REVIEW ARTICLE

Imatinib Therapy in Chronic Myeloid Leukaemia: Review of Mechanisms of Resistance and Therapeutic Options in Imatinib Failure

Ladu AI, Aisha MA

ABSTRACT

Chronic myeloid leukaemia is a triphasic, clonal malignancy, arising from the haemopoeitic stem cell. It is characterised by the presence of Philadelphia chromosome, which results from the reciprocal translocation between chromosome 9 and 22. The resulting oncogene- bcr-abl has proliferative activity and survival advantage against normal cell and this account for the clinical and laboratory manifestation of this myeloproliferative disorder. Imatinib, a tyrosine kinase inhibitor (TKIs) is currently the first line treatment, however, one third of patients develop resistance to it, thus necessitating alternative TKIs. Many factors are associated with the development of resistance to imatinib, such as mutation in the bcr-abl gene, increased production of the mutant protein and activation of alternative pathways amongst other causes. The aim of this review is to explore these factors, and to also evaluate current TKIs that are used as alternative in Imatinib resistant cases.

KEYWORDS: Imatinib Therapy, Resistance, Alternative.

Introduction

Chronic myeloid leukaemia (CML) is a clonal malignancy of the haemopoeitic stem cell (HSC), characterized by the presence of Philadelphia chromosome (Ph). This arises from reciprocal translocation between the Abelson gene (Abl) on the long arm of chromosome 9 and the breakpoint cluster region (bcr) on the long arm of chromosome 22 [t(9,22) (q34;q11.1)][1].

The resultant fusion oncogene (bcr-abl) codes for an abnormal protein with tyrosine kinase activity in excess of the wild type, consequently multiple signal transduction pathways are activated, such as, the STAT5/JAK, phosphatidylinositol 3 (PI3), the Ras, Raf and MAPK family of kinases. The result is the uncontrolled proliferation and gain of survival advantage of the malignant clones over normal cells[2].

Department of Haematology,
University of Maiduguri Teaching Hospital

Correspondence to:
DR. A.I LADU
Department of Haematology,
University of Maiduguri Teaching Hospital,
PMB 1414
Maiduguri, Borno State

In Ph-positive CML, the breakpoint in the BCR gene occurs in a small 5.8 Kb major breakpoint cluster region (M-bcr). This hybrid bcr-abl gene is transcribed into a novel 8.5 kb mRNA with a b3a2 and/or b2a2 junction that encodes a 210-kD fusion protein with enhanced protein tyrosine kinase activity[3]. The cellular form of Abelson leukaemia virus tyrosine kinase (c-Abl) is related to the src family of tyrosine kinases. It consists of about 1150 residues, with an "N" terminal cap of about 80 residues involved in autoinhibition. The fusion of the gene encoding c-Abl with the breakpoint cluster region (BCR) results in the formation of a chimeric protein with all of the c-Abl preserved except its N-terminal region. This fusion distorts the control mechanism that keeps the c-Abl in an inactive form and the enhanced tyrosine kinase activity results in chronic myeloid leukaemia[4-7].

The clinical course of CML is characterized by a chronic phase (CP), an accelerated phase

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(AP) and a blast crisis (BC). The last two stages are difficult to treat and associated with poor outcome[1]. However, the advent of Imatinib (IM), a tyrosine kinase inhibitor (TKI) two decades ago revolutionised the treatment of CML, and is currently the first line treatment for all phases of the disease. Given that the Bcr-Abl enzyme is necessary and sufficient for the initiation and propagation of CML, inhibition of the Abl kinase activity was an ideal target for a molecular-based therapy[8]. Imatinib is a potent and selective inhibitor of the abnormal kinase domain (KD), it competitively inhibits the binding of ATP onto its binding site on the bcr-abl domain (figure 1). Importantly, it binds to the KD in the inactive form, this makes Imatinib highly selective for the bcr-abl kinase compared to other agents (NB – the kinase are similar in their active form, but dissimilar when inactive). Imatinib displaces ATP and traps an inactive conformation of the kinase domain[9]. Unfortunately, about 1/3rd of patients may not respond, or may need to discontinue treatment due to intolerable side effects[2]. Multiple factors have been implicated in the mechanism of resistance to this agent. The aim of this review is to explore these factors, and to also evaluate current TKIs that are used as alternative in Imatinib resistant cases.

