MECHANISMS OF BCR–ABL IN THE PATHOGENESIS OF CHRONIC MYELOGENOUS LEUKAEMIA

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Abstract | Imatinib, a potent inhibitor of the oncogenic tyrosine kinase BCR–ABL, has shown remarkable clinical activity in patients with chronic myelogenous leukaemia (CML). However, this drug does not completely eradicate BCR–ABL-expressing cells from the body, and resistance to imatinib emerges. Although BCR–ABL remains an attractive therapeutic target, it is important to identify other components involved in CML pathogenesis to overcome this resistance. What have clinical trials of imatinib and studies using mouse models for BCR–ABL leukaemogenesis taught us about the functions of BCR–ABL beyond its kinase activity, and how these functions contribute to CML pathogenesis?

EXTRAMEDULLARY
Outside the bone marrow, as in the spleen, liver or lymph nodes.

COMPLETE CYTOGENETIC RESPONSE
Mononuclear bone-marrow cells shown to be negative for t(9;22)(q34;q11), as determined by cytogenetic analysis or fluorescence *in situ* hybridization analysis.

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Chronic myelogenous leukaemia (CML) results from the neoplastic transformation of a haematopoietic stem cell (FIG. 1). The hallmark genetic abnormality of CML is a t(9;22)(q34;q11) translocation, which was first discovered as an abnormal, small chromosome, named the 'Philadelphia chromosome'. This translocation generates the *BCR–ABL* fusion gene (reviewed in REF. 1). The initial chronic phase of this biphasic disease is characterized by a massive expansion of the granulocytic cell lineage, even though most, if not all, haematopoietic lineages can be produced from the CML stem cell. The median duration of the chronic phase is 3–4 years. Acquisition of additional genetic and/or epigenetic abnormalities causes the progression of CML from chronic phase to blast phase. This phase is characterized by a block of cell differentiation that results in the presence of 30% or more myeloid or lymphoid blast cells in peripheral blood or bone marrow, or the presence of EXTRAMEDULLARY infiltrates of blast cells.

Allogeneic stem-cell transplantation is the only known curative therapy for CML. However, most patients are not eligible for this therapy, because of advanced age (making them unable to tolerate the serious side effects of the treatment) or lack of a suitable stem-cell donor (reviewed in REF. 2). The discovery that BCR–ABL is required for the pathogenesis of CML, and that the tyrosine-kinase activity of ABL is essential for BCR–ABL-mediated transformation, made the ABL kinase an attractive target for therapeutic intervention (reviewed in REF. 3). Imatinib mesylate (Glivec, previously known as STI571 and CGP 57148) — a potent inhibitor of the tyrosine kinases ABL, ARG, platelet-derived growth factor receptor and KIT — has been shown to selectively induce apoptosis of BCR–ABL$^+$ cells[4–7], and is remarkably successful in treating patients with CML (reviewed in REF. 8). In newly diagnosed patients with CML in chronic phase, imatinib induces COMPLETE CYTOGENETIC RESPONSE in more than 80% patients. Patients with more advanced phases of CML also respond to imatinib, but this occurs much less frequently and treatment is less durable[8].

However, there are two major obstacles to imatinib-based therapies for patients with CML. One is the persistence of *BCR–ABL*-positive cells — this is known as 'residual disease', and is detected by a sensitive nested reverse-transcriptase PCR assay[9–11]. Suppression of the disease therefore relies on continuous imatinib therapy. The other major problem is relapse of the disease due to the emergence of resistance to imatinib (reviewed in REF. 12). Several mechanisms of resistance have been described, the most frequent of which are the appearance of point mutations in the *BCR–ABL* gene that impair the drug binding (comprehensively reviewed elsewhere[12–14]).

Summary

- Chronic myelogenous leukaemia (CML) results from the neoplastic transformation of a haematopoietic stem cell. Clinical and laboratory studies indicate that the fusion protein BCR–ABL is essential for initiation, maintenance and progression of CML, yet the transformation of CML from chronic phase to blast phase requires additional genetic and/or epigenetic abnormalities.
- Imatinib — an inhibitor of the tyrosine-kinase activity of BCR–ABL — has been successfully used to treat patients with chronic-phase CML, but residual disease persists and drug resistance emerges. It is therefore important to identify other factors involved in the pathogenesis of CML, to design alternative treatment strategies.
- Transgenic expression of BCR–ABL in mice leads to a myeloproliferative disorder that resembles the chronic phase of CML in patients. The ABL tyrosine-kinase activity is necessary but not sufficient to induce CML-like disease in mice. So, additional activities of BCR–ABL, beyond its kinase activity, are important for leukaemogenesis.
- BCR–ABL also interacts with oncogenic transcription factors to induce a form of acute myelogenous leukaemia that resembles the blast phase of CML, indicating that disease progression involves cooperation between BCR–ABL and mutations that disrupt haematopoietic gene transcription.
- CML progenitor cells seem to be refractory to imatinib therapy, indicating that the biology of haematopoietic stem/progenitor cells and tumour microenvironment are likely to contribute to the disease development and maintenance.

The fact that the resistance to imatinib is most commonly associated with point mutations in the kinase domain of BCR–ABL further demonstrates the importance of this activity in the pathogenesis of CML. On the other hand, the persistence of *BCR–ABL*-positive cells in patients on imatinib therapy indicates that inhibition of the ABL kinase activity alone might not be sufficient to eradicate the leukaemia cells. Identification of additional essential components in the pathogenesis of CML remains crucial for developing improved therapies for CML.

Numerous signalling pathways are active in BCR–ABL-expressing cells. Most of these were discovered through analysis of cultured cells. But the development of CML is a complex process that involves not only the signalling pathways mediated by BCR–ABL, but also factors provided by host cells and other components of the *in vivo* environment. It is therefore important to identify and validate potential therapeutic targets involved in BCR–ABL leukaemogenesis *in vivo*. What have studies in mouse models taught us about CML pathogenesis, response to therapy and mechanisms of minimal residual disease?

BCR–ABL and its oncogenic activities

ABL is a non-receptor tyrosine kinase that is expressed in most tissues (FIG. 2). In cells, the ABL protein is distributed in both the nucleus and cytoplasm of cells and can shuttle between the two compartments. It transduces signals from cell-surface growth factor and adhesion receptors to regulate cytoskeleton structure (reviewed in REFS 15,16). Mice with a homozygous disruption of the *Abl* gene — either through a null mutation or a deletion of the carboxy-terminal third of the protein — are variably affected, but phenotypes include an increased incidence of perinatal mortality, lymphopaenia and osteoporosis. ABL-null mice are also smaller, with abnormal head and eye development[17,18]. ABL shares functions with its homologue, ARG, during development[19]. ARG-deficient mice develop normally, but mice deficient for both ABL and ARG suffer from defects in neurulation and die before embryonic day 11.

BCR is also a signalling protein that contains multiple modular domains (FIG. 2). BCR-deficient mice develop normally, although their neutrophils have been shown to produce excess levels of oxygen metabolites following their activation[20]. The fusion of BCR sequences to ABL during the translocation associated with CML increases the tyrosine-kinase activity of ABL, and brings new regulatory domains/motifs to ABL, such as the growth factor receptor-bound protein 2 (GRB2) SH2-binding site (FIG. 3). Depending on the precise breakpoints in the translocation and RNA splicing, different forms of BCR–ABL protein with different molecular weights (p185 BCR-ABL, p210 BCR–ABL and p230 BCR–ABL) can be generated in patients.

