



University of Southern Denmark

Monitoring multiple myeloma patients treated with daratumumab

teasing out monoclonal antibody interference

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Published in:

Clinical Chemistry and Laboratory Medicine

DOI:

[10.1515/cclm-2015-1031](https://doi.org/10.1515/cclm-2015-1031)

Publication date:

2016

Document version

Final published version

Citation for pulished version (APA):

McCudden, C., Axel, A. E., Slaets, D., Dejoie, T., Clemens, P. L., Frans, S., Bald, J., Plesner, T., Jacobs, J. F. M., van de Donk, N. W. C. J., Moreau, P., Schechter, J. M., Ahmadi, T., & Sasser, A. K. (2016). Monitoring multiple myeloma patients treated with daratumumab: teasing out monoclonal antibody interference. *Clinical Chemistry and Laboratory Medicine*, 54(6), 1095-1104. <https://doi.org/10.1515/cclm-2015-1031>

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Monitoring multiple myeloma patients treated with daratumumab: teasing out monoclonal antibody interference

DOI 10.1515/cclm-2015-1031

Received October 21, 2015; accepted February 10, 2016; previously published online March 30, 2016

Abstract

Background: Monoclonal antibodies are promising anti-myeloma treatments. As immunoglobulins, monoclonal antibodies have the potential to be identified by serum protein electrophoresis (SPE) and immunofixation electrophoresis (IFE). Therapeutic antibody interference with standard clinical SPE and IFE can confound the use of these tests for response assessment in clinical trials and disease monitoring.

Methods: To discriminate between endogenous myeloma protein and daratumumab, a daratumumab-specific immunofixation electrophoresis reflex assay (DIRA) was

developed using a mouse anti-daratumumab antibody. To evaluate whether anti-daratumumab bound to and shifted the migration pattern of daratumumab, it was spiked into daratumumab-containing serum and resolved by IFE/SPE. The presence (DIRA positive) or absence (DIRA negative) of residual M-protein in daratumumab-treated patient samples was evaluated using predetermined assessment criteria. DIRA was evaluated for specificity, limit of sensitivity, and reproducibility.

Results: In all of the tested samples, DIRA distinguished between daratumumab and residual M-protein in commercial serum samples spiked with daratumumab and in daratumumab-treated patient samples. The DIRA limit of sensitivity was 0.2 g/L daratumumab, using spiking experiments. Results from DIRA were reproducible over multiple days, operators, and assays. The anti-daratumumab antibody was highly specific for daratumumab and did not shift endogenous M-protein.

Conclusions: As the treatment of myeloma evolves to incorporate novel monoclonal antibodies, additional solutions will be needed for clinical monitoring of patient responses to therapeutic regimens. In the interim, assays such as DIRA can inform clinical outcomes by distinguishing daratumumab from endogenous M-protein by IFE.

Keywords: complete response; daratumumab; immunofixation electrophoresis; monoclonal antibody; multiple myeloma.

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Introduction

Multiple myeloma (MM) is an incurable disease characterized by the presence of malignant plasma cells that secrete high levels of a monoclonal immunoglobulin protein (M-protein) [1, 2]. The International Myeloma Working Group (IMWG) has established criteria for clinical response

to treatment in MM, which include changes in serum/urine M-protein levels by serum protein electrophoresis (SPE) and immunofixation electrophoresis (IFE), percentage of bone marrow plasma cells, and free light chain (FLC) ratios [3–5]. For a patient to be classified as having a complete response (CR) by IMWG criteria, the serum and urine must be negative for M-protein, as determined by IFE and SPE, and bone marrow plasma cells must be $\leq 5\%$. In serum FLC-only patients, CR is defined as a normal FLC ratio in addition to the other criteria required to classify a CR [4]. For the more robust, deeper classification of stringent complete response (sCR), all of the criteria for CR must be met, along with a normal FLC ratio and absence of clonal plasma cells in the bone marrow, as measured by 2- to 4-color flow cytometry or immunohistochemistry.

The treatment of MM is evolving with the introduction of therapeutic monoclonal antibodies (mAbs) [6–8]. Since SPE and IFE are used to quantify and characterize the clonal nature of immunoglobulins, respectively, these assays are subject to interference from therapeutic mAbs [9, 10]. Experiments with spiked samples demonstrated that all mAbs evaluated could be detected by SPE and IFE, down to 0.1 g/L [10]. Interference on serum IFE from treated patients has been reported with several mAbs, including siltuximab, ofatumumab, and daratumumab [1, 9, 10], and similar interference has been observed with elotuzumab [7, 11]. The IMWG criteria for achieving CR specify no detectable M-protein by IFE and SPE [3]; thus, antibody interference can have a clinically important impact on the assessment of response to treatment and may result in underestimation of CR rates for mAb therapies. As therapeutic mAbs become utilized in myeloma, methods are needed to assess clinical responses, particularly CR/sCR, in light of this potential interference.

