Journal of Molecular Diagnostics, Vol. 5, No. 2, May 2003

Review

Clinical Applications of BCR-ABL Molecular Testing in Acute Leukemia

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Recent advances in molecular genetics impact the health care and outcome of patients with acute lymphoblastic leukemia (ALL). BCR-ABL, a common molecular defect in adult ALL, is a valuable tumor marker whose detection influences prognosis and clinical management decisions. Molecular methods such as fluorescence *in situ* hybridization (FISH), reverse-transcriptase polymerase chain reaction (rtPCR), and real-time quantitative rtPCR can be used to detect the chimeric BCR-ABL gene or its transcripts. These molecular assays improve our ability to measure residual disease and to estimate risk of relapse. On the horizon are gene expression profiles that will likely provide additional information beyond what is obtainable with current clinical and laboratory approaches. *(J Mol Diagn 2003, 5:63–72)*

In 1960, Nowell and Hungerford[1] reported the discovery of what came to be known as the Philadelphia chromosome (Ph^1) in association with chronic myelogenous leukemia (CML). Their short report entitled "Minute chromosome in human chronic granulocytic leukemias" was the first to demonstrate a leukemia-specific genetic abnormality. In the ensuing decades, the pathology of the Ph^1 has been studied and it is now known to represent an abnormally shortened (derivative) chromosome 22 resulting from translocation with chromosome 9.[2] The t(9;22) is found in over 90% of CMLs, in a lesser proportion of acute lymphoblastic leukemias (ALL) or biphenotypic acute leukemias, and in rare cases of *de novo* acute myelogenous leukemia (AML).

The break on chromosome 22q11.2 usually occurs in the major breakpoint cluster region (M-BCR), in the minor breakpoint cluster region (m-BCR), or rarely at other nearby sites. The break on chromosome 9q34 involves the ABL gene, named after Abelson murine leukemia virus where a viral version of the ABL gene was first discovered. The translocation brings the 5′ end of the BCR gene into juxtaposition with the tyrosine kinase domain of the ABL gene to produce a hybrid gene retaining tyrosine kinase activity.[3] Depending on whether the M-BCR or m-bcr breakpoint is involved in the translocation, transcription of the hybrid gene results in chimeric mRNA encoding a 210 kd BCR-ABL fusion protein or a 190 kd BCR-ABL fusion protein, respectively. The reciprocal ABL-BCR translocation also forms a chimeric gene that is capable of being transcribed, but the pathological significance of this reciprocal chimeric gene product is uncertain.

Laboratory Tests for t(9;22)

Conventional cytogenetics is the recommended test for detecting t(9;22) in newly diagnosed leukemia patients. Chromosome banding analysis has the advantage of high specificity and an ability to detect alternate or additional cytogenetic defects that are valuable in diagnosis and prognosis. However, cytogenetic analysis requires viable marrow cells or more than 10% blasts in the peripheral blood to reliably culture the cells and visualize metaphases. Occasionally fibrosis interferes with marrow aspiration, yielding few analyzable metaphase cells. The number of cells examined determines the sensitivity of karyotyping. A typical examination of 20 cells carries a sensitivity of one in 20, or 5%. Cryptic t(9;22) occurs in about 5% of CML cases and also in a small proportion of ALLs, resulting in false negative karyotypic interpretation in metaphase spreads of cells that actually contain the translocation at the molecular level.[4]

Fluorescence in Situ *Hybridization*

Fluorescence *in situ* hybridization (FISH) allows detection of the BCR-ABL translocation in either metaphase or interphase cells. Because interphase cells are suitable,

Accepted for publication February 14, 2003.

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FISH can be applied directly to blood leukocytes and other non-dividing cells. Moreover, FISH detects cryptic and complex BCR-ABL rearrangements such as three-way translocations or breaks outside of the usual major and minor cluster regions.[4] Dual color FISH utilizes two probes, one of which hybridizes to the 5′ end of the BCR region and the other to the 3′ end of the ABL region. Fluorescent microscopy is used to visualize these probes which are typically labeled with red and green fluorochromes to produce two red and two green spots representing the two copies of chromosomes 9 and 22 in normal cells. In cells harboring a BCR-ABL translocation, a red and green probe are juxtaposed to produce a yellow fluorescent signal. Typically, 200 interphase or metaphase nuclei are evaluated, yielding a sensitivity of about 1 in 200, or 0.5%.

