Prognostic significance of treatment response in CML in view of current recommendations for treatment and monitoring

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Nikolas von Bubnoff

***Abstract*:** The use of small-molecule kinase inhibitors has redefined the management of cancer. Chronic myelogenous leukaemia (CML) has become the paradigm for targeted cancer treatment. Imatinib has become the gold standard in the treatment of CML with excellent and durable responses and minimal side effects. Molecular diagnostics constitute an integral part of the routine monitoring. Results of cytogenetic analysis and quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) indicate suboptimal response or treatment failure and guide treatment. New Abl kinase inhibitors such as nilotinib or dasatinib are options after the failure of or intolerance to imatinib, and both are available for first-line treatment of newly diagnosed CML. This review focuses on the prognostic significance of achieving a response at specific time points in patients with CML treated with imatinib, nilotinib or dasatinib in view of available data and current treatment recommendations.

***Keywords*:** CML, matinib, myeloid neoplasms

Introduction

The Philadelphia chromosome, first described as a shortened chromosome 22 [Nowell and Hungerford, 1960], results from a reciprocal translocation between the long arms of chromosomes 9 and 22 [Rowley, 1973], and is present in approximately 95% of chronic myelogenous leukaemia (CML) patients and up to 20% of adult acute lymphoblastic leukaemia (ALL) [Faderl *et al.* 1999; Sawyers, 1999]. The Philadelphia translocation gives rise to the oncogenic BCR-ABL fusion protein that is characterized by a constitutively active tyrosine kinase. BCR-ABL is sufficient to cause CML in mice [Daley *et al.* 1990], and its transforming capacity strictly depends on tyrosine kinase activity [Lugo *et al.* 1990]. This made BCR-ABL an attractive target for therapeutic intervention in CML and Ph+ ALL. The 2-phenylaminopyrimidine class of small-molecule kinase inhibitors was identified using a high-throughput screen of compound libraries at Ciba-Geigy (now Novartis) [Zimmermann *et al.* 1996]. The phenylaminopyrimidine CGP57148B (Imatinib-mesylate, hereafter imatinib), a derivative of the initial lead compound, was found to inhibit autophosphorylation of Abl and Bcr-Abl [Buchdunger *et al.* 2000; Beran *et al.* 1998; Zimmermann *et al.* 1997; Druker *et al.* 1996]. Preclinical studies demonstrated activity in *Bcr-Abl* positive cell lines and in animal models [le Coutre *et al.* 1999; Druker *et al.* 1996; Zimmermann *et al.* 1996]. Based on these observations, clinical trials in *BCR-ABL* positive CML were initiated in 1998.

Phase 2 clinical trials demonstrated the activity of imatinib in chronic-phase as well as in accelerated-phase and blast crisis CML [Kantarjian *et al.* 2002a; Sawyers *et al.* 2002; Talpaz *et al.* 2002] and lead to the approval of imatinib for the treatment of CML in 2002. Activity was reported in patients with chronic phase CML and interferon (IFN) resistance or intolerance [Hochhaus *et al.* 2008b]. A phase 3 clinical trial (IRIS trial) documented the superiority of imatinib over IFN in combination with low-dose cytarabine in patients with newly diagnosed, untreated chronic phase CML with respect to haematologic, cytogenetic and molecular responses [Hughes *et al.* 2003; O'Brien *et al.* 2003], and also with respect to overall survival [Roy *et al.* 2006]. After 8 years, 55% of patients randomized to receive imatinib were still on study

Correspondence to:
Nikolas von Bubnoff, MD
III. Medizinische Klinik und Poliklinik, Klinikum rechts der Isar, Technische Universität München, Ismaningerstraße 22, 81675 München, Germany
n.bubnoff@lrz.tum.de

medication, and the overall survival rate for patients randomized to imatinib (intention to treat) was 85% or 93% when only CML-related deaths were considered [Deininger *et al.* 2009]. Imatinib side effects were mainly considered as mild or moderate [Druker *et al.* 2006; O'Brien *et al.* 2003]. Similar results were reported outside of the setting of a clinical trial [de Lavallade *et al.* 2008] and established imatinib 400 mg daily as standard treatment for patients with CML in the chronic phase [NCCN, 2010; Baccarani *et al.* 2009a]. While imatinib leads to sustained responses in the majority of chronic-phase CML cases, responses in advanced-phase CML are usually short lived [Sawyers *et al.* 2002; Talpaz *et al.* 2002]. Therefore, patients in blast crisis should proceed to allogeneic haematopoietic stem cell transplantation as soon as a haematologic response has been achieved [NCCN, 2010; Baccarani *et al.* 2009a]. For patients in accelerated phase, there is a correlation between complete cytogenetic response (CCyR) achieved with imatinib 600 mg per day (approximately 20% of patients [Palandri *et al.* 2009]) and survival for more than 5 years [Kantarjian *et al.* 2005]. These patients should be closely monitored, and in the case of a loss of haematologic or cytogenetic response, should be submitted to stem cell transplantation. For patients failing imatinib treatment, approved second-line Abl kinase inhibitors are available. Both nilotinib and dasatinib have been demonstrated to induce haematologic and cytogenetic responses in imatinib-intolerant and imatinib-resistant CML [Cortes *et al.* 2008; Hochhaus *et al.* 2008a; Guilhot *et al.* 2007; Kantarjian *et al.* 2007b; le Coutre *et al.* 2007] and have been approved for the treatment of imatinib-resistant or imatinib-intolerant CML. Moreover, both nilotinib and dasatinib in the setting of first-line treatment in chronic-phase CML demonstrated higher rates of cytogenetic and molecular responses at 12 months and lower rates of progression to accelerated or blast crisis [Kantarjian *et al.* 2010a; Saglio *et al.* 2010]. The availability of alternative ABL kinase inhibitors and the need for specialized diagnostic tools makes the clinical management of CML more complex. Prognostic factors have been identified that facilitate treatment decisions, and these include baseline as well as response-related factors.

Assessing response in CML

Determining the response to treatment is the prerequisite for the recognition of a treatment failure. According to the techniques used for monitoring, three levels of response can be discriminated (Figure 1) [Baccarani *et al.* 2006; Hughes *et al.* 2006; Talpaz *et al.* 1986]. With decreasing leukaemic burden, the primary finding will be the normalization of blood cell counts (haematologic response). Later on, the decrease of Philadelphia-positive metaphases in the bone marrow indicates cytogenetic response. It is important to note that the well-established association between cytogenetic response and positive outcomes (see the following) is based on conventional cytogenetic analysis of at least 25 bone marrow metaphases. Fluorescence in situ

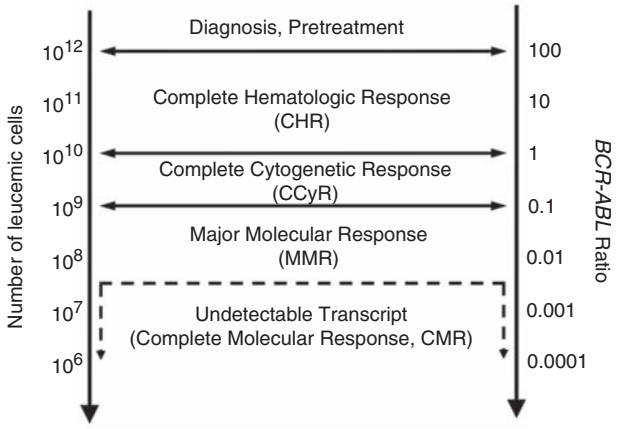

Figure 1. Relationship between leukaemia burden, response and number of *BCR-ABL* transcripts in the peripheral blood of chronic myelogenous leukaemia patients (adapted according to Baccarani *et al.* [2006]). CHR, complete haematologic response; CMR, complete molecular response; CCyR, complete cytogenetic response; MMR, major molecular response.

hybridization (FISH) can be used to assess cytogenetic response if marrow metaphases cannot be obtained, but as 200 peripheral blood interphase cells should be scored, low-level FISH positivity does not exclude CCyR established by conventional bone marrow cytogenetic analysis and therefore should not be used to define treatment failure. Molecular response is reflected by a decrease of *BCR-ABL* transcripts in peripheral blood or bone marrow using quantitative real-time polymerase chain reaction (qRT-PCR) and is at least 3 logs more sensitive than conventional cytogenetics [Branford *et al.* 1999]. The magnitude of molecular response is expressed as the ratio of *BCR-ABL* to a control gene or as log reduction compared with either the pretreatment value or a standard [Hughes *et al.* 2006]. Efforts of harmonizing the methodology for detection of BCR-ABL transcripts and expressing results have lead to the calculation of lab-specific correction factors allowing conversion of PCR results into the internationally uniform and comparable international scale [Branford *et al.* 2006; Hughes *et al.* 2006]. There is a good correlation between bone marrow cytogenetics and peripheral blood transcript levels, with a *BCR-ABL* ratio of 10% or less being equivalent to major cytogenetic response (MCyR) and 1% or less being equivalent to CCyR (Figure 1 and Table 1) [Ross *et al.* 2006]. The high sensitivity of PCR allows disease activity and treatment response below the level of CCyR to be monitored. In the case of a negative test result for quantitative PCR, nested PCR allows a qualitative detection of *BCR-ABL* transcripts with a one log higher sensitivity compared with qRT-PCR [Hughes *et al.* 2006]. However, the presence of a complete molecular response (CMR; undetectable *BCR-ABL* transcripts by qRT-PCR and/or nested PCR in two consecutive samples with sensitivity $>10 \times 10^4$ [Baccarani *et al.* 2009a]) is also dependent on test sensitivity, which might vary between different assays and laboratories [Hughes *et al.* 2006].

