Clinical Medicine Insights: Oncology

EXPERT REVIEW

Moving on up: Second-Line Agents as Initial Treatment for Newly-Diagnosed Patients with Chronic Phase CML

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Abstract: The treatment of chronic myelogenous leukemia (CML) was revolutionized by the development of imatinib mesylate, a small molecule inhibitor of several protein tyrosine kinases, including the ABL1 protein tyrosine kinase. The current second generation of FDA-approved ABL tyrosine kinase inhibitors, dasatinib and nilotinib, are more potent inhibitors of BCR-ABL1 kinase in vitro. Originally approved for the treatment of patients who were refractory to or intolerant of imatinib, dasatinib and nilotinib are now also FDA approved in the first-line setting. The choice of tyrosine kinase inhibitor (ie, standard or high dose imatinib, dasatinib, nilotinib) to use for initial therapy in chronic-phase CML (CML-CP) will not always be obvious. Therapy selection will depend on both clinical and molecular factors, which we will discuss in this review.

Keywords: CML, TKI, kinase, inhibitor, imatinib, dasatinib, nilotinib

Introduction

Chronic myelogenous leukemia (CML) is a relatively rare disease, with an estimated 4,000–5,000 new cases being diagnosed annually in the United States.[1] Nevertheless it has repeatedly served as a "keyhole" disease, providing insights that have dramatically affected the broad fields of oncology, cell biology, and molecular biology. The sensitivity of CML to the tyrosine kinase inhibitor (TKI) imatinib is certainly one of the most dramatic features of the disease. Imatinib is now a well-known entity with obvious, profound activity against CML, and the traditional dose (400 mg daily) has been the standard initial treatment for this disease. Its therapeutic benefits have also been extended to gastrointestinal stromal tumors,[2] dermatofibrosarcoma protuberans,[3] systemic mastocytosis,[4,5] and hypereosinophilic syndrome.[6] These entities also express mutant kinases that are sensitive to the inhibitory effects of imatinib.

While the vast majority of CML subjects benefit from imatinib therapy, it is also obvious that a substantial minority of patients with CML fail to benefit fully from this agent due to toxicity, lack of efficacy, or poor compliance. And there is no evidence that most patients with CML are being cured. Thus, at the end of the first decade of use of TKIs, it is appropriate to ask whether the treatment of CML can be further optimized. New therapies are available, as well as new insights into stem cell biology, kinase biochemistry, and medicinal chemistry. Further the validation of cytogenetic and molecular surrogate markers for efficacy assessment allows for improved efficiency in the identification of improved treatments. This review will focus on the application of newer agents to the initial treatment of chronic phase (CP) CML.

Imatinib for First-Line Therapy of CMl: Proof of Concept for TKIs

CML was the first cancer to be associated with a specific genetic anomaly, the Philadelphia chromosome. Molecular characterization of this aberrant chromosome fragment identified the t(9;22) translocation that produces the BCR-ABL1 chimeric tyrosine kinase. The BCR-ABL1 kinase is both necessary and sufficient to produce CML-CP, unlike most malignancies that require multiple genetic mutations to produce a fully transformed phenotype. This remarkable dependency on a specific, mutant protein has allowed CML to be the ideal "proof of concept" experiment to demonstrate the efficacy of small molecule kinase inhibitors. The introduction of imatinib for CML treatment in 2001 has been a seminal event in the field of molecular oncology, and has provided dramatic benefit to CML patients.

The pivotal phase III International Randomized Study of Interferon and STI571 (IRIS) trial compared the combination of recombinant interferon alfa and low-dose cytarabine to imatinib. After a median follow-up of 19 months, the estimated rate of a major cytogenetic response (MCyR) was 87.1% in the imatinib group and 34.7% in the group given interferon alfa and cytarabine ($P < 0.001$). The estimated rates of complete cytogenetic response (CCyR) were 76.2% and 14.5%, respectively ($P < 0.001$).[2] At eight years of treatment, imatinib continues to demonstrate both efficacy and safety for the 304 (55%) patients remaining on study treatment.[8] Estimated event-free survival (EFS) at 8 years was 81% and freedom from progression to accelerated-phase or blast crisis (AP/BC) was 92%. The rate of major molecular response (MMR) increased from 24% at six months and 39% at 12 months, to a "best observed" MMR of 86% at 8 years. Estimated overall survival (OS) was 85% at 8 years. These data suggest that for patients who initially respond to imatinib, responses can be maintained on long-term therapy, with a low side-effect profile. These studies have established imatinib (400 mg daily) as the standard therapy for CML.

