Monitoring treatment of chronic myeloid leukemia

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In this issue of the journal two articles deal with different but related aspects of monitoring the treatment of chronic myeloid leukemia (CML) in the era of tyrosine kinase inhibitor: the standardization of residual disease assays and the search for *cABL* kinase domain mutations.[1,2] In particular, the paper by Ernst *et al.* reports on the role of kinase domain mutations in the prognosis of CML patients. The article shows that these mutations are predictive of relapse and may be detectable in some cases several months before relapse. Their assay could, therefore, provide clinical benefit by prompting early therapeutic interventions.[1] Since the introduction of the first tyrosine kinase inhibitor, imatinib mesylate (Gleevec, Novartis Pharma), it has become clear that optimal treatment of patients was based on appropriate monitoring of the effect of therapy, which was emerged as a relevant and sensitive part of the therapeutic strategy. Nowadays other, second generation tyrosine kinase inhibitors have become available (Dasatinib, Sprycel, Bristol-Myers Squibb; Nilotinib, Tasigna, Novartis Pharma), and several other interesting agents are in advanced, clinical phase evaluation.[3-6] Furthermore, the options for treatment of CML are not limited to tyrosine kinase inhibitors. Allogeneic stem cell transplantation is still an established modality of treatment that can cure a substantial proportion of patients.[6,7] Interferon-α is very effective in patients at low risk and may help to improve the results obtained with tyrosine kinase inhibitors.[8,9] Other targeted agents are currently being investigated, and cytotoxic chemotherapy can still help in the management of some particular conditions.[5,6] The wide range of currently available treatment options increases both the complexity of the therapeutic strategy to ensure the highest quality of life and longest survival for all patients and the cost of the treatment itself. There is, therefore, an increasing need for ever more sophisticated and expensive techniques to monitor the results of treatment at molecular level. Consequently, it is difficult to interpret the esults of monitoring correctly outside a specialized and trained clinical setting. It should also be recognized that because of the rapid evolution of treatment and because there are many different therapeutic options for the patients who fail to benefit from imatinib, do not achieve a suboptimal response, or do not tolerate the drug, it is difficult to trace the boundaries between monitoring in clinical practice and in clinical research. Tracking the effects of treatment at a molecular level is essential to expand knowledge, and this is essential for planning the best treatment. The correct monitoring of treatment of CML, which nowadays comprises assays of imatinib as well as cytogenetic and molecular evaluation of residual disease and the search for *cABL* kinase domain mutations, should be performed at regular intervals. If possible, this should always carried out at the same laboratories, and standard procedures should be adopted each time. Response definitions should also be standardized to a common language. These issues, which have been highlighted and discussed in detail elsewhere,[10,11] are summarized in Table 1.

Cytogenetic monitoring

Monitoring the percentage of Philadelphia chromosome-positive cells is the best validated system for the assessment of the response to interferon-α and tyrosine kinase inhibitors, since the cytogenetic response is the best surrogate marker of survival.[3,6] For patients who achieve a complete cytogenetic response to interferon-α, the 10-year survival is about 75%.[8] For patients who achieve a complete cytogenetic response to imatinib, the 5-year survival rate is close to 100%.[12] The response is conventionally determined by chromosome banding analysis of marrow cell metaphases. A panel of experts appointed by the European LeukemiaNet recommended that at least two cultures should be performed, one for 24 hours and another for 48 hours.[13] The number of metaphases that is required to assess the response was arbitrarily fixed at 20.[3,8,11] Although it is obvious that the accuracy of the test may depend on metaphase number, we suggest that the definition of cytogenetic response, and particularly complete cytogenetic response, should be based more on confirmation of the test results than on metaphase number. Two sequential tests should, therefore, be performed, the second being confirmatory of the first. Furthermore, if there are fewer than 20 metaphases, the cytogenetic response can be validated by determining the level of *BCR-ABL* transcripts, as discussed in the next section, and by molecular cytogenetics, or fluorescence *in situ* hybridization (FISH). FISH can be performed on metaphases (high mitotic index metaphases or hypermetaphase FISH, HM-FISH,[14] or more frequently and more conveniently on interphase cells (IP-FISH).[15-18] Several reports strongly suggest that all FISH data correlate very significantly with chromosome banding data,[14-18] as well as with *BCR-ABL* transcript levels.[14,18] Moreover, FISH can detect deletions of the long arm of chromosome 9 and variant translocations. However, almost all studies reporting the results of the treatment of CML with interferon-α or tyrosine kinase inhibitors have used chromosome banding data, and have reported responses in terms of percentages of marrow cell metaphases.[3,8,11] There are no shared, standardized and validated definitions of the

