Has MRD monitoring superseded other prognostic factors in adult ALL?

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Running title:
MRD as main prognostic factor in adult ALL

Keywords:
Acute lymphoblastic leukemia, ALL, Minimal residual disease, MRD, flow cytometry, PCR
ABSTRACT

Significant improvements have been made in the treatment of acute lymphoblastic leukemia (ALL) during the past two decades, and measurement of submicroscopic (minimal) levels of residual disease (MRD) is increasingly used to monitor treatment efficacy. For a better comparability of MRD data there are ongoing efforts to standardize MRD quantification using real-time quantitative polymerase chain reaction (RQ-PCR) of clonal immunoglobulin and T-cell receptor gene rearrangements, RQ-PCR-based detection of fusion gene transcripts or breakpoints, and multiparameter flow cytometric immunophenotyping. Several studies have demonstrated that MRD assessment in childhood and adult ALL significantly correlates with clinical outcome. MRD detection is particularly useful for evaluation of treatment response, but also for early assessment of an impending relapse. Therefore MRD has gained a prominent position in many ALL treatment studies as tool for tailoring therapy with growing evidence that MRD supersedes most conventional stratification criteria at least for Ph-negative ALL. Most study protocols on adult ALL follow a two step approach with a first classical pretherapeutic and a second MRD-based risk stratification. Here we discuss whether and how MRD is ready to be used as main decisive marker and whether pretherapeutic factors and MRD are really competing or complementary tools to individualize treatment.
INTRODUCTION

Treatment outcome in ALL patients depends on a combination of multiple factors such as properties of the leukemic cells (e.g. proliferative capacity, susceptibility to drugs and other escape mechanisms), host factors (e.g. general fitness and concomitant diseases, treatment compliance, host pharmacodynamics and pharmacogenetics), and treatment given to eradicate the disease. Many of intrinsic leukemia cell and host factors have already been elucidated with immunologic and molecular methods and are ongoing translated into providing prognostic information (Table 1). Based on retrospective analyses of large cohorts of patients, conventional pretherapeutic risk criteria, including age, elevated white blood cell count (WBC) at diagnosis, adverse immunophenotypic features and cytogenetic as well as molecular aberrations provide the basis for upfront risk stratification in current treatment protocols. It has to be acknowledged, however, that these advances in our understanding of ALL biology in the past have only to a limited extent been accompanied by improved survival of adult ALL patients with relapse still being the main clinical problem. Source of these relapses is the persistence of minimal residual disease (MRD) that is undetectable by standard diagnostic techniques. Several studies have shown that detection of MRD in childhood and adult ALL is an independent risk parameter of high clinical relevance, both in de novo and relapsed ALL as well as in ALL patients undergoing stem cell transplantation (SCT). Consequently, an increasing number of treatment protocols use MRD as a tool for treatment stratification. In addition, post remission MRD monitoring is also used to predict an impending relapse and to start pre-emptive salvage treatment in time. Therefore, MRD is not only a prognostic factor, but challenges the traditional concept of defining remission and relapse. Prerequisite for application of MRD for treatment tailoring is an adequate, sensitive and standardized MRD methodology. The focus of this Perspective is therefore to highlight pros and cons of the different MRD techniques, to present the published experience of MRD analysis and MRD guided treatment in adult ALL and to discuss the value of MRD in different clinical settings compared to other prognostic factors.
DOES METHODOLOGY OF MRD DETECTION MATTER? PROS AND CONS OF DIFFERENT TECHNIQUES

MRD assessment relies on the identification of specific molecular or immunophenotypic markers on the leukemia cells. Flow cytometry (FCM) is applied to detect combinations of cell markers that are present on the leukemic but not on normal bone marrow cells. PCR is used to detect leukemia-specific fusion transcripts (e.g. BCR-ABL) or clone specific immunoglobuline (Ig) or T-cell receptor (TCR) genes.

Each of these techniques has its own strengths and limitations that are summarized in Table 2 and are described below in more detail.

**Multiparameter flow cytometry**

MRD measurement by FCM is based on the detection of leukemia-associated immunophenotypes (LAIP) that can be used to distinguish them from normal hematopoietic cells. LAIPs usually describe a subpopulation of cells of a given lineage at a particular differentiation stage with aberrant molecular expression patterns, asynchrony, and/or profound over- or underexpression of molecules. Therefore-unlike molecular methods- FCM needs the identification of a cluster of events for definition of MRD positivity. Using 4-colour flow cytometry LAIPs can be identified in about 90% of B-precursor and more than 95% of all T-ALL patients reaching detection limits of $10^{-3}$ to $10^{-4}$.5;16-22

The major advantage of FCM is its rapidity, which allows reporting of quantitative results within one day. This is specifically useful when MRD results are needed quickly to guide therapy. In addition, FCM allows the simultaneous assessment of cell qualities requisite for emerging targeted therapies in ALL.23-24 A potential pitfall of the method results from similarities between leukemic lymphoblasts and non-malignant lymphoid precursors in various phases of regeneration or chemotherapy-induced alterations that may lead to false positivity.18;25 In addition, phenotypic shifts frequently occur in leukemic cells during induction among others due to steroid-induced gene expression modulation.23-28

In times of targeted therapies (e.g. anti-CD19, anti-CD20, anti-CD22 or anti-CD33 treatment) also completely unknown marker shifts may occur potentially influencing detectability of residual leukemic cells. Therefore, interpretation of MRD FCM data requires a deep understanding of the expression patterns of benign hematogones and leukemic cells within different treatment phases and should be restricted to reference laboratories even if FCM is broadly available in many hematology centers.16
Also careful standardization of experimental setup and interpretation is needed in order to obtain comparable MRD results in multicenter studies which is one topic of international study groups. Additional efforts focus on improving the method and its sensitivity through usage of more colors (≥ 8), inclusion of new markers, possibly more specific for leukemic cells and development of new software for fast and easy automated data analysis.

