CMLS, Cell. Mol. Life Sci. 61 (2004) 2897–2911
1420-682X/04/232897-15
DOI 10.1007/s00018-004-4271-0

CMLS Cellular and Molecular Life Sciences

Abl: the prototype of oncogenic fusion proteins

G. Saglio* and D. Cilloni

Dept. of Clinical and Biological Sciences, University of Turin, San Luigi Hospital, Gonzole 10, 10043 Orbassano-Torino (Italy), Fax: +39 11 9038636; e-mail: giuseppe.saglio@unito.it

Abstract. Since it was first recognized, chronic myeloid leukemia (CML) has always represented a unique model to understand the molecular mechanisms underlying the onset and progression of a leukemic process. CML was the first recognized form of cancer to have a strong association with a recurrent chromosomal abnormality, the t(9;22) translocation, which generates the so-called Philadelphia (Ph)-chromosome. Twenty years later, this abnormality was shown to cover a specific molecular defect, a hybrid *BCR-ABL* gene, strongly implicated in the pathogenesis of the disease through the production of a protein with a constitutive tyrosine-kinase activity. Although we still lack a complete definition of all the transformation pathways activated by Bcr-Abl, the recent introduction into clinical practice of tyrosine kinase inhibitor represents a major breakthrough to the management of CML and, furthermore, promises to usher in molecularly targeted therapy for other types of leukemia, lymphoma and cancer.

Key words. c-Abl; BCR-ABL; Ph-chromosome; CML; tyrosine-kinase.

Introduction

Tyrosine kinases (TKs) are a group of ~100 proteins which act to transfer phosphate from ATP to tyrosine residues on specific cellular proteins and which are deeply involved in controlling important cellular functions, including proliferation and the differentiation processes [1]. Due to the key role played in these vital routes, TKs are generally tightly controlled by various physiological mechanisms. However, alterations of the function of TKs are among the most frequent findings in human cancer, and at least three general mechanisms have been described by which TKs may become constitutively activated and lead to development of neoplastic diseases: overexpression, activating mutations and chromosomal translocations.

Concerning the understanding of the latter mechanism, certainly a pivotal role has been played by the study of the Philadelphia chromosome (Ph-chromosome) translocation and of its molecular consequences, activation of the Abl TK, which represents a primary event in the genesis of chronic myelogenous leukemia (CML) and of other types of human leukemias.

Indeed, since it was first recognized as a specific disease more than 150 years ago [2], CML has always been regarded as an ideal model to study the pathogenesis of the leukemia processes in humans and the ways by which we can try to cure them.

CML is a myeloproliferative disease, initially characterized by abnormal expansion of a clonal hematopoiesis still capable of achieving terminal differentiation [3]. CML exhibits a characterististic biphasic clinical course: the initial chronic phase, which can last for some years, originates as an indolent disease but is ineluctably followed by an acute leukemia, termed blast crisis, that is marked by the emergence within the clonal hematopoiesis of fully transformed cell clones arrested at an early stage of differentiation, either myeloid or lymphoid.

The presence of a Ph-chromosome in the cells of the CML patients has for many years been the only cytogenetic abnormality known to be associated with a specific malignant disease in humans [4]. Later it was recognized that the Ph-chromosome is the result of a reciprocal translocation between the long arms of chromosome 9 and 22, t(9;22)(q34;q11) [5]; finally, in the 1980s, the molecular defect associated with this cytogenetic abnormality was identified, and it was established that the Ph-chromosome results in the juxtaposition of parts of the

* Corresponding author.

BCR and *ABL* genes, which are normally located respectively on chromosome 22 and chromosome 9, to form a new hybrid *BCR-ABL* gene [6, 7]. The corresponding chimeric protein (P210) [8] has a causative role in the neoplastic transformation of the pluripotent stem cells, giving origin to the enormous expansion of the myeloid compartment which characterizes the chronic phase of the disease. However, in spite of the fact that the structural organization and molecular biology of the *BCR-ABL* gene as well as that of the normal *ABL* and *BCR* genes have been subjects of intensive investigation in the last 20 years, many questions concerning the mechanisms by which the hybrid gene is formed and transforms the hemopoietic stem cells still remain unanswered. Moreover, a further degree of complexity is represented by the fact that the Ph-chromosome may also be found in leukemias other than CML and associated with a wide spectrum of hematological phenotypes, ranging from that of apparently 'de novo' acute lymphoblastic leukemia to indolent chronic myeloproliferative disorders [9, 10].

In the late 1980s, the data accumulated on the role of *BCR-ABL* in the onset and in the progression of CML showed *BCR-ABL* to be most attractive target for molecularly targeted therapy. Therefore, attempts to decrease the amount of the *BCR-ABL* transcripts and/or to inhibit the TK activity of the oncoprotein were initiated, and this process finally ended with the discovery and development of imatinib mesylate [11]. This small chemical compound, which that, at micromolar concentrations inhibits the kinase activity of Bcr-Abl by competing with ATP for its binding site, was shown to inhibit cellular growth and to induce apoptosis of the leukemic cells both in vitro and in vivo [12]. The importance of imatinib goes beyond the exceptional therapeutic results obtained with its use in CML and in other Ph-positive leukemias and represents the real starting point of the so-called molecularly targeted therapy.

The purpose of this review is to summarize the most recent advances and problems still open concerning the mechanisms leading to the Abl TK activation in human leukemias. As the recognition of these mechanisms is having a major impact on the clinical arena, even diagnostic and therapy aspects will be considered.

Structure and function of normal c-ABL

The proto-oncogene *c-ABL* belongs to the family of the non-receptor TKs and was originally identified for its homology with v-ABL, a component of oncogenic viruses able to induce an acute neoplastic transformation in the mouse [13].

The *c-ABL* gene is expressed ubiquitously in cells of various tissues, and the c-Abl protein may be found both at the cytoplasmic and nuclear level [14]. The pattern of expression and the endocellular localization of the c-Abl protein suggest that this molecule may play a key role in cellular biology and exert multiple functions in various cell compartments, but the exact role of Abl still needs further clarification.

Studies on the transgenic mouse have led to the discovery that in the homozygous deletion of the *ABL* gene, the absence of the c-Abl protein is incompatible with the normal development of the animal: mice die in the prenatal period with a serious growth delay, hypoplasia of the lymphoid organ and adrenal failure [15].

c-Abl kinase has been implicated as both a negative and a positive regulator of cell growth, depending on the cellular compartments (cytoplasmic or nuclear), on its phosphorylation state and perhaps, also on the level of expression.

Several studies suggest a positive role of c-Abl in cell cycle regulation. In quiescent cells, nuclear c-Abl is kept in an inactive state by binding to the retinoblastoma protein (pRB) [16]. Phosphorylation of pRB by cyclin D disrupts this complex and results in activation of c-Abl TK in S-phase, during which c-Abl is able to stimulate the transcriptional activity of factors such as CREB and E2F-1 and to promote the activity of RNA polymerase II [17]. Therefore, it appears probable that the ABL protein is part of the general mechanism of regulation of gene transcription during the cell cycle. This is consistent with the observation that fibroblasts derived from c-Abl knockout mice exibit a consistent delay in S-phase entry in response to a platelet derived growth factor (PDGF) stimulus [18].

c-Abl plays a key function in the signalling pathways regulating growth factor-induced proliferation also at cytoplasmic level. The existence of a TK signalling cascade involving receptor (PDGFR) and non-receptor TKs (c-Src/c-Abl) important for mitogenesis and growth factor-induced c-Myc expression was recently described [19]. Along this pathway c-Abl is situated downstream of Src and contributes to transmitting the mitogenic signal activating c-Myc directly or through the Ras/Erk pathway (see below).

Within this context, a complex functional interdependence between c-Abl and lipid signalling pathways involving phospholipase C-γ1 (PLC-γ1) has been discovered [20]. PLC-γ is important in cell migration, membrane ruffling and also mitogenesis [21]. PLC-γ1 is also critically important in cytoskeletal reorganization and cell adhesion and, similarly to c-Abl, localizes to membrane ruffles and translocates to the plasma membrane after growth factor stimulation [21]. The bidirectional link between PLC-γ1 and the c-Abl recently uncovered shows that PLC-γ1 is required for c-Abl activation by PDGFR and Src and that c-Abl functions downstream of PLC-γ1, as c-Abl inactivation blocks PLC-γ1 function. PLC-γ1 and c-Abl form a complex that is enhanced by PDGF

stimulation, but after activation c-Abl phosphorylates PLC-γ1 and, in turn, negatively modulates its function [20, 22].

In contrast to the previously described positive role in stimulating proliferation, overexpression of wild-type c-Abl in fibroblasts has been shown to be able to induce cell cycle arrest in the G1 phase of the cell cycle [23]. The growth inhibitory effect of c-Abl requires nuclear localization of the c-Abl protein and its TK activity and is likely to relate to the ability of c-Abl to induce apoptosis in response to genotoxic stress as that due to ionizing radiation, which is known to activate c-Abl [24]. Activation of the c-Abl kinase by DNA damage has been shown to require ATM, which regulates cell cycle checkpoints, DNA repair and apoptosis in response to DNA damage [24]. Cells lacking c-Abl can activate cell cycle checkpoints and DNA repair, but show defects in apoptosis. This c-Abl function is dependent on the presence of wild-type p53, Rb and p73 (a functional homologue of the p53 tumor suppressor) proteins, although a proapoptotic activity of c-Abl independent of the presence of these factors has also been reported [25]. Therefore, multiple and different signalling pathways may contribute to the induction of apoptosis through activation of c-Abl under cellular stress conditions, but the complete scenario still lacks definition.

In order to understand the possible functions of c-Abl at both the cytoplasmic and nuclear level, it is also important to consider the different domains present in the c-Abl protein, as shown in Figure 1, which represent the basis for its interaction with other proteins.

As in other non-receptor TKs, the Abl protein possesses at the N-terminal extremity the SH3 and SH2 domains (from SRC homology region 3 and 2), which are the docking sites of proteins that contain, respectively, proline-rich sequences interacting with the SH3 region and phosphotyrosine residues interacting with the SH2 region [26]. Molecules such as SOS, which are capable of converting G proteins (e.g. Ras, Rho, Rac) from the inactive form, linked to GDP, into the active form linked to GTP, belong to the first group. Molecules such as Shc, Crkl, Nck, Bp-1 and Grb2, which lack enzyme activity but possess both phosphotyrosine and SH3 and SH2 sequences, belong to the second group; these proteins probably play a role of 'adaptors', allowing the formation of complexes capable of putting enzymes and specific substrates in contact with each other. On the basis of these domains, it is deducible that c-Abl, like its oncogenic counterpart Bcr-Abl, is capable of activating the transduction of a mytogenic signal through a pathway that involves the activation of Ras and that, in sequence, includes the activation of Raf, Mek1 and Mek2, and Erk [27]. The latter kinase is capable of phosphorylating transcription factors such as Fos and Jun, both triggering cellular proliferation [28].

