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Authors

Van Etten, Richard A
Mauro, Michael
Radich, Jerald P
et al.

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Advances in the biology and therapy of chronic myeloid leukemia (CML): Proceedings from the 6th Post-ASH International CML and Myeloproliferative Neoplasms Workshop

Richard A. Van Etten[1], **Michael Mauro**[2], **Jerald P. Radich**[3], **John M. Goldman**[4], **Giuseppe Saglio**[5], **Catriona Jamieson**[6], **Simona Soverini**[7], **Carlo Gambacorti-Passerini**[8], **Rüdiger Hehlmann**[9], **Giovanni Martinelli**[7], **Danilo Perrotti**[10], **David T. Scadden**[11], **Tomasz Skorski**[12], **Ayalew Tefferi**[13], and **Tariq I. Mughal**[1]

[1]Tufts Medical Center, Boston, MA, USA

[2]Oregon Health and Science University, Portland, OR, USA

[3]Fred Hutchinson Cancer Research Center, Seattle, WA, USA

[4]Imperial College London, London, UK

[5]University of Turin, Orbassano, Italy

[6]University of California San Diego, La Jolla, CA, USA

[7]University of Bologna, Bologna, Italy

[8]University of Monza, Monza, Italy

[9]University of Mannheim, Mannheim, Germany

[10]The Ohio State University, Columbus, OH, USA

[11]Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

[12]Temple University, Philadelphia, PA, USA

[13]Mayo Clinic, Rochester, MN, USA

Abstract

Following the 53rd annual meeting of the American Society of Hematology in San Diego in December 2011, a group of clinical and laboratory investigators convened for the 6th post-ASH International Workshop on chronic myeloid leukemia (CML) and myeloproliferative neoplasms (MPN). The workshop took place on the 13th–14th December at the Estancia, La Jolla, California, USA. This report summarizes the most recent advances in the biology and therapy of CML that were presented at ASH and discussed at the Workshop. Preclinical studies focused on the CML stem cell and its niche, and on early results of deep sequencing of CML genomes. Clinical advances include updates on 2nd and 3rd generation TKIs, molecular monitoring, TKI discontinuation studies, and new therapeutic agents. A report summarizing the pertinent advances in MPN has been published separately.

Keywords

Chronic myelogenous leukemia; dasatinib; imatinib mesylate; nilotinib; tyrosine kinase inhibitor

Correspondence: Richard A. Van Etten, Molecular Oncology Research Institute, Tufts Medical Center, 800 Washington Street, Box 5609, Boston, MA 02111 USA, Tel 617-636-8110, Fax 617-636-7060, Rvanetten@tuftsmedicalcenter.org.

Introduction

Chronic myeloid leukemia (CML) is arguably the human malignancy that is the best understood in terms of its underlying biology and pathophysiology, and it is difficult to dispute that it is also one of the most successfully treated cancers. Of course, these two features are inherently linked, as it was recognition of the BCR-ABL1 tyrosine kinase as the direct cause of the disease that ultimately led to the development of the ABL1 tyrosine kinase inhibitors (TKIs) that have so dramatically altered the prognosis of patients with CML. Despite this tremendous progress, there is plenty of room for advancement in the preclinical and clinical science of CML. Results from clinical trials where TKI therapy is deliberately discontinued have shown these drugs are not curative in the majority of CML patients, and a large body of research suggests this might be due to the persistence of leukemia-initiating or leukemia "stem" cells that (LSCs) are relatively resistant to killing by TKIs. Furthermore, TKI treatment is much less effective in patients with advanced phase CML or with Philadelphia positive (Ph^+) acute leukemia. Here, we review the latest developments in CML biology and therapy that were presented at the 2011 ASH meeting. In the preclinical arena, much current effort is focused on understanding the molecular pathways that regulate LSC survival and on mechanisms of genomic instability and disease progression. Recent clinical developments include follow-up on phase III trials of second generation ABL1 TKIs as initial therapy in CML, the continued progress of so-called "pan-ABL1" inhibitors that have activity against the BCR-ABL1 gatekeeper T315I mutant, updates on TKI discontinuation trials in CML, and preliminary results from trials combining TKIs with second agents designed to eliminate CML stem cells.