This classic dual-color single-fusion FISH (S-FISH) assay is highly accurate for analyzing metaphases, but is hampered in its application to interphase cells by the coincidental overlap of BCR and ABL signals in about 4% of normal nuclei.[5] Because it is prone to false positivity, quantification below 10% is generally considered unreliable.[6–9] To improve assay specificity, alternate strategies were applied in the design of newer DNA probes. For example, extra-signal FISH (ES-FISH) employs a larger 650-kb ASS-ABL probe (Vysis, Downer's Grove, IL) which targets an area spanning the ABL gene and the adjacent arginino-succinate synthetase (ASS) gene. Since this probe spans both sides of the ABL breakpoint cluster regions, assay specificity is improved. In a validation study conducted at our institution, 30 non-leukemic patients were assayed for the presence of the BCR-ABL fusion using interphase ES-FISH. The background level of fusion signals in marrow samples was less than or equal to 5% with a confidence limit of 95%, indicating a marginal improvement in assay specificity. ES-FISH is also touted for its ability to distinguish M-BCR from m-BCR based on the appearance of the spot pattern.[10] (See Figure 1.)

Another strategy to improve assay specificity is dual-fusion FISH (D-FISH). This involves applying probes that span the common breakpoint regions of both ABL and BCR genes so that one may visualize both the BCR-ABL and the ABL-BCR fusion signals. In a commercial version of this system (Vysis, Downers Grove, IL) the large ASS-ABL probe mentioned above is combined with a large probe spanning a distance of about 1.5 mb on both sides of the BCR gene, thus providing a second confirmation of the translocation and reducing the rate of false positives to less than 0.5%.[4,11–13] In addition, D-FISH, like S-FISH and ES-FISH, can detect cryptic and complex BCR-ABL translocations that are missed or undecipherable by conventional cytogenetics.[4]

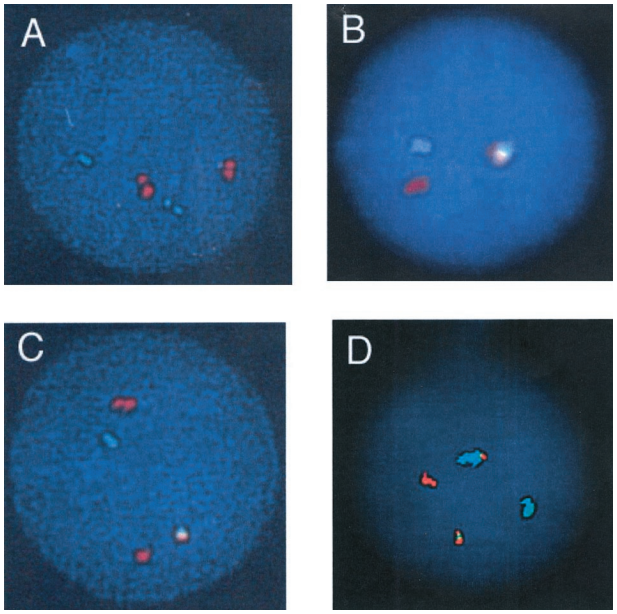

Figure 1. FISH using the Vysis Extra Signal (ES) probe reveals a normal result yielding two green chromosome 22 and two red chromosome 9 signals (**A**); one green, one red, and one red-green fusion signal, representing a pattern that could be artifact due to coincidental juxtaposition of red and green signals, or it could represent BCR-ABL translocation with concomitant loss of chromosome 9 material proximal to the breakpoint, or finally it could represent an insertion of bcr into the chromosome 9 long arm at the abl locus (**B**); typical findings in CML (p210 breakpoint) yielding one green, one red, one residual red, and one red-green fusion signal (**C**); and typical findings in ALL (p190 breakpoint) yielding one green, one red, and two red-green fusions (**D**).

Southern Blot Analysis

Southern blot analysis reliably identifies BCR gene rearrangement using probes targeting either the M-bcr or m-bcr breakpoint. While this is quite helpful in confirming the BCR defect associated with CML or ALL, the Southern blot method suffers from high cost and slow turnaround time. In addition, the assay is not sensitive for detecting minimal residual disease since tumor levels below about 5% are not detectable.

Amplification Technology

Reverse transcriptase polymerase chain reaction (rtPCR) is the most sensitive method described to date for detecting BCR-ABL. Instead of targeting chromosomal DNA, the assay targets the more abundant chimeric RNA transcripts produced from the fused genes. Chromosomal DNA is an impractical target not only because it is less abundant than RNA but also because the breakpoint regions span such large segments of intronic DNA that multiple PCR primer sets would be required to detect every possible translocation. On the other hand, the chimeric RNA is remarkably homogeneous from case to case, thus allowing reliable detection of nearly all disease-associated translocations (Figure 2).

