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Daratumumab in Combination With Lenalidomide Plus Dexamethasone Results in Persistent Natural Killer (NK) Cells With a Distinct Phenotype and Expansion of Effector Memory T Cells in POLLUX, a Phase 3 Randomized Study

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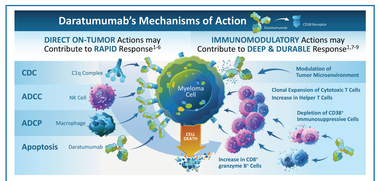
INTRODUCTION

- Daratumumab (DARA) is an anti-CD38 monoclonal antibody that is approved in the United States and Europe for the treatment of patients with relapsed/refractory multiple myeloma (RRMM)[1,2]:
  - As monotherapy in heavily pretreated multiple myeloma patients who received ≥3 prior lines of therapy, including a proteasome inhibitor and an immunomodulatory drug, or who are double refractory to these agents
  - In combination with lenalidomide and dexamethasone or bortezomib and dexamethasone for multiple myeloma patients who received ≥1 prior therapy
  - In combination with pomalidomide and dexamethasone for multiple myeloma patients who received ≥2 prior therapies, including lenalidomide and a proteasome inhibitor (United States only)
- DARA has direct on-tumor mechanisms of action that include complement-dependent cytotoxicity,[3] antibody-dependent cellular cytotoxicity (ADCC),[3] antibody-dependent cellular phagocytosis,[4] apoptosis,[3] and direct enzymatic inhibition[5] (**Figure 1**)
- DARA (16 mg/kg) single-agent, phase 1/2 translational studies (MMY2002[7] and GEN501[8]) revealed an additional, novel immunomodulatory mechanism of action that can induce lysis of myeloid-derived suppressor cells, regulatory B cells, and a subpopulation of regulatory T cells (T_{reg}s; $CD4^+CD25^+CD127^{dim}$) to promote T-cell activity[9]
- Natural killer (NK) cells were reduced in these studies with no effect on DARA efficacy or safety[10]



DARA, daratumumab; CDC, complement-dependent cytotoxicity; ADCC, antibody-dependent cellular cytotoxicity; NK, natural killer; ADCP, antibody-dependent cellular phagocytosis.

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Figure 1. DARA mechanisms of action.

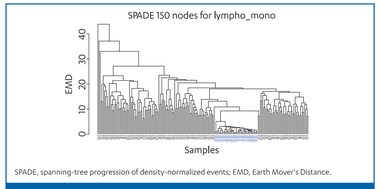
- Current limitations of flow cytometry warrant incorporation of next-generation, high-parameter, single-cell analysis platforms that allow exploration of the dynamic immune system at a greater resolution
- Mass cytometry by time-of-flight (CyTOF®) was used to assess the effects of DARA on patients in POLLUX (ClinicalTrials.gov Identifier: NCT02076009), a phase 3 study evaluating the effects of DARA in combination with a standard of care regimen (lenalidomide plus dexamethasone [Rd]) for RRMM patients, to yield a more comprehensive phenotypic and functional profile of immune cell subpopulations

OBJECTIVE

- Patient blood samples were analyzed at baseline (Cycle [C] 1 Day [D] 1) and after treatment (C3D1) with Rd or DARA plus Rd (DRd) using CyTOF® to further investigate DARA immune modulation inclusive of promoting adaptive T-cell responses and immunophenotypical changes in persisting NK cells

METHODS

- POLLUX is a multicenter, randomized (1:1), open-label, active-controlled, phase 3 study evaluating the efficacy of DRd versus Rd in patients with RRMM
- Whole blood samples from RRMM patients in POLLUX were collected:
  - At baseline (DRd, n = 40; Rd, n = 45)
  - After 2 months of therapy (DRd, n = 31; Rd, n = 33; with matched baseline sample)
- Samples were stained with a metal-conjugated antibody panel and evaluated by CyTOF®[11]
- Sufficient-quality (>10K singlet events) samples were then clustered into nodes of similar cellular events using the spanning-tree progression of density-normalized events (SPADE) algorithm,[12] followed by gating into immune populations via Cytobank® software
- Raw data were used for data distribution exploration
- Quality control of this sample set, including ordinal embedding,[13] clustering of the Earth Mover's Distance[12] of the percent of cells, and subsequent tree structure, revealed the absence of technical batch effects and the expected clustering of control samples (in blue; **Figure 2**)



SPADE, spanning-tree progression of density-normalized events; EMD, Earth Mover's Distance.

Figure 2. Dendogram illustrating the clustering of patient and control samples based on Earth Mover's Distances.

