MINI-REVIEW

Chronic Myeloid Leukemia - Prognostic Value of Mutations

Bushra Kaleem*, Sadaf Shahab, Nuzhat Ahmed, Tahir Sultan Shamsi

Abstract

Chronic myeloid leukemia (CML) is a stem cell disorder characterized by unrestricted proliferation of the myeloid series that occurs due to the BCR-ABL fusion oncogene as a result of reciprocal translocation t(9;22) (q34;q11). This discovery has made this particular domain a target for future efforts to cure CML. Imatinib revolutionized the treatment options for CML and gave encouraging results both in case of safety as well as tolerability profile as compared to agents such as hydroxyurea or busulfan given before Imatinib. However, about 2-4% of patients show resistance and mutations have been found to be one of the reasons for its development. European Leukemianet gives recommendations for BCR-ABL mutational analysis along with other tyrosine kinase inhibitors (TKIs) that should be administered according to the mutations harbored in a patient. The following overview gives recommendations for monitoring patients on the basis of their mutational status.

Keywords: Chronic myeloid leukemia - BCR-ABL mutations - tyrosine kinase inhibitors

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Introduction

Chronic myeloid leukemia (CML), a malignant haematopoeitic stem cell disease, characterized by the occurrence of the Philadelphia chromosome which is thought to be a definitive diagnostic marker for CML and is present in almost 90% of the patients (Hagop et al., 2002). This chromosome results due to the balanced reciprocal translocation t (9; 22) (q34; q11). The fusion of the Abelson murine leukemia (ABL) gene on chromosome 9 with the breakpoint cluster region (BCR) gene on chromosome 22 gives rise to the BCR-ABL oncogene that encodes an oncoprotein (Saad et al., 2009).

The breakpoints in the BCR gene has been shown to be clustered in three regions, (a) a 5.8 kb region spanning exons 12-16 (e12-e16), called the major breakpoint cluster region (M-bcr) that codes a 210 kDa chimeric protein (p210), (b) a 55 kb sequence of the first intron (e1-e2) called the minor breakpoint cluster region (m-bcr) that encodes a 190 kDa chimeric protein (p190) and finally intron 19, called μ-bcr forming a resultant fusion transcript of 230 kDa protein (p230) (Fausel, 2007; Yuan et al., 2014).

Studies show an association between the genetic modifications within the precursor haematopoietic stem cells that may lead to the development of chronic myeloid leukemia (Meggyesi et al., 2011; Gulzar et al., 2012).

This oncoprotein is an active tyrosine kinase in the ABL region that promotes unrestricted growth and replication by causing deviations in proliferation and resists apoptosis through multiple downstream path¬ways, such as Janus kinase signal transducer and activator of transcription (Jak-STAT), MYC etc. (Pavan et al., 2013).

Even after being described for about more than 150 years ago, the steps taken with regards to its treatment were not rewarding enough for a better half of the century. The initial effectual treatment options for chronic myeloid leukemia included radiation therapy to the spleen and conventional chemotherapeutic drugs, mainly Busulfan and Hydroxyurea which helped control the rapid myeloproliferation leading to significantly improved quality of life during the indolent chronic phase (CP) of the disease. However, it did not have any important role in either preventing or delaying the progression towards accelerated phase or the blast crisis and thus had a limited effect on overall survival (OS) (Michele et al., 2006; Hehlmann et al., 2007; Bjorkholm et al., 2011; Kantarjian et al., 2012).

The introduction of allogeneic stem cell transplantation became the first major step forward in terms of treatment of CML, as about 50% of the patients who were eligible for alloSCT were cured as proven by them being both BCR-ABL negative as well as Philadelphia negative after the transplant. However the drawback in case of the transplantation was that it gave the best results in patients who were less than 40 years old, while CML being a disease of the elderly had median age at diagnosis of 60 years. The development of chronic graft-versus-host-disease was another major hurdle in this treatment option (Michele et al., 2006; Hehlmann et al., 2007; Baccarani et al., 2009; Pavlu et al., 2011; Baccarani et al., 2013).

