

Review

Minimal Residual Disease in Multiple Myeloma—Current Approaches and Future Clinical Implications

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Abstract: The prognosis and clinical outcomes for patients with multiple myeloma have improved significantly over the past two decades. A substantial number of patients now achieve complete remission after induction therapy, and more sensitive methods are needed to assess response. Minimal or measurable residual disease (MRD) has been incorporated in many clinical trials as well as in clinical practice. The importance of MRD assessment and correlation between MRD negativity and prolonged progression-free and overall survival has been confirmed in numerous clinical trials and several meta-analyses. Recent studies have even suggested that MRD negativity can partly overcome the impact of the negative prognostic factors such as high-risk cytogenetics or adverse revised international scoring system (R-ISS) stage. MRD can be measured in the bone marrow via imaging and via emerging blood-based techniques. The most common methods are multicolor flow cytometry and next-generation sequencing of bone marrow samples. Using these methods in optimal settings, MRD negativity with a sensitivity level of 10^{-6} can be detected. In this review, we discuss the benefits and limitations of these techniques as well as the clinical implications.

Keywords: multiple myeloma; minimal residual disease; flow cytometry; next-generation sequencing



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1. Introduction

Over the past two decades, the introduction of novel agents and advanced therapies has transformed the management of multiple myeloma [1–7]. These advances are reflected in a higher proportion of patients achieving deep and durable responses from treatment as well as longer survival [8–12]. The majority of patients now achieve a complete response after induction therapy with triplet or quadruplet therapy [5,6,13–15]. Achieving a complete remission (CR) significantly increases progression-free survival (PFS) and overall survival (OS) [16–18]. Similarly, the rate of minimal residual disease (MRD) negativity has increased substantially; up to 55–70% in trials using modern combination therapies in the front line setting [2,5,14]. Additionally, in the relapse refractory setting, treatment with bispecific antibodies and chimeric antigen receptor (CAR) T-cells have shown high CR rates and MRD negativity [19–21]. Importantly, the correlation between minimal residual disease (MRD) negativity and longer progression-free survival (PFS) and overall survival (OS) has been demonstrated in clinical trials and several meta-analyses [8–10]. Furthermore, the significance of sustained MRD negativity has been recognized and was further demonstrated in a recent longitudinal study [22].

Serum-based markers, including monoclonal proteins on serum electrophoresis, were previously used as the only method for response assessment, but as more patients were achieving deeper responses, more sensitive methods were called for. This led to the development of bone marrow-based assays with a higher level of sensitivity and the possibility of assessing deeper response levels. The International Myeloma Working Group (IMWG) published a white paper in 2016 with the definitions and recommendations for MRD assessments (Table 1) [23,24]. The minimum sensitivity level recommended by

the IMWG is the detection of 1 neoplastic cell in 100,000 (10^{-5}) cells, while 1–2 cells in 1,000,000 (10^{-6}) is ideal [23,25]. The IMWG recommends MRD assessment in the bone marrow using multicolor flow cytometry (MCF) or next-generation sequencing (NGS). The IMWG is agnostic as to which method is used as long as it is validated for the specific sensitivity level [25]. These two methods, MCF and NGS, have separate benefits and pitfalls, which are discussed in this review. Additional methods for response and MRD assessment include imaging to detect extramedullary disease. Moving forward, blood-based assays such as mass spectrometry and circulating tumor cells and liquid biopsies can come to replace bone marrow-based assays, providing easier access and more convenience for patients.

Table 1. IMWG MRD criteria [23].

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 presence of a clone is defined as less than two identical sequencing reads obtained after DNA sequencing of bone marrow aspirates using the LymphoSIGHT platform (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher. (Note: LymphoSIGHT has been replaced by ClonoSEQ).
Sustained MRD-negative	MRD negativity in the marrow (NGF or NGS, or both) and by imaging as defined below, confirmed minimum of 1 year apart. Subsequent evaluations can be used to further specify the duration of negativity (e.g., MRD-negative at 5 years).
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 a preceding PET/CT or decrease to less mediastinal blood pool SUV or decrease to less than that of surrounding normal tissue.

