# Identification of Friend or Foe: The Laboratory Challenge of Differentiating M-Proteins from Monoclonal Antibody Therapies

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**Background:** Since the first monoclonal antibody (mAb) therapy hit the market in 1996, the number of disorders treated with this class of therapeutics has seen tremendous growth, with over 50 antibody-based therapeutics currently approved for use in the US and Europe. This class of therapeutics recently made profound progress in the treatment of refractory multiple myeloma (MM). Treating MM with the mAbs will challenge the laboratory's ability to differentiate exogenous mAbs being used to treat patients from endogenous mAbs associated with disease.

**Content:** An overview of the therapeutic mAbs (t-mAbs) developed for the treatment of MM is provided. The anticipated impact of these therapies on patient care, laboratory testing, and clinical research is discussed. Efforts underway to develop strategies and technologies to help laboratories address the growing challenge of mAb interferences are reviewed.

**Summary:** Laboratories can implement risk mitigation strategies at the preanalytical and postanalytical phase of testing to reduce the likelihood of reporting false-positive M-protein results in patients receiving t-mAbs. However, at the analytical phase of testing, current laboratory methods are ill-suited to differentiate between residual disease and residual drugs. Mass spectrometry–based methods might be best positioned to aid laboratories with the rapidly evolving landscape of MM treatment.

## IMPACT STATEMENT

Patients being screened for monoclonal gammopathies, and those diagnosed with MM and currently undergoing treatment with t-mAbs will benefit from raised awareness of the potential for false-positive M-protein results due to t-mAbs. Here, an informational resource is provided to foster a better understanding of when t-mAbs are likely to be encountered and how the associated risk can be mitigated. This article reviews the emerging role of mass spectrometry as a method of measuring M-proteins and why this is less likely to be negatively affected by t-mAbs compared to current testing methods.

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Multiple myeloma (MM)[2] is the second most common hematological malignancy and makes up approximately 2% of deaths from all cancers. Nearly all cases of MM are preceded by a nonsymptomatic condition known as monoclonal gammopathy of undetermined significance (MGUS), which is defined, in part, by the presence of a monoclonal protein (M-protein) (*1*). In MGUS, it is thought that the immune system generates an abnormal response to antigenic stimulation that leads to a limited clonal proliferation of plasma cells (PCs). Although not clearly defined mechanistically, a "second hit" occurs, leading to a more permanent expansion of a malignant clonal PC. MGUS may progress into a phase known as smoldering multiple myeloma (SMM), where the clonal PC population expands leading to an increased concentration of the circulating M-protein and other features; however, these patients remain clinically asymptomatic. Patients with MGUS convert to MM at a rate of approximately 1%/year, whereas patients with SMM convert to MM at a rate of approximately 10%/year over the first 5 years, which levels out to 1%/year after 10 years (*2*).

MM is considered an incurable disease, which ultimately relapses regardless of the treatment approach (*3*). Newly diagnosed MM patients typically have good responses to initial therapy, resulting in periods of stable disease and symptom control. However, invariably, the disease will eventually progress. At this point, the initial frontline therapy can be reused or alternative frontline therapies can be initiated. In about half of these instances, a clinical response will be achieved. Typically, subsequent relapses occur at increasing frequency and increased likelihood of encountering disease that is unresponsive to frontline therapies (*4*). After multiple rounds of therapy with diminished responses, a patient is considered to have refractory disease.

The last several years has seen the emergence of a new set of therapies active against refractory MM. Two of these new therapies, daratumumab (Darzalex®) and elotuzumab (Empliciti™), were granted Food and Drug Administration (FDA) approval for treatment of relapsed/refractory MM in November of 2015 based on promising clinical trial results in the heavily treated refractory MM population (*5*, *6*). These therapies are now being investigated as either single-agent therapies or as part of the current frontline treatment strategies in active clinical trials (see clinicaltrials.gov).