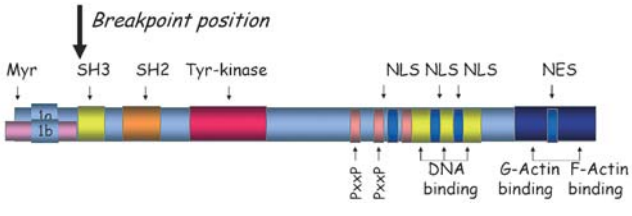

Figure 1 Functional domains of ABL coding sequences: Myr, myristilation site; SH3 and SH2, Src Homology Domain 3 and 2; Tyr-kinase, tyrosine kinase domain; NLS, nuclear localization sequence; NES, Nuclear export sequence; PxxP, praline-rich regions.

The C-terminal extremity of the c-Abl protein contains a domain of interaction with F-actin [29], through which the c-Abl protein certainly plays a key role in the physiology of the cytoskeleton. The homozygous deletion of this region in the transgenic mouse is incompatible with life, resulting in a phenotype overlapping that induced by the deletion of the entire *ABL* gene [30]. The exact function of this interaction is unknown; several data, however, suggest an important role of c-Abl in the mechanisms which regulate the variations of the cellular morphology and the intercellular adhesion [31]. In addition, c-Abl has also been shown to inhibit migration of fibroblasts through the regulation of the Crk/CAS complexes. c-Abl phosphorylates Crk, which results in disruption of Crk-CAS complexes [32].

The c-Abl protein is the only non-receptor TK to possess a DNA binding domain in the C-terminal region of the molecule [33]. It also contains specific sequences which allow the nuclear localization of the protein (NLS) and its nuclear extrusion (NE) [34]. These structural data are in agreement both with the nuclear localization of c-Abl and with continuous shuttling between the nucleus and the cytoplasm. The possible function of the c-Abl protein at the nuclear level has been previously discussed.

Mechanisms leading to the t(9;22) translocation which gives rise to the Ph-chromosome

Although we know that leukemogenesis in humans is caused by a number of recurrent chromosomal translocations, we still lack any substantial information on the genesis of these translocations.

Concerning the t(9;22)(q34;q11) translocation which gives rise to the Ph-chromosome, we know that radiations may play a role, since accidental exposure to radiation has been demonstrated to be significantly associated with an increased risk of developing a Ph-positive CML [35], and high-dose irradiation of myeloid cell lines in vitro has been shown to be able to induce the expression of *BCR-ABL* fusion transcripts like those present in CML [36]. In addition, t(9;22) translocation and *BCR-ABL* fusion is probably a very frequent event that only very sporadically leads to the development of a leukemia phenotype, as

suggested by the finding that small amounts of *BCR-ABL* transcripts can be found in a high percentage of adult normal subjects [37]. However, at present, no clear molecular basis favoring the rearrangement between the *BCR* and *ABL* genes has been identified, and only hypothetical mechanisms have been proposed. The close proximity of the *BCR* and *ABL* genes in hematopoietic cells in interphase has been suggested to be a potential mechanism that may favor translocations between the two genes [38]. In addition, a 76-kDa duplicon on chromosome 9 near to the *ABL* gene and on chromosome 22 near the *BCR* has been recently identified [39]. Duplicons are chromosome-specific duplications, ranging from a few to 200 kb in length, which have been implicated in the formation of new genes over evolutionary time and which, due to misalignment during meiosis, can be responsible for genetic diseases known as genomic disorders [40]. At present, the possible role of duplicons in triggering mitotic changes in somatic cells, finally leading to the genesis of recurrent chromosomal translocations such as those present in human leukemias, is purely speculative. The duplicon identified on chromosomes 9 and 22 could have a role in the initial step of the t(9;22) translocation process, simply by drawing together the specific chromosomal regions containing the *BCR* and the *ABL* genes and favoring exchanges between them. Very sporadically, this exchange could result directly in the formation of a functional *BCR-ABL* rearrangement, able to lead to the synthesis of a sufficient enough amount of Bcr-Abl protein to confer a growth advantage to the Ph-positive clone and finally ending in its expansion. However, the finding of variant translocations also involving other chromosomes in addition to chromosome 9 and 22 and the presence of sporadic cases with aberrant *BCR-ABL* transcripts showing the presence of sequences of different origin interposed between the *BCR* and *ABL* exons suggest that the genesis of the Ph-chromosome and that of the consequent *BCR-ABL* rearrangement can sometimes represent the final result of a complex and multistep event [41].

BCR/ABL rearrangement

Although the presence of a Ph-chromosome translocation always parallels the presence of a *BCR-ABL* rearrangement, there is variability at the molecular level concerning the type of rearrangement between *BCR* and *ABL*. As a consequence, *BCR-ABL* hybrid genes can generate different types of fusion transcripts and proteins, which show a preferential but not exclusive association with different leukemia phenotypes [9, 10].

All *BCR-ABL* fusion genes contain a 5′ portion derived from *BCR* sequences and a 3′ portion which, with very few exceptions, includes the entire *ABL* gene sequence with the exclusion of the first 26 codons, corresponding to the two alternative 5′ exons, that normally generate two variant protein products denoted as type Ia and Ib. The type Ib variant contains a consensus sequence for N-terminal myristylation [42].

In CML, the breakpoints on chromosome 22 are restricted to a central region of the BCR gene called mBCR (major breakpoint cluster region), which contains five exons originally numbered from 1 to 5 and corresponding to BCR exons 10 to 14; two different types of BCR/ABL junction may be present [6, 7]. In the first BCR exon 13 is joined to ABL exon 2 (e13a2 junction, previously b2a2), whereas in the second, BCR exon 14 is spliced to ABL exon 2 (e14a2 junction, previously b3a2) [43]. The two chimeric messenger RNAs (mRNAs) differ by the presence of the BCR exon 14 sequences [75 bp), and the corresponding P210 proteins differ by 25 amino acids.

The Ph-chromosome may also be present in a consistent percentage of ‘de novo’ acute lymphoblastic leukemia (ALL), particularly in elderly patients [44]. In ~70% of these cases, a shorter Bcr-Abl hybrid protein (P190] is detected [45]. In these cases the breakpoint is located in the first intron of the *BCR* gene joining the first *BCR* exon to *ABL* exon 2 (e1a2 junction) [46]. Both in vivo and in vitro studies suggest that P190 is characterized by a higher transforming activity than P210, and this may explain why P190 is preferentially associated with an acute leukemia phenotype [47].

However, the relationship existing between the type of Bcr-Abl fusion protein and the leukemia phenotype is an intriguing question which, at present, remains largely unsolved. Actually, although the P190 fusion protein is almost exclusively associated with an acute leukemia phenotype, mainly lymphoid (Ph-positive ALL), there are also rare chronic phase CML cases which instead of P210 express exclusively P190 [10]. These P190 CMLs appear frequently to have a chronic myelomonocytic leukemia-like (CMML) phenotype [48]. The discovery that at diagnosis all CML patients express a variable amount of transcripts with an e1a2 junction (that leading to the P190 protein) as a consequence of alternative splicing between *BCR* exon 1 and *ABL* exon 2 within the original e13a2 or e14a2 transcripts leading to P210 makes the question more complex [49].

Finally, a longer type of *BCR-ABL* transcript in which the breakpoint takes place at the very 3′ end of the *BCR* gene and joins *BCR* exon 19 with *ABL* exon 2 (e19a2) was originally described some years ago [50]; this transcript contains the same portion of *ABL* sequences as the other more common types of *BCR-ABL* transcripts, but includes almost all the *BCR* coding sequences and results in a fusion protein of 230 Kda in molecular weight (P230) [50]. Interestingly enough, this type of *BCR-ABL* rearrangement has been often associated with a very mild form of CML, denominated Ph-positive neutrophilic CML (Ph+ N-CML), showing clinical and hematological

features overlapping those of classical CML and chronic neutrophilic leukemia (CNL) [51]. However, cases associated with a classical CML phenotype or even an acute leukaemia phenotype also have been described. Moreover, other rare types of *BCR-ABL* transcript have been occasionally described in CML patients, but their common denominator is that all the fusions found are able to maintain the *ABL* portion in the correct reading frame, even through the interposition of anomalous sequences extraneous to the *BCR* and *ABL* coding regions [39]. In conclusion, an exclusive association between the type of *BCR-ABL* rearrangement and the leukemia phenotype cannot be established; however, there is a clear association between certain types of *BCR-ABL* transcripts and the leukemia phenotype. In addition, in the cases in which the same fusion is associated with different types of leukemia (i.e. p210 in CML and in ALL, p190 in ALL and in CML), other still unknown factors, such as different level of expression, could be implicated [9, 10].

Mechanisms of activation of the c-ABL proto-oncogene

The presence of a Bcr-Abl fusion protein certainly plays a key role in the mechanism of neoplastic transformation of Ph-chromosome-positive cells. The definitive evidence of this assumption derives from transfection studies of normal hemopoietic bone marrow progenitors of mice, with retroviral vectors allowing the expression of the P210-type *BCR-ABL* transcripts. In this manner, Daley et al. [52] were able to reproduce a CML-like disease, which frequently and rapidly switched into an acute lymphoid leukemia, thus mimicking the characteristic biphasic course of the human disease. However, the molecular pathways by which the Bcr-Abl proteins are able to induce transformation still remain in part elusive in spite of the large body of data accumulated in recent years.

A substantial increase TK activity, compared to that of normal Abl, is the leading feature of all known forms of rearranged Abl proteins endowed with transforming properties, both in humans and in mice. Moreover, there is a direct relationship between the level of TK activity observed and the transforming potential, suggesting that Abl enzymatic activity is the key factor in inducing and maintaining the neoplastic phenotype [47].

