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Foundation Day celebration of the Institute

Imatinib resistant Chronic Myeloid Leukemia and therapeutic approach

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Summary : A revolution in medical science was marked with the advent of imatinib, a site specific drug for the management of patients with chronic myeloid leukemia (CML). Imatinib mesylate (also known as Glivec, Gleevec, STI-571, CGP57148), an orally administered 2-phenylaminopyrimidine derivative approved by the FDA in 2001 for treatment of CML and is highly effective in treating the early stages of chronic myeloid leukemia, while remission induced in advanced phase were observed to be relatively short lived. The primary cause of resistance in CML patients is the mutations in the BCR/ABL kinase domain. This review discusses the different mechanisms leading to Imatinib resistance and various treatment options to over ride imatinib resistance.

Introduction

Chronic myeloid leukemia is a clonal hematopoietic stem cell disorder, characterized by excess proliferation of myeloid progenitors that retain the capacity for differentiation during the stable or chronic phase of the disease. Hallmark of this disease is a unique chromosome, known as the Philadelphia chromosome (Ph), which results from the reciprocal translocation between the long arms of chromosomes 9 and 22 [t(9;22) (q34;q11)] [Fig. 1 & 2] that leads to the fusion of the breakpoints cluster (BCR) and human ABL1 genes [Fig.3]. The root cause of this disease is still unknown. The majority of patients with CML have no history of excessive exposure to ionizing radiations or carcinogens, but the incidence does increase progressively with exposure to increasing doses of radiation. The incidence of CML in United states and most of the western countries is about 1.5 per 1,00,000 population per year and accounts for about 15% of all cases of leukemia; about 4500 new cases are diagnosed in the United States each year (1). CML is slightly more frequent in men than in women (incidence ratio: 1.4 to 2.2:1), but the course of the disease is the same. The median age is about 50; it is rare in children and the incidence increases progressively with age.

Molecular biology and pathophysiology of chronic myeloid leukemia.

Chronic myeloid leukemia is probably the most extensively studied human malignancy. The breakpoints within the ABL (Abelson tyrosine kinase) gene at 9q34 can occur anywhere over a large (greater than 300Kb) area at its 5'end, either upstream of the first alternative exon Ib, downstream of the second alternative exon Ia, or more frequently, between the two. Regardless of the exact location of the breakpoint, splicing of the primary hybrid transcript yields a mRNA molecule in which BCR sequences are fused to ABL exon a2. In contrast to ABL, breakpoints within BCR localize to 1 of 3 so-called breakpoint cluster regions (bcr). In most patients with CML and in approximately one third of patients with Ph-positive acute lymphoblastic leukemia (ALL), the break occurs within a 5.8-kb area spanning BCR exons 12-16 (originally referred to as exons b1-b5), defined as the major breakpoint cluster region (M-bcr). Because of alternative splicing, fusion transcripts with either b2a2 or b3a2 junctions can be formed. A 210-kd chimeric protein (P210BCR-ABL) is derived from this mRNA.

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In the remaining patients with ALL and rarely in patients with CML, characterized clinically by prominent monocytosis, the breakpoints are further upstream in the 54.4-kb region between the alternative BCR exons e2' and e2, termed the minor breakpoint cluster region (m-bcr). The resultant e1a2 mRNA is translated into a 190-kd protein (P190BCR-ABL). Recently, a third breakpoint cluster region (μ-bcr) was identified downstream of exon 19, giving rise to a 230-kd fusion protein (P230BCR-ABL) associated with the rare Ph-positive chronic neutrophilic leukemia (2).

The targets for BCR-ABL include members of the Ras, phosphotidylinositol-3 kinase (PI3K)/Akt, and Jak/Stat signaling pathways, which regulate cell proliferation and apoptosis. BCR/ABL abrogates cell dependence on external growth factors by upregulating interleukin-3 production and alters the cell adhesion properties by modulating expression and activation of focal adhesion kinase and associated proteins. The kinase also has diverse effects on the DNA repair response, which may promote additional chromosomal alterations and mutations involved in the progressive nature of late-stage CML (3).

The disease characteristically develops in 3 phases. Left untreated, the initial chronic phase lasts approximately 3 to 6 years; the disease then progresses, often through an accelerated phase, to a terminal blastic phase. Most patients are diagnosed in chronic phase, which is characterized by an increased number of leucocytes and/or platelets and a bone marrow blast count less than 10%. The acclerated phase may be marked by 1 or more of the following: increasing splenomegaly and leukocytosis, an increase of basophils to 20% or greater, thrombocytopenia, and clonal evolution. In the blastic phse, for which the median survival is 2 to 4 months, 30% or more of blood and bone marrow cells are blasts, and myeloid precursors may also form tumors in the lymph nodes, skin, and bone. (3).

Since the description of BCR/ABL oncogene, a myraid of treatment options have been explored in CML, including arsenic trioxide, spleenic irradiation, busulphan, hydroxycarbamide, stem cell transplantation, and interferon α. Among these, interferon α showed superior activity compared with other chemotherapeutic agents. It can promote long term survival, but the proportion of such patients is small. Stem cell transplantation can cure the disease, however, as the average age of onset is >50 years, this factor, combined with the inability to identify suitably matched donors in all cases, limits this option to a minority of patients (4).

Imatinib therapy in patients with CML

Imatinib was developed in the late 1990s by Biochemist Nicholas Lydon, a former researcher for Ciba Geigy (presently Novartis, Basel, Switzerland) and Brian Druker, an oncologist from Oregon Health and Science University, and the clinical trials were led by Charles Sawyers, confirming its efficacy in CML.

Structural analyses have revealed that imatinib is sandwich between the N-lobe and C-lobe of the ABL kinase domain and penetrates through the central region of the kinase. The pyrimidine and pyridine rings of imatinib occupy the region where an adenine ring of ATP normally binds. The rest of the molecule wedges itself into a hydrophobic cage between the activation loop and the C-helix of the N-lobe, specifically recognizing the inactive and unphosphorylated conformation of ABL. Much of the remarkable efficacy of imatinib appears to derive from its ability to stabilize the inactive conformation through an 'induced fit' mechanism. The potency of

imatinib against the activated form of BCR-ABL presumably arises from the dynamic nature of kinase molecules, which transiently switch between inactive and active forms, allowing imatinib to trap the kinase in its inactive conformation (5).

