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Chronic Myeloid Leukemia 2011: Successes, challenges, and strategies – Proceedings of the 5th Annual BCR-ABL1 positive and BCR-ABL1 negative myeloproliferative neoplasms workshop

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Abstract

This report is based on the presentations and discussions at the 5th annual BCR-ABL1 positive and BCR-ABL1 negative myeloproliferative neoplasms (MPN) workshop, which took place immediately following the 52nd American Society of Hematology (ASH) meeting in Orlando, Florida on December 7th-8th, 2011. Relevant data which was presented at the ASH meeting as well as all other recent publications were presented and discussed at the workshop. This report covers front-line therapies of BCR-ABL1-positive leukemias, in addition to addressing some topical biological, pre-clinical and clinical issues, such as new insights into genomic instability and resistance to tyrosine kinase inhibitors (TKIs), risk stratification and optimizing molecular monitoring. A report pertaining to the new therapies and other pertinent preclinical and clinical issues in the BCR-ABL1 negative MPNs is published separately.

Introduction

For patients with BCR-ABL1-positive leukemias, which comprise of all the Philadelphia (Ph) chromosome-positive and some Ph-negative leukemias, the introduction of the original tyrosine kinase inhibitor (TKI), imatinib mesylate (IM) into the clinics in 1998, resulted in being a classic therapeutic landmark (1,2). After 12 months of therapy with IM, 69% of
patients with CML-CP achieve a complete cytogenetic response (CCyR) and after 8 years of follow-up, such response rates increases to 83%. This remarkable activity translates into an estimated overall survival of 93% (when only CML-related deaths are accounted for), which is substantially higher than that achieved by any previous medical treatment, including allogeneic stem cell transplantation (allo-SCT) (1-3). The success in the treatment of patients with CML in advanced phases and the Ph-positive acute lymphoblastic (ALL) leukemia has also been improved with the addition of IM to cytotoxic drugs, though less remarkably (4,5). The adverse events attributable to IM appear to be relatively mild, but not innocuous, and generally easily manageable (6,7).

Conversely, about 35% of all patients with CML-CP and substantially higher proportions with CML in advanced phases and Ph-positive ALL cannot tolerate IM or have a leukemia that becomes resistant or refractory to IM. Recent observations suggest that about 18% of IM-treated patients do not achieve a CCyR, and 10% who do will lose such response over time. Furthermore about 26% of patients are intolerant of IM (6). Novel risk stratification methods and optimal molecular monitoring can be used to judge response and predict future risk of progression for patients with CML-CP. These are complemented by recent insights into the mechanisms of resistance to TKIs as well as by knowledge gained regarding aspects of the cellular and molecular biology of BCR-ABL1-positive cells, such as their underlying genomic instability.

Given the limited activity of TKI therapy in advanced phases of the disease, the most immediate goal of CML therapy is the prevention of progression, which has been associated with the achievement of deep responses at early time points during the course of TKI therapy. In this regard, the use of second generation TKIs (i.e. nilotinib, dasatinib, bosutinib) as frontline therapy has led to an increase in the number of patients capable of achieving CCyR during the first year of therapy. The activity of these agents as frontline therapy for patients with CML-CP has been tested in a series of ongoing randomized, multicenter: nilotinib (ENESTnd), dasatinib (DASISION and Intergroup Trial SO325), and bosutinib (8-10).

Both dasatinib and nilotinib have been recently approved for front-line use for patients with CML-CP in many other countries around the world; dasatinib is also approved for Ph-positive ALL in USA and Switzerland. Both of these drugs are more potent BCR-ABL1 inhibitors with demonstrated efficacy in patients resistant to or intolerant of imatinib and are active against most BCR-ABL1 mutations, with the notable exception of the T315I mutation (11,12). The increased potency, and a safety profile which so far appears to be equivalent if not better than that of IM, further supports the use of these drugs as front-line treatment for patients with CML-CP. Bosutinib, while still not approved as a CML therapy, has been recently shown promising activity in both the frontline and the post-imatinib failure settings. Looking ahead, other novel agents, such as ponatinib and DCC-2036, are being assessed for the treatment of patients carrying the T315 mutation, as well as for those who have failed therapy with more than two TKIs (13-15). As novel TKIs are added to the CML armamentarium, the front-line treatment algorithm for patients with this malignancy needs to be reassessed. Furthermore, IM is rapidly approaching the end of its patent life, a fact that will undoubtedly factor into the treatment decision making process. Ongoing efforts are assessing the potential role of generic IM.

Some Topical Preclinical and Basic Science Issues in CML

The enormous clinical success of TKI therapy has turned CML into a truly chronic disease, as a recent study found that CML patients who achieve CCyR within 2 years on IM have an overall survival rate that is no different from the general population (16). This, in turn,
predicts that the population of CML patients in the U.S. will exceed 250,000 by 2040. The ultimate goal of oncologists is to cure cancer, and the focus of much of basic and preclinical CML research has now turned to this area. We now recognize that TKI therapy is not curative in the majority of CML patients, as results from the French STIM study demonstrate that over half of CML patients in complete molecular remission (CMR) on imatinib relapse quickly when TKI therapy is stopped (17). It is postulated, but not proven, that these relapses are a consequence of quiescent CML stem cells that are resistant to killing by conventional TKIs (18). Indeed, these malignant progenitors can be detected in bone marrow (BM) from CML patients in CCyR on IM (19). Results presented at the 2010 ASH meeting demonstrated that BCR-ABL1 positive clonogenic progenitors, including LTCIC, can also be found in CML patients in CMR (20), whose disease is undetectable by conventional RT-PCR. Hence, there is much current interest in identifying targets and strategies for eliminating leukemic stem cells (LSC) in CML.

