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Ph-positive leukemias in the era of modern cytogenetics, molecular biology, tyrosine kinase inhibitors and hematopoietic stem cell transplantation

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Summary

The review is devoted to the complex problems of modern cytogenetic and molecular diagnostics of Ph-positive leukemias, their treatment in era tyrosine kinase inhibitors and hematopoietic stem cell transplantation at the condition of cytogenetic and molecular monitoring.

Keywords: Ph-positive leukemias, diagnosis, treatment, tyrosine-kinase inhibitors, ABL-BCR mutations, hematopoietic stem cell transplantation, cytogenetic and molecular monitoring, prognosis

Introduction

The Philadelphia (Ph[1])-chromosome was discovered in 1960 year [1] and for a long time it was considered to be a specific marker of chronic myeloid leukemia (CML). Later it was found that Ph1 is the result of reciprocal translocation between chromosomes, i.e. t(9;22)(q34;q11) [2]. In fact, this translocation results in the formation of two hybrid genes, BCR-ABL on the Ph[1] chromosome and ABL-BCR on 9q+. The chimeric BCR-ABL fusion product is a tyrosine kinase. Its activity accounts for more than 95% of CML cases [3-5], and 10–20% of adults and about 2–5% of children with B-cell acute lymphoblastic leukemia (ALL) [6,7]. Occasional bona fide Ph[1]-positive (Ph+) cases among acute myeloid leukemias (AML) [8,9], lymphomas [10], multiple myeloma [11], myelodysplastic syndromes [12,13], 'essential thrombocythemia' [14-16], and chronic neutrophilic leukemia (CNL) [17] have also been published.

The treatment of CML has been recently revolutionized by the development of imatinib mesylate (IMT, Gleevec, STI571) and other tyrosine kinase inhibitors (TKIs), which report to be targeted inhibitors of BCR-ABL [18-20].

I. Laboratory Tests

1.1. t(9;22)(q34;q11) translocation detection

Translocation (9;22) in newly diagnosed leukemia patients is detected using conventional cytogenetics (CC) supplemented by chromosome banding analysis (Fig. 1), and supported by reverse transcriptase polymerase chain reaction (RT-PCR) [21]. The chromosome banding analysis remains the best first-line genetic test for assessing any new acute leukemia because it screens for t(9;22) as well as for alternate and nonrandom additional genetic defects, including +der(22)t(9;22), 9p abnormalities, i(17q), -7, +8, +19, +21, and so on [21].

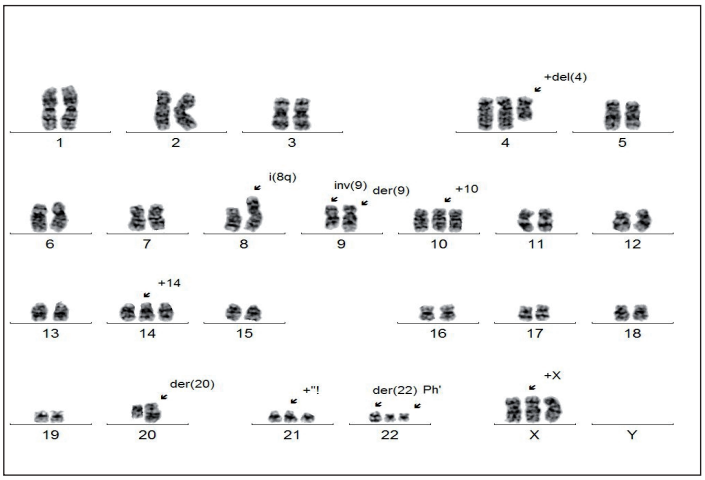

Figure 1. Karyotype of bone marrow cell from a female patient with Ph-positive acute lymphoblastic leukemia, relapsed after allogeneic stem cell transplantation, made in Germany. It illustrates two Ph-chromosomes with typical translocation t(9;22)(q34;q11) and multiple additional numeral and structural chromosome changes. 2n=52, XX, +X, t(9;22)(q34;q11), +der(22) t(9;22), +del(4)(q27), -8, +i(8)(q10), +10, +14, -20, +der(20q+), +21.

1.2. Fluorescence in situ hybridization

One of the sensitive approaches for diagnosis of Ph+ leukemias is fluorescence in situ hybridization (FISH). The FISH technique can be applied directly to non-dividing cells, it detects both cryptic and complex BCR-ABL rearrangements, including three-way translocations as well as some breaks outside of the usual major and minor cluster regions (Fig. 2). Sensitivity of the method is about 1 cell in 200 tested, or 0.5%. A problem with around 1% of CML patients who have a Ph1-negative karyotype because of a cryptic BCR/ABL1 fusion is that it can only be located by FISH at chromosome 22q11, 9q34 or a third chromosome [22-24].

