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Treatment of multiple myeloma with monoclonal antibodies and the dilemma of false positive M-spikes in peripheral blood

Kazunori Murata^{*,a}, Samuel I McCash^a, Brittany Carroll^a, Alexander M Lesokhin^b, Hani Hassoun^b, Nikolett Lendvai^b, Neha S Korde^b, Sham Mailankody^b, Heather J Landau^c, Guenther Koehne^c, David J Chung^c, Sergio A Giral^c, Lakshmi V Ramanathan^a, and Ola Landgren^b

^aClinical Chemistry Service, Department of Laboratory Medicine, Memorial Sloan Kettering Cancer Center, 1275 York Ave. New York, NY, 10065

^bMyeloma Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, 1275 York Ave. New York, NY, 10065

^cBone Marrow Transplant Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, 1275 York Ave. New York, NY, 10065

Abstract

Objectives—To characterize the effect of three humanized IgG κ monoclonal antibodies (daratumumab, isatuximab, and elotuzumab) on the interpretation of results generated by protein electrophoresis, immunofixation, free light chain, and heavy/light chain assays performed on human serum.

Methods—Healthy volunteer serum and serum from multiple myeloma patients were supplemented with clinically relevant concentrations of each of the three monoclonal antibodies. These specimens then underwent analysis via serum protein electrophoresis, immunofixation, serum free light chain quantification, heavy/light chain quantification, total IgG, and total protein. In addition, serum specimens from patients who had undergone treatment with elotuzumab for multiple myeloma underwent similar analysis.

Results—Addition of the study drugs to serum from both the healthy donor as well as multiple myeloma patients resulted in a visible and quantifiable M-protein on SPEP and a visible IgG κ band by IFE. Increases were also noted in total IgG, IgG κ , and IgG κ /IgG λ -ratios. Analysis of serum from multiple myeloma patients receiving study drug showed similar findings with an

*Corresponding Author, Kazunori Murata, Memorial Sloan Kettering Cancer Center, 1275 York Ave, New York, NY 10065; muratak@mskcc.org.

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AUTHORSHIP

KM and OL designed the study. KM performed the experiments. KM, SM, OL, and BC reviewed data and wrote the manuscript. AML, HH, NL, NSK, HJL, GK, DC, and SAG recruited patients for the study. All authors reviewed and approved the final version of the paper.

additional IgG κ band and quantifiable M-protein with similar migration patterns in specimens drawn after administration.

Conclusion—The treatment of multiple myeloma patients with monoclonal antibodies results in a visible and quantifiable M-protein that has the potential to falsely indicate poor response to therapy.

Keywords

elotuzumab; daratumumab; isatuximab; electrophoresis; immunofixation; myeloma

INTRODUCTION

The initial observation from a clinical study that therapeutic monoclonal antibodies can interfere with SPEP and IFE was made in patients receiving the monoclonal antibody siltuximab which targets IL-6[1]. Although the observation was first done in this setting, the clinical implications were limited since the drug is not FDA approved for the treatment of multiple myeloma. However, in 2015, the first monoclonal antibodies were approved by the FDA for the treatment of multiple myeloma: the humanized IgG κ monoclonal antibodies targeting CD38 (daratumumab) and SLAMF-7 (elotuzumab); and more drugs are under development (e.g., isatuximab) [2-4]. Because clinical response criteria for multiple myeloma include assessment of monoclonal-(M)-protein, the use of humanized IgG κ monoclonal antibodies has the potential to interfere with clinical response criteria. Indeed, based on current response criteria for multiple myeloma, a complete response (CR) includes the elimination of detectable M-protein in peripheral blood [5]. Therefore, the use of humanized IgG κ monoclonal antibodies in a patient with IgG κ multiple myeloma may result in a detectable M-protein which may be misinterpreted at response evaluation of peripheral blood, as residual disease. (i.e. false positive residual disease).

