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Best Practices for the Assessment of Measurable Residual Disease (MRD) in Multiple Myeloma

Discussants



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Abstract: Therapeutic advances in multiple myeloma have led to durable, deep remissions in a subset of patients. However, outcomes of patients achieving a complete response are not homogeneous. In recent years, measurable residual disease (MRD) has emerged as a prognostic biomarker. While several technologies have been evaluated to detect MRD, two assessment technologies are most frequently utilized in patients with multiple myeloma. Next-generation flow (NGF) uses flow cytometry to identify malignant plasma cells through the presence of immunologic markers located on the cell surface. Next-generation sequencing (NGS) analyzes for the presence of sequences in immunoglobulin genes that were previously identified as markers of that specific patient's plasma cell malignant clone. Both methods are included in criteria for MRD by the International Myeloma Working Group, which defines MRD negativity as less than 10^{-5} . Recently, the NGS-based clonoSEQ[®] Assay obtained clearance from the US Food and Drug Administration, with a limit of detection of less than 10^{-6} given proper sample input. Based on available evidence correlating attainment of MRD negativity with outcomes, MRD assessment has been incorporated into ongoing clinical trials. Analyses will provide additional insight into the correlation between MRD and outcome. This monograph examines the available trial data and provides recommendations on how to incorporate MRD assessment into clinical management.

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Assessment of Measurable Residual Disease (MRD) in Multiple Myeloma: A Review of the Data

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Depth of Response in Multiple Myeloma

Like other hematologic malignancies, multiple myeloma shows a direct correlation between the depth of response achieved with therapy and patient outcomes, most notably, survival.¹ Historically, response evaluation in multiple myeloma was based on the assessment of serum and urine monoclonal protein concentrations via protein electrophoresis and immunofixation, which served as surrogate markers for disease burden. This assessment evolved to allow the inclusion of serum-free light-chain values. Bone marrow plasma cell quantitation was also included, performed on either biopsies or aspirates. Traditionally, a complete response required bone marrow assessment with fewer than 5% plasma cells, irrespective of their clonal nature. Further refinement of this definition added in serum-free light-chain values and immunohistochemical clonal assessment to define a stringent complete response.²

In recent years, the treatment landscape for multiple myeloma has substantially evolved. The introduction of highly active classes of agents has led to improvements in rates and depth of response. The higher rates of complete response achieved with newer therapeutic strategies prompted the need for more granular response criteria to better describe deeper responses. In 2016, the International Myeloma Working Group (IMWG) reported new consensus criteria to redefine multiple myeloma disease response that included an emphasis on incorporation of measurable residual disease (MRD).²

Introduction to MRD

Therapeutic advancements have driven the need for significant improvements in the evaluation of residual disease. These advancements provide opportunities to learn more about how to best implement changes in clinical practice. One important advancement in multiple myeloma is related to the use of MRD, the evaluation of residual

disease present in the patient. While several techniques are available, two techniques are the most commonly utilized to evaluate for MRD: next-generation sequencing (NGS) and next-generation flow cytometry (NGF). A third method, allele-specific oligonucleotide quantitative polymerase chain reaction (aso-PCR) has also been extensively studied, but has less frequent utilization in the multiple myeloma patient population and is therefore not further discussed in this monograph.²

NGS is an *in vitro* technique used to analyze sequences of a patient's immunoglobulin genes. One NGS test, the clonoSEQ Assay[®] (Adaptive Biotechnologies; Seattle, WA), was cleared by the US Food and Drug Administration (FDA) in September 2018 for the detection and monitoring of MRD in bone marrow samples from patients with multiple myeloma or B-cell acute lymphoblastic leukemia (ALL).^{3,4} The FDA reviewed the clonoSEQ Assay through the *de novo* premarket review pathway, a regulatory pathway used for the evaluation of novel devices of a new type associated with low-to-moderate risk. The clonoSEQ Assay uses multiple primer sets to amplify gene segments and identify immunoglobulin rearrangements, within IgH (VDJ), IgH (DJ), IgK, and IgL receptor gene sequences, as well as translocated BCL1/IgH (J) and BCL2/IgH (J) sequences.⁵ The rearrangements of these immunoglobulin genes are not specific to myeloma, but occur in all B cells. Therefore, this rearrangement pattern can provide a cancer cell-specific measure that is unique to that cell and its progeny within the patient. These gene rearrangements must be established in a baseline identification sample obtained at the time of diagnosis. After PCR amplification of the immunoglobulin loci, the DNA is sequenced by synthesis, and the resulting reports are analyzed to determine the presence of the predetermined clonotype.

Like traditional flow cytometry, NGF involves the detection of cell surface markers using fluorescently labeled antibodies, in which cells are quantified as posi-

Table 1. Comparison of NGS and NGF Techniques for the Assessment of MRD in Multiple Myeloma

| | NGS (clonoSEQ [Adaptive Biotechnologies]) | NGF (EuroFlow Consortium) |
|---|---|---|
| FDA-cleared | Yes (2018) | No |
| Baseline sample required | Yes; obtained at diagnosis or from a time point with detectable disease | No |
| Method | Specific DNA sequences or BCR rearrangements are identified and detected by comparison with baseline sample | Abnormal (clonal) plasma cells are identified in any sample by their distinct immunophenotypic pattern vs normal plasma cells |
| Applicability | >92% of patients | >95% of patients |
| Availability | In the United States, performed by a single company at a central laboratory location | Performed at few institutions with EuroFlow implemented |
| Turnaround time | Days (sample must be shipped to and processed by a single, central laboratory) | Hours to days (samples can be performed locally) |
| Required sample amount and sensitivity | Up to 20 µg of DNA (~3 million cells), sensitivity <10 ⁻⁶ | 2 × 10 ⁷ nucleated cells (for sensitivity of 2 × 10 ⁻⁶) |
| Compatible with archived specimens | Yes | No |
| Limit of detection^a | 6.8 × 10 ⁻⁷ | <2 × 10 ⁻⁶ based on the identification of ≥20 abnormal plasma cells among 10 ⁷ events |
| Limit of quantification | 1.8 × 10 ⁻⁸ | <5 × 10 ⁻⁶ based on identification of ≥50 abnormal plasma cells among 10 ⁷ events |

^aLimit of detection refers to the background of nontarget cells in which a target cell can be detected.

FDA, US Food and Drug Administration; MRD, measurable residual disease; NGF, next-generation flow; NGS, next-generation sequencing.

Data from Kumar S et al. *Lancet Oncol.* 2016;17(8):e328-e346² and Bal S et al. *Br J Haematol.* 2019;186(6):807-819.⁷

tively labeled fluorescent cells pass (or flow) in front of a detector. Most prominently developed by the EuroFlow Consortium, the NGF technique has gone through several iterations and improved over time to allow for a higher number of fluorochromes that can be used simultaneously, resulting in an increased number of cells that can be rapidly interrogated.⁶ Many surface markers have been described for the identification of plasma cells and for distinguishing clonal multiple myeloma plasma cells from normal plasma cells. These markers include CD138, CD38, CD45, CD56, CD19, and cytoplasmic κ and λ immunoglobulin light chains. Additional markers that may be of value include CD20, CD27, CD28, CD81, CD117, and CD200.² Currently, the NGF technique has not received FDA clearance for MRD detection.

Each technique has several benefits and limitations associated with its use. Table 1 compares some of the salient features of each of these techniques.

