Deep Insight Section

Monitoring of minimal residual disease in acute myeloid leukemia

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Introduction

Modern treatment of patients with acute myeloid leukemia includes intensive induction and consolidation therapies as well as autologous and allogeneic stem cell transplantation. This results in long-term disease control in most patients with good prognostic features while the majority of other patients are prone to relapsing and resistant leukemia. Based on the most powerful prognostic parameters, i.e. cytogenetics, age, and AML as secondary disease (Schoch et al., 2003; Schoch et al., 2004), patient groups with highly differing prognosis can be identified. However, for many patients and within the large group of patients with an intermediate prognosis in particular an improvement of prognostication can be achieved by use of therapy-dependent parameters. Minimal residual disease (MRD) carrying the potential of leukemic regrowth and overt relapse is considered an ideal target for the definition of treatment-dependent prognostic parameters.

General aspects on minimal residual disease (MRD)

Different courses of this minimal residual disease (MRD) are depicted in figure 1. Consecutive elements of antileukemic therapy may lead to repeated reductions of the amount of residual leukemic cells until eventually cure from the disease is achieved. In contrast, residual leukemic cells may become resistant to antileukemic therapy in other cases leading to increasing levels of MRD while the patient still is in complete remission as assessed cytomorphologically. In most of the latter cases the regrowth of the leukemic population results in the occurrence of clinical relapse. Taking these issues into account, the level of MRD is anticipated not only to have substantial impact on the course of the disease and to become a valuable therapy-dependent prognostic factor but also to be increasingly used for the selection of risk-adapted treatment strategies.

Along this line, the recently "Revised Recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia" now include the definition of treatment failure based on the reappearance of molecular and cytogenetic abnormalities (Cheson et al., 2003). In addition, the flow cytometric evaluation of AML at diagnosis is recommended in order to indentify leukemia-associated aberrant immunophenotypes (LAIP) useful for the quantification of MRD.
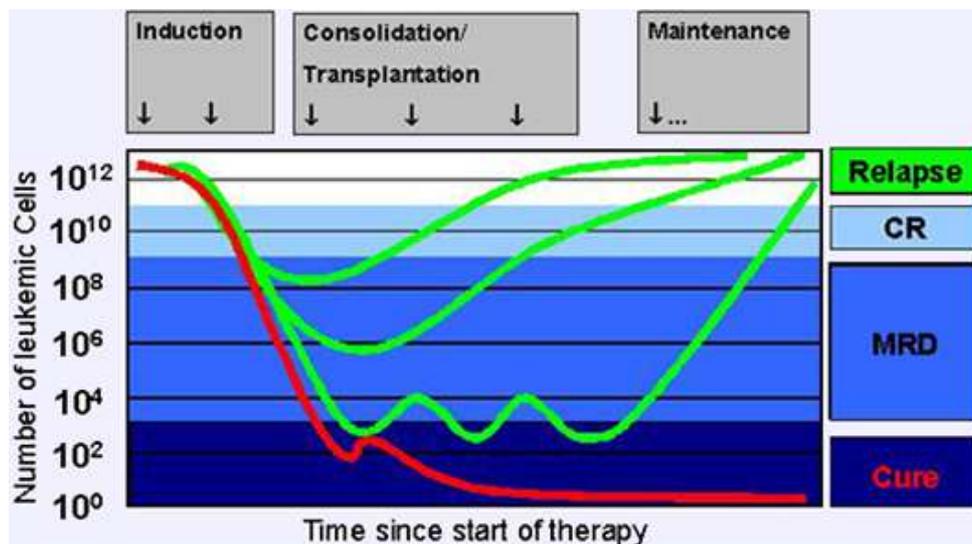
Figure 1: Leukemia treatment outcome. The red line indicates a sequential reduction of the leukemic cell mass with the exception of a slight increase before the second consolidation therapy. Eventually, cure is achieved. The green lines indicate cases with relapses of AML in which increasing MRD levels are present before relapse. The light blue area refers to the cytomorphologic finding of 1% to 5% bone marrow blasts which is compatible with complete remission. The intermediate blue area refers to a 0% bone marrow blasts count with MRD levels detectable by MFC or QRT-PCR. The dark blue area refers to a 0% bone marrow blasts count with MRD levels below the sensitivity of MFC and QRT-PCR.

Clinical use of monitoring MRD in AML

The two most important aspects of monitoring of MRD in AML are its use as a stratification parameter and as a tool for the early detection of impending relapse. In the first setting, patients are divided based on distinct levels of MRD into two or more groups with different risks of relapse and probabilities of long-term remission. The resulting stratification models can be used in randomized clinical trials to delineate the relative efficacy of investigational treatment approaches within prognostically homogeneous subgroups of AML. In the second setting, the aim of monitoring of MRD is the identification of cases with a very high risk of relapse who then can be treated much earlier and more effectively by salvage protocols as compared to patients in overt relapse.

A further possible application is the use of the

reduction of MRD as a surrogate marker for long-term efficacy of newly developed and evaluated treatment approaches.