cytogenetic response based on IP-FISH data. In particular, IP-FISH negativity may range from less than 1% to 5 or 6% depending on reagents and laboratories.[14-18] The GIMEMA CML Working Party performed a prospective study of more than 400 patients with CML receiving imatinib, comparing chromosome banding analysis with IP-FISH (*BCR-ABL* extra-signal probe, dual color-dual fusion probe, counting a minimum of 200 cells and defining the cytogenetic response as complete when the percentage of positive cells was less than 1%). Of 549 tests negative by chromosome banding analysis, 19% were positive by IP-FISH. Of 453 tests negative by IP-FISH, only 2% were positive according to chromosome banding analysis. These data suggest that IP-FISH is more sensitive than chromosome banding for the detection of Philadelphia positive cells. This is not surprising since 20 or more cells were investigated with chromosome banding, whereas 200 or more were used in IP-FISH. Therefore, IP-FISH could be used instead of chromosome banding analysis. It could also be more convenient because it is performed on blood cells, thus avoiding marrow aspiration. However, the issue of monitoring cytogenetic response with IP-FISH on blood cells is still open to debate; in fact, there are not enough studies reporting a comparison of IP-FISH on marrow and blood cells,[16] and chromosome banding analysis is necessary to detect additional chromosome abnormalities in Philadelphia-positive cells or other chromosome abnormalities in Philadelphia-negative cells. A patient who has not achieved or has lost a complete cytogenetic response should always be studied with chromosome banding analysis of marrow cells. The value of regular bone marrow cytogenetics in stable cytogenetic and molecular responders has been challenged.[19] These patients could be monitored by IP-FISH on blood cells unless otherwise suggested by clinical and laboratory findings, e.g. cytopenia or dysplasia.[5,20-22]

Monitoring BCR-ABL transcript levels

Given the limited significance of qualitative reverse transcription polymerase chain reaction (RT-PCR) in the follow-up of CML patients, several groups had developed quantitative PCR assays based on competitive PCR strategies to estimate the amount of residual disease in patients under interferon-α therapy or after allogeneic bone marrow transplantation who were able to achieve complete cytogenetic remission, but remained RT-PCR-positive.[23,24] The data obtained showed that the level of minimal residual disease correlated with the probability of relapse in complete cytogenetic responders to interefon as well as in patients who underwent allogeneic bone marrow transplatation.[25,26] In the latter group, competitive PCR was also used to adapt treatment and to determine the optimum time to initiate donor lymphocyte infusion and to monitor the response.[27,28] However, the competitive PCR methods were labor intensive, time-consuming, difficult to standardize and not suitable for large-scale analyses.

Table 1. Definitions of the responses to imatinib (slightly modified from ref. #10).

	Optimal response	*Suboptimal response*	*Failure*
3 months	CHR	<CHR	No HR
6 months	≥PCgR	Minor CgR	No CgR
12 months	CCgR	PCgR	<PCgR
18 months	MMolR	< MMolR	<CCgR
Any time	Stable CCgR and MMolR	Loss of MMolR[2] Mutations[1] ACA in Ph^+ cells[2]	Loss of HR Loss of CgR Mutations[1]