In past years, considerable effort has been devoted to understanding the critical sequences of the *ABL* gene, whose mutations, deletions or rearrangements might influence enzymatic activity and consequently trigger its oncogenic potential [53]. The leukemogenic activity of Bcr-Abl proteins is due to the fact that the normally regulated TK activity of the Abl protein is constitutively activated by the junction with the N-terminal portion coded by the *BCR* gene. Bcr acts by causing dimerization of the Bcr-Abl proteins and in this way promoting a transphosphorylation process [54]. The uncontrolled kinase activity of Bcr-Abl mimics the functions of normal Abl by interacting with effector proteins and finally determines an uncontrolled cellular proliferation, decreased adherence of the leukemia cells to the bone marrow stroma and reduced sensitivity to apoptotic stimuli.

Clearly, however, to consider Bcr-Abl only a constitutively activated form of normal Abl is too simplistic: Bcr-Abl may have different binding properties with respect to its normal counterpart. Bcr-Abl may, for example, also bind directly to Grb2. Because this association involves the Bcr portion of the protein, which is absent in Abl, this connection is unique to the human oncogene [55].

The kinase domain of Bcr-Abl is also differently regulated with respect to c-Abl, which is indeed tightly regulated in vivo [56].

The lack of Abl oncogenic activity, even following overexpression, reveals the presence of mechanisms of inhibition able to restrain constitutive activation of Abl. Both intra- and intermolecular interactions are implicated in this process and regulate the physiological activity of Abl.

A very important domain in the regulation of Abl activity is the SH3. Deletions or mutation of the SH3 domain stimulate Abl kinase activity in vivo, and several candidate inhibitors of Abl kinase activity that bind to the Abl SH3 domain and inhibit kinase activity in vivo have been identified, such as Pag/Msp23, AAP1 and the Abi adaptors [57–59]. In the nucleus, the retinoblastoma protein (Rb) binds to Abl during the G1 phase of the cell cycle. In particular, Rb binds to the ATP-binding portion of the Abl kinase domain, and this interaction inhibits Abl kinase activity [16]. Moreover, intramolecular interactions between the SH3 domain and the region connecting the SH2 and catalytic domains of Abl have been shown to be important in negative regulation of Abl kinase activity [60, 61]. Finally, Abl kinase activity is also regulated by phosphorylation of specific residues in the activation loop [62].

On the other hand, activated Abl is rapidly downregulated by the ubiquitin-dependent proteasome machinery, and this mechanism prevents high levels of momentarily activated Abl kinase activity from persisting in cells, thereby causing the harmful consequences of deregulated Abl kinase activity [63].

The situation is quite different in human *BCR-ABL* fusion transcripts, where only the first 26 *ABL* codons are replaced by *BCR* sequences and no other mutations are usually detectable in the *ABL* coding sequence. Thus, most of the SH3 region is intact, suggesting that the mechanism operating to induce the increased TK activity observed is mainly due to the dimerization properties of the Bcr portion [54]. However, data also show that AAP1 fails to bind to the SH3 region of the Bcr-Abl proteins, suggesting that the N-terminal Bcr sequences may interfere with

AAP1 binding to SH3, perhaps altering the spatial configuration of the molecule [64]. This is in keeping with the observation that rare CML cases carrying *BCR-ABL* transcripts in which the SH3 domain is absent because of an unusual breakpoint position within the *ABL* gene do not differ significantly in the leukemia phenotype with respect to the cases carrying the usual type of transcripts [65]. However, even the rearrangement with genes other than *BCR* may be able to induce an increased TK activity in the fusion protein; Golub et al. [66] reported a rare case of *TEL-ABL* rearrangement in a patient carrying an undifferentiated type of acute leukemia, showing multiple cytogenetic abnormalities; the corresponding hybrid protein was constitutively phosphorylated and endowed with very high TK activity. As in all the classical forms of *BCR-ABL* rearrangements, only the first 26 *ABL* codons were removed in the *TEL-ABL* chimeric transcripts, therefore leaving intact the SH3 region; in vitro studies have documented that the HLH (helix-loop-helix) domain of the *TEL* gene included in the hybrid protein has a critical role in increasing Abl TK activity. This could occur through the formation of oligomers which, in turn, might interfere with the SH3 binding of inhibitory proteins [67].

Mechanisms of transformation activated by BCR/ABL proteins

Despite of the large body of data accumulated in recent years the molecular pathways by which Bcr-Abl proteins induce transformation still remain in part elusive; as we have previously seen, this is in part a consequence of the incomplete knowledge that we have on the physiological function of the *ABL* proto-oncogene product. Nevertheless, it is progressively becoming evident that a multiplicity of molecular interactions is implicated in the transforming activity of Bcr-Abl hybrid proteins, inducing perturbations of various molecular pathways at different levels, from membrane to nucleus.

Effects of BCR/ABL on signal transduction

One of the critical signalling pathways constitutively activated in CML hematopoiesis is that controlled by Ras proteins and their relatives [68]. TK activity of P210 maintains p21 Ras in an active state, bound to GTP. Ras activation results from interaction of P210 with other cytoplasmic proteins, which function as adaptor molecules, to create multiprotein signalling complexes. The amino-terminal *BCR*-encoded sequences of Bcr-Abl contain a tyrosine-phosphorylated site that binds the SH2 domain of the adaptor protein Grb2 [55]. The P210-Grb2 ligand recruits Sos, a Ras-guanine nucleotide-releasing-protein (GNRP), which is constitutively associated with the Grb2 SH3 domain [68]. In turn, the Bcr-Abl-Grb2-Sos complex stimulates conversion of the inactive GDP-bound form of Ras to its active GTP-bound state [68]. Moreover, the RAS-controlled pathway is enhanced by the phosphorylation of the Shc gene products p46 and p52, which, together with the induction of a Shc-Grb2 complex, has the potential to further activate RAS-mediated signalling [69]. The critical role of intermediate adaptor proteins, consisting of SH2/SH3 domains, but lacking a catalytic domain, as potential substrates for P210 Bcr-Abl TK-activity is substantiated by heavy phosphorylation of the Crkl product, the most prominent tyrosine-phosphorylated protein in Ph-positive cells, which functions as specific ligand binding for Abl and, in CML cells, may link P210 to the Phospo-inositol-3-phosphate (PI-3) kinase pathway [70–72].

Finally, the P210-activated pathways, through p21 Ras, activate Raf, Mek1 and Mek2 and Erk kinases and elicit early nuclear events, transactivating transcriptional genes such as *FOS* and *JUN* [73].

The importance of the activation of the Ras signalling pathway in CML has been pointed out by several in vitro studies showing that blocking the *RAS* function suppresses the transforming properties of Bcr-Abl fusion proteins [74]. This is in agreement with the low incidence of *RAS* mutations encountered in CML [75], whereas a high incidence of *RAS* mutations is usually detected in *BCR-ABL*-negative acute and chronic myeloproliferative disorders [76].

Another postulated nuclear ‘target’ of the transforming activity of the P210 protein is represented by the proto-oncogene *MYC*, which is expressed at a high level in CML cells. *MYC* activation, however, seems to be independent of activation of the *RAS* pathway; in fact, in vitro complementation study seems to reveal that the *ABL* SH2 region is probably directly involved in the mechanism of *MYC* upregulation [77].

Activation of the transcription factor NF-kB/Rel by Bcr-Abl has also been demonstrated, but the mechanisms remain obscure. Bcr-Abl induces NF-kB/Rel nuclear translocation, which is at least partially due to increased IkBa degradation [78].

STAT1 and *STAT5* are constitutively activated in *BCR-ABL*-positive cell lines from CML patients and in primary cell samples from CML patients [79]. This finding led to the attractive model that constitutive activation of Stats by Bcr-Abl confers the cytokine independence characteristic of *BCR-ABL*-positive cells. In normal cells, nuclear translocation of Stats occurs only after cytokine binding to receptors and is mediated by activation of the receptor-associated Jak kinases. Bcr-Abl does not activate Stats by a Jak-dependent pathway as Jak kinases are not consistently activated in *BCR-ABL*-positive cells [80]. It has been recently suggested that Stat activation by Bcr-Abl may be mediated by direct association of Stat

SH2 domains with phosphorylated tyrosines on Bcr-Abl, but there is no proof of this [79].

All reported activated signalling pathways converge into a unique terminal point: loss of control of proliferation and expansion of the hematopoietic progenitors clonally derived from the primitive Ph-positive stem cell. In addition, all the above-mentioned activities may be implicated in deregulating the balance between the proliferation and differentiation processes of Ph-positive cells.

Impaired adhesion of Ph-positive cells to bone marrow stroma induced by BCR/ABL

An increasing set of data suggests the well known defective adhesion capacity of the CML cells is also implicated. This action may derive from the fact that the Bcr-Abl proteins may form multimeric complex with adhesion proteins such as paxillin and are able to bind to F-Actin, thus suggesting a direct action on cytoskeleton function [81, 82]. Since adhesive interactions represent the tool for extensive 'cross-talk' between cells, they ensure the regulated state of a large number of cell functions such as growth and differentiation.

Part of the adhesion defect of Ph-positive hematopoiesis is mediated through abnormalities of phosphatidyl-inositol (PI)-linked surface receptors, which are involved in the reduced adhesion capacity of CML progenitors to the bone marrow stromal compartment [83]. In particular, the deficient expression of one PI-linked cell surface cytoadhesion molecule, lymphocyte-associated-antigen three (LFA 3), has been associated with abrogation of immune-mediated control on the size of Ph-positive clones [84]. LFA 3 (identified by the monoclonal antibody CD 58] is a widely expressed cell surface protein whose only known function is to act as the binding ligand for the T cell surface protein CD2. CD2/LFA 3 adhesive interaction between a subset of human T cells and early (CD34+) hematopoietic progenitors plays a role in controlling the size of the actively cycling stem cell pool. Through LFA3-deficient expression, the Ph-positive stem cell compartment may escape this immune-mediated growth regulation.

However, the most relevant consequences of changes in the adhesion properties of CML hematopoiesis arise from muddled interactions of Ph-positive progenitors with the hematopoietic microenvironment. The intrinsic defect in adhesion to the bone marrow microenvironment of CML hematopoiesis was first described by Gordon et al., who observed the failure of Ph-positive hematopoietic progenitors to adhere to preformed stromal layers [85]. Normally, adhesion of the hematopoietic stem cell to special 'niches' within the bone marrow microenvironment is crucial for maintenance of its quiescent state. The adhesive ligand involved at this very early stage of hematopoiesis results from adhesive interactions of β1 integrins VLA-4 and VLA-5, the homing lymphocyte receptor (CD44) and the cell surface proteoglycan receptor with distinct functional domains of fibronectin, one component of the hematopoietic microenvironment extracellular matrix [86–88]. Hematopoietic inhibitory factors macrophage inflammatory protein α (MIP1α) and transforming growth factor β (TGF-β) have also been proposed as soluble messengers responsible for contact-mediated inhibitory effects on cell proliferation [87].

