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Role of minimal residual disease assessment in multiple myeloma

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Abstract

Multiple myeloma (MM) is a hematologic malignancy characterized by clonal proliferation of plasma cells. MM is a heterogeneous disease, featured by various molecular subtypes with different outcomes. With the advent of very efficient therapies including monoclonal antibodies, bispecific T cell engagers and chimeric antigen receptor T cells (CAR T cells), most of MM patients have now a prolonged survival. However, the disease remains incurable, and a subgroup of high-risk patients continue to have early relapse and short survival. Novel and highly sensitive methods have been developed allowing to detect minimal residual disease (MRD) during or after treatment. Achievement of MRD negativity is a strong and independent prognosis factor in both prospective randomized clinical trials and in real-world setting. While MRD assessment is now a validated endpoint in clinical trials, its incorporation in clinical practice is not yet established and its potential impact on guiding therapy remains under deep evaluation. We discuss in this chapter, the different available methods allowing MRD assessment and the role of MRD evaluation in multiple myeloma management.

Multiple myeloma is a heterogeneous disease featured by more than ten distinct molecular subtypes associated with variable outcomes(1). The therapeutic landscape of MM has dramatically changed over the last 5 years. The incorporation of monoclonal antibodies first in relapse setting and more recently in frontline in triplet or quadruplet regimen, the approval of chimeric antigen cell (CAR) T cell therapy and soon of bi-specific monoclonal antibodies or T cell engagers have revolutionized MM treatment and prognosis(2-4). With more than 14 FDA approved drugs, the treatment options are now various and most of MM patients have now prolonged survival(2). However, MM remains incurable and therefore, the ability to identify high risk (HR) patients and to appropriately sequence therapy based on disease characteristics and response to treatment is critical. Along with plasma cell molecular and cytogenetics characteristics, response to treatment is another major prognosis factor and its assessment is an essential part of patient care. The definition of hematologic response has evolved in the past 20 years with the incorporation of novel highly sensitive methods to allow comparison of treatment strategies in clinical trials. International consensus criteria defining hematologic response in MM have been established first in 1998 and revised in 2016, especially to incorporate the free light chain dosage. The original definition of a complete response (CR) only required bone marrow (BM) with less than 5% plasma cells, irrespective of their clonal nature while the 2016 criteria defined CR as negative immunofixation on the serum and urine, disappearance of any soft tissue plasmacytomas and <5% plasma cells in bone marrow aspirates. Stringent CR was defined as CR plus normal FLC ratio and absence of clonal cells in bone marrow (BM) biopsy by immunohistochemistry (κ/λ ratio $\leq 4:1$ or $\geq 1:2$ for κ and λ patients, respectively, after counting ≥ 100 plasma cells). Achievement of complete hematologic response (CR) is overall associated with significantly prolonged period free survival and overall survival (5). However, not all patients achieving CR have a good prognosis as some patients achieving CR unfortunately have early relapse or progression. The concept of minimal residual disease (MRD) which refers to the ability to detect a very small number of malignant plasma cells during or after treatment was adopted in the international myeloma working group (IMWG) consensus criteria in 2015 to provide more accurate hematologic response assessment. Since then, MRD evaluation has been shown to significantly improve hematologic response evaluation and to improve prognostic stratification after therapy in newly diagnosed - transplant and non transplant eligible- and in relapse disease. The prognosis role of MRD is now well documented and demonstrated in

several retrospective and prospective studies and MRD negativity is now an established criteria in MM clinical trials. However, the impact of MRD assessment on treatment decisions remains to be determined and is currently under deep investigation in several randomized clinical trials(6-16). In addition to bone marrow (BM) based MRD evaluation, novel techniques utilizing whole body imaging and blood-based evaluation have been developed and will likely improve MRD evaluation in MM patients. Here, we describe the different available methods to assess MRD and discuss the clinical applications and challenges of MRD utilization in clinical practice.

