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Serum Proteomic Analysis of Multiple Myeloma Subjects Treated With Daratumumab Monotherapy

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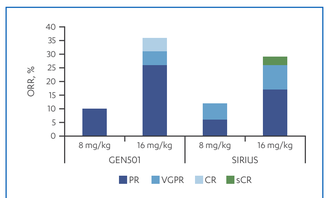
INTRODUCTION

Daratumumab

- Daratumumab is a first-in-class, human anti-CD38 IgG1 monoclonal antibody in clinical development across the multiple myeloma (MM) disease spectrum
- High levels of CD38 are expressed on myeloma cells[1,2]
- The antimyeloma activity of daratumumab is mediated through a number of mechanisms of action, including complement-dependent cytotoxicity, antibody-dependent cell-mediated toxicity, antibody-dependent cellular phagocytosis, and apoptosis via cross-linking[3,4]
- Two studies, GEN501 (ClinicalTrials.gov Identifier: NCT00574288) and SIRIUS (NCT01985126), demonstrated that single-agent daratumumab had a tolerable safety profile and promising efficacy in patients with relapsed or refractory MM (**Figure 1**)[5,6]



ORR, overall response rate; PR, partial response; VGPR, very good partial response; CR, complete response; sCR, stringent complete response.

Figure 1. Overall response rates with daratumumab monotherapy in GEN501 and SIRIUS.

SomaLogic Platform

- The SOMAscan Assay (SomaLogic, Inc., Boulder, CO) utilizes an aptamer-based technology to measure >1,100 native proteins in complex matrices by transforming each individual protein concentration into a corresponding SOMAmer reagent concentration, which can then be quantified using microarrays or quantitative PCR
- SOMAmer reagents cover a broad array of proteins that are associated with cellular processes and disease pathophysiology (**Figure 2**)

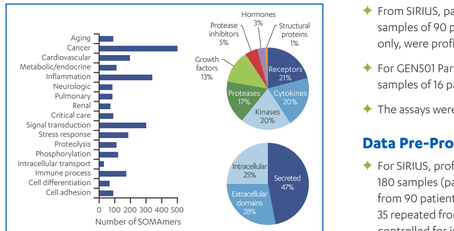


Figure 2. Proteins measured by the SomaLogic Platform.

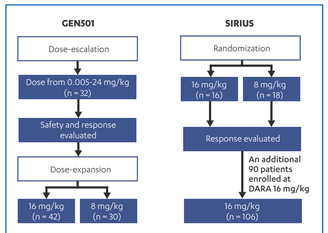
OBJECTIVES

- To further investigate the mechanism of action of daratumumab in relapsed and refractory MM through changes in protein expression
- To identify predictive and/or pharmacodynamic protein markers of clinical response to daratumumab

METHODS

Study Designs

- GEN501 was a phase 1/2, open-label, multicenter study conducted in 2 parts (**Figure 3**)
  - Part 1 was a dose-escalation phase
  - Part 2 was a dose-expansion phase
- SIRIUS was a phase 2, open-label, international, multicenter study of Simon 2-stage design (**Figure 3**)



DARA, daratumumab.

Figure 3. Study design for GEN501 and SIRIUS.

- 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 proteasome inhibitors (PIs), immunomodulatory drugs (IMiDs), chemotherapy, and autologous stem cell transplantation
  - 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
- A dosing schedule of 16 mg/kg weekly for 8 weeks, every other week for 16 weeks, and then monthly thereafter was established as the recommended dose

Analysis Methods

- From SIRIUS, paired baseline and on-treatment serum samples of 90 patients, plus another 15 patients at baseline only, were profiled
- For GEN501 Part 2, paired baseline and on-treatment serum samples of 16 patients were tested
- The assays were performed at SomaLogic, Inc.

