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Measurable residual disease in multiple myeloma and in acute myeloid leukemia, an evolving topic

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Abstract

Minimal or measurable residual disease (MRD) is a term that refers to the submicroscopic tumor disease persisting after therapy. Sensitive immunophenotypic and molecular techniques are used to detect the small amount of residual tumor cells, conferring a detection capacity clearly more sensitive of common cytomorphologic techniques. MRD evaluation now represents an important tool in the study of solid tumors and of hematological malignancies. Concerning hematological malignancies, MRD evaluation was particularly developed in the study of multiple myeloma and acute myeloid leukemia, representing in these diseases a precious biomarker to quantify response to treatment, to evaluate the chemosensitivity/chemoresistance of the disease and to have a prognostic prediction on disease outcome. The finding that MRD evaluation may have a prognostic value, predicting the risk of relapse, stimulated interest in the introduction of MRD in clinical trials, either as a clinical endpoint or as a tool to guide treatment decisions. However, the clinical use of MRD requires a standardization of the techniques used for its detection, the use of multiple techniques and the development of a consistent accuracy and reproducibility. Finally, prospective clinical trials are required to assess the real clinical benefit potentially deriving from the introduction of MRD evaluation into clinical studies.

Key words

- hematologic malignancies
- multiple myeloma
- acute myeloid leukemia
- measurable residual disease
- flow cytometry

INTRODUCTION

Measurable residual disease (MRD, also known as minimal residual disease) in neoplastic diseases can be defined as the amount of residual tumor cells that remains in the body after the end of treatment. The objective of cytoreductive or of new targeted therapies consists in the complete eradication of all tumor cells; however, a significant proportion of patients display a residual number of resistant cells that represent the MRD and that are responsible for disease relapse. Historically, the response to treatment was based on cytologic examination of tumor biopsies with a detection limit of 10^{-1}-10^{-2}. It is evident that using a traditional technology, such as cytology, there is an intrinsic limitation to detect low levels of residual tumors; whole detection, however, is of fundamental importance at clinical level.

The development of new techniques of high-sensitivity able to quantify tumor cells, even when present in low or very low amounts, has revolutionized the detection of residual tumor cells. Techniques such as multiparameter flow cytometry (MFC), reverse transcription quantitative polymerase chain reaction (RQ-PCR), digital droplet polymerase chain reaction (dd-PCR), amplico-based next generation sequencing (NGS), panel directed- NGS and whole-exome or whole-genome NGS have reached sensitivities up to 10^{-6} and allow to detect even a very minor residual tumor cell population, providing a much more accurate definition of the response to therapy.

Dramatic progresses have been made in the last years in the treatment of patients with hematological malignancies. Although these progresses, not all patients respond equally to the treatments due to disease heterogeneity and intrinsic or acquired resistance to antitumor drugs used to treat these patients. In the treatment of these patients, it is particular important to distinguish between patients who really respond to treatment with virtual disease eradication from those responding in only a partial way to these treatments with a residual and variable amount of tumor cells. The consistent progresses made in the definition of the recurrent cellular and molecular abnormalities observed in these tumors offered the unique opportunity to detect and quantify even small amounts of cells surviving to treatments [1]. Particularly, efficient techniques have been developed

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for evaluation of MRD in seven hematological malignancies, including chronic myeloid leukemia (CML), chronic lymphoid leukemia (CLL), follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL), multiple myeloma (MM), acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) [1]. Molecular techniques based on polymerase chain reaction were developed and standardized for all these diseases, while MFC techniques were used for MRD detection in MM, ALL, AML and CML.

In some of these diseases, the evaluation of MRD was of fundamental importance at clinical level. Thus, the monitoring of MRD in CML patients based on the quantification of the BCR-ABL1 transcript was essential for the definition of the best individual algorithm treatment and for a selection of patients who may discontinue tyrosine kinase inhibitors [2].

In ALL, MRD was evaluated by different techniques, ranging from MFC, allele-specific and mutation specific RQ-PCR and NGS techniques; these studies unequivocally supported the clinical utility of MRD evaluation as a parameter predicting clinical outcome, providing criteria for the selection of patients for intensified treatments and for MRD-targeted therapy [3].

CLL is a disease whose therapy was in continuous evolution during the last years, a condition that required the support of an assay, such as MRD, providing fast information on therapeutic efficacy. In CLL, MRD can be evaluated with a high level of sensitivity by MFC, RQ-PCR and NGS; MRD status was adopted in numerous clinical trials in CLL patients and showed that a MRD-negative status was associated with a better PFS and OS [4, 5]. Undetectable MRD was considered a main objective in some clinical studies [6].

Although is undoubted that MRD evaluation represents a precious tool for oncology clinical studies, it is also evident that MRD assays require not only a good sensitivity, but also careful procedure of standardization and the formulation of international scientific guidelines generated by experts in the specific field and institutional guidelines formulated by regulatory agencies.

In this review we analyze the progress made in the clinical use of MRD evaluation in MM and AML, considered as paradigmatic for an understanding of the contribution of MRD to clinical progress in both the understanding and treatment of these diseases.

DETECTION OF MRD IN MULTIPLE MYELOMA

Dramatic progresses have been made in the last years in the therapy of multiple myeloma (MM), leading to a significant improvement of the outcome of these patients (*Table 1*). Thus, many therapeutic strategies are capable of inducing a significant rate of complete responses. This progress rendered particularly important the accurate definition and the sensitive detection of MRD to better stratify the risk and the need for supplementary treatments of MM patients achieving complete response (CR). In fact, a significant proportion of CR patients' relapse, thus indicating that low, but clinically significant levels of MRD remain in the majority of patients attaining CR. This explains the absolute need of developing highly sensitive techniques able to detect deeper responses than CR, as recently indicated by the International Myeloma Working Group (IMWG) [7].

The key role of MRD detection in MM patients is strongly supported by a meta-analysis carried out in 14 clinical studies and on a total of 1273 patients: in fact, in these patients an MDR-negative status after treatment for newly diagnosed MM was associated with long-term survival [8]. An updated analysis extended to 8098 MM patients for progression-free survival (PFS) analysis and 4297 patients for overall survival (OS) analysis confirmed these results showing that compared with MRD positivity, the achievement of MRD negativity was associated with a significant improvement of both PFS and OS [9]. Importantly, MRD negativity was associated with improved OS independently of the disease status (newly diagnosed or relapsed disease), MRD sensitivity level, cytogenetic risk, method used for MRD assessment and the level of the clinical response at the time of MRD evaluation [9].

According to Burgos *et al.* techniques used for evaluation of MRD in MM can be divided into those able to detect extramedullary disease (such as positron emission tomography/computed tomography, PET/CT) and those able to detect intramedullary disease (such as molecular detection of immunoglobulin gene rearrangements or multiparameter flow cytometry (MFC) immunophenotyping) [10].