As a consequence, impaired adhesion to the stromal microenvironment allows CML progenitors to cycle continuously, independent of physiological stimuli that induce cell cycle arrest on the normal counterpart as was first observed by Eaves et al. [89].

In conclusion, the complete biochemical pathway underlying the adhesion defect of Ph-positive progenitors has not at present been identified. It seems to involve both direct interactions of Bcr-Abl with molecules playing a key role in the cytoskeleton organization and indirect interactions mediated by other proteins. It is also not known whether the previously described interaction between PLC-γ1 and normal c-Abl [20, 22], entails dysregulation of this pathway in the disturbance of the cell adhesion observed in Bcr-Abl transformed cells.

Inhibition of apoptosis by Bcr-Abl

Whereas we know that normal c-Abl plays a key part in the cellular response to genotoxic stress and is likely to have a proapoptotic function, oncogenic Bcr-Abl TK is a potent inhibitor of apoptosis [24]. This may in part be explained by the fact that one of the most striking differences between normal c-Abl and Bcr-Abl is their different subcellular localizations. The c-Abl protein is found in both the nucleus and the cytoplasm and can shuttle between these two compartments because of the presence of nuclear localization and nuclear export domains. By contrast, Bcr-Abl is exclusively cytoplasmatic and seem to be unable to enter the nucleus where normal c-Abl exerts its proapoptotic activity [24]. Interestingly, BCR-ABL is retained in the cytoplasm mainly because of its constitutively activated TK. But if the kinase is inhibited in vitro with imatinib, and its nuclear export is simultaneously blocked with leptomycin B, the oncoprotein may enter the nucleus; and if imatinib is subsequently removed and the trapped nuclear BCR-ABL reactivates its TK activity, apoptosis is induced [90].

Furthermore, Bcr-Abl can also inhibit apoptosis at the cytoplasmic level by activating the PI3K/AKT pathway. Indeed, Bcr-Abl, but not c-Abl, associates with and activates PI3K at the cytoplasmic level [91]. BCR-ABL may activate PI3K by more than one pathway, because Crk and Crkl have been involved in connecting activated Bcr-Abl with PI3K [92] and, as shown in other systems, p21ras may also activate PI3K [93]. Recent work, however, sug-

gests that Gab2 represents a fundamental factor in linking Bcr-Abl to PI3K activation and that Tyr177 of Bcr-Abl recruits the scaffolding adaptor Gab2 via a Grb2/Gab2 complex [94]. Akt kinase is certainly an important effector of Bcr-Abl-activated PI3K [95] and its activation is dependent on the products of PI3K, phosphatidylinositol-3,4-bisphosphate (PIP_2), and phosphatidylinositol-3,4,5-trisphosphate (PIP_3) [96]. As known, once active, Akt exerts many cellular effects through the phosphorylation of downstream substrates such as Bad, caspase 9, Ask1 and Mdm2 that regulate the apoptotic machinery [97], finally leading to prolonged survival and expansion of the abnormal clone.

Bcr-Abl can be implicated in genetic instability

The same mechanisms responsible for inhibition of apoptosis [24] could also be responsible for the rapid accumulation of those additional genetic mutations that may lead to disease progression, representing the natural evolution of CML clinical history. Indeed, inhibition of apoptosis following severe genetic damage may favor this phenomenon [24]. At the moment, however, it is not clear whether the genetic instability is caused by Bcr-Abl or whether the *BCR-ABL* rearrangement is the consequence of a previously existing genetic instability [98]. The finding of several cytogenetic abnormalities in Ph-chromosome-negative cells reemerging in patients who reach complete Ph-negativity after therapy with imatinib may support the second hypothesis, but this matter is still highly controversial [99, 100].

Mechanisms of disease progression

The natural history of CML ends, in the great majority of cases, with an acute leukemia phase which is usually fatal in a few months. Understanding the mechanisms which trigger the transition from chronic to acute phase is of utmost importance, because the timing of this event is the major determinant of patient survival in CML. A number of factors have been implicated as causative agents of acute transformation: however, no consistent molecular pathways leading to the acute leukemia phenotype have been recognized so far.

When the disease progresses to acute phase, ~80% of patients show additional chromosomal abnormalities in their leukemic cells [101]. Furthermore, a number of molecular changes have been found, including p53 mutations, *RB* deletion and *RAS* activation [102, 103]. However, no specific alteration has been observed even if the deletion of another tumor suppressor gene, p16 or cyclin-dependent kinase 4 inhibitor (*CDK4N2*), seems associated with lymphoid transformation [104]. On the other hand, it is apparent that an important role in transition to the acute phase is played by the duplication of Ph-positive chromosome or by an increase in the expression of the *BCR-ABL* transcript, which in some cases has been reported to precede the progression of the disease [105]. Overall, the genetic alterations observed suggest that a large spectrum of molecular mechanisms may be involved in clonal progression of CML; thus, the *BCR-ABL*-transformed cell seems to be endowed with an intrinsic genetic instability, leading to a progressive accumulation of genetic lesions which at the end lead to the appearance of an acute leukemia phenotype. Wada's report [106] on microsatellite instability in the blastic but not in the chronic phase of the disease is consistent with this hypothesis; however, others [107] have obtained negative results. At present, it is not clear which is the molecular mechanism involved in inducing the genetic instability observed; however, we can speculate, on the basis of the known nuclear function of normal Abl protein, which is compromised in *BCR-ABL*-transformed cells, that spontaneous mutations are not able to trigger activation of the apoptotic death pathway in Ph-positive cells, as occurs in normal cell populations; hence, a progressive alteration of the biological behavior of chronic phase cells ensues, leading to acute transformation. From this perspective, the *BCR-ABL* rearrangement appears to be not only the causative agent of the chronic phase of CML but seems also to represent the primary mechanism that eventually leads to disease progression.

Therapeutic and diagnostic implications

The data previously presented clearly show that *BCR-ABL* plays a primary role in the onset and progression of CML and led to the development of imatinib mesylate, the first kinase inhibitor to be developed for clinical use (Glivec, Novartis) [11, 12]. Imatinib, which, as will be described below, is extraordinarily effective in treating CML, represents the final step of a long conceptual evolution in the strategy to cure CML.

The natural course of CML generally comprises three different phases (chronic, accelerated and blast crises). The chronic phase may persist for several years, but once the disease enters into accelerated or blast crisis phases, which, if not prevented, represent the natural evolution of the disease, life expectancy is reduced to only few months [108]. This is because these phases of the disease are more resistant to all types of therapy. With so-called conventional chemotherapy (mainly hydroxyurea), control of white blood cell and platelet counts (hematological remission) is generally achieved, but this therapy does not significantly alter the progression of the disease [109].

At the moment, despite the great advances in the therapy of CML that we are facing in our days with imatinib, the only ascertained curative therapy for CML still remains

allogenic bone marrow transplantation (BMT). In fact, after having had an unmanipulated allogeneic BMT from a human lymphocyte antigen (HLA)-identical sibling, a CML patient in the first chronic phase has only a 10–20% probability of relapsing within 5 years [110]. Therefore, in a high percentage of cases BMT may definitively cure CML patients. We now know that BMT is capable of curing CML mainly through a graft versus leukemia (GVL) mechanism [111]. The latter factor is frequently capable of determining complete eradication of the Ph-positive clone, but sometimes it is only capable of eliminating its spread. In the latter case, reappearance of the disease can take place owing to a major spreading force of the leukemic clone, as occurs when the disease is in an accelerated or blastic phase, or to the reduction of capacity of the effect GVL of the donor marrow (T-depletion, immunosuppressive therapy). One of the consequences (confirmed by clinical data) is that transplant offers greater probabilities of success when it is done in a early phase of the disease [110]. It is also significant that an increment of the effect of GVL using infusion of lymphocytes from the donor in relapsed patients can determine a new remission [112].

In the mid-1980s, with the introduction CML of interferon-α (IFN-α), the first biological agent capable of inducing cytogenetic remission in patients with CML, it became clear that the degree of tumor load reduction during therapy is an important prognostic factor for CML patients [109]. Whereas the hematological response, which corresponds to 1 log reduction of the leukemia cell burden, does not represent a sufficient therapeutical goal in CML per se, as patients still 100% Ph-positive invariably progress to a blastic phase and die from its complications, the degree of cytogenetic remission, that, if complete, indicates ~2 log reduction of the leukemia cell load, has been shown to be a strong prognostic indicator [109], and it has often been suggested in clinical trials as a possible surrogate marker for overall survival. The cytogenetic response is established on the basis of the proportion of residual Ph-positive metaphases and is defined as complete (0% of Ph-positive metaphases), partial [1–33%), minor [34–66%) or minimal [67–99%), whereas a major response represents the sum of the complete and partial cytogenetic responses. Only major (complete and partial) cytogenetic remissions have been shown to be associated with increased survival, whereas the impact on prognosis of minor or minimal cytogenetic responses remains negligible [109].

When imatinib mesylate, was introduced in CML therapy at the end of last century, it immediately showed extremely promising results [113].

A phase 2 study performed to characterize the efficacy and safety profiles of imatinib in a large group of patients with chronic-phase CML in whom previous interferon therapy had failed showed that Imatinib is able to induce complete hematologic responses in 95% and major cytogenetic responses in 60% of patients and to reduce progression to the accelerated or blast phases [114]. More recently, the results of a randomized study performed on more than 1000 patients in the first chronic phrase clearly demonstrated that imatinib is significantly better than interferon plus Ara-C for chronic-phase CML when measured either as time to progression, cytogenetic response, or tolerance [115]. In particular, a major cytogenetic response was observed in almost 80% of patients.

The ctivity of imatinib has also been observed in patients whose CML is in the blastic phase, but a high rate of relapse has been described in these patients [116]. Relapses are usually caused by the emergence of resistant clones, and resistance also remains a significant problem for a percentage of CML patients in chronic phase [117].

Resistance can be defined as acquired, when loss of established hematologic or cytogenetic response to imatinib occurs, or as primary, when no response to imatinib is achieved. Acquired resistance is usually associated with restoration of Bcr-Abl kinase activity [117] and may be due to different mechanisms: mutations in *BCR-ABL* that affect its drug interaction [118–122], increased expression of *BCR-ABL* [123], decreased 'in vivo' availability or decreased intracellular levels of imatinib [124, 125].