Molecular and cytogenetic diagnostics at the time of imatinib failure

Molecular mechanisms that frequently cause clinical resistance to imatinib include *BCR-ABL* gene amplification and protein overexpression [Hochhaus *et al.* 2002; Gorre *et al.* 2001], clonal cytogenetic evolution [Cortes *et al.* 2003; Marktel *et al.* 2003; Hochhaus *et al.* 2002] and most importantly, mutations of the BCR-ABL kinase domain that lead to structural changes that affect drug binding [Branford *et al.* 2002; Hochhaus *et al.* 2002; Roche-Lestienne *et al.* 2002; Shah *et al.* 2002; von Bubnoff *et al.* 2002; Gorre *et al.* 2001]. Importantly, not only treatment failure itself but also molecular mechanisms leading to resistance can be identified by molecular diagnostic procedures that are routinely performed during treatment monitoring: conventional cytogenetic analysis (clonal cytogenetic evolution), FISH (*BCR-ABL* gene amplification),

Table 1. Response definitions according to the ELN recommendations [Baccarani *et al.* 2009a].

CHR (complete hematologic response)	WBC count <10 g/l	
	Platelet count <450 g/l	
	Differential without immature myeloid cells and <5 % basophils	
	Nonpalpable spleen	
Cytogenetic response (at least 25 BM metaphases counted)	Complete (CCyR)	0% Ph+
	Partiall (PCyR)	1–35% Ph+
	MajorCyR (MCyR)	0–35% Ph+
	Minor CyR	36–65% Ph+
	Minimal CyR	66–95% Ph+
	No CyR	>95% Ph+
Molecular response (*BCR-ABL* transcripts PB)	Complete (CMR)	Not detectable (qRT- and/or nested PCR) in two consecutive samples; sensitivity $>10^4$)
	Major (MMR)	*BCR-ABL*/control gene ≤0.10

CHR, complete haematologic response; CMR, complete molecular response; CCyR, complete cytogenetic response; CyR, cytogenetic response; MMR, major molecular response; PB, peripheral blood; PCR, polymerase chain reaction; pCyR, Partial cytogenetic response; WBC, white blood cell; qRT, quantitative real-time; Ph+, Philadelphia positive.

denaturing high-performance liquid chromatography (DHPLC; screening for *BCR-ABL* gene mutations; sensitivity 0.1–10%) and conventional sequencing of the BCR-ABL kinase domain (sensitivity approximately 20%). Direct sequencing is sufficiently sensitive to detect clinically significant mutant leukaemia subpopulations, and at the present time still is considered as the technology of choice. DHPLC-based methods offer the advantage of sensitive, high-throughput capacity screening for multiple mutations at one time in an automated, cost-effective way [Deininger *et al.* 2004; Irving *et al.* 2004; Soverini *et al.* 2004]. This technology increasingly complements conventional sequencing. However, a mutation detected by HPLC still needs to be confirmed by conventional sequencing. Detection of low-level mutant disease clones using highly sensitive assays such as allele-specific PCR (sensitivity as low as 0.01%) is of low clinical significance.

Sequencing analysis for the presence of *BCR-ABL* mutations that mediate inhibitor resistance should be initiated in the case of treatment failure and in the case of repeated qRT-PCR results demonstrating an increase of *BCR-ABL* transcripts. The factor increase that should trigger mutation analysis is not yet established [Kantarjian *et al.* 2009; Press *et al.* 2007; Hughes *et al.* 2006]. If a rise in transcripts is due to expansion of a mutant clone, the proportion of transcripts carrying the mutation might very well be above the threshold of detection of conventional sequencing although the absolute transcript level might be low.

Prognostic significance of biologic, molecular and cytogenetic parameters at baseline

In CML, initial workup at the time of diagnosis gives information that is of prognostic relevance and predictive with respect to response to treatment and overall survival (see Table 2).

First, disease phase at diagnosis predicts both response to imatinib and overall survival. In chronic phase, overall survival for patients randomized to first-line imatinib in the IRIS trial (intention to treat) at 8 years was 85% or 93% when only CML-related deaths were considered [Deininger *et al.* 2009]. In contrast, overall survival was 53% at 4 years and 43% at 7 years, respectively, in the accelerated phase [Palandri *et al.* 2009; Kantarjian *et al.* 2005], and 11% at 3 years in blast crisis [Palandri *et al.* 2008].

Table 2. Baseline prognostic factors in chronic myelogenous leukaemia.

Factor	Prognostic significance
Disease phase	HR, CR, PFS, OS
Sokal, Hasford score[#]	CR, PFS, OS
CCA Ph+	PFS, OS
hOCT-1 activity*	CR, MR, PFS, OS

[#]Sokal or Hasford score does not affect outcome once CCyR is achieved.
*Measured in peripheral blood mononuclear cells, not routinely available.
CCA Ph+, clonal chromosomal abnormalities in Philadelphia-positive cells; CR, cytogenetic response; HR, haematologic response; MR, molecular response; PFS, progression-free survival; OS, overall survival.

Second, in chronic-phase CML the baseline prognostic scores proposed by Sokal and colleagues [Sokal *et al.* 1984] and Hasford and colleagues [Hasford *et al.* 1998] are predictive for response and overall survival in patients treated with imatinib [Baccarani *et al.* 2009b; Marin *et al.* 2008; Druker *et al.* 2006; Hughes *et al.* 2003]. Both scores were developed in the era of hydroxyurea/busulfan (Sokal) and IFN (Hasford) and in terms of biology at least in part reflect features of accelerated-phase disease. In the IRIS trial, CCyR rates at 12 months for Sokal low, intermediate and high patients were 78%, 68% and 51%, respectively [Hughes *et al.* 2003], and overall survival at 6 years was 94%, 87% and 76%. However, pretreatment Sokal score did not significantly affect outcome, once a CCyR was achieved [Druker *et al.* 2006]. Interestingly, first-line treatment of chronic phase CML with novel Abl kinase inhibitors such as nilotinib or dasatinib might at least in part overcome the adverse effect of intermediate or high-risk Sokal score. Looking at the ENEST phase 3 trial, which compares imatinib 400 mg OD to nilotinib at 300 mg and 400 mg BID in newly diagnosed chronic-phase CML, CCyR rates at 12 months among patients with Sokal high-risk score were 74% with nilotinib at 300 mg BID, 63% among those receiving 400 mg of nilotinib BID, and 49% with imatinib, and MMR rates were 41% for 300 mg nilotinib BID, 32% for 400 mg nilotinib BID, and 17% with imatinib 400 mg [Saglio *et al.* 2010]. Similar results were observed in the DASISION phase 3 trial, which compares imatinib 400 mg daily with dasatinib 100 mg daily in newly diagnosed chronic phase CML. Here, the rates of CCyR by 12 months among patients with Hasford high-risk score were 78% with dasatinib

and 64% with imatinib, and the corresponding MMR rates at 12 months were 31% with dasatinib and 16% with imatinib [Kantarjian *et al.* 2010a].

Third, attempts have been made to correlate disease-specific molecular and cytogenetic features at baseline with outcome. In particular, 5–10% of patients with chronic-phase CML present with clonal chromosomal abnormalities (CCAs) in Ph+ cells [Sokal *et al.* 1988; Kantarjian *et al.* 1985; Swolin *et al.* 1985]. The finding of CCAs at baseline or in the course of treatment was found to be associated with disease progression and inferior overall survival in the pre-imatinib era [Sokal *et al.* 1988; Kantarjian *et al.* 1985; Swolin *et al.* 1985], as well as in CML patients in chronic and accelerated phase treated with imatinib [Marin *et al.* 2008; Cortes *et al.* 2003; Marktel *et al.* 2003], although response to imatinib therapy in one study was a stronger independent predictor for survival than clonal evolution at diagnosis [Cortes *et al.* 2003]. Current recommendations define CCAs as a 'warning' at baseline and as treatment failure during imatinib treatment [Baccarani *et al.* 2009a]. The significance of CCA in Ph- cells is less clear. CCAs in Ph- cells may occur in a transient manner, and current recommendations identify CCAs in Ph- cells as a 'warning' feature, based on rare instances of progression to myelodysplasia or acute myeloid leukaemia [Zaccaria *et al.* 2010, 2007; Baccarani *et al.* 2009a; Abruzzese *et al.* 2007; Deininger *et al.* 2007; Jabbour *et al.* 2007; Kovitz *et al.* 2006]. Recently, the human organic cation transporter-1 (hOCT-1) was found to be involved in the imatinib import into the cell [Thomas *et al.* 2004]. Three studies demonstrated a correlation of hOCT-1 gene expression or activity in CML mononuclear cells at baseline and response to imatinib [Marin *et al.* 2010; White *et al.* 2007b; Crossman *et al.* 2005], and baseline hOCT-1 activity was also found to be associated with overall and progression-free survival [White *et al.* 2010]. In addition, it has been demonstrated that low hOCT-1 activity can be overcome by increasing the imatinib dose [White *et al.* 2010, 2007b]. These data suggest that patients with low baseline hOCT-1 activity in mononuclear cells might benefit from imatinib dose increase or treatment with nilotinib or dasatinib, which both do not utilize hOCT-1 [Giannoudis *et al.* 2008; Hiwase *et al.* 2008; White *et al.* 2006]. Thus, determination of hOCT-1 activity should be prospectively evaluated in future clinical trials and might be incorporated in future treatment recommendations. Other pharmacologic markers that have been proposed to be of prognostic significance and/or predictive for response include measurements of plasma and intracellular imatinib levels. Plasma levels of imatinib and its major metabolite N-desmethyl-imatinib can be measured using HPLC and mass spectrometry [Larson *et al.* 2008; Picard *et al.* 2007; Titier *et al.* 2005]. Several reports investigated the significance of imatinib plasma levels for response. In two reports, higher imatinib plasma levels correlated with achieving CCyR and major molecular response (MMR), and in one report not surprisingly also with adverse events [Larson *et al.* 2008; Picard *et al.* 2007], while one report did not find such an association [Forrest *et al.* 2009]. Interestingly, imatinib plasma levels did also correlate with clinical benefit (complete response, partial response, stable disease, time to progression) in patients with unresectable or metastatic gastrointestinal stromal tumours (GISTs) [Demetri *et al.* 2009]. Both in CML as well in GIST patients, a mean trough imatinib plasma level of approximately 1000 ng/ml or above was associated with response [Demetri *et al.* 2009; Larson *et al.* 2008; Picard *et al.* 2007]. Thus, monitoring of imatinib plasma concentrations in patients with CML might be useful in poorly responding patients, in patients with suspected insufficient compliance and in patients with unusually severe toxicity. However, in chronic-phase CML patients with suboptimal response to or failure of imatinib and documented 'suboptimal' plasma levels, it is currently not clear whether increasing the imatinib dose would be equivalent to switching the treatment to nilotinib or dasatinib. The measurement of intracellular imatinib levels or inhibition of *BCR-ABL* would integrate drug absorption, tissue distribution, drug influx and efflux in leukaemic cells. One study reported a correlation of dephosphorylation of CRKL (which is phosphorylated by BCR-ABL and thus indirectly reflects BCR-ABL kinase activity) measured in peripheral blood mononuclear cells, and the subsequent MMR rate in chronic phase patients treated with imatinib at 600 mg daily [White *et al.* 2007a]. However, both intracellular measurement of nonradiolabeled imatinib and measurement of p-CRKL is technically challenging and thus implementation in daily routine seems unrealistic. In addition to pharmacokinetic markers and predictors of response, specific gene