IMWG—International Myeloma Working Group; MRD—minimal residual disease; NGF—next-generation flow; NGS—next-generation sequencing.

MRD has important clinical implications and is more and more being incorporated in clinical trials and clinical decision making. MRD-driven therapy is already being discussed as a basis for decisions on up-front autologous stem cell transplants as well as the length of maintenance therapy. Therefore, it is of great importance that MRD assessment is accurate and sensitive. In this review, we discuss the two different bone marrow-based assays for MRD detection and the implications of using MRD in clinical practice.

2. Methods for Measuring MRD

2.1. Multicolor Flow Cytometry

Multicolor flow cytometry (MCF) uses an antibody panel to detect and quantify immunophenotypically aberrant and clonal plasma cell populations in bone marrow aspirate samples [11,26]. Early MCF protocols included different combinations of 4–8 color flow cytometry with varying sensitivity, usually around 10^{-4} , and there was a lack of standardization between methods and centers. MCF is done on fresh bone marrow samples and can be done in virtually any patient throughout the disease course without the need for knowledge of the baseline immunophenotype. The immune profile of the aberrant plasma cells is stable over time with possible minor alterations from clonal evolution [24]. As techniques for MRD have been refined, most MCF protocols include ≥ 8 colors for surface and intracellular antigen, and there has been an effort to standardize and validate MCF protocols across institutions and countries. This has resulted in increasing levels of sensitivity, and per the most recent IMWG guidelines, labs that report MRD by MCF should be able to detect a minimum sensitivity of 10^{-5} and up to the current highest sensitivity of 2×10^{-6} [25].

One of the more commonly used MCF protocols for plasma cell disorders is the EuroFlow panel (next-generation flow, NGF) which has been extensively validated to

overcome many of the former limitations associated with MCF [27]. EuroFlow is based on an 8-color 2-tube panel (10 colors in total, Table 2) as well as software tools for automated plasma cell detection. With this approach, MRD with sensitivity to 2×10^{-6} can be detected in a majority of samples [28]. The assay includes antibodies to detect the most common surface and cytoplasmic markers of abnormal plasma cells as well as internal controls to detect mast cells, erythrocytes, and B-cell precursors to assess sample quality and hemodilution [27]. In a recent validation study of the EuroFlow panel in 458 patients in the PETHEMA/ GEM2014MAIN trial, the sensitivity was reported as $<2 \times 10^{-6}$ in 1% of samples, 2×10^{-6} to $<10^{-5}$ in 88% of samples, $\geq 10^{-5}$ to $<10^{-4}$ in 99.9% and $\geq 10^{-4}$ in 100% of samples [28]. In this validation study, 14 of 1114 samples had significant hemodilution and were considered inadequate for analysis [28].

Table 2. EuroFlow antibody panel [27].

CD19	CD81
CD27	CD117
CD38	CD138
CD45	Cyt- κ
CD56	Cyt- λ

In addition to EuroFlow, several centers have developed MCF protocols, which also have been thoroughly validated. Memorial Sloan Kettering Cancer Center developed a 10-color 1-tube approach, which was validated against EuroFlow and found to be equally accurate and sensitive ($<2 \times 10^{-6}$) [29]. Other centers in the US, UK, and Australia have also developed MCF protocols that have been thoroughly validated, using 1 or 2 tubes and 10–12 antigen combinations in the clinical setting [30].

One of the advantages of MCF is that it can be used in any patient at any time point; the baseline profile does not need to be identified. It has a quick turnaround time and can therefore be used to inform clinical decision making in real time. MCF can also be used for comprehensive immune profiling of the bone marrow microenvironment, including T-cells, NK-cells, B-cells, and macrophages [31,32]. Limitations of MCF include the need for direct processing, usually within 24 h in order to preserve viable cells (recommended $>85\%$ for optimal quality). Quite a large sample, 10 million cells, is needed for optimal sensitivity with the EuroFlow assay [28]. Furthermore, patients receiving daratumumab and other anti-CD38 antibodies could pose an additional problem as daratumumab can interfere with MCF analyses and plasma cell identification [33]. Several assays, including EuroFlow, are working to overcome this with alternative CD38 multi-epitope and nanobody conjugates as well as other plasma cell markers, including CD229, CD319, and VS38c [30,33–35].

