Chronic Myeloid Leukemia – An Overview

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Abstract: ***Chronic myeloid leukemia (CML) is a clonal stem cell disease caused by an acquired somatic mutation that fuses, through chromosomal translocation, the abl and bcr genes on chromosomes 9 and 22, respectively. It is the commonest adult leukemia in India and the first cancer in which a consistent chromosomal abnormality was observed. Chronic Myeloid Leukemia (CML) is best described disease resulting from the t (9; 22) (q34, q11.2) and other oncogenic BCR fusions. CML typically begins in the chronic phase, and over the course of several years progresses to an accelerated phase and ultimately to a blast crisis. The major modality of treatment of CML, inhibitors of the tyrosine kinase (TKI), the commonest used is Imatinib. The results in patients failing or intolerant to imatinib intrigued the investigators for searching new modifications and work with second generation TKI for CML patients. Second generation TKIs consisted of nilotinib, dasatinib and bosutinib. Future trials should: i) compare the newer TKIs with high-dose imatinib as front-line treatment in newly diagnosed Chronic phase-CML patients; ii) examine the option of discontinuing TKIs after the achievement of complete molecular response; and iii) evaluate novel therapeutic strategies, such as combination or consecutive use of different TKIs, as well as combinations with agents which influence the quiescent stem cell compartment.***

Keywords: Chronic myeloid leukemia, Philadelphia chromosome, tyrosine- kinase inhibitors (TKI), abl and bcr genes, translocation.

1. Introduction

CML is a myeloproliferative disorder characterized by a specific cytogenetic abnormality, the Philadelphia chromosome (Ph), a balanced translocation, involving a fusion of the Abelson oncogene (ABL) from chromosome 9q34 with the breakpoint cluster region (BCR) on chromosome 22q11. The molecular consequence of this translocation is the generation of a BCR-ABL fusion oncogene, which translates into Bcr-Abl oncoprotein with increased tyrosine kinase activity. (1)

CML is the commonest adult leukemia in India and the annual incidence ranges from 0.8–2.2/100,000 population in males and 0.6–1.6/100,000 population in females in India. The median age of diagnosis is 38-40 years. Though CML is predominantly a disease affecting adults, a minority of patients are children and young adults. (17,18)

Leukemia that start in early myeloid cells - the cells that become white blood cells (other than lymphocytes), red blood cells, or platelet-making cells (megakaryocytes)-are called myeloid leukemia. These are also known as myelocytic, myelogenous, or nonlymphocytic leukemia. CML is caused by an acquired somatic mutation that fuses, through chromosomal translocation, the *abl* and *bcr* genes on chromosomes 9 and 22, respectively. CML is the first cancer in which a consistent chromosomal abnormality the Philadelphia chromosome was described by Nowell in 1962. (18)

Normally, the bone marrow makes blood stem cells (immature cells) that become mature blood cells over time. A blood stem cell may become a myeloid stem cell or a lymphoid stem cell. A lymphoid stem cell becomes a white blood cell. A myeloid stem cell becomes one of three types of mature blood cells:

- Red blood cells that carry oxygen and other substances to all tissues of the body.
- Platelets that form blood clots to stop bleeding.
- Granulocytes (white blood cells) that fight infection and disease.

