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High-parameter Mass Cytometry (CyTOF®) Evaluation of Relapsed/Refractory Multiple Myeloma (MM) Patients (Pts) Treated With Daratumumab Supports Immune Modulation as a Novel Mechanism of Action

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INTRODUCTION

- Daratumumab (DARA) is a human monoclonal antibody that targets CD38 and has a direct on-tumor and immunomodulatory mechanism of action[1-4] (MOA; **Figure 1**)
- Previous assessments of flow cytometry data reflect a reduction in CD38$^+$ myeloma cells in patients with relapsed/refractory multiple myeloma (MM), as well as increased T-cell expansion and reduction in immune-regulatory populations, both of which are suggestive of immune modulation as an additional MOA[5]
- Current limitations of flow cytometry warrant incorporation of next-generation, high-parameter tools that can better visualize the dynamic components of the immune system
- Next-generation mass cytometry (CyTOF®), which allows high-parameter evaluation of immune systems, was used to assess the effects of DARA alone or in combination with a standard of care regimen (lenalidomide plus dexamethasone) on a more comprehensive profile of immune cell subpopulations

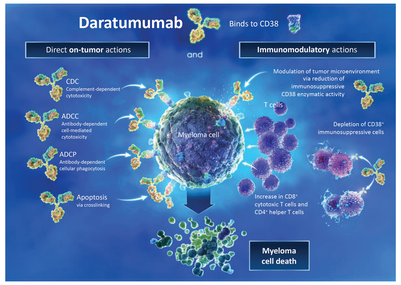


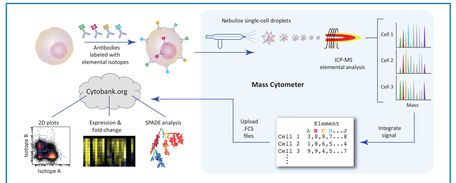
Figure 1. Mechanism of action of DARA.

OBJECTIVE

- To identify novel aspects of disease biology that contribute to efficacy and depth of response upon treatment with DARA alone or in combination with standard of care regimens

METHODS

- Relapsed/refractory MM patient samples were collected and analyzed from:
  - SIRIUS (32 patients; whole blood [WB] only, at baseline and Cycle [C] 3 Day [D] 1)[6]
  - GEN501 (5 patients; WB and bone marrow [BM] at baseline and 2 months on study and end of treatment)[7]
  - GEN503, a study of DARA in combination with lenalidomide and dexamethasone (9 patients; WB and BM at baseline and 3 months on study)[8]
- Samples were stained with a fluorochrome or metal-conjugated antibody panel and then were evaluated by flow cytometry or CyTOF® (**Figure 2**),[9] respectively
- Sufficient-quality (>10K singlet events) samples were then clustered into nodes (similar cellular events) using the spanning-tree progression of density-normalized events (SPADE) algorithm, followed by gating into immune populations via Cytobank® software
  - Stable lineage markers were exclusively used to cluster cellular events into common nodes for WB samples. For BM samples, CD138 was also included for clustering (**Table 1**)



From Bendall et al. *Science*. 2011;332(6030):687-696.
ICP-MS, inductively coupled plasma mass spectrometer; SPADE, spanning-tree progression of density-normalized events.

Figure 2. CyTOF® technology.

Table 1. CyTOF® Antibody Panel Markers

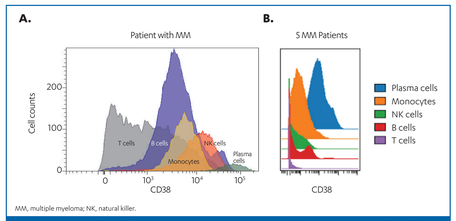
Stable markers		Transitional markers
CD45	CD66b	CD38
CD11b	CD127	BCMA
CD14	HLA-DR	CTLA4
CD3	CD45RA	OX40
CD45	CD45RO	Granzyme B
CD8	CD34	CD59
CD19	CD123	CD55
CD20	CD15	CD137
CD16	CD28	CD138 (WB)
CD56	CD25	CD69
CD1c	CD138 (BM)	VISTA
CD33		PD1

BM, bone marrow; WB, whole blood.