Targeting CML stem cells

Several research groups have utilized mouse models of CML to implicate genetically several signaling pathways in the maintenance of LSC in vivo. Müschen and colleagues had shown previously that BCL6 is a transcriptional repressor of p53 and ARF whose activity is suppressed by BCR-ABL1 in Ph^+ lymphoblastic leukemia cells, whereas BCL6 induction following TKI treatment contributes to LSC drug resistance [1]. They have now extended these results to CML, implicating BCL6 as a downstream effector of active FOXO signaling in CML LSC that contributes to their survival and resistance to TKIs [2]. A similar strategy was used to implicate HIF1α, a master transcriptional regulator of the cellular response to hypoxia, in the survival of CML LSCs. Conditional deletion of *Hif1a* in a mouse CML model caused cell cycle arrest and apoptosis in LSCs, accompanied by a failure in secondary transplantation of the disease [3]. While both BCL6 and HIF1α are now validated as potential targets for eradication of CML LSCs, the therapeutic potential of inhibiting the JAK-STAT pathway in CML is less clear. Elegant genetic studies by Hantschel and colleagues using conditional *Jak2*-deficient mice have shown that BCR-ABL1 is the kinase directly responsible for activating STAT5 in CML cells [4], but deletion of STAT5 itself does not appear to eliminate CML stem cells or prevent progression to blast crisis in a mouse retroviral CML model [5].

Other research has focused on primary human CML progenitors and assessment of CML LSCs by xenotransplantation into immunodeficient (NOD-*scid Il2rgc*$^{-/-}$, NSG) recipient mice. Work from Bhatia and colleagues showed previously that the stress-related deacetylase SIRT1 was induced by BCR-ABL1 in a conditional *BCR-ABL1* transgenic mouse CML model, and its deletion or pharmacologic inhibition attenuated leukemogenesis [6]. Extending these studies to human CML, siRNA knockdown of SIRT1 or treatment with the SIRT1 inhibitor tenovin-6 and imatinib increased apoptosis in $CD34^+CD38^-$ quiescent ($CFSE^{high}$) CML progenitors and impaired engraftment of NSG mice [7]. Full transcriptome

RNA sequencing studies from Jamieson and co-workers have shown that BCR-ABL1-expressing LSC acquire several new signaling abnormalities during progression to blast crisis, including a switch towards expression of long (pro-survival) compared with short (pro-apoptotic) splice isoforms of BCL2, and related family members BCLX and MCL1 [8] in the setting of enhanced RNA editing, together with enhanced expression of the Shh transcriptional activator GLI2 and decreased expression of the GLI3 transcriptional repressor [9]. These effects were most pronounced in the marrow niche, indicative of microenvironmental responsiveness of $CD123^+$ (IL-3Rα) blast crisis LSC resulting in enhanced survival and self-renewal through JAK/STAT signaling. These pathways could be pharmacologically targeted with a novel pan-BCL2 protein family inhibitor (sabutoclax), a smoothened antagonist (PF-04449913), and a selective JAK2/FLT3 inhibitor (SAR302503; Sanofi Oncology), with selective effects on LSC versus normal hematopoietic progenitors.

Previous work from Perrotti and colleagues demonstrated decreased activity of the phosphatase PP2A in CML blast crisis through upregulation of its inhibitor SET, and identified the therapeutic potential of the PP2A activator and immunosuppressive sphingosine analog FTY720 in advanced CML [10]. Recent studies have extended this paradigm to hematopoietic progenitors expressing the $JAK2^{V617F}$ mutant tyrosine kinase, but with a twist, as PP2A activation depends on the $JAK2^{V617F}$– and PI3Kγ–induced phosphorylation of SET at Ser 24 [11]. Unlike its immunosuppressive activity, the anti-leukemic mechanism of FTY720 in CML and MPN does not require sphingosine kinase 2-dependent phosphorylation, motivating the development of non-immunosuppressive derivatives of this drug for leukemia development. A novel, specific, cell penetrating peptide (OP449) that binds to SET and antagonizes SET's inhibition of PP2A was also selectively cytotoxic to CML cell lines and primary progenitors [12], further validating the SET-PP2A axis as a novel target for therapy in CML.

