REVIEW PAPER

Volume-6 | Issue-10 | October-2017 | ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

WORKING WITH CML: IMATINIB TREATMENT AND DISEASE EVALUATION.

Oncology

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KEYWORDS

Introduction

Imatinib (also known as "Gleevec" or "Glivec"), a tyrosine kinase inhibitor, was called as "magical bullet," when it revolutionized the treatment of CML in 2001. Imatinib was invented in the late 1990s by biochemist Nicholas Lyndon then working for Ciba-Geigy (now Novartis), and its use to treat CML was driven by Brian Druker, an oncologist at the Dana-Farber Institute. The first clinical trial of Imatinib took place in 1998 and the drug received FDA approval in May 2001. Lyndon, Druker, and the other colleagues were awarded the Lasker-DeBakey Clinical Medical Research Award in 2009 for "converting a fatal cancer into a manageable condition" and the Japan Prize in 2012 for their part in "the development of a new therapeutic drug targeting cancer-specific molecules."

The ultimate goal of treatment for CML is prevention of blast crisis, because, once this occurs, the prognosis is dismal. Although a rapid reduction of the white cell count can be achieved with chemotherapy (i.e., busulfan or hydroxyurea) in CP CML, these drugs usually fail to prevent disease progression. IFN-α was the first agent proven capable of modifying the biological history of CML by prolonging survival in patients who achieved complete hematologic response (CHR) and major cytogenetic response (MCR): <35% of Ph+ cells [01]. A second breakthrough in the treatment of CML occurred when 60–80% of patients undergoing allo-BMT in CP were reported to be disease free at 5 years [02,03,04]. However, both IFN-α and allo-BMT have considerable treatment-induced toxicity that attenuated the initial enthusiasm for these results, and novel, less toxic therapeutic strategies are being explored. In 2001, Druker et al. [05] reported the first Phase I study with imatinib mesylate (STI571, Gleevec), a specific inhibitor of BCR/ABL oncogenic activity. In this study, the authors demonstrated that high rates of CHR and MCR were achieved with relatively few side effects in patients with CML refractory or intolerant to IFN-α, opening new avenues for molecularly targeted therapies in this disease. Subsequently, encouraging results were also obtained for CML patients in BP.

1. Mechanism of Imatinib action

BCR/ABL is an ideal target for molecular targeted therapy, because this fusion protein is present in all of the CML cells, is absent from non-malignant cells, and is necessary and sufficient to induce leukemia. Imatinib mesylate is a 2-phenylaminopyrimidine tyrosine kinase inhibitor (Fig.01) with specific activity for ABL, platelet derived growth factor receptor, c-kit, and Albeson-related gene [06]. The pharmacological basis of this interaction has been elucidated by crystallographic studies. Imatinib mesylate binds to the amino acids of the BCR/ABL tyrosine kinase ATP binding site and stabilizes the inactive, non-ATP-binding form of BCR/ABL, thereby preventing tyrosine autophosphorylation and, in turn, phosphorylation of its substrates (Fig.02). This process ultimately results in "switching-off" the downstream signaling pathways that promote leukemogenesis. Preclinical in vitro and in vivo data indicated an impressive selective activity of imatinib mesylate on cells expressing BCR/ABL, and supported a rapid transition of this compound from the bench to the clinic.

2. Imatinib response in CML

Being an inhibitor of BCR-ABL, the advent of Imatinib rapidly and dramatically modified the treatment of CML and led to important changes in management [07]. The initial landmark studies by Druker et al. showed high response rates to Imatinib in patients with advanced CML [08] and those pretreated with IFN-α [08,09]. The IRIS study, a landmark study in CML, by O'Brien et al. compared Imatinib and the combination of interferon-alpha (INF-α) with cytarabine in a randomized trial in 1106 CP-CML patients [10]. Imatinib induced complete hematologic response (CHR) in 95.3% patients and complete cytogenetic response (CCR) in 73.8% patients. In addition, patients on Imatinib had a better quality of life [11]. On the basis of these results, Imatinib received FDA approval in 2001. At 6-year follow up of IRIS trial, Imatinib induced CHR in 98% of patients in chronic phase and CCR in 87% patients [12].

At molecular level, the goal of therapy with Imatinib is achievement of major molecular response (MMR). Obtaining MMR was associated with significantly better long term remission duration and progression-free survival (PFS). At 60-month follow-up, achievement of CCR and MMR by 12 months was associated with a PFS of 97% compared to 89% for patients with CCR but with less than MMR. Early molecular response predicted better outcome: progression of disease correlated with failure to achieve a 1-log reduction in transcript level by 3 months and a 2-log reduction by 6 months [12].

3. Imatinib dosage

A dose of 300 mg orally (PO) daily was sufficient to achieve a CHR in almost all chronic phase CML patients, but standard dose which is 400 mg daily is well explored and appears to be easily tolerated. Doses lower than 400 mg are discouraged and cautioned for the possible development of resistance [13]. The superiority of 400 mg imatinib PO daily over recombinant interferon-α and low-dose cytarabine was confirmed in a prospective randomized international study (International Randomized Study of Interferon and STI571 [IRIS]) [10]. In this phase III clinical trial, 1106 patients were randomized to imatinib at a dose of 400 mg daily or interferon alpha plus cytarabine. At a follow up around 60 months, imatinib was superior to recombinant interferon alpha for efficacy, with a complete hematologic response rate of 95% versus 55%, a complete cytogenetic response rate of 76% versus 15% and progression-free survival (PFS; survival free from progression to AP/BC) at 19 months of 97% versus 91% ($p < 0.001$).

Patients in accelerated phases of CML who were treated with imatinib at 400 or 600 mg/d were evaluated for hematologic and cytogenetic response, time to progression, survival and toxicity. In comparison with 400 mg, imatinib doses of 600 mg/d led to more cytogenetic responses (28% compared to 16%), longer duration of response (79% compared with 57% at 12 months), time to disease progression (67% compared with 44% at 12 months), and overall survival (OS: 78% compared with 65% at 12 months), with no clinically relevant increase in toxicity [12,14]. Thus, a daily dose of 600 mg was likely to be more effective than 400 mg for AP/BC patients.

Since at higher concentrations imatinib may inhibit more effectively BCR-ABL and some mutants, studies were initiated to test higher doses also in chronic phase. In patients with both prior hematologic and cytogenetic resistance to 400 mg of imatinib daily, increasing the dose to 800 mg resulted in a complete hematologic remission in 65% of patients and a complete cytogenetic response in 18% of treated patients [14]. Compared with standard-dose imatinib, 800 mg daily dose of imatinib was associated with significantly better complete cytogenetic response ($p = 0.0005$), major molecular response ($p = 0.00001$) and complete molecular response ($p = 0.001$) at the cost of frequent myelosuppression [15].

