Annals of Medicine. 2006; 38: 512–521

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TRENDS IN CLINICAL PRACTICE

Monitoring of minimal residual disease in leukemia, advantages and pitfalls

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Abstract
The term 'minimal residual disease' (MRD) defines the level of disease detectable in patients in clinical remission during therapy, below the detection limit of conventional methods. Very sensitive methods can be used, able to identify one leukemic cell out of 10,000 normal lymphocytes. *In vivo* measurements of leukemia cytoreduction reflect the combined effect of clinical and biological variables, thus providing direct information on the effectiveness of treatment in each patient. Thus, these methods can potentially be used for tailoring treatment and personalize the cure. Although MRD studies are becoming an integral part of the modern management of patients with leukemia, several parameters are critical for the application and interpretation of MRD studies, including therapeutic context, timing of sampling, target genes and sensitivity of the polymerase chain reaction (PCR) assay, inter-laboratory standardization (particularly relevant in multicenter studies), selection of patients, retrospective or prospective nature of the study. Methodologies and pitfalls as well as results of clinical uses of MRD will be reviewed in this article by selecting significant examples of its clinical impact in the management of patients with leukemia.

Key words: *Leukemia, minimal residual disease, treatment stratification*

Introduction

Treatment response in patients with acute leukemia is influenced by several factors, including cell lineage, maturation stage, karyotypic and molecular abnormalities. In addition, size of the tumor burden, dosage of drugs and their interaction, pharmacokinetic and pharmacogenetic variables are important factors. Although some of these parameters showed to be predictive and have been used for risk classification of patients, they variably contribute to the risk of relapse; thus they cannot be used to individualize treatment decisions.

In vivo measurements of leukemia cytoreduction reflect the combined effect of clinical and biological variables, thus providing direct information on the effectiveness of treatment in each patient. Along this view, these methods can potentially be used for tailoring treatment and personalizing the cure.

Continuous clinical remission (CCR) in patients with acute leukemia, either lymphoid (ALL) or myeloid (AML) is conventionally defined to be less than 5% of bone marrow (BM) blasts by morphologic assessment. However, due to the relatively low sensitivity of morphologic detection, this description can be associated even with a leukemia burden of up to 1×10^{10} blasts.

As shown in Figure 1, the term 'minimal residual disease' (MRD) has been used to define the residual disease detectable in patients in CCR, below the detection limit of the available conventional methods.

Several techniques have been developed over the past 10–15 years to complement morphology in assessing response to treatment, including immunologic, molecular, fluorescent *in situ* hybridization (FISH), *in vitro* drug response and colony assays (1–3). This improvement drastically changed the definition of 'remission', which now depends on the sensitivity of the detecting methodology.

Despite notable progress with these methods, their sensitivities vary considerably, and several critical issues must be resolved before MRD determinations can be routinely considered in clinical decision making.

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ISSN 0785-3890 print/ISSN 1365-2060 online
DOI: 10.1080/07853890600978123

Several parameters are critical for the interpretation of MRD studies, including therapeutic context, timing of sampling, target gene and sensitivity of the PCR assay, inter-laboratory standardization (particularly relevant in multicenter studies), selection of patients, and retrospective or prospective nature of the study. Methodologies and pitfalls as well as results of clinical uses of MRD will be reviewed in this article by selecting significant examples of its clinical impact in the management of patients with leukemia.

Key messages

- Clinical use of minimal residual disease (MRD): 1) identification of subgroup of patients with different kinetic of early tumor reduction; 2) identification of impending relapse; 3) identification of patients with different outcome within genetically homogeneous subgroups.
- The methodology, the timing and the sensitivity required for informative MRD studies depend mainly on the clinical question, but also from the expertise and the facilities available. Accordingly, appropriate and well designed prospective clinical trials are mandatory before any clinical application.
- Although numerous methodologies and new technologies are now available to monitor MRD in leukemia, standardization and quality control are still needed to apply molecular diagnostic procedures in oncohematology. This is particularly true in efforts to assure reproducible results within multicenter international studies.