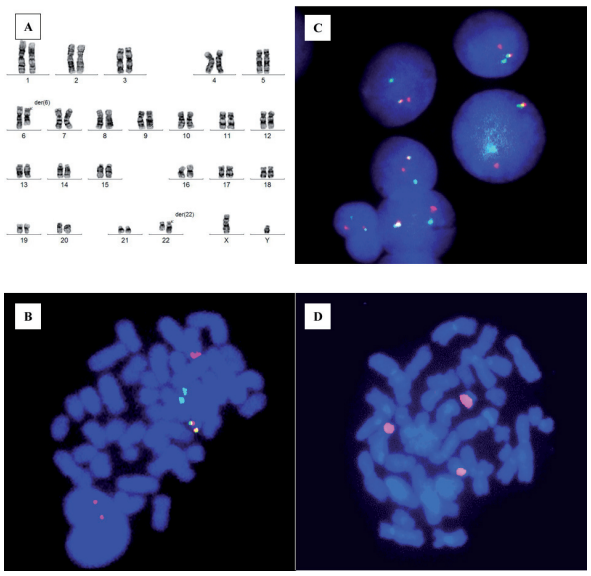

Figure 2. Karyotype (A) and fluorescent in situ hybridization (FISH) analysis findings on chromosomes (B, D) and interphase nuclei (C) from a patient with chronic myeloid leukemia, illustrating atypical translocation of t(6;22)(q21;q11), which was associated with formation of fusion ABL/BCR gene on chromosome der(22)t(6;22). 2n=46, XY, t(6;22)(q21;q11). The fusion gene (yellow color) (B, C) was evidenced by means of fluorescent probes to ABL (red) and BCR (green) genes. On the other hand, the atypical translocation t(6;22) was evidenced (D) by means of fluorescent probe WCW22 directed to chromosome number 22. After FISH each chromosome plate contains three brightly stained signals, including those on der(22)t(6;22) (small signal), unchanged chromosome 22 (right big signal) and der(6)t(6;22) (left big signal). Magnification x900.

1.3. Southern blot analysis

Southern blot analysis reliably identifies BCR gene rearrangement using probes targeting either the M-bcr or m-bcr breakpoint. Although this technique is quite helpful in confirming the BCR defect associated with CML or ALL, the Southern blot method is time-consuming and costly [22].

1.4. Amplification technologies

RT-PCR is the most sensitive of today‘s methods for detecting BCR-ABL. One of the advantages of RT-PCR is an ability to differentiate p210 from p190 forms of BCR-ABL [22]. It should be mentioned here that the recently developed real-time quantitative PCR (RQ-PCR) technique allows the assessment of trends in the BCR-ABL load over time and, hence, the chance to control minimal residual disease (MRD) [25].

1.5. Analysis of BCR-ABL kinase domain (KD) mutations

There are several molecular techniques that can detect BCR-ABL kinase domain (KD) mutations in Ph+ leukemias that are resistant to tyrosine kinase inhibitors (TKI). A more effective technique among them is a recently modified liquid chromatography (D-HPLC) [26-33].

1.6. Gene expression profiles and micro array analysis

A major advantage of array technology is the ability to evaluate an expression of thousands of genes simultaneously. For this purpose RNA is extracted from the patient’s sample and converted to labeled cRNA or cDNA before being applied to an array of complementary probes that allows the successful differentiation of BCR-ABL cases from other forms of ALL [34].

II. Laboratory and clinical findings in Ph-positive disorders

2.1. Structure of the BCR-ABL fusion genes and their transcripts

The breakpoint in the ABL gene occurs within a >300-kb segment at the 5’end of the gene, either upstream of the first alternative exon Ib, between exons Ib and Ia, or downstream of axon Ia. In the vast majority of CML patients and in about one third of ALLs, the breakpoint in the BCR gene is revealed within a 5.8-kb region known as the major breakpoint cluster region (M-bcr), spanning 5 exons historically named b1 to b5, now known to be exons e12 to e16 of the BCR gene. Regardless of the position of the ABL breakpoint, processing of the primary BCR-ABL transcript usually results in hybrid BCR-ABL mRNA molecules with e14a2 and or e13a2 junction encoding a $p210^{BCR-ABL}$ fusion protein [7]. In two thirds of ALLs and in very rare cases of CML and AML patients, the breakpoint in BCR falls further upstream, in the long (54.4 kb) intron between the two alternative exons e2’ and e2, known as the minor bcr (m-bcr). In these circumstances, exons e1’ and e2’ are removed by splicing, and the hybrid BCR-ABL transcript contains an e1a2 junction, and is translated into a smaller 190-kD BCR-ABL fusion protein named $p190^{BCR-ABL}$. Thus, e14a2 (b3a2) and/or e13a2 (b2a2) fusion transcripts and p210BCR-ABL protein[s] are more characteristic for CML. In contrast, the $p190^{BCR-ABL}$ protein arising from the minor BCR rearrangement producing the e1a2 and/or e1a3 fusion transcript is seen in the majority of cases of Ph+ ALL. Meantime, the expression of e13a2 and/or e14/a2 fusion transcripts is noted in ALL, especially in adult patients [35,36]. Furthermore, in Ph+ ALL patients some atypical BCR-ABL transcripts, including e1a3, e13a3, and e6a2, have recently been detected [36]. Additionally, occasionally cases of CML with e1a2, e19a1 and e19a2 transcripts [33,37,38] have been reported; the clinical significance of which remains unclear.

2.2. Prevalence of BCR-ABL

2.2.1. CML

The vast majority of CML patients have a p210 breakpoint of ABL-BCR gene, and they retain this genotype when their disease progresses to blast crisis (BC). There is also a possibility for coexistent p190 and p210 transcripts in 8% patients with accelerated-phase or BC CML [39].

It is important to clarify whether the position of the breakpoint within the M-bcr region (5' M-bcr v 3' M-bcr), or the type of fusion transcript (for instance, e13a2 v e14a2) somehow influences the disease phenotype or not. The debate has been going on for several years, with some evidence in favor [33,40,41] and some against [42,43] a possible link between M-bcr breakpoint location and disease features, that remains to be cleared up.