Other recent work from Skorski and colleagues has focused on the mechanisms of genomic instability in CML progenitors and its role in disease progression and TKI resistance. CML progenitors have increased levels of reactive oxygen species (ROS), which triggers oxidative DNA damage and DNA double-strand breaks, both of which are subjected to error-prone repair mechanisms in the leukemic cells. In Ph^+ cells, activated Rac signals to the mitochondrial respiratory chain complex III to enhance production of ROS, and inhibiting Rac or genetic inactivation of complex III decreases ROS levels and DNA damage [13]. In the conditional *BCR-ABL1* transgenic mouse CML model, the source of genetic instability in CML originates from the earliest (LSK) stem/progenitor cells [14], and because these cells have defects in RAD51-mediated homologous recombination repair [15], they may be more dependent on the alternative RAD52 pathway to repair DSBs. Genetic deletion of *Rad52* reduced the frequency of CML LSCs in mice, while inhibition of RAD52 function with a peptide aptamer reduced the clonogenic potential and proliferation of $CD34^+$ cells from CML patients, but not from normal donors [16].

Focus on the CML stem cell niche

Although the bulk of preclinical investigation in CML is currently focused on the leukemic cells, there is increasing appreciation that the hematopoietic stem cell niche can play an important role in the malignant phenotype, offering additional opportunities for therapeutic intervention. Scadden and colleagues recently described a novel myelodysplastic-like syndrome that is induced in mice upon osteoprogenitor-specific deletion of Dicer1, the ribonuclease required for processing microRNAs [17]. Subsequent lineage-marking studies have defined a marrow osteoblast progenitor that can self-renew, lending further support to the concept of niche-induced oncogenesis. The Scadden group demonstrated previously that genetic and pharmacologic manipulation of the osteoblastic niche increases the number and

mobilization of normal HSC [18], and are now extending these studies to the leukemic stem cell niche. Activation of osteoblasts through expression of a constitutively active parathyroid hormone (PTH) receptor or by administration of PTH impaired the function of the LSC niche and decreased LSC frequency in a mouse CML model, but interestingly had the opposite effect on AML induced by the *MLL-AF9* fusion oncogene [19], providing some of the first biological evidence of differences between normal and leukemic niches.

Other studies from Kyoto demonstrated that Galectin-3 is induced in CML cells via interaction with the bone marrow microenvironment, and overexpression of Gal-3 increased the homing and engraftment of CML cells in NSG mice [20]. The group of Valent identified CD26 (dipeptidylpeptidase IV) as specifically expressed on CML LSC, which may function to increase LSC mobilization by disrupting the CXCR4/CXCL12 LSC-niche interaction [21]. Although the study of the LSC niche is in its infancy, these and other studies demonstrate the promise of this line of investigation.

Next-generation genetics in CML

Application of next-generation sequencing and genomic analysis techniques to the study of CML continues to yield important new information on pathogenesis and response to treatment. Ultra-deep amplicon sequencing can be used as a platform to perform *BCR-ABL1* kinase domain mutation screening with a sensitivity of 0.1%, a cost comparable to conventional Sanger sequencing, and the advantage to quantitatively follow the dynamics of mutated subclones over time and detect emerging *BCR-ABL1* mutant subclones earlier than D-HPLC or conventional sequencing [22]. This may offer particular advantages for monitoring of Ph^+ ALL patients, who are highly prone to develop resistance and mutations while on TKI therapy.

Another outstanding example of genomics and genetics in CML was reported by the group of Ong and co-workers, who performed massively parallel DNA sequencing of paired-end ditags to identify genetic factors associated with TKI resistance in CML patient samples. They discovered a novel deletion polymorphism in the *BIM* gene that was common in East-Asian (12.3%) but not African or Caucasian (0%) populations, which correlated with TKI resistance both clinically and in vitro. Pharmacologic restoration of BH3 activity (using the BH3-mimetic drug, ABT-737) re-sensitized the cells to imatinib [23]. This study serves as a paradigm for investigating constitutional differences in response to targeted therapy in other cancers.