Radioimaging techniques play an important role in the diagnostic procedures of MM to assess both medullary and extramedullary disease. Low-dose whole body computed tomography is a sensitive technique to assess the osteolytic bone disease, superior in its sensitivity to other conventional techniques of skeletal survey in the detection of bone disease [11, 12]. Conventional magnetic resonance imaging (MRI) was shown to be superior to ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET-CT) for the detection of small focal lesions and diffuse marrow infiltration; however, FDG-PET-CT had the advantage to provide more quantitative measures [13]. A peculiar technique of MRI, whole-body diffusion-weighted MRI (WB-DWI), based on a non-ionizing radiation modality is suitable for measurement of disease burden and treatment response in MM [13]. WB-DWI offers the advantage compared to standard MRI to be more sensitive and quantitative; furthermore WB-DWI allows the evaluation of skeletal complications and does not require intravenous contrast [13]. FDG-PET-CT imaging was shown to give 11% of false negative results in MM patients, due to the low expression of the hexokinase-2 gene in PET false-negative cases [14].

Multiparametric flow cytometry (MFC) is one of the techniques that allows to detect the intramedullary extent of MRD in MM patients. This technique is based on the identification of myelomatous plasma cells according to aberrant phenotypic features and to the presence of light-chain clonality. MFC evolved from a phase I technology with a 10^{-4} sensitivity to a more sensitive technique developed by Euro-Flow, next-generation flow cytometry (NGF) with a sensitivity of $2\times10^{-6.9}$. MFC technique is based on the labeling of

bone marrow cells with a panel of monoclonal antibodies: the immunophenotype of normal plasma cells was $138^{+}45^{+}19^{+}56^{-}$, whereas the phenotype of myelomatous plasma cells was $138^{+}45^{-}19^{-}56^{+}$; this technique allows the detection of both normal and neoplastic plasma cells [15]. Rawstrom *et al.* have used this first-generation assay of MFC to evaluate the outcome of MM patients undergoing autologous stem cell transplantation (ASCT) and showed that this technique helped to define early after transplantation patients with MRD-positive, needing additional treatment strategies [15].

San Miguel *et al.* reported the MFC detection of neoplastic plasma cells using a more extended panel of monoclonal antibodies; they defined the phenotype of normal plasma cells as 38^{+++}, 56^{-}, 45^{+}, 20^{-}, 28^{-}, 33^{-}, 117^{-} [16]. Using this first-generation MFC technique they showed that ASCT induced a greater reduction of the number of residual neoplastic plasma cells compared to high-dose chemotherapy alone and that after ASCT the coexistence of normal and neoplastic plasma cells was observed, a condition similar to that observed in monoclonal gammopathies of undetermined significance [16].

At variance with most routine diagnostic tests currently used for the evaluation of response to treatment in MM, MFC suffered from large intra-laboratory variations in terms of sensitivity, sample preparation, data acquisition and analysis. However, a recent study provided evidence that full standardization of interlaboratory MM MRD evaluation is feasible and compatible with the generation of highly concordant and reproducible MRD data [17].

The comparison of the detection of MRD in MM undergoing ASCT using first-generation MFC and allelic-specific real-time PCR showed that the first technique is less sensitive than the second technique; however, in patients with detectable MRD using both techniques, the percentage of tumor cells estimated by the two techniques was similar [18].

The introduction of a second-generation 8-color multiparameter-flow cytometry allowed to improve the sensitivity of MFC technique for MRD detection; the application of this technique to the study of elderly MM patients allowed to define three groups of patients according to MRD levels: i) MRD-negative ($<10^{-5}$); ii) MRD-positive (range from 10^{-5} to 10^{-4}); MRD-positive ($\geq 10^{-4}$) [19]. The standardization of the 8-color flow-cytometry, the so-called Next Generation FLOW (NGF) allowed an additional improvement of both the sensitivity and reproducibility of this technique [20]. The EuroFlow PCD 8-color panel included the analysis of 12 different markers: CD38, CD138, CD45, CD19, CD27, CD28, CD56, CD81, CD117, Cylgκ, Cylgγ and β2-microglobulin [20]. Using this technique, multicenter analysis of bone marrow samples from 110 MM patients showed that NGF-MRD was significantly more sensitive than conventional 8-color flow-MRD [20].

The possible clinical uses of MRD evaluation in MM patients is reported in *Table 2*.

Terpos *et al.* have evaluated by NGF cytometry 52 patients with sustained complete remission (≥2 years) after frontline therapy: 45% of patients were MRD-positive at the level of 10^{-5} and 17% at 10^{-6} level [21]. All patients who relapsed during the follow-up were MRD-positive, including those with ultra-low tumor burden [21]. Paiva *et al.* have recently reported the results observed in a large set of MM patients monitored by NGF for MRD status and treated in the context PETHEMA trial with high-intensity chemotherapy, ASCT and consolidation chemotherapy [22]. The NGF assay achieved a median limit of detection of 2.9×10^{-6} [22]. 45% of these patients achieved a MRD-negative status after consolidation therapy: 7% of these patients experienced disease progression and 50% of these patients displayed extramedullary disease [22]. Patients MRD-negative by NGF assay displayed a 88% decrease of the risk of death [22]. These findings strongly support the NGF assay of MRD in clinical evaluation of the efficacy of MM treatment.

In MM, as well as in other tumors, tumor cells can be detected in peripheral blood. A recent study showed that circulating plasma cells are detected in MM patients and can be studies by NGF cytometry [23].

Interestingly, combining detection of MRD by DW-MRI and functional imaging by DW-MRI improved prediction of outcome of MM patients, double-negativity defining patients with excellent prognosis and double-positivity patients with dismal prognosis [24].

The study of MRD by NGF was of fundamental importance not only as a prognostic measure of outcome, but also as a tool to better understand the mechanisms of treatment resistance in MM patients. Goicoechea *et al.* have evaluated MRD with the NGF technique in MM patients with standard and with high-risk cytogenetic abnormalities enrolled in the PETHEMA trial [25]. In patients with MRD-negative, both those pertaining to the standard and to the high-risk groups, progression-free survival and overall survival rates were greater than 90% after 36 months of follow-up [25]. MRD-positivity was associated with a median time of progression-free survival of two and three years in high-risk and standard risk patients, respectively [25]. The NGF technology was used also to explore the whole-exome sequencing of paired diagnostic and MRD tumor cells, showing remarkable difference between the two groups of patients: standard-risk MM patients showed greater clonal selection, whereas high-risk MM patients showed acquisition of new mutations [25]. The characterization of clones of MRD tumor cells may represent an important tool to understand the molecular mechanisms of MRD resistance.

