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A Novel Approach to Remove Interference of Therapeutic Monoclonal Antibody with Serum Protein Electrophoresis

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Abstract

Objectives: Multiple myeloma (MM) is characterized by malignant growth of plasma cells, usually producing a monoclonal antibody (mAb). New treatments for MM include therapeutic monoclonal antibodies (tmAbs), but patients treated with tmAb demonstrate interference on serum electrophoresis (SPE) and immunoprecipitation electrophoresis (IEP). Evaluation of treatment efficacy and determination of MM remission include SPE and IEP which identifies mAb, but cannot differentiate between disease associated mAb and tmAb. We hypothesized that tmAb could be removed from patient sera before testing by SPE and IEP to provide accurate diagnoses for clinicians.

Design and Methods: We developed the Antigen Specific therapeutic monoclonal Antibody Depletion Assay (ASADA), that utilizes magnetic beads coated with the cognate antigen of the tmAbs, to deplete two different tmAb (daratumumab, elotuzumab) from saline and patient sera and assessed for complete removal of tmAb by SPE and IEP.

Results: We found that tmAb could be efficiently removed from saline and patient sera. ASADA demonstrated acceptable analytical specificity and sensitivity in IEP. Recovery of appropriate quantitative values by SPE was demonstrated with clinically acceptable precision. A single bead cocktail could be used to treat both daratumumab and elotuzumab.

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Declarations of Interest

All authors are inventors on a patent application that includes this work, in keeping with the intellectual property requirements of their institution.

Conclusions: This demonstrates proof of principle that ASADA can be used to remove current and future tmAb from patient sera, regardless of platform. This research provides for accurate diagnosis, disease monitoring, and remission status in MM patients being treated with tmAb.

Keywords

Daratumumab; monoclonal gammopathy; therapeutic monoclonal antibody; plasma cell myeloma

1. Background

Multiple myeloma (MM) is an incurable hematologic malignancy. Traditional therapies, including immunomodulatory drugs, proteasome inhibitors, cytotoxic agents and autologous hematopoietic stem cell transplant, have prolonged the average survival to 4–5 years [1,2]. However, this may be further improved by the introduction of therapeutic monoclonal antibodies (tmAb) approved by the Food and Drug Administration (FDA) and the National Institute for Health and Care Excellence (NICE), daratumumab and elotuzumab [3–7]. Daratumumab is a human IgG1/ κ tmAb against plasma cell surface antigen CD38, and elotuzumab is a humanized IgG1/ κ targeting a self-ligand receptor, signaling lymphocytic activation molecule family member 7 (SLAMF7). Due to its efficacy and response rate [6,8], daratumumab was recently approved by the FDA as a frontline therapy for newly diagnosed MM and by NICE for second line therapy [5,9].

As daratumumab gains popularity in treating MM, it complicates the monitoring of myeloma patients. MM is typically monitored by the detection of the disease-associated mAb as an M-protein by serum protein electrophoresis (SPE) and immunoprecipitation (IEP), as well as the percentage of bone marrow plasma cells, and free light chain ratios [6,10–12]. As tmAbs, daratumumab and elotuzumab can be readily detected by SPE and IEP [9–13]. We recently reported that about 11 % of our total SPE and IEP cases had daratumumab interference in determination of tmAb and disease associated mAb. Identifying daratumumab by its electrophoretic migration pattern is inefficient, only 46.7 % of the cases with suspected daratumumab due to the characteristic migration pattern were actually on daratumumab [14]. Additionally, there are many new tmAb in development that will require tmAb and disease associated mAb differentiation [15]. Therefore, new assays that can remove tmAb or are not subject to the interference of tmAb are needed.

Currently, Sebia's Hydrashift 2/4 daratumumab is the only reagent approved by the FDA to mitigate daratumumab interference on Sebia's semi-automated gel platform HYDRASYS 2 [16,17]. It is a gel shift assay that uses an anti-daratumumab antibody to form a complex with daratumumab and thereby shift the daratumumab migration pattern during electrophoresis. This requires the patient sample to be tested in duplicate, in the presence and absence of anti-dara antibody for SPE and/or IEP. If a band in question migrates to a different position in the presence of anti-dara antibody, the band is most likely caused by daratumumab [17].