Imatinib as a single agent has shown impressive activity in the chronic phase of CML patients, with >95% complete haematological response and >73% complete cytogenetic remission (6). However, patients treated in the later stages of the disease do not respond as well, with only about 65% displaying haematological responses (7).

Resistance to Imatinib

The emergence of resistance to imatinib has dampened the enthusiasm for this drug. The rate of relapse and resistance appear to correlate with disease stage and the incidence increases as CML progresses. Resistance to imatinib can be divided into primary resistance, in which patients show lack of efficacy to this TkI from the start of the therapy, and secondary resistance, also known as acquired resistance, is defined as a loss of haematologic, cytogenetic, or molecular response, as well as progression to advanced phases of CML. Resistance can further be divided into haematologic (lack of normalization of peripheral blood counts), cytogenetic (persistence of Ph chromosome) and molecular (persistence of BCR/ABL1 transcripts by reverse transcriptase polymerase chain reaction[RT-PCR]).

Patients can be classified according to their response to treatment, which can be considered optimal, suboptimal, or failure. Failure indicates that primary resistance patients in this category should be recommended a change in therapy to second-generation TKI. Patients with suboptimal response need close monitoring, and a dose escalation from 400mg to 800mg is justified (8).

Mechanisms of Imatinib Resistance

Imatinib has become the standard first line therapy for CML. Indeed, imatinib induces complete remission in virtually all patients with the indolent chronic phase of CML who are treated immediately upon diagnosis (7). When treated during the more aggressive stage of blast crisis, most of the patients ultimately evolve drug-resistance disease. Various mechanisms are postulated for imatinib resistance which can be broadly divided into BCR/ABL independent and BCR/ABL dependent mechanisms of Imatinib resistance.

BCR/ABL Independent Mechanisms of Resistance

Quiescent CML stem cell

Quiescent CML stem cells account for approximately 0.5% of the CD34+ population and are characterized by intrinsic resistance to imatinib. Jorgensen and Holyoake (9) noticed that though CD34+38- HSCs carried a single copy of BCR/ABL, still exhibited a significant (10-fold) increase in BCR/ABL kinase level compared with total mature mononuclear cells. This study was in consensus with the earlier studies by Copland et al (10).

Although rationally designed TKIs have proven to be effective in disease management, nevertheless, they do not offer a cure owing to the persistence of insensitive HSCs. One possible explanation for this molecular persistence is oncogene over-expression at the message, protein and kinase activity levels that do not overcome by possible sub-therapeutic levels of TKI within HSCs. Another possible explanation given by Corbin et al (11) which suggests that CML stem cells can survive after complete BCR-ABL inhibition and are therefore not oncogene addicted. This finding may explain why primitive leukemic cells are present in the BM of patients with established complete

cytogenetic response during the course of 5 years on imatinib (12). Not only imatinib but other TKIs (nilotinib, dasatinib and bosutinib) fail to induce cell death in primitive stem/progenitor cells. Nevertheless, introduction of novel agents such as the putative FTI BMS-214662 will make significant advances towards the goal of elimination of the diseased stem cell population, preferentially targeting quiescent leukemic cells over normal. Further elucidation of its true mechanism of action will potentially inform drug development for use in other leukemias and stem cell-derived cancers.

Pharmacokinetics

Pharmacokinetics is widely studied in CML patients with drug resistance. Pharmacokinetic data indicates that patients receiving 400 mg imatinib per day have plasma concentrations constantly higher than 1µmol/L (13). An increased plasma concentrations (>20umol/L) have been observed among the patients who are receiving higher doses (600-800 mg) of imatinib. Since imatinib is metabolized largely by the cytochrome p450 isoenzymes P3A4 (CYP3A4) and P3A5 (CYP3A5), differences in the concentrations of CYP3A4/A5 or drugs that can inhibit or induce said enzymes have the potential to greatly affect the levels of imatinib in plasma (8). In two separate studies by Amirimani B et al (14) and Angelini S et al (15), investigating the influence of in vivo CYP3A4 activity on the achievement of molecular response to imatinib found that higher activity significantly correlated with higher complete molecular response rates at 12 months. This observation might find a rationale in the fact that the main IM metabolite formed by CYP3A4, the N-desmethylated piperazine derivative (CGP74588), is pharmacologically active, has potency and selectivity similar to those of IM, and longer terminal half-life. It may be hypothesized that the presence of the variant allele leads to a higher CGP74588 amount. Another intriguing hypothesis is that CGP74588 can be subjected to a different transport process which would be in line with the observation of higher cellular uptake of CGP74588 as compared to IM (16), obviously no definitive explanation can be given and further studies on the pharmacokinetics of CGP74588 would be needed.

It has been proposed that binding of α-1-acid glycoprotein (AGP) which is an acute phase plasma protein, can inhibit the activity of imatinib (8, 17). Therefore, imatinib present in the plasma could be mostly bound to AGP and thus biologically inactive. To test this hypothesis, blood samples from two patients who were resistant to imatinib were incubated with erythromycin (which competes with imatinib for binding of AGP) for 1 hour before separation of mononuclear cells and lysis. The activity of imatinib on BCR/ABL autophosphorylation was restored, indicating that most plasma imatinib is bound to AGP, however, not active (13).

Furthermore, when patients who were being treated with imatinib received a simultaneous infusion of clindamycin, another molecule known to bind AGP, a large decrease in total plasma concentrations of imatinib were observed within a short time. This finding also shows that most imatinib present in plasma is not in equilibrium with tissues and therefore there is a gradient between plasma and tissues (13). Although the exact concentration of imatinib inside the cells (the final target of the drug) is not known, this further suggests that plasma concentrations of the drugs are not a reliable indicator of the concentration present inside the leukemic cells.