Currently many atypical BCR-ABL transcripts are documented in patients with CML, including e1a3, e6a2, e8a2, e13a3, e14a3, e19a2 and others [7,36,44]. The most frequent transcripts among them are e6a2 and e1a3 [36]. The clinical course of Ph+ CML with BCR breakpoints outside the above three main cluster regions (e.g. with e1a3) was more benign [45-48], although myeloid BC CML developed in most of them [36,38,47,49]. It has been actively discussed that the poor prognosis of leukemias in the patients with atypical ABL/BCR transcripts might be associated with their lacking the (GET)/Abl-like domain of BCR [50]. This conclusion was based on the findings of a case in Ph-negative CML, where BCR-ABL fusion was recognized by FISH staining of metaphase chromosomes only [51], and wherein e6a2 atypical ABL-BCR fusion, encoding a hybrid p195BCR-ABL protein [50] was detected.

2.2.2. ALL

The BCR-ABL fusion gene is found in around 25% of adult ALL cases [36,52-54] and in 2% of children [55-56]. Minor breakpoint transcripts (e1a2) encoding for p190 protein, are found in 59% to 70% of positive cases, whereas major breakpoint transcripts (e13a2 or e14a2) encoding for p210 protein are detected in 23% to 30% of cases [57]. Thus, the typical BCR-ABL mRNA transcripts are e1a2, e13a2, and e14a2. At the same time 3% to 19% of ALL patients can express both p210 and p190 fusion transcripts [35,52-54,57]. Preliminary data show a high prevalence of p210 copies with respect to copies of the p190 transcript in p190/p210+ cases [35]. Finally, atypical BCR-ABL transcript products are revealed in 1–2% of all Ph+ ALL cases, and include such atypical transcripts as e1a3 (most often), e6a2, e13a3, and e19a2 [7,36,58,59].

The presence of BCR-ABL in ALL is interesting for two reasons. First, the patients with Ph+ ALL have a poor prognosis under conventional therapy [52] and are, therefore, considered high-risk patients and primary candidates for both intensified therapy regimens and alloSCT [36]. Secondly, BCR-ABL positive patients can have a significantly better prognosis under treatment regimens with ABL TKIs such as imatinib [60], dasatinib [61,62], nilotinib [63] etc., and should take them whenever possible.

2.2.3. Clinical significance of BCR/ABL isoforms

The majority of BCR-ABL positive adult [52] and childhood ALLs [55,56,64] have common or pre-B cell immunological variants with frequent co-expression of CD10, CD13, and CD33 antigens. A special analysis of gene expression patterns showed PILRB, STS-1 and SPRY genes to be overexpressed, whereas TSPAN16 and ADAMTSL4 genes are under-expressed in $p190^{BCR-ABL}$-positive cases relative to $p210^{BCR-ABL}$-positive ALLs [54,57]. No difference was seen in the overall survival of patients with $p190^{BCR-ABL}$-positive vs $p210^{BCR-ABL}$-positive ALL. On the basis of this data a gene expression- and interaction-based outcome predictor consisting of 27 genes (including GRB2, GAB1, GLI1, IRS1, RUNX2, and SPP1) has been constructed which, in turn, correlated with overall survival (p=0.0001) in BCR-ABL-positive adult ALL, independent of age (p=0.25) and WBC count at presentation (p=0.003) [54].

The overall response to induction therapy (without TKIs) as well as the type of transplants did not differ significantly between patients with p190+ and p210+ transcripts, although the probabilities of DFS and OS are dependant on BCR/ABL isoforms [35]. Multivariate analysis performed in BCR/ABL+ patients showed that among the variables analyzed (i.e., age, white cell count, expression of CD34, CD10, CD13 and CD33, and type of BCR/ABL transcript), the presence of the p190 fusion transcript was the only powerful independent prognostic factor that favorably influenced disease-free survival (p=0.031) [35]. Besides this, the p190 fusion appears to be the only independent prognostic factor with regard to DFS and OS [35,65].

2.3. Treatment of BCR-ABL+ leukemias with TKIs

2.3.1. CML

The deregulated tyrosine kinase activity of the BCR-ABL and represents an extremely attractive target for therapeutic intervention. The first original Abl tyrosine kinase inhibitor (TKI) imatinib mesylate (imatinib, IMT) has been developed for clinical use as a result of collaboration between Brian Druker [66-69] and investigators at Ciba-Geigy [70]. Its efficacy was evidenced both on cell lines and the great material collected from patients with CML [69,71-83]. With a median follow-up of 19 months [77], the estimated rates of complete hematologic remission (CHR) for patients whose initial treatment was imatinib were 96%; major cytogenetic response (MCyR) was 87%; and from CCyR-treated patients, 76%. In patients with accelerated phase CML, imatinib results in a CHR rate of 82% and a MCyR rate of 24% [84]. However, in BC patients CML responses to IMT were of short duration [68,71], although around 7% patients remain alive after a median observation time of 6 years [85]. In this case imatinib results in a CHR rate of 8–11%, having a MCyR response rate of 16% only, and the median time to relapse in responding patients with BC was about 3 months [86].

The striking efficacy of IMT in CML [25,72,74,75,78,79,87-89] has established this therapy as a new standard for care for the disease. However, resistance is an emerging problem. which has prompted the design of several second-generation TKIs. To overcome the resistance to imatinib [90-93], high-dose IMT therapy was used [94] as well as alternative second-generation TKIs, including dasatinib, nilotinib, INNO-406, MK-0457, and bosutinib (BST).

Dasatinib (BMS-354825; Bristol-Myers Squibb) [68,92] is active against many of the kinase domain mutants responsible for imatinib resistance [95,96]. One remarkable exception appears to be the T315I mutation. As shown in a special study of 21 Ph+ patients who failed to respond or relapsed during dasatinib therapy, all patients but one had mutations at residue 315 and/or at residue 317 [30].