Amplification assays are capable of detecting one affected cell among 100,000 or so normal cells. The exceptional sensitivity of rtPCR makes it well suited for assessing minimal residual disease following therapy. A downside that has hampered its implementation in clinical laboratories is the possibility of false positive results due to contamination of a negative specimen by amplicons produced from prior positive amplification reactions.[14] Recent technological improvements such as

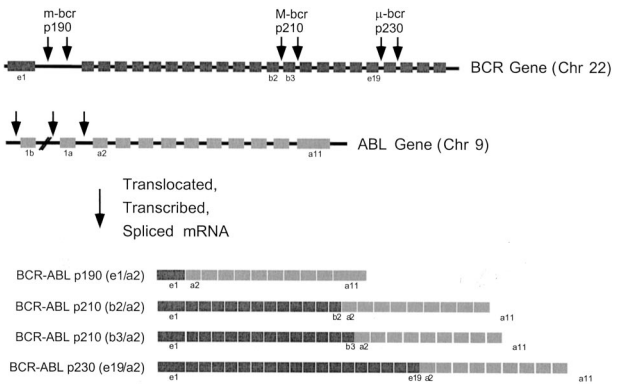

Figure 2. Diagrammatic representation of the BCR and ABL genes and the corresponding BCR-ABL fusion transcripts. **Arrows** designate three different breakpoint cluster regions in the BCR gene. Breaks in the M-BCR are most commonly associated with CML, the m-BCR with ALL, and the μ-BCR with chronic neutrophilic leukemia. ABL breakpoints usually occur between exons 1a and 2, resulting in fusion transcripts containing the tyrosine kinase domain of the ABL gene and variable portions of the BCR gene.

chemical destruction of wayward amplicons (eg, uracil-N-glycosylase system), or real-time detection of products to avoid amplicon manipulation, help minimize the possibility of amplicon contamination.

Another cause for concern is physiological positive results that mimic tumor-associated positive results. Spurious positive results for BCR-ABL rtPCR are identified in blood samples from up to 75% of normal individuals.[15,16] The majority of these normal individuals express only minute amounts of BCR-ABL transcripts that were detected by highly sensitive assays capable of detecting 1 in 100 million tumor cells. Typical clinical assays are a thousand-fold less sensitive and very rarely yield positive results in healthy individuals. Nevertheless, it is advisable to repeat any positive test when looking for residual disease and to compare the size of the amplicons with those known to be produced from the patient's tumor. Newly available quantitative rtPCR assays now make it possible to assess trends in the BCR-ABL load over time. These quantitative tests are further described in the section on minimal residual disease.

False negative amplification results are also a concern, particularly since RNA is the substrate for rtPCR assays, and RNA is notoriously subject to degradation by ubiquitous RNase enzymes. It is therefore advisable to stabilize RNA immediately on sample collection and to use appropriate control tests to confirm that amplifiable cDNA is present in each patient specimen.

Gene Expression Profiles and Microarray Analysis

Microarrays have been used to survey gene expression in ALL samples. A major advantage of array technology is the ability to evaluate expression of thousands of genes simultaneously. In a typical analysis, RNA is extracted from the patient sample and converted to labeled cRNA or cDNA before being applied to an array of complementary probes. Yeoh et al[17] reported using 12,600 probe sets to study 327 diagnostic marrow samples from childhood ALL patients. They could distinguish BCR-ABL cases from other forms of ALL, and they also identified additional subsets of patients at high risk of relapse based on their gene expression profiles. The findings are promising and will be followed by more studies aimed at defining a smaller panel of probe tests that is informative for diagnosis, classification, prognosis, and predicting response to therapy.

Prevalence of BCR-ABL in Acute Leukemia

The reported prevalence of the Ph[1] in adult ALL is 20 to 23% by conventional cytogenetic testing.[18–20] More sensitive rtPCR techniques reveal a prevalence of 27 to 30%.[20–22] In a study by Gleissner et al,[23] rtPCR testing for BCR-ABL failed or produced false negatives in 19 of 212 patients (9%). This was due to insufficient material in 10 cases, ambiguous results in Ph[1]-negative patients in six cases, and false negative test results in three cases. Cytogenetic analysis failed or was falsely negative in 48 patients (23%) including 32 non-analyzable and 16 false negative test results. Despite the possibility of failure, chromosome banding analysis remains the best first-line genetic test to assess any new acute leukemia because it screens for t(9;22) as well as for alternate or additional genetic defects. When negative or inconclusive results are obtained by cytogenetics, then FISH or rtPCR are recommended to determine whether BCR-ABL is present.