Model systems for studying BCR-ABL leukaemogenesis. Several biological model systems have demonstrated that *BCR–ABL* is an oncogene that promotes CML pathogenesis. These model systems are also important tools for elucidating the molecular mechanisms of CML formation, and to identify potential therapeutic targets. One type of model system measures the transforming potential of BCR–ABL in cultured cells. Expression of BCR–ABL has been shown to transform established mouse fibroblast cell lines, factor-dependent haematopoietic cell lines and primary bone-marrow cells[21]. Expression of BCR–ABL in human CD34$^+$ cells causes increased proliferation in response to growth factors, increased growth-factor-independent survival, reduced adhesion to fibronectin and reduced chemotaxis to stroma-derived factor-1α[22,23]. These properties of BCR–ABL-expressing human haematopoietic progenitor cells mimic those of CML progenitor cells isolated from patients.

Development of leukaemia is a complex process that involves both the effects of BCR–ABL within the haematopoietic cells, and the interactions of BCR–ABL-expressing cells with the rest of the *in vivo* environment. So, in addition to studies in cultured cells, animal models of CML are needed to study *in vivo* pathogenesis, the role of the environment in leukaemogenesis, and to identify therapeutic targets. The most commonly used animal models are mice with haematopoietic cells that express BCR–ABL — through transgenic, knock-in or retroviral transduction techniques.

Expression of BCR–ABL in mouse bone-marrow cells by retroviral transduction and bone-marrow transplantation (BMT) methods was shown to induce a MYELOPROLIFERATIVE DISORDER (MPD) that closely resembled CML[24–26]. However, in earlier BMT experiments, mice that received bone-marrow cells tranduced with p210 *BCR–ABL* also developed other haematopoietic neoplasms, such as B-CELL ACUTE LYMPHOBLASTIC LEUKAEMIA (B-ALL) and macrophage tumours. More importantly, the low efficiency of induction of MPD and poor

CD34
A cell-surface protein expressed by haematopoietic stem cells (HSCs), haematopoietic progenitor cells and endothelial cells, and used as a marker to isolate human HSCs.

MYELOPROLIFERATIVE DISORDER
Characterized by high peripheral-blood counts with granulocyte predominance, hepatosplenomegaly (enlarged liver and spleen) and pulmonary haemorrhages, owing to extensive granulocyte infiltration.

B-CELL ACUTE LYMPHOBLASTIC LEUKAEMIA
Characterized by high peripheral-blood cell counts, bloody pleural effusion (chest-cavity fluid) that contains B-lymphoblastic cells, and an enlarged lymph node.

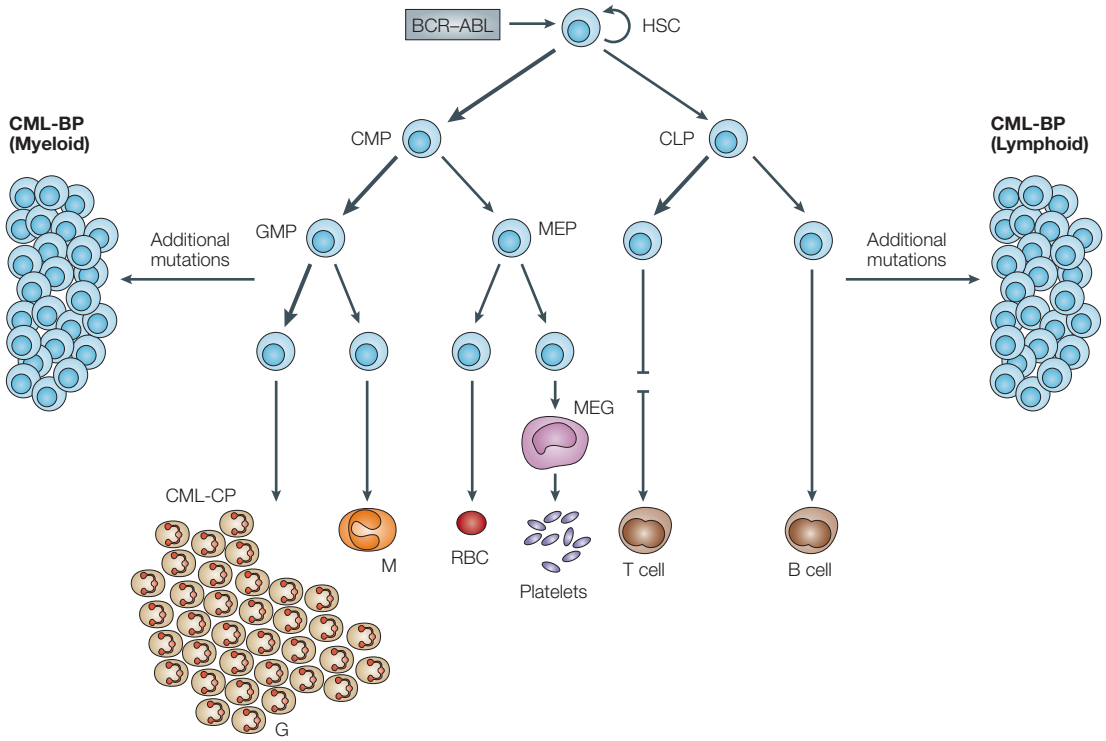

Figure 1 | **The development of chronic myelogenous leukaemia.** Chronic myelogenous leukaemia (CML) is a biphasic disease, initiated by expression of the *BCR–ABL* fusion gene product in self-renewing, haematopoietic stem cells (HSCs). HSCs can differentiate into common myeloid progenitors (CMPs), which then differentiate into granulocyte/macrophage progenitors (GMPs; progenitors of granulocytes (G) and macrophages (M)) and megakaryocyte/erythrocyte progenitors (MEPs; progenitors of red blood cells (RBCs) and megakaryocytes (MEGs), which produce platelets). HSCs can also differentiate into common lymphoid progenitors (CLPs), which are the progenitors of lymphocytes such as T cells and B cells. The initial chronic phase of CML (CML-CP) is characterized by a massive expansion of the granulocytic-cell series. Acquisition of additional genetic mutations beyond expression of BCR–ABL causes the progression of CML from chronic phase to blast phase (CML-BP), characterized by an accumulation of myeloid (in approximately two-thirds of patients) or lymphoid blast cells (in the other one-third of patients). Although the CML stem cell is multipotent, production of B cells from the neoplastic clone occurs only at low levels, and only rare T-cell precursors can be detected. This indicates that lymphopoiesis, particularly the development of T cells, is compromised by BCR–ABL expression[118].

reproducibility in these earlier models hindered the usefulness of this method in studying the biology of BCR–ABL in CML. Nonetheless, these experiments confirm the oncogenic activity of BCR–ABL and its role in CML.

Subsequently, we and others began using a murine stem-cell retroviral vector to express the *BCR–ABL* oncogene in haematopoietic stem/progenitor cells[27,28]. Expression of the transgene in the bone-marrow cells of mice induced an MPD that resembled the chronic phase of human CML with 100% efficiency[29–31]. Common features of the disease include increased numbers of peripheral-blood cells (with a predominance of granulocytes), splenomegaly, extramedullary haematopoiesis in liver and pulmonary haemorrhages, owing to extensive granulocyte infiltration in the lung. In addition, the disease is mostly polyclonal and can be transplanted to secondary-recipient mice. The ability of BCR–ABL to induce a CML-like MPD in mice much more efficiently and reproducibly in this model than in previous models was probably due to efficient expression of the transgene in the correct cell type. This model system has been used to identify targets both within and downstream of BCR–ABL.