Daratumumab, a human IgG1 κ mAb, binds with high affinity to a unique CD38 epitope, inducing tumor cell death through a variety of mechanisms, including complement-dependent cytotoxicity, antibody-dependent cell-mediated cytotoxicity, antibody-dependent cellular phagocytosis, and induction of apoptosis [12–15]. Additionally, subpopulations of regulatory T cells, regulatory B cells, and myeloid-derived suppressor cells with high CD38 expression are sensitive to daratumumab [16]. Cytotoxic T cell activation, expansion, and increased T cell clonality have been observed after monotherapy treatment in relapsed or refractory disease, suggesting a possible immunomodulatory role for daratumumab in MM [16].

In GEN501, a phase 1/2 study of patients with relapsed or refractory MM, daratumumab monotherapy was well tolerated, and 36% of patients receiving daratumumab at 16 mg/kg achieved at least a partial response (PR) or better

[6]. SIRIUS, a phase 2 study, examined daratumumab in patients with at least three lines of prior therapy or double refractory MM [8]. Overall response rate (ORR) was 29% and responses deepened with continued treatment; median overall survival was 17.5 months (95% confidence interval, 13.7–not estimable) in these heavily pretreated patients (median of 5 prior lines of treatment) [8]. On the basis of these studies, daratumumab was recently approved in the United States for the treatment of patients with MM who have received 3 or more lines of prior therapy including a proteasome inhibitor (PI) and immunomodulatory drug (IMiD), or are double refractory to a PI and an IMiD [17]. Daratumumab is also being investigated in phase 3 clinical studies in combination with other therapeutic agents in patients with MM.

At the recommended dosing schedule (16 mg/kg weekly for 8 weeks, then every 2 weeks for 16 weeks, and every 4 weeks thereafter), daratumumab reaches peak serum concentrations of approximately 915 $\mu\text{g/mL}$ (0.915 g/L) at the end of the weekly dosing period [18], making it readily detectable on most SPE/IFE assays [1]. As a human IgG κ immunoglobulin, daratumumab may be detected by IFE and can thus be misinterpreted as a myeloma-associated M-protein, thereby interfering with the response criteria [19].

To help distinguish daratumumab from endogenous M-protein in serum IFE, the daratumumab-specific immunofixation electrophoresis reflex assay (DIRA) was developed to confirm suspected daratumumab interference and to allow separation of daratumumab bands from residual endogenous M-protein. DIRA relies on the use of an anti-daratumumab antibody that binds daratumumab and alters its migration on IFE. The present study describes the validation of DIRA for clinical trial testing, which included determination of the assay's limit of sensitivity, specificity, and reproducibility. This assay is currently being utilized in clinical trials to distinguish daratumumab from endogenous M-protein by IFE and has triggered additional clinical response assessments to confirm CRs in myeloma patients treated with daratumumab.

Materials and methods

Serum sample collection

Human serum samples from patients with MM or healthy donors were acquired from a commercial source (Bioreclamation, Westbury, NY, USA) or from daratumumab-treated patients ($n=33$). Serum samples from clinical trials of daratumumab as monotherapy (GEN501 and SIRIUS) or as combination therapy with lenalidomide in an

ongoing study (GEN503; ClinicalTrials.gov Identifier: NCT01615029) were collected in 2.5 or 8.5 mL serum separator tubes (Becton Dickinson, Franklin Lakes, NJ, USA) and centrifuged at 1300–2000×g for 10–15 min, after 30 min at room temperature, to allow complete blood clotting/cooling. Serum samples were collected and shipped (frozen) to a central laboratory (BARC, Ghent, Belgium) for SPE and IFE or subsequent DIRA testing. Patients with low-level (<5 g/L) or negative SPE but repeated positive IgGκ IFE were flagged as having potential daratumumab interference, and were utilized for validation and DIRA testing. Samples were based on suspected interference rather than predefined time points. Clinical trials were approved by the independent Institutional Review Boards at study sites in accordance with the Declaration of Helsinki and consistent with Good Clinical Practices. All patients provided written informed consent.

Anti-daratumumab antibody

A murine anti-daratumumab antibody clone (5–3–9–4) (Johnson & Johnson, New Brunswick, NJ, USA) was produced from a hybridoma cell line (Genmab, Utrecht, The Netherlands). Supernatants from cultured cells were concentrated using tangential flow filtration (Millipore, Billerica, MA, USA), purified by MabSelect-Sure (GE Healthcare, Marlborough, MA, USA), and dialyzed into Dulbecco's phosphate-buffered saline, pH 7.2 (Life Technologies, Grand Island, NY, USA).

IFE and SPE

Immunofixations were performed on semi-automatic Hydrasys or Hydrasys 2 using Maxikit Hydragel 4IF or 9IF (Sebia, Norcross, GA, USA). SPE was performed on Capillarys using the Capillarys Protein 6 kit (both from Sebia). Both IFE and SPE were performed according to the manufacturer's specifications.

DIRA

For DIRA, anti-daratumumab or saline was spiked into baseline or daratumumab-treated patient serum, incubated at room temperature for 15 min, and separated by electrophoresis according to the standard IFE methods described previously. One lane of each baseline and daratumumab-treated patient serum was fixed as a reference and anti-human, anti IgG, or κ (Sebia) antisera were applied to detect heavy and light chains. Upon completion of electrophoresis and staining, gels were assessed for (1) migration of control daratumumab with anti-daratumumab, (2) lack of migration of baseline M-protein with the addition of anti-daratumumab, (3) a shift in the migration pattern of the putative daratumumab band relative to the daratumumab control in daratumumab-treated serum samples, and (4) the presence or absence of a non-daratumumab M-protein band. The absence of remaining disease M-protein was defined as a DIRA-negative result. The presence of remaining disease M-protein qualified as a DIRA-positive result.