At the 2010 ASH meeting, several groups reported on using next-generation and deep sequencing technologies to interrogate CML patient genomes to identify new pathogenetic targets in CML. Comparative whole transcriptome sequencing of a CML patient who progressed to myeloid blast crisis identified 8 missense mutations in novel genes, including IDH2 and protein kinase D2 (21). Deep sequencing of 40 blast crisis CML patients (25 myeloid, 10 lymphoid, 5 unspecified) revealed frequent IKZF1, RUNX1, and ASXL1 mutations that developed during disease progression (22). Further studies will be necessary to determine the role of these mutations in disease progression and assess their suitability as targets for therapy.

Additional research efforts focused on specific signaling pathways that might represent targets for elimination of LSCs in CML. BCL6, a zinc finger protein that functions as a proto-oncogene in diffuse large B cell lymphoma, was shown to be required for maintenance of CML stem cells in the retroviral mouse model, and incubation of human CML progenitors with a peptide BCL6 inhibitor decreased engraftment of immunodeficient NSG mice (23). Stearoyl-CoA desaturase 1 (Scd1), an endoplasmic reticulum enzyme catalyzing the biosynthesis of monounsaturated fatty acids from saturated fatty acids, was identified as a potential tumor suppressor gene in CML stem cells, as CML-like leukemia induced from Scd1–/– BM had higher levels of functional LSCs, whereas treatment of leukemic mice with the PPARγ agonist rosiglitazone increased Scd1 expression and decreased LSCs (24).

Several previous studies have implicated the Hedgehog (Hh) pathway in the maintenance of CML stem cells in mouse retroviral models. A recent study defined the phenotype of leukemia-initiating cells in a conditional transgenic mouse model of CML, and demonstrated that treatment of mice with the Hh inhibitor LDE225 together with the TKI nilotinib decreased phenotypic CML LSCs in spleen, but not BM, and further decreased engraftment of NSG mice with human CD34+ CML progenitors (25). Given the recent launch of clinical trials of Hh pathway antagonists in refractory Ph-positive leukemia, further preclinical studies of these agents are warranted to aid in their clinical development. The possible role of JAK2 in the maintenance of quiescent, TKI-resistant BCR-ABL1-expressing stem cells in CML was also explored by several groups, where JAK2 may be activated by an extrinsic pathway through stroma-mediated cytokines (26), or through an intrinsic pathway via inhibition of a phosphatase, PP2A (27). These results open the possibility of targeting JAK2 in CML either through a specific JAK2 TKI, or the PP2A activator FTY720. Another study presented at ASH tested the activity of a novel high potency pan-Bcl-2 inhibitor, 97C1, and demonstrated that this drug could effectively kill stem cells from blast crisis CML in vitro and in vivo (28), possibly through combined inhibition of Bcl2, BclX, and Mcl1.
Together, these exciting basic and preclinical studies continue to define CML as perhaps the best understood human cancer, and offer the hope that one day we will be able to eradicate the leukemia and cure patients without the need for lifelong drug therapy.

**Risk Stratification of patients with BCR-ABL1 Positive CML**

The goal of risk stratification is to distinguish patients with a higher risk of progression on current therapies. Patients with CML-CP deemed as having a high probability of resistance or progression can be offered more aggressive therapy, for example the use of initial second generation TKI, be enrolled in a clinical trial, or be considered for allo-SCT. In addition, patients at high risk could be monitored closely, with considerations for alternative or more aggressive therapy if strict optimal response criteria are not met.

**Clinical**

Though both the Sokal and Hasford prognostic scores were derived from clinical experience before the TKI-era, they appear to work well in predicting response to TKI therapy. At presentation, patients with CML present with highly variable lengths of time from BCR-ABL1 acquisition. Prior to therapy, unopposed BCR-ABL1 signaling promotes proliferation and genetic instability. Furthermore it is possible that the longer the period of time elapsed between CML initiation and TKI therapy, the greater the likelihood of additional molecular mutations, which may lead to the more advanced phases of the disease. The clinical and laboratory features that serve as surrogates of time from disease initiation are the spleen size and the white blood cell count (WBC). While the rate and which the spleen grows and the WBC increases vary among different patients, these parameters provide a gross estimate of disease burden. These and other clinical parameters constitute the basis for the Sokal (age, platelet count, spleen size, blast percentage) and Hasford (Sokal factors plus eosinophil and basophil percentage) risk scores, which have been shown to be effective in predicting response to TKI therapy but not survival.

**Functional studies**

TKI are among the class of drugs potentially imported and exported by drug influx and efflux pumps, respectively. IM can be imported by OCT-1, and exported by ABCB1 and ABCG2. *In vitro* studies measuring imatinib uptake in mononuclear cells by OCT-1 activity demonstrated that high OCT-1 activity was associated with higher response rates (in survival, event-free survival, molecular response, and mutation rate) compared to patients whose cells had low OCT-1 activity (29-31). Other studies have suggested that mRNA OCT-1 levels (which can be measured in a much easier and reproducible manner than IM uptake) also correlate with response (32). It is of interest that neither of the second-generation TKIs (dasatinib and nilotinib) is transported by OCT-1; rather, the activity of ABC efflux pumps may be involved in maintaining intracellular drug levels for these two TKIs.