Bone marrow based MRD assessment

Next generation flow cytometry

First MRD evaluation was performed by multiparametric flow cytometry (MFC), a worldwide available technique able to identify monotypic plasma cells in the bone marrow even if present at low level(17, 18). Conventional MFC MRD approaches usually use 4 to 10 cell markers (colors) but is limited by relatively low sensitivity, absence of standardization and lack of reproducibility. Therefore, next generation flow cytometry (NGF), a more sensitive method, was developed and standardized by EuroFlow to overcome most of conventional MFC limitations (19-21). NGF is based on a more efficient sample preparation protocol for acquisition of up to 10 million BM cells and uses 8 to 12 colors characterizing most of cellular subtypes and normal plasma cells (CD138 and CD38) and aberrant plasma cell markers (CD20, CD56, CD19, CD45, CD27, CD28, CD33, and CD117). Additionally, intra-cytoplasmic markers for κ or λ immunoglobulin light chains are used to confirm monotypic or clonal cells. Importantly, NGF high sensibility allows detecting one abnormal plasma cell out of $10^{\wedge-6}$ cells and does not require a sample at the time of diagnosis (21). It is also adapted to patients receiving anti-CD38 therapy despite potential interference with plasma cell detection(21). Standardized data analysis methods allow an increased sensitivity and reliability. The clinical impact of high-sensitivity MRD detection by NGF has been validated in randomized clinical trials and in real world patients with multiple myeloma (19, 22). In the GEM/PETHEMA trials, only 7% of patients achieving MRD-negativity (MRD < 2 □ $10^{\wedge-6}$ cells) were reported to have disease progression with half of those patients progressing with extra-medullary disease. Achievement of MRD negativity was associated with

an 82% and 88% reduction in the risk of progression and death (HRs of 0.18 and 0.12, P < 0.001), respectively. Importantly, MRD negativity overcame the poor prognostic value of high-risk cytogenetics at diagnosis(19). EuroFlow NGF approach is now validated by the IMWG as the reference flow cytometry method to evaluate MRD negativity after therapy (5). The EuroFlow process, which uses 2-tube 8-color methodology, is broadly used in Europe, Asia and the US. However, several groups, especially in the US, have developed other methods, using single-tube, 10- or 12-color methods that are similarly efficient, more cost-effective and in agreement with the IMWG and NCCN guidelines(23).

Next generation sequencing (NGS)

High-throughput DNA sequencing methods developed to study B or T cell receptors repertoire have been applied to MM. These methods can identify 1 malignant cell in 1 million analyzed cells ($10^{\wedge-6}$). The Adaptive Biotechnologies (clonoSEQ) NGS assay is currently the only assay cleared by the food and drug administration (FDA) for MRD evaluation in BM from patients with multiple myeloma. NGS allows detecting clonotypes that are defined by the sharing of identical immunoglobulin gene sequence reads with a frequency ≥ 5%. This strategy requires initial BM sample to identify the predominant clone and allows detecting the myeloma clone in 90 to 92% of myeloma patients (16). The prognosis value of achieving NGS based MRD negativity has been demonstrated in several randomized trials. In the IFM 2009 trial, MRD negativity was a strong prognostic factor for both progression-free survival (adjusted hazard ratio, 0.22; 95% confidence interval, 0.15-0.34; $P < .001$) and overall survival (adjusted hazard ratio, 0.24; 95% confidence interval, 0.11-0.54; $P = .001$). Patients who were MRD negative had a higher probability of prolonged progression-free survival than patients with MRD positive disease, cytogenetic risk profile, regardless of the treatment arm or International Staging System disease stage at diagnosis. The level of MRD correlated with outcome, and the deeper the level of MRD ($<10^{\wedge-6}$) the better the prognosis (16). A pooled analysis investigating for associations between patients achieving complete response or better (≥ CR) with MRD-negative status and progression-free survival (PFS) from 4 randomized clinical trials, confirmed that relapse/refractory MM and transplant ineligible newly diagnosed MM (TIE NDMM) patients achieving ≥CR with MRD negativity had a significant PFS benefit (NDMM and RRMM hazard ratio [HR] 0.20, P < .0001; TIE NDMM and RRMM ≤2 PL HR 0.20, P < .0001) (14).

Remarkably, achievement of MRD negativity is, independently of the study arm, associated with best and similar outcome in newly diagnosed MM patients transplant eligible, transplant ineligible as well as in relapse refractory disease(14, 15). These data strongly support the concept that achieving MRD negativity may be more important than how one reached that state.

BM MRD assessment requires to repeat invasive procedure and is limited by the known patchy nature of MM and possibility of extra-medullary disease that are inherent to MM. Therefore, alternative approaches including blood or Imaging based MRD evaluation assessment have been developed (24).

Blood-based MRD assessment

Circulating plasma cells

The presence of circulating plasma cells (CPC) in peripheral blood (PB) can be detected in most MM patients and is associated with poor prognosis. Different methods have been used to assess the presence of CPC. The standard EuroFlow NGF is reliable and requires a small volume of blood. Other methods are available that use a plasma cell enrichment method, which requires a larger blood sample and is more sensitive but also more complex. NGF has been used to identify and track CPC in MM patients with interesting results. However, while CPC detection appears to be a powerful prognosis factor, CPC is unlikely to be a good MRD marker. Indeed, a comparison between NGF in BM and PB after therapy in a real world case series of 137 patients showed that 40% of patients achieving blood based MRD negativity had BM MRD positive disease, strongly suggesting that blood NGF based MRD evaluation is a less sensitive MRD marker than BM MRD(22, 25, 26).