The purpose of our study was to characterize the effect of three humanized IgG κ monoclonal antibodies (daratumumab, isatuximab, and elotuzumab) on the interpretation of results generated by protein electrophoresis, immunofixation, free light chain, and heavy/light chain assays performed on human serum.

MATERIALS AND METHODS

Pure daratumumab, isatuximab, and elotuzumab were obtained from their respective manufacturers (Jansen, Sanofi, and Bristol Myers Squibb). Daratumumab and elotuzumab were provided in powdered form ready for clinical use and reconstituted, while isatuximab was provided from the manufacturer as a 5mg/mL solution. Serum from a healthy male volunteer was obtained, and supplemented with varying concentrations of each of the three monoclonal antibodies. The concentrations that were evaluated were chosen to approximate the serum C_{max} values attained by each of the drugs that were available based on available literature on Phase 1/2 studies (Daratumumab: 993 μ g/mL after Dose 7 at 16 mg/kg (Daratumumab FDA submission documents), Elotuzumab: 563 μ g/mL after Dose 4 at 20 mg/kg[6]). Due to the lack of publicly available information on Isatuximab, a concentration similar to that of Daratumumab was chosen. Concentrations twice the C_{max} were also

included to examine the effects of extremely high concentrations of monoclonal antibody on the IgG and heavy/light chain assay quantitation.

The dilute nature of the isatuximab solution provided by the manufacturer required the addition of a significant volume of isatuximab solution to the serum solution in order to attain the desired drug concentration. This resulted in the isatuximab spiked specimens that were approximately 30% PBS by volume. Therefore, an additional aliquot of volunteer serum was diluted with saline (70% serum, 30% saline) to serve as a control for the diluting effect of the isatuximab solution on the spiked specimens.

In addition, serum from two multiple myeloma patients, each with a diagnosis of IgG κ multiple myeloma, were obtained. One of the patients was in complete remission (CR), while the other had a visible M-protein. Aliquots of serum from both patients were supplemented with either elotuzumab or daratumumab. Spiking experiments were not performed for isatuximab due to the highly dilute nature of the provided antibody.

All specimens underwent analysis via serum protein electrophoresis (Sebia Capillarys 2, Sebia USA, Norcross, GA, USA), serum immunofixation (Sebia Hydrasis, Sebia USA, Norcross, GA, USA), serum free light chains and heavy/light chain assays (Freelite and Hevylite, The Binding Site, Birmingham, UK), and nephelometric IgG quantitation and total protein (Siemens USA, Tarrytown, NY, USA). All M-proteins visible by serum protein electrophoresis were quantified using the Sebia Phoresis (Sebia USA, Norcross, GA, USA). For myeloma patient specimens, M-spikes caused by the patient's clone and spikes caused by the mAb antibodies were quantitated separately.

Furthermore, to expand our work and to include *in vivo* samples from patients treated in our clinic, we assessed serum specimens drawn from patients (N=3) who had undergone treatment by elotuzumab for multiple myeloma. All three patients were on a protocol in which they received 10mg/kg of elotuzumab once a week. These specimens were drawn at various points during the course of their treatment. Patient clinical information was deidentified for this study, and use of the data was approved by our institutional review board.

RESULTS

In vitro studies

Analysis of the healthy donor serum demonstrated no visible monoclonality by either protein electrophoresis or immunofixation (Figure 1a). Addition of clinically relevant concentrations of either daratumumab (Fig 1b), isatuximab (Fig 1c), or elotuzumab (Fig 1d) to the serum specimen, all resulted in a visible and quantifiable M-protein by protein electrophoresis as well as a visible monoclonality by immunofixation. The addition of any of the three monoclonal antibodies to healthy donor serum resulted in noticeable increases in total IgG, and IgG κ via heavy/light chain assay as well as IgG κ /IgG λ ratios relative to the unspiked serum specimens (Table 1), however, the increases did not result in an abnormal IgG κ /IgG λ ratio. No clinically significant increases were noted in the quantitation of kappa free light chains (Freelite), or free κ /free λ -ratios.