Assessment of MRD in AML by multiparameter flow cytometry, general aspects

The detection and quantification of MRD by multiparameter flow cytometry MFC relies on the presence of leukemia-associated aberrant immunophenotypes (LAIP) on leukemic cells in patients with AML (Kern et al., 2003). These LAIP are present in all of or in a subset of the leukemic cells and are present at very low frequencies or even absent in normal bone marrow cells. Figure 2 shows the course of MRD during therapy as identified by the aberrant expression of CD56 on CD33+CD34+ bone marrow blasts.
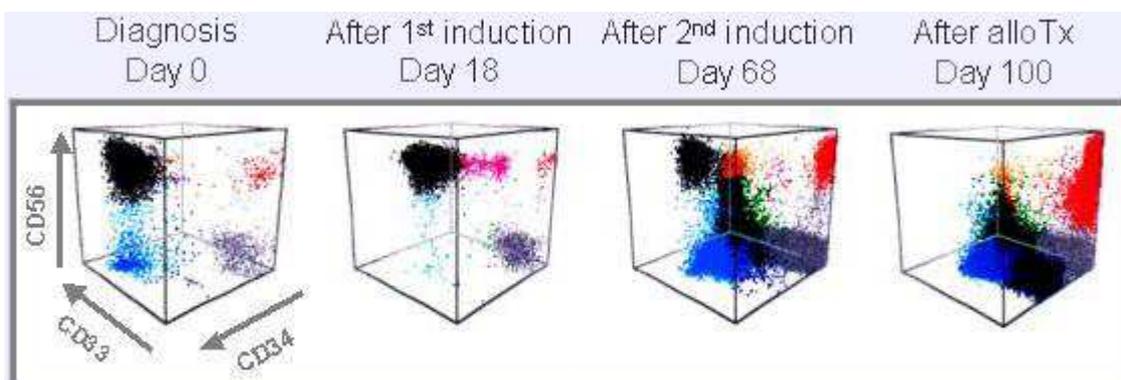

Figure 2: Course of MRD. The number of LAIP-positive cell decreases with each consecutive course of treatment. LAIP-positive cell are absent after allogeneic stem cell transplantation.

Given that at diagnosis of AML the LAIP present in an individual case has been identified, MRD during the course of treatment and follow-up can be assessed by the quantification of the frequencies of these cells by MFC. Due to the phenotypic heterogeneity of AML it is essential to apply a comprehensive panel of combinations of monoclonal antibodies at diagnosis in order to allow the detection of an LAIP. Furthermore, the comparison of leukemic bone marrow to normal bone marrow samples is most important in order to maximize specificity.

LAIPs can be grouped into four different categories according to the kind of aberration:

a. "cross-lineage" expression (expression of lymphoid antigens on AML cells),

b. lack of expression of an antigen (an antigen which is expressed during normal granulocytic or monocytic differentiation is not expressed),

c. overexpression (expression of antigens with an expression intensity higher than observed in normal bone marrow or expression of antigens normally not expressed in bone marrow), and

d. asynchronous expression of antigens (co-expression of antigens which during normal granulocytic and monocytic differentiation are expressed sequentially but not simultaneously).

In addition, leukemic cells frequently display an aberrant light scatter pattern which reflects mainly the size (forward-light scatter; FLS) and the granularity (orthogonal-light scatter; OLS) of the cells.

Applicability and sensitivity of MRD quantification by MFC

Besides the determination of the prognostic impact of MRD levels in AML in general one of the key issues in immunologic monitoring in AML is the balance between maximizing the applicability to all patients and the sensitivity and specificity of the method.

Due to the phenotypic heterogeneity of AML within individual cases, which occurs as more than one population or, in most cases, as a wide range of expression intensity of various antigens, the inclusion of all AML cells into one LAIP is possible in some cases only (e.g. CD34+CD56+CD33+) while in other cases only a part of the total population of AML cells can be included into one LAIP (e.g. CD64++CD4++CD45++). Clearly, the portion of AML cells which can be covered by a LAIP is dependent on the degree of aberration in the leukemia-associated immunophenotype as compared to the respective immunophenotype in normal bone marrow cells.

Thus, in AML patients with leukemic cells displaying a less aberrant LAIP the choice with regard to immunologic MRD monitoring is:

a. to include only a portion of all cells into one LAIP which is absent in normal bone marrow cells,

b. to include all leukemic cells into one LAIP but at the same time to loose sensitivity and specificity due to the overlap between leukemic and normal immunophenotypes, and

c. to not include these AML patients with less aberrant immunophenotypes into the monitoring program.

From the technical point of view the latter approach is the most prefered one and has been adopted in a variety of analyses on the prognostic impact of immunologically quantified MRD in AML. Thus, the AML cells in more than 25% of all patients analyzed at diagnosis were considered not to carry a LAIP (Venditti et al., 2000; San Miguel et al., 2001; San Miguel et al., 1997).

Although these studies consistently demonstrate the high prognostic impact of immunologically determined MRD levels in AML they do not address the issue of optimizing the applicability of this approach by improving the panel of antibodies and thus the capability of identifying LAIPs in a larger number of patients. New data in this regard suggest that maximizing this applicability is possible even to 100 percent of all AML patients with the resulting sensitivity as analyzed by normal bone marrow samples amounting to a median of 0.05% (Kern et al., 2003). In fact, data are available to prove that MRD levels determined in this way have significant impact on the patients' prognosis (Kern et al., 2004).