**Genetic studies**

Single nucleotide polymorphism (SNP) arrays measure the association of a SNP with a known phenotype (e.g., response to TKI), while mRNA expression arrays measure gene expression and pathways associated with response. The most direct evidence of the impact of specific polymorphisms on response derives from the study of the polymorphisms of the ABCB1 (MDR1) gene. In a study involving 90 patients with CML, those with a homozygous allele 1236 TT polymorphism had higher plasma imatinib levels and a higher frequency of molecular responses; the authors did not demonstrate effect on intracellular imatinib levels (33).
Several groups have searched for genes associated with progression and resistance to TKI therapy in CML. The findings thus far suggest: (a) there is a robust gene signature which is differentially expressed in advanced phase disease compared to CP. The major differences in gene expression occur during the transition from CP to accelerated phase (AP); few genes are further deregulated in the transition from AP to blast phase (BP); (b) there are biologically relevant gene and pathway targets that may targets for prevention of CML progression. For example, progression is associated with deregulation of genes involved in the WNT/catenin, differentiation, glucose metabolism, RNA processing, DNA repair, and apoptosis pathways. Among the most upregulated genes in progression were WT1 and PRAME, both of which are potential targets for T cell directed immunotherapy; (c) There appears to be significant overlap in the genes involved in progression and resistance. Recently, 6-gene signature has been shown to tightly correlate with progression. This six-gene set was applied to new patients in CP and advanced phase disease, as well as cases of newly diagnosed CML and patients with CP that relapsed on IM therapy. The score predicted progression and mapped with response to IM therapy in patients in CP (i.e., newly diagnosed CP disease had a low progression score, while cases of relapsed CP disease had a high score). Two cases of newly diagnosed CML who were resistant to IM had a high progression score, further suggesting an overlap of the biology of progression and resistance.

Further proof of the power of this classifier was demonstrated when it was applied to patients with CML-CP undergoing allo-SCT. The expression of the 6-gene set was retrospectively analyzed in 169 CML-CP cases, and a score was calculated. Cases with a low progression score pre-transplant had a relapse rate of only 5%, while those with a high score, suggesting a relatively blast-like genetic profile), had a relapse rate of 20%. This work suggests that gene expression signatures can be derived from retrospective samples and that such approach might permit the real-time assignment of a relapse risk.

**Optimizing molecular monitoring of patients with BCR-ABL1 Positive Leukemias**

**Early BCR-ABL1 response**

An important predictor of long-term response to TKI therapy is the depth of response at early time points. The Adelaide group have demonstrated that BCR-ABL1 mRNA levels assessed by PCR after only 3 months of therapy is strongly associated with achievement of CCyR, major molecular response (MMR) and progression free survival (35). The measurement of BCR-ABL1 transcripts by QPCR is most relevant in patients that have achieved a CCyR. After 7 years of follow-up in the IRIS (International Randomized Study if Interferon versus STI571) trial,(5) No patients achieving CCyR and MMR at 18 months has progressed to AP or BP. The rate of progression for those that had a CCyR but less than 3 log reduction in BCR-ABL1 was only 3%. Subsequent studies have confirmed the IRIS PCR data and demonstrate that patients with a deeper molecular response at the time of initial CCyR, or a >3-log reduction of BCR-ABL1 during CCyR, have very low odds of progression and a superior PFS compared to patients with an inferior response (36-40).

**What should one do with a rising BCR-ABL1 PCR?**

First, the PCR assessment should be repeated. The BCR-ABL1 QPCR may rise in a patient for a number of reasons. One possibility relates to compliance, especially in the context of an expensive drug (i.e. any TKI) and a patient with a good molecular response and/or in the presence of chronic insidious side effects (a situation where the temptation to enjoy a “drug holiday” is strong). Secondly, results may “wobble” due to sampling error (especially in the presence of a very low tumor burden), and the intrinsic variability of the test itself. In most
labs, however, a 5-10 fold change in the QPCR is likely “real.” However, it is not known how BCR-ABL1 levels vary in patients naturally over time while on TKI therapy. CML is known to have cyclic oscillations with peaks and troughs occurring at even 1-2 month intervals, and this has not been studied in cases with residual disease (41-43). Of course, the last and most worrisome, possible explanation for an increase in BCR-ABL1 is impending relapse. Minor changes in BCR-ABL1 levels should not trigger any change in therapy. However, loss of MMR, never achieving MMR, or experiencing an increase in BCR-ABL1 mRNA transcript levels > 1-log should be very closely monitored.

**Dasatinib as Frontline Therapy for Patients With CML-CP**

Dasatinib is an orally available potent dual kinase inhibitor, inhibiting the BCR-ABL1 and SRC kinases (44,45). Following the success in the treatment of patients with CML-CP resistant/refractory or intolerant to IM, the drug was approved for the treatment of all phases of CML with intolerance or resistance/refractiveness to IM and all patients with Ph-positive ALL. Dasatinib was noted to overcome most mechanisms of resistance to IM, with the exception of the T315I mutation. The drug thereafter entered an international randomized phase 3 trial comparing it with IM for front-line therapy of newly diagnosed patients with CML-CP (46-51). A total of 519 such patients were recruited into the DASISION (Dasatinib vs Imatinib Study in Treatment-Naïve CML Patients) trial and the initial results were published in June 2010 and presented following an 18 month follow-up at the ASH meeting in December 2010 (Table 1) (9,52).

