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Molecularly targeted treatment of chronic myeloid leukemia: beyond the imatinib era

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TABLE OF CONTENTS

1. ABSTRACT

Chronic myeloid leukemia cells contain a BCR-ABL oncoprotein with an enhanced tyrosine kinase activity, which is considered to be the principal 'cause' of the leukemia. Though the precise mechanisms underlying the leukemogenesis remains enigmatic, the use of imatinib to inhibit the dysregulated kinase activity has proved remarkably successful in clinical practice. Imatinib was the first small molecule developed to inhibit BCR-ABL tyrosine kinase activity and its success introduced the current era of molecularly targeted therapies for a number of other malignancies. In patients with chronic myeloid leukaemia who develop resistance to imatinib, the Bcr-Abl signaling pathway is often re-established. This has led to the emergence of a number of alternative treatment strategies designed to target the leukemic cell which are resistant to imatinib.

2. INTRODUCTION

Chronic myeloid leukemia (CML) is a clonal multilineage myeloproliferative disorder, which originates in a single abnormal hematopoietic stem cell (1). The leukemia cells have a consistent cytogenetic abnormality, the Philadelphia (Ph) chromosome, and contain a BCR-ABL fusion gene and its corresponding protein, $p210^{BCR-ABL}$, which is considered to be the principal 'cause' of chronic-phase CML (2). This oncoprotein has enhanced protein tyrosine kinase activity, which is presumed to be responsible for its oncogenic activity. The discovery that this excessive kinase activity could be inhibited in a highly specific manner has proved to be a major landmark in the treatment of patients with CML (3). In the past seven years results of clinical studies using the tyrosine kinase inhibitor, imatinib mesylate (Gleevec in US; Glivec in Europe), as a single-agent treatment for patients with CML

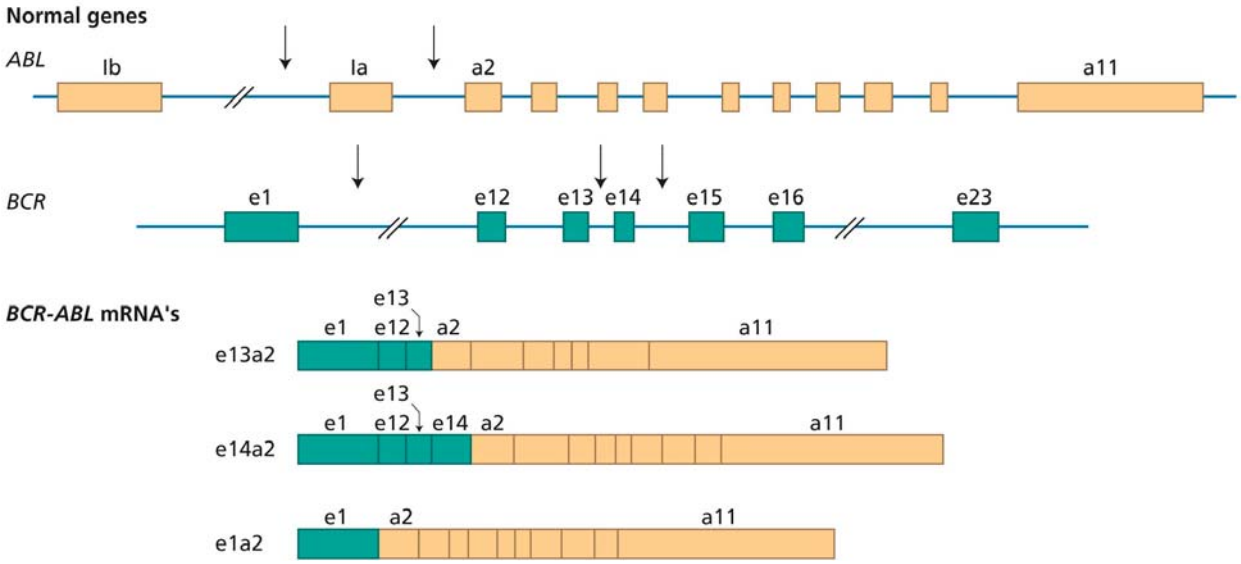

Figure 1. Schematic representation of the various breakpoints in the ABL and BCR genes and the encoded proteins in the BCR-ABL positive leukemias. The genes are shown at top and the RNA transcripts and corresponding proteins below. The arrow show the possible sites on breakage in the ABL gene (above) and the possible sites of the two alternative breaks in M-BCR (below) that are characteristic of CML (namely $p210^{BCR-ABL}$ with an e13a2 junction or $210^{BCR-ABL}$ with an e14a2 junction. Breaks m-bcr and µ-bcr are characteristic of Ph-positive acute lymphoblastic leukemia and Ph-positive chronic neutrophilic leukaemia respectively [published, with permission from Goldman & Mughal, Chronic Myeloid Leukaemia, Ed: Hoffbrand, Tuttenham & Catovsky, Blackwell Science, Oxford, UK (2005)].

have shown that most patients achieve hematological and cytogenetic response. The drug reduces substantially the number of leukemia cells in a patient's body and promises to prolong survival in comparison with older agents (4). Complete molecular responses are, however, quite rare and allogeneic hematopoietic stem cell transplantation (SCT), which is associated with an appreciable risk of morbidity and mortality, remains the only treatment known to result in long-term leukemia-free survival for the majority of patients eligible for the procedure (5). In this paper we review the role of tyrosine kinase inhibition for treating CML and the impact of imatinib and some of the newer treatment modalities on decision-making strategy for the newly diagnosed patient.