**PCR analysis of Ig and TCR gene rearrangements**

Ig and TCR genes rearrangements represent fingerprint-like DNA regions of individual lymphoid cells and its descendants making them to attractive targets for clone specific PCR. Prerequisite is the molecular characterization of leukemia-specific Ig/TCR gene rearrangements for each individual patient which is possible for vast majority (>95%) of B- and T-lineage ALL. Quantification of these target genes is nowadays performed with real-time quantitative (RQ)-PCR that allows for a higher degree of automation and sample throughput without the need of post-PCR manipulation of samples. Similarly to rearranged immune genes, also some leukemia specific translocations (in particular MLL rearrangements) can be used as targets for DNA based RQ-PCR analysis after the rearrangement break point at the DNA level has been characterized. Sensitivity is determined exactly for each assay and generally reaches 1:10^4 to 1:10^5 which is about 0.5 to 1.0 log higher than for published FCM based MRD assays.

Right from the start, DNA based MRD diagnostics has been highly standardized through the efforts of the EuroMRD Group. Guidelines are published for determination of linearity, sensitivity, specificity, and reproducibility for each clone-specific assay. Usage of DNA as analytical sample allows long shipping times, sample storage and retrospective cumulative PCR analyses of sample batches. Nevertheless there are some caveats including clonal evolution of Ig/TCR genes which may lead to false negativity. It is therefore recommended to use at least two independent targets. On the other hand, false positive MRD results cannot be completely excluded as massive regeneration of normal lymphoid progenitors might lead to low levels of nonspecific amplification. In addition, the method needs the time consuming initial characterization of the leukemic Ig/TCR rearrangements with a panel of different PCR and sequencing reactions. However, recent advances in sequencing technology (next generation sequencing) will probably accelerate this process in the near future and even add a new molecular technology for MRD assessment.
**PCR analysis of fusion transcripts**

Leukemia specific gene fusions represent ideal targets for MRD detection because they are linked to the oncogenic process and are therefore highly specific. Only few translocations (mainly MLL rearrangements\(^3\) and \textit{SIL-TAL1} translocations) are analyzed on DNA level, the broadest routine clinical use is the quantification of aberrant BCR-ABL mRNA transcripts in Philadelphia (Ph)-positive ALL.

Advantages of RQ-PCR to evaluate fusion transcripts in Ph-positive ALL include the high sensitivity of up to \(10^{-6}\) due to presence of multiple specific mRNA-transcripts per cell. Furthermore, these transcripts allow the usage of the same primer/probe-combinations for RQ-PCR of many patients thus keeping the costs for the individual analyses low. In addition, there is a broad experience in BCR-ABL PCR assays because it is widely used for MRD monitoring in CML. However, methodologies are not fully comparable for both entities (dominance of minor break-point transcript in Ph-positive ALL compared to major breakpoint cluster region in CML, differences in relevant MRD thresholds with higher sensitivities being required in Ph-positive ALL).

A crucial point for BCR-ABL PCR is the sample quality, which is adversely influenced by long transport times resulting in degradation of RNA. In addition, lack of standardization of RNA extraction, cDNA synthesis, selection of housekeeping genes and variation in the way RQ-PCR analyses are carried out, lead to substantial differences in results and carry the risk of false negativity. Also false positivity cannot be ruled out e. g. due to cross-contamination and is observed in external quality controls even among experienced laboratories. There are ongoing efforts to standardize methodology and interpretation to allow a better comparability of PCR results within different clinical trials.\(^4\)

**Proposal for a uniform description of MRD results**

An important step towards a better comparability of MRD data is the standardized and uniform description of MRD results. Irrespective of the methodology used a testing result may be (1) negative, (2) positive without being quantifiable due to the rarity of the event and (3) positive at a quantifiable level (Figure 1).

Concerning Ig/TCR-PCR these results are defined by the EuroMRD Group based on the general performance, linearity, reproducibility and background of the individual assay.\(^3\) Also for BCR-ABL and FCM MRD there are ongoing international efforts to define generally accepted guidelines for standardized analysis and interpretation of results.\(^3\)
Based on these efforts also a uniform MRD terminology referring to terms that are already established for cytomorphology with definitions of complete MRD response, MRD persistence and MRD relapse seems feasible 16 (Figure 1).
MRD FOR ASSESSMENT OF REMISSION AND RELAPSE COMPARED TO OTHER PROGNOSTIC FACTORS

The two major applications of MRD in adult ALL are (1) the assessment of response to initial therapy for definition of MRD-based risk groups and (2) the subsequent monitoring of remission patients to detect MRD reoccurrence and allow a pre-emptive salvage treatment.

**MRD for initial remission assessment**

The most significant application of MRD for *de novo* ALL is the sensitive assessment of treatment efficacy in patients reaching a complete morphological remission, thereby refining initial risk stratification. Compared to childhood ALL with reports on several thousand patients\(^2\,4^5\), MRD in adult ALL has been studied less extensively. Nevertheless, also in adult ALL large studies have shown that initial MRD kinetics is highly predictive for outcome (Table 3).