Serum from a multiple myeloma patient in CR with no visible monoclonality by immunofixation or protein electrophoresis (Fig 1e), as well as a patient with a visible M-spike and monoclonal band (Fig 1h), were analyzed by protein electrophoresis and immunofixation after addition of daratumumab or elotuzumab. The specimens supplemented with daratumumab (Fig 1f) as well as elotuzumab (Fig 1g) demonstrated monoclonal bands visible by both protein electrophoresis and immunofixation in serum from the remission patient. In serum from the patient with a pre-existing M-protein, the specimen supplemented with daratumumab showed a slight increase in the M-protein peak (Fig 1i, Table 1), while the specimen supplemented with elotuzumab (Fig 1j) showed two distinct M-protein peaks by protein electrophoresis and two monoclonal bands by immunofixation. Quantitation of total IgG, IgG κ , IgG κ /IgG λ ratios, free light chains, and free light chain-ratios resulted in findings similar to that found in supplemented healthy human serum (Table 1).

In vivo studies

To expand our work to include *in vivo* samples, stored serum specimens from three patients receiving elotuzumab were analyzed by protein electrophoresis and immunofixation. Patient A, a multiple myeloma patient with free κ light chain-myeloma, had a visible IgG κ band as well as a corresponding M-protein that was not caused by the monoclonal free κ light chain band at both 1 week post dose 4 (Fig 2a) as well as at 1 week post dose 12 (Fig 2b). Patient B, with IgA λ disease, had an additional faint IgG κ band visible by immunofixation at both 1 week post dose 3 (Fig 2c) and at 1 week post dose 6 (Fig 2d). Patient C, with IgA λ disease, had no additional visible M-protein or monoclonal bands prior to receiving elotuzumab (Fig 2e), but had an additional M-protein by protein electrophoresis and an IgG κ band visible by immunofixation at both 1 week post dose 3 (Fig 2f) and at 1 week post dose 4 (Fig 2g). The electrophoretic pattern of the additional visible M-proteins on both SPEP and IFE were noted to be similar to the electrophoretic pattern seen in the *in vitro* spiking studies on healthy serum. Also worth noting, the visible bands caused by elotuzumab became more pronounced with increasing numbers of doses, indicating accumulation of elotuzumab in the patient's peripheral blood.

The reporting of an additional M-protein along with the appearance of an additional IgG κ band by immunofixation (Fig 2a-b) had no effect on the clinical management of the Patient A. Patients B and C were taken off protocol shortly following dose 6 and 4, respectively, due to progression despite treatment with elotuzumab. Both patients had progressive increases in their M-spike values (Table 2) which were determined not to be due to elotuzumab based on review of SPEP and immunofixation results (Fig 2c-g).

Discussion

Current International Myeloma Working Group (IMWG) Uniform response criteria for multiple myeloma[5] state that in order for patients to be classified as being in CR, laboratory testing should show absence of M-protein by immunofixation in serum and urine, while classification into very good partial response (VGPR) requires serum and urine M-protein detectable by immunofixation but not on electrophoresis. The results from our study clearly demonstrate that all three of the new therapeutic monoclonal antibodies for the

treatment of multiple myeloma are capable of generating M-protein detectable by protein electrophoresis and immunofixation. This leads to a dilemma in the assessment of treatment response. To highlight these issues, a clarification of the definition of CR was recently proposed by the IMWG[7]. The updated definition stipulates that the presence or absence of monoclonal bands by immunofixation or SPEP refers only to the patient's original M-protein secreted by the pathologic plasma cells.

Serum protein electrophoresis and immunofixation results are typically reviewed by a pathologist or doctoral level scientist prior to release into a patient's medical record. However, there is significant variation in how results are reported due to lack of standardization[8]. One such example of relevance to this study is that many laboratories report out all visible M-protein values and monoclonal bands without reference to the monoclonal protein secreted by the cancerous plasma cells. This is particularly true of SPEPs and IFE's performed at reference laboratories, which typically do not have access to patient medical records.