3. BCR-ABL TYROSINE KINASE

The ABL and BCR genes, located on chromosomes 9 and 22 respectively, are normal genes whose precise functions are not entirely established. The ABL gene encodes a tyrosine kinase whose activity is normally tightly regulated. Both these genes are transected in the formation of the t(9;22)(q34;q11) reciprocal translocation, which generates the 22q-, or Philadelphia (Ph) chromosome, that characterises CML cells. Thus 3' sequences of the ABL gene are transferred to the derivative 22 chromosome and 3' sequences of the BCR gene are transferred to the derivative 9 chromosome. These events generate two fusion genes: BCR-ABL on the Ph chromosome and the ABL-BCR on chromosome 9q+. The BCR-ABL gene expresses a 'fusion' mRNA and a $p210^{BCR-ABL}$ protein that has a much greater ABL-associated tyrosine kinase activity than its normal counterpart. Depending on the precise position of the breakpoint in the BCR gene, the fusion protein can vary in size from 190 kD to 230kD (Figure 1) (6). Most CML patients have leukemia cells that express the $p210^{BCR-ABL}$. Though the mechanism underlying the enhanced tyrosine kinase activity of this oncoprotein is not fully defined it seems to be due in part to loss of normal autoinhibition (7,8). Studies using animal models demonstrated that insertion of the BCR-ABL gene into murine stem cells induces a CML-like disease and thus provides strong support for the causal link between the BCR-ABL protein and the induction of CML (9). The BCR-ABL protein is found in the leukemia cells of all CML patients; rare patients who have hematologically acceptable CML without a BCR-ABL gene (known as BCR-ABL negative CML) are now classified as 'myeloproliferative syndrome, unclassifiable'.

Much attention has focused on determining how the BCR-ABL oncoprotein actually transforms normal stem cells. Currently three possible mechanisms have been implicated, not necessarily mutually exclusive (10). First, the oncoprotein may constitutively activate mitogenic signaling and 'autonomously' expand the progenitor cell pool. Second, it may reduce the adherence properties of progenitor cells and thereby allow them to escape physiological inhibitory mechanisms in the marrow microenvironment. Third, the oncoprotein may act by opposing apoptosis. Although much is known of the abnormal interactions between the BCR-ABL oncoprotein and other cytoplasmic molecules, it is not yet clear which signal transduction pathways, such as the RAS-MAP kinase, the JAK-STAT and the PI3 kinase pathways, are