In addition to the Hydrashift assay, mass spectrometry based assays are not subject to tmAb interference and can identify tmAbs based on their accurate molecular mass, circumventing the need to develop additional biological reagents [18–20]. However, they require expensive

equipment and extensive expertise to implement. Other assays that are not platform-specific, do not require expensive equipment, and have the potential to detect or remove different tmAbs at the same time are highly desirable.

Here we report a novel approach, Antigen Specific therapeutic monoclonal Antibody Depletion Assay (ASADA), that utilizes magnetic beads coated with the cognate antigen of the tmAbs to deplete the respective tmAb in patient samples. This method is highly specific, simple to use, and circumvents the need to develop new anti-sera for each new tmAb, more importantly, it allows depletion of various tmAbs with a single assay.

2. Materials and Methods

2.1. Patient samples

Patient samples sent for SPE and IEP to the University of Pittsburgh Medical Center Immunopathology Laboratory were tested by ASADA. Serum samples were collected in serum separator tubes and were processed at the central laboratory with centrifugation at 1932 g for 7 minutes at room temperature on the Beckman Coulter automated line. The samples were then delivered to the Clinical Immunopathology Laboratory for SPE and IEP. This work was carried out under the auspices of the UPMC Quality Assurance Board # 1072.

2.2. Materials and reagents

Daratumumab (Janssen Pharmaceuticals, Inc. NJ, USA) and elotuzumab (Bristol-Myers Squibb, New York City, NY, USA) were purchased from the research pharmacy at UPMC. Daratumumab is in 20 mg/mL solution. Elotuzumab is reconstituted with water to obtain a concentration of 25 mg/mL per manufacturer instructions. Both drugs were stored at 2–8 °C and used before their expiration date. Stability was confirmed by continued appearance of the monoclonal band at the appropriate location by electrophoresis.

Magnetic beads were from Invitrogen (Dynabeads, Catalog No 10104D, Carlsbad, CA). His-tag human CD38 protein (Catalog No 10818-H08H) and his-tag human SLAMF7 (11691-H08H) were from Sino Biological Inc. (Wayne, PA). Both recombinant proteins were purchased in lyophilized aliquots and were reconstituted within 1 week of use. All reagents were used before their expiration date. Bead binding of antigen was verified by performance of a Pierce bicinchoninic acid assay (Thermo Fisher Scientific, Waltham MA, USA) to determine protein concentration before and after antigen binding to the beads. tmAb spiked patient sera was utilized as a control to ensure beads coated with cognate antigen continued to remove the anticipated concentration of tmAb.

2.3. ASADA

We coated Dynabead His-Tag magnetic beads with His-tagged CD38 or SLAMF7, and utilized the coated beads to deplete daratumumab and/or elotuzumab.

The magnetic bead His-Tag depletion was optimized using 100 µl (0.20 g/L) his- tagged human CD38 to coat 25 µl, 50 µl, or 100 µl beads to achieve a CD38: daratumumab molar ratio of 1:1, 1:1.6, or 1:2 respectively. Briefly, beads were thoroughly resuspended before

transferring to a microcentrifuge tube. The beads were collected by placing the microcentrifuge tube on a magnet for 2 minutes then the supernatant was discarded. His-tagged human CD38 or SLAMF7 100 μ l (0.20 g/L) in 1 $\times$ binding buffer were added to the beads and incubated on a roller for 10 min at room temperature. The beads were collected and washed four times with 300 μ l wash buffer. After the final wash, 100 μ l patient serum, daratumumab- or elotuzumab-spiked blank serum or saline solution were added to the beads and rotated overnight at room temperature to deplete daratumumab or elotuzumab in the samples. The supernatant serum was then collected for SPE and IEP after placing the tubes on magnetic plate for 2 min. Naïve beads not coated by CD38 or SLAMF7 were used as a control for each sample.