2.2. Next-Generation Sequencing

Next-generation sequencing (NGS) takes advantage of the unique V(D)J sequences created during B-cell development [36–43]. In the immunoglobulin heavy chain (*IGH*) variable region, rearrangement occurs in the variable (V), diversity (D), and joining (J) gene segments creating a unique V(D)J sequence (Figure 1) [36]. This is followed by somatic hypermutation and class-switch recombination resulting in a mature immunoglobulin sequence. The V(D)J sequence in the malignant clone is not aberrant or contributing to the tumorigenesis per se but is more abundant at baseline compared to the background V(D)J sequences and can thus be used to determine clonality [37,38]. As there are $\sim 10^{11}$ possible variations of *IGH* rearrangements, the likelihood that there would be an identical V(D)J sequence independently in the malignant cell clone and any normal plasma cell in the same bone marrow sample is extremely low [37]. The V(D)J sequence is preserved throughout the disease course and clonal evolution, making it optimal for MRD tracking over time [44].

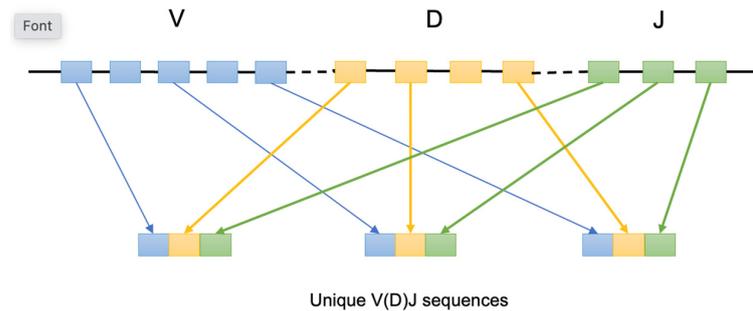


Figure 1. Schematic V(D)J rearrangement. The V(D)J rearrangement occurs during B-cell development by joining the V, D, and J segments. Additionally, junctional diversity, somatic hypermutation, and deletions and insertions contribute to the unique V(D)J sequence in each cell. These unique V(D)J sequences can be targeted and captured using NGS.

The two leading assays are ClonoSEQ by Adaptive and LymphoTrack by Invivoscribe, where ClonoSEQ is the only one that so far has been FDA approved. Both assays use a multiplex PCR step with locus-specific primers, different between the two assays, to amplify DNA encompassing the CDR3 region containing the V(D)J sequence. The PCR step is followed by next-generation sequencing and sophisticated bioinformatic computational analysis [41]. ClonoSEQ contains primers for the *IGH* complete (*IGH*), *IGH* incomplete (*IGH_D*), and light chain *IGK* and *IGL* loci [38,41,42]. In each tumor cell, it is possible to obtain up to eight sequences as there are four loci and two alleles [45]. The light chain loci do not have the *IGH_D*, and the clonotype sequence may therefore be less unique, something that is taken into consideration in the analytical algorithm for MRD assessment [38,45]. LymphoTrack has specific primers for the *IGH* gene; FR1, FR2, FR3, and Leader which targets a conserved upstream sequence to overcome somatic hypermutation. Cases without detectable *IGH* V(D)J clonotype are also be sequenced for *IGK* [39,46].