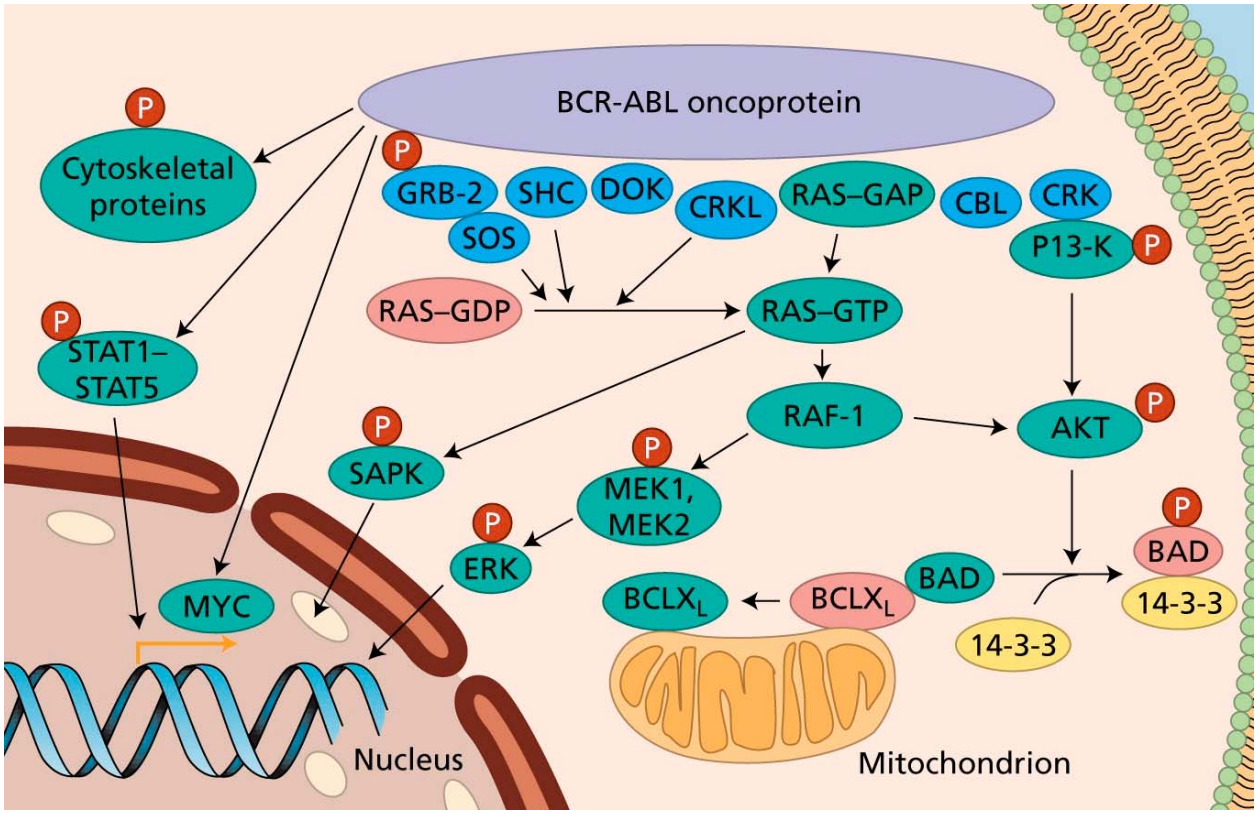

Figure 2. Signal transduction pathways involved in CML. Schematic representation of the cellular effects of BCR-ABL are exerted through the interactions with various proteins that transduce the oncogenic signals responsible for the activation or repression of gene transcription, of mitochondrial processing of apoptotic responses, of cytoskeletal organization and of the degradation of inhibitory proteins. The key pathways implicated so far are those involving RAS, mitogen-activated protein (MAP) kinases, phosphatidylinositol 3-kinase (PI3-K) and MYC. Note the STAT pathway to the left, the RAS/RAF/1-MEK1 pathway centrally and the PI3-K/AKT pathways to the right. Molecules known to be phosphorylated by activated BCR-ABL are marked with the letter 'P'[published, with permission from Goldman & Mughal, Chronic Myeloid Leukaemia, Ed: Hoffbrand, Tuttenham & Catovsky, Blackwell Science, Oxford, UK (2005)].

critically involved in mediating the malignant phenotype (Figure 2).

4. TARGETING BCR-ABL TYROSINE KINASE ACTIVITY

In the early 1990s a series of small molecules were developed that had significant inhibitory activity against the BCR-ABL tyrosine kinase. Initial efforts yielded the tyrphostins (for tyrosine phosphorylation inhibition), AG1112 and AG 568, which induced inhibition of the ABL kinase in a CML blast crisis cell line (11). Another tyrphostin, AG 957, restored the defective β1 integrin-mediated adhesion seen in CML and also exerted an inhibitory effect on Ph positive cells in myeloid colony growth *in vitro* (12). Another compound, herbimycin A, a natural benzoquinoid ansamycin antibiotic, inhibited ABL tyrosine kinase in murine cells transduced with the human BCR-ABL gene and preferentially inhibited the Ph positive cell growth (13). Imatinib mesylate , a molecule based on the 2-phenylaminopyrimidine class of compounds, then called CGP 57148B (later known as STI571, Gleevec or Glivec, Novartis Pharma, Basel, Switzerland) was developed as a result of collaboration between Brian Druker and Novartis (then Ciba-Geigy) (3,14). Imatinib occupies the ATP-binding pocket (P-loop) of the BCR-ABL protein and so prevents access to ATP, thereby preventing phosphorylation of any substrate; imatinib also makes contact with parts of the kinase domain outside the P-loop (Figure 3).

Preclinical studies confirmed that imatinib was highly effective in blocking the tyrosine kinase activity of ABL. It inhibited the proliferation of CML cell lines and clonogenic cells from patients with chronic phase CML, but did not affect normal cells. It also inhibited the enzyme activity of three related tyrosine kinases, the platelet-derived growth factor receptor (PDGFR), the stem-cell factor receptor (c-kit), and ARG (Abl-related gene) (15,16,17), but had little effect on the majority of tyrosine kinases, including other members of the SRC and JAK kinase families, c-FMS, FLT3, vascular endothelial epithelial growth factor receptors, epidermal growth factor receptors and HER-2/neu. The restricted activity of imatinib led to efforts to characterize the structural mechanism for this selective inhibition that targets the ABL