expression signatures of leukaemic cells at baseline might be associated with treatment response or progression [Diaz-Blanco *et al.* 2007; Frank *et al.* 2006; Radich *et al.* 2006; Villuendas *et al.* 2006; Zheng *et al.* 2006]. However, the prognostic or predictive value of gene expression studies remains to be determined. As stated above, highly sensitive PCR assays allow the detection of low-level (as low as 0.01%) BCR-ABL kinase domain mutations associated with imatinib resistance [Ernst *et al.* 2009]. However, even in the setting of accelerated phase or blast crisis, low-level detection of mutations neither at baseline nor in patients responding to imatinib treatment without concomitant rise in *BCR-ABL* transcript levels correlated with response, relapse, progression-free survival or overall survival [Sherbenou *et al.* 2007; Willis *et al.* 2005; Roche-Lestienne *et al.* 2002]. Thus, mutational analysis at baseline or in patients responding to treatment is not useful.

Long-term survival in CML requires cytogenetic response

Hydroxyurea and busulfan can induce haematologic but not cytogenetic responses in chronic-phase CML [Hehlmann *et al.* 2003; The Italian Cooperative Study Group on Chronic Myeloid Leukemia, 1998]. A meta-analysis demonstrated a survival benefit at 4 years for hydroxyurea compared with busulfan (53.6% *versus* 45.1%) [Chronic Myeloid Leukemia Trialists' Collaborative Group, 2000]. Later on, treatment with alpha IFN alone or in combination with low-dose cytarabine-induced haematologic responses in 70–80% of cases and durable CCyRs (for the definition see Table 1) in 5–15% of cases, with higher rates of cytogenetic responses achieved by IFN in combination with low-dose cytarabine [Baccarani *et al.* 2002; Bonifazi *et al.* 2001; Guilhot *et al.* 1997; Hehlmann *et al.* 1994]. A survival advantage of IFN-based therapy to hydroxyurea or busulfan was demonstrated in a meta-analysis (5-year survival 57% *versus* 42%) [Chronic Myeloid Leukemia Trialists' Collaborative Group, 1997], and survival at 10 years was 72% with IFN-based therapy when a CCyR was attained [Bonifazi *et al.* 2001]. Thus, already in the pre-imatinib era, long-term survival in nontransplant patients was linked to cytogenetic response.

It was therefore not unexpected to see that in patients with chronic-phase CML treated with imatinib, outcome was determined by haematologic and cytogenetic response to first-line imatinib at specific time points. Based on these observations, recommendations for the management and monitoring of CML patients receiving imatinib were developed [NCCN, 2010; Baccarani *et al.* 2009, 2006; Hochhaus *et al.* 2006], including criteria defining suboptimal response and treatment failure which are based on haematologic and cytogenetic response to first-line imatinib in patients with chronic-phase CML (Table 3).

Table 3. ELN response criteria [Baccarani *et al.* 2009a] and prognostic significance of suboptimal response or treatment failure at 3, 6, 12 and 18 months imatinib in early chronic-phase chronic myelogenous leukaemia.

Time	Failure	Suboptimal response
3 months	<CHR#*	No CR+
6 months	No CR+#*	<PCyR#
12 months	<PCyR#*	<CCyR#*
18 months	<CCyR#*	<MMR
Anytime	Loss of CHR#*	Loss of MMR
	Loss of CCyR#*	*BCR-ABL* mutation$
	BCR-ABL Mutation§	
	CCA Ph+#*	

+Low probability of subsequent CCyR.
#Predictive for progression-free survival.
*Predictive for overall survival (see the text for details).
§Exchange poorly imatinib sensitive.
$Exchange imatinib sensitive.
CHR, complete haematologic response; CCA Ph+, clonal chromosomal abnormalities in Philadelphia-positive cells; CCyR, complete cytogenetic response; CR, cytogenetic response; MMR, major molecular response; PCyR, partial cytogenetic response.

Prognostic significance of response to imatinib in CML

Importantly, it became evident very soon that most criteria for suboptimal response and treatment failure at 3, 6, 12 and 18 months in chronic-phase CML patients receiving imatinib as defined by the original and revised European Leukemia Net (ELN) recommendations [Baccarani *et al.* 2009a, 2006] indeed proved to identify patients with inferior outcome with respect to overall survival, progression-free survival, gain of a CCyR, or loss of a previously achieved CCyR. Haematologic imatinib failures in early chronic phase are rare (<5% of cases) [Marin *et al.* 2008; O'Brien *et al.* 2003]. In contrast, primary cytogenetic failures are more prevalent and were reported to occur in 3–18% at 6 months [Marin *et al.* 2008], 15–27% at 12 months [Marin *et al.* 2008; Druker *et al.* 2006] and

23–49% at 18 months [Marin *et al.* 2008; Roy *et al.* 2006]. Lack of a complete haematologic response (CHR) at 3 months and lack of any cytogenetic response (Ph+ >95%) at 6 months, lack of a partial cytogenetic response (PCyR; Ph+ >35%) at 12 months, and lack of a CCyR (Ph+ ≥1%) at 18 months indicate primary haematologic or cytogenetic failure (see also Table 3) and were associated with inferior progression-free survival [Kantarjian *et al.* 2008; Marin *et al.* 2008; Druker *et al.* 2006; Roy *et al.* 2006]. Patients without any cytogenetic response (>95% Ph+) at 3 and 6 months had a low probability of achieving a CCyR later on [Alvarado *et al.* 2009; de Lavallade *et al.* 2008]. Looking at the IRIS study, for patients not achieving a MCyR at 12 months (failure according to ELN), a significant disadvantage was also seen for overall survival and moreover, a similar significant disadvantage was seen for patients failing CCyR after 12 months (suboptimal response according to ELN) [Roy *et al.* 2006]. A single institution analysis performed by the Hammersmith group confirmed these findings demonstrating that treatment failure at 3, 6 and 12 months affected overall survival [Marin *et al.* 2008]. In addition, it was shown that suboptimal responders either at 6 months (less than PCyR) or 12 months (less than CCyR, see Table 3) had a significantly inferior progression-free survival, a lower probability of CCyR, and in the case of 12 months suboptimal responders also worse overall survival after 5 years (98% *versus* 85%). Similar findings were reported from a single-institution analysis performed by the Houston group [Kantarjian *et al.* 2008], although this analysis mainly included patients receiving an increased imatinib dose of 800 mg instead of the standard dose of 400 mg. Looking at the 18 months time point, failure to achieve a CCyR constitutes a treatment failure according to ELN, and in the IRIS study was associated with inferior overall survival after 8 years (74.9% *versus* 94.9%) [Deininger *et al.* 2009]. These findings substantiate the importance for early monitoring and demonstrate the prognostic significance of the ELN response criteria. Criteria for suboptimal response are very close to failure with respect to prognostic significance, and therefore the consequence in either case will rather be a switch to nilotinib or dasatinib than a dose increase of imatinib.

Acquired treatment failure (secondary resistance) denotes loss of a previously achieved haematologic or cytogenetic remission or progression to accelerated phase or blast crisis despite continued imatinib treatment [Baccarani *et al.* 2009a]. In early chronic-phase CML patients receiving imatinib treatment, the annual rates of secondary resistance (loss of CHR, loss of MCyR, progression to accelerated phase/blast crisis) or death in the IRIS study continuously decreased from the second (7.5%) to the sixth year (0.4%) and was 1.3% in the eighth year, including 2.8%, 0% and 0.4% with progression to accelerated phase or blast crisis at the second, sixth and eighth year [Deininger *et al.* 2009]. Looking at all of the 457 IRIS patients who achieved a CCyR, 82 patients (18%) had a documented loss of CCyR during treatment and 15 (3%) progressed to accelerated phase/blast crisis [Deininger *et al.* 2009]. Any loss of haematologic or cytogenetic response predicted shorter progression-free and overall survival [Marin *et al.* 2008].

Several studies have established a correlation between achieving a MMR (*BCR-ABL* ratio ≤0.1%) and improved durations of CCyR [Marin *et al.* 2008; Press *et al.* 2007; Iacobucci *et al.* 2006; Cortes *et al.* 2005]. In the IRIS study, the probability of loss of CCyR at 7 years was 3% for patients in MMR at 18 months compared to 26% for patients with CCyR without MMR [Hughes *et al.* 2010]. Two studies demonstrated a small, but significant advantage in progression-free survival for patients achieving a MMR at 12 [Hughes *et al.* 2010], or 18 months [Kantarjian *et al.*, 2008, Hughes *et al.*, 2010]. However, achieving MMR at 12 or 18 months was not demonstrated to be predictive for overall survival [Hughes *et al.* 2010; Kantarjian *et al.* 2008; Marin *et al.* 2008; Druker *et al.* 2006; Iacobucci *et al.* 2006; Cortes *et al.* 2005]. These findings might well indicate that MMR can be regarded as a 'safe haven' [Hughes *et al.* 2008], and suggest that cytogenetic analysis is dispensable once a CCyR has been attained and confirmed, provided that regular molecular monitoring can be assured [Baccarani *et al.* 2009a; Ross *et al.* 2006].