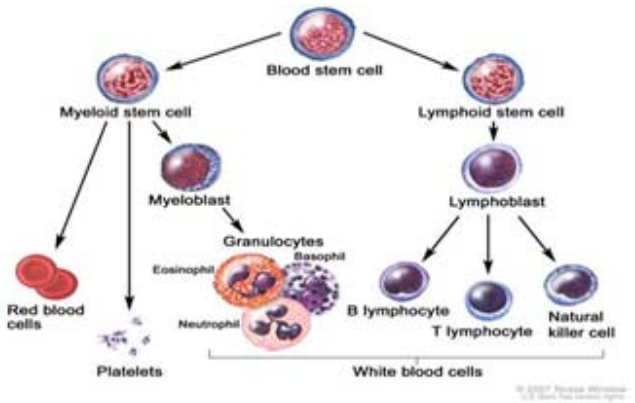

Figure 1: Clonal proliferation

CML is often suspected on the basis of a complete blood count, 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. A bone marrow biopsy is often performed as part of the evaluation for CML, and CML is diagnosed by detecting the Philadelphia chromosome. This characteristic chromosomal abnormality can be detected by routine cytogenetics, by fluorescent in situ hybridization, or by PCR for the bcr-abl fusion gene. Controversy exists over so-called *Ph-negative* CML, or cases of suspected CML in which the Philadelphia chromosome cannot be detected. Many such patients in fact have complex chromosomal abnormalities that mask the (9; 22) translocation, or have evidence of the translocation by FISH or RT-PCR in spite of normal routine karyotyping. The small subset of patients without detectable molecular evidence of bcr-abl fusion may be better classified as having an undifferentiated myelodysplastic / myeloproliferative disorder, as their clinical course tends to be different from patients with CML.

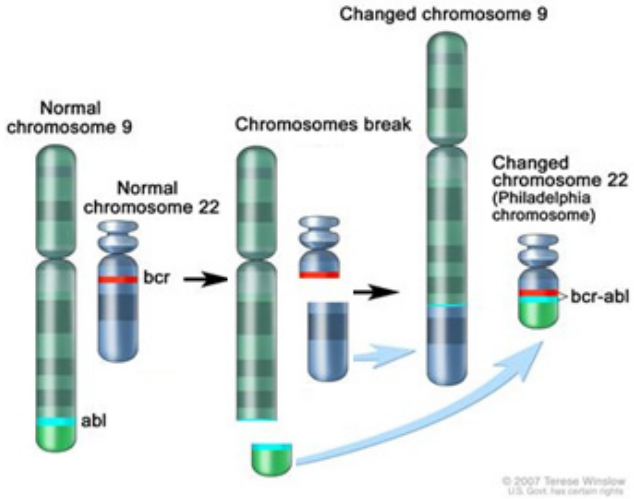

Figure 2: Translocation 9:22

In this translocation, parts of two chromosomes (the 9th and 22nd by conventional karyotypic numbering) switch places. As a result, part of the BCR ("breakpoint cluster region") gene from chromosome 22 is fused with the ABL gene on chromosome 9. This abnormal "fusion" gene generates a protein of p210 or sometimes p185 weight. Because abl carries a domain that can add phosphate groups to tyrosine residues (a tyrosine kinase), the *bcr-abl* fusion gene product is also a tyrosine kinase. The fused BCR-ABL protein interacts with the interleukin 3beta(c) receptor subunit. The BCR-ABL transcript is continuously active and does not require activation by other cellular messaging proteins. In turn, BCR-ABL activates a cascade of proteins that control the cell cycle, speeding up cell division. Moreover, the BCR-ABL protein inhibits DNA repair, causing genomic instability and making the cell more susceptible to developing further genetic abnormalities. The action of the BCR-ABL protein is the pathophysiologic cause of chronic myelogenous leukemia.

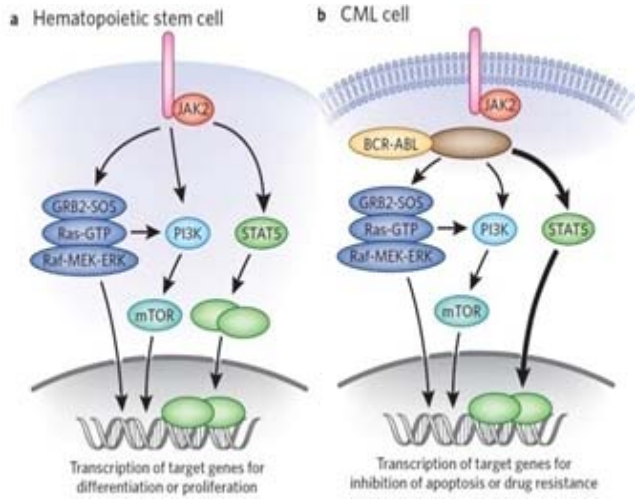

Figure 3: Normal hematopoietic stem cells following activation of cytokine (IL-3 or GMCSF) receptors