Because of the dramatic clinical effects of imatinib, coupled with a high proportion of cytogenetic and molecular responses, and the marked improvement in overall survival for CML patients,[9,10] investigators are beginning to ask whether CML can be cured by TKIs. The initial results from the Stop Imatinib (STIM) trial have been presented recently.[11] This trial documents the persistence of molecular remission after stopping imatinib, in subjects who had achieved a complete molecular response (CMR) lasting at least 2 years. At 12 months after imatinib withdrawal, 59% of subjects had lost their previous molecular remission, with almost all relapses occurring within 6 months of drug cessation. However, 41% continued to maintain a molecular remission, resulting in a distinct "break" in the slope of the relapse-free survival curve (Fig. 1). All patients who relapsed responded to reintroduction of imatinib. Low Sokal score, male sex, and duration
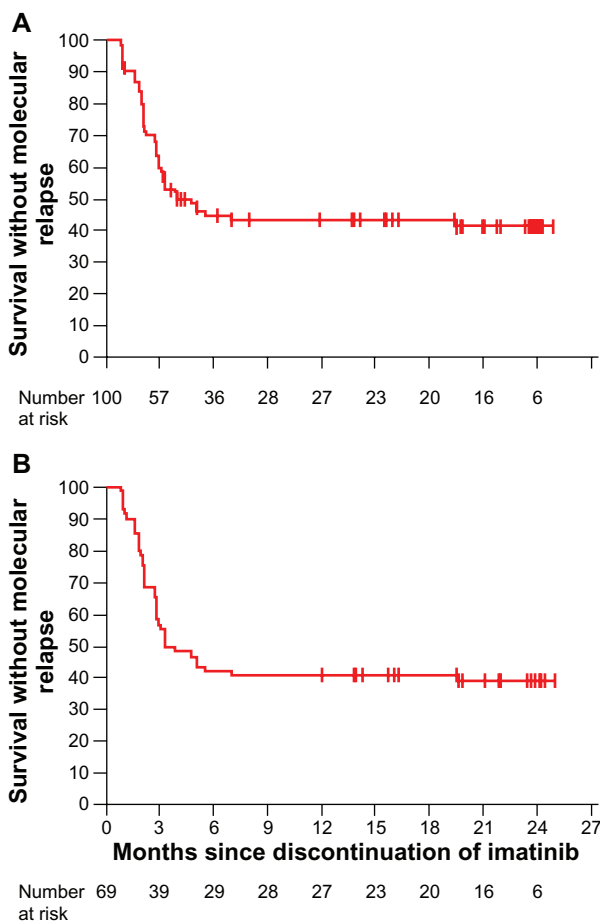
Figure 1. Maintenance of complete molecular response after discontinuing imatinib therapy. Panel A: All subjects. Panel B: Subjects with at least 12 months of follow-up. Reproduced from[11] with permission.

of imatinib treatment were factors predictive of CMR maintenance after the drug was withdrawn. These data suggest that patients who are exposed to imatinib for longer periods of time might be more likely to maintain CMR[12] and more importantly, at least some patients with CML may actually be cured by imatinib.[13]

Second-Line Agents for CML Patients Who Fail Imatinib Therapy

In spite of the dramatic benefits for imatinib documented in the IRIS and STIM trials, a substantial minority of patients fail to benefit fully from this agent due to toxicity, lack of efficacy, or poor compliance. Approximately 6% of patients on the IRIS study stopped treatment due to imatinib toxicity by 8 years. Imatinib lacked sufficient efficacy in another 16%. And the STIM study demonstrates that most patients with CML are still not being cured. To overcome these

limitations, additional TKIs have been studied in CML-CP patients resistant to standard-dose imatinib. Agents with activity in this setting are likely to overcome at least some degree of imatinib resistance and may be better for first-line therapy. Three such agents (dasatinib, nilotinib, and bosutinib) have been studied in phase II and phase III trials for either CP or accelerated/blast phase (AP/BP) of CML. These agents have been the subject of multiple reviews, and the reader is referred to these sources for details.[14–17] The salient features of each agent are presented in Table 1. Nilotinib uses the same molecular scaffold as imatinib, while dasatinib and bosutinib are structurally completely different.

The activity of dasatinib for both CP and AP/BP CML was documented in the series of START trials. The START-R study of subjects with CML-CP that had failed standard dose imatinib (400 mg daily) randomized participants to high-dose imatinib (800 mg daily) or dasatinib (70 mg BID;[18]). At a minimum follow-up of two years, the MCyR rate for dasatinib vs. imatinib was 53% vs. 33% ($P = 0.017$). Similarly the CCyR rate was 44% vs. 18% ($P = 0.0025$) and the MMR rate was 29% vs. 12% ($P = 0.028$). The estimated progression-free survival (PFS) favored dasatinib as well, with the mean PFS not reached at 30 months in the dasatinib arm, but a PFS of about 3 months in the imatinib arm. Twenty-three percent of subjects in the dasatinib arm stopped treatment due to adverse events which were mostly drug-related.

Nilotinib has also been evaluated via a phase II trial in CML-CP subjects who were refractory or intolerant of imatinib.[19] The clinical activity of nilotinib was very similar to that seen with dasatinib. Thus, with a minimum follow-up of two years, the MCyR rate was 59%, CCyR rate was 44%, and the MMR was 28%. PFS was not reached at 36 months. Nineteen percent of subjects went off treatment due to adverse events.

Bosutinib is a dual ABL1/SRC TKI, which, like nilotinib and dasatinib, is somewhat more potent than imatinib in vitro, and which retains activity against several imatinib-resistant ABL1 mutants. In preliminary data from the phase II portion of a phase I/II study investigating bosutinib in patients with CML-CP and who were resistant or intolerant to imatinib, 75 of 96 evaluable patients (78%) achieved a complete hematologic response (CHR), 47 of 106 (44%) achieved an MCyR, and 35 (33%) achieved a CCyR.[20] A MMR was

Table 1. Comparison of BCR-ABL1 TKIs approved or in phase III clinical trials for CML-CP.

Agent	Spectrum of kinases inhibited at nanomolar concentration	BCR-ABL1 binding site	Relative potency for BCR-ABL1 vs. imatinib	Toxicity	ABCG2 substrate
Imatinib	BCR-ABL1, c-KIT, PDGFR, c-FMS	Inactive	1	Fatigue, edema, myalgia, cytopenias, hypophosphatemia	+
Dasatinib	>100 kinases, incl. BCR-ABL1, PDGFR, c-KIT, SRC family, EPHA family, BTK, BMX, c-FMS,	Active, inactive	200–300	Fatigue, effusions, hypocalcemia, hypophosphatemia, increased lipase and amylase, immuno-suppression, cytopenias	+
Nilotinib	BCR-ABL1, c-KIT, PDGFR, c-FMS	Inactive	10–30	Hyperbilirubinemia, fatigue, increased amylase and lipase, hypocalcemia, hypophosphatemia, cytopenias	+
Bosutinib	ABL1, SRC family	N/A	1–10	GI, fatigue, cytopenia	–

achieved in 27 of 85 patients (32%), of which 15 (18%) were complete molecular responses (CMR).

