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Original Article

Monitoring of response to therapy with imatinib mesylate in Chronic Myeloid Leukemia in chronic phase (CML-CP)

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ABSTRACT

Background: The BCR-ABL tyrosine kinase is a well validated therapeutic target in Chronic Myeloid Leukemia (CML). Imatinib mesylate (IM), a tyrosine kinase inhibitor is highly effective in the treatment of chronic phase CML. BCR – ABL transcripts have been well established as a molecular marker to document response to therapy in CML. Periodic monitoring of this marker helps in evolving therapeutic strategies with IM and also in diagnosing early relapse. This study was undertaken to monitor therapeutic response to IM in CML in chronic phase (CML-CP) by assessing BCR-ABL by real time quantitative PCR (RQ-PCR) techniques and to determine the effectiveness of the Indian generic IM.

Methods: One hundred consecutive patients of CML in chronic phase (CML-CP) were treated with an Indian generic of IM. Eighty-five patients were evaluable at 12 months of therapy. At entry, diagnosis of CML-CP was confirmed by FISH and RQ-PCR. Response to therapy was monitored by assessing BCR-ABL levels by RQ-PCR at 6 and 12 months of therapy. Regular follow up of patients was done to evaluate the safety profile of IM used in these patients. **Results:** Complete hematological response (CHR) rates at 3, 6, 9 and 12 months were 92%, 94%, 100% and 100% respectively. The total molecular response at 12 months was 43.52% of which complete molecular response (CMR) was noted in 17.64% and major molecular response (MMR) was observed in 25.88%. A cumulative survival probability of 0.8 was observed.

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Conclusion: The Indian generic molecule of IM is effective in the treatment of CML-CP. The cost of Indian generic molecule is less than Rs. 10,000 per month there by making this affordable for large number of CML-CP patients in India.

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Introduction

Chronic Myeloid Leukemia (CML) is the commonest leukemia among adults in India. Its incidence varies from 0.8 to 2.2 per 100,000 population in males and 0.6 to 1.6 per 100,000 population in females accounting for nearly half of all adult leukemias.¹ Imatinib mesylate (IM), a specific inhibitor of BCR-ABL tyrosine kinase has dramatically changed the management of CML in the last decade. Of all non-transplant therapies IM induces the highest rate of complete hematological response (CHR), cytogenetic response and molecular response.² FISH was the initial test used for detecting BCR-ABL translocation since it was easier to perform and was readily available in many labs including ours. Though FISH is a sensitive method for diagnosis of CML, it gives false positive results when used for monitoring of response to IM therapy. BCR-ABL by quantitative reverse transcriptase PCR techniques (RQ-PCR) has been accepted worldwide as the standard for molecular monitoring of CML patients.³ The sensitivity and specificity of RQ-PCR ranges from 95 to 98%.⁴

This study was undertaken to monitor the therapeutic response to IM in patients with CML in chronic phase (CMLCP) by BCR-ABL RQ-PCR techniques and to study the incidence of adverse effects during therapy.⁵ The primary objective was to study the effectiveness of the Indian generic IM by determining the decrease or disappearance of BCR-ABL transcripts (molecular response). Incidence of relapse and emergence of IM resistance were also evaluated.

Material and methods

This study was conducted at the Department of Clinical Hematology and Bone Marrow Transplant, in conjunction with the Department of Haemato-Pathology and Molecular Medicine, Army Hospital (Research and Referral), Delhi Cantt. One hundred consecutive patients of CML were enrolled between October 2006 and March 2008. The patients were included in the study after confirming the diagnosis of CML-CP on the basis of clinical and hematological criteria. This hospital caters to referral patients, mainly consisting of serving Armed Forces personnel and their dependent family members, representing a heterogeneous mix of multi-ethnic patients from all over the country. All good clinical practice guidelines were observed and ethical clearance was obtained for this study from institutional ethics committee. All patients diagnosed as CML-CP on the basis of clinical and hematological criteria and were positive for BCR-ABL by either FISH or RQ-PCR and consented for IM therapy were included in the study. All these patients were given IM as primary therapy and were instituted

an Indian generic IM (Veenat[®] from NATCO Pharma Ltd). Imatinib was started at a dose of 400 mg once a day for adults and 375 mg/m²/day for children. All patients of CML who did not satisfy the above criteria, CML-CP patients treated earlier with other modalities of treatment (Interferon and Busulfan), patients presenting in accelerated phase/blast crisis and cases of CML with pregnancy were excluded from the study. Patients who had myelofibrosis at diagnosis were excluded from the study as it put them in the category of accelerated phase. The patients were clinically evaluated and periodically followed up at 1,3,6,9 and 12 months.⁵