In normal hematopoietic stem cells following activation of cytokine (IL-3 or GMCSF) receptors, JAK2 phosphorylates STAT5 at a critical tyrosine residue close to the SH2 domain. Subsequently, STAT5 dimerizes and translocates to the nucleus, where the dimers bind DNA at target gene promoters. (**b**) In CML cells, BCR-ABL phosphorylates STAT5 at the same critical tyrosine residue close to the SH2 domain, inducing the same downstream events independently of JAK2. (Nature: ref).

The *bcr/abl* gene product is an oncogenic protein that localizes to the cytoskeleton and displays an up-regulated tyrosine kinase activity that leads to the recruitment of downstream effectors of cell proliferation and cell survival and consequently cell transformation.

2. Cytogenetic Study

This characteristic chromosomal abnormality can be detected by routine cytogenetics, by fluorescent in situ hybridization. Chronic Myeloid Leukemia (CML) is the best described disease resulting from the t(9;22) (q34,q11.2). In this translocation, parts of two chromosomes (the 9th and 22nd by conventional karyotypic numbering) switch places.

Figure 4: The abnormality seen by Nowell & Hungerford on chromosome 22. Now Known as the Philadelphia Chromosone

This chromosomal rearrangement leads to the well-known BCR-ABL fusion that promotes tyrosine kinase activity. Other oncogenic BCR fusions have also been found, such as platelet-derived growth factor receptor-alpha gene (PDGFRA) (4q12) and fibroblast growth factor receptor 1 (FGFR1) (8p12), which cause myeloproliferative disorders (MD). An extremely rare case of atypical CML that was found to be breakpoint cluster region (BCR)-Abelson (ABL) 1 (BCR-ABL1) -negative, due to the BCR-JAK2 fusion resulting from a t (9; 22) (p24, q11.2) translocation. To the best of our knowledge, this is the first case reported in Brazil and the sixth in the world. (15)

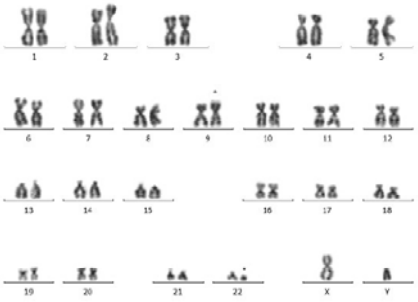

Figure 5: Conventional cytogenetics showing46, XY, t (9; 22) (p24; q11.2) karyotype

Conventional karyotyping was performed again and the 46, XY, t (9; 22) (p24; q11.2) karyotype was observed in all metaphases examined. (15)

3. Molecular Study

Cytogenetic analysis is still the standard technique for defining the response of patients to treatment, but this technique suffers from serious limitations. Bone marrow metaphases are required and aspiration and cultivation of proliferating cells is not always sufficient. Furthermore, this technique is relatively insensitive since typically a maximum number of only 20–50 metaphases are analyzed. Levels of BCR-ABL mRNA can be detected prior to cytogenetic or hematologic relapse. Quantitative PCR data are used to initiate donor lymphocyte transfusions for treatment of relapse and to monitor its response. With the real-time PCR, the direct measurement of the amount of PCR product and also reflects the dynamics of the reaction is enabled. Although the final amount of PCR product at the plateau phase usually does not correlate well with the number of starting molecules in the reaction, the time-point (cycle number) at which the fluorescence for a particular sample rises above the background (crossover point or threshold cycle) is a much more accurate indicator of the initial number of PCR targets. Amplification and specific detection of fusion transcripts by real-time PCR is possible by simultaneous amplification and fluorescence melting curve analysis using non-specific labeling. Controversies of *Ph-negative* CML cases, or cases of suspected CML in which the Philadelphia chromosome cannot be detected are seen. Many such patients in fact have complex chromosomal abnormalities that mask the (9; 22) translocation, or have evidence of the translocation by FISH or RT-PCR in spite of normal routine karyotyping. [20] The small subset of patients without detectable molecular evidence of bcr-abl fusion may be better classified as having an undifferentiated myelodysplastic/ myeloproliferative disorder, as their clinical course tends to be different from patients with CML.