In childhood ALLs, the incidence of BCR-ABL translocation is much lower at only 2 to 4% depending on the method used for identification.[24–27] BCR-ABL transcripts are also present in 31 to 35% of biphenotypic acute leukemia in adults and children,[22,28] in 6% of de novo adult AML,[22] and in 1% of childhood AML.[29]

Clinical and Immunological Correlates at Initial Diagnosis

The majority of BCR-ABL positive adult and childhood ALLs have a typical pre-B cell immunophenotype with co-expression of CD10 (CALLA). Indeed, when results from five different studies were combined, including 170 adults and children, 94% of BCR-ABL positive ALL had a pre-B cell immunophenotype.[20,24,26,30,31] In a study by Gleissner et al[23] on 875 adult ALL patients, the prevalence of BCR-ABL positivity among CD10-expressing adult pre-B ALL was 37% whereas in the CD10-negative cohort it was only 2%. The incidence of BCR-ABL in T-cell leukemias varies among studies from 0% to 5%.[20,23,24,26,30,31]

Coexpression of myeloid markers, as defined by CD13 or CD33 in at least 20% of blasts, is found in 27 to 29% of BCR-ABL positive adult and childhood ALL.[23,30,32] Myeloid markers are more frequently seen in BCR-ABL positive than in BCR-ABL negative ALL.[23,26] There was no correlation between myeloid co-expression and p190 *versus* p210 breakpoint,[20,30] nor was there a correlation with WBC count, blast count, hemoglobin, or hematocrit.[31] Some investigators suggest that myeloid antigen

expression has an adverse impact on survival in BCR-ABL positive ALL patients while others do not.[19,30,32]

Studies comparing the presenting clinical features of BCR-ABL positive *versus* negative ALL have also produced conflicting results. Some investigators found no significant difference in age, presenting WBC count, percentage of blasts, splenomegaly, or hepatomegaly[20,26,30] while others found that BCR-ABL positive children[24,25] and adults[23] had higher WBC counts and older age. Recently, Gleissner et al reported that hepatosplenomegaly was more common in BCR-ABL negative adult ALL patients.[23]

The Significance of p210 versus *p190 Breakpoint*

An advantage of rtPCR and of certain FISH assays is the ability to differentiate p210 from p190 forms of BCR-ABL. Nearly all CML patients have a p210 breakpoint, and they retain this genotype if their disease progresses to blast crisis. In contrast, the majority of de novo Ph^1-positive acute leukemias harbor a p190 breakpoint. Therefore, patients whose acute leukemia harbors a p210 breakpoint should be evaluated to distinguish de novo acute leukemia from blast crisis of CML. This distinction can be difficult or impossible unless the patient had a prior white cell count or signs and symptoms of CML, or unless the patient reverts to chronic phase after treatment.

Among children and adults with Ph^1 positive ALL, approximately 75% have p190 and 25% have a p210 breakpoint in the BCR locus.[20,23,26,30] Approximately 3% of adult ALL patients express both p210 and p190 fusion transcripts.[23] This is explained by alternative splicing whereby M-BCR breaks produce both p210 and, at a lower level, p190 transcripts.[23,33] Most studies show no correlation between the type of breakpoint and clinical parameters or prognosis,[20,26,30,34] while a few studies showed patients with p210 transcripts were likely to be older.[23,35] Some investigators found a tendency for p210 positive patients to do better[36–38] or worse[23] than p190 patients. In Ph^1-positive AML patients, the p210 breakpoint is present in about half while the rest have a p190 breakpoint. AMLs harboring a p190 breakpoint usually have a monocytic (FAB M4 or M5a) phenotype.[18,39]

As noted above, alternative splicing can result in p190 transcript production from template DNA having a p210 breakpoint, and therefore CML or ALL (M-BCR breakpoint) patients may have low levels of p190 transcripts, usually not comprising over 10% of the total BCR-ABL mRNA.[33,40–43] In one study, p210 transcripts were detected in all CML patients in accelerated phase or blast crisis, and coexisting p190 transcripts were found in 8%.[42] None of these patients had p190 mRNA at the time of initial diagnosis. The association of p190 detection with relapse was reported by Serrano et al in a study of 55 CML patients who underwent marrow transplant.[43] All 14 patients who relapsed had become p190 positive by the time of cytogenetic relapse. In contrast, the 41 patients who remained in remission consistently tested negative for p190 but not necessarily for p210. Radich et al[37] studied 36 ALL patients at multiple timepoints after marrow transplant. Among 23 patients with at least one positive rtPCR result, 8 had p210 alone, 10 had p190 alone, and 5 had both types of BCR-ABL transcripts. The patients with p190 transcripts detectable after transplant were more likely to relapse than were patients with p210 alone or with undetectable BCR-ABL. This could be because p190 protein has stronger tyrosine kinase activity and is associated with a more aggressive leukemia in animal model systems.