Daratumumab is a human monoclonal IgG κ antibody with high affinity for the PC surface protein, CD38. Malignant PCs express high levels of CD38, which is thought to play an important role in cell adhesion and maintaining a stromal environment supportive of PC proliferation and survival (*7*, *8*). Anti-CD38 monoclonal antibodies (mAbs) have been proven to be active against myeloma cells in preclinical studies (*9*). Daratumumab elicits tumor killing through a variety of mechanisms including induction of apoptosis through Fc-mediated cross-linking, complement mediate cell killing, antibody-dependent cell-mediated cytotoxicity, and antibody-dependent phagocytosis (*10*–*12*). Early-phase 1/2 clinical trials demonstrated that daratumumab in combination with lenalidomide was effective against relapsed and refractory MM and achieved an objective response rate (ORR) of 88% (*13*). Subsequently, daratumumab was investigated as a single agent in a heavily treated cohort of patients with relapsed/refractory MM. Of 42 patients receiving the highest dose tested (16 mg/kg), an ORR of 36% was achieved including 2 patients who achieved complete remission (*5*). It is noteworthy that no dose-limiting toxicities were encountered

[2] **Nonstandard abbreviations:** MM, multiple myeloma; MGUS, monoclonal gammopathy of undetermined significance; M-protein, monoclonal protein; PC, plasma cell; SMM, smoldering multiple myeloma; FDA, Food and Drug Administration; mAb, monoclonal antibody; ORR, objective response rate; t-mAb, therapeutic mAb; PEL, protein electrophoresis; IFE, immunofixation; CR, complete response; DIRA, daratumumab-specific immunofixation electrophoresis reflex assay; HC, heavy chain; LC, light chain; ESI, electrospray ionization; MS, mass spectrometry.

and, to date, a maximum tolerated dose of daratumumab has yet to be encountered (*5*). This strong body of evidence led to accelerated FDA approval of daratumumab for patients who had previously received 3 prior lines of treatment. Daratumumab quickly affected the treatment structure for refractory MM and may also penetrate the frontline therapy market. Importantly, the success of daratumumab validated the application of therapeutic mAbs (t-mAb) as a treatment approach for MM, opening the door for further development of additional t-mAbs for the treatment of MM.

Several days after the FDA approved daratumumab, elotuzumab was also approved for the treatment of refractory MM. Elotuzumab targets CS1/SLAMF7, a subunit of the CD2 cell surface glycoprotein predominantly expressed on the surface of plasma cells (*14*). Elotuzumab is also a humanized IgG κ mAb. Phase 1 clinical trials indicated that elotuzumab was effective in combination with either bortezomib or lenalidomide in patients with refractory MM (ORR of 48% and 82%, respectively) (*15*). Follow-up phase 2/3 trials provided similar results (*16*). A large phase 3 trial (ELOQUENT-2 trial) compared elotuzumab in combination with dexamethasone/lenalidomide to dexamethasone/lenalidomide alone. Both primary end points of this trial showed that progression-free survival and the ORR were superior for the elotuzumab arm with no added toxicity (*6*). This led to FDA approval of elotuzumab in combination with dexamethasone/lenalidomide for treatment of relapse and/or refractory MM.

While daratumumab and elotuzumab pioneered the use of monoclonal immunotherapies for treatment of refractory MM, there are now a number of additional t-mAbs in the pipeline for management of MM (Table 1). Isatuximab is a monoclonal IgG κ antibody that targets CD38 cells at an epitope distinct from daratumumab. In phase 1 trials, isatuximab as a single agent generated favorable results in patients with heavily treated, relapsed MM (*17*). Dose escalation studies

**Table 1. Properties of t-mAbs being investigated for the treatment of MM.[a]**

| Drug | Commercial name | Target | Dose, mg/kg | $C_{max}$,[b] mean μg/mL | $C_{min}$, mean μg/mL | Half-life, days | LC molecular weight, Da | Isotype | Phase |
|---|---|---|---|---|---|---|---|---|---|
| Daratumumab | Darzlex | CD38 | 16 | 915 | 537 | 21 | 23380 | IgG1 κ | FDA |
| Elotuzumab | Empliciti | CS1/SLAMF7 | 10 | 334 | 194 | N/A | 23432 | IgG1 κ | FDA |
| Rituximab | Rituxan | CD20 | 12.5[c] | 430 | 92 | 19 | 23035 | IgG1 κ | FDA |
| Pembrolizumab | Keytruda | PD-1 | 10 | 327 | 117 | 25.8 | 23744 | IgG4 κ | III |
| Siltuximab | Sylvant | IL-6 | 11 | 308 | 84 | 18 | 23210 | IgG1 κ | II |
| MOR202 | Not assigned | CD38 | 16 | Ongoing studies | Ongoing studies | Ongoing studies | Unknown | IgG1 λ | I/IIA |
| Isatuximab | Not assigned | CD38 | 20 | Ongoing studies | Ongoing studies | Ongoing studies | Unknown | IgG1 κ | I/IIA |