Patient demographics along with detailed history, period of follow up, past medication for CML, presence of any co-morbid medical/surgical conditions, occurrence of any adverse effects to IM were meticulously recorded in all patients as shown in Table 1. The risks/benefits involved were discussed with all the patients following which informed consent was obtained. The follow up patients were subjected to a detailed clinical examination.

All patients were worked up at presentation with complete blood counts (CBC), baseline coagulogram and for routine biochemical parameters (BUN (blood urea nitrogen), blood sugar, serum creatinine, uric acid and electrolytes). Peripheral blood samples were collected in EDTA for BCR-ABL FISH and/or RQ-PCR. FISH for BCR-ABL was performed using the dual colour dual fusion VYSIS probes. RQ-PCR for BCR-ABL transcripts and ABL as housekeeper gene was done using a Corbett Research real time PCR machine and using kits from Ipsogen and Applied Biosystems using the taqman principle. It was a multiplex RQ-PCR for b3a2 and b2a2 transcripts i.e. the 210 bp product. Bone marrow aspiration and biopsy was done in all patients from posterior superior iliac spine for baseline parameters and to document presence/absence of myelofibrosis. The BCR-ABL: ABL ratio was calculated and expressed as a percentage. The baseline or the BCR-ABL: ABL ratio of our Laboratory was calculated by calculating the median of 30 cases of CML-CP at diagnosis and this was standardized in March 2007 and was used to calculate the log reduction at 6 months and 12 months.⁵ BCR-ABL FISH was the available test modality at our centre for diagnosis in the first 40 CML-CP patients at entry. This was subsequently replaced by quantitative RQ-PCR for all patients. The initial 40 patients were evaluated for molecular response by BCR-ABL FISH at 3 months and later by RQ-PCR at 6 and 12 months (Tables 4 and 5). As the molecular monitoring was started after 3–6 months of diagnosis, 3 log reduction in the transcript level from the baseline calculated was taken as major molecular response (MMR). The base line BCR-ABL: ABL ratio for our lab was 80% and thus the conversion factor for converting to the IRIS baseline of 100% and the MMR i.e. 3 log reduction (0.1%) was calculated as 1.25 to express the results of our lab on the

Table 1 – Patient characteristics (n = 100).

1 M: F	1.4: 1
2 Median age (range)	40 yrs (9–80 yrs)
3 Median follow-up	9 months (6–18 months)
4 Median time to diagnosis is	2 months (2–12 months)
5 Splenomegaly >10 cm	31
6 Splenomegaly 1–9 cm	69
7 Hepatomegaly	55
8 Total number of patients having low lap score	74
10 Number of patients with grade I/II fibrosis on bone marrow biopsy	11
11 Co-morbid illness	01 (Type 2 diabetes mellitus)

International scale. However, as we did not have access to the IRIS standard, we affiliated to a reference centre for conversion factor; a 3 log reduction from baseline was taken as MMR in the study. The data of clinical examination on first visit is depicted in Table 1. Bone marrow aspiration and biopsy was done at entry only. Complete blood counts were monitored every three-month. BCR-ABL: ABL ratio was monitored at baseline, 6 and 12 months as shown in tables (Tables 2 and 3). The Sokal score was calculated at baseline and 53% had a score more than 1.2 i.e. high-risk category (Table 5).