Drug efflux and influx mechanism

Several other cellular mechanisms of resistance to imatinib have also been identified, but the possible importance of drug-transporter proteins that affect drug concentration, such as Permeability –glycoprotein (Pgp), human organic cationic transporter (hOCT) and ATP-binding cassette G2 has only been recently appreciated. The generally accepted action of MDR1 (or adenosine triphosphate-binding cassette transporter ABCB1) is to reduce intracellular drug accumulation through Pgp-mediated efflux, thus hampering the achievement of effective drug levels at the target site. Galimberti et al and Mahon FX et al (18, 19) separately showed that those patients who failed to attain a major cytogenetic response or progress exhibited MRD1 gene overexpression. In contrast to this study, Ferrao et al (20) have shown that over expression of MDR1 in K562 cells does not confer resistance to imatinib in vitro. Association of single nucleotide polymorphisms in MDR1 gene and response to imatinib has been reported. Dulucq et al, (21) have demonstrated the usefulness of three single nucleotide polymorphisms (1236C>T, 2677G>T/A, 3435C>T) for the identification of CML patients who may or may not respond optimally to imatinib (21).

Inhibition of imatinib influx through the hOCT1 has also been proposed to be an important factor for regulating intracellular imatinib availability. The above mentioned transporters regulate in concert the active transport of imatinib in and out of the cell and potentially play an important role in pharmacogenetics of imatinib. Modification of Imatinib regimen by increasing the standard dose from 400mg/day to 600-1000mg/day is suggested, in some patients having an altered expression of transporter proteins. A recent study on hOCT1 polymorphisms found that the hOCT1 SNPs M420del and M408V alter imatinib uptake and M420del modifies clinical outcome in imatinib-treated chronic myeloid leukaemia (22).The authors claimed that pre-therapy determination of hOCT-1 activity identifies the patients at highest risk for achieving a suboptimal response, and those likely to respond well to standard dose imatinib. (23).

Cellular signalling

It is hypothesized that pathways affected by BCR/ABL, which also contribute to the malignant transformation, are involved in drug resistance. Yamada et al (24) reported the involvement of STAT 5 as a critical transcription factor, which confers imatinib resistance on leukemic cells through the transcription of TERT and MDR1. In a recent study, analysis of CML cancer cell lines and CML patient samples revealed the upregulation of PRL-3. Inhibition of bcr/abl signalling either by imatinib or by RNAi silencing BCR-ABL reduced PRL-3 levels and increased cleavage of PARP. In contrast, the amount of PRL-3 protein remained constant or even increased in response to imatinib treatment in drug resistant cells expressing P210 T315I. (25). Tibullo D et al (26) reported that nuclear translocation of heme oxygenase-1 confers resistance to Imatinib in chronic myeloid leukemia cells. Imatinib was able to increase the formation of cellular reactive oxygen species (ROS) in CML cell lines and this effect was reversed by HO-1 induction or the addition of N-acetylcisteine (NAC). The protective effect of HO-1 on imatinib-induced cytotoxicity involved the nuclear translocation of HO-1 following proteolytic cleavage. Hurtz C et al (27) identified the BCL6 proto-oncogene as critical effectors downstream of FoxO in self-renewal signaling of CML-initiating cells. BCL6

represses Arf and p53 in CML cells and is required for colony formation and initiation of leukemia.

BCR/ABL dependent mechanisms of resistance

Mutations within the ABL kinase domain (point mutations and frame shift mutations)

The mutation in the ABL kinase domain and other domains which regulate the conformation of ABL kinase domain is the major cause of imatinib resistance.

Some critical questions in CML therapy are (i) Genetic mutations in chronic myelogenous leukemia: when to check and what to do? (ii) What are the clinically important mutations in the disease i.e., both on BCR/ABL and outside BCR/ABL? (iii) What is the laboratory assessment for progression of the disease to be undertaken and at what time point? Mutation analysis testing should be considered in patients with Accelerated and Blastic Phase of the disease and in patients who have an increase in bcr-abl transcript levels that result in loss of a major molecular response, as well as in patients who demonstrate suboptimal response to therapy.

To date, mutations have been detected that sterically hinder drug occupancy of the active site, alter the deformability of the highly conserved phosphate binding P loop, and influence the conformation of the activation loop surrounding the active site (28). Mutations are clustered mainly into four main groups, (i) Imatinib-binding site, (ii) Nucleotide-binding site for ATP, (iii) Activation loop and (iv) Hydrophobic patch between helices E,F and I in the terminal lobe of the enzyme (13).

Studies done all over the world for screening of mutations in BCR/ABL kinase domain have reported many novel mutations with different frequencies occurring in the population. The first mutation (T315I) linked to imatinib resistance was reported by Gorre et al (29) in a cohort of relapsed patients from USA. Subsequently, soon after the report of T315I, several other mutations were identified in closely juxtaposed residues. Studies in German population, by Von Bubnoff et al. (30) revealed five distinct mutations in the BCR/ABL domain (sample size 7) which resulted in exchange of amino acids within the ATP-binding site (in six patients) or in the activation loop (1 patient) of BCR/ABL kinase domain. Their study strongly suggests that a patient could be resistant to STI-571 by acquisition of different individual point mutations within the ATP-binding pocket or activation loop of BCR/ABL. Hughes (31) from Australia, reported nine mutations in BCR/ABL kinase domain which account more than 85% of the mutations causing imatinib resistance. The greatest degree of resistance has been associated with the T315I mutation and point mutations in the P-loop domain, i.e., G250E, Q252H, Y253F, and E255K/V.

Rare cases of splicing events inducing deletions or insertions of multiple nucleotides in the ABL kinase domain, leading to imatinib resistance have been described. Hayette et al (32) reported a novel frame shift mutation acquired at the moment of imatinib resistance, consisting in an insertion of 12nts, and leading to the conservation of open reading frame.

Mutations outside the kinase domain

The studies done in-vitro have shown that mutations outside the kinase domain in the neighbouring linker, SH2, SH3, and Cap domains can confer imatinib resistance (33). In the context of ABL, these domains have an autoinhibitory effect on kinase activity, and mutations in this region can activate the enzyme. In an attempt to determine the frequency and relevance to resistance of regulatory domain mutations, Sherbenou

et al (34) screened for such mutations in a cohort of consecutive CML patients with various levels of response. They found mutation T212R conferred resistance to tyrosine kinase inhibitor and was associated with relapse, whereas most other mutations did not affect drug sensitivity.