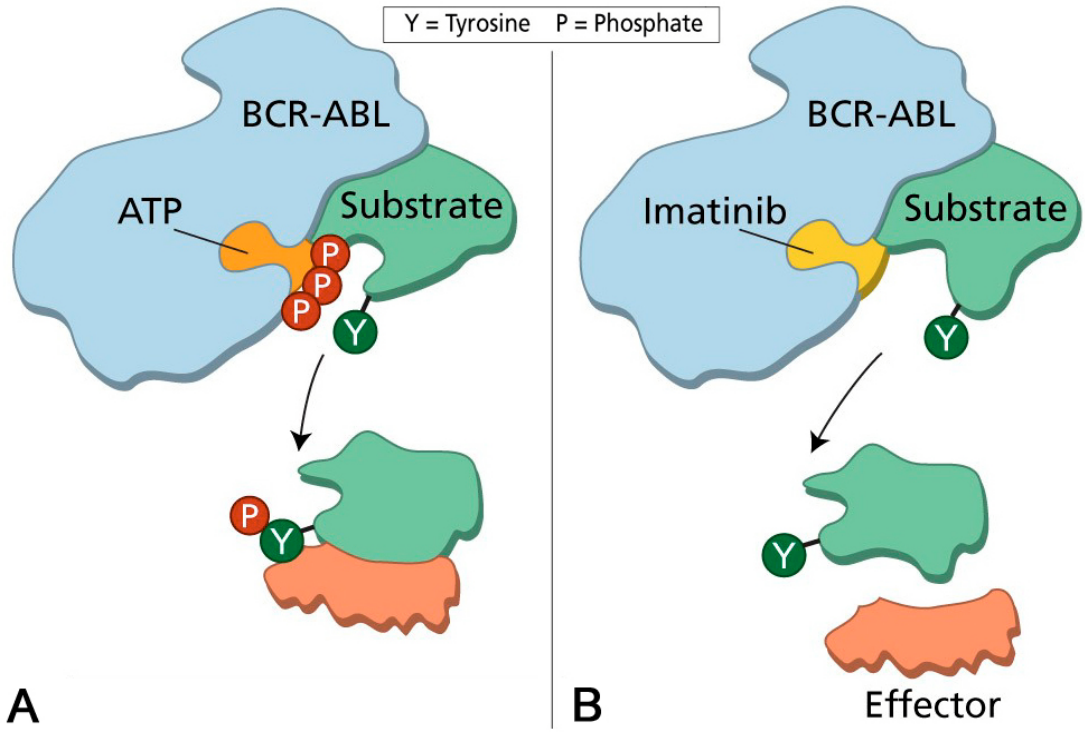

Figure 3. Mode of action of imatinib. The phosphorylation of a substrate is shown schematically. ATP occupies the pocket in the ABL component of BCR-ABL oncoprotein. The substrate then detaches itself from the BCR-ABL oncoprotein and makes functional contact with a further downstream effector molecule. When imatinib occupies the ATP binding site, it prevents phosphorylation of the substrate. This molecule in turn fails to make contact with the effector protein and the signal transduction pathway that would otherwise transmit the 'leukemia signal' is interrupted [published, with permission from Goldman & Mughal, Chronic Myeloid Leukaemia, Ed: Hoffbrand, Tuttenham & Catovsky, Blackwell Science, Oxford, UK (2005)].

kinase but leaves unaffected for example the SRC tyrosine kinase with which ABL shares many physicochemical features. It appears that imatinib achieves its high degree of specificity by targeting and stabilizing the activation loop of ABL protein, thereby maintaining the enzyme in its inactive state. The conformation of this loop is distinct amongst the various tyrosine kinases and may explain the high affinity and high specificity of imatinib for the ABL kinase (18).

Imatinib has now been tested in a variety of malignancies, both hematological and solid tumors, in which the tyrosine kinases may play a significant pathogenetic role. Impressive results have been noted in patients with gastrointestinal stromal tumours with a mutated c-kit gene, in patients with a rare form of chronic myeloid leukemia characterised by t(5;12) translocations involving the ETV6-PDGFRB fusion gene and in a subset of patients with eosinophilic leukemia characterized by a FIP1L1-PDGFRA fusion gene (19,20).

4.1. Current clinical results of Bcr-Abl tyrosine kinase inhibition

Imatinib was first used to treat patients with CML in the chronic phase as well as those in the more advanced phases of the disease in 1998 (21). Later patients with Ph positive acute lymphoblastic leukemia (ALL) were also included in the early studies. The drug caused a rapid reversal of the clinical and hematological abnormalities. Adverse effects were relatively rare, but nausea, myalgia, edema, diarrhea and rashes did occur (22). Fluid retention was observed in some patients. Some patients developed laboratory evidence of hepatotoxicity which was generally reversible on dose reduction. As the follow-up period for patients on imatinib increases, further side effects are becoming apparent. For example, possible infertility of men, heralded by the emergence of gynecomastia and low testosterone has been observed (23). Serious adverse events, such as potentially fatal cerebral edema and bone marrow necrosis, have been reported (24,25). Very little information is currently available on the safety of imatinib in pregnancy and it probably should be avoided in pregnant patients until more data is acquired (26). There have also been speculation of a potential increase in the frequency of genitourinary tumors in murine models, but not in humans studied so far (27). Nonetheless, further careful observations are required.

Preliminary clinical results of comparing imatinib with the combination of interferon alfa and cytarabine in a prospective randomized study (the IRIS study) have now been reported (4). Newly diagnosed chronic phase patients were randomly allocated to receive imatinib alone (400 mg/daily) or interferon-alfa plus cytarabine; with a follow-

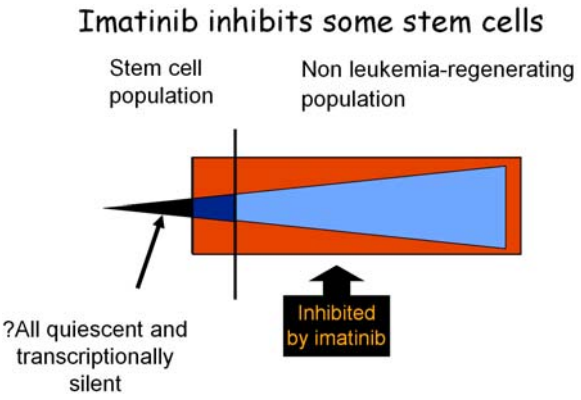

Figure 4. Imatinib and quiescent stem cells. A schematic model suggesting how some CML stem cells are not eradicated by imatinib and are capable of re-establishing the disease.

up of 19 months the projected incidence of complete cytogenetic response (CCyR) was 76.2% versus 14.5% ($p< 0.001$) respectively in these two groups (28). Cytogenetic and molecular monitoring of patients who received imatinib as primary therapy showed that those who achieved 3 log or greater reduction in BCR-ABL/ABL ratio at 18 months had a 100% progression-free survival (PFS), a result significantly superior to PFS for those who achieved lesser degrees of transcript reduction. In patients who maintained their CCyR, there was further reduction in transcript numbers during the period of follow-up (median 18 months); in 12 (4%) of 333 evaluable patients, transcript numbers were undetectable on at least one occasion (29). These findings suggest that the level to which BCR-ABL transcript numbers are reduced may well predict the duration of survival.

A smaller study from the Houston group reported the results of treating patients at diagnosis with a higher dose of imatinib, namely 800 mg/day (30). This dose is tolerated somewhat less well than 400 mg, but transcript numbers fell substantially more rapidly than in control patients treated with 400 mg/day. The incidence of PCR negativity was also substantially higher. The results suggest that the optimal dose for treating CML with imatinib as a single agent may be 800 mg/day, a suggestion now being tested in prospective studies.

