Mitigating the Interference of Daratumumab with Immunofixation Electrophoresis: A Single-center Experience Using the Hydrashift 2/4 Kit

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ABSTRACT **Background:** Multiple myeloma (MM) accounts for approximately 10% of hematological malignancies. The monoclonal immunoglobulin G kappa (IgG-κ) daratumumab can bind to CD38 on MM cells and be detected in serum immunofixation (IF), causing pitfalls in M-protein quantification.

Objectives: To determine the efficacy of mitigating the interference of IgG MM treated with daratumumab.

Methods: Levels of Ig, free light chains (FLC) kappa (κ) and lambda (λ), serum protein electrophoresis (SPE)/IF, and Hydrashift 2/4 assays were assessed following manufacturer's instructions in three patients.

Results: Patient 1 was a 70-year-old male diagnosed with IgG-λ MM. The IF distinguished two monoclonal bands (IgG-κ and IgG-λ). With the Hydrashift assay, the daratumumab–anti-daratumumab immune complex shifted the IgG-κ to the α zone, suggesting that the monoclonal IgG-κ band corresponded to daratumumab. Patient 2 was a 63-year-old male with IgG-κ MM who was receiving daratumumab once every other week. SPE/IF assay revealed a faint monoclonal IgG-κ band in the γ zone. A stronger monoclonal band was observed after administration. The IgG-κ band disappeared on the Hydrashift assay, while the daratumumab–anti-daratumumab complex appeared as a broad smear in the α-region. Patient 3, a 63-year-old male diagnosed with IgG-λMM, was receiving daratumumab once every other month. The IF assay showed two distinct bands (IgG-κ and IgG-λ) post-daratumumab administration. The shift to the α zone of the IgG-κ bands on the Hydrashift assay confirmed that the additional band observed post-infusion was due to the daratumumab.

Conclusions: The Hydrashift assay can help distinguish daratumumab from endogenous M-spike.

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Multiple myeloma (MM) is the second most prevalent hematological malignancy, accounting for approximately 10% of all cases of cancer. It is characterized by the proliferation of neoplastic plasma cells in the bone marrow leading to excessive production of monoclonal protein (M-protein) [1].

The most typical clinical manifestations are hypercalcemia, renal failure, anemia, and bone disease (commonly summarizes by the acronym CRAB) [2].

Most MM cases evolve from an asymptomatic pre-malignant stage termed monoclonal gammopathy of undetermined significance (MGUS). It is characterized by the presence of a serum M-protein concentration lower than 3 g/dl and less than 10% clonal plasma cells in bone marrow as well as by the absence of MM-related end-organ damage [3].

MGUS could progress to a more advanced, but still asymptomatic, phase known as smoldering multiple myeloma (SMM) characterized by the presence of a serum M-protein level higher than 3 g/dl and/or up to less than 60% of bone marrow plasma cell infiltration, with no clinical CRAB criteria [3,4]. SMM could subsequently transform into active MM with a rate of 10% per year over the first 5 years following diagnosis, and 1% to 3% per year thereafter [5].

According to the International Myeloma Working Group (IMWG), the detection, quantification, and typing of the M-spike by serum protein electrophoresis (SPE) and immunofixation (IF) methods are critical for the evaluation and follow-up of patients with MM [5].

Over the past decade, treatment options for MM have evolved substantially, resulting in improved survival rates among MM patients. In relation to patient characteristics, disease biology, and tumor burden, most newly diagnosed MM patients can achieve durable responses with known novel drug combinations, resulting in prolonged duration of responses and improved control of symptoms and quality of life [4]. Nevertheless, MM remains an incurable disease despite improved treatment options [1].

In 2015, the U.S. Food and Drug Administration (FDA) approved daratumumab, a therapeutic monoclonal antibody (t-mAb), for the treatment of relapsed or refractory MM. Daratumumab is a fully human immunoglobulin G kappa (IgG-κ) t-mAb that can bind to the highly expressed glycoprotein CD38 on MM cells [6]. It is thought to induce MM cell death through

several mechanisms, including direct cellular apoptosis, complement-dependent cytotoxicity, antibody-and cell-mediated cytotoxicity, and antibody-dependent cellular phagocytosis [6]. Given its potent anti-myeloma activity, daratumumab-based combinations are being tested in front line settings [7].

