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# Can we incorporate MRD assessment into clinical practice in AML?

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ABSTRACT

Measurable residual disease (MRD) can be assessed either by flow cytometry or molecular techniques. It has been proven to be highly prognostic in quite a number of prospective clinical studies. The recently published ELN MRD recommendations aim harmonize the approaches to MRD assessment in order to improve its overall quality. The predictive value leading to the usage as a surrogate endpoint for survival which would be instrumental for faster drug approvals has still to be proven. Nevertheless, many AML centers use MRD status to inform treatment.

## 1. Introduction

The goal of treatment in AML is the achievement of CR after which in many studies a survival advantage in intensively treated patients has been shown [1]. The same is true for low intensity treatment with Hypomethylating Agents (HMAs) [2]. The limitations of defining remission by cytomorphology are several and in comparison with immunophenotyping both false -negativity and positivity are quite often observed [3]. ELN 2017 recognizes CR without measurable residual disease (MRD) as an even better predictor of outcome in comparison to CR and suggests that this should now be the new standard [4].

## 2. Methods

The method mostly applied to assess MRD is by multiparameter flow cytometry (MPFC) either according the leukemia aberrant phenotype method (LAIP) approach, which defines LAIPs at diagnosis and can be followed along the patient journey, and the different from-normal (DfN) approach, which identifies aberrant differentiation/maturation profiles at follow-up. The DfN approach is especially useful if a diagnostic sample is not available [5].

If an optimally composed large panel of antibodies is used the differences between these two approaches are minimal. ELN advices to combine both methods to get the best out of two worlds and suggest to name it “LAIP-based DfN approach”. MPFC is applicable in over 90% of AMLs [6].

Molecular MRD assessment is now generally accepted if done by real-time PCR-based approaches aiming for sensitivities between $10^{-4}$ and $10^{-6}$ and is applicable in 40–50% of AMLs [7]. In reality only NPM1 and the CBF leukemias are used as targets for real time qPCR. Digital PCR will further increase the sensitivity of the molecular MRD assessment.

Next generation sequencing (NGS) for MRD assessment is in theory as broadly applicable as MPFC and will in the future, when methods improve the sensitivity, become an important method for MRD assessment [8].

The recently published ELN MRD recommendations aim to harmonize the approaches to MRD assessment in order to improve its

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overall quality [6]. Agreement on measurement and practical application of MRD has been achieved providing guidance for use in clinical practice. Application of molecular and/or MPFC MRD according the technical ELN guidelines for every clinical trial at all times of evaluation of response is now strongly recommended. Agreements or attempts towards agreements have been reported on antibody panels, thresholds, time points for MRD assessments, definitions for molecular remission, molecular progression, and molecular relapse, calculation and reporting of MRD burden. Still a lot of unsolved issues are identified and are currently tackled by the same group of international AML MRD experts.

## 3. Clinical studies

### 3.1. MPFC assessed MRD

Many single institute non-randomized retrospective studies generally with few patients have been published [9] Although patient population, treatment and MRD assessment varied a cut off of 0.1% in most of these studies had a prognostic value. Now also prospective studies in younger and elderly AML treated intensively showed independent and significantly prognostic value in terms of CIR and OS.

- MPFC based MRD was assessed in a multicenter, multinational study by HOVON/SAKK investigators [10]. In this trial of 517 adults with AML between 18 and 60 years of age, MRD was assessed centrally using serial bone marrow samples from diagnosis, after one and two cycles of induction therapy, and after consolidation. Laboratory assessment of MRD was made independently of clinical data and patients were treated according to protocol without knowledge of MRD-related data. Lower levels of MRD were associated with better outcomes than higher levels, and MRD levels ≥ 0.1% of white blood cells after the second cycle of chemotherapy were associated with higher risk of relapse in multivariate analysis.
- The UK NCRI group assessed MRD using flow cytometry in 427 patients older than 60 years of age [11]. MRD negativity after the first cycle of chemotherapy, defined as absence of LAIPs, conferred significantly better 3-year survival after CR. MRD-positive patients had increased relapses and higher risk of early relapse (median time to relapse, 8.5 v 17.1 months, respectively). In multivariate analysis, MRD status was again an independent prognostic indicator.
- Also the MD Anderson group reported similar results in a study in 166 intensively treated patients [12]: 79% became negative for MRD after induction, which was associated with an improvement in relapse-free survival and overall survival. In a multivariate analysis including age, cytogenetics, response and MRD, achieving an MRD-negative status was the most important independent predictor of RFS and OS
- The NCRI prospectively assessed MFC MRD in 2450 younger adult patients with AML [13]. Survival outcomes from patients in partial remission and in CR but MRD-positive after cycle 1 were similar, underlining the prognostic value of MRD even after one cycle of chemotherapy.