Stability of LAIP at relapse

A prerequisite for the validity of immunologic monitoring in AML is the stability of the LAIP between diagnosis and relapse which has been anticipated not to be present in a subset of patients given that even changes in karyotype occur in about one third of cases (Kern et al., 2000; Kantarjian et al., 1988).

Published data in this regard suggest that with a few exceptions the LAIP is detectable also at relapse, however, the populations covered by the LAIP at relapse in some cases represent only less than 10% of all leukemic cells and the intensity of aberrantly expressed antigens may have changed (Oelschlagel et al., 2000; Macedo et al., 1996; Reading et al., 1993; Drach et al., 1992; Campana et al., 1990; Voskova et al., 2004).

Prognostic impact of immunologically quantified MRD in AML

Depending on the selection of applied antibodies and on the aberrantly expressed genes the sensitivity in most AML cases ranges between 0.1% and 0.01% of all nucleated bone marrow cells (Campana and Coustan-Smith, 1999; Coustan-Smith et al., 1993). The studies conducted in patients after achievement of a

complete remission indicate that the amount of residual disease for the majority of patients are in the same range and thus assure the feasibility of using MFC for the quantification of MRD (Venditti et al., 2000; San Miguel et al., 2001; San Miguel et al., 1997; Kern et al., 2004; Nakamura et al., 2000).

Published data on the detection of MRD by MFC suggest that in a portion of all patients an estimation of the prognosis is possible based on the amount of residual disease (Venditti et al., 2000; San Miguel et al., 2001; San Miguel et al., 1997; Kern et al., 2004; Nakamura et al., 2000; Sievers et al., 1996; Coustan-Smith et al., 2003).

In all studies on adult AML a prognostic impact of MRD levels determined by MFC has been shown.

- San Miguel et al. demonstrated in 53 patients in complete remission that an MRD level of 0.5% following induction therapy and an MRD level of 0.2% following consolidation therapy allowed to separate patients into groups with significantly different relapse-free survival (San Miguel et al., 1997).

- In a similar study Venditti et al. could demonstrate that an MRD level of 0.045% following induction therapy and of 0.035% following consolidation therapy had an independent impact on both event-free survival and overall survival (Venditti et al., 2000).

- A different approach was attempted in another study on 22 patients in whom an asynchronous coexpression of CD15 and CD117 has been detected in at least 5% of all leukemic cells (Nakamura et al., 2000). An association between the amout of these cells at 10 months after start of induction therapy on the one hand and the duration of the remission on the other hand was shown.

- An extension of one of these studies revealed a prognostic impact of MRD level between 0.01% and 0.1%, between 0.1% and 1%, and higher than 1% at the time of achievement of complete remission after induction therapy. The separation of patients according to these limits resulted in significant differences in the overall survival and identified flow cytometrically quantified MRD level as an independent prognostic marker in a multivariate analysis (San Miguel et al., 2001).

- Furthermore, a study performed in childhood AML demonstrated the applicability of flow cytometric quantification of MRD in 85% of all cases and identified the MRD levels after both induction 1 and induction 2 as independent prognostic markers with regard to overall survival in multivariate analyses (Coustan-Smith et al., 2003).

While the studies discussed above cases were selected according to the degree of aberrancy of LAIPs and only 50% to 80% of all cases were thus included, two recent reports indicate that prognostication by flow cytometric assessment of MRD is possible in the large majority of patients with AML (Kern et al., 2004).

Thus, the MRD levels both during aplasia after induction therapy as well as during CR (before and after consolidation therapy) were shown to strongly correlate with the prognosis of the patients. In 106 unselected patients MRD levels at day 16 after start of induction therapy were significantly related to the achievement of CR (p=0.0001), event-free survival (p<0.0001), overall survival (p=0.003), and relapse-free survival (p=0.0003). Importantly, the prognostic impact of day 16 MRD was independent of cytogenetics and other prognostic parameters with regard to achievement of CR, event-free survival, and relapse-free survival. An additional analysis of 58 patients before and 62 patients after consolidation therapy revealed similar results with independent prognostic impact of MRD levels at both check points.

These data are in line with previous reports and in addition demonstrate that the flow cytometric assessment of MRD

a. may be extended to virtually all patients with AML and

b. yield powerful prognostic parameters applicable as stratification parameters in clinical trials.

Improvement of MRD quantification by CD45 gating

The studies published so far on the detection of MRD using MFC applied three fluorochromes and different triple-combinations of monoclonal antibodies. This implies that the basic parameters used in all combinations for the identification of the blast population are the light scatter characteristics, i.e. forward-light scatter (FLS) and orthogonal-light scatter (OLS). A significant step forward has been the introduction of the simultaneous detection of four fluorochromes and the use of CD45-gating. Since CD45 is differentially expressed not only between different lineages of hematopoietic differentiation but also within these lineages during the process of maturation it is capable, in combination with OLS, of clearly differentiating blasts from other cells (Borowitz et al., 1993) as is shown in figure 3.