Limit of sensitivity

Ten commercial MM samples were spiked with 0.25, 0.5, and 1.0 g/L daratumumab with and without anti-daratumumab in a 1:1 ratio to

determine the effectiveness and reproducibility of anti-daratumumab to shift daratumumab bands. Ten additional MM serum samples and 10 normal human serum (NHS) samples were spiked with a wider range of clinically relevant concentrations of daratumumab (0, 0.1, 0.2, 0.25, and 0.5 g/L) with and without anti-daratumumab in a 1:1 ratio. Two independent reviewers evaluated the results.

The limit of sensitivity was defined as the lowest level of daratumumab detectable by at least one parameter (daratumumab IgG, daratumumab + anti-daratumumab complex IgG, daratumumab κ, or daratumumab + anti-daratumumab κ by IFE; daratumumab or daratumumab + anti-daratumumab by SPE) in all samples tested.

Specificity

To demonstrate that the anti-daratumumab antibody did not shift endogenous M-protein migration, commercially available serum samples from patients with MM (n=51) were spiked with daratumumab, anti-daratumumab, or daratumumab + anti-daratumumab (0.5 g/L and 1 g/L; 1:1 ratio) and were analyzed by IFE. Additionally, a subset (n=35) evaluated fixed concentrations of 1 g/L anti-daratumumab and 0.5 g/L daratumumab. Gels were assessed by determining whether there was a shift in daratumumab, with no corresponding shift in M-protein with anti-daratumumab alone. In addition, in each DIRA assay, control serum samples from patients prior to treatment with daratumumab were spiked with anti-daratumumab and evaluated for a shift of endogenous M-protein on IFE.

Reproducibility

Three independent runs of 10 commercial samples spiked with 0.25, 0.5, and 1 g/L daratumumab and 10 samples from daratumumab-treated patients with M-protein ≤5 g/L, by SPE, were performed using DIRA. The results were assessed for reproducibility by two independent reviewers. The reviewers' evaluations were standardized using predefined assessment criteria. These criteria, as well as the reviewers' responses to a single sample, are shown in Table 1. Inter-day and inter-operator reproducibility was evaluated using three commercial MM samples on three separate days by two operators, and interpreted by two independent reviewers.

Results

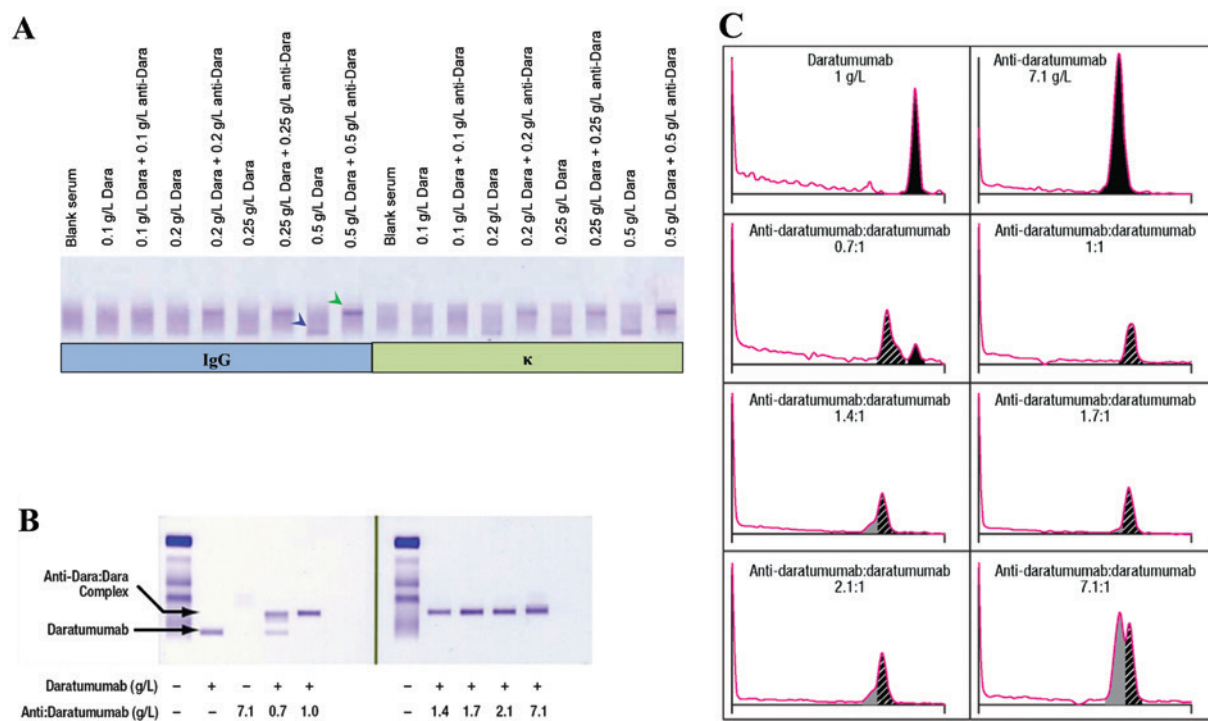
Daratumumab can be shifted with anti-daratumumab

