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Review

Measurable Residual Disease Assessment in Multiple Myeloma: How Deep Is Enough?

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Abstract: The introduction of new and more effective therapeutic options for Multiple Myeloma (MM) has significantly deepened and prolonged patients' remission. As currently used treatment protocols induce high rates of complete responses, Measurable Residual Disease (MRD) assessment has become essential to enhance the evaluation of treatment efficacy. Detection of MRD has improved with the development of highly sensitive and standardized techniques such as Next Generation Flow or Next Generation Sequencing, complemented by functional imaging techniques. These advances offer a valuable opportunity to further optimize criteria of response to treatment. Currently, extensive data demonstrate that MRD status is a valuable prognostic factor of survival. Since MRD represents a real measurement of disease burden, its incorporation in clinical trials to guide treatment decisions will certainly translate into clinical benefits. Sustained MRD negativity can be used to consider optimal candidates for treatment discontinuation, whereas MRD positive high-risk patients may have access to novel immunotherapeutic strategies such as bispecific drugs or CAR T cell therapy. In this review, we describe the available techniques to detect MRD, address the current data regarding MRD as a surrogate endpoint within clinical trials, examine how MRD can be introduced into the clinical management of MM patients, and discuss the future of MRD monitoring.

Keywords: multiple myeloma; measurable residual disease; prognostic factor; surrogate endpoint

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1. Introduction

Multiple Myeloma (MM) therapy has improved considerably in the past years, with the development of multi-drug regiments and the inclusion of novel agents such as immunomodulatory drugs (IMiDs), proteasome inhibitors (PIs), and monoclonal antibodies (mAbs) [1–5]. These new treatment approaches have led to high complete remission (CR) rates, associated with longer progression free survival (PFS) and prolonged overall survival (OS) [6,7]. Furthermore, increased knowledge regarding the biology of this disease, the mutational landscape, and the interplay between myeloma cells and the bone marrow (BM) microenvironment have introduced new insights to tackle this hematological malignancy in a more effective way [8–10]. The prospect of eliminating the malignant plasma cells and of converting MM into a fully curable disease seems within reach. Alongside innovations regarding new treatments, the development of highly sensitive techniques to evaluate its efficacy is critical. Criteria for response to therapy can no longer solely rely on traditional methods such as serum immunofixation and determination of plasma cells by morphology, which lack the sensitivity and discrimination to fully define disease status and accurately predict patient response. Therefore, the inclusion of measurable residual disease (MRD) detection to monitor patients and assess the true depth of response, opens the field towards a more personalized treatment management in MM.

This review will focus on the current available methods to detect MRD, the relevance of MRD assessment in the treatment of MM, the benefits MRD evaluation can bring to clinical research, the questions MRD can help answer in the clinical setting, and the future directions for MRD testing.

2. Methods for BM MRD Detection

The development and validation of methods for MRD detection have evolved greatly in the last years. Technological advances, with the introduction of more accurate and sensitive approaches, have paved the way towards a more effective monitoring of MRD. Currently, BM MRD evaluation and imaging-based techniques are recommended by the International Myeloma Working Group (IMWG) as the most appropriate tools to assess low levels of disease [11].

2.1. Flow Cytometry-Based MRD

Multiparameter flow cytometry (MFC) immunophenotyping is one of the most frequently used methods to study plasma cell (PC) disorders. This technique is available worldwide, allowing for the fast evaluation of a high number of cells, discriminating, characterizing, and quantifying both neoplastic and normal PCs at low frequency, by using a combination of well-defined surface and cytoplasmatic markers and modern flow cytometers. The combination of anti-CD38 and anti-CD138 mAbs are used to specifically identify PCs, and the unequivocal presence of an aberrant phenotype is commonly evaluated with discriminatory markers such as CD19, CD56, CD45, CD27, CD117, CD81, and cytoplasmic Igκ and Igλ.

Identification of the minimum number of aberrant PCs remaining after treatment is of great utility to monitor therapy efficacy and predict outcome in MM patients, and MFC is a sensitive method for analyzing the PC compartment [12]. In 2008, a study by the Spanish group showed that MRD negative patients at day 100 after Autologous Stem Cell Transplant (ASCT) had longer PFS (median 71 vs. 37 months; $p < 0.001$) and OS (median not reached vs. 89 months; $p = 0.002$). An absence of residual clonal PCs by MFC evaluation was the most relevant independent prognostic factor [13]. Similarly, MM patients that maintained MRD positivity by MFC 3 months after ASCT had shorter PFS. Being MRD positive, despite a negative immunofixation result, was associated with a worse outcome [14]. In the French IFM 2009, MRD negativity evaluated by MFC was likewise associated with higher PFS and OS at a sensitivity level of 10^{-4} [15]. Taken together, these results reflect the higher prognostic value and clinical relevance of MRD assessment by MFC.

The ability to quantify residual disease using MFC assays and the level of sensitivity reached have evolved over the years. First generation assays using 4 to 6 colors were progressively replaced by 2nd generation MFC using 8 colors, and now it is standard to use panels of two tubes with 8 colors or even one tube with 10 colors. The EuroFlow Consortium has developed and validated a standardized Next Generation Flow (NGF) methodology [16] included in the International Myeloma Foundation consensus criteria [17]. By using an 8-color two-tube panel, BM processing applying a bulk-lysis procedure, and a minimum recommended number of 10^7 cells/tube, has allowed this methodology to reach an increased level of sensitivity between 10^{-5} and 10^{-6}, when enough cells are evaluated ($\geq 5 \times 10^6$ per tube). Additionally, new software tools have been developed for automatic analysis with normal and malignant reference databases, speeding the process and avoiding biases potentially introduced by individual operator assessment [18]. Both the limit of detection and the limit of quantification can be determined based on the identification of at least 20 and 50 neoplastic PCs among 10^7 nucleated cells, respectively, discriminating between positive and negative MRD samples. A direct comparison between the 8-color two-tube and 10-color one-tube assays was performed, showing concordance of 98% between the two approaches. The first assay has the advantage of acquiring a higher number of cells and the possibility to have a confirmatory tube; the second assay is less time consuming and affordable [19]. The possibility of new improvements in this field leading to an increase

of the level of sensitivity is not to be excluded and alternative single-tube MFC approaches have also been presented [20,21].

Normal PCs display a heterogeneous immunophenotype according to their maturation process. Knowing the expression pattern in different conditions, from reactive to regenerating BM samples, enables a clear distinction from aberrant PCs [22]. Likewise, it renders possible to identify neoplastic PCs in follow-up samples, even upon phenotypic shifts [23]. As such, one major advantage of NGF is that a diagnostic sample is unrequired. Although the introduction of new anti-CD38 Ab treatments for MM, such as daratumumab or isatuximab, might hamper the use of this marker to specifically detect PCs, this can be overcome with the use of multi-epitope CD38 Abs [24].

Additionally, a broad detection of the different cell populations present in a sample can be done, including those that permit the identification of potential hemodiluted samples (mast cells, nucleated red blood cells, myeloid, and B-cell precursors). This intra-assay quality control is especially important as hemodiluted BM aspirates may induce a false negative result. The risk can be minimized if the first BM aspirate is sent to MRD evaluation by NGF, as recommended by the recently published work on consensus on performing and reporting MRD evaluation [25].

Determining the depth of MRD negativity and the level of sensitivity that is clinically relevant is especially important. The PETHEMA/GEM2012MENOS65 trial published in 2020 showed that patients with undetectable MRD after being treated with lenalidomide–bortezomib–dexamethasone (RVd) induction, followed by consolidation with ASCT and RVd, had an 82% reduced risk of progression or death (Hazard Ratio (HR) = 0.18; 95% Confidence Interval (CI) 0.11 to 0.30; $p < 0.001$). An MRD negative response defined by NGF identified a group of patients with a lower risk of progression, confirming a cut-off of 10^{-6} as being clinically relevant [26]. In the EMN02/HO95 MM Phase 3 trial, newly diagnosed MM (NDMM) patients, who had reached CR prior to maintenance therapy and retained it during the maintenance period, had a significant reduced risk of progression or death when MRD was negative by NGF evaluation every 6 months. After a median follow-up of 75 months, the 5-year PFS was 66% in MRD negative versus 31% in MRD positive patients (HR = 0.39; $p < 0.001$), and the 5-year OS was 86% versus 69%, respectively (HR = 0.41; $p < 0.001$) [27]. Despite using lesser sensitive MFC assays, several studies have reported a better outcome for patients reaching MRD negativity [13]. Rawstron et al., demonstrated a benefit in OS per log reduction of MRD levels as evaluated by MFC (5.9 years–10^{-2}–10^{-3}; 6.8 years–10^{-3}–10^{-4}; >7.5 years-10^{-4}) [28]. More recent trials confirmed that achieving undetectable MRD at a sensitivity of 10^{-5}–10^{-6} was able to overcome the worse outcome in high-risk patients at baseline, though these patients require more intense regimens to reach these levels of MRD [26,27,29,30]. Deep response to treatment may therefore shift patients with an adverse prognosis to a more favorable one, defining a more accurate risk stratification.