The second major success came in the form of human recombinant interferon-alfa (rIFNα), which gave complete cytogenetic response (CCyR) in 15% to 30% of patients, and also provided with much better survival benefit over conventional chemotherapy (Hagop et al., 2010; Pavan et

*Haematology, Medical Sciences, National Institute of Blood Diseases and Bone Marrow Transplantation, Karachi, Pakistan *For correspondence: bushra_kaleem_lgmd@hotmail.com*

al., 2013; Talpaz et al., 2013).

The advent of imatinib mesylate (IM) in the late 90's created a revolution in terms of the treatment of patients with chronic myeloid leukemia (Hehlmann et al., 2007; Thomas et al., 2009). Imatinib Mesylate (IM) the selective inhibitor of the BCR-ABL tyrosine kinase, competitively targets the adenosine 5'-triphosphate binding site of the Abl Kinase Domain and thus blocks downstream signal transduction pathways leading to growth arrest and apoptosis (Hagop et al., 2010; Yasser et al., 2013). Imatinib has become the standard treatment for CML as it is extremely safe with minimal side-effects. A 7-year follow-up of the International randomized study of interferon versus STI571 (IRIS) study showed that the overall survival (OS) in CML patients who received IM as the first line drug was 86% and the progression to advanced stage of the disease (i.e. either the accelerated or the blastic phase) was reduced by 93% (Stephen et al., 2010).

However, a small proportion of patients in the chronic phase of the disease and significant number of patients in the advanced phase showed refractoriness to the treatment either in the initial stage or acquired resistance after initial response, thus resulting in relapse (Hochhaus et al., 2007; Thomas et al., 2009). Of all the reasons for the acquired resistance to occur, most common cause is the emergence of point mutations within the BCR-ABL kinase domain which leads to an impairment in the Imatinib binding by creating an obstacle at the site where the Imatinib or by preserving the configuration of BCR-ABL which has reduced Imatinib binding affinity (O'Hare T et al., 2007). Analysis of CML patients found to be resistant to Imatinib had the presence of more than 90 different BCR-ABL kinase domain mutations that encoded more than 50 different amino acid substitutions, although some are definitely more frequent than others. Different mutations lead to variable degrees of resistance to Imatinib (Corbin et al., 2003; Apperley JF 2007). The other reasons for the occurrence of acquired resistance are the clonal evolution that also speeds up the progression to the advanced stage, drug bioavailability, augmentation of the BCR-ABL fusion gene, transporter genes of Imatinib or tyrosine kinases such as Src family kinases being overexpressed, and harmful effects as a result of drug non-compliance or dose reduction (Michele et al., 2006; Sarit et al., 2011; Zaidatul et al., 2014).

Response and Monitoring to TKIs

European Leukemianet established guidelines for the assessment of the response(Table1 a) and the time period for the assessment of the response(Table1b) to the TKI therapy. Table 2 defines the category the patient would be placed in according to the time period and the type of response.

Optimal response means continue with the same treatment. Warning means to closely monitor the patient's response. Failure means to switch to another drug. CHR-Complete Haematological Response represented by Platelet count <450x109/L, WBC count <10x109/L, differential

Table 1 (a). Response to TKI Therapy of Patients According to the Guidelines Provided by the European Leukemianet 2013 (Lahaye et al. 2005; Hughes et al 2006; Hughes 2006; NCCN 2010; Michele et al 2013)

HAEMATOLOGIC RESPONSE	CYTOGENETIC RESPONSE	MOLECULAR RESPONSE (BCR-ABL1 transcripts)
Complete:		
✓ Platelet count < 450 x 10^9/L;	✓ Complete: Ph+ 0%	✓ MR4.5: ≤ 0.003%
✓ WBC count < 10 x 10^9/L;	✓ Partial: Ph+ 1%-35%	✓ MR4.0: ≤0.01%
✓ Differential without immature granulocytes and with less than 5% basophils;	✓ Minor: Ph+ 36%-65%	✓ Major3.0: ≤ 0.10%
✓ Nonpalpable spleen	✓ Minimal: Ph+ 66%-95%	✓ Relapse: > 0.5-1.0%
	✓ None: Ph+ >95%	