[a] t-mAbs were selected based on current or prior clinical trials investigating their use in patients with MM.
[b] $C_{max}$, maximum concentration of drug achieved in serum after dosing; $C_{min}$, minimum concentration of drug achieved in serum after dosing.
[c] The highest dose of rituximab is 1000 mg, which was calculated as ~16 mg/kg based on a worldwide average body weight of 60 kg.

indicated that high doses (>10 mg/kg) led to a substantial response in 11% of participants, with an ORR of 33% (*18*, *19*). No dose-limiting toxicities were reported at the highest tested dose of 20 mg/kg. Additional clinical trials are underway looking at isatuximab in combination with carfilzomib, a new generation proteasome inhibitor, as a treatment for refractory MM as well as in combination with bortezomib, cyclophosphamide, and dexamethasone. A third anti-CD38 t-mAb, which is a fully humanized IgG λ antibody, MOR202, is currently in phase 1/2 clinical trials as both a single agent therapy and in combination with pomalidomide or lenalidomide plus dexamethasone (*20*). The highest dose tested (16 mg/kg) did not demonstrate any dose-related toxicities.

The recommended dosing schedule of daratumumab is 16 mg/kg administered weekly for 8 weeks and then biweekly for 16 weeks; finally, at 25 weeks onward, dosing occurs every 4 weeks or until disease progression occurs. Pharmacokinetics studies demonstrated a mean serum $C_{max}$ of 915 µg/mL at the completion of the weekly dosing schedule. The mean trough concentration (predose) at the completion of the weekly dosing schedule was 573 µg/mL (*21*). Elotuzumab is recommended at a dose of 10 mg/kg given weekly for 8 weeks and then biweekly until disease progression occurs. Steady-state trough concentrations were 194 µg/mL (*22*). There are limited data available for other mAbs in the pipeline for treatment of MM, but if these t-mAbs are approved at similar doses and scheduling, they will likely produce concentrations of t-mAb in circulation in the range of those observed for daratumumab given they are structurally similar IgG mAbs.

Monitoring MM patients typically includes regular assessment for the presence of residual disease by measuring the disease-associated M-protein, typically by protein electrophoresis (PEL) and/or immunofixation (IFE). The limit of detection of M-proteins by PEL is approximately 0.1 g/dL and by IFE it is approximately 0.02 g/dL. However, in many MM patients, there is disease or treatment-associated hypogammaglobulinemia, which lowers the limit of detection of M-proteins. In hypogammaglobulinemia patients, M-proteins have been detected at concentrations <0.01 g/dL by IFE. Therefore, during the treatment course and several months after the last infusion of anti-CD38 t-mAbs, there is a strong likelihood that the t-mAb will be seen on IFE gels. In the case of anti-CD38 t-mAbs, depletion of the PC population is likely to reduce concentrations of circulating antibodies, since PCs contribute a significant percentage to the overall pool. Even before the widespread use of t-mAbs in MM, several case studies reported the detection of t-mAbs by traditional electrophoresis methods, including siltuximab and ofatumumab and, more recently, daratumumab and elotuzumab (*23–26*). Given the recent FDA approval of the latter 2 therapies and their potential role as frontline therapies, the likelihood of encountering interferences in the clinical laboratory will increase substantially in coming years. In contrast to FDA-approved anti-CD38 t-mAbs used for treatment of MM, most FDA-approved t-mAbs used to treat diseases other than MM (e.g., infliximab, adalimumab, bevacizumab, etc.) are not administered at sufficiently high doses to achieve steady-state concentration in circulation that would be detectable by IFE if administered according to the recommended dose with proper blood collection. In addition, non-MM directed t-mAbs are not anticipated to induce hypogammaglobulinemia. Lastly, the prevalence of non-MM direct t-mAbs in the population being tested for M-proteins is currently limited. Therefore, not all t-mAbs carry the same degree of risk for causing interferences on PEL or IFE.

