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REVIEW

Molecular Monitoring as a Path to Cure Acute Promyelocytic Leukemia

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ABSTRACT

Acute promyelocytic leukemia (APL) is a molecularly well-defined disease, characterized by a specific chromosomal translocation; the improvement in biologic and clinical knowledge and subsequent introduction of molecularly targeted therapies have transformed the management of APL, with survival rates now exceeding 80%. Minimal residual disease (MRD) assessment in APL is the most important tool for its treatment; the prognostic role of the molecular detection of promyelocytic leukemia retinoic acid receptor α (PML-RARα) transcript after consolidation therapy in the early identification of the following hematologic relapse is now well established and guides preemptive therapy. First experiences performed with a qualitative polymerase chain reaction (PCR) approach were replaced with more accurate real-time quantitative PCR (RQ-PCR), which guarantees a numeric quantification of MRD. The identification of arsenic trioxide (ATO) as a valid therapy not only in relapsed patients but also as an alternative to standard therapy alone or in association with all-trans-retinoic acid enlarges the setting of validation of MRD evaluation in APL patients, considering a possible different clearance of PML-RARα with innovative therapy different from the standard ones. MRD monitoring demonstrated its validity also in the setting of relapsed patients with interesting results in the autologous and allogeneic stem cell transplantation setting or with the use of other biological agents. The aim of this review is to report and discuss the actual state of the art of MRD in APL.

Keywords: Acute promyelocytic leukemia; All-trans-retinoic acid; Arsenic trioxide; Minimal residual disease; Molecular monitoring; Quantitative real-time polymerase chain reaction

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INTRODUCTION

Acute promyelocytic leukemia (APL) is a molecularly well-defined disease, characterized

by the presence of a specific chromosomal translocation—t(15;17) (q22;q21), which leads to the aberrant expression of the promyelocytic leukemia retinoic acid receptor α (PML-RARα) fusion gene (Fig. 1) [1]. In about 2% of cases, other aberrant translocations, such as t(11;17)(q23;q21), t(11;17)(q13;q21) and t(5;17)(q35;q21), and from an interstitial deletion event on chromosome 17 can involve fusions between retinoic acid receptor α (RARα) and other partner genes, such as the promyelocytic leukemia zinc finger (PLZF), nuclear mitotic apparatus protein (NuMA), nucleophosmin, and signal transducer and activator of transcription 5b (STAT5b) [2–6]. The aberrant expression of PML-RARα led to the exaltations of oncogenic signaling and consequently to the neoplastic transformation of myeloid cells [7].

An exquisite sensitivity to anthracyclines by APL blasts was shown for the first time by Jean Bernard in 1973, but those first encouraging results in APL treatment were impaired by the persistent and so far unresolved problem of early deaths and absence of valid alternative therapeutic strategies in case of relapse or refractory disease [8]. The evidence that all-trans-retinoic acid (ATRA) is highly effective in the induction of blast differentiation and its subsequent use in clinical practice induced a dramatic improvement in the outcome of APL, especially when associated with standard chemotherapy [9–11]. Further studies have widened the molecular knowledge around APL pathogenesis and have also demonstrated that arsenic trioxide (ATO) exhibits a significant antileukemic effect in relapsed and low-/intermediate-risk newly diagnosed patients [12, 13, 15]. It is of paramount importance that the baseline correctly identifies the fusion partner gene, as it is crucial to define the eventual sensitivity to molecularly targeted therapy: in fact, it has been demonstrated that morphological cases such as APL associated with PLZF and STAT5b are resistant to ATRA, while ATO activity is restricted only in cases of PML-RARα alteration [2, 14].