BCR/ABL amplification

In a study, 3 out of 11 CML patients in blast crisis who relapsed after initially responding to imatinib were shown to have multiple copies of the BCR-ABL gene by fluorescence in situ hybridization (FISH) (29). In another study, 7 out of 55 patients showed a more than 10-fold increase in BCR-ABL transcript levels and 2 out of the 32 patients evaluated were found to have genomic amplification of BCR-ABL by FISH (35). In the latter 2 patients, resistance was primary and not acquired. Over expression of Bcr-Abl leads to resistance by increasing the amount of target protein needed to be inhibited by the therapeutic dose of the drug. It is also possible that a transient over expression of Bcr-Abl may be an early phenomenon in the establishment of imatinib resistance, preceding the emergence of a dominant clone with a mutant kinase domain, as suggested by kinetic studies in cell lines (36).

Strategies to overcome imatinib resistance

The strategies to overcome imatinib resistance in chronic myeloid leukemia patients is summerized in Fig.4.

Targeting tyrosine kinase activity using second-generation and third-generation tyrosine kinase inhibitors

Second-generation TKIs: Several clinical observations have shown impressing results against second-generation Abl inhibitors nilotinib (AMN107, developed by Novartis Pharmaceuticals, Basel, Switzerland) and dasatinib (BMS-354825, developed by Bristol-Myers Squibb, Princeton, USA). Nilotinib, like imatinib, requires the Abl protein to be in the inactive conformation for optimal binding. Nilotinib was found to be 10 to 25 fold more potent as compared to imatinib in the reduction in both autophosphorylation and proliferation (37) and significantly active against 32 / 33 imatinib-resistant BCR–Abl mutants (38). Dasatinib is a dual-specific SRC and ABL inhibitor, structurally unrelated to imatinib that is able to bind and inhibit both the active and inactive conformations of Abl, resulting in 100 to 300 fold higher activity than imatinib. Shah et al. (28) recently demonstrated that dasatinib has 100-fold increased activity against the Abl kinase compared to imatinib and retains activity against 14 of 15 imatinib-resistant BCR–ABL mutants in vitro.

Third-generation TKIs: The second-generation TKI, dasatinib, holds great promise for the management of practically all imatinib-resistant mutations except for the T315I mutation. T315I currently remains the most troublesome mutant, and there are no effective treatment options for patients harbouring this mutation. Preclinical research has demonstrated that AP24534 (ponatinib), an orally active pan inhibitor of Bcr–abl, can inhibit the entire spectrum of mutations that cause resistance to other Bcr–abl inhibitors including the most resistant T315I (39). Ponatinib is currently in phase II trials and showed impressing results against T315I. Carbon–carbon triple bond linker is the key structural feature of ponatinib that makes hydrophobic contact with the side chain of I315, allowing inhibition of the T315I mutant. It also acts as an inflexible connector that makes obligatory correct positioning of the two binding segments into their binding pockets. Its inhibitor profile incorporates multiple contact points to confer very high potency

and balances the overall binding affinity.

The extensive network of optimized molecular contacts leads to high potency and renders binding less susceptible to disruption by single-point mutations (25). Another third-generation TKI, bosutinib (SKI-606), is a dual Src– abl inhibitor that is active in the low nanomolar range against Bcr–abl (40). Bosutinib is now in phase III clinical trials and has shown good activity in patients resistant to imatinib or other TKIs in phase II (41). At nanomolar concentrations, it inhibits the autophosphorylation of both Abl and Src kinase, resulting in inhibition of cell growth and apoptosis. Because of dual mechanism of action, this agent shows activity in resistant CML disease, other myeloid malignancies, and solid tumours. It seems to cause fewer side effects because it selectively inhibits the faulty proteins in the leukemic cells and does not affect similar proteins in normal cells as much as the earlier drugs do. Rebastinib (formerly called DCC-2036, Deciphera Pharmaceuticals), is a novel and potent TKI which binds to a novel region called the switch pocket, preventing BCR/ABL1 from adopting an active conformation. Efficacy against multiple imatinib-resistant BCR/ABL1 mutants has been demonstrated both in vitro and in vivo. Importantly, DCC-2036 retained its full potency against the T315I mutant in preclinical efficacy studies. The drug is currently in phase I study designed to find the maximal tolerated dose (MTD) when administered daily as a single-agent on a 28-day cycle. The preliminary results from 30 patients with CML in various phases, including 11 patients with T315I mutation, suggested that Rebastinib was well tolerated and has anti-leukemic activity in subjects with refractory CML and T315I positive disease. (42). Recently, two novel compounds were reported, ONO12380 (43) and MK-0457 (44), to inhibit Bcr–abl kinase activity through a distinct mechanism in cell lines expressing the T315I mutation.

Combination therapy

TKIs alone can induce remission in CML but do not eliminate leukemia stem cells, which remain a potential source of relapse. Monotherapy leads to the generation of mutants. Combination therapy involves the combination of two or more anticancer drugs of which one is mostly a TKI. Treatment with histone deacetylase inhibitor (HDACis) combined with imatinib effectively induced apoptosis in quiescent CML progenitors resistant to elimination by imatinib alone and eliminated CML stem cells capable of engrafting immunodeficient mice. The interaction between imatinib and HDACis inhibited genes regulating hematopoietic stem cell maintenance and survival (45). Some clinical studies have demonstrated that the combination of imatinib and interferon α (IFNα) is superior than either therapy alone, perhaps because unlike imatinib, IFNα preferentially targets CML stem cells (46). At 12 months, the rate of superior molecular response was significantly higher (30%) among the patients receiving imatinib and peg-interferon alfa-2a than those receiving 400 mg of imatinib alone (14%) (47).

Targeting pathways downstream of BCR–ABL

The Ras / Raf pathway is intimately linked to BCR–ABL through the adaptor molecules Grb2 (growth factor receptor–bound protein 2) and Crkl, inhibition of which with a farnesyl transferase inhibitor (FTI), such as lonafarnib, would theoretically reduce nuclear transcription (9). PI3K and its downstream targets, including the serine / threonine kinases AKT, mTOR, and p70S6 kinase, play a key role in the regulation of cell survival and

proliferation. Recently, it was shown that activation of the PI3K /mTOR pathway by Bcr–abl contributes to increased production of reactive oxygen species (48), thus linking PI3K /mTOR to mechanisms that have been implicated in genomic instability and imatinib resistance. Signal transduction through this pathway can be blocked by inhibitors such as LY294002 (9) or wortmannin, which specifically target PI3K. Cellular response to PI3K inhibition includes induction of apoptosis and inhibition of proliferation.

