

## Detecting minimal residual disease in multiple myeloma

### Challenges and prospects for the integration of novel approaches in clinical practice

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#### ABSTRACT

Relapse rates in multiple myeloma (MM) remain high, even in patients who achieve complete response (CR). Lower minimal residual disease (MRD) levels correlate strongly with longer progression-free survival, however the accuracy of MRD detection is strongly dependent upon the sensitivity of the techniques used. This review presents available data on MRD assays and their potential value in tailoring MM treatment. Available research shows that depth of response as assessed with current MRD evaluation methods is a powerful predictor of outcome, and may be more important than the manner in which a deep response was achieved.

These recent findings form the basis of the Terry Fox Pan-Canadian Multiple Myeloma Molecular Monitoring (M4) Study, which aims to identify optimal approaches for incorporating current methods of MRD testing into clinical practice. The application of leading-edge MRD detection technologies to the development of personalized treatment strategies is expected to improve patient outcomes while avoiding the costs and toxicities associated with ineffective or unnecessary therapies.

**Keywords:** multiple myeloma, minimal residual disease, molecular monitoring, personalized treatment

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#### INTRODUCTION

Multiple myeloma (MM) is characterized by the accumulation of monoclonal plasma cells in the bone marrow. For many years, the standard methods of disease monitoring included repeated bone marrow biopsies to assess the abundance of plasma cells, and quantification of serum and urine monoclonal immunoglobulin using electrophoresis. However, the limited sensitivity of these assays is widely recognized.<sup>1,2</sup> Current definitions of therapeutic response and relapse for MM are summarized in the guidelines put forth by the International Myeloma Working Group (IMWG).<sup>3</sup>

Treatment for MM is becoming increasingly effective, with more patients achieving a conventionally defined complete response (CR) to therapy, longer remission durations, and improved overall survival (OS) rates. Yet, despite achieving CR, relapse rates for MM patients remain high, indicating the presence of residual disease not detected by the current standard clinical assessment. As modern treatment regimens achieve improved outcomes, newer technologies and definitions of response are required to assess disease status in MM patients.<sup>4</sup> The inclusion of minimal residual disease (MRD) detection in the IMWG guidelines demonstrates recognition by the myeloma community of the need for better MRD monitoring to inform clinical practice.

Lower MRD levels correlate strongly to longer progression-free survival (PFS).<sup>5</sup> A review by Munshi and colleagues of 496 MM patients showed that MRD-positive

status was predictive of shorter PFS, with a median PFS for MRD-negative patients of 60 months compared to 39 months for MRD-positive patients.<sup>6</sup> Attal and colleagues (2017) randomly assigned 700 MM patients to receive lenalidomide, bortezomib and dexamethasone (RVD), with or without immediate high-dose melphalan plus stem-cell transplantation, followed by lenalidomide maintenance.<sup>7</sup> While this study demonstrates superior PFS with immediate transplant, OS is, so far, equivalent; intriguingly, outcomes in MRD-negative patients are similar regardless of treatment assignment. Korde and colleagues (2015) found a high rate of MRD-negative remission in 45 MM patients treated with frontline carfilzomib-lenalidomide-dexamethasone, without transplant.<sup>8</sup> These results demonstrate that some patients who achieve MRD-negative remission with highly active novel therapies do very well without immediate transplant.

Accuracy of MRD detection in MM patients is strongly dependent upon the sensitivity of the techniques used. Several such techniques are currently being examined, with the most promising including: 1) multiparameter flow cytometry (MFC); 2) allele-specific oligonucleotide quantitative PCR (ASO-qPCR) and next-generation sequencing (NGS); and 3) positron emission tomography (PET). Further evidence is required to support the utility of these techniques to inform clinical decision-making. This review will discuss the evolution of MM disease monitoring, including current data regarding MRD assays, to provide insight into challenges being faced in tailoring MM treatment and the potential value of making MRD detection technologies available to patients.

### MULTIPARAMETER FLOW CYTOMETRY (MFC)

MFC employs fluorochrome-labelled antibodies for immunophenotyping to quantify the complement of plasma cells in bone marrow aspirates. MFC allows for accurate distinction between abnormal and normal plasma cells. The sensitivity of MFC for determining MRD is dependent on the number of cells analyzed and the capability of the antibody panel to distinguish normal from abnormal plasma cells.