Applying this approach in the context of immunologic MRD monitoring has been shown to improve the sensitivity of the method by almost one log (Kern et al., 2004). Future clinical trials have to
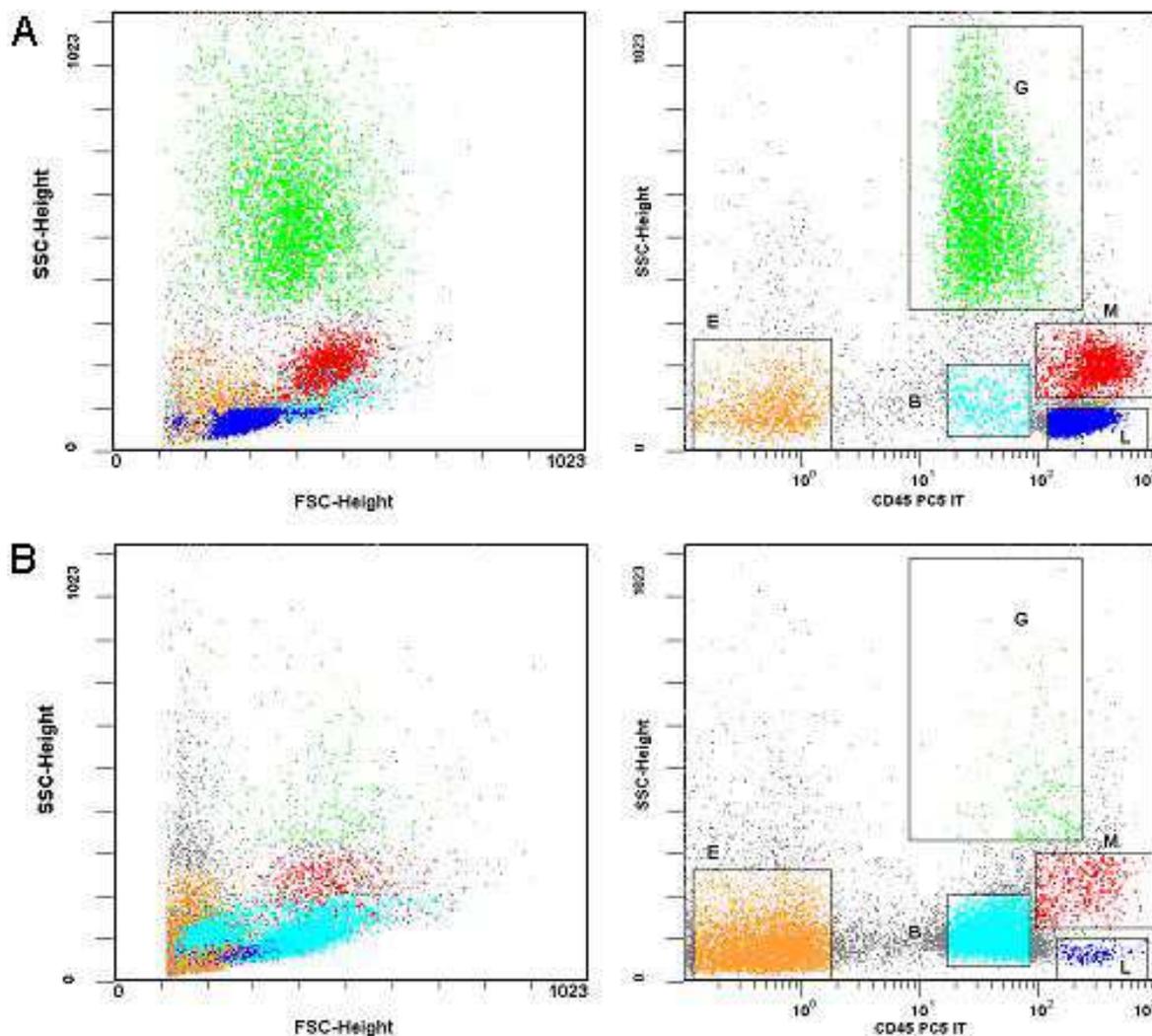
Figure 3: Improvement of separation of populations by CD45 gating. A=normal bone marrow (left: FLS-OLS plot, right: CD45-OLS plot), B=AML bone marrow (left: FLS-OLS plot, right: CD45-OLS plot), G=granulocytes, M=monocytes, L=lymphocytes, E=erythrocytes, B=blasts. CD45-OLS gating allows to isolate bone marrow blasts from all other populations which is not possible by FLS-OLS gating.

validate the translation of these data into improved prognostic parameters.

Summary of MRD quantification by MFC in AML

Flow cytometric quantification of MRD in AML is feasible in the large majority of patients and results in powerful prognostic parameters which are independent of pretherapeutic parameters like cytogenetics, age, and AML occuring as a secondary disease. A comprehensive panel of monoclonal antibodies is necessary to allow the detection of a LAIP in each patient at diagnosis. The use of CD45-gating may improve the prognostic power of MRD measurements. Further large-scale clinical trails will determine the optimum time points for MRD assessment and the relative value of MFC-based and PCR-based methods of MRD quantification.