A chief advantage of NGS compared with NGF is the number of cells required to achieve a given threshold to attain “MRD-negative” status. Because of the specificity of the gene rearrangements assessed by NGS, the background or noise of the assay is zero for the most

unique rearrangements.⁵ This characteristic allows for high sensitivity, such as 1⁻⁶, using only a million (10⁶) cells. Validation of NGF technology beyond 10⁻⁵ is performed at site-specific locations. In addition to the small specimen volume required, NGS is advantageous owing to specimen flexibility (it can be used with archived frozen specimens), and its robust limit of detection (1 cancer cell in a background of more than 1 million). Additionally, because the NGS test is validated and performed by a central laboratory in which it is already FDA-cleared, there is no requirement for multiple validations of the assay across laboratories. Limitations of NGS include the requirement for a baseline patient sample for identification and detection, and the need to send the specimen out to a laboratory for processing, which can result in a longer turnaround time (days) relative to NGF (24-48 hours).⁷

In addition to rapid sample turnaround time (24-48 hours), NGF could potentially be performed at individual institutions, with a higher degree of patient applicability. However, a higher sample volume requirement with NGF is a challenge when using bone marrow specimens. Additionally, in order to be performed at individual institutions, the technique must be cross-validated for each

laboratory. However, EuroFlow™ is not widely available and has a lower limit of detection of 2×10^{-6} (with >20 observed events).⁷ In fact, the vast majority of US institutions performing flow cytometry for assessment of multiple myeloma are not using EuroFlow, and the sensitivity of those assays may not be comparable.

Interpreting MRD Assessment

MRD status is often described as MRD-negative or MRD-positive. MRD negativity can simply be considered as the absence of detection of the cancerous plasma cell within a particular number of total nucleated bone marrow cells (determined by the sensitivity of the assay). For example, an NGS assay with a sensitivity reported as 10^{-6} means that no clonal sequences were detected in a sample containing up to or beyond 1,000,000 bone marrow cells. Likewise, an NGF assay is typically reported with a sensitivity reported as 10^{-5} , meaning there is less than 1 malignant plasma cell among 100,000 bone marrow cells.

Multiple myeloma is a notably heterogeneous and patchy disease. These tests are performed using bone marrow samples, which can vary greatly in quality/quantity and thereby significantly impact the performance of the assay. Studies have demonstrated that using the first “pull” is extremely important to maintain the overall integrity of the assay. Traditionally, the first pull had been reserved for morphology testing. However, it is now recommended that the first pull be used for MRD analysis. Unfortunately, this recommendation is yet to be widely adopted.^{2,7} Additionally, myeloma cells may leave the bone marrow microenvironment to cause extramedullary disease, which is not evaluated with bone marrow MRD assessment. Ultimately, assessment of the blood or circulating free DNA will be an important feature in the overall evaluation for MRD, as complete eradication of intramedullary and extramedullary disease is necessary to prevent disease relapse.^{2,7}

The limit of detection is an important consideration, as is the sensitivity of that assay for evaluating MRD. Several studies have shown that as the sensitivity of the assay improves, the discriminatory power of the MRD quantitation in terms of its translation to prognosis and progression-free and overall survival differs. For example, the IFM/DFCI (Intergroupe Francophone du Myélome/ Dana-Farber Cancer Institute) 2009 trial (described in greater detail below) evaluated a novel combination regimen in the context of an up-front vs deferred transplant.⁸ This study established the novel combination for use in up-front management of multiple myeloma. At the time of this study, NGS was not available; instead, first-generation flow cytometry was performed on patient samples

at different time points before and after maintenance therapy. Using these data, the investigators concluded that the patients who were MRD-negative at a sensitivity of 10^{-4} had improved progression-free survival. As NGS techniques became more widely available, stored samples were re-approached using an NGS platform for MRD detection at a sensitivity of 10^{-6} .⁹ In the follow-on study, the investigators showed that using this newer approach with higher sensitivity for MRD detection was more prognostic, with a reduction in the risk of progression and death of approximately 80%.

MRD as a Prognostic Factor

Several meta-analyses have now shown that for patients with multiple myeloma, MRD after initial therapy is prognostic for progression-free survival, as well as overall survival. A meta-analysis from 2017 included 21 studies: 14 reported prognostic information related to progression-free survival (PFS) and 12 assessed the prognostic impact of MRD (first-generation flow cytometry, sensitivity 10^{-4}) on overall survival.¹⁰ Progression-free survival was a median of 54 months among patients with MRD-negative status vs 26 months among those with MRD-positive status (hazard ratio [HR], 0.41; 95% CI, 0.36-0.48; $P < .001$; Figure 1). Overall survival was a median of 98 months vs 82 months, respectively (HR, 0.57; 95% CI, 0.46-0.71; $P < .001$; Figure 2).

The traditional approach to response assessment is now being questioned in the setting of MRD. The definition of complete response refers to the absence of monoclonal protein and clonal cells in the bone marrow. There are patients without a complete response who are MRD-negative. Detectable levels of monoclonal protein may persist owing to the variable kinetics of clearance from the serum. This phenomenon has been observed in recent studies, most notably in chimeric antigen receptor (CAR) T-cell therapy, where response occurs early. These patients are MRD-negative within the bone marrow, but will often have detectable levels of M protein.^{7,11,12}

Even among patients with a traditionally defined complete response (a lack of detectable paraprotein and clonal plasma cells within the bone marrow), MRD still adds useful prognostic information. Among patients with a complete response according to the traditional criteria but who remain MRD-positive, outcome is as poor as that in patients who do not have a complete response. The same meta-analysis showed that MRD was superior to traditional definitions of complete response in predicting survival.¹⁰ Among patients with a complete response, median PFS was 56 months for MRD-negative patients vs 34 months for MRD-positive patients (HR, 0.44; 95% CI, 0.34-0.56; $P < .001$). For this cohort, the median over-

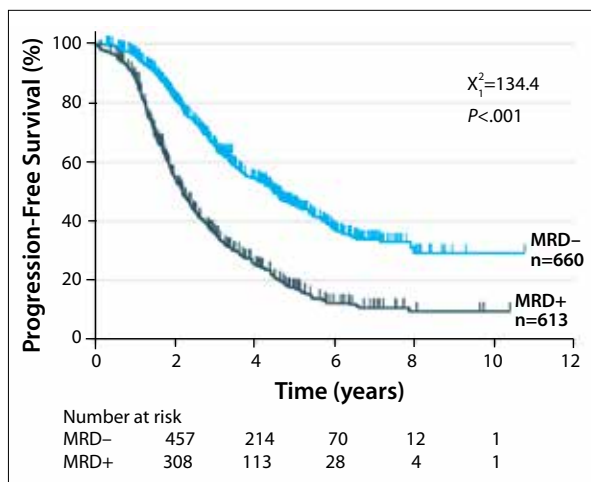


Figure 1. Progression-free survival according to MRD status in a meta-analysis of trials of patients with multiple myeloma. PFS, progression-free survival. Adapted from Munshi NC et al. *JAMA Oncol.* 2017;3(1):28-35.¹⁰

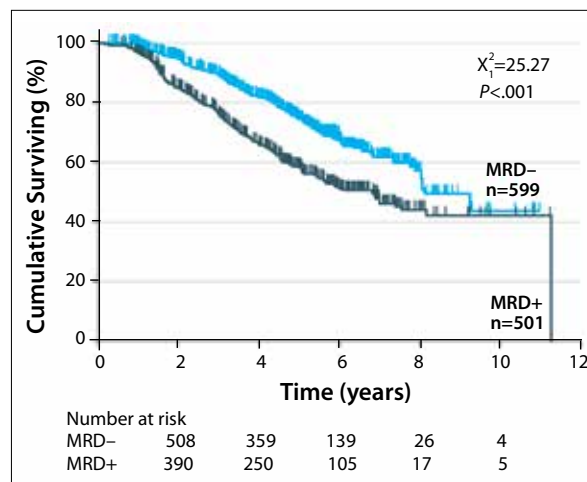


Figure 2. Overall survival according to MRD status in a meta-analysis of trials of patients with multiple myeloma. Adapted from Munshi NC et al. *JAMA Oncol.* 2017;3(1):28-35.¹⁰

all survival was 112 months for MRD-negative patients vs 82 months for MRD-positive patients (HR, 0.47; 95% CI, 0.33-0.67; $P < .001$).