Clinical experience in treatment of IMT-resistant or refractory CML patients with dasatinib and nilotinib has been recently re-

viewed by Goldman [75]. As a result, MHR rates with dasatinib among intolerant or resistant to imatinib patients were 63% for accelerated phase CML (follow-up > or =9 months), 34% for patients with myeloid BC CML, and 35% for those with lymphoid BC CML (follow-up > or =12 months; START-B and START-L trials) [92]. In another study, CHR responses with dasatinib were achieved in 81% IMT-resistant patients with CP CML, while MCyR was demonstrated in 57% of patients [97]. As for high resistance to dasatinib, it was related to the T315I and F317L Abl KD mutations [30,98,99].

The next of the second-line BCR-ABL TKI is nilotinib (formerly AMN107), which is 20 to 50 times more active than imatinib against CML cells [87,93]. The first experience with nilotinib in 119 IMT-resistant patients with CML showed the following. Of 30 patients at BP of the disease, 13 (39%) experienced HR and 9 patients (27%) had a CyR, of whom 6 had a MCyR (Ph+ cells in metaphase, ≤35%). Of 46 patients with accelerated phase CML 33 had a HR and 22 had a CyR. Lastly, 11 of 12 patients with the CP CML had a CHR [90]. As for the resistance to nilotinib, it was again associated with T315I and F317L ABL KD mutation [30] that allows a choice of such TKIs as INNO-406 or MK-0457 for treatment patients with the above unfavorable mutations [30,75,98,99].

2.3.2. ALL

The treatment of Ph+ ALL has also changed dramatically since imatinib was introduced [100-105]. Early phase I and II studies of imatinib in Ph+ ALL revealed its significant, albeit unsustain, activity against relapsed or refractory leukemias [73,81,106]. On the other hand, combined with chemotherapy, or even as a single agent, it can produce CR rates of 90% or higher in newly diagnosed patients [6,85,100-102,104,105,107-109]. However, these responses were not durable (median, 9 months) [60], whereas further intensification of chemotherapy has had no substantial impact on the unfavorable course of de novo Ph+ adult ALL or in patients who fail first-line therapy [110,111]. A special study of alternating vs concurrent schedules of imatinib and chemotherapy as front-line for Ph+ ALL demonstrated that co-administration of imatinib and induction cycle 2 results in a CHR rate of 95% and RT-PCR negativity for BCR-ABL in 52% of patients, compared with 19% in patients in the alternating treatment cohort (p=0.01). Further, in each cohort, 77% of patients underwent alloHSCT in CR1. As a result, both schedules of imatinib had acceptable levels of toxicity to patients, but concurrent administration of IMT and chemotherapy had resulted greater anti-leukemic efficacy [109].

It should be noted also, that the Ph+ ALL is very characteristic for elderly patients, wherein it has a poor prognosis, with a low CHR rate, high induction mortality, and short remission duration [112]. On the other hand, imatinib as a single agent for elderly patients is toxic enough whereas a potentially curative alloHSCT is not generally applicable [110]. Therefore, it is noteworthy, that the overall CHR rate in the group of elderly patients where imatinib was used for remission induction, was significantly higher (93.5%) than that in patients wherein for induction remission was used multi-agent chemotherapy (50%, p=0.0001) [112].

Despite the similar cytogenetic and molecular characteristics, the resistance to imatinib in patients with Ph+ ALL develops earlier and more frequently than that in CML, and therefore second-line TKIs are necessary. In part, CHR and CyR may be achieved with dasatinib [68,90], and nilotinib [113], which appear to be ineffective in cases with T315I and F317L KD mutations [99].

Analogous with CML, the best drugs for treatment of patients who seem to be resistant to or intolerant to imatinib, were dasatinib, nilotinib, or bosutinib [6,63,91]. According to published data [90,92], dasatinib induced CHRs in 42% of patients with IMT-resistant Ph+ ALL (follow-up > or =12 months), which is even higher than those in patients with myeloid and lymphoid variants of BC CML.

2.4. BCR-ABL kinase domain (KD) mutations

2.4.1. CML

More than 90 different point mutations encoding for distinct single amino acid substitutions in the BCR-ABL KD have been so far identified, but 10 of them occur in >80% of the cases [20]. The presence of KD mutations has been studied mainly in the advanced phase, and in CP patients when they become resistant to imatinib [113]. Meantime, a special screening for the mutation status of 319 newly-diagnosed patients with CP CML showed the identification of a mutation without other evidence of imatinib resistance to be highly predictive for loss of CCyR (RR, 3.8; p=0.005) and for progression to advanced phase (RR, 2.3; p=0.01). On the basis of this data it was concluded that mutations in the P-loop (excluding residue 244) were associated with a higher risk of progression than mutations elsewhere [114]. Although T315I was the most frequent mutant to emerge, other mutations like T315A, V299L, and F317I were detected that demonstrated retention of sensitivity to imatinib and potentially to nilotinib [30,115]. Among patients with CP CML who develop (secondary) resistance to imatinib, 30% to 50% have one or more BCR-ABL KD mutations [116-120]. Some DK mutations (V299L, T315I, and F317L/I) were greatly specific for dasatinib [122-125], and others (G250E, Y253H, E255K, T351I, or F311I) for NLT [122] or MK-0457 [126]. Serial investigations showed that the longest period between detection of a mutation and subsequent relapse is observed in patients harboring M351T mutations [125]. In contrast, patients harboring T315I or P-loop mutations demonstrated a 5–25-fold need for immediate withdrawal of imatinib [28,29].