Daratumumab is given as a weekly infusion in cycles 1–2 (weeks 1 to 8), every 2 weeks in cycles 3–6 (weeks 9 to 24), and every 4 weeks from cycle 7 until disease progression. Its half-life is concentration and time-dependent with a linear elimination time of 18 ± 9 days [1].

The administration of a t-mAb can be detected as a distinct monoclonal band in serum IF. Moreover, IgG-κ M-proteins may co-migrate with daratumumab, generating pitfalls in the M-protein quantification, since pharmacokinetic studies show that peak plasma concentrations of daratumumab reach up to 1 g/L [8]. That result may lead to misinterpretation and may affect response assessment according to the IMWG criteria.

Hydrashift 2/4 is a commercial kit from Sebia (France), which was recently approved by the FDA. It was designed to overcome this possible interference by forming a specific complex anti-daratumumab–daratumumab and shifting the daratumumab band to the alpha zone in IF gel.

We report our results using the Hydrashift assay in three cases of IgG-κ MM treated with daratumumab at different time intervals.

PATIENTS AND METHODS

The study was approved by the ethics committee of the Samson Assuta Ashdod University Hospital, protocol number AAA-0071-19.

Whole blood samples were collected from three patients with IgG-κ MM, who were receiving daratumumab in relapsed/refractory settings. Samples were collected prior to daratumumab administration and on days +1, +7, and +20 after infusion. Serum was separated from cells by centrifugation at 3500 RPM for 10 minutes within 1 hour of collection, and then stored at -20°C. Before analysis, the aliquots were thawed and mixed well.

Levels of IgG, IgM, IgA, and free light chains (FLC) kappa (κ) and lambda (λ) were assessed following manufacturer's kit instructions ((Roche Cobas 6000® and Freelite® Roche Cobas® C system [Switzerland], respectively).

SPE, IF, and Hydrashift 2/4 assays were tested with Sebia hydrasys 2 according to manufacturer's instructions.

RESULTS

PATIENT 1

A 70-year-old male patient with a previous diagnosis of IgG-λMM was admitted to our clinic for daratumumab treatment. The drug was already being administrated intravenously once a week at a dose of 16 mg/kg.

At admission, serum levels of immunoglobulins were below normal and those of FLC-κ were within the normal range. FLC-κ concentration was above normal values, with a consequently low FLC-κ/λ ratio [Table 1].

SPE assay revealed a single band in the gamma (γ) zone at baseline and one day post-daratumumab infusion [Figure 1A, lines 2 and 3, respectively].

The IF distinguished two monoclonal bands (IgG-κ and a weak IgG-λ) [Figure 1B, left panel]. At the Hydrashift assay, the daratumumab-anti-daratumumab immune complex shifted the IgG-κ to the α zone, while the monoclonal IgG-λ band remained in the α zone [Figure 1B, right panel].

These results suggest that the monoclonal IgG-κ band corresponds to daratumumab, identifying the presence of the treatment-related oligoclonal band.

PATIENT 2

A 63-year-old male with IgG-κMM and hypogammaglobulinemia was admitted to our institute. He was already receiving daratumumab once every other week.

At admission, IgG and IgM levels were below the reference values, while IgA and FLC levels were within normal ranges [Table 1].

Before the first daratumumab infusion at our center, the SPE assay revealed a faint monoclonal band in the γ zone [Figure 1B, line 2]. A stronger monoclonal band was observed immediately after the administration [Figure 2A, line 3]. It was attenuated again a week later [Figure 2A, line 4].

Figure 1. Serum protein electrophoresis (SPE)

[A] Patient 1. A single band is observed in the g zone (highlighted with a box) both before (line 2) and a day after daratumumab administration (line 3). Line 1 corresponds to normal control.

[B] Patient 2. The monoclonal band in the g zone (highlighted with a box) was faint before the daratumumab infusion (line 2), stronger a day after administration (line 3, arrow), and attenuated a week later (line 4). Line 1 corresponds to normal control

[C] Patient 3. No evidence of M-spike was observed at SPE before (line 2). A faint band was then visible after a day (line 3, arrow), with no evidence at 14-days (line 4), and 30-days (line 5) after daratumumab administration. Line 1 corresponds to normal control.