Despite the increased sensitivity of NGF approaches, patients with undetectable MRD still progress, particularly those who present extramedullary disease or single focal lesions. This emphasizes the importance of combining it with complementary techniques such as high-precision imaging; a subject that will be discussed further in this review.

2.2. Molecular-Based MRD

Over the last decade, we have witnessed an improvement in molecular methods for MRD monitoring, particularly with the introduction of Next Generation Sequencing (NGS) techniques. Indeed, this molecular-based MRD assessment has replaced other laborious and low-applicable techniques, such as allele-specific quantitative polymerase chain reaction (ASO-qPCR) [31–33].

NGS is based on the use of consensual primers that amplify and sequence all rearranged immunoglobulin genes (IgH (VDJ), IgH (DJ), IgK, and IgL receptor gene sequences, translocated BCL1/IgH (J) and BCL2/IgH (J) sequences) on aberrant BM PCs. A baseline sample is required to identify the dominant sequence(s) used to monitor MRD after

treatment. This sequencing method has shown a sensitivity up to 10^{-6} and is applicable in more than 90% of myeloma cases [34]. This technique has also been applied in other hematological neoplasms such as acute lymphoblastic leukemia, mantle cell lymphoma, and chronic lymphocytic leukemia [35–37].

In the context of MM, several groups have shown the efficacy of MRD assessment to predict patient outcome in CR and independently of disease risk. Martinez-Lopez et al., in 2014 demonstrated that deep sequencing of immunoglobulin rearranged genes can effectively identify and quantify neoplastic PCs in the BM of MM patients. More importantly, this method was shown to be a prognostic biomarker of time-to-tumor progression (TTP) and OS. In this study, three groups of patients were identified based on MRD levels: high ($<10^{-3}$), intermediate (10^{-3} to 10^{-5}), and low ($>10^{-5}$), with significantly different TTPs of 27, 48, and 80 months ($p = 0.003$ to 0.0001), respectively [38]. Another study by Avet-Louiseau et al., published in 2015, evaluated NGS sensitivity using BM aspirates from the IFM/DFCI2009 clinical trial (NCT01191060). This trial included NDMM patients receiving RVd induction therapy followed by consolidation therapy with ASCT plus RVd or RVd alone and had MRD evaluation of gene rearrangement in PC performed before and after maintenance. Researchers found that, by establishing a cut-off of 10^{-6}, patients below this value presented a 3-year PFS of 83% pre-maintenance and 90% post-maintenance. Notably, these values were similar when restricted to MM patients in CR, demonstrating that deep sequencing through NGS can predict PFS, even in patients treated with state-of-the-art strategies [15]. These results were further confirmed by Perrot et al., who demonstrated that an MRD negative status is indeed a powerful prognostic biomarker of both PFS (adjusted HR = 0.22; 95% CI, 0.15–0.34; $p < 0.001$) and OS (adjusted HR = 0.24; 95% CI, 0.11–0.54; $p = 0.001$) [39]. Overall, these studies suggest that deeper responses are associated with better patient outcome.

Furthermore, Takamatsu et al., showed that NSG-based MRD detection in autograft and BM samples from MM patients who received high-dose melphalan plus ASCT had significant prognostic value. Briefly, MRD negative patients ($<10^{-6}$) by NGS showed significant better PFS (96%, $p < 0.001$) and OS (100%, $p = 0.04$) compared with MRD positive patients. Patients who underwent ASCT treatment using novel therapeutic agents and whose autografts were MRD negative ($<10^{-7}$) by NGS had significant better PFS compared with untreated patients ($p = 0.001$). Overall, these results demonstrate a strong correlation between NGS-based MRD negativity and PFS. Conversely, ASO-qPCR showed no significant benefit compared to NGS. However, this study lacked a standardized therapy and sample size, therefore these results should be further confirmed in other prospective studies to draw stronger conclusions [40]. Nevertheless, several other randomized trials such as ALCYONE, MAIA, CASTOR, or POLLUX have demonstrated that, independently of the treatment scheme, patients who achieved MRD negativity through NGS techniques had significantly higher PFS [1,41,42]. Although BM-based MRD evaluation using NGS has shown a strong prognostic value that correlates with the majority of patients' outcomes, this is not witnessed in a cohort of patients who, despite being MRD negative, eventually relapse [43,44]. This reflects the need to further improve currently available techniques for MRD assessment in terms of sensitivity and specificity to better stratify this group of patients.

Despite NGS being an extremely sensitive MRD detection method with the advantage that samples do not require immediate processing, contrary to NGF, some pitfalls are starting to arise. The fact that it is labor intensive, time consuming, more expensive, and less available, combined with the presence of somatic mutations which are genetic hallmarks of MM and may confound NGS results, giving rise to false-negative results due to clonal evolution [45,46]. A major disadvantage is the lack of standardization and harmonization across laboratories, including analysis of results, characteristics of tested patients, or time points assessed [47]. Therefore, it is of utmost importance that MRD testing procedures are uniformed so that data can be compared between institutions, allowing large-scale analysis and introduction into routine clinical practice [48]. An international group of 21 laboratories with expertise in NGS—EuroClonality-NGS consortium—is currently

working on its standardization and has already validated assays for MRD assessment in Acute Lymphoblastic Leukemia [49].

So far, most of the published data relies on the use of the clonoSEQ platform (Adaptive Biotechnologies) [50] as the only assay cleared by the FDA for MRD assessment. The clonoSEQ Watch Registry (NCT04545333)—a prospective multicenter observational study of adult patients—is currently collecting data regarding the use of this assay in the management of lymphoid malignancies, including MM. Tests to develop non-commercial alternatives are ongoing. Recently, Martinez-Lopez et al., successfully described and validated a novel in-house NGS method to assess MRD in MM patients. This technology demonstrated high reproducibility with a sensibility of 10^{-5} and can be fully automated, minimizing variations across laboratories. Results showed a global concordance of 89% between in-house NGS data and flow cytometry (Spearman correlation r = 0.8; $p < 0.001$) [51].

Despite the data available on the performance of each BM-based methodology, information is still lacking regarding concordance between NGF and NGS. Results from the FORTE trial showed a concordance between the two methods of 86% at 10^{-5} (n= 335; r = 0.61) and 78% at 10^{-6} (n = 56; r = 0.77). In pre-maintenance samples, however, MRD assessed by MFC revealed a higher percentage of positive cases, with a discordance of 1–10% at the same level of sensitivity [50]. In the CASSIOPEIA trial, concordance was also high (92.9%) [52]. Additional clinical trials with extended follow-up periods will be needed to tackle this issue.

Taken together, NGS results are very promising, although more studies are required before widespread implementation in the routine clinical practice.

2.3. Imaging Techniques-Based MRD

Whole body imaging techniques such as 18F-Fluorodeoxyglucose positron emission tomography/computed tomography (18F-FDG PET/CT) and Magnetic Resonance Imaging (MRI) provide important complementary information regarding the presence of residual disease. MM is a heterogenous disease with patchy infiltration [53,54], and most patients present focal lesions detectable by imaging techniques [55,56] that constitute a challenge for intramedullary MRD methods that use a single BM aspirate.

At present, recommendations for imaging during follow-up and response evaluation of MM patients include whole-body CT or PET/CT, depending on availability of initial results at baseline for comparison. PET/CT is currently the standard of care to evaluate and determine metabolic response after treatment [57,58]. This technique combines the imaging of a molecular process (usually FDG tracer uptake) with morphologic images obtained by CT and is especially useful when addressing extramedullary disease [59,60]. MRI can detect bone involvement and the presence of extramedullary disease and provides details on soft tissue disease and patterns of BM infiltration [61]. Both MRI and PET/CT techniques showed similar results at identifying bone lesions at diagnosis, but only normalization before maintenance therapy, detected by PET/CT, showed a predictive role for PFS and OS [62]. Evaluation of treatment efficacy by PET/CT has been shown to correlate with prognosis in MM [63]. Conventional MRI might not be the optimal approach to evaluate MRD, as focal lesions remain positive for several months after therapy, even in patients who respond to treatment [64]. However, Diffusion-Weighted MRI (DWI), a functional MRI technique that measures water molecule movement in tissues, could provide useful information on residual focal lesions. This technique still requires standardization, and further studies are needed to compare DWI with 18F-FDG PET/CT and to evaluate DWI results in the context of MM after therapy [65,66]. Optimization of PET/CT is also needed. Efforts were made to standardize this technique, particularly with the Deauville scores (standardized uptake value cut-offs using the liver background as reference to identify a complete metabolic response) [67]. Erroneous results might arise due to a low expression of the hexokinase enzyme responsible for the glycolysis of the FDG tracer used [68]. Alternatives to assess disease activity in MM are currently being evaluated, such as 11C-Methionine [67]. Other PET tracers under investigation include peptide tracers such as 68Ga-Pentixafor targeting CXCR4, presently

being evaluated in a Phase 2 ongoing clinical trial in NDMM patients (NCT04561492), and lipid tracers such as choline or acetate. These new tracers and molecular imaging biomarkers are currently being included in clinical trials before they can be fully adopted in clinical practice [69,70].

