CyTOF®: A New Tool to Decipher the Immunomodulatory Activity of Daratumumab

Journal: Cytometry Part A – Commentary

Title
CyTOF®: a new tool to decipher the immunomodulatory activity of daratumumab

Authors: [1]Barbara Castella, [2]Roberto Mina, [2]Francesca Gay

Affiliations

[1] Laboratorio di Immunologia dei Tumori del Sangue, Centro Interdipartimentale di Ricerca in Biologia Molecolare, Università degli Studi di Torino, Torino, Italy

[2] Myeloma Unit, Division of Hematology, University of Torino, Azienda Ospedaliero-Universitaria Città della Salute e della Scienza di Torino, Torino, Italy

Corresponding author: Dr. Francesca Gay, MD, PhD, Myeloma Unit, Division of Hematology, University of Torino, Azienda Ospedaliero-Universitaria Città della Salute e della Scienza di Torino, via Genova 3, 10126, Torino. Tel. 0039 0116334279. E-mail: fgay@cittadellasalute.to.it. ORCID ID: 0000-0002-8619-412X

Funding

No funding was provided for this contribution.

Conflict of Interest Disclosure

RM has received honoraria from Amgen, Celgene and Janssen. FG has received honoraria from Amgen, Bristol-Myers Squibb, Celgene, Janssen, and Takeda; has served on the advisory boards for Amgen, Bristol-Myers Squibb, Celgene, Janssen, Roche, and Takeda. BC declares no competing financial interests.

Keywords: Anti CD38, monoclonal antibody, CyTOF, Multiple Myeloma

CD38-targeted therapy is emerging as one of the most effective immunotherapies ever developed for the treatment of Multiple Myeloma (MM) (1). CD38 is a transmembrane glycoprotein that serves as a receptor with a multifunctional ecto-enzymatic activity, which is highly expressed on plasma-cells, but also on several other cells belonging to the hemopoietic as well as to the non-hemopoietic tissues (2).

Daratumumab, a fully human IgG1-kappa monoclonal antibody, is the first-in-class antibody targeting CD38. It has rapidly become a cornerstone of the anti-MM treatment, showing activity both as single agent in heavily pre-treated MM patients and in combination with other agents.

To date, daratumumab has been approved for newly diagnosed MM patients not eligible for autologous transplant in combination with bortezomib-melphalan-prednisone (D-VMP), and for relapsed/refractory MM patients as monotherapy or in combination with lenalidomide-dexamethasone (D-Rd), bortezomib-dexamethasone (D-Vd) (in the European Union), and pomalidomide-dexamethasone (D-Pd) (in the United States). Despite the great efficacy displayed by daratumumab, all responding patients ultimately acquire resistance to the drug and progress while on therapy; a better knowledge of its mechanisms of action and of the various mechanisms of resistance is essential for optimal development and use of these treatment options.

CD38-targeted therapy represents a unique opportunity to concurrently impact on tumor cells and tumor microenvironment (2), since CD38 is expressed on myeloma cells, immune effector cells [natural killer (NK) cells, cytotoxic T cells, B cells, Vγ9Vδ2 T cells] and inhibitory cells, such as regulatory T cells (Tregs), regulatory B cells (Bregs) and myeloid-derived suppressor cells (MDSCs). Daratumumab exploits classic Fc-dependent immune effector mechanisms, including antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), complement-dependent cytotoxicity (CDC), and apoptosis upon secondary cross-linking, which are dependent on CD38 expression on the tumor cells (3).

Since CD38 is expressed on various immune cell populations, the potential immunomodulatory effects of daratumumab monotherapy in RRMM patients have recently been investigated, showing a multifaceted immunomodulatory role for daratumumab through the eradication of CD38-positive immune suppressor cells (e.g. Tregs, Bregs and MDSCs) (4). The disappearance of an immunosuppressive contexture results in CD4+ and CD8+ T cell expansion, alloreactive functional T-cell responses, increased T-cell clonality, and in a potentially better host's anti-tumor immune response. Moreover, it has been shown that daratumumab may also reduce adenosine production in the bone marrow microenvironment through the modulation of CD38 enzymatic activity both on tumor cells and on cytotoxic T cells, thus promoting their antitumor activity (5).