The other fundamental technique used for the evaluation of intramedullary MM disease consists in the molecular assessment of immunoglobulin gene rearrangements. As observed for flow cytometry, there was a similar evolution for molecular studies of detection of immunoglobulin gene rearrangements, moving from an initial allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) more complex and less sensitive technique to a more sensitive next generation sequencing techniques with a sensitivity in the order of $10^{-6.9}$. The ASO-PCR detects rearranged B-cell receptor genes on the basis of the identification of clonotypic sequences; this technique is specific and sensitive, but has the

considerable disadvantage of being technically complex and of limited applicability. The development of high throughput sequencing technologies, using amplification and sequencing of immunoglobulin gene segments using consensus primers, improved of about 1 log the sensitivity of detection of immunoglobulin gene rearrangements and showed a good applicability, greater than 90%. MM patients who were MRD-negative by NGS displayed a significantly better survival than those who were MRD-positive [26].

Using this deep-sequencing technology, Perrot *et al.* provided evidence that in a large group of MM patients treated with lenalidomide, bortezomib and dexamethasone molecular MRD negativity was a strong prognostic factor predicting a prolonged overall survival, regardless of cytogenetic risk profile and disease stage at diagnosis [27].

In MM, as well as in many other tumors, tumor cells are not only resident in bone marrow but circulate also and release tumor DNA that can be found in peripheral blood. Mazzotti *et al.* have explored whether plasma could replace bone marrow for assessment of MRD in MM using deep sequencing [28]. However, the results of this study failed to show an association between circulating tumor DNA and bone marrow for MRD by NGS using only immunoglobulin gene rearrangements [28].

All the clinical trials that included the evaluation of MRD using a sensitive and standardized technique have reached the conclusion that MM patients achieving a MRD-negative status, either after chemotherapy treatments or ASCT, displayed a better PFS and OS compared to those with MRD-positivity [29, 30]. The available data were sufficiently clear to convince regulatory medicinal agencies, such the European Medicine Agency (EMA) that MRD measured by a standardized method with a quantitative lower limit set of at least 10^{-5} can be used as an intermediate endpoint in randomized controlled trials [31]. In line with this view, some ongoing clinical trials have as main objectives the study of MRD in MM: the trial NCT04108624 aims to assess for MRD in MM at a deeper level by combining novel imaging and laboratory techniques, to determine if patients who are MDR-negative by multiple evaluation and discontinue post-transplant maintenance therapy, and to determine is liquid biopsy is a more accurate and less invasive sampling technique for MM; the trial NCT04140162 aims to determine whether a double duratumamab-based regimen (induction and consolidation) is able to increase the proportion of MM patients reaching a MRD-negative status.

There is now consistent evidence that MRD negativity is a superior prognostic factor than conventional CR for MM patients. However, many questions related to the clinical use of MRD assays remain open.

One of these problems is related to the optimal threshold of MRD detection. Although initial studies have proposed the ideal threshold of MRD detection at 10^{-5}, however, there is now evidence that a more sensitive set-up limit of 10^{-6} is more relevant at clinical level: in fact, Paiva *et al.* using MFC [22] and Perrot *et al.* using NGS [27] showed that patients achieving MRD negativity at the level of 10^{-6} have longer PFS periods in comparison with those that are MRD negative at 10^{-5}. Future studies will evaluate whether ultra-sensitive techniques with a limit detection in the order of 10^{-7} may further improve the prognostic predictive capacity of MRD.

In spite of the consistent improvements of the sensitivity of MRD assays and the clear clinical impact of achieving MRD negativity at 10^{-6}, disease relapses still occur in a significant proportion of patients. Thus, Paiva *et al.* using NGF reported that 7% of patients with MRD negative status at 10^{-6} displayed disease relapse after a median follow-up period of 40 months post-consolidation therapy; Perrot *et al.* showed that 29% of MM patients with MRD negativity by NGS at 10^{-6} after a follow-up of 38-55 months after randomization [27]. Interestingly, the analysis of MM patients participating to the CASSIPOETH study showed a 61.9% concordance between MRD negativity and PET-CT radioimaging post-consolidation: 6.8% of all patients displayed PET-CT positivity with a MRD negativity [32]. This finding implies the necessity of evaluating treatment responses by both MRD assays and functional radioimaging, particularly in patients with extramedullary disease [32]. Other studies confirmed the need of combining PET-CT radioimaging with MRD assay to provide an accurate prognostic evaluation of these patients [33]. The problem of disease relapse in patients with a MRD-negative status after ASCT is specifically under evaluation in the ongoing REMNANT clinical study, proposing to compare the treatment of these patients either just after MRD positivization or after disease progression [34].

It is important to identify therapies and regimens that drive sustained MRD negativity and can improve long-term outcomes. A sustained negativity of MRD may be operationally defined as a negative MRD status confirmed for one or more than one year. The detection of a sustained MRD negativity is of fundamental importance in clinical studies in newly diagnosed MM patients not eligible for ASCT and in patients with refractory/relapsing disease. The introduction in therapy of the anti-CD38 monoclonal antibody Duratumumab, in association with standard of care drug combinations, allowed in a part of patients sustained clinical responses. Recently, Avet-Loiseau reported the results on the long-term evaluation of MRD status in the POLLUX and in the CASTOR clinical trials involving the treatment of refractory/relapsing MM patients with Daratumumab/Lenalidomide/Dexamathasone and Duratumumab/Bortezomib/Dexamethasone, respectively [35]. After a follow-up of more than 50 months, the MRD negativity status was 32.5% in the POLLUX trial and 15.1% in the CASTOR trial; in these two studies, patients who achieved a MRD negative condition displayed improved PFS compared with patients who achieved MRD negative status but did not maintain MRD durability [35]. These observations support the view that achieving sustained MRD negativity predicts long-term outcomes in refractory-relapsing MM patients [35].

MM treatment has considerably changed and improved during the last two decades. The current par-

Table 1
Sentivity of the various techniques that can be used to detect the presence of multiple myeloma or acute myeloid leukemia cells

Technique	Multiple myeloma	Acute myeloid leukemia
Morphology	$1\text{-}5x10^{-2}$	$1\text{-}5x10^{-2}$
Cytogenetics	$1\text{-}5x10^{-2}$	$1\text{-}5x10^{-2}$
FISH	$1x10^{-2}$	$1x10^{-2}$
MFC	$1x10^{-4}\text{-}2x10^{-6}$	$1x10^{-4}\text{-}1x10^{-5}$
RT-PCR	$1x10^{-5}\text{-}1x10^{-6}$	$1x10^{-3}\text{-}1x10^{-6}$
NGS	$1x10^{-5}\text{-}1x10^{-6}$	$1x10^{-3}\text{-}1x10^{-5}$

adigm for transplant-eligible newly diagnosed MM patients implies chemotherapy induction treatments [VRd (Bortezomib, Lenalidomide, Dexamethasone) or DuraVTD (Duratumumab, Bortezomid, Thalidomide, Dexamethasone)], induction of stem cell mobilization and autologous stem cell transplantation [ASCT), followed by consolidation and maintenance [36]. Among patients achieving a CR, patients exhibiting a MRD-positive status were associated with a reduced PFS and OS compared to those with a MRD-negative condition and outcomes similar to those observed in patients exhibiting a partial response [8].