**Ph-negative ALL**

Several studies reported on the independent prognostic value of MRD in adult Ph-negative ALL performing either Ig/TCR-RQ-PCR\(^1\,3\,9\,4^6\,4^7\) or FCM\(^1^2\,2^2\,4^8\) (Table 3). Concordantly, different European study groups demonstrated that MRD persistence measured at different time-points 1-6 months after initial diagnosis is associated with a poor prognosis.\(^1\,3\,9\,2^2\,4^6-4^8\) Brüggemann et al.\(^3\) and Vidrias et al.\(^2^2\) additionally identified a small subset of patients with a very rapid tumor clearance (low-level/undetectable MRD after 2 weeks of therapy) and an excellent prognosis. The presently largest MRD study on adult ALL was recently published by Gökbuget et al.\(^4^6\) and analysed MRD in Ph-negative patients with standard risk (SR, n=434) and high risk (HR, n=146) features. A complete MRD response after induction 2 and/or consolidation 1 was associated with a comparable clinical benefit irrespective of pretherapeutic risk factors. MRD was the only parameter with significant prognostic impact in multivariate analysis. The biological differences between SR and HR patients were reflected by the significant different proportion of patients reaching a complete MRD response between SR and HR patients with about 20 percent point lower rates of MRD negativity in HR patients.

Whether or not rare factors like t(4;11) may retain their prognostic significance is hard to state as these patients are diluted within the whole population. Cimino and colleagues analyzed a limited number of adult MLL-AF4-positive patients demonstrating the prognostic impact of MRD also for this rare biologic entity.\(^4^9\)
**Ph-positive ALL**

In Ph-positive ALL most published studies focus on detection and quantification of BCR-ABL transcripts. In the pre- tyrosine kinase inhibitors (TKI) era, the level of MRD after induction and/or consolidation treatment turned out to be a powerful indicator of prognosis although data differed concerning the discriminative value of different time-points for MRD assessment\(^{10,50}\) (Table 3). Introduction of TKI substantially changed treatment outcome and MRD kinetics. In a study of Lee et al.\(^{51}\) MRD assessment after induction chemotherapy did not retain its prognostic significance when followed by imatinib treatment. Conversely, a reduction of BCR-ABL transcript levels of at least three log after the first 4-week imatinib therapy was identified as the most powerful predictor of a better disease free survival (DFS) and overall survival (OS) rate after SCT (4-year DFS 82.1% versus 41.7%, \(p=.009\), 4-year OS 82.3 versus 48.6, \(p=.007\)). These findings indicate that poor response to chemotherapy may be compensated by subsequent administration of imatinib. Leguay et al.\(^{52}\) presented data of the GRAAL AFR03 study that imatinib combined with high dose chemotherapy improved molecular remission rate before transplantation and led to an improved outcome. In contrast, Yanada et al.\(^{53}\) failed to establish an association between an early MRD response to imatinib combined chemotherapy and outcome and concluded that relapse risk may depend on factors unrelated to initial treatment response (e. g. outgrowth of pre-existing subclones with resistance mutations.\(^{54}\))

**Post remission MRD monitoring**

A second important application of MRD is post remission monitoring of patients reaching complete MRD response for early detection of an impending relapse.

**Ph-negative ALL**

Raff at al.\(^{55}\) was the first to demonstrate within the German Multicenter ALL Study Group (GMALL) trials that molecular relapse defined as reconversion to quantifiable molecular MRD positivity was followed by a clinical relapse after a median time of 4.1 months in Ph-negative ALL. Remarkably, low-level, non-quantifiable MRD was not necessarily related to a subsequent relapse confirming this MRD value as a sort of grey area.\(^{16}\) A recent update of Raff’s study by Gökbüget et al.\(^{46}\) confirmed the close correlation between conversion to quantifiable MRD positivity and subsequent relapse: in 34 patients with conversion to quantifiable MRD positivity (“MRD relapse”, \(n=13\) during first year of treatment,
n=21 subsequently) the probability of continuous complete remission (CCR) was 21±9% at five years. If patients with MRD-based SCT in first CR were excluded the probability of CCR was only 0% (5±5% at 3 years).

**Ph-positive ALL**

In Ph-positive ALL several studies published already in the 1990s showed a significant relationship between conversion to MRD positivity and subsequent relapse in the pre-TKI era. Yanada and colleagues confirmed these findings also for Ph-positive patients being treated with imatinib-combined chemotherapy. In a prospective study on 100 adult patients with Ph-positive ALL showed an MRD elevation in CR. Of these, 12/13 who had not undergone allogeneic (allo) SCT experienced a relapse whereas only 3/16 patients who underwent allo SCT relapsed. The authors concluded that an increase in MRD is predictive of a subsequent relapse, but such patients can be successfully treated with allo SCT.
MRD BASED TREATMENT INTERVENTION

Treatment stratification according to initial MRD response

The objective of measuring MRD response to initial therapy is to adjust treatment with the ultimate goal to improve outcome of MRD-HR patients and to reduce toxicity in MRD-LR patients without worsening their prognosis.

Several clinical trials on adult ALL implemented MRD into their treatment stratification: The PETHEMA ALL-AR-03 trial focuses on HR Ph-negative ALL and passes on SCT in first complete remission in case of standard cytologic response (<10% blasts in BM on day 14) and MRD <5x10\(^{-4}\) after early consolidation. In contrast, patients with a slow cytologic response and/or MRD >5x10\(^{-4}\) after early consolidation receive allo SCT further on. Preliminary results indicate that the prognosis of HR patients with adequate response to induction and adequate clearance of MRD is not worsened by avoiding allo SCT. The combined MRD level after induction and consolidation therapy was the main prognostic factor for CR, DFS and OS although a part of MRD-LR patients received a MRD-based de-escalated therapy.

Within the GMALL 07/03 trial patients with persistent MRD >10\(^{-4}\) after induction (day 71) and/or first consolidation (week 16) were allocated to the MRD-HR group and qualified for allo SCT. Gökbüget et al. recently showed first results of MRD based treatment intensification in these patients: 120/504 evaluated patients (24%) were allocated to the MRD-HR group (89 SR and 31 HR patients defined by conventional criteria). In 47% of these patients, SCT was realized in first CR with the SCT rate being significantly higher in HR compared to SR patients (71% vs. 39%, p<.002). The probability of CCR after 5 years was significantly higher for patients receiving an MRD-directed SCT in first CR compared to those without SCT in first CR (66±7% vs. 12±5%, p<.0001). This also translated into a better OS at 5 years (54±8% vs. 33±7%, p=.06).