Blood chemistry, including liver function tests, blood urea nitrogen, creatinine, uric acid and blood sugar levels were measured every three months. Toxicities encountered during therapy were chronicled and dose modifications were done as per the toxicity and hematological and molecular responses. Hematological monitoring for CHR were done monthly for first 3 months and thereafter 3 monthly, unless otherwise indicated. CHR was maintained through 6 and 12 months in all cases.

Response to therapy with IM were defined as: (a) Complete hematological response (CHR)⁴: Platelet count < 450,000/mm³; WBC 10,000/mm³; differential count showing no immature granulocytes, no basophilia; and nonpalpable spleen. (b) Hematological relapse^{5,6}: Development of constitutional features; hepatosplenomegaly; leukocytosis; Presence of blasts; promyelocytes; basophils and thrombocytopenia on PBS after having achieved complete hematological remission. (c) Molecular response^{4,5}: Complete molecular response (CMR): BCR-ABL transcripts not detectable; Major molecular response (MMR): A reduction in BCR-ABL transcript levels of >3 log (1000-fold reduction) from the laboratory-specific pretreatment standard baseline was defined as a MMR. All patients who had achieved CHR and were clinically stable but did not

Table 3 – Log reduction of BCR-ABL/ABL ratio at 6 and 12 months while on imatinib.

Log reduction/BCRABL/ABL ratio	At 6 months n = 63	At 12 months n = 85
Zero = CMR	03 (4.76%)	22 (25.8%)
≥3 log reduction = MMR	04 (6.3%)	15 (17.6%)
>0.2 and <3 log reduction	34 (1.58%)	35 (41.17%)
2 log reduction	07 (11.11%)	5 (5.8%)
>1 and <2 log reduction	05 (7.9%)	2 (2.3%)
<1 log reduction	10 (15.8%)	6 (7.05%)
Total	63 (100%)	85 (100%)

CMR = complete molecular response, MMR = major molecular response.

fall in to either MMR/CMR at 12 months have been defined as IR. (d) Molecular relapse^{7,8}: BCR-ABL/ABL levels showing two fold rise on three consecutive occasions at least 01 month apart; or the ratio showing a five fold rise on two consecutive samples.

A database was created in MS Excel and SPSS version 14.0 was used for statistical analysis. Survival analysis was carried out taking the entire duration of follow up from the time of inclusion in the study till the last day of the study period with death being taken as an event.

Results

The first 40 patients were FISH positive at diagnosis. The remaining 60 patients were positive for BCR-ABL RQ-PCR at diagnosis. By RQ-PCR, BCR-ABL: ABL levels in these patients at baseline varied from 30% to 100%. These discrepancies in testing for BCR-ABL: ABL at recruitment were primarily due to the availability of these testing modalities in the lab at those points of time and initial problems of standardization which was finally achieved in March 2007.

Table 4 shows the molecular response pattern calculated as per universal method of log reduction of transcript ratio as compared to the median baseline value for our laboratory. The values were available for 63 and 85 patients at 6 months and 12 months respectively. There was no evidence of primary drug resistance as all patients went into complete haematological remission. MMR rates at 06 and 12 months were 6.3% and 25.8% respectively, while CMR was achieved by 4.76% and 17.6% of the patients respectively.

The adverse events noted during therapy of CML-CP with IM were mainly hypo pigmentation in 40%, facial puffiness in 10%, weight gain in 15%, leucopenia and thrombocytopenia in 11% each, muscle cramps in 10%, and skin rashes in 9%.

Table 2 – BCR-ABL: ABL Levels by RQ-PCR on imatinib therapy.

RQ-PCR% (BCR-ABL: ABL%)	Number of patients at 6 months (n = 63)	Number of patients at 12 months (n = 85)
0–20	41 (65.07%)	72 (84.70%)
21–40	12 (19.04%)	5 (5.88%)
41–60	3 (4.76%)	4 (4.70%)
61–80	7 (11.10%)	4 (4.69%)

Table 4 – Sokal score at diagnosis (n = 100).

Sokal score	Frequency	Percent
<0.8	9	9.0
0.8–1.2	38	38.0
>1.2	53	53.0
Total	100	100.0

Table 5 – Drug toxicity.