The prognostic impact of the MRD status after cycle 2 was again confirmed and remained significant (relapse: HR 1.88 survival: HR 1.77).

- Also in studies with less intensively treated patients MRD appeared to be of prognostic value [14].
- Pre-transplant MRD status has been shown of highly prognostic value. Patients who are MRD neg before transplant both in the ablative and non-myeloablative setting perform much better than those who are MRD pos. These observations are further supported by a meta-analysis of 19 studies evaluating pre-HSCT MRD (mainly assessed by MPFC) [15]. Until yet, this leaves us with the unsolved question whether we should defer an allogeneic transplant procedure in MRD negative patients while performing such in MRD positive AML patients. A highly relevant question is if MRD conversion from positive to negative or deepening of the response pre transplant will improve outcome. Preliminary data point in that direction. Unfortunately, in one study such conversion from MRD positivity pre-transplant to MRD negativity after myeloablative conditioning, did not substantially improve relapse rate or OS [16].

Recently midostaurin (RYDAPT), a first generation FLT3m inhibitor [17], and CPX-351 (Vyxeos), a liposomal formulation of daunorubicin and cytosine-arabinoside in a fixed 1:5 M composition [18], were approved by FDA. Both drugs showed an overall survival benefit in comparison to standard intensive chemotherapy. Remarkably, in both studies the outcome of patients who were transplanted in first complete remission was better in the arms with the experimental drug as compared to the standard of care control arms. It could be hypothesized that a deeper response pre-transplant due to either midostaurin or Vyxeos is responsible for this. Unfortunately, in both studies no MRD assessment was included in the study design.

### 3.2. qPCR assessed MRD

Currently, validated molecular MRD targets in AML include the PML-RARA translocation in APL, core-binding factor (CBF) translocations, and mutations in NPM1 [19–21].

Ivey et al. showed that the presence of MRD, as determined by quantitation of NPM1-mutated transcripts assessed in 346 patients with NPM1-mutated AML who had undergone intensive treatment in the National Cancer Research Institute AML17 trial, provided powerful prognostic information independent of other risk factors [22]. Persistence of NPM1-mutated transcripts in blood was

observed in 15% of the patients after the second chemotherapy cycle and, compared to absence of such transcripts, was associated with a greater risk of relapse after 3 years of follow-up (82% vs. 30%; hazard ratio, 4.80) and a lower rate of survival (24% vs. 75%; hazard ratio for death, 4.38). The presence of minimal residual disease was the only independent prognostic factor for death in multivariate analysis.

### 3.3. Next-generation sequencing assessed MRD

P. Valk et al. carried out targeted next-generation sequencing at diagnosis and after induction therapy (during complete remission) in 482 patients between 18 and 65 years of age. At least one mutation was detected in 430 out of 482 patients (89.2%). Mutations persisted in 51.4% of those patients during complete remission [23]. After exclusion of persistent DTA mutations (DNMT3A, TET2, and ASXL1), compared to those in whom no mutations persisted, the persistence of molecular MRD was associated with a significantly higher relapse as (55.4% vs. 31.9%), as well as with lower rates of relapse-free survival (36.6% vs. 58.1%) and overall survival (41.9% vs. 66.1%).

Multivariate analysis confirmed that the persistence of non-DTA mutations during complete remission conferred significant independent prognostic value with respect to the rates of relapse, relapse-free survival and overall survival. Thol et al. reported more or less similar data in 116 AML patients undergoing allogeneic HSCT in CR [24]. Targeted resequencing at diagnosis identified a suitable mutation either in peripheral blood or bone marrow for follow up in over 90% of the patients. In 12 patients persistence of an ancestral clone was found. After transplant, 45% of patients were MRD-positive. The cumulative incidence of relapse (CIR) was higher in MRD-positive as compared to MRD-negative patients (5-year CIR, 66% vs 17%), whereas non-relapse mortality was not significantly different. In multivariate analysis, MRD positivity was the strongest independent negative predictor of CIR (HR, 5.68; $P < .001$), Thus, also NGS-based MRD is emerging and appears to be widely applicable to AML patients, although more data are warranted and sensitivity should be improved, it seems highly prognostic for relapse and survival.

### 3.4. Limitations of MRD testing and possibilities for improvement

All currently used MRD-test lack sensitivity or specificity to accurately predict relapse risk certainly not at the level where individual therapy decisions are made. 20–40% of AML patients who are MRD negative relapse whereas some with a positive MRD-test are cured. The reasons are probably multifactorial and can be due to: insufficient sensitivity of the assay, immunophenotype switches, inhomogenous distribution of leukemic blasts in the bone marrow, an inadequate bone marrow sample or not taken into account the importance of leukemic stem cells (LSC) [25].