Recently, a cooperative German-Italian randomized trial demonstrated that the chemotherapy-free approach with ATRA plus ATO is superior to the association of ATRA plus standard chemotherapy in low-intermediate-risk APL [15]. New therapeutic strategies and refinement of molecular standardization have

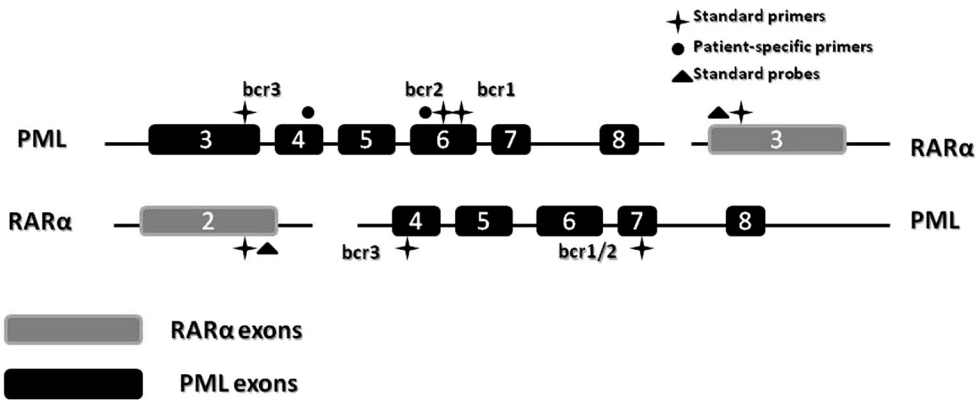

Fig. 1 Schematic representation of the PML/RARalpha hybrid with distinct isoforms. *PML* promyelocytic leukemia, *RARα* retinoic acid receptor α

transformed APL from an aggressive fatal disease into one of the most curable neoplasms; indeed, a low percentage of patients (10–15%) failed to obtain a durable remission, and few patients were refractory to initial therapy [16]. To prevent morphologic relapse, potentially fatal because it is associated with concomitant coagulopathy, minimal residual disease (MRD) monitoring has been successfully standardized for the early identification of relapse [17].

Molecular methodologies have deeply changed the diagnostic and therapeutic approach to acute leukemias; during the last 3 decades diagnosis and follow-up criteria have evolved from simple morphologic evaluation to highly sensitive molecular methods. The standard reverse transcriptase polymerase chain reaction (RT-PCR) represents an important methodology for diagnosis assessment, but it was demonstrated to be less suitable for MRD monitoring during follow-up, because it was only informative about the disease status (positive or negative) and provided no real quantification of MRD.

The introduction of real-time quantitative PCR (RQ-PCR) overcame the limits of previous methodologies with sensitive and accurate quantification of gene expression. Parallel amplification of the target gene and one or more control genes represented a crucial innovation. The amplification of a control gene, mostly represented by the Abelson gene, avoided false-negative results related to suboptimal amplification of PCR results [18].

The aim of this review is to highlight the principal indications and new insights in MRD monitoring in APL and to overview the rationale of MRD-based preemptive therapy. This article is based on previously conducted studies and does not involve any new studies of human or animal subjects performed by any of the authors.

MINIMAL RESIDUAL DISEASE MONITORING IN NEWLY DIAGNOSED PATIENTS TREATED WITH STANDARD REGIMENS

A specific molecular hallmark in APL and the possibility to monitor the MRD with accurate and standardized RT-PCR allow strict molecular monitoring during the frontline treatment and follow-up phase.

Cooperative groups in Italy [Gruppo Italiano Malattie EMatologiche dell'Adulto (GIMEMA)] and Spain [Programa para el Tratamiento de Hemopatias Malignas (PETHEMA)] have largely contributed to the correct definition of the standard of care in APL. In 1997, the GIMEMA group introduced a combination of ATRA and anthracycline as first-line induction therapy (AIDA trial), followed by three cycles of standard chemotherapy consolidation; after the third consolidation, a qualitative evaluation of MRD showed the absence of the PML/RARα transcript in 98% of patients. This protocol guaranteed event-free survival (EFS) rates of 83% at 1 year and 79% at 2 years [19]. The PETHEMA group introduced a similar trial slightly different from AIDA by the absence of cytarabine and etoposide in consolidation. RT-PCR MRD evaluation was performed at the end of induction treatment and consolidation, with a complete molecular remission rate of 51% and 93%, respectively. In this trial similar overall survival (OS) (82%) and EFS (79%) compared to the AIDA protocol were reported: the authors concluded that cytarabine and etoposide have a minor role in the treatment of newly diagnosed APL [20].

