TO THE EDITOR:

A universal solution for eliminating false positives in myeloma due to therapeutic monoclonal antibody interference

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Multiple myeloma (MM) is a malignant plasma cell disorder resulting in expansion of clonal plasma cells that encode for a unique monoclonal immunoglobulin (M-protein). The M-protein is derived from recombination and somatic hypermutation events occurring at both the heavy- and light-chain loci in the precursor B cell. As a result, the M-protein has a distinct amino acid sequence and corresponding molecular mass that can serve as a patient-specific M-protein biomarker.1 The traditional methods of M-protein detection, protein gel electrophoresis and capillary electrophoresis, have limited resolution, impeding their ability to separate multiple bands. This has resulted in the inability to accurately stratify therapeutic responses for some immunoglobulin G (IgG) κ MM patients whose M-protein comigrates with the monoclonal therapeutic antibody (t-mAb) + Unique t-mAb

Retention Time

977.04 m/z

23,468 Da

Isatuximab

979.21 m/z

23,423 Da

Elotuzumab

975.31 m/z

23,381 Da

Daratumumab

Figure 1. Determining t-mAb from M-protein by using unique parameters from liquid chromatography and mass measurements. Extracted ion chromatograph of the +24 charge state for each t-mAb with the indicated retention time (left). Corresponding accurate molecular mass of each respective t-mAb (middle). Representative examples of miRAMM results for patient samples with M-proteins comigrating with the indicated t-mAb (right).

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used for treatment. Misclassification of therapeutic responses in IgG MM patients receiving daratumumab and elotuzumab is of concern, as studies involving drug effectiveness are dependent on accurate clinical response classification. Given the growth in use of t-mAbs in the treatment of MM patients and the introduction of combination t-mAb therapeutic approaches, this analytical limitation is likely to be exacerbated. A method to circumvent this limitation was recently developed for daratumumab. The daratumumab-specific reflex assay utilizes current gel electrophoresis methods but incorporates a gel-shift assay to "shift" the migration of daratumumab away from endogenous M-proteins to alleviate confusion over interpreting gel electrophoretic patterns in patients receiving daratumumab. However, this assay is only applicable to daratumumab and maintains the other analytical limitations of electrophoretic methods.

Recently, mass spectrometry (MS) approaches have been developed to identify M-proteins using high-resolution molecular mass measurements that achieve superior sensitivity compared with traditional methods. Several reports have demonstrated that MS can provide a solution for resolving t-mAb interferences in MM patients. We assessed the analytical ability of previously described MS method termed monoclonal immunoglobulin rapid accurate mass measurement (miRAMM) to positively identify endogenous M-proteins in the context of therapeutic levels of daratumumab, elotuzumab, and isatuximab in patient sera.

To test the effectiveness of miRAMM to resolve interferences, serum immunoglobulin enrichment was performed using nanobodies targeting the heavy chain of IgG, and light chains were reduced from the heavy chains as previously described. An Eksigent Ekspert 200 microLC (Foster City, CA) was used to separate light chains prior to ionization and detection using a SCIEX TripleTOF 5600 quadrupole time-of-flight as previously described. Data analysis was performed using Analyst TF v1.6 and PeakView version 2.2. The mass spectra of the multiply charged light-chain ions were deconvoluted to accurate molecular mass using Bio Tool Kit version 2.2 plug-in software. Mass measurement accuracy was estimated to be 15 ppm over the course of this analysis. Retention times and molecular masses of daratumumab, elotuzumab, and isatuximab light chains were established using residual drug form the manufacturer. Deconvoluted mass spectra were reviewed manually.