According to the three largest retrospective studies [28,117,127], the incidence of the T315I mutation in IMT-resistant patients was 4%, 11%, and 19%, respectively whereas the frequency of P-loop mutations in the same series of patients was much higher, being 28%, 46%, and 39%, respectively. In general, survival of patients with this mutation remains dependent on the stage of the disease, it having an indolent course in many CP patients [98]. Meantime it is expected that patients with the T315I mutation will also reveal resistance to the treatment with either dasatinib or nilotinib [29,30,68,87,128]. That is why INNO and ON012380, which are binding regions of BCR-ABL other than the ATP binding pocket, are recommended first of all for these patients [30]. It should be noticed that except for the lack of response to second TKIs (p=0.002), other patient characteristics, including outcomes between patients with T315I and those with other or no mutations were similar.

So the prognostic significance of such KD mutations as T315I, and P-loop region of ABL is undoubted. It is also important that T315I and P-loop mutations were preferentially presented in accelerated phase and of BC CML, compared to other types of mutations in CP CML (p=0.003). In addition, overall survival (OS) after imatiunib initiation was significantly worse for P-loop (28.3 months) and for T315I (12.6 months), than that for other mutations (p=0.0004). Meantime, multivariate analysis of data from CP patients demonstrated a worse OS for P-loop mutations only (p=0.014), but a worse progression-free survival (PFS) for T315I mutations (p=0.014).

The vast number of mutation reports published later have demonstrated number of IMT-resistant patients with different amino acid substitution to be greatly high [89]. Many of these BCR-ABL DK mutations are associated with a relatively modest increase in resistance to imatinib, which may be overcome by dose increase only [30,51]. On the contrary, mutations such as T315I, and those falling within the P-loop region, i.e., G250E, Y253F/H, and Ee55K/V, confer a highly resistant phenotype [28-30]. An analysis of BCR-ABL mutations by means of denaturating high-performance liquid chromatography found them in 127 of 297 (43%) of the available Ph+ patients. These mutations were revealed in 27% of CP CML patients (14% treated with IMT frontline; 31% treated with IMT post INF-α failure), 52% of accelerated-phase patients, 75% of myeloid BC patients, and 83% of lymphoid BC/Ph+ ALL patients. It should be mentioned that mutations were associated in 30% of patients with primary resistance to imatinib (44% hematologic and 28% cytogenetic), and in 57% of patients with acquired resistance (23% patients who lost CyR; 55% patients who lost HR; and 87% patients who progressed to accelerated phase/BC). On the basis of these findings the following conclusions were drawn. Firstly, amino acid substitution of seven residues (M244V, G250E, V253F/H, E255K/V, T315I, M351T, and F359V) accounts for 85% of all resistance-associated mutations. Secondly, the search for mutations is important both in case of IMT failure and in case of a loss of response at the hematologic or cytogenetic level.

It is interesting to note that in the majority of the resistance patients with CML, mutations were detectable at various intervals prior to hematologic relapse (range 0.9–44.2 months), which was registered at a median of 12.9 months after the start of imatinib therapy. On the other hand, BCR-ABL mutations first became detectable at a median of 5.8 months (range 0–30.5) after commencing imatinib. The difference between time to hematologic relapse and earliest detection of a mutation was highly significant (p<0.0001).

Since BCR-ABL KD mutations in some of CML and Ph+ ALL patients can be found without clinical evidence of resistant disease [26,129-131], the questions arises as to when tests for DK mutations should be done and by what methods. According to international recommendations, BCR-ABL KD mutation screening in chronic phase CML is only recommended for patients with inadequate initial response to TKIs or those with evidence of loss of response. Mutation screening is also recommended at the time of progression to accelerated or BC CML [26,132,133]. Criteria for inadequate initial response (i.e., primary resistance) include lack of CHR, mCyR (66-95% Ph+ metaphases in BM) or lack of MCyR at 3, 6, and 12 months respectively which are similar to the criteria adopted by the European Leukemia Net [89]. Criteria for loss of response to TKI (i.e., secondary resistance) are also based on cytogenetic and/or hematological relapse, with variable use of molecular relapse criteria. One proposed molecular trigger for mutation testing is a tenfold or greater increase in BCR-ABL transcript levels, although smaller rises in BCR-ABL transcript levels may also be predictive of mutation development.

2.4.2. ALL

In comparison with CML, BCR-ABL KD mutations in relapsed Ph+ ALL occur much more frequently (80% to 90% of cases), especially in patients who have been treated with TKIs as initial or ongoing therapy [26,32,33,134]. However, more sensitive detection methods reveal low levels of a point mutation clone in some Ph+ ALL cases before exposure to TKIs. The latter convinces that resistant clones in Ph+ ALL may precede TKI selection [134].

A study of the ABL KD mutation status in newly diagnosed Ph+ ALL patients showed that IMT-resistant TK domain mutations were detected in 38% patients even before exposure to IMT. Although the frequency of the mutant allele was low in such patients at the time of relapse, the same mutation was present as the dominant clone in 90% of the relapsing cases [134].

2.5. Hematopoietic stem cell transplantation (HSCT)

2.5.1. CML

Until the advent of imatinib, alloHSCT was considered to be the only known curative therapy for patients younger than 50 years having suitable HLA-identical siblings, HLA-matched family members, or HLA-matched volunteer unrelated donors [75]. Although the morbidity and indeed mortality attributable to the procedure were both appreciable, the probability of survival could be predicted with reasonable accuracy via the scoring system developed for the European Group for Blood and Marrow Transplantation (EBMT) [135]. Graft-versus-leukemia (GvL) effects contribute substantially to the curative potential of this approach [25,136]. However, relapse occurs in approximately 5% to 20% of patients transplanted in chronic phase. While some patients remain in a stable MRD status, others rapidly progress to overt clinical relapse [137]. In order to re-induce MolR through augmenting or re-establishing the GvL effect, cessation of immunosuppression [138] and, particularly, donor lymphocyte infusion (DLI) are standard approaches for patients with relapse after alloHSCT [139,140]. Unfortunately, this approach is frequently associated with substantial risk, mainly induction of graft-versus-host disease (GvHD) or BM suppression [139-144], which in large surveys have been observed in approximately 48% and 18% of patients [25,145]. As a result, over 80% of patients with low risk of GvHD (i.e., 0–2) had a probability of being alive at 5 years [146] and the probability of subsequent relapse for patients free of detectable CML was extremely low [147].