In advanced-phase CML, primary haematologic failure occurs more frequently and in phase 2 clinical trials was reported in 18–30% of patients with accelerated phase and in 60% of patients with blast crisis. After 4 years, resistance to imatinib had emerged in 45–70% (accelerated phase) and 90% (blast crisis) [Palandri *et al.* 2009; Kantarjian *et al.* 2005, 2002b, 2002c;

Ottmann *et al.* 2002; Druker *et al.* 2001]. Importantly, in accelerated phase approximately 20% of patients treated with imatinib at 600 mg daily achieve a CCyR [Palandri *et al.* 2009], and these patients in phase 2 trials survived more than 5 years [Palandri *et al.* 2009; Kantarjian *et al.* 2005]. Thus, accelerated-phase patients achieving CCyR should be continuously monitored and should be submitted to allogeneic transplant if cytogenetic or haematologic response is lost.

Together, haematologic and cytogenetic response determine progression-free and overall survival, and regular monitoring of imatinib treatment in CML is indispensable to confirm adequate response and to identify patients with suboptimal response or treatment failure early enough to make appropriate treatment changes [Baccarani *et al.* 2009a]. Patients who continue with imatinib despite the lack of cytogenetic response face the risk of progression to accelerated phase and blast crisis. In contrast, achieving a CCyR is associated with excellent progression-free survival, provided that imatinib is continued without dose reduction or interruptions [Kantarjian *et al.* 2008; Marin *et al.* 2008; Druker *et al.* 2006; Roy *et al.* 2006; O'Brien *et al.* 2003]. These findings underscore the importance of a regular monitoring particularly in the beginning of treatment. However, treating physicians and patients must also be aware that nonadherence to imatinib is prevalent [Darkow *et al.* 2007]. Poor adherence might be a frequent cause for suboptimal outcome in CML treated with oral tyrosine kinase inhibitors, and in the setting of stable CCyR in chronic-phase CML patients receiving imatinib was a strong predictor of not achieving a subsequent MMR [Marin *et al.* 2010]. Moreover, improving adherence may not only optimize outcomes of treatment but also reduce the economic burden, since adherence was found to be inversely associated with healthcare costs excluding imatinib [Darkow *et al.* 2007].

New BCR-ABL inhibitors after imatinib failure: baseline and response-related prognostic factors

The finding of clinical resistance to imatinib triggered the development of novel ABL kinase inhibitors. Preclinical models revealed a higher inhibitory activity of these drugs against wild-type *BCR-ABL* in cell lines and animal models, and also demonstrated activity of these novel compounds against many of the known imatinib-resistant *BCR-ABL* exchanges. Examples include nilotinib (AMN107) [Weisberg *et al.* 2005] and dasatinib (BMS354825) [Shah *et al.* 2004]. Both compounds have been demonstrated to induce haematologic responses in imatinib-intolerant and imatinib-resistant CML [Kantarjian *et al.* 2011; Apperley *et al.* 2009; Cortes *et al.* 2008; Hochhaus *et al.* 2008a; le Coutre *et al.* 2008; Giles *et al.* 2007;] and demonstrated superior rates of cytogenetic and molecular responses at 12 months compared with imatinib given as first-line treatment in chronic-phase CML [Kantarjian *et al.* 2010a; Saglio *et al.* 2010]. Both compounds have been approved for the treatment of imatinib-resistant or imatinib-intolerant CML, and recently also for the first-line treatment of CML. Baseline prognostic factors with respect to response to nilotinib or dasatinib have not been identified for patients with imatinib-intolerant disease but, however there is evidence for baseline prognostic factors in imatinib-resistant patients. Specifically, one study established and validated a scoring system based on cytogenetic response to imatinib, Sokal score and recurrent neutropenia during imatinib treatment [Milojkovic *et al.* 2010]. The three resulting risk categories predicted a cumulative incidence of CCyR at 2.5 years of 100%, 52.2% and 13.8% [Milojkovic *et al.* 2010]. In a second study, three risk categories based on previous cytogenetic response to imatinib, and performance status predicted the 12-month probability of achieving a MCyR to nilotinib or dasatinib (64%, 36% and 20%), and in addition discriminated 2-year rates for event-free survival (78%, 49% and 20%) and overall survival (95%, 85% and 40%) [Jabbour *et al.* 2011]. In addition, it was demonstrated that haematologic resistance to imatinib, clonal cytogenetic evolution, and the presence of specific, but not any BCR-ABL kinase domain mutations mediating imatinib resistance affects response to nilotinib and dasatinib [Milojkovic *et al.* 2010; Hochhaus *et al.* 2008a; Shah *et al.* 2008; Tam *et al.* 2008; Kantarjian *et al.* 2007a]. Both inhibitors display a largely nonoverlapping profile of resistance mutations that well correlates to mutations that were predicted by *in vitro* studies [Ray *et al.* 2007; Bradeen *et al.* 2006; von Bubnoff *et al.* 2006; Burgess *et al.* 2005; O'Hare *et al.* 2005]. Of note, two studies demonstrated that patients with history of a *BCR-ABL* resistance mutation might have a higher likelihood of developing further mutations receiving second- and third-line

tyrosine kinase inhibitors, and noted that some patients fail several lines of Abl tyrosine kinase inhibitors without ever developing a BCR-ABL kinase domain mutation [Soverini *et al.* 2009; Cortes *et al.* 2007], probably reflecting a difference in biology of the disease. Regarding response to nilotinib or dasatinib after imatinib failure, two studies demonstrated that the probability of achieving a CCyR is low if a patient does not achieve any cytogenetic response at 3 months and less than a minor cytogenetic response at 6 months [Milojkovic *et al.* 2010; Tam *et al.* 2008]. Both studies included patients in chronic phase, with 28% and 23% of the patients displaying additional cytogenetic abnormalities. In addition to the predictive value of cytogenetic response at 3 and 6 months for achieving a subsequent CCyR, a significant progression-free survival and overall survival advantage was demonstrated for patients achieving at least a minor cytogenetic response at 3 months [Milojkovic *et al.* 2010] and a MCyR at 6 [Milojkovic *et al.* 2010] or 12 months [Tam *et al.* 2008], respectively. Thus, both studies demonstrate that early cytogenetic response to nilotinib or dasatinib after imatinib failure predicts outcome. For patients with CML in advanced phase or Ph+ ALL treated with nilotinib or dasatinib after imatinib failure, a recent analysis identified patients failing a complete haematologic response at the time of achievement of a MCyR due to incomplete neutrophil or platelet recovery to have a particular poor outcome, pointing to the prognostic significance of recovery of Ph- haematopoiesis [Fava *et al.* 2009]. Overall survival at 2 years in these patients was similar to that of patients failing a MCyR.

Imatinib *versus* new BCR-ABL inhibitors as first-line treatment

As outlined above, the outcome of chronic-phase CML patients treated with imatinib is determined by response to treatment. A patient who rapidly achieves CCyR or MMR will have a lower risk of treatment failure. Second, although long-term outcome with imatinib in chronic-phase CML is impressive, one has to be aware that approximately 25% of patients in chronic-phase CML fail imatinib, and only 63% of the patients in the IRIS study were in CCyR and on study treatment after 6 years [Hochhaus *et al.* 2009]. As already pointed out, not achieving a CCyR determines inferior progression-free and overall survival, and of all patients in the IRIS study who achieved CCyR, 18% lost CCyR, and 3% progressed to accelerated phase or blast crisis [Deininger *et al.* 2009]. With these presumptions in mind, it might well be that superior rates of CCyR and MMR observed at 12 months with nilotinib [Saglio *et al.* 2010] or dasatinib [Kantarjian *et al.* 2010a] given as first-line treatment in chronic-phase CML will translate into long-term outcomes superior to imatinib. In the short term, both drugs reduced the rate of early progression to accelerated phase or blast crisis (in the case of dasatinib not yet statistically significant). On the other hand, it will be interesting to analyse whether baseline prognostic parameters (as described previously) allow the identification of subgroups that either do or do not gain benefit from frontline treatment with nilotinib or dasatinib. In addition, it might well be that treatment can be initiated with imatinib, and early response monitoring allows the identification of patients at risk for failing treatment which then would be switched to nilotinib or dasatinib. Unfortunately, there will probably never be a trial comparing the strategy of switching suboptimal imatinib responders/failures *versus* upfront nilotinib or dasatinib. Thus, treating physicians and patients might discuss and decide on an individual basis, taking into account specific circumstances and probably also costs. Some patients with ongoing complete molecular responses may be able to discontinue imatinib without subsequent relapse [Mahon *et al.* 2010]. Thus, long-term costs do not necessarily need to be higher with nilotinib or dasatinib, since rapid and deep remissions might lead to a higher proportion of patients qualifying for investigational treatment discontinuation. However, discontinuation of any Abl kinase inhibitor is not recommended outside the setting of a clinical trial. Alternative frontline treatment strategies might include induction-maintenance concepts with initial debulking using nilotinib or dasatinib and response-guided maintenance treatment using continued nilotinib or dasatinib, imatinib or alpha IFN, depending on the pace and quality of response. The Italian GIMEMA is currently examining the feasibility and response rates of a rotating regime of nilotinib and imatinib for frontline treatment of CML and Ph+ ALL [Saglio *et al.* 2009]. In addition to alternating strategies, the upfront combination of alpha IFN with imatinib is currently being investigated in France, Germany and Italy. The French SPIRIT study reported superior molecular response rates in the combination arm [Preudhomme *et al.* 2010]. This observation

was confirmed in a retrospective analysis by the Italian GIMEMA [Palandri *et al.* 2010]. In contrast, the German CML IV study so far did not detect differences in response rates between imatinib and imatinib + alpha IFN [Hehlmann *et al.* 2009]. The difference might well be attributed to disparities in adherence to the combination and the formulation of IFN. It will be interesting to see whether improved molecular responses seen in the SPIRIT trial will translate into superior long-term outcome. Finally, a small study from Germany suggests that alpha IFN maintenance might sustain CCyR and MMR achieved during induction with imatinib and IFN.