An initial challenge with these assays was the detection of the baseline V(D)J sequence; the first versions of the Adaptive/Sequentia NGS assay had a baseline capture rate of around ~80%. The assay has to account for the vast number of possible variations of V(D)J sequences as well as somatic hypermutation, which can affect the annealing of primers and decrease the capture rate. Both NGS assays have been optimized to overcome many of the issues with the baseline detection rate, including strategies to increase the resilience to somatic hypermutation as well as the addition of inline controls. The NGS assays are highly sensitive for MRD tracking (10^{-6}) provided that the initial clonotype is identified, and V(D)J sequencing has been included in numerous clinical trials for assessment of MRD [38,39,43,45,47–49]. An advantage of NGS is that the assay does not require live cells and can thus be run on stored or frozen samples. Current versions of the NGS assays have a high baseline capture rate, >93–95%, provided that the bone marrow sample contains sufficient genomic DNA [39,45,46]. The tumor cell content is recommended to be 5% or more for successful clonotype identification [30,46]. In a recent study by Ho et al. using LymphoTrack, the baseline clonotype detection rate was below 80% in samples with <5% plasma cell content while it was as high as 98% in samples with $\geq 10\%$ plasma cells [46]. The limiting factor in sensitivity is often sample quality and tumor content rather than technical limitations [30,39,40,46]. Provided that the baseline clonotype is identified, the clonotype can be detected in virtually 100% of follow-up samples provided that the tumor clone is present [45,46].

In addition to MRD tracking, NGS is used to interrogate the genomic landscape in multiple myeloma both on a discovery level and for individual risk profiling. The more complex genomic aberrations and structural variants are assessed using whole-genome sequencing, however, so far mainly for research purposes. Several groups have however developed targeted NGS panels to assess the genomic aberrations more suited for clinical practice [50,51]. As an example, a targeted NGS assay (myTYPE) for detection

of *IGH* translocations, copy number gains and losses, and somatic mutations in multiple myeloma was found to have a >99% sensitivity and specificity compared to FISH [50]. Using this targeted assay, the tumor-specific V(D)J sequences could be identified and were found to be identical to the V(D)J clonotype found using LymphoTrack in 93% of samples [40]. Furthermore, Rustad et al. used bulk RNA sequencing to identify clonal CDR3 sequences that can be used for MRD tracking [44]. These advances indicate that various NGS assays can be used to identify the clonal V(D)J sequence and that NGS can be used for several aspects of diagnosis and tracking throughout the disease course, including MRD assessment.

2.3. Comparison of Multicolor Flow Cytometry and Next-Generation Sequencing

MCF and NGS both have pros and cons in terms of utility as well as sensitivity, and several authors have published head-to-head comparisons. Medina et al. compared EuroFlow versus NGS (LymphoTrack) and found a high level of concordance, $R^2 = 0.905$ [52]. Similarly, Ho et al. found a 93% concordance between NGS and high sensitivity MCF [46]. Both MCF and NGS have a high sensitivity level; however, MCF requires a higher number of cells, around 10 million, while 3 million cells can be enough to achieve the highest level of sensitivity with NGS [53]. As mentioned above, MCF has a quick turnaround time, and samples can be analyzed without knowing the baseline immunophenotype, while limitations include the need for quick processing and the required high volume of bone marrow. Benefits of NGS include the high sensitivity and the possibility to run analyses on stored samples (Table 3). The limitations for NGS include the need for the baseline sequence to be known as well as the longer turnaround time, usually 5–7 days. Nevertheless, the prognostic impact of MRD negativity has been corroborated as long as the sensitivity is high regardless of the method [9,46,52].

Table 3. Comparison of multicolor flow cytometry vs. V(D)J sequencing using NGS for MRD assessment.

Multicolor Flow	V(D)J Sequencing
Rapid turnaround	Days to weeks turnaround
No need for baseline sample	Need for baseline V(D)J sequence
Need for fresh samples	Can be done on fresh or stored samples
Sensitivity 2×10^{-6}	Sensitivity 1×10^{-6}
10 million cells needed	3 million cells needed
Applicable to nearly all patients	Not applicable to all patients (up to ~95%)
Technique readily available	Technique not widely available
Part subjective interpretation	More objective interpretation

3. Clinical Implications

3.1. Importance of MRD Negativity

MRD status has prognostic value and has been incorporated as an endpoint in the majority of ongoing clinical trials. MRD negativity with a sensitivity of preferably 10^{-6} is strongly associated with a longer PFS and OS in patients with multiple myeloma. Additionally, there are ongoing efforts to incorporate MRD negativity as a surrogate endpoint which could lead to a more rapid readout of clinical trials.