Although, in the past, there were concerns around the limitations of MFC (e.g. lack of specific plasma cell markers, low numbers of plasma cells in bone marrow aspirates post-treatment), it is now a key tool in the management of MM and can aid in diagnosis and prognosis, as well as disease monitoring. Efforts are underway to globally standardize antibody panels, the specific types of fluorochromes used, and the number of cells analyzed.<sup>9,10</sup>

First-generation studies with MFC highlighted the sensitivity of the assay for MRD detection. A study in Spain employing three 6-colour MFC to detect MRD in 45 patients 3 months post autologous stem cell transplant (ASCT) found plasma cells in approximately one-third of patients with negative immunofixation results.<sup>11</sup> This study also found that patients who tested MRD-negative had better outcomes than those who were MRD-positive.<sup>11</sup> These data have been supported by multiple subsequent studies using three 6-colour MFC,<sup>12-18</sup> further emphasizing the association between depth of response and outcome.

Variability in methodology and insufficient sensitivity (1 in 10<sup>4</sup> cells detected) were limitations of earlier techniques for detecting MRD in MM patients. The development of ≥8-colour immunophenotyping through MFC has addressed these concerns, increasing sensitivity to 1 in 10<sup>5</sup> cells. This simultaneous assessment of millions of cells allows for a fast and efficient way to distinguish normal from abnormal plasma cells in MM patients. Variability in methodology has been addressed through development of the EuroFlow method.<sup>19</sup> The EuroFlow method utilizes two 8-colour cytometer tubes that combine surface antigens for the identification of phenotypically aberrant clonal plasma cells, and cytoplasmic κ and λ light chain expression, to confirm clonality.<sup>19</sup> Software algorithms have been developed for automated identification of clonal plasma cells. The 2-tube approach also allows for confirmation in a second independent measurement. Sample quality can be assessed by monitoring other bone marrow cells. The EuroFlow approach has been extensively validated,<sup>20</sup> confirming the improved reliability, consistency and sensitivity of the method. Further standardization through prospective studies is warranted, and should examine optimal combinations of reagents: some studies have shown that the staining profiles of the same CD markers differ according to the distinct combination of reagents employed.<sup>21</sup> A promising means of overcoming this concern is identified in the recent study conducted in 2017 by Flores-Montero et al, which establishes a novel EuroFlow-based next-generation flow approach (NGF-MRD) for even more sensitive (10<sup>-5</sup>-10<sup>-6</sup>) and standardized MRD detection in MM.

MFC has many advantages as outlined by Chatterjee and colleagues<sup>22</sup>: 1) it allows simultaneous analysis of multiple parameters; 2) a number of cell types can be studied within a relatively short period of time; 3) sensitivity is high; 4) quantitative analysis of antigen density is possible; 5) surface and intracellular antigens can be studied; 6) DNA ploidy can be analyzed; and 7) concurrent study of the non-plasma cell bone marrow compartment can be performed. MFC can be used to assess MRD even in cases where a baseline sample has not been analyzed, and is applicable to virtually all patients, in contrast to allele-specific oligonucleotide PCR techniques. MFC is becoming more widely available and affordable, as laboratories become equipped with ≥8-colour cytometers. MFC strikes a balance between effectiveness and accessibility, making it an attractive tool for MRD and the tailoring of treatment in MM patients.

### IMMUNOGLOBULIN GENE SEQUENCING APPROACHES TO MRD

Malignant plasma cells have specific clonal rearrangements in the immunoglobulin heavy (IgH) and light (IgL) genes,<sup>23</sup> which can be exploited by molecular techniques for the diagnosis, prognosis and, especially, monitoring of MM. One of the first molecular techniques to be used was allele-specific oligonucleotide PCR (ASO-PCR) of variable/diverse/joining (VDJ) heavy chain rearrangements<sup>24</sup> to allow for qualitative assessment of MM patients. This technique was quickly replaced by ASO-qPCR; unlike ASO-PCR,