A stringent complete response is used to further define those patients who attain a normal free light-chain ratio. However, it is known that the free light-chain ratio can be confounded by many factors, such as end-organ function (specifically, renal dysfunction) or suppression of the uninvolved chain (a common occurrence). Therefore, patients may have an abnormal free light-chain ratio even when they are MRD-negative.¹³⁻¹⁵

To date, MRD has essentially been shown to be a snapshot in time of what is happening in a representative sample of bone marrow. It is unclear whether patients who attain MRD negativity may be candidates for treatment discontinuation. It is difficult to rely on a single MRD measurement to pursue discontinuation of therapy, particularly in light of available testing modalities, with their respective limitations. As the sensitivity of the MRD assay improves, the ability to discriminate these outcomes will likely increase. Sustained MRD negativity at different time points paired with additional imaging modalities may be used in conjunction to consider optimal candidates for treatment discontinuation.¹⁶⁻¹⁸

Clinical Trials

GEM2000/GEM2005 Trials: First-Generation Flow Cytometry

The PETHEMA/GEM trial (Programa Español de Tratamientos en Hematología/Grupo Español de Mieloma) examined 241 patients enrolled in either the GEM2000

or GEM2005 <65 y trials for potential prognostic markers.¹⁹

All patients were in a complete response at 100 days after high-dose therapy/autologous stem cell transplant (HDT/ASCT; day +100). An MRD assessment at day +100 after HDT/ASCT was performed using a first-generation flow-based assay (sensitivity 10^{-4} to 10^{-5}). Immunophenotyping showed persistent MRD in 36% of the 241 patients who were in a complete response at day +100 post-HDT/ASCT. Twenty-nine patients (12%) had an unsustained complete response defined as progressive disease within the first year after HDT/ASCT, with a low median overall survival of 39 months. These patients showed a higher incidence of persistent MRD compared with patients who remained in a complete response (66% vs 32%; $P = .001$).

The rates of 3-year time to progression were 86% among patients who had an immunophenotypic complete response at day +100 vs 58% among those who did not ($P < .001$).¹⁹ The rates of overall survival at 3 years were 98% vs 80%, respectively ($P = .001$). Multivariate analysis identified cytogenetic risk (HR, 17.3; $P = .002$) and immunophenotypic complete response at day +100 post-HDT/ASCT (HR, 8.0; $P = .005$) as predictive markers for an unsustained complete response. Overall best outcomes in this study were noted for patients achieving an immunophenotypic complete response and standard cytogenetics (3-year time to progression, 94%; 3-year overall survival, 100%). The worst outcomes were noted for patients with persistent MRD (no immunophenotypic complete response) and high-risk cytogenetics (3-year time to progression, 0%; 3-year overall survival, 32%).

The authors concluded that among patients attaining a complete response, the presence of high-risk cytogenetics or lack of an immunophenotypic complete response (MRD, flow cytometry, sensitivity 10^{-4} to 10^{-5}) was able to identify patients at risk for early progression.

PETHEMA/GEM 2012: Next-Generation Flow

At the 2018 American Society of Hematology Annual Meeting, a Spanish group presented results from a series of 390 patients enrolled in the PETHEMA/GEM2012 trial (6 induction cycles with bortezomib, lenalidomide, and dexamethasone followed by ASCT and 2 courses of consolidation with bortezomib, lenalidomide, and dexamethasone).²⁰ MRD was prospectively assessed following induction, transplant, and consolidation, using EuroFlow NGF. The results suggested that achieving MRD negativity may overcome the poor prognosis of high-risk cytogenetics. Among patients achieving MRD negativity ($<2 \times 10^{-6}$), 3-year PFS rates were similar for those with standard-risk fluorescence in situ hybridization (FISH), t(4;14), and del(17p) (90%, 100% and 89%, respectively; $P > .05$).

The IFM/DFCI 2009 Trial: Flow Cytometry

The IFM/DFCI 2009 study was a randomized, open-label, phase 3 clinical trial that evaluated the efficacy and safety of a novel induction regimen consisting of lenalidomide, bortezomib, and dexamethasone (RVD) to understand the differences between up-front and deferred HDT/ASCT in patients younger than 65 years with newly diagnosed multiple myeloma. Patients assigned to a deferred HDT/ASCT arm received 8 treatment cycles of RVD (3 cycles as induction and 5 cycles as consolidation).⁸ Patients assigned to an up-front HDT/ASCT arm received 3 cycles of RVD, followed by high-dose melphalan plus stem cell transplant (consolidation), followed by 2 additional cycles of RVD. All patients in both arms then received maintenance lenalidomide for 12 months.

Bone marrow samples were collected following consolidation and maintenance from patients who achieved a complete response or very good partial response. The samples underwent MRD assessment by 7-color flow cytometry sensitivity at a level of 10^{-4} . A total of 65% of patients were MRD-negative in the deferred transplant group compared with 79% in the up-front transplant group ($P < .001$).

The IFM/DFCI 2009 study demonstrated that up-front transplant was associated with a significant benefit in PFS compared with RVD alone in patients with newly diagnosed multiple myeloma.⁸ Progression-free survival was significantly prolonged among patients who were MRD-negative vs MRD-positive (adjusted HR, 0.30; 95% CI, 0.23-0.37; $P < .001$).

Overall survival at 4 years after randomization was 82% in the deferred transplant arm vs 81% in the up-front transplant arm (HR, 1.14; $P = .43$). Like progression-free survival, overall survival was also significantly longer among patients who were MRD-negative vs MRD-positive (adjusted HR, 0.34; 95% CI, 0.22-0.51; $P < .001$).

The authors concluded that up-front transplant with RVD induction significantly improved rates of MRD negativity and PFS compared with a deferred transplant approach in patients with newly diagnosed multiple myeloma.⁸ Importantly, MRD-negative status was associated with significantly prolonged overall survival, regardless of the treatment arm. These findings further demonstrated that MRD negativity could serve as an important goal for patients with newly diagnosed multiple myeloma.