The study of MRD status in MM patients eligible for ASCTC may provide important information at various stages of the whole treatment procedure. The evaluation of MRD status may help to define the optimal chemotherapy induction regimen preceding ASCT; thus, the results of IFM/DFC/2009 study have provided that MM patients undergoing upfront ASCT after 3 cycle of RVd induction displayed a better response compared to the patients undergoing delayed ASCT after 8 cycles of RVd induction; however, in spite this finding, patients achieving a MRD negativity at $\leq 10^{-6}$ in both arms of the study showed a similar OS, thus, suggesting that early ASCT did not provide additional benefit in cases achieving a MRD negativity status [37]. The study by Paiva *et al.* [22] provided evidence that MM patients achieving a MRD-negativity either before or after ASCT display a similar OS. The FORTE trial compared various induction pre-transplantation regimens (KRd 4 cycles, KRd 12 cycles, KCD) and two maintenance regimens (KR *vs* R) [34]. The results of this study showed that: KR maintenance induced a higher rate of conversion from MRD-positivity to MRD-negativity; the outcomes of patients that were MRD-negative at 10^{-5} by MFC and NGS were similar; MRD-negative patients receiving 4d KRd-ASCT exhibited a longer PFS than patients receiving 12d KRd-ASCT; KR compared to R in the maintenance regimen significantly prolonged PFS in patients achieving a MRD-negative condition before maintenance [38]. The analysis of a large phase III clinical trial (EMN02/H095 MM) showed that MRD negativity was associated with reduced risk of disease progression or disease-related death in all subgroups treated, including also patients at high-risk; in the 1-year MRD maintenance population, 42% of patients MRD-positive at pre-maintenance became MRD-negative after lenalidomide treatment [39].

The current standard of care for MM patients not eligible for ASCT implies three therapeutic options based on three different regimens: VRd, DaraRd (Daratumumab, Lenalidomide, Dexamethasone) or DaraVMP (Duratumumab, Bortezomib, Melphalan, Prednisone). In these MM patients ineligible for transplantation the introduction of the anti-CD38 monoclonal antibody Duratumumab elicited a clear benefit in terms of reduced risk of disease progression or death. In these patients, durable MRD negativity (i.e., lasting for at least 12 months) was associated with improved PFS and clinical outcomes [40]. A similar conclusion was reached in the MANHATTAN clinical study carried out to assess the safety and effectiveness of Duratumumab, Carfilzomib, Lenalidomide and Deaxamethasone combination therapy for newly diagnosed MM patients, in the absence of high-dose melphalan chemotherapy and ASCT [41]. In this nonrandomized clinical study, the primary endpoint consisted in the achievement of MRD negativity, an objective reached in 29 of 41 patients, with a median time to MRD negativity corresponding to 6 cycles [41].

MRD is currently evaluated by NGS or by NFC in bone marrow and, therefore, it is not surprising that it is influenced by the quality of bone marrow samples. Bone marrow tissue may be obtained either by needle aspiration or by biopsy; bone marrow biopsy is better than bone marrow aspiration because it allows to obtain a higher number of tumor cells, less diluted by blood than bone marrow aspirates [42, 43]. Ideally, bone marrow aspirates should be obtained at multiple sites [42, 43]. To ensure a better detection of tumor cells some studies have used the immune magnetic $CD138^+$ cells enrichment, a procedure commonly used in MM patients for baseline FISH analysis to obtain a concentrated source of neoplastic cells [42, 43].

Another important problem is related to the definition of optimal time points of MRD detection. These time points have not been yet standardized. In this context, most information derives from clinical trials carried out in transplantation-eligible MM patients, where MRD was evaluated after high-dose melphalan therapy and ASCT. Since MRD is a measure of the tumor cells present after the end of the therapeutic effects of a treatment, it is evident that the optimal time for MRD evaluation at the level of bone marrow during the course of treatment is directly related to the dynamics of response to a particular therapeutic regimen [44].

MRD IN THE PROGNOSIS AND TREATMENT ASSESSMENT OF AMLs

AMLs are a heterogeneous group of hematological malignancies, characterized by a complexity of molecular alterations and clonal development. In the last years, considerable progresses have been made in the characterization of the molecular abnormalities underlying AMLs, with the identification of recurrent chromosomal alterations and of gene mutations, allowing the classification of these leukemias in various subgroups,

characterized by different genetic alterations and response to current treatments [45, 46]. This molecular classification identified some major molecular subtypes: i) AMLs characterized by peculiar translocation events leading to the formation of fusion genes and correspondent fusion proteins, including inv(6), t(15;17), t(8;21), inv(3); ii) AMLs exhibiting chromatin-spliceosome gene abnormalities, including mutations of genes involved in RNA splicing, chromatin and transcription; iii) AMLs characterized by *TP53* mutations, complex karyotype alterations and copy-number alterations; iv) AMLs displaying mutations of the nucleophosmin 1 (NPM1) gene; v) AMLs characterized by double CEBPA mutation [45, 46]. The genes most frequently mutated in AMLs are represented by: mutations of the tyrosine kinase membrane receptor Flt3, more frequently (about 30% of adult AMLs) with Flt3-Internal Tandem Duplication (FLT3-ITD) and less frequently (about 10%) with FLT3-Tyrosine Kinase Domain (FLT3-TKD) mutations; mutations of the *NPM1* gene observed in 30-35% of cases; mutations of the methyltransferase *DNMT3A* gene (20-30% of AMLs); *NRAS* (15-20% of cases); mutations of the transcription factor *RUNX1* (15% of AMLs); the methylcytosine dioxygenase 2 *TET2* gene (15-20% of AMLs); the isocitrate dehydrogenase 2 (*IDH2*) gene (10-15% of AMLs) and *IDH1* gene (5-10%) [46]. The identification of genetic abnormalities in AMLs was of fundamental importance for the understanding of leukemia pathogenesis, for the identification of new therapeutic targets and for the identification of biomarkers suitable to monitor the response to anti-leukemia therapy [47].