Recent studies have demonstrated the importance of combining PET/CT with MRD BM techniques such as NGF or NGS to evaluate treatment response. A low agreement between BM MRD and imaging techniques was previously reported in the IFM/DFCI 2009 trial, with 26% of MRD negative patients by MFC (sensitivity 10^{-4}) and positive by PET/CT, and by the CASSIOPET sub-study of CASSIOPEIA, with 10.5% of MRD-negative patients by NGF (sensitivity 10^{-5}) being PET/CT positive [62,71]. A study by Rasche et al., revealed that a proportion of MRD negative patients by NGF eventually relapsed with extramedullary disease, and patients who were MRD negative by both NGF and PET/CT had better PFS than those only MRD negative by NGF. There are also more discrepant cases among the relapsed/refractory MM (RRMM) group (50% versus 12% in the NDMM patients) [72]. Patients with persistent 18F-FDG PET/CT lesions after induction and subsequent ASCT had shorter 4-year estimates of PFS (32% versus 47%; $p = 0.02$) and OS (66% versus 79%; $p = 0.02$) [73]. BM involvement may not be uniform and BM examination, usually done in the easily accessible pelvic bone, may not account for residual focal lesions in other sites that can be detected by imaging techniques. The clonal heterogeneity that characterizes MM, with the existence of multiple clones and distinct molecular and biological traits, further accentuates the need to combine both approaches. Recent data regarding genetic spatial tumor heterogeneity represented by focal lesions highlight the existence of sub-clones with gene expression signatures associated with disease aggressiveness and drug resistance, strengthening the hypothesis that these focal lesion sub-clones are at the origin of MM relapse [74].

Present recommendations by the IMWG on the use of MRD in clinical trials state that both BM MRD assessment and functional imaging by PET/CT should be evaluated simultaneously [25]. A comparison of the currently recommended methods to measure MRD is summarized in Table 1.

Table 1. Characterization of currently available techniques for MRD evaluation in MM recommended by the IMWG.

	Next Generation Flow (NGF)	**Next Generation Sequencing (NGS)**	**PET/CT**
Applicability	~100%	~90%	~90% (specifically extramedullary disease)
Availability	High (≥8 colors needed)	Low (commercial and academic platforms)	Intermediate
Sample at diagnosis	Not obligatory	Obligatory (identification of dominant clone)	Not obligatory (required for identification of focal lesions or extramedullary disease
Number of cells required	10×10^6 cells	$1–3 \times 10^6$ cells	NA
Sample processing	Fresh samples Processing within 24–48 h	Fresh/frozen samples	NA
Patchy sample	Impact	Impact	No impact
Whole sample characterization	Yes (global cell characterization)	No	No
Clonal evaluation	Not possible	Possible (identification of minor clones)	Possible (requires focal lesion biopsy)

Table 1. *Cont.*

	Next Generation Flow (NGF)	Next Generation Sequencing (NGS)	PET/CT
Quantitative method	Yes	Yes	Yes
Standardization	Yes (EuroFlow Consortium)	Yes (Adaptive Biotechnologies; FDA approved)	No (ongoing)
Sensitivity	10^{-5}–10^{-6}	10^{-5}–10^{-6}	High (4 mm)
Time to results	3–4 h	7 days	2 h
Complexity	Cytometry skills; automated analysis available	Bioinformatic support	Nuclear medicine support
Reproducibility	High	High	Moderate

Abbreviations: NGF—Next Generation Flow; NGS—Next Generation Sequencing; PET/CT—Positron Emission Tomography/Computed Tomography; NA—Not Applicable; FDA—Food and Drug Administration.

The combination of multiple approaches will undoubtedly provide solid and substantial information to support clinical decisions, namely the safe and effective discontinuation of treatment in a specific cohort of patients. Currently, there are ongoing clinical studies (NCT04108624) to assess MRD in MM patients and to determine whether patients who are MRD negative by multiple modalities (novel imaging and laboratory techniques) can safely and effectively discontinue post-transplant maintenance therapy after receiving at least one year of maintenance therapy.

Overall, neither NGF- nor NGS-based methods, in combination with imaging techniques, associate undetectable MRD with better outcomes, revealing that the depth of response and level of sensitivity achieved is more important than the technique itself.

3. Future of MRD Testing: Beyond BM Assessment

The most sensitive methods currently available to assess MRD in MM require an invasive BM aspiration. To overcome this concern, as well as the spatial heterogeneity of MM and the frequency of MRD assessment, alternative less invasive approaches are being investigated. The use of liquid biopsies using peripheral blood as a source for circulating tumor-derived components is a promising option to tackle these issues, including the high sensitivity detection of circulating tumor plasma cells (CTPC), circulating cell-free tumor DNA (cfDNA), and serum monoclonal immunoglobulins [75–78].

The same strategy used for BM PC analysis can be applied to the detection of neoplastic PC present in PB. CTPC have been detected in a high percentage of MM cases at diagnosis [79] and the release of CTPC from the BM to PB has been associated with decreased survival [80]. Furthermore, at different treatment timepoints, a higher CTPC frequency seems to have clinical utility as an unfavorable prognostic factor in MM patients [81–83] and is also associated with a greater risk of progression in pre-clinical stages such as monoclonal gammopathy of undetermined significance (MGUS) and smoldering MM (SMM) [84,85]. Recently, Gonsalves et al., revealed that the presence of $\geq$5 CTPC/μL at diagnosis in patients with Revised International Staging System (R-ISS) Stage I and II that were re-classified as R-ISS IIB had worse prognosis than those with R-ISS Stage III (time to next treatment: R-ISS I, 40 months; R-ISS II, 30 months; R-ISS IIB, 21 months; R-ISS III, 20 months; OS: R-ISS I, not reached; R-ISS II, 72 months; R-ISS IIB, 45 months; R-ISS III, 47 months) [86]. Another study addressing the prognostic value of CTPC in NDMM patients revealed that the combination of R-ISS Stage III and a level of CTPC $\geq$ 0.105% was able to stratify a subgroup of patients associated with aggressive disease (OS = 11.5 months versus 33 months; $p < 0.001$; PFS = 10 months versus not reached; $p < 0.001$) [87]. These results emphasize the utility of CTPC as a complementary prognostic tool to improve MM patient risk stratification. It is well established that CTPC can be detected with a high throughput NGF strategy [88,89]. In the study by Sanoja-Flores et al., the NGF assay was able to detect and quantify CTPC in 17% of MM patients in CR, identifying a subgroup with

lower PFS (HR = 7.4; 95% CI, 3.0–18.2; $p < 0.0001$). Although all patients with detectable CTPC were MRD positive in the BM, sensitivity is still suboptimal and 40% of patients who were positive in the BM had undetectable neoplastic PC in PB [90]. However, these results already hint that CTPC may reflect the ability for disease dissemination, providing an early indication of progression. In fact, CTPC analysis revealed specific features in terms of phenotype and subclonal mutations, different from the PC normally present in the BM [91]. In fact, besides quantification, CTPC can be evaluated using molecular approaches and eventually used as a biomarker for response to treatment [78,92,93]. The detection of CTPC might be useful to provide a complete image of the genomic landscape of MM patients, including detection of mutations and copy number variations by whole exome sequencing. In a study by Garcés et al., the mutations present in BM myeloma cells and extramedullary plasmacytoma were also detected in CTPC (86% and 87%, respectively). A high concordance between the genetic profile of matched CTPC and BM myeloma cells was observed (82%), including mutations in genes frequently altered in MM (83%), as well as copy number alterations (95%) [94]. However, MRD evaluation relying solely on CTPC is far from a routine clinical application and needs further technical and standardization improvements.

cfDNA has been explored as a liquid biopsy in cancer [95], including MM [96], but its relevance in MRD assessment is still being discussed. Although cfDNA can be used to effectively reconstitute the genetic landscape and clonal heterogeneity of MM in the BM [97], there are dissonant results regarding its applicability as an MRD monitoring tool. Studies using NGS to compare MRD in paired cfDNA from PB and BM samples showed that 69% of patients who were MRD positive in the BM had undetectable MRD in the PB [92], and that it failed to detect IgH rearrangements in 66% of patients in CR [98]. Even with the commercially available NGS kit used for MRD monitoring, a low concordance (49%) with BM results was obtained [92]. These results could be mostly due to decreased sensibility to detect specific Ig gene rearrangements in PB when there is a low disease burden in the BM and to technical limitations of this methodology to target DNA fragments that are longer than cfDNA. More encouraging results from a long-term study using cfDNA detected by ASO-qPCR found a specificity of 83.3% and a sensitivity of 66.7%, with a significant correlation between no or low (positive non-quantifiable) cfDNA level and the number of patients reaching CR ($p = 0.012$) [99]. Recently, our group published a review that summarizes the current knowledge regarding the use of CTPC and cfDNA to evaluate MRD [100]. Thus far, these methodologies do not seem sufficiently mature or sensitive for widespread MRD assessment. Frequent MRD monitoring in blood is an attractive approach, and further characterization of CTPC and cfDNA can offer insights into the molecular evolution of MRD during tumor progression with potential therapeutic repercussions.