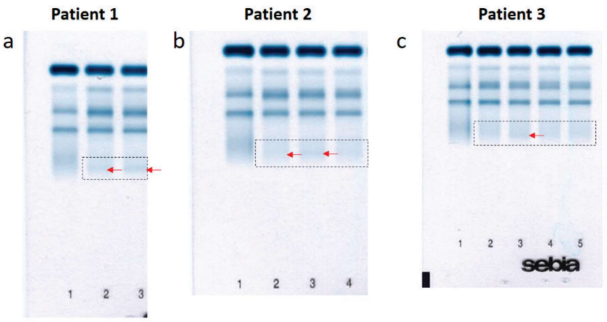


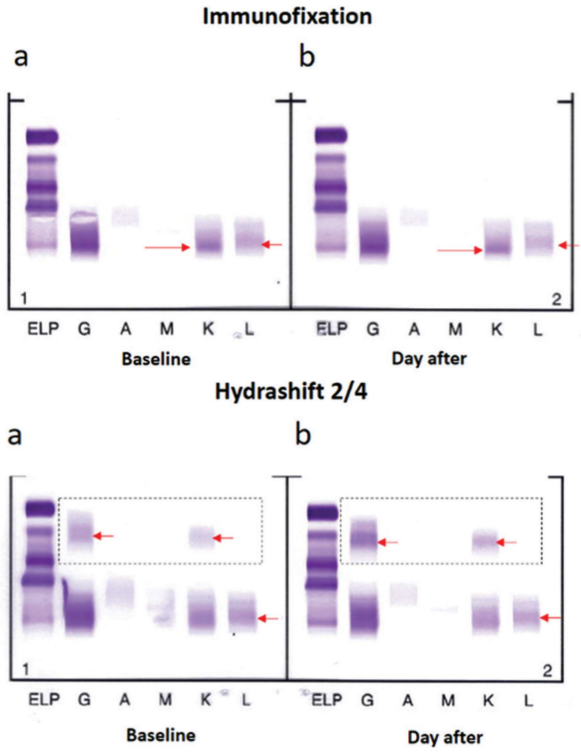
Table 1. Laboratory characteristics at baseline and after treatment

	Total protein (6.4–8.3 gr/dl)	IgG (700–1600 mg/dl)	IgM (40–230 mg/dl)	IgA (70–400 mg/dl)	FLC (3.3–19.4 mg/dl)	FLC κ (5.7–26.5 mg/dl)	κ/ λ Ratio (0.25–1.65)
Patient 1							
Baseline	6.2	501	26	25	5.2	70.84	0.07
Post-treatment	5.7	500	24	23	3.85	62.1	0.06
Patient 2							
Baseline	5.6	398	8	79	23.53	17.77	1.32
Post-treatment	5.9	466	9	86	n/a	n/a	n/a
7 days post-treatment	5.5	388	7	74	21.5	16.03	1.34
Patient 3							
Baseline	6.8	526	23	26	6.49	13.75	0.47
Post-treatment	6.5	559	22	25	6.54	13.59	0.48
14 days post-treatment	6.7	513	19	23	7.32	14.07	0.52
30 days post-treatment	6.3	517	25	21	6.73	13.4	0.5

At the same time points, the IF assay confirmed the presence of a monoclonal IgG-κ band [Figure 2B, left panel]. When the samples were run on the Hydrashift assay, the IgG-κ band disappeared and the daratumumab–anti-daratumumab complex appeared as a broad smear in the α region. The results confirmed that the patient was in complete remission, demonstrating that the IgG-κ band on the IF was due to daratumumab [Figure 2B, right panel].

Figure 2. Immunofixation (left) and Hydrashift 2/4 (right) assays

[A] Patient 1
Left: IgG k and IgG l monoclonal bands (arrows) were detected by immunofixation both before (a) and a day after (b) daratumumab administration.
Right: Daratumumab–anti-daratumumab complex appears as a band smear in the A zone (highlighted with a box) both before (a) and a day after (b) daratumumab administration, remaining only a monoclonal IgGl band (arrow).



PATIENT 3

A 63-year-old male with a diagnosis of IgG-λMM was referred to our institute while receiving a daratumumab once every other month. Immunoglobulin levels were below the normal range, while FLC levels were normal [Table 1].

No obvious evidence of M-spike was observed at SPE before treatment [Figure 2B, line 2]. A faint band was then observed after administration [Figure 2B, line 3] with no evidence of it after 14 and 30 days from the daratumumab infusion [Figure 2C, lines 4 and 5, respectively].