In addition, multiple newer approaches are in clinical or preclinical development for treatment of CML-CP resistant to available treatment. Such agents may provide advantages for selected patients (for review, see[21,22]). Some newer therapies involve combinations of imatinib or other single TKIs with agents targeting other signaling molecules or pathways. These may include inhibitors of downstream kinases,[23,24] histone deacetylase inhibitors,[25] regulators of alternative splicing,[26] HSP90 inhibitors,[27] scaffold protein antagonists[28] and inhibitors of downstream transcriptional factors,[29,30] among others. Most single agent therapies may be considered kinase domain inhibitors of the BCR-ABL1 protein. An exception is the switch inhibitor DCC-2036 that binds to critical residues involved in switching the kinase between the active and inactive conformations.[31] These agents have shown activity with purified proteins or cell lines. Thus there are many agents that may eventually be incorporated at earlier stages in the CML treatment continuum.

Second Line TKIs as First-Line Therapy

Agents approved for second-line use have now been tested for initial therapy. The major findings are summarized in Table 2.

Dasatinib

Following the demonstration of substantial activity for dasatinib against refractory/relapsed CML-CP, this TKI has now been evaluated for first line therapy. An initial phase II trial involved 62 patients with newly-diagnosed chronic phase CML.[32] Among 50 patients with at least 3 months of followup, the CCyR rate was 94% and the MMR rate reached 82% by 18 months. Responses were very rapid, with most CCyRs occurring by 6 months of treatment. Treatment was well tolerated. Estimated 24-month EFS was 88%. These encouraging results were followed by the phase III Dasatinib versus Imatinib in Newly Diagnosed Chronic-Phase Chronic Myeloid Leukemia (DASISION) trial.[33] Five hundred nineteen patients with newly diagnosed CML-CP were randomly assigned to receive dasatinib (100 mg once daily) or imatinib (400 mg once daily). The primary endpoint was CCyR at 12 months. After a minimum follow-up of 12 months, the rate of CCyR observed on at least one assessment significantly favored dasatinib (83% vs. 72%, $P = 0.001$). The rate of MMR was also higher with dasatinib than with imatinib (46% vs. 28%, $P < 0.0001$). In addition, responses were achieved in a shorter time with dasatinib. The safety profiles of the two treatments were similar. Five percent of dasatinib-treated subjects withdrew from treatment due to toxicity, while 4% of imatinib-treated patients did similarly.

Table 2. Comparison of dasatinib, nilotinib, high-dose imatinib, and bosutinib as first-line treatment of CML-CP, in terms of response at 12 months, PFS, and toxicity.

Agent, schedule (vs. imatinib 400 mg QD)	CCyR* (vs. imatinib 400 mg QD)	MMR* (vs. imatinib 400 mg QD)	PFS* (vs. imatinib 400 mg QD)	Comments	Refs
Dasatinib 100 mg QD	77% vs. 66%	46% vs. 28%	96% vs. 97% at 12 months (NS)	Higher incidence of pleural effusions in dasatinib arm. No difference between each arm in % of patients who DC'd drug because of AE.	33
Dasatinib 100 mg QD	82% vs. 69% (NS)	59% vs. 43%	97% vs. 95% at 12 months (NS)	Only 51% of subjects had CCyR data available	34**
Nilotinib 300 mg BID	80% vs. 65%	44% vs. 22%	N/A	Higher incidence of rash, increased bilirubin and AST/ALT in nilotinib arm.	35
Imatinib 400 mg BID	64% vs. 58% (NS)	49% vs. 41% (NS)	86% vs.72% at 36 months (NS)	Median average daily dose 720 mg daily	41
Imatinib 400 mg BID	69.9% vs. 65.6% (NS)	46.4% vs. 40.1% (NS)	97.4% vs. 95% at 18 months (NS)	Average daily dose 620 mg daily. 66.8% of patients had dose interruption >5 d vs. 37.6%.	40
Imatinib 400 mg BID	62.9% vs. 49.4%	59% vs. 44%	94% vs. 94% at 36 months (NS)	Only 16.7% of patients in high-dose treatment arm could tolerate full-dose.	39
Imatinib 400 mg BID	47.8% vs. 37.3% (NS)	32.1% vs. 25.4% (NS)	97.3% vs. 93.9% at 12 months (NS)	Only 54.4% of patients tolerated high-dose imatinib.	42

Notes: *Values statistically significant unless otherwise noted. **Preliminary data.

Dasatinib was approved for first-line treatment of newly diagnosed patients CML-CP in October 2010.

An additional randomized study comparing standard dose imatinib with dasatinib for newly diagnosed CML-CP is the S0325 Intergroup Trial, carried out by four North American cooperative groups between December 2006 and February 2009. Initial results were recently presented.[34] The primary endpoint was the frequency of >4 log reduction in BCR-ABL1 transcripts at 12 months. Molecular response at 12 months was deeper in the dasatinib arm (median 3.3 log reduction in BCR-ABL1 transcript vs. 2.8 with imatinib, $P = 0.048$) as was MMR (59% vs. 43%; $P = 0.042$); however, the proportion achieving >4 log or >4.5 log reductions did not differ significantly. CCyR rate at 12 months, as well as PFS and overall survival were not different between the two arms. A higher proportion of dasatinib-treated patients experienced grade 4 hematologic and non-hematologic toxicities. Followup is continuing to determine if the deeper molecular response in the dasatinib-treated subjects will lead to improved long-term outcomes.