Complete hematologic response (CHR) is identified by a white blood cell count $<10x10^9$/L, a differential with <5% basophils and no immature granulocyte precursors, a platelet count $<450x10^9$/L and a non-palpable spleen. Cytogenetic response (CgR) is classified by chromosome banding analysis of at least 20 marrow cell metaphases as complete (no Ph^+), partial (1-34% Ph^+), minor (35-65% Ph^+), minimal (66-94% Ph^+), and none (95% Ph^+). Molecular response is defined as major (MMolR) if the ratio of BCR-ABL to the housekeeping gene is ≤ 0.1% according to the international scale (see text and ref. #9). [1]BCR-ABL kinase domain mutations with low (suboptimal) or high (failure) level of insensitivity to imatinib. [2]To be confirmed on two occasions, unless associated with HR or CgR loss.

More recently, real-time quantitative RT-PCR (RQ-PCR) assays were developed to monitor the kinetics of residual *BCR-ABL* transcripts over time.[29,30] Variables in the quantitative PCR assay (quality and quantity of the RNA and the reverse transcription step) may be controlled by quantification of transcripts of a control gene (*ABL*, *BCR*, *G6PD* or *β2-microglobulin*) as an internal standard.[31] Furthermore, a first standardization study and the introduction of rigorous, internationally accepted controls have meant that the results of RQ-PCR have become a robust basis for routine therapeutic decisions.[32] This has become essential in the imatinib era.

Molecular studies within the context of the IRIS study first showed that the amount of residual disease at 12 months, established by RQ-PCR in terms of log reduction of *BCR-ABL* transcripts with respect to the median value observed at diagnosis, is statistically significant in predicting event-free survival and the risk of disease progression for newly diagnosed CML patients achieving a complete cytogenetic response under imatinib therapy.[33] Indeed, achieving more than a 3-log reduction in the level of *BCR-ABL* transcripts (later defined as a major molecular remission) was found to identify a group of patients with very sporadic losses of response and virtually no risk of progression.[33] These results were further confirmed after 5 years of follow-up of the CML patients enrolled in the IRIS study.[12] Another important aspect (obtained outside the IRIS study in which only one sample after 12 months of therapy was scheduled) is that early reduction of *BCR-ABL* mRNA transcript levels may predict cytogenetic response in CML patients treated with imatinib, and that this parameter can also identify groups of patients with different risks of progression.[34] Further confirmation of these data could be particularly important since high dose imatinib has been shown to improve and to accelerate the achievement of complete cytogenetic response and of major molecular remission. However, whether earlier achievement of these responses is important for progres-

sion-free survival is at present not yet known.[5,35]

As a consequence of these data, RQ-PCR monitoring is now considered an integral part of the management of CML patients treated with imatinib (or with other tyrosine kinase inhibitors), and failure to achieve a major molecular response by 18 months after starting imatinib therapy is considered a suboptimal response requiring careful re-evaluation, and possible reassessment, of therapy.[11] However, diversity of molecular approaches could make comparison of results between different laboratories and studies problematic, and the need for harmonization of both procedures and expression of results has prompted international efforts to establish recommendations on the generation and interpretation of molecular data in CML. Various prerequisites to achieve optimal sensitivity and standardization have been agreed upon and published.[10,36-38] It was pointed out that, much more conveniently for patients, peripheral blood may replace bone marrow aspirates as the source of cells to be analyzed for minimal residual disease. However, to ensure the optimal sensitivity of the assay, a minimum amount of 10 mL of blood should be drawn from patients and used as the source of buffy coat cells for the analysis. In addition, an international reporting scale (IS) was proposed whereby the absolute *BCR-ABL* value representing major molecular response is standardized at *BCR-ABL* 0.1% (Figure 1).[10] A value of 1.0% is approximately equivalent to the achievement of a complete cytogenetic response.