Circulating cell free DNA (cfDNA) for MRD assessment

cfDNA based methods often referred as “liquid biopsy” allow tracking genomic aberrations such as tumor mutations, copy number aberration or translocation present in circulating cfDNA isolated from blood plasma (27-29). Multiple studies showed a high concordance of somatic mutations and copy-number alterations between BM and cfDNA of patients with multiple

myeloma (30-33). However, the low level of circulating tumor DNA is a significant challenge and most of current methods are not sensitive enough. Ultradeep targeted sequencing has significantly improved the detection of cfDNA but its sensitivity relates to the number of tumor mutations available to track and have only been evaluated in few clinical studies to date. In a study which compared blood and BM evaluation, with NGS and cfDNA in 42 patients, there was only 49% consistency and poor correlation between the two methods. Similar to CPC detection, BM MRD was more often positive and suggested lack of sensitivity of cfDNA approach(34). Novel and more sensitive methods are needed before cfDNA can be utilized as a standard approach.

Single-cell RNA sequencing

Single-cell RNA sequencing (scRNA-seq) is another powerful technology largely used in research. It allows transcriptomic analysis at a single cell level and can detect rare malignant cells (35). Ongoing research are investigating if this approach could even allow to select therapy based on transcriptomic features and clonal heterogeneity(36, 37). However, its availability, its relative complex workflow, reproducibility and cost are significant challenges that need to be addressed before considering it in clinical practice (38). This approach is also limited as it can currently only evaluate a certain number of cells much lesser than with flow or NGS MRD based methods assessments. Therefore, the absence of detection of malignant cells would not necessarily correspond to negative MRD and scRNAseq appears more as a potential complimentary method that may help tailoring therapy to target MRD positive cells rather than to determine MRD status.

Mass spectrometry (MS) methods

Instead of tracking the malignant residual plasma cell, MS methods have been applied to detect the monoclonal immunoglobulin produced by the malignant plasma cells. This very sensitive method can detect the presence of a monoclonal immunoglobulin at much lower concentration level than standard serum immunofixation. MS can also distinguish therapeutic monoclonal antibodies from myeloma monoclonal immunoglobulin and can identify post-translational modifications relevant for patients with monoclonal gammopathy of clinical significance especially AL amyloidosis (39-41). Several MS methods have been developed. Matrix-assisted

laser desorption/ionization mass spectrometry (MALDI-TOF MS) and the MASS-FIX assay have been shown to be particularly efficient and both more sensitive and specific than serum protein electrophoresis and immunofixation (40, 42, 43). Another MS approach was initially developed by the Binding Site company in collaboration with the MAYO clinic. The MAYO clinic went on to develop their own approach using a commercial assay while the Binding Site assay, which has more selective probes, was sold to ThermoFisher and is used in individual labs. Methods using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) are currently under clinical investigation and are not yet FDA-approved. Other MS methods focusing on quantifying unique clonotypic peptides (MS-MRD) derived from the variable region of the monoclonal immunoglobulin by enzymatic digestion followed by LC-MS/MS have been developed and provide great sensitivity. MS-MRD demonstrated a 1,000-fold higher sensitivity compared to serum protein electrophoresis (SPEP) and can be used to monitor MRD patients.(40, 44-46) While some preliminary studies suggest that MS may be a strong predictor of PFS (47), additional studies are needed to incorporate these assays into clinical practice. Especially, the persistence of the monoclonal immunoglobulin in the serum in context of bone marrow MRD negativity detected by serum immunofixation, or mass spectrometry has been reported in different studies and may be related to the immunoglobulin half-life rather than to a MRD positive disease. Therefore, incorporation of MS needs to be clarified.