The Impact of BCR-ABL on Prognosis of ALL

While early reports showed a lower response to induction chemotherapy in Ph^1-positive patients,[24] this has not been the case with the use of modern therapies. In a large study of 1322 children with ALL, nearly all Ph^1-positive (97%) and Ph^1-negative (98%) patients achieved complete remission after induction therapy.[25] But those who had Ph^1-positive leukemias were more likely to relapse. The estimated 4-year event-free survival was only 20% for Ph^1-positive as compared with 76% for Ph^1-negative patients. The four-year overall survival was 56% for Ph^1-positive and 85% for Ph^1-negative patients. The poor prognosis in children with Ph^1 positive ALL was confirmed by Schrappe et al,[30] where complete remission was achieved in 90% but the 4-year event-free survival was only 38%, and 4-year overall survival was 48%.

Analogous to the situation in childhood ALL, young adults consistently have a worse prognosis when their ALL harbors BCR-ABL translocation. Gleissner et al[23] found that 68% of BCR-ABL positive adult ALL patients achieved complete remission compared to 85% of BCR-ABL negative patients, with a higher frequency of early relapses in BCR-ABL positive patients. The median overall survival was 11 months for BCR-ABL-positive and 30 months for BCR-ABL-negative patients. The 3-year overall survival was 15% for BCR-ABL-positive and 47% for BCR-ABL-negative patients. Kantarjian et al, using a hyper-CVAD regimen to treat adult ALL, confirmed that the complete remission rate for Ph^1-positive patients was not different from that of the whole cohort (91%), but their 5-year survival was only 7% as compared to 39% for the entire study population.[19] The findings may not extend to the elderly, however, based on work from Onciu et al[44] showing that ALL patients over 59 years of age did not display an association between Ph^1 and poor prognosis.

The prognosis of biphenotypic acute leukemia (BAL) is worse than either ALL or AML. In a study of 23 adult BAL patients by Legrand et al, complete remission was achieved by 48% as compared to 65% of adult AML and 81% of adult ALL patients.[22] The median overall survival was 7.5 months for BAL, 11 months for AML, and 12 months for ALL. Killick et al, [45] in a report of 25 adult and pediatric BAL patients, found that prognosis was strongly related to t(9;22) status and age less than 15 years. The two-year overall survival for adults was 17% and for children was 75%. There was no difference in survival between children with Ph^1-negative BAL and those with

Ph[1]-negative AML or ALL suggesting that the worse prognosis in BAL may be related to BCR-ABL positivity.

Impact of BCR-ABL on Selecting Therapy for ALL

Because of the high rate of relapse and low overall survival in BCR-ABL positive acute leukemia patients, detection of this genetic abnormality is considered an indication for allogeneic BMT in first remission.[27,46,47] In a report of 30 children with Ph[1]-positive ALL, 29 achieved complete remission.[25] Fifteen of these patients underwent allogeneic BMT (five were related, seven were unrelated, and three were unspecified); 10 of these transplants occurred at the time of first complete remission. After follow-up for a median of 39 months, there were 6 event-free survivors among these 10 patients and only 2 event-free survivors among the other 20 patients, underscoring the utility of BMT in first remission.

In another study involving 61 children with BCR-ABL positive ALL, 28 underwent marrow transplant (23 were related, 5 were unrelated).[30] The four-year event-free survival for the whole group was 38%, 61% for patients who were transplanted, and 28% for patients who received only chemotherapy. Unrelated donor transplantation was associated with lethal toxicity in four of five patients. On the other hand, 15 of the 19 patients who received matched-related marrow transplant remained in first complete remission, and their four-year event-free survival was 83%.