Patients were randomized to receive either IM 400mg daily (n=260) or dasatinib 100mg daily (n=259). The primary objective of the study was confirmed CCyR at 12 months. Patients were stratified by Hasford risk score, which resulted in equal distributions of low, intermediate, and high risk scores in each arm of the trial. Dose escalation to IM 400 mg twice daily and to dasatinib 140 mg once daily was permitted for patients with suboptimal responses. After a median follow-up of 18 months, more patients required dose interruptions amongst those treated with dasatinib (56%) compared to those receiving IM (39%). However, the rates of confirmed CCyR were superior in those patients receiving dasatinib therapy, both at 12 (77% vs 67%, p=0.0086) and at 18 months (78% vs 70%, p=0.0366). At 3 months, CCyR rates were 54% with dasatinib vs 31% with IM, increasing to 73% vs 59%, respectively, at 6 months. MMR rates by 12 months were significantly higher with dasatinib compared with IM (46% vs 28%, P<0.0001). Among the subgroup of patients who achieved MMR, median time to MMR was 8.3 months for dasatinib and 11.8 months for IM.

Among patients treated with dasatinib, only 6 (2.3%) progressed to advanced phase CML compared with 9 (3.5%) (p= not significant) among those receiving IM. Notably, these data were reported on an intention-to-treat (ITT) fashion and patients were followed for transformation to AP or BP for up to 60 days after dasatinib discontinuation. Patients developing clonal evolution without any additional criteria for CML-AP were not considered as having transformed to AP, which may have potentially underestimated the risk of transformation in both arms of the study.

After a median follow-up of 18 months, 81% of patients continue to receive dasatinib while 80% continue to receive IM therapy. Therapy was well tolerated with both TKIs, with grade 3-4 non-hematologic drug-related toxicities occurring in ≤1%. Twelve percent of patients treated with dasatinib developed pleural effusion, but only 4 (1.5%) discontinue therapy for such toxicity. Rates of grade 3-4 anemia (11% vs 7%) and neutropenia (22% vs 20%) were similar but more patients treated with dasatinib developed grade 3-4 thrombocytopenia compared with those treated with IM (19% vs 10%).
Overall, the results reported by the DASISION studies suggest that frontline therapy with dasatinib renders higher response rates with a comparable toxicity profile compared to IM. It remains unknown whether these higher rates of early response will translate into improved EFS and/or OS rates. Thus far, no differences in OS have been observed between the dasatinib (97%) and the imatinib (99%) arms.

The interim results from the SWOG S0325 study were also reported at the ASH meeting and the workshop (53). This was a randomized study of imatinib at 400 or 800 mg vs dasatinib 100 mg in newly diagnosed CP CML patients. At 12 months, the CCyR and MMR rates were 69%/32% for imatinib 400 vs. 82%/47% for dasatinib- essentially independently confirming the DASISION results.

Nilotinib as a Frontline Therapy for Patients with CML-CP

Following the success observed in studies assessing the role of nilotinib, an ABL1 TKI structurally and biologically similar to IM, the drug was evaluated in the front-line use in patients with CML in CP (54-59). The ENESTnd [Evaluating Nilotinib Efficacy and Safety in Clinical Trials-Newly Diagnosed Patients (ENESTnd)] trial is the only phase 3, randomized, open-label, multicenter study comparing the efficacy and safety of nilotinib with IM (Table 2) (8,60,61). 846 patients with CML in CP were randomly assigned 1:1:1 to nilotinib 300 mg twice daily (n = 282), nilotinib 400 mg twice daily (n = 281), or IM 400 mg/day (n = 283). The primary endpoint was MMR at 12 months, and patients were stratified by Sokal risk score, which resulted in equal distributions of low, intermediate, and high Sokal risk scores in each arm of the trial.

Initial results were published in June 2010 and presented at the ASH meeting in December 2010, following a 24 months’ follow-up (62). IM dose escalation to 400 mg twice daily was permitted in the IM arm to ensure that therapy was optimized for patients with suboptimal responses to standard-dose IM. More patients remained on study in both nilotinib arms (84% for nilotinib 300 mg twice daily, 82% for nilotinib 400 mg twice daily) than in the IM arm (79%). On an ITT basis, the MMR rate at 12 months was significantly higher for nilotinib 300 mg twice daily (44%, $P < .0001$) and nilotinib 400 mg twice daily (43%, $P < .0001$) than for IM (22%); for evaluable patients only, the MMR rates were again higher for nilotinib 300 mg twice daily (51%, $P < .0001$) and nilotinib 400 mg twice daily (50%, $P < .0001$) than for IM (27%). On an ITT basis, the cumulative rates of CCyR by 12 months were significantly higher for nilotinib 300 mg twice daily (80%, $P < .0001$) and 400 mg twice daily (78%, $P < .0005$) than for IM (65%); for evaluable patients, the CCyR at 12 months was higher for nilotinib 300 mg twice daily (93%) and nilotinib 400 mg twice daily (93%) than for IM (76%). Responses were rapidly achieved with nilotinib, with 6-month MMR rates (ITT) of 33%, 30%, and 12% and 9-month MMR rates of 43%, 38%, and 18% for nilotinib 300 mg twice daily, nilotinib 400 mg twice daily, and IM, respectively.