- Classic lineage markers were used exclusively to cluster cellular events into common nodes (**Table 1**)

Table 1. CyTOF® Antibody SPADE Clustering Markers

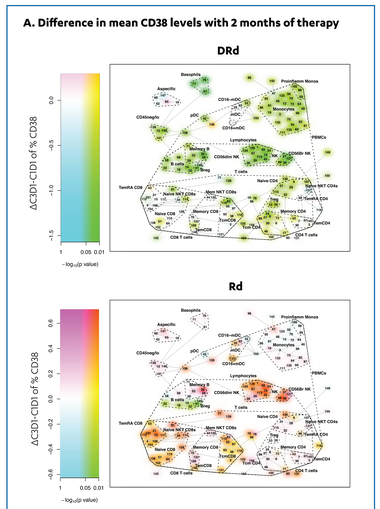
Lineage markers		Markers of interest
CD45	CD27	CD38
CD11b	CD66b	Caspase 3
CD14	CD127	CTLA4
CD3	HLA-DR	Granzyme B
CD4	CD45RA	CD57
CD8	CD45RO	CD137
CD19	CD34	CD138
CD20	CD123	CD69
CD16	CD15	VISTA
CD56	CD28	PD-1
CD11c	CD25	TIM3

CyTOF®, cytometry by time-of-flight; SPADE, spanning-tree progression of density-normalized events; HLA-DR, human leukocyte antigen-D related; CTLA4, cytotoxic T-lymphocyte–associated protein 4; VISTA, V-domain immunoglobulin suppressor of T-cell activation; PD-1, programmed death-1; TIM3, T-cell immunoglobulin mucin-3.

- Differential analysis of population fractions and marker intensity, over time and between treatment groups, derived raw *P* values from *t* tests for longitudinal samples (paired and unpaired) and baseline samples (unpaired) analyses
  - Single cell–level bootstrap adjusted *P* values were corrected for multiple dependent hypothesis testing
- Results were visualized using SPADE trees and Radviz projections,[14] 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 an empty node for 1 patient sample in the respective data set
- CyTOF® data were correlated with clonality, T-cell fraction, and richness of the T-cell repertoire data[15] of these same patients generated with immunoSEQ (Adaptive Biotechnologies, Seattle, WA)

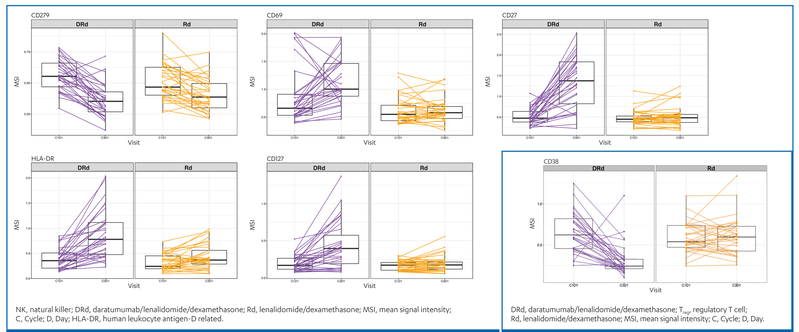
RESULTS

- Consistent with previous DARA randomized monotherapy studies,[10,16] a decrease in CD38 expression across cell types (**Figure 3A**) occurred in DRd patients after 2 months of therapy
- DRd treatment resulted in a reduction of NK cells and an increase in T cells (**Figure 3B**)
- NK cells that persisted with DRd treatment have a distinct phenotype, suggesting that these cells remain competent to elicit ADCC (**Figure 4**):
  - Decreased expression of programmed death-1 (PD-1; CD279)
  - Increased expression of human leukocyte antigen-D related (HLA-DR), CD69, CD127, and CD27
- These effects were negligible or not observed with Rd and may affect the adaptive immune response
- Consistent with observations from DARA monotherapy and combination therapy studies,[16] $CD38^+$ T_{regs}, a cell population that potently suppresses T-cell proliferation,[9] were exclusively decreased by DRd (**Figure 5**)



DRd, daratumumab/lenalidomide/dexamethasone; Rd, lenalidomide/dexamethasone; C, Cycle; D, Day; Monos, monocytes; mDC, myeloid dendritic cell; pDC, plasmacytoid dendritic cell; PBMC, peripheral blood mononuclear cell; BR, bright; NK, natural killer; B_{reg}, regulatory B cell; TemRA, effector memory $CD45RA^+$ T cell; NKT, natural killer T cell; Tem, effector memory T cell.
Note: Nodes are colored by decrease (cyan; green, if significant) or increase (magenta; red, if significant).

Figure 3. Overall DRd- and Rd-mediated changes on (A) CD38 expression on immune cell populations and (B) total immune cell populations.