Methodological approaches

Several methods of MRD detection have been recently developed and evaluated (1–3). These include cell-culture systems, fluorescence *in situ* hybridization (FISH), Southern blotting, immunophenotyping and PCR techniques. However, most of these techniques have limited sensitivity, specificity, or applicability (i.e. cell-culture systems and Southern blotting). Reliable techniques to detect MRD should have the following features: 1) sensitivity of at least 10^{-3} (1 malignant cell within 10^3 normal cells), but sensitivities of 10^{-4} to 10^{-6} are preferred; however, the sensitivity depends on the clinical question to be addressed by the MRD assessment; 2) ability to discriminate between malignant and normal cells (no false-positive results); 3) stability of leukemia-specific markers; if the marker at diagnosis is lost or changes during follow-up, false-negative results can occur; 4) reproducibility between laboratories; this is essential for multicenter treatment protocols and must be carefully undergone to standardization and quality control round; 5) rapid collection of results; MRD data must be obtained in time for a clinical usefulness; 6) a method of quantifying MRD.

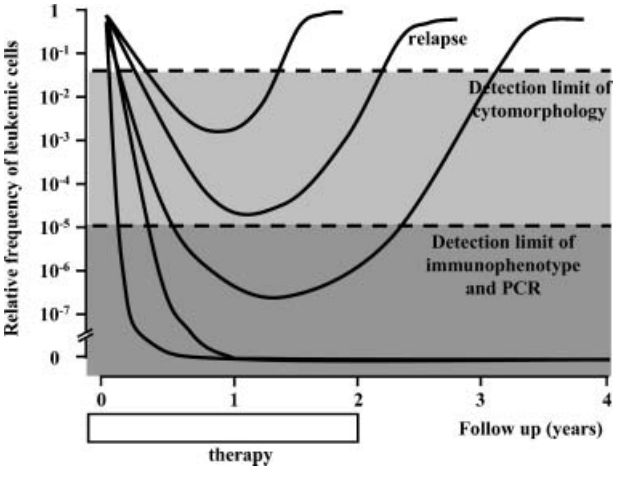


Figure 1. Diagram of the frequencies of leukemic cells in peripheral blood or bone marrow of patients with acute leukemia, during and after chemotherapy. The detection limit of different techniques for minimal residual disease (MRD) monitoring is indicated.

Either in lymphoid and myeloid leukemia, the most reliable methods for MRD detection include flow cytometric profiling of aberrant immunophenotypes, PCR amplification of fusion transcripts and chromosomal breakpoints, and, in ALL, PCR amplification of antigen-receptor genes. These approaches are widely used for MRD monitoring because they are sufficiently specific, sensitive (10^{-4} to 10^{-6}), quantitative, and relatively easily applicable (1–7).

Flow cytometric detection relies on the identification of aberrant immunophenotypes (reviewed in (4,5)). Acute leukemia can be considered as malignant counterparts of cells in immature stages of hematopoiesis. Aberrant immunophenotypes are the result of cross-lineage antigen expression, maturational asynchronous expression of antigens, antigen overexpression or absence of antigen expression, and/or ectopic antigen expression (4,5).

Molecular assays have been applied to the identification of three types of 'clone-specific' targets in leukemia: breakpoint fusion regions arising from chromosomal translocations, patient-specific

sequences reflecting unique recombinations of antigen receptor genes, and aberrantly expressed genes.

Somatically acquired chromosomal translocations or inversions occur frequently in lymphoid and myeloid leukemia (8,9). In most of the cases, the consequence of these structural rearrangements is that discrete segments of two different genes may be joined as a result of the translocation, creating a fusion gene encoding a chimeric protein. Breakpoint fusion regions of chromosomal aberrations can be employed as unique tumor-specific PCR targets for MRD detection.

Amplification of such hybrid sequences with 'standard range' PCR on tumor DNA is only feasible when the breakpoints of different patients cluster in relatively small breakpoint areas of preferably < 2 kb. However for most of the translocations, the breakpoints of different patients are scattered over large areas of up to 200 kb. The precise breakpoint recombination site at the DNA level is specific for each patient and difficult to determine, thus making the chimeric mRNA, after its reverse transcription into cDNA, as the preferred target for PCR analysis (reviewed in (10)). The PCR analysis of fusion genes is based on the design of oligonucleotide primers at the opposite sides of the breakpoint fusion regions, so that the PCR product contains the tumor-specific fusion sequences (Figure 2). This approach requires the extraction of total or messenger RNA from BM mononuclear cells, reverse transcription of RNA into cDNA and molecular assay by PCR, followed by agarose gel electrophoresis. The sensitivity of the method is specific for each target and can be assessed by amplification of serial dilution of diagnostic RNA into RNA from healthy individuals. A single PCR test is sufficiently sensitive (1 leukemia cell in 10^2 to 10^3 normal cells) to detect fusion transcripts at diagnosis (10). A higher sensitivity is required for MRD assessment during follow-up analyses and can be achieved by a second round of PCR ('nested' PCR) using internal primers (10). In this way, 1 leukemia cell in 10^4 to 10^5 normal cells can be detected for most transcripts. Extra primer sets must be designed to cover fusion gene transcripts with different exon compositions (10).