The IFM/DFCI 2009 Trial: NGS Analysis

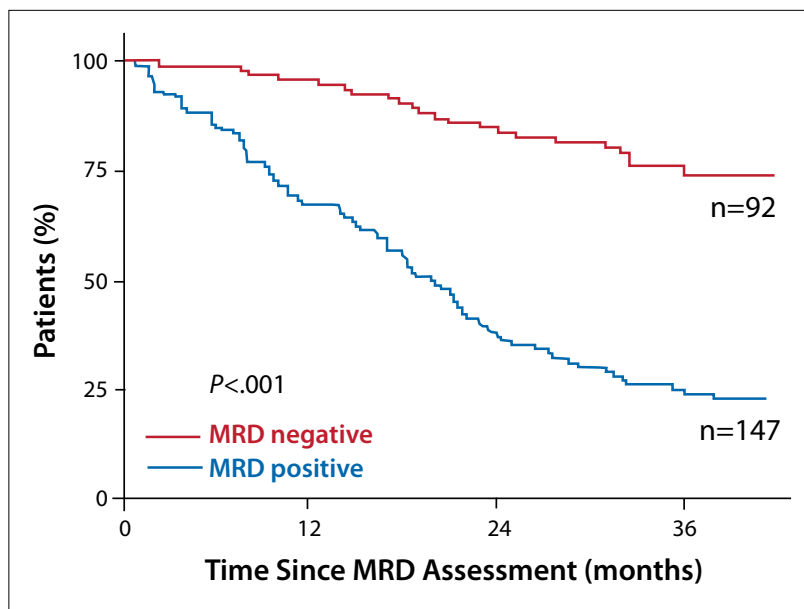
When the IFM/DFCI 2009 trial was initially designed in 2008, the NGS technique for assessing MRD was not available.⁸ For this reason, MRD was initially determined via first-generation flow as described above. When the NGS technique became available, archived specimens from the IFM/DFCI 2009 trial were reanalyzed to evaluate the prognostic value of MRD as assessed by NGS during the maintenance therapy phase.²¹ DNA was extracted from the archived bone marrow specimens and analyzed. Tumor DNA obtained from CD138-positive cells at enrollment was used to provide the baseline identification sample for each patient. MRD status was determined with a sensitivity of 10^{-6} . MRD-negative status was defined as a level of less than 10^{-6} . A total of 509 patients were assessed for MRD status with NGS.

Overall, MRD-negative status was achieved in 127 patients (25%) at least once during maintenance therapy.^{8,21} MRD-negative status was reported in 20% of the RVD-alone arm and 30% of the RVD-plus-transplant arm (adjusted odds ratio for undetectable MRD, 1.65; 95% CI, 1.10-2.49; $P = .02$). MRD status was not significantly impacted by the patient's cytogenetic risk profile (high vs standard risk).

Progression-free survival and overall survival were significantly longer in patients who were MRD-negative vs MRD-positive during maintenance therapy (Figure 3).^{8,21} Survival analyses demonstrated similar rates of PFS and overall survival for patients who maintained their MRD-negative status at both measurements compared with patients who achieved MRD negativity only after 12 months of maintenance therapy. Both of these groups showed significantly improved survival when compared with patients who were either MRD-positive at both assessments or who became MRD-positive following maintenance therapy.

Although there were several limitations to this study,

Figure 3. An analysis of patient samples from the IFM/DFCI 2009 trial using next-generation sequencing showed that progression-free survival after maintenance therapy was higher among MRD-negative patients. IFM/DFCI, Intergroupe Francophone du Myélome/Dana-Farber Cancer Institute; MRD, measurable residual disease. Adapted from Perrot A et al. *Blood*. 2018;132(23):2456-2464.⁹



including missing data and the use of stored specimens (less likely to be the first “pull” sample), the authors concluded that the use of a more sensitive technique—NGS—was able to further discriminate outcomes within the same cohort of patients compared with flow cytometry (sensitivity 10^{-4} to 10^{-5} ; Figure 4).²¹

MRD in Clinical Practice

In the future, it may be possible to use MRD as an actionable indicator to identify deeper levels of remission beyond the complete response category (Figure 5).⁷ Currently, MRD testing in routine practice is limited to providing prognostic information, similar to conventional cytogenetics and FISH analysis, which identify patients more likely to have inferior outcomes. In the future, MRD may be used by physicians and patients to help balance the risks and benefits of different postremission management strategies. Intervention or intensification of therapy in this subset of patients have not been systematically evaluated. There are several ongoing clinical trials with MRD as the primary endpoint, as well as studies evaluating risk-adapted strategies to modify therapy to eradicate MRD. These studies will provide additional evidence regarding how the target of MRD negativity can be a goal of treatment.

Incorporation of MRD into Ongoing and Future Clinical Trials

The importance of prospectively incorporating and uniformly reporting MRD in ongoing and future clinical

studies cannot be overstated. As newer therapies lead to deeper and more prolonged responses, patients with multiple myeloma are living longer. Studies designed with overall survival as the primary endpoint are the most meaningful. However, as survival continues to improve, even longer durations of follow-up will be required to reveal clinically significant differences among treatments. An alternative approach is to identify a surrogate endpoint. To be successful, the surrogate endpoint must be more rapidly achievable and assessable in smaller studies with fewer patients and shorter follow-up. Ideally, surrogate endpoints will help ensure that patients have greater access to clinical trials of novel agents and regimens. A surrogate marker requires a test that is biologically plausible and specific, demonstrates proportionality, and is universally applicable. MRD may be one such surrogate endpoint for future clinical trials.

MRD-guided change of therapy is another important question that must be examined in the context of clinical trials. This strategy has gained traction in other hematologic malignancies, such as ALL and CML. For example, among some pediatric patients with ALL who have achieved MRD-negative status, there is the potential to avoid an allogeneic stem cell transplant. Patients who remain MRD-positive may require further intensified therapy with additional agents, such as blinatumomab. However, in order to drive these treatment decisions, it will be necessary to study MRD in clinical trials that share a uniform reporting system, follow clearly defined methodology, and recognize the sensitivity of the assay. It will also be important to standardize the time points that will be tested. Several ongoing clinical trials will determine the

Figure 4. In an analysis of data from the IFM/DFCI 2009 trial, MRD below 10^{-6} was associated with better outcomes than MRD at levels of 10^{-5} or 10^{-4} . IFM/DFCI, Intergroupe Francophone du Myélome/Dana-Farber Cancer Institute; MRD, measurable residual disease. Adapted from Perrot A et al. *Blood*. 2018;132(23):2456-2464.⁹

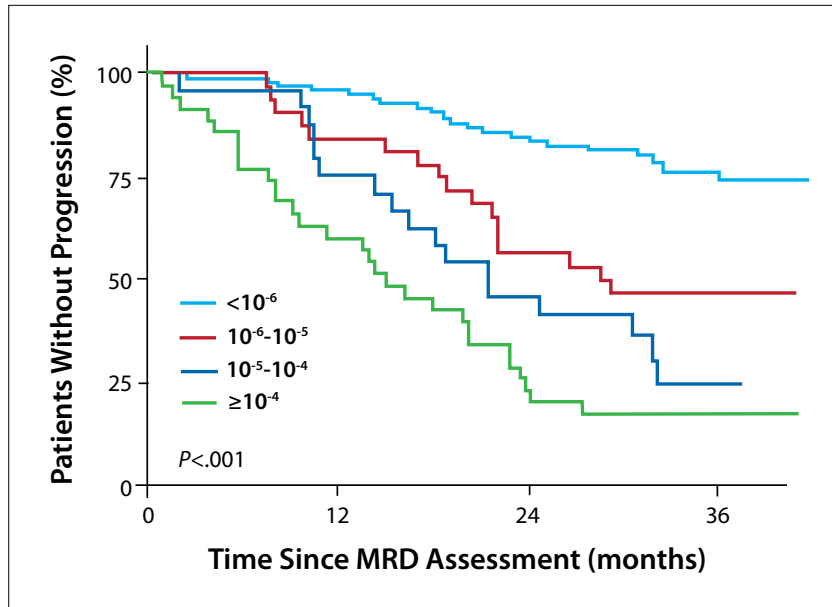
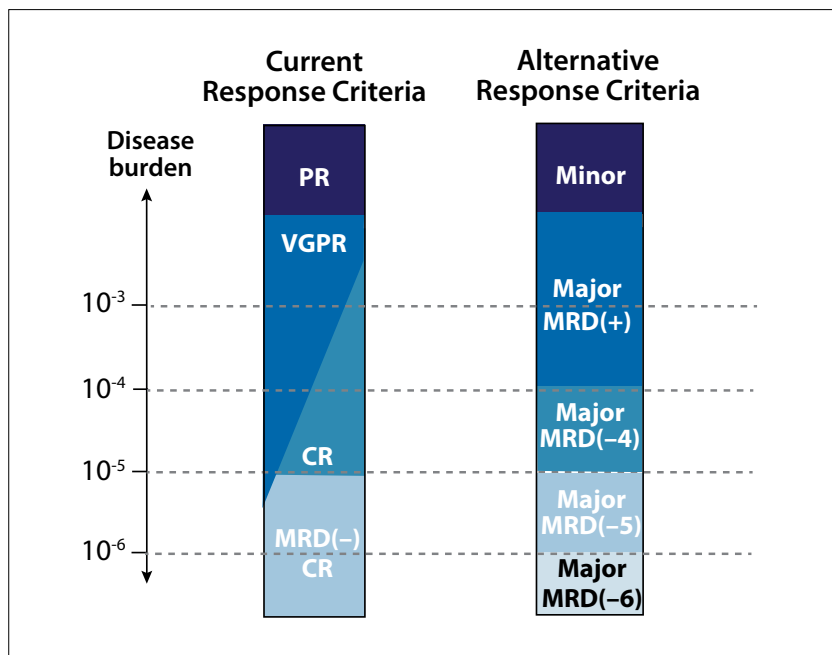