Nilotinib

Another recent randomized, prospective trial has compared nilotinib with standard dose imatinib for initial therapy of CML-CP.[35] In the phase III Evaluating Nilotinib Efficacy and Safety in Clinical Trials-Newly Diagnosed Patients (ENESTnd) trial, nilotinib at a dose of either 300 mg or 400 mg twice daily was compared with imatinib 400 mg once daily in patients with newly diagnosed CML in the chronic phase. The rate of MMR at 12 months was the primary end point. The MMR rate for nilotinib-treated subjects was significantly better than for imatinib patients (44% [300 mg/d] or 43% [400 mg/d] vs. 22%; $P = < 0.001$). In addition, the rate of CCyR by 12 months was significantly higher for nilotinb (80% for the 300 mg dose and 78% for the 400 mg dose) than for imatinib (65%; $P < 0.001$). The proportion of patients who stopped treatment due to adverse events was similar in each treatment arm. In June 2010, the FDA approved nilotinib for the treatment of adult patients with newly diagnosed Philadelphia chromosome positive chronic myeloid leukemia (Ph + CML) in chronic phase.

Bosutinib

This new SRC/ABL1 inhibitor has recently completed accrual for the phase III randomized Bosutinib Efficacy and Safety in Chronic Myeloid Leukemia (BELA) study, involving 502 newly diagnosed CML-CP. Subjects were assigned to bosutinib 500 mg or imatinib 400 mg daily with a primary endpoint of CCyR at 12 months. A preliminary report was presented as ASH 2010, and showed activity in CML-CP, as well as novel toxicities.[36] Because of the lack of mature clinical data in peer-reviewed publications, bosutinib will not be discussed further in this review.

High-dose imatinib

An additional approach to enhancing first-line therapy for CML-CP is to use higher doses of imatinib. Multiple studies have demonstrated that molecular or cytogenetic outcomes for CML-CP depend on dose intensity. Promising phase II studies suggested that high-dose imatinib therapy (600–800 mg daily) could have a higher and faster rate of meaningful responses than standard doses (400 mg daily). The GIMEMA CML Working Party completed the first prospective phase II trial to evaluate the efficacy and tolerability of high-dose imatinib in previously-untreated CML patients.[37] Seventy-eight patients were treated with imatinib 400 mg twice daily. CCyR rates at 12 and 24 months were 88% and 91%; moreover, at 12 and 24 months 56% and 73% of CCyR patients achieved a MMR. For the same risk category, these response rates were faster than those documented in the IRIS trial. The Rationale and Insight for Gleevec High-Dose Therapy (RIGHT) trial also demonstrated similar promising results.[38]

Subsequently, four randomized, prospective trials evaluated a high-dose imatinib regimen in CML-CP. Using a dose escalation design, the German CML Study Group reported results comparing tolerability-adapted imatinib 800 mg/d versus 400 mg/d versus 400 mg/d plus inteferon-α, in newly-diagnosed CML patients.[39] Initial treatment in all study arms was imatinib 400 mg once daily. However, if no CHR was reached after two months or no MCyR was reached after six months, a dose increase was permitted. A significantly higher rate of MMR at 12 months occurred with tolerability-adapted imatinib 800 mg/d than with imatinib 400 mg/d (59% vs. 44%, $P < 0.001$) or than with imatinib 400 mg/d plus IFN-α (59% vs. 46%, IP = 0.002). Three other studies

have used a more traditional study format; none found an improvement in major endpoints with higher dose imatininb. Cortes *et al.* randomly assigned 476 newly-diagnosed patients with CML in a 2:1 fashion to receive either high-dose or low-dose imatinib.[40] Seventy percent of subjects had low Sokal scores. At 12 months, differences in MMR and CCyR rates were not statistically significant. However, MMR and CCyR did occur faster at six months among patients assigned to imatinib 800 mg daily. Baccarani *et al.* have evaluated the use of high-dose imatinib as front-line treatment in high-risk CML-CP.[41] This European LeukemiaNet study compared imatinib 400 mg daily to 800 mg daily in 216 newly diagnosed patients with CML and high Sokal scores. The CCyR at 1 year was 58% and 64%, respectively, which was not statistically significant. There were no differences detectable in the cytogenetic response at three and six months, in the molecular response rate at any time, as well as in the rate of other events. Moreover, only 28% of patients in the high-dose arm could tolerate the full dose. Finally, the ISTAHIT study from the Central European Leukemia Study Group has reported insignificant increases in CCyR and MMR at 12 months, in patients treated with 800 mg daily of imatinib, compared with those receiving 400 mg daily.[42] This study also noted faster responses with the higher dose, but only 45.6% of subjects could tolerate the 800 mg dose.

Treatment for Newly Diagnosed CML-CP: are Second-Line TKIs First in Line?

Randomized, prospective studies have failed to confirm an advantage for high-dose imatinib as first-line therapy in CML-CP. In addition, the phase III data for bosutinib are preliminary and mixed as to a potential benefit. Thus we will not consider these further as current treatments for CML-CP. However, we may consider that dasatinib and nilotinib may now be viable alternatives to standard dose imatinib.

How can one decide among the alternative first-line therapies for CML in CP? Potential points to consider include the strength of the data supporting the approval of dasatinib and nilotinib as alternative first line therapies. We will also want to review the drawbacks to standard dose imatinib therapy, including mechanisms for imatinib resistance and toxicity. We will then examine if the newer agents offer a significant benefit in terms of avoiding these limitations to imatininb therapy.