According to various molecular criteria the European Leukemia Net stratified AMLs into three risk subgroups, with favorable prognosis (comprising t(15;17), t(8;21), inv(6), biallelic mutated *CEBPA* and *NPM1* mutant without *FLT3-ITD*), intermediate prognosis (encompassing *NPM1* mutant with *FLT3-ITD*^{low}, t(9;21) and various cytogenetic abnormalities not classified as favorable or adverse) and adverse prognosis (comprising monosomy 7 and 5, deletion of long arm (q) chromosome 7, abnormalities of 3q, 17p and 11q, multiple cytogenetic abnormalities, *NPM1* wt and *FLT3-ITD*^{high}, *TP53* mutations associated with complex karyotype, *ASXL1* mutations, t(6;9) and t(3;3) groups [48]. Prognostic stratification of AML patients at diagnosis had strong clinical implications in that it allows to allocate after remission patients with high-risk and intermediate-risk AMLs to allogeneic stem cell transplantation. The standard therapy for adult AML patients involves treatment with induction chemotherapy with cytarabine and an anthracycline; the majority of patients achieve a morphological remission with this treatment, but their prognosis remains poor in that more than 50% of these patients relapse [49, 50].

For a long time, the evaluation of "complete response" (CR) to therapy was based on the morphological evaluation of bone marrow and a threshold of 5% or less was required for assessment of CR [50] (*Table 1*). Progresses in the techniques of multicolor flow-cytometry allowing the identification of leukemia-associated immune phenotypes and in the molecular detection of leukemia-specific molecular alterations by quantitative PCR, next generation sequencing and digital droplet PCR have allowed the detection of MRD, a measure of response to therapy much more stringent than CR [51].

A consensus document from the European Leukemia Network MRD working party reported the key clinical and scientific issues in the measurement and application of MRD in AML, stressing the need of a qualitative and quantitative standardization of the flow cytometry and molecular protocols used to evaluate MRD in AML [50] For flow cytometry blood evaluation of MRD a value of 0.1% as the threshold to distinguish MRD-positive from MRD-negative patients was recommended [52].

Voso *et al.* have analyzed the applications, the advantages and the limitations of molecular methods used for AML MRD detection [51]. Quantitative RQ-PCR methods are sensitive and specific and are used in the detection of the fusion genes observed in some AMLs and NPM1-mutant AMLs; NGS offers the advantage to be potentially applicable to all leukemic patients and detect the potential of combined mutations assessment

Table 2
Possible clinical use of MRD evaluation during the clinical course of multiple myeloma

Clinical phase	Potential clinical utility
Patients with newly diagnosed MM eligible for ASCT	Evaluation of MRD status at various times after ASCT
Transplant-inelegible newly diagnosed MM patients	Evaluation of the percentage of patients with post-therapy and sustained MRD negativity during and after maintenance therapy
Elderly frail MM patients with newly diagnosed disease not-eligible for high-dose chemotherapy or ASCT	Evaluation of MRD negativity rate following various treatments
Previously diagnosed patients with MM on lenalidomide maintenance post SCT	Evaluation of the conversion rate to MRD negativity
Relapsed/refractory MM patients treated with multiple lines of therapy	MRD negativity before and after experimental treatments
Relapsed MM patients previously treated with ASCT	Evaluation of MRD negativity rate at various time points after experimental treatments
Patients with relapsed and lenalidomide refractory MM	Evaluation of MRD negativity ratre in patients who achieved CR with experimental treatments

for MRD evaluation; digital droplet PCR is a technique based on amplification of target genes without a reference standard curve, providing an absolute quantification: this technique is highly sensitive and specific and is increasingly used for MRD evaluation [51].

Several studies have strongly supported the clinical utility of MRD detection in AMLs (*Table 3*). In this context, particularly instructive were two studies. Freeman *et al.* evaluated the CR and MRD status (as assessed by MFC) in a large set of adult AML patients undergoing standard induction chemotherapy: about 31% of these patients (and 42% of those with good and intermediate risk) achieved a CR/MRD-negative status and their survival was significantly longer than that observed for CR/MRD-positive patients [53].

A second fundamental study carried out by Jongen-Lavrencic *et al.* implied the analysis of 482 AML patients with targeted next generation sequencing: i) 89% of these patients displayed at least one mutation; ii) mutations persisted in 51.4% of these patients; iii) the detection of mutations associated with clonal hematopoiesis, such as mutations of *DNMT3A, TET2* and *ASXL1* genes was not correlated with disease relapse; iv) after exclusion of these clonal-related mutations the presence of a MRD positivity was clearly associated with a reduced relapse rate, relapse-free survival and overall survival compared to the presence of MDR negativity; these differences remained statistically significant in both univariate and multivariate analysis [54].

Very recently, Tsai *et al.* have investigated the prognostic impact of NGS MRD detection in a cohort of 335 *de novo* AML patients at two time points after chemotherapy: after induction chemotherapy and after the first consolidation cycle [55]. Excluding *DNMT3A, TET2* and *ASXL1* mutations, MRD was detected in 46% of patients at the first time point and in 29% at the second time point [55]. Patients with detectable NGS MRD at either time point had a higher incidence of relapse and a shorter survival; however, NGS MRD evaluation after consolidation therapy was more predictive of outcomes than the one after induction chemotherapy [55]. Thus, the evaluation of NGS MRD after first consolidation therapy can help to individually predict the clinical outcome of AML patients [55].

Other fundamental studies have shown the predictive value of outcome of MDR status measured prior to myeloablative allogeneic stem cell transplantation (ASCT): MRD negativity before ASCT was associated with a clearly better survival compared to that observed in MRD-positive patients [56, 57]. Promising targets of MRD prior to allogeneic stem cell transplantation are represented by *NPM1, FLT3-ITD* and *IDH1/IDH2* mutations [58].

AML is a very heterogeneous disease and this requires to analyze the strategy for the measurement of MRD in the different AML subsets. Here we will analyze the evidence supporting *NPM1* mutations as a suitable biomarker for evaluation of MRD status in *NPM1*-mutant AMLs.

NPM1-mutant AMLs represent about 30% of adult AML and are now recognized as a distinct entity in the 2017 World Health Organization (WHO) classification of hematopoietic neoplasms [59]. NPM1 is a multifunctional nucleolar protein with shuttling properties, delocalized in the cytoplasm following the mutations observed in AMLs, usually involving the exon 12 of the gene [60]. *NPM1* mutations are always heterozygous and frequently co-occur with other mutations. In animal models *NPM1* mutations cooperate with *DNMT3A* and *FLT3-ITD* mutations to promote leukemia development [47]. The prognostic impact of *NPM1* mutations in AML is dependent upon the co-mutation pattern and the allelic ratio of *NPM1* mutations [60]. Concerning the co-mutation, the presence of *NPM1* confers a relatively favorable prognosis in the absence of *FLT3-ITD* co-mutations; it was proposed that only *NPM1-mutant/FLT3-ITD*^{high}, but not *NPM1-mutant/FLT3-ITD*^{low} double mutants AMLs are associated with a negative prognosis, but this point remains controversial [60]. Concerning the variant allelic ratio (VAF), it was shown that *NPM1*-mutant AMLs with a high allelic ratio (≥0.44) display a shortened overall survival following standard treatment compared to *NPM1*-mutant AML with a low-allelic ratio (≤0.44) [61].

