Introduction
CML is a clonal stem cell disorder arise from abnormal pluripotent stem cell characterized by the acquisition of an oncogenic BCR/ABL fusion protein resulting from a reciprocal translocation t(9; 22). CML is often referred to as disease of 'first' as it was the first disease in which the term leukemia was first utilized, with consistently associated with recurring chromosomal abnormality, to be the direct result of a specific gene fusion, and to have first targeted therapy. Translocation t(9; 22) is also known as Philadelphia chromosome according to the city where it was first identified by Nowell and Hungerford in 1960. Objective of this review article is to discuss about accuracy of diagnosis, early & appropriate initiation of therapy and monitoring of CML patient in a comprehensive way to assess the optimal haematological, cytogenetic & molecular response in timely manner.

Epidemiology
CML accounts for 15%-20% of leukaemia cases in adults. The worldwide incidence of CML is 1-2 cases per 100,000 populations with a slight male predominance. Median age at diagnosis is 50 years, but in Bangladesh most CML patients are from a younger age group (31-40 years) with 86%, 11% and 3% in chronic phase, accelerated phase and blastic phase respectively. Radiation exposure has been implicated as a risk factor evident by increased CML incidence in survivors of the atomic bomb explosions in Japan, though in many cases etiological factors cannot be found.

Pathophysiology
CML is a myeloproliferative neoplasm (MPN) that share several distinct features with other MPN. It is a clonal disorder that arises from abnormal pluripotent stem cell or early progenitor cell characterized by dysregulated production of myeloid cell with fairly normal differentiation and tendency to progress to acute leukemia. The genetic hallmark is the presence of BCR-ABL fusion protein resulting from t(9; 22). Most often, it is a result of reciprocal translocation between Abelson (ABL) oncogene on 9th chromosome and the Break point cluster region (BCR) on 22 chromosomes. This leads to increased tyrosine kinase phosphorylation activities. Probably these changes are responsible for proliferation defects in myeloid precursor cells in CML leading to excessive proliferation of all stages of myeloid cells.

Figure-1: Mechanisms of BCR-ABL activity in CML and blast crisis, leading to stimulation of proliferation of genetic instability, DNA damage and impaired DNA repair. Reactive oxygen species induced by BCR-ABL are thought to mediate DNA damage and genetic instability. [Ref: Blood 2012;120(4):p738]

Disease course and clinical presentation
Traditionally CML evolves into three stages, chronic phase, accelerated phase and blast crisis. Chronic phase may be asymptomatic and sometimes diagnosed on routine check-up. Common symptoms of CML are fatigue, tiredness, weight loss, night sweats, early satiety, abdominal pain & fullness because of enlarged spleen. More infrequently patients present with bleeding, fever, bone pain, lymph node enlargement. Rarely features of leucostasis such as priapism, neurological deficit, retinal haemorrhage, gout, splenic rupture may be presenting problems. About 95% patients have enlarged spleen and size of spleen usually correlates with the magnitude of leukocyte count in blood and the size of spleen has a significant role in CML risk score. 10% patients may present as blastic crisis at diagnosis which usually mimics the clinical features of acute leukaemia de novo and may present with extramedullary infiltration (chloroma).

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Diagnosis

Diagnosis of CML rest on clinical history, physical examination, peripheral blood smear, bone marrow biopsy and demonstration of Philadelphia chromosome by karyotype or FISH and/or BCR-ABL fusion protein detection by PCR. The quickest and cheapest way to diagnose a suspected case of CML is to assay the peripheral blood smear. In chronic phase there is marked granulocytic leucocytosis (generally >50 x 10^9/L, with a range 20-500 x 10^9/L). Blood smear reveals all spectrums of granulocytic series including myeloblast to mature neutrophils, predominant cells are myelocytes (myelocyte bulge) and neutrophils in chronic phase. Granulocytes are morphologically normal, shows no evidence of dysplasia. Myeloblasts do not exceed more than 3%. Absolute basophilia is universal finding and gives a diagnostic clue. Many patients also have eosinophilia. Platelets are increased or normal. Bone marrow in chronic phase is typically hypercellular, a marked granulocytic predominance with full spectrum of granulocytes and precursors with blast <5% of total nucleated cells are seen. Megakaryocytes are increased and characteristically with small & hypolobulated nucleus. Increased reticulin fibrosis may be seen in up to 40% cases. In pihperipheral blood & bone marrow aspirate differential count are key components of determining disease stage. NAP score is low in CML and help to differentiate from leukaemoid reaction.