Major MR3.0 mean at least 3 log reduction in BCR-ABL Transcripts and expressed according to the International Scale

Table 1 (b): Monitoring of TKI Therapy of Patients According to the Guidelines Provided by the European Leukemianet 2013 (Lahaye et al. 2005; Hughes et al 2006; Hughes 2006; NCCN 2010; Michele et al 2013)

MONITORING OF HAEMATOLOGICAL RESPONSE	MONITORING OF CYTOGENETIC RESPONSE	MONITORING OF MOLECULAR RESPONSE
Initial:	Initial:	Initial:
Every 2 weeks until complete response achieved.	Every 6 months until complete response achieved.	Every 3 months.
		In case of failure, suboptimal response, or transcript level increases mutational analysis required.
Follow-up:	Follow-up:	
Every 3 months	Every 12 months	

Table 2. Guidelines for response monitoring according to European Leukemianet 2013(Hughes T et al 2006; Hughes T 2006; NCCN 2010; Michele B et al 2013)

Response	Time Period	Haematologic Response	Cytogenetic Response	Molecular Response
Optimal Response	03 Months	CHR	PCyR (Ph+:≤35%metaphases)	BCR-ABL1 <10%
	06 Months	-	CCyR (Ph+:0% metaphases)	BCR-ABL1 <1%
	12 Months	-	-	BCR-ABL1 <0.1%
	>12 Months or any other time	-	-	BCR-ABL1 ≤0.1%
Warning/ Suboptimal response	03 Months	-	Ph+: 36-95%metaphases	BCR-ABL1 >10%
	06 Months	-	Ph+: 1-35%Metaphases	BCR-ABL1 1-10%
	12 Months	-	-	BCR-ABL1 >0.1-1%
	>12 Months or any other time	-	CCA/Ph– (–7, or 7q–)	-
Failure	03 Months	Non-CHR	None: (Ph+:>95%metaphases)	-
	06 Months	-	Ph+:>35% metaphases	BCR-ABL1 >10%
	12 Months	-	Ph+:>0%metaphases	BCR-ABL1 >1%
	>12 Months or any other time	Loss of CHR	✓Loss of CCyR ✓Mutations ✓ CCA/Ph+	Confirmed loss of MMR*

Optimal response means continue with the same treatment. Warning means to closely monitor the patient's response. Failure means to switch to another drug. CHR-Complete Haematological Response represented by Platelet count <450x109/L, WBC count <10x109/L, differential without immature granulocytes and <5% basophils and nonpalpable spleen; PCyR:Partial Cytogenetic Response; CCyR:Complete Cytogenetic Response. Cytogenetic response assessed in 500 interphase cells by Fluorescence in situ hybridization(FISH) or metaphases observed in 20 nuclei. Molecular response is assessed by RT-PCR of the RNA extracted from the blood cells and expressed in ratio of BCR-ABL210/ABL according to the International Scale(IS). CCA/Ph–:clonal chromosome abnormalities in Ph– cells. CCA/Ph+:clonal chromosome abnormalities in Ph+ cells. *:In 2 consecutive tests, of which one with a BCR-ABL1 transcripts level ≥1%.

without immature granulocytes and <5% basophils and nonpalpable spleen; PCyR, Partial Cytogenetic Response; CCyR, Complete Cytogenetic Response. Cytogenetic response assessed in 500 interphase cells by Fluorescence in situ hybridization (FISH) or metaphases observed in 20 nuclei. Molecular response is assessed by RT-PCR of the RNA extracted from the blood cells and expressed in ratio of BCR-ABL210/ABL according to the International Scale (IS). CCA/Ph-, clonal chromosome abnormalities in Ph- cells. CCA/Ph+, clonal chromosome abnormalities in Ph+ cells. *, In 2 consecutive tests, of which one with a BCR-ABL1 transcripts level ≥1%.