For beads coated with both CD38 and SLAMF7, 100 μ l beads were incubated with his-tagged human CD38 and SLAMF7, 100 μ l each (0.20 g/L).

2.4. SPE and IEP

SPE and IEP were performed on the Helena SPIFE 3000 analyzer (Texas, USA) according to manufacturer's protocol with all reagents recommended by the manufacturer. Helena Electrophoresis Sample Handler was used to automatically dilute and load serum samples (ESH). Serum total protein is established by using a digital refractometer (Index Instruments U.S., Inc). Antisera to IgG, IgA, IgM, Kappa and Lambda for IEP are from the SPIFE ImmunoFix Kits.

3. Results

3.1. Optimization of ASADA

Therapeutic monoclonal antibodies are directed against specific antigens and we hypothesized that we might be able to leverage this binding to eliminate monoclonal antibody interference in SPE and IEP. We used daratumumab as our first model system with its antigen CD38. We attempted the Antigen Specific therapeutic monoclonal Antibody Depletion Assay (ASADA) with daratumumab spiked in saline (0.40 g/L). Cmax for daratumumab is 0.90 g/L and most patients in our institution are tested at minimum after the first half life [21]. Different volumes of CD38-coated beads were used to deplete a known amount of daratumumab to find the optimal molar ratio of CD38: daratumumab. As shown in Fig 1, daratumumab is visible as a distinct monoclonal band by SPE and as IgG/ κ by IEP when antigen naïve beads were used. With CD38-coated beads, the depletion of daratumumab increases as the CD38: daratumumab molar ratio increases. With a 2:1 of CD38: daratumumab molar ratio, a complete visible depletion of daratumumab was achieved while a 1.6:1 left a faintly visible band in the IgG lane. Therefore, the 2:1 molar ratio was considered the optimal molar ratio and was used in further experiments.

3.2. ASADA optimization for daratumumab or elotuzumab in serum

We next tested the ability of ASADA to successfully deplete daratumumab or elotuzumab in the more complex milieu of serum. We tested hypo-, normo- and hyper-gammaglobulin serum spiked with daratumumab or elotuzumab (0.40 g/L) using ASADA. When antigen naïve beads were used as controls, daratumumab exhibited a light cathodal band by SPE

(Fig. 2A) and cathodal IgG/ κ by IEP (Fig. 2B). CD38-coated beads with CD38: daratumumab molar ratios at 2:1 completely removed the daratumumab monoclonal band by both SPE and IEP, even in hypogammaglobulin serum where the detection limit of daratumumab is lower than that of normo- or hyper-gammaglobulin serum [14]. The same results were achieved with serum spiked with elotuzumab, using its antigen SLAMF7, by both SPE (data not shown) and IEP (Fig. 3).

3.3. Sensitivity, precision, and specificity of ASADA

ASADA is a sample treatment that must be tested before assay implementation. As an assay ASADA introduces a 20% dilution of the sample in its current implementation. We first assessed neat samples compared to naïve and CD38/SLAMF7 ASADA and found only minor changes in visual intensity of the SPE and IEP (Supplemental Fig. 1). Patients with hyper-, normal, and hypo- gammaglobulin regions were spiked with varying concentrations of daratumumab to assess the sensitivity of ASADA (Fig. 2). We found that daratumumab was visible and specifically removed by ASADA at 0.80 g/L in hypergammaglobulinemia, 0.40 g/L in normal gammaglobulin levels, and 0.20 g/L in hypogammaglobulinemia in SPE (Fig. 2C) and IEP (Fig. 2D).

We assessed precision of ASADA in SPE by spiking hypergammaglobulinemic and hypogammaglobulinemic patient sera with daratumumab and treating with ASADA for both naïve beads and CD38 beads (Table 1). SPE were quantified using the total protein measurement from the unprocessed sample. Differences between the original sample quantitation and ASADA treated sample quantitation demonstrated clinically acceptable accuracy (Table 1).