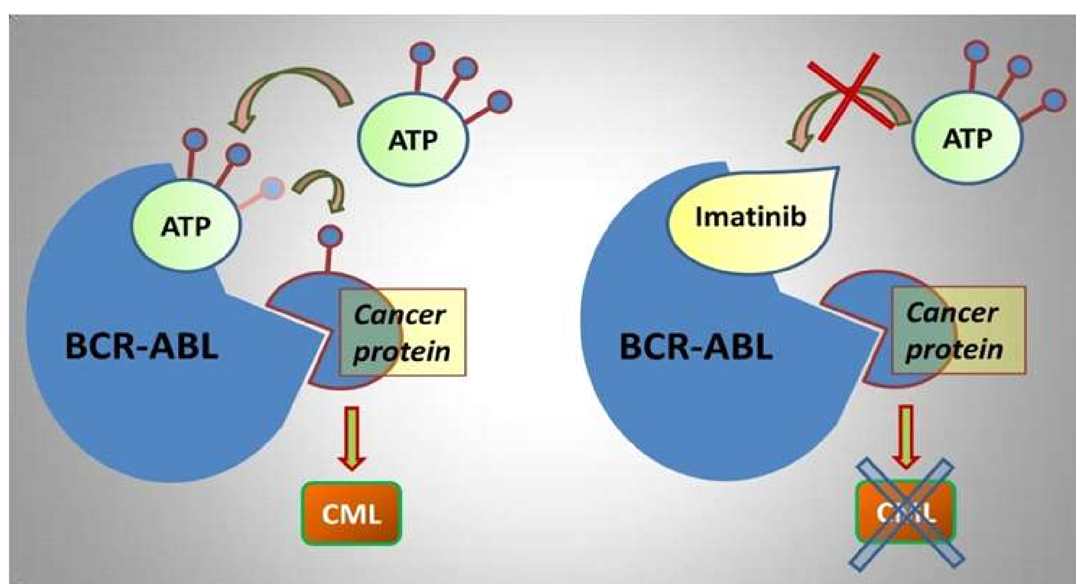

Figure 1: The mechanism of action of Imatinib. It binds to the bcr-abl ATP binding site and thereby prevents phosphorylation of the leukaemic protein (*adapted from www.intechopen.com*)

Response to imatinib treatment:

Ninety percent of patients with chronic phase CML achieve haematologic responses to imatinib, and about 50% of these patients achieve cytogenetic response using standard cytogenetic assays. Approximately 60% of patients with advanced-stage CML (blast crisis) respond to imatinib, but nearly all these patients relapse despite continued therapy[10]. The landmark 'IRIS' (international randomized study of interferon vs ST1571) trial, where newly diagnosed patients with CML were randomized to treatment with Imatinib (ST1517) or interferon, established Imatinib 400mg as the first line drug for patients with CP CML [11]. Certain criteria are used to assess if there is optimal response following treatment with imatinib. Complete haematological response (CHR) indicates a normal peripheral blood count after 3 month of therapy. Cytogenetic response can either be partial cytogenetic response (pCyR) (Ph

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chromosome levels less than 35% at 6 month) or complete cytogenetic response (CCyR) (absence of Ph positive metaphases within the bone marrow at 12month)[2]. Cytogenetic response to treatment can be monitored using either conventional cytogenetic assessment of bone marrow metaphase, or FISH using peripheral blood or marrow metaphase or interphase cells. TKIs are highly effective at reducing the number of tumour cells in the bone marrow and should be monitored by RT PCR analysis for BCR ABL1 transcripts in marrow or blood and/or karyotypic analysis of the bone marrow, typically at 3, 6 and 12 months[10]. Molecular response is assessed as a ratio of BCR ABL1 transcripts to ABL1 transcripts and it is expressed as BCR ABL1% on a log scale, where 10%, 1%, 0.1%, 0.01%, and 0.001% correspond to a decrease of 1, 2, 3, 4, and 5 logs respectively below the baseline. A major molecular response (MMR) is defined as BCR ABL1 transcript level of < 0.1% or less than pre-treatment levels[11].