Surrogate endpoints

For both dasatinib and nilotinib, the data favoring their use as first line therapies are based on surrogate endpoints and relatively short followup periods. Survival endpoints have not been reported for either agent in first-line use.[33,35] Therapeutic studies of CML have consistently used endpoints developed by the European Leukemia Network and validated in multiple studies. Thus CCyR and MMR are recognized as surrogate endpoints that predict for an improvement in progression-free survival, a lower chance of loss-of-response, and less frequent transformation to more aggressive disease. Interventions which achieve improvements in these two parameters, compared to a standard therapy, are associated temporally with an improvement in overall survival of CML.[7] However, early achievement of CCyR does not always translate into improvement at 12 months in either CCyR or MMR.[40,43] Thus, while some uncertainty exists as to the best time for assessment of CCyR and MMR, the times used in the first-line, randomized trials of dasatinib and nilotinib are consistent with proposed optimal values.[44] The quality of the data for improved outcomes associated with early use of dasatinib or nilotinib generally supports their first-line use. There are discrepancies between the DASISION and S0325 trials of first-line dasatinib, though both use validated surrogate endpoints. Both report an improvement in MMR at 12 months. However DASISION found a statistically significant improvement in CCyR at 12 months ($P = 0.001$ compared with imatinib therapy) whereas thus far S0325 has not found such an effect ($P = 0.097$). One possible explanation for the difference is the fact that CCyR data were available for only 51% of the subjects in the S0325, possibly impairing the power to detect differences.

Toxicity

Imatinib, dasatinib, and nilotinib are generally well tolerated. Nevertheless significant toxicities can occur and may impact compliance, which can in turn impair treatment outcome (see below). In the phase III studies of first-line treatment of CML-CP using dasatinib or nilotinib, similar proportions of patients stopped treatment due to adverse events, regardless of the TKI. In the ENESTnd trial (nilotinib vs. imatinib) the proportion of subjects stopping therapy due to AEs was 5% (nilotinib 300 mg/d), 9% (nilotinib 400 mg/d), and 7% (imatinib 400 mg/d).

The DASISION trial (dasatinib vs. imatinib) documented that 5% of subjects receiving dasatinib stopped therapy due to AEs, whereas 4% of the imatinib-treated patients terminated treatment due to toxicity.

Common toxicities to all three TKIs include nausea, fatigue, and rashes. Rarely are these more than grade 1–2, and they are unlikely to lead to drug interruption. Cytopenias are also common with all three agents. However, grade 3–4 cytopenias affect only about 10%–20% of patients, and the incidence does not differ among subjects treated with any of the drugs. All three agents also produce a variety of metabolic anomalies, particularly hypophosphatemia or hypocalcemia. Concurrent use of bisphosphonates may exacerbate this problem. Unique toxicities of each TKI also are usually no more than grade 1 or 2 in severity. Nevertheless they could alter the choice of agent to use in specific patients.

Fluid retention

Fluid retention is a common side-effect with imatinib use. First-line dasatinib and nilotinib treatment are associated with lower rates of edema. However, pleural or pericardial effusions are a distinct side-effect of dasatinib. Patients with pre-existing effusions or ascites from any cause are probably not optimal candidates for first-line dasatinib therapy, but if use of this agent is necessary, dose limitations, low-dose glucocorticoid therapy, or diuretics may be needed.

Bleeding

Dasatinib has been found to produce a platelet function defect due its inhibition of SRC-family kinases. The qualitative and quantitative effects of this coagulopathy resemble those of aspirin.[45] In leukemia trials involving dasatinib, major bleeding events were usually associated with high-grade thrombocytopenia. In addition there is a greater risk of bleeding with advanced stage of CML. In patients with CML-CP, gastrointestinal bleeding is uncommon. In one study the overall incidence was 5%, but grade 3 or greater hemorrhage occurred in only 0.4% of subjects.[46] Dasatinib could be a less-attractive option for subjects with an ongoing coagulopathy, use of anti-coagulants, or low platelet counts.

Cardiac toxicity

Both dasatinib and nilotinib have been associated with prolongation of the QT interval,[47,48] whereas no similar

concern exists for imatinib.[46] In the case of nilotinib, this is associated with a "black box" warning on the prescribing information sheet. For both agents, correction of any associated hypokalemia or hypomagnesemia is mandated prior to TKI therapy. Treatment with either agent is not recommended for patients with congenital long QT syndrome, and it is discouraged for patients with obligate use of certain agents that can also prolong QT intervals, such as anti-arrhythmia agents, azole anti-fungal agents, and quinolone antibiotics.

Gastrointestinal side-effects

Diarrhea is also fairly common with imatinib and can be managed with antidiarrheal medication. Upper gastrointestinal symptoms such as heartburn may be alleviated if taken with food and water. In contrast, nilotinib and dasatinib cause less gastrointestinal side-effects, though nilotinib must be taken on an empty stomach because its absorption is altered with food. Dasatinib can be taken with or without food.

Nilotinib has a disproportionate incidence of hyperbilirubinemia, compared with dasatinib and imatinib. As many as 9%–15% of CML subjects in phase II nilotinib trials have been observed to have grade 3–4 hyperbilirubinemia. The elevation is primarily unconjugated bilirubin, and may be associated with the UGT1 A1*28 polymorphism of a key enzyme in bilirubin conjugation.[49] Since the implications of this anomaly are unknown, it would be best to avoid using nilotinib in subjects with a history of Gilbert's syndrome or anomalies of bilirubin conjugation.

Both nilotinib and dasatinib can produce striking elevations of amylase and lipase, at times equivalent to grade 3 or 4 toxicity. Gastrointestinal symptoms are infrequent in these subjects, and anatomic or radiographic abnormalities of the pancreas have not usually been demonstrated. The abnormal enzyme patterns usually resolve during withdrawal of the TKI.