All three monoclonal antibodies have a characteristic migration pattern via protein electrophoresis, with daratumumab running at the cathodal end of the gamma fraction, while isatuximab and elotuzumab run closer to the middle of the gamma fraction. This pattern may sometimes assist the laboratorian in determining whether the band being visualized is due to one of the monoclonal therapeutics. However, in practice, it is not always possible to use electrophoretic mobility solely as a means to distinguish whether the band is caused by a genuine M-protein or a mAb drug. This is because each individual myeloma patient has an M-protein with a unique electrophoretic migration pattern, some of which may closely approximate that of the mAb drugs. This difficulty is even more pronounced in patients in complete remission, with no visible pathologic M-protein visible by immunofixation.

One potential strategy for circumventing the issues created by treating multiple myeloma patients with mAb therapies has been recently proposed [9-11]. This approach makes use of an anti-idiotypic monoclonal antibody targeted to the variable region of daratumumab. Binding of this antibody to daratumumab changes the electrophoretic mobility of this therapeutic antibody. Thus, pretreatment of a patient specimen with this antibody reagent will shift the visible bands caused by daratumumab compared to an untreated specimen, allowing the reviewing pathologist to distinguish a pathologic M-protein from one caused by a therapeutic mAb. Substantial development has been completed with the DIRA assay[12]. Similar assays are currently under development for elotuzumab and isatuximab. These assays are anticipated to become commercially available eventually. However, none have gained FDA approval as of this writing. Furthermore, the DIRA assay is labor-intensive, and also requires additional lanes of immunofixation, significantly increasing costs for the laboratory. It may also significantly increase turnaround time.

Other potential approaches beyond anti-idiotypic monoclonal antibodies include the use of mass spectrometry for the quantitation of patient monoclonal proteins[10, 13-15]. Alternatively, this approach can also be applied to the detection and quantitation of therapeutic monoclonal immunoglobulins in serum, including infliximab, adalimumab, eculizumab, vedolizumab, and rituximab. A method utilizing Electrospray Time of Flight

mass spectrometry has been developed which are able to detect these mAbs at concentrations well below the detection threshold of SPEP and IFE [16]. However, both of these technologies are still in their infancy, and it will take many years before use of such technology becomes standard practice.

The appearance of therapeutic monoclonal antibodies on serum protein electrophoresis and immunofixation have been previously reported[1], in the setting of therapeutic administration of Siltuximab to a multiple myeloma patient. More recently, a study by Ruinemans-Koerts et al[17] has demonstrated the effect of 8 separate mAb therapeutics on serum protein electrophoresis and immunofixation assays. However, our study is, to our knowledge, the first study to examine the effect of a range of mAb drugs that are specifically targeted towards the treatment of multiple myeloma.

One strategy that may be used, and is in use at our institution, is to ensure that review of all SPEP and IFEs are performed in conjunction with a record of the patient's original disease clone requiring monitoring. Our laboratory currently maintains a digital library of all SPEP/IFE's searchable by medical record number which is available to all reviewing scientists/pathologists. Using this method, the reviewing scientist/pathologist is able to determine, based on the electrophoretic mobility and immunoglobulin isotype, whether the M-protein visible on the SPEP/IFE is consistent with the patient's previous history of monoclonal gammopathy. At our institution, every effort is made by the reviewing physician/scientist to identify a patient's original pathologic clone, including the immunoglobulin subtype and its electrophoretic mobility, either through review of a patient's initial SPEP/IFE results or through review of patient medical records. This ensures that any M-protein values as well as the presence of any monoclonal bands visible by immunofixation can be appropriately identified as a recurrence of the patient's myeloma vs bands caused by oligoclonal reconstitution or other therapeutic monoclonal antibodies. It is however important to keep in mind that this strategy also has its limitations. For example, some patients arrive at an institution having been previously treated for their myeloma. These patients often have no discernible M-protein in their serum or urine, either by SPEP or IFE. In such cases, our reviewing scientists and pathologists make every attempt to correlate SPEP/IFE results with patient medical history, pathology and/or flow cytometry results, or even external medical records, if available. As an additional measure, implementing a change in the test ordering system to require physicians/practitioners ordering SPEP/IFE's to state whether a patient is currently on or has a history of receiving monoclonal antibody therapy may also be helpful. This will alert the laboratory when a specimen is received from a patient receiving therapeutic monoclonal antibodies.