Initial development of transgenic and knock-in mouse models of CML was problematic. The generation of conventional transgenic and knock-in mice, through expression of BCR–ABL from the *BCR* promoter, caused embryonic lethality owing to the toxicity of this activated tyrosine kinase during embryonic development[32,33]. Transgenic mice that expressed BCR–ABL from a metallothionein-inducible promoter or by a *TEC* (haematopoietic-specific) promoter primarily developed T-CELL ACUTE LYMPHOBLASTIC LEUKAEMIA (T-ALL), rather than CML[34–37]. Although the progeny of one of the *TEC*-controlled BCR–ABL mice developed mild granulocyte hyperplasia and a THROMBOCYTOSIS after a greatly extended latency of about 1 year, this system does not effectively model CML.

Studies with transgenic mice created through expression of *BCR–ABL* under control of a tetracycline-responsive promoter revealed that in order for CML to arise, it is crucial to express this oncogene in the proper cell type. Using this system, expression of BCR–ABL in B-cell lymphocytic and megakaryocytic precursors resulted in the development of B-ALL and megakaryocytic myeloproliferative syndrome[38,39]. When the oncogene was expressed specifically in

T-CELL ACUTE LYMPHOBLASTIC LEUKAEMIA
Characterized by high peripheral-blood cell counts, bloody pleural effusion that contains T-lymphoblastic cells and enlarged thymus.

THROMBOCYTOSIS
Increased numbers of platelets in the peripheral blood.

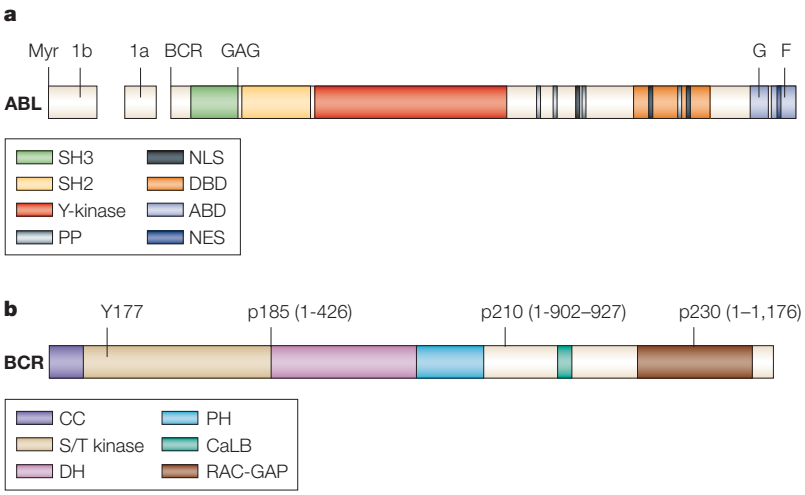

Figure 2 | **The ABL and BCR proteins.** There are several important domains that make up ABL and BCR proteins. **a** | Two isoforms of ABL (human types 1a and 1b) are generated by alternative splicing of the first exon, one of them (1b) contains a myristoylation modification site (Myr). (Myristoylation is a process that attaches the fourteen-carbon saturated fatty acid myristate to the amino-terminal glycine of proteins.) Apart from the alternatively spliced sequences, the amino-terminal half of ABL contains tandem SRC homology 3 (SH3), SH2 and the tyrosine-kinase (Y-kinase) domains. These domains can assemble into an auto-inhibitory structure, in which the SH3 and SH2 domains function as a 'clamp' that holds the kinase in the 'off' state[119,120]. In ABL1b, the myristoyl group at the extreme end of the amino-terminal segment also binds to the tyrosine-kinase domain and functions as a 'latch' that keeps the SH3–SH2 clamp in place[119,121]. In its carboxy-terminal region, ABL contains four proline-rich SH3 binding sites (PPs), three nuclear localization signals (NLSs), one nuclear exporting signal (NES), a DNA-binding domain (DBD), and an actin-binding domain (ABD). This actin-binding domain contains binding sites for both monomeric (G) and filamentous (F) forms of actin. The points in ABL that fuses with BCR and GAG (for v-Abl) are indicated. **b** | BCR contains a coiled-coil (CC) oligomerization domain, a serine/threonine (S/T) kinase domain, a Dbl/CDC24 guanine-nucleotide exchange factor homology (DH) domain and a pleckstrin homology (PH) domain, a putative calcium-dependent lipid binding site (CaLB) and a RAC guanosine triphosphatase-activating protein (RAC-GAP) domain. BCR also contains binding sites for growth factor receptor-bound protein 2 (GRB2) at tyrosine 177 (Y177), as well as for the GRB10, 14-3-3 and the ABL proteins, through its SH2 domain. p185, p210 and p230 indicate the points at which BCR most commonly fuses to ABL — these forms are associated with acute lymphoblastic leukaemia, chronic myelogenous leukaemia (CML) and a milder form of CML, respectively. The number of amino acids in each form are indicated in parentheses.

haematopoietic stem cells through use of a mouse stem cell leukaemia (SCL) enhancer to regulate expression, the mice developed a chronic-phase CML-like disease. Some mice also succumbed to superimposed B-cell lymphoblastic disease, indicating lymphoblast crisis[40]. This recently developed inducible genetic model of CML should be useful for studying leukaemogenic mechanisms in haematopoietic stem cells during disease initiation and progression. Together, these studies have indicated that BCR–ABL expression alone is sufficient to induce chronic-phase CML in mice (BOX 1). What then is the role of BCR–ABL in blast transformation of CML?

Other genetic defects associated with CML. Additional cytogenetic and molecular changes are frequently found in patients with CML during the progression of the disease from chronic to blast phase[41]. Some of the genetic changes found in leukaemic cells isolated from patients with blast-phase CML include mutations in *TP53*, *RB*, and *CDKN2A* (also known as $p16^{INK4A}$), or overexpression of genes such as *EVI1* and *MYC*. Additional chromosome translocations are also observed, such as t(3;21)(q26;q22), which generates *AML1–EVI1* — this fusion gene is also called *AML1–MDS1–EVI1*, and is hereafter referred to as *AME*. Other CML-associated fusion genes include *AML1–ETO*, which results from the t(8;21)(q22;q22) translocation; *NUP98–HOXA9*, which results from the t(7;11)(p15;p15) translocation; and *CBFβ–SMMHC*, which results from inv(16)(p13;q22). (For reviews of these translocations, see REFS 41–44.) Does the transformation of CML from chronic phase to blast phase require cooperation between *BCR–ABL* and these additional genetic abnormalities? Investigating the role of these fusion-gene products in leukaemogenesis, along with the potential cooperation between BCR–ABL and these secondary genetic defects, is crucial for understanding the molecular mechanism of CML-blast transformation. Studies have tested whether BCR–ABL and the AME fusion protein cooperate to induce blast-phase CML using the mouse BMT model.

AML1 (also known as RUNX1 and CBFα2), part of which is fused to EVI1 in AME, is the DNA-binding subunit of core binding factor (CBF). It forms a heterodimeric transcription factor with the non-DNA-binding CBFβ subunit[45]. AML1 and CBFβ are essential for definitive haematopoiesis[45]. The EVI1 component of AME is a transcription factor with two sets of zinc-finger domains[46]. *EVI1* was first identified as a gene whose expression was activated by retroviral integration in mouse myeloid tumours[47], and its product is essential for embryonic development[46]. Using a BMT approach, it was shown that expression of AME can induce an acute myelogenous leukaemia (AML) with a long latency (5–13 months) in mice[48]. In an analysis of the direct effect of AME on haematopoiesis during the 4-month pre-leukaemia stage, expression of AME alone in mouse bone-marrow cells did not block myeloid differentiation *in vivo*, indicating that additional mutations were required for induction of AML by AME[49]. Interestingly co-expression of BCR–ABL and AME in mouse bone-marrow cells, by retroviral transduction, rapidly induced a form of AML that resembles blast-phase CML[49]. So the block in myeloid differentiation and transformation of CML from chronic phase to blast phase involves cooperation between BCR–ABL and defects in haematopoietic gene transcription[49]. A similar finding was made in a BMT model that expressed both the BCR–ABL and NUP98–HOXA9 fusion proteins[50].