More patients achieved complete molecular responses with nilotinib 300 mg twice daily (13%) and nilotinib 400 mg twice daily (12%) than with IM (4%). These higher responses were also associated with significantly fewer progressions with nilotinib than with IM. After 24 months of follow-up, the cohort randomized to receive either dose of nilotinib continued to demonstrate better responses compared to IM, and a significant overall survival advantage was noted for nilotinib 400 mg twice daily (98.9%, $P = 0.03$) vs IM (96.7%). Parenthetically the FDA-approved dose for nilotinib in the US is 300 mg twice daily and not 400mg twice daily. It is also of interest that in this study, patients on the nilotinib 300 mg twice daily arm who were suboptimal responders were taken off study since the study design did not allow for a dose escalation of nilotinib to 400 mg twice daily. Such patients went off study and entered an ‘extension study’ where a dose escalation was permitted!
In general, therapy was well tolerated in all the study cohorts and treatment discontinuation due to adverse events was observed in 5%, 9%, and 7% of patients on nilotinib 300 mg twice daily, nilotinib 400 mg twice daily, and IM, respectively. Grade 3-4 thrombocytopenia was more common in the nilotinib cohort, compared to IM cohort, who experienced more neutropenia and anemia. Grade 3-4 laboratory abnormalities with nilotinib such as elevated levels of lipase, alanine aminotransferase, aspartate aminotransferase, total bilirubin, and glucose were seen less often than those reported in earlier phase 2 nilotinib studies; there were no occurrences of QTc prolongation >500 msec in any of the nilotinib arms. Other grade 3-4 toxicities were reported in <1% of the study patients, with the exception of rash, which was observed in 3% of the nilotinib 400mg twice daily cohort twice-daily arm (63,64).

**Bosutinib as Frontline Therapy for Patients with CML-CP**

Bosutinib (SKI-606) is a next generation TKI with a dual mechanism of action, targeting the ABL1 and SRC kinases (65-67). The drug appears to exert minimal inhibitory activity against PDGFR or c-kit, making it less likely to be associated with serious hematological toxicity (68,69). Following initial studies assessing bosutinib's role in the treatment of patients with CML-CP intolerant or resistant/refractory to IM, the drug entered an international randomized, phase III, open-label trial to evaluate its role in the front-line setting, compared to standard dose IM (70-73). In the phase I/II trial, 299 patients with CML-CP intolerant or refractory/resistant to IM were recruited and 46% of the evaluable patients were noted to have achieved a CCyR (60). The principal grade 3-4 toxicities were diarrhea (8%), rash (9%), hypermagnesemia (11%), and abnormal liver function (alanine transaminase (10%); grade 3-4 hematological toxicity included thrombocytopenia (23%), neutropenia (14%) and anemia (9%). Bosutinib was also noted to overcome a range of diverse mutations associated with IM resistance, but not the gatekeeper T315I mutation.

The preliminary results from a front-line randomized phase III study comparing the activity and toxicity of imatinib were presented at the 52nd ASH meeting (10). The study enrolled 502 newly diagnosed patients with CML-CP. An intent to- treat analysis showed that at 1 year, the MMR was 39% with bosutinib vs 26% with IM (P = .002). The study failed to meet its primary endpoint of superior CCyR with bosutinib at 1 year: 70% for bosutinib vs 68% for IM, a difference that was not statistically significant. However, at 1 year, treatment failure rates were 3% on the bosutinib arm compared to 10% on the IM arm (p = 0.001).

In this randomized phase III study, bosutinib was associated with more diarrhea, nausea, vomiting, and rash compared with IM; the most frequent grade 3 and 4 adverse events were diarrhea (8%) and rash (2%). More muscle cramps, bone pain, and periorbital edema were associated with IM therapy. Treatment discontinuation was reported in 29% of patients treated with bosutinib and 20% of those receiving IM.

Bosutinib has also been tested as a third-line option in an open-label, phase I/II study of patients with CML-CP intolerant or resistant/refractory to IM and a second generation TKIs (74). A total of 118 patients were included in this study. At week 24, 26% of patients achieved a major CyR, including 13% with CCyR; the cumulative CCyR rate was 22%. The toxicity profile appeared to be similar to that observed in earlier studies, with the exception of diarrhea, which was noted in 83% of patients. These results indicate that bosutinib is active in a fraction of patients who have previously failed currently approved TKIs in CML-CP.

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Management of Patients with Ph-positive ALL and CML in Lymphoid Blast Phase

The introduction of TKIs, in particular dasatinib, for the treatment of patients with CML in advanced phases and Ph-positive ALL has improved the response rates and the prognosis of these patients (75-80). Historically these patients have fared poorly and long term remission and possible cure, could only be accorded to a small minority of patients who were suitable for an allo-SCT (81,82). Single-agent IM accorded a modest degree of success in this setting, but most patients still succumb to the disease due to lack of response and development of resistance (83-87). In fact, a significant proportion of patients with Ph-positive ALL develop kinase domain mutations after an initial response to IM (88). Studies of high dose IM versus standard dose IM have shown a trend towards better cytogenetic response rates with the former approach (89). Combinations of IM or the second generation TKI dasatinib, with cytotoxic chemotherapy has proven to be more effective in managing patients with Ph-positive ALL with a number of recent reports that 2 and 3 year survival comparable to that achievable by allo-SCT may be possible with such combinations (82).