To determine whether a shift in daratumumab could be detected by SPE and IFE, spiking experiments were performed, with varying concentrations of daratumumab with or without anti-daratumumab added to myeloma serum or NHS and analyzed by SPE or IFE. Daratumumab was effectively detected and shifted with anti-daratumumab in all samples tested. (Figure 1A and data not shown). To evaluate the amount of anti-daratumumab needed to

Table 1: Concordance of reviewer assessments of the same sample across multiple experiments based on predefined acceptance criteria.

	Lane	Run 1	Run 2	Run 3
Reviewer 1				
Migration of Dara + anti-Dara in control?	4 vs. 3	Y	Y	Y
Migration of endogenous M-protein at baseline?	6 and 10	N	N	N
Migration of Dara in \geq PR due to the disappearance of Dara (DD) or the appearance of Dara + anti-Dara complex (AC)?	8 vs. 7 and 12 vs. 11	Y	Y	Y
Presence of M-protein after migration of Dara?	8 and 12	N	N	N
M-protein (M) or Dara (D)?		D	D	D
Conclusion		Negative	Negative	Negative
Reviewer 2				
Migration of Dara + anti-Dara in control?	4 vs. 3	Y	Y	Y
Migration of endogenous M-protein at baseline?	6 and 10	N	N	N
Migration of Dara in \geq PR due to the disappearance of Dara (DD) or the appearance of Dara + anti-Dara complex (AC)?	8 vs. 7 and 12 vs. 11	Y	Y	Y
Presence of M-protein after migration of Dara?	8 and 12	N	N	N
M-protein (M) or Dara (D)?		D	D	D
Conclusion		Negative	Negative	Negative

Dara, daratumumab; Y, yes; N, no; PR, partial response.

**Figure 1:** Daratumumab can be identified on IFE/SPE and can be shifted with anti-daratumumab.

Daratumumab can be detected by IFE (A), anti-daratumumab antibody can bind and shift daratumumab migration pattern on IFE (B); 1:1 ratios of daratumumab:anti-daratumumab are enough to completely shift daratumumab on IFE. Similarly, on SPE, a 1:1 ratio of daratumumab:anti-daratumumab was able to completely shift daratumumab (C). Daratumumab and daratumumab:anti-daratumumab complex are indicated by the blue and green arrows, respectively. IFE, immunofixation electrophoresis; SPE, serum protein electrophoresis; Dara, daratumumab.

completely shift daratumumab on IFE and SPE, varying ratios of anti-daratumumab were spiked into serum containing 1 g/L daratumumab, the maximum predicted

concentration in patient serum after weekly dosing [15]. A 1:1 ratio of daratumumab:anti-daratumumab or excess anti-daratumumab completely shifted daratumumab on

IFE (Figure 1B). Excess mouse anti-daratumumab was not detected by human antiserum. Densitometry of SPE lanes showed a 1:1 ratio of daratumumab:anti-daratumumab was necessary to completely shift daratumumab; excess daratumumab or anti-daratumumab were also visualized as protein peaks since SPE does not discern total protein from human or mouse antibodies (Figure 1C). Because of its specificity for human antibody and its greater sensitivity, IFE was used to develop DIRA.

DIRA distinguishes daratumumab from endogenous M-protein

Among patients enrolled in daratumumab clinical studies, a residual IgG κ band or a faint IgG κ band that appeared over time was often observed on IFE. Daratumumab interference was suspected and had the potential to mask CRs. DIRA was developed to distinguish daratumumab from endogenous M-protein in patients with low measurable M-protein by SPE (≤ 5 g/L) and IgG κ band by IFE. Exploratory analyses utilized samples with a higher range of SPE to help refine criteria for implementation and validation of the assay. Figure 2 shows a schematic that outlines the samples, controls, and loading in a typical DIRA.

DIRA evaluates patient samples prior to (baseline) and after treatment when daratumumab interference is suspected. DIRA requires 12 sample lanes and uses a protein fixative and 2 antisera (IgG and κ ; Figure 2A). Lanes 1 and 2 comprise baseline and post-treatment samples with total protein fixative and display the migration patterns of all serum proteins at baseline and post-treatment. Lanes 3 and 4 are controls containing daratumumab and daratumumab + anti-daratumumab in saline, respectively. Lanes 5 and 6 (with anti-IgG antisera) include the baseline sample alone and with anti-daratumumab, respectively, to characterize endogenous M-protein migration and to demonstrate that anti-daratumumab alone has no effect on endogenous M-protein. Lanes 7 and 8 (with anti-IgG antisera) include the post-treatment sample alone and with anti-daratumumab, respectively, to characterize daratumumab and to determine whether disease M-protein remains. If the entire remaining band shifts with the addition of anti-daratumumab, indicating that endogenous M-protein is absent and that only daratumumab remains, the result is determined to be DIRA negative (similar to a standard IFE-negative result; Figure 2B). If the band only shifts partially, indicating that endogenous M-protein remains, the result is determined to be DIRA positive (similar to a typical IFE-positive result; Figure 2B).