Analytical specificity was confirmed using sera from patients not on tmAb but with disease associated monoclonal proteins that co-run with tmAb. tmAb was then added to patient sera and samples were treated with ASADA for daratumumab and elotuzumab (Supplemental Fig. 1). Disease associated mAb remained and reductions in M-spike concentration were noted after ASADA (Supplemental Fig. 1).

3.4. ASADA in specimens from patients receiving daratumumab therapy

To determine if ASADA could successfully and specifically deplete daratumumab in the clinical laboratory, we tested remnant specimens from patients whose initial IEP results indicated the possibility of daratumumab treatment (known cathodal IgG/ κ bands; N=18 patient specimens). SPE (Fig. 4A and Supplemental Fig. 2) and IEP (Fig. 4B and C) results demonstrated that the cathodal bands completely disappeared after ASADA in samples 1–5, 10, 12–18. Review of electronic medical records confirmed daratumumab therapy of these patients. In contrast, ASADA for daratumumab failed to remove the cathodal bands in samples 6, 7, 9, and 11. Chart reviews revealed that these patients were not on daratumumab therapy, demonstrating that these cathodal bands represented endogenous mAbs and appropriate specificity of the assay. The patient from sample 8 was on daratumumab therapy, however, the high concentration endogenous IgG/ κ co-migrated with daratumumab causing the persistence of cathodal IgG/ κ band after ASADA. These results demonstrated the specificity of ASADA for daratumumab. Four samples were also re-tested in a second run

(samples 1,2,4,5 correspond to 13,14,16,17 respectively), which achieved the same results, confirming the repeatability of the assay.

3.5. ASADA for both daratumumab and elotuzumab

The advantage of ASADA is that it can be multiplexed with multiple antigens used to coat the solid support. To test the hypothesis that ASADA can be used as a single assay for depletion of two different mAbs, we coated the beads with CD38 and SLAMF7, to deplete either daratumumab or elotuzumab in serum samples. The double-coated beads depleted either daratumumab or elotuzumab in spiked serum (0.40 g/L) as evidenced by SPE (Fig. 5A) and IEP (Fig. 5B).

4. Discussion

With current and future tmAbs as promising therapies for MM and other diseases, monitoring for MM by SPE and IEP becomes complicated [15]. Currently, most tmAbs are monoclonal IgG/ κ , including daratumumab and elotuzumab. Although daratumumab has a characteristic far-gamma electrophoretic migration pattern, our previous work demonstrated that it may be insufficient to identify daratumumab by its migration pattern alone [14]. Elotuzumab and tmAb currently in clinical trials have migration patterns that are less obvious and more commonly co-run with patient disease mAb (19). Moreover, several tmAbs are under development or clinical trials for the treatment of MM are not IgG/ κ mAbs [22]. All these aspects render an accurate estimation of MM therapeutic response by SPE and IEP a continuous challenge, though serum free light chain analysis has been shown to be unaffected by tmAb treatment [14].

To mitigate this problem, assays to remove daratumumab interference using anti-daratumumab specific antisera to treat patient samples have been developed. Such assays are specific to daratumumab and will have no effect on a different tmAb. To remove the interference of another tmAb, a new antisera will need to be developed and approved by governing agencies. Assays not subject to tmAb interference, such as mass spectrometry assays, are not currently available to most hospital laboratories due to the requirement of expensive equipment and extensive expertise [18–20]. Therefore, an assay that does not require the development of new antisera, is capable of removing the interference of multiple tmAbs with a single reagent, and does not require specialized equipment, is highly desirable.