The International Myeloma Working Group establishes standardized response criteria to aid in the evaluation of novel therapies as well as to aid in the treatment decisions and/or prognosis (*27*). The traditional International Myeloma Working Group definition of complete response (CR) requires that the patients' serum and/or urine must

have no detectable M-protein found by either IFE or PEL. Therefore, substantial responses (CR status or better) could be misclassified due to the failure to differentiate between residual disease and t-mAbs. This scenario could also potentially affect clinical trials that use relapse from CR or MRD status as a disease-free survival end point. In the ELOQUENT-2 trial, which assessed elotuzumab in combination with lenolidamide/dexamethasone, the rate of CR was lower in the elotuzumab/lenolidamide/dexamethasone arm than in the control lenolidamide/dexamethasone arm, likely due to elotuzumab interferences on PEL/IFE (*6*). In clinical trials of daratumumab, a gel-shift IFE assay termed DIRA (daratumumab-specific immunofixation electrophoresis reflex assay) was used to mitigate interferences. In at least 2 cases, patients who would have been classified as having only achieved partial responses were properly classified as achieving CR because of the ability of DIRA to distinguish between daratumumab and residual M-proteins (*5*). In the most recent IWMG recommendations, it was suggested that laboratories, particularly those involved in clinical trials, have a mechanism in place to differentiate t-mAbs from endogenous M-proteins (*27*). Accordingly, as part of the process of FDA approval, the labeling for both daratumumab and elotuzumab includes disclaimers in the usage claims that warn of the potential appearance of these t-mAb on PEL and IFE assays (*21*, *22*).

Most of the approved t-mAbs are either humanized or fully human IgG antibodies. The most commonly used subclass is $IgG_1$, mostly due to its potent effector function. In some cases, $IgG_2$ or $IgG_4$ subclasses (e.g., eculizumab) have been used when a lack of specific cellular activity is desirable. To date, there are no $IgG_3$ t-mAbs approved, likely due to limited stability, challenges of large-scale purification, and the many allotypes across different populations. Currently, t-mAbs are also exclusive of both IgA and IgM classes. Given the fact that the vast majority of t-mAbs are of the IgG κ isotype and IgG κ M-proteins are the most common isotype encountered in the laboratory, there is the potential for failure to differentiate between the 2 and issue an erroneous positive report (*28*).

With current technologies used for the detection, identification, and isotyping of M-proteins, there is limited ability to recognize the presence of mAb therapeutics at the analytical phase of testing (*29*). The only solution available without new technology would be to use the specific migration pattern of t-mAbs to recognize them. However, there is a chance of encountering comigrating IgG κ M-proteins; therefore, the use of migration distances to positively identify t-mAbs is of limited utility. The question then becomes, how can the laboratory mitigate the risk of reporting a falsely positive result that incorrectly indicates the presence of an endogenous IgG κ M-protein? Timing of blood collection is particularly important if the intention is to evaluate a patient for the presence of residual disease when the patient is receiving a t-mAb. Ideally, blood collection should occur at trough immediately before the next infusion. The concentration of mAbs can be expected to be highest immediately after an infusion. It is known that blood collections shortly after infusion of t-mAbs can lead to interferences in laboratory assays (*30*).