Mutations in *BCR-ABL* appear to be the most common mechanism of resistance and can be divided into groups according to the *BCR-ABL* regions where they occur; they can be in the imatinib-binding site (as one of the most frequent, the T315I), in the P-loop or in other regulatory regions [117]. Emergence of mutations in the P-loop have been reported to hold a very poor prognosis with respect to the others, with most of the patients carrying these mutations progressing to blast crisis shortly after mutation detection [122]. It seems that, at least in some cases, mutations conferring resistance to imatinib are already present prior to treatment and are selected by the drug [126]. Further evidence in support of this hypothesis comes from the finding that the emergence of mutations conferring resistance is significantly associated with duration of the disease prior to imatinib treatment [122].

Concerning the other mechanisms, the binding of imatinib to α1-acid-glycoprotein may reduce the availability of the drug in vivo [124] and increased levels of *BCR-ABL* due to amplification of the *BCR-ABL* gene has been described [125]. Mutations in *BCR-ABL* are not commonly seen in primary refractory patients to imatinib, suggesting the presence of a different mechanism of resistance in these cases [117–122]. In these cases, secondary oncogenic abnormalities may lead to the emergence of *BCR-ABL*-independent pathways that may support proliferation or resistance to apoptosis, diminishing the efficacy of imatinib therapy [127].

Combination of imatinib therapy with conventional cytotoxics and newer biological therapies able to affect other significant molecular pathways could eventually overcome this resistance, and several attempts in this direction are presently ongoing. A lower incidence of resistance is expected with combination therapies, but this needs to be tested [128].

Molecular monitoring of continuos complete remission (CML) patients

The very high percentage of CCR obtained in CML patients treated with imatinib at diagnosis emphasized the importance of molecular methods to monitor the response of CML patients.

Molecular remission was traditionally defined on the basis of detection of residual *BCR-ABL* transcripts by conventional qualitative nested RT-PCR (reverse transcription-polymerase chain reaction). Indeed, data on the prognostic significance of achieving molecular remission, as defined above, have been obtained mainly in cohorts of patients subjected to allogeneic BMT, the only category of patients able to achieve this condition in a consistent percentage of cases [129, 130]. For all the other patients treated in a different way and in particular for those treated with IFN-α, in which the number of absolute molecular remissions in terms of persistent PCR negativity was very low [131], the gold standard for evaluating patient response to treatment remained conventional cytogenetic analysis (CCyR), as the simple non-quantitative RT-PCR analysis was too sensitive to discriminate sufficiently between patients characterized by a residual volume of disease that could be in a range of 4 log. Peripheral blood fluorescence in situ hybridization (FISH) for the *BCR-ABL* translocation was also reported to be an easy and sensible method for serial monitoring of CML patients [132] and is the only method able to disclose the presence of deletions on the derivative chromosome 9q+, whose presence has been reported to be associated with a worse prognosis for patients treated with IFN-α [133], but has not been able to replace the more diffuse conventional cytogenetic methods.

The development of simple and reliable methods for quantitative PCR has recently changed this picture.

Even in the past, in view of the very limited value of qualitative PCR, several groups had already developed quantitative PCR assays based on competitive PCR strategies to estimate the amount of residual disease in patients able to achieve a complete cytogenetic remission, but remaining RT-PCR-positive [134, 135]. The data obtained showed that the level of minimal residual disease correlate with the probability of relapse in complete cytogenetic responders to IFN [131] as well as in patients who underwent allogeneic BMT [136]. In the latter group, competitive PCR was also used for adapting treatment and for determining the optimum time point to initiate donor lymphocyte infusion and to monitor the response [137, 138]. However, competitive PCR methods are labor intensive, time consuming, difficult to standardize and not suitable for large-scale analysis. With real-time quantitative RT-PCR (RQ-PCR), the variables in the quantitative PCR assay (quality and quantity of RNA and the reverse-transcription step) may be controlled by quantification of transcripts of a control gene (*ABL*, *G6PD* or β2-microglobulin) as an internal standard [139]. Moreover, standardization and the introduction of rigorous, internationally accepted controls have been established to make RQ-PCR a robust and routine basis for therapeutic decisions [140, 141]. These advancements are particularly needed in light of the extremely positive therapeutic results obtained with the use of imatinib mesylate in CML therapy. In fact, ~75% of patients with newly diagnosed chronic-phase CML treated initially with imatinib achieve CCyR, and imatinib also induces Ph negativity, though less frequently, in patients treated in advanced phases of the disease [114, 115].

Very recent studies show that the amount of residual disease at 12 months established by RQ-PCR in terms of log reduction of BCR-ABL transcripts with respect to the pretherapy copy number is statistically significant in predicting the risk of disease progression for newly diagnosed CML patients achieving CCyR under imatinib therapy [142]. A lower risk of losing CCyR has been also demonstrated in CML patients resistant or intolerant to IFN who subsequently obtained a CCyR with imatinib therapy and that by RQ-PCR analysis were demonstrated to achieve a higher reduction of *BCR-ABL* transcript amounts [143].

Other important conclusions recently reached show that early reduction of *BCR-ABL* mRNA transcript levels predicts cytogenetic response in CML patients treated with imatinib [144] and that this parameter can also identify groups of patients with a different risk of progression, as the incidence of progression, defined by hematologic, cytogenetic or quantitative PCR criteria, was significantly higher in patients who failed to achieve a 1 log reduction by 3 months or a 2 log reduction by 6 months [145].

Conclusions

Since CML was shown to be associated with the Ph-chromosome and the hybrid *BCR-ABL* gene, the dissection of these alterations has constituted the basis for a continuous research effort to understand the molecular basis of the disease and to improve its diagnosis and surveillance. More recently, this has also finally led to a major step in the therapy of the disease that is of paramount importance because it opens a new era in cancer treatment, that of

molecularly targeted therapy. This is clearly in line with the vision that the importance of CML and of its molecular lesion, the *BCR-ABL* rearrangement, goes well beyond their real incidence in human leukemias, as they represent a model for investigating and curing cancer. Although much has been achieved, many important issues pertaining to the biology and treatment of CML still remain unresolved, but the feeling is that at least some of these problems will find a solution within the next few years.

Acknowledgements. This work has been supported by grants from the CNR (Progetto Finalizzato Oncologia), MURST-COFIN 2003, AIL (Associazione Italiana contro le Leucemie), AIRC (Associazione Italiana per la Ricerca sul Cancro) and by Regione Piemonte.

1 Robinson D. R., Wu Y. M. and Lin S. F. (2000) The protein tyrosine kinase family of the human genome. Oncogene **19:** 5548–5557
2 Geary C. G. (2000) The story of chronic myeloid leukaemia. Br. J. Haematol. **110:** 2–11
3 Goldman J. M, Melo J. V. (2003) Chronic myeloid leukemia–advances in biology and new approaches to treatment. N. Engl. J. Med. **349:** 1451–1464
4 Nowell P. C. and Hungerford D. A. (1960) A minute chromosome in human chronic granulocitic leukemia. Science **32:** 1497–1501
5 Rowley J. D. (1973) A novel consistent chromosome abnormality in chronic myelogenous leukemia detected by quinacrine fluorescence and Giemsa staining. Nature **243:** 290–203
6 Groffen J., Stephenson J. R., Heisterkamp N., de Klein A., Bartram C. R. and Grosveld G. (1984) Philadelphia chromosome breakpoint are clustered within a limited region, bcr, on chromosome 22. Cell **33:** 93–99
7 Heisterkamp N, Stam K., Groffen J., de Klein A. and Grosveld G. (1985) Structural organization of the bcr gene and its role in the Ph translocation. Nature **315:** 758–761
8 Ben-Neriah Y., Daley G. Q., Mes-Masson A. M, Witte O. N. and Baltimore D. (1986) The chronic myelogenous leukemia-specific p210 protein is the product of the bcr-abl hybrid gene. Science **233:** 212–214
9 Melo J. V. (1996) The diversity of Bcr-Abl fusion proteins and their relationship to leukemia phenotype. Blood **88:** 2375–2384
10 Saglio G., Pane F., Martinelli G. and Guerrasio A. (1997) *BCR/ABL* transcripts and leukemia phenotype**:** an unsolved puzzle. Leuk. Lymphoma **26:** 281–286
11 Buchdunger E., Zimmermann J., Mett H., Meyer T., Muller M., Druker B. J. et al. (1996) Inhibition of the Abl protein-tyrosine kinase in vitro and in vivo by a 2-phenylaminopyrimidine derivative. Cancer Res. **56(1):** 100–104
12 Druker B. J., Tamura S., Buchdunger E., Ohno S., Segal G. M., Fanning S. et al. (1996) Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat. Med. **2(5):** 561–566
13 Abelson H. T. and Rabstein L. S. (1970) Cancer Res. 30**:** 2213–2222
14 Pendergast A. M. (2002) The Abl family kinases**:** mechanisms of regulation and signaling. Adv. Cancer Res. **85:** 51–100
15 Tybulewicz, V. L. J., Crawford C. E., Jackson P. K., Bronson R. T. and Mulligan R. C. (1991) Neonatal lethality and lymphopenia in micewith a homozygous disruption of the c- abl proto-oncogene. Cell **65:** 1153–1163
16 Welch P. J. and Wang J. Y. (1993) A C-terminal protein-binding domain in the retinoblastoma protein regulates nuclear c-Abl tyrosine kinase in the cell cycle. Cell **75:** 779–790
17 Baskaran R., Wood L. D., Whitaker L. L., Canman C. E., Morgan S. E., Xu Y. et al. (1997) Ataxia telangiectasia mutant protein activates c-Abl tyrosine kinase in response to ionizing radiation. Nature **387:** 516–519
18 Plattner R., Kadlec L., DeMali K. A., Kazlauskas A. and Pendergast A. M. (1999) c-Abl is activated by growth factors and Src family kinases and has a role in the cellular response to PDGF. Genes Dev. **13:** 2400–2411
19 Furstoss O., Dorey K., Simon V., Barila D., Superti-Furga G. and Roche S. (2002) c-Abl is an effector of Src for growth factor-induced c-myc expression and DNA synthesis. EMBO J. **21(4):** 514–524
20 Plattner R., Irvin B. J., Guo S., Blackburn K., Kazlauskas A., Abraham R. T. et al. (2003) A new link between the c-Abl tyrosine kinase and phosphoinositide signaling through PLC-gamma1. Nat. Cell Biol. **5(4):** 309–319
21 Rebecchi M. J. and Pentyala, S. N. (2000) Structure, function and control of phosphoinositide-specific phospholipase C. Physiol. Rev. **80:** 1291–1335
22 Plattner R., Koleske A. J., Kazlauskas A. and Pendergast A. M. (2004) Bidirectional signaling links the Abelson kinases to the platelet-derived growth factor receptor. Mol. Cell Biol. **24(6):** 2573–2583
23 Sawyers C. L., McLaughlin J., Goga A., Havlik M. and Witte O. (1994) The nuclear tyrosine kinase c-Abl negatively regulates cell growth. Cell. **77(1):** 121–131
24 Wang J. (2000) Regulation of cell death by the Abl tyrosine kinase. Oncogene **19:** 5643–5650
25 Barila D., Mangano R., Gonfloni S., Kretzschmar J., Moro M., Bohmann D. et al. (2000) A nuclear tyrosine phosphorylation circuit**:** c-Jun as an activator and substrate of c-Abl and JNK. EMBO J. **19:** 273–281
26 Scheijen B. and Griffin J. D. (2002) Tyrosine kinase oncogenes in normal hematopoiesis and hematological disease. Oncogene **21:** 3314–3333
27 Puil L., Liu J., Gish G., Mbamalu G., Bowtell D., Pelicci P. G. et al. (1994). BCR-ABL oncoproteins bind directly to activators of Ras signalling pathway. EMBO J. **13:** 764–773
28 Raitano A. B., Halpern J. R., Hambuch T. M. and Sawyers C. L. (1995) Proc. Natl. Acad. Sci. USA **92:** 11746–11750
29 McWhirter J. R. and Wang J. Y. J. (1993) An actin-binding function contributes to transformation by the Bcr-Abl onncoprotein of Philadelphia chromosome-positive human leukemias. EMBO J. **12:** 1533–1546
30 Schwartzberg P. L., Stall A. M., Hardin J. D., Bowdish K. S., Humaran T., Boast S. et al. (1991) Mice homozygous for *ABL* m1 mutation shows poor viability and depletion of selected B and T population. Cell **65:** 1165–1176
31 Woodring P. J., Hunter T. and Wang J. Y. (2003) Regulation of F-actin-dependent processes by the Abl family of tyrosine kinases. J. Cell. Sci. **116:** 2613–2626
32 Kain K. and Klemke R. (2001) Inhibition of cell migration by Abl family tyrosine kinases through uncoupling of Crk-CAS complexes. J. Biol. Chem. **276:** 16185–16192
33 Wen S. T., Jackson P. K. and Van Etten R. A. (1996) EMBO J.,15**:** 1583–1595
34 Taagepera S., McDonald D., Loeb J. E., Whitaker L. L., MacLeod A. K., Wang J. Y. J. et al. (1998) Proc. Natl. Acad. Sci. USA **95:** 7457–7462
35 Preston D. L., Kusumi S., Tomonaga M., Izumi S., Ron E., Kuramoto A. et al. (1994) Cancer incidence in atomic bomb survivors. III. Leukemia, lymphoma and multiple myeloma, 1950–1987. Radiat. Res. **137:** Suppl: S68–97
36 Deininger M. W., Bose S., Gora-Tybor J., Yan X. H., Goldman J. M. and Melo J. V. (1998) Selective inductio of leukemia-as-