Although the treatment with imatinib is less toxic, it may not cure the disease and, being used for a long time makes it very expensive. Additionally, resistance to imatinib can also occur in the course of the therapy. Therefore, alloHSCT for the treatment of some CML patients remains necessary [25]. Firstly,

transplanted HSC have anti-leukemic activity and contribute to maintaining remissions. Secondly, the effect of imatinib may be increased by potential side effects of DLI and vice versa [148]. Since suppression of MRD with imatinib can theoretically allow re-establishment of complete chimerism as well as restore full GvL effects, this approach has been recently tested successfully in clinical practice [37,77,149]. Additionally, a special molecular investigation showed that 70% of patients with CML who relapsed after alloHSCT can achieve CMolR after imatinib treatment and DLI [139,140,142,148].

2.5.2. ALL

The poor outcome with conventional chemotherapy made alloHSCT an attractive option for patients with Ph+ ALL [100,101,108,109,150-157]. The first study of allo BMT in 10 patients with Ph+ showed that 4 patients died of transplant-related complications, while 6 patients survived the transplant and remained in CR (median follow-up; 19 months) [158]. A retrospective analysis in 67 Ph+ ALL patients aged 5–49 years who underwent alloHSCT, showed that 21 of them (31%) were in continuous CR≥2 years after transplant [159]. There were no significant differences in TRM, DFS, and relapse rate between patients who had been transplanted in first or later CR. However, 2-year DFS was much lower in patients who had never achieved CR before transplant. Better results were reported later [151,154,160,161]. For instance, Snyder et al [151] reported a 65% DFS rate (15/23) at 3 year post-transplant, whereas the estimated relapse rate during that period was only 12%. According to data from the French Bone Marrow Transplantation Society [162], in a study where 76 adult patients were in CR1 at the time of transplant, 2-year OS was demonstrated in 50% of patients, whereas the 2-year relapse incidence rate was 37%. In the largest prospective alloHSCT study (conducted during the pre IMT era), 5-year OS was superior in those who underwent MRD alloHSCT compared with chemotherapy or autologous HSCT [163,164]. Unfortunately, despite its efficacy, currently only 20% to 60% of patients actually undergo alloHSCT. Moreover, even after alloHSCT in CR1 the probability of relapse for Ph+ ALL patients is approximately 30%, which together with a high transplant-related mortality of 20% to 40% highlights the limitations of current therapy [109,165,166].

The recent appearance of TKIs in clinical practice has changed the up-front treatment paradigm and appears to affect the outcome after HCST [167]. In contrast with CML, wherein TKIs have changed our attitude to HSCT as the treatment of first choice [168], TKIs in Ph+ ALL appear to be seen only as a “bridge to transplant”, helping more patients to achieve and maintain sufficiently durable remission before [156] or after [63] HSCT. The latter is desirable to undergo in CR1. In such a case the long-term survival rates may be greater than 35%. Furthermore, the results can be essentially improved after first-line imatinib interim treatment [107,157]. The data suggests that the 3-year estimated probabilities of relapse, DFS, and OS were 3.5% vs 47.3% in controls (p=0.02), and 78.1% vs 38% in controls (p=0.001), respectively, without much difference in the transplant-related toxicities. In this context, other studies have also demonstrated the feasibility of giving imatinib following alloHSCT for BCR-ABL+ ALL either preemptively or to treat any MRD detected after transplant prior to relapse instead of using a donor lymphocyte infusion [169,170]. The management of patients with Ph+ ALL relapsing after alloHSCT represents a major challenge with limited chances of success. The efficacy of DLI in Ph+ ALL is much lower that that in CML [171], which is explained by the diverse immune escape mechanisms of ALL blasts from GVL effects of DLI [172]. On the other hand, the addition of cytoreductive chemotherapy to DLI does not seem to improve the outcome of relapsed ALL patients [173], whereas a combination of TKIs and DLI may be greatly effective [63].

2.6. Monitoring treatment and/or minimal residual disease

2.6.1. CML

2.6.1.1. Blood counts and bone marrow karyotyping

Complete blood counts should be performed at least weekly until they have stabilized, with greater intervals thereafter. Once CHR has been documented, monitoring continues with karyotyping of bone marrow metaphases, which is recommended at 6, 12, and 18 months, or until CCyR has been achieved [38,89,174,175]. If there are fewer than 20 metaphases, the cytogenetic response can be validated by determining the level of BCR-ABL transcripts and by molecular cytogenetics, or FISH. Of note is that FISH can be performed on metaphases or more frequently and more conveniently on interphase cells (IP-FISH) [38,176]. In fact, all FISH data correlates very significantly with chromosome banding data [177], as well as with BCR-ABL transcript levels [176,177]. Moreover, FISH can detect deletions of the long arm of chromosome 9 and variant translocations.

2.6.1.2. Monitoring BCR-ABL transcript levels

Molecular monitoring of BCR-ABL transcript levels with RQ-PCR has become an integral part of management of patients with CML [132,178-180]. This is particularly relevant in the era of imatinib therapy, when residual levels of leukemia usually fall below the level of detection via bone marrow cytogenetic analysis. Indeed, even small increases in the BCR-ABL level can identify patients with TK domain mutations that lead to IMT resistance.