Somatic rearrangement of immunoglobulin (Ig) and T cell receptor (TcR) gene loci occurs during early differentiation of any B and T cell, by joining the germline variable (V), diversity (D) and joining (J) gene segments. By this process, each lymphocyte gets a specific combination of V-(D-) J segments that codes for the variable domains of Ig or TcR molecules. The uniqueness of each rearrangement further depends on random insertion and deletion of nucleotides at the junction sites of V, (D) and J gene segments, making the junctional regions of Ig and TcR genes as 'fingerprint-like' sequences. This combined sequence constitutes a specific signature of each lymphocyte. Due to the clonal origin of the neoplasm, each malignant lymphoid disease will represent the expansion of a clonal population with a specific Ig/TcR signature. Therefore, junctional

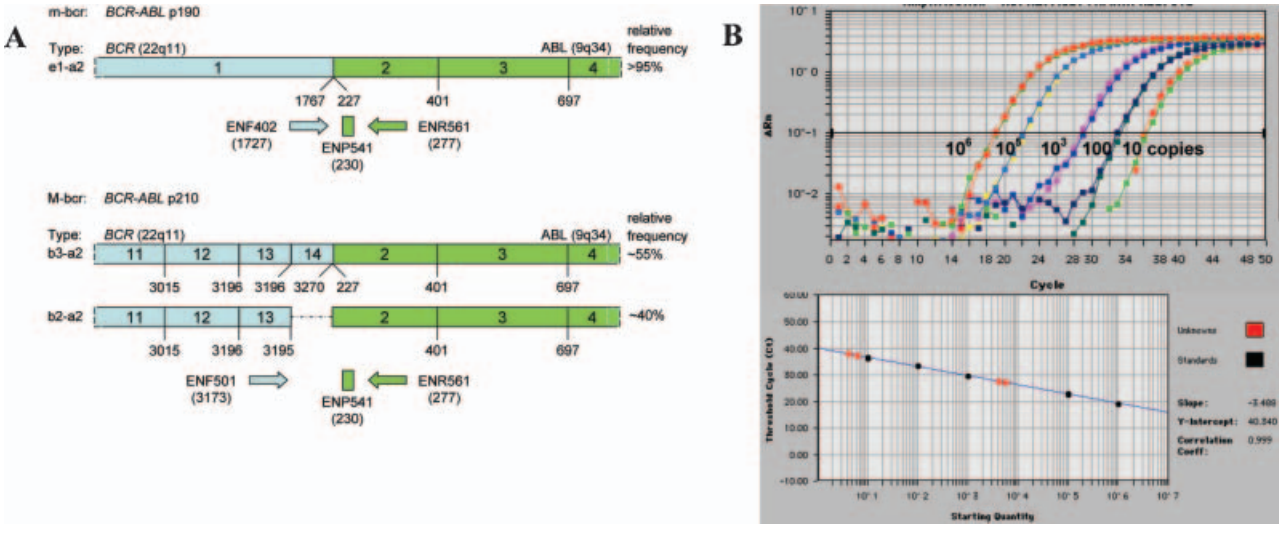
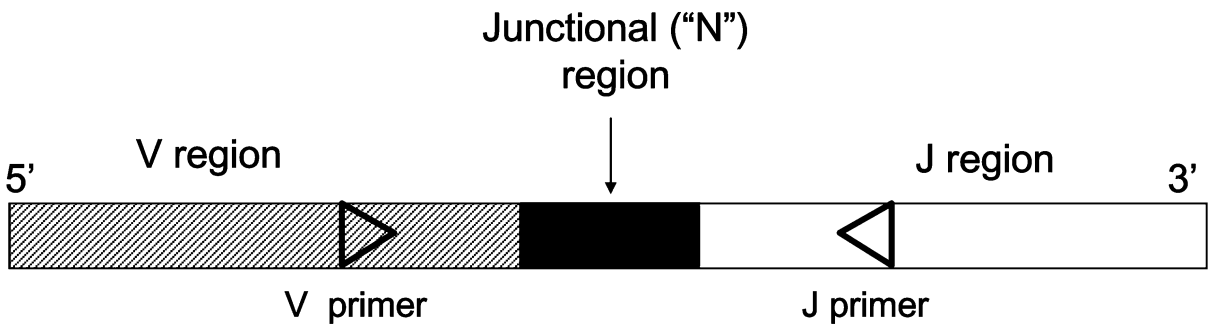