The immunomodulatory functions of daratumumab contributed to elucidate the significant clinical benefits and enhanced survival observed. However, given the expected increasing use of daratumumab in early lines of therapy, a deeper and extensive analysis of its effects on frequency and functional role of additional immune cell populations is urgently needed in order to identify possible resistance mechanisms. All the available reports about the impact of daratumumab treatment on immune CD38+ cells were based on the use of flow cytometry tools, which strongly limited the number of immune cell subpopulations that could be investigated at once.

In the paper recently published in «Cytometry Part A» (6), Adams et al. reported how they extended and complemented their previous findings (4, 7, 8) using the Cytometry by Time of Flight (CyTOF®), a novel platform for high-dimensional phenotypic and functional analysis of single cells. CyTOF® is a next-generation flow cytometry platform with several technological advances, as compared to the conventional fluorescence-based flow cytometry (9, 10). The CyTOF® system does not depend on the detection of fluorescence, which requires compensation for spillover into adjacent channels, but on the use of elemental metal isotopes conjugated to monoclonal antibodies, thus identifying discrete isotope peaks without substantial overlap. Moreover, the platform allows for the detection of 42+ unique parameters rather than the 8–12 parameters included in a standard flow cytometry panel, and it can be customized for the analysis of both phenotypic and functional markers. By taking advantage of the resolution, sensitivity and dynamic range of mass spectrometry on a time-scale that allows the

measurement of 1000 individual cells per second, this configuration offers a new and valuable approach to high-content cytometric analysis.

Adams et al. identified new modifications in immune cell profile that correlated with the efficacy and depth of response observed during treatment with daratumumab in RRMM patients. As already demonstrated by their previous flow cytometric analyses, they pointed out a decreased expression of CD38 on several immune cells (plasma cells, NK cells, monocytes, B cells, and T cells) in daratumumab-treated patients. Although NK cells disappeared after daratumumab treatment, the remaining NK cells displayed an activated phenotype both in peripheral blood and in bone marrow, increasing CD69, CD127, CD25, CD27, and CD137 expression and decreasing CD45RA and granzyme B expression. These new observations corroborated their earlier findings (8) according to which, although NK cells were reduced after daratumumab treatment, they were not completely depleted and could still contribute to ADCC, clinical efficacy, and infections control. Recently, Wang and colleagues (11) discovered a fratricide mechanism for daratumumab-mediated NK-cell depletion and provided a potential therapeutic strategy to overcome this side effect in daratumumab-treated MM patients. It would be fascinating to pursue – through the analysis of combinatorial expression of multiple phenotypic markers with the CyTOF® platform – the influence of daratumumab treatment on diverse functional properties of NK cells, thus discerning between immunoregulatory NK cells ($CD56^{bright}/CD16^{dim/neg}$) and cytotoxic NK cells ($CD56^{dim}/CD16^{bright}$). Daratumumab treatment could affect the immunoregulatory NK-cell subset reducing its number, but could also promote the cytotoxic NK-cell compartment by increasing its CD107a and CD69 expression and IFNγ secretion. Accordingly, the best attractive partners to combine with daratumumab are immunomodulatory drugs (IMiDs), since they are able to enhance or prolong the functionality of cytotoxic NK cells.

Moreover, Adams et al. observed a reduced percentage of CD38+ basophils and a shift toward an increased killing ability of the cytotoxic T cells after daratumumab monotherapy treatment, thus further confirming the previously reported daratumumab-mediated depletion of immune-suppressive populations.

These results highlighted the importance of CyTOF® and related methodologies in obtaining a deeper understanding of the mechanism of action of daratumumab as monotherapy and in combination with other agents.

At once, the CyTOF® platform could be very useful to identify cellular mediators of resistance to anti-CD38 therapy and to define potential mechanisms to restore sensitivity to daratumumab. Several factors contributing to the development of resistance to the immunomodulatory activities of daratumumab in MM patients have been already elucidated, such as the expression levels of complement inhibitory proteins CD55 and CD59 on MM cells (7) and immunogenetic factors involved in the effector cell function (e.g. the Fcγ receptor polymorphisms) (12).