Modulating the levels of imatinib in Plasma

Imatinib is metabolized largely by the cytochrome p450 isoenzymes like CYP3A4 and CYP3A5. Differences in the concentrations of CYP3A4/CYP3A5 or drugs that can inhibit or induce said enzymes have the potential to greatly affect the levels of imatinib in plasma (8). Also, in this regard, dose escalation of imatinib (600–1000 mg/ d) can overcome resistance to standard-dose imatinib (400 mg/ d) in some patients with CML. In case of P-loop mutations, escalation of the dose of imatinib is not recommended owing to highly resistant nature of these mutations (41).

CML vaccines

In recent years, a series of DNA vaccines have been developed based on different aspects to target the CML cells. Lucansky et al. (49) demonstrated the use of a plasmid carrying either the complete BCR–ABLl fusion gene or fragment thereof coding for a 25-amino-acid sequence- long junction zone (bcr–abl 25 amino acids) linked with genes coding for a variety of immuno stimulatory factors, as the DNA vaccines in mice. Peptide vaccines derived from amino acid sequences crossing the b3a2 fusion breakpoint have proved their efficacy in patients with chronic-phase CML (50). These vaccines elicit class I restricted cytotoxic T lymphocytes and class II responses.

Destabilizing BCR–ABL protein

Stability of Bcr–abl is dependent upon its ability to form complex with HSP90 and co-chaperone protein p23. HSP90 antagonist such as 17-allylamino-17-demethoxygeldanamycin (17-AAG) interfere with Bcr–abl chaperoning such that the mature protein would be unable to fold correctly into its quaternary structure, leaving it susceptible to proteasome degradation (9).

NIIH Experiences

Our experience on CML patients suggest that majority of them are in younger age group as compared to the studies done on western populations. The factors which could be responsible for this younger age presentation are likely to be genetic and/or environmental with early risk exposure.

At the time of disease presentation, majority of the patients were in chronic phase of the disease followed by accelerated and blast crisis. Patients in chronic phase were started with the standard dose of imatinib (400mg/day) and patients with advanced phase were given a higher dose of 600mg imatinib/day. All the patients were monitored closely at the interval of three months for the response milestones achieved according to the European Leukemia.net. At the end of 18 months, the patients were categorised on the basis of response to treatment. 55% of the patients responded whereas 45% showed resistance or suboptimal response to imatinib. The response rate in our study was found to be very poor as compared to the western population, where it is reported to be more than 75%. The factors contributing to the low response rate could be attributed to the poor hygiene and ignorance about the patient care.

The incidence of mutations in the ABL KD in our

study groups was found to be 39%, whereas in the regulatory region (SH3-SH2), the incidence of occurrence of mutation was 5.4%. The frequency of mutations was found to be more in the P-loop, accounting to 41% of the total mutations. The most common and the highly resistant mutation found in the P-loop was Y253H/F with a frequency of 17% and the PPHv2 damaging score of 1. Four novel mutations were detected, two in KD and two in regulatory regions.

Conventional cytogenetics is routinely carried out in the department of cytogenetics. Almost 70% of the CML patients show the 'Classical Philadelphia Pattern' i.e., t(9;22)(q34;q11). Chromosome 9q deletions and chromosome 22q deletions are found in almost 25% of the CML patients whereas, variant translocations are found in 5% of the cases (Fig.5).

Single nucleotide polymorphisms associated with imatinib response were also studied in imatinib resistant patients. Of the many SNPs of CYP3A4, CYP3A5 and hOCT gene studied, only CYP3A5*3 and AA genotype for M408 SNP were found to be associated with poor imatinib response. Increase in dosage of imatinib or change of therapy to second generation TKI was seen to be beneficial for patients with these polymorphisms.

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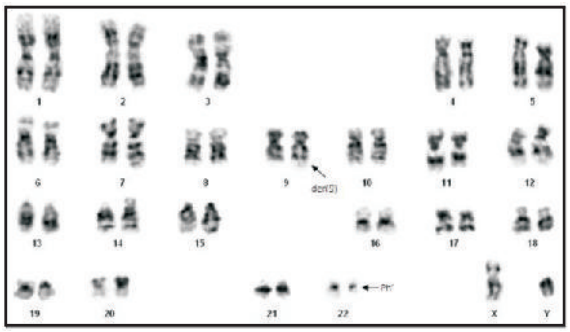

Fig. 1. G banded karyotype showing classical Philadelphia pattern 46, XY t(9;22)(q34;q11)

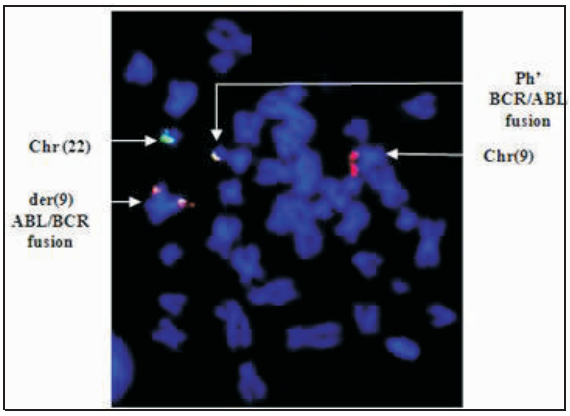

Fig. 2. FISH showing double reciprocal translocation using BCR/ABL dual colour, double translocation probes.