Fenaux et al. investigated the role of maintenance therapy in APL patients [9]. Patients in complete remission (CR) were randomized to receive 2-year maintenance therapy with continuous low-dose chemotherapy (6-mercaptopurine, methotrexate), intermittent ATRA (15 days every 3 months), a combination of both agents or no maintenance therapy. The authors concluded that a maintenance therapy combining chemotherapy and intermittent ATRA reduced the incidence of relapse in APL patients [9].

Although the association of ATRA and anthracyclines guaranteed high response rates, a minority of patients did not reach CR or early relapse. A predictive model was introduced to identify risk of relapse earlier. Three risk categories were proposed considering the clinical characteristics at diagnosis [high risk: white blood cell count (WBC) $\geq 10 \times 10^9$/l; intermediate risk: WBC $\leq 10 \times 10^9$/l and platelet count $\leq 40 \times 10^9$/l; low risk: WBC $\leq 10 \times 10^9$/l and platelet count $\geq 40 \times 10^9$/l] [21], and consolidation therapy was adapted to reduce the toxicity and rate of mortality in CR. It should be mentioned that, at the present time, the prognostic relevance based on platelet levels and the WBC assumes a minor role and is not yet considered strictly prognostic with the possible exception of the WBC. At the same time, a specific correlation between MRD positivity and the subsequent risk of clinical relapse has been reported [17, 22–27]; in particular, it has become clear that the assessment at the end of consolidation is the more appropriate point for performing MRD evaluation compared to the end of induction. Otherwise, molecular characterization of morphologic-resembling APL with an atypical transcript clarifies the correlation between the molecular pattern and clinical resistance to treatment [2–6].

Considering the prognostic value of molecular monitoring for the early identification of relapse, in 1999 the GIMEMA group showed for the first time that molecularly guided preemptive therapy confers an advantage compared to patients treated at the time of hematological overt relapse; they reported cumulative 2-year progression-free survival (PFS) of 85% in patients preemptively treated based on molecular relapse, which was statistically significant when compared with the results from previous series in which treatment was initiated in the presence of hematological relapse (2-year PFS: 44%). Molecular relapse was considered as the reappearance of a positive RT-PCR (sensitivity 10^{-4}) in two consecutive tests performed on bone marrow (BM). The small number of patients and absence of quantitative evaluation of PML-RARα did not allow stratification of the risk of relapse [28]. In 2007, the PETHEMA group also confirmed that salvage therapy in the presence of molecular relapse guaranteed a better outcome compared to treatment at the time of hematological relapse [29] (Table 1). Also in this study MRD was assessed with qualitative RT-PCR; it is remarkable that the RT-PCR methods used in those studies showed an inferior sensitivity (10^{-4}) of almost two logs compared to other techniques used for other types of leukemia in the same period. This difference is largely attributable to the intrinsically lower sensitivity of RT-PCR for the PML/RARα transcript in respect to other AML genes, such as BRC-ABL [30] or AML/ETO [31]. This inferior sensitivity can explain the high probability of subsequent hematological relapse demonstrated in different experiences [27, 32]. Conversely, when more sensitive MRD detection for PML-RARa methods is used (10^{-6}), a clear correlation between molecular and hematological relapse became less clear.