Figure 2. Schematic representation of the strategy to detect by PCR a fusion gene generated by a chromosomal translocation and minimal residual disease (MRD) quantification by RQ-PCR method. Panel A: schematic diagram of the *BCR-ABL* p190 and p210 fusion transcripts generated by two alternative *BCR* breakpoints of the t(9;22) translocation. Alternative splicing and relative frequency are also indicated. The red arrows indicate the relative position of the primers used for RQ-PCR detection. In pink is the fluorescent 'TaqMan' probe. Panel B indicates a typical dilution curve of a positive sample (top) to define a standard curve (bottom) for quantitative assessment of unknown samples amplified for *BCR-ABL* transcript.



Patient	Marker	Junction		Junctional sequence		Sensitivity (10-x)
AC05	Vg3-Jg1.3	0 / 5 /-3	TCTATTACTGTGCCACCTGGGACAGG	CGGGT	TTATTATAAGAAACTCTTTGGCAG	4
AE32	Vg3-Jg1.3	0/10 /-22	TCTATTACTGTGCCACCTGGGACAGG	CCCCACCAGA	GGCAG	4
AS07	Vg3-Jg1.3	-9 /16 /-10	TCTATTACTGTGCCACC	CCGCGGATAATGGTCA	AAGAAACTCTTTGGCAG	4
AL24	Vg2-Jg1.3	-8 /3 /-8	TCTATTACTGTGCCACCT	CGA	TATAAGAAACTCTTTGGCAG	5

Figure 3. Variability of the N-junctional V(D)J region of Ig/TcR gene rearrangements as patient and clone-specific target for minimal residual disease (MRD) detection.

regions can be used as leukemia-specific targets for PCR analysis of MRD either in acute or chronic lymphoid leukemia (Figure 3) (reviewed in (11,12)).

In addition to Ig/TcR gene rearrangements and chromosome translocations, several other genetic aberrations in hematologic malignancies can be used as MRD-PCR target. A reference example is the Wilms' tumor gene (*WT1*), a tumor suppressor gene coding for a zinc-finger transcription factor located on chromosome 11p13, which was originally identified for its involvement in the pathogenesis of the Wilms' tumor. It functions as a potent transcriptional repressor of several growth factors, and its expression is strongly regulated in a time- and tissue-specific manner. By contrast, *WT1* is highly expressed in most acute leukemia, including ALL, AML and myelodysplastic syndromes (13,14). Its level of expression is associated with the presence, persistence, or reappearance of leukemia hematopoiesis, thus proving to be a reliable marker for MRD detection.

Quantification of MRD

Independently of the method of choice, an accurate quantification of residual leukemia cells during a certain treatment course is highly requested, in order to have a precise insight of the kinetics of response to drugs, or to predict impending relapse.

One of the major advantages of immunophenotyping for detection of MRD is that it gives direct quantitative information. The measurement of MRD by flow cytometry is usually performed by relative estimation (percentage) of leukemia cells on total mononuclear cells or on total nucleated cells (reviewed in (4)). The sensitivity depends on the numbers of acquired events. The number of cells analyzed for each set of markers in clinical samples is usually less than 10^6. Because a specific cluster of at least 10–20 dots is required to reliably interpret flow cytometric features, the maximum sensitivity of the assay would be 0.001% (or 1 leukemia cell in 10^5 normal cells), but a more realistic sensitivity, according to practical routine conditions is 0.01% (or 1 leukemia cell in 10^4 normal cells). In this concern, rigorous control of technical aspects of the procedures is critical and needs attention (reviewed in (4)); minimal spurious signals may be a source of major errors when studying MRD. Moreover, false signals can be due to defects of the buffers used, sample carryover and nonspecific binding of antibodies or fluorochromes to cells. Quantification of MRD by immunophenotyping can also be assessed by absolute quantification (number of leukemia cells per volume of BM) instead of relative estimation, thus allowing a better quantitative evolution of the disease over time due to the variation in nucleated cell counts at different time points.