More recent studies have incorporated dasatinib during induction, consolidation, and maintenance for patients with relapsed or newly diagnosed Ph-positive ALL (90-92). In a single center study, 23 patients with relapsed Ph-positive ALL and 9 patients with CML in lymphoid blast crisis were recruited (91). Complete responses were observed in 95% of patients, with 79% achieving CCyR. Remarkably, MMR was noted in 21% and CMR in 51%.

In the newly diagnosed Ph-positive ALL setting, the same investigators examined the efficacy and safety of adding dasatinib 50 mg twice daily or 100 mg once daily for the first 14 days of each of 8 cycles of alternating hyper-CVAD, and high-dose cytarabine and methotrexate. (92). Patients who achieved a complete remission then received maintenance daily dasatinib and monthly vincristine and prednisone for 2 years, followed by dasatinib indefinitely. A total of 35 newly diagnosed patients with Ph-positive ALL with a median age of 53 years (range, 21-79 years) were recruited. At the time of the publication in September 2010, 33 of 35 patients (94%) achieved a complete remission. Following a median follow-up of 14 months (range, 4-37 months), the estimated 2-year survival was 64%.

These results are very promising although not dissimilar from those obtained at the same institution with hyper-CVAD in combination with high dose imatinib. Further follow-up is warranted to confirm the role of dasatinib in the management of patients with Ph-positive ALL. At present, patients with this disease are still recommended to undergo allo-SCT in first complete remission (93,94). Dasatinib, in contrast to IM and nilotinib, is able to cross the blood-brain barrier and may afford an advantage compared to other TKIs regarding the prevention of CNS relapse (95).

Many patients with CML in advanced phases and Ph-positive ALL fail to respond or lose their response as a consequence to developing kinase domain mutations. Prominent amongst the latter in patients with Ph-positive ALL is the T315I mutation, which is resistant to all currently available TKIs. The prognosis of such patients is poor and many efforts are now assessing a number of drugs in this scenario (Table 3).

One such drug is ponatinib (previously known as AP24534), a rationally designed inhibitor of BCR-ABL1 that binds both active and inactive conformation of the enzyme and is active against a broad array of BCR-ABL1 mutants (including T315I) as well as other kinases such as VEGF, FGF, c-KIT, and SRC (96). In cellular proliferation assays, the IC50 of ponatinib
against T315I BCR-ABL1 is 11 nM, in stark contrast to >3125 nM, >2000 nM, and >200 nM for IM, nilotinib, and dasatinib, respectively.

The result of the first phase I study of this agent was reported during the 52nd ASH meeting (15). Sixty seven patients including 57 with CML (42, 7, and 8 with chronic, accelerated, and blast phase disease, respectively), 3 with Ph-negative ALL and 7 with other leukemias were treated. The prior treatment for the 57 patients with Ph-positive leukemias were IM in 96%, dasatinib in 89%, and nilotinib in 55% with 95% and 64% failing 2 or more prior TKIs or 3 or more prior TKIs, respectively. The T315I mutation was present in 38% of patients with a variety of other mutations in other patients including 26% with no mutations.

The dose of ponatinib was escalated from 20 mg orally daily to 30, 45, and 60 mg orally daily and 45 mg was chosen as the dose for phase II studies with the dose limiting toxicity being pancreatic toxicity (elevation of enzymes and pancreatitis). One of 22 patients treated at 45 mg daily had grade 3 rash as dose-limiting toxicity. Overall, 48 patients with Ph-positive leukemia were evaluable for response. Of 32 evaluable patients with CML in CP, 30 (94%) had complete hematologic response (CHR), and 20 (63%) had major cytogenetic response (MCyR): 12 CCyR, 8 partial CyR (PCyR). Of 20 CML-CP cytogenetic responders, 18 remain on treatment (mean duration 326 [range 142–599] days) without progression, and 2 patients treated at 4 and 15 mg progressed after PCyR. Of 11 CML-CP patients with T315I mutation, 11 (100%) had CHR, 9 (82%) had MCyR (8 CCyR). Of 16 evaluable patients with advanced phase CML or Ph-positive ALL, 5 (31%) had major hematologic response (MHR), 3 (19%) had MCyR, 1 (6%) had minor CyR. Of 9 patients with advanced phase CML or Ph-positive ALL with the T315I mutation, 3 (33%) had MHR, 2 (20%) had MCyR. Responses were also observed in heavily refractory patients with no mutations as well as in those carrying a variety of mutations. Overall, 13 of 60 (22%) patients with Ph-positive leukemia achieved MMR, including 12/42 (28%) patients with CML-CP, 6/15 (40%) with T315I mutation confirmed at baseline, 10/40 (25%) with starting doses ≥3 0 m g.

Pharmacodynamic analysis demonstrated sustained inhibition of CrkL phosphorylation above 15 mg. The authors concluded that ponatinib has an acceptable safety profile at therapeutic dose levels. A multinational phase II study (the PACE study) is currently evaluating the activity of this agent in Ph-positive leukemias.

Resistance to TKIs and Novel Insights into Genomic Instability of CML Stem Cells

Although the TKIs currently in clinical use (IM, dasatinib and nilotinib) eventually eliminate the majority of leukemia progenitor cells (LPCs) in patients responding to the treatment, their effect on leukemia stem cells (LSCs) is considered negligible. Patients with CML-CP harbor ~5×10⁷ leukemia cells displaying innate resistance to imatinib (97). These cells may accumulate additional genetic aberrations causing acquired resistance to TKIs and progression to BP (98,99).