Conclusions

In chronic-phase CML, treatment decisions today are mainly based on imatinib response-related prognostic factors that predict outcome and can be determined by routine monitoring. The challenge of the coming years will be to integrate nilotinib and dasatinib in the first-line treatment in a reasonable and also cost-effective way. Unfortunately current and future trials are intended to replace imatinib rather than to complement it. On the other hand, the availability of nilotinib and dasatinib simplifies matters in patients that do not achieve optimal response to imatinib. The ELN categories of suboptimal response and failure might well amalgamate since the outcome of both categories is similar, and a switch to nilotinib or dasatinib for the majority of patients failing imatinib would be more appropriate than to increase the imatinib dose. The second challenge will be the identification of patients that may safely temporarily or permanently discontinue ABL kinase inhibitor treatment. In the future, the majority of CML patients may not die from their leukaemia, and some of them may not do so even after treatment discontinuation.

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References

Abruzzese, E., Gozzetti, A., Galimberti, S., Trawinska, M.M., Caravita, T., Siniscalchi, A. *et al.* (2007) Characterization of Ph-negative abnormal clones emerging during imatinib therapy. *Cancer* 109: 2466–2472.

Alvarado, Y., Kantarjian, H., O'Brien, S., Faderl, S., Borthakur, G., Burger, J. *et al.* (2009) Significance of suboptimal response to imatinib, as defined by the European LeukemiaNet, in the long-term outcome of patients with early chronic myeloid leukemia in chronic phase. *Cancer* 115: 3709–3718.

Apperley, J.F., Cortes, J.E., Kim, D.W., Roy, L., Roboz, G.J., Rosti, G. *et al.* (2009) Dasatinib in the Treatment of Chronic Myeloid Leukemia in Accelerated Phase After Imatinib Failure: The START A Trial. *J Clin Oncol* 27(21): 3472–3479.

Baccarani, M., Cortes, J., Pane, F., Niederwieser, D., Saglio, G., Apperley, J. *et al.* (2009a) Chronic myeloid leukemia: an update of concepts and management recommendations of European LeukemiaNet. *J Clin Oncol* 27: 6041–6051.

Baccarani, M., Rosti, G., Castagnetti, F., Haznedaroglu, I., Porkka, K., Abruzzese, E. *et al.* (2009b) Comparison of imatinib 400 mg and 800 mg daily in the front-line treatment of high-risk, Philadelphia-positive chronic myeloid leukemia: a European LeukemiaNet Study. *Blood* 113: 4497–4504.

Baccarani, M., Rosti, G., de Vivo, A., Bonifazi, F., Russo, D., Martinelli, G. *et al.* (2002) A randomized study of interferon-alpha *versus* interferon-alpha and low-dose arabinosyl cytosine in chronic myeloid leukemia. *Blood* 99: 1527–1535.

Baccarani, M., Saglio, G., Goldman, J., Hochhaus, A., Simonsson, B., Appelbaum, F. *et al.* (2006) Evolving concepts in the management of chronic myeloid leukemia. Recommendations from an expert panel on behalf of the European Leukemianet. *Blood* 108: 1809–1820.

Beran, M., Cao, X., Estrov, Z., Jeha, S., Jin, G., O'Brien, S. *et al.* (1998) Selective inhibition of cell proliferation and BCR-ABL phosphorylation in acute lymphoblastic leukemia cells expressing Mr 190,000 BCR-ABL protein by a tyrosine kinase inhibitor (CGP-57148). *Clin Cancer Res* 4: 1661–1672.

Bonifazi, F., de Vivo, A., Rosti, G., Guilhot, F., Guilhot, J., Trabacchi, E. *et al.* (2001) Chronic myeloid leukemia and interferon-alpha: a study of complete cytogenetic responders. *Blood* 98: 3074–3081.

Bradeen, H.A., Eide, C.A., O'Hare, T., Johnson, K.J., Willis, S.G., Lee, F.Y. *et al.* (2006) Comparison of imatinib mesylate, dasatinib (BMS-354825), and nilotinib (AMN107) in an N-ethyl-N-nitrosourea (ENU)-based mutagenesis screen: high efficacy of drug combinations. *Blood* 108: 2332–2338.

Branford, S., Cross, N.C., Hochhaus, A., Radich, J., Saglio, G., Kaeda, J. *et al.* (2006) Rationale for the recommendations for harmonizing current methodology for detecting BCR-ABL transcripts in patients with chronic myeloid leukaemia. *Leukemia* 20: 1925–1930.

Branford, S., Hughes, T.P. and Rudzki, Z. (1999) Monitoring chronic myeloid leukaemia therapy by real-time quantitative PCR in blood is a reliable alternative to bone marrow cytogenetics. *Br J Haematol* 107: 587–599.

Branford, S., Rudzki, Z., Walsh, S., Grigg, A., Arthur, C., Taylor, K. *et al.* (2002) High frequency of point mutations clustered within the adenosine triphosphate-binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (STI571) resistance. *Blood* 99: 3472–3475.

Buchdunger, E., Cioffi, C.L., Law, N., Stover, D., Ohno-Jones, S., Druker, B.J. *et al.* (2000) Abl protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-kit and platelet-derived growth factor receptors. *J Pharmacol Exp Ther* 295: 139–145.

Burgess, M.R., Skaggs, B.J., Shah, N.P., Lee, F.Y. and Sawyers, C.L. (2005) Comparative analysis of two clinically active BCR-ABL kinase inhibitors reveals the role of conformation-specific binding in resistance. *Proc Natl Acad Sci U S A* 102: 3395–3400.

Chronic Myeloid Leukemia Trialists' Collaborative Group (1997) Interferon alfa *versus* chemotherapy for chronic myeloid leukemia: a meta-analysis of seven randomized trials. *J Natl Cancer Inst* 89: 1616–1620.

Chronic Myeloid Leukemia Trialists' Collaborative Group (2000) Hydroxyurea *versus* busulphan for chronic myeloid leukaemia: an individual patient data meta-analysis of three randomized trials. *Br J Haematol* 110: 573–576.

Cortes, J., Jabbour, E., Kantarjian, H., Yin, C.C., Shan, J., O'Brien, S. *et al.* (2007) Dynamics of BCR-ABL kinase domain mutations in chronic myeloid leukemia after sequential treatment with multiple tyrosine kinase inhibitors. *Blood* 110: 4005–4011.

Cortes, J., Kim, D.W., Raffoux, E., Martinelli, G., Ritchie, E., Roy, L. *et al.* (2008) Efficacy and safety of dasatinib in imatinib-resistant or -intolerant patients with chronic myeloid leukemia in blast phase. *Leukemia* 22: 2176–2183.

Cortes, J.E., Talpaz, M., Giles, F., O'Brien, S., Rios, M.B., Shan, J. *et al.* (2003) Prognostic significance of cytogenetic clonal evolution in patients with chronic myelogenous leukemia on imatinib mesylate therapy. *Blood* 101: 3794–3800.

Cortes, J., Talpaz, M., O'Brien, S., Jones, D., Luthra, R., Shan, J. *et al.* (2005) Molecular responses in patients with chronic myelogenous leukemia in chronic phase treated with imatinib mesylate. *Clin Cancer Res* 11: 3425–3432.

Crossman, L.C., Druker, B.J., Deininger, M.W., Pirmohamed, M., Wang, L. and Clark, R.E. (2005) hOCT 1 and resistance to imatinib. *Blood* 106: 1133–1134, author reply 1134.

Daley, G.Q., Van Etten, R.A. and Baltimore, D. (1990) Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. *Science* 247: 824–830.

Darkow, T., Henk, H.J., Thomas, S.K., Feng, W., Baladi, J.F., Goldberg, G.A. *et al.* (2007) Treatment interruptions and non-adherence with imatinib and associated healthcare costs: a retrospective analysis among managed care patients with chronic myelogenous leukaemia. *Pharmacoeconomics* 25: 481–496.

de Lavallade, H., Apperley, J.F., Khorashad, J.S., Milojkovic, D., Reid, A.G., Bua, M. *et al.* (2008) Imatinib for newly diagnosed patients with chronic myeloid leukemia: incidence of sustained responses in an intention-to-treat analysis. *J Clin Oncol* 26: 3358–3363.

Deininger, M., O'Brien S, G., Guilhot, F., Goldman, J., Hochhaus, A., Hughes, T. *et al.* (2009) International randomized study of interferon vs STI571 (IRIS) 8-year follow up: sustained survival and low risk for progression or events in patients with newly diagnosed chronic myeloid leukemia in chronic phase (CML-CP) treated with imatinib. In: *Proceedings of the 51st ASH Annual Meeting*, New Orleans, LA. *Blood* 114: 462a.

Deininger, M.W., Cortes, J., Paquette, R., Park, B., Hochhaus, A., Baccarani, M. *et al.* (2007) The prognosis for patients with chronic myeloid leukemia who have clonal cytogenetic abnormalities in Philadelphia chromosome-negative cells. *Cancer* 110: 1509–1519.

Deininger, M.W., McGreevey, L., Willis, S., Bainbridge, T.M., Druker, B.J. and Heinrich, M.C. (2004) Detection of ABL kinase domain mutations with denaturing high-performance liquid chromatography. *Leukemia* 18: 864–871.

Demetri, G.D., Wang, Y., Wehrle, E., Racine, A., Nikolova, Z., Blanke, C.D. *et al.* (2009) Imatinib plasma levels are correlated with clinical benefit in patients with unresectable/metastatic gastrointestinal stromal tumors. *J Clin Oncol* 27: 3141–3147.

Diaz-Blanco, E., Bruns, I., Neumann, F., Fischer, J.C., Graef, T., Rosskopf, M. *et al.* (2007) Molecular signature of CD34(+) hematopoietic stem and progenitor cells of patients with CML in chronic phase. *Leukemia* 21: 494–504.

Druker, B.J., Guilhot, F., O'Brien, S.G., Gathmann, I., Kantarjian, H., Gattermann, N. *et al.* (2006) Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *N Engl J Med* 355: 2408–2417.