Assessment of MRD in AML by quantitative PCR, general aspects

Quantitative RT-PCR (QRT-PCR) serves a highly sensitive method capable of quantifying MRD in patients with AML which has resulted in powerful prognostic parameters (Marcucci et al., 2001; Schnittger et al., 2003; Buonamici et al., 2002; Guerrasio et al., 2002; Gallagher et al., 2002; Krauter et al., 2003). While in most studies leukemia-specific fusion gene associated with recurrent cytogenetic translocations were targeted several other genetic alterations like length mutation of FLT3 and partial tandem duplications of MLL have been increasingly used as targets for MRD quantification (Schnittger et al., 2004). As a consequence, the general applicability of QRT-PCR for quantification of MRD has been increased from 20% to 50% of all patients with AML

and may increase to the vast majority of all patients if the overexpression of WT1 and EVI1 and other potential targets are confirmed to result in clinical meaningful data (Cilloni et al., 2003; Ogawa et al., 2003).

PCR-targets for MRD detection

Targets that are available for MRD detection in AML include
1. fusion-gene transcripts,
2. breakpoint regions around chromosomal or molecular rearrangements, and
3. aberrantly expressed genes.

With the exception of aberrantly expressed genes most of these targets are highly specific and have no background signal in normal cells. The applicability of these different targets varies per disease. In AML the fusion transcripts were the most frequently applied targets so far. However, the use of breakpoint specific assays and aberrantly expressed genes as targets for MRD detection in AML are of growing importance, especially to increase the number of cases that can be monitored by sensitive PCR techniques.

Quantification of a target

Whereas with MFC the real number of malignant cells in a population can be assessed, the QRT-PCR measures the mRNA expression level of an AML-specific gene relative to one or more other reference genes.

The RNA gain per cell may greatly vary due to the time from sampling to RNA preparation, stabilisation of sample, cell count per µl, and other factors. In addition, the cDNA yield may vary mainly due to differences in daily used enzyme lots and inhibitors in the sample.

To normalize for such quality variation each transcript to be quantified has to be compensated with the expression of one or more reference genes. As reference gene usually so-called housekeeping genes are being used that are believed to be expressed at similar levels in all different cell types. An attractive feature of this implementation of reference genes in comparison to standard RT-PCR is that the final sensitivity of each individual assay can be documented and poor quality samples can be eliminated. It is still a matter of debate which housekeeping gene is the most stable and most suitable one in AML.

The Europe Against Cancer (EAC) program initiated a comparison of 14 different potential control genes.

Based on the absence of pseudogenes and the level and stability of expression, three genes were finally

selected:
- Abelson (ABL),
- beta-2-micoglobulin (B2M), and
- beta-glucuronidase (GUS) (Beillard et al., 2003).

Out of these three, ABL was the only one that did not differ significantly between normal and leukemic samples at diagnosis and therefore was proposed to be used as reference in leukemic patients (Weisser et al., 2004).

Quantification of fusion genes in AML

The PML-RARA , AML1-ETO , and CBFB-MYH11 fusion genes are the three most common ones in AML. Although all are associated with a prognostically favorable subgroup of AML even in these subtypes 10-30% of cases are prone to treatment failure and early identification of these high risk patients is important to adjust treatment strategies. A number of studies dealing with the quantification of these fusion transcripts have shown the importance of MRD detection in these AML subtypes (Marcucci et al., 2001; Schnittger et al., 2003; Buonamici et al., 2002; Guerrasio et al., 2002; Gallagher et al., 2002; Krauter et al., 1999; Cassinat et al., 2000; Krauter et al., 2001).

Transcript ratios at diagnosis

Like for all MRD analyses the first time point to measure the expression of a fusion gene must be the diagnosis of AML in order to assess the molecular subtype of the fusion gene as this may vary tremendously at the molecular level. In addition, this value is mandatory as the basis value for all following MRD time points.