Recent studies have shown that the pool of granulocyte/macrophage progenitor (GMP) cells from patients with blast-phase CML is expanded, and that these cells have increased levels of BCR–ABL and nuclear β-catenin, leading to self-renewal capacity[51]. These results indicate that GMP cells might be transformed into leukaemic stem cells during blast-phase CML, and β-catenin could be a common downstream target of the genetic abnormalities associated with CML during myeloid blast crisis. It is not clear at present whether the increase in nuclear β-catenin levels is caused by co-expression of *BCR–ABL* and its cooperating oncogenes.

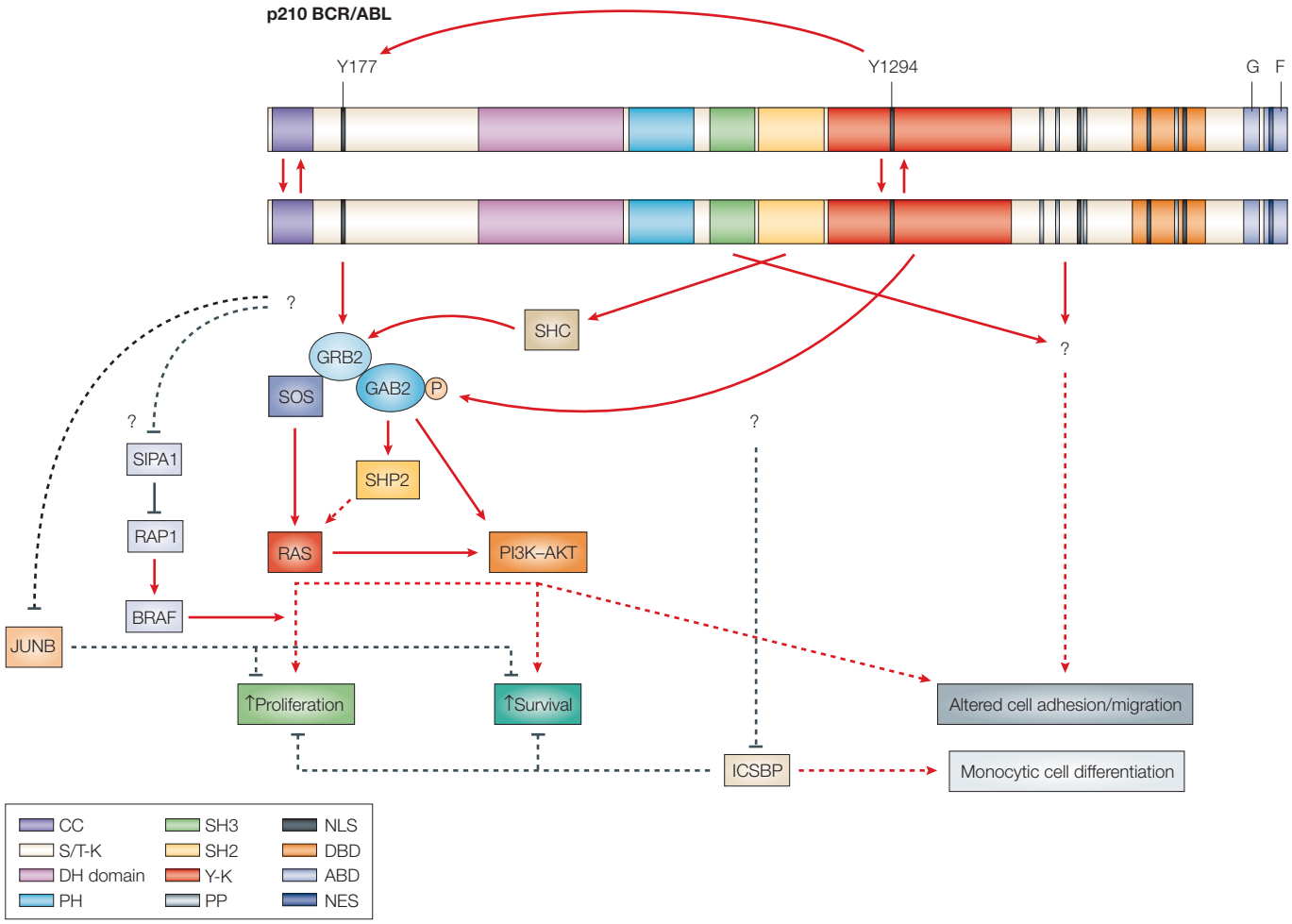

Figure 3 | **Leukaemogenic signalling of BCR–ABL.** The p210 form of BCR–ABL is shown in this diagram (see Fig. 2 for fusion point, which occurs after the pleckstrin homology (PH) domain of BCR). The BCR–ABL proteins can form dimers or tetramers through their CC domains, and trans-autophosphorylate (indicated by up and down arrows between protein structures). Phosphorylation at the Y177 residue generates a high-affinity binding site for growth factor receptor-bound protein 2 (GRB2). GRB2 binds to BCR–ABL through its SH2 domain and binds to SOS and GRB2-associated binding protein 2 (GAB2) through its SH3 domains. SOS in turn activates RAS. Following phosphorylation (P) by BCR–ABL, GAB2 recruits phosphatidylinositol 3-kinase (PI3K) and SHP2 proteins. The SH2 domain of ABL can bind SHC, which, following phosphorylation can also recruit GRB2. The ABL SH3 domain and the SH3 binding sites in the carboxy-terminal region can bind several proteins that involve regulations of cell adhesion/migration. Interferon consensus sequence binding protein (ICSBP), also known as interferon regulatory factor 8, negatively regulates proliferation and survival of myeloid cells by inducing differentiation of monocytic cells. JUNB inhibits cell proliferation and survival, partly by antagonizing the RAS downstream target JUN. SIPA1 (signal-induced proliferation-associated gene-1) is a RAP1 GAP that keeps RAP1 inactive. BCR–ABL can promote cell proliferation and survival partly by activating the RAS, SHP2 and PI3K–AKT signalling pathways. It can also downregulate transcription of ICSBP and JUNB, and might also inhibit SIPA1. Red arrows indicate direct interactions and/or activations. Black arrows indicate negative regulations. Broken arrows indicate multiple steps. ABD, actin-binding domain; CC, coiled-coil; DBD, DNA-binding domain; DH, Dbl/CDC24 guanine-nuleotide exchange factor homology; NES, nuclear exporting signal; NLS, nuclear localization signal; PP, proline-rich SH3 binding site; S/T-K, serine/threonine kinase; Y-K, tyrosine kinase.