Resistance of CML

Resistance to imatinib can be labeled as primary or intrinsic when there is no Complete Haematologic Response by 3 months, any cytogenetic response by 6 months, partial cytogenetic response by 12 months or complete cytogenetic response by 18 months. Resistance is referred to as secondary or acquired when there is a loss of previously gained haematological, cytogenetic or molecular response. It also may include the progression to accelerated or blast phase after a period of sustained complete haematological response, occurrence of clonal chromosomal abnormalities, or any other BCR-ABL kinase domain mutations (Hughes et al., 2006; Michele et al., 2006; Simona et al., 2011; Michele et al., 2013; Zafar et al., 2013). Approximately 50% of the CML patients presenting with either primary or secondary resistance to the Tyrosine Kinase Inhibitors (TKI) therapy have been due to the presence of the BCR-ABL kinase domain mutations (Hochhaus et al., 2002; Lahaye et al., 2005; Branford et al., 2006; Soverini et al., 2006; Jabbour et al., 2006; Sarit et al., 2011).

European Leukemianet (ELN) and European Treatment and Outcome Study gives the following recommendations with regards to the BCR-ABL mutational analysis (Figure 1), (Simona et al., 2011)

Various methods exist for the detection of mutations each having their own specificity and sensitivity. The most common involved are direct sequencing (sensitivity, 15-25%), sequencing after subcloning of PCR products (sensitivity, 9%), Denaturing High-Performance Liquid Chromatography (D-HPLC) (sensitivity, 0.1-10%), pyrosequencing and double gradient denaturing

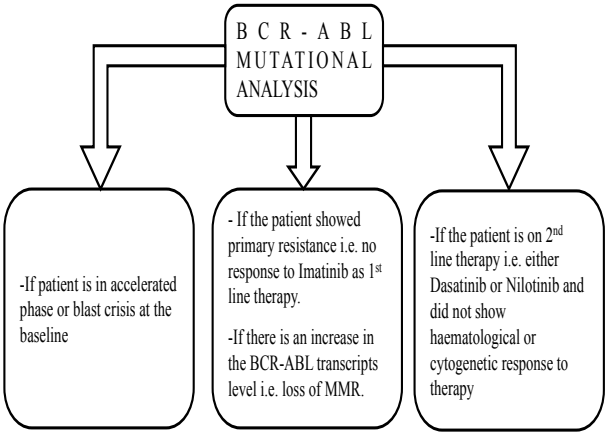

Figure 1. Recommendations for Mutational Analysis

electorphoresis (sensitivity, 5% both). A few other techniques involved in the mutational analysis are Flouorescence PCR and Peptide Nucleic Acid-based Clamping and Allele-Specific Oligonucleotide PCR (ASO-PCR) both with much more better sensitivity (Alderborn et al., 2000; Gorre et al., 2001; Branford et al., 2002; Roche-Lestienne et al., 2002; Shah et al., 2002; Branford et al., 2003; Kreuzer et al., 2003; Roche et al., 2003; Deininger et al., 2004; Irving et al., 2004; Thomas et al., 2005; Sorel et al., 2005; Willis et al., 2005; Khorashad et al., 2006). The method used decides the range of mutations that will be detected. For example, mutations in the BCR-ABL kinase domain will be detected in a patient by direct sequencing only if they are present in greater than 10-20% of the leukemic cells (Hughes et al., 2006). Caution is thus required at the time of result interpretation.

