



CANCER NEWS

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Focus Area:
**CHRONIC MYELOGENOUS
LEUKEMIA**



**Rajiv Gandhi Cancer Institute
and Research Centre**

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From the Desk of Director Research

Hematological malignancies, also referred to as liquid tumors, constitute approximately 9.5% of total tumor burden in India with a mortality rate of 10–20% per year. Chronic myeloproliferative neoplasms are a group of stem cell disorders characterized by overproduction of mature white cells [Chronic myelogenous leukemia (CML)], red cells (polycythaemia vera), and platelets (essential thrombocythaemia), suggesting that the maturation of neoplastic cell line is normal. CML accounts for about 20% of all leukemias affecting adults. It typically affects middle-aged individuals, rarely adolescents or children and is higher in men than in women. These diseases are progressive and exhibit transition from one disease to another over a period of time and may result in bone marrow failure or transformation to acute leukemia in terminal phase.

CML was first described in 1845 in Edinburgh as a case of “hypertrophy of the spleen and liver in which death took place from suppurative of the blood”. In 1960, a genetic link to this cancer was found by Peter Nowell and Davind Hungerford and this became the first neoplastic disease associated with a chromosomal aberration. It was called the ‘Philadelphia Chromosome’, after the name of city in which it was discovered. This later proved to be the genetic signature caused by chromosomal translocation and was observed to be present only in malignant cells of CML, somehow providing them a growth advantage over normal leukocytes. The 1980’s led to the discovery of translocation partners BCR (Breakpoint Cluster Region) on chromosome 22 and ABL (Abelson) gene on chromosome 9, followed by a breakthrough that the fusion protein causes unregulated tyrosine kinase activity which is critical for transformation of cells.

Symptoms are not specific in CML and include weight lost, asthenia, fever, night sweats and malaise. However, the diagnosis is unexpected being based on abnormal blood counts and differential in 40% of cases. Patients present in either of the 3 phases: chronic phase, accelerated phase or blast crisis. The hallmark in diagnosis is splenomegaly, leukocytosis with basophilia, immature granulocytosis and thrombocytosis. Thus peripheral blood examination with bone marrow interpretation is the key investigations for diagnosis. The confirmation is done by cytogenetics demonstrating translocation t(9;22) (q3.4;q1.1) and by reverse transcriptase polymerase chain reaction showing BCR ABL transcripts.

Treatment of CML was historically based on busulfan, then on hydroxyurea. Inteferon alpha was the gold standard before tyrosine kinase inhibitors (TKIs) were introduced. Imatininb is now, a known first line treatment worldwide, followed by nilotinib and dasitinib which are approved as second line therapies. More than 90 different point mutations have been reported in patients who develop resistance to imatinib and cause treatment failure. Response to treatment or moving on to another treatment is determined by the degree of cytogenetic response (CgR) and on detection of BCR ABL Kinase Domain point mutations. Cytogenetic monitoring, using chromosome banding analysis of marrow cell metaphases, is employed and responses are classified as complete, minor, minimal and none, depending on the number of Ph+ metaphases. Response evaluation to treatment is assessed at 3 months, followed by every six months until complete CgR (CCgR) is achieved. This is followed by evaluation at every 12 months unless a regular molecular monitoring for transcript level is done. Approximately 33% of patients achieve a CCgR while others have drug resistance or drug-related toxicities. Hence, newer drugs are being developed to overcome such limitations. Ponatinib, a pan-BCR-ABL TKI that targets multiple point mutations, including T315I, is notoriously associated with imatinib and multiple second-generation TKI failures. Novel interventions pave the way to improve patient outcomes and address the common mechanisms of resistance in the treatment of CML.

The present issue of the Cancer News highlights the newer advances in the field of CML cancer and features the regular articles, such as Special Feature, Guest Article, Perspective and In Focus. We are grateful to Dr Anupam Chakrapani, Senior Consultant, Clinical Haematology and BMT, Tata Medical Centre Kolkata for the "Guest Article"; Dr Neeraj Sidharthan, Associate Professor, Clinical Haematology and Stem Cell Transplant, Amrita Institute of Medical Sciences, Kochi for the "In Focus".

Suggestions/comments from the readers are welcome.

Dr D C Doval

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SPECIAL FEATURE

CML: CHANGING LANDSCAPE IN INDIAN SCENARIO

In India, chronic myeloid leukaemia (CML) is the most common adult leukemia, accounting for 30% to 60% of all adult leukemias. CML is characterized by excessive tyrosine kinase activity resulting from a chimeric BCR- ABL fusion protein and balanced reciprocal translocation t(9;22) typically between chromosome 9 and 22.

Beginning of the 21st century coincided with a dramatic change in the CML landscape. The discovery by Dr. Brian Druker and Nicholas Lydon to make targeted therapy by targeting t(9;22), led to the discovery of STI571 “Imatinib” which quickly proved to be the “magic bullet”.

As per the registry data available from various centres in India the incidence of CML is one of the commonest adult leukemia in Indian population. The male preponderance and median age reported is a decade younger compared with the age presented in European (median age 55years) and American (median age 66 years) literature. The most common signs and symptoms are splenomegaly, hepatomegaly, fatigue, weakness, dragging pain, pallor or sometimes asymptomatic seen in 30% cases. The percentage of patients presenting in chronic phase varies from 85%-97%, while in European data, the presentation of CML in chronic phase has been reported to be as high as 96%.

Diagnosis and monitoring of CML has been a challenging issue all over the world. With the advent of good cytogenetics and molecular laboratory services, most of the patients can

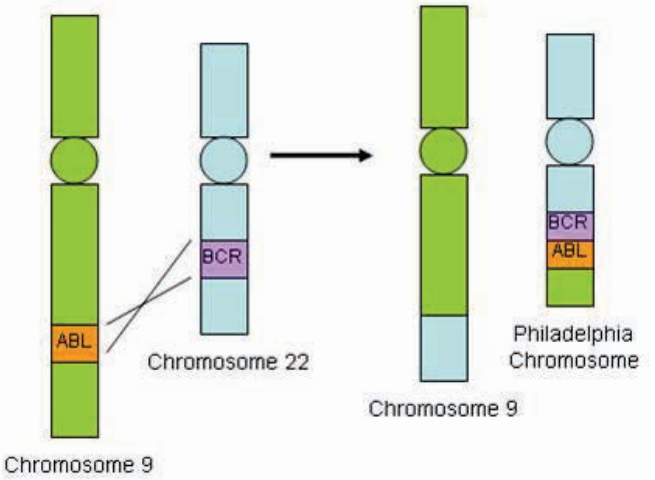


Fig. 2. Diagrammatic representation of translocation of chromosome 9 and 22

avail these services in India at very economical rates. In some part of rural India, diagnosis of CML is still based on peripheral smear examination, bone marrow examination, LAP score and clinical examination of splenomegaly.

The recommended starting dose of Imatinib (IM) in the IRIS study was 400 mg daily, regardless of the size of the patient, and this remains the “standard” dose. The cumulative best complete cytogenetic response (CCyR) rate was 82%; 63% of all patients randomized to receive imatinib and still on study treatment showed CCyR at last assessment. The estimated event-free survival at 6 years was 83%, and the estimated rate of freedom from progression to AP and BC was 93%. The estimated overall survival was 85% or 93% when only CML related deaths were considered. This 8-year update of IRIS underscores the efficacy and safety of imatinib as first-line therapy for patients with CML.

The Eutos and Sokal score appears to influence the probability of achieving (complete cytogenetic response) CCyR and major molecular response (MMR) in the IMera.

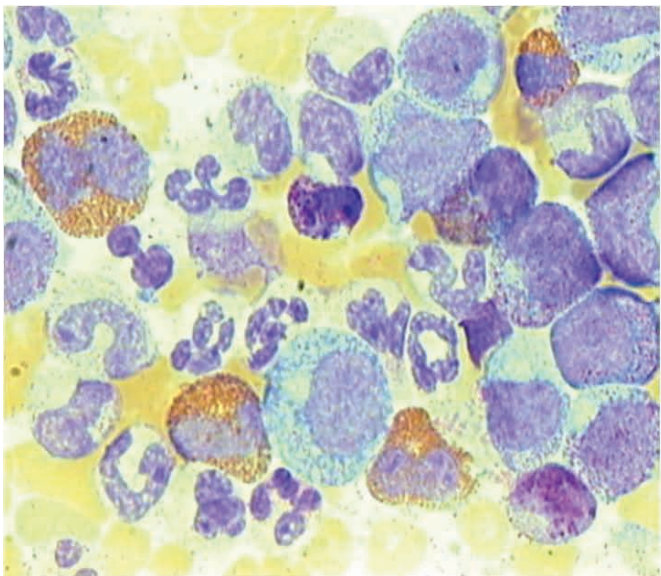
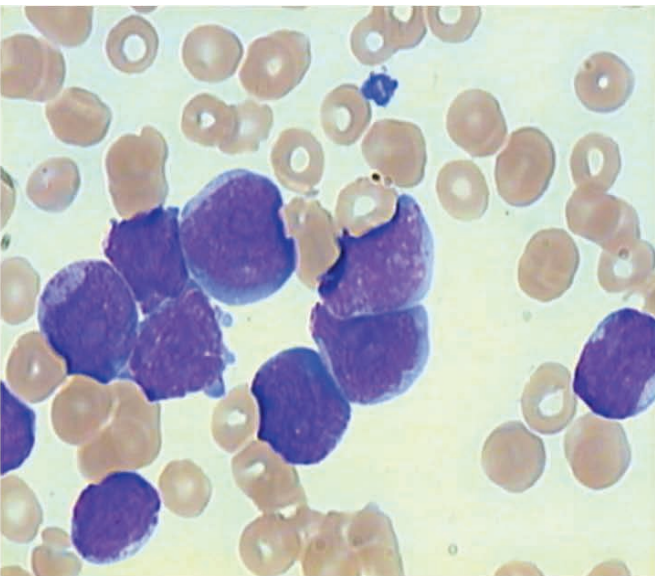


Fig. 1. (a) CML in chronic phase (peripheral smear)



(b) CML in blast crisis (peripheral smear)

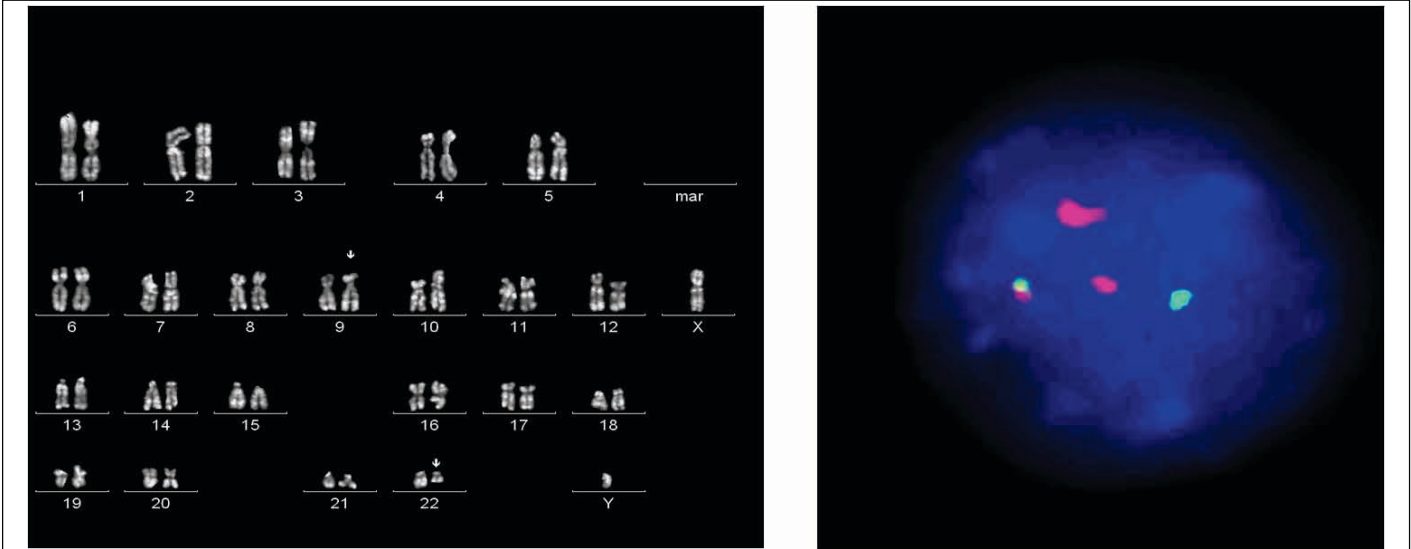


Fig. 3. (a) Karyotyping (Cytogenetic study)

(b) Fluorescence in situ Hybridization in case of CML

Response criteria laid down by European leukemia Net (ELN) and World Health Organization (WHO) provide useful tool in measuring the responses in patient on TKIs.