Improperly timed blood collections could readily result in detection of a t-mAb, even by the most insensitive electrophoresis-based methods. It is important to note that any patient receiving a t-mAb, regardless of dose, is at risk for this error if blood is drawn proximal to an active infusion site or shortly after an infusion if the patient happens to have a workup ordered that included PEL and/or IFE. CLSI guidelines provide recommendations on drawing blood below (distal to) an intravenous catheter, indicating that the intravenous catheter should be turned off >2 min and that a tourniquet be placed between the intravenous site and the blood-draw site (CLSI document H3-A6). Given the dearth of data evaluating how long it takes equilibrium to be met postinfusion for

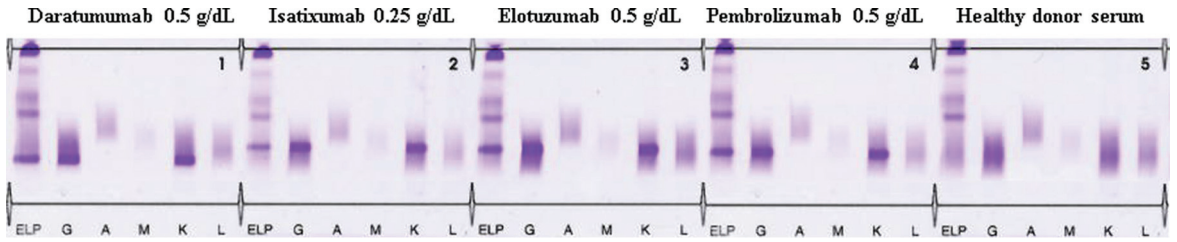


**Fig. 1. Migration patterns of T-mAbs currently being investigated in the treatment of MM.**
The indicated concentrations of t-mAbs were spiked into healthy adult pooled sera. Immunofixation was performed using 9IF gels from Sebia using standard laboratory techniques. Supratherapeutic concentrations were used to ensure readers could readily visualize the migration patterns on scanned IFE images. Lower concentrations (≤0.1 g/dL) are more likely to be encountered clinically. G, IgG; A, IgA; M, IgM; K, kappa; L, lambda.

t-mAbs and the increased risk for reporting false positives by electrophoresis and IFE, it seems prudent to avoid collecting blood during or shortly after infusion of t-mAbs when testing includes a PEL and/or IFE workup.

Improving the general awareness among clinicians and laboratorians that mAbs can positively interfere with both PEL and IFE is imperative. Timing blood collections to occur before the next infusion can mitigate some of the risk. Appreciating that the dose of t-mAb that a patient may be receiving and the pharmacokinetics of that therapy can allow one to reasonably predict whether the mAb is likely to be detected by PEL and/or IFE. Review of the electronic medical record for information related to past history of t-mAb infusions, as well as confirming the timing of infusion and sample collection with the staff performing the blood collection will reduce the risk of t-mAb interferences. Also, if a new IgG κ clone is detected in a MM patient, the laboratory could include a disclaimer in the report stating that the appearance of a new IgG κ M-protein could be indicative of a t-mAb. However, this does not provide a solution for cases where M-proteins comigrate with a t-mAb; this distinction will be impossible to resolve with confidence using traditional testing methods.

## Identification of t-mAb interferences at the analytical phase of testing

The ability to differentiate between endogenous M-proteins and t-mAbs is limited when using standard PEL, capillary electrophoresis, and IFE. Each t-mAb has a specific migration pattern, which could be used to approximate the likelihood that a distinct band found on PEL, capillary electrophoresis, or IFE corresponds to a t-mAb (Fig. 1). Migration pattern alone is not strong or conclusive evidence that a band corresponds to a specific t-mAb. A further confounder is found in those laboratories using capillary electrophoresis and/or immunosubstraction. Capillary electrophoresis may note different migration patterns compared with gel-based electrophoretic separation due both to the different instrumentation used and the corresponding differences in the electrochemical properties of the buffer system. Given the limited analytical solutions to the problem of t-mAb interferences, newer approaches that either serve as adjunct testing methods using traditional platforms or incorporate new technologies must be developed to address this challenge.

### DIRA

DIRA was developed to enable the proper classification of responses in patients receiving daratumumab therapy during clinical trials (*31*).

