

# Therapeutic drug monitoring and tyrosine kinase inhibitors (Review)

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**Abstract.** The therapeutic activity of drugs can be optimized by establishing an individualized dosage, based on the measurement of the drug concentration in the serum, particularly if the drugs are characterized by an inter-individual variation in pharmacokinetics that results in an under- or overexposure to treatment. In recent years, several tyrosine kinase inhibitors (TKIs) have been developed to block intracellular signaling pathways in tumor cells. These oral drugs are candidates for therapeutic drug monitoring (TDM) due to their high inter-individual variability for therapeutic and toxic effects. Following a literature search on PubMed, studies on TKIs and their pharmacokinetic characteristics, plasma quantification and inter-individual variability was studied. TDM is commonly used in various medical fields, including cardiology and psychiatry, but is not often applied in oncology. Plasma concentration monitoring has been thoroughly studied for imatinib, in order to evaluate the usefulness of TDM. The measurement of plasma concentration can be performed by various analytical techniques, with liquid chromatography-mass spectrometry being the reference method. This method is currently used to monitor the efficacy and tolerability of imatinib treatments. Although TDM is already being used for imatinib, additional studies are required in order to improve this practice with the inclusion of other TKIs.

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## 1. Introduction

Therapeutic drug monitoring (TDM) is the measure of the drug concentration in the blood, in order to adapt the dosage (1,2). TDM is already used for drug classes such as antibiotics, immunosuppressants and, more recently, antiretroviral drugs (1). TDM allows for the determination of the actual drug concentration in the body. To study the pharmacokinetics of a molecule, three parameters are typically measured: The residual concentration, serum peak and area under the curve (AUC). The residual concentration (C<sub>0</sub>) refers to the drug concentration measured just before the administration of the next dose. This measurement is characterized by its easy sample collection and low cost; however, it can sometimes lead to dose modifications being performed too frequently (1). The serum peak drug concentration is the maximum concentration of the drug reached in the body, in a steady state. This is achieved by the accumulation of the molecule in the body until an equilibrium is reached, in which the concentration peak no longer increases (1,3). The third parameter is the measured AUC, which refers to the index of the total drug exposure. The measurement of AUC helps to determine whether the kinetics of the patient are classic, thus highlighting inter-individual variability in the metabolism or excretion of the drug.

The pharmacokinetic parameters of a drug are influenced by several factors: Genetic (slow and fast metabolizers),

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physiological (age), pathological (renal or liver failure) and environmental (food) (4). These factors are important role in the pharmacokinetics of a molecule, since at the same dosage, the plasma concentration of a drug can differ from patient to patient, leading to variations in the therapeutic responses obtained, as well as the occurrence of adverse effects (4). Anticancer drugs are good candidates for TDM, since they exhibit different parameters that are required for a molecule to be eligible for TDM: Narrow therapeutic window between efficacy and toxicity; significant inter-individual variability (bioavailability and metabolism); therapeutic concentration effect; associations between plasma concentration and toxic/therapeutic effect; drug interactions or intercurrent diseases that may interfere with the kinetics, and finally, the possibility of interpreting the plasma concentrations using the appropriate quantification method (2). However, TDM is rarely applied in clinical oncology, mainly due to a lack of pharmacokinetic data and optimal values. The limited use of TDM may also be due to certain analytical difficulties, either technical or faced during the interpretation of the results, such as the presence of an active metabolite, and determination of the free vs. tumor concentration of the drug (2).

Tyrosine kinase inhibitors (TKIs) are a class of targeted therapy used in the treatment of malignant diseases (5,6). The most well known TKI is imatinib, which is used in patients with chronic myeloid leukemia (CML) (5,6). TKIs compete with adenosine triphosphate (ATP) at its binding site (5,6). This inhibits tyrosine kinase, which is involved in several pathways, including tumor cell growth and proliferation, as well as suppression of apoptosis and promotion of angiogenesis (5). TKIs have been demonstrated to be efficient in monotherapy and in combination with chemotherapy. There are currently 17 Food and Drug Administration (FDA)-approved TKIs (7,8) for various indications (9), as indicated in Table I.

Imatinib mesylate (Gleevec® or Glivec®; Novartis), the oldest TKI, is indicated for the treatment of CML and gastrointestinal stromal tumors (GIST) (6). Imatinib mesylate is a competitive inhibitor of the fusion protein breakpoint cluster region-Abelson oncoprotein (BCR-ABL), which is responsible for the increased proliferation of leukemic cells and their resistance to apoptosis (10).