It has been shown in a number of studies using different housekeeping genes that transcript ratios may vary up to two orders of magnitude at diagnosis (Marcucci et al., 2001; Schnittger et al., 2003; Krauter et al., 2003; Krauter et al., 1999; Krauter et al., 2001). A recent large study based on 349 patients showed in AML1-ETO-, CBFB-MYH11-, and PML-RARA-positive patients that transcription levels at diagnosis are correlated with clinical outcome independently of other parameters (Schnittger et al., 2003). With a range of 2.5 orders of magnitude these variations were most prominent in AML1-ETO-positive cases. This variation can only be explained by high differences in expression activity of the malignant cells between individual patients.
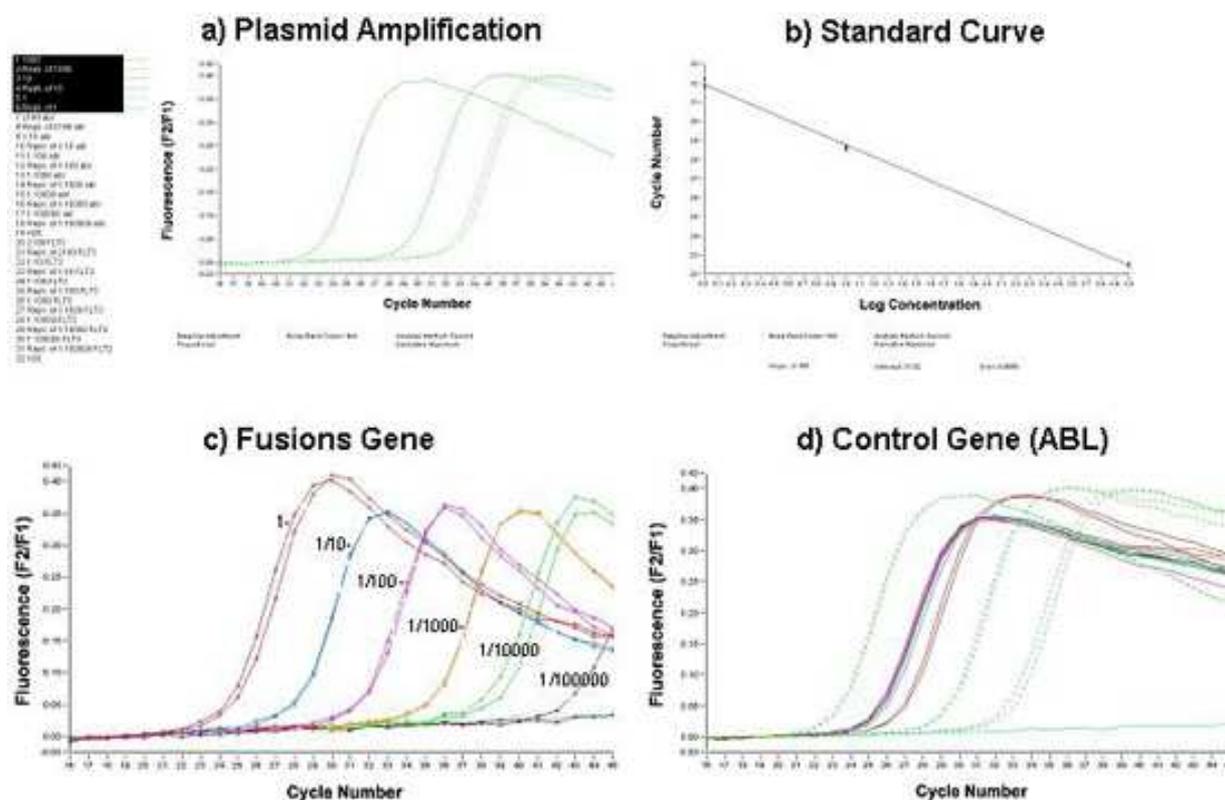
Figure 4: Quantification by real time PCR. a) Amplification of defined plasmid dilutions containing the target to be quantified. b) Standard curve that is constructed from the plasmid dilution. c) Limited dilution series of a fusion gene positive in a fusion gene negative sample, indicating the sensitivity of the assay. d) Assessment of the ABL control gene in the same samples as depicted in "c".

Transcription reduction during therapy and its impact on prognosis

The kinetics of reduction of the transcript ratio after the first and second induction therapy were found to have no impact on OS or EFS while the transcript levels after consolidation treatment had (Schnittger et al., 2003; Guerrasio et al., 2002).

- In contrast, Krauter et al., (Krauter et al., 2003) suggested a minimum of 2 log reduction directly after induction therapy to be a prerequisite for ongoing remission.
- In another study all patients with CCR ultimately obtained PCR negativity and the authors suggested that the "cure" of M4eo is strictly associated with CBFB-MYH11 negativity (Guerrasio et al., 2002). In addition, they defined a threshold of one CBFB-MYH11 copy/10,000 ABL copies in CR to discriminate high and low risk patients.
- In a further report focussing on inv(16)/CBFB-MYH11-positive AML the authors suggested based on a limited number of patients a critical transcript ratio of 0.25% at any time in remission to be predictive for relapse (Buonamici et al., 2002).

- In a recent study on CBF leukemias that included 37 AML1-ETO- as well as CBFB-MYH11-positive AML a cut of level of 1% of the initial ratio at all time points after induction therapy discriminated two prognostically different groups (Krauter et al., 2003). For PML-RARA-positive AML it was shown that patients with lower than a 5 log reduction had a 4-fold risk of relapse (Gallagher et al., 2002).
- A further recent study showed that even lower levels than 0.25% have been detected in three patients after previous negativity all of whom subsequently relapsed shortly thereafter (Schnittger et al., 2003). Therefore, the different thresholds defined by different groups may be too insensitive and due to the high interpatient variability of transcript levels general cut-off levels may not be appropriate. It seems more informative to monitor the kinetics in single patients and to use an increase of transcript levels as predictor for relapse.

Due to very variable time points of MRD testing that were applied in different studies the most predictive time point and the time span between follow up assessments are still unknown and must be defined in prospective and multicenter trials.