Immunosuppression

A potential toxicity of dasastinib is immunosuppression. Many activating signals for T cells involve SRC family kinases and BTK kinase, which can be inhibited at clinically achievable concentrations by dasatinib. Most studies of the effects of dasatinib on immune functioning have involved immortalized or normal primary cells treated ex vivo. Various effects have been described, such as inhibition of TLR signaling, inhibition of T cell activation, reduction in cytokine release from basophils, and suppression of Treg function.[50–53] The results or relevance of these potentially immunosuppressive and anti-inflammatory effects in vivo are not clear. One report describes an increased incidence of infections typically seen in immunosuppressed patients, in 12/16 subjects on dasatinib trials.[54] Blood obtained from those subjects before and after dosing with dasatinib was examined for activation of T cell and basophil functions. These ex vivo experiments showed transient and erratic suppression of IgE-dependent activation of blood basophils and TcR-dependent activation of T-lymphocytes. During a recent clinical trial using dasatinib therapy for castration-resistant prostate cancer, we performed serial assays to detect evidence of drug-induced immunosuppression.[55] Whole blood was repeatedly obtained before or 2–3 hrs after dasatinib administration during several weeks of treatment. Phytohemagglutinin (PHA) was added to the whole blood and incubated for 2 hrs. Messenger RNAs were then isolated by a previously-described method,[56] involving plate filtration, lysis, reverse transcription, and on-membrane amplification via RT-PCR to quantify PHA-stimulated cytokine mRNAs. Results for a typical subject are shown in Figure 2. An immediate 1- or 2-log reduction in PHA-induced stimulation of GM-CSF, GZMB, CD40L and IL-2 expression was seen in this patient, and in all other evaluable subjects (n = 11). This suppressive effect of dasatinib on T cell functions persisted throughout the treatment period in most patients, but PHA stimulation of cytokine release returned to normal following the end of dasatinib therapy. These data suggest that real impairment of T cell function is a common feature of dasatinib therapy in cancer patients. Subjects who are already chronically immunosuppressed might be best treated with an alternative TKI.

Proinflammatory effects

While the immunosuppressive function of dasatinib has been described most often, there are also data to suggest that dasatinib can also mediate a pro-inflammatory effect. Pleural effusions in dasatinib-treated subjects characteristically have abundant lymphocytes, at
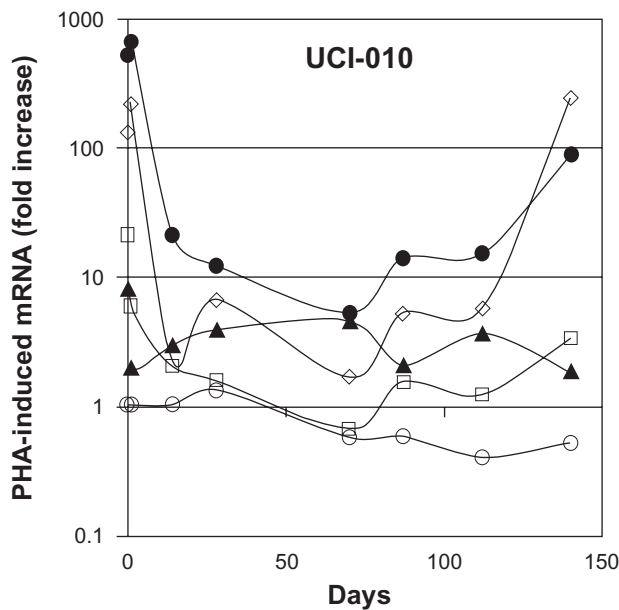
Figure 2. Serial analysis of PHA-induced cytokine mRNA expression in a subject treated with dasatinib for prostate cancer. Solid circle: GM-CSF mRNA; open diamond: IL-2 mRNA; solid triangle: GZMB mRNA; open square: CD40 L mRNA; open circle: ACTB mRNA. All values were normalized to day 0 ACTB (= 1). Dasatinib (100 mg/d) was administered from day 1 to day 135.

times associated with a peripheral blood lymphocytosis as well.[57] An increase in peripheral blood cytotoxic T cells has been described in some reports of dasatinib-treated patients.[58] A pro-inflammatory effect by dasatinib may be responsible for reports of panniculitis in dasatinib-treated patients. Two female patients with CML-CP developed severe panniculitis after exposure to dasatinib.[59] In one patient, withdrawal and reintroduction of dasatinib together with prednisone successfully controlled the panniculitis. However, the rash did not respond to steroids in the second patient. Onset of lupus and rheumatoid arthritis has also been described during dasatinib therapy.[54,60]

Compliance

When deciding among imatinib, dasatinib, or nilotinib as the initial first-line therapy for a patient with CML, physicians will no doubt have to consider patient adherence as well as cost. Cost will likely become more of an important health care issue when generic imatinib becomes possible in 2015.[61]

While formal clinical trials identify no significant differences among the three TKIs in terms of the percent of patients who stop treatment due to adverse events, these data may not represent the situation in routine clinical care. Patients on trials are likely to

be more highly motivated to comply with treatment. However, during routine care, toxicities and costs may become limiting. Patients who claim to be continuing treatment with imatinib may have suboptimal outcomes due to compliance issues. In the ADAGIO study, up to a third of subjects were considered to be non-compliant with the prescribed imatinib regimen, while only 14.2% of subjects were perfectly compliant.[62] Patients with a suboptimal response had significantly higher mean percentages of imatinib not taken (23.2%) than did those with optimal response (7.3%). Additionally, Marin et al.[63] found a highly significant correlation between the probability of major molecular response at 6 years, and the adherence rate (< or = 90% or > 90%) in subjects who had achieved a CCyR on imatinib therapy. Dose density has also been shown to significantly impair the achievement and maintenance of key cytological and molecular endpoints that predict for good outcomes.[64] Because of the concern about poor compliance and underdosing, some investigators are now advocating frequent monitoring of imatinib trough blood levels.[65,66]

Dasatinib and nilotinib may also be subject to societal barriers to optimum use. A comparison of the total cost of therapy for dasatinib and nilotinib was recently presented.[67] Patients treated with dasatinib experienced higher levels of healthcare resource utilization and medical service costs, particularly related to hospitalizations. Dasatinib patients were also observed to have more frequent ER visits and outpatient visits compared to nilotinib patients, although the differences were not statistically significant. Total medical service costs during the study period averaged $18,477 for dasatinib patients and $6,571 for nilotinib patients, with an unadjusted cost difference of $11,905, which was statistically significant. Interestingly, though nilotinib requires twice daily dosing and dasatinib is recommended as a once daily dose, the dasatinib cohort exhibited lower levels of treatment adherence compared to the nilotinib cohort. One possible explanation for this finding may be related to the safety profiles of each drug, as adverse events can disrupt treatment.