Second generation TKIs as first-line therapy for patients with CML in chronic phase

Nilotinib (Tasigna; Novartis), an oral TKI designed as a chemical modification of imatinib, received regulatory approval for first line use in patient with CML in chronic phase (CML-CP) in the US in September 2010, and in the UK in December 2011. This was based on the 12 months' follow-up of the ENESTnd trial (*Evaluating Nilotinib Efficacy and Safety in Clinical Trials-Newly Diagnosed Patients*), a phase III, randomized, open-label, multicenter study, comparing the efficacy and safety of nilotinib at two different dosages (300 mg and 400 mg twice daily) versus imatinib 400 mg/day in patients with newly diagnosed CML-CP [24]. Major molecular response (MMR) at 12 months, the selected primary endpoint, was significantly higher at 12 months in the intention-to-treat analysis for nilotinib 300 mg twice daily (44%, $P < .0001$) than for imatinib (22%). These higher responses were also associated with significantly fewer progressions to the advanced phases with nilotinib, compared with imatinib. They also remained durable at the 24 months' analysis [25].

Following a minimum follow-up of 36 months, the cumulative rates of MMR appear to increase for all groups of patients (Table I), but remain statistically significantly higher for the nilotinib versus imatinib-treated patients: nilotinib 300mg twice daily (73% ($p<0.001$); nilotinib 400mg twice daily (70% ($p<0.001$); imatinib (53%) [26,27]. Importantly, there appeared to be no changes in the toxicity of either dose of nilotinib, or of imatinib. No new progressions to the advanced phases were observed in the third year of treatment and the differences between the number of progressions observed in both nilotinib arms were lower with respect to those observed in the imatinib arm and remain significant not only for patients still in the core study ($p=0.006$, nilotinib 300 mg BID vs imatinib; $p=0.019$ nilotinib 400 mg BID vs imatinib), but also including those patients who discontinued from the study, in an intention to treat analysis ($p=0.05$, nilotinib 300 mg BID vs imatinib; $p=0.009$ nilotinib 400 mg BID vs imatinib). More patients achieved $CMR^{4.5}$ with nilotinib 300 mg twice daily (32%; $p<0.0001$) and nilotinib 400 mg twice daily (28%; $p=0.0004$) than with imatinib (15%). Similar efficacy and safety data has been noted in the updated single center (MD Anderson Cancer Center, Houston) phase II study and the phase III Italian (GIMEMA) study [28,29].

Dasatinib (Sprycel; Bristol-Myers Squibb) is an oral dual ABL and SRC kinase inhibitor that received regulatory approval for first-line use in CML-CP in September 2010. This was based on the 12-month results of the DASISION (*Dasatinib versus Imatinib Study in Treatment-NaÔve CML Patients*) trial, which showed a confirmed CCyR (the selected primary end-point) of 86% for the dasatinib-treated cohort compared to 73% for the imatinib-arm ($p=0.0002$) [30]. Following a minimum follow-up of 24 months, the rates of cumulative CCyR remained higher, though no longer statistically significant (86% versus 82%; Table I) [31]. MMR rates by 12 and 24 months were significantly higher with dasatinib compared with imatinib (46% and 64% versus 28% and 46%, respectively; $p<0.0001$). Among the subgroup of patients who achieved MMR, median time to MMR was 15 months for dasatinib and 36 months for imatinib; $CMR^{4.5}$ was achieved in 17% of dastinib and 8% of imatinib-treated patients ($p=0.002$). Transformation to the advanced phases was noted in 2.3% of the dasatinib and 5.0% of the imatinib-treated patients. The updated safety analysis confirms a comparable toxicity profile for dasatinib compared to imatinib by 24 months of minimum follow-up.

Bosutinib (Bolusif; Pfizer), an oral dual ABL and SRC kinase inhibitor, is chemically different from both dasatinib and nilotinib. It is currently being assessed for potential first line therapy in patients with CML-CP in the BELA (*Bosutinib efficacy and safety in CML*) trial, a randomized, phase III, open-label study of bosutinib versus imatinib 400mg/day in newly diagnosed patients with CML-CP (Table I). At the 12-month landmark analysis, the CCyR for bosutinib-treated patients was 70%, compared to 68% for imatinib [32]. Following a minimum follow-up of 24 months, the cumulative rates of CCyR and MMR were 87% with bosutinib versus 81% with imatinib, and 67% with bosutinib versus 52% with imatinib, respectively ($p = 0.002$) [33]. It is of interest that a lower treatment failure rate was observed in bosutinib-treated patients (4%) compared to those treated with imatinib (13%); additionally there were fewer progression events on bosutinib (2%) versus imatinib (5%). Bosutinib was recently approved for use in the U.S. for relapsed/refractory CML. The 24 month BELA data showing improved molecular response and protection from progression (Table I) lend some support for the drug's candidacy for regulatory approval in the frontline setting. Bosutinib was associated with higher incidences of gastrointestinal toxicities, in particular grade 3/4 diarrhea, which was noted in 12% of patients. Grade 3/4 liver function abnormalities were also more common in the bosutinib-arm compared to imatinib (23% versus 4% ALT increase, 12% versus 4% AST increase). Interestingly, the incidence of grade 3/4 neutropenia was less frequent with bosutinib compared to imatinib (11% vs 24%).

