Advances in the treatment of haematological malignancies: optimal sequence of CML treatment

A. Hochhaus
Ill. Medizinische Klinik, Medizinische Fakultät Mannheim der Universität Heidelberg, Mannheim, Germany

introduction

Chronic myelogenous leukaemia (CML) has been considered a model for other cancers based on its multi-step evolution with three stages: its association with a defined cytogenetic translocation t(9;22)(q34;11), the elucidation of molecular pathogenesis and the successful development of a molecular therapy. Targeting of the BCR-ABL tyrosine kinase with the selective inhibitor imatinib has induced remissions with almost complete disappearance of any signs and symptoms of CML. This therapeutic success has triggered an intensive search for target structures in other cancers and has led to the development of numerous inhibitors of potential targets which are being studied in preclinical and clinical trials.

medical treatment of cml

Therapy was palliative during the first century of CML treatment, which included splenic irradiation, various cytostatic agents, of which busulfan was standard for almost three decades, and intensive combination therapy. Treatment intention became curative with the introduction of stem cell transplantation (SCT) in the 1970s. A prolongation of survival could be achieved by interferon alpha (IFN) in combination with hydroxyurea or low-dose cytarabine (ara-C), particularly in low-risk patients and in patients who achieve a cytogenetic remission.

The introduction of imatinib marks a major advance in CML treatment with regard to efficacy and minimizing adverse reactions. Its mechanism of action is blocking the ATP-binding site of the BCR-ABL tyrosine kinase with high affinity and high specificity. After imatinib had been shown to inhibit BCR-ABL-positive cell lines in vitro, phase I trials started in 1998 and phase II trials in 1999. Imatinib showed good efficacy and tolerability in patients who had failed IFN treatment. The beneficial effect of imatinib was demonstrated in chronic phase (CP), accelerated phase (AP) and in blast crisis (BC), as well as in Ph+ acute lymphoblastic leukaemia (ALL).

Long-term studies on the use of imatinib for up to 6 years in 454 CP CML patients who were haematologically or cytogenetically resistant/refractory or intolerant to IFN confirmed safety and durability of imatinib. Patients were administered imatinib 400 mg/d and evaluated for best major and complete cytogenetic response (MCR and CCR), time to progression to AP or BC, and overall survival (OS). Following a median duration of 65 months treatment with imatinib, MCR rate was 67% and CCR rate was 57%; 28 patients (6%) achieved CCR after the third year of therapy. At 6-years follow up (median 75 months), OS was 76%, prompting the suggestion that the annual CML mortality rate (≤10–20% in the pre-imatinib era) is now <5% in the first 5–6 years of imatinib therapy post-IFN failure. Progression-free survival was 65%. Dosing was increased for suboptimal response in 56% of patients and 44% of patients remained on imatinib therapy. Cyto genetic response at 12 months was predictive for long-term prognosis. Patients who achieved at least a MCR at 12 months had an estimated 6-year survival rate of 90% compared with 64% for other response categories. Importantly, side effects at 6 years follow-up are the same as previously reported and no worrisome long-term side effects were observed. The overall incidence of serious cardiac events was 0.8% (comparable with age-controlled matched population) and no drug-related cardiac events were reported between 2002 and 2006 of the study [1, 2].

In a phase III study in 1106 non-pretreated patients in early phase CML randomized between imatinib and IFN in combination with low dose ara-C (IRIS trial), the imatinib group achieved complete haematological remissions (CHR) in 98%, MCR in 92% and CCR in 87% of patients (60 months data, update 2006). Time to CHR was much shorter with imatinib (~90% after 3 months) than with IFN. Tolerability of imatinib was excellent: only 1–2% experienced grade 3–4 toxicity in contrast with IFN-treated patients in which up to 24% experienced severe fatigue or depression. Major parameters with prognostic impact were any cytogenetic response after 6 months, MCR after 12 months, and CCR after 18 months of therapy. Importantly, annual relapse rate is continuously decreasing to <1% in the fifth year of therapy [3].

