ORIGINAL ARTICLE

CELLULAR AND MOLECULAR RESPONSES OF SAUDI CHRONIC MYELOID LEUKAEMIA PATIENTS TO IMATINIB (STI-571): TEN YEAR EXPERIENCE

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Background: The cyto-genetic hallmark of chronic myeloid leukaemia (CML), the Philadelphia chromosome (Ph), is the first consistent chromosomal abnormality that has been associated to a certain cancer type. In CML, Philadelphia chromosome is present leading to resistance to cell death and rapid proliferation. The aim of this study is to evaluate the different responses, toxicity and survival of Saudi CML patients to imatinib mesylate. **Methods**: All newly diagnosed CML patients who were treated with imatinib were included in this study. We investigated haematological, and molecular and cyto-genetic responses by CBC, FISH and RT-PCR respectively. Cell proliferation and apoptosis were assayed using AUC and TUNEL respectively. **Results:** Of the 12 cases, 9 (75%) were males and 3 (25%) were female. Four (33%) of the cases were diagnosed incidentally and 8 cases (67%) presented mainly with fatigue (75%), fever (58%), and splenomegaly (83%). Signs of bleeding and rashes were rare at presentation. The majority of patients had low risk (8, 67%), and 33% had intermediate risk; but none of them had high risk CML. At the last follow up, 11 (92%) were in remissions. One patient (8%) was in remission after 3 years, 4 (33%) were in remission after 6 years, one was in remission after 7 years and 5 (42%) were in remission after 10 years. Only one patient had incomplete major molecular response (MMR) to imatinib after 12 years. The majority of the patients (10, 83%) were in MMR after 6 years and 42% of them were in MMR after 10 years of therapy. Adverse effects of imatinib were not reported by the patients. Imatinib treatment resulted in the reduction of proliferation and induction of apoptosis of CML CFU-GM cells. **Conclusion:** Imatinib mesylate is capable of treating Philadelphia chromosome-positive CP-CML without any adverse effects.

Keywords: Imatinib, Philadelphia, CML, responses

INTRODUCTION

The cytogenetic hallmark of chronic myeloid leukaemia (CML), the Philadelphia chromosome (Ph), is the first consistent chromosomal abnormality that has been associated to a certain cancer type. The Ph chromosome was first reported by Peter Nowell and David Hungerford in the University of Pennsylvania in Philadelphia, United States after studying peripheral blood samples of two CML patients.[1] CML arises as a consequence of a rare mutational event resulting in a reciprocal translocation between the long arms of chromosomes 9 and 22. Approximately 90–95% of CML patients are Ph chromosome positive in cytogenetic analysis of the bone marrow samples.[2,3]

CML is an acquired clonal haemopoietic stem cell disease, with a chronic phase (CP), an accelerated phase, and a blast crisis phase (BC).[4] Understanding the key cellular and molecular mechanisms involved in the BC phase of CML represents a vital goal in patient management because, once BC has been reached, treatment becomes extremely difficult, and prognosis poor.

Anti-apoptotic defects,[5–8] high levels of proliferation,[9–10] insensitivity to negative regulators[11–12] and defects in the adhesion mechanisms[13–15] are considered to result from expression of the BCR-ABL fusion protein.[16–17] These defects are therefore likely to be responsible for CML myeloid expansion.

Imatinib, (formerly STI-571, also called imatinib mesylate) is a rationally designed TKI which gained a UK license in 2001. It is a tyrosine kinase inhibitor that preferentially blocks the activity of ABL tyrosine kinases, in particular $p210^{BCR-ABL}$ and is the first targeted therapy for patients with CML and other tumours.[18–21] It may lead to the inhibition of the signalling cascades downstream of BCR-ABL and inhibition of cell proliferation.[22–23]

Clinical experience has taught us the responses of CML patients may be broadly categorised into three groups according to whether they achieve a complete cytogenetic response (no BCR-ABL transcripts detectable by RT-PCR) or a haematological response (reduction in the white cell count):

1. Complete cyto-genetic responders. In these patients the Ph+ clone will be suppressed and haemopoietic cells in the blood and bone marrow will be Ph- and normal. This result suggests that CML clone was sensitive to imatinib and that there was sufficient

normal haemopoiesis to sustain haemopoietic activity.
2. Haematological responders. In these patients the white blood cell count will fall in response to imatinib, but no cytogenetic improvement will appear. This implies that the CML clone was sensitive but that there was insufficient normal haemopoiesis to sustain cytogenetic response.
3. Non responders. In these patients the white cell count does not respond fully to imatinib therapy, indicating that the CML clone is insensitive to this drug.