4. How CML is staged?

CML is often divided into three phases based on clinical characteristics and laboratory findings. In the absence of intervention, CML typically begins in the *chronic* phase, and over the course of several years progresses to an *accelerated* phase and ultimately to a *blast crisis*. Blast crisis is the terminal phase of CML and clinically behaves like an acute leukemia whose progression can be stopped by drug treatment.

During chronic phase, patients are usually asymptomatic or have only mild symptoms of fatigue, left side pain, joint and/or hip pain, or abdominal fullness. The duration of chronic phase is variable and depends on how early the disease was diagnosed as well as the therapies used. In the absence of treatment, the disease progresses to an accelerated phase.

The accelerated phase are variable; the most widely used criteria are those put forward by investigators at M.D. Anderson Cancer Center, by Sokal et al., and the World Health Organization. The WHO criteria are most widely used, and define the accelerated phase by any of the following:

- 10–19% myeloblasts in the blood or bone marrow
- >20% basophils in the blood or bone marrow
- Platelet count <100,000, unrelated to therapy
- Platelet count >1,000,000, unresponsive to therapy
- Cytogenetic evolution with new abnormalities in addition to the Philadelphia chromosome
- Increasing splenomegaly or white blood cell count, unresponsive to therapy

The patient is considered to be in the accelerated phase if any of the above is observed in the patient. The accelerated phase is significant because it signals that the disease is progressing and transformation to blast crisis is imminent. Drug treatment often becomes less effective in the advanced stages. Blast crisis is the final phase in the progression of CML, and behaves like an acute leukemia, with rapid progression and short survival. Blast crisis is diagnosed if any of the following are present in a patient with CML:

- >20% myeloblasts or lymphoblasts in the blood or bone marrow
- Large clusters of blasts in the bone marrow on biopsy
- Development of a chloroma (solid focus of leukemia outside the bone marrow). (16)

5. Therapeutic Approach

CML has a biphasic or triphasic clinical course: 90% of the patients are diagnosed in the chronic phase, but the disease eventually evolves to a blastic phase unless successfully treated. Approximately two-thirds of the patients manifest an accelerated phase. A distinct feature of the disease progression is the appearance of additional cytogenetic abnormalities in the Ph+ cells. (6)

The major modality of treatment of CML inhibitors of the tyrosine kinase, the commonest used is Imatinib. These are chemical competitive inhibitors of ATP which is required for phosphorylation of tyrosine residues of downstream proteins in the signaling pathways of ABL tyrosine kinase. Newer 2 generation tyrosine kinase inhibitors like Nilotinib and Dasatinib are used in patients resistant to Imatinib. By blocking the function of the tyrosine kinase protein, the drug effectively reduces the abnormal effects of this "bad" *bcr-abl* gene, (i.e. the Philadelphia chromosome). In addition, the drug can also cause direct death of the *bcr-abl* - expressing cells to die (apoptosis). (21, 18)

The pivotal International Randomized Study of Interferon and STI571 (IRIS) established imatinib as first-line treatment in chronic phase (CP) CML.(8) It showed that 69% of patients given front-line imatinib treatment achieved complete cytogenetic response (CCyR) after 12 months of treatment, 57% of them also achieving a major molecular response (MMR). However, 7.9% progressed to accelerated phase (AP) or blastic crisis (BC). (7)

Despite the excellent results obtained in the IRIS trial, 40-45% of patients discontinue imatinib for various reasons. These include also unsatisfactory therapeutic outcomes in

16% of patients defined as failure to achieve response by a specific time point (i.e. complete hematologic response, CHR) at three months, or primary resistance, or the loss of initial response (e.g. loss of secondary resistance). [10]

These results in patients failing or intolerant to imatinib intrigued the investigators for searching new modifications and work with second generation TKI for CML patients. Clinical trials showed an advantage in 2nd generation TKIs when used as second-line treatment in patients with CP-CML. Newer agents showed higher rates of cytogenetic and molecular responses.