utilizing molecular markers to optimize outcome

Molecular monitoring of BCR-ABL transcript levels with quantitative reverse transcriptase–polymerase chain reaction (RT–PCR) technology in patients who have achieved CCR has become an important asset of long-term CML management. Real time PCR using specific fluorescent probes and standard procedures with internal controls allow a rapid and accurate analysis. Early reduction of BCR-ABL transcript levels predicts...
cytogenetic response and favourable clinical course in imatinib-treated CP CML patients. Low levels of residual disease have been associated with continuous remission. The degree of molecular response correlates directly with progression-free survival. The persistence of BCR-ABL transcripts even after prolonged imatinib treatment in most patients argues against a prospect of cure by imatinib alone and for additional therapeutic measures. Similar observations have been made earlier after allografting. Transplanted patients with complete disappearance of BCR-ABL transcripts within 6–12 months have been found to have excellent prospects for a successful transplantation outcome and probably cure.

However, the lack of standardization of the methodology represents a major barrier in the comparison of data generated in different studies. Therapeutic response can be expressed in three ways: (i) calculation of the ratio of messenger RNA (mRNA) transcripts of target to reference gene, e.g. ratio BCR-ABL/ABL; (ii) individual calculation of the relative molecular response: i.e. comparison of the minimal residual disease (MRD) level after therapy versus pre-therapeutic level; and (iii) use of a lab-specific reference point, e.g. a pool of diagnostic samples for calculation of the log reduction. In the IRIS trial, a >3-log reduction after 18 months of imatinib therapy was accompanied by a 96% relapse-free survival after 60 months and defined as ‘major molecular response’ (MMR). The reference sample, however, is not available for widespread distribution. There is a relationship between the log reduction approach and the ratio of BCR-ABL to total ABL transcripts. Using standardized methods for the quantification of BCR-ABL and ABL transcript with an identical plasmid dilution for both transcript types, a 3-log reduction is in the Mannheim lab actually equivalent to a ratio BCR-ABL/ABL = 0.12%. The use of external calibrators, which are in preparation, has been suggested to overcome the marked international heterogeneity of molecular data [4, 5].

stem cell transplantation

Although allografting is still considered to be the only potentially curative approach to CML, transplantation numbers have dropped significantly in the imatinib era due to treatment-related morbidity and mortality. A trend towards lower mortality rates after related donor transplantations has been noted, but overall mortality seems to remain unchanged due to the higher proportion of unrelated donor transplantations and an increased age of transplanted patients. The European Group for Blood and Marrow Transplantation (EBMT) score [6] allows the recognition of patients with especially low or high transplantation risks. Current management of newly diagnosed CML patients therefore has to include the evaluation of patients according to disease risk profile and transplantation risk.

In a prospective randomized comparison of primary haematopoietic SCT versus best available drug treatment (IFN-or imatinib-based therapies) conducted by the German CML study group, 621 patients with CP CML were stratified for eligibility for transplantation. With an observation time of up to 11 years (median 7.8, minimum 5+ years) OS was superior for patients with no related donor, superiority being confined to low-risk patients. The general recommendation of allogeneic SCT as first-line treatment option for all patients in CP CML can no longer be maintained [7].

treatment optimization trials

Randomized trials were designed by national study groups in Germany, France, and the UK to compare imatinib single agent at 400 mg with imatinib in various combinations (IFN, ara-C) and dosages (600 and 800 mg). The German CML Study IV [8] started recruitment in July 2002. By May 2007, 1000 patients had been randomized. The study compares imatinib at 400 mg versus the combination of imatinib plus IFN versus imatinib plus low-dose ara-C versus imatinib after IFN failure. The sequential treatment concept of imatinib after IFN failure is supported by the modes of action of the two drugs. IFN has been shown to induce a T-cell response against proteinase 3 which is associated with complete cytogenetic remission. No such response has been observed with imatinib which may even inhibit T-cell activation [9]. After imatinib failure allogeneic transplantation is recommended for all patients who have a donor and are eligible for the procedure.