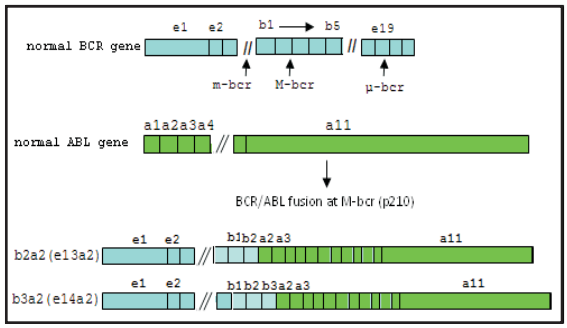

Fig. 3. Diagram showing two types of BCR/ABL fusion transcripts

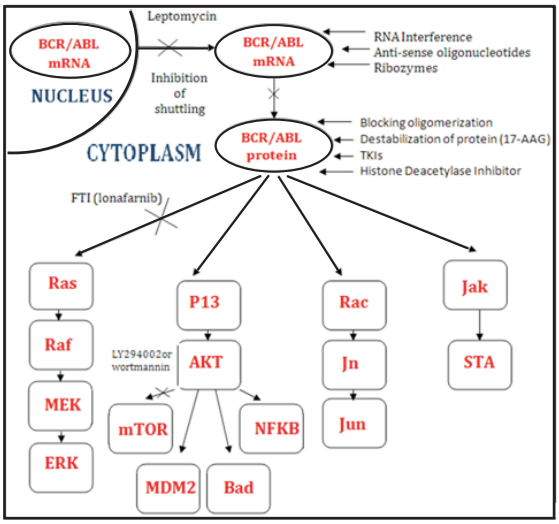

Fig. 4. Strategies to overcome Imatinib resistance at various levels

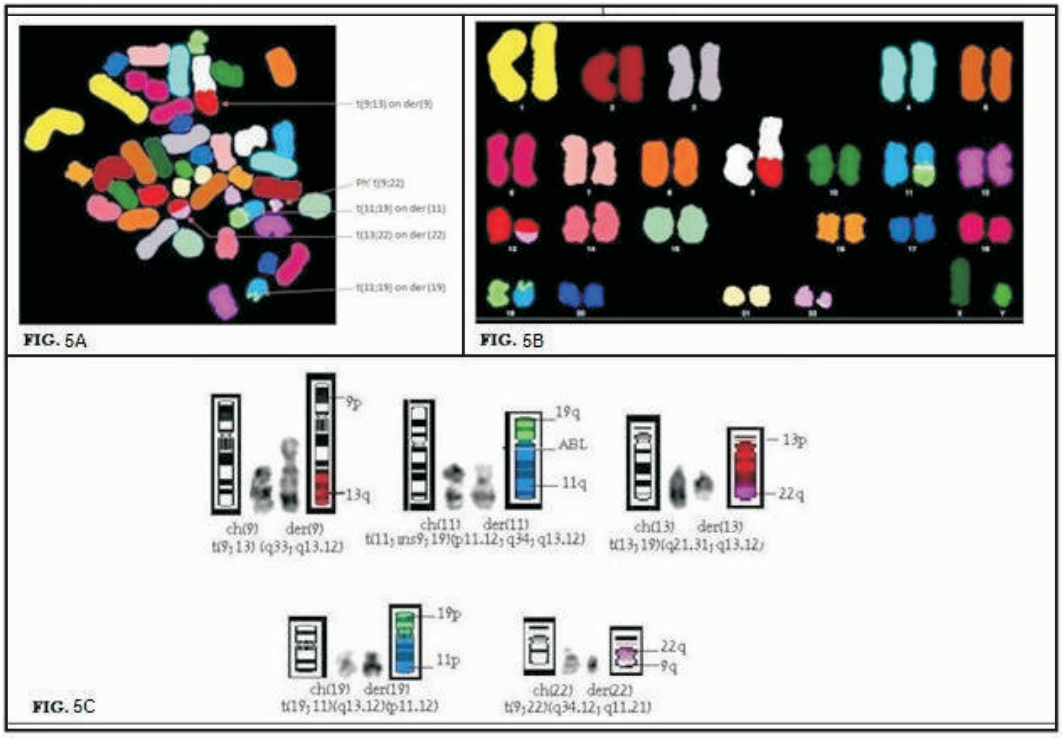

Fig. 5. A and B, SKY showing 5-way translocation 46,XY,t(9;11;13;19;22)(9q34.12;11p11.12;13q21.31;19q13.12;22q11.21). C, Partial karyotyping translocation breakpoints on chromosomes 9, 11, 13, 19, and 22.

LIBRARY INFORMATION SCIENCE

Vijay Padwal, ALIO

GENERAL INFORMATION

ICMR has renewed the subscription to the J-Gate and JCCC@ICMR and Medical e-journals like Lancet, Nature, Science and NEJM for all the ICMR institutes. J-Gate has lauched the new JCCC platform viz; "J-Gate Plus" which have several new features and functionalities. The new platform enable each and every beneficiary institute to create its own account in the JCCC and participate in the process of document delivery. Library being a key source between the readers plays a vital role in collection, development and dissemination of scientific and technical information to meet the future needs of the Institute. Library is equipped with high speed WI-FI internet facility and digital resources. The NIIH Library was planning for the digitalization and fully automation of the library resources by implementing the Digital Library Software and Advanced Library Automation Software. The library would like to point out some new upgraded tips for the J-Gate Plus database as mentioned below.

J-Gate Database:

J-Gate is an electronic gateway to global e-journals. It is lauched in 2001 by Informatics India Limited. It provides seamless access to millions of journal articles available online offered by 11428 publishers. It presently has a massive database of journal literature, indexed from 36,987 e-journals with links to full text at publisher sites. It provides two types of product/services:

i) Table of Content (TOC)
ii) Database

J-Gate Features

Truly e-journal portal
Portal with largest number of e-journals
Access to 4,833 e-only-journals
17,009 open-access online journals
Links to 5,320,569 open-access articles
Full-text link to largest number of publisher sites
Union List linkage
Comprehensive journal classification
Easy-to-use search functionalities

J-Gate Benefits

- J-Gate Database consists of more extensive and comprehensive content than TOC.
- Classification on three levels of subject category.
- Subject-wise indexing.
- Search by Title, Author, Subject Categories, Keywords, Year and any combination of these.
- Provides basic bibliographic data with abstracts (where available).
- Author Address and e-mail where available.
- Link to full-text (both open-access and subscription).
- Link to Union List for finding availability.
- Daily updating with progressive accumulation.

Publications

Annual report of the Institute for the year 2013-2014 compilation is in progress.