Bassan et al. described an MRD-oriented therapy for all t(4;11)\(^{neg}\)/t(9;22)\(^{neg}\) ALL patients within the Northern Italy Leukemia Group (NILG). MRD positive patients (defined as MRD >10\(^{-4}\) before induction-consolidation cycle 6 and MRD positivity before cycle 8) were allocated to allogeneic or autologous SCT, whereas MRD negative patients received standard maintenance regardless of classical risk factors. 4-year DFS was 76% in MRD-LR patients versus only 24% in the MRD-HR group despite of treatment intensification.
The first phase II clinical study prospectively analysing an MRD-based targeted therapy administered Blinatumomab monotherapy in patients with MRD failure or MRD reappearance (defined as quantifiable MRD >1x10^{-4} after end of first consolidation). Sixteen out of 20 evaluable patients (15 patients with MRD failure, 5 patients with MRD relapse) became MRD negative after one cycle of Blinatumomab treatment. Twelve of the 16 MRD responders had never achieved MRD negativity before. MRD negativity translated into an ongoing haematological remission in all 16 MRD negative patients within a median observation time of 405 days (1-year RFS probability 78%). One patient was censored due to withdrawal of informed consent during second treatment cycle, 8 of these patients consecutively underwent allo SCT without any treatment related mortality, 7 patients did not receive any further consolidation treatment, indicating the possibility of an improved outcome with this MRD-guided treatment even if data can only be compared to historical controls.

**Pre-emptive treatment in case of MRD recurrence**

MRD assessment is also used for pre-emptive treatment intervention in case of MRD recurrence. In the GMALL 07/03 trial salvage treatment in intended to be started at time of re-occurrence of quantifiable MRD. In a recent publication of Gökbuget et al.\textsuperscript{46} 10 out of 34 patients with MRD relapse underwent MRD triggered SCT in ongoing first CR. Three-year probability of CCR was 80±18% compared to only 5±5% in patients without transplantation. Other study groups also perform post-remission MRD monitoring for selected patient subgroups with some of them drawing clinical consequences in case of high level MRD (reviewed in\textsuperscript{16}). Besides SCT, also targeted therapies are investigated to improve outcome in patients with an MRD relapse.

In Ph-positive ALL, post remission MRD monitoring is mainly used to tailor treatment after SCT. Wassmann et al.\textsuperscript{63} investigated the effect of imatinib to decrease the relapse probability in case of re-conversion to MRD positivity after SCT. BCR-ABL transcripts became undetectable by both quantitative and nested RT-PCR in 15/29 (52%) patients. This was associated with a sustained remission whereas MRD persistence 6-10 weeks after start of imatinib treatment correlated with an almost certain relapse.
PROPOSAL FOR SELECTION OF SAMPLING TIME-POINTS AND METHODOLOGY

Optimal sampling time-points and sampling frequency have to be defined according to the individual protocol depending on the treatment protocol and the stratification aim. In Ph-negative ALL post-induction MRD assessment (after 2-4 months of treatment) is considered to have the most important role for evaluation of initial treatment response and MRD-based risk-stratification in de novo ALL. An MRD assessment during induction (after about 2 weeks of treatment) additionally identifies patients with a rapid tumour clearance and a particular good outcome. Concerning postremission monitoring of MRD for early relapse identification the GMAALL proposes three-monthly intervals for a total of three years as the majority of clinical relapses occur within this time and reconversion to MRD-positivity precedes a clinical relapse with a median time of 4.1 months between first quantifiable MRD positivity and relapse.

In Ph-positive ALL the value of MRD for initial remission assessment is more limited in the era of TKI whereas MRD assessment is frequently used for post-remission monitoring. However, compared to Ph-negative ALL relapse kinetics seem to be more rapid with median time between MRD elevation and relapse of only 2-3 months with or without application of TKI. Therefore, sampling frequency is recommended to be higher than in Ph-negative ALL.

Comparisons of MRD results obtained by different methodologies show that different techniques cannot be considered fully interchangeable. The main difference between FCM and Ig/TCR-PCR seems to be sensitivity with both methods quantifying single signals/leukemic cell. In contrast, BCR-ABL-PCR measures multiple transcripts/cell with copy numbers potentially varying during course of treatment. As there is evidence of multi-lineage involvement of Ph-positive cells also target cells may not be fully concordant to other MRD techniques. Therefore, choice of the MRD method in a particular protocol should be guided by the question to be answered, the experience gained in former MRD trials, the available technical expertise, the logistics, and whether or not treatment intervention is planned according to MRD results.
CAN PRETHERAPEUTIC RISK ASSESSMENT BE SKIPPED IN ADULT ALL?

The topic of this Perspective is to debate whether MRD superseded other risk factors. To answer this question it is essential to illuminate the value of MRD within different clinical settings but also to discuss pretherapeutic factors that partly changed their meaning from prognostic to predictive classifiers.

Classical risk factors versus MRD – the conventional concept of risk-oriented therapy

During the past three decades, treatment of adult ALL patients comprised an induction/consolidation chemotherapy followed either by additional consolidation cycles and maintenance treatment (partly supported by auto SCT) or allo SCT. Although there was also a discussion on optimization of chemotherapy elements the main therapeutic decision was whether or not to apply allo SCT which on the one hand leads to a reduced relapse rate but on the other hand is accompanied by severe side effects and a considerable treatment related mortality. Data exist for both treatment paradigms: Collaboration from the UK Medical Research Council (MRC) and the Eastern Cooperative Oncology Group (ECOG) demonstrated the superiority of allo SCT at least in younger patients.65 On the other hand pediatric-inspired chemotherapy regimens show favorable outcomes in adolescents restraining indication of allo SCT.66-68 Most protocols struck a balance between both approaches generally recommending SCT for patients with clinical risk factors and a high risk of postchemotherapy relapse.