Table 1 Summary of molecular monitoring reported in clinical trials

Reference	Number of evaluated patients	MRD status at the end of the third consolidation (no. of patients)	Relapse rate on the basis of MRD status	Efficacy of preemptive therapy for obtaining mCR
Lo-Coco et al. [17]	35/35 (100%)	Positive: 13	11/13 (84.6%)	Not assessed
		Negative: 22	0/22 (0%)	
Miller et al. [22]	32/32 (100%)	Positive:13	13/13 (100%)	Not assessed
		Negative: 19	3/19 (15%)	
Huang et al. [23]	62/97 (64%)	Positive: 11	5/11 (45%)	Not assessed
		Negative: 51	0/51 (0%)	
Fukutani et al. [24]	27/27 (100%)	Positive: 13	10/13 (77%)	Not assessed
		Negative: 14	0/14 (0%)	
Burnett et al. [26]	76/239 (32%)	Positive: 7	4/7 (57%)	Not assessed
		Negative: 69	39/69 (27%)	
Diverio et al. [27]	163/163 (100%)	Positive: 21	20/21 (95%)	Not assessed
		Negative: 142	8/142 (6%)	
Lo-Coco et al. [28]	14/253 (5%)	14 patients with positive MRD selected from AIDA trials	–	CR: 12/14 (85%)
Esteve et al. [29]	16/549 (3%)	16 patients with positive MRD selected from LPA96 and LPA99 trials	–	CR: 14/16 (87%)

APL acute promyelocytic leukemia, *CR* complete remission, *mCR* molecular complete remission, *MRD* minimal residual disease

Tobal et al. showed that some APL patients in long-term remission may show RT-PCR MRD positivity without ever experiencing a further hematological relapse [33].

In order to overcome limitation bias related to qualitative PCR, standardized quantification of the PML/RARα copy number based on RQ-PCR has become the new alternative for MRD monitoring. Also with RQ-PCR, Santamaria et al. confirmed the correlation between high levels of normalized copy numbers of the PML-RARα transcript and risk of relapse when it was evaluated at the end of consolidation (high risk of relapse when the normalized copy number is >10, persistence in hematological remission when the value is <1) [34]. These data confirmed that the prognostic relevance of MRD became effective when it was identified after consolidation therapy, and it is concordant with what was demonstrated in previous experiences [17, 32, 35]. RQ-PCR offers several advantages compared to the qualitative method: Flora et al. [36] demonstrated that RQ-PCR enhanced sensitivity and reduced the risk of sample contamination. Moreover, it allowed performing quality control of the process by the quantification of an independent control

gene amplification (ABL gene), avoiding obtaining a false negativity related to a suboptimal amplification of the PCR result. Finally, the quantification of the gene transcript allowed evaluating the increase of the MRD over time, which is essential for making the correct treatment decision [37, 38].

The validation of the novel RQ-PCR approach came from a large UK study that analyzed samples from 406 patients receiving ATRA and anthracycline-based therapy, most of them enrolled in the AML 15 trial [39]. A total of 6727 BM and peripheral blood (PB) samples were analyzed. With the higher sensitivity of RQ-PCR, 95% of patients achieved complete molecular remission at the end of consolidation; patients with a positive RQ-PCR were preemptively treated with ATO. Quantitative MRD assessment in BM was shown to be the most powerful predictor of relapse-free survival (RFS) in multivariable analysis [hazard ratio 17.87; 95% confidence interval (CI) 6.88–46.41], superior to the presenting WBC (hazard ratio 1.02; 95% CI 1.00–1.03). Also in this article the evaluation of MRD in PB was not significant because of the lack of sensitivity, which limits the opportunity to use PB for monitoring [40].

Regarding the management of high-risk patients, a risk-tailored treatment has been established; high-risk patients benefit from a more intensive consolidation therapy. Data from two PETHEMA trials (LPA96 and LPA99) showed that an increased dose of anthracyclines enhanced the antileukemic efficacy [41]. Although risk stratification was performed using clinical parameters, it is of interest that six out of seven MRD-positive patients were high risk, and a linear correlation between clinical and molecular parameters has been shown [43]. The GIMEMA trial AIDA-2000 confirmed the validity of a risk-tailored therapy and demonstrated that the introduction of cytarabine in consolidation had a favorable role [44].