This study focuses on the clinical presentation, haematological response, molecular response, survival and adverse effects of imatinib mesylate at the last visit of CML patients at our institution.

MATERIAL AND METHODS

Twelve patients, nine males and three females in the age group of 17–65 years who were diagnosed as chronic phase of chronic myeloid leukaemia (CP-CML) at our hospital since 2001 who were treated with imatinib mesylate. All patients were diagnosed according to clinical symptoms and signs, peripheral blood smear findings, bone marrow aspiration and biopsy findings and reverse transcriptase quantitative polymerase chain reaction (RT-PCR). The dose of imatinib administered was 400 mg/day orally. They were followed closely at the oncology clinic during the period of 2002–2012. During this period, clinical examination, complete blood count (CBC) and peripheral smears were done every 3 months. The RT-PCR was done at the last visit. The patients were considered as having clinical response if they were free of symptoms and signs of CML. They were considered as having responded at the haematological level if they achieved normal haemoglobin, leukocyte, and platelet indices at the last visit as shown in Table-1 and 2.

Peripheral blood and bone marrow samples obtained from patients in CP-CML who were scheduled to receive imatinib therapy. Samples obtained before treatment and after treatment from those patients who become complete cytogenetic responders. Normal control materials obtained as bone marrow from individuals who are donating cells for allogeneic transplantation and as blood from normal volunteers. Written informed consent and Research Ethics Committee approval was obtained in all cases.

Monitoring of this disease quantitated using Real-Time PCR in the forthcoming protocol, using IgH and TCR genes as genomic targets. Reverse transcriptase standardised in our laboratory for the p190 and p210 fusions. This technique is used to monitor all children who are receiving imatinib and provideds information about the kinetics of response to treatment.

BCR-ABL protein and phosphorylated tyrosine kinase activity was detected during SDS-PAGE and quantitated via densitometry.

Apoptosis, where appropriate detected using TUNEL and flow cytometry (Annexin-V labelling). Fluorescence *in situ* hybridisation (FISH) analysis of CD34+ cells and of colonies grown *in vitro* was done.

This technique has been used to discriminate between Ph+ and Ph- cells at different stages of maturation and colonies grown in myeloid colony assay. $CD34^+$ cells were separated using MiniMACS magnetic immuno-affinity columns as directed by the manufacturer (Milteny Biotec). Cells and colonies will be transferred into multicompartmentalised slides and air dried by evaporation. The cells will then be probed using a mixture of BCR sequences labelled with Spectrum Green and ABL sequences labelled with Spectrum Orange (Vysis) as directed by the manufacturer. Ph+ cells will be identified by the coincidence of green and orange signals. FISH combined with immunophenotyping will be used to determine the relative frequencies of Ph+ and Ph- cells in CD34+ cell subcompartments (CD33±, CD38±, HLA-DR±). Finally CD34+ cells will be stained for proliferating cell nuclear antigen (PCNA) and probed by FISH to measure the frequencies of quiescent versus proliferative Ph+ and Ph- cells.

Preparation of Human cells to cell culture:

Mononuclear cells (MNCs) were obtained by density gradient centrifugation over Ficoll-Hypaque (1.077 g/ml) (Nyegaard). After washing with HBSS (GibcoBRL), cells were resuspended in HBSS. To calculate the concentration of nucleated cells, the sample was diluted with 3% acetic acid to lyse red blood cells and an aliquot placed in a haemocytometer counting chamber. The cell concentration was adjusted to 10×10^6/5 ml with 15% FCS in minimal essential medium (MEM) (GibcoBRL). The cell suspension was placed in a 25 Cm^2 plastic flask for 2 hours incubation at 37 °C in humidified 5% CO_2 in air to remove adherent monocytes and macrophages. Non adherent MNCs were harvested for culture.