Some Recent Additions (Apr2013-Mar 2014) :

Books	-	28
Journals(International)	-	39
(National)	-	16
E-Journals	-	22
Bound Volumes	-	-
CD-ROM/DVD	-	36

NIIH Happenings

Dr K Ghosh, Director

1. Invited as a member to the Scientific Advisory Committee Meeting of Valsad Raktdan Kendra, Valsad from 9th to 10th January 2014.
2. Delivered Dr. Sunil Chandra Bose Oration, at West Bengal Academy & Technology 2014, Kolkata on 17th January 2014. The topic of the Oration was "Cardiology Seen through the Window of Haematology".
3. Delivered S.K.Menon Memorial Oration Award, at SMSMC, Jaipur on 31st January 2014. The topic of the Oration was "Crisis of Medical Education in India".
4. Invited to deliver a talk on "Viruses and Man" at the National Institute of Virology Foundation day, Pune on 7th February 2014.
5. Invited as an expert to the Selection Committee Meeting of UPSC to Select Grade III (Pathology) candidates at UPSC, New Delhi on 23rd February 2014.
6. Attended the Project Review Committee Meeting of Stem Cell at ICMR, New Delhi on 26th February 2014.
7. Invited to deliver a talk on "Haemolgobinopathy" at Foundation Day Celebration of RMRC, Jabalpur on 28th February 2014.
8. Invited as an expert member to Annual Day Research Meeting of Hinduja Hospital, Mumbai on 1st March 2014.
9. Invited as an examiner to conduct MD Transfusion Medicine Examination at PGIMER, Chandigarh from 3rd to 4th March, 2014.
10. Received the "Ranbaxy Award 2012" at New Delhi on 22nd March 2014.
11. Invited as an External Examiner to conduct Viva Voce examination of Ph.D. thesis at Arabian Gulf University, Baharain from 23rd to 26th March 2014.
12. Attended the Meeting of JNU in respect to post graduation studies to be conducted by ICMR at New Delhi on 11th April 2014.
13. Invited as an expert for Scientific Advisory Committee for Surat Raktdan Kendra, Surat on 19th April 2014.
14. Attended the Scientific Advisory Group Meeting at ICMR, New Delhi on 23rd April, 2014.
15. Attended the Technical Resource Group Meeting at NACO, New Delhi from 24th to 25th April 2014.
16. Attended the Annual Hemophilia Meeting on 25th to 26th April 2014.

Department of Hematogenetics

Dr Roshan B Colah, Scientist F

1. Visited Bulsar Raktadan Kendra, Valsad in relation to New Born Screening programme among the tribal groups of South Gujarat from 9th to 10th January 2014.
2. Invited to delivered a talk on "Thalassemia: Can we reduce the National burden" at the International Conference on Human Genetics and 39th Annual Meeting of Indian Society of Human Genetics held at Ahmedabad from 22nd to 25th January, 2014.
3. Attended the Red Cross Blood Transfusion Sub-Committee meeting on 28th January, 2014.
4. Attended the Tribal Health Research Forum meeting held on 5th April 2014 at RMRC, Belgaum.
5. Attended the Hemoglobin Update Bio-Rad users meet on 22nd April, 2014

Dr Malay Mukherjee, Scientist D

1. Visited Bulsar Raktadan Kendra, Valsad in relation to New Born Screening programme among the tribal groups of South Gujarat from 9th

to 10th January 2014.

2. Attended International Conference on Human Genetics and 39th Annual meeting of Indian Society of Human Genetics held at Ahmedabad from 22nd to 25th January 2014 and presented a paper "Genetic variations in the UGT1A1 and OATP2 genes and their association with neonatal hyperbilirubinemia".
3. Attended "Tribal Health Research Forum Meeting" held at RMRC, Belgaum on 5th April 2014.
4. Attended "4th Hemoglobin Update Bio-Rad users Meet" held at Mumbai on 22nd April, 2014.

Dr P S Kedar, Technical Officer

1. Invited as a Faculty member and delivered a lecture on "Red cell Enzymopathy" in the workshop on "Enzymology" held at Haffkine Institute for Training Research and Testing, Mumbai from 18th to 19th January 2014.
2. Presented a paper entitled "Molecular characterization and pathophysiology of hereditary non-spherocytic hemolytic anemia and methemoglobinemia associated with red cell enyzmopathies in India at the International Conference on Human Genetics and 39th Annual Meeting of Indian Society of Human Genetics held at Ahmedabad from 22nd to 25th January, 2014.
3. Presented a paper entitled "Primaquine induced methemoglobinemia in plasmodium vivax malaria patients associated with a novel mutation (R57W) in NADH-Cytochrome B5 Reductase gene" at the 37th Annual Conference of Mumbai Hematology Group held at ACTREC, Navi Mumbai from 22nd to 23rd March 2014.

Following staffs and students have attended and presented papers in the 37th Annual Conference of Mumbai Hematology Group held at ACTREC, Navi Mumbai from 22nd to 23rd of March 2014.

1. Dipti Upadhye : Presented a paper entitled "HbM Boston and HbM Hyde Park in two Indian families – a rare cause of methemoglobinemia".
2. Pallavi Mehta : Presented a paper entitled "Implications of associated alpha globin gene triplication on β thalassaemias" and awarded 2nd prize.
3. Vrushali Pathak : Presented a paper entitled "H blood group antigen: A possible receptor in plasmodium falciparum invasion" and awarded 2nd prize.
4. Harshada Kangne : Presented a paper entitled "Association of variant alleles of endothelial nitric oxide synthase (eNOS) and Mannose binding lectin (MBL2) gene with acute chest syndrome and infections in sickle cell disease patients from western India".
5. Dr. Khushnuma Italia : Presented a poster entitled "A twin pregnancy in HbE-β thalassaemia".
6. Dr. Prashant Warang : Presented a poster entitled "Use of microplate for screening various haematological disorders".

Ms Vrushali Pathak undergone a training on "In vitro malarial parasite culturing and testing for sensitivity of P. falciparum isolates to anti-malarials" at National Institute of Malaria Research, New Delhi from 15th to 25th April 2014.

Department of Transfusion Medicine

Dr A C Gorakshakar, Scientist E

1. Attended 101st session of Indian Science Congress held at Jammu from 3rd to 7th February 2014 and

delivered a talk entitled "Genetic Disorders in India - Development of indigenous kit for early detection of beta thalassaemia and sickle cell anemia" in Public Outreach Programme organized by ICMR on 5th February.

2. Delivered Dr J.G.Parekh Oration on a topic entitled "Blending Genes and Geography in Hematology Research" at 37th Annual Conference of Mumbai Hematology Group held at ACTREC, New Mumbai from 22nd to 23rd March 2014.
3. Attended a Conference on "Good Clinical Practice" organized by Maharashtra University of Health Sciences, Nashik, held at KEM Hospital, Mumbai on 5th April 2014.
4. Awarded Second Prize (Dr Satoskar Prize) for poster presentation on a paper entitled "Nanodiagnostic approach for blood group detection" at 7th International Annual Conference on "Clinical Pharmacology - Translational Research: Patient to Public Health" held at Mumbai from 17th to 20th April 2014.