Toxicity	Number of patients (n = 100)
1 Hypo pigmentation	40
2 Weight gain	15
3 Leucopenia, thrombocytopenia	11
4 Muscle cramps/fatigue facial puffiness	10
5 Skin rashes (hyper pigmentation)	9
6 Fullness of stomach	6
7 Anemia, elevated transaminases	5
8 Pedal edema	3
9 Raised uric acid, blood sugar & gum/mucosal bleeding	2
10 Decreased libido	1
11 Urea/creatinine abnormalities	nil

Two patients showed evidence of molecular relapse, which was followed by hematological relapse. Of these two patients one had achieved MMR at 6 months but showed evidence of molecular relapse at 12 months with BCR-ABL: ABL ratio rising to 10%, which was confirmed on repeat testing. The second patient had achieved complete molecular response at 6 months and had molecular relapse at 12 months with BCR-ABL: ABL ratio rising to 49.70%. They developed hematological relapses followed by disease progression to myeloid blast crises. They were successfully treated with Idarubicin and Ara-C. None of the patients treated with IM developed any serious adverse effects and there were no deaths due to IM toxicity.

Survival analysis shows a cumulative survival probability of almost 0.8 at the end of 65 months of follow up (Fig. 1).

Discussion

IM has been used in the West since 1998 and several trials have demonstrated its efficacy to achieve CMR and MMR, with an acceptable and manageable side effects profile.^{6,7,9,10} Our study included 100 consecutive patients diagnosed with CML-CP from October 2006 to March 2008. All these patients were

treated with Indian generic molecule of IM in a standard dose 400 mg/day (~260 mg/m²). The responses were monitored for hematological, cytogenetic and molecular response.

The results were compared with published trials; mainly with IRIS III (International Randomized Study of Interferon and ST1571)³ and an Indian study.⁴ In our study complete hematological response (CHR) was achieved at 3, 6, 9 and 12 months in 92%, 94%, 100% and 100% of the patients respectively, which is comparable with the studies mentioned *ibid*.^{3,4}

In our study, the molecular response by real time PCR at 6 months showed a median BCR-ABL: ABL ratio of 12% which compares well with the reported Indian data of 11%.⁴ In the IRIS III study 39% patients were in MMR at 18 months,³ where they had considered MMR as patients showing >3 log reduction (<0.1%) in the BCR-ABL: ABL; which is comparable to our results. The median BCR-ABL: ABL ratio at 12 months was 1% in our study as compared to 3.8% in the other Indian study,⁴ the difference possibly could be due to the small sample size (16 patients) in the other study. The sensitivity of RQ-PCR for BCR-ABL: ABL^{5,6} is nearly a log higher at 10⁻⁵ to -6 (95–98%) in comparison to cytogenetics. We studied the molecular responses as reductions from an absolute baseline (common to all) rather than a relative baseline (individualized) to ensure that patients with the same level of response have the same degree of residual disease. Though we have used the term CMR it should be understood that it means only undetectable levels of BCR-ABL with a sensitivity of 4.5 log below the standardized baseline.⁵ Recognizing that this level of response reflects the limits of current detection methods, undetectable BCR-ABL should not be equated with eradication of BCR-ABL expression or cure. Cases in which patients with undetectable BCR-ABL discontinued imatinib therapy and then subsequently relapsed reinforce that the lack of detection of BCR-ABL transcripts is neither a cure nor a reason to discontinue imatinib therapy.^{11,12}

Most centers carry out molecular monitoring only after complete cytogenetic response is achieved. However at our centre though we initially used FISH for diagnosis and early monitoring, but since then we have been using only RQ-PCR for molecular monitoring. In the early days we used to report as copies/ml but soon shifted to the BCR-ABL/ABL ratio as a percentage using the M BCR fusion Quant kit by Ipsogen. As seen in the various studies we also found that a 2-log reduction in BCR-ABL/ABL ratio corresponded to complete cytogenetic response. We found that if a 1-log reduction occurred at three months there was a very high chance of MMR at 12 months. At times fluctuations occurred in the values in patients in MMR and we confirmed a rise in transcripts by repeating the RQ-PCR on fresh blood sample.