Figure 5. The current criteria and alternative criteria to assess response among patients with multiple myeloma. CR, complete response; MRD, measurable residual disease; PR, partial response; VGPR, very good partial response. Adapted from Bal S et al. *Br J Haematol*. 2019;186(6):807-819.⁷



optimal conditions that must be met to consider cessation of therapy. These steps will help ensure the optimal use of MRD assessment in clinical trials and, subsequently, in clinical practice.

Disclosure

Dr Bal has no conflicts of interest to report.

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Insights into the Assessment of Measurable Residual Disease (MRD) in Patients With Multiple Myeloma

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Defining MRD

The term MRD has been used for many years. However, different sets of data use various definitions of this term. Previous studies have typically referred to “minimal” residual disease, whereas some newer reports have replaced this word with “measurable.” Use of the word “measurable” is preferable, as it emphasizes that assays provide a one-dimensional continuous variable measurement of residual disease. Much of the early literature (and even some current literature) defines MRD as positive or negative. There is some consensus that the positive/negative thresh-

old can be set at 10^{-5} . Some of the earliest MRD studies incorporated assays that reported only positive vs negative status, using a limit of detection of approximately 10^{-4} . More recent advancements in technology, such as the introduction of NGS,¹ now provide a limit of detection below 10^{-6} . These points are important to consider while navigating the literature and when communicating with clinicians throughout the multiple myeloma community.

Importance of Standardization in MRD

There has been some attempt to standardize the defini-

Table 2. IMWG Criteria for MRD^a

| Term | Definition |
|----------------------------------|---|
| Sustained MRD negative | MRD negativity in the marrow (NGF, NGS, or both) and by imaging as defined below, with a confirmed minimum of 1 year apart. Subsequent evaluations can be used to further specify the duration of negativity (eg, MRD negative at 5 years) |
| Flow MRD negative | Absence of phenotypically aberrant clonal plasma cells by NGF on bone marrow aspirates using the EuroFlow standard operation procedure for MRD detection in multiple myeloma (or validated equivalent method), with a minimum sensitivity of 1 in 10^5 nucleated cells or higher |
| Sequencing MRD negative | Absence of clonal plasma cells by NGS on bone marrow aspirate, in which the presence of a clone is defined as <2 identical sequencing reads obtained after DNA sequencing of bone marrow aspirates using the clonoSEQ platform (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher |
| Imaging plus MRD negative | MRD negativity as defined by NGF or NGS, plus disappearance of every area of increased tracer uptake found at baseline or during a preceding PET/CT; or decrease to less mediastinal blood pool standardized uptake value, or decrease to less than that of the surrounding normal tissue |

^aAll require a complete response in addition to the criteria defined here.

CT, computed tomography; IMWG, International Myeloma Working Group; MRD, measurable residual disease; NGF, next-generation flow; NGS, next-generation sequencing; PET, position emission tomography.

Data from Kumar S et al. *Lancet Oncol*. 2016;17(8):e328-e346.²

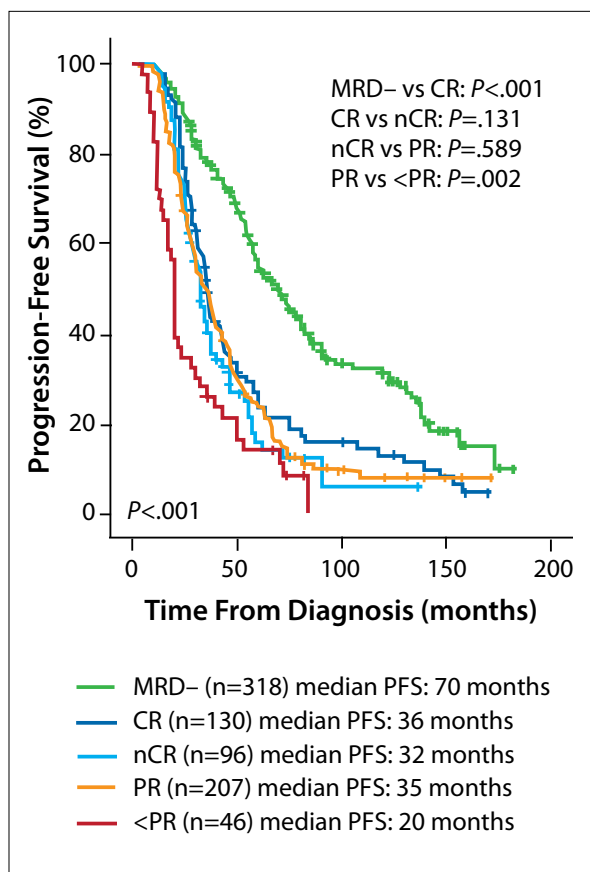


Figure 6. Assessment of measurable residual disease provides a powerful prognostic tool and is more sensitive than conventional response assessment. CR, complete response; MRD, measurable residual disease; nCR, near complete response; PR, partial response; VGPR, very good partial response. Adapted from Lahuerta JJ et al. *J Clin Oncol.* 2017;35(25):2900-2910.³

tion and testing of MRD. The IMWG has defined each of these MRD criteria definitions (Table 2).² Note that the IMWG does not favor either NGF or NGS technology because the defined 10^{-5} sensitivity threshold should be obtainable with either.

MRD provides a powerful prognostic tool and is more sensitive than conventional response assessment (Figure 6).³ However, the use of this measurement does have some potential drawbacks in this setting. Multiple myeloma can be an anatomically heterogeneous disease, with areas within the bone marrow showing a high discrepancy in the burden of disease. Similarly, there may be areas outside the bone marrow that have disease involvement, but are not accessed and therefore not represented in the sample obtained for the MRD assay.

Research has evaluated whether MRD assessment in

the bone marrow can be used with imaging techniques (particularly positron emission tomography [PET]/computed tomography [CT] scans). Studies show mostly agreement between the 2 measures. Patients with a high disease burden in the bone marrow tend to show areas of FDG uptake on the PET/CT scan, whereas those with undetectable MRD in the bone marrow generally have a negative PET/CT scan. This association is not exact. Some MRD-negative patients still have a positive PET/CT scan, and these patients have a worse prognosis than those who are MRD-negative and PET/CT-negative. The IMWG also includes a category of imaging-plus-MRD-negative complete response that is defined as both MRD-negative (with a threshold of $<10^{-5}$) and an absence of focal uptake on PET/CT. These patients have the best outcomes.