CONCLUSION

The treatment of multiple myeloma patients with monoclonal antibodies results in a visible and quantifiable M-protein that has the potential to falsely indicate poor response to therapy. Both laboratorians and clinicians need to be aware of the possibility of interference by these agents when evaluating responses in multiple myeloma patients receiving these therapies. Strategies are urgently needed to overcome this issue as monoclonal antibody therapy is likely to become part of standard of care for relapsed and even newly diagnosed multiple

myeloma patients in the near future. Until the methods previously discussed become readily available, it is strongly suggested that laboratories make efforts to ensure that SPEP/IFE results from patients treated with monoclonal antibody therapy are always correlated with past history of monoclonal gammopathy.

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Highlights

- Monoclonal antibodies used for the treatment of myeloma cause false positive M-protein bands in serum which can be visualized by serum protein electrophoresis and immunofixation electrophoresis
- False positive bands can be seen in sera from patients receiving elotuzumab
- Monoclonal antibody drugs have no effect on serum free light chain quantitation, but increase IgG, IgG κ , and IgG κ /IgG λ -ratios by heavy/light chain assays

Figure 1.

Serum protein electrophoresis and immunofixation of specimens supplemented with various anti-myeloma therapeutic mAbs.

a-d: Serum from a healthy volunteer (a), with 834 ug/mL isatuximab (b), with 707 ug/mL daratumumab (c), with 625 ug/mL elotuzumab (d).

e-g: Serum from a multiple myeloma patient in remission (e), with 707 ug/mL daratumumab (f), with 625 ug/mL elotuzumab (g)

h-j: Serum from a multiple myeloma patient with a visible M-spike by protein electrophoresis and immunofixation (h) , with 707 ug/mL daratumumab (i), with 625 ug/mL elotuzumab (j)

Figure 2.

Serum protein electrophoresis and immunofixation of serum drawn from patients treated with elotuzumab.

a, b: Myeloma patient A serum 1 week post dose 4 (2a), 1 week post dose 12 (2b)

c, d: Myeloma patient B serum 1 week post dose 3 (2c), and 1 week post dose 6 (2d)

e-g: Myeloma patient C serum pre dose 1 (2e), 1 week post dose 3 (2f), 1 week post dose 4 (2g)

Table 1
Laboratory test results of serum specimens supplemented with therapeutic anti-myeloma monoclonal antibodies