Lanes 9 through 12 contain the same samples as lanes 5 through 8, but are probed with anti- κ antisera.

Validation of DIRA

For clinical validation, the sensitivity, specificity, and reproducibility of DIRA, in both commercial and daratumumab-treated myeloma serum samples, were evaluated. Sensitivity was determined by evaluating 10 myeloma and 10 NHS samples spiked with a range of daratumumab \pm anti-daratumumab by SPE and IFE. Due to the potential for daratumumab or the daratumumab-anti-daratumumab complex to comigrate with M-protein with either IgG or κ antisera, sensitivity was defined by detection by at least one parameter (daratumumab or daratumumab + anti-daratumumab complex with IgG or κ by IFE; daratumumab or daratumumab + anti-daratumumab complex by SPE). The sensitivity per sample was defined by the lowest level of daratumumab that could be detected by any of these parameters. In myeloma serum samples, the sensitivity of DIRA was determined to be 90% for 0.1 g/L daratumumab and 100% for 0.2 g/L by IFE. In NHS, the sensitivity of DIRA by IFE was 80% for 0.1 g/L daratumumab and 100% for 0.2 g/L. By SPE, sensitivity was determined to be 30% for 0.1 g/L and 100% for 0.2 g/L in MM serum and 100% at 0.2 g/L in NHS. Therefore, the sensitivity of DIRA for daratumumab is ~ 0.2 g/L. Typically MM patients are immunosuppressed, such that background polyclonal interference has not been an issue to date. In spiked NHS samples, it was not possible to consistently identify residual daratumumab below 0.2 g/L. While IFE and thus DIRA is not a quantitative assay, determining the lower range of sensitivity demonstrated daratumumab can be detected and DIRA is functional within the range of predicted serum concentrations in treated patients.

The specificity of DIRA relies on the specificity of the anti-daratumumab. Accordingly, DIRA includes control lanes containing baseline serum samples that have been spiked with or without anti-daratumumab (Figure 2, Lanes 5 and 6). In commercial samples spiked with 0.5 or 1 g/L daratumumab, the antibody was shifted by anti-daratumumab at both concentrations in all samples (51 of 51 [100%]). No shift in M-protein occurred with the addition of anti-daratumumab alone in any of the samples. When only anti-daratumumab was spiked into the serum, a weak polyclonal smear appeared in the lanes with IgG antisera in four of 51 (8%) samples. However, this did not interfere with the interpretation of DIRA, as the band corresponding to daratumumab:anti-daratumumab complex was distinctly visible and the smear was not observed