Whole-body Imaging and MRD assessment

While imaging studies do not allow to detect active disease at the single cell resolution, relatively novel whole body imaging techniques including positron emission tomography with computed tomography (PET/CT) and MRI allow a better characterization of bone lesions and extramedullary disease (EMD). PET/CT and whole-body MRI have been evaluated to assess residual disease after therapy (48-51). Both methods are very sensitive and have been shown to complement BM based MRD assessment considering the patchy nature of MM and its spatial heterogeneity. Patients presenting with extra-medullary lesions or with para-medullary plasmacytomas are at higher risk of developing EM disease even in context of BM MRD negativity (52). ^{18}Fluorodeoxyglucose (^{18}F-FDG)-PET is a very sensitive method to identify active disease and several studies showed that PET-positive lesions after completion of therapy is associated with poorer outcome (93–96) while FDG-PET/CT negativity after ASCT in patients

achieving complete remission (CR), predict a lower risk of progression or death (53-56). Patients obtaining PET/CT normalization upon therapy have comparable prognosis to patients without baseline increased metabolism, suggesting the value of treating until suppression of glucose metabolism (56). In the FORTE trial, a high concordance between PET/CT and NGS (84%) and between PET/CT and MFC (93%) at $10^{\wedge-5}$ in the identification of BM residual disease was reported. By contrast, there was discrepancy in the assessment of MRD in patients with focal lesions in ~33–37% of cases, suggesting that PET/CT alone might not be accurate enough(57). Similarly, in the CASSIOPET study, a significant concordance between bone marrow and PETCT-based MRD assessment was reported in 109 patients, but the data suggested a higher sensitivity for the bone marrow-based MRD method(58). A standardized definition of PETCT complete metabolic response has been proposed considering the uptake of the liver as threshold and is currently under confirmation in independent prospective series of patients (48). A significant challenge related to ^{18}FDG PETCT relates to the 10% to 15% of MM patients with no FDG-avid lesions due to lack of hexokinase enzyme, which is responsible for FDG trapping in the myeloma cells or to the absence of identified lesions (59). New PET/CT tracers including CD38, radiolabeled antibodies and VLA-4 (NCT03804424) represent new methods potentially more sensitive that are under investigation, (13, 60, 61). In this line, conjugating daratumumab with the positron emitting radioisotopes Copper-64 (64Cu) and Zirconium-89 (^{89}Zr) has allowed for the creation of immunoPET tracers. ^{89}Zr-Daratumumab has demonstrated the ability to detect MM cells or lesions when not detected by ^{18}FDG-PET/CT and other clinically standard imaging methods(62, 63). However, the lower availability of these newer tracers, interpatient heterogeneity regarding specific targets and lack of prospective data remains an important challenge to be addressed.

Similar to PETC/CT, presence of residual lesions after high dose chemotherapy followed by autologous stem cell transplant (HDCSCT) identified by whole body MRI (wbMRI) is associated with adverse prognostic significance (64-66). MRI seems to be more sensitive at diagnosis to identify myeloma lesions than PETC/CT. In a prospective study comparing PETCT and wbMRI in 60 patients, WB-MRI showed significantly higher detection of focal lesions at all anatomic sites (except ribs, scapulae, and clavicles) and of diffuse disease at all sites. However, MRI is not able to differentiate between vital and necrotic tissue within preexisting osteolytic lesions(64)

and therefore PETCT positivity may be more accurate to assess MRD and to predict patient outcome (67-69). A more sensitive MRI based method called diffusion weighted imaging (DWI), is a promising alternative allowing more accurate detection of active lesions and is under investigation(70, 71). Whether MRD is assessed with PETCT or wbMRI, the complementary role of imaging studies to BM based MRD evaluations is significant and strategies to include both are being evaluated.

Based on the increasing amount of MRD data, the availability, reproducibility and standardization of MRD methods, MRD assessment has become an important and validated criteria in clinical trials. MRD assessment is now used for patient selection, risk stratification or enrichment of the trial population, and as an endpoint. MRD assessment will likely contribute to expedite drug development(13). However, using MRD assessment to guide therapy and MRD incorporation in clinical practice is not yet validated. We here discuss some of the most significant challenges that have been or need to be addressed.

1. Which MRD method to use?

As discussed above, MRD assessment using BM based methods remains the gold standard with increasing data regarding whole-body imaging. The availability, cost, prognostic power, and consistency are important factors to consider. Regarding BM based MRD, NFC and NGS are the two methods of choice to evaluate BM MRD and both have been shown to constitute a strong prognosis marker in MM. Table 1 is summarizing the characteristics of these methods. Comparison between flow cytometry and NGS methods have been performed in randomized clinical trials. In the phase II multicenter randomized FORTE trial reported, 86% of correlation with MRD at a sensitivity of $10^{\wedge-5}$ in $\geq$ CR patients was reported (72). In the phase III CASSIOPEIA trial, MFC and NGS were consistent in 83.5% with a sensitivity of $10^{\wedge-}5$ (73). A direct comparison between NGF and NGS (not clonoseq platform) was also reported in a study of 106 patients showing a high correlation ($R^2 = 0.905$)(74). As NGS and NGF are comparable, each method should be considered based on local availability. Complementary imaging methods to assess bone and extra-medullary MRD include currently whole-body MRI and PET/CT imaging and provide additional information. While BM and WB imaging-based methods have relatively good concordance and provide additional information particularly regarding extra-

medullary disease and high risk MM. Whole-body imaging should therefore be used in combination to BM based MRD in patients with HR or EM disease. Table 2 is summarizing the characteristics of WB imaging methods. Some studies are now being reporting supporting the benefit of combining both MRD methods in patients care(71).