Table I: WHO criteria for the diagnosis of accelerated phase & blast crisis

<table>
<thead>
<tr>
<th>Accelerated Phase:</th>
<th>Blast Crisis:</th>
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<tbody>
<tr>
<td>presence of one or more features</td>
<td>presence of one or more features</td>
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<tr>
<td>Blast in pihperipheral blood or bone marrow: 10-19%</td>
<td>Blast: 20% or more in pihperipheral blood or bone marrow</td>
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<tr>
<td>Basophil in pihperipheral blood: 20% or more</td>
<td>Cluster or large foci of blast in bone marrow</td>
</tr>
<tr>
<td>Platelet: &lt;100x10^9/L (unrelated to therapy) or &gt; 1000x10^9/L</td>
<td>Extramedullary blast infiltrates: Granulocytic sarcoma or chloroma</td>
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<tr>
<td>Progressive splenomegaly &amp; increasing WBC count</td>
<td>Cytogenetic evolution</td>
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Accelerated phase (AP) of CML is characterized by blasts counts of 10-19% in peripheral blood and/or bone marrow, basophils ≥ 20%, thrombocytosis not responding to therapy or persistent thrombocytopenia not related to therapy, increasing spleen size and WBC count despite therapy, as well as new clonal evolution. Any one of these features is consistent with accelerated phase.

In Blastic phase blasts comprise ≥ 20% or there is extramedullary blast proliferation or large clusters of blast in bone marrow.

The genetic hallmark of CML is Philadelphia chromosome. Ph1 chromosome is detected by Karyotyping, FISH analysis & RT-PCT test. Up to 95% of CML demonstrate Ph1 Chromosome, remaining minority have variant translocations involving other chromosome (e.g. 9; 14; 22) and rest are cryptic translocations of 9q34 & 22q11.2 that can't be identified by routine cytogenetic. These are referred to as 'Ph1-neg' and require FISH analysis to identify the BCR-ABL1 fusion gene or RT-PCR to identify the BCR-ABL1 fusion mRNA.

RT-PCR can identify different length product of fusion protein such as chimeric BCR-ABL proteins of 210, 190 and 230 kDa. 210 kDa fusion protein is present in vast majority of CML, whereas p190 kDa is found in Ph1+ve ALL. Although t(9; 22) is hallmark of CML, but it is not exclusive of CML. 20-30% of ALL and some of de novo AML also express t(9; 22). Therefore, final interpretation and diagnosis require correlation with the clinical, morphology and molecular findings.

Treatment

Treatment of CML has several options for individual patients depending on their stage of disease. The treatment paradigm at the present time is whether or not to recommend transplantation to a patient. Before the era of Tyrosine Kinase Inhibitors (TKI) the medications available for CML were hydroxyurea, busulphan & interferon. But the only curative option was allogeneic bone marrow transplantation. Then TKI was discovered as magic drug which changed the protocol of treatment modality in CML. Hydroxyurea (20-40 mg/kg/day) can be used to reduce white blood cell counts while awaiting confirmation of a suspected diagnosis of CML in a patient with significant leucocytosis or in patients with systemic symptoms or with symptomatic splenomegaly.8,9