Purification of human $CD34^+$ cells $CD34^+$ cells were separated using MiniMACS magnetic immuno-affinity columns as directed by the manufacturer (Milteny Biotec). Cells from pooled day 7 CFU-GM were incubated for 15 min at 4 °C with 100 µl reagent A1 (blocking FcR), 100 µl of reagent A2 (hapten-conjugated anti $CD34^+$ Mab) and 300 µl of MiniMacs buffer (MM) containing calcium and magnesium-free PBS (GibcoBRL), 0.5% human serum albumin (Immuno AG) and 5 mM EDTA (Gibco BRL). Following a single wash in 10 ml MM, cells were re-suspended with 100 µl reagent B (anti-hapten Mab conjugated to beads) and 400 µl of MM buffer and incubated for 15 min at 4°C. Cells then were washed again in 10 ml MM and gently but thoroughly re-suspended in 1 ml of MM buffer. The labelled cells

were loaded onto the affinity column held in a magnet and $CD34^+$ cells washed through with 4×0.5 ml of MM buffer. After removal from the magnet, the $CD34^+$ cells were vigorously expelled from the column with 1 ml MM buffer. The purity is about 85–98%, confirmed by flow cytometry.

All recombinant cytokines were obtained from First Link. The concentration of each cytokine used was optimized in the laboratory.

Non-adherent MNCs were mixed with 3 ml of methylcellulose (MC) (1×10^5 cells/ml) (Stem Cell Technologies) containing 10% FCS and supplemented with recombinant human cytokines {IL-3 (5 ηg/ml), G-CSF (100 ηg/ml), GM-CSF (1 ηg/ml) and SCF (20 ηg/ml)}. After being mixed well, 1 ml aliquots were plated out into three 35 cm^2 diameter petri-dishes. The dishes were then incubated at 37 °C in humidified 5% CO_2 in air for 7 days. Colonies consisting of 50 cells or more were scored under an inverted microscope.

After 7 days of incubation, 120 colonies consisting of 50 cells or more were plucked individually from MC using a sterile eppendorf pipette and thoroughly dispersed in separate wells of a 96 well plate, each containing 100 µl of MC plus FCS and supplemented with the four recombinant human cytokines. The surrounding wells were filled with sterile water to prevent evaporation during incubation. Plates were incubated for a further 7 days at 37°C in humidified 5% CO_2 in air. Then each well was scored for secondary colonies of more than 50 cells. The results were analysed by using Microsoft Excel version 5.0. They were plotted as the cumulative distribution of the proportion of primary CFU-GM producing more than n secondary colonies. Both axes are expressed on a logarithmic scale. This step allows good data fit, so that the Area-Under-the Curve (AUC) can be measured using the Trapezium Rule. This is done because the distribution of secondary colonies is highly skewed rather than being normally distributed. Therefore the median and not the mean is the correct value to use. However, for cases where more than 50% of the replated colonies do not yield secondary colonies, the median would be zero and uninformative. The AUC is calculated to provide an overall measure of progenitor cell self-renewal capacity. The AUC is calculated to provide an overall measure of progenitor cell self-renewal capacity. The sensitivity to Imatinib is given by the ratio between the AUC of the control culture and the AUC of the culture incubated in the presence of Imatinib. Data from the Ph- colonies will be compared with data from normal blood and bone marrow controls. In the case of complete cytogenetic responders we will study samples obtained by mobilisation, in addition to blood and bone marrow, and compare results with samples from normal stem cell donors who have been subjected to the same mobilisation regimen.

Cells were washed twice with phosphate-buffered saline (PBS) buffer and lysed at 4×10^4 cells/ml in cell lysis buffer (20 mM HEPES, pH 7.4, 0.25% NP-40 containing protease inhibitor cocktail; Boehringer Mannheim, Indianapolis, IN) for 10 minutes on ice. Equal amounts of lysate (equivalent to $5X10^5$ cells) were subjected to SDS-PAGE to 12% polyacrylamide gels. Proteins were transferred to Hybond-P (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom) membranes and reacted with first antibody for 2 hours at room temperature. After they were washed, membranes were probed with a horseradish peroxidase–conjugated secondary antibody and reacted with ECL reagent (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom). Anti–β-actin blot was made in parallel as a loading control. Signals were detected by a Phosphor Imager (Storm 860, version 4.0; Molecular Dynamics, Sunnyvale, CA) and quantified by Scion Image software (Scion, Frederick, MD). Results were expressed as 1^{st} Ab/β-actin ratios.