It currently remains unknown whether the higher rates of early response seen with these second generation TKIs will translate into improved EFS and/or OS rates in comparison with imatinib. Thus far, no differences in OS have been observed in the ENESTnd, DASISION, or BELA trials.

Discontinuing TKI therapy in CML

The challenge of how long to continue TKI therapy remains unresolved. To address this, Mahon and colleagues in Bordeaux conducted a study, STIM (*Stop Imatinib*), for patients who had achieved a sustained CMR on imatinib for ≥ 2 years and then discontinued the drug [34,35]. They observed that about 40% of the patients remained in $CMR^{>4.0}$ whilst the others relapsed. In those who had a molecular relapse, a remission was re-established on re-starting imatinib. This interesting observation raises the possibility that imatinib may have the capacity to eradicate CML in some cases, though not in others. This study also identified patients with a low Sokal risk score, male sex, and longer duration of imatinib treatment as potential prognostic factors for the maintenance of CMR after discontinuing imatinib.

Efforts are also assessing the potential to discontinue dasatinib or nilotinib. Rea and colleagues investigated the possibility of a patient remaining in CMR or MMR after a sustained $CMR^{4.5}$ of ≥ 2 years whilst on second-line therapy with dasatinib or nilotinib after imatinib failure in a cohort of 35 patients [36]. They observed a loss of molecular response in 27.2% of patients within the first 6 months after TKI therapy had been discontinued. This could mean that the so-called “complete molecular response” observed with second generation TKIs may be associated with a greater possibility of ‘success after treatment discontinuation’, now defined as *not losing MMR*.

Recent work has also demonstrated that CML progenitors and primitive stem cells have substantial lower *BCR-ABL1* expression that could be an additional mechanism for TKI resistance [37,38]. The authors speculate that this property would mean that the use of more potent TKIs would not necessarily increase the chance of eliminating CML stem cells; instead, alternative therapies would be required.

In efforts to optimize the probability of achieving a CMR and therefore possible discontinuation of TKI therapy, efforts are also assessing the merits of switching TKIs. The Australasian Leukaemia and Lymphoma Group reported an improvement in the overall rates of molecular response and a low risk of progression in an update of their TIDEL II study [39]. This study was designed to assess the notion of improving response by an early switch from imatinib at 400 or 600 mg/day to nilotinib. Another study, the ENESTcmr, an adjunctive study to the ENESTnd, is assessing the potential to convert long term imatinib treated patients with disease still detectable after ≥24 months of therapy to undetectable *BCR-ABL1* levels by switching to nilotinib [40]. By 12 months of study therapy, $CMR^{4.5}$, the primary endpoint, was achieved in 23% of the 202 eligible patients who have been switched to nilotinib 400 mg BID versus 10.7% for patients who continued imatinib 400 or 600 mg. Rates of confirmed CMR at 12 months were increased in those switched to nilotinib in comparison to maintaining imatinib but to a lesser degree (p=0.108).

New candidate drugs for CML

Ponatinib (formerly called AP24534, Ariad Pharmaceuticals) is a rationally designed BCR-ABL1 inhibitor that binds both active and inactive conformations 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 [41]. The preliminary results of PACE (Ponatinib Ph+ ALL and CML Evaluation), a phase II study in which 449 patients who were either resistant or intolerant to dasatinib or nilotinib, or had a T315I mutation were enrolled

appear promising [42]. Amongst the 271 patients with CML-CP, 207 were either resistant or intolerant to dasatinib or nilotinib and 64 patients had a T315I mutation. 47% of all patients in CP were able to achieve the primary end-point of a major cytogenetic responses (MCyR). 39% of these patients achieved a CCyR, 33% from resistant/intolerant to dasatinib or nilotinib group and 58% from the T315I cohort; the corresponding MMR results were 19%, 15% and 33%, respectively. The toxicity data confirmed grade 3 (or more) pancreatitis in 6%. Clearly, longer follow-up is required to establish the precise place of ponatinib in the management of patients with CML who are intolerant or resistant to dasatinib or nilotinib. Ponatinib appears to have a role in the management of patients with the T315I subclone and is now being assessed as a potential salvage therapy for second-generation TKI failures.

