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Review article

Breakthrough in the treatment of chronic myeloid leukemia

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Abstract

Chronic myeloid leukemia (CML) was once a life-threatening disease in which the clonal expansion of leukemic myeloid cells occurs as a result of reciprocal chromosomal translocation between long arm of chromosome 9 and 22 (Philadelphia chromosome). The resulting fusion protein BCR-ABL shows constantly activated ABL tyrosine kinase activity which is essential for growth of leukemic cells. Specific inhibition of this activated tyrosine kinase by imatinib (Gleevec, Novartis) has completely changed the curability of the disease (Druker et al., 2001). Imatinib is designed to inhibit the binding of ATP to the ABL tyrosine kinase so as to abolish the phosphorylation of the downstream signal proteins.
Chronic myeloid leukemia is one of the diseases of which the molecular mechanism of development has been best dissected. And the discovery of imatinib is truly a milestone and the victory for modern molecular medicine. Its amazing efficacy has transformed a disease with a formidable prognosis, CML, into a chronic condition with safety well established. Imatinib now being a standard therapy for CML, the history of the study of the molecular mechanism of CML, the discovery of the drug, and the related questions are discussed.

Key words: CML, Philadelphia chromosome, BCR-ABL, tyrosine kinase, imatinib

Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by excessive accumulation of apparently normal myeloid cells. Its annual incidence is 1.0-1.5 per 100,000 persons and the median age of onset is 50-60 years. Children are rarely affected. Symptoms at presentation may include lethargy, weight loss, unusual bleeding, sweats, anemia, and splenomegaly, but 50% of patients are asymptomatic at the time of diagnosis by the blood test. CML is characterized by the presence of the Philadelphia (Ph) chromosome which results from a (9; 22) (q34; q11) reciprocal translocation. It juxtaposes the c-abl oncogene (*ABL1*) gene on chromosome 9 with the breakpoint cluster region (*BCR*) gene on chromosome 22, generating *BCR-ABL* oncogene. It represents the constitutively activated c-abl tyrosine kinase, which gives growth advantage to a clonal leukemogenic hematopoietic cell. Before the advent of new drugs (c-abl tyrosine kinase inhibitors), the main topic of this article, all patients with the chronic phase of CML (CML-CP), which represents the initial condition of the disease, the massive accumulation of apparently normal myeloid cells, progressed spontaneously to advanced phase CML after a median interval of approximately 5 years. The advanced phase is divided into an initial accelerated phase (AP), during which patients may still respond to treatment for some months or sometimes years, and a subsequent, more aggressive, blastic phase (BP). The blastic phase of CML (CML-BP) is characterized by the condition called the "blast crisis", a proliferation of more than 20% of myeloblasts or lymphoblasts in the blood or bone marrow. And its symptoms are very similar to those of acute leukemia. Patients with CML-BP have a median survival of approximately 6 months. Therefore, according to this natural course of the disease, CML was indeed once a life-threatening disease. In the past, before c-abl tyrosine kinase inhibitors (TKIs) treatment was introduced, antimetabolites (e. g., cytarabine, hydroxyurea), alkylating agents, interferon alfa 2b, and steroids were used. But the effect of these drugs was limited and far from satisfactory.

Oncogenic potential of BCR-ABL fusion protein

CML was the first malignancy to be linked to a clear genetic abnormality, the chromosomal translocation known as the Philadelphia chromosome. Using a probe specific for the Philadelphia translocation breakpoint domain of one CML patient, a 46 kb human DNA fragment was molecularly cloned in 1984. Further analysis showed that the breakpoints were clustered within the 5.8 kb region of the DNA, for which it was named the "breakpoint cluster region (BCR)" on chromosome 22 (Groffen et al., 1984). Later, this region was revealed to be within the gene named BCR. The counterpart gene of this reciprocal translocation is a gene known as v-abl Abelsone murine leukemia viral oncogene homolog (ABL1) on chromosome 9. As a result of this translocation, a new BCR-ABL fusion protein, p210, is formed. This BCR-ABL fusion protein, p210, is an oncogenic protein, which encodes a constitutively activated tyrosine kinase (Lugo et al., 1990), the stimulant for proliferation of myeloid cells to produce CML.