4. Adverse effects of Imatinib

Adverse events with imatinib are generally mild, with rare grade 3 or 4 toxicities. Myelosuppression (neutropenia, thrombocytopenia, anemia) is the most frequent adverse event (16.7% in the IRIS study). These are manageable with supportive therapy with growth factors and blood transfusions without the need for interruption of imatinib. Mild elevation in liver enzymes (5.3%) and peripheral edema (4.5%) were other notable side effects. Decreasing fluid intake or diuretics are helpful for managing fluid retention. Other side effects may include gastrointestinal intolerance, rash, myalgia, arthralgia and drug interactions due to inhibition of the P450 pathway. In smaller series, changes in bone and mineral metabolism and congestive heart failure were associated with imatinib treatment. Little information about possible teratogenicity of imatinib in human beings is available; use of contraception during imatinib treatment is recommended.

5. Imatinib resistance in CML

Despite a major clinical advance in the treatment of CML, Imatinib resistance has become a challenging problem. The existence of patients resistant to Imatinib was evident soon after the introduction of the drug into clinical practice. Initial responses were lower in patients with advanced-phase disease and responses tended to be transient in most responders with advanced-phase disease [16,14].

Primary imatinib resistance is defined as an inability to achieve CHR at 3 months and MCR at 6 months. Primary resistance may be caused by differential drug metabolism and/or drug transport. Acquired or secondary resistance is defined as progression to advanced disease or loss of response with a 5–10-fold increase in BCR-ABL transcripts. Acquired resistance may be caused by mutations in the BCR-ABL kinase domain, amplification of the BCR-ABL fusion gene, over-expression of drug transporter genes, and over-expression of tyrosine kinases such as the SRC family kinases [17,18]. Second-line treatment options include higher doses of Imatinib, a second-generation tyrosine kinase inhibitor (TKI), or allogeneic stem cell transplant (allo-SCT) [19,20]. In other words, the phenomenon of Imatinib resistance broadly manifests via two types of mechanisms: (1) BCR-ABL dependent and (2) BCR-ABL independent.

5.1 BCR-ABL1 dependent mechanisms

BCR-ABL1 dependent pathways include kinase domain (KD) mutations and BCR-ABL1 amplification (genomic or transcriptional, with the former being more common), which are seen in 40-90% [21] and ~10% of cases of resistance, respectively.

5.1a Kinase domain mutations

This is the most common cause of resistance, and is evident almost exclusively in secondary resistance. Akin to antibiotic resistance that develops in bacteria, these mutations are not induced by imatinib, but rather are selected for by the drug. Intriguingly, it has been proposed that via BCR-ABL1 induction of reactive oxygen species, the oncoprotein attempts to escape inhibition by self mutation. This also suggests a possible role for the addition of antioxidants to therapy. More than 50 different mutations have been described; however, there are 7 that currently account for ~85% of all mutations. Mutations span >700bp in the region encoding the KD, affecting amino acids 240 through 500. Their frequency ranges between 40-90% of resistant patients [21] with this rather large range related to variables such as which patient populations are studied, what methods are used to detect these mutations, the definition of resistance and the phase of disease. Typically, mutations may be seen in ~10%, ~30%, ~60% and ~90% in early chronic phase, late chronic phase, accelerated phase and blast crisis, respectively.

Four main point mutation sites in the kinase domain that result in imatinib resistance have been reported: i) the direct binding site, ii) the phosphate binding-loop (P-loop), iii) the activation-loop (A-loop) and iv) the catalytic-loop (C-loop) (Fig.03 & Fig.04) [21].

i) The direct binding site T315 is essential for hydrogen bonding between imatinib and ABL; when the amino acid (aa) at 315 is substituted to Isoleucine from Threonine, hydrogen bonding between Threonine at aa315 and Imatinib is lost (Fig.05). Moreover, the bigger side-chain of Isoleucine (red-dotted circle) becomes an obstacle for the binding of Imatinib to the ATP-binding pocket. Furthermore, the T315I mutation induces a conformational change in several amino acid residues, which are important for the binding of imatinib and BCR-ABL (the allosteric effect). Accordingly, T315I yields imatinib resistance more strongly than other point mutations [21].

ii) P-loop is called the induced fit site because of its conformational change accompanied by imatinib binding [22]. This induced-fit enables imatinib to make a hydrogen bond with tyrosine 253 (Y253) that is intensified by other hydrophobic amino acids surrounding it. Therefore, the point mutations at Y253 including Y253F and Y253H interfere with imatinib binding to Y253. iii) Imatinib can only bind to inactive ABL in which kinase-active site is closed by the activation-loop (A-loop); this loop is involved in ABL specificity and resistance. iv) Mutations within the C-loop such as M351T also induce a conformational change of active ABL, while mutations within the A-loop prompt ABL to form a more active conformation [21].

Among these mutations, Y253H, E255K, T315I, and M315T are observed more frequently. Also, multiple mutations causing imatinib resistance may be detected simultaneously. When multiple mutations are detected, mutations may be present in different BCR-ABL molecules (polyclonal mutation) or in a single BCR-ABL molecule (compound mutation). Compound mutations have been reported to often cause stronger resistance to TKIs [23].

Other than the ATP-binding site, mutation of the SH3-SH2 domain of BCR-ABL (T212R) has been reported to be involved in resistance [24]. Alternatively spliced BCR-ABL mRNA with a 35-bp insertion (35INS) between ABL kinase domain exons 8 was also reported in imatinib-resistant patients which insertion resulted in a frame shift leading to the addition of 10 residues and truncation of 653 residues due to early termination [25]. However, it was also reported that this 35INS insertion/truncation mutant is kinase-inactive and does not contribute to tyrosine kinase inhibitor resistance in CML [26].

Pre-existing mutation: BCR-ABL KD mutations can exist in the newly diagnosed CML-CP patients and may affect the outcome of imatinib treatment. There are limited data available from imatinib-naive patients in CML-CP regarding the incidence of BCR-ABL KD mutations, and the correlation of these mutations with the therapeutic response in unselected patients has not been established [27]. To clarify the meaning of pre-existing mutaions in CML patients, the examination of mutation on CML stem cell may be useful. Previous studies indicated that a small population of CD34+ CML (stem/progenitor) cells are less responsive to imatinib and other TKIs, and act as a reservoir for the emergence of imatinib-resistant subclones [28]. Pre-existing ABL kinase domain mutations in CD34+ cells examined by multiplex allele-specific PCR were detected in 32/100 patients and included F311L, M351T, and T315I and all patients with pre-existing BCR-ABL mutations exhibited imatinib resistance [29].

5.2. BCR-ABL1 independent mechanisms

As part of this mechanism, several cellular and molecular entities have been found contributing towards this sort of resistance. They include Efflux and Influx transporter proteins: e.g. ABCB1/ P-glycoprotein (Pgp), ABCG2/ breast cancer related protein (BCRP) and organic cation transporter-1 (OCT-1); Inhibitor Apoptosis Proteins (IAPs): e.g. Survivin and X-linked inhibitor of apoptosis protein; Transcription factors: e.g. NF-κB and Fox-O series (mainly Fox-O3a factor).

The multidrug resistance (MDR) phenotype related to increased expression of efflux pumps, such as ABCB1/ P-glycoprotein (Pgp) and ABCG2/ breast cancer related protein (BCRP), is one of the most studied mechanisms of resistance in CML. More recently, the decrease in influx transporters, such as the organic cation transporter-1 (Oct-1), has also emerged as a mechanism responsible for inefficient drug uptake and consequent treatment failure [30,31].