Rebastinib (formerly called DCC-2036, Deciphera Pharmaceuticals), is a novel and potent TKI which binds to a novel region called the switch pocket, thereby preventing BCR-ABL1 from adopting a conformationally active state. Efficacy against multiple imatinib-resistant BCR-ABL1 mutants has been demonstrated both *in vitro* and *in vivo*. Importantly, DCC-2036 retains full potency against the T315I mutant in preclinical efficacy studies [43]. The drug is currently in a phase I study designed to find the maximal tolerated dose (MTD) when administered daily as a single-agent on a 28-day cycle. The preliminary results from 30 patients with CML in various phases, including 11 patients with the T315I mutation: 19 in CP, 8 in accelerated phase (AP) and 3 in blast phase demonstrate responses in CP patients: one MMR in a patient with T315I mutation, one CCyR, and one partial cytogenetic response. Hematologic responses were also seen in two patients in AP. These preliminary results suggest that DCC-2036 is well tolerated and has anti-leukemia activity in subjects with refractory CML and T315I positive disease [44]. Pharmacokinetics results are consistent with inhibition of BCR-ABL1 signaling in this first-in-man study of a switch pocket TKI.

Molecular Monitoring, Defining ‘CMR’, and the 3-months Molecular Response as a Predictor

The remarkable success accorded by TKIs for the management of patients with CML-CP has lead to many efforts designed to identify molecular markers to facilitate identification of patients who might require an alternative treatment to optimize their outcome. While early cytogenetic response may be similarly predictive, a critical mass of data is now available supporting early *molecular response* as a clear predictor of outcome and impending risk [45].

When after starting TKI therapy is measuring *BCR-ABL1* transcripts most clinically informative? In the seminal randomized trial of imatinib in newly diagnosed CML patients (IRIS), the *BCR-ABL1* transcript level at 12 months was found to be associated with better progression free survival, and this prompted investigators to use the 12-month *BCR-ABL1* level (especially the achievement of an MMR) as the endpoint for subsequent clinical trials [46]. Thereafter it was shown that the achievement of MMR at 12 months was associated with a good chance of reaching a CMR and with a very low risk of relapse [47,48]. All of this is fairly intuitive- ‘less disease is better’ - and hence the IRIS trial gave rise to a relevant ‘new’ landmark of response, namely the MMR threshold. However, during the 12-plus months that must elapse in order to judge ideal depth of response, a number of events may occur and the opportunity to identify a significant number of ‘at-risk’ cases may be lost. Parenthetically, a re-analysis of imatinib clinical data suggests that, for the 12-month landmark, CCyR is all that matters and MMR is not important [49].

Clearly a principal current goal of CML therapy is the continued pursuit of a ‘path to cure’, seeking a means to place patients into optimal remissions *without sustained therapy.* This

quest has been complicated by the lack of clarification regarding the threshold of detection of *BCR-ABL1* transcripts using current technology its implications. The most recent and lowest 'threshold' of CMR- 'complete molecular response'- is controversial as clinical benefit derived from achieving such a response is yet to be determined. As currently defined, 4.5 logs below standard baseline, 'CMR' may be numerically measurable in some specialized laboratories yet beyond the reach of a less experienced laboratory; this makes determination of a 'stable CMR' ambiguous. Some debate focuses on defining an accepted definition of patients eligible for a treatment discontinuation study. The recent experience of the Hammersmith group suggested that the PCR assay sensitivity and the threshold (CMR^4, $CMR^{4.5}$, or CMR^5) used to define 'stable CMR' were important issues for discontinuation studies [50].