Roles of BCR–ABL domains in leukaemogenesis

In addition to the ABL kinase domain, there are other important domains/motifs in BCR–ABL, such as those that regulate the kinase activity of ABL or connect to other downstream signalling pathways. The relative importance of various domains of BCR–ABL in neoplastic transformation have been examined in the biological model systems described above, including transformation of immortalized fibroblast cell lines, growth-factor-dependent haematopoietic cell lines and primary bone-marrow cells. The advantage of using cell lines is the relative ease of obtaining a large number of clonally derived cells for biochemical analysis, genetic manipulations and biological examinations. However, cell lines are limited in that they are not likely to include all the physiologically relevant components of BCR–ABL-mediated leukaemogenesis. The existence of unknown genetic abnormalities in established cell lines might also obscure the function of the gene under study. Inconsistent results have been obtained from cell line studies in assessing the role of certain functional domains or motifs, such as the GRB2 SH2-binding site at Y177 of BCR and of the SH2 domain of ABL, in transformation by BCR–ABL (reviewed in REF. 52). The importance of the different domains/motifs of BCR–ABL should

Box 1 | **Mutations necessary for the induction of chronic myelogenous leukaemia**

Although expression of BCR–ABL is sufficient to transform haematopoietic cells and to induce a chronic myelogenous leukaemia (CML)-like myeloproliferative disorder in mice, it remains unclear whether BCR–ABL alone is sufficient to induce CML in humans. Several observations indicate that additional genetic abnormalities might be required for the induction of CML. CML occurs in all age groups, but incidence increases with age, peaking in patients in their 50s and 60s. A mathematical model, based on epidemiological data, predicts that three mutations in a stem cell are necessary for chronic disease to develop, and that progression to blast crisis is caused by only one more mutation, in either a stem or committed cell[114]. Exposure to ionizing irradiation can increase risk of acquiring CML, but disease usually develops after a prolonged latent period (median latent period of 4–11 years, depending on the types of exposure), indicating that several mutations might be necessary for CML[115]. Finally, the *BCR–ABL* fusion gene can be detected at a very low level in the blood cells of about 30% of healthy individuals, but only a small percentage of people develop CML[116,117]. However, these observations do not prove that mutations other than BCR–ABL are required for the induction of chronic-phase CML — alternative explanations for these observations exist. For example, the *BCR–ABL* fusion gene detected in blood cells of healthy individuals might be generated in non-self-renewing differentiated cells, rather than haematopoietic stem cells. Further studies are necessary to examine whether the *BCR–ABL* fusion gene is present in haematopoietic stem cells of healthy individuals. In addition, haematopoietic stem cells of older people might have a higher rate of t(9;22)(q34;q11) translocation. As CML progenitor cells retain the ability to undergo terminal differentiation in the chronic phase of the disease, the accumulation of the 10^{12} leukaemic cells (typically observed in patients) from a single transformed haematopoietic stem cell might simply take a long time, rather than require additional mutations.

therefore be validated in *in vivo* models of CML. The mouse BMT system described above is an excellent system for the *in vivo* structure–function analysis of BCR–ABL (reviewed in REFS 21,53).

Various mutant forms of BCR–ABL have been expressed in mice using the BMT model. Mice that express a form of BCR–ABL with a point mutation in the ATP-binding site of ABL, which inactivates its kinase activity (refered to as kinase-deficient BCR–ABL), do not develop leukaemia, even when the fusion protein is expressed in the long-term repopulating haematopoietic stem/progenitor cells. This indicates that the ABL kinase activity is absolutely essential for BCR–ABL leukaemogenesis *in vivo*[29]. This result is consistent with the finding that the kinase activity of ABL is required for BCR–ABL-mediated transformation in cultured cells. It also confirmed that the ABL kinase is an effective target for treating CML.

Deletion of the SH3 domain of ABL results in a mutant form of the protein with increased tyrosine-kinase activity, and expression of this truncated protein can transform both fibroblast and haematopoietic cell lines *in vitro*. However, it only induces lymphoid leukaemia/lymphoma formation with a greatly extended latency in mice[54,55]. A mutant form of BCR–ABL with a deletion of the SH3 domain does, however, still effectively induce a fatal MPD[55]. These findings indicate that activation of ABL's kinase alone (through loss of SH3) is not sufficient to cause a CML-like MPD, and that other functional domains/motifs of BCR–ABL are required for the induction of CML-like disease.

The amino-terminal coiled-coil (CC) oligomerization domain of BCR is an important activator of the kinase activity of ABL, and also promotes the association of BCR–ABL with actin fibres (FIG. 3)[56]. A mutant form of BCR–ABL that lacks the BCR-CC domain (ΔCC-BCR–ABL) failed to induce the MPD in mice, but, rather, induced a T-cell leukaemia/lymphoma only after a long latent period[57–59]. Reactivation of the kinase activity of ABL by mutating its SH3 domain (through deletion or a P1013L point mutation), rescued the ability of ΔCC-BCR–ABL to induce the a CML-like MPD in mice, albeit with a lesser efficiency[29,59]. These results demonstrate that the BCR-CC domain is essential for the induction of myeloproliferative disorder by BCR–ABL in mice, mainly owing to its ability to activate the kinase activity of ABL.

Another important motif in the BCR region of BCR–ABL is the GRB2-binding site. GRB2 binds SOS — a guanine-nucleotide exchanger of RAS — as well as the scaffolding adapter GRB2-associated binding protein 2 (GAB2). Formation of this complex depends on BCR phosphorylation at tyrosine 177 (REFS 60,61), leading to activation of RAS, and recruitment of SHP2 and phosphatidylinositol 3-kinase (PI3K)[60–62] (FIG. 3). Mutation of the tyrosine-177 residue of BCR–ABL to phenylalanine (Y177F) largely abolished its ability to bind GRB2, yet did not affect the kinase activity of ABL[60,61]. In the BMT model for CML, a Y177F mutant form of BCR–ABL has a greatly reduced ability to induce MPD in mice. Mice expressing BCR–ABL Y177F eventually developed T-ALL or abdominal T-cell lymphomas after a prolonged latent period[57,58,63] (TABLE 1). These results demonstrate that phosphorylation at Y177 is required for the induction of MPD by BCR–ABL.

Another tyrosine phosphorylation site that is located in the activation loop of ABL's kinase domain (this corresponds to Y1294 in p210 BCR–ABL; FIG. 3) along with the SH2 domain of ABL also contribute to the activation of the RAS pathway[64]. The SH2 domain of ABL is believed to activate RAS, at least partially, through binding to SHC, which, following tyrosine phosphorylation, can recruit GRB2[64] (FIG. 3). The mechanism of Y1294 in activation of the RAS pathway is not known. Mutations in the SH2 domain of ABL reduce the ability of BCR–ABL to induce a CML-like MPD in mice[65,66] (TABLE 1). A Y1294F point mutation also attenuated leukaemogenesis by BCR–ABL[21,59]. These data indicate that both ABL's SH2 domain and its phosphorylation site at Y1294 contribute to the overall leukaemogenic strength of BCR–ABL.

The carboxy-terminal region of ABL is required for the proper function of normal ABL and for the lymphoid leukaemogenicity of v-Abl[17,67]. However, deletion of ABL's actin-binding domain or the entire carboxy-terminal region downstream of the ABL kinase domain did not affect the ability of BCR–ABL to induce CML-like MPD in mice[21,68]. So, the function of these domains is dispensable in BCR–ABL-mediated leukaemogenesis.

It is evident that certain domains/motifs of BCR–ABL bear overlapping functions. Deletions of both ABL's SH3 domain and the carboxy-terminal proline-rich SH3-binding sites (ABL-PP), but not single mutations of each, block the ability of BCR–ABL to stimulate spontaneous cell migration on fibronectin-coated surfaces, and greatly reduced BCR–ABL leukaemogenicity in mice[69]. Deletions of both the ABL SH3 and SH2 domains in BCR–ABL also showed more severe defects in mice than mutating either single domain[70].

The structure–function analysis of BCR–ABL, using mouse models of CML (TABLE 1), has shown that the activation of the tyrosine-kinase activity of ABC is necessary but not sufficient to induce CML-like disease. Domains/motifs of BCR–ABL outside of the ABL kinase catalytic site could be crucial determinants of both the severity and lineage specificity of BCR–ABL leukaemogenesis. What signalling pathways do these crucial domains link to, and might these also be effective therapeutic targets for CML?