First generation TKI

Tyrosine kinase inhibitors (TKI) are the first choice of drug. Imatinib mesylate is the first ever among the TKI. The standard treatment of patients with chronic-phase CML continues to be imatinib at 400 mg/day.10 Approximately 30% of patients with chronic-phase CML on imatinib experience grade 3-4 myelosupression, most commonly neutropenia, followed by thrombocytopenia. Blood should be monitored weekly for the first month.
after initiating imatinib. In the chronic phase, it is recommended to hold imatinib if the absolute neutrophil count (ANC) falls to $<$1.0x10^9/L or the platelets drop to $<$50.0x10^9/L. Treatment may be restarted once the ANC has recovered to 1.5x10^9/L and the platelet count to 100.0x10^9/L. Patients should be restarted on 400 mg/day unless they had profound myelosuppression or significantly delayed recovery. In these patients, restarting imatinib at 300 mg/day and then escalating to 400 mg/day in a few months is recommended. Other common side effects include oedema (50%), most commonly periportal but also pleural or pericardial effusions, ascites or anasarca, nausea (68%) and vomiting (50%), diarrhoea (49%), muscle cramps (46%), skin rash (39%), and bone pain or arthralgia (20 to 40%) and hepatotoxicity (<5%).

Drug resistance is associated with the reactivation of BCR-ABL signal transduction. Among those patients who start imatinib in the early chronic, late chronic and accelerated phases of CML, respectively, 12, 32, and 62% develop resistance mutations within 2 years of starting treatment. Resistance is associated with either a single amino acid substitution in the adenosine triphosphate (ATP)-binding region of BCR-ABL or, occasionally, with progressive BCR-ABL gene amplification. Mutations have been observed in different amino acids scattered throughout the ABL kinase domain and render the kinase variably less sensitive to imatinib. Therefore, second generation TKI were to emerge.

Second generation TKI
Selective Abl inhibitor, nilotinib and the highly potent dual Src/Abl inhibitor, dasatinib are two excellent examples of new TKIs. Nilotinib is 30 times and dasatinib is 300 times potent than imatinib. Dasatinib is a thiazolecarboxamide that is structurally unrelated to Imatinib and binds to an active and inactive conformation of the Abl enzyme, whereas nilotinib is an aminopyrimidine that is a structural derivative of imatinib and, like imatinib, binds only to the inactive conformation of the Abl kinase domain. Dasatinib was approved in 2006 by the U.S. Food and Drug Administration (FDA) and is indicated for the treatment of adults with newly diagnosed chronic phase CML and accelerated, or myeloid or lymphoid blast-phase CML with resistance or intolerance to prior therapy including imatinib. The approval was based on the response to this drug in imatinib-resistant or -intolerant patients. Dasatinib does not rely on a conformational change of ABL for binding and thus less susceptible to develop resistance.

The efficacy of dasatinib versus imatinib in the frontline therapy of CML also was investigated in a phase III randomized open label DASISION trial involving patients with newly diagnosed chronic phase CM, treatment with dasatinib, administered at a dose of 100 mg once daily, was compared with the current standard first-line therapy, imatinib, administered at a dose of 400 mg once daily. After a minimum follow-up of 12 months, the rate of confirmed complete cytogenetic response was higher with dasatinib than with imatinib (77% vs. 66%, P=0.007), as was the rate of complete cytogenetic response observed on at least one assessment (83% vs. 72%, P=0.001). The rate of major molecular response was higher with dasatinib than with imatinib (46% vs. 28%, P<0.0001). The present recommended starting dose of dasatinib is 100 mg once daily for chronic phase CML and 140 mg for advanced phase.

Response Criteria
Complete haematological response (CHR) is defined by a white cell count $<$10.0x10^9/L with no immature granulocytes and $<$5% basophils on differential; platelet count $\leq$450.0x10^9/L and spleen not palpable. Cytogenetic response; is assessed by chromosomal banding analysis of marrow cell metaphase with at least 20 metaphases analysed. Cytogenetic response is classified according to the percent Philadelphia chromosome positive cells into no response (>95%), minimal (66 to 95%), minor (36-65%), major (1-35%) and complete response (no Ph chromosome positive cells). For patients with an inadequate number of metaphases, Complete Cytogenetic Response (CCyR) can also be documented by FISH of blood interphase cell nuclei of at least 200 nucleuses.