it provides a quantitative assessment of disease status, allowing for more sensitive detection of gene rearrangements in plasma cells, detecting 1 abnormal plasma cell in  $10^5$  cells.<sup>25-31,18</sup> Analysis of these gene rearrangements requires a baseline measure with repeated sampling at varying times during the disease course to determine depth of response to treatment. Combining ASO qPCR with primer panels for the detection of myeloma-specific IgH rearrangements allows for the selective amplification of clonal IgH rearrangements.<sup>31</sup> Study results from patients in whom MRD testing was feasible (primers could be matched to their specific clonotype) showed strong, significant correlation between MFC and ASO-qPCR; with negative ASO-qPCR status, in turn, showing significantly improved PFS.<sup>18</sup> One of the drawbacks of this technology in MM disease monitoring is that ASO-qPCR is only feasible in 42% to 75% of patients; patient-specific primer design is also resource-intensive.<sup>32,18</sup> Fluorescence ASO PCR has been shown to increase the proportion of assessable patients, however, it is less sensitive than ASO-qPCR.<sup>30</sup>

Next generation sequencing (NGS) is promising as a replacement for ASO-qPCR in the detection of clonal plasma cells in MM patients. With NGS, different clonotypes in a patient sample are determined through amplification of genomic DNA using locus-specific primers designed for IgH-VDJ, IgH-DJ, or IgK, followed by sequencing of the immunoglobulin gene DNA. This method is highly sensitive (1 in  $10^6$  cells), is feasible in 95% of patients, and can be used on both bone marrow aspirates and blood.<sup>33,34</sup> Similar to ASO-qPCR, NGS compares samples taken throughout the course of the disease to a baseline sample to monitor the progression of disease. Improved PFS is seen in patients achieving a MRD-negative response to therapy.<sup>35,36,8</sup> NGS also eliminates the need for patient-specific primers. Limitations of this approach are high cost, availability (not all centres have access to NGS technologies), and the need for a baseline sample for identification of clonal Ig sequences.

## DETECTION OF EXTRAMEDULLARY DISEASE WITH PET, CT, AND MRI

The approaches described above for the detection and measurement of tumour burden rely on bone marrow assessment; the patchy distribution of MM in the bone marrow increases the likelihood of a false negative. Further, these methods also limit assessment of disease to the bone marrow, yet extramedullary disease has been found in up to 10% of MM patients.<sup>37-42</sup> Assessment of extramedullary disease relies strongly on imaging, including magnetic resonance imaging (MRI) and positron emission tomography (PET) and computerized tomography (CT) scans, alone or in combination (PET/CT).

MRI is a sensitive tool for the detection of bone marrow infiltration by MM cells even before bone marrow destruction is present.<sup>43-45</sup> Studies have shown that the number of focal lesions detected by MRI post therapy is negatively correlated to survival outcome.<sup>46-48</sup> However, the usefulness of MRI for the assessment of MRD after therapy remains unclear. There are several limitations to the use of MRI in myeloma disease monitoring, including the lack of availability of whole-body MRI; the time needed to conduct a scan; contraindications

in some patients; and critically, the inability to distinguish between active and non-active lesions post therapy.

PET/CT is a potent combination of structural and activity-based assessment of the skeletal compartment. Recently, <sup>18</sup>F-fluorodeoxyglucose (<sup>18</sup>F-FDG) PET has been shown to be a powerful tool in the assessment of tumour metabolic activity and of the effect of therapy on this metabolism.<sup>48-52</sup> As such, it can be effectively used to determine the response to treatment in as little as 7 days following commencement of therapy.<sup>51</sup> Studies have shown that the presence of 3 or more active lesions 7 days after the start of therapy is indicative of poorer outcomes.<sup>49</sup> As such, <sup>18</sup>F-FDG PET can potentially help tailor patient-specific treatment regimens by detecting nonresponders early. Suppressing focal FDG-avid lesions with treatment before ASCT is associated with better survival outcomes, whereas the persistence of maximum standardized uptake values ( $SUV_{max}$ ) greater than 4.2 after therapy commencement predicts early relapse.<sup>50,52</sup> Patients who were PET/CT disease negative at day 100 post-ASCT had better PFS and OS than those who tested positive.<sup>52</sup> Significantly shorter PFS and OS are seen when there is extramedullary disease and/or increased FDG uptake at baseline. The extent of FDG PET/CT involvement predicts shorter time-to-progression (TTP) and PFS.<sup>50</sup> Patients achieving a conventional CR but still showing positive PET/CT scans had a significantly shorter PFS compared with those who were PET/CT-negative.<sup>52</sup> Overall, the data from these studies show an inferior outcome for patients with positive PET scans, even in those who achieved deep responses, highlighting the relevance of this assessment method in MM patients.<sup>48,50,52</sup>