imatinib resistance

Early investigations into the underlying mechanism of imatinib resistance in cell lines revealed BCR-ABL gene amplification and BCR-ABL over-expression [10–12]. However, the most frequent mechanisms of resistance are clonal evolution, i.e. BCR-ABL-independent activation of alternative pathways, and point mutations in the BCR-ABL gene which create enzymes that are less sensitive towards inhibition by imatinib [13–16]. In retrospective studies P-loop and T315I mutations translated into significantly worse OS as compared with other mutations in patients who continued imatinib therapy [17–19].

In patients with advanced disease or long-lasting CP, imatinib resistance may arise through a very small background level of BCR-ABL mutations which exist prior to imatinib treatment, and which increase in number under therapy due to the selective pressure of imatinib. Several studies also suggest that drug-resistant BCR-ABL point mutations can arise during imatinib treatment. Such acquired resistance usually involves re-emergence of BCR-ABL tyrosine kinase activity, which suggests that the mutant BCR-ABL protein is still a putative target for inhibition in imatinib-resistant patients [17, 20–23]. Although imatinib potently inhibits the production of differentiated leukaemic cells, leading to high rates of haematological and cytogenetic remissions, it fails to deplete leukaemic stem cells [24, 34].

The dose increase of imatinib has been shown to improve response in patients with accelerated disease [25]. Kantarjian et al. have reported in a historical comparison that higher cytogenetic and molecular remission rates can be achieved in shorter time intervals with an imatinib dosage of 800 mg daily as compared to 400 mg in CP CML with the disadvantage of higher rate of adverse effects, in particular myelosuppression and fluid retention [26].
methods to predict potential resistance mutants

The development of highly sensitive, PCR-based screening assays has greatly facilitated the detection and identification of point mutations in imatinib-resistant patients, and could be useful for prediction of the most ideal course of treatment for these patients as well as patients resistant to the second generation BCR-ABL inhibitors [18, 27–29]. The highly sensitive allele-specific-oligonucleotide PCR method was used for detection of the first set of BCR-ABL point mutations shown to simultaneously exist in an imatinib-resistant CML patient. A denaturing-high-performance liquid chromatography (D-HPLC)-based assay has been successful in identifying point mutations in CML patients who showed cytogenetic resistance to imatinib.

Therapy surveillance by molecular methods has become a crucial part of the clinical management of CML patients [30]. The goal of detection and quantification of residual disease by RT–PCR and to early detection of mutations is to allow timely therapeutic interventions to optimize therapy. International standardization of the methodology is required to establish a sound basis for therapeutic decisions [5]. The prognostic value of early detection of imminent resistance to kinase inhibitors by sensitive methods to allow early therapeutic interventions needs to be evaluated.

imatinib in combination

Combinations of imatinib with other drugs have been extensively analyzed in vitro and have shown that a number of drugs are synergistic with imatinib. The feasibility of the combinations of imatinib with pegylated IFNs and low dose ara-C has been shown in phase I and II studies [31, 32].

The emergence of resistance has led to a search for downstream targets of the BCR-ABL kinase that may mediate the altered growth properties of BCR-ABL-transformed cells. Identification of signalling pathways downstream of ABL tyrosine kinase may increase our understanding of the pathogenesis of CML and suggest strategies to improve clinical treatment of the disease.

Farnesyltransferase inhibitors enhance the anti-proliferative effects of imatinib against BCR-ABL-expressing cells, including imatinib-resistant cells. Early clinical studies using a combination of imatinib and farnesyltransferase inhibitors in advanced phase CML patients demonstrated feasibility but showed only moderate activity, probably due to clonal evolution with novel molecular or cytogenetic aberrations in addition to BCR-ABL not responding to farnesyltransferase inhibitors [33].