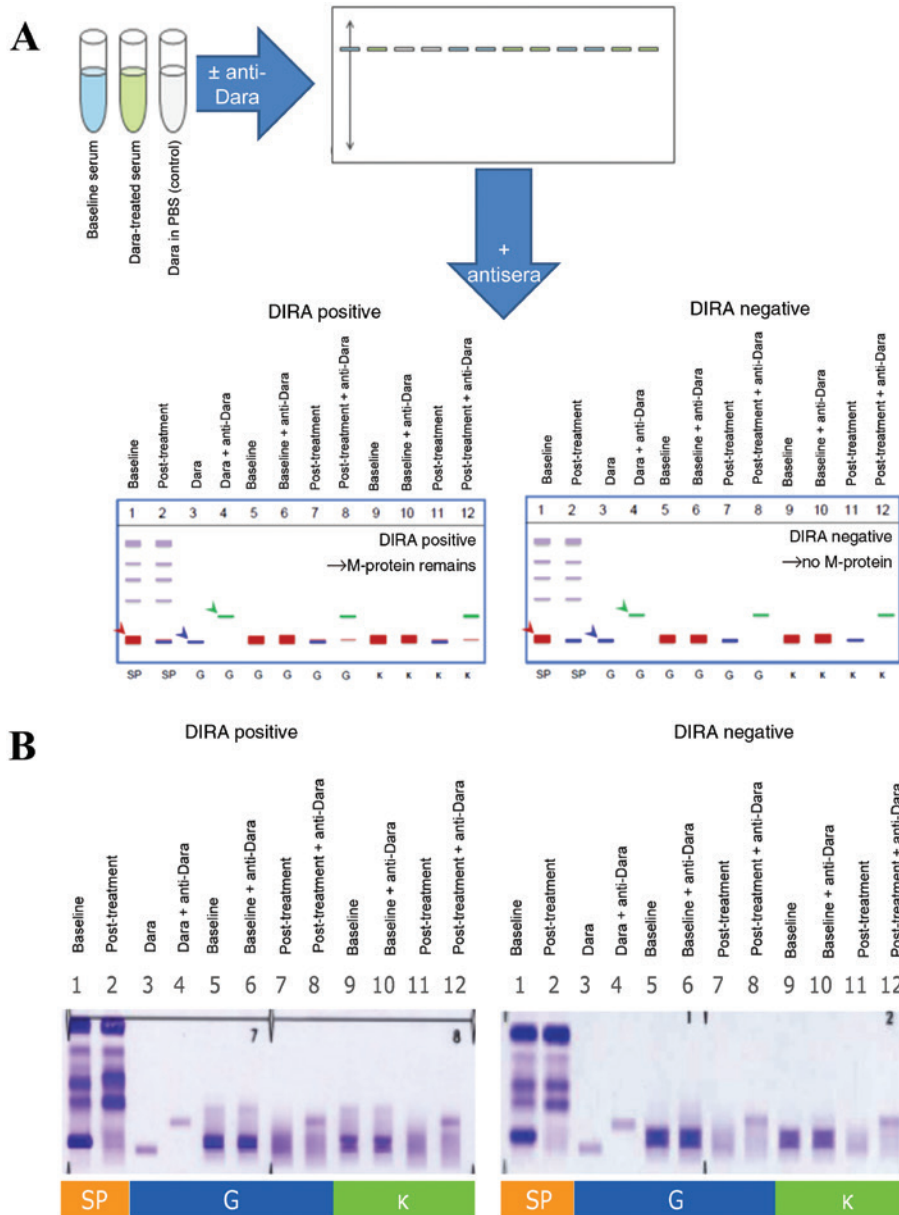


Figure 2: Daratumumab-specific IFE reflex assay.

Baseline (prior to treatment) serum samples are run \pm anti-daratumumab next to serum samples from a post-treatment time point with suspected daratumumab interference, \pm anti-daratumumab, to determine whether the remaining M-protein band shifts completely with anti-daratumumab. Both IgG and κ antisera are used for staining and fixation (A). DIRA positive, similar to IFE positive, indicates that endogenous M-protein (in red, and indicated by a red arrow in lane 1) remains. DIRA negative, similar to IFE negative, indicates that only daratumumab (in blue, and indicated by a blue arrow in lane 3) is remaining and endogenous M-protein is no longer detected (A). The DIRA template utilized daratumumab \pm anti-daratumumab as controls for migration of the therapeutic antibody and the daratumumab-anti-daratumumab shifted complexes (in green, and indicated by a green arrow in lane 4). In patient samples, baseline and post-treatment serum \pm anti-daratumumab were compared to determine whether M-protein remained after shifting daratumumab (B). DIRA-positive results showed M-protein, whereas DIRA-negative results showed only a shift in daratumumab but no remaining M-protein (lanes 8 and 12). IFE, immunofixation electrophoresis; M-protein, monoclonal immunoglobulin protein; DIRA, daratumumab-specific immunofixation electrophoresis reflex assay; Dara, daratumumab; PBS, phosphate buffered saline; SP, total serum protein fix; G, IgG antisera; κ , kappa antisera.

when daratumumab was present. While experienced reviewers consistently identify the faint residual band in the DIRA assays, it may be difficult to identify in Figure 2B. This is a known issue with faint bands on agarose gels;

scanned gels do not have the same resolution or detail as the physical version [20]. Therefore, anti-daratumumab appears highly specific for daratumumab. Specificity of the anti-daratumumab antibody, along with false-negative

and false-positive rates of DIRA, will be evaluated further in a randomized, phase 3 clinical study of daratumumab versus control.

The reproducibility of DIRA was assessed by performing the assay on daratumumab-treated patient samples in triplicate. In all daratumumab-treated patient samples (10/10), results were consistent across three independent experiments. To determine inter-operator and inter-day reproducibility, DIRA was performed on commercial samples spiked with daratumumab \pm anti-daratumumab, by two operators on three separate days. Further, results were evaluated by two independent reviewers. Concordance among reviews was demonstrated in 100% of assays. Reviewers' responses to a set of predetermined assessment criteria are shown for a single patient sample over three separate experiments (Table 1).

DIRA Plus

To ensure 1 g/L anti-daratumumab was sufficient to shift daratumumab in patient serum samples for which daratumumab concentration data were not available or SPE measurements were higher than the average range of daratumumab concentrations, samples from 14 daratumumab-treated patients were tested using increased concentrations of anti-daratumumab (a modification known as "DIRA Plus"; Figure 3). For these assays,

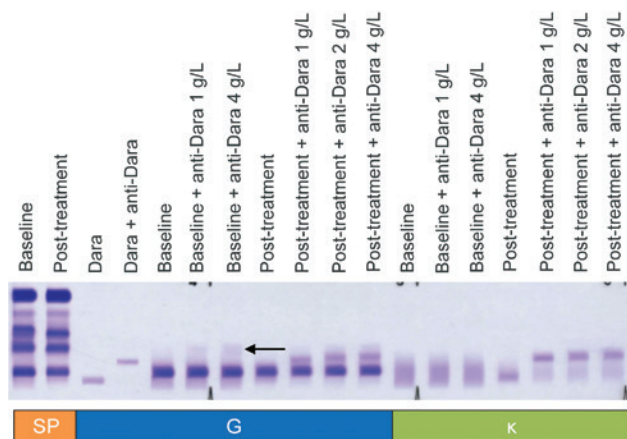


Figure 3: DIRA Plus for the evaluation of patients with serum concentrations of daratumumab above the normal range.