Table II summarizes the results of several studies presented at ASH 2011 demonstrating that very early cytogenetic and *BCR-ABL1* PCR responses could be highly predictive of clinical outcomes [51–54]. Thus, measuring *BCR-ABL1* transcripts at 3 months could identify patients with a relatively poor outcome, whether the endpoint is overall survival (OS), event- or failure-free survival (EFS, FFS), or major or complete molecular response (MMR, CMR). For the majority of these studies, the critical cut off point seemed to be at the 10% international scale (IS) level, where patients with a *BCR-ABL1* transcript numbers >10% IS fared poorly compared to those whose disease burden was ≤10% IS. The implication is that patients who fail to reach this milestone should be switched to alternative therapy with another TKI, proceed to transplant, or be enrolled in an innovative clinical trial. It is of interest that the updated results of the TIDEL II study support the notion of switching patients on imatinib 400 mg/day, but not imatinib 600 mg/day, who had >10% IS transcripts at 3 months [39,55].

The question of how and when to measure disease response is also relevant when patients are treated initially with 2nd generation TKIs, and at ASH 2011, new data obtained from both arms of the DASISION study were presented [56,57]. These data show that a level of *BCR-ABL1* transcripts of more than 10% after 3 months of therapy is useful to identify those patients who are not performing well not only on imatinib 400 mg therapy, but also and even in a more pronounced way on dasatinib 100 mg daily therapy.

Mutation Analysis: Mass spectroscopy and beyond

BCR-ABL1 is associated with genetic instability [58]. Thus, unopposed BCR-ABL1 signaling is in part responsible for the mutation and gross genetic changes that occur with disease progression. Clinically point mutations may be associated with resistance, but in some cases, the mutant clone is only a small proportion of the total resistant population. In addition, in some cases, mutations predicted to be sensitive to a particular agent are not, and vice versa. This has led to speculation that while mutations might often been the source of resistance, they may at other times be not-so-innocent bystanders, sentinels showing that that genetic damage (and hence, progression and resistance) has occurred [59].

It has previously been shown that using ultra-sensitive assays that cases with T315I mutations at relapse have the resistant clones very early in their course. The German CML group has now demonstrated that the reverse is also true [60]. When these investigators tested 40 patients with a ligation PCR assay capable of detecting one T315I mutation in a background of 10^5 wild type alleles, the detection of any T15I allele at a rate of 10^{-5} (that is, less than or equal to one mutant allele in 100,000 normal alleles) precluded MMR. On the contrary, a detection rate below 10^{-5} was always associated with MMR.

The fact that sensitive mutation detection by mass spectroscopy identified patients destined for a poor response was also presented at ASH this year [61]. 221 patients with IM failure

were placed on a second generation TKI, and analyzed for ABL1 mutations. This technique (with 0.1% sensitivity) detected roughly 50% more mutations than standard direct sequencing. Patients with multiple mutations did significantly worse than patients with only one or zero mutations, with inferior rates of CCyR, MMR, and FFS. In addition, far more patients with multiple mutations went on subsequently to develop new mutations (Table III). Thus, it appears with better techniques, the molecular monitoring of CML can predict patients likely to have a poor outcome.

Conclusions

The introduction of TKIs in the management of CML has clearly constituted a very remarkable development in the story of CML. Imatinib is highly effective in reducing the burden of leukemia in a patient's body and the early clinical results with the second generation TKIs, dasatinib, nilotinib and bosutinib, suggest that each may be superior to imatinib in terms of the rapidity of the responses obtained. A number of problems and issues do however remain. As pointed out earlier (Table I), there is as yet no significant difference in progression-free or overall survival for patients treated initially with imatinib versus either nilotinib or dasatinib. In this context, the cost of TKI therapy, the duration of which may be lifelong, may be become an important health care issue as generic imatinib becomes available by 2014. Each of these agents may be associated with the development of adverse events, some of which can be serious. A small but significant minority of patients fail imatinib and also fail second generation TKIs. Others fail after initial therapy with second generation TKIs, though some of these could theoretically be rescued by allogeneic stem cell transplantation. The issue of how best to improve the current use of TKIs is therefore urgent, and clinical trials are needed to guide the management of CML patients over the next decade [62]. It is possible that the overall response rate would be improved by third generation TKIs such as ponatinib. It is possible also that adding a new inhibitory molecule (targeting for example a signal transduction pathway) to an existing TKI could make an important difference to the overall response rate or could possibly increase the proportion of patients who appear to have been cured after discontinuation of all therapy. A very important challenge that remains is the development of agents effective in the management of CML in accelerated or blastic phase, where currently available TKIs have limited efficacy. *Pari passu*, significant advances have been made in understanding the cellular and molecular biology of CML, in particular in understanding the molecular pathways that regulate LSC survival and the mechanisms underlying genomic instability and disease progression. As the limitations of kinase inhibitor therapy in CML and other cancers become more apparent, we will increasingly return to studies of the molecular pathophysiology of this fascinating myeloproliferative neoplasm to inspire future treatment strategies.