As further support for transplant of Ph[1]-positive ALL during first remission, a study of 32 such children was conducted.[26] Thirty achieved complete remission, of whom eight then received allogeneic marrow, three received autologous marrow, and 19 received chemotherapy alone. Subsequently, three of the latter patients received allogeneic marrow and one received autologous marrow. All patients relapsed except six of the eight patients who received allogeneic marrow during first remission. There were no long-term survivors among relapsed patients despite treatments including allogeneic transplant.

Two long-term prospective studies confirm that adult ALL has a poor prognosis.[48,49] The highest risk was in patients who were over 35 years of age, Ph[1]-positive, had leukocyte counts above 30 $\times$ 10(9)/L, had null ALL or undifferentiated leukemia, or required longer than four weeks to achieve complete remission. A few studies specifically addressed the role of allogeneic BMT in Ph[1]-positive patients. Improved survival data were reported by Forman et al[50] in 10 such patients of whom six were alive and well for a median of 19 months. Snyder et al[51] showed that 23 Ph[1]-positive adult ALL patients who were transplanted in first remission had improved disease-free survival (65% at 3 years). Mitterbauer et al[21] reported that allogeneic transplant was more effective than chemotherapy alone in reducing BCR-ABL levels and in achieving long term remission. Among adults treated with stem cell transplant in first remission for Ph[1] ALL, Dombret et al[38] showed that those who achieved negativity for BCR-ABL before transplant had improved remission duration and survival after transplant.

Gleevec Therapy for BCR-ABL Leukemias

Imatinib (Gleevec), which targets the ABL tyrosine kinase hyperactivity of the BCR-ABL oncoprotein, has been shown to be effective in the treatment of CML or CML in myeloid or lymphoid blast crisis.[52,53] Unfortunately, Gleevec resistance sometimes develops after an initial response. A study by Gorre et al[54] showed that Gleevec resistance in nine CML patients was associated with reactivation of tyrosine kinase activity. In three patients, there was BCR-ABL chimeric gene amplification, while the other six patients acquired a point mutation in the tyrosine kinase domain of BCR-ABL (Thr315Ile).

Gleevec is also temporarily effective in treatment of BCR-ABL-positive ALL as shown in a recent report of 21 patients who had initially relapsed or failed to respond to standard chemotherapy.[55] With Gleevec therapy, 13 of these patients were classified as good responders (12 achieved complete hematological remission and one achieved partial remission with peripheral hematological recovery). Marrow aspirates of 9 of the 21 patients (5 good responders and 4 resistant patients) were obtained before and after therapy. Direct sequencing of cDNA representing a 714 bp segment of ABL encoding the ATP-binding site and the kinase activation loop showed an acquired point mutation at position 1127 (Glu255Lys) in 6 patients (4 good responders and 2 resistant patients). A seventh resistant patient acquired a point mutation at nucleotide 1308 (Thr315Ile). Other reports have confirmed the acquisition of point mutations in the ATP-binding domain of BCR-ABL in ALL and CML patients who became resistant to Gleevec.[56] Screening for these mutations may allow therapy to be altered before frank relapse.

Minimal Residual Disease Detection

Detection of minimal residual disease is beneficial to patients with ALL, and advances in laboratory technology provide new and more powerful ways to detect low level disease.[57–59] Most investigators have applied one of three approaches: 1) PCR amplification of rearranged IgH or TCR genes using custom-designed tumor-specific primers and probe; 2) multiparameter flow cytometry; or 3) Quantitative PCR of tumor-associated translocation breakpoints. All three approaches have merit. Targeting the IgH or TCR genes is feasible in nearly all cases of ALL, but the development of customized IgH or TCR probes is costly and labor-intensive. Flow cytometry is quite helpful assuming that the leukemic clone has an aberrant phenotype not prevalent in normal cells in the sample.

Analysis of tumor-specific translocations is a promising alternative for evaluating minimal residual disease in those patients whose tumor harbors a translocation for which a reliable laboratory assay is available.[60,61] BCR-

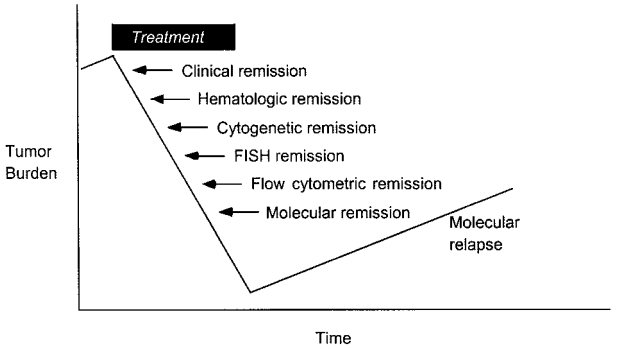

Figure 3. Laboratory tests are capable of detecting tumor burden at different levels. Up to a billion leukemic cells may remain in a patient who is in hematological remission, underscoring the importance of using more sensitive assays to detect and measure minimal residual disease. Theoretically, rising levels permit early intervention so that the number of cell divisions is minimized and the accompanying risk of secondary genetic events is likewise reduced.