BCR-ABL is comprised of four regions that are

Table 3. Functions and Mutations of Regions of BCR-ABL Domain

REGIONS	IMPORTANCE	MUTATIONS	FREQUENCY	REFERENCES
P-Loop	P-loop repositions itself in a way that it folds over the drug which leads to a much stronger binding of the drug to the kinase domain.	G250E*/R E255K/V* Q252H*/R Y253H*/F M244V L248V	36-48%	Branford S et al.2003;Jabbour E et al.2006;Shundong C et al. 2008;Ravandi F 2011
Contact Binding Site	Found in the imatinib binding site and interacts directly with the imatinib via hydrogen bonds or Van der Waals' interactions.	T315I*/A F311L/I F317L*/V	4-19% 1.50% 2.80% 5%	Jabbour E et al.2006; Soverini S et al. 2006; Nicolini FE et al. 2006; Elias J et al. 2008; Elias J et al. 2011; Ravandi F 2011;Shweta S Shweta S andSarjana D 2013 Soverini S et al.2006; Simona S et al.2007; Thomas E et al.2009; Jamshid SK et
Catalytic Domain/SH2 Domain		M351T* E355G/D F359V*	3.20% 11% 15% 7% 11% 16%	Nikolas von B et al.2005;Jamshid SK et al.2013;Shweta S and Soverini S et al.2006; Jamshid SK et al.2013; Shweta S and Sarjana
Activation loop (A-Loop)	It has characteristic active and inactive configurations (In the inactive form, Imatinib has occupied the ATP binding site and thus has competitively inhibited ATP binding to the kinase domain.	H396R*/P F382L L387M V379I	2.20% 2.50%	Soverini S et al.2006;Shweta S and Sarjana D 2013

*Most frequent mutations

composed of vastly preserved series of amino acids and its activity depends on the configuration of these amino acids.

Table 3 gives the function of all the four regions as well as the mutations that have been detected in these specific areas over the years by the employment of various techniques.

The mutations if present may lead to resistance by following mechanisms, (Shah et al., 2000; Apperley, 2007; Elias et al., 2008; Elias et al., 2011),

i) Direct inhibition by creating an alteration in the amino acid that is involved in binding the drug to the kinase for eg. T315I, F317L and F359C/V. *ii)* Indirect inhibition by creating an alteration in the BCR-ABL conformation for eg. G250E, Q252H, Y253H and E255K/V. *iii)* One another mechanism leading to resistance due to mutation is the maintenance of the active configuration of BCR-ABL for eg. M351T and H369R/P.

Of the numerous mutations discovered over the years, the most common ones observed are T315I, M244V, G250E, Y253H, E255K, F317L, M351T, F359V and H396R. The most resilient mutation thus far has been T315I. It accounts for about 4-19% of all the mutations detected in patients who showed resistance to the TKI therapy and is known as the gatekeeper mutation as it induces resistance by forming a hydrogen bond with the Imatinib through the insertion of the amino acid Isoleucine and thus prevents the binding of the Imatinib to BCR-ABL sterically (Gorre et al., 2001; Corbin AS et al., 2002; Deininger 2005; Ian et al., 2006; Michael 2006; Elias et al., 2008; Elias et al., 2011). Infact it is the only mutation which shows no response to both the first generation drugs i.e Imatinib as well as the second generation drug i.e , Dasatinib or Nilotinib (Nicolini 2007; Simona et al., 2011). Recent studies have shown the discovery of a pan BCR-ABL inhibitor Ponatinib which seems to overcome the resistance created by the notorious T315I mutation (Zhou et al., 2011; Karunakar et al., 2013). However extensive studies are still required to analyze its effect to gain Complete Cytogenetic Response in patients receiving the drug. Until the time the results get established and the drug receives an approval from FDA, the option available for the patients with T315I mutation seem to be the allogeneic stem cell transplantation (Michele, 2013).

Besides the T315I mutation, the other mutations that are known to confer resistance as well as progression to the advanced stage are mutations lying in the P-Loop. The known mutations of the P-Loop are G250E, Q252H, Y253F/H, E255K/V. The P-Loop mutations have been observed to be the most commonly occurring mutations along with T315I (Branford et al., 2003; Jabbour et al., 2006; Shundong and Delong 2008). The P-Loop (A.As, 248-256) is a highly preserved area of the BCR-ABL Kinase domain and is involved in the process of binding of the phosphate group of the ATP (Matti, Peter, Alfred, 1990; Schindler et al., 2000). Studies show that the presence of the P-Loop mutations has been frequently seen in patients who were in the advanced phase of the disease. Also if the patients were found to be in the chronic phase, the presence of the P-Loop mutations eventually led to progression to the advanced stage at a much faster rate as compared to those who did not have these mutations. Thus their presence means a poorer prognosis and early progress to the advanced disease (Branford et al., 2003; Simona et al., 2005; Soverini et al., 2006). Also studies show that the P-Loop mutations are resistant to Nilotinib and so should be given Dasatinib after they have shown no response or failure to Imatinib therapy (Baccarani et al., 2009; Elias et al., 2010; Baccarani et al., 2013; Michele et al., 2013; Michele et al., 2014).