Oncogenic potential to myeloproliferative disease of this abnormal BCR-ABL fusion protein was confirmed in several p210 expression methods in mice (Daley et al., 1990; Pear et al., 1998). Retroivirus induced p210 BCR-ABL fusion protein was introduced into the bone marrow cells. These cells were transplanted into mice to efficiently reproduce CML-like myeloproliferative disease. These results showed the oncogenicity of BCR-ABL fusion protein and revealed the essential role of this fusion protein in the course of CML development. The fused BCR-ABL protein interacts with the interleukin 3 beta (c) receptor subunit. The BCR-ABL transcript is continuously active and does not require activation by other cellular messaging proteins. In turn, BCR-ABL activates a cascade of proteins that control cell cycle, speeding up cell division. Moreover, the BCR-ABL protein inhibits DNA repair, causing genomic instability and making the cell more susceptible to developing further genetic abnormalities.

BCR-ABL tyrosine kinase as a therapeutic target

Since BCR-ABL tyrosine kinase was identified as a key element of CML development, extensive search for specific BCR-ABL tyrosine kinase inhibitors (TKIs) was done in the late 1990s. The first of these to be developed was imatinib (marketed as Gleevec or Glivec; previously known as STI-571 or CGP57148B). The efficacy of imatinib was really excellent for newly diagnosed patients with CML in the chronic phase. Adverse effects of imatinib were minimal; the most common were nausea, myalgias, edema, and diarrhea (Druker et al., 2001). Thus, imatinib has been proven to inhibit the progression of CML, allowing the majority of patients (65–75%) to have a regrowth of their normal bone marrow. Several years ago, 5-year follow-up data on patients receiving imatinib for CML was published (Druker et al., 2006). According to this, the overall survival rate is 89% after five years. This rate is much better than that of the former standard treatment with interferon alfa plus cytarabine (a 5-year overall survival of 68 to 70%) and imatinib treatment has made CML is the first cancer in which a medical treatment can give the treated patients a normal life expectancy. But an estimated 7–20% of patients progressed to the accelerated phase or blastic phase of CML. To overcome imatinib resistance and to increase the responsiveness of TKIs, two novel agents, dasatinib and nilotinible, were later developed. So there are now three drugs available for first-line treatment of CML.

Human chronic myeloid leukemia stem cells are insensitive to imatinib

Imatinib therapy, which targets the oncogene product BCR-ABL, has transformed chronic myeloid leukemia (CML) from a life-threatening disease into a chronic condition. CML originates in a clonal hematopoietic stem cell (HSC) with the specific reciprocal translocation t(9; 22). Imatinib induces complete cytogenetic response (CCRs) in more than 80% of newly diagnosed patients in the chronic phase CML. But most patients have *BCR-ABL* transcripts. Even in patients with *BCR-ABL* transcripts undetectable by RT-PCR, active leukemia recurs when imatinib therapy is discontinued. This indicates that some leukemia cells persist in most patients even when they are free of overt CML. Therefore, the current recommendation is lifelong continuance of therapy, at considerable cost and sometimes despite significant side effects. It is concluded that most patients harbor residual leukemia cells, and disease recurrence usually occurs when imatinib is discontinued. Although imatinib inhibits BCR-ABL in CML stem and progenitor cells, human CML stem cells do not depend on BCR-ABL

activity for survival and are thus not eliminated by imatinib therapy (Corbin et al., 2011).