The precise mechanism underlying the persistence of minimal residual disease (MRD) in the majority of patients who have achieved a CCyR is unknown (31). Patients with measurable MRD state almost invariably 'relapse' with increasing numbers of BCR-ABL transcripts if for any reason imatinib treatment is interrupted, suggesting that small number of residual leukemia cells are capable of re-establishing the disease (Figure 4). It also explains why some patients relapse directly in blast crisis from a CCyR state (32). The 'complete success' of imatinib treatment would presumably require it to eliminate all leukemia cells. Current *invitro* studies, however, suggest that the residual leukemia cells (some of which may be 'quiescent' or transcriptionally silent stem cells) are insensitive to imatinib, even at doses up to 10 times the recommended therapeutic dose (33).

An interesting development is the observation of cytogenetically abnormal clones in Ph chromosome negative cells in the marrow of patients with Ph-positive disease who appear to be responding well to imatinib (34). Some patients have an associated myelodysplastic marrow picture, but thus far the survival of patients with clonal changes in Ph-negative cells seems not to be different from comparable patients without additional cytogenetic abnormalities. These Ph-negative clones were most prominent in patients who have received prior therapy, but they have been observed also in patients whose initial treatment was imatinib. The commonest abnormality was trisomy 8, but changes in chromosomes 5, 7 and others were also observed. In one study, polymerase chain amplification of the human androgen receptor (HUMARA) was used to study X-chromosome inactivation as a marker of clonality in patients on imatinib despite the presence of some Ph-negative clones; the workers concluded that imatinib restored a polyclonal pattern of hematopoiesis in the majority of responders (35). These observations provide support for the recommendation that patients responding well to imatinib should continue to be followed, perhaps indefinitely, both by serial molecular studies and by regular bone marrow cytogenetics.

4.2. Resistance mechanisms to imatinib

Clinical studies have shown that some BCR-ABL positive cells can become resistant to the inhibitory effects of imatinib. Acquired resistance has been seen especially in patients with CML in blast crisis where up to 70% of those in myeloid blast crisis and all of those in lymphoid blast crisis relapse within 6 months of responding to imatinib (36).

By using an antibody-based assay to measure the phosphorylation of BCR-ABL or substrates, some insights into the possible reasons for this resistance to imatinib have been established. These can conveniently be considered as BCR-ABL *independent* or BCR-ABL *dependent*. BCR-ABL independent resistance may arise if a CML cell acquires additional molecular changes that cannot be targeted by imatinib (37). Thus far, there is little known about such events. Conversely BCR-ABL dependent resistance may be due to changes that specifically involve the BCR-ABL oncoprotein. Such changes include amplification of the BCR-ABL fusion gene with associated overexpression of the protein or overexpression of the MDR-1 gene and P-glycoprotein which could lead to excessive expulsion of the inhibitor from the cell. Some plasma proteins, such as α-1 acid glycoprotein, or enzymes, such as P450 enzyme, may neutralize imatinib and render it ineffective (38,39).

The kinase domain of the Bcr-Abl oncoprotein is identical to the kinase domain of the normal Abl protein. It can be divided into four component parts, an ATP (phosphate) -binding pocket or P-loop, an 'intervening' sequence, a catalytic domain and an activating loop component (40-42). Point mutations in each of these

domains were identified initially in patients with CML in advanced phases and thereafter in patients treated with imatinib for chronic phase disease. Mutations which result in structural changes which prevent imatinib binding, but do not prevent pathological phosphorylation of the relevant substrates by the oncoprotein, tend to be multiple and confer polyclonal resistance to imatinib (43). Such mutations likely reflect selection by imatinib of mutations already present at low level before initiation of treatment rather than *de novo* acquisition during imatinib therapy (44-47). Mutations can also be detected in CD34+ cells from patients with CML in chronic phase who are in CCyR on imatinib and might be a potential source for a subsequent relapse (48). At present 53 different mutations have been identified in association with acquired resistance to imatinib (Sawyers CL, *personal communication*). Thus even when multiple additional genetic events predominate in the advanced stages of CML, the original molecular event still plays some role in maintaining the aggressively transformed phenotype. This highlights the importance of BCR-ABL in the pathogenesis of chronic phase disease and also in the subsequent evolution of CML.

Bcr-Abl kinase domain mutations may be detected by direct sequencing of a PCR-amplified fragment or, with a higher degree of sensitivity, by sequencing of multiple clones of bacteria transfected with the product of this PCR amplification. Both techniques, in particular the latter, are however somewhat laborious and an alternative that may be more suitable for routine screening of patients is the use of denaturing high performance liquid chromatography based on heteroduplex formation by PCR products amplified from wild type and mutant alleles. It appears that the precise position of the substitution within the kinase domain reflects the degree of resistance to imatinib. For example, the methionine to threonine substitution at position 351 (M351T) was associated with a moderate degree of drug resistance which could to some extent be overcome by increasing drug dosage (49,50). In contrast, a threonine to isoleucine substitution at position 315 (T315I) or a glutamic acid to lysine substitution at position 255 (E255K) was associated with resistance to imatinib that did not respond to higher drug doses (51).