M-protein peptides detected by mass spectrometry (MS)-based techniques in the serum and urine are also emerging as a possibility to monitor the MM burden [101]. Alternative methods to the standard serum protein electrophoresis, such as matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and liquid chromatography quadrupole time-of-flight mass spectrometry (LC-MS), have shown promising results in MRD detection [102,103]. MALDI-TOF-MS detects total mass distribution of denatured intact Ig light chains, whereas LC-MS is a clonotypic peptide method recognizing specific peptides of the M-protein complementarity determining region [104]. Both techniques are more sensitive than conventional assays, but are still time consuming, costly, and may not be valid for all patients. The role of MS in the group of non-secretory MM patients, for instance, is still to be determined. An alternative method, Quantitative Immunoprecipitation MS (QIP-MS), uses polyclonal antibody-based technology to detect and quantify intact Ig in serum based on their molecular mass. Each M-protein is specific to each PC clone and differentiated from mAbs drugs applied in the clinical setting, allowing their tracing over time [105]. This method is currently being evaluated for MRD assessment in the context of clinical trials and has shown good concordance with BM NGF MRD results [106,107]. Comparison between BM NGS MRD and PB MS MRD revealed that all discordant cases were negative by the first technique, which could be explained with

the presence of long half-life residual paraprotein. In fact, clearance of the monoclonal component can be a later occurrence and patients might not meet the criteria for CR despite achieving MRD negativity in the BM, thus not accurately reflecting the tumor burden [108]. In this regard, performing MRD evaluation in patients with a very good partial response (VGPR) according to the IMWG criteria to determine any deeper level of remission would be a more practical approach [109].

Routine use of liquid biopsy approaches for MRD assessment is still a long way ahead and requires further technical improvement, optimization, and clinical validation before it can replace BM MRD assessment. Ongoing studies regarding the concordance between PB and BM MRD assessment are essential to determine whether there is a true representation of disease status.

Liquid biopsies are certainly appealing as they allow a more frequent evaluation of disease burden and could play a complementary role to determine the precise timing for MRD monitoring, paving the way to a more personalized management of MM patients.

4. Clinical Relevance of MRD Evaluation in MM

Determining the response to treatment is crucial in monitoring MM patients. As data on MRD evaluation from different clinical trials becomes available, the evidence supporting its relevance in the management of MM is ever increasing. MRD testing has been in the spotlight for its role in comparing the efficacy of different treatment strategies and supporting clinical decisions towards an individualized approach to therapy in MM.

4.1. MRD Improve Definition of Response

In 2016, the IMWG revised response criteria introduced the definition of MRD in CR patients as the persistence or re-appearance of one myeloma cell in at least 10^5 normal cells [11]. Response to treatment is widely accepted as one of the most important indicators of survival, since, over the years, several studies have clearly demonstrated a correlation between prolonged survival and deeper responses. In 2008, Lahuerta et al., showed that the quality of response after ASCT, specifically CR, was significantly associated with longer event-free survival and OS in NDMM patients [7]. Similarly, Kapoor et al., showed that achieving stringent CR (sCR) after ASCT improved long-term outcome [110]. However, the conventional definition of CR was not able to distinguish between patients with different progression risks [111] and patients in CR and sCR still relapsed [6,7,110]. Moreover, conventional criteria of CR failed to identify patients at risk of early relapse. This was demonstrated by Paiva et al., that patients with unsustained CR within 1 year after ASCT, despite presenting similar CR rates at day 100 after high-dose therapy followed by ASCT, had a dismal outcome (median OS of 39 months; $p < 0.001$) [112]. It was the introduction of sensitive methodologies able to detect minimal levels of residual MM cells that allowed the improvement of CR definition. Previous studies using MFC with a cut-off of 10^{-4} already suggested that even lower levels of MRD would provide a more accurate outcome prediction [28]. Using NGS, Martinez-Lopez et al., were able to identify patients with high ($<10^{-3}$), intermediate (10^{-3}–10^{-5}), and low ($>10^{-5}$) levels of MRD with distinct TTP (27 versus 48 versus 80 months; $p = 0.003$ to 0.0001) [38].

Indeed, patients in CR can be further discriminated into those with detectable and undetectable MRD, with clear association of undetectable MRD with superior PFS and OS. In a study by the PETHEMA/GEM, it was already clear that CR patients with persistent MRD and patients in near CR/VGPR presented the same outcome in terms of both PFS (27 versus 29 months) and OS (59 versus 65 months). MRD negativity was associated with higher PFS (median 63 months; $p < 0.001$) and OS (median not reached; $p < 0.001$), surpassing CR in reducing risk of progression (PFS HR = 0.42 for MRD negativity versus HR = 0.67 for CR) and/or death (OS HR = 0.33, for MRD negativity versus HR = 0.58, for CR) [113]. Two meta-analyses demonstrated a positive correlation between MRD negativity assessed by immunophenotypic and molecular methods and prolonged PFS and OS. Munshi et al., showed a significant association of undetectable MRD with significantly

better PFS overall (HR = 0.41; 95% CI 0.36–0.48; $p < 0.001$) and in CR patients (HR = 0.44; 95% CI 0.34–0.56; $p < 0.001$). OS was also favorable in MRD negative patients overall (HR = 0.57; 95% CI 0.46–0.71; $p < 0.001$) and in CR patients (HR = 0.47; 95% CI 0.33–0.67; $p < 0.001$) (HR of 0.44 for PFS and 0.47 for OS) [114]. Landgren et al., found that MRD negativity (versus positivity) was associated with better PFS (HR = 0.35; 95% CI 0.27–0.46; $p < 0.001$) and OS (HR = 0.48; 95% CI 0.33–0.70; $p < 0.001$) [115].

These results demonstrate that MRD redefined response criteria in MM and undoubtedly establish the importance of achieving undetectable MRD for patient outcome.

4.2. MRD Is a Relevant Prognostic Factor

Undetectable MRD is clinically relevant regardless of the patient risk group [116]. However, it is even more important in those high-risk patients associated with unfavorable cytogenetic alterations and biological characteristics according to the R-ISS. The IFM2009/EMN02 trials demonstrated that achievement of MRD negativity was less frequent in patients with high-risk cytogenetics, namely with del(17p). High-risk patients according to R-ISS and cytogenetics, with detectable MRD after treatment, had shorter PFS compared with MRD negative patients (7 versus 67 months for R-ISS Stage III patients, HR = 0.12, 95% CI 0.05–0.31; $p < 0.001$; 15 versus 53 months for patients with high-risk cytogenetics, HR = 0.18, 95% CI 0.09–0.35; $p < 0.001$) [27]. Perrot et al., also showed that patients with high-risk cytogenetics and undetectable MRD had longer PFS than those with standard-risk cytogenetics but detectable MRD. Furthermore, irrespective of the R-ISS disease stage at diagnosis, the median PFS was the same when MRD was undetectable, with comparable survival rates. [39]. The PETHEMA/GEM2010 trial demonstrated that MRD status was an independent prognostic factor for TTP (HR = 2.7; $p = 0.007$) and OS (HR = 3.1; $p = 0.04$), with significant positive impact of MRD negativity on TTP of high-risk cytogenetic patients (HR = 12.6; $p = 0.01$) [117]. Furthermore, the PETHEMA/GEM2012 trial revealed that the worse PFS and OS was attained in R-ISS Stage III patients with detectable MRD [26]. Munshi et al., recently published a large meta-analysis showing the importance of attaining MRD negative status in improving long-term survival regardless of disease stage, risk factors, treatment setting, and method and sensitivity thresholds for MRD measurement. MRD negativity improved PFS (HR = 0.33; 95% CI 0.29–0.37; $p < 0.001$) and OS (HR = 0.45; 95% CI 0.39–0.51; $p < 0.001$) compared with MRD positivity, especially when MRD negativity was reached 12 months post-maintenance rather than pre-maintenance [118]. A recent study by Goicoechea et al., showed 36-month PFS rates above 90% in patients with standard- and high-risk cytogenetic abnormalities when achieving undetectable MRD [116]. Li et al., also found no significant difference in PFS and OS between MRD negative high-risk patients and MRD positive standard-risk patients (median PFS 45 versus 34 months; $p = 0.3$; 4 year OS 100% versus 83.6%; $p = 0.196$) [119].

These findings highlight that achieving MRD negativity could overcome adverse risk factors identified at diagnosis, substantially altering patient prognosis during treatment.