In the past, most PCR-based MRD studies used semi-quantitative methods for the detection of clone-specific translocations or Ig and TcR gene rearrangements (reviewed in (15,16)). Standard PCR techniques have the ability to amplify target DNA to a plateau of amplification, so that after 35–40 cycles it is not possible to precisely define the initial amount of target DNA. Semi-quantitative methods, such as dot blot hybridization using a patient-specific VDJ region probe, competitive PCR

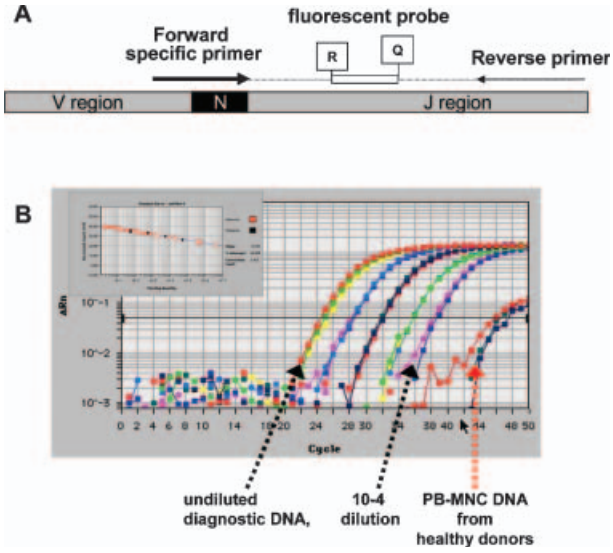


Figure 4. Schematic representation of the quantitative PCR of Ig/TcR rearrangements by TaqMan technology.

and limiting dilution PCR, are similarly based on post-PCR end point analysis. These techniques require serial dilutions and the analysis of multiple replicates, both of which introduce variability and cost, and are too difficult and too time-consuming to be performed routinely in the clinical laboratory. Real-time quantitative PCR technology (RQ-PCR) circumvents these problems and allows for quantitative assessment of residual disease.

RQ-PCR of Ig and TcR gene rearrangements can be used to quantify MRD levels by using allele-specific oligonucleotide (ASO) probes (Figure 4). Sensitivities of 1×10^{-3} to 1×10^{-5} are achievable with this strategy. Although initial assays used an ASO fluorescent probe to the junctional region, a more useful approach is to use a fluorescent probe complementary to the germline Ig and TcR gene segments, in combination with an ASO primer complementary to the junctional region (17). The ASO primer approach theoretically results in more sensitive MRD detection compared with use of germline primers, because no competition can occur with the amplification of similar rearrangements in normal cells. Although specific amplification can be distinguished from incidental nonspecific amplification, conditions with higher stringency of amplification may need to be used to overcome nonspecific amplification while maintaining the efficiency of the method.

Numerous publications have demonstrated the feasibility of the RQ-PCR approach to quantify chimeric transcripts resulting from chromosomal translocations occurring in ALL (reviewed in (18)). Although the principles of RQ-PCR are the same whether DNA or RNA is being analyzed, the reverse transcription (RT) step represents a major assay variable for accuracy of quantification and sensitivity when RNA is used. In fact, it is necessary to correct variations linked to differences in the RNA amount taken for the reaction or, more importantly, in efficiency (or inhibition) during reverse transcription. For this reason, the number of target gene copies has to be normalized using a ubiquitously and constantly expressed housekeeping gene as a reference (e.g. *ABL*, *B2M*, and *GUS*) (19). Thus, the number of chimeric transcripts will be expressed according to the number of copies of the reference gene transcripts.