2. When should MRD assessment be performed?

Another important challenge regarding MRD utilization relates to its timeline. A first question is whether MRD evaluation should be done early during frontline treatment (before SCT for example) or later on, after consolidation or during maintenance. Several trials have evaluated each of these time points and because of the dynamic nature of MRD which imply transition from MRD positivity to negativity and vice-versa, it has become clear that several evaluations are in fact more informative. These observations led to define the concept of “sustained” MRD negativity which appears to be the critical prognosis factor in MM(75). Having defined this concept led to another important question which is how much time between 2 time points should be used. It seems that longer time points will likely be associated with better outcome, but studies are investigating if 6 months, 1 year or 2 years of MRD negativity are more relevant to potentially impact treatment decision. In chronic myeloid leukemia (CML), strategies to stop therapy after 2 years of sustained MRD negativity have been developed, for example(76, 77). However, CML and MM are very different diseases biologically, and large phase 3 clinical trials are needed and ongoing to address this question. In future clinical practice, we anticipate that MRD assessment will be useful to evaluate the efficacy of a particular treatment strategy, before or after consolidation therapy or during maintenance in newly diagnosed myeloma and relapse/refractory disease. It is important to mention that the advent of cellular therapies including bi-specific antibodies and chimeric antigen receptor (CAR) T cell therapy have revolutionized patient outcome and are associated with dynamic and often dissociated patterns of MRD and serological residual disease. Therefore, the role of MRD evaluation in the context of these novel therapies remains to be fully validated(78).

3. How much is MRD evaluation useful in high-risk myeloma patients?

High risk (HR) MM are defined by the presence of del17p, t(4;14), t(14;16), low albumin, high beta 2 microglobulin and elevated LDH(79). MM patients experiencing early relapse after

frontline therapy have also the poorest outcome(80). Important studies are ongoing to improve treatment strategies in this subgroup of patients. Different results regarding clinical impact of MRD status in high-risk patients have been reported(81, 82). While achievement of MRD negativity is associated with clinical improvement in HR MM, it does not overcome its poor prognosis and HR MM may still have early progression. In addition, data from the large phase III trial (Myeloma XI) showed that high-risk molecular features had an adverse effect on PFS and OS even for those patients achieving MRD-negative status(83). BM MRD evaluation only evaluation is likely to be insufficient to fully assess MRD, in context of patchy disease and extramedullary disease as discussed above. Indeed, in a study comparing BM and Imaging based MRD assessment, 12% of patients who achieved BM MRD negativity by flow cytometry had positive PET/CT or whole-body diffusion-weighted MRI (WB-DWI-MRI) and had a shorter PFS in comparison to patients with both BM and imaging MRD negativity(56). Further data are needed to better interpret and use MRD status especially in high-risk patients and patients with EMD. Sustained MRD and combined BM and imaging based MRD assessment are important parameters to consider. Both sustained MRD negativity and combined bone marrow and imaging MRD studies (PET/CT or whole-body MRI) appear to be the most valuable approaches in HR MM patients. Results from randomized trials are expected to address this important question. Similarly, in context of relapse/refractory MM, while achievement of MRD negativity is associated with better outcome, most of the patients do experience relapse. This is well illustrated by the KarMMa trial that evaluated the efficacy and safety of Idecabtagene vicleucel (ide-cel) in patients with relapsed and refractory myeloma for example in which 26% of the patients achieved MRD negativity including 79% of patients achieving CR. However only 40% of patients achieving at least CR were in remission at 20 months of follow-up(84). It seems that sustained MRD negativity combined with whole-body imaging will be more relevant in that context.