The majority of patients achieve normal blood counts within 3 months, and more than 90% will have achieved complete hematological response (CHR) after start of treatment. Approximately 40% of previously untreated patients will have achieved a (major cytogenetic response) MCyR (<35% Ph-positive marrow metaphases) by 6 months, and 65% will have achieved a complete cytogenetic response (CCyR) after 1 year of therapy. A small minority of those who achieve CCyR will subsequently regain evidence of marrow Ph-positivity, and an even smaller proportion will eventually progress to advanced phase disease.

A significant proportion of patients sustain some degree of hematologic toxicity (cytopenia) after starting treatment with IM at 400 mg daily. This usually resolves over a period of time.

IM can cause a variety of non-hematologic toxic effects such as weakness, gastritis, muscle cramps, anorexia, vomiting, diarrhea, infraorbital edema or rarely more generalized edema, abdominal pain, pain in bones and joints, skin rashes, hepatic dysfunction.

There is preliminary evidence that the incidence of disease progression in responders diminishes with each successive year on IM and the incidence of toxicity decreases with duration of treatment. The best advice for individual patients responding to IM is that the drug should be continued indefinitely.

A very few patients showed resistance (primary or secondary) to imatinib. The issue confronting the clinician will be to decide that the patient has failed imatinib at the prescribed dose. Patient compliance seems to be an important consideration, and the clinician must do his/her best to exclude the possibility that the patient is not taking the drug nor is taking it at reduced or intermittent dosage.

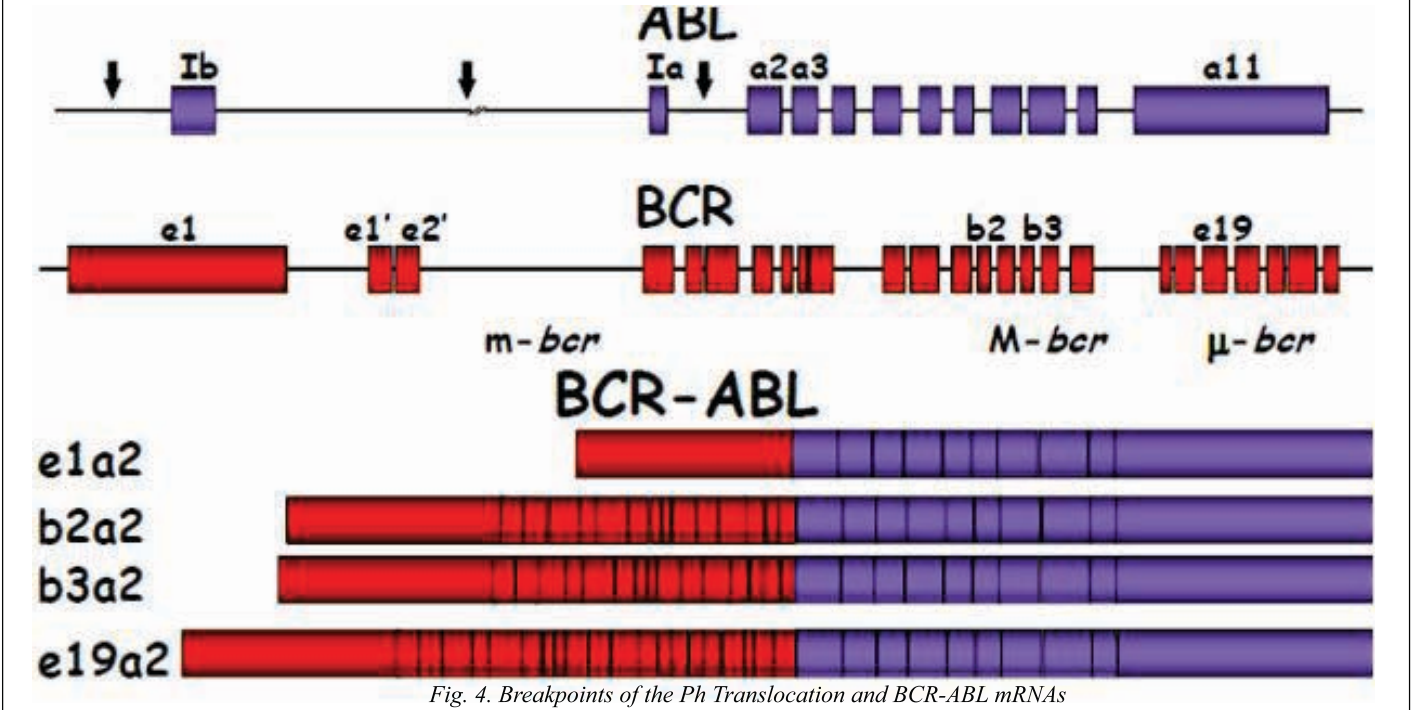


Fig. 4. Breakpoints of the Ph Translocation and BCR-ABL mRNAs

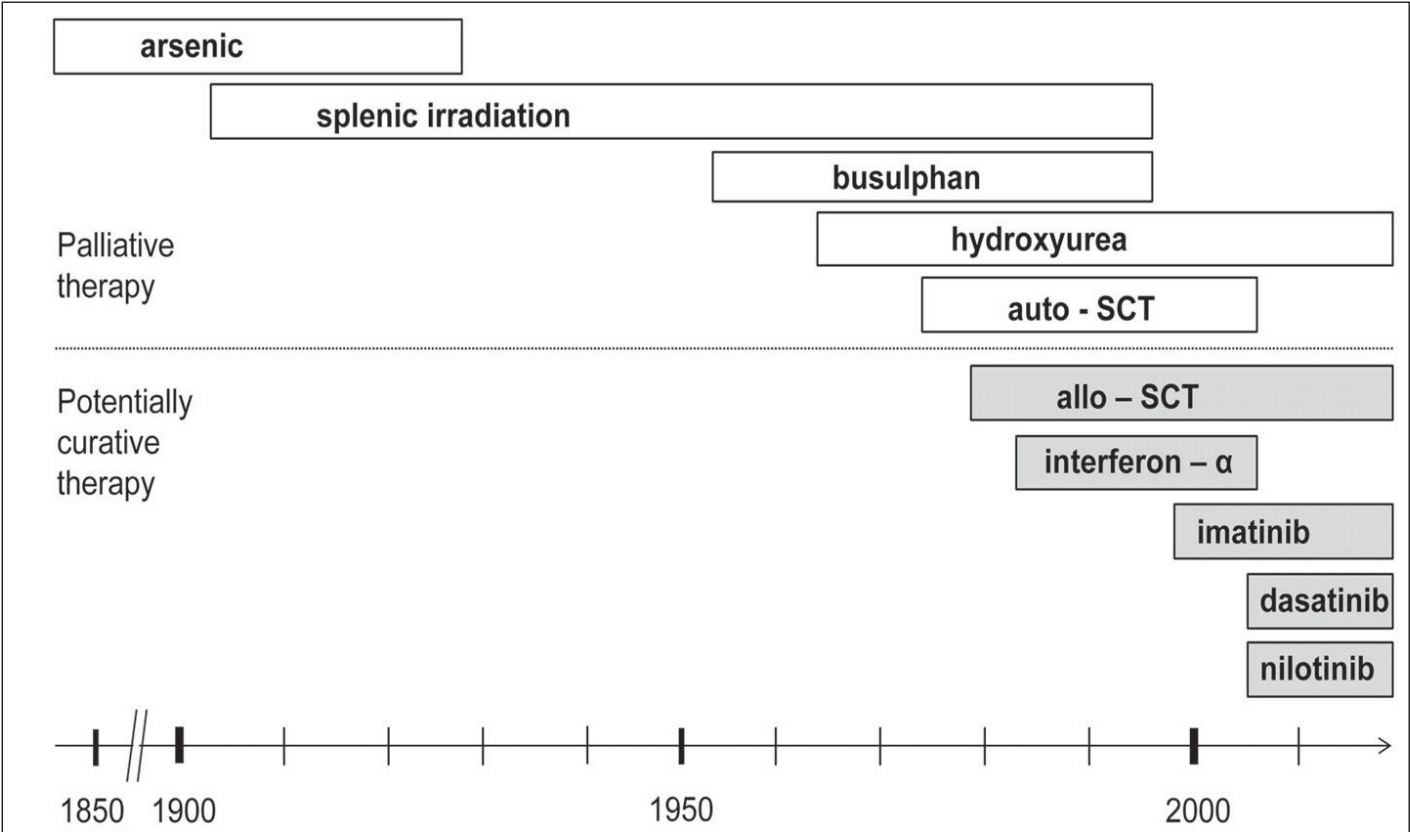


Fig. 5. A time line of chronic myeloid leukemia treatment

The next and easiest step will be to increase the dosage from 400 mg to 600 or 800 mg daily, and this may induce or reinstate cytogenetic responses, although they may not be prolonged. Also a patient who satisfies cytogenetic criteria for resistance to the highest feasible dose of IM should be considered for treatment with a second-generation TKI.

As per one study published in NEJM 2010 Nilotinib versus Imatinib for newly diagnosed CML, at 12 months, the rates of MMR for nilotinib (44% for the 300-mg dose and 43% for the 400-mg dose) were nearly twice that for imatinib (22%) ($P<0.001$ for both comparisons). The rates of CCR by 12 months were significantly higher for nilotinib (80% for the 300-mg dose and 78% for the 400-mg dose) than for imatinib (65%) ($P<0.001$ for both comparisons).

As per another study published in NEJM 2010, Dasatinib versus Imatinib in newly diagnosed chronicphase CML, after a minimum follow-up of 12 months, the rate of confirmed CCR was higher with dasatinib than with imatinib (77% vs. 66%, $P=0.007$), as was the rate of CCR observed on at least one assessment (83% vs. 72%, $P=0.001$). The rate of MMR was higher with dasatinib than with imatinib (46% vs. 28%, $P<0.0001$), and responses were achieved in a shorter time with dasatinib ($P<0.0001$).