MINIMAL RESIDUAL DISEASE MONITORING IN NEWLY DIAGNOSED PATIENTS TREATED WITH ATO IN FRONTLINE THERAPY

The efficacy of ATO in relapsed/refractory APL is well defined; during the last years, the possibility of its use in the first-line therapy was assessed, and this new approach opens a new scenario in the molecular monitoring of APL. It has been demonstrated that molecular clearance of APL blast cells using ATO as induction therapy is different compared to ATRA alone or ATRA plus chemotherapy regimens [43].

The variability in PML-RARα clearance between ATRA and ATO is probably influenced by the different mechanism of action of the two drugs on APL blast: while ATRA barely promotes blast differentiation, ATO at high concentration ($1–2 \times 10^{-6}$ M) induces apoptosis, mainly by activating the mitochondria-mediated intrinsic apoptotic pathway. Indeed, ATO at low concentrations ($0.25–0.5 \times 10^{-6}$ M) and with a longer treatment course promotes the differentiation of APL cells [44].

For this reason, data regarding MRD assessment derived from conventional treatment protocols may not be applicable in this particular setting.

First evidence of ATO efficacy in newly diagnosed patients came from developing countries where standard regimens are associated with significant economic costs that make them unaffordable. In 2006, an Iranian group published their experience with 111

patients with both newly diagnosed and relapsed APL patients who received ATO in monotherapy. Induction was performed with ATO at 0.15 mg/kg/day until hematological remission, followed by consolidation with the same schedule for 28 total infusions. The authors reported a 1- and 2-year disease-free survival (DFS) of 88.3% and 63.7%, respectively; in patients with relapsed disease, 19/24 (79%) obtained a second remission. MRD monitoring was performed with a semi-sensitive reverse transcription method on PB after the consolidation phase and 12 months after CR [45]. Long-term results after 5 years of this trial reported a morphologic CR rate of 85.8%, while DFS was 64.4 ± 4%. MRD was performed with the same method utilized in the first trial [44]. The Indian group produced a similar trial in which induction, consolidation and maintenance were performed with ATO as a single agent: in 2006 they reported an interim analysis in which hematologic CR was achieved in 86.1% of patients. At a median follow-up of 25 months, the 3-year EFS, DFS and OS were 74.87%, 87.21% and 86.11%, respectively. Side effects were mild and reversible [47]. The long-term follow-up showed that the 5-year EFS, DFS and OS were 69%, 80% and 74.2%, respectively [48], and confirmed the safety profile of ATO. Chendamarai et al. published on the specific role of MRD monitoring in ATO-treated patients as frontline therapy: the evaluation was performed using a quantitative RT-PCR on the PB sample [49]. They showed in multivariate analysis that a positive RQ-PCR at the end of the induction was associated with an increased risk of relapse. RQ-PCR negativity in low-risk patients (WBC $\leq 5 \times 10^9$/l; platelet count $\geq 20 \times 10^9$/l) was predictive of subsequent relapse. After the achievement of molecular remission, the MRD monitoring strategy predicted relapse in 60% of cases, with an overall sensitivity and specificity of 60% and 93.2%, respectively. Therefore, high-risk patients and those with RQ-PCR positivity after induction benefit from serial RQ-PCR monitoring for 3 years after completion of therapy [49]. Considering these results, it can be assumed that although ATO is efficacious in inducing morphological RC, the probability of relapse seems to be higher in this group in comparison with other standard approaches. Regarding ATO's role in consolidation for APL-naïve patients, Powell et al. investigated its role in a randomized trial: after a standard induction with ATRA, daunorubicin and cytarabine, patients were randomly assigned to receive consolidation therapy with two courses of ATRA and daunorubicin or two courses of ATO. They reported a better 3-year EFS and DFS in the ATO group in comparison with standard consolidation (80% vs. 63% and 90% vs. 70%, respectively) [50].

Recently ATO was successfully tested in first-line therapy as an alternative to the standard chemotherapy approach; first experiences using ATRA in association with ATO in untreated patients were reported by Estey et al. [51] and recently updated by Ravandi et al. [52], suggesting the potential efficacy and good safety profile of this association.