Pathways downstream of BCR–ABL

Many signalling proteins have been shown to interact with BCR–ABL through various functional domains/motifs (for example, GRB2, CRKL, CRK, SHC, 3BP2, ABL-interacting protein 1 and 2, and CRK-associated substrate (CAS)), and/or to become phosphorylated in BCR–ABL-expressing cells (for example, CRKL, CRK, SHC, docking protein 1, GAB2, CBL, CAS, signal transducer and activator of transcription 5 (STAT5), the p85 subunit of PI3K, phospholipase Cγ, synaptophysin, VAV1, RAS GTPase-activating protein, focal adhesion kinase, FES, paxillin and talin). (For reviews of these interactions, see REFS 42,71.) These proteins in turn activate a range of signalling pathways that activate proteins such as RAS, PI3K, AKT, JNK, SRC family kinases, protein and lipid phosphatases, and their respective downstream targets, as well as transcription factors such as the STATs, nuclear factor-κB and MYC (reviewed in REF. 71). BCR–ABL also induces expression of cytokines such as INTERLEUKIN-3 (IL-3), GRANULOCYTE COLONY-STIMULATING FACTOR and granulocyte–macrophage colony-stimulating factor (GM-CSF)[29,72]. Most of these findings were observed from experiments in *in vitro* systems, or from studies of the properties of cells derived from leukaemia patients with particular stages of disease. The importance of these signalling proteins and pathways in leukaemogenesis and their viability as therapeutic targets needs to be validated by *in vivo* model systems. Mouse models can be used to determine the involvement of these signalling pathways in CML pathogenesis and progression.

The leukaemogenic role of various factors that are activated by BCR–ABL expression has been effectively examined using knockout mice. Expression of BCR–ABL in bone-marrow cells, through retroviral transduction, still induced CML-like MPD in *Stat5a*$^{-/-}$*Stat5b*$^{-/-}$, *Cbl*$^{-/-}$ and *Il-3*$^{-/-}$*GM-CSF*$^{-/-}$ mice[73–75], so these proteins are not required for BCR–ABL-mediated leukaemogenesis. BCR–ABL expression also induced CML-like MPD in mice that lacked the SRC family kinases LYN, haematopoietic cell kinase and FGR, although it failed to induce B-ALL in these mice[76]. Interestingly, downregulating LYN expression by small interfering RNA induces apoptosis of human blast-phase CML cells, particularly the lymphoid blast cells[77]. Together these results indicate that LYN is important in the development of blast-phase CML — particularly for lymphoid blast crisis — but not for chronic-phase CML.

BCR–ABL also recruits the scaffold adapter GAB2 through GRB2. The major GRB2-binding site at Y177 of BCR–ABL was shown to regulate the tyrosine phosphorylation of GAB2 (REF. 62), indicating that GAB2 is a substrate of BCR–ABL. Consistent with the importance of Y177 function in BCR–ABL-mediated leukaemogenesis, BCR–ABL was unable to confer cytokine-independent growth (a characteristic of leukaemia cells) in primary myeloid cells isolated from *Gab2*$^{-/-}$ mice *in vitro*[62]. So, the inability of the Y177F mutant of BCR–ABL to induce leukaemia might be partially due to a failure to transmit appropriate signals through GAB2. GAB2 contains binding sites for the SH2 domains of the p85 subunit of PI3K and for SHP2 (REF. 62). The PI3K pathway has been implicated in a wide range of human cancers[78]. Mutations of the *SHP2* gene (also known as PTPN11) have also been found in approximately 50% of individuals with Noonan syndrome, a common human autosomal-dominant birth defect characterized by short stature, facial abnormalities, heart defects and possibly increased risk of leukaemia[79–81]. The PI3K and SHP2 signalling pathways could be required for BCR–ABL leukaemogenesis and therefore be effective therapeutic targets for CML.

SHP2 is required for normal activation of the RAS–ERK (extracellular signal-regulated kinase) pathway that most receptor tyrosine kinases and cytokine receptors signal through[82]. The mechanism of the activation of RAS–ERK pathway by SHP2 is not completely known. In addition to SHP2, RAS can be activated directly by BCR–ABL through the GRB2–SOS complex[60,64] (FIG. 3). Mutations that result in constitutive activation of RAS are associated with approximately 30% of all human cancers, including 20–30% of cases of AML, MPDs and myelodysplastic syndrome[83]. Recently, it was shown that expression of an oncogenic KRAS using a conditional knock-in line of mice efficiently induced an MPD that resembled human chronic myelomonocytic leukaemia (CMML)[84,85]. We found that expression of oncogenic NRAS in mice, through BMT, efficiently induced CMML- or AML-like disease in mice (R. Subrahmanyam, C. Parikh and R.R., unpublished observations). We

INTERLEUKIN-3
A haematopoietic growth factor that promotes survival and activation of many lineages of cells in the haematopoietic system — particularly in mast and basophil development and immunity in cases of parasitic infection.

GRANULOCYTE COLONY-STIMULATING FACTOR
Also known as granulocyte growth factor, it is important for the development of neutrophils and haematopoietic progenitors.

Table 1 | **Analysis of mutant forms of BCR–ABL**

Form of BCR–ABL*	Kinase activity	*In vivo* leukaemogenesis	References
Wild type	+	CML-like MPD	30–33
p185 BCR–ABL	+	CML-like MPD	33
p230 BCR–ABL	+	CML-like MPD	33
Kinase deficient	–	–	31
ΔSH3	+	CML-like MPD	44
ΔSH3–ABL	+	B-ALL and T-ALL (LL)	44
ΔCC	Greatly reduced	T-ALL (LL)	47–49
ΔCC/ΔSH3	+	CML-like MPD	47,49
CC-ABL	Significantly reduced	T-ALL (LL)	47
Y177F‡	+	T-ALL or abdominal T-cell lymphoma (LL)	47,48,55
ΔSH2	Significantly reduced	LPD→MPD§ (LL)‖	56,57
R1057K¶	+	LPD→MPD (LL)‖	56,57
Y1294F	+	LPD→MPD (LL)	22,49
ΔABD	+	CML-like MPD	59
ΔC′	+	CML-like MPD	22
p185/ΔSH3/ΔPP	+	Undetermined disease (LL)	58
ΔSH3/ΔSH2	Significantly reduced	–#	61

*The p210 form of BCR–ABL (Fig. 3), unless otherwise stated. ‡A point mutation that changes the tyrosine-177 residue (in the GRB2 SH2 binding site) to phenylalanine. §A biphasic disease, consisting of a transient B-cell lymphoproliferative disorder (LPD) followed by a fatal myeloproliferative disorder (MPD). ‖Data in Ref. 57 showed that BCR–ABL with SH2 domain mutations induced mainly B-ALL with a long disease latency. ¶A point mutation that changes the arginine residue in the phosphotyrosine-binding pocket of the ABL SH2 domain to a lysine residue. #In this study, wild-type BCR–ABL induced an acute myelogenous leukaemia (AML)-like disease in mice. Δ, deletion mutation; ΔC′, deletion of the entire carboxy-terminal region of ABC; B-ALL, B-cell acute lymphoblastic leukaemia; LL, Long latency; T-ALL, T-cell acute lymphoblastic leukaemia.

also found that co-expression of oncogenic NRAS and the Y177F mutant of BCR–ABL could rapidly and efficiently induce CML-like myeloproliferative disorder (R. Subrahmanyam and R.R., unpublished observations). So RAS seems to be a crucial downstream target of BCR–ABL, yet other signalling pathways activated by BCR–ABL restrict BCR–ABL-mediated leukaemogenesis in the granulocytic lineage.

