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CLINICAL TRIALS AND OBSERVATIONS

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An ATLAS to map MRD with peripheral blood

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In this issue of *Blood*, Kubicki et al[1] report on findings from the EXENT platform (Thermo Fisher)—a new assay that uses mass spectrometry (MS) to measure serum monoclonal protein—when used on samples from the ATLAS trial, which randomized patients with multiple myeloma to maintenance with carfilzomib, lenalidomide, and dexamethasone vs lenalidomide.[2] The authors compare its performance with established assays for measuring minimal residual disease (MRD) in the bone marrow, next-generation sequencing (NGS) by clonoSEQ (Adaptive Biotechnologies), or multiparameter flow cytometry, at the 10^{-5} threshold.

There are several advantages to using a blood-based MS assay to assess MRD. The most obvious one is that it avoids an invasive bone marrow biopsy and thus allows for frictionless, serial monitoring of disease. Moreover, peripheral blood may provide a systemic assessment of disease and avoids the limitations of heterogeneity in bone marrow sampling, such as in the case of macrofocal disease. Reliance on bone marrow also does not account for the possibility of extramedullary disease. Furthermore, emphasis on MRD end points will increase over time, motivated by the favorable voting in April 2024 by the US Food and Drug Administration's Oncology Drug Advisory Committee for use of MRD as an end point for accelerated approval.

Some background on MS-based approaches is helpful for understanding the work of Kubicki et al (see table). MS relies on the principle that myeloma cells produce an immunoglobulin with a unique amino acid sequence and therefore a distinct mass. The EXENT platform uses matrix-assisted laser desorption ionization (MALDI) to prepare the sample before time-of-flight (TOF) MS. At a sensitivity of 0.0015 g/dL, EXENT is about 100-fold more sensitive than conventional serum protein electrophoresis/serum immunofixation (SPEP/IFE).[10] The advantages of MALDI-TOF are speed and throughput, as compared with liquid chromatography, which is more labor intensive and inherently slower. Of note, the EXENT assay was previously known as quantitative immunoprecipitation (QIP)-MS and is now commercially available in Europe. It has already been evaluated in several trials, such as GEM2012MENOS65[5] and GMMG-MM5.[6] MASS-FIX (Mayo Clinic Laboratories) is another assay, using similar methodology as EXENT.[3] Given its performance, the Mayo Clinic reference laboratory has been using MASS-FIX rather than conventional SPEP/IFE for working up monoclonal gammopathies. MASS-FIX was also evaluated in the STAMINA study.[4]

The authors found that EXENT outperformed conventional SPEP/IFE, detecting a monoclonal protein in 21% of SPEP/IFE-negative samples, and it was able to stratify outcomes in patients who achieved a complete response. However, although EXENT was more sensitive than conventional SPEP/IFE, it was less sensitive than NGS, as 37% of NGS-positive samples were negative by EXENT. Nevertheless, MS can add value to bone marrow–based MRD, as patients with disease that was MRD negative in both peripheral blood and bone marrow had the best outcomes.

An important finding from the study is how the predictive value of peripheral blood MRD evolves over the course of a patient's treatment. Agreement between peripheral blood and bone marrow assessment was best after 18 cycles and less so at earlier time points. Some of this relates to an inherent limitation of peripheral blood MRD from the half-life of circulating monoclonal protein; for example, patients can be NGS negative in the bone marrow after CAR T-cell therapy yet still be in very good partial response while awaiting clearance of the monoclonal protein. Moreover, emergence of oligoclonal bands, different from the original monoclonal protein, after recovery from high-dose melphalan and autologous stem cell transplant may also be interpreted as "positive" on MS.

Another promising way of using MS is through a clonotypic-based approach. This approach leverages knowledge of the sequence of the baseline monoclonal protein combined with liquid chromatography to offer sensitivity that rivals that of the MALDI-TOF, down to as low as 1×10^{-5} g/dL, although the sensitivity may vary based on the peptide sequence.[7] MALDI-TOF imposes an intrinsic limitation to its sensitivity because it relies on the ability to detect a monoclonal protein above the polyclonal background. Indeed, the clonotypic approach can be even more sensitive than NGS for detecting residual disease.[8] Currently, there are 2 clonotypic assays that have recently become

Mass spectrometry assays for measuring peripheral blood MRD

Assay	Type	Platform	Sensitivity	Comments
MASS-FIX (Mayo Clinic)	Intact light chain	MALDI-TOF	0.01 g/dL	Commercially available through Mayo Clinic reference laboratory; has replaced SPEP/IFE in their workflow[3]; evaluated in STAMINA[4]
EXENT (Thermo Fisher), previously known as QIP-MS)	Intact light chain	MALDI-TOF	0.0015 g/dL	Commercially available in Europe; evaluated in, for example, GEM2012MENOS65[5] and GMMG-MM5[6]
M-Insight (Sebia)	Clonotypic peptide	LC-MS	2×10^{-5} to 5×10^{-4} g/dL	Commercially available in the United States; evaluated in IFM2009[7,8]
EasyM (Rapid Novor)	Clonotypic peptide	LC-MS	3×10^{-6} to 6×10^{-4} g/dL	Commercially available in the United States; evaluated in MCRN-001[9]

LC-MS, liquid chromatography mass spectrometry; MALDI-TOF, matrix-assisted laser desorption ionization–time of flight; QIP-MS, quantitative immunoprecipitation mass spectroscopy; SPEP/FIX, serum protein electrophoresis/serum immunofixation.

commercially available in the United States, M-Insight (Sebia)[8] and EasyM (Rapid Insight).[9] However, the requirement for a baseline serum sample to determine the clonotypic peptide sequence can be a limitation because a baseline sample is generally not available for patients already being treated. (Of note, the clonotypic peptide in initial studies could be determined by RNA sequencing of bone marrow aspirates, but this option is not currently available in the commercial platforms.) In contrast, assays using MALDI-TOF do not require an initial sample—a key advantage.

When will we use peripheral blood MRD testing in the clinic? The answer depends on use of MRD in general, as there is an ongoing question of whether the information from MRD testing is actionable. We anticipate data from ongoing trials, such as DRAMMATIC (NCT04071457) aimed to inform discontinuing maintenance or REMNANT (NCT04513639) to direct intervention at signs of an early molecular relapse, will provide a framework for using MRD results to guide treatment. We also await information on identifying the ideal level of sensitivity for making decisions and what time frame constitutes sufficient sustained absence of disease. Finally, what is the amount of monoclonal protein as measured by MS that matches MRD negativity at 10^{-5} or lower? The clonotypic peptide MS assays, which are beginning to become commercially available, offer sensitivity similar to that seen with NGS, so what is relevant to the latter may apply to the former. Currently, the number of patients evaluated by MS is quite small compared with NGS and multiparameter flow cytometry; thus, over time, we anticipate that MS data will become more robust so clinicians may gain confidence in using MS.

Kubicki et al provide valuable initial information on how to use the EXENT platform and how it may complement existing bone marrow–based MRD assays in addition to identifying optimal timing for its use. Given the rapid development of EXENT and other MS assays, the hope for patients of a "liquid" biopsy for MRD assessment instead of bone marrow aspiration is becoming a reality.

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