- Differential analysis of population fractions and marker intensity, over time and between response groups, derived raw *P* values from *t* tests; single-cell level bootstrap adjusted *P* values were corrected for multiple dependent hypothesis testing
- Results were visualized using SPADE trees and Radviz projections, a new method that allows for the comparison of populations and conditions while preserving the relation to original dimensions. Numbers (nodes) grayed out in SPADE trees were not included in the analysis due to a restricted parent-child population comparison or the existence of the empty node for 1 patient sample in the respective data set

RESULTS

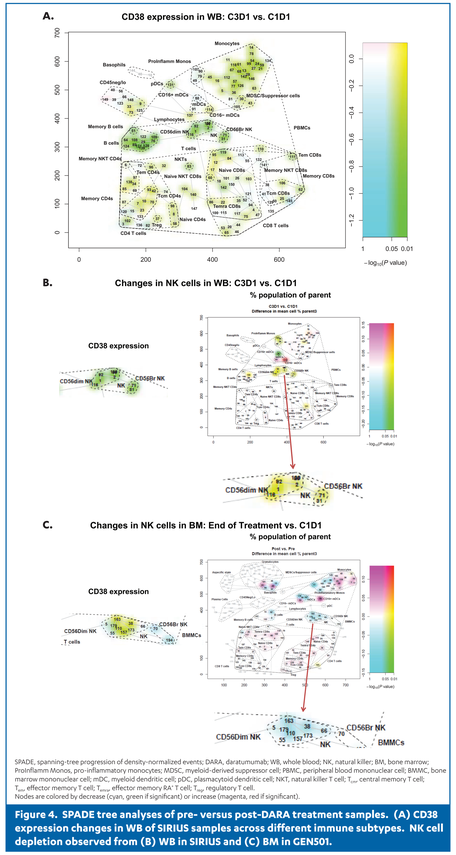
- Previous flow cytometry observations from MM bone marrow of SIRIUS and GEN501 were confirmed, including comparable CD38 marker intensity in natural killer (NK), monocyte, and B- and T-cell compartments (**Figure 3**)



MM, multiple myeloma; NK, natural killer.

Figure 3. Hierarchy of CD38 expression across immune subtypes, as assessed via flow cytometry[5] (A) and CyTOF® (B).

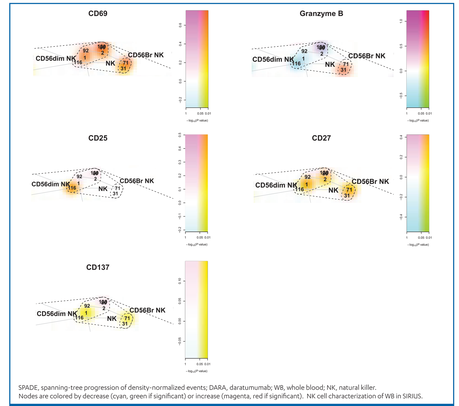
- Consistent with previous flow cytometry data from DARA-treated patients with MM, CD38 marker intensity decreased over time across many immune cell subtypes when assessed via CyTOF® in SIRIUS WB samples (**Figure 4A**)
  - Along with reduced CD38 expression, the NK cell population was depleted from WB in SIRIUS and BM in GEN501 (**Figures 4B** and **4C**)



SPADE, spanning-tree progression of density-normalized events; DARA, daratumumab; WB, whole blood; NK, natural killer; BM, bone marrow; Proinflamm Monos, pro-inflammatory monocytes; MDSC, myeloid-derived suppressor cell; PBMC, peripheral blood mononuclear cell; BMMC, bone marrow mononuclear cell; mDC, myeloid dendritic cell; pDC, plasmacytoid dendritic cell; NKT, natural killer T cell; T_{cm}, central memory T cell; T_{em}, effector memory T cell; T_{emra}, effector memory RA$^+$ T cell; T_{reg}, regulatory T cell.
Nodes are colored by decrease (cyan, green if significant) or increase (magenta, red if significant).

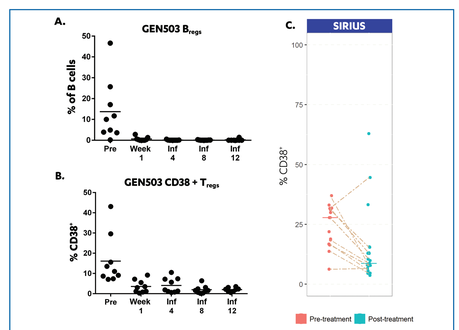
Figure 4. SPADE tree analyses of pre- versus post-DARA treatment samples. (A) CD38 expression changes in WB of SIRIUS samples across different immune subtypes. NK cell depletion observed from (B) WB in SIRIUS and (C) BM in GEN501.