Resistance to imatinib

Although a revolution in medical science was marked with the advent of imatinib, the emergence of resistance to imatinib has dampened the enthusiasm for this drug to some extent. The rate of relapse and resistance appear to correlate with disease stage, and the incidence increases as CML progresses. According to the five-year follow-up data on receiving imatinib for the initial stage of CML, the rate of complete cytogenetic response might reach 87% after 60 months according to Kaplan-Meier estimates. And only 7 % of patients showed imatinib resistance and progressed to the accelerated phase CML or blast crisis (Druker et al., 2006). Resistance to imatinib can be divided into primary resistance, in which patients show lack of efficacy to this tyrosine kinase inhibitor (TKI) from the start of the therapy, and secondary resistance, also known as acquired resistance, which is defined as a loss of hematologic (normalization of peripheral blood counts), cytogenetic (disappearance of Philadelphia chromosome), or molecular (undetectable *BCR-ABL* transcripts by RT-PCR) response. The mechanism of imatinib resistance is primarily associated with mutations within the tyrosine kinase domain of BCR-ABL fusion protein. The first mutation discovered with respect to imatinib resistance in American population was T315I. To date, 17 distinct point mutations in the ABL kinase domain conferring resistance to imatinib have been identified. In summary, as long as the treatment with imatinib begins in the chronic phase of CML, the prognosis of patients is very good, and most patients do not show resistance to imatinib. But for patients in the accelerated or blastic phase of CML, imatinib therapy is not completely effective. Strategies to overcome imatinib resistance should be considered in those cases.

How to overcome imatinib resistance

Now that imatinib therapy has been established as a standard treatment of CML, remaining endeavors are addressed to overcome imatinib resistance in its broad meaning. The main goals of present approaches can be classified into three categories. Firstly, to eradicate leukemia stem cells of CML, which remain potential sources of relapse. Secondly, to find a more effective way to treat CML-AP or CML-BP. And finally, to overcome imatinib resistance during the ordinary course of treatment of CML-CP.

Elimination of leukemic stem cells

Although imatinib successfully converts the life-threatening disease CML into a chronic condition, it is not a permanent cure of the disease because relapse is a common scenario with discontinuation of imatinib. The reason why it is so is the survival of quiescent leukemic stem cells, which are insensitive to imatinib. The bone marrow microenvironment supports survival of CML stem cells that are not oncogene addicted. Treatment with histone deacetylase inhibitor (HDA-Cis) combined with imatinib effectively induced apoptosis in quiescent CML progenitors resistant to elimination by imatinib alone (Zhang et al., 2010). Thus, combination therapy could be clinically more effective in the near future and might open the way to a complete cure.

Treatment of CML-AP or CML-BP

The molecular mechanism of the CML emergency is now fully dissected at the molecular level and is relatively simple. That is the main reason imatinib therapy is so successful. However, the molecular mechanism of progression of CML-CP to blast crisis is complicated and much more difficult to analyze. At present, its mechanisms underlying disease progression are still uncertain, but they most likely involve activation of oncogenic factors and/or inactivation of tumor suppressors. A plausible assumption is that BP is a multistep, time-dependent process initiated by both BCR-ABL-dependent and -independent DNA damage associated with inefficient and unfaithful DNA repair in CML-CP that leads to selection of one or more CML-BP clones. Indeed an increased level of BCR-ABL activity is known to cause genomic instability, which facilitates blastic transformation. In the past, CML-BP was often treated with drugs used for acute leukemias. But patients usually relapsed within a few months. The introduction of TKIs has improved the prognosis to some degree. The majority of CML-BP patients not previously treated with TKIs do initially respond to treatment with these agents. But, again, most still relapse within a few months of achieving seemingly complete hematologic or even cytogenetic response. With these rather dismal results of CML-BP and the good clinical outcome of imatinib treatment of CML-CP, one might reasonably conclude that the best approach to CML-BP would be prevention. But, it should be

noticed that there are still about 10% nonresponders to TKIs who inevitably progress to CML-AP/CML-BP, and about 15% of CML patients are already in CML-AP/CML-BP at the time of diagnosis. Second generation TKIs (dasatinib and nilotinib) with more potent tyrosine kinase inhibitory potential show better initial hematopoietic response to CML-AP/CML-BP than imatinib, but relapse is a common following scenario, as mentioned before. Third generation TKIs (bosutinib and ponatinib) are now in the pivotal phase II/III clinical trial. Combination therapy with imatinib is also in progress. At present, any CML-BP patient who does respond to modern therapy should proceed to allogeneic stem cell transplant, if possible, prior to relapse.