Druker, B.J., Sawyers, C.L., Kantarjian, H., Resta, D.J., Reese, S.F., Ford, J.M. *et al.* (2001) Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N Engl J Med* 344: 1038–1042.

Druker, B.J., Tamura, S., Buchdunger, E., Ohno, S., Segal, G.M., Fanning, S. *et al.* (1996) Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat Med* 2: 561–566.

Ernst, T., Gruber, F.X., Pelz-Ackermann, O., Maier, J., Pfirrmann, M., Muller, M.C. *et al.* (2009) A co-operative evaluation of different methods of detecting BCR-ABL kinase domain mutations in patients with chronic myeloid leukemia on second-line dasatinib or nilotinib therapy after failure of imatinib. *Haematologica* 94: 1227–1235.

Faderl, S., Talpaz, M., Estrov, Z., O'Brien, S., Kurzrock, R. and Kantarjian, H.M. (1999) The biology of chronic myeloid leukemia. *N Engl J Med* 341: 164–172.

Fava, C., Kantarjian, H.M., Jabbour, E., O'Brien, S., Jain, N., Rios, M.B. *et al.* (2009) Failure to achieve a complete hematologic response at the time of a major cytogenetic response with second-generation tyrosine kinase inhibitors is associated with a poor prognosis among patients with chronic myeloid leukemia in accelerated or blast phase. *Blood* 113: 5058–5063.

Forrest, D.L., Trainor, S., Brinkman, R.R., Barnett, M.J., Hogge, D.E., Nevill, T.J. *et al.* (2009) Cytogenetic and molecular responses to standard-dose imatinib in chronic myeloid leukemia are correlated with Sokal risk scores and duration of therapy but not trough imatinib plasma levels. *Leuk Res* 33: 271–275.

Frank, O., Brors, B., Fabarius, A., Li, L., Haak, M., Merk, S. *et al.* (2006) Gene expression signature of primary imatinib-resistant chronic myeloid leukemia patients. *Leukemia* 20: 1400–1407.

Giannoudis, A., Davies, A., Lucas, C.M., Harris, R.J., Pirmohamed, M. and Clark, R.E. (2008) Effective dasatinib uptake may occur without human organic cation transporter 1 (hOCT1): implications for the treatment of imatinib-resistant chronic myeloid leukemia. *Blood* 112: 3348–3354.

Giles, F., Larson, R., Kantarjian, H., le Coutre, P., Baccarani, M., Haque, A. *et al.* (2007) Nilotinib in Patients (pts) with Philadelphia Chromosome-Positive (Ph+) Chronic Myelogenous Leukemia in Blast Crisis (CML-BC) Who Are Resistant or Intolerant to Imatinib. In: *Proceedings of the 49th ASH Annual Meeting*, Atlanta, GA. *Blood* 110: 310a.

Gorre, M.E., Mohammed, M., Ellwood, K., Hsu, N., Paquette, R., Rao, P.N. *et al.* (2001) Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science* 293: 876–880.

Guilhot, F., Apperley, J., Kim, D.W., Bullorsky, E.O., Baccarani, M., Roboz, G.J. *et al.* (2007) Dasatinib induces significant hematologic and cytogenetic responses in patients with imatinib-resistant or -intolerant chronic myeloid leukemia in accelerated phase. *Blood* 109: 4143–4150.

Guilhot, F., Chastang, C., Michallet, M., Guerci, A., Harousseau, J.L., Maloisel, F. *et al.* (1997) Interferon alfa-2b combined with cytarabine *versus* interferon alone in chronic myelogenous leukemia. French Chronic Myeloid Leukemia Study Group. *N Engl J Med* 337: 223–229.

Hasford, J., Pfirrmann, M., Hehlmann, R., Allan, N.C., Baccarani, M., Kluin-Nelemans, J.C. *et al.* (1998) A new prognostic score for survival of patients with chronic myeloid leukemia treated with interferon alfa. Writing Committee for the Collaborative CML Prognostic Factors Project Group. *J Natl Cancer Inst* 90: 850–858.

Hehlmann, R., Berger, U., Pfirrmann, M., Hochhaus, A., Metzgeroth, G., Maywald, O. *et al.* (2003) Randomized comparison of interferon alpha and hydroxyurea with hydroxyurea monotherapy in chronic myeloid leukemia (CML-study II): prolongation of survival by the combination of interferon alpha and hydroxyurea. *Leukemia* 17: 1529–1537.

Hehlmann, R., Heimpel, H., Hasford, J., Kolb, H.J., Pralle, H., Hossfeld, D.K. *et al.* (1994) Randomized comparison of interferon-alpha with busulfan and hydroxyurea in chronic myelogenous leukemia. The German CML Study Group. *Blood* 84: 4064–4077.

Hehlmann, R., Jung-Munkwitz, S., Lauseker, M., Leitner, A., Pletsch, N., Shazi, S. *et al.* (2009) Randomized comparison of imatinib 800 mg vs. imatinib 400 mg +/- IFN in newly diagnosed BCR/ABL positive chronic phase CML: analysis of molecular remission at 12 months; The German CML-Study IV. In: *Proceedings of the 51st ASH Annual Meeting*, New Orleans, LA. *Blood* 114: 143–144a.

Hiwase, D.K., Saunders, V., Hewett, D., Frede, A., Zrim, S., Dang, P. *et al.* (2008) Dasatinib cellular uptake and efflux in chronic myeloid leukemia cells: therapeutic implications. *Clin Cancer Res* 14: 3881–3888.

Hochhaus, A., Baccarani, M., Deininger, M., Apperley, J.F., Lipton, J.H., Goldberg, S.L. *et al.* (2008a) Dasatinib induces durable cytogenetic responses in patients with chronic myelogenous leukemia in chronic phase with resistance or intolerance to imatinib. *Leukemia* 22: 1200–1206.

Hochhaus, A., Druker, B., Sawyers, C., Guilhot, F., Schiffer, C.A., Cortes, J. *et al.* (2008b) Favorable long-term follow-up results over 6 years for response, survival, and safety with imatinib mesylate therapy in chronic-phase chronic myeloid leukemia after failure of interferon-{alpha} treatment. *Blood* 111: 1039–1043.

Hochhaus, A., Fischer, T. and Beelen, D.W. (2006) Empfehlungen der DGHO für die Diagnostik und Therapie hämatologischer und onkologischer Erkrankungen. Chronische Myeloische Leukämie. http://www.dgho.de.

Hochhaus, A., Kreil, S., Corbin, A.S., La Rosee, P., Muller, M.C., Lahaye, T. *et al.* (2002) Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. *Leukemia* 16: 2190–2196.

Hochhaus, A., O'Brien, S.G., Guilhot, F., Druker, B.J., Branford, S., Foroni, L. *et al.* (2009) Six-year follow-up of patients receiving imatinib for the first-line treatment of chronic myeloid leukemia. *Leukemia* 23: 1054–1061.

Hughes, T., Deininger, M., Hochhaus, A., Branford, S., Radich, J., Kaeda, J. *et al.* (2006) Monitoring CML patients responding to treatment with tyrosine kinase

inhibitors: review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. *Blood* 108: 28–37.

Hughes, T., Hochhaus, A., Branford, S., Müller, M.C., Foroni, L., Druker, B. *et al.* (2008) Reduction of BCR-ABL transcript levels at 6, 12, and 18 months (mo) correlates with long-term outcomes on imatinib (IM) at 72 mo: an analysis from the International Randomized Study of Interferon *versus* STI571 (IRIS) in patients (pts) with chronic phase chronic myeloid leukemia (CML-CP). In: *Proceedings of the 50th ASH Annual Meeting*, San Francisco, CA. *Blood* 112: 129–130a.

Hughes, T.P., Hochhaus, A., Branford, S., Müller, M.C., Kaeda, J.S., Foroni, L. *et al.* (2010) Long-term prognostic significance of early molecular response to imatinib in newly diagnosed chronic myeloid leukemia: an analysis from the International Randomized Study of Interferon and STI571 (IRIS). *Blood* 116(19): 3758–3765.

Hughes, T.P., Kaeda, J., Branford, S., Rudzki, Z., Hochhaus, A., Hensley, M.L. *et al.* (2003) Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. *N Engl J Med* 349: 1423–1432.

Iacobucci, I., Saglio, G., Rosti, G., Testoni, N., Pane, F., Amabile, M. *et al.* (2006) Achieving a major molecular response at the time of a complete cytogenetic response (CCgR) predicts a better duration of CCgR in imatinib-treated chronic myeloid leukemia patients. *Clin Cancer Res* 12: 3037–3042.

Irving, J.A., O'Brien, S., Lennard, A.L., Minto, L., Lin, F. and Hall, A.G. (2004) Use of denaturing HPLC for detection of mutations in the BCR-ABL kinase domain in patients resistant to Imatinib. *Clin Chem* 50: 1233–1237.

Jabbour, E., Kantarjian, H.M., Abruzzo, L.V., O'Brien, S., Garcia-Manero, G., Verstovsek, S. *et al.* (2007) Chromosomal abnormalities in Philadelphia chromosome negative metaphases appearing during imatinib mesylate therapy in patients with newly diagnosed chronic myeloid leukemia in chronic phase. *Blood* 110: 2991–2995.

Jabbour, E., Kantarjian, H., O'Brien, S., Shan, J., Garcia-Manero, G., Wierda, W. *et al.* (2011) Predictive factors for outcome and response in patients treated with second-generation tyrosine kinase inhibitors for chronic myeloid leukemia in chronic phase after imatinib failure. *Blood* 117(6): 1822–1827.

Kantarjian, H.M., Cortes, J., O'Brien, S., Giles, F.J., Albitar, M., Rios, M.B. *et al.* (2002b) Imatinib mesylate (STI571) therapy for Philadelphia chromosome-positive chronic myelogenous leukemia in blast phase. *Blood* 99: 3547–3553.

Kantarjian, H.M., Giles, F.J., Bhalla, K.N., Pinilla-Ibarz, J., Larson, R.A., Gattermann, N. *et al.* (2011) Nilotinib is effective in patients with chronic myeloid leukemia in chronic phase after imatinib resistance or intolerance: 24-month follow-up results. *Blood* 117: 1141–1145.