This assay uses an anti-daratumumab antibody, which is incubated at room temperature with patient samples for 15 min before electrophoretic separation. The principle of the assay is that an anti-daratumumab antibody will bind tightly and specifically with daratumumab, forming a complex with a shifted migration profile (Fig. 2). Therefore, if upon addition of anti-daratumumab antibody, the potential M-protein now migrates entirely at a distance consistent with the daratumumab:anti-daratumumab complex, then the presence of residual M-protein can be excluded. In contrast, if a residual band remains and fails to "remigrate," then residual disease is likely still present. With this technique, a total of 12 lanes on an IFE gel are used for each patient, which includes controls and a patient sample (+/– anti-daratumumab). Two wells are fixed (a baseline sample and a posttreatment sample) as references. IFE is performed using both IgG and κ antisera. Daratumumab is identified by a reproducible shift in the migration distance of the distinct band in question by comparing the baseline samples (+/– anti-daratumumab) to the posttreatment samples (+/– anti-daratumumab). This assay has been reported to achieve 100% sensitivity and specificity at 0.2 g/dL daratumumab in known MM patients (*31*). DIRA has several limitations in that it has the attendant limitations of IFE and thus is nonquantitative. Interpretation of DIRA can be complex in cases of hypergammaglobulinema. Furthermore, in aggressively treated patients, oligoclonal responses can be challenging to interpret by IFE, which will likely be exacerbated when using DIRA (*32*). Nonetheless, development of DIRA enabled resolution of daratumumab interferences and more accurate estimations of the number of patients achieving CR status or better in clinical trials of daratumumab (*5*).

DIRA is specific to daratumumab and cannot resolve other t-mAb interferences. This is a problem, since there are a number of additional t-mAbs either already approved by the FDA for the treatment of MM or that may be approved in the near future (*5*, *6*, *29*, *33*). If gel-shift approaches are used for other t-mAbs, then this would require development and validation of numerous antidrug antibodies for each specific application in gel-shift type assays. Furthermore, while each drug manufacturer is likely to develop some methodology for ruling out drug interferences for use in clinical

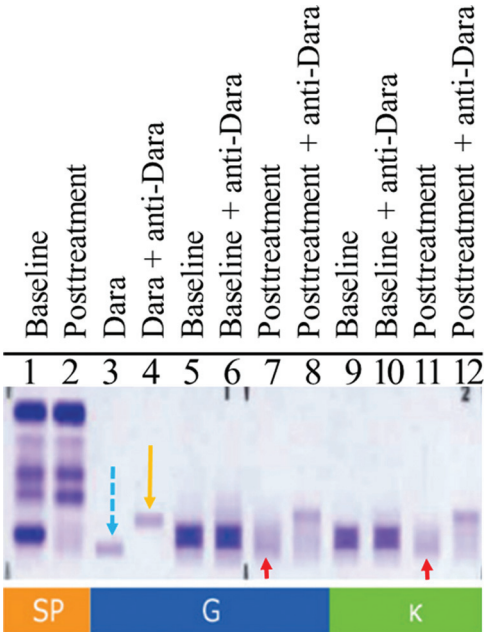


**Fig. 2. DIRA.**
In DIRA 12, IFE lanes are used. Two total serum protein fixation lanes are used indicated by the orange bar (lanes 1 and 2). Both IgG and κ antisera are used for staining and fixation, indicated by the blue bar and green bar, respectively. DIRA includes daratumumab (Dara) ± anti-daratumumab controls for migration of the t-mAb and the daratumumab:anti-daratumumab shifted complexes (lanes 3 and 4). Baseline serum samples are run ± anti-daratumumab next to serum samples taken posttreatment (lanes 5–12). The dashed blue arrow indicates the location of the daratumumab control (lane 3). The orange arrow represents the daratumumab:anti-daratumumab complex control (lane 4). The red arrows indicate the suspected daratumumab interference found in the posttreatment sample (lanes 7 and 11). Lane 12 is positive for the daratumumab:anti-daratumumab complex and negative for the suspected band, indicating this sample is DIRA negative. Figure adapted by authors with permission from Kricka et al. (*30*).

trials, the primary application of such assays is unlikely to be focused on the needs of clinical laboratories performing routine testing for M-proteins. Therefore, a more comprehensive solution may be useful.