NPM1 mutations represent a good candidate for MRD evaluation for three important properties: a high frequency; the stability at relapse; the absence of clonal hematopoiesis [60]. These properties have triggered numerous studies evaluating MRD in *NPM1*-mutant AMLs. A study by Gorello *et al.* reported the development of a quantitative PCR technique for quantification of *NPM1*-mutations: this technique was both sensitive and quantitative and allowed to define the level of *NPM1* mutations remaining after therapy [62]. Alternative methods for monitoring MRD in *NPM1*-mutant AMLs are based on digital droplet PCR or NGS [63, 64].

Table 3
Possible clinical use of MRD evaluation during the clinical course of acute myeloid leukemia

Clinical phase	Potential clinical utility
After induction therapy	MRD positivity may support therapeutic choices : i) an intensifying treatment at induction therapy ; ii) an extra treatment ; iii) a targeted therapy
At disease relapse	MRD status post-salvage therapy in relapsing patients is fundamental for prognostic stratification and HSCT choice
Before stem cell transplantation	MRD status may provide a fundamental tool for risk stratification and choice of optimal consolidation therapy (consolidation chemotherapy or stem cell transplantation)
After stem cell transplantation	MRD status may provide criteria for post-transplant therapeutic choices, such as targeted therapy or any other possible therapeutic intervention

In an initial study, Kronke *et al.* have evaluated the prognostic value of MRD in a group of AML patients with *NPM1* mutation: after double consolidation therapy, patients achieving a negative MRD status by quantitative RQ-PCR displayed after 4 years a clearly better survival than patients with a positive MRD status [65].

These findings were confirmed and extended by Ivey *et al.* who have explored the persistence of *NPM1*-mutated transcripts in the blood of 346 *NPM1*-mutated AML patients after second cycle of induction chemotherapy: 15% of these patients displayed persistence of *NPM1*-mutated transcripts and exhibited a significantly shorter overall survival than patients without detectable *NPM1*-mutant transcripts in their peripheral blood [66]. Importantly, in this study in 69/70 patients *NPM1* mutations were found at the time of relapse, thus supporting the stability of these mutations during disease evolution [66].

Several studies have all supported the predictive prognostic value of pretransplant NPM1 MRD levels in outcome after allogeneic stem cell transplantation. Thus, Kaiser *at al.* have shown that pre-transplant *NPM1* MRD levels >1%, as evaluated by quantitative RQ-PCR, are an independent prognostic factor for poor survival after allogeneic SCT [67]. Thiol *et al.* showed that pre-transplant MRD for *NPM1* mutations and *FLT3-ITD* mutations, as measured by NGS, are predictive of allogeneic SCT outcome [68]. Lussana *et al.* have reported the study of 89 patients with *NPM1*-mutant AML; after two cycles the MRD status was strongly associated with patient outcome. In MRD-negative patients, post-remission consolidation with allogeneic SCT did not result in an improved survival compared to conventional chemotherapy. In MRD-positive patients, overall survival was improved in patients treated with ASCT, compared to those receiving conventional chemotherapy [69].

Dillon *et al.* have analyzed in peripheral blood and bone marrow of 107 *NPM1*-mutant AML patients undergoing ASCT after standard consolidation chemotherapy for *NPM1*-mutant content using quantitative RQ-PCR [70] Using this approach, they have stratified patients as MRD-negative with an overall survival of 83% after 4.9 years of follow-up, MRD-low (<200 copies/10^5 *ABL* gene) with an overall survival 63% and MRD-high levels with 13% of overall survival [70]. Patients with *FLT3-ITD* co-mutations had poorer outcomes [70].

In addition to allogeneic SCT, in AML patients with good- or intermediate-risk AML autologous SCT is an alternative transplantation-based therapeutic approach; the pre-transplantation status is the most important determinant for eligibility to autologous SCT [71]. A recent study reported the study of 42 AML patients with *NPM1*-mutated AML undergoing autologous SCT: determinants of patient outcome were the *NPM1* MRD status and the $CD34^+$ mobilizing capacity, in those patients MRD-negative have a much better overall survival than MRD-positive patients and low CD34 mobilizer patients have a better survival than highly mobilizer patients [72]. Interestingly, patients MRD-negative and low CD34 mobilizers have a particularly good outcome, while those MRD-positive and CD34 highly mobilizers have a dismal prognosis [72].

In recent studies MRD was used as a tool to evaluate the efficacy of new drug combinations in *NPM1*-mutant AMLs. Thus, Kapp-Schwoerer *et al.* have evaluated the impact of gemtuzumab ozogamicin on MRD (*NPM1*-mutant transcript levels) and relapse risk in a large group of *NPM1*-mutated AMLs treated in the context of AML SG 09-09 trial [73]. In this study AML patients were treated with induction therapy alone or in combination with gemtuzumab ozogamicin. The achievement of a MRD-negative status in these patients was associated with a reduced relapse rate [73]. *NPM1*-mutant transcription levels were significantly lowered in the arm of patients treated with gemtuzumab ozogamicin, resulting in a significantly reduced rate of relapses [73].

In another study, Tiong *et al.* have evaluated the capacity of treatment based on low-intensity chemotherapy and venetoclax (a Bcl2 inhibitor) to lower *NPM1*-mutant levels in AML patients either with molecular persistence or with molecular relapse/progression after standard induction chemotherapy [74]. All the five patients with molecular persistence achieved durable molecular complete remission and 6/7 patients with molecular relapse/progression achieved a switch from a MRD-positive to a MRD-negative status [74]. In the ongoing phase II PEMAZA clinical trial (NCT 03769532) it is under evaluation the combination therapy of azacitidine and pembrolizumab (anti-PD1) to *NPM1*-mutated AML patients with MRD positivity and impending hematological relapse after conventional induction chemotherapy. Therefore, this is a trial based on MRD-guided treatment in *NPM1*-mutated AML patients.

