Monitoring Minimal Residual Disease in ALL and AML

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57.1 Monitoring MRD in ALL

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57.1.1 Introduction

In ALL evaluation of molecular treatment response, assessment of minimal residual disease (MRD) is a substantial independent predictor of outcome, as proven by randomized studies (Conter et al. 2010; Gökbuget et al. 2012; Bassan and Spinelli 2015). Consequently, MRD is implemented in virtually all clinical protocols in order to supplement or to redefine multifactorial risk strati-

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fication with optional customized treatment intensity. The detection of leukemic cells below the limit of classical cytomorphology is feasible either by disease-specific alterations of the immune phenotype or unique genetic features. Several competing and complementing MRD methods have been developed with preference application according to clinical protocols (Van der Velden et al. 2007; van Dongen et al. 2015).

57.1.2 MRD Assessment by IG/TCR Real-Time PCR

The discontinuous immune receptor genes provide the immune repertoire by somatic recombination of variable (V)-, diversification (D)-, and junction (J)- elements thus forming hypervariable CDR3 (complement determine region 3) regions during lymphocyte maturation. Such rearrangements can serve as clonal index of leukemia blasts originating from lymphoid precursor stages. Additionally, due to a relaxed regulatory control, leukemia blasts can harbor incomplete rearrangements and cross-lineage rearrangements and tend to accumulate simultaneously multiple rearrangements. Quantitative real-time PCR using junction complementary allele-specific oligonucleotides (ASO) frequently reaches a detection limit of 1E-05 with a quantitative range of 1E-04, is applicable to vast majority of cases, and has a high degree of standardization (Van der Velden et al. 2007).

57.1.3 MRD Assessment by Fusion Gene Transcript

Most frequent recurrent reciprocal translocations are in ALL t(9;22)(q34;q11) (*BCR-ABL1*), t(12;21)(p13;q23) (*ETV6-RUNX1*), and t(4;11) (q21;q22) (*MLL-AFF1*) with age stage associated preponderance in adults, childhood, and infant ALL, respectively. Derived chimeric fusion transcripts are validated marker for MRD detection by real-time PCR with an achievable detection limit of 1E-06. The methodology has been standardized by the Europe Against Cancer (EAC) program (Gabert et al. 2003).

57.1.4 NGS (Next-Generation Sequencing)

High-throughput sequencing (HTS) of immune receptor genes by next-generation sequencing (NGS) is a novel option for MRD. This methodology provides comprehensive qualitative and quantitative information regarding clonal consistence of the diagnostic sample and shares one protocol for index determination and MRD assessment without need of individual reagents. Potential sub- and new emerging leukemic clones also are covered. PCR steps during library construction can introduce bias effecting results internal controls and normalization calculations are necessary the generated data volume is high and data interpretation demand biostatistics expertise. Due to high sample capacity, NGS favors a centralized concept and service is available to commercial providers by academic centers (Kotrova et al. 2015).

57.1.5 Flow Cytometry

MRD by multicomponent flow cytometry (MFC) distinguishes leukemia-associated immune phenotypes (LAIP) and regular cells. LAIP consists of cell lineage maturation stage-specific (backbone) markers in combination with illegitimate markers. The standard four to six color approaches have been developed simultaneously by several centers. Therefore, the applied marker panels depend on study protocol. The consistently achieved detection limit is 1E-04. Recently, increase of specificity and sensitivity was enabled by high-throughput procedures demanding eight or ten color equipment. Here the options for targeted and visualized antigens allow simultaneous visualization of all developmental lymphocyte stages serving as background to distinguish leukemic cells. The EuroFlow Consortium validated available antibody panels and controls which can be applied in a standardized way, including automated gating with supportive software, data storage and comparison, accurate quantitative result, and option for IVD development. Similar to the NGS approach, the generated data volume is high and data interpretation demands biostatistics expertise; nevertheless, the concept allows decentralized data acquisition (Pedreira et al. 2013).

57.1.6 Limitations of MRD Assessment

The determined level of MRD always is a result of complex interrelation of baseline characteristics of tumor and patient, time point of MRD evaluation, therapeutic agents, course of clearance, and degree of therapy resistance. Several measurements therefore are mandatory. Adverse circumstances for MRD assessment are clonal selection and clonal evolution, since the associated index might be missed. Potentially impacted are leukemia with initial oligoclonality as observed in approximately 15% of B-ALL and up to 1000 subclones have been reported (Wu et al. 2016). Phenotypic plasticity under treatment and massive lymphocyte regeneration can cause false negativity or positivity, a solvable problem by applying mentioned high-throughput methodologies. Achievable detection limit is correlated with cell count of sample, and aplastic samples are challenging. Finally, all methodologies use different sample preparations, and analyses refer to different units, a circumstance which interferes result comparison.