sociated fusion genes by high-dose ionizing radiation. Cancer Res. **58:** 421–425
37 Biernaux C., Loos M., Sels A., Huez G. and Stryckmans P. (1995) Detection of major bcr-abl gene expression at a very low level in blood cells of some healthy individuals. Blood **86:** 3118–3122
38 Neves H., Ramos C., da Silva M. G., Parreira A. and Parreira L. (1999) The nuclear topography of ABL, BCR, PML and RARalpha genes**:** evidence for gene proximity in specific phases of the cell cycle and stages of hematopoietic differentiation. Blood **93:** 1197–1207
39 Saglio G., Storlazzi C. T., Giugliano E., Surace C., Anelli L., Rege-Cambrin G. et al. (2002) A 76-kb duplicon maps close to the BCR gene on chromosome 22 and the ABL gene on chromosome 9: possible involvement in the genesis of the Philadelphia chromosome translocation. Proc. Natl. Acad. Sci. USA **99(15):** 9882–9887
40 JiY., Eichler E. E., Schwartz S. and Nicholls R. D. (2000) Structure of chromosomal duplicons and their role in mediating human genomic disorders. Genome Res. **10:** 597–610
41 Johansson B., Fioretos T. and Mitelman F. (2002) Cytogenetic and molecular genetic evolution of chronic myeloid leukemia. Acta Haematol. **107:** 76–94
42 Chissoe S. L., Bodenteich A., WangY. F., Wang Y. P., Burian D., Clifton S. W. et al. (1995) Sequence and analysis of the human ABL gene, the BCR gene, and regions involved in the Philadelphia chromosomal translocation. Genomics **27:** 67–82
43 Shtivelman E., Lifshitz B., Gale R. P., Roe B. A. and Canaani E. (1985) Fused transcript of abl and bcr genes in chronic myeloid leukaemia. Nature **315(6020):** 550–554
44 Heim S. and Mitelman F. (1995) Cancer Cytogenetics. 2nd edn, Wiley-Liss, New York,
45 Chan L. C., Karhi K. K., Rayter S. I., Heisterkamp N., Eridani S., Powles R. et al. (1987) A novel abl protein expressed in Philadelphia chromosome positive acute lymphoblastic leukaemia. Nature **325:** 635–637
46 Fainstein E., Marcelle C., Rosner A., Canaani E., Gale R. P., Dreazen O. et al. (1987) A new fused transcript in Philadelphia chromosome positive acute lymphocytic leukaemia. Nature **330:** 386–388
47 Lugo T. G., Pendergast A. M., Muller A. J. and Witte O. N. (1990) Tyrosine kinase activity and transformation potency of *bcr-abl* oncogene products. Science **247(4946):** 1079–1082
48 Melo J. V., Myint H., Galton D. A. and Goldman J. M. (1994) p190 BCR-ABL chronic myeloid leukemia: the missing link with chronic myelomonocytic leukemia? Leukemia **8(1):** 208–211
49 Saglio G., Pane F., Gottardi E., Frigeri F., Buonaiuto M. R., Guerrasio A. et al. (1996) Consistent amounts of acute leukemia associated p190 bcr/abl transcripts are expressed by chronic myelogenous leukemia patients at diagnosis. Blood **87(3):** 1075–1080
50 Saglio G., Guerrasio A., Rosso C., Zaccaria A., Tassinari A., Serra A. et al. (1990) New type of BCR/ABL junction in Philadelphia chromosome-positive chronic myelogenous leukemia. Blood **76(9):** 1819–1824
51 Pane F., Frigeri F., Sindona M., Luciano L., Ferrara F., Cimino R. et al. (1996) Neutrophilic chronic myeloid leukemia**:** a distinct disease with a specific molecular marker (BCR/ABL with C3/A2 junction). Blood **88(7):** 2410–2414
52 Daley G. Q., Van Etten R. A. and Baltimore D. (1990) Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. Science **247:** 824–830
53 Pluk H., Dorey K. and Superti-Furga G. (2002) Autoinhibition of c-Abl. Cell **108:** 247–254
54 McWhirter J. R., Galasso D. L. and Wang J. Y. (1993) A coiled-coil oligomerization domain of Bcr is essential for the transforming function of Bcr-Abl oncoproteins. Mol. Cell. Biol. **13:** 7587–7595
55 Pendergast A. M., Quilliam L. A., Cripe L. D., Bassing C. H., Dai Z., Li N. et al. (1993) BCR-ABL-induced oncogenesis is mediated by direct interaction with the SH2 domain of the GRB–2 adaptor protein. Cell. **75(1):** 175–185
56 Hantschel O. and Superti-Furga G. (2004) Regulation of the c-Abl and Bcr-Abl tyrosine kinases. Nat. Rev. Mol. Cell. Biol. **5(1):** 33–44
57 Wen S. T. and Van Etten R. A. (1997) The PAG gene product, a stress-induced protein with antioxidant properties, is an Abl SH3-binding protein and a physiological inhibitor of c-Abl tyrosine kinase activity. Genes Dev. **11:** 2456–2467
58 Shi Y., Alin K. and Goff S. P. (1995) Abl-interactor–1, a novel SH3 protein binding to the carboxy-terminal portion of the Abl protein, suppresses v-abl transforming activity. Genes Dev. **9:** 2583–2597
59 Dai Z., Pendergast A. M. (1995) Abi–2, a novel SH3-containing protein interacts with the c-Abl tyrosine kinase and modulates c-Abl transforming activity. Genes Dev. **9:** 2569–2582
60 Hantschel O., Young M. A., Scheffzek K., Veach D., Bornmann W., Clarkson B. et al. (2003) Structural basis for the autoinhibition of c-Abl tyrosine kinase. Cell **112(6):** 859–871
61 Brasher B. B. and Van Etten R. A. (2000) c-Abl has high intrinsic tyrosine kinase activity that is stimulated by mutation of the src homology 3 domain and by autophosphorylation at two distinct regulatory tyrosines. J. Biol. Chem. **275:** 35631–35637
62 Dorey K., Engen J. R., Kretzschmar J., Wilm M., Neubauer G., Schindler T. et al. (2001) Phosphorylation and structure-based functional studies reveal a positive and a negative role for then activation loop of the c-Abl tyrosine kinase. Oncogene **20:** 8075–8084
63 Dai Z., Quackenbush R. C., Courtney K. D., Grove M., Cortez D., Reuther G. W. et al. (1998) Oncogenic Abl and Src tyrosine kinases elicit the ubiquitin-dependent degradation of target proteins through a Ras-independent pathway. Genes Dev. **12(10):** 1415–1424
64 Brasher B. B., Roumiantsev S. and Van Etten R. A. (2001) Mutational analysis of the regulatory function of the c-Abl Src homology 3 domain. Oncogene **20(53):** 7744–7752
65 Tiribelli M., Tonso A., Ferro D., Parziale A., Cambrin G. R., Scaravaglio P. et al. (2000). Lack of SH3 domain does not imply a more severe clinical course in Ph+ chronic myeloid leukemia patients. Blood. **95(12):** 4019–4020
66 Golub T. R., Goga A., Barker G. F., Afar D. E., McLaughlin J., Bohlander S. K. et al. (1996) Oligomerization of the ABL tyrosine kinase by the Ets protein TEL in human leukemia. Mol. Cell. Biol. **016(8):** 4107–4116
67 Okuda K., Golub T. R., Gilliland D. G. and Griffin J. D. (1996) p210BCR/ABL, p190BCR/ABL and TEL/ABL activate similar signal transduction pathways in hematopoietic cell lines. Oncogene **13(6):** 1147–1152
68 Cortez D., Reuther G. W. and Pendergast A. M. (1997) The BCR-ABL tyrosine kinase activates mitotic signaling pathways and stimulates G1-to-S phase transition in hematopoietic cells. Oncogene **15:** 2333–2342
69 Pelicci G., Lanfrancone L., Salcini A. E., Romano A., Mele S., Grazia Borrello M. et al. (1995) Constitutive phosphorylation of Shc proteins in human tumors. Oncogene. **11(5):** 899–907
70 Oda T., Heaney C., Hagopian J. R., Okuda K., Griffin J. D. and Druker B. J. (1994) Crkl is the major tyrosine-phosphorylated protein in neutrophils from patients with chronic myelogenous leukemia. J. Biol. Chem. **269(37):** 22925–22928
71 Sattler M., Salgia R., Okuda K., Uemura N., Durstin M. A., Pisick E. et al. (1996) The proto-oncogene product p120CBL and the adaptor proteins CRKL and c-CRK link c-ABL, p190BCR/ABL and p210BCR/ABL to the phosphatidylinositol–3′ kinase pathway. Oncogene **12(4):** 839–846