Risk assignment is conventionally based on indirect measures of leukemic burden and chemosensitivity, such as chromosome, molecular and immunophenotypic analyses at diagnosis (Table 1, Figure 2A). Compared to all these pretherapeutic factors, MRD directly measures chemosensitivity for individual patients and integrates different host, leukemia and treatment related components of treatment outcome.

In fact, most published data recognize MRD as most important independent prognostic factor in adult ALL that supersedes all other risk factors, at least for Ph-negative ALL. Prospective studies on MRD-oriented therapy also indicate that MRD-based treatment is safely possible, allo SCT may be avoided in MRD-LR Ph-negative patients and seems to be particularly active in MRD-HR patients. In addition MRD measurement helps to prevent the problem of stratifying patients with newly identified genetic risk factors that are too rare to allow a reasonable assignment to a special risk group.
However, available MRD data presented here are not fully consistent: Optimal sampling time-points and MRD threshold seem to differ between ALL subgroups, in particular in Ph-positive compared to Ph-negative ALL. In addition, multivariate analyses in published studies partially retain prognostic factors other than MRD as independent variables (interestingly, the “old fashioned” factor elevated WBC at diagnosis emerges repeatedly). As an additional restriction -as true also for all other prognostic factors- MRD assessment is not realizable in all patients due to technical limitations (e. g. lack of identification of leukemia specific markers, missing follow-up samples). Depending on the minimum technical requirements, the MRD methodology and the adherence to the protocol, MRD-based stratification using stringent criteria seems to be feasible in about 80 to 90% of patients.\(^2,69\)

It also must be admitted that no published study on adult ALL performed a randomized comparison between action and no action on MRD but only compared the results of MRD-based treatment to historical outcome data or to groups of patients that did not receive an MRD guided therapy for whatever reasons. Therefore, as already started in childhood ALL, also in adult ALL randomized trials have to be done to confirm these encouraging data. MRD excellently qualifies for such an approach because it not only serves as marker for initial treatment stratification but also allows for an ongoing monitoring (Figure 2B). Thereby MRD can serve as “safety net” enabling early re-intensification in case of MRD-based treatment de-escalation.

**Targeted therapies – prognostic factors become predictive**

Increased understanding of molecular mechanisms of cancer and availability of drugs targeting them is changing the meaning of particular leukemic markers from solely prognostic towards predictive factors (Table 1). In adult ALL, BCR-ABL positivity can serve as a model for this transformation: Formerly, the detection of the Ph chromosome during diagnostic workup of adult ALL prognosticated an extremely poor outcome with remission rates being at least 10% lower than in Ph-negative ALL and with a median survival of only 8 months\(^70\). The sole curative option was an allo SCT but even in this setting Ph-positivity formed the risk group with the poorest outcome, making Ph-positivity to a “poorly tractable therapeutic problem”.\(^71\) However, things changed with the implementation of TKIs as a targeted therapy: CR rates improved and also evidence of a survival benefit emerges. Now BCR-ABL is not only a prognostic marker but also predicts response to TKI. In addition, this new therapeutic
approach changes MRD kinetics in Ph-positive ALL. Whereas in the pre-TKI era studies suggested a
good correlation between MRD and outcome, MRD data in the TKI setting are more conflicting, and
optimal time-points and thresholds are still a matter of debate. A potential reason for the discrepancy
between initial MRD response and outcome is the existence of low-level \textit{BCR-ABL} kinase domain
mutations prior to treatment. \cite{54,72,73} Whereas the leukemic bulk may well respond to treatment the
small-sized resistant subclone may be the origin of relapse potentially necessitating MRD analysis of
subclones in the future.

Even though many of new potential predictive markers are not ready to be used as treatment targets
in clinical routine due to issues related to reproducibility, statistical significance, and practical
applications the possibilities of targeted therapy are intriguing. However, when implementing new
elements into treatment protocols, the sensitivity of leukemic cells to a particular drug is not known in
advance. Traditional risk groups may well respond differently to targeted therapies than they do to
classical chemotherapy potentially leading to unpredictable treatment responses in different subgroups
of ALL. E. g. CD20 expression in B-lineage ALL which is under debate to be an adverse prognostic
factor \cite{74,75} is increasingly targeted by protocols including rituximab. Data from the MD Anderson\cite{76}
indicate that addition of rituximab to polychemotherapy alters MRD kinetics and significantly improves
MRD response. However, the effect of the same rituximab dose seems to be different in SR compared
to HR patients. \cite{77} Therefore it is particular important to evaluate treatment success separately within
the different subgroups to identify sensitive subpopulations. Ignoring baseline risk factors means
obscuring a possibly relevant treatment effect in distinct biological subsets by diluting in the whole
population. Therefore, assessment of pretherapeutic markers cannot be skipped in diagnostic workup
of ALL even if MRD is closely monitored as pretherapeutic and MRD markers increasingly become
complementary tools to tailor therapy.
MRD AS NEW PRIMARY ENDPOINT