Branford and colleagues have shown that patients treated with imatinib for CP disease who have Abl kinase domain mutations have overall survivals significantly inferior to those of patients without mutations (52). If those with mutations are then subdivided according to whether or not the mutation is in the P-loop, those with P-loop mutations fare significantly worse that those with mutations elsewhere in the kinase domain. The same group showed that small rises in BCR-ABL transcripts levels (eg just a 2-fold increase in BCR-ABL/BCR levels) in patients in CCyR on imatinib may be associated with a high incidence of kinase mutations. The mechanism underlying this association has not yet been clarified, but to elucidate further the pathogenetic significance of such mutations the Los Angeles groups studied their incidence in CD34+ cells isolated from 13 patients in CCyR on imatinib (53). In 5 of the 13 patients they identified point mutations resulting in amino acid substitutions in the BCR-ABL kinase domain. Three of the mutations occurred at sites that have been previously identified as associated with imatinib resistance in an *in vitro* mutational screen. Two of these 5 patients subsequently relapsed, whereas no relapses were seen in the patients in whom mutations were not detected. The investigators introduced these mutations into wild-type *BCR-ABL* genes by site-directed mutagenesis and expressed the oncoprotein in TF-1 cells; varying degrees of resistance to imatinib were seen in these transfected cell lines. They speculated that the occurrence of such mutations in CML patients in CCyR on imatinib may permit small subsets of malignant precursors to persist in some patients and could thus be the cause of subsequent relapse.

4.3. Strategies to overcome the Bcr-Abl resistance

It is noteworthy that in CML cells which develop resistance to imatinib, the reactivation of the Bcr-abl signaling is a principal finding. This has led to many efforts focused on the re-inhibition of the Abl-kinase (54).

Attempts of dose escalation of imatinib have been shown to overcome clinical resistance in some patients, but not all, and appear to depend on the specific type of mutation (55,56). For example dose escalation is successful in mutations such as H386P, in contrast to T315I or E255K mutations (as discussed above). A variety of drug combinations with confirmed activity in CML, and in particular those which demonstrate synergism with imatinib, such as cytarabine, interferon alpha, homoharringtonine and decitabine, are currently being assessed (57).

Efforts in developing alternative inhibitors of Abl kinase activity have met a qualified success. These agents are often multi-kinases, in contrast to imatinib, being active against SRC and ABL kinases (58). The class of pyridopyrimidines, such as PD 173955, have shown a remarkable degree of activity in both imatinib-naïve and imatinib resistant mutants of Bcr-Abl, but currently appear to have an unfavorable clinical profile (59). Another family of compounds, the trisubstituted purines, in particular the compound dasatinib (BMS 354825; Bristol Myers Squibb Pharma), have demonstrated excellent responses in both in-vivo and in-vitro studies (60). A phase I study of dasatinib is currently in progress and the initial results appear promising. This agent appears to be effective in patients with a number of different ABL kinase domain mutations, but is not effective against the T315I mutation (61,62). Similarly, the new inhibitor AMN107 (Novartis Pharma), which is an improved version of imatinib, may prove to be effective in imatinib-resistant patients with CML. It has been shown to induce apoptosis in some cases of imatinib-resistant CML cells. Another agent, 17-allylaminogeldanamycin (17-AAG), which can lead to BCR-ABL protein degradation by inhibiting the heat shock protein 90 (hsp90), a molecular chaperone required for stabilization of Bcr-Abl, has just entered phase I studies (63). 17-AAG appears to have activity in patients with the E255K and T315I mutations. It also down-regulates Bcr-Abl mRNA, though the precise mechanisms remain unclear. Another novel tyrosine kinase inhibitor, PD166326