72 Salgia R., Pisick E., Sattler M., Li J. L., Uemura N., Wong W. K. et al. (1996) p130CAS forms a signaling complex with the adapter protein CRKL in hematopoietic cells transformed by the BCR/ABL oncogene. J. Biol. Chem. **271(41):** 25198–25203

73 Raitano A. B., Halpern J. R., Hambuch T. M. and Sawyers C. L. (1995) The Bcr-Abl leukemia oncogene activates Jun kinase and requires Jun for transformation. Proc. Natl. Acad. Sci. USA **92:** 11746–11750

74 Sawyers C. L., McLaughlin J. and Witte O. N. (1995) Genetic requirement for RAS in the trasformation of fibroblasts and hematopoietic cells by the BCR-ABL oncogene. J. Exp. Med. **181:** 307–313

75 Greenberger J. S. (1989) Ras mutations in human leukemia and related disorders. Int. J. Cell Cloning **7(6):** 343–359

76 Gaidano G. L., Guerrasio A., Serra A., Carozzi F., Rege-Cambrin G., Petroni D. et al. (1993) Mutations in the p53 and RAS family genes are associated with tumor progression of BCR-ABL negative chronic myeloproliferative disorders. Leukemia **7:** 946–953

77 Zou X., Rudchenko S., Wong K. and Calame K. (1997) Induction of c-myc transcription by the v-Abl tyrosine kinase requires Ras, Raf1 and cyclin-dependent kinases. Genes Dev. **11(5):** 654–662

78 Kirchnera D., Duysterc J., Ottmannd O., Roland M. Schmidt L., Bergmanna D. et al. (2003) Mechanisms of Bcr-Abl-mediated NF-kB/Rel activation. Exp. Hematol. **31:** 504–511

79 Ilaria R. L. Jr and Van Etten R. A. (1996) P210 and P190(BCR/ ABL) induce the tyrosine phosphorylation and DNA binding activity of multiple specific STAT family members. J. Biol. Chem. **271:** 31704–31710

80 Carlesso N., Frank D. A. and Griffin J. D. (1996) Tyrosyl phosphorylation and DNA binding activity of signal transducers and activators of transcription (STAT) proteins in hematopoietic cell lines transformed by Bcr/Abl. J. Exp. Med. **183(3):** 811–820

81 Salgia R., Li J. L., Lo S. H., Brunkhorst B., Kansas G. S., Sobhany E. S. et al. (1995) Molecular cloning of human paxillin, a focal adhesion protein phosphorylated by P210BCR/ABL. J. Biol. Chem. **270(10):** 5039–5047

82 Salgia R., Brunkhorst B., Pisick E., Li J. L., Lo S. H., Chen L. B. et al. (1995) Increased tyrosine phosphorylation of focal adhesion proteins in myeloid cell lines expressing p210BCR/ABL. Oncogene **11(6):** 1149–1155

83 Bhatia R., McGlave P. B., Dewald G. W., Blazar B. R. and Verfaillie C. M. (1995) Abnormal function of the bone marrow microenvironment in chronic myelogenous leukemia: role of malignant stromal macrophages. Blood **85(12):** 3636–3645

84 Upadhyaya G., Guba S. C., Sih S. A., Feinberg A. P., Talpaz M., Kantarjian H. M. et al. (1991) Interferon-alpha restores the deficient expression of the cytoadhesion molecule lymphocyte function antigen-3 by chronic myelogenous leukemia progenitor cells. J. Clin. Invest. **88:** 2131–2136

85 Gordon M. Y., Dowding C. R., Riley G. P., Goldman J. M. and Greaves M. F. (1987) Altered adhesive interactions with marrow stroma of haemopoietic progenitor cells in chronic myeloid leukemia. Nature **328(6128):** 342–344

86 Verfaille C. M., McCarthy J. B. and McGlave P. B. (1992) Mechanisms underlying abnormal trafficking of malignant progenitors in chronic myelogenous leukemia. Decreased adhesion to stroma and fibronectin but increased adhesion to the basement membrane components laminin and collagen type IV. J. Clin. Invest. **90:** 1232–1241

87 Verfaille C. M., Benis A., Iida J., McGlave P. B. and McCarthy J. B. (1994) Adhesion of committed human hematopoietic progenitors to synthetic peptides from the C-terminal heparin-binding domain of fibronectin**:** cooperation between the integrin 41 and the CD44 adhesion receptors. Blood **84(6):** 1802–1811

88 Verfaille C. M. (1997) Stem cells in chronic myelogenous leukemia. Hematology/Oncology Clinics of North America **11:** 1079–1114

89 Eaves A. C., Cashman J. D., Gaboury L. A., Kalousek D. K. and Eaves C. J. (1986) Unregulated proliferation of primitive chronic myeloid leukemia progenitors in the presence of normal marrow adherent cells. Proc. Natl. Acad. Sci. USA **83(14):** 5306–5310

90 Vigneri P. and Wang J. Y. (2001) Induction of apoptosis in chronic myelogenous leukemia cells through nuclear entrapment of BCR-ABL tyrosine kinase. Nat. Med. **7:** 228–234

91 Varticovski L., Daley G. Q., Jackson P., Baltimore D. and Cantley L. C. (1991) Activation of phosphatidylinositol 3-kinase in cells expressing abl oncogene variants. Mol. Cell. Biol. **11(2):** 1107–1113

92 Sattler M., Salgia R., Okuda K., Uemura N., Durstin M. A. and Pisick E. (1996) The proto-oncogene product p120CBL and the adaptor proteins CRKL and c-CRK link c-ABL, p190BCR/ABL and p210BCR/ABL to the phosphatidylinositol-3′ kinase pathway. Oncogene **12(24):** 839–846

93 Kauffmann-Zeh A., Rodriguez-Viciana P., Ulrich E., Gilbert C., Coffer P., Downward J. et al. (1997) Suppression of c-Myc-induced apoptosis by Ras signalling through PI 3-kinase and PKB. Nature **385:** 544–548

94 Sattler M., Mohi M. G., Pride Y. B., Quinnan L. R., Malouf N. A., Podar K. et al. (2002) Critical role for Gab2 in transformation by BCR/ABL. Cancer Cell. **1:** 479–492

95 Skorski T., Bellacosa A., Nieborowska-Skorska M., Majewski M., Martinez R., Choi J. K. et al. (1997) Transformation of hematopoietic cells by BCR/ABL requires activation of a PI–3k/Akt-dependent pathway. EMBO J. **16(20):** 6151–6161

96 Jain S. K., Susa M., Keeler M. L., Carlesso N., Druker B. and Varticovski L. (1996) PI 3-kinase activation in BCR/abl-transformed hematopoietic cells does not require interaction of p85 SH2 domains with p210 BCR/abl. Blood **88(55):** 1542–1550

97 Franke T. F., Kaplan D. R. and Cantley L. C. (1997) PI3K**:** downstream AKTion blocks apoptosis. Cell **88:** 435–437

98 Fialkow P. J., Martin P. J., Najfeld V., Penfold G. K., Jacobson R. J. and Hansen J. A. (1981) Evidence for a multistep pathogenesis of chronic myelogenous leukemia. Blood **58:** 158–163

99 O'Dwyer M. E., Gatter K. M., Loriaux M., Druker B. J., Olson SB, Magenis R. E. et al. (2003) Demonstration of Philadelphia chromosome negative abnormal clones in patients with chronic myelogenous leukemia during major cytogenetic responses induced by imatinib mesylate. Leukemia **17:** 481–487

100 Bumm T., Muller C., Al Ali H. K., Krohn K., Shepherd P., Schmidt E. et al. (2003) Emergence of clonal cytogenetic abnormalities in Ph-cells in some CML patients in cytogenetic remission to imatinib but restoration of polyclonal hematopoiesis in the majority. Blood **101:** 1941–1949

101 Bernstein R. (1988) Cytogenetics of chronic myelogenous leukemia. Semin. Hematol. **25:** 20

102 Ahuja H., Bar-Eli M., Arlin X., Advani S., Allen S. L., Goldman J. et al. (1991) The spectrum of molecular alterations in the evolution of chronic myelocitic leukemia. J. Clin. Invest. **87:** 2042–2047

103 Gaidano G., Serra A., Guerrasio A., Rege-Cambrin G., Mazza U. and Saglio G. (1994) Genetic analysis of p53 and RB1 tumor suppression genes in blast crisis of chronic myeloid leukemia. Ann. Hematol. **68:** 3–7

104 Serra A., Gottardi E., Della Ragione F., Saglio G. and Iolascon A. (1995) Involvement of cyclin-dependent kinase–4 inhibitor (CDKN2) gene in the pathogenesis of lymphoid blast crisis of chronic myelogenous leukemia. Br. J. Haemat. **91:** 625–629