Management of multiple myeloma is evolving toward approaches that will enable treatment discontinuation. It is likely that imaging will be used to identify MRD-negative patients who still have levels of disease that require continued therapy.

There are several hurdles to overcome in the standardization of MRD in multiple myeloma. Challenges stem from technical aspects of the assay, as well as the heterogeneity of the disease. It is also important to understand the differences between standardization of the analytical validation and standardization of the clinical validation. Standardization of the analytical validation is primarily affected by the assay itself, including its precision, accuracy, consistency, and limit of detection. In this regard, NGS seems to outperform NGF. FDA clearance of the clonoSEQ Assay involved a long process to develop a robust analytical validation that allows issuing of reports with the input of up to 20 μg of DNA (approximately 3 million cells). This sample input allows for a limit of detection (the number of observations needed to have 95% confidence that the result is reproducible) corresponding to 1.9 neoplastic cells or 6.8×10^{-7} , and a limit of quantification (the number of observations needed to quantify the result with an accuracy of 70% of the total error) of 2.4 neoplastic cells or approximately 1.8×10^{-6} .² It is important to emphasize that although the maximum input for the assay is approximately 3 million cells (20 μg of DNA), the test has been validated to run lower numbers of cells and can accurately identify 1 neoplastic cell out of 1 million. These are extremely low detection limits. During the FDA approval process, the manufacturer was able to demonstrate that the test essentially does not have a false-positive rate. There is a high concordance between the input of clonal cells and the readout of the test. Currently, this test can be reliably performed in more than 90% of patients with multiple myeloma. This rate is now reaching more than 95% in some studies.⁴

Advantages and Disadvantages of the Tests

One potential perceived disadvantage of the NGS test is that it is performed in a central laboratory, and results require more time as compared with in-house tests. Currently, the turnaround time for fresh samples is approximately 7 days. However, the MRD report is not necessarily time-sensitive. Processing through a single central laboratory provides the reassurance that the assay is consistently performed. The results obtained for patients in clinical practice are directly translatable to the results obtained in clinical trials and reported in the literature.

An advantage to NGF is that it can be applicable to more than 95% of patients. This is primarily because it does not require an identification sample from the time of diagnosis. The NGF assay requires a larger sample size of 2×10^7 nucleated cells (approximately ≥ 10 mL). The flow-based NGF assay requires a certain number of events to determine that a sample is positive for residual disease. For example, the limit of detection is thought to be 20 neoplastic cells, yielding to a sensitivity of 2×10^{-6} if a large enough sample is provided. The appeal of the NGF test is that most major medical centers have on-site flow cytometry capability, allowing results within hours. However, this can likewise be considered a limitation because it requires that the NGF test undergo analytical validation at each institution, as the flow cytometry assay for plasma cells performed at the vast majority of institutions is not NGF. This process has been well managed by the EuroFlow Consortium, which has established testing procedures, set quality standards and requirements for software and hardware, and standardized the assay tubes. However, few laboratories have been able to implement these EuroFlow standards in order to perform the NGF test at a similar level as that seen in the clinical studies used to generate the supportive clinical data. Many good-quality flow cytometry laboratories perform an in-house assay and report an MRD result without following the stringent steps implemented by the EuroFlow Consortium that are needed to have a robust analytical validation that can be used to support decision-making and novel drug development.

Consensus on MRD Time Points

The timing of MRD assessment is of critical importance. Historically, the typical pathway for multiple myeloma treatment dictates that patients continue therapy until disease progression or unacceptable toxicity. In the United States, most patients with newly diagnosed multiple myeloma will begin treatment with 4 cycles of triple or quadruple therapy. Those patients who are eligible will then proceed to transplant, followed by 2 cycles of con-

solidation. They will then receive maintenance therapy. In general, this treatment pathway is followed without any adjustments based on disease response. In reality, most patients will obtain, at a minimum, a very good partial remission with modern first-line regimens. By the end of consolidation therapy, most patients achieve a complete remission. Beyond this point, patients stay on the same treatment pathway, continuing the same maintenance therapy until they show clear signs of progression. However, this management course is likely suboptimal, as patients who are in remission but have a substantial disease burden are destined to have an early relapse. Conversely, those patients who are exquisitely good responders and show persistent MRD-negative status may be considered candidates for strategies of treatment discontinuation.

Much of the information on MRD status is drawn from patients who underwent autologous stem cell transplant. MRD status was obtained after transplant or consolidation, or after a certain duration of maintenance. Unfortunately, there is a paucity of information about the impact of MRD assessment taken at the end of 4 cycles of induction therapy. There are even fewer data sets regarding the assessment of MRD at multiple time points, which might provide insight into the association between MRD-negative status and long-term outcome. Guidelines from the IMWG and the National Comprehensive Cancer Network currently recommend MRD assessment at the end of each step of therapy: after induction, high-dose therapy/ASCT, consolidation, and maintenance (Figure 7).^{2,5}

In the future, it will be important for clinical trials in the newly diagnosed setting to include MRD assessment at the end of induction therapy and, in patients proceeding to transplant, following completion of the procedure. Another time point for assessment is during maintenance therapy, at a predetermined fixed time point. We need to have greater utilization of MRD testing—not only in clinical practice, but also in clinical trials—to provide information on what the kinetics of MRD clearance imply for long-term patient outcomes.

A better understanding of the role of MRD assessment at the various therapeutic time points will likely provide more direction regarding the best use of this test. For example, currently clinicians attempt to administer a fixed duration of induction therapy. However, the assessment of MRD at the end of induction might help identify which patients are candidates for extended induction or even the introduction of a newer agent before transplant. The time point at which there will likely be the most information to gain with MRD analysis is post-transplant, or post-transplant after a brief consolidation regimen. In this setting, the burden of residual disease