Standardization will be constantly maintained with the aid of certified reference material; this is not yet available but represents a critical factor for the generation of comparable quantitative data worldwide. Such material is being developed and assessed by an international collaborative group on the initiative of the European LeukemiaNet.[38] However, the production of reference material for *BCR-ABL* analysis that closely mimics patients' samples is a difficult task and is made more problematic by the intrinsic instability of RNA. Lyophilization of material may overcome the problems of stability and the introduction of standardized kits for routine clinical use with high-quality performance characteristics will probably simplify the entire standardization process.[39] Finally, the use of novel cartridge-based microfluidic systems that incorporate RNA extraction, reverse transcription, and quantitative PCR, may further facilitate the harmonization and standardization processes.[40]

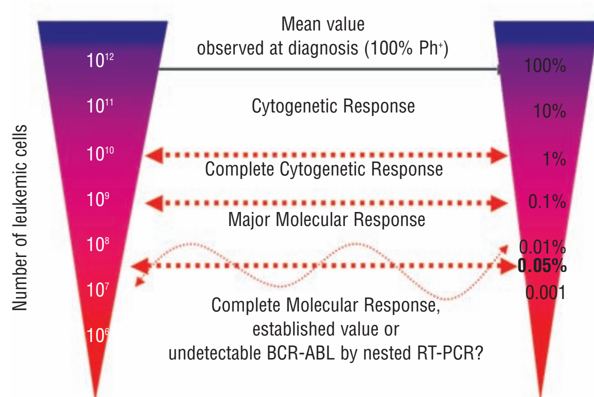


Figure 1. Relationship between the number of residual cells, the results of minimal residual disease assay and the cytogenetic response.

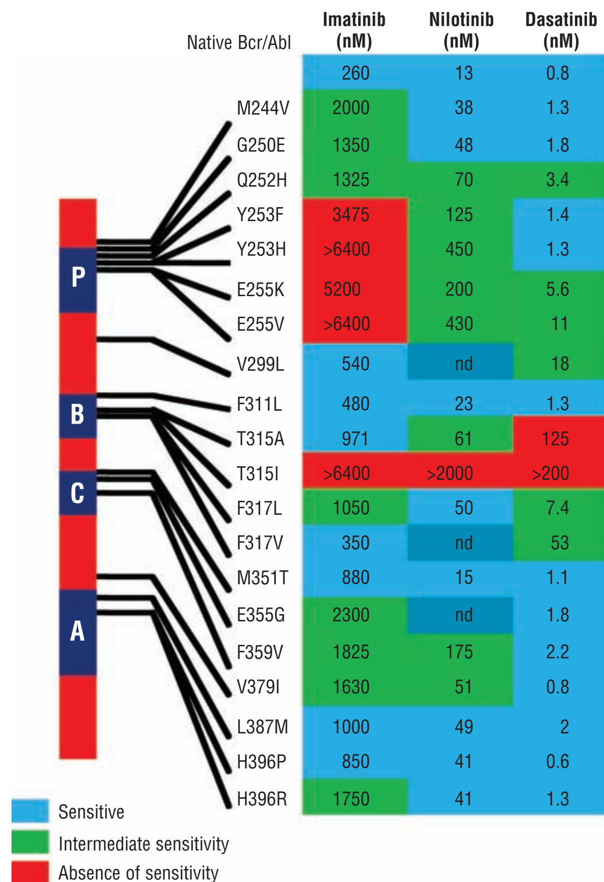
A higher rate of complete molecular responses (undetectable *BCR-ABL*) has been reported in patients receiving prolonged imatinib therapy. Complete molecular response must be better defined using strict PCR sensitivity criteria since this will probably help to identify patients with durable responses even after discontinuation of imatinib.[41] Although undetectable *BCR-ABL* does not necessarily mean eradication of leukemia, clinical trials with new kinase inhibitors of *BCR-ABL* will certainly establish complete molecular response as a clinical endpoint.