Resistance to imatinib treatment:
Failure to respond to imatinib can be classify as follow[2,12]:

- Primary resistance: defined as incomplete haematological response at 3/12, no CyR at 6/12 months, or less than partial or complete CyR in 12 and 18 month respectively.
- Secondary resistance (acquired): this is loss of the initial responsiveness to treatment. The risk factors include older patients, advanced disease or a high sokal score at presentation, previous treatment with interferon or new clonal evolution.
- Suboptimal response: patients respond to Imatinib treatment at a lower than expected rate
- Intolerance: this group of patients require the discontinuation of treatment due to intolerable side effects.

Mechanism of resistance to Imatinib
Various mechanisms have been implicated in imatinib resistance and these can be broadly divided into BCR-ABL dependent or independent causes as follow:

BCR-ABL dependent mechanisms include: ·
Mutations: Numerous mutation have been implicated in resistance to Imatinib (Figure 2); this can occur in various locations in the abl sequence, including Imatinib binding sites (T3151), ATP binding sites (p-loop), catalytic and activation sites[10,12]. Although the total number of known point mutations continues to grow, their clinical significance in an individual patient may vary, in addition, the frequency of mutation increases with disease progression. P-loop mutations account for majority of the mutation (48%), causes resistance by altering the conformation form required by imatinib to bind, associated with poor prognosis, and increase risk of disease transformation[13]. In samples obtained from imatinib- resistant patients, it was observed that a single nucleotide substitution (C→T)at position 944 of the Abl gene results in the substitution of amino acid threonine with isoleucine at position 315 of the abl protein sequence (Th^{315}→Ile^{315}; T315I); resulting in the elimination of a critical oxygen molecule required in the formation of hydrogen bonding between imatinib and the kinase domain; as well as steric hindrance to the binding of imatinib.T3151 mutation confers resistance to all TKIs in clinical use[14].

Increase production or expression of BCR-ABL transcript: Associated with secondary resistance and account for a minority of cases. The mechanism is not fully explained, but cells with increased transcript are less sensitive to imatinib in vitro[15].

Presence of alpha 1 acid glycoprotein (AGP): mediate resistance by decreasing the concentration of imatinib and thus inhibiting it at a dose dependent manner[12].

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BCR-ABL-independent mechanism:

- Increased expression of the SRC-family of kinase such as, Hck, Fgr, Lyn: these are involved in the regulation of cell proliferation and survival. Signalling pathways are activated irrespective of the BCR□ABL activity. They also act by stabilizing the conformation of KD thus preventing the effect of imatinib[12].
- Presence of embryonic stem cells: these CD38+ cells account for < 1% of the total stem cells, however, because they harbour the mutant clone and are in G0 phase, they serve as a reservoir for disease escalation[13].
- Clonal advancement: this indicates new cytogenetic abnormalities, and may be associated with progression to AP or BC. Common abnormalities include trisomy 8, isochromosome 17q and new Ph positive clones[16].
- Altered drug influx and efflux properties: affects bioavailability of imatinib by affecting its binding to the p-glycoprotein efflux pump. Uptake transporters have also been suggested to play a role in resistant cells[17].

Drug concentration: A trough level of imatinib above 1uM has been shown to correlate with the attainment of CCyR and MMR. In addition, the period of exposure to imatinib plays a critical role in therapeutic response[17].

Figure 2: Summary of the various BCR-ABL domain kinase mutations causing resistance to Imatinib. P loop (P), catalytic domain (C), activation loop (A)[10]

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WHAT ARE THE OPTIONS IN THE FACE OF RESISTANCE

Treatment options in cases of resistance to Imatinib include

1. Imatinib dose escalation: the use of higher doses of imatinib may overcome primary resistance arising from leukemia cells exposure to sub therapeutic levels of Imatinib[17]. An increase from the standard dose of 400mg has been suggested to be beneficial in such resistant cases however; this may not be suitable in patient intolerant to standard dose of Imatinib. There is paucity of studies in favour of dose escalation [18,19].

2. Individualized therapy: Patients that fail to respond to imatinib should be screened for compliance as well as an assessment of imatinib drug levels. Additionally, mutational assessments should be made to identify possible mechanisms of resistance. Studies have indicated that patients failing imatinib can often respond to a second or third-line TKI-based therapy, including those with and without identifiable KD mutations[19,,20,21].