ASADA utilizes magnetic beads coated with cognate antigens to deplete tmAbs, for example, CD38 for daratumumab depletion and SLAMF7 for elotuzumab depletion. Unlike developing new antisera, the soluble protein antigens are readily available. Coating the magnetic beads with soluble antigens is simple, and the beads (or any solid support) can be used to treat patient serum samples to deplete tmAbs. Therefore, this method is easy to use and has the potential to be a laboratory developed test. Furthermore, the specific antigen-antibody interaction between tmAbs and their cognate antigens allows for high assay specificity. We have shown that the coated beads as well as the control beads do not markedly reduce the visible intensity of protein bands other than daratumuamb (Fig. 4, Supplemental Fig. 1). We have also demonstrated that the sample pretreatment by ASADA provides acceptable precision and accuracy (Table 1) for SPE and appropriate analytical

specificity (Fig. 4, Supplemental Fig. 1) and analytical sensitivity (Fig. 2) for SPE and IEP. Due to the low C_{max} of elotuzumab (0.22 g/L) specimens of patients being treated with elotuzumab are rarely identified at our institution and we were unable to test any.

Further testing in patients dosed with elotuzumab is warranted. ASADA introduces a sample treatment step that is, at this stage, manual. This increases the potential for error as well as increasing testing time for samples requiring this treatment. There is economic advantage to reducing the quantity of antigen used in the assay, however, in clinical laboratory implementation it is vital that sufficient antigen be used to supercede the highest possible dosing at the earliest possible specimen draw timepoint. Finally, ASADA in the current formulation has a 20% dilution. This has a small effect on the visual intensity of the IEP, allowing for robust visual interpretation. In future work we anticipate designing buffers that are more concentrated allowing for addition of only 1–5% of the sample volume.

A major advantage of ASADA is its multiplex potential, as the beads can be coated with multiple antigens. In contrast to the current one assay, one drug paradigm, a bead cocktail can be used as one reagent to deplete current and new tmAbs as they enter use. We have proven this concept by demonstrating that beads coated with both CD38 and SLAMF7 can be used to deplete either daratumumab or elotuzumab (Fig. 5). Likewise, a mixture of beads each coated with CD38 or SLAMF7 can be used. With the anticipated emergence of new tmAbs for MM therapy, the cognate antigen of the new tmAb can be easily added to the bead cocktail and validated, avoiding the wait for the development of new assays.

Implementation of this assay as a laboratory developed test provides several avenues of effective use. Our preferred implementation strategy is to create a new test code within the hospital information system for patients on tmAb. A standard SPE and IEP will be available as well as an SPE and IEP for patients on tmAb. Samples ordered under the latter test code can be immediately treated with ASADA to ensure the fastest turn around of patient results. Alternately ASADA may be used as a confirmatory assay when an IgG kappa is concerning for tmAb rather than disease. This is a feasible, though inefficient approach. We have shown that only about half of cases suspected to have daratumumab interference were receiving daratumumab treatment [14]. Additionally, as new tmAb are introduced their electrophoretic pattern may be more complex to differentiate from disease. Turn around time and expense is also significantly increased under this model as preliminary testing must first be performed.

We have developed a new method to remove the interference of tmAbs on SPE and IEP that is simple to use, multiplexed, and highly specific. ASADA can be incorporated into diagnostic workflow as a laboratory developed test with one reagent to detect the presence of multiple potential tmAbs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

MM	Multiple Myeloma
SPE	Serum protein electrophoresis
IEP	Immunoelectrophoresis
mAb	Monoclonal antibody
tmAb	Therapeutic monoclonal antibody
ASADA	Antigen Specific therapeutic monoclonal Antibody Depletion Assay

5. Reference

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Highlights

- Therapeutic monoclonal antibodies cause interference in serum electrophoretic methods
- Antigens can be used to deplete therapeutic antibodies before testing
- Novel depletion assay (ASADA) removes therapeutic antibody interference
- ASADA can be multiplexed to provide a single test for all therapeutic antibodies

Fig 1. ASADA for daratumumab in saline.

Immunofixation results of Daratumumab spiked in saline (0.40 g/L) treated with naïve beads (left panel), and CD38-coated beads with different molar ratios of CD38: daratumumab (right panel).

Fig. 2. ASADA for daratumumab in serum with different levels of gammaglobulin.