Gene-knockout studies also revealed several key negative regulators of myelopoiesis. Mice with disruptions of the gene encoding interferon consensus sequence binding protein (ICSBP), also known as interferon regulatory factor 8, developed a CML-CP-like disease at 10–16 weeks of age, in addition to defects of viral and intracellular parasite immunity[86]. One-third of the mice also underwent blast crisis by 50 weeks of age. Consistent with the myeloid phenotype of the *Icsbp*-null mice, it was shown that ICSBP controls the development of myeloid cells by stimulating macrophage differentiation while inhibiting granulocyte differentiation, in both cases inhibiting cell growth[87] (FIG. 3). Expression of the ICSBP protein is significantly decreased in mice with BCR–ABL-induced CML-like disease, and forced expression of ICSBP inhibited the BCR–ABL-induced colony formation of bone-marrow cells *in vitro* and BCR–ABL-induced CML-like disease *in vivo*[88]. Downregulation of *ICSBP* transcripts was also found in patients with CML, and this reduction could be reversed by treatment with interferon-α[89]. These data indicate that ICSBP is a tumour suppressor and that downregulation of ICSBP is important for the pathogenesis of CML.

Consistent with the importance of the RAS signalling in promoting growth of myeloid cells, JUNB, an antagonist of the RAS downstream target JUN and negative regulator of cell proliferation and survival, was shown to act as a tumour suppressor in myeloid cells. *Junb*-null mice have severe vascular defects in the placenta, leading to early embryonic lethality[90]. However, inactivation of JUNB specifically in haematopoietic cells led to the development of a CML-like disease in mice, beginning at 4 months of age[91,92]. About 16% of mice further developed a syndrome that resembled blast crisis. More recent studies showed that JUNB inactivation in the long-term self-renewing haematopoietic stem cells of mice, but not in committed myeloid progenitor cells, could induce a CML-like myeloproliferative disorder. Furthermore, only JUNB-deficient long-term self-renewing haematopoietic stem cells from the diseased mice could induce CML-like disease in recipient mice after transplantation[92]. These results support the hypothesis that CML originates from haematopoietic stem cells. Downregulation of JUNB has also been observed in CML cells isolated from patients[93,94]. These results indicate that inactivation of JUNB could be important for CML development.

There is further support for the importance of RAS downstream signalling pathways in myeloid proliferation and survival. Targeted inactivation of SIPA1 (signal-induced proliferation-associated gene-1) — a principal RAP1 GTPase-activating protein in haematopoietic progenitors — led to a spectrum of myeloid disorders that resembled chronic-phase CML, blast-phase CML and

myelodysplastic syndrome by 1 year after birth in mice[95]. In pre-leukaemic SIPA1-deficient mice, there is a selective expansion of pluripotential haematopoietic progenitors. RAP1 is a close member of RAS-family GTPases and, like RAS, can activate the mitogen-activated kinase/ERK kinase (MEK)–ERK signalling pathway through activation of BRAF[96]. It is not clear whether expression or activity of SIPA1 is altered in CML cells, but it was shown that expression of BCR–ABL in a growth-factor-dependent haematopoietic cell line activated RAP1 and BRAF[97]. These results indicate that in addition to the well-established RAS signalling pathway, BCR–ABL might activate MEK–ERK signalling through a pathway involving RAP1 and BRAF (FIG. 3).

In mice, disruption of the genes encoding SH2-containing inositol-5-phosphatase (SHIP) also leads to a massive expansion of myeloid cells in the lung, bone marrow and spleen[98]. About half of these mice die at 14 weeks of age. Expression of BCR–ABL in growth-factor-dependent haematopoietic cells downregulates SHIP, so inactivation of SHIP somehow contributes to BCR–ABL leukaemogenesis[99]. However, in CML cells isolated from patients, SHIP expression seems to be differently altered in the early and late stages of differentiation[100]. In addition, SHIP-null primitive haematopoietic cells showed modestly reduced growth-factor independence. These results indicate that SHIP could have both positive and negative roles in haematopoiesis, depending on the cell context.

Residual disease in CML patients on imatinib

If the ABL tyrosine-kinase activity is essential for BCR–ABL-mediated leukaemogenesis, then why are all the leukaemic cells in patients with CML not eradicated by imatinib treatment? Can studies using mouse models of BCR–ABL-mediated leukaemogenesis provide useful information about the mechanisms of this residual disease?

In examining the role of the ABL kinase activity in mouse BMT models, bone-marrow cells that expressed an ABL-kinase-deficient form of BCR–ABL seemed to propagate normally in mice, although they did not become leukaemogenic[29]. Similarly, human $CD34^+$ cells that express the ABL-kinase-deficient form of BCR–ABL respond to cytokines like normal $CD34^+$ cells[22]. These experiments indicate that a kinase-deficient form of BCR–ABL is not toxic to haematopoietic cells. So how does the imatinib, which blocks the kinase activity of ABL, induce apoptosis in CML cells?

Cytokines maintain the homeostasis of normal haematopoietic cells, by regulating their proliferation, survival and differentiation. As described above, BCR–ABL can promote the survival and proliferation of myeloid cells by activating cytokine-receptor signalling. In addition, BCR–ABL induces overproduction of certain cytokines. These activities are likely to account for the massive expansion of haematopoietic cells that occurs during CML development. As the tyrosine-kinase activity of ABL is essential for most, if not all, of the activities of BCR–ABL in regulating cell survival and proliferation, inhibition of the ABL kinase could lead to inhibition of BCR–ABL signalling and reduced production of cytokines. The return to normal level of cytokine concentrations would not be able to support the excessive amount of haematopoietic cells, so the excess CML cells would die of growth-factor starvation.

Inhibition of the tyrosine-kinase activity of BCR–ABL by imatinib does not simply cause cells to revert to cytokine-dependent growth, however. Imatinib induces apoptosis of patients' leukaemic progenitor cells that are cultured in the presence of cytokines, with little effect on normal haematopoietic cells[4,5]. These results indicate that the cytokine signalling is disrupted in CML cells, and that the survival of CML cells has become BCR–ABL dependent. It is possible that BCR–ABL, in activating intracellular signalling pathways, might suppress the normal cytokine response through an unknown mechanism.

Although *in vitro* colony formation by CML progenitor cells is significantly inhibited by imatinib, not all CML cells are sensitive to the drug[4,5,101] — a subset of CML cells can grow in response to external cytokines independently of the tyrosine-kinase activity of BCR–ABL . Furthermore, $CD34^+$ progenitor cells isolated from nucleated bone-marrow cells of patients with a complete cytogenetic response (assayed on unsorted nucleated bone-marrow cells, in which $CD34^+$ cells are rare) to imatinib therapy tested positive for the presence of *BCR–ABL* when cells were analysed by fluorescence *in situ* hybridization[10]. Quantitative PCR assays have also demonstrated the level of the *BCR–ABL* transcript was higher in the $CD34^+$ cell population, indicating an enrichment of $BCR–ABL^+$ cells in the progenitor fraction. Colony-formation assays showed that $BCR–ABL^+CD34^+$ cells isolated from imatinib-treated patients retain functional committed and primitive progenitor capacity. So CML progenitor cells seem to be refractory to imatinib therapy, compared with more mature cells[10].