As regards to F317L mutation, it has been found that this mutation seems to be resistant to the second generation drug Dasatinib. Patients administered with this drug after no response or failure to Imatinib, showed reduced sensitivity to the drug (Thomas et al., 2009). So patients found with this mutation after the Imatinib failure have been recommended to be treated with another second generation drug, Nilotinib (NCCN 2010; Elias. et al., 2010; Michele et al., 2013; Thoralf. et al., 2013; Michele et al., 2014). M315T lies in the E-helix region and makes contact with the SH2 domain of the BCR-ABL . This contact leads to a reduced activity of the BCR-ABL kinase activity (Hantschel et al., 2003; Nagar et al., 2003; Nikolas et al., 2005; Willis et al., 2005).

F359V have been found to be resistant to the second generation drug Nilotinib. CML patients found to have this mutation should be given another second generation drug Dasatinib (Baccarani et al., 2009; Baccarani et al., 2013; Michele et al., 2013).

Figure 2 shows the strategic treatment plan for the patients according to their mutational status. Recent studies have indicated that if mutations are detected even at low levels several months before the response is lost, this would mean an eventual resistance and thus a prediction of relapse and progression to the advanced stage (Jamshid et al., 2008; Thomas et al., 2008).

Studies have shown that mutations leading to resistance to the TKIs therapy have much lower incidence in patients in Chronic Phase as compared to those who are in the Accelerated Phase or the Blast crisis (Lahaye et al., 2005; Jabbour et al., 2006; Soverini et al., 2006). Studies also show that mutations present in the Chronic phase leading to the progression to the accelerated phase or the blast crisis is much more frequent than in those patients who had no mutations in the chronic stage of the disease (Shah et al., 2002; Simona et al., 2005; Soverini et al., 2006).

The studies also show that the contribution of the mutations to the occurrence of primary resistance is less in comparison to the acquired one (Lahaye et al., 2005; Jabbour et al., 2006; Soverini et al., 2006). Studies show that the mutations are present in much higher numbers in patients who have been exposed to therapy like interferon and then administered with Imatinib as compared to those who are Imatinib-naïve (Willis et al., 2005; Soverini et al., 2006).

The presence of the mutations in the patients who have not yet been exposed to any therapy and were in the advanced stage disease at the baseline means that the mutations in these people were not due to the exposure to the drug. In fact, the presence of mutations in them is likely to be indicative of some consistent genetic instability as well as the possibility ofworsening of the