57.1.7 MRD in the Setting of HSCT

As all adult patients with ALL who relapse after initial chemotherapy have an absolute indication for allo-HSCT, pediatric patients are stratified into different treatment groups. Main prognostic determinants in these patients are the blast immune phenotype, time to relapse, and site of relapse. High-risk patients who experienced early isolated BM relapse, early relapse involving BM, and any BM relapse of T-lineage ALL have clear indication for HSCT. Intermediate-risk patients experienced early or late combined BM relapse and a late isolated BM relapse of a B-cell precursor (BCP).

ALL and very early and early isolated extramedullary relapse of either BCP-ALL or T-ALL have indication for HSCT if post induction MRD exceeds a threshold of 1E-03 (Eckert et al. 2013).

During the past decades, it could be clearly shown by several studies that the level of MRD immediately prior to transplant does have a clear prognostic impact on post-HSCT outcome (Knechtli et al. 1998). Retrospective studies in children with relapsed ALL revealed an important cutoff for post-HSCT outcome. Patients who received transplantation with an MRD load of ≥10 to 4 leukemic cells had a by far inferior prognosis than patients with lower MRD loads before transplant (Bader et al. 2009). Based on these findings, several studies are now underway investigating strategies to improve outcome in these ultrahigh-risk patients. Adaption of transplant approaches might allow successful transplantation (Leung et al. 2012).

Spinelli et al. showed that almost half of the patients with high levels of MRD before transplantation achieved molecular remission by day +100 (Spinelli et al. 2007). This finding indicates that MRD detection post transplant provides additional value to the MRD assessment prior to transplantation. It could be demonstrated in prospective clinical studies that the close monitoring of MRD by different approaches allows the prediction of relapse and may therefore form the basis of different intervention strategies making use of leukemia-specific targeted therapy (Bader et al. 2015; Balduzzi et al. 2014). Future perspectives will focus on MRD-guided intervention to prevent overt relapse (Rettinger et al. 2017).

57.2 Monitoring MRD in AML

Gert Ossenkoppele

57.2.1 Introduction

The possibility of defining residual disease far below the level of 5% leukemic cells is changing the landscape of risk classification. This so-called measurable/MRD approach at present establishes the presence of leukemia cells down to levels of $1:10^3$ to $1:10^6$ white blood cells, compared to 1:20 for morphology. Recently the ELN proposed a new response criterium: CR without minimal residual disease (CR_{MRD-}) is defined as CR with negativity for a genetic marker by RT-qPCR or CR with negativity by multicolor flow cytometry (MFC) (Döhner et al. 2017).

The reasons to apply MRD assessment in AML are (1) to provide an objective establishment of remission status; (2) to better predict outcome and guide post-remission treatment; (3) to identify early relapse as a robust post transplant surveillance, in order to enable early intervention; and (4) to be used as a surrogate endpoint to fasten drug testing and approval.

A recent ELN MRD consensus document was published with the aim to identify key clinical and scientific issues in the measurement and application of MRD in AML and to provide guidelines for the current and future use of MRD in clinical practice (Schuurhuis et al. 2018).

57.2.2 Methods for MRD Detection

57.2.2.1   MRD Detection by PCR
Real-time quantitative PCR (RT-qPCR) allows MRD detection in cases with chimeric fusion genes generated by balanced chromosomal rearrangements (Grimwade and Freeman 2014). Other genetic alterations can also be used for MRD detection including insertions/duplications, point mutations and gene overexpression. Apart from t(15;17) and *RUNX1–RUNX1T1* and *CBFB–MYH11*, currently *NPM1* is the best-validated molecular marker for MRD assessment. PCR assessment of MRD is in about 50% of patients in

principle possible. The methodology has been standardized for several molecular markers for clinical implementation in the Europe Against Cancer (EAC) program (Gabert et al. 2003).

57.2.2.2 Immune MRD by Multicolor Flow Cytometry

The basic principle is to identify at diagnosis leukemia-associated immune phenotypes (LAIP). These LAIPs consist of normally occurring markers, present in aberrant combinations in AML but in very low frequencies in normal and regenerating BM. The background levels of LAIP in normal and regenerating BM levels, in particular, although low, prevent specific detection of aberrancies with sensitivities higher than 1:10,000.