So far, data from several clinical trials have shaped MRD evaluation to be performed in specific timepoints for established treatment protocols. That is the case of NDMM patients less than 70–75 years old, for which the standard intensification approach is still high-dose melphalan with ASCT [120]. In 2008, the study by the PETHEMA/GEM group in NDMM patients who underwent ASCT, MRD evaluation by MFC was performed at day 100 after ASCT, with longer PFS (median 71 versus 37 months; $p < 0.001$) and OS (median not reached versus 89 months; $p < 0.002$) in MRD negative patients [13]. Likewise, in the Myeloma IX study, MRD was determined both post-induction with RVd and post-ASCT, with higher PFS in MRD negative patients at both timepoints compared with those that only became MRD negative post-ASCT, howbeit with no benefit in OS [121]. In the Phase 2 study of the French group, including NDMM treated with RVd followed by ASCT and lenalidomide maintenance, MRD was measured by MFC after induction revealing that 68% of patients achieved MRD negativity with an estimated 3-year PFS of 77% (95% CI, 57% to 88%) and OS of 100% [122]. Improved PFS and OS with high-dose melphalan consolidation plus

ASCT were obtained when compared with melphalan–prednisone–lenalidomide (median PFS 43 months versus 22.4 months, HR for progression or death = 0.44, 95% CI 0.32 to 0.61; $p < 0.001$; 4-year OS 81.6% versus 65.3%, HR for death = 0.55, 95% CI 0.32 to 0.93; $p = 0.02$) [123] and cyclophosphamide and dexamethasone plus lenalidomide (median 43.3 months (33.2–52.2) versus median 28.6 months (95% CI 20.6 to 36.7), HR for the first 24 months = 2.51, 95% CI 1.60 to 3.94; $p < 0{\cdot}0001$) [124]. The EMN02/HO95 study revealed, at a median follow-up of 60.3 months, a significant improvement in median PFS with ASCT compared with bortezomib–melphalan–prednisolone (VMP) (56.7 months (49.3 to 64.5) versus 41.9 months (37–5 to 46.9), HR = 0.73, 95% CI 0.62 to 0.85; $p = 0{\cdot}0001$) [125]. The results of the prospective multi-center PRIMeR study, that assessed MRD by MFC in the context of the STAMiNA trial, showed that MRD negative patients pre-maintenance and 1 year after ASCT had better PFS and longer OS, independent of the treatment arm. HR for progression or death in MRD negative versus MRD positive patients pre-maintenance and 1 year after ASCT were 0.48 and 0.22 ($p < 0.001$), and HR for overall mortality were 0.77 ($p = 0.52$) and 0.10 ($p < 0.001$), respectively [126].

The positive effect of attaining undetectable MRD even in NDMM patients ineligible for ASCT, particularly older and frail patients, is extremely relevant, as shown by the Spanish group [117]. Moreover, with the inclusion of daratumumab to the standard combination regiments in this group of patients, PFS and OS improved, and MRD negativity rates increased compared with standard therapy (27–24% versus 7%; $p < 0.001$) [1,3,42]. The MAIA Phase 3 trial comparing daratumumab–lenalidomide–dexamethasone (Dara-Rd) with lenalidomide–dexamethasone (Rd) [1], and the ALCYONE study comparing daratumumab–bortezomib–melphalan–prednisolone (Dara-VMP) with VMP [3] investigated outcomes using MRD negativity as a secondary endpoint, defined as the proportion of patients who were MRD negative at any timepoint using NGS with a sensitivity threshold of 10^{-5}. The concept of sustained MRD negativity was established as the maintenance of MRD negative results in BM more than 6 or 12 months apart. With a median follow-up of 36.4 months in MAIA and 40.1 months in ALCYONE, patients with sustained MRD negativity for more than 6 months showed better PFS compared with patients who did not maintain MRD negativity, both separately and when results from the two studies were pooled. This was also observed with sustained MRD negativity for longer than 12 months. Improved PFS was seen in patients in CR and MRD negative compared with those who were MRD positive or who achieved VGPR or worse (HR = 0.19; 95% CI 0.14 to 0.26; $p < 0.0001$). Time to subsequent treatment was higher in patients who were MRD negative at any of the follow-up times analyzed versus MRD positive patients and in patients with sustained MRD negativity for more than 6 or 12 months versus those without sustained MRD negativity [127]. Even in transplant eligible patients, the addition of daratumumab to bortezomib–thalidomide–dexamethasone (Dara-VTd) in pre-transplant induction and post-transplant consolidation improved depth of response, with higher rates of MRD negativity (64% versus 44%; $p < 0.0001$; at 10–5 sensitivity by MFC). Achievement of undetectable MRD was associated with prolonged PFS and such improvement was consistent across patients' baseline characteristics [5]. These studies demonstrate that regardless of treatment, MRD negativity is the major prognostic determinant, conferring a favorable prognosis.

A recent study by Ubieto et al., confirmed the prognostic relevance of MRD by showing that traditionally used parameters, such as myeloma cell count by morphology and serum-free light chain ratios, were irrelevant in patients with negative immunofixation. In addition, the current IMWG stratification had no prognostic value in transplant-eligible MM patients with persistent MRD [128]. The latest recommendations by the National Comprehensive Cancer Network include MRD assessment during follow-up as an indicator of prognosis [129]. Taken together, compelling data are being gathered regarding the importance of MRD negativity that may lead to the definition of new risk stratification tools.

Thus, attaining MRD negativity as a response to treatment, irrespective of the measurement technique or the therapy strategy, is indeed a robust predictor of PFS and OS.

5. MRD as a Surrogate Endpoint in Clinical Trials

With the continuous innovations introduced in MM treatment, the need to accelerate the drug approval process is increasingly important. Until now, PFS has been used as a surrogate endpoint for OS in clinical trials. However, as patients achieve prolonged responses, it is becoming a late endpoint, incompatible with the swiftness needed. In NDMM patients, achieving PFS is likely to take an average of seven years [130]. As the drug discovery landscape for MM changes, the introduction of validated surrogate endpoints instead of the traditional clinical trial endpoints is critically needed. MRD negativity is perhaps the strongest clinically significant prognostic factor associated with PFS, in both NDMM and RRMM patients, even more so than clinical staging or cytogenetics [40,115,131]. MRD is a specific measure of tumor burden, and knowledge regarding the role of MRD in the clinical course of MM has pushed its consideration as the best candidate to be used as a surrogate marker for drug approval. The use of MRD as a surrogate endpoint for PFS in MM is being evaluated and validated by the International Independent Team for Endpoint Approval of Myeloma MRD Initiative, a consortium that includes academic and pharmaceutical members [132]. Both the European Medicines Agency and the US Food and Drug Administration have issued draft guidelines [133,134]. MRD-guided therapy decisions require that key questions such as optimal sensitivity cut-off, definition of sustained MRD negativity, and best timing for MRD evaluation be specified in MM clinical trials. Very recently, recommendations on the harmonization in designing, performing, and interpreting MRD assessment in MM clinical trials were published to address these issues [25].

Treatment strategies based on MRD status should take into consideration the potential technical pitfalls that might hamper the correlation between MRD negativity and improvement in PFS, such as early evaluation after myeloablative conditioning treatments or sampling biases due to patchy infiltration. As previously mentioned, MM is characterized by spatial heterogeneity, and the random nature of BM aspirate sampling is a challenge for MRD analysis that could, in the future, be overcome through the inclusion of liquid biopsy approaches.

6. Open Questions That MRD Can Help Answer in the Clinical Setting

Advancements in the knowledge of residual disease in MM provide an important opportunity to determine how to better implement therapeutic changes in clinical practice. With the inclusion of increasingly effective therapies for MM, MRD represents an interesting means to guide treatment decisions.

6.1. Can MRD Redefine the Use of ASCT?

Thus far, current data suggest that attaining MRD negativity is more important than the actual means to achieve this profound depth of response. Consequently, the need for high-dose chemotherapy with ASCT is being questioned in a time when new and more effective treatments are being introduced. Results from the FORTE trial presented in 2019 showed similar MRD negativity rates, defined as $<10^{-5}$ by MFC, in patients who underwent ASCT with four induction cycles and four consolidation cycles with carfilzomib-lenalidomide–dexamethasone (KRd) and patients who received 12 KRd cycles without ASCT (58% versus 54%) [29]. Responses assessed before maintenance were well matched between groups, showing that approximately 65% of patients were MRD negative. Results from a Phase 2 study evaluating weekly KRd in combination with daratumumab (Dara-KRd) showed that 74% of NDMM patients achieved MRD negativity, with no progressions at a median follow-up of 10 months [131]. A large randomized multi-center trial was initiated to compare Dara-KRd with standard care for NDMM patients (NCT03290950).

Attal et al., (NCT01191060) showed that MRD negativity was higher in the group of patients submitted to ASCT than in the non-ASCT group (79% versus 65%; $p < 0.001$) [135]. Using MRD data evaluated by NGS, Perrot et al., found lower levels of MRD negativity in patients treated only with RVd than in those who underwent ASCT (adjusted odds ratio

for MRD negativity 1.65; 95% CI, 1.10–2.49; $p = 0.02$), but PFS was similar in both groups for patients with the same MRD status [39], raising the hypothesis that achieving MRD negativity after induction might inform decisions regarding the role of ASCT.