One gram per liter of anti-daratumumab was sufficient to migrate daratumumab in all samples (A). Higher concentrations of anti-daratumumab added to baseline serum (1 g/L and 4 g/L) resulted in the appearance of a faint, polyclonal smear (arrow) with IgG antisera. DIRA, daratumumab-specific immunofixation electrophoresis reflex assay; Dara, daratumumab; SP, total serum protein fix; G, IgG antisera; κ , kappa antisera.

anti-daratumumab concentrations of 1–4 g/L were used. In all cases (14 of 14 samples), 1 g/L of anti-daratumumab was sufficient to interpret DIRA. Anti-daratumumab concentrations of ≥ 1 g/L caused a weak, polyclonal smear to appear with no other change in the assay result versus the standard concentration of 1 g/L. Thus, the use of concentrations of anti-daratumumab > 1 g/L is neither warranted nor recommended.

Incorporation of DIRA into clinical testing

To automate the initiation of DIRA testing, especially for phase 3 clinical trials with large numbers of daratumumab-treated patients, an operational "algorithm" for triggering DIRA was devised. The algorithm stipulates that, if only IgG κ M-protein is detected on IFE and urine and FLC results are normal, then DIRA testing should be performed for patients demonstrating M-protein levels ≤ 2 g/L by SPE on 2 consecutive visits (Figure 4). If the results are DIRA negative, patients will have additional testing to confirm CR, including bone marrow evaluation of plasma cells. If the results are DIRA positive, indicating

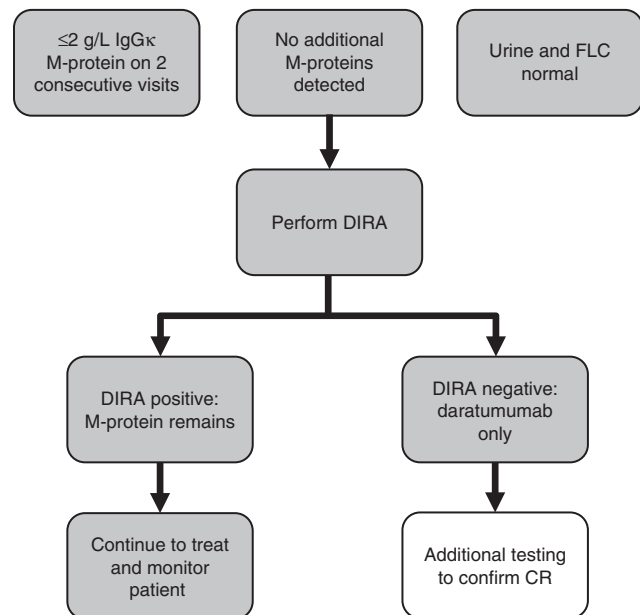


Figure 4: Testing algorithm to implement DIRA for clinical response assessment.

Samples from a patient with ≤ 2 g/L IgG κ M-protein on two consecutive visits, with normal urine and FLC and without additional M-protein, are good candidates for DIRA. For patients with DIRA-negative samples, additional testing to confirm a putative CR is warranted. DIRA, daratumumab-specific immunofixation electrophoresis reflex assay; M-protein, monoclonal immunoglobulin protein; FLC, free light chain; CR, complete response.

remaining disease M-protein, then no additional testing is warranted and disease monitoring will be continued (Figure 4).

Discussion

Daratumumab, a human anti-CD38 mAb, has demonstrated robust clinical efficacy in relapsed and refractory myeloma, including CRs in some patients. However, as a monoclonal immunoglobulin, daratumumab is detectable on the SPE and IFE assays that are used to monitor and characterize endogenous immunoglobulin protein. At the recommended 16 mg/kg dose and schedule, the mean (\pm standard deviation) maximum trough daratumumab concentrations was 0.573 ± 0.331 g/L, a concentration which can interfere with interpretation of the SPE and IFE assays (data on file). Current IMWG criteria for a CR include negative serum and urine protein electrophoresis and IFE, which is not possible when daratumumab is present at concentrations that fall within the therapeutic range. Therefore, DIRA was developed, validated, and implemented to distinguish daratumumab from myeloma M-protein.

DIRA utilizes a highly specific anti-daratumumab antibody to bind daratumumab and shift its migration on IFE gels. Patients with a single IgG κ band that is shifted completely by DIRA are considered to have no remaining M-protein (DIRA negative) and, thus, are candidates for additional IMWG-required confirmatory testing, including bone marrow assessment for plasma cells, to determine whether criteria for CR/sCR (as defined by the IMWG) are met. Patients with remaining endogenous M-protein on DIRA are considered to be DIRA positive, and disease monitoring is continued.