5.2a. ABCB1/P-glycoprotein in Imatinib resistance

The most common mechanism developed by tumor cells to escape a drug-induced death is displayed in intrinsic or acquired MDR phenotype by the over-expression of the drug-efflux protein ABCB1 [32,33]. ABCB1, a product of the ABCB1 gene, was first described in 1976 by Juliano and Ling, who observed a cell surface glycoprotein that altered drug permeability in hamster drug-resistant cells. Human cells also express ABCB1 on the cell surface, acting as a drug efflux pump and, consequently, decreasing intracellular drug concentration [34,35].

Studies in polymorphisms of ABCB1 have shown the importance of ABCB1 in CML treatment-resistance. This kind of study may provide information for the prediction of drug disposition in a specific way and promote better response to imatinib in CML patients [36,37]. Dulucq

et al. [38] analyzed 1236C>T, 2677G>T/A, and 3435C>T ABCB1 single nucleotide polymorphisms (SNPs) in CML patients treated with imatinib. The authors observed that allele G in 2677G>T/A polymorphism was associated with the worst response to imatinib. These studies show the importance of researching more about ABCB1 expression, function, and inhibition.

6. Evaluation and treatment monitoring in CML

CML is a malignant clonal disorder of hematopoietic stem cells that results in increases in not only myeloid cells but also erythroid cells and platelets in peripheral blood and marked myeloid hyperplasia in bone marrow. Accordingly, its evaluation for diagnosis as well as prognosis becomes imperative at peripheral blood and bone marrow levels with the help of following tools.

6.1. Complete Blood Count (CBC)

Laboratory diagnosis of CML is often suspected on the basis of the complete blood count (CBC), which shows increased granulocytes of all types, typically including mature myeloid cells. Basophils and eosinophils are almost universally increased; this feature may help differentiate CML from a leukemoid reaction.

In CML, leukocytosis is a common feature of CP, and the white blood cell (WBC) count can be as high as 1000x109/L, leading in rare instances to signs and symptoms of hyperviscosity, such as retinal hemorrhage, priapism, cerebrovascular accidents, tinnitus, confusion, and stupor. The manifestation of leukocytosis is predominantly of myeloid nature. People with CML often have: Decreased hemoglobin concentration; increased white blood cell count, often to very high levels and possible increase or decrease in the number of platelets depending on the severity of the person's CML.

6.2.Peripheral Blood Film Analysis (Morphological characteriz ation)

The PB smear analysis is a qualitative examination of the blood smear to detect clinically significant abnormalities in all cells, including leukocytes, erythrocytes, and platelets [39].

The significance of PB examination in distinguishing between the different hematological pathologies is further emphasized by how it helps in identification of different types of Myeloid Leukemias. WBC count is higher in CML than either CMML (Chronic myelomonocytic leukemia) or atypical CML, including WBCs greater than 200x109/L. The percentage of monocytes is generally greater than 10% in CMML, higher than in either CML or atypical CML. CML shows a prominent basophilia compared with CMML and atypical CML. Large numbers of immature granulocytes are typical of CML, with fewer numbers in atypical CML and even less in CMML. Greater numbers of circulating blasts are seen in atypical CML than in either CMML or chronic-phase CML. Finally, atypical CML has the most granulocytic dysplasia, which is generally absent in CML and may be present or absent in CMML.

Numerous subtle morphological abnormalities have been observed by light microscopy in CML granulocytes, erythrocyte precursors and megakaryocytes. These include hypersegmentation, hyposegm entation, abnormal lobulation and ring-shaped nuclei of the polymorphonuclear leukocytes, Pelger-like leukocytes, binucle- ate myelocytes, multinuclearity and karyorrhexis of the erythro- blasts, and large mononuclear forms, multiple small separated nuclei and microforms of the megakaryocytes [40,41,42,43]. The dysplastic changes occur in the chronic phase of CML more frequently than in normal subjects and become more prominent as the disease evolves into an accelerated or blastic phase; in particular, the appearance of hyposegmented neutrophils and micromegakaryocytes appears to herald blastic transformation [42,44].

Another abnormality occurring in CML is the presence of both eosinophilic and basophilic granules in the same cell [45,46]. Such hybridoid cells with dual granulation were found with varying frequency in all cases of CML examined and occurred in both mature segmented cells and immature non-segmented cells; these bigranulated cells are not found in normal subjects and are thought to demonstrate lineage infidelity in CML.

6.3. Bone Marrow examination

The bone marrow of a normal 70 kg adult contains approximately 1012 hematopoietic cells of which about one-half are granulocyte precursors, one-third to two-fifths are erythroblasts, and the remainder are other cells including megakaryocytes and lymphocytes [47,48,49,50].

Broadly, the bone marrow aspirates in a case of CML at diagnosis typically show hypercellular marrow with marked myeloid hyperplasia and M:E ratio usually more than 10:1.The maturation arrest in the myeloid series is variable with presence of a myelocyte peak and blast percentage varies with the phase of the disease. Megakaryocytes are increased with clustering and presence of dyspoietic changes in the form of nuclear hypolobations and micromegakaryocytes. Basophils are often increased. Eosinophilia and presence of sea blue histiocytes are usual findings. Trephine biopsy may show variable reticulin fibrosis. These features are due in part to the abnormal hematopoietic differentiation mediated by bcr-abl kinase activity as well as production of PDGF by the CML cells [51].

Deposition of connective tissue as detected by reticulin or PAS stains in bone marrow is not noted in most cases. Nevertheless, in some cases, deposition of connective tissue ranging from an increased number and thickness of fibers to multifocal areas of acellular connective tissue deposition reminiscent of idiopathic myelosclerosis. The deposition is typically around vessels and near megakaryocytes. Connective tissue deposition is associated with larger spleen sizes, increased blast percentages in the peripheral blood, decreased hemoglobin levels, and additional karyotypic abnormalities. As a result, it is not surprising that most studies have indicated that reticulin fiber deposition is associated with a worse prognosis, although a small set of patients with marked fibrosis and early stage CML has been reported to have a prolonged course.

6.4 Hematologic monitoring

A complete hematologic response (CHR) is achieved when laboratory values return to normal levels, with a white blood cell count <10,000/mm3, a platelet count <450,000/mm3, the presence of <5% myelocytes plus metamyelocytes, the presence of <5% basophils, the absence of blasts and promyelocytes in peripheral blood, and the absence of extramedullary involvement along with no splenomegaly [10,13]. European LeukemiaNet recommendations state that achievement of CHR within 3 months from the start of therapy is an optimal response [52] (Table 1). Nearly all patients with CML in chronic phase achieve a CHR with TKI therapy.

6.5. Cytogenetic analysis in CML

Chromosome aberrations in neoplasia are classified into primary and secondary abnormalities. Primary abnormalities are strongly nonrandom, correlated with a specific malignancy, may be the only cytogenetic abnormality, and may play a role in the initiation of the malignancy at the early stages. Secondary abnormalities are also nonrandom, but less disease specific, and are postulated to be later events contributing to the process of tumour progression.

In the clinical setting, cytogenetic analysis is an invaluable tool in the diagnosis, prognosis, and management of hematological malignancies. It also aids in the differential diagnosis between solid tumour types with common features.