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Table I

Updated response rates of the ENESTnd, DASISION, and BELA trials

Trial/ drug	N	12 mos					24 mos					36 mos				
		CCyR	MMR	CMR	PFS	OS	CCyR	MMR	CMR	PFS	OS	CCyR	MMR	CMR	PFS	OS
ENESTnd [References [24,25,27]]																
Nilotinib (300 bid)	282	80%*	55%*	11%*	99%*NS*	100%*NS*	87%*	71%*	25%*	99%*NS*	96%*NS*	NA	73%*	32%*	97%*NS*	95%*NS*
Nilotinib (400 bid)	281	78%*	51%*	7%*	99%*NS*	99%*NS*	85%*	67%*	19%*	99%*NS*	97%*NS*	NA	70%*	28%*	98%*	97%*NS*
Imatinib	283	65%	27%	1%	96%	100%	77%	44%	9%	96%	98%*NS*	NA	53%	15%	95%	94%
DASISION [References [30] and [31]]																
Dasatinib	258	83%*	46%*	NA	97%*NS*	99%*NS*	86%*NS*	64%*	17%*	94%*NS*	95%*NS*	NA	NA	NA	NA	NA
Imatinib	258	72%	28%	NA	96%	97%	82%	46%	8%	92%	95%	NA	NA	NA	NA	NA
BELA [References [32] and [33]]																
Bosutinib (500 qd)	250	70%*NS*	41%*	NA	98%*NS*	99%*NS*	87%*NS*	67%*	NA	NA	NA	NA	NA	NA	NA	NA
Imatinib	252	68%	27%	NA	96%	97%	81%	52%	NA	NA	NA	NA	NA	NA	NA	NA

Abbreviations: CCyR, complete cytogenetic remission; CMR, complete molecular remission; MMR, major molecular response; NA, not available; OS, overall survival; PFS, progression-free survival

*Statistically significant vs. imatinib 400 mg arm

*NS*Not significant vs. imatinib 400 mg arm

Table II

Three-month cytogenetic and molecular responses and outcomes

Drug	3 mo response	Level	Endpoints	Outcomes	Reference
IM	Cytogenetic (Ph^+)	CCyR	EFS (5 yr)	83% v. 35%	[51]
IM	Molecular (*BCR-ABL1*)	≤10% IS	cCCyR OS (8 yr)	91% v. 47% 93% v. 57%	[57]
IM ± IFN	Molecular (*BCR-ABL1*) Cytogenetic (Ph^+)	≤10% IS <35% Ph^+ (PCyR)	OS OS	97% v. 87% 95% v. 87%	[53]
NIL/DAS	Molecular (*BCR-ABL1*)	≤0.1% IS (MMR)	EFS FFS	94% v. 86% 95% v. 65%	[54]
DAS	Molecular (*BCR-ABL1*)	≤10% IS	CCyR (1 yr) MMR (2 yr) FFS (2 yr)	96% v. 27% 76% v. 16% 97% v. 83%	[56]
DAS	Molecular (*BCR-ABL1*)	≤10% IS	CCyR (2 yr)	97% v.59%	[52]

Abbreviations: IM, imatinib; IFN, interferon; NIL, nilotinib; DAS, dasatinib; CCyR, complete cytogenetic remission; cCCyR, continuous complete cytogenetic remission; PCyR, partial cytogenetic remission; EFS, event-free survival; OS, overall survival; FFS, failure-free survival; MMR, major molecular response; CMR, complete molecular response

Table III

Responses in patients with CML-CP and *ABL1* mutations detected by MS

No. of mutations	CCyR	MMR	New mutations	FFS (1.5 yr)
0/1	50%	31%	25%	51%
≥1	21%	6%	56%	33%

Abbreviations: MS, mass spectroscopy; FFS, failure-free survival