description	total protein (g/dL)	free kappa (mg/dL)	free lambda (mg/dL)	kappa/lambda ratio	patient disease M-spike value (g/dL)	mAb induced M-spike value (g/dL)	IgG (mg/dL)	IgGK (mg/dL)	IgGL (mg/dL)	Hevylite IgGK/IgGL ratio
reference ranges	6.3-8.1	0.33-1.94	0.57-2.63	0.26-1.65	N/A	N/A	650-1600	384-1207	191-674	1.12-3.21
CV (%)	1.5	2.3	3.1	N/A	N/A	N/A	1.1	2.56	7.32	N/A
healthy volunteer	6.7	0.56	1.14	0.49	N/A	0	703	521	380	1.37
healthy volunteer diluted with saline (70% serum/30% saline)	4.5	0.44	0.93	0.47	N/A	0	457	352	256	1.38
healthy volunteer serum with 834 ug/ml isatuximab	4.6	0.42	0.92	0.46	N/A	0.12	556	381	265	1.44
healthy volunteer serum with 707 ug/ml daratumumab	6.6	0.61	1.15	0.53	N/A	0.17	741	671	424	1.58
healthy volunteer serum with 625 ug/ml elotuzumab	6.7	0.6	1.09	0.55	N/A	0.21	764	546	364	1.50
healthy volunteer serum with 1667 ug/ml isatuximab	4.7	0.44	0.93	0.47	N/A	0.21	640	437	227	1.93
healthy volunteer serum with 1413 ug/ml daratumumab	6.5	0.67	1.17	0.57	N/A	0.23	784	727	416	1.75
healthy volunteer serum with 1250 ug/ml elotuzumab	6.5	0.68	1.11	0.61	N/A	0.26	788	601	346	1.73
myeloma patient #1	6.5	1.53	0.75	2.04	0	0	725	n/a	n/a	n/a
myeloma patient #1 serum with 707 ug/ml daratumumab	6.8	1.56	0.68	2.29	0	0.17	833	777	313	2.49
myeloma patient #1 serum with 625 ug/ml elotuzumab	6.7	1.55	0.71	2.18	0	0.2	813	754	281	2.69
myeloma patient #2	5.7	4.3	1.1	3.91	0.24	0	330	326	30	11.04

description	total protein (g/dL)	free kappa (mg/dL)	free lambda (mg/dL)	kappa/lambda ratio	patient disease M-spike value (g/dL)	mAb induced M-spike value (g/dL)	IgG (mg/dL)	IgK (mg/dL)	IgGL (mg/dL)	Hevylite IgGK/IgGL ratio
myeloma patient #2 serum with 707 ug/ml daratumumab	5.8	4.74	0.99	4.79	0.27	NQ*	397	440	35	12.43
myeloma patient #2 serum with 625 ug/ml elotuzumab	5.9	4.65	1.07	4.35	0.19	0.11	408	390	23	16.97

° unspiked control for the isatuximab spiked serum

* not quantifiable due to overlap of mAb M-spike and patient M-spike

Table 2

Laboratory test results of 3 myeloma patients treated with elotuzumab

description	myeloma type	total protein (g/dL)	free kappa (mg/dL)	free lambda (mg/dL)	kappa/lambda ratio	patient disease M-spike value (g/dL)	elotuzumab M-spike value (g/dL)	IgG (mg/dL)	IgGK (mg/dL)	IgGL (mg/dL)	Hevlyte IgGK/IgGL ratio
reference ranges		6.3-8.1	0.33-1.94	0.57-2.63	0.26-1.65	0	0	650-1600	384-1207	191-674	1.12-3.21
CV (%)		1.47	2.3	3.1	N/A	N/A	N/A	1.1	2.56	7.32	N/A
patient A: 1 week post dose 4	free κ	5.7	100.94	0.05	2018.80	not visible by SPEP	0.06	359	270.2	145	1.86
patient A: 1 week post dose 12		6.0	212.1	0.05	4242.00	not visible by SPEP	0.13	356	271.7	161.5	1.68
patient B: 1 week post dose 3	IgAλ	6.9	0.06	95.34	0.00	1.27	not visible by SPEP	294	206.1	258.6	0.80
patient B: 1 week post dose 6		9.3	0.07	147.7	0.00	4.68	not visible by SPEP	219	164.9	199.8	0.83
patient C: pre dose 1	IgAλ	7.4	0.05	3.7	0.01	1.64	N/A	243	182.8	192.7	0.95
patient C: 1 week post dose 3		8.0	0.05	7.16	0.01	2.39	0.1	233	179.8	160.5	1.12
patient C: 1 week post dose 4		8.0	0.05	8.4	0.01	2.65	0.1	201	168.1	139.2	1.21