In India, imatinib (glivec) is mainly available to patients via GIPAP (glivec international assisted programme). Now in India we also have many generic versions of imatinib available at very resonalble rates. We also have availability of second generation TKI's (dasatinib and nilotinib). Still majority of patients on second generation TKI can avail these

drugs due to access programme by pharmaceutical companies. These drugs are indicated in newly diagnosed, post IM failure or loss of response to imatinib. These drugs are also used in accelerated and blast crisis.

If the patient with IM-resistant leukemia, or CML in accelerated phase and CML in blast crisis, is relatively young and has an HLA-matched donor, the possibility of proceeding to allo-SCT should be considered. A few centers in India do offer allogeneic stem cell transplant as a curative approach in accelerated phase and blast crisis with success rate 40-60%.

The natural history of CML has changed in recent years, partly due to earlier diagnosis but mostly as a consequence of the availability of effective therapies that have the potential to eradicate the Ph chromosomepositive clone. As more than 90% of patients taking this drug are in stable cytogenetic remission for at least 5 years, this has resulted in increase in survival from CML. The availability of imatinib has changed the management of CML. Although the incidence rates of CML are low, its burden on population in terms of absolute numbers is quite high. Given the availability of reliable and simple diagnosis of CML, the public health goal should be to make sure the availability of proper diagnosis and management of CML across all the centers in urban and rural areas of developing world, so as to reduce the mortality. Currently, there have been limited studies to understand burden of CML in India.

It is important to observe the trends in incidence and mortality of CML over the years before celebrating success of recent diagnostic and management of CML.

References

1. Parikh PM, Charak BS, Banavali SD, Koppilar S, Giri NK, Kumar A, et al. Retrospective analysis of 224 cases of CML. Indian J Hematol. 1988;6:85–7.
2. Parikh PM, Iyer RS, Saikia TK, Gopal R, Advani SH. Philadelphia chromosome. J Assoc Physicians India. 1992;40:98–100.
3. Barbhaya SA, Parikh PM, Desai S. Bcr-c.abl detection by southern blotting in CML. Indian J Hematol. 1993;11:27.
4. Banavali SD, Parikh PM, Nanjangud G. Results of treatment of CML with a cisplatin based intensive treatment regimen (TMH 8802 protocol) Indian J Hematol. 1993;11:244–51.
5. Giles FJ, Hehlmann R, Hockhaus A, Parikh PM. Initial characterization of philadelphia chromosome chronic phase CML: A comparison between Asian and German patients on the International Oncology Study Group the German CML study group protocol. Int J Hematol. 1996;64:294.
6. Saikia TK, Advani SH, Parikh PM, Bapna A, Somjee S, Mukhopadhyay A, et al. Results of Allogeneic Bone Marrow transplant in Chronic Myeloid Leukemia (CML) following conditioning with Busulfan and cyclophosphamide. J Assoc Physicians India. 1999;47:770–3.
7. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med. 2001;344:1031–7.
8. O'Brien SG, Guilhot F, Goldman JM, Hochhaus A, Hughes TP, Radich JP, et al. International Randomized Study of Interferon versus STI571 (IRIS) 7-year follow-up: Sustained survival, low rate of transformation and increased rate of major molecular response (MMR) in patients (pts) with newly diagnosed chronic myeloid leukemia in chronic phase (CML-CP) treated with imatinib (IM) Blood. 2008;112:76.
9. Bhutani M, Kochupillai V. Hematological malignancies in India. In: Kumar L, editor. New York: Progress in Hematologic Oncology. Pub. The Advanced Research Foundation New York; 2003. p. 10.
10. A Hochhaus Six-year follow-up of patients receiving imatinib for the first-line treatment of chronic myeloid leukemia Leukemia (2009) 23, 1054–1061; doi:10.1038.
11. Giuseppe Saglio. Nilotinib versus Imatinib for Newly Diagnosed Chronic Myeloid Leukemia. N Engl J Med 2010; 362:2251–2259 June 17, 2010.
12. Hagop Kantarjian Dasatinib versus Imatinib in Newly Diagnosed Chronic-Phase Chronic Myeloid Leukemia. N Engl J Med 2010; 362:2260–2270 June 17, 2010.

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ACTIVITIES OF RGCI&RC

LYMPHOMA UPDATE 2016

The dawn of the 14 March saw a showery, windy morning. The conference lobby of the Hotel Crowne Plaza showed glimpses of an exhilarating crowd of pathologists, hematologists and clinical oncologists from distant places. The Conference was on “Lymphoma Update 2016, Essentials and Beyond”, the most awaited event of the year. Registration included 300 national and international delegates. The two day meet featured intense academic activities like lectures, slide seminars and poster presentation, keeping in mind the needs of the practicing pathologists and post graduate students. It brought together eminent international faculty and leading minds like- Dr Elaine S Jaffe, Senior Hematopathology Investigator, NIH, Bethesda USA; Dr Elias Campo, Research Director Barcelona, Spain, Dr Wing-Chung Chan, Professor Pathology, California, USA; Dr Bharat Nathwani, Director Pathology, California, USA. National faculty included personages like Dr Anita Borges, Director CoE, SRL, Mumbai, Dr Anil Handoo, Director Lab Services BLK Hospital, Delhi, Dr Jay Mehta, SRL, Mumbai; Dr Deepshikha Arora, Head Histopathology, Apollo Hospitals, New Delhi.

Day one commenced with brainstorming session on Diagnostic Approach to Lymphomas by Dr Deepshikha Arora, a specialist in oncopathology and transplant pathology, Apollo Hospitals, New Delhi. The session intensively covered the essentials in diagnosing a lymphoma. She emphasized that complete clinical data, a thorough morphological examination and choice of an appropriate immunohistochemical marker panel are the mainstay to a veridical diagnosis.



Inaugural of Lymphoma Update 2016

She asserted that both fundamental knowledge of normal lymphnode histology and histology of a lymphoma are unequalled partners. The 45-min session included normal architecture of lymphnode, different patterns of involvement of lymphoma, cellular constituents in lymphomas, reactive conditions mimicking lymphomas, how to shape a provisional diagnosis and working up of differential diagnosis. She concluded her talk by listing the final report contents of a clinically suspected lymphoma case. The session ended with an energetic audience interaction. Questions poured in with active faculty involvement.

The next session by Dr Jay Mehta complemented and crowned the previous session. He is a Consultant Histopathologist, CoE, SRL, Mumbai and has managed the histopathology laboratories of Tata Memorial Hospital, Mumbai, and Regional Cancer Centre, Trivandrum. He elaborated on the know-hows of immuno his to chemistry and pitfalls in the diagnosis of lymphoma. He discussed usage of antibody markers and their aberrant expression in various clinical settings like peripheral T cell NHL, plasmablastic lymphoma, Alk positive B cell Lymphoma. He concluded his session by a note of caution while reporting IHC and emphasized that multidisciplinary approach is required for a confirmed lymphoma diagnosis.

Post tea sessions included interesting and difficult case presentations by pathologists from various hospitals like-Medanta, Delhi; Max Hospital, Delhi; RCCC, Trivandrum; Artemis Hospital, Delhi; SRL, Mumbai, etc. The sessions lasted for a over 2 hours. Lunch session paralleled the poster exhibition with more than 30 participants exhibiting colourful and informative posters from all over the country. The best poster award had 2 nominees with the first position awarded to Dr. Namrata. P. Awasth from Dr RML Institute of Medical Sciences, Lucknow, titled "Flowcytometric Immunophenotyping and DNA Content Analysis in FineNeedle Aspirates of NHL" the second position was awarded to Dr. Meenakshi Chowdhary, RGCI, for the poster titled "Diagnostic Utility of Various Markers in B- ALL MRD".

Post lunch sessions included exhaustive coverage of flowcytometry in diagnosis of lymphomas by Dr Anil Handoo, Director Lab Services, BLK Hospital, New Delhi. He has served 10 years in the field of laboratory medicine with special interests in hematology and malignant flowcytometry. Flowcytometric immunopheno typing is useful in diagnosing lymphoma under the WHO classification system, where lymphoid neoplasms are separated into distinct clinical entities based upon morphology, immunophenotype, genetic abnormalities and clinical features. Flowcytometry can quantify the expression of proteins associated with a good or poor prognosis, detect multi-drug resistance, and measure cell proliferation, making it useful in measuring prognostic indicators in lymphoid neoplasia. Dr Handoo also discussed its limitations and obviated the need to use this technique in conjunction with other diagnostic modalities. The next session covered Diagnostic Approach to

Hodgkins Lymphoma and its Differential Diagnosis by Dr Anurag Mehta, Director Lab Services, RGCI, New Delhi. Dr Mehta a pioneer in oncopathology with special interests in Lymph Node pathology & Urogenital pathology, has also pursued Fellowship (WHO), in Lymph Node Pathology from MD Anderson Cancer Center, Houston, Texas, USA. He elaborated on various enigmas surrounding the diagnosis of the HL, its classical picture, the subtypes and differential diagnosis. His clinical case based depiction of perplexing situations faced by pathologists was appreciated by most present there. He also discussed cases where IHC was difficult to interpret or there was transformation in the subtypes. The discussion also included the category of gray zone lymphoma, a confounding factor rarely encountered. The sessions served as eye openers on the new facets of this disease. Day one consummated with another session of interesting case presentations lasting for an hour.

