A Universal Solution for Eliminating False Positives in Myeloma due to Therapeutic Monoclonal Antibody Interference

Running head: Identification of False Positive M-proteins

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TO THE EDITOR:

Multiple myeloma (MM) is a malignant plasma cell disorder resulting in expansion of clonal plasma cells which encode for a unique monoclonal immunoglobulin (M-protein). The M-protein is derived from recombination and somatic hyper mutation 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 (PEL) and capillary-electrophoresis (CZE), have limited resolution impeding their ability to separate multiple bands. This has resulted in the inability to accurately stratify therapeutic responses for some IgG kappa MM patients whose M-protein co-migrates with the monoclonal therapeutic antibody (t-mAb) used for treatment\(^2,^3\). Misclassification of therapeutic responses in IgG kappa MM patients receiving daratumumab and elotuzumab is of concern as studies involving drug effectiveness are dependent on accurate clinical response classification\(^4,^5\). 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 (DIRA) utilizes current gel electrophoresis methods but incorporates 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\(^6\).

However, this assay is only applicable to daratumumab and maintains the other analytical limitations of electrophoretic methods\(^7\).

Recently, mass spectrometry (MS) approaches have been developed to identify M-proteins using high resolution molecular mass measurements that achieve superior sensitivity compared to
traditional methods\textsuperscript{8-10}. Several reports have demonstrated that MS can provide a solution for resolving t-mAb interferences in MM patients \textsuperscript{11-13}. 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, isatixumab and elotuzumab in patient sera.

To test the effectiveness of miRAMM to resolve interferences, serum immunoglobulin enrichment was performed using nanobodies targeting the heavy chain (HC) of IgG and light chains were reduced from the heavy chains as previously described \textsuperscript{10}. 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 (Q-TOF) as previously described \textsuperscript{14}. Data analysis was performed using Analyst TF v1.6 and PeakView ver. 2.2. The mass spectra of the multiply charged LC ions were deconvoluted to accurate molecular mass using the Bio Tool Kit ver. 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 isatixumab light chains were established using residual drug form the manufacturer.

Deconvoluted mass spectra were reviewed manually.

Three t-mAbs were investigated: daratumumab and elotuzumab, both approved by the FDA and EMA, as well as isatuximab, currently in Phase III clinical trials. To establish the accurate molecular mass of the t-mAb-associated \(\kappa\) light chains (\(\kappa\)) as well as their retention times under miRAMM methodology, t-mAbs were diluted in normal human serum (NHS) 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 kappa M-protein migrating within the \(\gamma\)-region were collected and diluted.
to a range of concentrations (0.03 g/dL-1 g/dL) using NHS. 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)\textsuperscript{15,16}. Aliquots of these samples were then tested by IFE in a CLIA-certified laboratory using Hydrasys 9IF gels and by miRAMM. The Mayo Foundation Institutional Review Board (IRB) 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 $\kappa$LC mass of 23,380 +/- 1 Da with a retention time of 6.2 +/- 0.25 min; elotuzumab $\kappa$LC mass of 23,423 Da +/- 1 Da with a retention time of 5.7 +/- 0.25 min; isatuximab $\kappa$LC 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 which can be utilized 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 a one of the three 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$\kappa$ 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 (CR) there is also concern that t-mAbs may positively bias M-protein quantitation by protein electrophoresis. Residual patient sera with an endogenous M-protein co-migrating 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 co-migrating M-protein; 0.15 g/dL daratumumab and 0.15 g/dL co-migrating M-protein; and 0.03 g/dL daratumumab and 0.27 g/dL of co-migrating M-protein. These three samples were indistinguishable by PEL and IFE, including the reported M-spike which was 0.3 g/dL for all samples. In contrast, miRAMM readily separated the signal from daratumumab from that of the M-protein allowing for a more accurate quantitation (Figure 2). Consistent with this, we have identified serial serum collections in patients during daratumumab therapy that had M-protein concentrations of ~0.1 – 0.3 g/dL that persisted after several months of daratumumab therapy, miRAMM indicated that the M-protein concentration continued to decline while the steady state concentration of daratumumab either remained constant or increased (data not shown).

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 (13), miRAMM did not require protein sequence information prior to analysis. This greatly simplifies the application of miRAMM in the clinical lab. This should lead to improved accuracy in defining treatment responses and the amount of unnecessary follow up testing due to false positive results due to t-mAbs.
Authorship

Contribution: JRM, MAVW and DLM conceived and designed the study; JRM and MCK performed the experiments; JRM, MCK, DLM, KT analyzed the data; AD contributed materials. JRM and DLM wrote the manuscript; and all authors reviewed and approved the manuscript.

Conflict-of-interest disclosure: JRM and DLM have intellectual property and receive royalties related to the miRAMM.

References

15. FDA. daratumumab package insert; 2016.

**Figure Legends**

**Figure 1.** *Left panel*, extracted ion chromatograph of the +24 charge state for each t-mAb with the indicated retention time. *Center panel*, the corresponding accurate molecular mass of each respective t-mAb. *Right panel*, representative examples of miRAMM results for patient samples with M-proteins co-migrating with the indicated t-mAb.

**Figure 2.** A mixing study demonstrating the superior resolution of miRAMM as compared to SPEP and IFE for distinguishing and quantitating M-proteins in the presence of daratumumab. Residual patient sera with an endogenous M-protein co-migrating 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 co-migrating M-protein; 0.15 g/dL daratumumab and 0.15 g/dL co-migrating M-protein; and 0.03 g/dL daratumumab and 0.27 g/dL of co-migrating M-protein.
Figure 1

Unique t-mAb Retention Time + Unique t-mAb Light Chain Mass = Accurate t-mAb Identification

**Isatuximab**
- 977.04 m/z
- 23,488 Da
- M-protein

**Elotuzumab**
- 979.21 m/z
- 23,423 Da
- M-protein

**Daratumumab**
- 975.31 m/z
- 23,381 Da
- M-protein
Figure 2

[Diagram showing M-protein and Daratumumab levels with varying percentages of Dara: 10%, 50%, and 90%.]
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