Statistical analysis was carried out using Microsoft Excel spreadsheet and the Stat View SE+ graphics software. The probability of a significant difference between groups was determined by Mann-Whitney U test and Wilcoxon Signed rank test. Graphs were plotted using Cricket graph graphics package. Correlation was calculated using Spearman Rank test. The AUC was calculated using a Microsoft Excel spreadsheet. All software was run on a Macintosh computer.

RESULTS

Nine (75%) out of twelve patients with Philadelphia-chromosome positive chronic phase CML were males and 25% were females (Table-1). The most common symptoms and clinical signs with which the patients presented were fatigue 9 (75%), fever 7 (58%), rash 3 (25%), bleeding 2 (17%) and spleenomegaly 10 (83%). Four (33%) of patients were diagnosed incidentally. Eight (67%) of the patients had low risk disease, 4 (33%) of them had intermediate risk and none had high risk (WHO scoring system). Five (42%) of patients were in clinical, complete haematological and major molecular responses after 10 years, 1 (8%) after 7 years, 4 (33%) after 6 years and 1 (8%) after 3 years of therapy. One (8%) patient had clinical and complete haematological remission by 10 years of treatment but failed to have MMR. Imatinib mesylate was safe in our patients and no side effects were reported (Table-2).

We performed dose-response curve for the imatinib to determine the highest concentrations of imatinib that did not has suppressive effect on the colony formation (data not shown). Then, we used these obtained concentrations to study its effects on the proliferation of primary colonies. There is a significant reduction in the AUC of those treated with Imatinib

compared to untreated controls (p=0.05, n=10) (Figure-1 and 2). Interestingly, imatinib responses have no effect on NBM CFU-GM as it did for CML CFU-GM.

Use of *in vitro* assay systems, demonstrated clear differences between normal and CML cell proliferation, adhesion and responses to imatinib and IFN-α. The AUC in CML is significantly higher than normal, and is significantly reduced by imatinib or IFN-α compared to normal, but there are wide variations in both measurements. The tyrosine kinase inhibitor 'Imatinib, STI571' selectively inhibits the tyrosine activity of ABL and BCR-ABL proteins and the formation of CFU-GM colonies from CML patients. We found also that imatinib has a greater and more selective effect on the replating ability of CFU-GM from CML patients, than it has on primary colony numbers. In contrast to the suppression seen in CML CFU-GM, the replating ability of normal CFU-GM was significantly enhanced at low concentration of the compound (0.1 μM) when the differential effect was greater (Figure-3).

In some patients, increased amounts of lymphocytes were observed in follow-up samples during imatinib therapy. The morphology of lymphocytes varied in size and shape and also included immature forms. At the time of diagnosis none of the patients had increased amounts of lymphocytes in the bone marrow as the mean percentage of lymphocytes was 3%. After 9 months of imatinib therapy, the bone marrow cellularity had decreased and was normal in 9/12 patients. The median percentage of lymphocytes was 7%, but there was a marked variability between different patients. At the time of diagnosis, the CML patients had decreased number of B cells in the bone marrow compared to normal control values (10% of lymphocytes vs 29%), and no immature or maturating forms were detected. Patients with suboptimal response to imatinib also had decreased number of B cells in the bone marrow, whereas patients having optimal response and bone marrow lymphocytosis had normal or increased amounts of B cells. In T cells the CD4/CD8 ratio was normal and the proportion of regulatory T cells (Tregs) in bone marrow was also comparable in different groups. The numbers of DC were comparable to normal levels in patients with optimal response to imatinib treatment.

Baf3 cells were exposed to imatinib (20 ηM) for 24 hours and then lysates prepared for Western blot. Following SDS-PAGE electrophoresis, the samples were probed with anti-pCrkl and anti-actin. Crkl is an adaptor protein which plays an important role in BCR ABL mediated signal transduction. By this technique we demonstrated that in Baf3 cells imatinib exposure appeared to be associated with a decrease in BCR-ABL activity. BCR-ABL activity was effectively reduced with exposure to 150 ηM imatinib for 24 hour for comparison.

Patient samples CML CD34+ cells were cultured in GM mix in the presence and absence of imatinib. At each time point the viable cell count and percentage of apoptotic cells were calculated. With increasing concentration of imatinib there was an increase in the percentage of apoptotic cells staining positive for annexin-V alone (early apoptosis) and both annexin-V and 7-AAD (late apoptosis) (Figure-4).