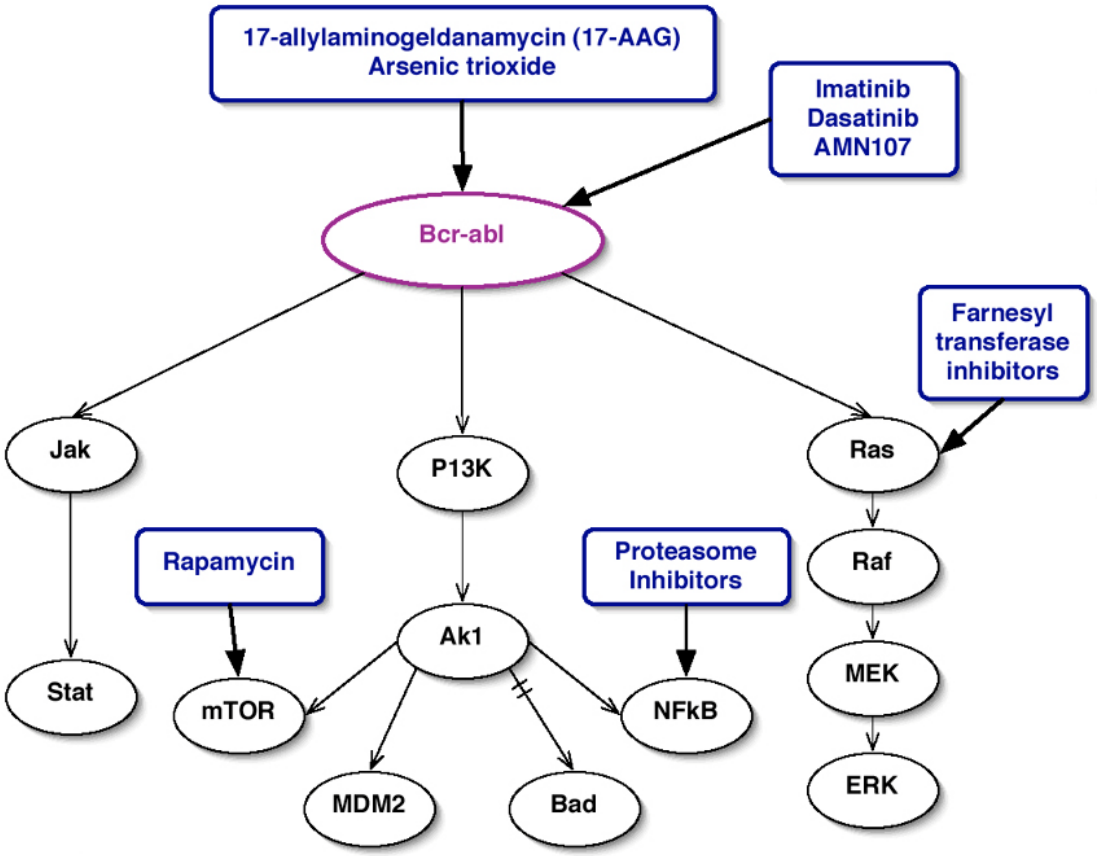

Figure 5. Signal tranduction pathways inhibited by imatinib and some of the other novel compounds currently in clinical trials.

also appears to have significant activity in patients with the H396P and M351T mutants (64). This agent also appears to be superior to imatinib in murine models. Arsenic trioxide, an agent known to down-regulate Bcr-Abl has also entered clinical trials recently (65). Figure 5 depicts the signal transduction pathways affected by some of these compounds.

Numerous studies have also tested specific inhibitors of signal transduction pathways downstream of Bcr-Abl alone and in combination with imatinib (66). Current evidence suggests that though several compounds appear to have some activity in overcoming STI571 resistance, such as farnesyl transferase inhibitors, for example SCH66336, and the proteasome inhibitor bortezomib, the merits of using them in combination with imatinib is not clear (67).

Very recently, a small molecule which is a non-ATP competitive inhibitor of BCR-ABL, ONO12380 (Onconova Therapeutics), has been shown to be active in all currently known imatinib-resistant murine CML cells, including T315I (68). This unique compound does not compete with ATP to inhibit BCR-ABL, but rather blocks substrate binding and acts synergistically with imatinib in wild-type BCR-ABL inhibition. In murine models, it appears to have very little toxicity at doses which appear to be maximally effective; importantly no myelosuppression has been noted so far. These observations should lead to efforts to target sites outside the ATP-binding domain in imatinib-resistant CML. ONO12380 is now in clinical trials.

4.4. Vaccines

Following the observation in 1992 of a unique aminoacid sequence of p210 at the fusion point that is immunogenic, many efforts were directed to explore the potential of developing an active specific immunotherapy strategy for patients with CML by inducing an immune response to this tumor-specific antigen (69). Workers have shown that peptides derived from the b3a2 junction avidly bind to four HLA class I or one HLA class II alleles and thereafter generate peptide-specific CD8 and CD4 T cells (70). Peptide vaccines derived from the b3a2 sequence were then investigated and the results for the most part have been rather mixed (71). Following the recent realization that a molecular remission and possible cure might not be possible with imatinib alone, efforts to explore the use of specific immunotherapy such as vaccine have been renewed. The fundamentals involve the generation of an immune response to the unique aminoacid sequence of p210 at the fusion point. Very recently an Italian study reported clinical responses to the BCR-ABL peptide vaccination, with 7 of 15 patients achieving a complete cytogenetic response (72). Importantly these investigators, in contrast to a number of previous studies, administered