Currently, the best molecular approach for monitoring BCR-ABL transcript levels is considered to be RQ-PCR [133,178,179]. The best gene for internal reference appears to be β-glucuronidase and not the widely-used ABL gene [180]. In fact, a failure to achieve a major MolR by 18 months after starting imatinib therapy is considered a suboptimal response requiring careful re-evaluation and possible reassessment of therapy [92]. Various prerequisites for achieving optimal sensitivity and standardization have been agreed upon and published [133,181,182]. According to recent international reporting scale, major MolR is established at BCR-ABL 0.1%, whereas a value of 1.0% is approximately equivalent to the achievement of CCyR [38]. From a practical point of view, it has been suggested that RQ-PCR must be performed every 3 months even in patients who achieve a MolR [179]. If, after alloHSCT, the attainment of BCR-ABL-negative status is conferred, the overall survival is significantly improved (57% vs 12%; p=0.006) [176]. In contrast, in the case where BCR-ABL levels increase, there is the possibility of identifying the patients who do not respond properly to the proposed therapy or even to start the

search for BCR-ABL mutations [183,184]. However, even considering that RQ-PCR is fundamental for monitoring patients with CCyR, there are other reasons to suggest that cytogenetics should not be completely replaced by RQ-PCR in the follow-up of CML patients. Firstly, additional chromosome abnormalities present at diagnosis or arising during the disease may have a prognostic influence [183]. Secondly, there is evidence of clonal cytogenetic abnormalities in the Ph-negative cells [185,186] which appeared after suppression of Ph-positive clone by imatinib and, in a majority of cases, could be a characteristic of future myelodysplasias [38].

2.6.1.3. Monitoring BCR-ABL kinase domain mutations

A mutation at the TK domain of the oncogenic BCR-ABL protein is frequently detected in patients with CML who fail to respond to TKIs or lose the response [187]. The best approach for screening for BCR-ABL mutations during therapy with TKIs is D-HPLC [28,31]. The studies show that mutations may be detectable several months before relapse, but the occurrence of BCR-ABL mutations during imatinib therapy is predictive of relapse.

2.6.2. ALL

2.6.2.1. Cytogenetic monitoring

In contrast to CML, cytogenetic changes in Ph+ ALL in the course of chemotherapy do not last long. Therefore, the main tasks of cytogenetics, including FISH, in these patients are: a) to verify a CCyR; or, in turn, b) to detect a resistance to chemotherapy, TKI or HSCT.

2.6.2.2. BCR-ABL transcript level monitoring

BCR-ABL RNA transcripts are suitable molecular markers for MRD analysis and for guiding therapy [25,188,189]. However, MRD analysis with RT-PCR is affected by intra- and inter-assay variability, which appear to reflect different proportions of leukemic blast cells. A special investigation of samples from 56 patients showed levels of BCR-ABL to be higher in bone marrow than in peripheral blood. In fact, they did not differ when normalized to 100% blasts. Moreover, the numbers of BCR-ABL transcripts per blast in 25 sequential BM and 8 sequential PB did not change significantly during evolution of Ph+ ALL. As for the mean number of p210 copies per blast concern they were 1.1 log higher than that for p190 (p=0.0006).

2.6.2.3. BCR-ABL kinase domain mutation monitoring

In contrast to CML, pre-existence of mutations including the T315I mutation did not adversely effect either the CR rate following imatinib or chemotherapy induction or the achievement of BCR-ABL negativity in response to combination therapy, when compared with patients exhibiting only non-mutated BCR-ABL at diagnosis [190]. In our opinion, this might be explained by the earlier use of high-doses chemotherapy followed by alloHSCT.

2.7. Prognosis

2.7.1. CML

In the TKI era the prognosis for CML patients has greatly improved. The majority of patients can now expect to survive 10 to 20 years [74,88,191]. It is even possible that some patients treated for a number of years can stop the imatinib and be regarded as cured of their leukemia [192]. Hence, any possibility of recognizing the minority of patients who respond poorly to imatinib at the earliest opportunity is very important [193]. Therefore, a series of empirical recommendations developed on behalf of the European LeukemiaNet (Table 1) [89] are used by clinicians to identify those CP CML patients responding poorly to imatinib at standard dosage. As for the other prognostically poor and verified factors in CML patients, they include the following: a) age; b) advanced stages CML; and c) BCR-ABL domain mutations, mainly, T315I.

Time	Failure	Suboptimal response	Warnings
Diagnosis	NA	NA	High risk
			del 9q+; addit chrom abnormalities (ACA) in Ph+ cells
3 months	No HR	Less than CHR	
6 months	Less than CHR	Less than partial CyR (Ph+ >35%)	
12 months	Less than partial CyR (Ph+ >35%)	Less than CCyR	Less than MMolR
18 months	Less than CCyR	Less than MMolR	
At any time	Loss of CHR; loss of CCyR; TK domain mutation	ACA in Ph+ cells; Loss of MMolR; TK domain mutation	Any rise in transcript level; ACA in Ph- cells

Table 1. Modified European LeukemiaNet definition of failure and suboptimal response for previously untreated, early CP CML patients treated with 400 mg IMT daily

Failure implies that the patient should be moved to other treatments whenever available; NA indicates not applicable; HR, hematological response; CHR, complete HR; CyR, cytogenetic response; CCyR, complete CyR; MMolR, major molecular response; ACA, additional chromosome aberrations, and TK tyrosine kinase