New score for risk stratification of AML

Based on a multicenter study with 131 patients (34 with AML1-ETO, 42 with CBFB-MYH11, and 55 with PML-RARA) evaluated at diagnosis as well as after consolidation therapy a prognostic score was established by combining the transcript ratio of both checkpoints (Schnittger et al., 2003). In all three AML subtypes a good risk group was identified by a transcription level at diagnosis of less than the 75%-percentile and an MRD level of less than the median in which no case with treatment failure was observed. In contrast, patients with either a higher transcription level at diagnosis or a higher MRD level after consolidation therapy did significantly worse.

Prediction of relapse

In a limited number of cases a molecular relapse was demonstrated to precede the hematologic relapse by some weeks (Marcucci et al., 2001; Krauter et al., 2003).

The largest study focussing on the prediction of relapse by an increase of a fusion gene detectable by PCR analyzed 142 patients at three or more different time points during and after therapy (Schnittger et al., 2003). Fifteen of these cases (10.6%) relapsed. In eight patients increasing transcript ratios indicated molecular relapses 1-5 months before hematologic and cytogenetic relapses occurred. An increase even from the undetectable to a marginal transcription level

significantly predicts relapse. Stressing the importance of small follow-up intervals, in all seven relapsing patients with follow up intervals larger than six months relapses were not detected beforehand by PCR. In general, follow-up assessments should be performed every three months in order to early identify impending relapse (Marcucci et al., 2001; Schnittger et al., 2003; Krauter et al., 2003).

FLT3-LM, MLL-PTD, and other genetic alterations

Besides the leukemia-specific fusion genes other genetic alterations have been evaluated as potential targets for quantification of MRD.

FLT3-LM (length mutation of FLT3) are detected in 20-25% of all AML and in approximately 40% of AML with normal karyotype or with other prognostically intermediate cytogenetic aberrations like trisomie 8, trisomy 11 and del(9q) , i.e. AML subgroups in which PCR was not applicable due to the lack of fusion genes (Schnittger et al., 2002). In a series of 97 paired diagnostic/relapse samples it could be shown that in the majority of cases (96%) the FLT3-LM was present at both time points (Schnittger et al., 2004). The sensitivity as estimated by limited dilution series ranged between 1:100 and 1:1,000. In six cases it was possible to detect FLT3-LM positivity after previous PCR-negativity 1-3 months before cytomorphological relapse. In another four cases a pending relapse was detectable by persistent PCR positivity of the FLT3-LM (Schnittger et al., 2004).
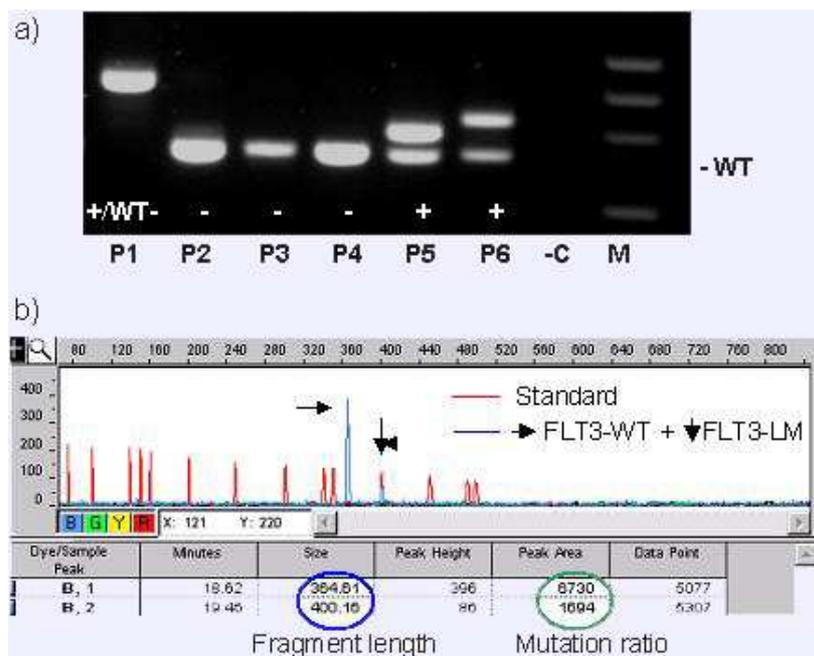