Kantarjian, H.M., Giles, F., Gattermann, N., Bhalla, K., Alimena, G., Palandri, F. *et al.* (2007b) Nilotinib (formerly AMN107), a highly selective BCR-ABL tyrosine kinase inhibitor, is effective in patients with Philadelphia chromosome-positive chronic myelogenous leukemia in chronic phase following imatinib resistance and intolerance. *Blood* 110: 3540–3546.

Kantarjian, H.M., O'Brien, S., Cortes, J.E., Smith, T.L., Rios, M.B., Shan, J. *et al.* (2002c) Treatment of Philadelphia chromosome-positive, accelerated-phase chronic myelogenous leukemia with imatinib mesylate. *Clin Cancer Res* 8: 2167–2176.

Kantarjian, H., O'Brien, S., Shan, J., Huang, X., Garcia-Manero, G., Faderl, S. *et al.* (2008) Cytogenetic and molecular responses and outcome in chronic myelogenous leukemia: need for new response definitions? *Cancer* 112: 837–845.

Kantarjian, H., Pasquini, R., Hamerschlak, N., Rousselot, P., Holowiecki, J., Jootar, S. *et al.* (2007a) Dasatinib or high-dose imatinib for chronic-phase chronic myeloid leukemia after failure of first-line imatinib: a randomized phase 2 trial. *Blood* 109: 5143–5150.

Kantarjian, H., Sawyers, C., Hochhaus, A., Guilhot, F., Schiffer, C., Gambacorti-Passerini, C. *et al.* (2002a) Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *N Engl J Med* 346: 645–652.

Kantarjian, H., Shah, N.P., Hochhaus, A., Cortes, J., Shah, S., Ayala, M. *et al.* (2010a) Dasatinib *versus* imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med* 362: 2260–2270.

Kantarjian, H.M., Shan, J., Jones, D., O'Brien, S., Rios, M.B., Jabbour, E. *et al.* (2009) Significance of increasing levels of minimal residual disease in patients with Philadelphia chromosome-positive chronic myelogenous leukemia in complete cytogenetic response. *J Clin Oncol* 27(22): 3659–3663.

Kantarjian, H.M., Smith, T.L., McCredie, K.B., Keating, M.J., Walters, R.S., Talpaz, M. *et al.* (1985) Chronic myelogenous leukemia: a multivariate analysis of the associations of patient characteristics and therapy with survival. *Blood* 66: 1326–1335.

Kantarjian, H., Talpaz, M., O'Brien, S., Giles, F., Faderl, S., Verstovsek, S. *et al.* (2005) Survival benefit with imatinib mesylate therapy in patients with accelerated-phase chronic myelogenous leukemia—comparison with historic experience. *Cancer* 103: 2099–2108.

Kovitz, C., Kantarjian, H., Garcia-Manero, G., Abruzzo, L.V. and Cortes, J. (2006) Myelodysplastic syndromes and acute leukemia developing after imatinib mesylate therapy for chronic myeloid leukemia. *Blood* 108: 2811–2813.

Larson, R.A., Druker, B.J., Guilhot, F., O'Brien, S.G., Riviere, G.J., Krahnke, T. *et al.* (2008) Imatinib pharmacokinetics and its correlation with response and

safety in chronic-phase chronic myeloid leukemia: a subanalysis of the IRIS study. *Blood* 111: 4022–4028.

le Coutre, P., Giles, F., Hochhaus, A., Apperley, J., Ossenkoppele, G., Haque, A. *et al.* (2008) Nilotinib in chronic myeloid leukemia patients in accelerated phase (CML-AP) with imatinib resistance or intolerance: 2-year follow-up results of a phase 2 study. In: *Proceedings of the 50th ASH Annual Meeting*, San Francisco, CA. *Blood* 112: 1108–1109A.

le Coutre, P., Mologni, L., Cleris, L., Marchesi, E., Buchdunger, E., Giardini, R. *et al.* (1999) In vivo eradication of human BCR/ABL-positive leukemia cells with an ABL kinase inhibitor. *J Natl Cancer Inst* 91: 163–168.

le Coutre, P., Ottmann, O.G., Giles, F., Kim, D.W., Cortes, J., Gattermann, N. *et al.* (2007) Nilotinib (formerly AMN107), a highly selective BCR-ABL tyrosine kinase inhibitor, is active in patients with imatinib-resistant or -intolerant accelerated phase chronic myelogenous leukemia. *Blood* 111: 1834–1839.

Lugo, T.G., Pendergast, A.M., Muller, A.J. and Witte, O.N. (1990) Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. *Science* 247: 1079–1082.

Mahon, F.X., Rea, D., Guilhot, J., Guilhot, F., Huguet, F., Nicolini, F. *et al.* (2010) Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial. *Lancet Oncol* 11: 1029–1035.

Marin, D., Bazeos, A., Mahon, F.X., Eliasson, L., Milojkovic, D., Bua, M. *et al.* (2010) Adherence is the critical factor for achieving molecular responses in patients with chronic myeloid leukemia who achieve complete cytogenetic responses on imatinib. *J Clin Oncol* 28: 2381–2388.

Marin, D., Milojkovic, D., Olavarria, E., Khorashad, J.S., de Lavallade, H., Reid, A.G. *et al.* (2008) European LeukemiaNet criteria for failure or suboptimal response reliably identify patients with CML in early chronic phase treated with imatinib whose eventual outcome is poor. *Blood* 112: 4437–4444.

Marktel, S., Marin, D., Foot, N., Szydlo, R., Bua, M., Karadimitris, A. *et al.* (2003) Chronic myeloid leukemia in chronic phase responding to imatinib: the occurrence of additional cytogenetic abnormalities predicts disease progression. *Haematologica* 88: 260–267.

Milojkovic, D., Nicholson, E., Apperley, J.F., Holyoake, T.L., Shepherd, P., Drummond, M.W. *et al.* (2010) Early prediction of success or failure of treatment with second-generation tyrosine kinase inhibitors in patients with chronic myeloid leukemia. *Haematologica* 95: 224–231.

NCCN (2010) NCCN Clinical Practice Guidelines in Oncology. Chronic Myelogenous Leukemia, Version 2.2010. National Comprehensive Cancer Network, http://www.nccn.org.

Nowell, P.C. and Hungerford, D. (1960) A minute chromosome in human granulocytic leukaemia. *Science* 132: 1497–1501.

O'Brien, S.G., Guilhot, F., Larson, R.A., Gathmann, I., Baccarani, M., Cervantes, F. *et al.* (2003) Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med* 348: 994–1004.

O'Hare, T., Walters, D.K., Stoffregen, E.P., Jia, T., Manley, P.W., Mestan, J. *et al.* (2005) In vitro activity of Bcr-Abl inhibitors AMN107 and BMS-354825 against clinically relevant imatinib-resistant Abl kinase domain mutants. *Cancer Res* 65: 4500–4505.

Ottmann, O.G., Druker, B.J., Sawyers, C.L., Goldman, J.M., Reiffers, J., Silver, R.T. *et al.* (2002) A phase 2 study of imatinib in patients with relapsed or refractory Philadelphia chromosome-positive acute lymphoid leukemias. *Blood* 100: 1965–1971.

Palandri, F., Castagnetti, F., Alimena, G., Testoni, N., Breccia, M., Luatti, S. *et al.* (2009) The long-term durability of cytogenetic responses in patients with accelerated phase chronic myeloid leukemia treated with imatinib 600 mg: the GIMEMA CML Working Party experience after a 7-year follow-up. *Haematologica* 94: 205–212.

Palandri, F., Castagnetti, F., Iacobucci, I., Martinelli, G., Amabile, M., Gugliotta, G. *et al.* (2010) The response to imatinib and interferon-alpha is more rapid than the response to imatinib alone: a retrospective analysis of 495 Philadelphia-positive chronic myeloid leukemia patients in early chronic phase. *Haematologica* 95: 1415–1419.

Palandri, F., Castagnetti, F., Testoni, N., Luatti, S., Marzocchi, G., Bassi, S. *et al.* (2008) Chronic myeloid leukemia in blast crisis treated with imatinib 600 mg: outcome of the patients alive after a 6-year follow-up. *Haematologica* 93: 1792–1796.

Picard, S., Titier, K., Etienne, G., Teilhet, E., Ducint, D., Bernard, M.A. *et al.* (2007) Trough imatinib plasma levels are associated with both cytogenetic and molecular responses to standard-dose imatinib in chronic myeloid leukemia. *Blood* 109: 3496–3499.

Press, R.D., Galderisi, C., Yang, R., Rempfer, C., Willis, S.G., Mauro, M.J. *et al.* (2007) A half-log increase in BCR-ABL RNA predicts a higher risk of relapse in patients with chronic myeloid leukemia with an imatinib-induced complete cytogenetic response. *Clin Cancer Res* 13: 6136–6143.

Preudhomme, C., Guilhot, J., Nicolini, F.E., Guerci-Bresler, A., Rigal-Huguet, F., Maloisel, F. *et al.* (2010) Imatinib plus peginterferon alfa-2a in chronic myeloid leukemia. *N Engl J Med* 363: 2511–2521.

Radich, J.P., Dai, H., Mao, M., Oehler, V., Schelter, J., Druker, B. *et al.* (2006) Gene expression changes associated with progression and response in chronic myeloid leukemia. *Proc Natl Acad Sci U S A* 103: 2794–2799.

Ray, A., Cowan-Jacob, S.W., Manley, P.W., Mestan, J. and Griffin, J.D. (2007) Identification of BCR-ABL

point mutations conferring resistance to the Abl kinase inhibitor AMN107 (nilotinib) by a random mutagenesis study. *Blood* 109: 5011–5015.

Roche-Lestienne, C., Soenen-Cornu, V., Grardel-Duflos, N., Lai, J.L., Philippe, N., Facon, T. *et al.* (2002) Several types of mutations of the Abl gene can be found in chronic myeloid leukemia patients resistant to STI571, and they can pre-exist to the onset of treatment. *Blood* 100: 1014–1018.