A recent meta-analysis with >8000 patients confirmed the prognostic benefit of MRD negativity [9]. The benefit of MRD negativity was seen irrespective of treatment, cytogenetic stage, or method of MRD assessment. The PFS and OS benefit with MRD negativity was observed in patients who achieved MRD negativity at any sensitivity level (Table 4) compared to MRD positive patients. Although as expected, this meta-analysis demonstrated that the PFS and OS benefit increased with the higher sensitivity level of MRD negativity,

and the largest benefit in PFS and OS was seen in patients who were MRD negative at the 10^{-6} sensitivity level [9].

Table 4. Progression-free survival and overall survival in relation to MRD sensitivity level in MRD-negative patients compared to MRD-positive patients [9].

	MRD Sensitivity	Hazard Ratio	Confidence Interval
Progression-Free Survival	10^{-4}	0.38	0.32–0.45 *
	10^{-5}	0.31	0.27–0.36 *
	10^{-6}	0.22	0.16–0.29 *
Overall Survival	10^{-4}	0.50	0.43–0.60 *
	10^{-5}	0.39	0.31–0.49 *
	10^{-6}	0.26	0.13–0.51 *

* All $p < 0.001$ vs. MRD-positive patients.

In this meta-analysis, the benefit of MRD negativity in terms of longer PFS and OS was not limited to patients who had achieved a completed response (CR) but was also seen in patients who were in a very good partial response (VGPR) [9]. In fact, the three-year PFS was similar in patients who achieved VGPR and CR and were MRD negative [9]. This indicates that MRD assessment should also be considered in patients in VGPR as novel therapies such as modern combination therapies and novel agents can have a rapid effect on clearing the bone marrow whilst the serological response for a variety of reasons may lag, one being the longer half-life of paraproteins. Additionally, monoclonal antibodies such as daratumumab may be detected as a low-level monoclonal band and cause false-positive serum electrophoresis and immunofixation results [54]. Importantly, in the meta-analysis, the benefit of MRD negativity was also significant in patients with relapsed/refractory disease [9]. This is encouraging as many novel therapies such as bispecific antibodies and CAR T-cell therapy can induce deep responses in the relapse setting [19–21].

Interestingly, a study by Medina et al. as well as the meta-analysis by Munshi et al. reported that MRD negativity was largely able to overcome the negative prognosis associated with high-risk cytogenetics [9,52]. However, 17p deletion remained a predictor of adverse prognosis in MRD negative patients [52]. Similarly, in the MASTER trial outlined below, MRD negative patients with zero or one high-risk cytogenetic feature had a similar prognosis, while two or more high-risk cytogenetic features still was associated with a poorer prognosis also in MRD negative patients [2].

As therapies for multiple myeloma are increasingly effective, analyses of PFS and OS can take a long time, and incorporating MRD as an endpoint can expedite the approval of new drugs. FDA approval of new drugs can be achieved either via regular approval or accelerated approval based on surrogate endpoints. For accelerated approval, a post-marketing trial is needed to confirm the outcome, and the drug can then achieve regular approval. Surrogate endpoints are approved by the FDA on a case-by-case basis based on the scientific rationale, relationship to the outcome, and clinical benefit [24]. Several workshops have been held with the FDA regarding MRD as a surrogate endpoint, but so far, the standardization and quality of the assays and data have not been robust enough to grant approval. The addition of the large meta-analysis by Munshi et al., the standardization of MCF, and the FDA approval of the ClonoSEQ assay may contribute to eventually approving MRD as a surrogate endpoint for accelerated drug approval.