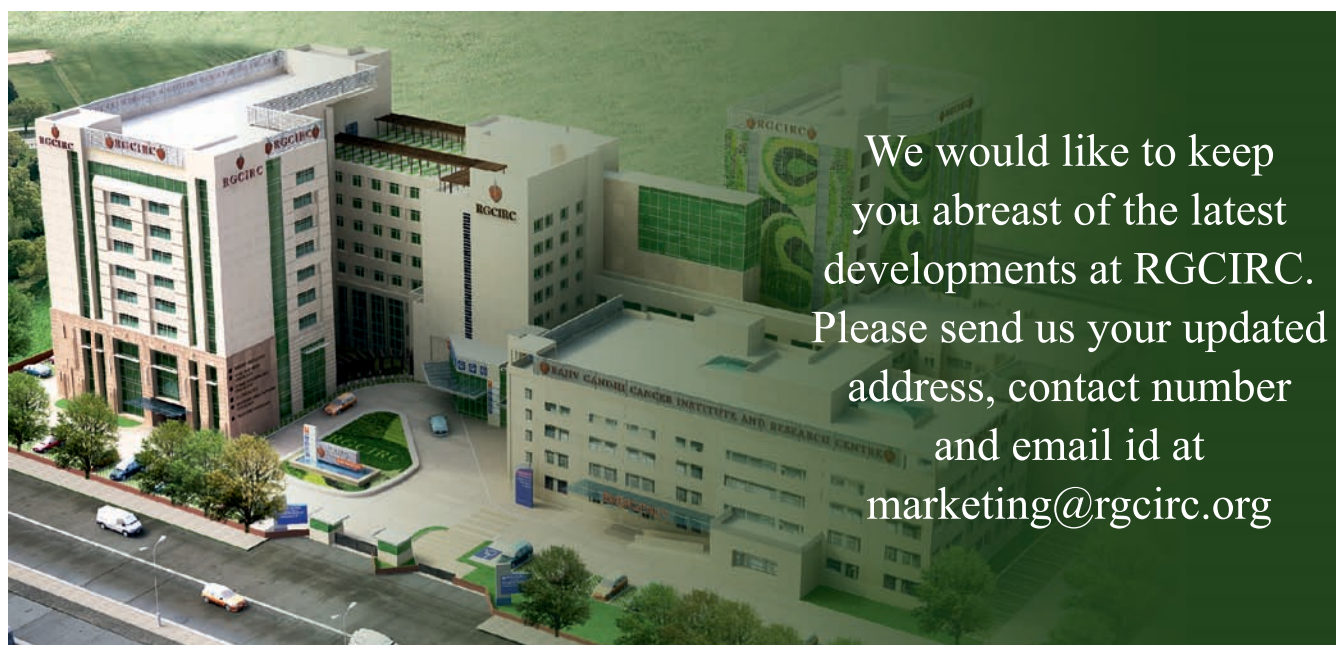
Day two was a bright and shiny day. The day commenced with the inauguration ceremony by lamp lighting and a welcome address by Dr Dewan, MD, RGCI. There were lectures by the members of the WHO think tank like Dr Elaine S Jaffe and Dr Wing Chang, Dr Elias Campo and Dr Bharat Nathwani. The academic session started with Dr Elaine S Jaffe, a Senior Hematopathology Investigator at NIH, Bethesda, USA and also on the editorial board of WHO Fascicle for Tumors for Hematopoietic and Lymphoid Tissue. She discussed the revised WHO classification and the new emerging concepts in 2008 classification. She elaborated on greater recognition of early lesions, like monoclonal B cell lymphocytosis (MBL), follicular lymphoma in situ (FL), mantle cell lymphoma in situ (MCL), and insisted that further brainstorming is required for borderline categories like DLBCL-BL and DLBCL-CHL. She highlighted that integration of molecular diagnostics like NGS studies, Nanostring, has helped in categorizing separately Double Hit and Double Expressor lymphomas with the former carrying a poor prognosis. She concluded by summarizing revisions in WHO 2016 - high grade B-cell lymphoma, with MYC and BCL2 and/or BCL6 rearrangements, High grade B-cell lymphoma, NOS (cases with blastoid features), Diffuse large B-cell lymphoma (DLBCL), NOS. The next session was by Dr Wing-Chung Chan, Professor Pathology, City of Hope Medical Centre, California, USA on Evolving Landscapes in DLBCL. He highlighted robust diagnostic assays that separate the GCB and ABC type of DLBCL using platforms like Q-RT PCR, immunohistochemistry (Han's algorithm, Choi's algorithm, Tally algorithm), nCounter technology, RNA protection based assay, RNA-sequencing. He said that classifying every case was difficult and may be we should personalize diagnosis and treatment, use comprehensive molecular characterization and then use this information to guide treatment. He focused on questions plaguing gray zone tumors. These are unique tumors that have overlapping characteristics of PMBL and cHL, and are difficult to diagnose with small biopsies, possibility of transformation and their management.

Post tea sessions included topics like EBV Associated Lymphoproliferative and Molecular Diagnostics in Lymphoma that were discussed through thought provoking presentations by Dr Elaine S Jaffe and Dr. Elias Campo. Dr Jaffe elaborated on how the role of EBV infection has been inculcated in the Current Concepts – WHO update (2016). Various types include - EBV - associated hemophagocytic lymphohistiocytosis (HLH) (non-neoplastic), Cutaneous CAEBV, Systemic CAEBV, T-cell or NK-cell, Systemic EBV+ T-cell lymphoma of childhood, Aggressive NKcell leukemia, Extranodal NK/T-cell lymphoma, nasal type. She concluded her session by a rare case presentation of a Mucocutaneous Ulcer – A Novel EBV Related Lesion, with plasmablastic lymphoma and PTLDMZL type as the closest differentials. Dr Elias Campo, Research Director, Hospital Clinic, Barcelona, Spain, spotlighted the applications of molecular diagnostics in lymphoma. The 45 min interaction was a rewarding experience to most present there. Dr Campo spoke incessantly about Heterogeneity of lymphoid neoplasms, and that Diagnosis of lymphoid neoplasms is an integrated process combining morphology, IHC and molecular techniques. He listed the various techniques like-IG and TCR clonality analysis, FISH, gene expression profiling using Sanger seq, next generation sequencing, nanostring. He discussed at length on the molecular subtyping of DLBCL-overall survival and molecular pathogenesis, clonality analysis of lymphomas, their challenges, limitations and pitfalls, so many mutations in lymphomas and epigenetic modifiers recurrently mutated in B-cell lymphomas.

Post lunch sessions included talks by Dr Wing Chan and Dr Elias Campo on diagnostic approach to T-cell Lymphomas, its mimickers and advances in understanding of mantle cell

lymphomas respectively. Dr Campo spoke illustratively on the mantle cell lymphoma, its genesis, architectural patterns, variants, clinical characteristics, molecular pathogenesis, pathogenesis of nodal vs non-nodal MCLs, indolent MCL and MCL in situ. He pointed out the possible oncogenic driver in MCL could be SOX 11 with other mutations being CCND1 and ATM, NOTCH1-2, BIRC3 mutations. The Day two academic session concluded by presentations on Mediastinal Lymphomas and NHL with Plasmacytic Differentiation by Dr Elaine S Jaffe and Dr Bharat Nathwani, respectively. Dr Jaffe discussed mediastinal Lymphomas, their differential diagnosis, practical and theoretical issues like molecular events that drive the transformation of Thymic B-cells. Dr Bharat Nathwani Director Pathology, National medical center, California, USA, discussed the diagnostic work-up in Non-Hodgkin lymphoma with plasmacytic differentiation, including the governing factors in this multistep process, enlisted lymphomas with plasma cell differentiation and used very depictive clinical case scenarios to illustrate these lymphomas. The closing remarks were made by Dr Gurudutt Gupta who thanked the patrons, advisory committee, chairpersons and the scientific committee for their untiring support. He also extended a special note of thanks to Dr Anurag Mehta who envisioned and conceptualized the entire meet. The summit ended with full glory and lot of take home messages for practicing pathologists who find lymphoma diagnosis arduous and exigent in their day-to-day practice.

(Dr Juhi Tayal, Clinical Pathologist, Dept of Research, Rajiv Gandhi Cancer Institute and Research Centre, Delhi)



We would like to keep you abreast of the latest developments at RGCIRC. Please send us your updated address, contact number and email id at marketing@rgcirc.org

GUEST ARTICLE

EVOLVING CONCEPT IN MANAGEMENT OF CML-MONITORING AND CHANGING THERAPY

Introduction

The incidence of chronic myeloid leukemia (CML) ranges between 10 and 15 cases/10⁶/year (age adjusted) without any major geographic or ethnic differences [1]. The median age at diagnosis ranges between 60 and 65 years in Europe, but is considerably lower in countries where the population is younger, like in India. The prevalence of CML is steadily rising due to the very substantial prolongation of survival that has been achieved with targeted therapy [2].The translocation of the ABL gene from chromosome 9 to 22 t(9;22) (q3.4;q1.1) leads to the formation of a onco-protein that has a strong, constitutively activated, tyrosine kinase activity, resulting in the activation of several downstream signals that transform hematopoietic stem cells [3]. The introduction of imatinib mesylate (IM) has revolutionized the treatment of chronic myeloid leukemia (CML). Progress in treatment of CML is so rapid, and is still marching so fast [1–3], that any recommendation on the management of CML can quickly become obsolete.

Diagnosis and Assessment of Prognosis

The hallmark of diagnosis is peripheral blood leukocytosis with basophilia and with immature granulocytes, mainly metamyelocytes, myelocytes and promyelocytes, and a few or occasional myeloblasts. The diagnosis must be confirmed by cytogenetics showing t(9; 22) (q3.4; q1.1), and by reverse transcriptase polymerase chain reaction (RT-PCR) showing BCR-ABL transcripts. There are three prognostic scoring systems: Sokal et al. [4], developed in 1984 in the era of conventional chemotherapy; EURO [5], derived in 1998 from IFN α -treated patients; and EUTOS [6], derived more recently (2011) from imatinib-treated patients. The EUTOS risk score is simpler, and in imatinib-treated patients have a prognostic value greater than Sokal and EURO.

Evolving Concept in Management

Current recommendations for the management of CML are basically addressed to the goal of achieving an at least MMR, with a life quality and a life duration close to normality, as much as possible. Several baseline factors and characteristics (Sokal, EUROS, or EUTOS) have been reported to influence the response to TKI and outcome. There are some other baseline high risk factors like, established: clonal chromosome abnormalities in Ph⁺ cells (ACA/Ph⁺); major route: +8, +Ph, i(17)(q10), ider(22)(q10), +19; provisional; transcript type (B3A2 vs B2A2, atypical transcripts);

Table 1: ELN guidelines 2013 for treatment of CML

Chronic phase	
First line	Imatinib 400 mg, or nilotinib 300 mg \times 2, or dasatinib 100 mg
Second line	In case of intolerance, switch to another TKI, taking into consideration the side effects of the first TKI, and comorbidities In case of failure of imatinib, switch to nilotinib, or dasatinib, taking into consideration the presence and the type of BCR-ABL KD mutation In case of failure of nilotinib or dasatinib, switch to dasatinib or nilotinib, taking into consideration the presence and the type of BCR-ABL KD mutation. Consider allo HSCT
Third line	In case of failure of two or three TKI, consider alloHSCT
Accelerated/blastic phase	
TKI naïve	Imatinib 600 or 800 mg, or nilotinib 400 mg \times 2 or dasatinib 140 mg, and consider allo HSCT
TKI pretreated	Switch to another TKI, consider chemotherapy and alloHSCT

Table 2: Response criteria ELN 2013

Time	Optimal	Warning	Failure
Baseline	NA	High risk or CCA/Ph+, major route	NA
3 months	BCR/ABL1 ≤10 % or Ph + ≤ 35 %	BCR-ABL1 > 10 % or Ph + 36–95 %	Non CHR or Ph+ >95 %
6 months	BCR-ABL1 ≤ 1 % or Ph + 0 (CCyR)	BCR-ABL1 1–10 % or Ph + 1–35 % (PCyR)	BCR-ABL1 > 10 % or Ph+ >35 %
12 months	BCR-ABL 1 ≤ 0.1 % (MMR)	BCR-ABL1 0.1–1 %	BCR-ABL1 > 1 % or Ph + ≥ 1 %

Transcripts level, Gene expression profile; polymorphisms of genes coding for proteins involved in drug metabolism and transport, and in apoptosis (BIM); expression level of genes involved in drug transport (MDR,hOCT1); Low-level BCR-ABL1 mutations which influences the outcome. So these groups of patients upfront 2nd generation TKI or Allo HSC SCT are under consideration.

In treatment of CML, the primary goal is to reach a major molecular response (3 log reduction <0.1%) but the CMR (4-5 log reduction <0.01% -0.001%) is prerequisite for drug interruption trial. Rise in 5 to 10 fold in transcript level in long-term follow up is indicator of early treatment failure. Mutation analysis by direct sequencing is mandatory before any switch in therapy. Regrettably, there are no studies comparing different TKIs in second-line. Therefore, the choice of the secondline TKI is guided by some patient characteristics, mainly age and comorbidities, by the type of side effects with the first TKI, and by the presence of BCR-ABL1 kinase domain point mutations, and also by drug availability and cost, and by doctor experience. For T315I mutation only sensitive drug is ponatinib.