- Although NK cells are depleted upon DARA treatment, in remaining NK cells, trends in decreased granzyme B and increased CD27 (suggestive of limited functionality) could be offset by increases in activation markers (CD69, CD25, and CD137; **Figure 5**). A similar observation was made in GEN501 BM
- Depletion of immune-suppressive populations in patients treated with DARA plus lenalidomide and dexamethasone is consistent with previous observations with DARA monotherapy (**Figure 6**)
  - In GEN503, both CD38$_{high}$ regulatory B cells and CD38$^+$ regulatory T cells (flow cytometry) were depleted rapidly by Week 1 of treatment and were sustained for a minimum of 2 months. CD38$^+$ regulatory T cells (CyTOF®) were also depleted in SIRIUS after 2 months of DARA treatment
- Radviz projections were used to identify drivers of T-cell phenotype changes after 2 months (C3D1 or Infusion 8) of DARA treatment. Plots of SPADE tree–defined T-cell populations reveal a shift to CD8 prevalence with granzyme B positivity that is suggestive of increased killing capacity. In both monotherapy studies, this shift was preferential in responders (**Figure 7**)



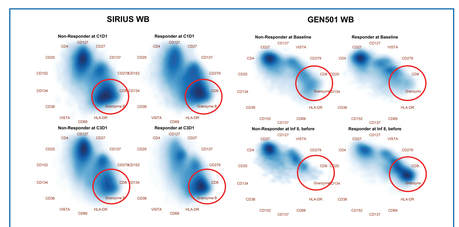
SPADE, spanning-tree progression of density-normalized events; DARA, daratumumab; WB, whole blood; NK, natural killer.
Nodes are colored by decrease (cyan, green if significant) or increase (magenta, red if significant). NK cell characterization of WB in SIRIUS.

Figure 5. SPADE tree analyses of pre- versus post-DARA WB treatment samples.



DARA, daratumumab; B_{reg}, regulatory B cell; T_{reg}, regulatory T cell; Inf, infusion.
Dotted lines indicate paired samples.

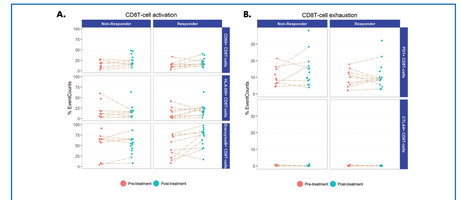
Figure 6. DARA as monotherapy or in combination with lenalidomide and dexamethasone depletes immune-suppressive populations: (A) B$_{regs}$, (B) CD38$^+$ T$_{regs}$, and (C) CyTOF® CD38$^+$ T$_{regs}$.



WB, whole blood; C, Cycle; D, day; Inf, infusion.
SIRIUS WB patients (17 nonresponders; 15 responders) and GEN501 WB patients (2 nonresponders; 2 responders).

Figure 7. T-cell population shifts to CD8 prevalence with granzyme B positivity in responders' WB from both monotherapy studies.

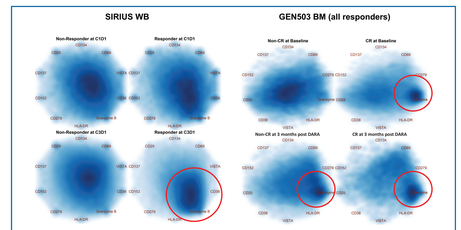
- CyTOF® manual gating that was performed on SIRIUS WB samples revealed changes in activation markers of CD38$^+$ T cells with a significant increase ($P = 0.017$, Wilcoxon) in granzyme B$^+$ production among responders (**Figure 8A**). Observed changes in markers associated with exhaustion were limited where %PD1$^+$ decreased after treatment for responders (baseline median 10.2-8.97 after treatment) and increased in nonresponders (baseline median 8.76-11.42 after treatment); however, both were nonsignificant (**Figure 8B**)



DARA, daratumumab.
Dotted lines indicate paired samples.

Figure 8. CD8 T-cell (A) activation and (B) exhaustion marker changes upon DARA treatment among SIRIUS responders and nonresponders.

- Baseline BM CD4 T-cell phenotypes (high granzyme B positivity) may contribute to the depth of response in patients who receive DARA plus lenalidomide and dexamethasone (**Figure 9**)
  - Of note, this T-cell phenotype was distinctly observed in SIRIUS WB responders after 2 months of DARA treatment



WB, whole blood; BM, bone marrow; C, Cycle; D, day; CR, complete response; DARA, daratumumab.
SIRIUS WB patients (17 nonresponders; 15 responders) and GEN503 BM patients (4 non-CRs and 2 CRs).

Figure 9. Potential predictive immune profiles.

CONCLUSIONS

- **CyTOF® analysis of patient samples from studies of DARA alone or in combination with a standard of care regimen confirm previous flow cytometry findings and corroborate DARA's immune-modulatory MOA**
- **T-cell changes in WB and BM towards a cytolytic, granzyme B$^+$ phenotype support adaptive response in patients and may contribute to depth of response**
- **Although these results are from a limited data set, they support exploration and use of these methodologies in future phase 3 studies of DARA in combination with other antimyeloma agents**

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DISCLOSURES

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