4. **Should MRD assessment be done only in patients achieving complete hematologic response?**

Another important point relates to which patient should be evaluated for MRD. In clinical trials, MRD investigations were performed either in MM patients achieving CR or stringent CR or in specific timepoints of a given therapeutic protocol (before stem cell transplant, before or during

maintenance for example). It was showed that patients achieving CR with MRD positivity had significantly worse outcome than patients achieving both CR and MRD negativity. However, several studies have reported MRD negative rates in MM patients achieving very good partial response (VGPR), and indeed, up to 25% of patients achieving MRD negativity assessed by either flow cytometry or NGS have persistent positive immunofixation and therefore are classified as VGPR(85). Importantly, retrospective and prospective studies showed that patients with positive IF and MRD negativity have similar outcome than patients with negative IF and MRD negativity. The discrepancy between positive IF and MRD negativity may be related to several reasons including extramedullary disease, bone marrow sample not representative of full bone marrow or long half-life of the monoclonal immunoglobulin(85). This is even more highlighted in studies utilizing MS (the sensitive assay to detect monoclonal immunoglobulin as described above). In a study, the monoclonal immunoglobulin was still detectable by MALDI-TOF in 69% of patients who achieved a conventionally defined CR and were bone marrow based NGF-MRD-negative after 100 days from ASCT (86). Finally, discordant MRD and IF results are frequently observed after CAR T cell therapy, with low rates of CR observed in patients achieving MRD negativity, particularly in the first 6 months after treatment, suggesting that MRD evaluation, which reflects the clearance of myeloma cells in the bone marrow, could be an independent prognosis marker in that setting(78). Therefore, assessing MRD in patients achieving $\geq$VGPR is very relevant and informative.

5. Should MRD status impact therapeutic decision?

MRD negativity is a very strong and now established prognosis marker. However, its impact on therapeutic decision remains to be determined. Several randomized clinical trials are currently ongoing to address this question (table 3). The goals of these trials are to determine if treatment should be adapted based on MRD status: intensification of treatment in case of MRD positivity, stop maintenance therapy in case of sustained MRD negativity or treatment change in case of MRD status conversion from negativity to positivity. The MASTER trial pioneered this strategy and evaluated the role of BM based MRD in treatment during consolidation. Patients received Daratumumab/carfilzomib/lenalidomide/dexamethasone (Dara-KRd) as induction therapy and BM MRD was performed by NGS at different time points (end of induction, after high dose chemotherapy followed by autologous stem cell transplant (HDCSCT), and every four cycles of

consolidation) to inform the use and duration of treatment with Dara-KRd. Treatment was stopped in patients who achieved two consecutive MRD-negative assessments. Among 123 included patients, 43% had none, 37% had 1, and 20% had 2 high risk cytogenetic features and 96% had BM MRD trackable by NGS. With a median follow-up of 25.1 months, 80% of patients reached MRD negativity (78%, 82%, and 79% for patients with 0, 1, and 2+ high risk cytogenetic, respectively), including 66% who reached MRD < 10^{-6}, and 71% who reached two consecutive MRD negative assessments during therapy, entering treatment-free surveillance. Two-year progression-free survival was 87% (91%, 97%, and 58% for patients with 0, 1, and 2 HRCA, respectively). Cumulative incidence of MRD resurgence or progression 12 months after cessation of therapy was 4%, 0%, and 27% for patients with 0, 1, or 2 HRCA, respectively(82). Similarly, a phase 3 clinical trial (GEM2012MENOS65) evaluated lenalidomide and dexamethasone maintenance with or without ixazomib in newly diagnosed myeloma patients, with treatment stopping after 24 cycles in case of bone marrow based MRD-negativity. Patients achieving MRD negativity after 24 months of maintenance therapy had a low progression rate (17.2%) at 4 years, strongly suggesting that the duration of maintenance therapy can be tailored based on MRD negativity(87). Accordingly, data regarding achievement of MRD negativity and early relapse have been reported but not yet published in high-risk MM patients included in the CASSIOPEIA trial(73). Although longer follow-up is needed, these trials already suggest that cessation of treatment may be feasible in patients with standard risk cytogenetic but not for patients with high-risk cytogenetic abnormalities. Improving MRD assessment maybe by combining bone marrow and imaging evaluation may be more relevant in high-risk cytogenetic patients in frontline. Another important observation has been reported in a phase 2 study evaluating MRD dynamics during lenalidomide maintenance. Patients who lost MRD negativity were more likely to progress than patients with sustained MRD negativity (HR infinite; p<0·0001) as expected but also and worse than patients with persistent MRD positivity (HR 5·88, 95% CI 1·18-33·33; p=0·015) at the 2-year landmark. These data suggest that the dynamic of the disease is another very important parameter to consider when considering using MRD as a therapeutic guide(88).