3. The second generation TKIs are the next line of treatment in cases of resistance to imatinib[19].

   Example of some second line TKIs:

   - **Dasatinib:** inhibits both bcr-abl and src family of kinases. Shown to have more activity against the bcr-abl (>350) compared with imatinib. Effective against all phases of CML, and has a good tolerability profile. It has been extensively evaluated in the START trial, a large multicentre that included both open and randomized studies; it showed sustained response against all subgroup of patients; cross-intolerance with imatinib has not been documented, thus beneficial in patients who are intolerable to Imatinib [22]. Not effective against T3151 mutation.
   - **Nilotinib:** an Imatinib analogue and has been shown to be effective in patients resistant or intolerant to imatinib. Active against all phases of the disease. Side effects include elevated lipase level and hyperglycaemia, dose modification required in patients with diabetes, liver or kidney disease[12].

4. The third generation TKI bosutinib, a dual Abl/Src inhibitor, with limited activity against c-Kit and the platelet-derived growth factor receptors (PDGFRs) has been used primarily in imatinib-resistant CP-CML patients, and after a median follow-up of 8 months, 79% have achieved a CHR, with 40% achieving a MCyR, and 29% a CCyR. Ninety-one percent of patients have maintained their MCyR over this brief interval [19]

Unfortunately, few options are available for patients who fail to respond to both first and second generation TKIs. In addition, none of the TKIs is effective against the T3151 mutation (Table 1). Stem cell transplantation is reserved as salvage treatment in patients who relapse or resistant to second generation TKIs, and currently the only potential cure for CML, however, the procedure is associated with high morbidity and mortality, availability of suitable donor also limits its use. Indicated in patients with primary resistance or advanced disease[23].

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Table 1: Summary of the various agents targeting the resistant bcr-abl clone:

Drug class	Mechanism	Effect on T3151	Comment	Trials	Reference (s)
2nd generation TKIs: Bosotunib	Dual kinase inhibitor	No	>200fold activity compared with IM	Phase 1	Burke et al [24]
Batetinib (INNO-406)	Non-ATP mediated kinase Inhibition.	No	>55 potency compared with imatinib Good toxicity profile.	Phase 1	Khoury et al [25]
3rd generation TKI: Ponatinib (AP24534)	Multiple kinase inhibitor-flt, src and bcr-abl	Yes	Good toxicity profile.	Phase 1/11	Bixby&Talpez [17]
Aurora KI (AT9238)-Danusertib	Multiple kinase inhibitor	Yes	Nil	Phase 1	Burke et al [24]
Heat shock protein inhibitor (hsp).eg 17AAG	Inhibition of hsp results in down regulation of bcr-abl activities& apoptosis	No	Synergistic effect with imatinib	Nil	Burke et al[24]
Arsenic trioxide	Induces apoptosis and proliferation of CML cells	No	Effective against M351T,Y253F mutation	Nil	Roy-Chowdhery and Talpaz[26]
Homoharringtonine. (HHT) Eg: omacetaxine, chemaxenine.	By product of plant alkaloid. Induces apoptosis	No	Nil	Phase 11	Meso&Chuah[27]

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Histone deacetylase inhibitors.eg Verinostat, SAHA.	Hyper acetylation of histone residues, leading to ↑ expression of p21&p27 and apoptosis	No	Increases sensitivity of mutant cells to imatinib	Nil	Meso&Chuah, [27]
Proteasome inhibitors.eg bortezomib	Cell cycle inhibition and induces apoptosis	No	Combination of low dose bortezomib and IM lead to anticancer	Nil	Meso&Chuah, [27]
Flavone and flavopiridol	Cyclin dependent kinase inhibitors	No	Additive effect with IM in resistant cells	Phase 11	Meso&Chuah, [27]
Farnesyltransferase inhibitor.eg Tipitarnib,lonatarnib	Ras/MAPK inhibitors	Partial	CHR achieved in IM resistant cells	Phase 11	Burke et al [24]
Raf -1- inhibitors	Multiple kinase inhibitor	No	Effective against IM resistance	Phase1/11	Burke et al [24]
MEK inhibitor.eg CI-1040	Nil	Partial- with arsenic and hsp)	Nil		Burke et al[24]
MTOR inhibitors.eg Rapamycin, sirolimus	Nil	Partial	Poor biochemical property	Phase1/11 (everolimus)	Burke et al [24]

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Monitoring Reponse to Treatment

Detection of mutation at an early stage can allow for modification of patients' treatment. In the last decade, various techniques that can detect the bcr-abl mutations have been developed, which has allowed for the on-going monitoring of patients to help identify those at risk of disease progression [28].