The next goal is to achieve a condition of treatment free remission (TFR)[7-9]. The early surrogate markers of TFR will be a rapid decline of BCR-ABL1 transcripts, a BCR-ABL1 transcripts level d”1 % within 3 months, d”0.1 % within 1 year, and d”0.01 % later on. It is likely that an extended use of second-generation TKIs that are more potent and induce faster and deeper molecular remissions, will bring more patients into TFR, It is likely that the earlier and the deeper the early molecular response, the higher will be the number of patients in TFR.

Bibliography

1. Hehlmann R, Hochhaus A, Baccarani M. European Leukemia Net. Chronic myeloid leukaemia. *Lance* 2007; 370: 342–350.

2. Björkholm M, Ohm L, Eloranta S et al. Success story of targeted therapy in chronic myeloid leukemia: a population-based study of patients diagnosed in Sweden from 1973 to 2008. *J Clin Oncol* 2011; 29: 2514–2520.

3. Quintás-Cardama A, Cortes J. Molecular biology of bcrabl1-positive chronic myeloid leukemia. *Blood* 2009; 113: 1619 –1630.

4. Sokal JE, Cox EB, Baccarani M et al. Prognostic discrimination in ‘good-risk’chronic granulocytic leukemia. *Blood* 1984; 63: 789–799.

5. Hasford J, Pfirrmann M, Hehlmann R et al. A new prognostic score for survival ofpatients with chronic myeloid leukemia treated with interferon alfa. *J Natl Cancer Inst* 1998; 90: 850–858.

6. Hasford J, Baccarani M, Hoffmann V et al. Predicting complete cytogenetic response and subsequent progression-free survival in 2060 patients with CML on imatinib treatment: the EUTOS score. *Blood* 2011; 118: 686–692.

7. Rousselot P, Huguet F, Rea D et al (2007) Imatinib mesylate discontinuation in patients with chronic myelogenous leukemia in complete molecular remission for more than 2 years. *Blood* 109:58–60.

8. Ross DM, Branford S, Seymour JF et al (2010) Patients with chronic myeloid leukemia who maintain a complete molecular response after stopping imatinib treatment have evidence of persistent leukemia by DNA PCR. *Leukemia* 24(10):1719–1724.

9. Mahon FX, Rea D, Guilhot J et al (2010) Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial.*Lancet Oncol* 11:1029–1035.

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PERSPECTIVE

ADVANCES IN BIOLOGY IN TREATMENT OF CHRONIC MYELOID LEUKEMIA

Introduction

Philadelphia chromosome was discovered in 1960 but it took almost 13 years to show that the Ph chromosome was the result of a t(9;22) reciprocal chromosomal translocation¹. After another 10 years, the translocation was shown between Abl proto-oncogene normally on chromosome 93 and Bcr for breakpoint cluster region gene on chromosome 22². The deregulated Abl tyrosine kinase activity was then defined as the pathogenetic principle³.

Molecular Anatomy of the BCR-ABL Translocation

The breakpoints within the Abl gene at 9q34 can occur anywhere over a large area at its 5' end whereas breakpoints within Bcr localize to 1 of 3 so-called breakpoint cluster regions (bcr). In most patients with CML, the break occurs within a 5.8-kb area, defined as the major breakpoint cluster region (M-bcr) giving rise to 210-kd chimeric protein. Rarely the minor breakpoint cluster region (m-bcr) results in a translated 190-kd protein or 230-kd fusion protein⁴.

Physiologic Function of the Translocation Partners

The Abl gene is the human homologue of the v-abl oncogene carried by the Abelson murine leukemia virus (A-MuLV), and it encodes a nonreceptor tyrosine kinase. Human Abl is a ubiquitously expressed 145-kd protein with 2 isoforms arising from alternative splicing of the first exon (Fig 1). Three SRC homology domains (SH1-SH3) are located toward the NH2 terminus.⁴ The SH1 domain carries the tyrosine kinase function.

The normal Abl protein influences decisions in regard to cell cycle and apoptosis⁵.

The 160-kd Bcr protein, like Abl, is ubiquitously expressed. Several structural motifs can be delineated (Fig 2). Bcr can be phosphorylated on several tyrosine residues, especially tyrosine 177, which binds Grb-2, an important adapter molecule involved in the activation of the Ras pathway. Interestingly, Abl has been shown to phosphorylate Bcr in COS1 cells, resulting in a reduction of Bcr kinase activity^{6,7}.

Mechanisms of BCR-ABL Mediated Malignant Transformation

Essential Features of the Bcr-Abl Protein: Mutational analysis identified several features in the chimeric protein that are essential for cellular transformation (Fig 4). In Abl they include the SH1, SH2, and actin-binding domains, and in Bcr they include a coiled-coil motif contained in amino acids 1-63, the tyrosine at position 177, and phosphoserine-threoninerich sequences between amino acids 192-242 and 298- 413 (Fig 2)^{4,8}.

Deregulation of the Abl Tyrosine Kinase:

Abl tyrosine kinase activity is tightly regulated under physiologic conditions. The SH3 domain plays a critical role in this inhibitory process. Its deletion or positional alteration activates kinase. Abl-1 and Abl-2 (Abl interactor proteins 1 and 2) activate the inhibitory function of the SH3 domain. Tyrosine phosphatases counterbalance and regulate the effects of tyrosine kinases under physiologic conditions, keeping cellular phosphotyrosine levels low⁴.

Altered Adhesion Properties

CML progenitor cells exhibit decreased adhesion to bone marrow stroma cells and extracellular matrix. CML cells express an adhesion-inhibitory variant of b1 integrin that is not found in normal progenitors.

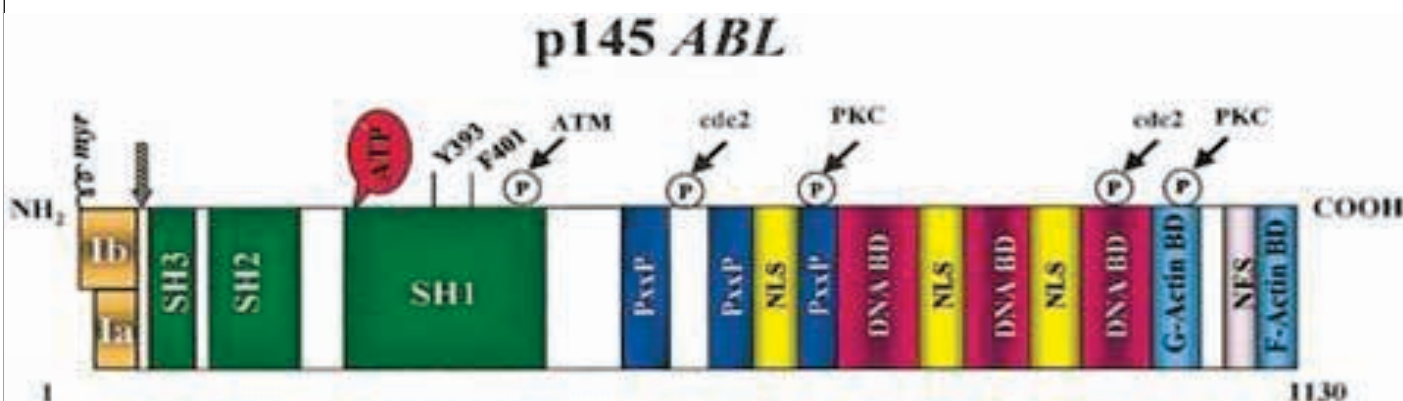


Fig. 1. Structure of the Abl protein. Type Ia isoform is slightly shorter than type Ib, Note the 3 SRC-homology (SH) domains situated toward the NH2 terminus. Y393 is the major site of autophosphorylation). The carboxy terminus contains DNA as well as G- and F-actin-binding domains.

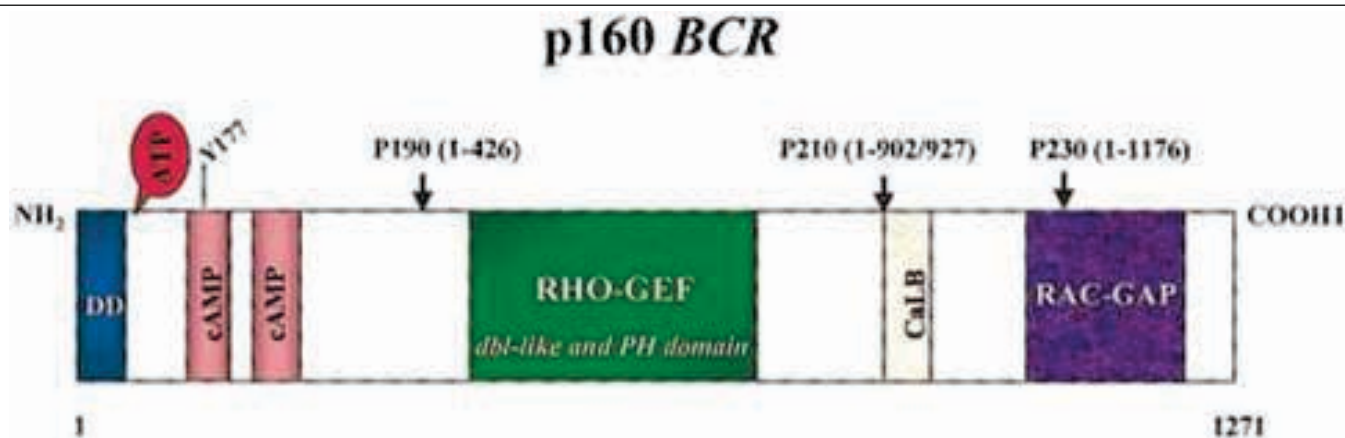


Fig. 2. Structure of the Bcr protein. Note the dimerization domain (DD) and the 2 cyclic adenosine monophosphate kinase homologous domains at the N terminus. Y177 is the autophosphorylation site crucial for binding to Grb-2. The center of the molecule contains a region homologous to Rho guanine nucleotide exchange factors (Rho-GEF) as well as dbl-like and pleckstrin homology (PH) domains. Toward the C-terminus a putative site for calcium-dependent lipid binding (CaLB) and a domain with activating function for Rac-GTPase (Rac-GAP) are found. Arrowheads indicate the position of the breakpoints in the Bcr-Abl fusion proteins

Activation of Mitogenic Signaling Ras and the MAP Kinase Pathways

Several links between Bcr-Abl and Ras have been defined. Ras activation is important for the pathogenesis of Ph-positive leukemias. This implies that the Ras pathway is constitutively active, and no further activating mutations are required. The third pillar of the MAP kinase pathway, p38, is also activated in Bcr-Abl transformed cells, and there are other pathways with mitogenic potential. It is also possible that Bcr-Abl uses growth factor pathways in a more direct way⁴.

Jak-Stat Pathway: Stat 5 activation appears to contribute to malignant transformation and its effect in Bcr-Abl transformed cells appears is anti-apoptotic and involves transcriptional activation of Bcl-xL.

PI3 Kinase Pathway: PI3 kinase activity is required for the proliferation of Bcr-Abl positive cells.127 Bcr- Abl forms multimeric complexes with PI3 kinase, Cbl, and the adapter molecules Crk and Crkl 95 in which PI3 kinase is activated.

Myc Pathway: Activation of Myc by Bcr-Abl is dependent on the SH2 domain, and the over-expression of Myc partially rescues transformation-defective SH2 deletion mutants whereas the over-expression of a dominant negative mutant suppresses transformation⁴.