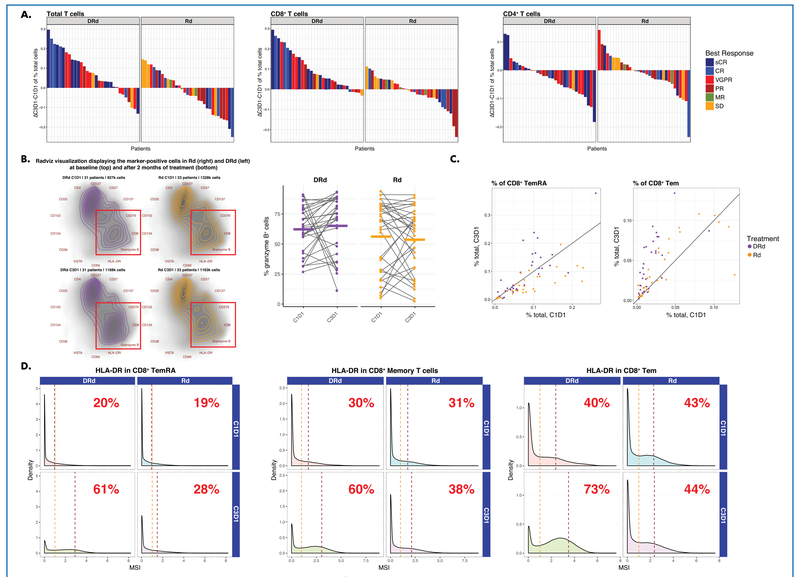
NK, natural killer; DRd, daratumumab/lenalidomide/dexamethasone; Rd, lenalidomide/dexamethasone; MSI, mean signal intensity; C, Cycle; D, Day; HLA-DR, human leukocyte antigen-D related.

Figure 4. Phenotypic changes observed in NK cells that persist upon DRd and Rd treatment.

DRd, daratumumab/lenalidomide/dexamethasone; T_{reg}, regulatory T cell; Rd, lenalidomide/dexamethasone; MSI, mean signal intensity; C, Cycle; D, Day.

Figure 5. DRd treatment reduces $CD38^+$ T_{reg}s.

- Interestingly, the proportion of T cells increased preferentially in deep responders (≈complete response) receiving DRd and correlated with a higher proportion of $CD8^+$ versus $CD4^+$ T cells (**Figure 6A**)
- $CD3^+$ T cells evaluated simultaneously for several markers of activation and exhaustion revealed a shift in composition towards $CD8^+$ granzyme B^+ T cells in response to DRd (**Figure 6B**)
  - This observation was corroborated by paired sample analysis of granzyme B in cytotoxic $CD8^+$ cells where this increase was distinct for DRd-treated patients (**Figure 6B**)
- Furthermore, DRd led to a higher proportion of effector memory T cells versus Rd (**Figure 6C**)
- In DRd-treated patients, greater increases in HLA-DR expression were observed than in Rd, particularly for effector memory $CD8^+$ T cells (**Figure 6D**)
  - Red percentages indicate the percentage of cells in the distribution above a mean signal intensity of 1

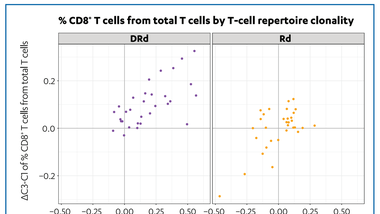


Density plots of HLA-DR expression on memory and effector memory $CD8^+$ T cells, before (top) and upon (bottom) treatment. DRd treatment (left panels) and Rd treatment (right panels). Vertical hashed orange lines are set at an MSI = 1, while vertical hashed purple lines are the MSI for 80% of the distribution. Red percentages indicate the percentage of cells in the distribution above an MSI of 1.

DRd, daratumumab/lenalidomide/dexamethasone; Rd, lenalidomide/dexamethasone; HLA-DR, human leukocyte antigen-D related; C, Cycle; D, Day; sCR, stringent complete response; CR, complete response; VGPR, very good partial response; PR, partial response; MR, minimal response; SD, stable disease; TemRA, effector memory $CD45RA^+$ T cells; Tem, effector memory T cells; MSI, mean signal intensity.

Figure 6. Effects of DRd and Rd on (A) T-cell correlation with response to treatment, (B) $CD3^+$ T cells, (C) effector memory T cells, and (D) HLA-DR expression on $CD8^+$ T cell subtypes.

- The expansion of $CD8^+$ T cells observed with CyTOF® is correlated with a clonality increase of the T-cell repertoire (**Figure 7**)



DRd, daratumumab/lenalidomide/dexamethasone; Rd, lenalidomide/dexamethasone; C, Cycle.

Figure 7. T-cell repertoire clonality.

CONCLUSIONS

- **DARA in combination with Rd specifically induced distinct and unique phenotypic changes in residual NK cells, suggesting that these cells may contribute to immune responses**
- **Of particular interest are the T-cell profile changes specifically induced by DRd, which include expansion of effector memory T cells, preferential increase in $CD8^+$ T cells in deep responders, and increased expression of activation markers**
- **The expansion of $CD8^+$ T cells observed in DRd-treated patients was correlated to an increase of clonality of their T-cell repertoire, suggesting an adaptive immune response**
- **This study supports the immunomodulatory mechanism of action of DARA and provides additional insight into changes in NK cells, T-cell subtypes, and activation status following DARA-based therapy**

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