Although PET/CT has been shown to have lower sensitivity than MRI, it has higher specificity and positive predictive value, lower negative predictive value, and was more accurate overall for the determination of remission status.<sup>53</sup> MRI is more sensitive at detecting diffuse marrow involvement, which can be difficult to distinguish from reactive marrow hyperplasia on PET.<sup>54</sup> However, <sup>18</sup>F-FDG PET is able to distinguish between active and non active focal lesions, allowing for more accurate measures of disease status post treatment than MRI.<sup>55</sup> Ongoing studies are using alternate tracers that show improved sensitivity in identifying small skull lesions, and are investigating the possibility of using anti-CD38 antibodies with radioisotope conjugates as an even more sensitive measure of MRD.<sup>56-58</sup> The drawback of <sup>18</sup>F-FDG PET scans for MRD monitoring is the lack of standardization for qualitative or quantitative evaluation (or both) for <sup>18</sup>F-FDG-PET/CT in MM, and a lack of intra-observer reproducibility in image interpretation.<sup>59</sup> Further prospective trials will allow fine tuning of the cutoffs used for defining therapeutic response on PET imaging.

## MRD MONITORING IN THE CURRENT ERA OF MYELOMA THERAPIES

Recent studies have emphasized the potential benefits of MRD monitoring in MM patients. By achieving MRD-negative status, regardless of the treatment regimen, patients may have better long-term outcomes. It may be

that not every patient requires high-dose chemotherapy to achieve MRD-negative status; indeed, better novel drug therapies are able to achieve MRD-negative status without the side effects of more aggressive therapy.


We now know that depth of response as assessed with current MRD evaluation methods is a powerful predictor of outcome, and that depth of response may be more important than the manner in which a deep response was achieved. Still, many questions remain. How do we incorporate MRD assessment with other prognostic markers in risk stratification of patients? Do all patients require intensive and/or continuous antimyeloma drug therapy to sustain a remission? Or can selected patients who achieve sustained MRD-negative remission deescalate their planned treatment without compromising long-term outcomes? Conversely, should we escalate or change therapy in some patients who do not enter a MRD-negative remission, to improve their prognosis? Studies to address these questions are underway.

### TERRY FOX PAN-CANADIAN MULTIPLE MYELOMA MOLECULAR MONITORING (M4) STUDY

The M4 study, supported by the Terry Fox Research Institute, is getting underway in 2017. This study will evaluate the optimal approach to incorporating current methods of MRD testing into Canadian and global clinical practice. Our team includes leading myeloma researchers who are developing new methods of characterization and monitoring disease. We will attempt to answer questions such as: How do PET, NGS and modern MFC compare and contrast in the measurement of MRD? At what points should each test be conducted? Should we examine not just the depth of the response, but also the rapidity of the response with serial testing? What is the most cost-effective way to apply these technologies in Canada? Can we develop protocols to explore the impact of limiting the duration and/or intensity of therapy in selected MRD-negative patients? Can we identify treatment strategies that improve outcomes for MRD-positive patients? How can we efficiently identify actionable oncogene mutations in MM patients in the most minimally invasive manner? What are the characteristics of myeloma cells that survive therapy and mediate relapse that we might exploit therapeutically? By applying leading-edge technologies to the development of more personalized treatment strategies and fleshing out the optimal application of the most developed methods of MRD monitoring, we expect to maintain or improve patient outcomes while avoiding the costs and toxicities of ineffective or unnecessary therapies.

### CONCLUSIONS

It is evident that with new myeloma treatments, patients can achieve deeper, more lasting responses; consequently, there is a need for novel, sensitive measures of treatment response and disease progression in MM patients. Minimal residual disease assessment with MFC, NGS and PET have shown great promise as predictors of outcome. Research should now focus on refining and standardizing these measures and on determining how they can be applied to

inform better patient management decisions. Efforts to study the refinement and application of these techniques are underway in Canada and around the world, holding out the promise of improving the lives of myeloma patients. 

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