Inhibition of Apoptosis

Bcr-Abl-positive cell lines are resistant to apoptosis induced by DNA damage. Bcr-Abl may block the release of cytochrome C from the mitochondria and thus the activation of caspases⁴. Another link between Bcr-Abl and the inhibition of apoptosis might be the phosphorylation of the pro-apoptotic protein Bad.

It is also possible that Bcr-Abl inhibits apoptosis by down-regulating interferon consensus sequence binding protein (ICSBP)^{9,10}.

Molecular Targets for Therapy

Based on the molecular and cell biology of CML therapeutic tools have concentrated on 3 main areas - the inhibition of gene expression at the translational level by “antisense” strategies, the stimulation of the immune system’s capacity to recognize and destroy leukemic cells, and the modulation of protein function by specific signal transduction inhibitors.

Perhaps the most exciting of the molecularly designed therapeutic approaches were the advent of signal transduction inhibitors (STI), which block or prevent a protein from exerting its role in the oncogenic pathway. The main transforming property of the Bcr-Abl protein is effected and direct inhibition of such activity seems to be the most logical means of silencing the oncoprotein.

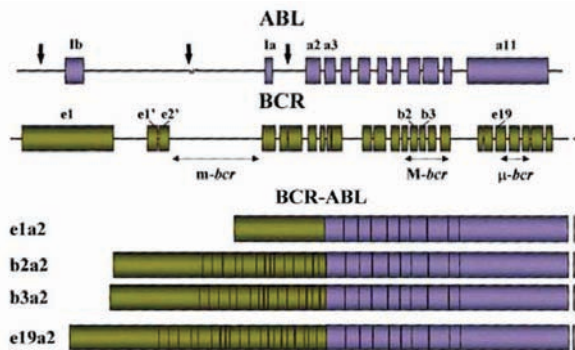


Fig. 3. Locations of the breakpoints in the Abl and Bcr genes and structure of the chimeric mRNAs derived from the various breaks.

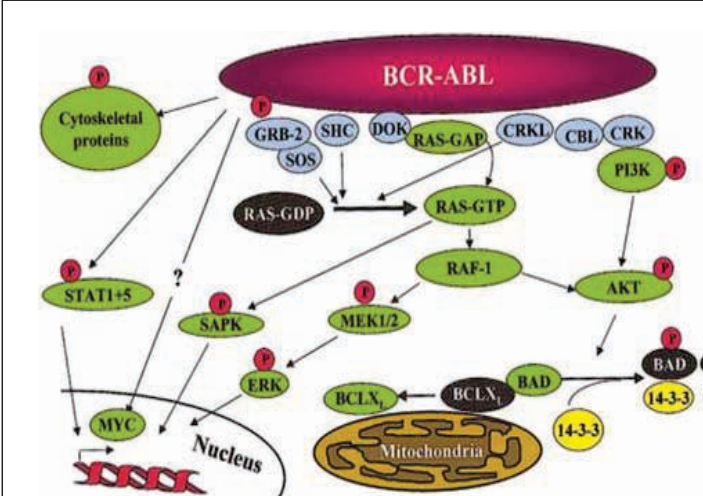


Fig. 4. Signaling pathways activated in Bcr-Abl-positive cells.

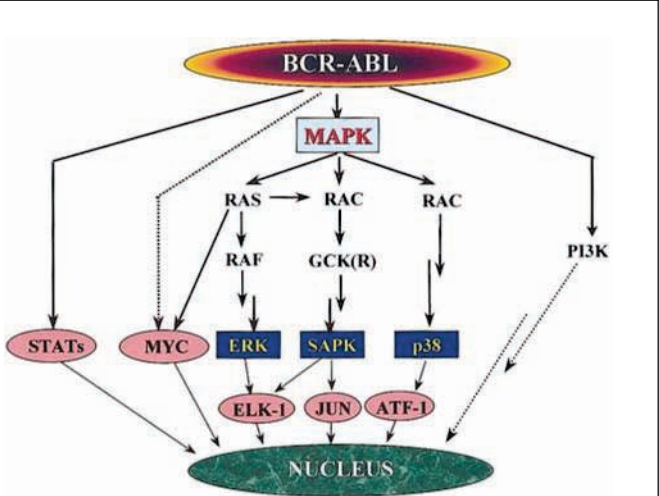


Fig. 6. Signaling pathways with mitogenic potential in Bcr-Abl-transformed cells.

The most promising of these compounds is the 2-phenyl lamino pyrimidine STI571, which specifically inhibits Abl tyrosine Kinase¹¹.

Current Biology of Chronic Myeloid Leukemia After Imatinib

Imatinib (2-phenylaminopyrimidine STI571) has been shown to induce a complete haematologic response in CP-CML patients. However, imatinib has been unable to completely eliminate Bcr-Abl-expressing leukemic cells due to problems such as disease persistence or relapse. Stem cells may escape imatinib-mediated apoptosis due to the inability of imatinib to attack quiescent stem cells (Table 1)¹².

Duplications

The development of imatinib-resistance was firstly described in 2000 through Bcr-Abl oncogene amplification.

Mutations

Mutations in the tyrosine kinase inhibitor binding site of Bcr/Abl is another important mechanism of drug resistance.

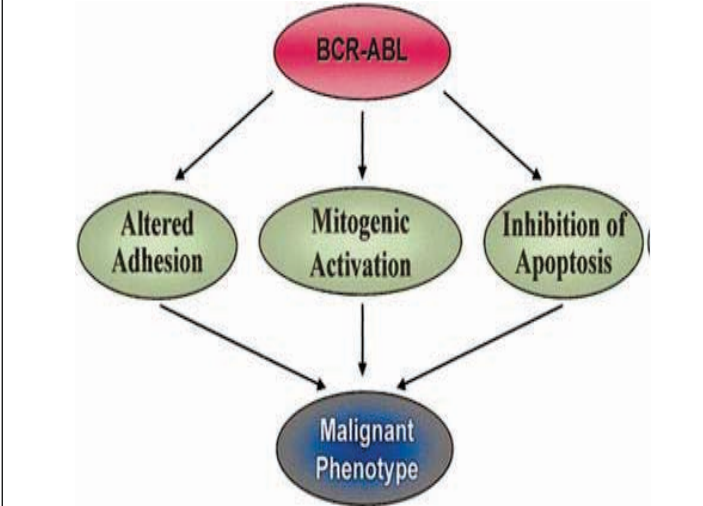


Fig. 5. Mechanisms implicated in the pathogenesis of CML

Mutational frequencies appear to increase in imatinib resistance and progress from CP to BP. The most frequently observed mutation identified in imatinibresistant CML patients is, T315I mutation.

Mutation-Independent resistance to Imatinib

Imatinib-resistance arises very rarely in patients that are treated with imatinib in early CP-CML. The Bcr/Abl fusion protein acts as an oncoprotein by activating several signaling pathways that lead to transformation. Myc, Ras, c-Raf, MAP/ERK, SAPK/JNK, STAT, nuclear factor kappa-B (NF-κB), phosphoinositole 3- kinase (PI-3K) and c-Jun are included as signal cascade molecules regulated by the Bcr/Abl activity. It has also been shown *in vitro* that mutations outside the kinase domain in the neighbouring linker, SH2, SH3, and Cap domains can confer imatinib-resistance¹³.

Other Target Pathways

Kinase domain mutations constitute 30-50% of there a son for imatinib-resistance. There are different molecular determinants that contribute to the sensitivity and resistance of tumor cells to imatinib-induced apoptosis. It has been suggested that p53 may be an important mediator of the imatinib induced apoptotic response, and, a deficiency in p53-signaling pathway antagonizes this response and mediates imatinib-resistance¹².

Conclusion and Future Perspectives

Discovery of the TKIs was the revolutionary therapy of CML However, TKIs are still unable to eradicate the disease due to the presence of a drug-insensitive stem cell population. CML stem cells do not depend on Bcr/Abl activity for survival and are less responsive to imatinib therapy and act as a reservoir for the emergence of imatinib-resistant subclones.

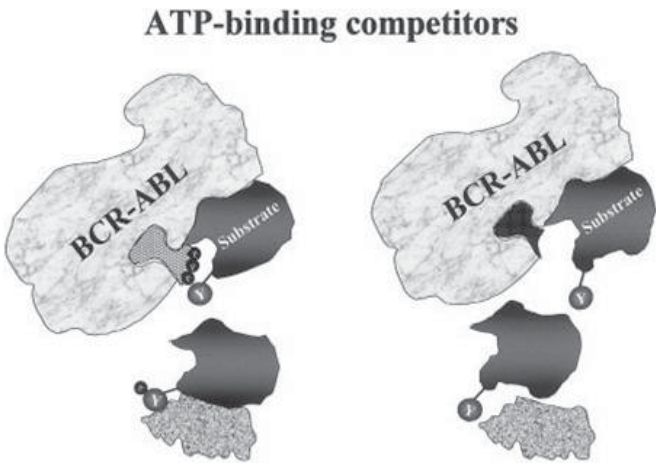


Fig. 7. Mechanism of action of tyrosine kinase inhibitors. The drug competes with ATP for its specific binding site in the kinase domain. Thus, whereas the physiologic binding of ATP to its pocket allows Bcr-Abl to phosphorylate selected tyrosine residues on its substrates (left diagram), a synthetic ATP mimic such as STI571 fits this pocket equally well but does not provide the essential phosphate group to be transferred to the substrate (right diagram). The downstream chain of reactions is then halted because, with its tyrosines in the unphosphorylated form, this protein does not assume the necessary conformation to ensure association with its effector.

Imatinib inhibited the Bcr/Abl activity to the same degree in all stem(CD34+CD38-CD133+) and progenitor (CD34+CD38+) cells, and in quiescent and cycling progenitors from newly diagnosed CML patients¹³.

The resistance is still a significant clinical problem. The detection of pre-existing mutations in primitive stem/ progenitor (CD34+) cells may have therapeutic and prognostic implications. Therefore, the determination of the molecular mechanisms of TKI resistance can provide an important role for the more effective treatment of CML patients.

References

1. Rowley JD. A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quina crine fluorescence and Giemsa staining [letter]. Nature. 1973;243:290-293.

2. Groffen J, Stephenson JR, Heisterkamp N, de Klein A, Bartram CR, Grosveld G. Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. Cell.1984;36:93-99.

3. Lugo TG, Pendergast AM, Muller AJ, Witte ON. Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. Science. 1990;247:1079-1082.

4. Michael W. N. Deininger, John M. Goldman, and Junia V. Melo. The molecular biology of chronic myeloid leukemia. BLOOD, 15 nov 2000, vol 96.

Kinase domain mutations	Mutation independent	Duplication	Other targets
● T315I	● PI3K/Akt	● Amplification in Abl sequence	● p53
● P-Loop -M244V -G250E -Y253 F/H	● MDR-1, Pgp		● bcl-2
● M351T	● HIF-1α		● JAK-2/STAT-5
● F359V	● Aberrant ceramide metabolism		● OCT-1
● SH2, SH3			
● Cap			

5. Lewis JM, Schwartz MA. Integrins regulate the association and phosphorylation of paxillin by c-Abl. J Biol Chem. 1998;273:14225-14230.

6. Ma G, Lu D, Wu Y, Liu J, Arlinghaus RB. Bcr phosphorylated on tyrosine 177 binds Grb2. Oncogene. 1997;14:2367-2372.

7. Liu J, Wu Y, Ma GZ, et al. Inhibition of Bcr serine kinase by tyrosine phosphorylation. Mol Cell Biol. 1996;16: 998- 1005.