TKI resistance may be induced not only by mutations in the kinase domain of BCR-ABL1, but also by mutations in other regions distant from the BCR-ABL1 kinase domain and by genetic aberrations affecting different genes (100). Cells harboring TKI resistant BCR-ABL1 kinase mutants appear to be more susceptible to accumulate additional aberrations, which may enhance their ability to evolve into more malignant clones (99,101,102). As expected genomic arrays revealed unusually high number of mutated genes in CML-BP, but even CML-CP cells harbor numerous, yet sporadic aberrations (103,104). The latter observation strongly suggests that genomic instability in CML is an early event. TKI resistant mutations in BCR-ABL1 kinase and additional chromosomal aberrations have been detected not only in LPCs, but also in LSCs suggesting that genomic instability occurs at the
level of LSC and/or LPC (105-107). Mutations detected in LSCs are likely to be passed onto successive generations of LPCs (106-108).

Genomic instability usually results from enhanced DNA damage and/or deregulated mechanisms of DNA repair (109-114). Much endogenous DNA damage arises from reactive oxygen species (ROS), which can cause oxidative damage to all nucleobases and free nucleotides (e.g., 8-oxoG) generating mismatches and DNA double-strand breaks (DSBs) (115,116). CD34+ CML cells display about 3-8 times more oxidized nucleobases and 4-8 times more DSBs than normal counterparts (117-119). Thus, elevation of ROS-induced oxidative DNA damage in CML cells appears to be a “driving force” of genomic instability (98,117,120).

Cellular DNA repair systems act to remove DNA damage and preserve the informational integrity of the genome (121). Since BCR-ABL1 kinase suppressed mismatch repair activity, elevated levels of oxidative DNA damage combined with inefficient mismatch repair activity may contribute to accumulation of point mutations in CML cells, including these in BCR-ABL1 kinase encoding TKI resistant mutants (122). Oxidative DNA damage can also generate DSBs that represent a “clear and present danger” to survival and genomic integrity. BCR-ABL1 kinase stimulates all three mechanisms of DSB repair: homologous recombination repair (HRR), non-homologous end-joining (NHEJ) and single-strand annealing (SSA) to enhance genomic instability. In leukemia cells HRR products incorporated point mutations, NHEJ resulted in more extensive deletions in some products and SSA generated large deletions (118,119,123-126). Thus, accelerated but unfaithful DSB repair may generate chromosomal aberrations, which are responsible for malignant progression of CML.

In conclusion, CML-CP LSCs and/or LPCs may display elevated levels of ROS-induced oxidative DNA damage and inefficient/unfaithful DNA damage repair mechanisms, which turn these cells into “ticking time-bombs”, eventually producing TKI resistant clones with an increased potential for progression to BP.

**Discussion**

The natural history of all BCR-ABL1 positive leukemias has been modified positively by the introduction of TKI therapy, which renders high rates of CCyR that translate into 8-year EFS and OS rates of approximately 80% and 85%, respectively. Second generation TKIs such as dasatinib and nilotinib produce CCyR and MMR at higher rates and at a much faster pace than IM. However, the follow-up of randomized studies involving the use of second generation TKIs used in the frontline setting is short and whether the higher response rates observed will translate into improved long term outcomes is yet unknown (127-129) Current results do, however, demonstrate either a trend towards, or a statistically significant improvement at 12 months in the rates of freedom from progression in the dasatinib-treated cohort in the DASISION trial, and the nilotinib-treated cohort in the ENESTnd trial, respectively, compared to the IM-treated cohorts.

Caution, however, must be drawn from some of the lessons learned from the IRIS and other trials with regards to the various timelines and goals of a specific therapy (128). These are discussed eloquently in a recent report by Kantarjian and colleagues (127,129). These authors also highlight the importance of the various differences in study designs, how the results are censored and perhaps more importantly, the selection of different primary endpoints (MMR in ENESTnd and “confirmed” CCyR in DASISION), which are evaluated at time points, which, sometimes, only a fraction of patients enrolled in the trial have been able to reach. The ENESTnd study reported MMR rates “at” 12 months of 44% for nilotinib
300 mg twice daily, while the DASISION trial reported best cumulative MMR rates “by” 12 months for dasatinib 100 mg once daily of 46%. It is possible that patients who may have either lost the response or discontinued treatment prior to 12 months would not be considered responders in the “at” analysis, but would be considered as responders in the “by” analysis. For instance, in ENESTnd, the MMR rate for nilotinib 300 mg twice daily was 55% “by” 12 months, which represents an increase of 11% in the MMR rate simply by reporting such response rates in a cumulative fashion. Furthermore, the results reported in these studies are derived after a “median” follow-up of 18 (DASISION) or 24 (ENESTnd) months, not after a “minimum” follow-up of 18 or 24 months, respectively, highlighting the immaturity of such results.

A further note of caution can, perhaps, be expressed by the notion that the longer term implications of the more robust responses, both CCyR and MMR, seen in the recent dasatinib and nilotinib front-line trials are unknown. This is best exemplified by initial analyses of the IRIS trial highlighting the critical importance of achieving MMR at 12 months in regards to PFS, which contrast with later analyses that minimizes the impact of such type of response at the same time point (129,131,132).