Data Pre-Processing

- For SIRIUS, profiling was performed in 2 batches of 180 samples (paired baseline and on-treatment samples from 90 patients) and 50 samples (all at baseline, 35 repeated from Batch 1); SomaLogic's standard workflow controlled for inter-plate variability
  - Samples from 35 patients, shared between the 2 batches, were used to rescale each of the Batch 2 sample SOMAmer values with the median of the 35 repeated-sample Batch 1/Batch 2 ratios for inter-batch variability correction
  - SOMAmer sequences that changed between the batches and SOMAmers with extremely large or small inter-batch calibration factors were removed from the analysis
  - The data from 35 samples repeated in Batches 1 and 2 were merged by calculating the mean for each protein
  - Site ID was determined to be significantly associated with the variables of interest (eg, demographics, response class, and sample time point; ≥7.37% variability explained; Wald test $P = 3.71\times10^{-9}$), and ComBat[7] was utilized to correct for site ID effects to reduce the impact of site-related effects within the data
- For GEN501 Part 2, paired baseline and on-study samples from 16 patients were run in 1 batch and the data were pre-processed with the SomaLogic standardization workflow

Statistical Analyses

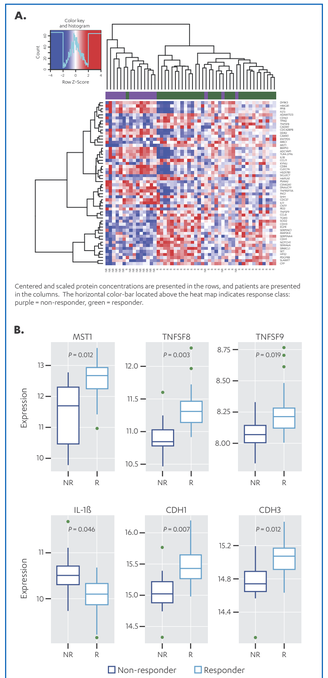
- Responders versus non-responders
  - Statistical comparison of protein concentration distributions in daratumumab responders (partial response [PR], very good PR [VGPR], complete response [CR], and stringent CR [sCR]) versus non-responders (progressed disease [PD]) was performed at both baseline and on treatment using 2 complementary methods: (i) Wilcoxon rank-sum test on each individual SOMAmer[8] and (ii) Limma analysis on all SOMAmers simultaneously[9]
  - All *P* values were adjusted using the Benjamini-Hochberg (BH) method for multiple hypothesis correction[10,11]
  - Wilcoxon-adjusted *P* values are reported here
- Baseline versus on treatment
  - Baseline versus on treatment protein levels were compared using 3 alternative statistical methods: (i) 2-way repeated-measures analysis of variance (ANOVA),[12] (ii) the Wilcoxon signed-rank test,[8] and (iii) the Friedman test[8]
  - All *P* values were adjusted to control false discovery rate using the BH method for multiple hypothesis correction
  - A 2-way repeated-measures ANOVA[12] was applied to determine if significant time-point:response-class interaction occurred for each SOMAmer; a modified Wilcoxon rank-sum test was applied as a post hoc test to specifically determine if responders and non-responders showed different treatment effects[8]
  - Significance values were adjusted using the BH method, and the null hypothesis was rejected when the adjusted *P* value <0.05

RESULTS

SIRIUS

Responders Versus Non-responders at Baseline

- A total of 51 proteins were identified as being significantly different at baseline between responders and non-responders (*P* values <0.05; **Figure 4A**)
  - Cytokines, cadherins, and tumor necrosis factor (TNF) family members were among the identified proteins
  - Of the 51 identified proteins, 30 were higher in responders and 21 were lower
- Macrophage-stimulating protein (MSP/MST1) expression was significantly higher in responders versus non-responders ($P = 0.012$; **Figure 4B**)
- TNF family members TNFSF8/CD30L/CD153 ($P = 0.003$; **Figure 4B**) and TNFSF9/4-1BBL/CD137L ($P = 0.019$; **Figure 4B**) also had significantly higher expression in responders versus non-responders
  - TNFs co-stimulate T cells[13]
- Expression of interleukin (IL)-1ß was significantly lower in responders compared to non-responders ($P = 0.046$; **Figure 4B**)
  - In the bone marrow microenvironment, high concentrations of IL-1ß elicit the release of IL-6, which promotes myeloma cell survival and expansion[14]
- E-cadherin (CDH1) and P-cadherin (CDH3) were shown to be increased in responders ($P = 0.007$ and 0.012, respectively; **Figure 4B**)
  - Soluble serum E-cadherin is an independent prognostic marker of poor survival for newly diagnosed patients with MM[15]
  - The role of CDH3 is similar to that of CDH1[16]