ABL is an appealing target considering that a significant proportion of ALLs harbor the translocation (about 28% of adult ALL; 3% of childhood ALL), and sensitive assays are available for detecting it by rtPCR. Such amplification assays are the most sensitive of all laboratory approaches, followed by flow cytometry, FISH, and cytogenetics, with associated detection thresholds of about 1 in 1 million, one in several thousand, one in several hundred, and 1 in 20, respectively. (See Figure 3.)

Real-time quantitative rtPCR techniques are now available that permit precise measurement of chimeric transcripts. Levels can be reported either in absolute terms or relative to a housekeeping transcript. Recent studies using this technology to detect BCR-ABL transcripts have shown good correlation with the results of cytogenetic analysis, FISH, Southern blot analysis, and conventional nested rtPCR.[62–64] Various platforms such as the ABI Prism 7700 (Applied Biosystems, Foster City, CA) or the LightCycler (Roche Diagnostics, Indianapolis IN) were able to reproducibly detect one positive cell from among 10,000 to 100,000 normal cells.[62–70]

Investigators have consistently demonstrated the prognostic value of molecular analysis of BCR-ABL transcripts in the management of ALL patients. After successful marrow transplantation, ALL patients tend to become rtPCR negative within six weeks.[71] Persistence of BCR-ABL transcripts is associated with a high risk of relapse, while those who remain rtPCR negative have a good prognosis.[37,71–73] Disease-free survival is related to the rtPCR status after marrow transplantation regardless of the presence of residual disease before transplantation.[73,74]

The predictive value of molecular follow-up has been confirmed in a study by Radich et al[37] of 36 adults and children who were transplanted for Ph^1-positive ALL. Patients in whom rtPCR was positive on one or more occasions after transplant were more likely to relapse, and the unadjusted relative risk of relapse for a positive test was 5.7. A positive p190 result was particularly indicative of a high risk of relapse while p210 positivity was not. Indeed, of 10 transplant patients who were rtPCR positive and relapsed, 9 had p190 either alone (7 patients) or in concert with p210 (2 patients). In a more recent study by Radich et al, [72] the ABI Prism 7700 real-time rtPCR platform was used to quantitate BCR-ABL in posttransplant samples from 12 CML patients who relapsed and 73 patients who remained in remission. BCR-ABL levels were a significant prognostic indicator in these divergent outcome groups.

There is reasonably good correlation between BCR-ABL rtPCR results from blood and marrow samples collected at the same time. Lin et al reported complete concordance in blood and marrow samples obtained on 23 occasions from CML patients following allogeneic BMT or interferon therapy.[75] Positive cases showed good correlation in the number of BCR-ABL transcripts per microgram of RNA ($r = 0.99$). Radich et al found a 91% correspondence in BCR-ABL rtPCR results between blood and marrow samples obtained on 605 occasions from CML patients who had undergone allogeneic BMT.[72] Evaluation of the occasional discordances showed marrow alone was positive in 36 cases while blood alone was positive in 18 cases. In an analogous study of ALL patients, Radich et al[37] reported a 74% concordance in samples obtained on 31 occasions from ALL patients who had undergone allogeneic BMT, with the few discrepancies involving marrow-only positivity in five cases and blood-only positivity in three cases. The findings suggest that either blood or marrow samples may be suitable for follow up of BCR-ABL associated leukemias. Surprisingly, archival formalin-fixed paraffin or acrylate-embedded marrow biopsies have recently been shown to be suitable sample types for BCR-ABL rtPCR.[76]