Dr Swati Kulkarni, Scientist C

1. Attended 37th annual conference of Mumbai Haematology Group held at ACTREC, New Mumbai from 22nd to 23rd Feb, 2014.

   Following students have attended and presented papers in the 37th Annual Conference of Mumbai Hematology Group held at ACTREC, Navi Mumbai from 22nd to 23rd of March 2014.

1 Harita Gogri : Presented a poster entitled "Spectrum of red cell allo antibodies in patients with different clinical conditions - Experience of a reference center" and awarded 1st prize.

2. Pearl Breganza : Presented a poster entitled "Genetic Studies among the Pathare Prabhus: An Indigenous Population from Mumbai" and awarded 2nd prize.
3. Bhavika Choudhary : Presented a paper entitled "Molecular Genotyping of Clinically Significant (Rh, Duffy, Kell and Kidd) Blood Group Antigens in Multitransfused Thalassemic Patients".

Department of Hemostasis

Dr. Shrimati Shetty, Scientist E

1. Invited to deliver a talk on "Management and Diagnostic challenges of haemophilia in Iindia" at the International Conference of Human Genetics and 39th Annual Meeting of ISHG, held at Ahmedabad from 22nd to 25th January 2014.

Students :

1. Ms. Patricia Pinto was awarded the 'Young Scientist Award' for the research paper entitled "Genetic and Non-Genetic Risk Factors for the development of FVIII Inhibitors in Indian Haemophilia A patients" at the 39th Annual Conference of the Indian Society of Human Genetics, held at Ahmedabad from 22nd to 25th January 2014.
2. Ms. Patricia Pinto presented a paper on "A Study of the *F8* Mutation Profile in Indian Severe Haemophilia A Patients with and without FVIII Inhibitors" at the 37th Annual Conference of the Mumbai Hematology Group held at ACTREC, Navi Mumbai from 22nd to 23rd March 2014.
3. Rucha Kiran Patil was awarded the HM Bhatia award at the 37th Annual Conference of Mumbai Hematology Group held at ACTREC, Navi

Mumbai from 22nd to 23rd March 2014, for the paper entitled "Risk Assessment of Thrombophilia Markers, New and Old, and Anticoagulation Therapy in Recurrent Pregnancy Loss".

Department of Cytogenetics

Dr..V.Babu Rao, Scientist D

1. Attended International conference on Human Genetics and 39th Annual meeting of Indian Society of Human Genetics, held at Ahmedabad, from 22nd to 25th January 2014 and delivered a lecture on "Chromosomal instability and molecular mutations in multi spectrum disease of Fanconi Anemia".
2. Attended conference on Cancer Genomics and 3rd annual conference of Molecular Pathologist Association of India, held at Mumbai from 14th to 15th February 2014. Chaired the session on Molecular Cytogenetics and delivered a lecture on "Molecular Karyotyping and diagnostic importance in hematological malignancies".
3. Attended as a chairman of Institutional Ethics Committee meeting at Surat Raktadan Kendra & Research Centre, Surat on 1st March 2014.
4. Delivered a guest lecture on "Recent advances in Human cytogenetics: Its importance in health and disease" in the CME on Developmental Genetics, held at MGM Medical college, Aurangabad on 7th March 2014.

Seema Korgaonkar, Technical Officer

1. Presented a paper entitled "Cytogenetic and Morphological Pattern in De-Novo Acute Myeloid Leukemia Patients" in the 37th Annual Conference of Mumbai Hematology Group held at ACTREC, Navi Mumbai from 22nd to 23rd of March 2014.

Students :

1. Shantashri Vaidya was awarded Young Scientist award for the research paper entitled "Understanding the interplay between BCR/ABL dependent and independent mechanisms of imatinib resistance" at the International Conference of Human Genetics and 39th Annual Meeting of the Indian Society of Human Genetics held at Ahmedabad from 22nd to 25th January 2014.

Department of Pediatric Immunology & Leukocyte Biology

Dr. Manisha Madkaikar, Scientist E

1. Invited to deliver a talk on 'Lymphocyte subset analysis for Primary Immunodeficiency Disorders' in the CME in haematology held at GCRI, Ahmedabad on 23rd Feb 2014.
2. Invited to deliver the following talks at the 2nd national conference on Primary Immunodeficiency Diseases held at Varanasi from 8th to 9th March 2014.

i. 'Clinical, molecular and diagnostic aspects of HLH'.

ii. 'Applications of flowcytometry for diagnosis of PID'

Following staffs and students have attended and presented papers in the 37th Annual Conference of Mumbai Hematology Group held at ACTREC, Navi Mumbai from 22nd to 23rd of March 2014.

1 Swati Garg : Presented a poster entitled "Expression of cell surface markers on hematopoietic stem cells: niche dependent

variations and comparison with acute leukemia initiating cells" and was awarded as best poster in clinical category.

2. Aparna Dalvi : Presented a poster entitled "CD40 deficiency, a rare cause of hyper IgM syndrome".
3. Manasi Kulkarni : Presented a poster entitled "Flow cytometry: Rapid detection of subtypes by flow cytometry; useful screening tests for Chronic Granulomatous Disease".

Department of Clinical and Experimental Immunology

Dr. Vandana Pradhan, Scientist B

1. Attended "Workshop on Good Clinical Practice" held at KEM Hospital, Mumbai, on 5th April 2014.

Others

1. Mr Suhas Shirshat, Technical Officer superannuated on 31st March 2014 after complication of 31 years of service.
2. Ms Reema Surve, Technical Assistant opted voluntary retirement on 1st April 2014 after completion of 21 years of service.

Faculty and Participants in the Annual Training Programme on Transfusion Medicine

Expert Committee Meeting for the Centre of Excellence on Primary Immunodeficiency Disorders

Mumbai Hematology Group Meeting

Farewell to Mr Suhas Shirsat, Technical Officer and Ms Reema Surve, Technical Assistant

Prof Neelam Marwaha receiving Dr H M Bhatia Memorial Oration from Dr V Ray

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