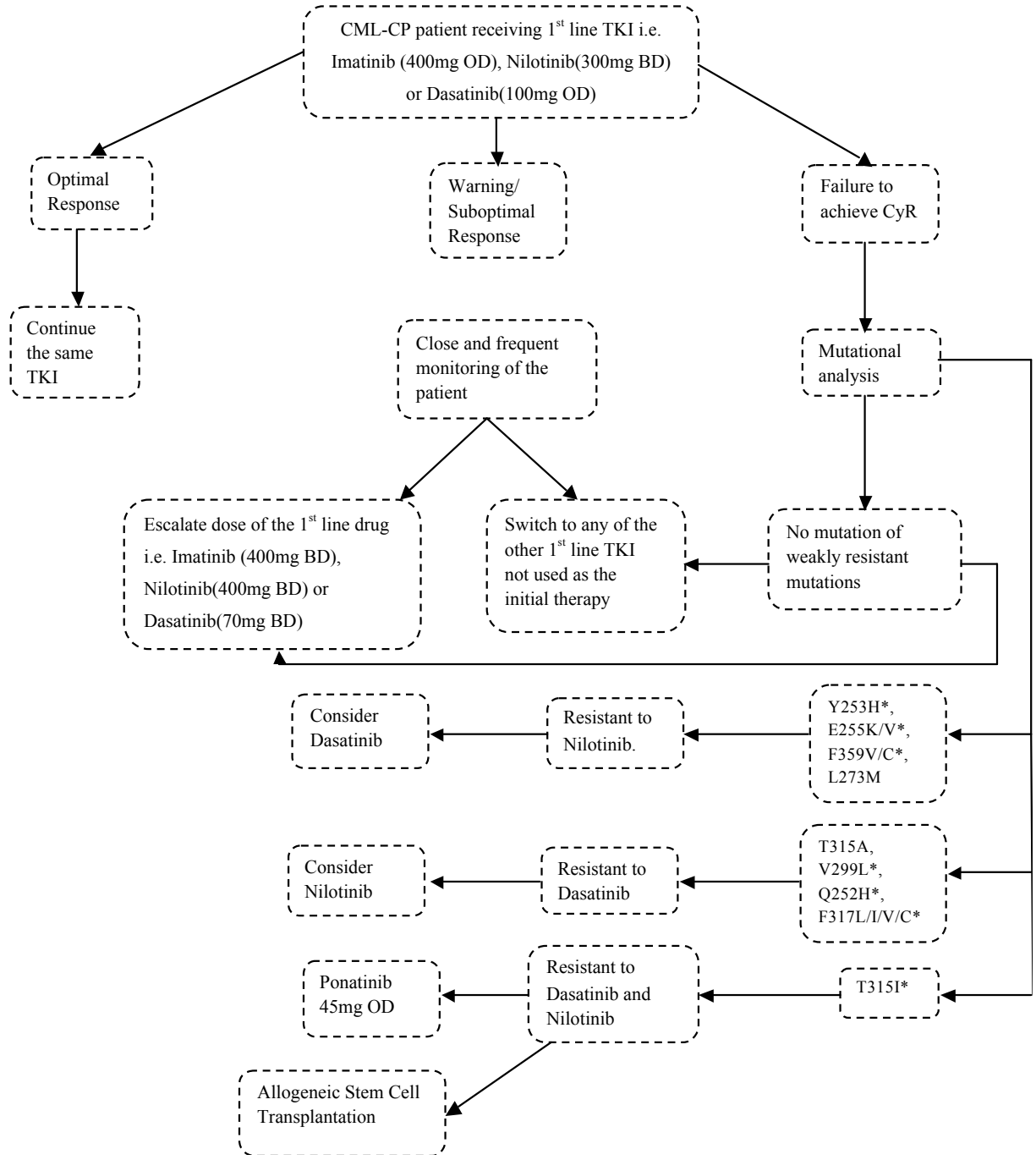

Figure 2. Strategic Treatment Plan for the CML Patients According to the Mutational Status

disease status and thus a poorer outcome (Hughes 2006; Elias et al., 2010).

Conclusions

The effect of mutations on the disease course has been under investigation for quite a while. European Leukemianet as well as NCCN has provided guidelines regarding the response monitoring of the patients. The algorithm provided by these bodies gives an uncomplicated way of monitoring the response of the patient and tailoring their treatment according to their mutational status. The ultimate goal of all these guidelines is to predict the response a patient might show depending on one's mutational status. Also, besides keeping in view the mutations the patient carries, considerable other factors like patient's Sokal score, his response to the Imatinib should also be kept in mind at the time the patient's future treatment is being decided.

Data have shown that certain mutations show resistance to second generation drug i.e. Dasatinib while another set of mutations are resistant to Nilotinib, another second generation drug. While T315I has shown resistance to both of them as well as the standard treatment i.e. Imatinib. Patients carrying T315I mutation have their question of treatment answered in the form of allogeneic stem cell transplantation or a more recently discovered third generation drug, Ponatinib which is currently under trial

and is awaiting FDA approval for patient administration. However, overall these set of mutations are less resistant to Dasatinib and Nilotinib as compared to Imatinib. Clinicians have begun to understand the importance of mutational analysis both for the monitoring the response of a certain TKI as well for the decision making at the time of switching to another TKI if the present TKI is not effective. However, extensive trial is in need for further understanding of the importance of the mutations encountered and their eventual significance both in terms of prognosis of the disease as well as the treatment plan.

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