6.5a. The standard Philadelphia translocation

CML starts when a "swapping" of chromosomal material (DNA) occurs between chromosomes 9 and 22 during cell division (Fig.06). Part of chromosome 9 goes to 22 and part of 22 goes to 9. This is known as a translocation and gives rise to a chromosome 22 that is shorter than normal. This new abnormal chromosome is known as the Philadelphia chromosome and cytogenetic studies reveal that it is present in about 90-95% of CML patients.

The swapping of DNA between the chromosomes leads to the formation of a new gene (an oncogene) called BCR-ABL [53]. This gene then produces the BCR-ABL protein, which is the type of protein called a tyrosine kinase. This protein causes CML cells to grow and reproduce out of control. In a very small number of CML patients, the leukemia cells have the BCR-ABL oncogene but not the Philadelphia chromosome. It is thought that the BCR-ABL gene must form in a different way in these people. In a very small number of people who seem to have CML, neither the Philadelphia chromosome nor the BCR-ABL oncogene can be found. They might have other, unknown oncogenes causing their disease and are not considered to truly have CML.

6.5b. Ph negative, BCR-ABL fusion positive CML

Small proportion of patients has a clinical picture consistent with CML, but no Ph chromosome can be cytogenetically observed. In these cases the chromosomal aberrations are submicroscopic and in conventional cytogenetic studies the cases seem to be Ph chromosome negative. These may also be called as cryptic translocations or masked Ph chromosomes 48. However, even though cytogenetically no abnormality may be observed, at the molecular level the pathogenic BCR-ABL fusion gene characteristic for CML is detectable. This condition is called Ph negative, BCR-ABL positive CML. The Ph negative, BCR-ABL positive cases do not otherwise differ from standard Ph positive patients except that the chromosomal mechanism of the fusion gene formation is instead of translocation most often insertion of 3´ABL or 5´BCR sequences to chromosome 22 or 9, respectively [54,55,56,57].

The "real" Ph negative cases that are also lacking BCR-ABL molecular rearrangement are regarded as separate entities: as chronic neutrophilic leukemia or atypical CML. These disorders are classified as either other chronic myeloproliferative or myelodysplastic/ myeloproliferative diseases according to WHO classification [58,59]. Usually these diseases are unresponsive to tyrosine kinase inhibitors and have a poor prognosis. Because of unresponsiveness to these inhibitors the name (regardless of the prefix "atypical") CML is slightly misleading.

6.5c. Deletions of the derivative chromosome 9

The discovery of deletions in the translocated chromosome 9, i.e. derivative chromosome 9, der(9), resulted from the development and further refinement of probes used in FISH assays. More distinguished probe sets were designed for detection of both the Ph chromosome and the der(9) in minimal residual disease analyses in order to reduce the number of false-positive findings that were relatively common when more conventional probes were used in the assay [60,61]. However, in some patient samples an aberrant signal pattern was unexpectedly observed. The fusion signal indicating the BCR-ABL gene in the Ph chromosome was visible, but der(9) chromosome was lacking a signal of its own. This finding was indicative for a deletion in der(9), a phenomenon not reported earlier. The presence of a deletion was further confirmed by PCR targeting microsatellite loci and additional FISH probes mapping on the deleted region [62,63].

Ever since the initial findings, large deletions in der(9) chromosome translocation region have been identified in 10-15% of CML patients in Western countries [62,63,64]. For an unknown reason the reported frequency of der(9) deletions is higher in Asian populations, being over 20% [65,66,67]. Patients with variant translocation have more often der(9) deletions than patients with standard translocation, as the approximate frequency is 40% [68]. Der(9) deletions have also been observed in Ph chromosome positive ALL, with a similar frequency as in CML [69].

The der(9) deletions have variable breakpoints and the size of the deleted region ranges from a few hundred kilobase pairs to several megabase pairs of DNA [62,63,70]. In most cases the deletions span the translocation breakpoint and contain material from both chromosomes 9 and 22. The other deletion types contain only chromosome 9 sequences upstream the ABL gene, or only chromosome 22 derived sequences, respectively [71,72].

6.5d. When are der(9) deletions formed?

The der(9) deletions are commonly regarded as occurring at the time of the Ph translocation, since distinct patient populations in different phases of CML have been found to exhibit nearly identical frequencies of cases with deletions. Likewise, paired samples taken at the time of diagnosis and at disease progression of the same patients have been analyzed with consistent results [62,68,73]. In few reports though, the deletion has been described as a secondary event, since cells with and without the deletion have been observed simultaneously [67,74], but the majority of the current literature is in support of simultaneous translocation and deletion events.

6.5e. The clinical impact of der(9) deletions

In the first published studies patients with der(9) deletions were found to confer poorer prognosis when compared to patients without deletions. Significant difference in overall survival was observed between the deleted or non-deleted groups. The deletion was found to be an independent prognostic factor and more powerful than Sokal or Hasford scoring systems [62,68,75]. The size of deletion was observed to confer prognostic significance: the larger deletions were associated with poor prognosis, whereas smaller ones have no prognostic impact [71,72].

The poor prognosis of der(9) deletions was discovered in patient populations treated mainly with interferon-alpha based regimens. Since the advent of imatinib the prognostic significance of der(9) deletions has been re-evaluated in a few studies. In one study, the rates of hematologic and cytogenetic response were lower in patients with deletions, although the difference was not significant when only newly diagnosed patients were selected for analysis [76]. In another study, no difference in response rates was observed, even if the der(9) deletion patients were receiving more often higher imatinib dose than cases without deletion [77]. This finding has also been confirmed in studies with equal imatinib dose [78,79]. However, results of deletion positive chronic phase CML patients treated with second generation tyrosine kinase inhibitors may indicate worse survival, but the reason for this disparity is not clear [80]. As the prognostic significance of der(9) deletion is not so clear in CML treatment, it has been considered as a warning sign being one candidate adverse prognostic factor [81].

6.5f. Clonal evolution

The majority of CML patients develop secondary (i.e. additional) clonal aberrations in Ph positive cells in advanced phases of the disease. Additional abnormalities can be detected in approximately 75-80% of CML patients in blast crisis. The appearance of secondary changes is a phenomenon called cytogenetic clonal evolution. Clonal evolution is thought to be reflecting genetic instability of the leukemic cells and may be a sign of disease progression [56,82].

Secondary chromosomal aberrations are clearly non-random, the most common ones being the isochromosome-17q, trisomy-8, additional Ph chromosome and slightly less frequently trisomy-19 [i.e. i(17)(q10), +8, +der(22) t(9;22) (q34;q11.2), +19]. The first three changes constitute over 90% of the CML cases in whom secondary chromosomal changes are being detected. These and other aberrations occurring at frequencies exceeding 5% are called "major route" abnormalities. Other, less frequently seen abnormalities are called "minor route" changes, being for example trisomy-21 and monosomies of chromosomes 7 and 17 [83].

The prognostic significance of specific secondary chromosome abnormalities is variable. Many studies have reported that blast crisis without secondary abnormalities might have a better prognosis. Other investigations have not found such a connection. In all, the prognostic impact of secondary abnormalities is heterogeneous and most likely linked to various other parameters, including time of appearance, types of aberrations and also treatment modalities.