MRD in clinical studies: reference examples

MRD as a surrogate marker for risk assignment of ALL patients

As shown in Table I, several ongoing studies have incorporated MRD evaluation for risk assignment of pediatric and adult ALL patients. Early retrospective studies and small prospective studies indicated that, in children and adults with ALL, detection of MRD at the end of remission induction treatment could predict outcome (reviewed in (15,16)). Review of published MRD studies showed that approximately 50% of children with ALL are MRD-positive at the end of induction treatment, and approximately 45% of these MRD-positive patients will ultimately relapse with the risk of relapse being proportional to the detected MRD levels (20–24). Multivariate analyses showed that the prognostic value of MRD-PCR levels of positivity after induction therapy is independent and superior to that of other clinically relevant risk factors, including age, blast count at diagnosis, immunophenotype at diagnosis, presence of chromosome aberrations, response to prednisone, and classical clinical risk group assignment, provided that MRD quantification is accurate and bone marrow samples are adequate (20–24). Based on these findings several prospective ongoing studies have incorporated the MRD evaluation at early time points as a surrogate marker for risk assignment of ALL patients. The ongoing cooperative AIEOP-BFM ALL2000 clinical protocol for childhood ALL (in Italy, Germany, Austria and Switzerland) is mainly based on MRD assessment at day +33 and day +78 by two Ig/TcR targets with sensitivity at least 10^{-4}. Concerning the preliminary series of more than 3500 patients enrolled into the study, MRD analysis was performed in more than 95% of cases. Considering failures due to target availability and sensitivity, availability of follow-up DNA, and

Table I. Ongoing European studies on the use on minimal residual disease (MRD) for risk assignment of acute lymphoid leukemia (ALL) patients (JJM van Dongen, personal communication).

Clinical Study	Intervention	MRD technique	Aimed sensitivity	Responsible PCR laboratories
Childhood ALL 1stCR				
BFM-AIEOP ALL 2000	+	RQ-PCR	$\leqslant 10^{-4}$	Heidelberg, Monza, Vienna, Zurich
ANZCHOG Study-8	+	RQ-PCR	$\leqslant 10^{-4}$	Sydney
BFM-HR-ALL	−	RQ-PCR	$\leqslant 10^{-4}$	Hannover
DCOG-ALL 10	+	RQ-PCR	$\leqslant 10^{-4}$	Rotterdam, Amsterdam
Interfant 99	−	RQ-PCR	$\leqslant 10^{-4}$	Rotterdam, Monza (+ several national centers)
MRC-ALL 2003	+	RQ-PCR	$\leqslant 10^{-4}$	Bristol, Sheffield, London, Leeds, Glasgow
COALL 07-03	planned	RQ-PCR	$\leqslant 10^{-4}$	Hamburg
FRALLE 2000	+	GeneScan	$\sim 10^{-3}$	Paris (St. Louis), Paris (Necker), Paris (Debré), Lille
EORTC-CLG 58951	+	GeneScan	$\sim 10^{-3}$	Paris (Debré), Brussels (AZ-VUB), Lille
NOPHO ALL-2000	+	RQ-PCR	$\leqslant 10^{-4}$	Stockholm, Copenhagen
MiniRisk (ALL-IC BFM)	−	RQ-PCR	$\leqslant 10^{-4}$	Prague, Israel, (Hong Kong)
Relapsed childhood ALL				
ALL-REZ BFM 2002	+	RQ	$\leqslant 10^{-4}$	Berlin, Sydney, Frankfurt
AIEOP REC 2003	+	RQ	$\leqslant 10^{-4}$	Monza, Padova
(pre-) BMT childhood ALL	+	RQ-PCR	$\leqslant 10^{-4}$	Bristol, Rotterdam, Prague, Frankfurt, Copenhagen, Monza (intervention in auto-BMT)
Adult ALL				
GMALL 06/99	+	RQ-PCR	$\leqslant 10^{-4}$	Kiel, Heidelberg, Frankfurt
GRALL 03/05	+(03)	RQ-PCR	$\leqslant 10^{-4}$	Paris (Necker), Paris (St. Louis), Paris (Debré), Lille, Brussels (AZ-VUB)
MRCUKALLXII	−	RQ-PCR	$\leqslant 10^{-4}$	London (Royal Free)
NIGL	−	RQ-PCR	$\leqslant 10^{-4}$	Bergamo

shifts to other treatment protocols, altogether it was possible to stratify according to MRD levels almost 75%–80% of eligible patients (unpublished data).