Serum protein electrophoresis (A) and immunofixation (B) results of daratumumab (0.40 g/L) spiked in hypo-, normo- and hyper-gammaglobulin serum in the presence of naïve beads (upper panel) and CD38-coated beads (lower panel). Analytical sensitivity of serum protein electrophoresis (C) and immunofixation (D) of daratumumab spiked at 0.20 g/L and 0.80 g/L in hypo-, normo- and hyper-gammaglobulin serum in the presence of naïve beads (upper panel) and CD38-coated beads (lower panel). Arrow: cathodal migration pattern of daratumumab band.

Fig. 3. ASADA for elotuzumab in serum with different levels of gammaglobulin. Immunofixation results of elotuzumab (0.40 g/L) spiked in hypo-, normo- and hypergammaglobulin serum in the presence of naïve beads (upper panel) and SLAMF7-coated beads (lower panel). Arrow: elotuzumab migrates in the middle of γ zone.

Fig. 4A. Serum protein and immunofixation electrophoresis of ASADA for daratumumab native patient samples.

Sample 1 and 13, 2 and 14, 4 and 16, 5 and 17 are four pairs of the same sample tested in two separate runs. Samples were from patients under known daratumumab therapy except for samples 6, 7, 9, 11. 1–18, number of samples. (A) Native patient samples with cathodal IgG/κ bands were used in ASADA for daratumumab. Ctr: depletion with naïve control beads. CD38: depletion with CD38-coated beads. Arrow, the cathodal bands that were tested in the ASADA assay.

Fig. 4B.

Immunofixation electrophoresis of native patient samples. Arrow, the cathodal IgG/κ bands present in all sample being tested in the ASADA assay.

Fig. 4C.
Immunofixation electrophoresis of native patient sample after ASADA for daratumumab.

Fig. 5. ASADA for both daratumumab and elotuzumab.

Serum protein electrophoresis (A) and immunofixation electrophoresis (B) of CD38 and SLAMF7 double-coated beads deplete daratumumab- (Dara) or elotuzumab- (Elo) spiked serum (bland serum spiked with 0.40 g/L of daratumumab or elotuzumab). Control (Ctr), depletion with naïve beads; Depletion (D), depletion with double-coated beads.

Table 1.

Precision and accuracy of SPE protein components after ASADA treatment.

		Albumin g/L	Alpha 1 g/L	Alpha 2 g/L	Beta g/L	Gamma g/L	M-spike naïve beads	M-spike CD38 beads
Hypergamma 0.80 g/L Dara	Original sample	36.4	2.3	8.9	8.8	19.7		
	Average (ASADA)	37.4	2.5	9.5	7.8	18.8	3.3	2.9
	CV	4%	6%	8%	9%	5%	2%	6%
	Original- ASADA	1.0	0.2	0.6	1.0	0.9		
		n = 8	n = 8	n = 8	n = 8	n = 8	n = 4	n = 4
Hypogamma 0.60 g/L Dara	Original sample	36.0	2.4	8.8	8.2	4.6		
	Average (ASADA)	35.5	3.1	10.0	5.7	5.8	1.4 ^a	1.1 ^a
	CV	3%	3%	4%	8%	16%	4%	4%
	Original- ASADA	0.5	0.7	1.2	2.6	1.2		
		n = 8	n = 8	n = 8	n = 8	n = 8	n = 4	n = 4
Hypogamma0.40 g/L Dara	Original sample	36.0	2.4	8.8	8.2	4.6		
	Average (ASADA)	35.4	3.1	10.2	5.8	5.6	1.3 ^a	1.0 ^a
	CV	3%	5%	5%	6%	14%	6%	14%
	Original- ASADA	0.6	0.7	1.4	2.4	0.9		
		n = 8	n = 8	n = 8	n = 8	n = 8	n = 4	n = 4

^aMeasurement range for SPE is not considered quantitatively accurate <2.0 g/L, numeric data is provided here for reference.