In times of targeted and sequential therapies definition of an adequate and discriminatory primary variable for judging treatment success and planning subsequent treatment steps becomes more and more important. OS - the classical “gold standard” for a primary endpoint- as reliable, objective, and easily determined parameter gets problematic in times of multimodal and sequential therapies including SCT. The other end-point -the CR rate- is less objective in particular in B-cell precursor ALL where the distinction between leukemic cells and hematogones is exceedingly difficult in bone marrow during recovery after chemotherapy or SCT. The value of CR assessment is also limited by the fact that current treatment protocols lead to CR rate of about 90% in adult ALL. In contrast, MRD offers itself as an endpoint in this setting as it is a clinical parameter that integrates different leukemia, host and treatment aspects into one highly sensitive parameter. As a prerequisite for a usage as primary endpoint there has to be (i) a plausible biological relationship between reduction of MRD and response of the disease to therapy, (ii) the prognostic value of the surrogate for the clinical outcome has to be validated and (iii) the evidence from clinical trials has to exist that treatment effects on the surrogate correspond to effects on the clinical outcome. A necessary technical precondition is the availability of a standardized technology for MRD measurement regarding both standardized analysis and interpretation. This is currently primarily fulfilled for MRD-assessment using DNA-based RQ-PCR-analysis according to EuroMRD-standards and related consensus definitions obtained at the second international symposium on MRD-assessment. Ten years of international quality controls with more than 40 participating labs demonstrated the robustness of the system with intra- and inter-assay variability of less than half a log. Also FCM based MRD quantification and RQ-PCR of BCR-ABL transcripts are increasingly standardized within different international collaborations. These analyses have to be performed in accredited specialized laboratories as tests used in decision-making in clinical trials generally have to conform to the OECD Guidelines on Good Laboratory Practice or the Clinical Laboratory Improvement Amendments. As shown in large-scale pediatric trials this does not hamper a broad application to all eligible ALL patients. While not yet generally accepted as a primary endpoint of clinical trials by the European Medicines Agency and the Food and Drug Administration, MRD measurement in combination with adaptive trial designs may overcome part of the current difficulties in evaluating the efficiency of new agents in ALL.
CONCLUSION

Published data on MRD assessment in adult ALL have shown a strong correlation between MRD response and outcome as well as the prognostic value of MRD reappearance for hematological relapse. In times of individualized targeted therapies MRD will not substitute baseline risk factors but evaluate their impact within different treatment regimens and will help to optimize treatment sequence. In this context, MRD has also to be considered as quantitative and objective extension of established endpoints of hematological remission and relapse more than a substitute of pretherapeutic risk factors.
ACKNOWLEDGEMENTS

The authors thank Nicola Gökbuget, Dieter Hoelzer and the participants of the German Multicenter Study Group for adult ALL for their close collaboration in the MRD studies. This work was in part supported by the Wilhelm Sander Stiftung (2001-074.1 and 2001-074.2) and the Deutsche Krebshilfe (702657Ho2).

AUTHORSHIP

M.B. wrote the first draft of the paper: T.R. contributed to the writing and prepared figures. M.B., T.R. and M.K. finalized the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interest.

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Table 1: Pretherapeutic factors associated with outcome in adult acute lymphoblastic leukemia

<table>
<thead>
<tr>
<th>Factor</th>
<th>Prognostic impact</th>
<th>Potential impact on targeted therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Worse outcome with advancing age&lt;sup&gt;84;85&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>White blood cell count at diagnosis</td>
<td>B: &gt;30 x 10&lt;sup&gt;9&lt;/sup&gt;/L (B) T: &gt;100 x 10&lt;sup&gt;9&lt;/sup&gt;/L (T)</td>
<td>High WBC associated with poor prognosis&lt;sup&gt;84;85&lt;/sup&gt;</td>
</tr>
<tr>
<td>Immunophenotype</td>
<td>CD20 expression</td>
<td>Conflicting data concerning prognosis&lt;sup&gt;74;75&lt;/sup&gt;</td>
</tr>
<tr>
<td>T versus B</td>
<td>Independent prognostic significance (T-ALL with better prognosis) mainly in early studies&lt;sup&gt;84;85&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Cytogenetics</td>
<td>t(9;22)/&lt;i&gt;BCR-ABL&lt;/i&gt;</td>
<td>Poor prognosis&lt;sup&gt;86;87&lt;/sup&gt;</td>
</tr>
<tr>
<td>t(4;11)/&lt;i&gt;MLL-AF4&lt;/i&gt;</td>
<td>t(8;14) Hypodiploidy* near triploidy Complex karyotype**</td>
<td>Poor prognosis&lt;sup&gt;86;87&lt;/sup&gt;</td>
</tr>
<tr>
<td>t(1;19)</td>
<td>Conflicting data concerning prognosis&lt;sup&gt;88;89&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>High hyperdiploidy***</td>
<td>Better prognosis&lt;sup&gt;86&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Specific molecular alterations</td>
<td>JAK mutations</td>
<td>Emerging significance of poor prognosis&lt;sup&gt;90&lt;/sup&gt;</td>
</tr>
<tr>
<td>IKZF Deletions/sequence mutations</td>
<td>Emerging significance of poor prognosis&lt;sup&gt;91;92&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>CRLF2 overexpression</td>
<td>Emerging significance (mainly childhood ALL) of poor prognosis&lt;sup&gt;92&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>ERG/BAALC expression</td>
<td>Conflicting data concerning prognosis&lt;sup&gt;93;94&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>NOTCH1 mutations</td>
<td>Conflicting data concerning prognosis&lt;sup&gt;95;96&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