From a practical point of view, it has been suggested that RQ-PCR must be performed every 3 months even in patients who achieve a major molecular response.[10] Confirmed and sequential increases in *BCR-ABL* levels could indeed help to identify the patients who do not strictly follow the proposed therapy or the few patients with an impending relapse, triggering the search for *BCR-ABL* mutations.[42,43]

Finally, even considering that RQ-PCR is fundamental for monitoring patients with complete cytogenetic responses, there are other reasons to suggest that cytogenetics should not be replaced by RQ-PCR in the follow-up of CML patients. Additional chromosomal abnormalities present at diagnosis or arising during the disease may have a prognostic influence;[44] several studies have also reported the occurrence of clonal cytogenetic abnormalities in the Philadelphia-negative cells which appeared after suppression of the Philadelphia-positive clone by imatinib and, in a minority of cases, could also lead to the appearance of myelodysplastic hematopoiesis.

Monitoring BCR-ABL kinase domain mutations

A mutation at the kinase domain of the oncogenic BCR/ABL protein is frequently detected in patients with CML who fail to respond to tyrosine kinase inhibitors or lose the response. Overall this is the clinically dominant and best-studied mechanism of imatinib resistance, with most of the mutations having already been validated *in vitro* and profiled for resistance to imatinib, nilotinib and dasatinib.[45-49] Indeed, mutations at the kinase domain may impair or reduce the effect of imatinib and the other tyrosine kinase inhibitors on the BCR/ABL protein, i.e. their main target in Philadelphia-positive cells. Importantly, the sensitivity of the different kinase domain mutations to the three main tyrosine kinase inhibitors, i.e. imatinib, nilotinib and dasatinib, established using various experimental approaches, shows a high degree of variability. In general, the second genera-



	Imatinib (nM)	Nilotinib (nM)	Dasatinib (nM)
Native Bcr/Abl	260	13	0.8
M244V	2000	38	1.3
G250E	1350	48	1.8
Q252H	1325	70	3.4
Y253F	3475	125	1.4
Y253H	>6400	450	1.3
E255K	5200	200	5.6
E255V	>6400	430	11
V299L	540	nd	18
F311L	480	23	1.3
T315A	971	61	125
T315I	>6400	>2000	>200
F317L	1050	50	7.4
F317V	350	nd	53
M351T	880	15	1.1
E355G	2300	nd	1.8
F359V	1825	175	2.2
V379I	1630	51	0.8
L387M	1000	49	2
H396P	850	41	0.6
H396R	1750	41	1.3

Figure 2. Sensitivity of BCR/ABL kinase domain mutations to tyrosine kinase inhibitors. Values of sensitivity are given as IC$_{50}$.

tion drugs, nilotinib and dasatinib, display greater activity against the mutations than imatinib.[48,50] Mutations may be categorized into four groups, based upon the crystallographic structure of cABL: (i) those which directly impair imatinib, binding to the catalytic domain of the oncogenic protein; (ii) those within the ATP binding site; (iii) those within the activation loop, preventing the kinase from achieving the inactive conformation required for imatinib binding; and (iv) those within the catalytic domain (Figure 2).[47,50] Second generation tyrosine kinase inhibitors are structurally different from imatinib. Because of this, their profile of resistance to cABL kinase domain mutations does not overlap that of imatinib. Nilotinib derives from the imatinib scaffold with topological modifications that improve its ability to fit the BCR/ABL kinase domain conformations and its potency against this protein. This translates into stronger inhibitory activity against the majority of the common kinase domain mutations which cause imatinib resistance.[51,52] Dasatinib, which is able to bind the active conformation of the oncogenic protein, inhibits BCR/ABL with a 300-fold greater potency with respect to imatinib. Therefore, most mutations associated with resistance to this drug are predicted to involve drug contact residues.[53] Nevertheless, the second generation tyrosine kinase inhibitors have also been shown to select for specific types of resistant mutants both *in vitro*[54] and *in vivo*, particularly when used sequentially on the onset of resistance.[55-57]