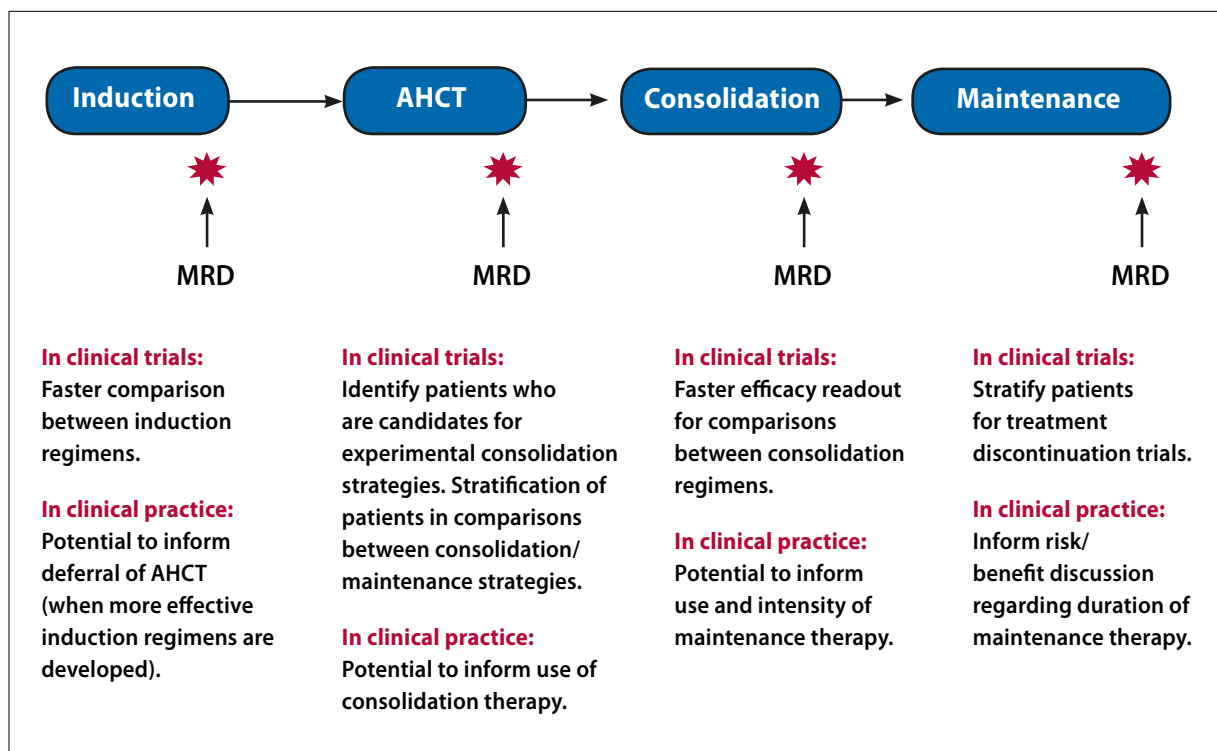


Figure 7. Time points for MRD assessment in multiple myeloma. AHCT, autologous hematopoietic stem cell transplant; MRD, measurable residual disease.

strongly predicts outcome, even among patients who are in complete remission according to traditional IMWG criteria. It is therefore important to design clinical trials in which the starting point is not failure of prior therapy, as indicated by the development of signs or symptoms of myeloma, or an increase in the M protein. Instead, the failure of prior therapy might be interpreted as persistence of a certain disease burden, which would enable the deployment of new treatment strategies at a point when the patient might appear to be in complete remission but has an MRD burden that is high enough to justify a new intervention. Conversely, the possibility of treatment discontinuation in patients with MRD below a certain threshold should be further explored.

MRD Sample Requirements

Assessment of MRD in multiple myeloma typically requires a bone marrow specimen. However, reports of MRD assays use diverse samples, leading to some confusion when interpreting the data. When assessing the bone marrow compartment, the first pull provides a good representation of the content of the bone marrow. As additional marrow volume is pulled, there is an increase in the likelihood that the specimen will be contaminated with

peripheral blood. This contamination has the potential to dilute the bone marrow components. Consequently, a sample that is obtained after multiple pulls can grossly underrepresent the true amount of disease burden. For that reason, it is of the utmost importance that the first pull that is obtained during a bone marrow assessment be sent for MRD analysis. This pull will provide the best representation of the marrow environment, and it will also be the least variable and most standardized. Consistent implementation of this directive will likely improve the robustness of data used to guide study designs and management strategies.

Reporting MRD

NGS MRD analysis provides a quantitative report that specifies how many cells contained the sequence that was initially identified as the hallmark of myeloma in an individual patient. The report states the number of cancer cells out of every 1 million cells. The confounding issue is that in the literature, and even in meeting presentations and abstracts, the data are sometimes simply reported as MRD-positive or MRD-negative, without clarifying what method was used and the threshold for negativity. This oversight leads to misinformation, and represents

one of the barriers to more widespread use of MRD in clinical practice for patients with multiple myeloma. Trial investigators, researchers, editors, and reviewers should ensure that all mentions of MRD are accompanied by full acknowledgement of the technique and the threshold for detection. Otherwise, the data might become impossible to interpret, or worse, misleading.

Incorporating MRD into Clinical Management

Currently, questions concerning the role of MRD in clinical care are often met with the response that more data are needed. Long-term implications of MRD status as an early assessment of outcome will likely be clarified by clinical trials that incorporate this measure for patient stratification and that investigate MRD assessment at multiple time points and with longer follow-up. However, disease burden is an important factor in treatment decisions. For example, clinicians might change a patient's induction regimen to achieve a deeper remission post-transplant, deploy a consolidation regimen in a patient without an optimal disease response by the end of transplant, or discontinue maintenance therapy in a patient who develops significant toxicity and has been in complete remission for several years. Some of these strategies lack supportive data from clinical trials. Estimation of the disease burden via a biomarker (eg, M protein) lacks analytical validation showing a correlation to outcomes. In contrast, MRD assessment provides an accurate assessment of disease burden through direct measurement. MRD burden above or below 10^{-6} , for example, has greater power in predicting outcome than a complete response or even a stringent complete response. Therefore, if a physician is going to use disease burden to guide clinical decision-making, MRD should be part of the assessment. As an example, I would assess MRD in a patient who develops fatigue or another quality-of-life issue after treatment with maintenance therapy for 2 or more years. Traditionally, the decision to stop therapy might have been based solely on toxicity, as well as the patient's preference. Now, the additional information about the MRD can be used to either encourage the patient to continue maintenance treatment longer (provided that the toxicity or quality-of-life burden is not too significant), or it could provide reassurance that the risk associated with discontinuing treatment is not high. Data from the IFM 2009 study provide a good estimation of the risk of progression once maintenance is discontinued. In that study, maintenance was discontinued by design after 12 months.^{6,7} The knowledge of MRD status at the end of maintenance and the risk of progression in MRD-positive vs MRD-negative patients can be used to predict expected outcomes of patients treated with modern induction therapy and limited dura-

tion maintenance once treatment is discontinued. Several ongoing studies are also addressing how MRD can guide maintenance therapy.⁸⁻¹⁰

Whether to proceed to transplant is another example of where MRD analysis may prove useful for decision-making, although this use is still a matter of intense debate. The goal might be for the patient to become MRD-negative after induction, which would allow deferral of transplant without impacting overall outcome. This strategy has the potential to spare some patients the toxicity of transplant. The concern, however, is that this approach might be used prematurely based on a misunderstanding of the data. The IFM 2009 trial is perhaps the best source of information for this approach.^{6,7} In one arm, patients who completed the induction therapy then proceeded to transplant and maintenance therapy. In the other arm, patients completed longer induction/consolidation therapy (8 cycles), deferred transplant, and proceeded to maintenance therapy. The rate of MRD negativity (measured after completion of transplant and consolidation, with a threshold of 10^{-6}) was just 30% among those patients who underwent autologous transplant. It is possible that the rate of MRD of less than 10^{-6} is likely far lower than 30%, and might even be less than 10% after induction. Among patients who underwent the prolonged induction therapy, the rate of MRD was 20%. This low rate was seen with RVD, an effective regimen, used for 8 cycles. The rate would presumably be even lower with 4 cycles of induction therapy. The use of MRD to guide the decision to proceed to transplant must be done in a safe way that does not affect patient outcomes. There is concern that physicians might use "MRD assays" with a much inferior limit of detection (eg, first-generation flow), and potentially inappropriately defer a therapy that could greatly impact a patient's remission. A longer induction period, with perhaps a more active regimen than RVD, may be required for patients to achieve MRD-negative status. This question is being evaluated in the DFCI 10-106 study, which is the sister study to IFM 2009.¹¹ In the DFCI 10-106 study, MRD will be assessed post-induction (and also post-transplant in the transplant arm).

Conclusion

MRD assessment is redefining how we understand treatment response in multiple myeloma and how we utilize measurement of the burden of residual disease to predict outcomes, deploy experimental therapies, or simply inform discussion with patients regarding the risks and benefits of subsequent therapies. Although broader availability of MRD testing raises new questions that

must be answered through well-planned trials, it also brings opportunities never before available in multiple myeloma.