The compensatory upregulation of multiple inhibitory immune checkpoints (ICP/ICP-L) can also promote the resistance to the immunomodulatory activities of CD38 antibodies. The concurrent expression of a wide array of ICP/ICP-L and CD38 on the same cells in the tumor microenvironment strongly suggests a cross-talk between these molecules and their downstream signaling pathways. Interestingly, the existence of such a cross-talk has recently been reported in NSCLC and melanoma mouse models (13). Preclinical results suggested that CD38 upregulation on tumor cells could represent an escape mechanism from the infiltrating cytotoxic T cells induced by anti-PD-L1/PD-1 therapy. The combination of anti-CD38 and anti-PD-L1 proved to be more effective than either monotherapy alone in suppressing primary tumor growth and metastases in these tumor models. Studies in syngeneic mouse models (colon adenocarcinoma, plasmacytoma and lung carcinoma) and *in vitro* assays confirmed that the combination of anti-CD38 and anti-PD-1 treatments can be more effective than targeting either pathway alone.

These preclinical premises suggested the development of clinical trials evaluating whether the anti-MM activity of CD38 antibodies could be enhanced with PD-1 or PD-L1 inhibitors.

Moreover, the upregulation of inhibitory receptors (PD-1, TIM-3, LAG-3, TIGIT) on effector cells could also involve daratumumab-resistant patients, since they are markers associated with a

functional exhausted phenotype. Some preliminary observations derived from the recent study by Adams et al. revealed that the percentage of PD1+CD8+ T cells decreased from baseline in daratumumab-responder patients but increased in non-responders, suggesting that the checkpoint receptor expression profile on effector cells could influence the clinical efficacy of daratumumab treatment and/or represent a remarkable mechanism of resistance. A systematic investigation of the peripheral blood and bone marrow "immunome" (immune cell profile) of daratumumab-treated MM patients could reveal an expansion of exhausted T cells with upregulation of the checkpoint inhibitors in resistant patients, suggesting the exploration of ICP-blocking strategies (anti-TIM3, anti-LAG-3, anti-TIGIT) as potential approaches to restore sensitivity to daratumumab.
A more effective use of daratumumab in the treatment of MM patients will likely depend on a deeper knowledge of the significant changes in the immune system and of the tumor-related features derived from daratumumab exposure, including its modulation of the immune system and the tumor-acquired mechanisms of resistance.

LITERATURE CITED

1. Lokhorst HM, Plesner T, Laubach JP, Nahi H, Gimsing P, Hansson M, Minnema MC, Lassen U, Krejcik J, Palumbo A, van de Donk NW, Ahmadi T, Khan I, Uhlar CM, Wang J, Sasser AK, Losic N, Lisby S, Basse L, Brun N, Richardson PG. Targeting CD38 with Daratumumab Monotherapy in Multiple Myeloma. N Engl J Med. 2015;373(13):1207-1219. doi: 10.1056/NEJMoa1506348
2. van de Donk NWCJ, Richardson PG, Malavasi F. CD38 antibodies in multiple myeloma: back to the future. Blood. 2018;131(1):13-29. doi: 10.1182/blood-2017-06-740944
3. van de Donk NWCJ, Usmani SZ. CD38 Antibodies in Multiple Myeloma: Mechanisms of Action and Modes of Resistance. Front Immunol. 2018;9:2134. doi: 10.3389/fimmu.2018.02134
4. Krejcik J, Casneuf T, Nijhof IS, Verbist B, Bald J, Plesner T, Syed K, Liu K, van de Donk NW, Weiss BM, Ahmadi T, Lokhorst HM, Mutis T, Sasser AK. Daratumumab depletes CD38+ immuneregulatory cells, promotes T-cell expansion, and skews T-cell repertoire in multiple myeloma. Blood 2016;128(3):384-394. doi: 10.1182/blood-2015-12-687749
5. Horenstein AL, Chillemi A, Quarona V, Zito A, Roato I, Morandi F, Marimpietri D, Bolzoni M, Toscani D, Oldham RJ, Cuccioloni M, Sasser AK, Pistoia V, Giuliani N, Malavasi F. NAD^+-Metabolizing Ectoenzymes in Remodeling Tumor-Host Interactions: The Human Myeloma Model. Cells 2015;4(3):520-537. doi: 10.3390/cells4030520
6. Adams HC 3rd, Stevenaert F, Krejcik J, Van der Borght K, Smets T, Bald J, Abraham Y, Ceulemans H, Chiu C, Vanhoof G, Usmani SZ, Plesner T, Lonial S, Nijhof I, Lokhorst HM, Mutis T, van de Donk NWCJ, Sasser AK, Casneuf T. High-Parameter Mass Cytometry Evaluation of Relapsed/Refractory Multiple Myeloma Patients Treated with Daratumumab Demonstrates Immune Modulation as a Novel Mechanism of Action. Cytometry Part A 2018; Dec 11 [Epub ahead of print]. doi: 10.1002/cyto.a.23693.
7. Nijhof IS, Casneuf T, van Velzen JF, van Kessel B, Axel AE, Syed K, Groen RW, van Duin M, Sonneveld P, Minnema MC, Zweegman S, Chiu C, Bloem AC, Mutis T, Lokhorst HM, Sasser AK, van de Donk NW. CD38 expression and complement inhibitors affect response and resistance to daratumumab therapy in myeloma. Blood 2016;128:959-970. doi: 10.1182/blood-2016-03-703439
8. Casneuf T, Xu XS, Adams HC 3rd, Axel AE, Chiu C, Khan I, Ahmadi T, Yan X, Lonial S, Plesner T, Lokhorst HM, van de Donk NWCJ, Clemens PL, Sasser AK. Effects of daratumumab on natural killer cells and impact on clinical outcomes in relapsed refractory