It is important to underline that an *in vitro* confirmation (cell proliferation assay or biochemical assay on purified enzyme) is required to ascribe clinical resistance to a kinase domain mutation. A practical measure of the sensitivity of a given mutation to a tyrosine kinase inhibitor is its IC$_{50}$ for that specific inhibitor.[58-60] At least 73 mutations leading to 50 amino acid substitutions have been so far described in CML resistant to imatinib therapy (Figure 2), but an IC$_{50}$ is available for imatinib for most of these and, in a more restricted number, for nilotinib or dasatinib[50,61] (Figure 2). There are two relevant consequences of IC$_{50}$ determination of ABL mutations. First, it is possible to modify the clinical strategy in treatment resistant patients with a kinase domain mutation on the basis of the IC$_{50}$ of drugs for the mutation, i.e. choosing whether to increase the dose of imatinib or replace it with a second generation tyrosine kinase inhibitor or, in the case of the pan-resistant T315I mutation, enroll the patient into a stem cell transplant program.[11] Second, when finding a mutation in a resistant patient, it is important to search for its IC$_{50}$ in a mutation database to link it to the clinical resistance,[50,59-62] since some rare mutations may be "innocent bystanders" that co-segregate with other mechanisms of resistance. A very recent report provides evidence that the highly immature (staminal) lin$^-$CD34$^+$ CD38$^-$ subpopulation of Philadelphia-positive cells is very unstable and accumulates, both *in vivo* and *in vitro*, several mutations at the kinase domain of the BCR/ABL oncogenic protein even before exposure to imatinib.[63] Interestingly, the progeny generated *in vitro* from this subpopulation of Philadelphia-positive cells acquires several different mutations at the kinase domain of the oncogenic protein both in the presence and in the absence of imatinib.[63] It therefore seems plausible that tyrosine kinase inhibitors do not directly induce mutations, but rather select them by giving a growth advantage to Philadelphia-positive subclones prior to the therapy or those originating in the highly unstable Philadelphia-positive stem cell compartment during tyrosine kinase inhibitor treatment. In this view, kinase domain mutations may be either a cause and/or a marker of resistance to tyrosine kinase inhibitors and may be of genetic instability of the Philadelphia-positive clone. The presence of kinase domain mutations may, therefore, be clinically relevant and the need to assay them has raised some important practical issues to be considered for optimal clinical monitoring of CML patients: when is it clinically useful to search for mutations during treatment and what are the most appropriate (and cost-effective) techniques to do this.

Several methods have been used to detect the presence of kinase domain mutations in CML patients (Table 2). Some of these give a quantitative or semi-quantitative

Table 2. Methods for detecting mutations.

Technique	*Sensitivity* (%)*	*Specific for single mutations*	*Mutation quantitation*	*Cost**/Diffusion*	*References*
Direct sequencing	15-25	No	No	$/++++	46,64,65
Subcloning and sequencing	10	No	No	$$/+	58
DHPLC	0.5-5#	No	No	$$$/++	68,69,70
Pyrosequencing	5	No	Semi-quantitative	$$/+	73
Double-gradient denaturing electrophoresis	5	No	No	$$/+	85
Fluorescence PCR and PNA clamping	0.2	Yes	Semi-quantitative	$$/+	86
ASO-PCR	0.1	Yes	No	$/+	66,67
Taqman-based RQ-PCR	0.1	Yes	Yes	$$$/+	87
Polymerase colony assay	0.01	No	Yes	$/+	88
Multiplex SNP and mass spectrometry	1.5-3	No	Yes	$$$$/+	89

**Sensitivity is expressed as the percentage of mutated sequences that can be detected by the method. **Cost estimate is also based on the price of the required instruments. #The highest sensitivity is obtained when mutated subfractions are isolated and then sequenced. DHPLC, denaturing high performance liquid chromatography; PCR, polymerase chain reaction; PNA peptide nucleic acid; ASO, allele-specific oligonucleotide; SNP, single nucleotide polymorphism.*