International standardization of molecular monitoring is highly desirable and will enable comparison of molecular response in trials where RQ-PCR is performed in different labs. The consensus meeting in Bethesda,¹³ proposed establishment of international scale to be applied to individual labs – set of clinical samples from local lab need to be sent to reference lab to develop conversion factor. It was suggested that every lab should follow a standardized procedure and the value of that lab be converted on the IS by getting a arithmetical conversion factor which would convert the baseline

Fig. 1 – Cumulative survival of all study participants.

value to 100% and the major molecular response to 0.100 (i.e. 3 log reduction) i.e. equivalent to the MMR value established in the IRIS trial.¹⁴

As we do not have access to the International standards from Adelaide and we follow certain methods to ensure the reliability and quality of the report provided by our lab we carry out the test within 24 h of collection of whole blood in EDTA. The latest consensus guidelines published by Foroni et al. in April 2011¹⁵ suggest that samples older than 72 h should not be used for patients being monitored for MRD. We use all the WBCs from 5 ml of peripheral blood collected in EDTA. We extract the whole RNA and use 2 µg for cDNA synthesis. We use Superscript III RT from Invitrogen for this step. The same volume of cDNA is used for both mutant and control gene test. We depend on the number of ABL copies as already stated. If copies are $\geq 10,000$; we report our result as reliable and sensitive. If value is $< 10,000$ and the BCRABL1 transcripts are negative this is not reported and test repeated with 10 ml blood sample. If the ABL value is > 5000 and $< 10,000$ and we get BCRABL1 transcripts in a case on monitoring for MRD we do not give the percentage and only state that patient has MRD. The latest guidelines also recommend the same. The standards we use are from Ipsogen i.e. 10^5 – 10^3 for ABL and 10^6 – 10^1 for BCRABL1. We run a standard curve for both the mutant and control gene at each run with a constant threshold. We check the slope (should be -3.20 to -3.60) and the R^2 (> 0.980) for each RQ-PCR run. We always include the ABL copy numbers in our report.¹⁴

We observed the Sokal to be much higher as compared to Western literature¹⁶ with 53% having a high risk score of > 1.2 probably as most of our patients had a large spleen and high platelet count at diagnosis. This data goes to show that most patients of CML-CP present late in the disease to the clinician in the Indian setting. 80.4% of the patients who did not go into major molecular response at 12 months had a Sokal score > 0.8 i.e. were either in high risk or moderate risk category.

Two of our patients showed molecular relapse at 12 months and progressed to hematological relapse requiring modification of drug therapy. These are the kind of patients who require kinase domain mutation analysis if and when available in our country. The most common adverse effects noticed in our study were hypo pigmentation and weight gain, where as in the IRIS study it was edema and nausea. The other small Indian study noted edema and arthralgia as their predominant adverse effects.⁴ There were no cases of primary drug resistance in our study population. Our study compares well with the famous IRIS-III CML-CP trial³ in all its monitoring parameters and toxicity profile and survival.

It has become imperative to highlight the fact that the original molecule IM (Gleevec) of Novartis was the drug used in all the clinical trials in the West and in the Indian study.^{4,7,8} The cost of this molecule was the main limiting factor for its use in India as it amounted to patients paying more than Rs. 100,000 every month, upon its launch in India in early 2000.¹⁷

The first generic molecule of IM was marketed by NATCO in India. A pilot trial of 10 patients was carried out by the same workers at Army Hospital (R&R) in 2002 (unpublished data)

and demonstrated the efficacy of this generic molecule in achieving molecular response comparable to the original molecule Gleevec, with similar toxicity profile.

Conclusion

Our study using the Indian generic molecule of IM (Veenat, NATCO) demonstrated comparable clinical, hematological and molecular responses with the parent molecule, Gleevec (Novartis). The drug was well tolerated and the adverse effects noted were manageable with supportive care. The cost of Indian generic molecule of IM is nearly a tenth of the cost of Gleevec, thereby making the Indian generic affordable for CML-CP patients in India.

Conflicts of interest

All authors have none to declare.

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