multiple myeloma. Blood Adv. 2017;1(23):2105-2114. doi: 10.1182/bloodadvances.2017006866
9. Bendall S, Simonds E, Qiu P, Amir el-AD, Krutzik PO, Finck R, Bruggner RV, Melamed R, Trejo A, Ornatsky OI, Balderas RS, Plevritis SK, Sachs K, Pe'er D, Tanner SD, Nolan GP. Single-cell mass cytometry of differential immune and drug responses across a human hematopoietic continuum. Science 2011;332(6030):687-696. doi: 10.1126/science.1198704
10. Spitzer MH, Nolan GP. Mass Cytometry: Single Cells, Many Features. Cell 2016;165(4): 780–791. doi: 10.1016/j.cell.2016.04.019
11. Wang Y, Zhang Y, Hughes T, Zhang J, Caligiuri MA, Benson DM, Yu J. Fratricide of NK Cells in Daratumumab Therapy for Multiple Myeloma Overcome by *Ex Vivo*-Expanded Autologous NK Cells. Clin Cancer Res. 2018;24(16):4006-4017. doi: 10.1158/1078-0432.CCR-17-3117
12. van de Donk NWCJ, Casneuf T, Di Cara A, Parren PW, Zweegman S, van Kessel B, Lokhorst HM, Usmani SZ, Lonial S, Richardson PG, Chiu C, Mutis T, Nijhof IS, Sasser AK. Impact of Fc gamma receptor polymorphisms on efficacy and safety of daratumumab in relapsed/refractory multiple myeloma. Br J Haematol. 2019;184(3):475-479. doi: 10.1111/bjh.15122
13. Chen L, Diao L, Yang Y, Yi X, Rodriguez BL, Li Y, Villalobos PA, Cascone T, Liu X, Tan L, Lorenzi PL, Huang A, Zhao Q, Peng D, Fradette JJ, Peng DH, Ungewiss C, Roybal J, Tong P, Oba J, Skoulidis F, Peng W, Carter BW, Gay CM, Fan Y, Class CA, Zhu J, Rodriguez-Canales J, Kawakami M, Byers LA, Woodman SE, Papadimitrakopoulou VA, Dmitrovsky E, Wang J, Ullrich SE, Wistuba II, Heymach JV, Qin FX, Gibbons DL. CD38-Mediated Immunosuppression as a Mechanism of Tumor Cell Escape from PD-1/PD-L1 Blockade. Cancer Discov. 2018;8(9):1156-1175. doi: 10.1158/2159-8290.CD-17-1033