The serine/threonine protein kinase mTOR is a downstream component of the PI3-Kinase/Akt pathway, and plays an important role in controlling cell growth and proliferation. The mTOR pathway is constitutively activated by BCR-ABL in CML cells. BCR-ABL-independent activation of the mTOR pathway has been demonstrated in vitro and in vivo [34]. The synergy between rapamycin and imatinib, occurring at doses well below typical serum levels obtained during monotherapy with each of these agents, represents a strong argument in favour of investigating the clinical activity of the combination.

characteristics of new bcr-abl inhibitors in clinical trials

The discovery of resistance mechanisms spurred the development of alternative therapies designed to override resistance to imatinib. Classes of these new inhibitors include selective ABL inhibitors, inhibitors of both ABL and SRC family kinases, Aurora kinase inhibitors, and non-ATP competitive inhibitors of BCR-ABL.

novel abl inhibitors

Several new ABL inhibitors have been reported, some of which have entered clinical trials [nilotinib (AMN107), dasatinib (BMS-354825) and bosutinib (SKI-606)]. Target structures of nilotinib are BCR-ABL, c-KIT and platelet-derived growth factor receptor (PDGFR), of dasatinib BCR-ABL, c-KIT, PDGFR and SRC, and of bosutinib BCR-ABL and SRC, but not the PDGFRs and c-KIT. For ABL inhibition, nilotinib, dasatinib and bosutinib are more potent than imatinib and have been shown to retain activity against most, but not all, imatinib-resistant BCR-ABL mutants.

Nilotinib (Novartis, East Hanover, NJ)

The phenylaminopyrimidine derivative nilotinib is ±30-fold more potent than imatinib as ABL inhibitor (IC50 < 30 nM) but has similar activity as imatinib against the receptor tyrosine kinases KIT (IC50 = 60 nM) and PDGFR (IC50 = 39 nM). In preclinical studies, nilotinib inhibited 32 of 33 mutant BCR-ABL forms resistant to imatinib [35, 36].

Results of a phase I study of nilotinib in 97 patients with imatinib-resistant CML in CP, AP and blast phase and nine patients with Ph+ ALL treated at doses ranging from 50 to 1200 mg daily have been reported [37]. In phase II trials in all phases of CML and Ph+ ALL after failure to imatinib, nilotinib was administered at a daily dose of 400 mg b.i.d. in imatinib-resistant and -intolerant patients with CP CML. Among a subset of 132 patients with at least 10-months follow up, CHR was reported in 77% of patients who did not have a CHR at baseline; MCR was observed in 49% of patients (32% complete), at a median 2.8 months. Rates of grade 3/4 non-haematological adverse events were >3% and most were transient. Thus, preliminary analyses demonstrate good efficacy and tolerability [38].

abl and src inhibitors

The SRC family of tyrosine kinases modulate multiple intracellular signal transduction pathways involved in cell growth, differentiation, migration and survival, many of which are involved in oncogenesis, tumour metastasis and angiogenesis. The family includes SRC, FYN and YES, which are ubiquitously expressed, and HCK, LYN, FGR, LCK and BLK, the expression of which is mainly restricted to haematopoetic cells. Further, BCR-ABL activates SRC kinases both through phosphorylation and direct binding. Analysis of patient samples taken prior to and after imatinib failure
revealed that activation of SRC-family kinases such as LYN and HCK occurs during disease progression, suggesting that over-expression of these tyrosine kinases might mediate BCR-ABL-independent imatinib resistance in some patients [39]. Therefore, simultaneously targeting BCR-ABL and SRC kinases could help to overcome imatinib resistance.