To overcome imatinib resistance in CML-CP

Considering the poor prognosis of CML-BP, preventing CML-CP from progressing to a more advanced stage is very important. Therefore, how to overcome imatinib resistance in CML-CP is one of the key questions in modern CML treatment. The main cause of imatinib resistance comes from mutations within *BCR-ABL*. The second generation TKI, dasatinib, holds great promise for the management of practically all imatinib-resistant mutation in *BCR-ABL* except for T315I mutation. Another second generation TKI, nilotinib, also failed to solve this problem. For the most troublesome mutant, T315I, third generation TKI, ponatinib, has promising preclinical research results and can inhibit the entire spectrum of mutations within *BCR-ABL*. Another, third generation TKI, bosutinib, is a dual Src-Abl inhibitor. Bosutinib is now in phase III clinical trials and has shown good activity in patients resistant to imatinib or other TKIs.

Mechanisms of blastic transformation of CML

The genetic lesions observed in CML-BP patients in the past, and now since the introduction of TKIs, mostly include the presence of additional chromosomes, gene deletions, gene insertions, and/or point mutations (including BCR-ABL mutations). But patterns differ in myeloblastic and lymphoblastic transformations. At the molecular level, the most common mutations detectable (other than those in the BCR-ABL kinase domain) occur at the loci of the tumor suppressor genes *P53* (20%-30% of cases) and the runt-related transcription factor gene (*RUNX1* also known as *AML1*) (38% of cases) in myeloid BP and at the loci of cyclin-dependent kinase inhibitor 2A/2B (*CDKN2A/B*) (50% of cases) and the Ikaros transcription factor (*IKZF1*) (55% of cases) in lymphoid BP. At present, any one secondary genetic aberration is unlikely to be identified as a cause of blastic transformation. In addition to genetic aberration seen in blastic transformation of CML, epigenetic changes are also seen, which are mostly dependent on the pleiotropic effect of constitutive BCR-ABL activity. The level of BCR-ABL activity starts to increase in CML-AP and greatly perturbs the CML transcriptome and changes the gene expression profile. Among the activated are $MAPK^{EPK1/2}$, MYC, JAK2, YES-1, LYN, hnRNP-E2, MDM2, STAT5, BMI-1, and BCL-2. On the other hand, P53, C/EBPα, and PP2A are inhibited. The relatively high BCR-ABL expression level in CML stem cells is likely to trigger combined genetic and epigenetic abnormalities and leads to enhanced proliferation/self renewal, increased genomic instability, inhibition of tumor suppressors, and blocked myeloid differentiation. With these complex mechanisms, blastic clones are finally selected to progress the disease.

Future perspective

Considering the complex mechanism of blast crisis, I believe the easiest way to our goal, the complete cure of CML, is definitely the development of more effective, and specific TKIs and administration of newly developed drugs before the progression of CML-CP. Indeed, second generation TKIs (nilotinib and dasatinib) showed impressive results not only in the treatment of patents resistant to imatinib but also in the treatment of newly diagnosed CML-CP patients (Table 1). The effective treatment of advanced stages of CML, CML-AP/CML-BP, will only be

Table 1 Compared responses to imatinib, nilotinib, and dasatinib in newly diagnosed CML-CP patients

Treatment	Complete cytogenetic response* (%)	Major molecular response* (%)
Imatinib 400 mg/day	72	22
Nilotinib 300 mg/day	80**	44**
Dasatinib 100 mg/day	83**	46**

*Complete cytogenetic response is defined as the absence of Ph-positive metaphases on the basis of G-banding and major molecular response is defined as a reduction in the BCR-ABL transcript level by at least 3 log (less than 0.1%) from the standardized base line level measured by quantitative RT-PCR assay. The rates of responses were measured at 12 months after initiation of each treatment.
**Statistically significant difference in nilotinib/dasatinib versus imatinib.

achieved when the critical biological process of progression of CML is identified, and the therapeutic methods impeding the process are developed. For a feasible future approach, other promising methods to target BCR-ABL are listed.