8. Pendergast AM, Muller AJ, Havlik MH, Maru Y, Witte ON. BCR sequences essential for transformation by the BCR-ABL oncogene bind to the ABL Sh2regulatory domain in a non-phosphotyrosine- dependent manner. Cell. 1991;66:161-171.

9. Gabriele L, Phung J, Fukumoto J, et al. Regulation of apoptosis in myeloid cells by interferon consensus sequence-binding protein. J Exp Med. 1999;190:411-421.

10. Hao SX, Ren R. Expression of interferon consensus sequence binding protein (ICSBP) is down regulated in Bcr-Abl-induced murine chronic myelogenous leukemia-like disease, and forced coexpression of ICSBP inhibits Bcr-Abl-induced myeloproliferative disorder. Mol Cell Biol. 2000; 20:1149-1161.

11. 206. Buchdunger E, Zimmermann J, Mett H, et al. Selective inhibition of the platelet-derived growth factor signal transduction pathway by a protein tyrosine kinase inhibitor of the 2-phenylaminopyrimidine class. Proc Natl Acad Sci U S A. 1995; 92:2558-2562.

12. Wendel HG, de Stanchina E, Cepero E, Ray S, Emig M, Fridman JS, Veach DR, Bornmann WG, Clarkson B, McCombie WR, Kogan SC, Hochhaus A, Lowe SW. Loss of p53 impedes the antileukemic response to BCRABL inhibition. Proc Natl Acad Sci U S A. 2006; 103:7444– 7449.

13. Melda Comert, Yusuf baran, and Guray Saydam. Changes in molecular biology of chronic myeloid leukemia in tyrosine kinase inhibitor era. Am j Blood Res.2013;3(3):191-200.

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CLINICAL TRIALS

Impact of a Switch to Nilotinib on Imatinib

The results from phase II ENRICH (Exploring Nilotinib to Reduce Imatinib Related Chronic Adverse Events) demonstrated that switching to nilotinib can mitigate imatinib-related chronic low-grade nonhematologic AEs in patients with chronic myeloid leukemia (CML) in chronic phase, in conjunction with acceptable safety and achievement of molecular responses. Three months after switching to nilotinib, 84.6% of the patients had overall improvement in imatinibrelated AEs (primary endpoint). Of 210 imatinib-related AEs identified at baseline, 62.9% had resolved within 3 months of switching to nilotinib. Of evaluable patients, most had improvements in overall quality of life after switching to nilotinib. At screening, 65.4% of evaluable patients had a major molecular response (Bcr-Abl1 dd 0.1% on the International Scale). After switching to nilotinib, the rate of the major molecular response was 76.1% at 3 months and 87.8% at 12 months. Treatment emergent AEs reported with nilotinib were typically grade 1 or 2; However, some patients developed more serious AEs, and 8 patients discontinued nilotinib because of new or worsening AEs.

(Clin Lymphoma Myeloma Leuk, 16 Feb 2016)

Lymphocytosis after Treatment with Dasatinib

The proliferation of clonal cytotoxic T-cells or natural killer cells has been observed after dasatinib treatment in small studies of patients with chronic myeloid leukemia (CML). The incidence, timing, and duration of lymphocytosis were determined. The duration of lymphocytosis was defined as the interval between the date of original detection and the date of last available follow-up sample that was positive for lymphocytosis. Overall, 1402 dasatinib-treated patients with newly diagnosed CML in chronic phase (CML-CP), CMLCP refractory/intolerant to imatinib, or with CML in accelerated or myeloid-blast phase were analyzed. Overall, lymphocytosis occurred and persisted in many dasatinib-treated patients in all phases of CML. Its presence was associated with higher response rates, significantly longer response durations, and increased overall survival, suggesting an immunomodulatory effect.

(Cancer, 21 Mar 2016)

NEW TECHNOLOGIES

New Nutrient Uptake Process Discovered

Medical researchers have found a novel nutrient uptake process that maintains the activity of murine chronic myelogenous leukemia (CML) stem cells. CML stem cells are reportedly responsible for the recurrence of CML, following the tyrosine-kinase inhibitor (TKI) therapy. It was observed that CML stem cells accumulate significantly higher levels of certain dipeptide species than the normal hematopoietic stem cells do. Once internalized, these dipeptide species act as nutrients for the CML stem cells and play a role in their maintenance. The pharmacological inhibition of nutrient uptake decreased CML stem cell activity *in vivo*. Hence, this nutrient supply and its downstream signalling pathway may offer novel candidate therapeutic targets for eradicating CML stem cells. An approach of using inhibitors to block the key nutrient uptake process specific to CML stem cells, in combination with TKI therapy, may offer actual therapeutic benefits to patients with CML.

(Nature Communications, 20 Aug 2015)

Novel Assay Detects Persistent Disease

The outcomes of chronic myeloid leukemia (CML) have dramatically improved as a result of tyrosine kinase inhibitor (TKI) treatment. However, for patients in “molecular remission,” uncertainties remain regarding whether they will relapse or if treatment should be discontinued. A team of researchers have validated an assay for quantifying very low levels of residual disease using targeted next-generation sequencing coupled with the use of a digital PCR dPCR platform. Thirty-six samples were taken from six patients with early CML who were thought to be in deep molecular remission, as indicated by RT-qPCR results. The sensitivity of new technique, dPCR assay was compared with three other methods currently used to measure residual CML, including reverse transcriptase-quantitative PCR (RTqPCR), quantitative PCR (qPCR) and reverse transcriptase-digital PCR (RT-dPCR). The novel assay detects the persistent disease in 81% of samples taken from a group of patients thought to be in remission. The researchers conclude that dPCR is the most sensitive method available for detection of residual-disease in CML and may prove useful in the management of TKI withdrawal.

(Journal of Molecular Diagnostics, March 2016)

RESEARCH AND DEVELOPMENT

BCR-ABL Kinase Domain Mutations

The authors document the characteristics of BCRABL kinase domain mutations (KDM) in the largest study from India comprising 385 patients and demonstrate that more than half (51.9%) of these patients have detectable abnormalities in the KD, both in adult and in pediatric chronic myelogenous leukemia (CML). These comprise singly occurring missense mutations (25.5%), polyclonal/compound point mutations (4.9%), and insertions/deletions (29.6%). Missense mutations were most commonly seen in the imatinib-binding region followed by the P-loop. The commonest mutation in the data set was T315I. Other common missense mutations were Y253H, M244V, and F317L. A high prevalence of Bcr-Abl exon7 deletion (p.R362fs*) was also seen (25.5% of the entire cohort), whereas the 35bp intron-derived insertion/truncation mutations were detected in 12 patients. The authors detected 11 novel mutations (seven missense mutations and four insertions/deletions).

(Leuk Lymphoma, 21 Mar 2016)

Evolutionary Dynamics

The t (9;22) translocation that causes chronic myeloid leukemia (CML) drives both transformation and the progression processes that eventually result in the disease changing to acute leukemia. Constitutively activated Bcr-Abl signaling in CML creates high levels of reactive oxygen species (ROS) that produce 8-oxo-guanine in DNA; this is mutagenic and causes chronic phase (CP) progression to blast phase (BP). The authors modeled three types of mutations involved in this progression: mutations that result in myeloid progenitor cells proliferating independently of external growth factors; mutations causing failure of myeloid progenitor cells to differentiate; and mutations that enable these cells to survive independently of attachment to marrow stroma. They further modeled tyrosine kinase inhibitors (TKIs) as restoring myeloid cell apoptosis and preventing ROS-driven mutagenesis, and mutations that cause TKI resistance. The authors suggest that the unusually low rate of resistance to TKI arises because these drugs deplete ROS, which in turn decrease mutation rates.

(AAPS J, 23 Mar 2016)

GLOBE SCAN

Mortality and Vascular Events

Tyrosine-kinase inhibitors (TKIs) can be associated with vascular events (VEs). The present study examined the event rates and mortality among elderly patients with and without chronic myeloid leukemia (CML). Linked surveillance, epidemiology, and end results cancer registry and medicare claims data were used to identify patients aged 66 years with an incident (index) diagnosis of CML from 2004 to 2009. A comparison cohort of patients without cancer was matched 1:1 to the CML cohort. The overall survival and rates of myocardial infarction (MI), stroke, pulmonary embolism (PE), and peripheral arterial disease (PAD) were analyzed. A total of 1466 patients with CML were identified and matched 1:1 to a noncancer cohort. Compared with the noncancer patients, those with CML had greater mortality (63% vs 23% died during the follow-up period; median survival, 23 vs > 84 months) and greater rates of MI (33.0 vs 11.9 per 1000 person-years), stroke (83.2 vs 43.0), PE (6.6 vs 2.6), and PAD (92.1 vs 59.3; $P < .01$ for all). Elderly patients with CML had greater mortality and greater rates of MI, stroke, PE, and PAD than did non-cancer patients.

(USA: Clin Lymphoma Myeloma Leuk, 6 Feb 2016)

Prevalence and Direct Medical Costs

After the introduction of tyrosine kinase inhibitors for chronic myeloid leukemia (CML), the survival of these patients has increased significantly. However, these new drugs are expensive and impose considerable expense to patients and governments. The authors estimated the incidence, prevalence and direct medical cost of CML in Iran. They used the National Cancer Registry data from 2006 to 2009 to estimate the incidence rate of CML (ICD-10 code C92.1). The 5-year prevalence of CML patients was about 2263 cases (2.98 per 100 000). The total direct medical cost of CML was \$23 089 323 and most of the cost (97%) was related to drug costs. The increased survival of CML patients and a possible increase in incidence of CML in Iran will most likely lead to a considerable rise in its prevalence and economic burden.

(Iran: Asia Pac J Clin Oncol, 17 Mar 2016)

IN FOCUS

TRANSPLANT IN CML: WHEN, WHOM AND HOW SOON

Chronic Myeloid Leukemia (CML) arises from hematopoietic stem cell as a consequence of a reciprocal translocation between chromosomes 9 and 22, giving rise to the Philadelphia chromosome with abnormally high BCR/ABL kinase activity. Chronic Phase (CP) is the early phase and in the current era, it is treated with tyrosine kinase inhibitors (TKIs) (eg, Imatinib, Nilotinib or Dasatinib) as first line therapy, with imatinib being the most popular first line drug in India. Disease phase sometimes progresses to a second: Accelerated Phase (AP) during which patients may respond to treatments for some months or even years or an aggressive state in the Blast Phase (BP) during which almost all of the treatment strategies fail with a median survival of 6 months.

TKIs provide an opportunity for complete cytogenetic and molecular remissions. A complete haematological remission is essentially when the CBC and smear becomes normal and spleen is not palpable. A complete cytogenetic response is when Ph positive metaphases are no longer detected on cytogenetic examination. A complete molecular response is when a quantitative polymerase chain reaction fails to detect BCR/ABL transcripts. Clinical, haematologic, cytogenetic and molecular responses have to be constantly monitored throughout treatment. TKI resistant BCR/ABL mutations occur and can cause disease progression. Extent of cytogenetic responses at various time points have been used to define treatment failures with imatinib and TKIs.

Allogeneic Stem Cell Transplantation (allo-SCT), which became popular in the 1980s has been a curative option. However, it was associated with considerable rates of morbidity and mortality. New strategies involving the combination of TKIs with transplantation have provided opportunities to control relapse following allo-SCT, overcome chronic graft versus host disease, and to induce second chronic phase in the patients with advanced disease stages prior to transplantation.