Ghatnekar et al. recently reported on a study assessing the cost-effectiveness of dasatinib versus high-dose imatinib for second-line treatment in chronic phase CML patients, resistant to lower doses of imatinib (≤600 mg) in Sweden.[68] A Markov simulation

model was adapted to Swedish treatment practice. The model was populated with efficacy data from clinical trials, resource utilization by expert opinion, published quality of life data and unit prices from official price lists. The results showed that chronic phase CML patients resistant to standard dose imatinib gain on average 0.67 life-years, or 0.62 quality adjusted life-years, when treated with dasatinib 140 mg daily compared with high-dose imatinib 800 mg daily. The incremental societal cost amounts to EUR 4250 during the lifetime period, or EUR 6880 per quality-of-life-year gained. In aggregate these data suggest that careful attention to patient motivation and insurance resources will be important in selecting among the possible TKIs for initial therapy.

BCR-ABL1 mutations

Resistance to imatinib and other TKI treatment is mediated through a variety of mechanisms, including BCR-ABL1 mutations affecting the kinase domain, BCR-ABL1 amplification and overexpression, the activation of alternative resistance pathways in the leukemic clone, and expression of drug resistance proteins. These mechanisms have been the subjects of many recent reviews, and the reader is referred to these sources for details.[21,69,70,71,72] In addition, pharmacokinetic/pharmacodynamic effects may result in lower trough levels of imatinib and poorer outcomes.[65] Quantitatively, mutations in the BCR-ABL1 chimeric gene account for the greatest fraction of identified resistance mechanisms. More than 50 or more mutations in the BCR-ABL1 sequence have been cataloged, primarily point mutations but also including frame shift mutations due to insertions or deletions.[73,74] Attention has focused most closely on those in the kinase domain, particularly the P-loop.[75]

De novo BCR-ABL1 mutations

Imatinib, dasatinib, and nilotinib differ substantially in terms of the spectrum of mutant BCR-ABL1 sequences that they inhibit, and also in terms of the mutations that arise during therapy with the individual TKIs. It might be surmised that the presence of aberrant BCR-ABL1 genotypes could profoundly affect the choice of initial therapy, but it is not clear that this is the case. Polymorphisms in the BCR-ABL1 sequence may be present at the point of diagnosis, before any therapy has been administered. These may be known "normal" polymorphisms in the BCR or ABL1 sequences, or may be non-functional or functional mutations that can affect the kinase activity of the enzyme. The traditional view has been that these mutations, including the multi-drug resistant T315I allele, may be present at diagnosis but seldom cause imatinib resistance.[76,77] Because of the infrequent finding of pre-treatment mutations, the inconsistency in predicting disease course, and the generally successful outcome from first-line imatinib therapy, the routine search for BCR-ABL1 polymorphisms at the time of diagnosis is not recommended.[78,79,80] However, a recent report has identified a high frequency (61.5%) of pre-treatment BCR-ABL1 mutations involving the kinase domain among patients with a high Sokal score, whereas patients with a low Sokal score were mutation-free.[81] The mutations frequently involved the P-loop and would be predicted to confer resistance to imatinib, and possibly nilotinib. Several of these patients failed initial and salvage TKI therapy and died. If confirmed by larger studies, the presence of pre-treatment mutations in CML-CP cells could inform the decision as to initial treatment.

Acquired BCR-ABL1 mutations

Most resistance mutations in the BCR-ABL1 kinase emerge during TKI therapy. If the three TKIs differed in their ability to suppress the emergence of mutant kinases, one could use this as a basis for preference. Extensive studies have been made of the spectrum of polymorphisms that emerge during imatinib therapy (for reviews, see[75,82,83]). "Hot spots" for mutations during imatinib therapy include the P-loop, the catalytic domain, the activating loop, and the T315 gatekeeper locus. Few data exist to describe mutations that arise during dasatinib or nilotinib therapy, and then only in the setting of second-line treatment.[84] Most of the dasatinib-associated mutations appear to be polymorphisms that do not directly confer dasatinib resistance. Known dasatinib-resistance mutations such as F317L and T315I appear to be a distinct minority. No reports have yet described the emergence of mutations during first line therapy with dasatinib or nilotinib. However, cross-resistance among possible mutants appears unlikely. P-loop mutants appear to be resistant to nilotinib and imatinib, but sensitive to dasatinib. Conversely, nilotinib seems to be more active against F317L than does dasatinib.[71,85,86] The T315I mutation

is highly resistant to all three TKIs, but fortunately only represents about 15% of all clinically-detected BCR-ABL1 mutations.