DIRA was highly specific, sensitive, and reproducible both in commercial samples spiked with daratumumab and in clinical samples from daratumumab-treated patients. The presence, or even excess, of anti-daratumumab did not affect the detection or migration of endogenous M-proteins. In the absence of daratumumab, a weak polyclonal smear was observed in IgG antisera lanes in four of 51 samples when anti-daratumumab was added, but the daratumumab:anti-daratumumab complex was still easily distinguishable by visual inspection and it did not interfere with the interpretation of DIRA. DIRA was always able to detect daratumumab by at least one parameter. The limit of sensitivity of DIRA was determined to be 0.2 g/L in serum from patients with myeloma. At this concentration and above, daratumumab interference with M-protein is predicted. Trough daratumumab concentrations throughout

the weekly and every 2 weeks dosing periods are typically above the DIRA sensitivity and may result in daratumumab detection by IFE. However, daratumumab trough concentrations during every 4 weeks dosing may fall below the DIRA sensitivity and may not interfere with M-protein monitoring during this time. Further, DIRA could be modified for patients with higher than average serum concentrations (DIRA Plus) by increasing the amount of anti-daratumumab used, although assay reliability decreased with increasing anti-daratumumab concentrations >1 g/L.

Reproducibility was assessed several different ways. Two independent reviewers scored all DIRA tests, and their assessments were always in agreement; a third reviewer was never required. Reproducibility tests were performed with 10 samples, and results for individual samples were consistent across multiple repetitions; similar results were obtained. Taken together, these findings indicate that DIRA is a robust test with high sensitivity, specificity, and reproducibility.

Despite these advantages, DIRA also has limitations. First, DIRA is not quantitative and interpretation by a trained operator is required. Although rare in myeloma, high polyclonal background signals may make it difficult to assess responses in some patients, leading to false interpretations. Second, DIRA is highly specific to daratumumab. Responses in patients receiving other antibodies cannot be resolved using DIRA. Other potential methods to address antibody interference, such as mass spectrometry, will be needed for patients receiving combinations of antibodies or patients requiring quantitative testing.

DIRA is important for determining response in daratumumab clinical studies, particularly for patients with IgG κ M-protein. Patients with non-IgG κ endogenous serum M-proteins (i.e. urine, FLC, IgA κ or λ , or IgG λ M-proteins) that were positive for IgG κ by IFE were readily detected using DIRA but, to meet current IMWG criteria, they also had to be evaluated to demonstrate that only daratumumab remained.

In phase 2 studies, an IgG κ band often appeared in SPE/IFE over the course of daratumumab treatment in patients originally classified as having non-IgG κ myeloma (IgA, IgM, IgE myeloma, or light-chain-only myeloma). It is likely that this band is indicative of daratumumab interference rather than a new plasma cell clone secreting an IgG monoclonal M-protein. In cases where the original myeloma clone was IgA or light chain only, reported as approximately 24% and 11% of patients in the general myeloma population, respectively [21], daratumumab was easily identified with DIRA and a lack of endogenous M-protein could be confirmed. However, 60% of patients with MM have IgG M-protein [21], and for those with IgG κ

the distinction between daratumumab and endogenous M-protein can be difficult. The most difficult cases to interpret were those in which the migration of daratumumab completely overlapped with M-protein and the entire band did not shift with anti-daratumumab. Waiting until M-protein measurements on SPE were lower, in accordance with the operational algorithm, reduced the number of these cases. IMWG has recently released a clarification to address antibody interference. In these updated guidelines, only the original myeloma clone(s) are required to be undetectable by SPE/IFE [22]. However, DIRA can still distinguish IgG κ clones from daratumumab.

The development and validation of DIRA offers a solution to mitigate daratumumab interference in IFE and improve clinical response assessment in daratumumab-treated patients. Until recently, the modest success of mAbs for the treatment of MM in the clinic did not necessitate a solution for mAb assay interference. Phase 1 and 2 studies of daratumumab as a monotherapy have yielded deep responses, including CRs and sCRs [6, 8], making it essential to establish a reliable method for distinguishing M-protein from daratumumab. As myeloma therapy evolves to incorporate additional mAb therapeutics, other methods to mitigate antibody interference on SPE/IFE will be needed. These alternative methods could include the incorporation of minimal residual disease (MRD) detection into formal clinical criteria for CR and sCR. MRD detection by 8- to 10-color flow cytometry could be susceptible to antibody interference as well, but standardized approaches using noncompeting antibodies may offer a solution. Utilization of methods such as polymerase chain reaction/next-generation sequencing could also be evaluated.

Conclusions

DIRA is an effective test with high sensitivity, specificity, and reproducibility to distinguish endogenous M-protein from daratumumab. DIRA is currently employed in daratumumab clinical trials to determine if patients with outcomes of very good PR should undergo confirmatory assessments for characterization of CR. These studies will provide functional validation of DIRA as a clinical tool.

Acknowledgments: This study was sponsored by Janssen Research & Development, LLC. Medical writing and editorial support were provided by Erica Chevalier-Larsen, PhD, of MedErgy, and was funded by Janssen Global

Services, LLC. Anti-daratumumab was originally generated and produced by Paul Parren, PhD, and Jeroen Lammerets van Bueren, PhD, at Genmab.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: CM received research support for this project from Janssen Pharmaceuticals. NWCJvdD has received research support from Janssen Pharmaceuticals, Amgen, and Celgene.

Employment or leadership: NWCJvdD serves on advisory boards for Janssen Pharmaceuticals, Amgen, and Celgene. AEA, PLC, SF, JB, JMS, TA, and AKS are employees of Janssen Research & Development, LLC. All other authors report no conflicts.

Honorarium: None declared.

Competing interests: This study was sponsored by Janssen Research & Development, LLC.

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