Imatinib, the first TKI, was followed by the second generation (2G) TKIs (nilotinib, dasatinib, bosutinib) which are used in imatinib resistance or intolerance. However, some of the second line TKIs mentioned have been approved for treatment in a first line setting in some developed

countries. 2G- TKIs (nilotinib, dasatinib, bosutinib) have been reported to be more potent BCR/ABL inhibitors than imatinib with more cytogenetic and molecular responses and less incidences of relapses when used upfront.

Despite the effectiveness of 2G-TKIs, secondary resistance to these drugs is often seen in the patients who initially fail imatinib. Rate of complete cytogenetic remission is only 40-50% in the patients who switch to 2G-TKIs after imatinib failure, and some of these patients may also lose their response to 2G-TKIs. Responses were neither deep nor durable when used in advanced disease like AP or BP. This is often as a result of TKI resistant BCR/ABL kinase domain mutations. Such patients should be considered for allo-SCT.

Allo-SCT in TKI Era

Allo-SCT is recommended only when patients develop resistance or intolerance to TKIs. Often high risk patients with co-morbidities, and advanced CML are referred for transplantation, resulting in increased rates of relapse, and transplant related mortality. Approximately 10- 20% of the patients die as a result of treatment related toxicity, and GVHD occurs in almost half of the patients who undergo transplantation.

The probability of remaining in complete cytogenetic remission is only 40-45% when the patients are switched to 2 G-TKIs as a result of imatinib resistance or intolerance. Allo-SCT should always be considered for such patients, and donor search should immediately begin in order to avoid delay. An ideal donor is Human Leukocyte Antigen (HLA) matched sibling. Since the probability of having such a donor is only 25-30%, matched unrelated donor/HLA- mismatched/haploidentical related donor allografts are also used.

Impact of Disease Phase on Allo-SCT

The effectiveness of allo-SCT in CML patients largely depends on the disease phase at the time of transplantation. CML-CP patients benefit the most from transplantation, with a disease free survival (DFS) rate of 80%, and overall survival rates of more than 80%. However, overall survival and DFS rates for advanced phase patients are 50% and 40% respectively. Copelan et al, demonstrated the allo-SCT outcomes of 335 CML patients, 229 of them were in CML-CP and 106 patients in advanced phase at the time of transplantation. Overall survival rates were 70.5% for chronic, 38% for accelerated, and 16% for blast phase patients. Relapse rates at three years in advanced phase were higher (32.5%) than the patients who were in chronic phase (21.4%) at the time of allo-SCT. The overall survival rates for CML-BP patients transplanted are just 10-20%.

Nonetheless a better clinical outcome can be achieved with the induction of a second chronic phase prior to allo-SCT. For CML-BP patients who are able to achieve a second chronic phase (with TKIs or chemo/ combination), the transplantation outcomes are similar to the accelerated phase patients.

GVHD and T Cell Depletions

Poor engraftment and GVHD are mediated by donor T cells in HLA-mismatched allo-SCT. T cell depletion (TCD) strategies were thus developed. TCD/CD34+ stem cell selection followed by a mega dosage of CD34+ cells can overcome GVHD, but results in delayed immune reconstitution; non-relapse mortality rate of up to 57% due to toxicity and infectious complications; with cytomegalovirus and invasive fungal infections being the most fatal ones. Hence, therapeutic regimens to control and manage post HLA-mismatched allo-SCT complications are required to make it a feasible approach. There is also a need for development and optimisation of better TCD alternatives. Strategies for HLA haplomatched transplants without T cell depletion, including post transplant cyclophosphamide, are being explored.

TKIs and GVHD

Imatinib, when administered post transplant, has been found to induce immunomodulatory effect that can decrease the probability and severity of chronic GVHD. It might be due to the fact that the drug inhibits the proliferation of T cells in vitro, blocks the platelet derived growth factors and transforming growth factor β pathways that lead to the development of chronic GVHD. Multicentre data is needed to validate this. It will also be interesting to see whether the 2G-TKIs have the same immunomodulatory effect.

Disease Relapse and Donor Lymphocyte Infusions

Another major limitation of allo-SCT is relapse. 5- 20% of the chronic phase patients and 30-60% of the patients who are in advanced phase at the time of transplantation, relapse. Increased incidences of relapse are observed with SCT strategies involving TCD, decreased quantity of donor T cells in allografts, and Reduced Intensity Conditioning (RIC) regimens. Donor Lymphocyte Infusions (DLIs) have been very effective in CML patients in tackling relapse especially as CML-CP with remission rates of 70-90% for incremental DLI. Using escalating doses of DLI also helps in decreasing GVHD.

DLI works by the GVL effect of donor T cells against CML cells. There is a risk for GVHD, especially when DLI is done in the first year after transplant. For advanced disease, the effectiveness declines.

TKIs Following Allo-SCT for Relapse

Post allo-SCT administration of TKIs can also overcome relapse and prolong disease-free survival in CML-CP and AP patients. Improved outcomes of CML patients have been reported with imatinib administration as maintenance post allo- SCT with RIC conditioning. The use of imatinib is feasible both with and without DLIs in controlling CML-AP relapse. Prospective studies are needed to validate and for CLBP the effect need to be studied in greater detail. In the present TKI era, most of the patients have already been exposed to imatinib, and therefore, they may be unresponsive to its therapy for controlling relapse post transplantation. 2G-TKIs provide a hope for such patients. However, resistance to TKIs which is mainly due to T315I mutation, is a concern. Ponatinib is a new drug which has activity against T315I mutation but post marketing ADRs reported, especially thrombosis is a safety concern. In the current era, various immunotherapy strategies are being explored.

Reduced Intensity Conditioning

Myeloablative conditioning regimens are high in toxicity and contraindicated in the comorbid or intensely pre-treated patients, the elderly and young adults who wish to preserve fertility. Hence, less toxic, RIC/nonmyeloablative preparative regimens are considered for them. RIC/nonmyeloablative strategies lead to faster immune reconstitution, reduce the duration of neutropenia, have less infectious complications and less risk of GVHD. However, increased rates of relapse have been observed with RIC regimens. Approximately 32-35% of the chronic phase RIC conditioned patients relapse following allo-SCT. Optimization of RIC regimens with low dosages of total body irradiation and 1G, 2G-TKI/DLI combination strategies could be considered to overcome/ prevent relapse and ensure prolonged disease-free survival.

Conclusion

Also SCT, a curative option in CML, has a role in advanced phase disease and in TKI resistance. Time required for donor search, preparatory regimen related toxicity, advanced disease stages, delayed immune restoration, severe GVHD and relapse are the major limitations in its safety and efficacy.

Early donor search and transplantation, induction of chronic phase in patients with advanced disease stages prior to allo-SCT, less toxic preparative regimens, intensive supportive care, and optimized TKI and allo-SCT combination strategies can result in improved clinical outcomes.

References

1. Afreen S, Safran ZA (2013) Allogeneic Stem Cell Transplantation for Chronic Myeloid Leukemia in the Era of Tyrosine Kinase Inhibitors- What are the Limitations? *J Carcinogene Mutagene* S14: 001. doi:10.4172/2157-2518.S14-001.
2. Radich JP, Gooley T, Bensinger W, et al. HLA-matched related hematopoietic cell transplantation for chronic-phase CML using a targeted busulfan and cyclophosphamide preparative regimen. *Blood*. 2003;102:31–35. [PubMed: 12595317].
3. Saussele S, Lauseker M, Gratwohl A, et al. Allogeneic hematopoietic stem cell transplantation (allo SCT) for chronic myeloid leukemia in the imatinib era: evaluation of its impact within a subgroup of the randomized German CML Study IV. *Blood*. 2010; 115:1880–1885. [PubMed: 19965667].
4. Devergie A, Reiffers J, Vernant JP, et al. Long-term follow-up after bone marrow transplantation for chronic myelogenous leukemia: factors associated with relapse. *Bone Marrow Transplant*. 1990; 5:379–386. [PubMed: 2088319].
5. Martin PJ, Clift RA, Fisher LD, et al. HLA-identical marrow transplantation during accelerated-phase chronic myelogenous leukemia: analysis of survival and remission duration. *Blood*. 1988; 72:1978–1984. [PubMed: 3058233].
6. Clift RA, Buckner CD, Thomas ED, et al. Marrow transplantation for patients in accelerated phase of chronic myeloid leukemia. *Blood*. 1994; 84:4368–4373. [PubMed: 7527674].
7. Goldman JM, Gale RP, Horowitz MM, et al. Bone marrow transplantation for chronic myelogenous leukemia in chronic phase. Increased risk for relapse associated with T-cell depletion. *Ann Intern Med*. 1988; 108:806–814. [PubMed: 3285744].
8. Tutschka PJ, Copelan EA, Klein JP. Bone marrow transplantation for leukemia following a new busulfan and cyclophosphamide regimen. *Blood*. 1987; 70:1382–1388. [PubMed: 3311203].
9. Clift RA, Buckner CD, Thomas ED, et al. Marrow transplantation for chronic myeloid leukemia: a randomized study comparing cyclophosphamide and total body irradiation with busulfan and cyclophosphamide. *Blood*. 1994; 84:2036–2043. [PubMed: 8081005].
10. Peggs K, Mackinnon S (2003) Imatinib mesylate-the new gold standard for treatment of chronic myeloid leukemia. *N Engl J Med* 348: 1048-1050.
11. Hochhaus A, O'Brien SG, Guilhot F, Druker BJ, Branford S, et al. (2009) Six-year follow-up of patients receiving imatinib for the first-line treatment of chronic myeloid leukemia. *Leukemia* 23: 1054-1061.
12. Chu S, McDonald T, Lin A, Chakraborty S, Huang Q, et al. (2011) Persistence of leukemia stem cells in chronic myelogenous leukemia patients in prolonged remission with imatinib treatment. *Blood* 118: 5565-5572.
13. Gado K, Matolcsy A, Csomor J, Kicsi D, Bodor C, et al. (2012) Long lasting complete molecular remission after suspending dasatinib treatment in chronic myeloid leukemia. *Exp Hematol Oncol* 1: 17-3619-1-17.
14. Horn M, Glauche I, Muller MC, Hehlmann R, Hochhaus A, et al. (2013) Model-based decision rules reduce the risk of molecular relapse after cessation of tyrosine kinase inhibitor therapy in chronic myeloid leukemia. *Blood* 121: 378- 384.
15. Cortes J, Goldman JM, Hughes T (2012) Current issues in chronic myeloid leukemia: monitoring, resistance, and functional cure. *J Natl Compr Canc Netw* 10 Suppl 3: S1-S13.
16. Crawley C, Szydlo R, Lalancette M, Bacigalupo A, Lange A, et al. (2005) Outcomes of reduced-intensity transplantation for chronic myeloid leukemia: an analysis of prognostic factors from the Chronic Leukemia Working Party of the EBMT. *Blood* 106: 2969-2976.
17. Weisser M, Ledderose G, Jochem Kolb H (2007) Long-term follow-up of allogeneic HSCT for CML reveals significant improvement in the outcome over the last decade. *Ann Hematol* 86: 127-132.
18. Pavlu J, Szydlo RM, Goldman JM, Apperley JF (2011) Three decades of transplantation for chronic myeloid leukemia: what have we learned? *Blood* 117: 755-763.
19. Kröger N (2011) Approaches to relapse after allogeneic stem cell transplantation. *Curr Opin Oncol* 23: 203-208.

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