Tailoring therapy based on BCR-ABL1 mutations

Insight into the potential for the 3 TKIs to suppress the emergence of specific mutations can be found in the studies of Bradeen, et al.[87] These investigators used an ENU mutagenesis strategy to treat murine lymphoblasts containing the p210 BCR-ABL1 protein. Selection of mutants was performed in the presence of various concentrations of imatinib, dasatinib, or nilotinib. The 26 recovered mutants represented 83% of the then-known clinical BCR-ABL1 mutations, demonstrating that the model faithfully reproduces the natural history of BCR-ABL1 mutations seen clinically. Mutations arose in wells treated with all three TKIs. The pan-resistant T315I mutation arose in the presence of any of the three inhibitors. P-loop mutations arose in the presence of either imatinib or nilotinib, but not dasatinib. Conversely the F317L mutation (and other F317 polymorphisms) associated with dasatinib resistance appeared only in the presence of that TKI. It remained sensitive to nilotinib and imatinib. Dasatinib selection was associated with the lowest total number of mutations, with nilotinib selection producing the next lowest. Imatininb therapy was associated with the greatest number and diversity of mutations, but almost all remained sensitive to the alternative TKIs. Combination therapy (dasatinib plus nilotinib, or dasatinib plus imatinib) eliminated the development of essentially all BCR-ABL1 mutations except T315I.

With the exception of T315I, do any of the described resistance-associated mutations make a practical difference to initial therapy? A recent mathematical analysis has examined the probability of treatment success based on the number of cross-resistant mutants in the Bradeen data.[88] The model postulates the existence of variant BCR-ABL1 kinases that are resistant to one, two, or three TKIs (such as imatinib, dasatinib, and nilotinib). In general, the mutations which confer resistance to the largest number of drugs in the combination are the ones which define how likely it is that the protocol fails. Based on this premise, Katouli and Komarova developed a counting strategy which can weigh different treatment strategies according to their cross-resistance properties, and find the protocols with the highest probability of treatment success.[88] In developing their algorithm, the authors constructed a data set allowing the determination of the number of possible resistant and cross-resistant mutants in the context of combing drugs and different concentrations. Notably, any single- or double-resistant mutation is ultimately manageable by the sequential or combined use of inhibitors. The initial choice of treatment is claimed to be less important because the limited cross resistance will not prevent ultimate success. At low (5 nm) and medium (10–25 nm) concentrations of dasatinib, certain mutations arose (F317C, F317V, V299L) that did not also confer resistance to imatinib or nilotinib, suggesting that dasatinib could be used as first-line therapy, with imatinib or nilotinib used later if necessary. An alternative view based on empiric, clinical data suggests that a more cautious approach is necessary. Mutations can arise rapidly during TKI therapy,[89] and second-line treatments are not nearly as effective as first line therapy when resistance is clinically apparent. Optimal first-line single (or combination) TKI therapy may be preferable to reliance on a sequential approach.

Resistance of CML stem cells to imatinib

CML persistence and relapse is associated with the presence of leukemia stem cells which are protected from the adverse effects of therapy. It has been speculated that a pool of CML stem cells may persist in a dormant state as a residual, resistant population, with the ability to repopulate the leukemic clone, even in patients who are in CMR.[82] CML stem cells are widely viewed as being resistant to BCR-ABL1 inhibition.[90,91] The recent observation from the STIM trial of a distinct "break" in the relapse-free survival curve after imatinib withdrawal suggests that sometimes imatinib therapy may be associated with clonal extinction of the leukemic stem cells. But in most cases resistance is the rule both in the laboratory and the clinic. The causes for this resistance are controversial and are usually attributed to the effects of growth factors, stromal signals, and alternative intracellular signaling mechanisms. Nilotinib[92] and dasatinib[93] have also been evaluated for their ability to inhibit primitive CML cells. Dasatinib has been of special interest due to its ability to inhibit multiple other kinases,

including SRC family members. Dasatinib targets an earlier progenitor population than imatinib and is likely more effective than imatinib within the stem cell niche.[93] Under some culture conditions dasatinib can suppress growth of CML long-term culture initiating cells more than does imatinib, at clinically achievable doses.[94] However, it does not appear to induce apoptosis or clonal extinction more than does imatinib. To improve on the intriguing results documented in the STIM trial, it is likely that combinations of TKIs (including non-BCR-ABL1-targeting agents) or combinations of a BCR-ABL1-targeted TKI with conventional cytotoxic agents will be needed.

Conclusions

Today, patients newly-diagnosed with CML-CP have more options when starting TKI therapy. Inevitably for physicians, with more options come more questions on how to choose the right TKI for each individual patient. Will it be standard imatinib, dasatinib, or nilotinib? The answer will depend on factors such as toxicities, compliance, cost, BCR-ABL1 mutation status, and the likelihood of being able to reach certain surrogate endpoints. It is likely that there is no universal answer.

Dasatinib demonstrates improved outcomes in surrogate markers for the most mature studies, compared with imatinib. Dasatinib may be especially attractive for patients with high Sokal scores, who are at risk for having pretreatment BCR-ABL1 mutations involving the P-loop. Preclinical models also suggest that dasatinib has the lowest rate of mutations developing during TKI therapy. However, toxicity and compliance may be problematic, especially in patients with co-morbidities and who take multiple medicines.

Nilotinib also has improved outcomes in surrogate markers compared with imatinib. Compared with dasatinib-treated patients, subjects receiving nilotinib may have less costs and improved compliance. However the relatively common P-loop mutations in BCR-ABL1 may limit its usefulness as a first- or second-line agent. As with dasatinib, toxicity may be an issue in patients with co-morbidities and who are on multiple drugs.

Imatinib is the only agent for which improved overall survival has been documented. Furthermore, the STIM trial suggests that with selected patients (male, low Sokal score) it remains a viable (and possibly curative) option for long-term treatment of CML-CP. If a generic version becomes available,

imatinib treatment may result in significant cost savings compared with other TKIs. Like dasatinib and nilotinib, imatinib therapy also may be complicated by poor compliance.

Each of the available agents has advantages and disadvantages, and only further randomized trials can provide definitive answers. It is likely that the next few years will bring new studies on combination TKI therapy involving BCR-ABL1-targeting agents combined with other kinase inhibitors,[95] or with chemotherapy agents.[96] In the meantime patients with CML-CP can be assured that there are already several excellent options for controlling their disease.

Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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