New quadruplet regimens are also being investigated in NDMM patients in the context of ASCT. In the CASSIOPEIA study (NCT02541383), the addition of daratumumab to VTd before and after ASCT was shown to improve depth of response and PFS or OS. Significant MRD negativity rates, at a 10^{-5} sensitivity threshold, were also detected in the Dara-VTd group ($p < 0.0001$), except in the high-risk patients [5]. In the GRIFFIN study (NCT02874742), MRD negativity after Dara-RVd was improved compared with RVd (51.0% versus 20.4%; $p < 0.0001$) [4]. A high rate of MRD negativity at a sensitivity of 10^{-5} by NGS was also achieved in the MASTER trial with Dara-KRd after consolidation, revealing that patients treated with this four-drug regimen without subsequent ASCT achieved similar MRD results as those with ASCT [136].

Thus, MRD status might be used to challenge the current paradigm regarding the role of high-dose chemotherapy and a transplant-based approach.

6.2. Can MRD Guide Maintenance Therapy?

The role of MRD in shaping maintenance treatment for MM is still an unanswered question. Two pooled analyses of two Phase 3 studies in patients receiving lenalidomide maintenance revealed an improvement in depth of MRD response, with 27% of MRD positive patients after consolidation becoming MRD negative during maintenance (sensitivity 10^{-4}–10^{-5}) [137,138]. In earlier trials, such as the Myeloma IX where MRD was assessed by MFC at a level of sensitivity of 10^{-4}, the percentage of patients with durable MRD negativity was higher in the group that received maintenance therapy with thalidomide compared with those who did not receive any maintenance treatment (96% versus 68%; $p = 0.026$). In the subsequent Myeloma XI study, higher PFS was reported in MRD negative patients who received lenalidomide maintenance [139]. In a retrospective observational study, Alonso et al., reported that 34.3% of patients who were MRD positive after induction achieved MRD negativity during maintenance with lenalidomide. Patients who did not achieve MRD negativity, but showed declining MRD values during maintenance, still obtained a benefit in survival, with no difference in PFS between these patients and those who ultimately obtained MRD negativity [140]. In the abovementioned FORTE trial, significantly more patients who were MRD positive at maintenance randomization became MRD negative with carfilzomib and lenalidomide (KR) versus lenalidomide after a median follow up of 31 months and a median duration of maintenance of 27 months (46% versus 32%; $p = 0.04$) [29]. Other studies have also highlighted the beneficial effect of prolonging maintenance therapy in MRD positive patients [141,142]. Hahn et al., demonstrated that MRD negativity one year after ASCT was prognostic of better PFS and OS ($p < 0.001$) [126]. Long-lasting MRD negativity might have a good prognostic effect, but its optimal duration is still a matter of debate. This was explored by Gu et al., in a study of MRD evaluated in post-induction and during 3 until 24 months post-ASCT. A better PFS and a significantly longer OS was identified in patients that maintained MRD negativity since post-induction with no progression until 24 months post-ASCT compared with those with MRD negativity at post-induction but who became MRD positive 24 months post-ASCT (PFS not reached versus 15.4 $\pm$ 2.4 months; $p = 0.000$; OS not reached versus 35.2 $\pm$ 18.6 months; $p = 0.000$). Interestingly, patients who were MRD negative 24 months after ASCT regardless of their MRD status post-ASCT showed similar OS [143].

Prospective clinical trials with the inclusion of long-term serial MRD measurements would help determine the clinical significance of MRD evolution and lead toward a tailored approach to maintenance treatment.

6.3. Can MRD Be Used to Decide Treatment Discontinuation?

Repeated MRD monitoring can be used as a tool to confirm sustained MRD negativity and to identify subgroups of patients with different prognosis. The study by Gu et al.,

already suggested that the pattern of MRD evolution is more informative than determining MRD in a single time point and that sustained MRD negativity throughout treatment is associated with better prognosis [143]. Whether patients with sustained MRD negative status during maintenance can discontinue or de-escalate treatment is another open question.

In the recently published results of the MASTER trial, MRD status was applied to inform about the efficacy and duration of maintenance with Dara-KRd after ASCT. In total, 80% of patients reached MRD < 10^{-5} and 66% reached MRD < 10^{-6}. A total of 34% of patients had MRD <10^{-5} post-induction, 70% post-transplant, and 80% at best response. A total of 71% of patients with confirmed MRD negative remission (two consecutive MRD results) received no further consolidation therapy, and cumulative incidence of MRD reappearance or progression after 12 months of maintenance cessation was between 0% and 27% for patients with one, two, or more high-risk cytogenetic abnormalities [136]. Currently, clinical trials are ongoing to address whether treatment can be safely reduced or even discontinued taking into consideration a sustained MRD status, without aggravating patient prognosis. In the EMN17 Perseus trial (NCT03710603), patients with at least two MRD negative samples 12 months apart during maintenance with daratumumab–lenalidomide (Dara-R) can hold back daratumumab maintenance after at least two years and continue with lenalidomide only. In the SWOG 1803 DRAMMATIC trial (NCT04071457) comparing Dara-R to lenalidomide alone as maintenance after ASCT, patients who are MRD negative after 2 years of maintenance are randomized to treatment discontinuation vs. continuation. In the "Monoclonal Antibody-Based Sequential Therapy for Deep Remission in Multiple Myeloma (MASTER)" study (NCT03224507) in NDMM patients who are eligible for transplant, MRD negative patients by NGS (10^{-5}) after induction therapy with Dara-KRd, followed by ASCT discontinue treatment, whereas patients who are MRD positive undergo further consolidation with Dara-KRd for up to two cycles until MRD negativity [136].

Results of these ongoing trials have the potential to avoid intensive therapy, preventing associated toxicity, and to change the current paradigm of treating patients until progression.

6.4. Can MRD Be Used to Intensify Treatment?

Another open issue is whether patients who are MRD positive would benefit from a treatment change or intensification with the purpose of achieving MRD negativity. In the IFM 2009 trial, no significant difference was observed in survival outcomes between patients who were MRD negative before maintenance and those who achieved MRD negativity during the first year of maintenance [39]. Response improvement over time under continuous therapy was observed in a retrospective study, where 34.3% of MRD positive patients after induction became MRD negative during maintenance [140]. The benefit of including new drugs in the therapeutic armamentarium against MM to attain deeper and more sustained responses is particularly important and is currently being examined in the AURIGA study (NCT03901963) with the association of daratumumab to lenalidomide during maintenance. The kinetics of MRD was also investigated in the GIMEMA trial, where patients receiving VTd after ASCT were monitored for MRD. This clinical trial revealed that the median duration of response was shorter in patients with MRD persistence (9 months); longer for those with MRD reappearance (38 months); and was not reached for patients in major MRD response (two consecutive MRD results < 10^{-4} by RQ-PCR) ($p < 0.001$). Moreover, results showed a median time lag between MRD reappearance and need for salvage treatment of 9 months [144]. Oliva et al., demonstrated that MRD progression, as measured by both MFC and ASO-qPCR every 6 months during maintenance, anticipated clinical relapse by a median of 9 months and biochemical relapse by a median of 4 months [145]. As reported in a randomized prospective study by Mina et al., early intervention with bortezomib and dexamethasone (Vd) induced disease stability or improvement in 82% of patients and delayed clinical progression by 11 months versus observation until clinical relapse (20.3 versus 9.5 months) [146].

Data regarding the approach to patients who lose MRD negativity is still scarce. The recently published study by Mohan et al., regarding the clinical implication of MRD loss revealed that MRD conversion occurred in 39% of the cohort of NDMM patients submitted to ASCT and at least 2 years of combined maintenance treatment at a median of 6.3 years. MRD conversion was associated with a higher risk of relapse, being able to predict clinical relapse in 70% of patients and preceding it by a median of 1 year (range 0-4.9). Noticeably, patients who converted from negative to positive MRD within the first 3 years after ASCT had lower PFS and OS compared with patients with sustained MRD negativity (PFS: HR = 4.5; 95% CI, 4.3–33.7; $p < 0.0001$; OS: HR = 5.7; 95% CI, 6.3–63.0; $p < 0.0001$) [147]. Treatment intervention upon signs of biochemical relapse rather than clinical symptoms can have a favorable impact on the outcome of these patients, as observed by the ENDEAVOR trial [148]. The hypothesis that outcome could be improved when considering an even lower level of disease, as determined by MRD assessment, is being addressed in the ongoing REMNANT trial (NCT04513639), where MRD negative patients after induction are randomized to start treatment if MRD becomes positive versus at the time of progressive disease [149].

These findings encourage the use of MRD as a marker to predict an imminent progression and to trigger an early therapeutic intervention to avoid the development of MM-related comorbidities.