Disclosure

Dr Costa has performed consulting for Amgen, Celgene, and Adaptive Biotechnologies. He has received honoraria from Amgen and Sanofi. He has received research grants from Amgen and Janssen.

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Best Practices for the Assessment of Measurable Residual Disease (MRD) in Multiple Myeloma: Further Observations

Luciano J. Costa, MD, PhD, and Susan Bal, MD

Luciano J. Costa, MD, PhD A major opportunity for MRD is to better understand the impact of each phase of therapy in multiple myeloma. For example, in the past, to understand what transplant strategy worked best with which combination of agents, it was necessary to perform a full clinical study and wait months to years to fully assess PFS and overall survival. Although randomized clinical trials remain the gold standard, they are becoming increasingly difficult to conduct because patients do not simply complete transplant and proceed to observation. They receive different types of maintenance therapy for different durations, making it difficult to isolate outcome back to transplant. Measurement of MRD now provides direct and objective linear measurement of disease burden that can be deployed before and after that phase of therapy. This allows for much faster comparison than a test between 2 conditioning regimens. The same can be said about consolidation therapy. MRD testing before and after these phases provides immediate, direct measurement of the impact of treatment on the disease burden. Previously, this type of measurement had been difficult

because most patients are in complete remission or very good partial remission using traditional criteria. Now their response can be more fully characterized with more sensitive techniques.

Susan Bal, MD Low burden of MRD positivity presents a unique opportunity to study novel treatment approaches. Patients who remain MRD-positive at the end of a defined period of therapy might be candidates for immunotherapeutic strategies. Immune strategies, such as bispecifics and CAR T-cell therapy, are safer and more effective in the context of lower-burden disease. A group of high-risk patients who remain MRD-positive according to a defined threshold after initial up-front management might be candidates for such studies.

Similarly, maintenance discontinuation should be explored among patients who demonstrate sustained MRD negativity at predefined time points. As Dr Costa mentioned, patients who demonstrate a persistent deep response may benefit from discontinuation of therapy.

Slide Library

Depth of Response in Multiple Myeloma

- Multiple myeloma shows a direct correlation between the depth of response achieved with therapy and patient outcomes, most notably survival¹
- Therapeutic advancements have driven the need for significant improvements in the evaluation of residual disease
- In 2016, the IMWG reported new consensus criteria for redefining multiple myeloma disease response that included an emphasis on incorporation of measurable residual disease (MRD)²

IMWG, International Myeloma Working Group.
 1. Palra E et al. *Blood*. 2015;125(26):3059-3066. 2. Kumar S et al. *Lancet Oncol*. 2016;17(10):e328-e346.

Assessment of Measurable Residual Disease (MRD) in Multiple Myeloma

- Next-generation flow (NGF) uses flow cytometry to identify malignant plasma cells through the presence of immunologic markers located on the cell surface
- Next-generation sequencing (NGS) analyzes for the presence of sequences in immunoglobulin genes that were previously identified as markers of that specific patient's plasma cell malignant clone
- In 2018, the FDA cleared the NGS clonoSEQ Assay[®] (Adaptive Biotechnologies; Seattle, WA) for the detection and monitoring of MRD in bone marrow samples from patients with multiple myeloma or B-cell ALL

ALL, acute lymphoblastic leukemia; FDA, US Food and Drug Administration.

Interpreting MRD Assessment

- MRD status is often described as MRD-negative or MRD-positive
- MRD negativity can be defined as the absence of detection of a cancerous plasma cell within a particular number of total nucleated bone marrow cells (determined by the sensitivity of the assay)
- An NGS assay with a sensitivity reported as 10⁻⁶ means that no clonal sequences were detected in a sample containing up to or beyond 1,000,000 bone marrow cells
- An NGF assay is typically reported with a sensitivity of 10⁻⁵, meaning there is less than 1 malignant plasma cell among 100,000 bone marrow cells

MRD as a Prognostic Factor

- Studies have shown that MRD after initial therapy is prognostic for progression-free survival, as well as overall survival, in patients with multiple myeloma¹⁻⁵
- In a meta-analysis of 21 studies¹:
 - Progression-free survival was a median of 54 months among patients with MRD-negative status vs 26 months among those with MRD-positive status (HR, 0.41; 95% CI, 0.36-0.48; P<.001)
 - Overall survival was a median of 98 months vs 82 months, respectively (HR, 0.57; 95% CI, 0.46-0.71; P<.001)
- MRD adds useful prognostic information even among patients with a traditionally defined complete response

HR, hazard ratio; 1. Munshi NC et al. *JAMA Oncol*. 2017;3(11):26-31. 2. Arora M et al. *Blood*. 2017;129(14):1711-1718. 3. Palra E et al. *Blood*. 2012;119(3):647-651. 4. Gower-Cherry et al. *Blood*. 2014;123(suppl 1):5. 5. Arora M, Lachkar H et al. *Blood*. 2015;126(suppl 21).

MRD and Complete Response

- A meta-analysis showed that MRD was superior to traditional definitions of complete response in predicting survival¹
- Among patients with a complete response:
 - Median PFS was 56 months for MRD-negative patients vs 34 months for MRD-positive patients (HR, 0.44; 95% CI, 0.34-0.56; P<.001)
 - Median overall survival was 112 months for MRD-negative patients vs 82 months for MRD-positive patients (HR, 0.47; 95% CI, 0.33-0.67; P<.001)

1. Munshi NC et al. *JAMA Oncol*. 2017;3(11):26-31.

Incorporation of MRD Into Clinical Trials

- The importance of prospectively incorporating MRD assessment into ongoing and future clinical studies cannot be overstated
- As newer therapies lead to deeper and more prolonged responses, patients with multiple myeloma are living longer. Prolonged durations of overall survival will require that clinical studies include years of costly and long-term follow-up to reveal clinically meaningful differences among treatments
- An alternative approach is to identify a surrogate endpoint to overall survival, and MRD may be one such endpoint

MRD Sample Requirements

- Assessment of MRD in multiple myeloma typically requires a bone marrow specimen
- It is of the utmost importance that the first pull obtained during a bone marrow assessment be sent for MRD analysis. This pull will provide the best representation of the marrow environment, and it will also be the least variable and most standardized
- Consistent implementation of this directive will likely improve the robustness of data used to guide study designs and management strategies

Reporting NGS MRD

- The NGS MRD report states the number of cancer cells out of every 1 million cells
- The confounding issue is that the data are sometimes simply reported as MRD-positive or MRD-negative, without clarifying what method was used and the threshold for negativity
- This oversight leads to misinformation, and represents one of the barriers to more widespread use of MRD in clinical practice for patients with multiple myeloma
- Trial investigators, researchers, editors, and reviewers should ensure that all mentions of MRD are accompanied by full acknowledgement of the technique and the threshold for detection

MRD Time Points

- Guidelines from the IMWG and the NCCN currently recommend MRD assessment at the end of each step of therapy:
 - After induction
 - After high-dose therapy/ASCT
 - After consolidation
 - After maintenance

ASCT, autologous stem cell transplant; NCCN, National Comprehensive Cancer Network.

Incorporating MRD Into Clinical Management

- Currently, MRD testing in routine practice is limited to providing prognostic information—similar to that from conventional cytogenetics and FISH analysis—that identifies patients more likely to have inferior outcomes
- This information can be used to inform physicians and patients when they are balancing the risks and benefits of different postremission management strategies

FISH, fluorescence in situ hybridization.

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