Comparable results have been obtained by using flow cytometry. In studies at St. Jude, flow cytometry was used to prospectively study MRD in 195 children with newly diagnosed ALL enrolled in a single chemotherapy program (TOTAL XIII) (25–27). Detectable MRD (i.e. $\geqslant$0.01% leukemia mononuclear cells) at each time point (day 19 of remission induction therapy, end of remission induction and weeks 14, 32 and 56 of continuation) was significantly associated with a higher rate of relapse. Dworzak et al. reported the correlation between MRD detected during treatment and outcome in 108 children enrolled in Berlin-Frankfurt-Munster 95 protocol in Austria (28). These investigators converted percentages of MRD into number of blasts among bone marrow nucleated cells. Sequential monitoring at day 33 and week 12 of treatment was found to be particularly useful: patients with persistent disease ($\geqslant$1 blast/μL) had a 100% probability of relapse, compared to 6% in all others. It was found, however, that the sensitivity of the test with the markers used was limited when regenerating bone marrow samples were studied.

In adult ALL, a considerable number of mainly retrospective MRD studies have been performed with different methods (reviewed in (16)). In adults the decrease of MRD occurs more slowly than in children, and fewer patients reach a negative MRD status. However, high MRD at any time point after induction is associated with a higher relapse risk and the prediction value increases at later time points (months 6–9). A recent report from the German adult study Group GMALL showed that MRD

quantification during treatment identified prognostic subgroups within the otherwise homogeneous standard risk ALL population who may benefit from individualized treatment (29).

MRD for tailoring treatment in acute promyelocytic leukemia (APL) patients

Acute promyelocytic leukemia (APL) is characterized by rearrangements of the retinoic acid receptor α (*RARA*) gene on chromosome 17q21 (reviewed in (30)). To date, five different partner genes have been identified, with the vast majority of cases having an underlying promyelocytic leukemia-retinoic acid receptor alpha (*PML-RARA)* fusion as the result of the t(15;17)(q22;q21) chromosomal translocation (30). Determination of the underlying molecular lesion is critical for appropriate management of APL, with presence of an underlying *PML-RARA* fusion gene predicting a favorable response to molecularly targeted therapies in the form of all-*trans* retinoic acid (ATRA) and arsenic trioxide (ATO) (6).

Presenting leukocyte count has been shown to be a key prognostic factor in clinical trials involving pediatric and adult patients with APL, with white blood cell (WBC) counts $> 10 \times 10^9$/L predicting a significantly increased risk of induction death and relapse risk (reviewed in (31)). Relapse risk amongst patients treated with ATRA and anthracycline-based chemotherapy with WBC $< 10 \times 10^9$/L is typically approximately 10%, as opposed to about 30% in patients with higher presenting WBC. This raises the possibility that some patients at low risk of relapse are being over-treated with current protocols leading to unnecessary morbidity, while patients at higher risk of relapse could benefit from additional therapy. There is evidence to suggest that more precise tailoring of therapy may be achieved through monitoring of minimal residual disease (MRD) which has been shown to provide an independent risk factor for relapse (6). Using conventional 'end point' PCR assays which achieve a sensitivity of approximately 1 in 10^4, Italian studies undertaken in children and adults in conjunction with the AIDA protocol have established that patients with *PML-RARA* transcripts still detectable at the end of consolidation (who account for less than 10% of cases overall) or those subject to a later recurrence of PCR positivity (molecular relapse) are destined to undergo subsequent hematological relapse, which may, however, in both instances be averted by additional therapy (32–34). Indeed, preliminary data from the Italian trials suggest that preemptive treatment at the time of molecular relapse may lead to improved survival in comparison to patients who are re-treated in frank hematological relapse (35).

Whilst MRD monitoring using conventional nested RT-PCR has provided valuable prognostic information, its clinical utility has been somewhat compromised by failure to detect residual disease in a significant proportion of APL patients who ultimately relapse (6). This may be a reflection of the relatively limited sensitivity of conventional assays and/or variation in RNA quality/quantity and efficiency of the reverse transcription (RT) step. Quantitative PCR approaches using hydrolysis (TaqMan) or hybridization probe technology afford a number of advantages in comparison to conventional 'end point' assays. In particular, quantitation of fusion gene and endogenous control gene transcripts enables more reliable determination of kinetics of molecular remission achievement or relapse and readily identifies poor quality samples that could potentially give rise to 'false-negative' results. Moreover, RQ-PCR assays are rapid, facilitate high throughput sample analysis, are highly reproducible and readily standardized, thereby lending themselves to MRD assessment in multicenter clinical trials. Optimized RQ-PCR protocols for detection of the *PML-RARA* fusion gene and ubiquitously expressed control genes have been established by the Europe Against Cancer (EAC) Group (18). These assays have subsequently been validated in relation to conventional nested RT-PCR in a series of 47 patients derived from the Medical Research Council (MRC) ATRA trial; this analysis established that the 'real-time' assay was marginally more sensitive and provided an independent predictor of relapse risk and overall survival (36). Other studies relating to MRD detection using RQ-PCR methodology in APL are encouraging (37) and in the relatively large series (n=123) relating to the randomized US Intergroup ATRA trial, the *PML-RARA* transcript level at the end of consolidation was found to be of prognostic value (38). The latter study also revealed that, despite the higher sensitivity of RQ-PCR, a significant number of patients who ultimately relapsed tested PCR-negative at the end of consolidation, underlining the importance of serial monitoring to increase the predictive value of this approach.