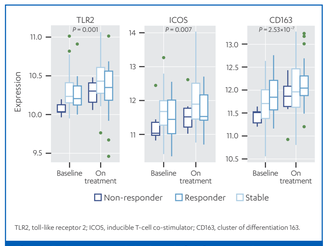
Centered and scaled protein concentrations are presented in the rows, and patients are presented in the columns. The horizontal color-bar located above the heat map indicates response class: purple = non-responder, green = responder.

MST1, macrophage-stimulating 1; TNFSF8, tumor necrosis factor subfamily 8; TNFSF9, tumor necrosis factor subfamily 9; NR, non-responder; R, responder; IL-1ß, interleukin-1ß; CDH1, cadherin 1; CDH3, cadherin 3.

Figure 4. Proteins with different concentration distributions between responders and non-responders at baseline.

Treatment-induced Response-independent Changes in Protein Expression

- 142 proteins were identified as having different concentrations at baseline versus on treatment by ANOVA, Wilcoxon rank-sum test, and Friedman test (BH-adjusted *P* values <0.05)
  - In all response classes (responders, stable, and non-responders), expression of immune-related proteins toll-like receptor 2 (TLR2), inducible T-cell co-stimulator (ICOS), and CD163 increased on treatment (**Figure 5**)



TLR2, toll-like receptor 2; ICOS, inducible T-cell co-stimulator; CD163, cluster of differentiation 163.

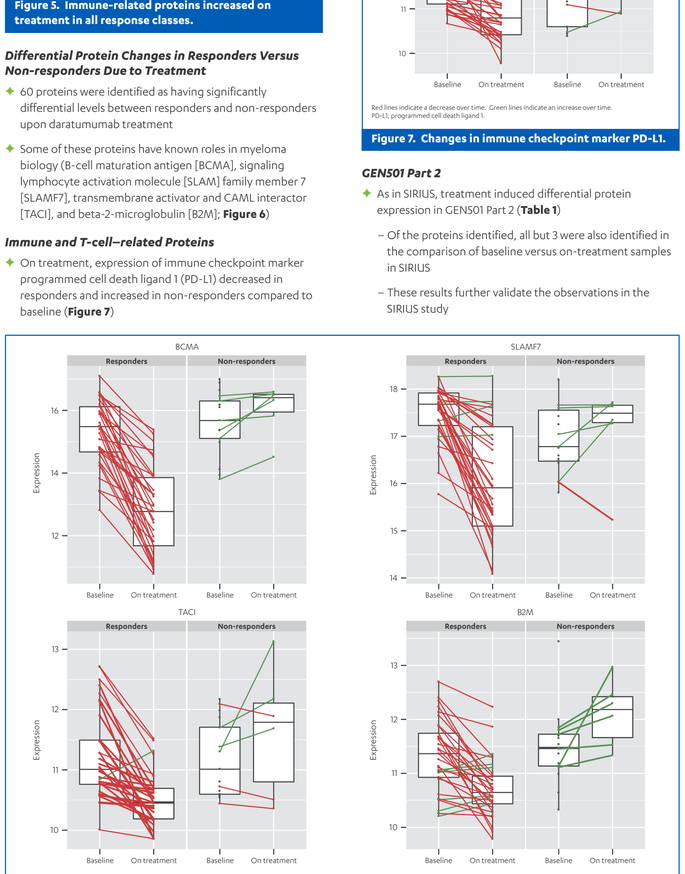
Figure 5. Immune-related proteins increased on treatment in all response classes.