Molecular response is assessed by quantitative PCR (Q-PCR) of the peripheral blood and defined according to the level of detection of the assay. The International Scale (IS) is expressed as the ratio of BCR-ABL1 transcript to ABL-ABL1 transcripts on a log scale. The log reduction refers to the decrease below the standard baseline that was used in the IRIS study. The IS assumes that all patients start at 100% and express results as log reduction in transcripts from this baseline. MR2 (roughly correspond to CCyR) means $\geq$2 log reduction from standardized baseline and detectable disease at a level of $\leq$1% on IS. MR3 (Major Molecular response) is $\geq$3 log reduction from standardized baseline and detectable disease at a level of $\leq$0.1% on the IS. MR 4 ($\geq$ 4 log reduction) is detectable disease at a level of $\leq$0.01% on the IS. This level of response requires that the assay being used is sensitive enough to detect a
single abnormal transcript amongst 10,000 normal ABL1 transcripts. MR 4.5 (≥4.5 log reduction) is detectable disease at a level of ≤0.0032% on the IS.18,19 Some earlier response criteria utilized the term 'Complete' molecular response when BCR-ABL1 transcript was undetectable and not quantifiable on two consecutive blood samples using an assay that had at least a 4 to 5 log range of detection.17,20 As Q-PCR assays have become increasingly sensitive, it became clear that the term 'Complete' molecular response is misleading and likely inaccurate since residual leukaemia stem cells may still be found in patients who achieve these levels of disease control.

**Table-II:** Schedule for monitoring CML patients in CP on TKI

<table>
<thead>
<tr>
<th>At diagnosis</th>
<th>Chromosome banding analysis (CBA) of marrow cell metaphases. FISH in case of Ph1 negativity (to identify variant, cryptic translocations). Qualitative PCR (to identify transcript type.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>At the initiation of therapy</td>
<td>Following initiation of therapy: CBC with differential every 1-2 weeks until complete haematological response is achieved. After achievement of complete haematological response CBC &amp; Chemistries on every 3 months. Failure to attain a complete haematological response by 3 months is an indication for change of therapy.</td>
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<tr>
<td>At 3 months: (after achieving CHR)</td>
<td>Quantitative real time PCR (QRT-PCR) to determine BCR-ABL1 transcripts level, to be performed every 3 months until an MMR (BCR- ABL1 ≤0.1 percent or MR3) has been achieved, then every 3 to 6 months.</td>
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<tr>
<td>At 12 months of Treatment</td>
<td>If do not achieve CCyR (&lt;MR 2) then bone marrow cytogenetic test should be done. Failure to achieve CCyR by 12 months is an indication for a change of therapy.</td>
</tr>
<tr>
<td>At failure or progression of disease</td>
<td>RQ-PCR, Mutational analysis and CBA of marrow cells meta phases, Immunophenotyping in blastic phase.</td>
</tr>
</tbody>
</table>

When there is evidence for a loss of response to therapy, the disease phase should be re-evaluated with a bone marrow biopsy with cytogenetics. In addition, patients with either an inadequate response to a TKI or in whom there is a loss of response. BCR-ABL1 kinase mutational analysis is recommended. A newly acquired mutation in BCR-ABL1 may trigger a change in treatment (e.g. dose increase, change to another TKI, HCT) depending upon the type of mutation found. Mutation analysis of BCR-ABL1 is recommended for all patients who fail to respond to a TKI. Only 40-50% of patients in chronic phase resistant to TKI therapy have detectable BCR-ABL1 kinase mutations, the mechanisms of resistant in other patients are poorly understood. Different mutations data help guide the choice of therapy in the future. Notable examples are; The T315I mutation has shown resistance to all currently available TKI except Ponatinib. The Y253H, E255K/V and F359V/C/I mutations are resistant to imatinib and nilotinib but sensitive to dasatinib. The F317L/V/I/C, V299L and T315A mutations are sensitive to nilotinib but intermediate sensitive to imatinib and dasatinib.21,22

**Monitoring for response and prognosis**

After diagnosis chronic phase remains stable for 3-5 years before progression to advanced phase. The rate of transformation to blastic phase is 5-10% each year during the first 2 years but increases up to 25% per year thereafter. Although there are several prognostic scores for CML e.g. Sokal score, Gratwohl score, Hasford score etc. in the era of TKI the most important prognostic indicator is the response to therapy. Currently, the CCyR rate to frontline TKI is 70-90% with 5-year PFS & OS between 80% and 95%. The clinical milestones of therapy are as follows: achieving CHR at 3 months, achieving at least MCyR at 12 months, and CCyR at 18 months.

