Liu, T. Ahmadi, and A.K. Sasser are employees of Janssen Research & Development. T. Plesner reports membership advisory boards for Janssen, Genmab, and Celgene; and research funding from Janssen, Celgene, Roche, and Novartis. N.W.C.J. van de Donk reports research funding from Janssen-Pharmaceuticals, Amgen, and Celgene. B. Weiss reports research funding from Janssen and Onclave; and consultancy for Janssen and Millennium. H. Lokhorst reports honoraria from Genmab, Janssen, and Amgen; and research funding from Genmab and Janssen. T. Mutis reports research funding from Janssen and Genmab. J. Krejcik and I. Nijhof report no conflicts.

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