*<sup>84</sup> < 44 chromosomes/leukemic cell
**<sup>91</sup> > 5 abnormalities
***<sup>96</sup> > 50 chromosomes/leukemic cell
<table>
<thead>
<tr>
<th>Technique</th>
<th>General statements</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flow cytometry</strong></td>
<td>Aberrant antigen expression</td>
<td>• Applicable for almost all ALL patients</td>
<td>• Immunophenotypic shifts of leukemic cells</td>
</tr>
<tr>
<td></td>
<td>Sensitivity depends on technology (number of colors) and on cell input</td>
<td>• Availability of methodology in many labs</td>
<td>• Expanded and altered precursor-B-cell compartment during regeneration</td>
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<tr>
<td></td>
<td></td>
<td>• Rapid</td>
<td>• Low cellularity during/after induction</td>
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<tr>
<td></td>
<td></td>
<td>• Quantitative</td>
<td>• Relatively high costs (depends on cell input, number of markers/colors and ulterior cytometer utilizations)</td>
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<td></td>
<td></td>
<td>• Additional information on benign cells</td>
<td>• Limited sensitivity/applicability using 3- to 4-color flow cytometry</td>
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<tr>
<td></td>
<td></td>
<td>• Additional information on malignant cells</td>
<td>• ≥ 6-color flow cytometry: extensive knowledge and experience for sensitive and standardized analysis needed</td>
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<td></td>
<td></td>
<td>• Identification and monitoring of treatment targets possible</td>
<td>• No sample asservation and retrospective analysis possible (on-site availability of expert operators necessary)</td>
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<td></td>
<td></td>
<td>• Growing standardization (mainly throughout Europe)</td>
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<tr>
<td><strong>RQ-PCR of immune gene rearrangements</strong></td>
<td>Real-time quantitative PCR</td>
<td>• Applicable for almost all ALL patients</td>
<td>• Time consuming marker characterization</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• High sensitivity</td>
<td>• Potential instability of targets (clonal evolution phenomena, therefore need for preferably two targets/patient)</td>
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<tr>
<td></td>
<td></td>
<td>• High degree of standardization</td>
<td>• Extensive knowledge and experience needed</td>
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<tr>
<td></td>
<td></td>
<td>• Accepted and uniformly used definition of quantitative range and sensitivity</td>
<td>• Relatively expensive</td>
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<td></td>
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<td>• Well established stratification tool in various clinical protocols</td>
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<td>• Most published data for evidence based treatment decisions</td>
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<td></td>
<td></td>
<td>• Stability of DNA (multicenter setting, shipment time)</td>
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<td></td>
<td></td>
<td>• Possibility of asservation and retrospective/batch analysis</td>
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</tr>
<tr>
<td><strong>RQ-PCR of fusion transcripts</strong></td>
<td>Targets: BCR-ABL (about 35% of adult B-cell precursor ALL) MLL rearrangements</td>
<td>• High sensitivity</td>
<td>• Useful only in a minority of patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Unequivocal link with leukemic/preleukemic clone</td>
<td>• Instability of RNA</td>
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<td></td>
<td></td>
<td>• Stability of target during course of treatment</td>
<td>• Uncertain quantitation due to unknown number of transcripts/cell (potential differences during course of treatment)</td>
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<td></td>
<td></td>
<td>• Fast</td>
<td>• False positivity due to cross contamination</td>
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<td></td>
<td></td>
<td>• Relatively cheap</td>
<td>• Standardization necessary</td>
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<thead>
<tr>
<th>Study</th>
<th>No. and risk group of pts</th>
<th>Treatment</th>
<th>MRD assessment/prognostic significance of MRD/ conclusions</th>
<th>Retaining prognostic significance of classical risk factors/comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vidriales 2003**</td>
<td>102 pts: - B- and T- lineage - CR after induction</td>
<td>According to clinical risk factors: - Chemo (n=65) - Plus auto SCT (n=14) - Plus allo SCT (n=23)</td>
<td>FCM (3 colors) during (d+14) and after (d+35) induction: - Prognostic influence of MRD at both tps - Poor prognosis in pts with MRD persistence ≥5x10^{-3} d+14 and/or ≥5x10^{-4} d+35 - Excellent prognosis in small subgroup of pts with early MRD clearance (≤3x10^{-4}, d+14)</td>
<td>MRD most relevant independent prognostic factor - Additional independent prognostic factors: age, WBC, Ph positivity</td>
</tr>
<tr>
<td>Brüggemann 2006</td>
<td>196 pts: - B- and T- lineage - SR ALL</td>
<td>- Chemo**</td>
<td>RQ-PCR (Ig/TCR) at 9 tps (d+11, d+24, d+44, d+71, w+16, w+22, w+30, w+41, w+52) - Prognostic influence of MRD at all tps - Excellent prognosis in small subgroup of pts with early MRD clearance (MRD negativity at d+14) - Poor prognosis in pts with MRD persistence (MRD &gt;10^{-3} from d+71 onwards)</td>
<td>Study population homogeneous concerning other prognostic factors - MRD only variable with significant impact on outcome - No prognostic impact of remaining variables (age, WBC, T- vs. B-lineage)</td>
</tr>
<tr>
<td>Holowiecki 2008*</td>
<td>116 pts: - B- and T- lineage - SR (n=34) and HR (n=82) ALL</td>