estimation of the relative proportion of the mutated clone, and the sensitivity in some cases is very high (1/10,000). However, the high cost of the instruments required limits the practical use of the most sensitive methods. Direct sequencing, which is now often performed using automated high-throughput instruments, is still the most widespread method applied in the routine monitoring of patients.[46,64,65] The main disadvantage of this technique is its low sensitivity (20%) which is responsible for false negative results. Techniques based upon the use of allele-specific PCR have a higher sensitivity, which can reach 0.1%. Their main disadvantage is that they are directed to the search for specific mutations and do not screen the entire kinase domain region of the *BCR/ABL* gene.[66,67] Various groups, including ours and the authors of the article published in this issue of journal, use denaturing high performance liquid chromatography (D-HPLC) as a routine approach to screen for the presence of kinase domain mutations which will then be confirmed by sequence analysis.[68-70] This method has been shown to be applicable to the routine monitoring of CML patients and to have a sensitivity of 1-5% in mutation detection. Nevertheless, from a clinical standpoint, the significance of very small subclones carrying kinase domain mutations has yet to be demonstrated. Indeed, the presence of low-titer mutations has been shown in retrospective studies in patients still responding to imatinib with long-lasting complete cytogenetic remission,[71,72] or even prior to treatment, tracing back the mutations detected at the time of relapse in patients treated with imatinib mainly in late chronic phase.[67]

Interestingly, a recent study on consecutive unselected CML patients found correlations between the pre-treatment presence of low-level mutations and both stage of disease and clonal cytogenetic evolution, but not with the probability of response to imatinib. Even the completely resistant T315I mutant, when detected at a low titer prior to treatment, did not prove to be selected during treatment.[66] These findings are confirmed by observations on the kinetics of the mutated clones in a small group of early and late chronic phase CML patients showing a lack of correlation between the residual disease and the presence of a predominant kinase domain mutation.[73] As a practical consequence, screening with high sensitivity methods for low-titer kinase domain mutations in newly diagnosed patients should be reserved for clinical investigations, since the long term significance of the pre-treatment low titer mutations that may constitute a biological marker of the clone's instability still needs to be assessed in prospective clinical trials. On the other hand, the issue of evaluating kinase domain mutations during follow-up deserves more attention. The identification of a specific type of mutation in relapsed patients seems to be of prognostic relevance since the different types of mutation correlate with the risk of evolution of relapsed patients.[74] The presence and the type of mutation with its specific IC_{50}, should, therefore, help re-evaluate treatment strategy, particularly now that second generation tyrosine kinase inhibitors are available. Screening for mutations should always be carried out in patients who fail to respond or who have a suboptimal response to imatinib, as defined by the recommendations of the European LeukemiaNet (Table 1). The GIMEMA co-operative group in Italy reported that the overall frequency of mutations evaluated in 297 resistant patients was 43%; the frequency did, however, range from 14% in the therapy-resistant patients who started imatinib in early chronic phase to 83% among patients with lymphoid blast crisis. Mutations are more frequent in patients with secondary resistance than in those with primary resistance (57% *vs.* 30%),[75] and, interestingly, 23% of patients who lost their cytogenetic response had a kinase domain mutation detectable by the DHPLC method.

The prognostic role of the detection of a kinase domain mutation in responders is still a matter of debate. Various groups showed that mutations detected in patients with stable complete cytogenetic responses have little prognostic significance.[71,72] However, the increase of residual disease measured by Q-PCR has been correlated with the presence of a kinase domain mutation. For the

moment, it is still difficult to define the extent of the increase required before performing mutation screening in patients in complete cytogenetic remission. In a first report on this issue, a two-fold increase of minimal residual disease was suggested as a threshold to screen for mutations.[42] However, this threshold appears too restrictive and is associated with too high a rate of negative results to be adopted as a routine strategy for determining whether to search for mutations in the clinical setting with a D-HPLC method. A more realistic approach would be to assay for mutations all those patients who show at least a one-log increase of minimal residual disease, those with a less pronounced but progressive increase over time, and those who never achieve a major molecular response and show increases of minimal residual disease.[76]