The value of MRD in the RRMM setting is also being explored. Several studies have demonstrated an improved PFS in RRMM patients who achieve MRD negativity [2,150]. In the aforementioned meta-analysis by Munshi et al., MRD negativity improved PFS (HR = 0.34; 95% CI 0.24 to 0.47; $p < 0.001$) and OS (HR = 0.28; 95% CI 0.18 to 0.45; $p < 0.001$) in the group of relapsed heavily pre-treated patients [118]. The POLLUX [42] and CASTOR [151] trials assessed MRD by NGS (sensitivity threshold of 10^{-5}) in patients at suspected CR, following confirmed CR (3 and 6 months in POLLUX; 6 and 12 months in CASTOR), and every 12 months after CR. With a median follow-up of 54.8 months in POLLUX and 50.2 months in CASTOR, the MRD negativity rate and the proportion of patients in CR achieving sustained MRD negativity for longer than 6 months were higher in daratumumab-based regimens compared with standard treatment (35.8 versus 9.2; $p < 0.0001$ for POLLUX; 36.1 versus 13; $p = 0.404$ for CASTOR). Similar results were observed for patients with sustained MRD negativity for longer than 12 months (28.4 versus 6.2; $p = 0.0001$ for POLLUX; 23.6 versus 0; $p = 0.0098$ for CASTOR). More than 80% of patients with sustained MRD negativity for more than 6 months had not progressed at 36 months [152]. The value of sustained MRD has been a matter of open discussion in recent years, and both POLLUX and CASTOR trials emphasized that the depth of response and the durability of MRD negativity is a favorable prognostic marker in RRMM patients.

In this particularly difficult-to-treat group of patients, how to approach the re-appearance of MRD is an additional unanswered clinical question.

7. MRD in the Context of New Therapies

Achieving deeper remissions is now frequently attainable. Since the first studies, new immunotherapeutic therapies such as chimeric antigen receptor T (CAR T) and T cell engagers have demonstrated the ability to achieve rapid MRD responses [153,154]. The emergence of new therapies against MM is particularly critical in the context of RRMM. Recent studies regarding mAbs and cellular products described exceptional rates of CR and MRD negativity among heavily pre-treated patients. Studies in the RRMM setting revealed a rate of MRD negativity between 5% and 30% of patients treated with an anti-CD38 mAb (daratumumab or isatuximab) in combination with IMiDs or PIs [155,156]. In the CARTITUDE-1 update presented at ASH 2021, with a median follow-up of 18 months, 91.8% MRD negativity was reported in patients with CR in a heavily pre-treated population after a single infusion of the anti-BCMA CAR T cell therapy ciltacabtagene–autoleucel (cilta-cel). Importantly, 44.3% of patients had sustained MRD negativity for $\geq$6 months and 18% for $\geq$12 months [157]. The Phase 2 CARTITUDE-2 study (NCT04133636) is

currently ongoing and will evaluate the overall MRD negativity rate in various clinical settings. Additionally, in a first-in-human study of another anti-BCMA CAR T cell therapy LCAR-B38M, included in the LEGEND-2 study and identical to the construct used in the CARTITUDE-1 (NCT03548207) clinical study, CR was achieved in 74% of RRMM patients, with 92.8% of CR patients being MRD-negative with a PFS rate of 71% at 18 months [158]. In the KarMMa study, using a CAR T product targeting the BCMA idecabtagene–vicleucel (ide-cel, bb 2121), at a median follow-up of 13.3 months, the overall response rate (ORR) was 73% with 33% CR; 26% of the patients in CR and 39% of the patients in VGPR or better achieved MRD negativity by NGS (sensitivity $< 10^{-5}$) [159]. Despite these impressive results, patients still relapse, with loss of BCMA expression as a potential underlying factor. A recent report by Da Via et al., provided an in-depth analysis of the molecular mechanisms behind alterations in the TNFRSF17 BCMA-encoding gene in a patient with irreversible loss of BCMA expression [160]. Results of the EVOLVE study using an investigational BCMA-directed CAR T cell product (Orva-cel) reported a median follow-up of 6.9 months with ORR of 92% and VGPR or better in 68% of the patients, with MRD negativity by NGS (sensitivity $< 10^{-5}$) in 84% at 3 months. Of note, MRD assessment was performed in patients achieving partial response (PR) or better and not in CR [161]. A systemic review and meta-analysis of 30 studies using anti-BCMA CAR T cell in MM confirmed an estimated response rate of 78.3% (95% CI 72.4 to 84.3%) [162]. Based on the information provided, Bravo-Perez et al., estimated the pooled proportion of patients achieving MRD negativity to be 58.7% (95% CI 44.4 to 73.1%) in patients treated with CAR T cell constructs [163]. The first-in-human study of the anti-B-cell maturation antigen BiTE molecule AMG420 for RRMM patients revealed a response rate of 70%, including 50% MRD negative complete responses at the maximum tolerated dose [164].

In the study using a new combination of BCL-2 inhibitor venetoclax (Ven) associated with daratumumab (Dara) and dexamethasone (d) (Dara-Vd) with or without bortezomib (B) for RRMM, an increase in ORR and VGPR or better in the arm without bortezomib was reported (ORR of 92% and 96% with 79% and 96% VGPR or better in the Dara-Vd + B and Dara-Vd arms, respectively). Patients achieving CR/sCR who also reached MRD negativity were significantly higher in the arm without bortezomib [165].

As previously stated, deeper responses are associated with prolonged survival, and the new agents that are currently being evaluated in ongoing clinical trials convey a new hope to obtain sustained MRD negativity.

8. Sustained MRD Negative Response: Role of the Immune System

One of the aspects that should be considered when considering the meaning of durable MRD negativity is the role of the immune microenvironment and the patient immunological profile. The equilibrium between the BM microenvironment and a residual MM clone that remains stable with no clinical implication is an interesting concept in the field of MM. The latest data on the cellular patterns within the BM microenvironment of MM patients, particularly those that maintain long-term responses, revealed features of active immune surveillance [166]. A deeper understanding of the interaction between MM cells and the immune system will eventually allow us to define specific immune profiles associated with a stable disease state versus an early relapse state.

Immune suppression is responsible for increasing the risk of infection in MM patients and it has also been associated with MM relapse and progression. Data are emerging to suggest an association of specific immune profiles with MRD status and outcome. In ASCT ineligible patients, immune profile concomitant with MRD assessment was able to identify three groups with distinct outcomes (OS at 3 years: poor 25%, intermediate 61%, and favorable 100%; $p = 0.01$). An increase in mature B cells was observed in patients with a more favorable outcome [117]. Ho et al., recently showed that higher peripheral CD19+ B cell counts before ASCT was correlated with better 2-year PFS (83% (highest quartile) vs 53% (lowest quartile); $p = 0.01$) and OS (93% (highest quartile) vs 63% (lowest quartile); $p = 0.0003$), particularly in MRD positive patients who were more impacted by

the level of B cells, implying a beneficial effect from an improved immune profile. At day 100 after ASCT, higher peripheral γδ and CD4+ central memory T cell counts correlated with improved 2-year OS (89% (highest quartile) vs 65% (lowest quartile); $p = 0.01$ and 95% (upper quartile) vs 47% (lowest quartile); $p = 0.0003$, respectively), only in MRD negative patients, suggesting that an improved immune profile is not sufficient to control higher tumor loads [167]. A study by Bhutani et al., addressing the role of the immune system in achieving MRD negativity, revealed distinct circulating NK, NK-T, and T cell phenotypes according to MRD status [168]. Papadimitriou et al., identified a specific peripheral blood immune profile in MRD positive patients with lower effector/effector memory CD4+ T cell and higher naïve CD4+ T cell subsets. These specific immune signatures were informative and predictive of BM MRD status (Area Under the Curve (AUC) = 0.8) [169].

Previous studies have addressed the immune profile in patients achieving long-term disease control. These patients seem to revert to a MGUS-like profile, showing a lower expression of regulatory T cells in the BM and recovery of BM and circulating B cell counts. Interestingly, patients who did not reach CR after HDT/ASCT but achieved a MGUS-like profile presented similar TTP ($p = 0.81$) and OS ($p = 0.24$) than those who reached CR [170,171]. Recently presented data on the cellular and immune microenvironment of MM patients treated with lenalidomide maintenance therapy, revealed that patients who reached sustained MRD negativity showed a normalization of the immune microenvironment. However, an immunosuppressive landscape was present before and remained after 1 year of maintenance therapy in patients with unsustainable MRD negativity [172]. The superior outcome of an MGUS-like profile was already confirmed by GEP70 [173,174]. Recently, MRD conversion from negative to positive was associated with different GEP subtypes, with short-time conversion (less than 5 years from diagnosis) being mostly HY subtype and longer-time conversion (10 years from diagnosis) by LB and CD-2 subtypes [147]. In fact, subtype CD-2, despite having lower rates of CR and MRD negativity, showed a comparable clinical outcome to the superior performing CD-1 subtype [175]. In such cases, clones remaining after therapy may not be able to drive disease relapse, being composed of immature clonotypic cells without most somatic mutations and copy number variations found in MM cells [166]. Thus, despite failure to achieve CR and MRD negative response, there is a subgroup of patients who might remain in stable disease without further treatment or compromise in outcome.

Incorporating immune profiling in clinical trials as an exploratory endpoint, along with MRD to assess depth of response, will help to accurately determine prognosis and ultimately help guide the management of MM patients.