The ongoing MIDAS trial (NCT04934475) is designed to evaluate the role of HDCSCT on the basis of MRD status after induction in newly diagnosed MM patients. In this trial, patients are

treated with 6 cycles of quadruplet regimen induction with isatuximab/carfilzomib/lenalidomide/ dexamethasone (Isa-KRd) and evaluated for BM based MRD (with a threshold of 10^{-5} cells) post-induction. Patients are next stratified into standard risk (MRD negativity < 10^{-5}) or high risk (MRD positivity> 10^{-5}). Patients achieving MRD negativity following induction are randomized to receive six additional cycles of Isa-KRd followed by maintenance or HDCSCT, followed by two cycles of Isa-KRD, and maintenance with lenalidomide for 3 years. High-risk patients defined by MRD positivity post-induction are randomly assigned to receive HDCSCT followed by two cycles of Isa-KRD versus tandem HDCSCT followed by isatuximab-iberdomide maintenance for 3 years. This ambitious trial will address if MRD status can be used to guide therapy and if HDCSCT remains the gold standard in patients achieving early MRD negativity after induction.

Several other randomized clinical trials are evaluating MRD based treatment decision. The PERSEUS trial (NCT03710603) will evaluate the possibility to stop daratumumab during maintenance in patients achieving sustained MRD negativity for 12 months and after a minimum of 24 months of maintenance and the benefit of restarting daratumumab in case of MRD conversion (from negative to positive) or confirmed loss of CR without IMWG disease progression criteria(89). The AURIGA trial (NCT03901963), randomly assigns patients who have achieved very good partial response but who are MRD-positive to receive daratumumab and lenalidomide versus lenalidomide maintenance for the primary endpoint of MRD conversion at 12 months from initiation of maintenance. The DRAMMATIC trial (NCT04071457) by SWOG (S1803) randomly assigns patients to receive daratumumab and lenalidomide versus lenalidomide maintenance post HDCSCT. After 2 years of Maintenance, MRD is assessed to guide further therapy. MRD positive patients will continue with the assigned treatment while MRD negative patients will be further randomized to either continue or discontinue the assigned treatment. The OPTIMUM trial (NCT03941860) by ECOG (EAA171) will randomly assign MRD positive patients who are receiving lenalidomide maintenance after HDCSCT to receive ixazomib or placebo in addition to continuing lenalidomide.

In conclusion, MRD assessment methods have significantly improved in the past two decades and allow identification of patients with deep hematologic response. Bone marrow-based

methods using NGF and NGS are to date the most available, standardized, and sensitive methods. Whole body imaging includes wbMRI and PET/CT are also very interesting and when combined with BM MRD assessment provide better evaluation especially in the setting of high risk cytogenetic and extramedullary disease. Achievement of MRD negativity is a very strong prognosis factor that is now an established endpoint in myeloma clinical trials. Persistent or sustained MRD negativity portends better outcome in newly diagnosed and relapsed refractory disease, including after CAR T cell therapy in myeloma and may allow discontinuation of therapy in patients without high risk cytogenetic. Several clinical trials are currently ongoing to establish if MRD can be used to guide therapy and to monitor disease activity.

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Bone marrow based MRD	Next Generation Flow Cytometry (NGF)	Next Generation Sequencing (NGS)
Bone marrow evaluation	Yes	Yes
Standardization	Euroflow	Clonoseq
Require evaluation at diagnosis	Not required	Required
Fresh sample	Yes	No
Cost	+	++
Applicability	Universal	~90% of patients
Sensitivity	$10^{\wedge-5}$-$10^{\wedge-6}$	$10^{\wedge-6}$

Table 1: Available methods to assess Bone marrow MRD in multiple myeloma.

Whole body imaging methods	PETCT	Whole Body MRI
Bone marrow evaluation	No	No
Standardization	Yes	Yes
Require evaluation at diagnosis	Not required* Negative in ~10% of MM patients	Not required*
Cost	++	++
Applicability	++	+
Sensitivity	++	+++

Table 2: Available whole body imaging methods to complement bone marrow MRD assessment in multiple myeloma. *Useful to have whole body imaging at diagnosis to evaluate response. Important to confirm if PET/CT positive at diagnosis.