There is a correlation between the type of secondary chromosome aberration with the phenotype of the blast crisis. Hyperdiploidy, trisomy 8, 19, 21, and i(17q) are associated with myeloid blast crisis. An extra Ph is seen in both myeloid and lymphoid blast crisis. Chromosome loss is seldom seen (-7 is the most common) compared to chromosome gain in blast crisis. These changes can precede the blastic phase by 2-4 months. Followup cytogenetic studies would be a valuable tool to monitor the progression of the disease, especially an impending blast crisis [84,85].

6.5g. Clonal chromosomal changes in Ph negative cells

Other clonal chromosome aberrations can also be detected in Ph chromosome negative cells. This relatively new finding was observed during cytogenetic monitoring of CML patients treated with imatinib. The patients have achieved cytogenetic response to the treatment, but unexpectedly other clonal chromosome aberrations were seen in Ph negative cells. The incidence of these other clonal aberrations is relatively low, being 2-15% of the imatinib treated patients depending on whether the patients have been studied from selected or unselected cohorts. A small fraction of patients with Ph negative abnormalities develop bone marrow myelodysplasia, or myelodysplastic syndrome. Most frequently observed chromosome abnormalities are numerical aberrations, mainly -Y, +8 and -7. Structural changes are observed less frequently, out of which deletions of long arms of chromosomes 7 or 20 (7q-, 20q-) are more commonly seen [86,87,88,89].

The mechanism of the formation of aberrant Ph negative clones is not clear. In small proportion of patients the presence of Ph negative clone

has been shown in samples preceding imatinib treatment. This would indicate the expansion of pre-existing clone after eradication of Ph positive cells by imatinib. However, this has not been detected in all patients, so direct effect of imatinib cannot be totally excluded, even though it is unlikely [88,90,91]. Ph negative clonal hematopoiesis has also been detected during dasatinib treatment, so the phenomenon is not restricted only to imatinib therapy, but concerns other tyrosine kinase inhibitors as well [92,93].

Just like the origin, the clinical significance of clonal aberrations in Ph negative cells is not unequivocal. The appearance of clonal abnormalities in Ph negative metaphases may be transient, occurring only once, but the cells may also persist or even increase in time. The prognostic impact of Ph negative clonal aberrations needs further clarification, but chromosome 7 changes, in particular monosomy 7, appears to have the greatest risk of developing myelodysplastic syndrome or acute myeloid leukemia. Aneuploidies of chromosomes Y and 8 (-Y and +8) seem more indolent [94]. In practice, periodical monitoring of CML patients with conventional cytogenetics has clear importance because of the variable clinical significance of these abnormalities.

6.5h. Ph chromosome in other hematologic malignancies

The Philadelphia chromosome is not however, exclusively detected in CML. It has also been reported in B-cell acute lymphoblastic leukemia (ALL) [95]. About 25% of adults and 5% of children diagnosed with ALL show Ph in chromosome analysis [96] In addition, rare cases (<1-4%) of acute myeloid leukemia present Ph chromosome at diagnosis [97,98]. Some of the cases may actually represent CML diagnosed not until in the lymphoid or myeloid blast crisis. The prognosis of Ph chromosome positive acute leukemia is poor when treated with conventional cytotoxic therapy. Some patients may be cured by allogeneic hematopoietic stem cell transplantation. The advent of broad-spectrum tyrosine kinase inhibitors may dramatically change the prognosis of Ph+ ALL.

6.6. Cytogenetic monitoring

Cytogenetic analysis remains the gold standard for response to treatment monitoring in CML.10 Conventional cytogenetics requires a bone marrow sample and evaluation of >20 metaphases for the Ph chromosome. Categories of cytogenetic response include no cytogenetic response, with >95% Ph+ metaphases; minimal cytogenetic response, with 66% to 95% Ph+ meta-phases; minor cytogenetic response, with 36% to 65% Ph+ metaphases; partial cytogenetic response, with 1% to 35% Ph+ metaphases; and complete cytogenetic response, with 0% Ph metaphases (Table 2).

Although cytogenetic studies are associated with a wide confidence interval because of the limited number of metaphases evaluated, the association between cytogenetic response and positive outcomes has been well established [81,12].

Monitoring the percentage of Philadelphia chromosome-positive cells is the best validated system for the assessment of the response to interferon-α and tyrosine kinase inhibitors, since the cytogenetic response is the best surrogate marker of survival [10,99]. For patients who achieve a complete cytogenetic response to interferon-a, the 10-year survival is about 75%.8 For patients who achieve a complete cytogenetic response to imatinib, the 5-year survival rate is close to 100% [100].

The response is conventionally determined by chromosome banding analysis of marrow cell metaphases. A panel of experts appointed by the European LeukemiaNet recommended that at least two cultures should be performed, one for 24 hours and another for 48 hours [101]. The number of metaphases that is required to assess the response was arbitrarily fixed at 20 [10,102,81]. Although it is obvious that the accuracy of the test may depend on metaphase number, it is desirable that the definition of cytogenetic response, and particularly complete cytogenetic response, should be based more on confirmation of the test results than on metaphase number. Two sequential tests should, therefore, be performed, the second being confirmatory of the first.

6.7. Molecular cytogenetic monitoring (FISH Analysis)

FISH makes use of differently labeled fluorescent DNA probes. In the first-generation FISH technique, two probes are utilized. One probe, specific for ABL, labeled orange, for example, hybridizes to the 3′ end of the ABL breakpoint region. The other probe, specific for BCR, labeled green, for example, hybridizes to the 5′ end of the BCR breakpoint. In BCR-ABL translocations, the 3′ portion of ABL joins the 5′ end of BCR, the orange signal overlies the green signal, and a yellow fusion signal is generated.

FISH has several advantages over cytogenetics. The specificity of the newer split signal assay is high. Also, unlike cytogenetics, which requires dividing metaphase cells, FISH can be performed on interphase nuclei in peripheral blood. It therefore may bypass the requirement for a bone marrow specimen. However, the percentage of BCR-ABLpositive nuclei determined by FISH using peripheral blood specimens seems to be lower than that using bone marrow.

If there are fewer than 20 metaphases, the cytogenetic response can be validated by determining the level of BCR-ABL fusion segment via molecular cytogenetics, or fluorescence in situ hybridization (FISH). FISH can be performed on metaphases (high mitotic index metaphases or hypermetaphase FISH, HM-FISH, [103] or more frequently and more conveniently on interphase cells (IP-FISH) [104,105,106,107]. Several reports strongly suggest that all FISH data correlate very significantly with chromosome banding data, [103,104,105,106,107] as well as with BCR-ABL transcript levels [103,107].

Moreover, FISH can detect deletions of the long arm of chromosome 9 and variant translocations. However, almost all studies reporting the results of the treatment of CML with interferon-a or tyrosine kinase inhibitors have used chromosome banding data, and have reported responses in terms of percentages of marrow cell metaphases.There are no shared, standardized and validated definitions of the cytogenetic response based on IP-FISH data. In particular, IP-FISH negativity may range from less than 1% to 5 or 6% depending on reagents and laboratories [103,104,105,106,107].