It was hypothesized that the bulk leukemic blast population detected by standard flow cytometric MRD may arise from LSC populations and that it is the LSC compartment that serves as a reservoir for relapse post treatment [26]. Therefore, there is a rational for inclusion of flow cytometric monitoring of LSC-enriched populations as a supplementary residual disease assay. Since the CD34 +CD38− LSC cells seem to have the highest leukemogenic ability and therapy resistance, we prospectively validated the prognostic relevance of the CD34 +CD38− LSC frequency, both at time of diagnosis and after induction therapy [27]. LSC-negative patients (n = 204) had better 3-yr CIR and 3-yr OS compared to LSC-positive patients (n = 98). Moreover, in multivariate analyses LSC status in CR patients was an independent predictor for both CIR (HR 1.87) and OS (HR 1.62) Combining LSC and.

MRD showed strongly reduced survival in MRDhigh/LSChigh patients (HR 3.62 for OS and 5.89 for cumulative incidence of relapse [CIR]) compared to MRDlow/LSChigh, MRDhigh/LSClow, and especially MRDlow/LSClow patients.

Moreover, in the NPM1mutant positive sub-group, prognostic value of golden standard NPM1-MRD by qPCR could be improved by addition of flow cytometric approaches.

The combined use of both delivers improved prognostic value for the risk of relapse compared to the individual techniques. The same was also found for combinations of NGS and flow based detection of MRD/LSC (unpublished).

The inhomogeneous expression of leukemic cells in the bone marrow could possibly be overcome by peripheral blood assessment of MRD [28]. This is currently under investigation in ELN setting.

Assessment of MRD from flow cytometry data requires extensive manual gating procedures, followed by interpretation of complex cell surface protein expression patterns of the leukemic bone marrow by comparison with normal bone marrow differentiation patterns. To reliably perform such analyses requires a high level of expertise, and inherently, the analyses are subjective to a more or lesser extent. These issues may be overcome by the application of computational approaches that automate the analyses required for MRD assessment [29,30].

### 3.5. Can MRD status already guide treatment?

It is without doubt that MRD is already being used in clinical practice to inform the care of individual patients.

In APL, by comparing two subsequent studies, it has been shown that therapy intensification based on MRD testing can prevent clinical relapse [31]. However in APL, given the very low relapse rate in current treatment strategies, MRD determination has become less important. In AML a number of studies clearly indicate that treatment intensification guided by MRD measurement may improve outcome. However randomized data to support this are currently lacking.

- Rubnitz et al. applied risk directed therapy in childhood AML. Risk was determined by risk profile at diagnosis and level of MRD after the first cycle of chemotherapy. MRD was measured by MPCF [32]. MRD status was used to intensify treatment and timing of

the second cycle of chemotherapy. The outcome was better if compared with other comparable trials performed in childhood AML, suggesting that this better outcome was due to the MRD based risk stratification strategy.

- Zhu et al. performed a risk adapted non randomized study in CBF AML. Patients were assigned to alloHSCT after the second consolidation treatment if MRD positive [33]. In case of MRD negativity the treatment consisted of chemotherapy or an autologous HSCT. Some patients decided not to follow the assigned treatment based on the MRD status. It was shown that the patients receiving treatment other than that assigned by the risk status did worse than those who completed their assigned treatment

### 3.6. Can MRD be used as a surrogate endpoint for survival to accelerate drug approval?

Once MRD status is accepted as a surrogate endpoint for survival, it would be helpful for the evaluation of effectiveness of new drugs and offers the possibility of speeding up drug approval or, the other way around, to stop the development of drugs that are potentially not successful. Based on the long interval between start of trials and the ultimate final outcome survival, there is an urgent need for more rapid surrogate endpoints. A few studies suggest that indeed MRD can serve as a surrogate for overall survival endpoints:

1. Prebet et al. showed that in CBF-AML a higher dosage of Daunorubicin (90mg/m2/3days) has a better clinical outcome than the standard dosage (60mg/m2/3days), and was associated with a significantly lower level of MRD [34].
2. Lambert et al., reported that the improved overall survival in good risk AML by the addition of gemtuzumab ozogamicin to standard induction therapy, correlated with MRD status [35].
3. In the recent HOVON/SAKK 102 study, where standard of care chemotherapy plus or minus clofarabin was compared, a subanalysis showed that OS and EFS significantly improved in the clofarabin arm in the ELN intermediate I prognostic risk AML (EFS: 26% vs 40%) [36]. The relapse rate also was reduced in the clofarabin arm (44% vs 35%). In parallel, it was found that clofarabin caused a significant reduction of MRD level in the clofarabin arm (0.014%) compared to the standard therapy arm (0.023%).