So, the first 3 months of starting treatment needs regular CBC check-up initially once weekly up to the WBC counts settle down and then twice weekly up to CHR. There are many schedules for monitoring CML patients on TKI. Following is one approach to monitoring disease in patients with newly diagnosed CML in chronic phase (Table II).
Apart from TKIs, allogeneic Hemopoietic Stem Cell Transplantation (HSCT) is the only curative option for CML. It is now estimated that patients <50 years of age can expect more than a 50% likelihood of long-term disease-free survival after allogeneic HSCT performed in first chronic phase. Younger patients with suitable stem cell donor who failed treatment with TKIs may be offered Allogeneic HSCT. Various clinical parameters influence the likelihood of long-term survival after allogeneic HSCT. Increasing patient age, increasing interval from diagnosis, prior busulfan therapy, and more advanced stage of disease negatively affect outcome. Possible major complications include graft versus host disease (GVHD), infection, and CMV reactivation, veno-occlusive disease (VOD) of liver and idiopathic pneumonitis. Transplant related mortality (TRM) is roughly 20% and chance of relapse is 15%. HSCT in overt blastic crisis is unsuccessful.

### Role of Allo-HSCT

### Duration of drug therapy

The exact duration of TKI therapy in CML patients continues to be a pertinent question. Most physicians would continue the drug for indefinite period. There is no convincing Data to discontinue the TKI. But some have opinion to discontinue the drug after 2 years of retaining deep molecular remission. TKI is continued indefinitely as long as it is tolerated and treatment milestones are met. If TKI is discontinued a substantial proportion of patients relapse since tumour cells remain in a quiescent state despite therapy. Discontinuation of TKI therapy should only be considered in the context of clinical trials with frequent molecular monitoring.

### Fertility issue and pregnancy outcome in CML

Tyrosine kinase inhibitors revolutionized the treatment of CML with increase in survival and significant improvement in quality of life thereby resulting in an increased number of patients willing to be father or mother. Imatinib has potential teratogenicity in animals, but the effect of exposure to imatinib during conception and pregnancy in humans is not known. A large description of over 180 women exposed to imatinib treatment during pregnancy has been published and pregnancy and foetal outcome data were reported in 125 (69%) women: 63 patients delivered normal live births (18/63 were under imatinib during their pregnancy), 9 infants were born with foetal abnormalities, 35 (28%) had an elective termination, and 18 (14.4%) showed spontaneous abortion.
After getting the result of French STIM study of discontinuing TKI after molecular response, it might be possible to stop imatinib for a period of time to allow the patient to conceive and carry the child without exposure to the drug. But there is always a risk of progression. However, female patients remaining in complete haematological, cytogenetic and major molecular responses for at least 2 years who are planned for pregnancy are advised to stop imatinib for 1 month prior to conception and 3 months after conception (first trimester). The male patients should stop therapy 1 month prior to conception of their wives for being the TKI molecules to be washed out from body. TKIs have significant effect on decreasing the sperm parameters, and decreased serum concentrations of LH and FSH. These potentially toxic effects on spermatogenesis are less prominent in patients treated with dasatinib compared to imatinib and nilotinib.

Conclusion

CML is a disease of excellent prognosis if treated with targeted therapy. Tyrosine Kinase inhibitors. Clinical and laboratory parameters are quite simple to diagnose the case as CML. Second generation TKIs has higher response rates with deeper and faster responses. Molecular monitoring of BCR-ABL1 transcripts for patients with chronic myeloid leukaemia (CML) is now used to assess response to tyrosine kinase inhibitors (TKIs), including treatment failure that mandates a change of therapy. The molecular monitoring is expensive and should emerge the less expensive but effective method for monitoring of CML. Allogeneic HSCT is to be reserved for those who have bad TKD mutations and decreased serum concentrations of LH and FSH. These potentially toxic effects on spermatogenesis are less prominent in patients treated with dasatinib compared to imatinib and nilotinib.

Reference