Current guidelines recommend that patient monitoring should include a baseline profile and regular monitoring as outlined below [29,30].

- Baseline assessment: these should include a blood bcr-abl transcript detection (RQ-PCR) to establish the diagnosis of CML; type of transcript present [e.g of common transcript are e13a2 (b2a2) and e14a2 (b3a2)], this allow for identification of any new transcript due to disease progression; bone marrow cytogenetic to detect presence of extra cytogenetic abnormalities or translocations.
- Response to treatment: for patients responding to treatment, monitoring entails twice monthly blood count, brc-abl transcript every 3/12 and marrow for cytogenetic analysis every 6/12.
- Establishment of CCyR: monitoring of bcr-abl transcript, importantly, to characterize the pattern of change, such as, whether it is declining, steady or rising. FISH can also be used to assess for CCyR.
- Mutation analysis: this is indicted in patients with rising levels of transcript, advance disease at presentation, loss of initial response or suboptimal response in patients with CP disease. Also important in deciding second line TKIs to use or if SCT should be offered.

Conclusion

Chronic myeloid leukaemia resulting from Philadelphia chromosome positivity can be reliably manage with imatinib, however, the malignant clone are unstable and prone to developing resistance. Patient with resistance can be offered treatment with second generation or third generation TKIs or salvage treatment with SCT, however, the outcome is poor with the latter. Various agents targeting the resistant bcr-abl clone are under evaluation, with the aurora kinase family showing positive result against the T3151 mutation.

References

1. Deininger M, Buchdunger E, Druker B. The development of imatinib as a therapeutic agent for chronic myeloid leukemia. Blood 2005; 105:2640-2653.
2. Hoffbrand AV, Moss PAH. Chronic myeloid leukeami in: Essential Haematology. Eds AV Hoffbrand and PAH Moss. 2016; 7th edition. Blackwell publishing Ltd.
3. Zhao Junmei, Yu Fengkuan, Song Yongping, Fang Baijun, Liu Yuzhang, Liu Lina, et al. Coexistence of P190 and P210 BCR/ABL transcripts in chronic myeloid leukemia blast crisis resistant to imatinib. Springerplus 2015; 4: 170
4. Van Etten R.A. Cycling, stressed-out and nervous: cellular functions of c-Abl. Trends Cell Biol. 1999;9: 179-186
5. Pluk H, Dorey K, Superti-Furga. Autoinhibition of c-Abl. Cell. 2002 ;108:247-259
6. Bhushan N, Oliver H, MathewA, Young K.S, Daren V, William B et al. Structural basis for the autoinhibition of c-Abl tyrosine kinase. Cell. 2003;112:859-871
7. Sawyers C.L. Disabling Abl- perspectives on Abl kinase regulation and cancer therapeutics. Cancer cell. 2002;1:13-15
8. Nagar B, Bowmmann W.G, Pellicena P, Schinder T, Veach D.R, Miller W.T et al. Crystal structures of the kinase domain of c-Abl in complex with the small molecule inhibitors PD173955 and imatinib (STI-571). Cancer Res. 2002;62:4236-4243
9. Schindler T,Sicheri F, Pico A, Gazit A, Levitzki A, Kuriyan J. Crystal structure of

This work is licensed under a Creative Commons Attribution 4.0 International License