**Dasatinib (Bristol Myers Squibb, New York, NY)**

Dasatinib is a highly potent oral inhibitor of SRC-family kinases and also a potent BCR-ABL inhibitor, and has additional activity against the c-Kit, PDGFR and Ephrin receptor tyrosine kinases. Dasatinib is very different to imatinib, both in terms of its chemical structure and kinase selectivity. The drug inhibits 21/22 mutant forms of BCR-ABL resistant to imatinib [36, 40, 41]. Based on positive data in phase I [42] and II trials, dasatinib received accelerated approval by the FDA and the EMEA in 2006 for the treatment of adults in all phases of CML with resistance or intolerance to imatinib, both in terms of its chemical structure and kinase selectivity. The drug inhibits 21/22 mutant forms of BCR-ABL resistant to imatinib [36, 40, 41]. Based on positive data in phase I [42] and II trials, dasatinib received accelerated approval by the FDA and the EMEA in 2006 for the treatment of adults in all phases of CML with resistance or intolerance to imatinib therapy. Full approval was also granted for the treatment of adults with Ph+ ALL with resistance or intolerance to prior therapy.

In a study of CP CML patients after resistance or intolerance to imatinib, 387 patients received dasatinib (70 mg b.i.d.) with dose escalation to 90 mg b.i.d. for patients with suboptimal response. Dose reductions to 50 and 40 mg b.i.d. were also allowed for toxicity or intolerance. At a median follow-up of 13 months, CHR was observed in 91% of patients and MCR in 58% of patients (47% complete). Grade 3/4 neutropenia or thrombocytopenia was reported in 49 and 48% of patients, respectively. Dose interruptions and reductions were required in the majority of patients, the average daily dose was 103 mg/day. Non-haematological toxicities included diarrhoea, headache, rash and pleural effusions [43]. A good activity of dasatinib was also demonstrated in advanced disease [44].

One of the primary safety concerns with the use of dasatinib has been the incidence of grade 3/4 pleural effusions. Approximately one-third of patients treated with dasatinib experienced pleural effusions which were even more frequently observed in patients with advanced CML receiving doses ≥140 mg daily. A change of the schedule from twice daily to once daily, and a starting dose of 100 mg/day did not change the efficacy, but improved the toxicity of the drug considerably [45].

**Bosutinib (SKI-606, Wyeth, Madison, NJ)**

Bosutinib has been developed as an inhibitor of Src-family kinases for the treatment of solid tumours, although, like dasatinib, it also targets BCR-ABL but not KIT or PDGFR. This compound inhibits SRC and ABL with an IC50 of 1.2 and 1 nM, respectively [46]. Bosutinib showed in vitro activity against all imatinib-resistant mutants, except T315I. In a phase I/II clinical trial in imatinib-resistant CML and Ph+ ALL, Bosutinib has shown evidence of efficacy at well-tolerated doses [47].

**INNO-406 (Innovive, New York, NY)**

INNO-406 (previously known as NS-187, CML-187) is a potent BCR-ABL and LYN dual tyrosine kinase inhibitor, structurally related to imatinib and nilotinib, originally developed by Nippon Shinyaku as a potential treatment for patients with CML. INNO-406 is >25-fold more potent than imatinib against BCR-ABL-positive unmutated or mutated leukaemia cell lines, except T315I. INNO-406 also suppresses the autophosphorylation of PDGFR and KIT at equivalent levels to imatinib and is also a potent inhibitor of LYN kinase, but it has no effect on c-SRC kinase activity [39, 48]. A phase I/II study is ongoing.

**Non-ATP competitive inhibitors of bcr-abl**

A potential alternative approach to ATP-competitive BCR-ABL inhibition is to use molecules that inhibit the kinase activity either by a non-ATP competitive allosteric mechanism or by prevention of the binding of substrates to the kinase. This strategy has the advantage in that the imatinib-resistant mutants are unlikely to be resistant to such inhibitors, due to the different binding sites. An example for this strategy represents ON012380 (Onconova Therapeutics, Princeton RI) [49], which has however not entered clinical trials so far.

**novel targeted agent may prove effective against T315I mutants**

Recent data have described a number of different mutations, although nearly all have limited impact of imatinib efficacy. However, one mutation—T315I—has been tagged as the gatekeeper for conferring resistance to imatinib as well as to the more potent second-generation tyrosine kinase inhibitors. To this end, there has been interest in identifying novel agents with activity against T315I clones.