The mechanism of resistance of CML progenitor cells to imatinib treatment is not known. It is possible that the intracellular concentration of the drug in these cells is low because of their unique efflux-pump activity. More potent BCR–ABL tyrosine-kinase inhibitors, or combinations of BCR–ABL kinase inhibitors, might overcome this problem. It is also possible that residual leukaemic cells express mutant forms of BCR–ABL that are resistant to imatinib. It has been shown that imatinib-resistant mutant forms of BCR–ABL exist in some patients even before imatinib treatment[102]. Furthermore, genetic defects other than t(9;22)(q34;q11) might cause resistance of CML cells to imatinib. However, rapid expansion of such mutant cells and relapse of the disease are expected. Therefore, this mechanism might not apply to most imatinib-treated patients with a durable and complete cytogenetic response.

The most worrisome possibility, with regard to minimal residual disease in patients undergoing treatment with imatinib, is that the survival and even proliferation of some CML progenitors is not fully dependent on BCR–ABL. This would mean that such kinase inhibitors might not be able to kill the cancer stem cells, even when they can fully inhibit the kinase activity of BCR–ABL.

There is some evidence to support this idea. BCR–ABL expression promotes cytokine-independent growth of haematopoietic cell lines, such as the murine myeloid cell line 32D and the human megakaryocytic cell line MO7e. Imatinib treatment did not inhibit the proliferation of these cells in the presence of IL-3 (for 32D cells) or GM-CSF (for MO7e cells), although it did induce apoptosis of BCR–ABL-expressing 32D cells and MO7e cells in the absence of cytokines. The proliferative capacity of BCR–ABL expressing 32D, but not MO7e cells, in the presence of imatinib, however, could be rescued by addition of exogenous cytokines[4]. So, imatinib just causes some cells to regain cytokine-dependent proliferation.

What does this mean for treatment of patients? Residual disease in CML patients on imatinib might be maintained, at least partially, by the normal haematopoietic environment. A study of human CML cells showed that imatinib functioned by inhibiting the abnormally increased proliferation rates of progenitor cells, rather than by selective induction of apoptosis[103]. Stem-cell quiescence could contribute to such imatinib resistance. So, haematopoietic cells might differ in terms of developing BCR–ABL dependency. In addition, the tyrosine-kinase-defective form of BCR–ABL retains the ability to dysregulate cell adhesion and migration[22,104]. The effects of these altered cell activities in the maintenance of residual disease in CML patients on imatinib therapy will require further study.

There are other ways in which CML cells might survive and proliferate even in the presence of a BCR–ABL kinase inhibitor such as imatinib. Recent studies have shown that expression of the AML-associated oncogene *MOZ–TIF2*, formed by a fusion of the gene encoding the acetyltransferase MOZ with the gene encoding the nuclear receptor coactivator TIF2, in committed myeloid progenitors such as common myeloid progenitors (FIG. 1) and GMPs (which have no self-renewing capacity) can induce leukaemia in a BMT mouse model[105]. However, expression of BCR–ABL in these same cell types, using the same model system, fails to induce disease. These results indicate that MOZ–TIF2, but not BCR–ABL, can confer properties of leukaemic stem cells to some haematopoietic progenitors. So it is possible that BCR–ABL-mediated leukaemogenesis relies on the self-renewal capacity of the original haematopoietic stem cell, and that the activity of BCR–ABL might be dispensable for the self-renewal capacity of CML stem cells.

Future directions

A range of new approaches and agents are being developed to improve therapy for patients with CML (reviewed in REFS 8,14,106,107). These include combination of targeted and conventional chemotherapeutic agents; new tyrosine-kinase inhibitors and agents that downregulate the BCR–ABL transcripts and/or protein (RNA interference); inhibitors of heat-shock protein 90 (a protein that regulates the stability and function of various oncoproteins including BCR–ABL); and arsenic trioxide, which has proven to be an effective therapy for patients with t(15;17)-associated acute promyelocytic leukaemia. Other strategies include inhibiting the downstream signalling pathways of BCR–ABL (such as the RAS and PI3K pathways), immunotherapy and nuclear entrapment of BCR–ABL.

Certain functions of BCR–ABL could render CML cells resistant to many conventional chemotherapies, such as by promoting cell survival in response to DNA damage (reviewed in REF. 108). Imatinib inhibition of BCR–ABL's kinase activity could re-sensitize these cells to these effects of chemotherapies. However, as some CML progenitors are quiescent (reviewed in REF. 109), it is not clear whether conventional chemotherapies could effectively eradicate all CML cells.

Additional BCR–ABL kinase inhibitors have been developed. Among them, BMS-354825 is a novel dual SRC and ABL kinase inhibitor with more than 100-fold greater potency than imatinib that has been shown to inhibit the kinase activity of 14 out of 15 imatinib-resistant forms of BCR–ABL[110]. A Phase I clinical trial has shown a remarkable safety and efficacy of BMS-354825 in patients with imatinib-resistant chronic-phase CML[111]. Further development of ABL tyrosine-kinase inhibitors will undoubtedly improve CML therapies. But it is not clear whether targeting BCR–ABL alone is sufficient for eradicating leukaemic cells.

Combining a BCR–ABL tyrosine-kinase inhibitor with inhibitors of its downstream signalling pathways could significantly improve CML therapy. Inhibitors that target various stages of the RAS signalling pathway, such as farnesyl transferase, RAF1, MEK1, PI3K, mammalian target of rapamycin (mTOR) and cyclin-dependent kinases (CDKs), have been shown to have synergistic or additive effects in preventing BCR–ABL-mediated transformation (reviewed in REF. 3). Farnesyl-transferase inhibitors, mTOR inhibitors (rapamycin and RAD001) and CDK inhibitors (flavopiridol) have entered clinical trials. Further identification of crucial signalling targets involved in the pathogenesis of CML is needed to achieve this goal. Additionally, allogeneic stem-cell transplantation can cure patients with CML because it elicits a graft-versus-leukaemia effect that eradicates cancer cells. Further elucidation of this mechanism of anti-CML immunity would help to selectively destroy leukaemic cells in patients.

Lastly, it might be possible to harness BCR–ABL activity to turn this oncoprotein into a killer of malignant cells. One such approach is through nuclear entrapment of BCR–ABL[112]. The ABL protein is distributed in both the nucleus and cytoplasm of cells and can shuttle between the two compartments. However, BCR–ABL localizes to the cytoplasm of transformed cells under physiological conditions, even though it contains both nuclear localization and nuclear-exporting signals[113] (FIG. 2). Inhibition of its kinase activity by imatinib rescued the capacity of BCR–ABL to shuttle between the nucleus and cytoplasm[112]. Inhibition of the nuclear export by leptomycin B (LMB) entrapped the kinase-inactive form of BCR–ABL in the nucleus. Reactivation of the BCR–ABL tyrosine kinase by removing imatinib

induced apoptosis of the transformed cells. This combined treatment with imatinib and LMB increased levels of apoptosis, compared with treatment with either drug alone, when tested in BCR–ABL-expressing haematopoietic cells *in vitro*. The nuclear entrapment approach might be a powerful treatment strategy for CML, although the therapeutic application of LMB is limited, owing to neuronal toxicity.

Studies of the molecular mechanisms of CML have led to the development of a powerful targeted therapy for this disease. Although the tyrosine-kinase activity of BCR–ABL is essential for the pathogenesis of CML, other functions of BCR–ABL, along with the biology of haematopoietic stem/progenitor cells and tumour microenvironment, are likely to contribute to the disease development and maintenance. These factors impose obstacles to therapy with imatinib as a single agent, but can also provide additional targets for developing pharmaceutical interventions for eradicating CML cells, particularly the CML stem cells.

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Competing interests statement

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