Table-1: Demographic characteristics

Characteristics	Value
Total numbers of patients	12
Male/female	9/3
Age: range	17–65
Fever	7
Fatigue	9
Bleedings	2
Rash	3
Splenomegaly	10
WHO score	
Low risk	8
Intermediate risk	4
High risk	0

Table-2: Response to imatinib mesylate therapy

Patients 12	Clinical response	CHR	MMR	CMR	Duration of treatment
5	5	5	4	1	9 years
1	1	1	0	1	7 years
4	4	4	3	1	6 years
1	1	1	0	1	3 years
1	1	1	0	0	10 years

CHR=complete haematological response, CMR=complete molecular response, MMR=major molecular response

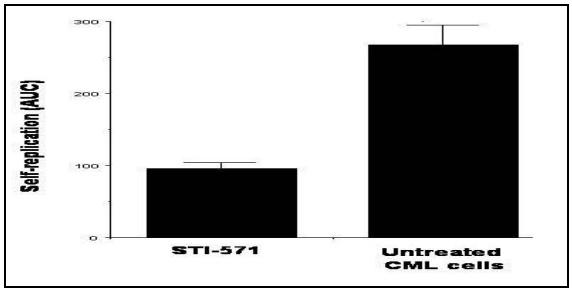

Figure-1: Self-renewal capacity (AUC) of CFU-GM from CML patients treated with STI-571

The results are compared to untreated cells. There is a significant reduction in the AUC of those treated with STI-571 compared to untreated controls (Wilcoxon Signed-rank test).

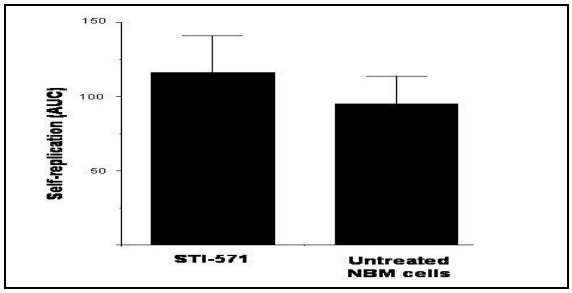

Figure-2: Self-renewal capacity (AUC) of CFU-GM from NBM controls treated with STI-571

The results are compared to untreated cells. There is no significant difference in the AUC of STI-571 compared to untreated controls (Wilcoxon Signed-rank test).

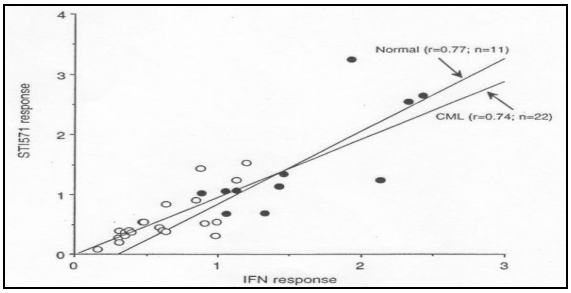

Figure-3: Correlation between the responses of NBM (solid symbols) and CML (open symbols) progenitors, as measured by the AUC assay to 50U/ml IFN-α and 0.1 μM STI571

Responses of both normal and CML cells showed strong correlations but NBM cells were stimulated, while most CML cells were inhibited.

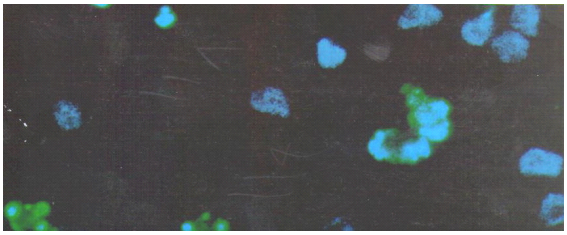

Figure-4: apoptotic CD34+ cells stained with TUNEL assay under immunofluorescent microscopy

Data is a representative of 3 experiments

DISCUSSION

Chronic myeloid leukaemia (CML) is a clonal myeloproliferative disorder of the pluripotent stem cells with an incidence of 1 per 100,000 in the west[24,25] whereas, in Saudi Arabia the true incidence is not known. All leukaemia in general account for 6.2% of all newly diagnosed cancers in year 2004, and only 4.6% and 6.7% were due to CML in male and female respectively.[26] The median age of patients suffering of CML is 67 years in the west with slight male predominance[27], however, in our study the median age was 39 years with male predominance.