MRD to monitor patients with chronic myelogenous leukemia (CML) and to reassess therapy

Chronic myeloid leukemia (CML) is a malignant disease of the hematopoietic system characterized by the presence of a reciprocal translocation between chromosomes 9 and 22 (reviewed in (39)), generating

the so-called 'Philadelphia' (Ph) chromosome. The chimeric breakpoint cluster region-Abelson (*BCR-ABL*) gene generates several types of fusion proteins, of which the $p185^{BCR-ABL}$ form is detectable in 85% of patients with Ph-positive ALL, whereas the $p210^{BCR-ABL}$ chimeric protein typical of CML is observed in approximately 10% of patients with ALL. The *BCR-ABL* fusion proteins are characterized by a constitutive protein tyrosine kinase (PTK) activity that is absent in the normal ABL protein. This dysregulated PTK activity, which results in changes of multiple signal transduction pathways, is crucial to the transforming activity of the BCR-ABL fusion proteins and their ability to cause leukemia *in vivo*. Therefore, inhibition of the PTK activity of this oncoprotein is a rational therapeutic approach for BCR-ABL expressing leukemia.

Imatinib mesylate (Imatinib) is an inhibitor of the protein tyrosine kinases associated with BCR-ABL, the platelet-derived growth factor (PDGF) receptor and c-Kit, but not of other members of the Type III receptor kinase family, such as Flt-3 and Fms (39). Imatinib shows selectivity for the ABL protein tyrosine kinase at the *in vitro*, cellular and *in vivo* levels (40). Its 1998 introduction in the treatment of patients with CML revolutionized management of those patients (42–44). However, nearly all patients with CML have persistent disease measurable by PCR and indicative of a reservoir of residual leukemia cells that may be a source of relapse (44,45). Relapse during imatinib is most often caused by mutations in the kinase domain of *BCR-ABL* that interfere with imatinib binding (reviewed in (46)). Recently the second generation of tyrosine kinase inhibitors are proven to be effective in patients with CML or Ph-positive ALL who cannot tolerate or are resistant to imatinib (47,48).

The molecular monitoring of the *BCR-ABL* fusion transcripts is currently considered an essential component of the clinical follow-up of a CML patient (reviewed in (7)). The methodology employed has evolved over the years. More recently the results were published of a consensus meeting that took place at the National Institute of Health (NIH) in Bethesda (MD, USA) in October 2005. It makes suggestions in order to achieve a better harmonization of the different methodologies for measuring *BCR-ABL* transcripts in CML (49). Moreover it recommends the use of serial RQ-PCR results rather than bone marrow cytogenetics or FISH for the *BCR-ABL* gene to monitor individual patients responding to treatment and to detect and report Ph-positive subpopulations bearing *BCR-ABL* kinases domain mutations.

Concluding remarks

MRD studies are becoming an integral part of the modern management of patients with leukemia. The selection of the methods to be used in each center depends on the clinical question but also on expertise and facilities available, and on collaborative links that can be established with laboratories that are proficient in MRD detection.

Each of the methodologies for studying MRD in children with acute leukemia has relative advantages and disadvantages. The sensitivity required for informative MRD studies depends on the clinical question, and accordingly appropriate and well-designed prospective clinical trials are mandatory.

Acknowledgements

We thank Fondazione Tettamanti and Comitato ML Verga, Associazione Italiana per la Ricerca sul Cancro (AIRC) and Fondazione Cariplo for supporting our MRD studies.

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