3.2. MRD-Driven Therapy

MRD can be incorporated in clinical decision making at various time points, for instance, to decide the duration of induction therapy, upfront autologous stem cell transplant (ASCT), as well as intensity and continuation of maintenance therapy. Several recent trials have incorporated MRD-driven therapy for clinical decision making for induction and upfront transplant. In the recently published MASTER trial, patients were treated with daratumumab, carfilzomib, lenalidomide, and dexamethasone (dara-KRD), followed by au-

tologous stem cell transplant with close monitoring of MRD status. Patients who achieved MRD negativity (10^{-5}) at two consecutive time points were allowed to go off therapy per protocol and with close MRD surveillance (MRD-sure). During the trial, 80% of patients achieved MRD negativity at the 10^{-5} level and 66% MRD negativity at the 10^{-6} level. In total, 71% had two consecutive negative MRD assessments and entered the MRD-sure phase. Around 6% of patients in the MRD-sure phase had a resurgence of MRD positivity; of these, 4%, 0%, and 27% had 0, 1, or 2+ high-risk cytogenetic abnormalities [2]. In phase I/II trial by Korde et al., the number of cycles of induction therapy with carfilzomib, lenalidomide, and dexamethasone (KRD) was individualized based on MRD status [55]. Using this approach, 60% of patients achieved MRD negativity (see Figure 2).

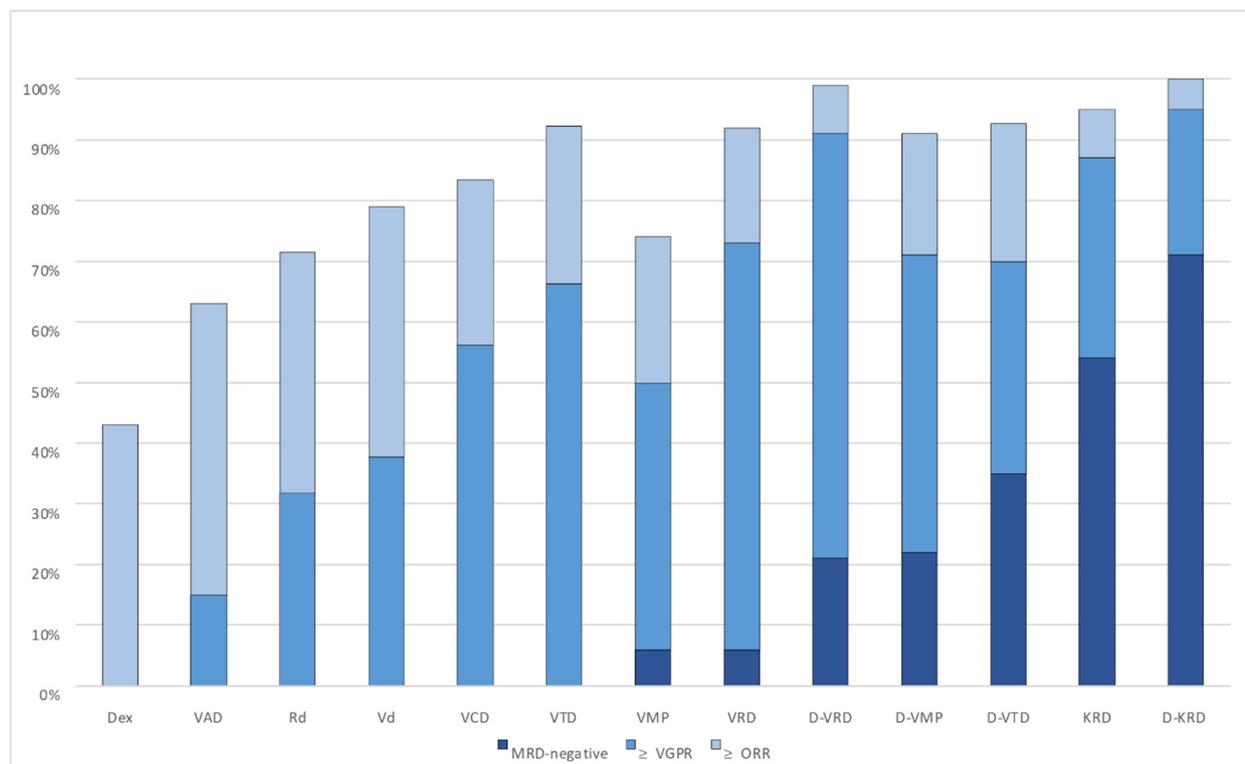


Figure 2. Depth of response and MRD negativity in relation to first-line treatment regimen for multiple myeloma [2,3,5,6,13,14,48,55–58].