The old conventional chemotherapy of CML has been either Busulphan or hydroxyurea with many adverse effects, median survival of less than 3 years, haematological remission of less than 80% and no cytogenetic remission.[24,28] Allergenic stem cell transplantation is the only curative treatment for CML but not always feasible or available with high mortality rate and adverse effects. Interferon Alfa was the treatment of choice before tyrosine kinase inhibitors (TKI).

Nowadays, the standard frontline therapy of CML patients is imatinib 400 mg daily.[29] The inhibition of BCR-ABL tyrosine kinase is predictable, selective and effective therapy for Philadelphia chromosome positive CML. The adverse reactions are less and manageable compared to the old options of therapy.

In the present study, we investigated whether imatinib treatment behave in the similar manner as other populations. Twelve patients were followed up for 10 years. The results demonstrated that none of our patients complains of any adverse effect even though the duration of therapy was long, ranging from 3–10 years and 90% had a duration more than 6years. These results are on line with many local reports from Saudi Arabia.[30–32]

Studies have shown that after 6 years of follow-up, 86–88% of patients with Ph+ CML who were treated with imatinib mesylate remained in MMR, which was defined as a 3-log reduction in the quantity of the Bcr-Abl transcript and has emerged as a new target response to achieve and is gaining acceptance as a surrogate for long-term benefit in clinical studies.[33,34]

In our study, even though small one, 83% of our patients are in MMR after 6 years and 42% are in MMR by 10 years of treatment and none of them has complained of side effects or has discontinued imatinib mesylate therapy. Our study is on agreement with several studies which showed that an overall survival rate of 95.2% after 8 years.[35,36]

To investigate whether the self-replication of CML CFU-GM could be reduced to the levels of NBM CFU-GMs, STI571 was incubated at various concentrations. There was a decrease in the CML CFU-GMs replication to the levels seen in NBM, But did not affect the NBM replication. These results are consistent with Gordon's group.[37–39]

These findings showing that imatinib suppresses progenitor cell proliferation, as reflected by the AUC obtained in the colony replating assay, by CML but not by normal CFU-GM confirms the link between p210 expression, enhanced progenitor cell renewal and responses to therapeutic agents in CML. The idea that different downstream signalling pathway may be activated to different extents and commensurately inactivated by treatment with imatinib is supported by our preliminary findings on the effects of pathway inhibitors in individual patients. Our data have implicated the PI3-kinase pathway and suggest that downstream target (e.g., ERK and p70) are differently affected in different patients. These initial results support imatinib as a less toxic and orally administrated alternative to IFN-α therapy of CP-CML. Two reports showed that STI571 induced apoptosis in CML cells[40,41] as our results revealed.

It could be inferred that the presence of fully functional genes regulating both cell cycle and apoptosis, will maintain the balance between the rate of cell division and apoptosis of any population in vivo. Therefore, any malfunction or loss of any one of the above mentioned genes, may lead to an increase in their self-replication.[42,43]

CONCLUSION

Imatinib mesylate is effective and safe even after 10 years of treatment and follow-up of patients suffering of Ph+ CML.