105 Gaiger A., Henn T., Horth E., Geissler K., Mitterbauer G. and Maier-Dobersberger T. (1995) Increase of BCR-ABL chimeric mRNA expression in tumor cells of patients with

chronic myeloid leukemia precedes disease progression. Blood **86(6):** 2371–2378
106 Wada C., Shionoya S., Fujino Y., Tokuhiro H., Akahoshi T., Uchida T. et al. (1994) Genomic instability of microsatellite repeats and its association with the evolution of chronic myelogenous leukemia. Blood **83:** 3449–3456
107 Silly H., Chase A., Mills K. I., Apfelbeck U., Sormann S., Goldman J. M. et al. (1994) No evidence of microsatellite instability or consistent loss of heterozygosity at selected loci in chronic myeloid leukemia blast crisis. Leukemia **8:** 1923–1928
108 Wadhwa J., Szydlo R. M., Apperley J. F., Chase A., Bua M., Marin D. et al. (2002) Factors affecting duration of survival after onset of blastic transformation of chronic myeloid leukemia. Blood **99(7):** 2304–2309
109 Chronic Myeloid Leukemia Trialists' Collaborative Group (1997) Interferon alfa versus chemotherapy for chronic myeloid leukemia: a meta-analysis of seven randomized trials. J. Natl. Cancer Inst. **89:** 1616–1620
110 Gratwohl A., Hermans J., Niederwieser D., Frassoni F., Arcese W., Gahrton G. et al. (1993) Bone marrow transplantation for chronic myeloid leukemia**:** long-term results. Chronic Leukemia Working Party of the European Group for Bone Marrow Transplantation. Bone Marrow Transplantation **12(5):** 509–16
111 Horowitz M. M., Gale R. P., Sondel P. M., Goldman J. M., Kersey J., Kolb H. J. et al. (1990) Graft-versus-leukemia following bone marrow transplantation. Blood **75(3):** 555–562
112 Cullis J. O., Jiang Y. Z., Schwarer A. P., Hughes T. P., Barrett A. J. and Goldman J. M. (1992) Donor leukocyte infusions for chronic myeloid leukemia in relapse after allogeneic bone marrow transplantation. Blood **79:** 1379–1381
113 Druker B. J., Talpaz M., Resta D. J., Peng B., Buchdunger E., Ford J. M. et al. (2001) Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N. Engl. J. Med. **344:** 1031–1037
114 Kantarjian H., Sawyers C., Hochhaus A., Guilhot F., Schiffer C., Gambacorti-Passerini C. et al. (2002) Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. N. Engl. J. Med. **346:** 645–652
115 O'Brien S. G., Guilhot F., Larson R. A., Gathmann I., Baccarani M., Cervantes F. et al. (2003) Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N. Engl. J. Med. **348:** 994–1004
116 Druker B. J., Sawyers C. L., Kantarjian H., Resta D. J., Reese S. F., Ford J. M. et al. (2001) Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N. Engl. J. Med. **344:** 1038–1042
117 Gambacorti-Passerini C. B., Gunby R. H., Piazza R., Galietta A., Rostagno R. and Scapozza L. (2003) Molecular mechanisms of resistance to imatinib in Philadelphia-chromosome-positive leukaemias. Lancet Oncol. **4:** 75–85
118 Gorre M. E., Mohammed M., Ellwood K., Hsu N., Paquette R., Rao P. N. et al. (2001) Clinical resistance to STI–571 cancer therapy caused by BCRABL gene mutation or amplification. Science **293:** 876–880
119 Branford S., Rudzki Z., Walsh S., Grigg A., Arthur C., Taylor K. et al. (2002) High frequency of point mutations clustered within the adenosine triphosphate-binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (STI571) resistance. Blood **99(9):** 3472–3475
120 Hochhaus A., Kreil S., Corbin A. S., La Rosee P., Muller M. C., Lahaye T. et al. (2002) Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. Leukemia **16:** 2190–2196
121 Shah N. P., Nicoll J. M., Nagar B., Gorre M. E., Paquette R. L., Kuriyan J. et al. (2002) Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. Cancer Cell **2:** 117–125
122 Branford S., Rudzki Z., Walsh S., Parkinson I., Grigg A., Szer J. et al. (2003) Detection of BCR-ABL mutations in patients with CML treated with imatinib is virtually always accompanied by clinical resistance and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. Blood **102(1):** 276–283
123 Le Coutre P., Tassi E., Varella-Garcia M., Barni R., Mologna L., Cabrita G. et al. (2000) Induction of resistance to the Abelson inhibitor STI571 in human leukemic cells through gene amplification. Blood **95(5):** 1758–1766
124 Gambacorti-Passerini C. B., Rossi F., Verga M., Ruchatz H., Gunby R., Frapolli R. et al. (2002) Differences between in vivo and in vitro sensitivity to imatinib of Bcr/Abl+ cells obtained from leukaemic patients. Blood Cells Mol. Dis. **28(3):** 361–372
125 Mahon F. X., Belloc F., Lagarde V., Chollet C., Moreau-Gaudry F. and Reiffers J. (2003) MDR1 gene overexpression confers resistance to imatinib mesylate in leukemia cell line models. Blood **101(6):** 2368–2373
126 Roche-Lestienne C., Soenen-Cornu V., Grardel-Duflos N., Lai J. L., Philippe N., Facon T. et al. (2002) Several types of mutations of the Abl gene can be found in chronic myeloid leukemia patients resistant to STI571 and they can pre-exist to the onset of treatment. Blood **100(3):** 1014–1018
127 Donato N. J., Wu J. Y., Stapley J., Lin H., Arlinghaus R., Aggarwal B. B. et al (2004) Imatinib mesylate resistance through BCR-ABL independence in chronic myelogenous leukemia. Cancer Res. **64:** 672–677
128 Ross D. M. and Hughes T. P. (2004) Cancer treatment with kinase inhibitors**:** what have we learnt from imatinib? Br. J. Cancer **90(1):** 12–19
129 Hughes T. P., Morgan G. J., Martiat P. and Goldman J. M. (1991) Detection of residual leukemia after bone marrow transplant for chronic myeloid leukemia**:** role of polymerase chain reaction in predicting relapse. Blood **77(4):** 874–878
130 Guerrasio A., Martinelli G., Saglio G., Rosso C., Zaccaria A., Rosti G. et al. (1992) Minimal residual disease status in transplanted chronic myelogenous leukemia patients**:** low incidence of polymerase chain reaction positive cases among 48 long disease-free subjects who received unmanipulated allogeneic bone marrow transplants. Leukemia **6(6):** 507–512
131 Hochhaus A., Lin F., Reiter A., Skladny H., Mason P. J., van Rhee F. et al. (1996) Quantification of residual disease in chronic myelogenous leukemia patients on interferon-a therapy by competitive polymerase chain reaction. Blood **87:** 1549–1555
132 Cuneo A., Bigoni R., Emmanuel B., Smit E., Rigolin G. M., Roberti M. G. et al. (1998) Fluorescence in situ hybridization for the detection and monitoring of the Ph-positive clone in chronic myelogenous leukemia**:** comparison with metaphase banding analysis. Leukemia **12:** 1718–1723
133 Sinclair P. B., Nacheva E. P., Leversha M., Telford N., Chang J., Reid A. et al. (2000) Large deletions at the t(9;22) breakpoint are common and may identify a poor-prognosis subgroup of patients with chronic myeloid leukemia. Blood **95(3):** 738–743
134 Cross N. C. P., Feng L., Chase A., Bungey J., Hughes T. P. and Goldman J. M. (1993) Competitive polymerase chain reaction to estimate the number of BCR-ABL transcripts in chronic myeloid leukemia patients after bone marrow transplantation. Blood **82:** 1929–1936
135 Lion T., Henn T., Gaiger A., Kalhs P. and Gadner H. (1993) Early detection of relapse after bone marrow transplantation

in patients with chronic myelogenous leukaemia. Lancet **341:** 275–276

136 Lin F., van Rhee F., Goldman J. M. and Cross N. C. P. (1996) Kinetics of increasing BCR-ABL transcript numbers in chronic myeloid leukemia patients who relapse after bone marrow transplantation. Blood **87:** 4473–4478

137 van Rhee F., Lin F., Cullis J. O., Spencer A., Cross N. C. P., Chase A. et al. (1994) Relapse of chronic myeloid leukemia after allogeneic bone marrow transplant: the case for giving donor leukocyte transfusions before the onset of hematologic relapse. Blood **83:** 3377–3783

138 Raanani P., Dazzi F., Sohal J., Szydlo R., van Rhee F., Reiter A. et al. (1997) The rate and kinetics of molecular response to donor leucocyte transfusions in chronic myeloid leukaemia patients treated for relapse after allogeneic bone marrow transplantation. Br. J. Haematol. **99:** 945–950

139 Emig M., Saussele S., Wittor H., Weisser A., Reiter A., Willer A. et al. (1999) Accurate and rapid analysis of residual disease in patients with CML using specific fluorescent hybridization probes for real time quantitative RT-PCR. Leukemia **13:** 1825–1832

140 Beillard E., Pallisgaard N., van der Velden V. H. J., Bi W., Dee R., van der Scoot E. et al. (2003) Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using 'real-time' quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR) – a Europe against Cancer Program. Leukemia **17(12):** 2474–2486

141 Gabert J., Beillard E., van der Velden V. H. J., Bi W., Grimwade D., Pallisgaard N. et al. (2003) Standardization and quality control studies of 'real time' quantitative reverse transcriptase polymerase chain reaction (RQ-PCR) of fusion gene transcripts for residual disease detection in leukemia – a Europe against Cancer Program. Leukemia **17(12):** 2318–2357

142 Hughes T. P., Kaeda J., Branford S., Rudzki Z., Hochhaus A., Hensley M. L. et al. for the International Randomised Study of Interferon versus STI571 (IRIS) Study Group (2003) Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid Leukemia. N. Engl. J. Med. **349(15):** 1423–1432

143 Rosti G., Martinelli G., Bassi S., Amabile M., Trabacchi E., Giannini B. et al. (2004) Molecular response to imatinib in late chronic phase chronic myeloid leukemia. Blood **103(6):** 2284–2290

144 Merx K., Muller M. C., Kreil S., LahayeT., Paschka P., Schoch C. et al. (2002) Early reduction of BCR-ABL mRNA transcript levels predicts cytogenetic response in chronic phase CML patients treated with imatinib after failure of interferon-a. Leukemia **16:** 1579–1583

145 Branford S., Rudzki Z., Harper A., Grigg A., Taylor K., Durrant S. et al. (2003) Imatinib produces significantly superior molecular responses compared to interferon alfa plus cytarabine in patients with newly diagnosed chronic myeloid leukemia in chronic phase. Leukemia **17(12):** 2401–2409