Second generation TKIs consisted of nilotinib, dasatinib and bosutinib. Like imatinib, nilotinib binds an inactive conformation of *BCR–ABL1*; with a 30-50 fold increased binding affinity. [11] Dasatinib binds to a distinct, although overlapping, binding site within the ATP-binding pocket and is 325-fold more potent than imatinib. Bosutinib binds to a confirmation of ABL1 that is transitional between the active and inactive conformations and is approximately 25-fold more potent than imatinib *in vitro*. [12, 13]

With IFN-alfa-based therapy, 30-70% of patients achieved a cytogenetic response, which was major in 20-50 %.[3] Fayad et al. first reported the occurrence of cytogenetic abnormalities in the Ph-negative cells in three patients responding to IFN-alfa. One of these patients developed 5q- in one analysis and a complex karyotype in a subsequent analysis, the second developed +18p11, and the third developed deletion 11q21. From the cited literature, it was seen the first patient developed a myelodysplastic syndrome 86 months later and the other patients remained in complete haematological and cytogenetic response at the time of the report (6 months and 33 months, respectively) after these abnormalities first were noted [5]. The pathogenesis and clinical significance of these abnormalities is still unclear. To date, all patients reported have been previously treated with chemotherapy. [5]

Previous treatment with idarubicin and cytosine arabinoside (Ara-C) has been suggested as a risk factor. [5] Most patients have been treated with Ara-C in combination with IFN-alfa many years. The majority of patients who have failed to respond to IFN-alfa have received other agents, most frequently homoharringtonine before imatinib became available. Another important observation is that patients treated with high-dose imatinib (i.e., 800 mg/day) have not developed these abnormalities. Although few patients have so far received the higher doses, it is possible that a faster and more effective elimination of the Ph malignant clone, as noted with high-dose imatinib, may decrease the risk of development of chromosomal abnormalities in the Ph-negative stem cells. [4] Longer follow-up on a larger population is required to clarify this issue further.

Future trials should: i) compare the newer TKIs with high-dose imatinib as front-line treatment in newly diagnosed CP-CML patients; ii) examine the option of discontinuing TKIs after the achievement of complete molecular response; and iii) evaluate novel therapeutic strategies, such as combination or consecutive use of different TKIs, as well as combinations with agents which influence the quiescent stem cell compartment. [1]

Imatinib and other drugs that target the BCR-ABL protein have proven to be very effective, but by themselves these drugs don't help everyone. Studies are now in progress to see if combining these drugs with other treatments, such as chemotherapy, interferon, or cancer vaccines might be better than either one alone. One study showed that giving interferon with imatinib worked better than giving imatinib alone. The 2 drugs together had more side effects, though. It is also not clear if this combination is better than treatment with other TKIs, such as dasatinib and nilotinib. A study going on now is looking at combing interferon with nilotinib.[16] Another approach is use of a cancer vaccine -- a substance injected into the body that boosts the immune system and causes it to attack certain cells. Several vaccines are now being studied for use against CML; like a vaccine called *CMLVAX100* was given along with imatinib and seemed to increase its effectiveness. Research is on going with different kind of vaccines for this. [16, 22]

6. Conclusion

In CML, during the reciprocal translocation a segment of *ABL* gene (9q34) is moved into one of at least 3 well characterized breakpoints of the *BCR* gene. In short, the routine monitoring along with cytogenetic evaluation allows the patients and clinicians about the long term aspects of disease control information for cml treatment. Additionally, karyotypic abnormalities predict an advanced stage of the disease and a poorer response to Imatinib. The drug therapies progress is helping patients with CML to fight against this cancer and do a good management in follow ups, survival rate and remission. There is still a lot to be done so that the new strategic drugs can reach out to people for the treatment.

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