As our efforts in improving on the front-line therapies continue, we can anticipate an improvement in the way progression and resistance to TKI risk is classified, based on the emerging tools. These tools may include of set of different levels of genetics-mutated genes that become evident in studies utilizing whole genome sequencing; micro RNAs; and gene expression. In addition, the advent of RNA sequencing may uncover new cryptic translocations, or splicing variants, which define disease biology. Pari passu, the BCR-ABL1 molecular monitoring by PCR is becoming routine and largely supplanting conventional cytogenetics. In the future cytogenetics will likely still be useful to define new clonal abnormalities associated with advanced phase disease (that is, until whole genome sequencing becomes cheap and fast enough). BCR-ABL1 PCR on peripheral blood will thereafter be used to monitor disease, which bone marrows only performed in cases of inadequate response or relapse, both demonstrated by PCR. Recent studies, such as the French group's STIM (Stop IM) trial, suggest the notion that achieving a complete molecular remission (CMR) may predict for greater durability of response and perhaps be used to interrupt TKI therapy (17,133). The STIM and other similar recent studies suggest that in about 40% of patients who achieve a CMR, IM could be discontinued safely. Conversely, about 60% of patients relapse at a molecular level within 6 months of IM being discontinued. This would make CMR an attractive target to both clinical researchers and patients. More sensitive molecular methods will thus spring up to more deeply probe the depths of CMR, identifying patients with really good CMR v. those CMR by the older PCR assays. This should pave the way towards further efforts to achieve ‘high quality’ CMR and thereby eliminate minimal residual disease and facilitate treatment discontinuation safely. It is tempting to speculate that such a strategy may represent a ‘cure’ for patients with CML-CP.

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81. Rousselot P, Cayuela JM, Recher C, et al. Dasatinib (Sprycel) and chemotherapy for first-line treatment in elderly patients with de novo Philadelphia positive ALL: results of the first 22 patients included in the EWALL-Ph-01 trial (on behalf of the European Working Group on Adult ALL (EWALL)). Blood. 2008; 112(Suppl. 1) Abstract 2920.


Table I
Demographics and Efficacy of Dasatinib (DASISION Trial)

<table>
<thead>
<tr>
<th></th>
<th>Dasatinib 100mg once daily (n= 259)</th>
<th>Imatinib 400mg once daily (n= 260)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median duration of treatment</td>
<td>14.0 months</td>
<td>14.3 months</td>
</tr>
<tr>
<td>Median age</td>
<td>46 years</td>
<td>49 years</td>
</tr>
<tr>
<td>Range</td>
<td>(18-84 years)</td>
<td>(18-78 years)</td>
</tr>
<tr>
<td>Hasford Risk (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>Intermediate</td>
<td>48</td>
<td>47</td>
</tr>
<tr>
<td>High</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Prior Imatinib (%)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>MMR (evaluable) (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>By 12 months</td>
<td>46</td>
<td>28 (P&lt;0.0001)</td>
</tr>
<tr>
<td>CCyR (evaluable) (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>By 12 months</td>
<td>83</td>
<td>72 (P&lt;0.001)</td>
</tr>
</tbody>
</table>

(Adapted, with permission, from Kantarjian et al, Dasatinib versus Imatinib in newly diagnosed chronic myeloid leukemia, N Engl J Med, 2010;362:2260-2270)
Table II
Demographics and Efficacy of Nilotinib (ENESTnd trial)

<table>
<thead>
<tr>
<th></th>
<th>Nilotinib 300mg twice daily (n=282)</th>
<th>Nilotinib 400mg twice daily (n=281)</th>
<th>Imatinib 400mg once daily (n=283)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median duration of treatment</td>
<td>13.8 months</td>
<td>13.8 months</td>
<td>13.8 months</td>
</tr>
<tr>
<td>Median age Range</td>
<td>47 years (18-85 years)</td>
<td>47 years (18-81 years)</td>
<td>46 years (18-80 years)</td>
</tr>
<tr>
<td>Sokal Risk (%)</td>
<td>Low 37</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Intermediate 36</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>High 28</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>Prior Imatinib (%)</td>
<td>13</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>MMR (%)</td>
<td>By 6 months 44</td>
<td>43</td>
<td>22 (P&lt;0.001, &lt;0.001)</td>
</tr>
<tr>
<td></td>
<td>By 12 months 55</td>
<td>51</td>
<td>27 (P&lt;0.001, &lt;0.001)</td>
</tr>
<tr>
<td>Confirmed CCyR (%)</td>
<td>By 6 months 67</td>
<td>63</td>
<td>45 (P=NR)</td>
</tr>
<tr>
<td></td>
<td>By 12 months 80</td>
<td>78</td>
<td>72 (P&lt;0.0001, &lt;0.0005)</td>
</tr>
</tbody>
</table>

(Adapted, with permission, from Saglio et al, Nilotinib versus Imatinib for newly diagnosed chronic myeloid leukemia, N Engl J Med, 2010;362:2251-2259)
Table III
Candidate agents in investigation, for patients with BCR-ABL1 positive leukemias intolerant/refactory/resistant to second generation TKIs or harboring the T315 mutation.

<table>
<thead>
<tr>
<th>Novel kinase inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Ponatinib (AP24534)</td>
</tr>
<tr>
<td>• DCC-2036</td>
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<tr>
<td>• PHA-739258</td>
</tr>
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<table>
<thead>
<tr>
<th>Omacetaxine (Homoharringtonine)</th>
</tr>
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Other approaches

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<th></th>
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<tbody>
<tr>
<td>• HSP-90 inhibitors</td>
</tr>
<tr>
<td>• Histone deacetylase inhibitors</td>
</tr>
</tbody>
</table>