Differential Protein Changes in Responders Versus Non-responders Due to Treatment

- 60 proteins were identified as having significantly differential levels between responders and non-responders upon daratumumab treatment
- Some of these proteins have known roles in myeloma biology (B-cell maturation antigen [BCMA], signaling lymphocyte activation molecule [SLAM] family member 7 [SLAMF7], transmembrane activator and CAML interactor [TACI], and beta-2-microglobulin [B2M]; **Figure 6**)

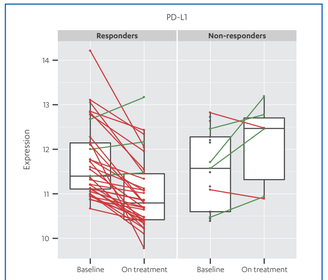
Immune and T-cell–related Proteins

- On treatment, expression of immune checkpoint marker programmed cell death ligand 1 (PD-L1) decreased in responders and increased in non-responders compared to baseline (**Figure 7**)
  - PD-L1/CD274 is the ligand for the receptor PD-1; the engagement of PD-1 on T cells leads to reduced T-cell function and increased regulatory T-cell development[17]



Red lines indicate a decrease over time. Green lines indicate an increase over time.
BCMA, B-cell maturation antigen; SLAMF7, signaling lymphocyte activation molecule family member 7; TACI, transmembrane activator and CAML interactor; B2M, beta-2-microglobulin.

Figure 6. Proteins associated with tumor load that decreased in responders and increased in non-responders.



Red lines indicate a decrease over time. Green lines indicate an increase over time.
PD-L1, programmed cell death ligand 1.

Figure 7. Changes in immune checkpoint marker PD-L1.

GEN501 Part 2

- As in SIRIUS, treatment induced differential protein expression in GEN501 Part 2 (**Table 1**)
  - Of the proteins identified, all but 3 were also identified in the comparison of baseline versus on-treatment samples in SIRIUS
  - These results further validate the observations in the SIRIUS study
- Two proteins, VCAM1 and GSN, were identified by all 3 applied statistical tests (shown in red text in **Table 1**)

Table 1. Proteins Exhibiting Differential Expression Between Baseline Versus On-Treatment Samples by Statistical Test

Wilcoxon	ANOVA:Visit	Friedman
VCAM1	**VCAM1**	**VCAM1**
GSN	**GSN**	**GSN**
CXCL12	**THBS4**	CXCL12
IGHM	**MMP3**	
PRTN3		
PLG		
THBS4		
MMP3		
PIGR		

Boldface and underlined text indicates that the SOMAmer was also identified in the comparison of baseline versus on-treatment samples in SIRIUS.
VCAM1, vascular cell adhesion molecule 1; GSN, gelsolin; CXCL12, C-X-C motif chemokine 12; IGHM, immunoglobulin heavy constant mu; PRTN3, proteinase 3; PLG, plasminogen; THBS4, thrombospondin 4; MMP3, matrix metalloproteinase 3; PIGR, polymeric immunoglobulin receptor.

CONCLUSIONS

- **Several proteins with differential expression between responders and non-responders at baseline were identified, many of which are associated with MM or CD38**
- **142 proteins were identified as differentially expressed between baseline and on treatment**
  - **Two proteins involved in T-cell stimulation (TLR2 and ICOS) increased in all patients**
- **60 proteins were identified as differentially expressed between daratumumab responders versus non-responders over time; these proteins include markers of tumor burden, which decrease in responders and increase in non-responders**
- **On treatment, PD-L1 expression decreased in responders and increased in non-responders compared to baseline**
  - **The relevance of these findings in the context of the observed immunomodulatory effect of daratumumab (Poster 3037) will be investigated in future studies**
- **Confirmation of these observations in a larger sample size and using other methods of detection is underway**

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DISCLOSURES

T. Casneuf, J. Bald, T. Ahmadi, and A.K. Sasser are employees of Janssen Research & Development, LLC. A. Lysaght is an employee of and holds stock in immuneering Corporation. C. LeFave is an employee of LabConnect, LLC. B. Weiss reports consultancy for Janssen and Millennium, and research funding from Janssen and Onclave. N.W.C.J. van de Donk reports research funding from Janssen Pharmaceuticals, Amgen, and Celgene. H.M. Lokhorst reports honoraria from Amgen, Janssen, and Genmab, and research funding from Janssen and Genmab.

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