A patient who has not achieved or has lost a complete cytogenetic response should always be studied with chromosome banding analysis of marrow cells. The value of regular bone marrow cytogenetics in stable cytogenetic and molecular responders has been challenged [108]. These patients could be monitored by IP-FISH on blood cells unless otherwise suggested by clinical and laboratory findings, e.g. cytopenia or dysplasia [89,94,109,110].

6.8. Molecular Analysis

There are three broad scenarios in which molecular testing is indicated in CML, i.e. at diagnosis, for monitoring during and following therapy, and in the emerging area of detecting kinase domain mutations.

6.8a. Molecular testing for initial diagnosis- Qualitative PCR (Transcript typing)

Cytogenetics serves to identify the presence of the t(9;22) translocation in only approximately 95% of cases of CML, but can identify the presence of other chromosomal abnormalities. In one-half of the patients with a normal karyotype, the BCR-ABL1 fusion transcript is detectable at the molecular level. This discrepancy is due to a submicroscopic genetic fusion. Therefore, in those patients with a normal karyotype, who have the clinical and hematological profile of CML, molecular testing serves a primary role in CML diagnosis.

At the molecular level, the translocation t(9;22)(q34;q11.2) consistently involves exon 2 of the ABL gene, but occurs in different exons of the BCR gene. The fusion involving the major breakpoint region (MBR) between exons 12 and 13 (or e13a2; formerly called b2a2) or 13 and 14 (or e14a2; formerly called b3a2) leads to expression of a transcript that codes for a 210kD fusion protein (p210BCR-ABL1). The breakpoint in the minor breakpoint region (m- BR) between alternate exon1 and exon2 (e1a2) results in a smaller transcript coding for a 190 kD protein (p190BCR-ABL1) [111].

Most adult CML have the b2a2 or b3a2 transcript (p210BCR-ABL1), whereas the e1a2 (p190BCR-ABL1) is usually present in acute lymphoblastic leukemia and the lymphoid blast crisis of CML. Chronic phase CML with p190 BCR-ABL1 have a myelomonocytic morphology. Less commonly, the break point involves the μ-BCR (micro-BCR) or exon19 (e19a2) resulting in a p230 BCR-ABL1 kD product [112]. Co-expression of the p210 BCR-ABL1 and p190 BCR-ABL1 encoding transcripts can occur as a result of alternative splicing in the MBR of BCR [113,114,115,116].

Different transcripts can be identified by qualitative multiplex RT-PCR [117]. In this assay, RNA is isolated from the patient bone marrow or peripheral blood, reverse transcribed into cDNA, and subjected to PCR amplification using primers specific for the different regions of the BCR and ABL exons. The primer set is designed to detect the different fusion products as well as a segment of the normal BCR gene that is not involved in the translocation and constitutes the control for sample cDNA quality. PCR products are analyzed by electrophoresis and ultraviolet transillumination of ethidium bromide-stained gels that depict PCR products of different lengths for each kind of transcript detected [117]. However, the limit of detection for this assay is at least 1 in 100,000 cells, which is suitable for diagnosis but is not sensitive enough for monitoring molecular response during treatment.

6.8b. Quantitative molecular analysis

The qualitative RT-PCR has little role in minimal residual disease assessment as the estimation of disease load is clinically more relevant than mere detection of the disease. Therefore, the serial quantitative assays that assess the kinetics of tumor clearance (response) or reappearance (relapse) have greater predictive value with quantitative real-time PCR (q-PCR) having clearly emerged as the preferred modality for post-therapeutic monitoring.

6.8c. Minimal Residual Disease (MRD) Evaluation by Quantit ative PCR

Quantitative reverse transcriptase PCR (q-PCR) performed on real time platforms can detect presence of abnormal transcripts with a sensitivity of 1 in 104 or 1 in 105 cells, and is currently the most sensitive laboratory technique for detection of the BCR-ABL1 transcript [118,119,120,121]. q-PCR is particularly useful for subsequent monitoring of patients who have achieved CCR. The assay is equally sensitive on peripheral blood and bone marrow samples and either specimen can be used for the assay, as long as the follow up is done using the same sample type for comparable results [122]. The levels of BCR-ABL transcript in the peripheral blood by q-PCR show excellent congruity with those of metaphase cytogenetics [122].

q-PCR involves extraction of total RNA from the peripheral blood or bone marrow specimen, reverse-transcription of the mRNA so obtained into cDNA, and quantitative (real time) co-amplification of the target BCR-ABL cDNA and cDNA of an internal control gene (to control for RNA integrity, sample preparation, extraction and loading). Standard curves are constructed by serial dilutions of known amount of cloned plasmid containing the fusion DNA, or from serial dilutions of K562 cells in normal DNA. The value of BCR-ABL transcript extrapolated from the standard curve is expressed as a normalized ratio of the BCR-ABL transcript to the control gene transcript [123,121].

Despite the fact that q-PCR is currently performed by most laboratories nation-wide, there is a lack of uniformity in the way the results are obtained and the data expressed. This inherent variability is due to several factors that affect the performance of q-PCR including methods of specimen transport, storage, RNA extraction procedures, reverse transcription and PCR efficiency, and the type of real-time platform used. The use of different control genes by the laboratories can also significantly alter the BCR-ABL/control gene ratios.

To develop international treatment guidelines it is essential that the molecular end-points for therapy protocols be comparable. For harmonizing the molecular monitoring of CML by q-PCR, recommendations were made at the CML meeting at the National Institutes of Health in Bethesda in 2005 to establish guidelines for specimen collection and transport, appropriate RNA quality, methodology for reverse transcription, PCR amplification efficiency, use of suitable control genes, test sensitivity for reporting negative results, quality assurance of the assay, generation of international reference, and a standardized method for expressing the result on an international scale [122]. To achieve this objective, a proposal was made to develop laboratory-specific conversion factors to convert the results obtained locally to a standardized international scale (expressed as % or IS units) [124].

The international scale was derived from the IRIS study in which the baseline median transcript level in 30 pre-treatment patient samples by q-PCR performed independently in the three principal laboratories was defined as 100% BCR-ABL level. Using this international scale, 1% BCR-ABL level correlated with the state of complete cytogenetic remission, and 1 log below this level (0.1% BCR-ABL) was defined as major molecular remission (MMR). In the IRIS study, no patient in MMR had positive Ph1 on conventional karyotyping [122]. According to the current guidelines laid by the NCCN and the European Leukemia Net [125,126,127], optimum response to imatinib therapy is indicated by attainment of MMR by 18 months of start of therapy (Table 3).

Data from several recent studies have shown that time to response is important and the probability of a favorable long-term outcome is best for patients who have the best responses at 3 months after the start of therapy [128,129,130]. Using receiver operated characteristic curves, [130] authors have confirmed that the BCR-ABL1 transcript ratio of less than 9.84% at 3 months correlates with best outcome, and compared with current NCCN and ELN cytogenetic milestones, molecular determination of transcript levels at 6 and 12 months levels was the only independent of overall survival ($p < 0.001$ for both time points). These data have not yet been translated into guidelines for testing. It is clear that the success of these predictive time points depends on standardization of methodology for determining the BCR-ABL1 transcript and subsequent reporting of the results. The first World Health Organization International Genetic Reference Panel for quantitation of BCR-ABL mRNA was approved by the Expert Committee on Biological Standardization of the World Health Organization in November 2009 and is now available commercially [131,132]. It consists of 4 dilutions of lyophillized preparations of K562 cells with assigned, fixed BCR-ABL/control gene values according to the IS. This development is a major milestone towards standardized molecular monitoring of therapeutic response to tyrosine kinase inhibitors in CML.