Targeting pathways downstream of BCR-ABL

The RAS/RAF pathway is intimately linked to BCR-ABL through the adapter molecules GRB2 and CRKL. Inhibitors of these molecules, such as a farnesyl transferase inhibitor, theoretically block the pathway. P13K pathway is also in the BCR-ABL downstream. It causes increased production of reactive oxygen species and genomic instability. It can be blocked by inhibitors such as LY294002 or wortmannin.

Targeting mRNA of BCR-ABL

RNAi technology is a powerful tool in silencing gene function. The use of RNAi with breakpoint-specific short-interfering RNA has been shown to selectively inhibit BCR-ABL dependent proliferation and enhance imatinib effect (Wohlbold et al., 2003).

Conclusion

As a student, in 1988, I attended the last lecture of the former head and professor of the division of hematology, department of internal medicine. I still cannot forget the concluding remarks in that lecture. He said "Although great progress has been made in the treatment of acute leukemia, there has been no progress at all in the treatment of CML during these 50 years of my career in this university." After the discovery of the Philadelphia chromosome by Nowell and Hungerford in 1960, CML may be the most extensively studied human cancer. But, at that time, surely few might have expected that a breakthrough would be achieved within 20 years. A remarkable breakthrough in cancer therapy came with the introduction of imatinib to the treatment for CML. Because CML is a hematopoietic stem cell disorder, newly developed methods to overcome the imatinib resistance at the CML stem cells level are most eagerly awaited.

References

Corbin AS, Agarwal A, Loriaux M, Cortes J, Deininger MW, Druker BJ: Human chronic myeloid leukemia stem cells are insensitive to imatinib despite inhibition of BCR-ABL activity. J Clin Invest 121: 396-409, 2011

Daley GQ, Van Etten RA, Baltimore D: Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. Science 247: 824-830, 1990

Druker BJ, Guilhot F, O'Brien SG, Gathmann I, Kantarjian H, Gattermann N, Deininger MW, Silver RT, Goldman JM, Stone RM, Cervantes F, Hochhaus A, Powell BL, Gabrilove JL, Rousselot P, Reiffers J, Cornelissen JJ, Hughes T, Agis H, Fischer T, Verhoef G, Shepherd J, Saglio G, Gratwohl A, Nielsen JL, Radich JP, Simonsson B, Taylor K, Baccarani M, So C, Letvak L, Larson RA; IRIS Investigators.: Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. N Engl J Med 355: 2408-2417, 2006

Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, Lydon NB, Kantarjian H, Capdeville R, Ohno-Jones S, Sawyers CL: Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med 344: 1031-1037, 2001

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 36: 93-99, 1984

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

Pear WS, Miller JP, Xu L, Pui JC, Soffer B, Quackenbush RC, Pendergast AM, Bronson R, Aster JC, Scott ML, Baltimore D: Efficient and rapid induction of a chronic myelogenous leukemia-like myeloproliferative disease in mice receiving P210 bcr/abl-transduced bone marrow. Blood 92: 3780-3792, 1998

Wohlbold L, van der Kuip H, Miething C, Vornlocher HP, Knabbe C, Duyster J, Aulitzky WE: Inhibition of bcr-abl gene expression by small interfering RNA sensitizes for imatinib mesylate (STI571). Blood 102: 2236-2239, 2003

Zhang B, Strauss AC, Chu S, Li M, Ho Y, Shiang KD, Snyder DS, Huettner CS, Shultz L, Holyoake T, Bhatia R: Effective targeting of quiescent chronic myelogenous leukemia stem cells by histone deacetylase inhibitors in combination with imatinib mesylate. Cancer Cell 17: 427-442, 2010