Ross, D.M., Branford, S., Moore, S. and Hughes, T.P. (2006) Limited clinical value of regular bone marrow cytogenetic analysis in imatinib-treated chronic phase CML patients monitored by RQ-PCR for BCR-ABL. *Leukemia* 20: 664–670.

Rowley, J.D. (1973) Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature* 243: 290–293.

Roy, L., Guilhot, J., Krahnke, T., Guerci-Bresler, A., Druker, B.J., Larson, R.A. *et al.* (2006) Survival advantage from imatinib compared with the combination interferon-alpha plus cytarabine in chronic-phase chronic myelogenous leukemia: historical comparison between two phase 3 trials. *Blood* 108: 1478–1484.

Saglio, G., Abruzzese, E., Alimena, G., Bocchia, M., Carella, A., Fanin, R. *et al.* (2009) Prospective exploratory phase II studies of a rotating regime of nilotinib and imatinib for frontline treatment of Philadelphia POSITIVE (Ph+) chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL). In: *Proceedings of the 51st ASH Annual Meeting Abstracts*, New Orleans, LA. *Blood* 114: 4972.

Saglio, G., Kim, D.W., Issaragrisil, S., le Coutre, P., Etienne, G., Lobo, C. *et al.* (2010) Nilotinib *versus* imatinib for newly diagnosed chronic myeloid leukemia. *N Engl J Med* 362: 2251–2259.

Sawyers, C.L. (1999) Chronic myeloid leukemia. *N Engl J Med* 340: 1330–1340.

Sawyers, C.L., Hochhaus, A., Feldman, E., Goldman, J.M., Miller, C.B., Ottmann, O.G. *et al.* (2002) Imatinib induces hematologic and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crisis: results of a phase II study. *Blood* 99: 3530–3539.

Shah, N.P., Kantarjian, H.M., Kim, D.W., Rea, D., Dorlhiac-Llacer, P.E., Milone, J.H. *et al.* (2008) Intermittent target inhibition with dasatinib 100 mg once daily preserves efficacy and improves tolerability in imatinib-resistant and -intolerant chronic-phase chronic myeloid leukemia. *J Clin Oncol* 26: 3204–3212.

Shah, N.P., Nicoll, J.M., Nagar, B., Gorre, M.E., Paquette, R.L., Kuriyan, J. *et al.* (2002) Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. *Cancer Cell* 2: 117–125.

Shah, N.P., Tran, C., Lee, F.Y., Chen, P., Norris, D. and Sawyers, C.L. (2004) Overriding imatinib resistance with a novel ABL kinase inhibitor. *Science* 305: 399–401.

Sherbenou, D.W., Wong, M.J., Humayun, A., McGreevey, L.S., Harrell, P., Yang, R. *et al.* (2007) Mutations of the BCR-ABL-kinase domain occur in a minority of patients with stable complete cytogenetic response to imatinib. *Leukemia* 21: 489–493.

Sokal, J.E., Cox, E.B., Baccarani, M., Tura, S., Gomez, G.A., Robertson, J.E. *et al.* (1984) Prognostic discrimination in "good-risk" chronic granulocytic leukemia. *Blood* 63: 789–799.

Sokal, J.E., Gomez, G.A., Baccarani, M., Tura, S., Clarkson, B.D., Cervantes, F. *et al.* (1988) Prognostic significance of additional cytogenetic abnormalities at diagnosis of Philadelphia chromosome-positive chronic granulocytic leukemia. *Blood* 72: 294–298.

Soverini, S., Gnani, A., Colarossi, S., Castagnetti, F., Abruzzese, E., Paolini, S. *et al.* (2009) Philadelphia-positive patients who already harbor imatinib-resistant Bcr-Abl kinase domain mutations have a higher likelihood of developing additional mutations associated with resistance to second- or third-line tyrosine kinase inhibitors. *Blood* 114: 2168–2171.

Soverini, S., Martinelli, G., Amabile, M., Poerio, A., Bianchini, M., Rosti, G. *et al.* (2004) Denaturing-HPLC-based assay for detection of ABL mutations in chronic myeloid leukemia patients resistant to Imatinib. *Clin Chem* 50: 1205–1213.

Swolin, B., Weinfeld, A., Westin, J., Waldenstrom, J. and Magnusson, B. (1985) Karyotypic evolution in Ph-positive chronic myeloid leukemia in relation to management and disease progression. *Cancer Genet Cytogenet* 18: 65–79.

Talpaz, M., Kantarjian, H.M., McCredie, K., Trujillo, J.M., Keating, M.J. and Gutterman, J.U. (1986) Hematologic remission and cytogenetic improvement induced by recombinant human interferon alpha A in chronic myelogenous leukemia. *N Engl J Med* 314: 1065–1069.

Talpaz, M., Silver, R.T., Druker, B.J., Goldman, J.M., Gambacorti-Passerini, C., Guilhot, F. *et al.* (2002) Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a phase 2 study. *Blood* 99: 1928–1937.

Tam, C.S., Kantarjian, H., Garcia-Manero, G., Borthakur, G., O'Brien, S., Ravandi, F. *et al.* (2008) Failure to achieve a major cytogenetic response by 12 months defines inadequate response in patients receiving nilotinib or dasatinib as second or subsequent line therapy for chronic myeloid leukemia. *Blood* 112: 516–518.

The Italian Cooperative Study Group on Chronic Myeloid Leukemia. (1998) Long-term follow-up of the Italian trial of interferon-alpha *versus* conventional chemotherapy in chronic myeloid leukemia. *Blood* 92: 1541–1548.

Thomas, J., Wang, L., Clark, R.E. and Pirmohamed, M. (2004) Active transport of imatinib into and out of cells: implications for drug resistance. *Blood* 104: 3739–3745.

Titier, K., Picard, S., Ducint, D., Teilhet, E., Moore, N., Berthaud, P. *et al.* (2005) Quantification of imatinib in human plasma by high-performance liquid chromatography-tandem mass spectrometry. *Ther Drug Monit* 27: 634–640.

Villuendas, R., Steegmann, J.L., Pollan, M., Tracey, L., Granda, A., Fernandez-Ruiz, E. *et al.* (2006) Identification of genes involved in imatinib resistance in CML: a gene-expression profiling approach. *Leukemia* 20: 1047–1054.

von Bubnoff, N., Manley, P.W., Mestan, J., Sanger, J., Peschel, C. and Duyster, J. (2006) Bcr-Abl resistance screening predicts a limited spectrum of point mutations to be associated with clinical resistance to the Abl kinase inhibitor nilotinib (AMN107). *Blood* 108: 1328–1333.

von Bubnoff, N., Schneller, F., Peschel, C. and Duyster, J. (2002) BCR-ABL gene mutations in relation to clinical resistance of Philadelphia-chromosome-positive leukaemia to STI571: a prospective study. *Lancet* 359: 487–491.

Weisberg, E., Manley, P.W., Breitenstein, W., Bruggen, J., Cowan-Jacob, S.W., Ray, A. *et al.* (2005) Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. *Cancer Cell* 7: 129–141.

White, D.L., Dang, P., Engler, J., Frede, A., Zrim, S., Osborn, M. *et al.* (2010) Functional activity of the OCT-1 protein is predictive of long-term outcome in patients with chronic-phase chronic myeloid leukemia treated with imatinib. *J Clin Oncol* 28: 2761–2767.

White, D.L., Saunders, V.A., Dang, P., Engler, J., Venables, A., Zrim, S. *et al.* (2007b) Most CML patients who have a suboptimal response to imatinib have low OCT-1 activity: higher doses of imatinib may overcome the negative impact of low OCT-1 activity. *Blood* 110: 4064–4072.

White, D., Saunders, V., Grigg, A., Arthur, C., Filshie, R., Leahy, M.F. *et al.* (2007a) Measurement of in vivo BCR-ABL kinase inhibition to monitor imatinib-induced target blockade and predict response in chronic myeloid leukemia. *J Clin Oncol* 25: 4445–4451.

White, D.L., Saunders, V.A., Dang, P., Engler, J., Zannettino, A.C., Cambareri, A.C. *et al.* (2006) OCT-1-mediated influx is a key determinant of the intracellular uptake of imatinib but not nilotinib (AMN107): reduced OCT-1 activity is the cause of low in vitro sensitivity to imatinib. *Blood* 108: 697–704.

Willis, S.G., Lange, T., Demehri, S., Otto, S., Crossman, L., Niederwieser, D. *et al.* (2005) High-sensitivity detection of BCR-ABL kinase domain mutations in imatinib-naive patients: correlation with clonal cytogenetic evolution but not response to therapy. *Blood* 106: 2128–2137.

Zaccaria, A., Testoni, N., Valenti, A.M., Luatti, S., Tonelli, M., Marzocchi, G. *et al.* (2010) Chromosome abnormalities additional to the Philadelphia chromosome at the diagnosis of chronic myelogenous leukemia: pathogenetic and prognostic implications. *Cancer Genet Cytogenet* 199: 76–80.

Zaccaria, A., Valenti, A.M., Donti, E., Gozzetti, A., Ronconi, S. and Spedicato, F. (2007) Persistence of chromosomal abnormalities additional to the Philadelphia chromosome after Philadelphia chromosome disappearance during imatinib therapy for chronic myeloid leukemia. *Haematologica* 92: 564–565.

Zheng, C., Li, L., Haak, M., Brors, B., Frank, O., Giehl, M. *et al.* (2006) Gene expression profiling of CD34+ cells identifies a molecular signature of chronic myeloid leukemia blast crisis. *Leukemia* 20: 1028–1034.

Zimmermann, J., Buchdunger, E., Mett, H., Meyer, T., Lydon, N. and Traxler, P. (1996) (Phenylamino)pyrimidine (PAP) derivatives: a new class of potent and highly selective PDGF-receptor autophosphorylation inhibitors. *Bioorg Med Chem Lett* 6: 1221–1226.

Zimmermann, J., Buchdunger, E., Mett, H., Meyer, T. and Lydon, N.B. (1997) Potent and selective inhibitors of the ABL-kinase: phenylaminopyrimidine (PAP) derivatives. *Bioorg Med Chem Lett* 7: 187–192.