One caveat to molecular monitoring is that it cannot assess the clonal evolution of disease. For this assessment, cytogenetic karyotypic analysis is required and should be performed every six to twelve months throughout the monitoring process regardless of the therapeutic modality employed. The emergence of cytogenetically abnormal (but Ph-negative) clones with the use of imatinib, with an incidence of ~5%, further underscores the need for periodic conventional cytogenetic analysis.

6.9 Kinase domain Mutation analysis: use of molecular techniques

Various techniques are available for detection of the ABL kinase mutation. There is no consensus and no recommendation regarding the best routine assay, but efforts to harmonize the testing for mutations of ABL kinase are currently in progress [133]. Mutations can be reliably detected by nested-PCR amplification of the translocated ABL kinase domain, followed by direct sequencing of the entire amplified kinase domain. This is the most widespread method for routine monitoring and is suitable for detecting known and unknown mutations. The sensitivity of the assay enables detection of mutations in samples containing at least 15%–20% of mutated clones. A lower percentage of mutated clones may cause false negative results. Sub-cloning and sequencing, which are based on selection and expansion of specific clones, followed by direct sequencing, can detect a lower level of mutated clones compared to regular direct sequencing. However, this technique is cumbersome and is not applicable for routine monitoring. Assays based on allele-specific PCR (approximately 0.1% sensitivity), DHPLC (denaturing high performance liquid chromatography), or MALDI-TOF-MS (matrix assisted laser desorption/ionization time-of-flight mass spectrometry) [134] are more sensitive but are suitable primarily for finding mutations already detected by sequencing and not for screening the entire kinase domain for unknown mutations.

Mutations are found mostly in accelerated phase or blast crisis patients and rarely in chronic-phase patients [135]. In some cases, mutations found in patients with low level disease burden may be transient or unstable. Moreover, early publications demonstrated that the most resistant T315I mutation, found at low levels in pretreated patients, was not selected during therapy [136] and mutations found in patients with stable CCyR have little prognostic significance. In practice, screening for mutations is justified when an increase in the BCR-ABL1 transcripts is measured by RQ-PCR (especially when passing the MMR level) or in any advanced-phase disease such as in CP patients who do not achieve the appropriate cytogenetic response [126].

6.10a. Mutation analysis in treatment naive patients

There are a few reports of ABL1 kinase mutations present in newly diagnosed treatment naïve patients. Most of them are in patients who

present in accelerated phase or blast crisis at diagnosis [137,138, 136,139]; they are rare in chronic phase treatment naïve patients [27,135]. Given the rare occurrence, and lack of any clinical evidence of an adverse outcome from mutations in chronic phase patients, routine testing of all chronic-phase patients for mutations is not recommended at this time. The present recommendation to perform BCR-ABL (kinase domain) KD mutations in treatment naïve patients is restricted to those who present with advanced disease in accelerated phase or blast crisis [139].

6.10b. Mutation analysis in patients on Imatinib as first line therapy

As per the NCCN practice guidelines [125] and ELN recommenda tions [126,127], testing for BCR-ABL KD mutations in patients on imatinib as first line therapy is to be performed in the following circumstances: 1) Failure to respond or suboptimal response at the defined time points i.e. complete hematological remission at 3 months, minor cytogenetic remission at 6 months, and CCyR at 12 months of initiation of imatinib therapy [125,126]. Twenty-nine percent of chronic phase non-responders and 16% of patients with suboptimal response harbor BCR-ABL KD mutations [140,141,142]. 2) Confirmed loss of previously attained optimum response demonstrated by loss of MMR or disease progression [136]. Fluctuations in levels of BCRABL transcript may occur when the levels are very low and are not considered clinically significant. A trend of increasing BCR-ABL transcript level by analysis of sequential samples, or a two-fold (or one log) increase in BCR-ABL transcript level is more consistent with definitive loss of MMR and predictive of relapse [143].

6.10c. Mutation analysis in patients on second-line TKIs

KD mutations arise under selective drug pressure. Thus, it is not unexpected to see emergence of additional resistant mutations in patients who are put on second-generation TK inhibitors following resistance to imatinib [144]. Mutations resistant to dasatinib (V299L, T315A, F317L/V/I/C) or to nilotinib (E255K/V, Y253H, F359V/C/I) have been described in patients developing secondary response failure with second-generation TK inhibitors [145,146]. The data on resistant mutations to the second generation TKIs is limited; currently KD mutation testing is done in event of failure to respond, or suboptimal response, similar to that for imatinib. Preliminary data show a better projected one-year survival in patients receiving dasatinib as second or subsequent-line therapy if they attain MCyR at 12 months, and suggest alternative therapies if there is no cytogenetic response in 3 to 6 months [147,148].

6.11. Tyrosine kinase inhibitor (TKI) Response monitoring paradigm

It is imperative to follow patient responses closely and recognize suboptimal response or treatment failure at the earliest to perform additional tests and make necessary adjustments to treatment regimen. The NCCN and the European Leukemia Net recommends the haematological, cytogenetic and molecular monitoring to be carried as tabulated in (Table 4).

Imatinib failure is defined as no hematological response at 3 months, incomplete hematological response or no cytogenetic response at 6 months, less than partial cytogenetic response at 12 months, less than complete cytogenetic response at 18 months, loss of complete hematological or cytogenetic remission, or detection of highly resistant mutations of BCR-ABL at any time. Suboptimal response is defined as incomplete hematological response at 3 months, less than partial cytogenetic response at 6 months, less than complete cytogenetic response at 12 months, less than major molecular remission at 18 months, loss of major molecular response, mildly resistant mutations, or other chromosome abnormalities. The NCCN and ELN criteria for remission, suboptimal response and failure for previously untreated patients on standard dose of imatinib are tabulated in (Table 5).

Conclusion

Use of Imatinib in the treatment of CML has certainly been a great clinical success and immensely instrumental in taming the disease to the extent that in many cases the prospects of treatment free management of CML are being explored. While Imatinib continues to prove a boon to patients in being the first-line TKI therapy for CML (and cancer therapy overall), approximately 15–25% of patients do not respond to initial imatinib treatment or are intolerant to imatinib, which is termed as primary resistance. Hematologic, cytogenetic and molecular monitoring of CML along with BCR-ABL1 mutational analysis have become integral to the routine management of CML. The information that each type of test provides is essential to confirm a diagnosis, determine the disease stage, assess response to treatment, and monitor for signals of disease progression – all of which can be used to identify patients who might require further evaluation, closer follow-up, and additional intervention, and to guide clinical decisions.