If such correlations are specific, studies in which no clinical benefit was found for a new therapy when compared to standard therapy, should have no differences in MRD in the two arms. This was indeed found in the previous HOVON/SAKK42a study in which standard chemotherapy treatment was compared to the standard therapy plus growth factor G-CSF (with the aim to increase standard therapy effectiveness by promoting entrance of cells into cell cycle) [37]. There was no difference in survival in the two treatment arms and, in line with that, no difference in MRD levels after the first and second cycle of induction therapy (Table 1).

Now many new studies investigating promising new agents include MRD as an outcome measure. It is expected that, once these studies prospectively show the value of MRD as a predictive instead of a prognostic marker for outcome, the regulatory authorities will accept MRD as a surrogate endpoint for survival resulting in faster drug approvals.

### 3.7. Intervention

In many centers frequent MRD assessments are clinical reality, this can result for example after early detection of relapse post-transplant in either stopping immune suppressive treatment, early application of DLI or other interventions to slow down leukemic growth or to try to increase the graft vs leukemia effect.

In the prospective phase II RELAZA2 study, patients undergoing allo-HSCTwere prospectively screened for MRD either by the quantification of NPM1 mutation level, leukemia-specific fusion genes or donor chimerism (DC) analysis of $CD34^{+}$ cells on peripheral blood cells [38]. Patients in CR with residual disease above the threshold defining imminent relapse got six cycles of AZA (75 mg/m sc, days 1–7) followed by a MRD risk-adapted AZA-based therapy for up to 18 additional months. After six cycles of treatment, 58% achieved an overall response. Major response was observed in 19 responders (61%) with a decline of MRD below a predefined threshold (increasing $CD34^{+}$ DC to ≥ 80% or mutation level ≤ 1%). The findings of this RELAZA2 study demonstrate that MRD monitoring can identify patients who are likely to relapse and that MRD-guided treatment with a hypomethylating agent could be an effective strategy to prevent or substantially delay relapse with an acceptable safety profile in MRD-positive patients with AML.

Whether conversion of MRD positivity to MRD negativity pre-transplant will be of benefit for the outcome after an alloSCT is not clear currently. Randomized studies are warranted to answer that question.

**Table 1**
Median MRD levels (% and range) defined in patients treated without G-CSF or with G-CSF.

| | 1st cycle | 2nd cycle |
|---|---|---|
| GCSF- | 0.038% (0–16[a]) n = 78 | 0.022% (0–15[a]) n = 83 |
| GCSF+ | 0.040% (0–4.2) n = 86 | 0.023% (0–21[a]) n = 100 |
| p-value | 0.94 | 0.83 |

[a] Percentages above 5% (morphological cut-off for CR) can occur since flow cytometrically defined blast count do not always match morphological blast count.

**Fig. 1.** MRD-guided treatment in HOVON trials.

### *3.8. How can MRD status be implemented as part of the decision making process to consolidate a CR?*

The options to maintain CR after induction are either continuation with chemotherapy or an autoSCT or assignment of patients to an alloSCT [39,40]. AlloSCT reduces relapses but non-relapse mortality and morbidity may counterbalance this beneficial effect. The ELN recommendations on alloSCT propose risk assessment as a dynamic process during treatment, incorporating both disease-related and transplant-related factors for the decision to proceed either to allogeneic HSCT or to apply a non-transplant strategy [41]. Relapse risk should be counterbalanced by the estimated treatment related mortality: alloSCT might be advised if a 10% disease free survival can be expected. These recommendations have been provided under conditions when MRD was not as routinely available as it is now. Introducing MRD now into the recommendations who to transplant in CR1, might be used to upstage (presence of MRD) or downstage (no MRD). This is how HOVON-SAKK currently use MRD in their treatment algorithm (Fig. 1). This treatment strategy is supported by preliminary data from the NCRI suggesting that alloSCT in CR1 has a beneficial effect in intermediate risk patients in the presence but not the absence of MRD [13].

## 4. Conclusion

MRD is a rapidly emerging field and adds additional value to the traditional definition of CR. Methodologies are improving and further standardization and/or harmonisation of these are on the way. Although in many centers and major AML trial groups, MRD is already used for informing patient care, randomized studies are lacking. We are on the brink of using MRD as a surrogate endpoint for survival which would be very helpful in drug development.

### Practice points

- MRD assessment changes the definition of Complete Remission.
- MRD assessment in AML should be included in every clinical trial
- MRD status can be used to inform treatment decisions

### Research agenda

- NGS to measure MRD should be further investigated
- MRD as a surrogate endpoint for survival has to be proven
- Automatically analysis of flow determined MRD should be developed
- Combinations of different methods to assess MRD should be further investigated

### Disclosure

No relevant conflicts with any commercial interest.

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