2.7.2. ALL

The prognosis of Ph+ ALL — whatever the fusion protein — is poor in both adults and children [70,194]. Detailed analysis of prognostic-related factors showed that deletion of the IKZF1 gene [195-199], as well as the secondary chromosome aberrations at diagnosis [200-204], in addition to t(9;22), had a significant association with shorter RFS. The latter has been reported in both before the imatinib [177,178]] and during the imatinib era [21]. Partly, there is data from 80 Ph+ ALL patients treated with imatinib, which was followed by alloHSCT in 60 of them. In fact it was found that the 2-year RFS for those who had undergone alloHSCT during CR1 was 62.6±7.5% compared to 62.1±12.3% for those who had not undergone alloHSCT. When alloHSCT was considered as a time-dependent covariant, it was shown to have no significant effect on RFS. On the other hand, a statistically significant negative impact on RFS for +der(22)t(9;22) and 9p abnormalities ($p<0.001$ and $p<0.005$) [21] was revealed, although it had not been demonstrated earlier [204].

Conclusions and future directions

The study of patients with Ph+ leukemias allowed a clarification of many sides of the nature and pathogenesis of leukemias [127,128], and helped to develop new therapeutic approaches [208,209]. The treatment of these disorders was partly revolutionized with TKIs. As a result, nowadays the vast majority of the patients with CML can be treated without alloHSCT. As far as HSCT is concerned, this must be reserved for those CML patients who become resistant to TKIs. The main reason for the resistance formation appears is considered to be mutations of BCR-ABL KD, primarily T315I. For achieving effect in relapsed after HSCT patients may be effectively used DLI which is the ideal approach for treating these CML patients [164].
In contrast to CML, alloHSCT is the only known curative modality for patients with Ph+ ALL under 55 years old. Naturally, it must be coupled by high dose chemotherapy and TKIs before transplantation [157,170]. If such therapy is unsuccessful, testing for BCR-ABL domain mutations is necessary. Besides other prognostic factors, the following are considered to be important: a) additional chromosome changes, including the 2nd Ph-chromosome and 9p abnormalities; b) deletion of genes (i.e., IKAROS) needed for natural differentiation of B-cells; c) some cryptic chromosome changes; and d) micro-deletions of many important genes, which respond for natural differentiation of lymphoid and myeloid hematopoietic cells. The latest investigations appear to be directed onto: a) the development of new techniques of mutation status testing; b) the active introduction into clinical practice of TKIs of second- and third-lines; c) the further elucidation of mutated genes' participation in both pathogenesis and resistance formation to treatment of Ph+ leukemias. As a result, one can expect that discoveries in these regions will allow the development of a concepts for target therapy of Ph+ leukemias, enabling more effective treatment than ever before.

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Ссылка: Клеточная терапия и трансплантация, том 1, номер 4

Ph-позитивные лейкозы в эру современной цитогенетики, молекулярной биологии, ингибиторов тирозин-киназ и трансплантации гемопоэтических стволовых клеток

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Резюме

Обзор посвящён анализу сложных проблем современной цитогенетической и молекулярной диагностики Ph–позитивных (Ph+) лейкозов, их лечению в эру блокаторов тирозин-киназ (БТК) и аллогенной трансплантации гемопоэтических стволовых клеток (аллоТГСК). Весомое место в обзоре уделено проблемам мутационного статуса вновь образованного гена ABL-BCR и большой вариабильности транскрибируемых с него молекулярных продуктов. Несмотря на то, что малый транскрипт (e1a2), ответственный за образование белка p190, свойственен большинству больных Ph+ ОЛЛ (как взрослых, так и у детей), он встречается также у четверти больных хроническим миелолейкозом (ХМЛ). Наоборот, ответственные за продукцию более крупного, характерного для большинства больных ХМЛ p210 протеина, e13a2 и e14a2 транскрипты имеют место также у четверти больных с Ph+ ОЛЛ. Наконец, у 3% - 19% наблюдений оба транскрипта могут быть представлены одновременно. С другой стороны, появились данные о большом количестве точечных мутаций различных участков гена *ABL-BCR*, в том числе у нелеченых ранее больных. Большинство этих мутаций, за исключением T315I и F317L, на результатах терапии Ph+ лейкозов БТК отражается мало, в то время при наличии в клетках отмеченных выше двух мутаций резистентность к терапии БТК становится доминирующей.

Одним из важных моментов большого успеха аллоТГСК при Ph+ лейкозах является высокая чувствительность Ph+ клеток к антилейкемическому действию трансплантата и к вливаемым донорским лимфоцитам. Несмотря на это, в эру активного использования в клинике БТК аллоТГСК при ХМЛ отошла на второй план, не утратив своего излечивающего значения у плохо переносящих БТК или резистентных к ним больных. В отличие от ХМЛ, аллоТГСК у больных с Ph+ ОЛЛ должна проводиться без проволочек, причём крайне желательно в состоянии полноценной ремиссии (т. е. без признаков минимальной остаточной болезни). Для достижения этой цели всех больных с Ph+ ОЛЛ желательно направлять в хорошо оборудованные специализированные центры сразу же после постановки диагноза, поскольку только с использованием современных цитогенетических и молекулярно-биологических методик имеются реальные возможности: а) для лучшего контроля эффекта лечения желательной комбинации высокодозной химиотерапии и БТК, б) для выбора наилучших режимов кондиционирования и профилактики РТПХ; и, наконец, в) для оценки реального прогноза этих заболеваний.

Ключевые слова: Ph-позитивные лейкозы, цитогенетика, молекулярная биология, блокаторы тирозин-киназ, трансплантация гемопоэтических клеток