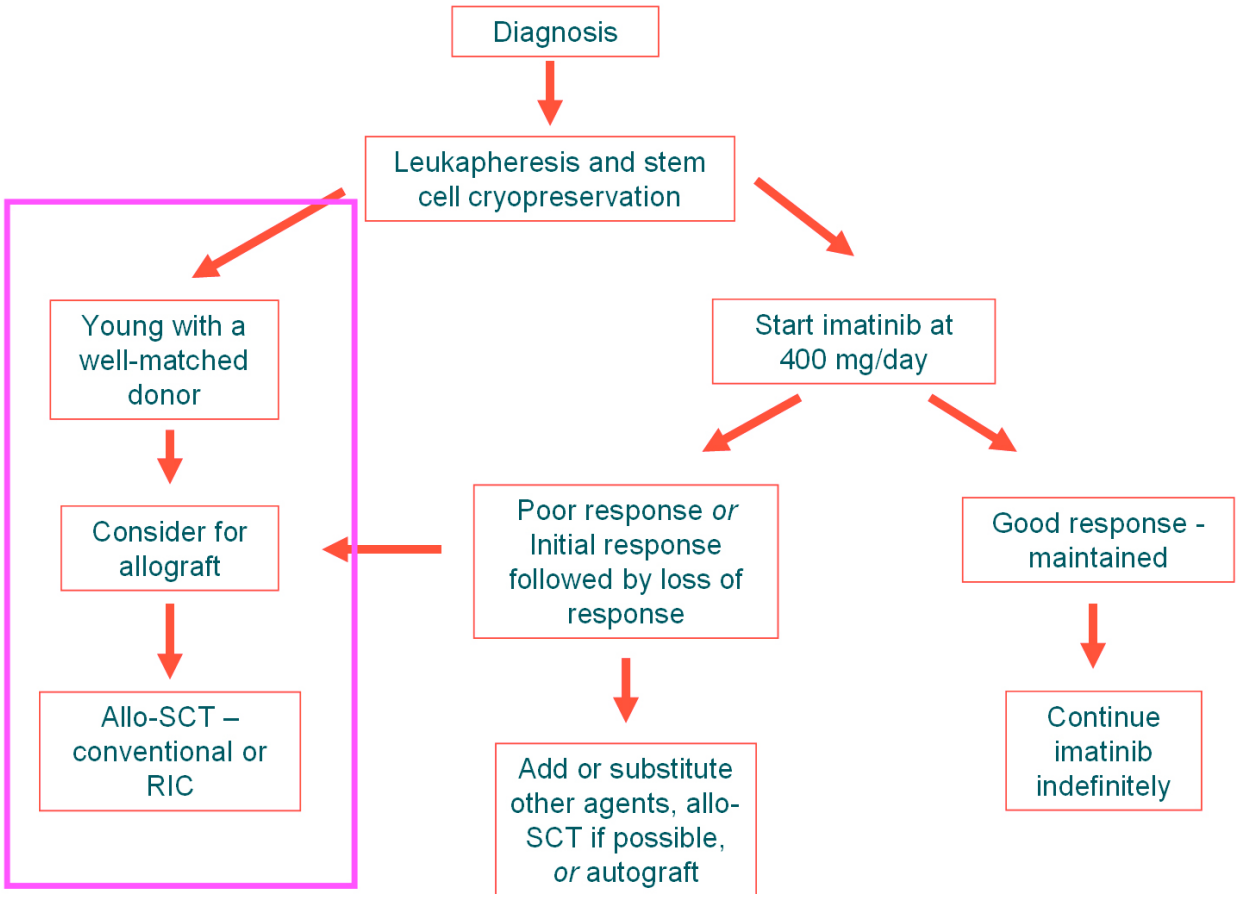

Figure 6. A suggested therapeutic algorithm for the management of a patient with CML in 2005. Algorithm shows a possible approach to the management of a patient with newly diagnosed CML in chronic phase. The great majority of newly diagnosed patients should probably be treated first with imatinib Those who respond well should continue on the drug indefinitely. Patients who respond less well or who lose their response should be considered for other therapy. A minority of young patients with suitable donors may be eligible for an intial treatment by allografting.

GM-CSF as an immune adjuvant. Furthermore the small study enrolled patients with HLA alleles known to bind avidly to the fusion peptides and patients had measurable residual disease only. This study may well prove to be a significant landmark and further efforts are now being directed. If these results can be confirmed, vaccine development against BCR-ABL and other CML-specific antigens could become an attractive treatment following having achieved a minimal residual disease status with imatinib. Other targets for vaccine therapy now understudy include peptides derived from the Wilms tumor-1 protein and proteinase-3, both of which are overexpressed in CML cells (73,74).

Another vaccine strategy which may prove useful for patients who do not achieve a CCyR to imatinib is a heat shock protein 70 (hsp70) peptide complex. Heat shock proteins are anti-apoptotic proteins which appear to play a major role in the immune response to CML and a number of other malignancies. They do this by inducing maturation of dendritic cells, and by induction of innate immune response including natural killer cell activation and cytokine secretion and activation of CD8+ and CD4+ lymphocytes (75,76). In CML cells, Bcr-Abl expression is accompanied by an up-regulation of hsp70, leading to induction and activation of 5 (STAT5) and Bcl-x_l. This in turn leads to the activation of several apoptosis signaling pathways, including caspase-9 and caspase-3. Hsp70 inhibits apoptosis both upstream and downstream and recent observations suggest that attenuated levels of hsp70 might be a useful target for reversing BCR-ABL mediated resistance. A current study is exploring the use of autologous hsp70-peptide complexes to immunize patients who appear to be either resistant to imatinib or fail to achieve a CCyR. Preliminary results following the recruitment of 11 patients was encouraging with 2 of 5 patients who had completed the study achieving a complete molecular remission (77).

5. CONCLUSIONS AND FUTURE DIRECTIONS

Despite the impressive levels of complete cytogenetic remission induced in patients with CML by the tyrosine kinase inhibitor, imatinib, it is now clear that most patients will not achieve a durable molecular remission and will presumably not be cured. One possible approach to disease

eradication would therefore be to integrate imatinib therapy with an allogeneic stem cell transplant, the only known therapy known to cure this leukaemia (78). We propose an algorithm (Figure 6) whereby most patients should receive imatinib therapy soon after diagnosis and an allograft is offered to the cohort not achieving a suitable response on imatinib; it is conceivable that a small group of patients with high-risk (for example by Euro staging system) disease and a desire to proceed with an allograft can be identified, who can be considered for a transplant as early as possible.

Imatinib has unequivocally established the principle that molecularly targeted treatment can work and the field is now open with a host of small, relatively nontoxic agents entering the laboratory at an unprecedented rate. The usefulness of imatinib should now be addressed in other malignancies where, unlike CML in which there seems to be a single molecular target, the number of possible molecular targets seems to be large. So far it has been found to be useful in gastrointestinal stromal tumors where it targets mutated c-kit and in a variety of non-Ph-positive myeloproliferative disorders where it targets the platelet derived growth factor receptor (PDGFR). These facts have provided important insights of the value of imatinib in blocking other critical signaling events. Understanding these events and the molecular pathogenesis of resistance to imatinib have paved the way for a number of other small molecule compounds which are now in clinical trials. Compounds such as dasatinib have already been shown to have significant activity in selected patients who are resistant to imatinib.

Finally the notion that graft-versus-leukemia effect is the principal reason for success in patients with CML subjected to an allograft has renewed interest in immunotherapy and it possible that combinations of kinase inhibitors and immunotherapies will be tested in the future (79).

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