It is theorized that minimal residual disease after marrow transplantation could be controlled with relatively modest intervention. A recent report showed that residual BCR-ABL transcripts in two ALL patients were rendered undetectable following acute graft *versus* host disease. Either donor lymphocyte infusion (DLI) or rapid reduction of immunosuppression caused this graft *versus* host effect, and it was associated with lasting disease remission.[77] Similarly, it has been shown that CML patients who achieve complete remission after DLI have prolonged survival. In a study of 39 CML patients who achieved cytogenetic remission with DLI, the overall probability of survival was 87% at 1 year, 76% at 2 years, and 73% at 3 years (median follow-up of 40 months after DLI).[78] The use of rtPCR positivity as the criterion for relapse following allogeneic BMT and as the indication for DLI has been investigated. Mughal et al presented data on 20 CML patients in molecular relapse who were treated with DLI.[79] After a median follow-up of 42 months, these patients were in continuous molecular remission. Their actuarial probability of survival at 10 years after BMT was 100% and their probability of relapse was 0%. The actuarial probability of survival at 10 years for 63 control CML patients who had been continuously PCR negative after allogeneic BMT was 97% (median follow-up of 8.4 years after allogeneic BMT). The availability of such effective measures for detecting and dealing with minimal residual disease is promising.

Currently there are no definitive guidelines on when or how frequently to monitor BCR-ABL for purposes of minimal residual disease detection in ALL patients. If we

extrapolate from the extensive literature on monitoring BCR-ABL in CML patients, we infer that the type of test and frequency of its application depends on which therapy is used and on its curative *versus* palliative intention.[73] CML treated with hydroxyurea, busulfan, or other conventional non-curative therapy relies on cytogenetics to monitor residual disease and to detect markers of progression, while more sensitive molecular tests such as FISH are only marginally more informative, and rtPCR is probably not needed at all.[9] When interferon therapy is used to treat CML, the European consortium recommends that once the number of Ph^1-positive cells has fallen below 10% by karyotype then quantitative rtPCR should be performed every 3 months.[81] Interferon should be continued until the BCR-ABL/ABL ratio is lower than 0.02%.[82] After stem cell transplant, the European consortium recommends quantitative rtPCR be done every 4 weeks for as long as BCR-ABL is detectable, then at intervals of 3 months and, after 1 year of undetectable levels, at up to 6-month intervals.[81] If levels increase, immediate retesting helps to confirm and direct intervention. This group has defined molecular relapse as a 1 log (10-fold) or greater increase in BCR-ABL determined by a minimum of three consecutive quantitative rtPCR tests regardless of the interval between these tests. Lin et al defined molecular relapse as >50 transcripts per microgram of RNA or increasing values on serial testing.[83] A more recent report from the same institution by Olavarria et al suggested the following criteria: A) the BCR-ABL/ABL ratio is >0.02% on three consecutive occasions over 1 month apart; or B) the ratio is >0.05% on two consecutive samples; or C) the transcript number is rising in three consecutive samples, with at least two samples having ratios above 0.02%.[84]

The National Comprehensive Cancer Network (NCCN) recommends less frequent monitoring of residual disease in CML.[85] According to their recent practice guidelines, after hematological remission is achieved following allogeneic marrow transplant, cytogenetics and rtPCR should be done every 6 months for the first 2 to 3 years and yearly thereafter.[85] If karyotype is positive at any timepoint, the patient should be treated for relapse with interferon or donor lymphocyte infusion (DLI). If rtPCR is positive but karyotype is negative, the patient should be observed and retested every 6 months for 2 years and then every 12 months. After treatment of relapse, the guidelines recommend rtPCR testing every 6 months for 2 years and yearly thereafter, with cytogenetic testing at any rtPCR-positive timepoint. Several clinical trials are underway to further determine the utility and recommended frequency of laboratory monitoring of CML, particularly now that Gleevec has been added to the armamentarium. Of note, one mechanism of Gleevec resistance is amplification of the BCR-ABL chimeric gene, leading to overexpression of the fusion transcript rather than the anticipated decrease expected with successful therapy.[54]

Conclusion

Patients with pre-B ALL should be tested for BCR-ABL at the time of diagnosis to identify those who have the translocation and who therefore carry a worse prognosis and are candidates for treatment with intensive induction regimens and marrow transplantation. Transplantation during first remission appears to benefit such patients. Studies are underway to assess alternative treatment programs such as Gleevec for their role in disease management.

Karyotype should be initially performed on all suspected ALL patients. While karyotyping has the advantage of being able to detect a large variety of genetic alterations, it is also vulnerable to false negative results. FISH and rtPCR are helpful for detecting occult t(9;22) and for differentiating p190 and p210 breakpoints. Amplification strategies are quite sensitive and thus well suited for follow-up to detect minimal residual disease. One caveat, however, is that trends in BCR-ABL test results rather than a value obtained at a single timepoint should be taken into consideration before making clinical management decisions. The recent advent of quantitative BCR-ABL rtPCR testing permits precise measurement of low levels of BCR-ABL that appear to reflect trends in tumor burden.

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