<td>- Chemo (n=54) - Auto SCT (n=27) - Allo SCT (n=35)</td>
<td>FCM (3 colors) after induction and cons: - Poor prognosis in SR and HR pts with MRD &gt;10^{-3} after induction and being treated with chemotherapy alone. - Higher proportion of pts with MRD &gt;10^{-3} in HR pts than in SR pts - No prognostic impact of MRD on T-ALL - No significant impact of MRD after cons</td>
<td>MRD after induction most relevant independent prognostic factor - Additional independent prognostic factors: age and WBC</td>
</tr>
<tr>
<td>Bassan 2009</td>
<td>112 pts: - B- and T- lineage - all risk groups</td>
<td>According to MRD and clinical risk factors**: - Chemo (n=51) - Auto SCT (n=25) - Allo SCT (n=21) - No additional treatment (relapse, toxicity: n=15)</td>
<td>RQ-PCR (Ig/TCR and/or fusion genes) at 3 tps (w+10, w+16, w+22) - Good prognosis in pts with MRD &lt;10^{-4} (w+16) and any MRD negativity (w+22) irrespective of classical risk group - Poor outcome in pts with MRD persistence without differences related to original clinical risk class - Early MRD (w+10) predicted late MRD (w+16/w+22)</td>
<td>Combined MRD information at w+16 and w+22 most relevant independent prognostic factor - Additional independent prognostic factor: WBC - Allo SCT not needed in HR pts with MRD LR profile</td>
</tr>
<tr>
<td>Patel 2010****</td>
<td>159 pts: - B-lineage ALL</td>
<td>Irrespective of risk group: - Chemotherapy (n=94) - Auto SCT (n=25) - Allo SCT (n=40)</td>
<td>DNA fingerprinting/RQ-PCR (Ig/TCR) at 3(-5) tps (w+5, w+10, w+17, for pts without SCT; also w+28, w+39): - Prognostic influence of MRD in pts receiving chemotherapy or auto SCT as post-remission treatment - Most discriminative tps: w+10, w+17 - No significant impact of MRD in pts with allo SCT</td>
<td>No multivariate analysis performed</td>
</tr>
<tr>
<td>Study</td>
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</tbody>
</table>
| Gökbuget 2012 | 580 pts: B- and T- lineage ALL  
- SR (n=434)  
- HR (n=146) pts | - Chemo (n=425)  
- Allo SCT (n=155) according to MRD (only SR ALL) and clinical risk factors | RQ-PCR (Ig/TCR) at 2 tps (d+71, w+16)  
- Prognostic influence of MRD at d+71 and w+16  
- Complete MRD response rate higher in SR vs. HR pts (d+71: 77% vs. 51%), clinical benefit of complete response comparable for SR and HR pts  
- MRD-based allo SCT in MRD-HR patients:  
  ▪ SCT performed in half of the pts  
  ▪ Improved DFS and OS for pts with SCT | - Multivariate analysis: MRD only parameter with significant prognostic impact |
| Ph-positive ALL |                            |                                                                                                                  | RQ-PCR (Ig/TCR) at 2 tps (d+71, w+16)  
- Prognostic influence of MRD at d+71 and w+16  
- Complete MRD response rate higher in SR vs. HR pts (d+71: 77% vs. 51%), clinical benefit of complete response comparable for SR and HR pts  
- MRD-based allo SCT in MRD-HR patients:  
  ▪ SCT performed in half of the pts  
  ▪ Improved DFS and OS for pts with SCT | - Multivariate analysis: MRD only parameter with significant prognostic impact |
| Pane 2005     | 45 Ph+ pts                | - Chemo followed by allo SCT (in case of available donor, n=20)  
- No TKI | RQ-PCR (BCR-ABL) at 2 tps (end of ind, end of first cons)  
- Heterogeneous sensitivity to treatment  
- Better DFS and OS in good molecular responders (>2 log reduction after ind + >3 log reduction after cons 1) compared to poor molecular responders | - Age and WBC did not differ between both MRD groups |
| Ottmann 2007  | 49 Ph+ pts: Elderly pts (54-79 years) | - Randomized ind: chemo vs. TKI  
- Cons: chemo + TKI | RQ-PCR (BCR-ABL) at 2 tps (end of ind, end of first cons)  
- Heterogeneous sensitivity to treatment  
- MRD response in pts with TKI ind compared to chemo:  
  ▪ better initial MRD response in TKI group  
  ▪ no difference in median MRD level after cons  
- MRD negativity at any time associated with more favourable outcome | - Lower WBC at diagnosis in MRD negative pts |
| Yanada 2008   | 100 Ph+ pts**** | - Ind. chemo+TKI  
- Donor: allo SCT in CR1 (n=60)  
- No donor: cons chemo+TKI (n=37) | RQ-PCR (BCR-ABL) at d+28, d+63 (end of ind), end of cons 1 (+later tps)  
- No impact of rapid MRD clearance (MRD negativity at d+28, d+62 or after cons 1) on relapse free survival | - No MRD assessment/prognostic significant of classical risk factors/comments |
| Lee 2009      | 52 Ph+ pts                | Treatment sequence:  
- Ind chemo  
- 4 w TKI  
- Cons chemo  
- 4 w TKI  
- Allo SCT in CR1 (n=48) or non-CR (n=4) | RQ-PCR (BCR-ABL) after ind chemo, first TKI, after cons chemo, after second course TKI:  
- No prognostic impact of MRD after ind chemo prior to TKI  
- Better disease free survival and lower relapse rate in pts with ≥3 log reduction after first imatinib course | - MRD after TKI most significant independent prognostic factor  
- Chronic GVHD only additional variable with an independent prognostic impact |

* Only study of Bassan et al. includes Ph-positive pts.  
** Eleven pts with SCT censored at time of SCT  
*** Clinical risk factors for assignment to allo SCT independent of MRD: presence of t(9;22) or t(4;11)  
**** Update from an interim report of Mortuza et al.  
***** Including 1 resistant pt and 2 early deaths
Figure 1: Proposals for definition of MRD terms in ALL. According to [16]
Figure 2: Prospective therapeutic shifts according to conventional pretreatment stratification criteria and MRD. (A) Potential treatment decisions based on pretreatment factors. (B) Potential treatment decisions based on MRD. Cons: Consolidation. DLI: Donor lymphocyte infusion. SCT: Stem cell transplantation. TD: Treatment decision. TKI: Tyrosine kinase inhibitor.
Has MRD monitoring superseded other prognostic factors in adult ALL?

Monika Brüggemann, Thorsten Raff and Michael Kneba