In the IMF2009 trial, patients who achieved MRD negativity in both treatment groups; lenalidomide, bortezomib, dexamethasone (RVD), or RVD followed by autologous stem cell transplant (ASCT), had longer PFS compared to MRD positive patients in both groups [47,59]. Even though this was a post hoc analysis, these findings have led to an ongoing discussion regarding the need for upfront ASCT in patients who achieve MRD negativity after induction therapy [60]. In the FORTE trial, patients who received KRD induction followed by ASCT had a slightly higher MRD negativity rate compared to patients treated with 12 cycles of KRD without ASCT [14]. In the DETERMINATION trial, which also compared RVD vs. RVD followed by ASCT, the PFS was similar in patients who achieved MRD negativity in both groups [61]. Additionally, as three- and four-drug induction therapies are becoming increasingly effective and resulting in high MRD-negative rates (Figure 2), it is important to continue discussing and further personalizing upfront ASCT to avoid overtreatment and possible toxicities especially in the non-high-risk patient population.

Diamond et al. highlighted the importance of the dynamics of MRD in phase 2 single-arm study with lenalidomide maintenance [22]. In this trial, patients were treated with lenalidomide maintenance for up to five years with annual MRD monitoring. At the two-year landmark, MRD negative patients who converted from being MRD negative to MRD

positive were more likely to progress, both in comparison to patients who had sustained MRD negativity and those with persistent MRD positivity [22]. There are ongoing trials that incorporate MRD assessment for clinical decision making aiming to stop maintenance in patients with sustained MRD negativity.

4. Summary and Future Directions

MRD negativity is a strong prognostic factor that can overcome many of the initial factors associated with poor prognosis. MRD-driven therapy will become increasingly utilized and help guide clinical decisions on autologous stem cell transplants as well as intensity and duration of maintenance. Currently available assays are highly sensitive, and recent studies have demonstrated the significance of reaching MRD negativity at the 10^{-6} level as well as the importance of sustained MRD negativity.

Moving forward, as treatments are becoming more and more effective, MRD assessments with even higher sensitivity levels are needed. This can be achieved by integrating blood and bone marrow assessments with sensitive imaging, which adds information on the skeletal and extramedullary disease, see articles by Thoren as well as Hillengass et al. in this issue of Hemato [62]. New imaging techniques, e.g., PET MRI, and PET targeting tumor cell markers such as CD38, BCMA, and CXCR4, can help guide initial diagnosis and increase sensitivity of imaging-based MRD assessment [30]. Several blood-based assays, e.g., mass spectroscopy and liquid biopsies for analysis of circulating tumor cells, are undergoing clinical validation [63–68]. Mass spectroscopy using either MALDI-TOF or liquid chromatography (LC-MS) has significantly higher sensitivity for detecting monoclonal proteins compared to serum electrophoresis [63,69]. Mass spectroscopy has been found to have similar sensitivity and a high level of concordance when compared to bone marrow based MRD assessments [63,64,69,70]. There are current efforts for validation of V(D)J sequencing for MRD evaluation of peripheral blood samples rather than bone marrow samples. Blood-based assays are more convenient for the patient and can help guide MRD surveillance and the timing of bone marrow assessments. Moreover, analysis of circulating tumor cells and circulating tumor DNA can add information on tumor genomics and actionable targets.

Importantly, there is an ongoing evaluation of whether MRD can be used as a surrogate endpoint in clinical trials and FDA approval of new drugs. Standardization, quality, and optimal timing of MRD assessment have been of concern in regard to using MRD as a surrogate endpoint. Substantial efforts have gone into standardizing protocols for MRD assays, and the recent large meta-analysis confirmed the strong correlation between MRD negativity and prolonged PFS and OS [25]. MRD assessment is a powerful tool that can inform clinical decisions to intensify or deescalate therapy based on the individual risk profile to further optimize and tailor treatment for multiple myeloma patients.

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