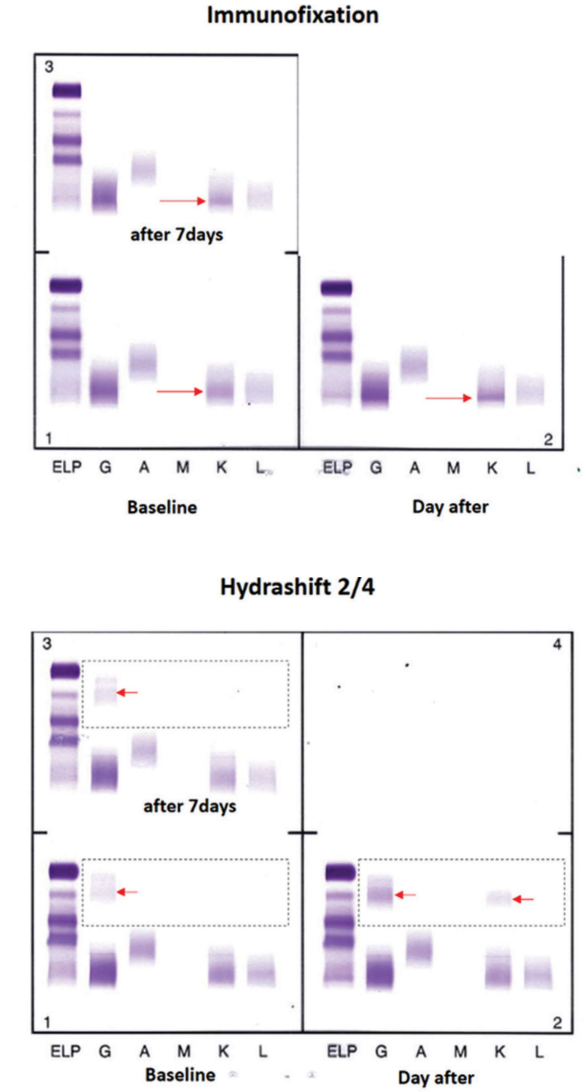
The IF assay showed a weak IgG-λ band at baseline, two distinct bands (IgG-κ and IgG-λ) post-daratumumab administration, and again only aIgG-λ band detected after 14 and 30 days, respectively [Figure 2B, left panel].

The shift to the α zone of the IgG-κ bands on the Hydrashift assay confirmed that the additional band observed post-infusion was due to the daratumumab [Figure 2C, right panel].

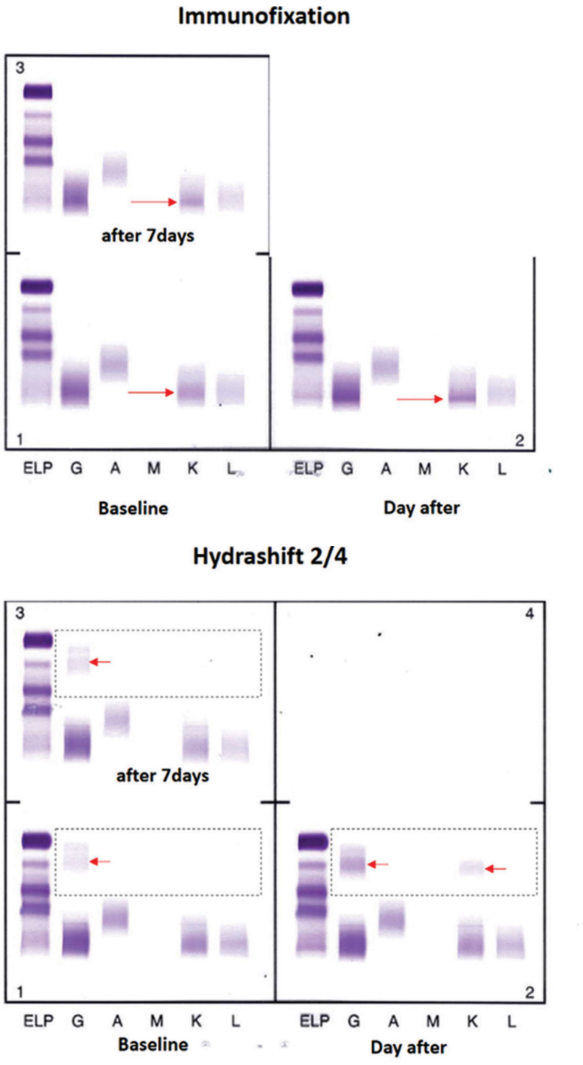
DISCUSSION

This study was designed and conducted at the end of 2018, following the College of American Pathologists external quality control. According to the results, more than half of the 900 par-