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REFERENCES

1. Nowell PC, Hungerford DA. Chromosome studies in human leukemia. II. Chronic granulocytic leukemia. J Natl Cancer Inst 1961;27:1013–35.
2. Gorusu M, Benn P, Li Z, Fang M. On the genesis and prognosis of variant translocations in chronic myeloid leukemia. Cancer Genet Cytogenet 2007;173(2):97–106.
3. Bose S, Deininger M, Gora-Tybor J, Goldman JM, Melo JV. The presence of typical and atypical BCR-ABL fusion genes in leukocytes of normal individuals: biologic significance and implications for the assessment of minimal residual disease. Blood 1998;92(9):3362–7.
4. Deininger MW, Goldman JM, Melo JV. The molecular biology of chronic myeloid leukemia. Blood 2000;96:3343–56.
5. Mandanas RA, Boswell HS, Lu L, Leibowitz D. BCR-ABL confers growth factor independence upon a murine myeloid cell line. Leukemia 1992;6:796–800.
6. Sirard C, Laneuville P, Dick J. Expression of bcr-abl abrogates factor-dependent growth of human hematopoietic M07E cells by an autocrine mechanism. Blood 1994;83:1575–85.
7. Cortez D, Kadlec L, Pendergast AM. Structural and signaling requirements for BCR-ABL-mediated transformation and inhibition of apoptosis. Mol Cell Biol 1995;15:5531–41.
8. Daley GQ, Baltimore D. Transfection of an interleukin 3-dependent hematopoietic cell line by the chronic myelogenous leukemia-specific P210 protein. Proc Natl Acad Sci USA 1988;85:9312–6.
9. Udomsakdi C, Eaves CJ, Swolin B, Reid DS, Barnett MJ, Eaves AC. Rapid decline of chronic myeloid leukemia cells in long-term culture due to a defect at the stem cell level. Proc Natl Acad Sci USA 1992;89:6192–6.
10. Marley SB, Lewis JL, Scott MA, Goldman JM, Gordon MY. Evaluation of “discordant maturation” in chronic myeloid leukaemia using cultures of primitive progenitor cells and their production of clonogenic progeny (CFU-GM). Br J Haematol 1996;95:299–305.
11. Chasty RC, Lucas GS, Owen-Lynch PJ, Pierce A, Whetton AD. Macrophage inflammatory protein-1 alpha receptors are present on cells enriched for CD34 expression from patients with chronic myeloid leukemia. Blood 1995;86:4270–7.
12. Cashman JD, Eaves CJ, Sarris AH, Eaves AC. MCP-1, not MIP-1alpha, is the endogenous chemokine that cooperates with TGF-beta to inhibit the cycling of primitive normal but not leukemic (CML) progenitors in long-term human marrow cultures. Blood 1998;92:2338–44.
13. Gordon MY, Dowding CR, Riley GP, Goldman JM, Greaves MF. Altered adhesive interactions with marrow stroma of haematopoietic progenitor cells in chronic myeloid leukaemia. Nature 1987;328:342–4.
14. Verfaillie CM, McCarthy JB, McGlave PB. Mechanisms underlying abnormal trafficking of malignant progenitors in chronic myelogenous leukemia. Decreased adhesion to stroma and fibronectin but increased adhesion to the basement membrane components laminin and collagen type IV. J Clin Invest 1992;90:1232–41.
15. Vijayan KV, Advani SH, Zingde SM. Chronic myeloid leukemic granulocytes exhibit reduced and altered binding to P-selectin: modification in the CD15 antigens and sialylation. Leuk Res 1997;21(1):59–65.
16. Rowley JD. A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature 1973;243:290–3.
17. Bartram CR, de Klein A, Hagemeijer A, van Agthoven T, Geurts vanKessel A, Bootsma D, *et al*. Translocation of c-Abl oncogene correlates with the presence of a Philadelphia chromosome in chronic myelocytic leukaemia. Nature 1983;306:277–80.
18. Dasmahapatra G, Nguyen TK, Dent P, Grant S. Adaphostin and bortezomib induce oxidative injury and apoptosis in imatinib mesylate-resistant hematopoietic cells expressing mutant forms of Bcr/Abl. Leuk Res 2006;30(10):1263–72.
19. Borthakur G, Kantarjian H, Daley G, Talpaz M, O'Brien S, Garcia-Manero G, Giles F, *et al*. Pilot study of onafarnib, a farnesyl transferase inhibitor, in patients with chronic myeloid leukemia in the chronic or accelerated phase that is resistant or refractory to imatinib therapy. Cancer 2006;106:346–52.
20. Jorgensen HG, Allan EK, Mountford JC, Richmond L, Harrison S, Elliott MA, *et al*. Enhanced CML stem cell elimination in vitro by bryostatin priming with imatinib mesylate. Exp Hematol 2005;33:1140–6.
21. Shah NP, Tran C, Lee FY, Chen P, Norris D, Sawyers CL. Overriding imatinib resistance with a novel ABL kinase inhibitor. Science 2004;305(5682):399–401.
22. Mukai M, Che XF, Furukawa T, Sumizawa T, Aoki S, Ren XQ, *et al.* Reversal of the resistance to STI571 in human chronic myelogenous leukemia K562 cells. Cancer Sci 2003;94557–63.
23. Nicolini FE, Hayette S, Corm S, Bachy E, Bories D, Tulliez M, *et al*. Clinical outcome of 27 imatinib mesylate-resistant chronic myelogenous leukemia patients harboring a T315I BCR-ABL mutation. Haematologica 2007;92:1238–41.
24. Kumar L. Chronic myelogenous leukaemia (CML): An update. Natl Med J India 2006;19:255–63.
25. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108.
26. Kingdom of Saudi Arabia, Ministry of Health, National Cancer Registry. Cancer Incidence Report. 1999–2000. Available at: http://www.kfshrc.edu.sa/oncology/files/ncr99_00.pdf
27. Miller BA, Ries LAG, Hankey BF, Harras A, Edwards BK, (Eds). SEER Cancer Statistics Review 1973–90. (NIH Pub No. 93-2789). Bethesda MD: National Cancer Institute; 1993. p. 1–44.
28. Dieninger MW, O'Brien SG, Ford JM, Druker BJ. Practical management of patients with chronic myeloid leukemia receiving imatinib. J Clin Oncol 2003;21:1637–47.
29. Baccarani M, Dreyling M, ESMO Guidelines Working Group. Chronic myeloid leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010;(Suppl 5):165–7.
30. Khalil SH, Abu-Amero KK, Al Mohareb F, Chaudhri NA. Molecular monitoring of response to imatinib (Glivec) in chronic myeloid leukemia patients: experience at a tertiary care hospital in Saudi Arabia. Genet Test Mol Biomarkers 2010;14(1):67–74.
31. Aleem A, Siddiqui N. Chronic myeloid leukemia presenting with extramedullary disease as massive ascites responding to imatinib mesylate. Leuk Lymphoma 2005;46:1097–9.
32. Al-Qurashi F, Ayas M, Al Sharif F, Ibrahim E, Sahovic E, Al Mahr M, Chaudhri N, *et al*. Second allogeneic bone marrow transplantation after myeloablative conditioning analysis of 43 cases from single institution. Hematology 2004;9(2):123–9.
33. Gupta A, Prasad K. Hematological and Molecular Response Evaluation of CML Patients on Imatinib. J Assoc Physicians India 2007;55:109–13.
34. Mauro MJ. Defining and Managing Imatinib Resistance. Hematology Am Soc Hematol Educ Program 2006;(1):219–25.
35. Hochhaus A, O’Brien SG, Guilhot F, Druker BJ, Branford S, Foroni L, *et al*. Six-year follow-up of patients receiving imatinib for the first-line treatment of chronic myeloid leukemia. Leukemia 2009;23:1054–61.
36. Gambacorti-Passerin C, Antolini L, François-Xavier M, Guilhot F, Deininger M, Fava C, *et al.* Multicenter Independent