Clinical trial	Patient population	Treatment scheme
UMCC 2018.056 (NCT04140162)	Phase 2 Study With Minimal Residual Disease (MRD) Driven Adaptive Strategy in Treatment for Newly Diagnosed Multiple Myeloma (MM) With Upfront Daratumumab-based Therapy	This phase 2 trial will test whether the combination of DaraRd (daratumumab + lenalidomide + dexamethasone) as induction therapy, followed by DRVd (daratumumab + lenalidomide + bortezomib + dexamethasone) consolidation therapy, if needed, will result in more patients achieving minimal residual disease (MRD)-negative status, relative to the standard of care. Consolidation therapy will be administered only to those patients with MRD-positive status after induction therapy.
MIDAS NCT04934475	MInimal Residual Disease Adapted Strategy (MIDAS) Phase 3 clinical trials in newly diagnosed MM patients	IFM 2020-02 will enroll patients eligible for ASCT less than 66 years. All patients will receive induction based on 6 cycles (28-day) of KRD-Isatuximab (Isa-KRD), in order to achieve deep responses and high MRD negativity rates. Patients will be classified at diagnosis according to cytogenetics (standard vs high-risk cytogenetics defined by the LP score including 17p deletion, t(4;14), del(1p32), gain 1q, trisomy 21 and trisomy 5.
PERSEUS (NCT03710603)	Phase 3 clinical trial Daratumumab, VELCADE (Bortezomib), Lenalidomide and Dexamethasone Compared to VELCADE, Lenalidomide and Dexamethasone in Subjects With Previously Untreated Multiple Myeloma	A Phase 3 Study Comparing Daratumumab, VELCADE (Bortezomib), Lenalidomide, and Dexamethasone (D-VRd) vs VELCADE, Lenalidomide, and Dexamethasone (VRd) in Subjects with Previously Untreated Multiple Myeloma Who Are Eligible for High-dose Therapy. MRD-negative subjects will stop daratumumab after sustained MRD negativity for 12 months & after a min. of 24 months of maintenance. Daratumumab should be restarted at recurrence of MRD or confirmed loss of CR without disease progression.
DRAMMATIC (NCT04071457)	Phase 3 clinical trial Lenalidomide +/- Daratumumab/rHuPh20 as Post-ASCT Maintenance for MM w/MRD to Direct Therapy Duration (DRAMMATIC)	In this trial, patients who received HDCSCT are randomized between Lenalidomide for 2 years and Lenalidomide + Daratumumab. After 2 years of Maintenance, MRD is assessed to guide further therapy. MRD-positive patients will continue with the assigned treatment. MRD-negative patients will be further randomized to either continue or discontinue the assigned treatment.
EMN20 (NCT04096066)	Phase 3 clinical trial. A Trial That Compares Two Treatments in Newly Diagnosed Myeloma Patients Not Eligible for Transplant (KRd vs Rd)	This protocol is a randomized, multicenter study designed to determine the MRD negativity and the PFS of KRd treatment regimen. Patients will be randomized in a 1:1 ratio to receive carfilzomib-lenalidomide-dexamethasone (KRd - Arm A) or lenalidomide-dexamethasone (Rd - Arm B). Patients will be stratified basing on international staging system (ISS) and fitness status using a web-based procedure completely concealed to study participants. Patients will be treated until disease progression or intolerance to the therapy. The only exception is for patients enrolled in KRd arm who achieve at least a VGPR during the first year of treatment and in sustained MRD negativity (MRD negative at least at 10-5 after one and two years of therapy): these patients will stop carfilzomib administration after 2 years, whereas treatment with lenalidomide and dexamethasone will be continued.
MASTER-2 (NCT05231629)	Phase 2 clinical trial. Sequential Therapy in Multiple Myeloma Guided by MRD Assessments (MASTER-2)	This research study will determine the proportion of patients with lowest minimal residual disease (MRD) response obtainable after receiving 6 cycles of study treatment. Minimal residual disease is multiple myeloma cells below the level of 1 cancer cell out of 100,000 in the bone marrow. For patients who become MRD "negative" (i.e. less than 1 cancer cell out of 100,000) at the end of 6 cycles of therapy, this study will study if that good response can be maintained with 3 additional cycles of treatment instead of use of autologous hematopoietic cell transplantation (AHCT). For patients who are MRD "positive" at the end of 6 cycles of therapy, this study will answer whether more patients can become and remain MRD "negative" with AHCT plus teclistamab in combination with daratumumab when compared with patients who undergo AHCT followed by lenalidomide (an established anti-myeloma drug) plus daratumumab.
RADAR (EudraCT 2019-001258-25)	Phase 3 clinical trial. Risk-Adapted therapy Directed According to Response comparing treatment escalation and de-escalation strategies in newly diagnosed patients with multiple myeloma (NDMM) suitable for stem cell transplant.	All participants will receive the same initial induction treatment and during this time will have genetic tests to determine whether they have ‘standard-risk’ or ‘high-risk’ disease. Following this chemotherapy treatment participants will receive AHCT. After induction treatment participants will be allocated to a second stage treatment group based on their genetic risk, high-risk or standard-risk, and on how well the myeloma has responded to the initial treatment. Each treatment group will then receive different combinations of medication to investigate their benefit. Treatment will comprise of combinations of isatuximab, bortezomib, cyclophosphamide, lenalidomide and dexamethasone.

Table 3: Ongoing prospective clinical trials evaluating MRD adapted therapy