All this evidence demonstrated that ATO is effective also in first-line therapy, either as induction or consolidation therapy, and not only in the setting of relapsed patients. Lo-Coco et al. demonstrated that frontline therapy with ATRA and ATO for low-/intermediate-risk patients might be superior to the standard association of ATRA plus chemotherapy, with a better safety profile. In this experience the authors also investigated whether ATO can induce a different pattern of clearance of the molecular burden; MRD evaluation with

RT-PCR was also performed after the induction phase in addition to conventional measurement at the end of the third consolidation, but no statistical differences were noted between the ATO and standard group [15]. The identification of this novel targeted therapy raises new questions about the correct management of APL: the ATRA-ATO association in frontline therapy seems to be effective, but for high-risk patients, according to Sanz [21], the use of a chemotherapeutic approach probably cannot be abandoned tout court. Future experiences may better clarify the role of ATO in first-line therapy for high-risk patients.

MRD MONITORING IN RELAPSED PATIENTS

The rationale of molecular monitoring in APL patients is to detect disease relapse early and consequently to provide a preemptive intervention; the preemptive approach has been validated and shown to be a significant advantage in the long-term outcome, even though ATO therapy has not yet been identified as the optimal therapy for relapsed patients [28, 29]. Two studies showed the predictive value of the molecular detection of relapse: Diverio et al. prospectively detected 21 positive PCRs in the entire cohort of 163 untreated patients receiving the AIDA protocol. Of them, 20/21 experienced a further hematological relapse with a short time interval (3 months, range 1–14) [27]. Similarly, Jurcic et al. demonstrated that in 7/10 cases of molecular relapse (either after the first or subsequent remission), a further hematological relapse occurred [32].

These data, associated with the evidence that most relapses occur during the first 3 years after consolidation, led to the indication in current guidelines that molecular monitoring should be performed every 3 months for the first 3 years after the end of consolidation [9, 11, 13–52]. With the increased sensitivity of last generation RQ-PCR tools, concerns about the possibility of monitoring MRD by PB were posed: although some studies proposed interesting experiences with MRD monitoring with PB, lack of validation impaired the real clinical utility of this approach. In particular, a comparative analysis between PB and BM evaluation showed the superiority of BM with an average 1.5 log sensitivity [34, 40].

The optimal treatment of relapsed APL was progressively refined together with the improvement of first-line therapy: in the AIDA and PETHEMA studies [19, 20], treatment strategies at molecular relapse were different (autologous stem cell transplantation, further chemotherapy schedules) and case tailored, and different authors have approached this problem. In the pre-ATO era, salvage therapy with ATRA conferred only a partial advantage in the outcome of patients: Lo-Coco et al. reported that early intervention when molecular relapse occurred with ATRA was efficacious (2-year OS of 92%), but suboptimal results were obtained when the same regimens were administered in overt hematological relapse (44%) [28].

The introduction of ATO as a specific therapy for relapsed APL improved the clinical outcome of relapsed patients: preliminary evidence of ATO efficacy was provided by Chinese groups in the 1990s [53]. In 1999 Niu et al. demonstrated the efficacy of ATO in APL patients (11 newly diagnosed and 47 relapsed patients). Surprisingly, ATO guaranteed 85.1% ORR, but with a 2-year DFS of 41.6%. Furthermore, a molecular assessment was performed with RT-PCR, but the high percentage of molecular

positivity (14/15) probably was largely sustained by the premature evaluation performed immediately after the achievement of the hematological response [54].

These results were subsequently confirmed by other experiences [55–57], and, consequently, ATO was approved as the standard therapy for relapsed APL [13], with reported CR rates ranging between 80–90%.

Regarding the molecular clearance of PML-RARα with ATO, recently Shen et al. [58] evaluated the clearance of PML-RARα in patients with APL with RQ-PCR: 61 patients were randomized to receive ATRA, ATO or their combination. Although CR was similar in the three groups (≥90%), the median time to achieve morphologic and molecular CR was significantly shorter in the combination arm, and this difference also lasted after consolidation. In particular, clearance of PML-RARα was higher with ATO monotherapy compared to ATRA, but only few data were available in the literature.