Assessment of Outcomes in Chronic Myeloid Leukemia Patients Treated With Imatinib. J Natl Cancer Inst 2011;103:553–61.
37. Marley SB, Deininger MW, Davidson RJ, Goldman JM, Gordon MY. The tyrosine kinase inhibitor STI571, like interferon-alpha, preferentially reduces the capacity for amplification of granulocyte-macrophage progenitors from patients with chronic myeloid leukemia. Exp Hematol 2000;28(5):551–7.
38. Marley SB, Davidson RJ, Lewis JL, Nguyen DX, Eades A, Parker S, *et al*. Progenitor cells from patients with advanced phase chronic myeloid leukaemia respond to STI571 in vitro and in vivo. Leuk Res 2001;25:997–1002.
39. Marley SB, Davidson RJ, Goldman JM, Gordon MY. Effects of combinations of therapeutic agents on the proliferation of progenitor cells in chronic myeloid leukaemia. Br J Haematol 2002;116(1):162–5.
40. Oetzel C, Jonuleit T, Gotz A, van der Kuip H, Michels H, Duyster J, *et al*. The tyrosine kinase inhibitor CGP57148 (ST1571) induces apoptosis in BCR-ABL-positive cells by down-regulating Bcl-X. Clin Cancer Res 2000;6:1958–68.
41. Gambacorti-Passerini C, le Coutre P, Mologni L, Fanelli M, Bertazzoli C, Marchesi E *et al*. Inhibition of the ABL kinase activity blocks the proliferation of BCR/ABL+ leukemic cells and induces apoptosis. Blood Cells Mol Dis 1997;23: 380–94.
42. Alenzi FQ. Is there a link between apoptosis and chronic leukemia. J Cell Sci Ther 2012;3:1–3.
43. Alenzi FQ, Wyse RK, Tamimi W, Bamaga M, Lotfy M. A close link between Fas, p53 and Apaf-1 in chronic leukemia. Saudi Med J 2007;28:1296–99.

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