[B] Patient 2
Left panel: IF showed a weak monoclonal IgGK band (arrows) before (1), stronger a day after (2), and faint after 7 days of daratumumab infusion (3). Right panel: Daratumumab–-anti-daratumumab complex appears as a smear in the A zone (highlighted with a box) both before (1), a day (2), and a week after (3) the daratumumab administration.



[C] Patient 3
Left panel: IF showed a weak monoclonal IgGl band at baseline (arrow), and a IgG k and a IgG l the day (marked by arrows). Only the IgG l was still visible after 14- and 30-days of daratumumab infusion (arrows). Right panel: Daratumumab–anti-daratumumab complex appears a day after treatment as a smear in the A zone (highlighted with a box). The bands were mainly visible a day after treatment (arrows).



ticipating laboratories in the world were not aware of the interference of the monoclonal t-mAb (such as daratumumab) in quantification and typing of the M-spike by SPE and IF assays.

In the present study, we reported three cases of MM showing different scenarios during treatment with daratumumab.

In the first patient (IgG-λMM), SPE results detected the presence of a single monoclonal band both before and after the daratumumab infusion. However, an additional IgG-κ band was then revealed by the IF. This situation may suggest that the M protein co-migrates with daratumumab. The Hydrashift assay was helpful in identifying the presence of the treatment-related band. Only the IgG-λ protein had diagnostic relevance.

In the second case, a patient in complete remission, Hydrashift 2/4 was able to demonstrate in that the monoclonal band detected even after 7 days after the last dose was a consequence of daratumumab administration.

The last situation showed a disparity between the results of the IF immediately after administration of daratumumab (two different monoclonal bands) and those observed 14 and 30 days later (only one IgG-λ monoclonal band). Hydrashift 2/4 was

helpful in confirming the presence of a treatment-related monoclonal band at the earlier time point.

We found that the Hydrashift assay was easy to performed using existing laboratory instruments. The assay addresses the false positive interference of daratumumab on IF. The test may be most useful when M protein levels are approximately equal to or less than the concentration of daratumumab (approximately 1g/L) [7].

Proper interpretation of SPE/IF results is crucial for monitoring the treatment of patients with MM. Complete response, defined by negative IF and less than 5% bone marrow plasma cells, has been accepted as a relevant surrogate marker of survival [9].

Although MM remains incurable, management of the disease has been transformed during the last decade, with the introduction of novel agents, such as proteasome inhibitors (bortezomib, carfilzomib, ixazomib), immunomodulatory agents (thalidomide, lenalidomide, and pomalidomide),and more recently monoclonal antibodies (daratumumab, elotuzumab) [10]. The use of these frontline agents has resulted in 5-year survival rates as high as 80% [11].

Daratumumab is a fully human anti-CD38 IgG1- κmAb. Preclinical studies showed that daratumumab may kill myeloma cells by complement-mediated cytotoxicity, by antibody-dependent cellular cytotoxicity, and by antibody-dependent cellular phagocytosis [12,13]. Since it was approved by the FDA in 2016, case reports identifying a new small γ fraction abnormality in SPE and the identification of an IgG-κ on IF have been common. Daratumumab is given in high enough concentrations that if a blood sample is drawn within a couple of days after the infusion, it will appear as a band on SPE/IF. If the original MM patient clone is of a different isotype, such as an IgA-λ, the finding of a new IgG-κ during follow-up should prompt the laboratory to report it as a potential t-mab (the appearance of a new IgG-κ disease close would be much rarer). The most challenging scenario would be when the endogenous IgG-κ and the t-mab co-migrate on SPE.

Several strategies have been suggested to help properly interpret IF results for patients who are receiving daratumumab [14,15]. The Hydrashift 2/4 daratumumab assay is a useful tool to confirm the source of the IgG-κ band on IF.

This assay also becomes critically important for patients receiving upfront therapy with daratumumab-based regimens, as a significant proportion will be expected to achieve complete remission [16,17].