### Mass spectrometry–based approaches

Each t-mAb, as is the case with endogenous human antibodies, is comprised of 2 identical heavy chains (HCs) and 2 identical light chains (LCs), and each have a unique amino acid sequence ascribing it a distinct molecular mass (Table 1). With the advent of electrospray ionization (ESI) and high-resolution mass spectrometers, mass measurement error of <1 Da is achievable for intact mAbs (approximately 150 000 Da), as well as the HC (approximately 55 000 Da) and LC (approximately 23 000 Da) components. Pharmaceutical manufacturers have used these inherent properties to confirm the identity/purity of mAbs in the development and production phase of t-mAbs, which can be used to meet regulatory requirements of demonstrating a purity profile (*34*). Consequentially, mass spectrometry (MS) has a place in assessing critical quality attributes. Intact analysis of IgG antibodies has been reported using both MALDI and ESI coupled to different mass analyzers (*35*). The clinical utility of these methods were also subsequently recognized for monitoring patients with MM using ESI-TOF MS (*36*).

Robust MS-based methods have been developed and subsequently refined for identifying mAbs in human serum that provide improved sensitivity compared to current clinical assays for monitoring M-proteins (e.g., PEL and IFE) (*36*). This technology, coined miRAMM (monoclonal-immunoglobulin-rapid-accurate-mass measurement), uses microflow liquid chromatography–ESI-TOF MS to measure the accurate molecular mass of the LC portion of mAbs, which serves as a surrogate marker of the intact mAb. Serum antibodies are purified offline, reduced to release the LCs from HCs, separated using microflow liquid chromatography, and analyzed by either ESI-TOF MS or MALDI-TOF MS. The mass spectra of multiply charged LC ions generated during ESI are converted to their molecular mass to produce the mass distribution of LCs. The presence of a peak above the polyclonal background is consistent with the presence of an M-protein, similar to current gel-based methodologies with the added benefit of improved resolution and accurate mass measurements within 1 Da (*36*). This technology has high concordance with gel-based PEL as well as IFE in large patient cohorts using both MALDI-TOF and ESI-TOF MS (*37*, *38*). In MM patient samples, the molecular mass of the LC component of disease-associated M-proteins is constant over many years and can be used to track patient-specific disease. The utility of this technique for readily identifying a variety of t-mAbs used in the treatment of disorders of the immune system was recently demonstrated (*39*). Infliximab, adalimumab, eculizumab, vedolizumab, and rituximab were all readily identified by their LC molecular masses when spiked into patient serum at physiologically relevant concentrations. This approach has potential to be useful for accurately quantitating M-proteins in the presence of comigrating t-mAbs (*37*, *40*). Therefore, accurate molecular mass measurement of patient sera for the detection of both endogenous M-proteins and t-mAbs is a promising application of mass spectrometry.

A practical early application of this technology would be its use as a reflex test in patient samples positive for the presence of a previously undocumented monoclonal IgG κ M-protein, or in patients with IgG κ MM who are actively undergoing treatment with a t-mAb (e.g., daratumumab or elotuzumab), where assessing treatment response is important. To date, there are no clinically orderable mass spectrometry assays offered in CLIA-certified laboratories to rule out t-mAb interferences. It is likely these technologies will be first introduced in the setting of large regional or national reference laboratories. Eventually, as

smaller laboratories become accustomed to MS and these MS-based assays are optimized and further developed, this approach may be more broadly implemented. While the use of MS can overcome the problem of t-mAb interferences, to date, there have not been studies assessing the clinical sensitivity and specificity of this methodology for the detection of mAb interferences.

t-mAbs primarily used in the treatment of MM (e.g., daratumumab) pose the greatest risk for interference in PEL and IFE, but the growing list of conditions treated with t-mAb increases the likelihood that screening for MGUS may occur in a patient who is receiving a t-mAb for an unrelated condition. For most t-mAbs, the recommended dosing is insufficient to achieve peak concentrations that would be detectable by IFE. However, preanalytical collection errors could result in nonphysiological concentrations of mAb therapeutic that would be misidentified as an M-protein. Unfortunately, if this preanalytical error is undetected and the laboratory fails to recognize that the patient is currently receiving a t-mAb, the presence of an M-protein could be incorrectly reported. This step could result in unnecessary additional testing, follow-up medical consultation, or invasive procedures and undue stress associated with a positive finding. Clinical laboratories need to be weary of encountering t-mAbs during routine screening and monitoring of M-proteins.

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