9. Ideal Threshold for MRD: How Deep Should We Go?

Continuous advancement of the treatment options for MM has dramatically increased patient outcome. The sensitivity limit of the current available techniques is, in fact, determinant, as MRD-negative strictly means that disease is undetectable below a certain level. Therefore, "minimal" is now replaced with "measurable" as there is no guarantee that the disease was eliminated. Along with improvements in the techniques to evaluate MRD, we are now closer to achieving an unprecedented level of response. What is the depth for MRD detection needed to enhance patient management?

One simple answer could be that the assay with the highest sensitivity should be considered as standard. As previously stated, several studies using both NGF and NGS showed an association between the level of sensitivity and the rate of PFS, clearly defining subsets of patients with different risks of relapse [26,38,176].

There is now ample consensus regarding MRD as the most powerful prognostic tool in MM, clearly surpassing the traditional definition of CR. The recent large meta-analysis by Munshi et al., showed that undetectable MRD is associated with favorable outcomes in various disease and treatment settings [118]. These data confirm the advantage of using MRD as a surrogate for PFS and OS, setting the stage to use this knowledge for determining treatment strategies.

Currently, MRD-guided treatment decisions are taken in other hematological diseases such as acute lymphoblastic leukemia and chronic myeloid leukemia [177,178]. Thus far, this is not the case in MM, where treatment strategies are not adapted according to MRD results. An increasing number of clinical trials currently being opened include MRD negativity as a primary endpoint or use MRD assessment to guide treatment decisions, as summarized in Table 2. Results from these clinical trials will eventually lead to changes in this treatment paradigm.

Table 2. Examples of clinical trials that use MRD testing to guide treatment decisions [179].

Title	Phase	Objective	Study Population	Time Point Assessment	Decision	Treatment	Primary Outcome	MRD Method
MASTER trial (NCT03224507)	2	Decision to begin maintenance	NDMM after Dara-KRd and ASCT	End of consolidation	MRD+	Maintenance Dara-KRd	MRD negativity rate	NGS (10^{-5})
					MRD-	Observation (2x MRD-)		
DART4MM study (NCT03992170)	2	Effect of Dara on MRD positive patients	MRD+ patients >VGPR	Maintenance (24 weeks)	MRD+	Dara (80 weeks)	MRD negativity rate	NGF (10^{-5})
					MRD-	Stop treatment		
AURIGA study (NCT03901963)	3	Guide maintenance	MRD+ patients after ASCT	Maintenance	MRD+	Dara-R	MRD negative status	NGS (10^{-5})
					MRD-	R		
NCT04140162	2	Guide consolidation after initial therapy	NDMM after Dara-Rd induction	After induction	MRD+	Consolidation Dara-RVd	MRD negativity rate	NGS (10^{-5})
					MRD-	Maintenance Dara-R/R		
DRAMMATIC study (NCT04071457)	3	Guide maintenance after initial therapy	NDMM after randomization to Dara-R versus R following ASCT	Maintenance (2 years) (R versus Dara/rHuPH20)	MRD+	Continue maintenance	OS	NGS (10^{-5})
					MRD-	Continue versus stopping maintenance		
REMNANT study (NCT04513639)	2–3	Guide early treatment of relapse	NDMM MRD- after VRd and ASCT	After consolidation (MRD assessed every 4 months)	MRD+	Dara-Kd	MRD negativity rate; PFS; OS	NGF (10^{-5})
					MRD-	Observation (until PD)		
MRD2STOP (NCT04108624)	NA	Guide maintenance cessation	Patients at CR after ASCT during maintenance	Maintenance ($\geq$ 1 year)	MRD+	Continue maintenance	MRD conversion rate; PFS; OS	NGS (exploring 10^{-7}) or NGF and PET/CT
					MRD-	Stop maintenance		
PERSEUS (NCT03710603)	3	Guide maintenance after initial therapy	NDMM after Dara-VRd during maintenance (Dara-R)	Maintenance (2 years)	MRD+	Dara-R	PFS	NGS (10^{-5})
					MRD-	R (sustained MRD- 12 months)		
PREDATOR-MRD (NCT03697655)	2	Role of Dara in MRD	RRMM (1–2 prior lines therapy)	Maintenance	MRD+	Dara	EFS	NGF (10^{-5})
					MRD-	Observation		
NCT03490344	2	Effect of Dara on MRD positive patients	Patients in $\leq$VGPR after induction with/without ASCT	Maintenance (Dara-R)	–	–	MRD negativity rate	NGF (10^{-5})
NCT04221178	NA	Guide maintenance cessation	MRD- patients under maintenance	Maintenance ($\geq$ 3 years)	MRD+	Continue	MRD negativity rate after 1 year	NGF (10^{-5})
					MRD-	Stop maintenance		
NCT02659293	3	Guide duration of maintenance	NDMM patients after ASCT	Maintenance (end cycle 6)	MRD+	KRd (cycle 5–36)	PFS	NGS (10^{-5})
					MRD-	KRd (cycle 5–8)		
NCT02389517	2	Effect of Ixa on MRD positive patients	MRD+ patients after ASCT	Maintenance (Ixa-Rd versus R)	–	–	MRD negativity rate	NGF (10^{-5})
NCT02969837	2	Guide duration of maintenance	NDMM	Maintenance	MRD+	E-KRd (6 cycles) + E-Rd (until PD)	MRD negativity rate	NGS (10^{-5})
					MRD-	E-Rd until PD		

Table 2. *Cont.*

Title	Phase	Objective	Study Population	Time Point Assessment	Decision	Treatment	Primary Outcome	MRD Method
NCT04096066	3	Guide maintenance therapy	NDMM (≥ 65 years)	Maintenance (KRd versus Rd)	MRD+	KRd	MRD negativity rate; PFS	NGF or NGS (10^{-5})
					MRD-	Rd (after 2 years)		
NCT04140162	2	Guide consolidation therapy	NDMM after Dara-Rd induction	After Induction	MRD+	DRVd consolidation	MRD negativity rate	NGF or NGS (10^{-5})
					MRD-	Dara-Rd maintenance		
NCT05091372	2	Effect of Belantamab mafodotin on MRD	NDMM MRD+ patients after ASCT	Maintenance	–	–	MRD negativity rate	NGF or NGS (10^{-5})
MILESTONE (NCT04991103)	2	Guide consolidation/maintenance therapy	NDMM after Dara-VRd	After Induction	MRD+	Proceed to ASCT and Maintenance	MRD negativity rate	NGS (10^{-5})
					MRD-	Defer ASCT		
MIDAS (NCT04934475)	3	Guide consolidation therapy	NDMM after Isa-KRd	After Induction	MRD+	ASCT+Isa-KRd versus ASCT	MRD negativity rate	NGS (10^{-5})
					MRD-	Isa-KRd versus ASCT+Isa-KRd		
MASTER-2 (NCT05231629)	2	Guide consolidation/maintenance therapy	NDMM after Dara-VRd	After Induction	MRD+	ASCT+Dara-Tec versus ASCT+Dara-R	Depth of response; sustained MRD negativity	NGS (10^{-5})
					MRD-	Dara-VRd+ Dara-R versus ASCT+ Dara-R		
HEME-20 (NCT05192122)	1	Guide maintenance cessation	NDMM MRD- after ASCT	Maintenance (3 years)	MRD+	Continue maintenance	Sustained MRD negativity	NGS (10^{-5})
					MRD-	Stop maintenance		

Abbreviations: MRD—Measurable Residual Disease; NDMM—Newly Diagnosed Multiple Myeloma patients; RRMM—Relapsed/Refractory Multiple Myeloma patients; NGF—Next Generation Flow; NGS—Next Generation Sequencing; ACST—Autologous Stem Cell Transplant; Dara—Daratumumab; R—Lenalidomide; d—Dexamethasone; Ixa—Ixazomib; K—Carfilzomib; E—Elotuzumab; Tec—Teclistamab; PD—Progressive Disease; OS—Overall Survival; EFS—Event Free Survival; NA—Not Applicable.

10. Conclusions

The results arising from multiple clinical trials have shown that a deeper and durable response is associated with prolonged survival and that undetectable MRD has a prognostic impact in NDMM and RRMM patients. However, MRD assessment, despite providing valuable information with high accuracy regarding the quality of response, is yet to attain the deserved spotlight in clinical practice. With the development of more innovative and effective therapies for MM, and the simultaneous efforts to standardize testing and reporting of MRD data, results of prospective, ongoing, and future MRD-based clinical trials will help to overcome the apparent reluctancy in implementing MRD to drive treatment decisions. We anticipate that MRD will prove to have a pivotal role in correctly defining and ultimately improving MM patient outcome.

The impact of MRD should also take into consideration the new therapy regimens available for both NDMM and RRMM patients. It is expectable that different therapies, with diverse mechanisms of actions and applied in different disease settings, could require distinct levels of MRD for optimal prognosis and treatment decisions. Supported by results from clinical trials, clinical practice can take advantage of the knowledge apported by sustained MRD to define monitoring guidelines and to drive treatment decisions. We believe that establishing MRD monitoring as a surrogate for OS and a biomarker to assess therapy efficacy, combined with baseline risk factors, will certainly help to establish an individualized and tailored approach for MM treatment.

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