Figure 5: Semiquantitative and quantitative analysis of FLT3-LM. a) Semiqualitative conventional agarose gel electrophoresis. The wildtype allele (WT) is the smallest fragment. All that are larger than this fragment indicate FLT3-LM. Patients P1, P5, and P6 are positive for a FLT3-LM. P1 in addition has a loss of the WT-allele. –C: H2O control. M: molecular weight marker. b) Fragment analysis for quantification of the FLT3-LM. In red the standard. In blue the FLT3-WT and the FLT3-LM.
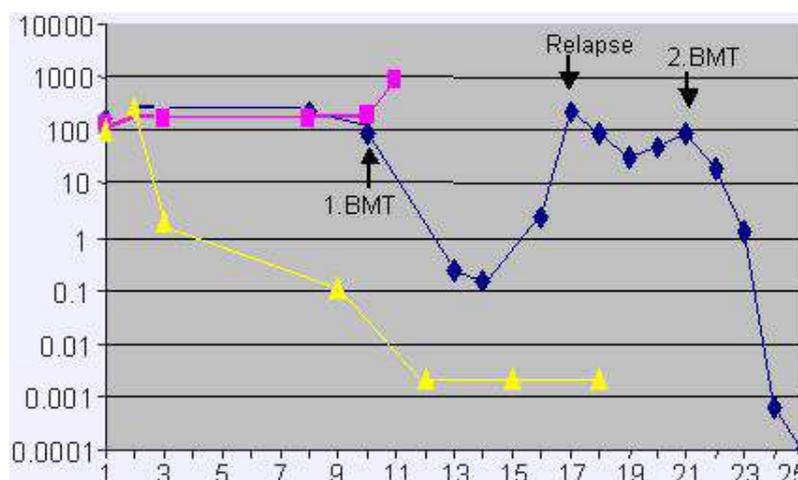
Figure 6: Examples of follow up with MLL-PTD in three patients. In pink a resistent patient. In blue a case with bad response to standard chemotherapy that underwent bone marrow transplantation. In yellow a good responder.

A high expression level of MLL-PTD (partial tandem duplications of MLL) is present in unselected AML at diagnosis in 6.5% and in AML with normal karyotype in 10% (Schnittger et al., 2003; Schnittger et al., 2000). A real time PCR assay has been recently developed which is sensitive and quantitative and reflects treatment response as well as early detects relapse (Schnittger et al., 2004). However, the presence of positive cells in normal bone marrow in the range between 0.01% and 0.05 % may indicate a limited sensitivity.

Additional genetic mutations which are currently analyzed with regard to their usefullness for MRD quantification include overexpression of WT1 and EVI1 (Cilloni et al., 2003; Ogawa et al., 2003).

Summary of MRD quantification by QRT-PCR in AML

Quantification of MRD in AML by QRT-PCR is feasible in 50% of patients and results in powerful prognostic parameters, in particular in patients assigned a favorable prognosis by pretherapeutic parameters. A QRT-PCR analysis targeting specific genetic alterations and even a screening analysis must be performed at diagnosis in order to identify and quantify the target that may be used for MRD assessment. Further large-scale clinical trials will determine the optimum time points for MRD assessment and the relative value of QRT-PCR-based and MFC-based methods of MRD quantification.

Comparison of multiparameter flow cytometry and quantitative RT-PCR

Given the powerful applicability of both MFC-based and QRT-PCR-based quantifications of MRD to estimate the patients prognosis it becomes increasingly important to assess the respective value and superiority of either method for distinct clinical settings.

However, data published on this issue are scarce. Preliminary data indicate that for the majority of cases both methods reveal comparable results (Kern et al., 2003). Thus, out of 372 follow-up assessments from 144 patients with AML analyzed by both methods in parallel both at diagnosis and at follow-up 61% were found to give concordant results with regard to positivity and negativity, respectively. Another 19% were found positive by QRT-PCR and negative by MFC and 20% vice versa demonstrating differences in the sensitivity between both methods for different targets. QRT-PCR was superior to MFC in most cases with CBF leukemias due to the very good sensitivity of the fusion transcripts CBFB-MYH11 and AML1-ETO. Conversely, MFC was superior to QRT-PCR in many cases with acute promyelocytic leukemia due to the highly sensitive LAIP HLA-DR-CD33+CD34+ which, however, occurs in only 20% of all cases with acute promyelocytic leukemia.

With regard to the quantitative comparison of data obtained by both methods there were also highly significant correlations which is also true for the comparison of the courses of MRD as determined by both methods in individual patients. The performed evaluations in addition indicate that MRD levels determined at different check points by both methods correlate with the prognosis, however, additional analyses are needed to get conclusive results on the topic which method to apply at which check point for which subentity of AML.

Overall, QRT-PCR due to the high sensitivity is anticipated to be most important in CBF leukemias while MFC due to its applicability to virtually all patients with AML is anticipated to be more important in the remaining cases. Additional studies will have to be performed to substantiate these data (Coustan-Smith et al., 2003). Clearly, approaching these issues should be led by the search for the clinically most informative and therefore most useful application in order to help

optimizing the therapeutic management of patients with AML.

Conclusions

Based on published data it is anticipated that the quantification of MRD will significantly improve the estimation of the prognosis in patients with AML and as a consequence will play a major role as a stratification parameter to guide the risk-adapted therapy of the disease.

In cases with CBF leukemias, i.e. AML with t(8;21) and inv(16)/t(16;16), QRT-PCR is more sensitive as compared to MFC and thus will be used for MRD quantification preferably in these cases.

In contrast, MFC offers an applicability with a clinically relevant sensitivity to virtually all patients with AML and thus is considered an ideal tool for the use of MRD quantification in large AML populations including all subtypes of the disease. Technical improvement like the use of five or more colors in MFC and the identification of additional leukemia-specific genetic targets which can be quantified by QRT-PCR will lead to a further increase of the applicability and sensitivity of both methods for MRD quantification.

Future studies will define which methods at which check point are most useful for the management of patients with AML.

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