Hck in complex with a src family-selective tyrosine kinase inhibitor. Mol. Cell. 1999:3;639-648
10. Shah NP, Nicoll JM, Nagar B, Gorre ME, Pacquette RL et al. Multiple BCR BCRABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. Cancer Cell. 2002;2:117-125
11. O'Brien SG, Guihot F, Larson RA, Gathmann I. Imatinib compared with interferon and low dose cytarabine for newly diagnosed chronic phase chronic myeloid leukaemia. The New England Journal of Medicine. 2003; 348 (11): 994-1004.
12. Pablo R, DiPersio JF. Therapy options in Imatinib failures. The Oncologists. 2008; 13: 424-434.
13. Zaharieva MM, Amudov G, Konstantinov SM, Guenova ML., 2013. Modern Therapy of Chronic Myeloid Leukemia, Leukemia, Prof. Margarita Guenova (Ed.), ISBN: 978-953-51-1127-6, InTech, DOI: 10.5772/55505. Available from: http://www.intechopen.com/books/leukemia/modern-therapy-of-chronic-myeloid-leukemia
14. Gorre ME, Mohammed M, Ellwood K. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science. 2001;293:876–880.
15. Milojkovic D, Apperley J. Mechanism of resistance to imatinib and second generation tyrosine inhibitor in chronic myeloid leukaemia. Clinical Cancer Research. 2009; 15: 7519.
16. Jabbour E, Knatarjian MH, Lynne V, O'Brein S. Chromosomal abnormalities in Philadelphia chromosome-negative metaphases appearing during Imatinib mesylate therapy in patients with newly diagnosed chronic myeloid leukaemia in chronic phase. Blood. 2007; 110; 2991-2995.
17. Bixby D, Talpaz M. Seeking the causes and solutions to imatinib-resistance in chronic myeloid leukaemia. Leukaemia. 2011; 25:7-22.
18. Knatarjian MH, O'Brein S, Cortes J. Survival advantage with imatinibmesylate therapy in chronic-phase chronic myelogenous leukaemia (CML-CP) after interferon-alpha failure and in late CML-CP, comparison with historical controls. Clinical Cancer Research. 2004; 10:68-75.
19. Dale B, Moshe T. Mechanism of resistance to tyrosine kinase inhibitors in chronic myeloid leukaemia and recent therapeutic strategies to overcome resistance. ASH Education. 2009:1;461-476
20. Soverini S, Colarossi S, Gnani A. Resistance to dasatinib in Philadelphia-positive leukemia patients and the presence or the selection of mutations at residues 315 and 317 in the BCR-ABL kinase domain. Haematologica. 2007;92:401–404
21. Kantarjian H, Giles F, Bhalla K. Nilotinib in chronic myeloid leukemia patients in chronic phase (CML-CP) with imatinib (IM) resistance or intolerance: longer follow-up results of a phase II study [Abstract]. J Clin Oncol (Meeting Abstracts). 2009;27:7029.
22. Apperley JF, Cortes JE, Kim DW, Roy L. Dasatinib in the treatment of chronic myeloid leukaemia in accelerated phase after imatinib failure: the START A trial. Journal of Clinical oncology. 2009; 27(210):3472-3479.
23. Oehler VG, Gooley T, Snyder D, Johnston. The effects of Imatinib mesylate treatment before allogeneic transplantation for chronic myeloid leukaemia. Blood. 2007;109: 1782-1789.
24. Burke AC, Sword RT, Kelly K, Giles FJ. Current status of agents active against the T3151 chronic myeloid leukaemia phenotype. Expert opinion Emerg Drug. 2011; 16 (1): 85-103.
25. Khoury HJ, Cortes J, Knatarjian MH, Gambacorti-Passerini. Bosutinib is active in chronic phase chronic myeloid

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leukaemia after imatinib and Dasatinib and/or nilotinib therapy failure. Blood. 2012; 119:3403-3412.

26. Roychowdry S, Talpaz M. Managing resistance in chronic myeloid leukaemia. Blood. 2011; 25 (6):279-290.

27. Melo JV, Chuah C. Novel agents in chronic myeloid leukaemia therapy: tyrosine kinase inhibitors and beyond. American society of haematology education programme. 2008; 427-435.

28. Hughes T, Deininger M, Hochhaus A, Branford S. Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: review and recommendation for harmonizing current methodology for directing BCR-ABL transcript and kinase domain mutations and for expressing results. Blood. 2006; 108: 28-37.

29. National comprehensive cancer network. NCCN clinical practice guidelines in oncology: chronic myeloid leukaemia. 2008. Available (online) at [http://www.nccn.org/professional/physician_gls/pdf/cml.pdf.] Accessed March 5, 2014.

30. Hughes T, Branford S. Monitoring disease response to tyrosine kinase inhibitor therapy in CML. Haematology. 2009; 477-487.

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