The optimal consolidation therapy after ATO-induced second remission is still a controversial point, and this is largely due to the restricted number of patients with resistant disease. Interesting data were reported by Lo-Coco et al. with gemtuzumab ozogamycin in the second or more advanced molecular relapse or in patients in first molecular relapse not eligible for conventional therapies. MRD evaluation was assessed after two doses at 6 mg/m^2; all patients achieved a molecular CR after the third dose with a median duration of molecular response of 15 months [59].

The best transplant procedure in relapsed/refractory APL was not identified: autologous HSCT guarantees a better safety profile but is free from any graft versus leukemia effect; furthermore, the hypothetical risk of stem cell harvest contamination may impair the use of this procedure [13]. For this reason, MRD evaluation before the stem cell harvest is a critical point and should guide the following therapeutic strategies. Meloni et al. demonstrated the need for an MRD evaluation on stem cell harvesting before autologous HSCT, because all patients who underwent this procedure with positive MRD relapsed, while none of the negative patients showed the same behavior [60].

According to the current guidelines, it seems reasonable that the choice of the allogeneic HSCT should be reserved for patients who failed to achieve a second CR or for patients with a short first molecular remission [52, 61]. The MRD monitoring also has a place during follow-up in patients who have undergone allogeneic transplantation, and it is interesting that in a paper by Lo-Coco et al. [62] the subsequent negativity of MRD was obtained without the use of ATRA or ATO, but only with the enhancement of the graft versus leukemia effect obtained by reducing immunosuppressive therapy.

CONCLUSION

Unlike other acute myeloid leukemias, biological and clinical improvements in the APL understanding have revolutionized the outcome of a traditionally fatal disease. The homogeneity of the molecular hallmark in APL patients allowed confirming the diagnosis of APL and further introducing the possibility of preemptive therapy that has demonstrated its validity in reducing mortality. The assessment of molecular remission in BM is now the standard of care in APL treatment, considering the evidence cited above, although the effect on the mortality and morbidity of sequential monitoring has not been evaluated in a randomized trial. Some authors have raised the question whether standard MRD

monitoring for low-risk patients has significance after the post-consolidation time point, considering the low risk of relapse and costs related to molecular monitoring after this time point [63]. Some considerations should be made regarding this critical point: it has to be considered that also some low-risk patients can have a slow clearance of the molecular burden and as in high-risk patients an early discontinuation of molecular monitoring can induce a higher rate of relapses. Moreover, all these considerations born from the results obtained with standard therapy, including antracyclines and ATRA, also have to be considered; the introduction of a novel treatment strategy with the association of ATRA and ATO could induce a different clearance of the molecular burden; subsequently, the time for MRD assessment could be completely different from what has been demonstrated. Burnett et al. recently published an MRD evaluation in ATRA plus ATO as frontline therapy: the results demonstrated that all patients (including high-risk patients) treated with ATRA plus ATO who achieved a molecular remission did not experience a further relapse of disease. If these data are confirmed by other experiences, it may redefine the role of MRD monitoring in this setting of patients [64].

Although the role of MRD monitoring is well established, it is limited in particular settings: about 10% of morphologic-resembling APL presented an aberrant rearrangement pattern involving other responsible genes, in some cases (PLZF-RARα and STAT5b- RARα) with a well-known insensitivity to retinoids [65]. Currently, there are virtually no data on molecular monitoring in PLZF-RARα and STAT5b-RARα-positive diseases, which have been associated with a poor prognosis, although Jovanovic et al. reported the possibility of MRD monitoring in this subset of patients and designed a specific RQ-PCR for MRD evaluation, with interesting results [66]. In conclusion, MRD monitoring remains an important tool in APL management; cooperative randomization trials could provide more information regarding the optimal MRD management, considering the advent of novel and less toxic therapeutic strategies.

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