Monitoring drug plasma levels

With some exceptions, leukemia and cancer treatment are not modulated or adapted according to drug plasma levels. In the case of imatinib, the recommended dose is not even calculated according to body weight or surface, because pharmacokinetic studies showed no differences in peak and trough plasma levels and the area under the curve.[77] Since the first recommended dose of imatinib (400 mg daily) was not the maximum tolerated dose,[3] and since it was reported that in accelerated phase a 600 mg dose was better than 400 mg,[78] the issue of the dose of imatinib has remained open. On the one hand, it has stimulated comparison of 400 mg *vs.* 600 or 800 mg daily in still ongoing clinical studies.[5,11] On the other hand, it has led to investigations of whether different drug plasma levels may affect efficacy and toxicity. A retrospective analysis of the pharmacokinetic data collected during the IRIS study suggests that the rate and the rapidity of achieving cytogenetic responses correlate with the mean trough plasma level of imatinib.[6] In an independent, prospective study of 68 patients treated with imatinib 400 mg daily, it was confirmed that the mean trough plasma levels of imatinib were significantly higher in the group of patients who achieved complete cytogenetic responses than in the group of non-responders (1123±617 ng/mL *vs.* 694±556 ng/mL, p=0.03).[79] In the same study, it was also found that a trough plasma level of 1002 ng/mL was significantly associated with the likelihood of achieving a major molecular response.[79] Such a plasma concentration is about double the imatinib concentration that is required to inhibit 50% of the biochemical activity of BCR-ABL *in vitro*. The plasma concentration of imatinib depends on drug metabolism through the cytochrome P450 system (CYP 3A4 and CYP 3A5),[77] so that several drugs can influence the concentration in either direction. The intracellular concentration of imatinib is also influenced significantly by several drug transporters: these include, in particular, the anion transporter hOCT1, which brings imatinib into the cells,[80] but also other proteins belonging to the ATP binding cassette family, which aid the egress of imatinib out of the cells.[81-84] Therefore, monitoring the plasma concentrations of imatinib and other tyrosine kinase inhibitors, which was overlooked for several years, is assuming ever more importance, and is advised in cases of treatment failure, suboptimal response (see Table 1), low compliance and severe side-effects. A European network of dedicated laboratories will soon be operational. These will be run by European LeukemiaNet and coordinated by François-Xavier Mahon in Bordeaux (*mathieu.molinard@pharmaco.u.bordeaux2.fr*).

Conclusions

Progress in the treatment of cancer and leukemia requires an ever increasing committment and diligence in monitoring the response to treatment, minimal residual disease, and the course of the disease. For CML, blood counts and differential were sufficient until the introduction of interferon and allogeneic stem cell transplantation, which began to require cytogenetic and molecular monitoring. With the introduction of tyrosine kinase inhibitors, both quantitative and qualitative cytogenetic and molecular monitoring has become mandatory in all patients, and drug plasma level assays will very soon become necessary. This may have an impact on costs. The current cost of tyrosine kinase inhibitors ranges between 25,000 and 50,000 euro per year, depending on the drug and the dose. The cost of cytogenetic analysis ranges between 150 and 200 euro for chromosome banding analysis of marrow cells, and between 200 and 250 for IP-FISH of blood cells. The cost of RQ-PCR ranges between 200 and 250 euro. Performing three cytogenetic tests and four RQ-PCR per year may cost between 1250 and 1600 euro per year, corresponding to a small fraction of the cost of the drug, from a minimum of 2.5% (assuming 1250 euro for the tests, and 50,000 euro for the drug) to a maximun of 6.4% (assuming 1600 euro for the tests and 25,000 euro for the drug). The identification of a *BCR-ABL* kinase domain mutation may cost 700 to 1000 euro, and is performed only in the case of suboptimal response or failure. Therefore, monitoring is not only important for ensuring that a patient is receiving the best treatment, but is also convenient from a pharmacoeconomic point of view. Continuing the treatment, or changing to another treatment without testing, is much more expensive than testing, and results in a waste of money. The tests must be performed in dedicated laboratories using standardized methods and reagents, and the interpretation of the tests requires specifically trained and experienced staff. Imatinib, the other tyrosine kinase inhibitors, and any new targeted agents may be magic bullets only if they are fired with a good rifle, and carefully aimed.

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