Figures

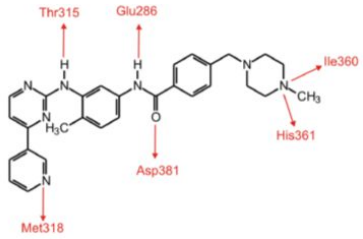

Fig. 01: Structure of Imatinib. ***Reprinted from Weisberg et.al., 2006.***

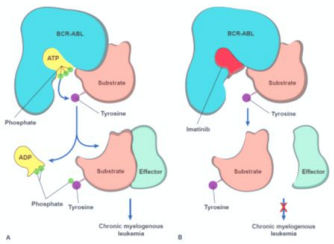

Fig. 02: Mechanism of Imatinib action (A) The phosphorylation and activation of tyrosine residue after binding of ATP in the kinase domain (KD) of BCR-ABL oncoprotein. (B) Prevention of phosphorylation and activation of tyrosine residue when Imatinib binds to the KD. ***Reprinted from Savage DG et.al. New Engl J Med. 2002;346:683-693.***

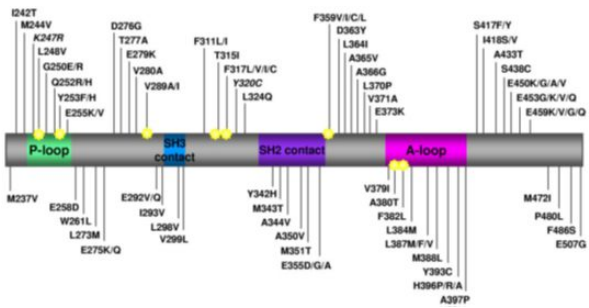

Fig. 03: Map of the amino acid substitutions in the BCR-ABL KD identified in clinical samples from patients reported to be resistant to Imatinib in all published papers from 2002-2009. Yellow star indicates amino acid position reported to be directly involved in Imatinib binding. K247R & Y320C are shown in italics for being reportedly SNPs. ***Reprinted from Review article on CML by Simona Soverini et.al. Blood. 2001;118(5):1208-1215.***

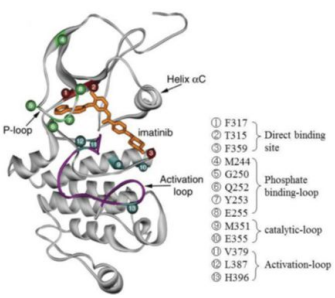

Fig. 04: Ribbon representation of the Kinase Domain of ABL complexed to Imatinib (gold colour) depicting resistant mutations as indicated by numbers. ***Reprinted from Review article on CML by Shinya Kimura et.al. J Hematol Transfus. 2014;2(3):1022.***

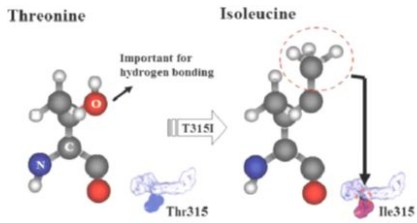

Fig. 05: The structures of Threonine & Isoleucine. Substitution of the amino acid (aa) at 315 is from Threonine to Isoleucine. Reprinted from *Review article on CML by Shinya Kimura et.al. J Hematol Transfus. 2014;2(3):1022.*

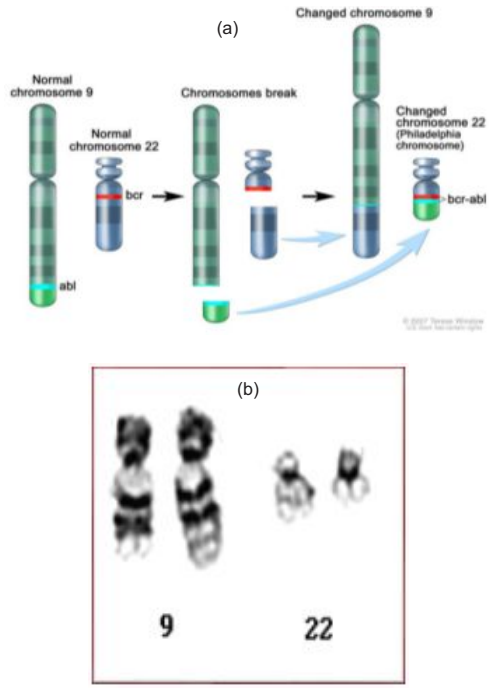

Fig. 06: Translocation t(9;22)(q34;q11.2)- (a). A piece of chromosome 9 and a piece of chromosome 22 break off and trade places to give rise to the Philadelphia chromosome carrying the BCR-ABL fusion gene responsible for CML. *Courtesy Terese Winslow. (b) actual karyotypic representation (SKIMS Lab.)*

Tables

Table 1: Complete hematologic response to TKI therapy of patients as per European LeukemiaNet guidelines, 2013.

Complete Hematologic Response
Platelet count: >450 x 109/L
WBC count: <10 x 109/L
Differential without immature granulocytes and basophils less than 5%
Non-palpable Spleen

Table 2: Cytogenetic response to TKI therapy of patients as per European LeukemiaNet guidelines, 2013.

Cytogenetic Response
Complete: Ph+ 0%
Partial: 1-35%%
Minor: Ph+ 36-65%
Minimal: Ph+ 66-95%
None: Ph+ >95%

Table 3: Molecular response to TKI therapy of patients as per European LeukemiaNet guidelines, 2013.

Log Molecular Response (BCR-ABL transcripts)
MR 4.5: ≤0.003%
MR 4.0: ≤0.01%
Major (MMR) 3.0: ≤0.10%
Relapse: >0.5-1-0%

MR: Molecular response; MMR: Major molecular response.

Table 4: Monitoring of TKI therapy of patients as per the guidelines of the European LeukemiaNET, 2013.

Hematological-Response Monitoring	Cytogenetic-Response monitoring	Molecular-Response monitoring
Initial	Initial	Initial
Every 2 weeks until complete response	Every 6 months until complete response	Every 3 months
Follow-up	Follow-up	Follow-up
Every 3 months	Every 12 months	Failure, sub-optimal response or increase in transcript level requires Mutational analysis

Table 5: TKI Response evaluation as per European LeukemiaNet recommendations.

Response	Time Period	Hematologic Response	Cytogenetic Response	Molecular Response
Optimal Response	03 Months	CHR	PCR (Ph+:≤35%)	BCR-ABL<10%
	06 Months	-------	CCR (Ph+:0%)	BCR<1%
	12 Months	-------	-------	BCR-ABL<0.1%
	>12 Months	-------	-------	BCR-ABL<0.1%
Suboptimal Response/ Warning	03 Months	-------	Ph+: 36-95%	BCR-ABL>10%
	06 Months	-------	Ph+: 1-35%	BCR-ABL 1-10%
	12 Months	-------	-------	BCR-ABL>0.1-1%
	>12 Months	-------	Clonal Evolution	-------
Failure	03 Months	NO CHR	Ph+:>95%	-------
	06 Months	-------	Ph+:>35%	BCR-ABL>10%
	12 Months	-------	Ph+:>0%	BCR-ABL>1%
	>12 Months	Loss of CHR	Loss of CCR/ Mutations/ Clonal Evolution	Confirmed Loss of MMR

CHR: Complete hematologic response. CCR: Complete cytogenetic response.
PCR: Partial cytogenetic response. MMR: Major molecular response.

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