**aurora kinase inhibitors: MK-0467 (VX-680, Merck, Blue Bell, PA)**

The Aurora family of serine/threonine kinases is essential for mitotic progression. MK-0457, originally developed by Vertex Pharmaceuticals as VX-680, is a potent Aurora kinase inhibitor. Besides being a potent inhibitor of all three Aurora kinases and FLT3, MK-0457 is also a moderate to strong inhibitor of normal and mutated ABL and JAK2. MK-0457 inhibits the proliferation of BaF3 cells harbouring the BCR-ABL T315I mutation with an IC50 of ~100 nM and inhibits the autophosphorylation of wild-type ABL with an IC50 of 360 nM.

In a phase I study in imatinib-refractory patients with CML and the T315I mutation, continuous 5-day intravenous treatment with MK-0457 every 2–3 weeks resulted in one major haematological response and one CCR with acceptable tolerability at 3 months follow up. A variety of dose levels have been investigated, ranging from 8 to 32 mg/m2/h, but no drug-related non-haematological grade 3 adverse events have been observed and the maximum tolerated dose has not yet been established. Myelosuppression is consistently observed and appears to be dose-related [50].

**conclusions**

The advent of selective tyrosine kinase inhibitors has significantly changed CML therapy. However, despite...
promising results, patients should be identified in whom treatment requires optimization, either by dose escalation of imatinib or combination with other drugs. In case of resistance, novel tyrosine kinase inhibitors are available within clinical trials.

The introduction of imatinib has marked an important and revolutionary step, but the long-term outcome of this treatment cannot yet be assessed. In contrast, allogeneic SCT holds the promise of cure, but with definite toxicity and mortality. From a randomized study the superiority of drug treatment overall and specifically in low-risk patients is evident and significant. There is no hint so far that the years lost early due to transplant-related mortality will be compensated in the course of the transplant group later on [7].

The discovery of the nature and prevalence of BCR-ABL mutations prompted the development of second generation kinase inhibitors. The advances in our understanding of resistance against BCR-ABL targeted therapy have important implications in the development of new targeted treatments of other malignancies, such as lung cancer, where mutations of the EGFR kinase are associated with response or resistance to erlotinib.

In addition to haematological and cytogenetic monitoring, molecular surveillance of response and resistance is essential for therapeutic decisions. Molecular-cytogenetic monitoring is expensive and requires appropriate resources and sophisticated facilities. However, the cost of monitoring is negligible by comparison with the cost of treatment, whether it is a targeted agent or SCT. The current therapeutic and diagnostic progress makes treatment more effective but not necessarily easier. Thus the treatment of CML should be provided under the guidance of an experienced centre, offering and asking patients to be registered on investigational studies. This is necessary to ensure that all the data, clinical and biological, that are required to answer the present questions, are collected and analysed in an accurate and timely manner, for the benefit of the subsequent patients and for further progress in the treatment of leukemia [5, 30].

acknowledgements

The review was supported by the Competence Network ‘Acute and chronic leukemias’, sponsored by the German Bundesministerium für Bildung und Forschung (Projektträger Gesundheitsforschung; DLR e.V.–01 GI9980/6) and the Development.

references

32. Hochhaus A, Fischer T, Brümmendorf T et al. Imatinib (Glivec®) and Pegylated Interferon α2a (Pegasys®) (Phase VII Combination Study in Chronic Phase Chronic Myelogenous Leukemia (CML)). Blood 2002; 100 (Supp.1): 164a–165a.
42. Golas JM, Arndt K, Etienne C et al. SRC-606, a 4-anilino-3-quinoxalinecarbonilide dual inhibitor of Src and Abl kinases, is a potent antiproliferative agent against chronic myelogenous leukemia cells in culture and causes regression of K562 xenografts in nude mice. Cancer Res 2003; 63: 375–381.