If no diagnosis sample is present, one can make use of "different from normal" approach which uses a standard fixed antibody panel to recognize leukemic cells based on their difference with normal hematopoietic cells (Loken et al. 2012).

Currently, immune MRD aberrancies may be detected in over 90% of AML cases at diagnosis.

57.2.3 MRD in Clinical Studies

Despite a multitude of prognostic factors at diagnosis, the outcome of patients is still highly variable and not individually predictable. It thus seems that prognosticators at diagnosis will not enable clinicians to reach the ultimate goal of truly individualized risk assessment. Treatment parameters may be more useful (Ossenkoppele and Schuurhuis 2013).

Two large, prospective, multicenter studies have identified flow cytometry-based MRD as an independent prognostic indicator in adults with AML (Freeman et al. 2013; Terwijn et al. 2013). Flow cytometry-based MRD was assessed in a multicenter, multinational study in adults with AML between 18 and 60 years of age by HOVON/SAKK investigators (Terwijn et al. 2013). Patients were treated according to protocol, without knowledge of MRD-related data. In this study, 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 (Freeman et al. 2013). MRD negativity after the first cycle of chemotherapy 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).

An example indicative for the usage of molecular MRD was recently published by Ivey et al. (2016) who showed in a large study by NCRI that the presence of MRD, assessed by Q-PCR of *NPM1*-mutated transcripts, provided powerful prognostic information independent of other risk factors. Persistence of *NPM1*-mutated transcripts in blood was present in 15% of the patients after the second chemotherapy cycle and was associated with a greater risk of relapse after 3 years of follow-up than was an absence of such transcripts (82% vs. 30%; hazard ratio, 4.80) and a lower rate of survival (24% vs. 75%; hazard ratio for death, 4.38).

Many other studies point in the same direction that MRD status after two cycles of chemotherapy is highly predictive independently from other prognostic factors for outcome (Hourigan et al. 2017). However surrogacy for survival has not been proven yet (Ossenkoppele and Schuurhuis 2016).

57.2.4 Pretransplant MRD

Evidence is accumulating that the presence of MRD assessed by multicolor flow cytometry immediately prior to allogeneic HCT is a strong, independent predictor of post transplant outcomes in AML (Buckley et al. 2017; Walter et al. 2015). In a recent update, Araki et al. showed that in 359 adults, the 3-year relapse rate was 67% in MRD-positive patients, compared to 22% in MRD-negative patients, resulting in OS of 26% vs. 73%, respectively (Araki et al. 2016). This applies for the myeloablative as well as for the non-myeloablative transplant setting.

Also molecular MRD as measured by RT-PCR in NPM1-mutated AML has a significant impact on outcome after allo-HSCT (Balsat et al. 2017).

Performing an allo-HSCT in case of a suboptimal reduction (<4 $\log_{10}$) of NPM1 levels after chemotherapy resulted in improved overall survival. In patients with optimal (≥ 4 $\log_{10}$) reduction of NPM1 levels after chemotherapy, allo-HSCT had no significant effect on survival. However, no prospective studies using MRD to guide postremission therapy are available at the time of this publication. Regardless, it is clear that novel treatment strategies before, during, and after transplant are urgently needed to improve outcomes in AML. Thereby depth of response prior to transplant, as measured by level of MRD, has emerged as one of the most important predictors of transplant outcome. Randomized trials are warranted to determine if MRD-guided preemptive therapy is associated with improved outcome.

Currently no clinical trial including transplantation trials should be performed without including MRD assessment.

57.2.5 Future Developments

New technologies are emerging to assess MRD. Quantifying leukemic stem cells is such a promising approach (Terwijn et al. 2014; Zeijlemaker et al. 2016). Next-generation sequencing for MRD assessment can, theoretically, be applied to all leukemia-specific genetic aberrations. In a recent HOVON study, it was shown that persistence of gene mutations in CR appeared to be a highly significant independent prognostic value for relapse and overall survival (Jongen-Lavrencic et al. 2018).

Key Points

- MRD should be included in the definition of CR.
- MRD positivity is an independent predictor of relapse after chemotherapy in AML patients and a negative predictor for ALL patients.
- Pretransplant MRD positivity is highly indicative for relapse.
- MRD assessment should be implemented in every clinical trial.

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