CONCLUSIONS

The use of a Hydrashift 2/4-daratumumab kit could help successfully distinguish daratumumab from endogenous M-spike. It is essential to inform the laboratory of the treatment protocol to avoid misinterpretations of SPE and IF results. Based on this study, we recommend drawing a blood sample just before the next infusion to decrease the possibility of seeing the therapeutic monoclonal antibody t-mAbs in IF. Guidelines and recommendations are necessary to properly implement the Hydrashift assay into laboratory workflows and routine clinical practice.

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References

1. Tzogani K, Penninga E, Schougaard Christiansen ML, et al. EMA review of daratumumab for the treatment of adult patients with multiple myeloma. *Oncologist* 2018; 23 (5): 594-602.
2. Nakaya A, Fujita S, Satake A, et al. Impact of CRAB symptoms in survival of patients with symptomatic myeloma in novel agent era. *Hematol Rep* 2017; 9 (1): 16-18.
3. Mateos MV, Landgren O. MGUS and smoldering multiple myeloma: diagnosis and epidemiology. *Cancer Treat Res* 2016; 169: 3-12.
4. Mills JR, Murray DL. Identification of friend or foe: the laboratory challenge of differentiating M-proteins from monoclonal antibody therapies. *J Appl Lab Med An AACC Publ* 2017; 1 (4): 421-31.
5. Rajkumar SV, Dimopoulos MA, Palumbo A, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. *Lancet Oncol* 2014; 15 (12): e538-e548.
6. Sanchez L, Wang Y, Siegel DS, Wang ML. Daratumumab: a first-in-class CD38 monoclonal antibody for the treatment of multiple myeloma. *J Hematol Oncol* 2016; 9 (1): 51.
7. Thoren KL, Pianko MJ, Maakaroun Y, Landgren CO RL. Distinguishing drug from disease by use of the Hydrashift 2/4 Daratumumab Assay. *J Appl Lab Med* 2019; 3 (5): 857-63.
8. Van De Donk NWCJ, Otten HG, El Haddad O, et al. Interference of daratumumab in monitoring multiple myeloma patients using serum immunofixation electrophoresis can be abrogated using the daratumumab IFE reflex assay (DIRA). *Clin Chem Lab Med* 2016; 54 (6): 1105-9.
9. Martinez-Lopez J, Blade J, Mateos MV, et al. Long-term prognostic significance of response in multiple myeloma after stem cell transplantation. *Blood.* 2011; 118 (3): 529-34.
10. Naymagon L, Abdul-Hay M. Novel agents in the treatment of multiple myeloma: A review about the future. *J Hematol Oncol* 2016; 9 (1): 1-20.
11. Moreau P, Attal M, Facon T. Frontline therapy of multiple myeloma. *Blood* 2015; 125 (20): 307684.
12. de Weers M, Tai Y-T, van der Veer MS, et al. Daratumumab, a novel therapeutic human CD38 monoclonal antibody, induces killing of multiple myeloma and other hematological tumors. *J Immunol* 2011; 186 (3): 1840-8.
13. van der Veer MS, de Weers M, van Kessel B, et al. Towards effective immunotherapy of myeloma enhanced elimination of myeloma cells by combination of lenalidomide with the human CD38 monoclonal antibody daratumumab. *Haematologica* 2011; 96 (2): 284-90.
14. Axel AE, McCudden CR, Xie H, Hall BM, Sasser AK. Development of clinical assay to mitigate daratumumab, an IgG1κ monoclonal antibody, interference with serum immunofixation (IFE) and clinical assessment of M-protein response in multiple myeloma. *Cancer Research* 2014; 74 (19 Suppl): 2563.
15. McCudden C, Axel AE, Slaets D, et al. Monitoring multiple myeloma patients treated with daratumumab: Teasing out monoclonal antibody interference. *Clin Chem Lab Med* 2016; 54 (6): 1095-04.
16. Palumbo A, Chanan-Khan A, Weisel K, et al. Daratumumab, bortezomib, and dexamethasone for multiple myeloma. *N Engl J Med* 2016; 375 (8): 754-66.
17. Dimopoulos MA, Oriol A, Nahi H, et al. Daratumumab, lenalidomide, and dexamethasone for multiple myeloma. *N Engl J Med* 2016; 375 (14): 1319-31.