Bataller *et al.* recently reported the results of a study involving 114 *NPM1*-mutated AML patients achieving CR after induction chemotherapy; in the post-remission phase, patients exhibiting molecular failure (33/114) or hematological relapse (13/114) were treated with MRD-based pre-emptive intervention: two-years OS of patients with molecular failure 86% and of patients with hematological relapse was 42% [75]. These authors showed also that quantitative NPM1 detection was predictive of leukemia-free survival (LFS): patients with an MRD ratio *NPM1*mut/ABL1=<0.05 displayed a two-year LFS of 77%, compared to a LFS of 40% for patients with a MRD *NPM1*mut/ABL1>0.05 [75].

Although the data concerning the *NPM1* mutational status in relapsing patients support a consistent genetic stability of these mutations, a recent study by Hollein *et al.*, based on the study of 104 relapsing *NPM1*-mutant AMLs reported that 14 of these patients relapsed with $NPM1^{WT}$ AML [76]. Several findings supported the view that *NPM1*-mutated AMLs that relapse with wild-type *NPM1* is a distinct disease compared to the rest of *NPM1*-mutated AMLs: blood counts at diagnosis were very different between patients with $NPM1^{mut}$ and $NPM1^{wt}$ relapse (30 *vs* 3×10^9/L); $NPM1^{mut}$ relapse occurred earlier than $NPM1^{wt}$ relapse (14 *vs* 43 months); *DNMT3A* mutations are more frequent in patients with $NPM1^{wt}$ relapse [76].

The difficulties to use MRD testing in AML clinical studies

There is no doubt that MRD assays have improved our ability to measure the level of response to treatment beyond the limitations of morphological analysis. When introduced in clinical trials, the various techniques of MRD detection in AML, either based on immunophenotypic or molecular parameters, may give a strong contribution by providing: a sensitive measure of effectiveness; a surrogate endpoint in these studies; a clear rationale for their use to guide treatment [77, 78]. These three objectives are of increasing complexity and require not only a high sensitivity and standardization of MRD assays to detect residual neoplastic disease, but also the capacity to predict the outcome of individual patients. Thus, concerning the first objective, it is possible to conclude that MRD assays are able to improve the definition of treatment effectiveness in AML patients.

The evaluation of treatment effectiveness by MRD assays allowed the identification of AML patients displaying MRD-positivity: these patients are considered at a high-risk of relapse. Future studies should develop specific trials aiming to identify specific treatments that could reduce the relapse risk in patients with a positive MRD test. In this context, a number of promising targets for a MRD-directed therapy have been identified and are under current investigation [58, 79].

However, the clinical potential utility of the detection of a MRD-positivity is highly variable for different AML subtypes. In fact, the clinical utility of the detection of MRD-positivity is related to two fundamental variables: the positivity of MRD assay must be precedent to the clinical relapse, giving a sufficient lapse of time for alternative therapies to try to prevent disease relapse; the availability of alternative therapies potentially effective. The first point implies the variability in the kinetics of relapse for different AML subtypes. Thus, Ommen *et al.* reported that the relapse kinetics is remarkably slower in *CBFB-MYH11* AMLs than in *RUNX1-RUNXT1*, *PML-RARA* and *NPM1*-mutated AMLs; this finding implies the need of a different timing of sampling of blood in these leukemias for MRD assay to have chances to detect MRD-positivity with a sufficient lapse of time before clinical relapse [80].

According to these observations it was suggested an individualized follow-up for different AML subtypes in remission. This individualized follow-up implies not only a different timing of sampling but also a different frequency of sampling in different AML subsets [81]. Thus, the European Leukemia Net MRD work group has recommended optimal time points for MRD evaluation by PCR and MFC for different molecular targets according to evidences deriving from specific studies: thus, the most relevant time points for MRD evaluation in *PML-RARA* and *RUNX1-RUNXT1*-positive AMLs using specific PCR assay is at the end of the consolidation treatment, while in *NPM1*-mutant AMLs is after 2 cycles of chemotherapy [52, 82, 83].

In line with these conclusions, a recent study by Puckrin *et al.* evaluated whether monitoring of MRD every 3 months for two years after chemotherapy treatment could predict and prevent morphologic relapse in 114 patients with core-binding factor AMLs [71]. However, the results of this study provided evidence that MRD evaluation was able to detect impending relapse in only 25% of patients [84]. This finding implies the need to develop alternative strategies for monitoring of MRD in these patients [84]. Furthermore, other studies showed that the kinetics of relapse showed heterogeneity within molecular subgroups of AMLs, according to the co-mutation pattern: thus, AML with partial tandem duplications (PTD) within the *MLL* gene displayed a slower relapse kinetics than AMLs with *MLL* translocations; however, *MLL-PTD* showed a consistent heterogeneity in their relapse kinetics, dictated by the presence of *RUNX1* or *FLT3-ITD* mutations accelerating relapse timing [85]. Finally, targeted DNA sequencing for residual disease is clearly more informative after than during initial induction chemotherapy [86].

RUNX1-RUNXT1 transcript levels after treatment represent the best biomarker to monitor MRD in t(8;21) AMLs and are a marker to predict relapse. The combination of *KIT* mutation, the only gene with prognostic significance in t(8;21) AMLs, with MRD status improves risk stratification and treatment guidance [87].

MRD detection was introduced as a major endpoint in some recent clinical studies involving new therapeutic approaches based on immunotherapy. AML patients, compared with normal controls, display increased inhibitory coreceptor expression on CD8 cells, involving molecules such as PD1, TIM3 and LAG3 [88]. High PD1, PDL1 and PDL2 expression in AML was associated with poor overall survival [89] IN *NPM1* and *FLT3*-mutated AMLs, high PDL1 expression predicts a poor outcome [90]. These observations have supported the study of immune check inhibitors in AML patients. Anti-CTLA4 monoclonal antibody elicited a significant clinical response in 4/12 AML patients relapsing after allogeneic SCT; interestingly, all these patients displayed an extramedullary disease [91]. Monotherapy with anti-PD1 antibodies induced only modest clinical responses in AML patients [92].

To improve the response rate to anti-PD1 of AMLs, the anti-PD1 drug Nivolumab was administered in combination with induction chemotherapy or the hypomethylating agent azacitidine. In a phase II study, newly diagnosed AML patients were treated with induction chemotherapy, followed by nivolumab up to 1 year: 77% of patients achieved a CR and 53% displayed a MRD-negative status, as assessed by MFC [93]. In relapsing/refractory AML patients treated with azacitidine, complete responses were observed in 22% of cases [94]. A phase II pilot study evaluated nivolumab as maintenance therapy and not eligible for SCT; the large majority of these 14 AML patients had a MRD-positive status and 1 of these patients switched to MRD-negative status during maintenance therapy [95]. A randomized, phase II clinical trial (NCT02275533) is evaluating a maintenance therapy based on nivolumab to eliminate MRD and to prevent relapse in AML patients in CR after standard chemotherapy.