Three t-mAbs were investigated: daratumumab, elotuzumab (both of which have been approved by the US Food and Drug Administration and European Medicines Agency), and isatuximab (which is currently being investigated in phase 3 clinical trials). To establish the accurate molecular mass of the t-mAb–associated k light chains (kLCs) as well as their retention times under miRAMM methodology, t-mAbs were diluted in normal human serum to a final concentration of ~0.5 g/dL and tested over at least 3 different runs to confirm reproducibility of retention times and mass accuracy. Once these parameters were established, a cohort of residual patient serum with IgG M-protein migrating within the g-region was collected and diluted to a range of concentrations (0.03 g/dL to 1 g/dL) using normal human serum. Aliquots were made and then spiked with daratumumab (n = 48), elotuzumab (n = 72), or isatuximab (n = 72) at concentrations that mimic expected steady-state serum concentrations (0.01 g/dL to 0.1 g/dL) (based on standard dosing schedules). Aliquots of these samples were then tested by immunofixation electrophoresis (IFE) in a Clinical Laboratory Improvement Amendments–certified laboratory using Hydrasys 9IF.
gels and by miRAMM. The Mayo Foundation institutional review board approved this retrospective study.

The goal of this study was to evaluate the effectiveness of high-resolution MS to differentiate between endogenous disease-associated M-proteins and t-mAbs used in the treatment of MM. On the basis of 3 different, separate measurements for each antibody spiked into serum, analytical parameters were established for each t-mAb as follows: daratumumab, kLC mass of 23 380 ± 1 Da with a retention time of 6.2 ± 0.25 min; elotuzumab, kLC mass of 23 423 Da ± 1 Da with a retention time of 5.7 ± 0.25 min; and isatuximab, kLC mass of 23 488 Da ± 1 Da with a retention time of 6.5 ± 0.25 min. Thus, each t-mAb has both a unique retention time and mass that can be used to differentiate it from an endogenous M-protein (Figure 1).

Reviewers were provided with miRAMM mass spectra to determine if the respective profiles were consistent with the presence of 1 of the 3 t-mAbs, an endogenous M-protein, or both. Using miRAMM, the t-mAb and the endogenous M-protein were correctly differentiated in 100% (192/192) of samples tested regardless of migration patterns observed by IFE.

To corroborate these results, residual serum samples were obtained from patients with a history of IgG MM with detectable M-proteins who were actively receiving daratumumab (n = 17), elotuzumab (n = 2), or isatuximab (n = 2). The endogenous M-protein was readily differentiated from the t-mAb in all 21 cases using miRAMM. While the major concern for t-mAb interferences is the risk of false reporting the presence of an M-protein in patients in complete response, there is also concern that t-mAbs may positively bias M-protein quantitation by protein electrophoresis. Residual patient sera with an endogenous M-protein comigrating with daratumumab was mixed with different concentrations of daratumumab to achieve a 0.3 g/dL final concentrations with the following compositions: 0.27 g/dL daratumumab and 0.03 g/dL comigrating M-protein, 0.15 g/dL daratumumab and 0.15 g/dL comigrating M-protein, and 0.03 g/dL daratumumab and 0.27 g/dL of co-migrating M-protein. These 3 samples were indistinguishable by protein gel electrophoresis and IFE, including the reported M-spike, which was 0.3 g/dL for all samples. In contrast, miRAMM readily differentiated in 100% (192/192) of samples tested regardless of migration patterns observed by IFE.

This study highlights the ability of miRAMM to distinguish t-mAb from residual M-proteins. The potential for an M-protein to have the same retention time and LC mass within ±0.2 Da as a t-mAb is feasible but small. In addition, unlike other tryptic MS approaches, miRAMM did not require protein sequence information prior to analysis. This greatly simplifies the application of miRAMM in the clinical laboratory. This should lead to improved accuracy in defining treatment responses and the amount of unnecessary follow-up testing because of false-positive results due to t-mAbs.

Authorship

Contribution: J.R.M., M.A.V.W., and D.L.M. conceived and designed the study; J.R.M. and M.C.K. performed the experiments; J.R.M., M.C.K., D.L.M., and T.K. analyzed the data; A.D. contributed materials; J.R.M. and D.L.M. wrote the manuscript; and all authors reviewed and approved the manuscript.

Conflict-of-interest disclosure: J.R.M. and D.L.M. have intellectual property and receive royalties related to miRAMM. The remaining authors declare no competing financial interests.

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