Given the capacity of azacitidine to stimulate CTLA4

expression in AML patients, another trial evaluated the association of azacitidine, nivolumab and ipilimumab (a monoclonal antibody anti-CTLA4) on refractory/relapsed AML patients [96]. 43% of 20 treated patients showed a CR with an OS at 1 year of 58%; however, this drug association was accompanied by a consistent toxicity [96]. Finally, an ongoing phase II clinical trial (NCT04214249) is evaluating whether blockade of PD1 added to standard chemotherapy is able to target MRD in AML patients; this randomized study treatment with intensive chemotherapy alone or in association with anti-PD1 Pembrolizumab as frontline therapy in AML patients.

One of the most relevant and potentially useful contributions of MRD detection in AML patients would consist in providing a guide for SCT and for the type of SCT in AML patients achieving a CR status after consolidation therapy. Several studies have explored this topic. In a retrospective study, Versluis *et al.* reported the results on 547 AML patients achieving a CR after consolidation therapy and all explored for MRD status by MFC before post-remission therapy: 52% received allo-SCT, 19% auto-SCT and 29% a third cycle of chemotherapy [84]. 19% of these patients were MRD-positive after induction chemotherapy and their OS was poorer after post-remission therapies compared to that of patients with an initial MRD-negative condition [97]. Importantly, allo-SCT significantly reduced the rate of relapse compared with chemotherapy or auto-SCT, an effect similarly observed in MRD-negative and MRD-positive patients [97].

Recently, the results of a prospective study, GIMEMA AML 1310 trial of risk-adapted, MRD-directed therapy for young AML patients were reported. This trial involved the treatment of AML patients with favorable-risk after consolidation therapies with auto-SCT, of AML patients with poor-risk AML with allo-SCT and of AML patients with intermediate-risk AML received either auto-SCT or allo-SCT depending on the post-consolidation levels of MRD [98]. This study involved the analysis of MRD by MFC in 342 AML patients achieving a CR post-consolidation therapy. Two-year OS in the favorable-risk group was 74% and, in the poor-risk group was 42%; in the intermediate-risk AMLs, OS was 79% in the MRD-negative group and 70% in the MRD-positive group [85]. The absence of a significative difference in OS among intermediate-risk AMLs receiving auto-SCT and intermediate-risk AMLs receiving allo-SCT, supports the view that MRD status is a valuable biomarker for risk-stratification of this group of AML patients [98].

The decision to recommend or not a SCT to an AML patient in first remission remains a complex choice. This choice is particularly challenging for AML patients in first remission with a MRD-negative status and is related to the decision to transplant or not, to the type of SCT, auto-SCT or allo-SCT, and to the type of conditioning regimen, myeloablative conditioning (MAC) or reduced-intensity conditioning (RIC). This choice cannot be guided only by the MRD status but must consider criteria related to the risk category of the AML subtype and to several patient-specific clinical features.

Retrospective analysis performed on a very large set of AML patients suggested that allo-SCT with myeloablative conditioning regimens should be the preferred choice for MRD-positive patients and that MRD-negative patients should be treated with transplantation procedures involving reduced-intensity conditioning regimens, avoiding the toxicities of the myeloablative conditioning regimens [99].

The role of conditioning regimen was explored in a recent phase III study in a group of AML patients in morphologic remission after induction chemotherapy, explored by ultradeep NGS sequencing for 13 commonly mutated genes in AML and randomly assigned to allo-SCT after MAC or RIC [87]. In patients with no mutations, the OS of patients undergoing either MAC or RIC was similar; however, in patients MRD-positive MAC compared to RIC resulted in a reduced relapse rate (19% *vs* 67%) and survival (after 3-years, 61% *vs* 43%) [100]. The results of this study supported the view that MAC rather than RIC in MRD-positive AML patients before allo-SCT resulted in an improved survival.

The choice at the level of individual AML patients cannot be based only on the MRD status as a predictor of relapse risk but must be based on a number of covariates, including white blood cell counts at diagnosis, number of chemotherapy cycles to achieve first remission, cytogenetic and mutation profiles and global risk evaluation according to ELN [101]. This type of approach is strongly justified by the observation that the accuracy and precision of MRD in predicting outcomes of therapy in AML is limited and must be carefully evaluated using standardized methods and using more than one technique (i.e., MFC and RQ-PCR) [101]. Furthermore, in clinical studies MRD results are reduced in terms of negative and positive, where the positivity may correspond from few to many AML cells. Finally, the impact of MRD-testing in the context of SCT must be evaluated in prospective studies.

It is important to note that a recent systematic review and meta-analysis based on 81 studies involving a total of 11,151 AML patients provided evidence that the estimated 5-year disease-free survival was 64% for patients with a negative MRD status, compared to 25% for those with a positive MRD status; the estimated overall survival was 68% for patients without MRD and 34% for those with MRD [102]. The findings of this meta-analysis support the view that achievement of MRD negativity represents a fundamental therapeutic objective and is associated with a better disease-free survival and overall survival in patients with AML. These observations also support the evaluation of MRD status as a fundamental end-point for evaluation of new drugs or treatments for the therapy of AMLs.

In conclusion, the studies on MRD have shown a clear association between MRD positivity and adverse outcomes, thus supporting the role of MRD as a routine biomarker in both current clinical practice and clinical trials [103]. The use of MRD as a surrogate efficacy-response biomarker is a potentially important strategy to accelerate drug development/approval [103]. The assessment of MDR after induction inten-

sive chemotherapy represents an important prognostic factor for risk stratification of patients and for guiding therapeutic choices in some AML subsets, such as intermediate-risk patients: MRD-negative patients are selected to receive autologous stem cell transplantation, whereas MRD-positive patients are selected for allogeneic stem cell transplantation. Future studies will be required to demonstrate whether treatment effects on MRD, such as the timing of therapeutic intervention with respect to MRD assessment (at the moment of MRD detection or at overt disease recurrence) may improve outcomes.

CONCLUSION

In conclusion, the studies in MM and AML patients strongly support the clinical utility of MRD detection as a tool to obtain an evaluation on the quality of response to treatment. However, MRD assessment is challenging and requires MRD assay optimization and standardization. Different sensitive techniques for MRD assessment are currently evaluated and a combination of different techniques seems to provide the accurate results. There is no doubt in these pathological conditions that patients in histological complete remission with an MRD-test-negative have better outcomes than those with an MRD-test-positive. These results have justified the inclusion of MRD evaluation in clinical trials involving new therapeutic approaches in MM and AML. However, it is evident that when MRD role in clinical studies moves from a passive role (i.e., a measure of the extent of treatment effectiveness) to an active role (i.e., a tool to guide treatment choices), a careful standardization, a consistent sensitivity and reproducibility of MRD assays are strictly required.

Conflict of interest statement

The Authors declare no conflict of interest.

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