In the last decade, second generation TKIs have been introduced into routine practice. Among them, dasatinib (Sprycel®; Bristol-Myers Squibb) and nilotinib (Tasigna®; Novartis), are used for the treatment of CML and acute lymphoblastic leukemia (ALL), if imatinib fails (10).

Nilotinib shares the same mechanism with imatinib, but has a higher inhibitory activity (11). In a phase II clinical trial, out of the 321 patients with CML treated with 400 mg nilotinib twice a day, 226 (70%) were imatinib-resistant and 95 (30%) imatinib-intolerant. The main adverse events observed at the 24-month follow-up were rash (31%), pruritus (26%) and nausea (25%). Adverse events led to the discontinuation of the treatment for 53 patients. At 24 months, 59% of the patients had achieved a major cytogenetic response and 45% a complete response. At 48 months, the rate of progression-free survival was 57% (11).

Dasatinib is a multi-kinase inhibitor prescribed to patients with CML and ALL, who are intolerant or resistant to imatinib (6). A study was conducted on 387 patients with

CML in chronic phase who were resistant or intolerant to imatinib to estimate the rate of major cytogenetic response at 6 and 8 months. Patients were treated with dasatinib at a dose of 70 mg twice daily, in chronic phase (12). A complete hematologic response was obtained in 90% of patients (80% of imatinib-resistant patients and 97% of imatinib-intolerant patients). Dasatinib also induced a major cytogenetic response in 52% of patients (39% of imatinib-resistant patients and 80% of imatinib-intolerant patients), which was maintained in 96 and 100% of the above patients, respectively, during 8 months (12). In that study, dasatinib was well tolerated, with only 9% of patients discontinuing their treatment after 8 months, due to adverse effects. Dasatinib has been approved by the FDA for the treatment of CML and Philadelphia chromosome (Ph)-positive ALL (13).

Bosutinib (Bosulif®; Pfizer) is another TKI that has been recently approved for the treatment of patients at different stages of CML (14). Like dasatinib, bosutinib is an inhibitor of BCR-ABL and sarcoma oncoprotein (14). In a study involving 288 patients with chronic phase CML (200 of which were resistant or intolerant to imatinib) treated with 500 mg bosutinib daily, a major cytological response was reported at 24 months in 31% of patients (33% of imatinib-resistant patients and 27% of imatinib-intolerant patients) (15). The most frequently observed non-hematologic adverse events were diarrhea (84%), nausea (44%), rash (44%) and vomiting (35%). Grade 3-4 neutropenia was observed in 18% of patients, thrombocytopenia in 24% of and anemia in 13% (15).

Sunitinib (Sutent®; Sugen) is a multitarget TKI approved for the treatment of advanced renal cell carcinoma (RCC) and GIST, following disease progression or in case of intolerance to imatinib (6). A total of 63 patients with metastatic RCC previously treated with cytokines were enrolled in a phase II study (16). Out of the 63 patients, 25 achieved partial remission (40%). Median time to disease progression was 8.7 months. Observed toxicities included asthenia (38%), decline of cardiac ejection fraction (13%) and elevated serum lipase without clinical pancreatitis (24%).

Other TKIs are also used for various pathologies; for example, gefitinib (Iressa®; AstraZeneca), erlotinib (Tarceva®; Genentech), afatinib (Giotrif®; Boehringer Ingelheim) or crizotinib (Xalkori®; Pfizer) can be used for the treatment of non-small cell lung cancer (NSCLC), lapatinib (Tyverb®; GlaxoSmithKline) for the treatment of human epidermal growth factor-positive breast cancer, and regorafenib (Stivarga®; Bayer) for the treatment of metastatic colorectal cancer.

## 2. Practical issues in TDM: Application for routine analysis

The current reference analytical method for the measurement of anticancer agent concentration in the plasma is liquid chromatography with tandem mass spectrometry (LC-MS/MS) (7). High-performance liquid chromatography (HPLC) is a technique used for the analytical separation of the constituents present in a complex matrix (17). To identify the different molecules, the chromatography is coupled to a detector. There are several types of detectors; the most frequently used for TDM are MS, MS/MS and ultraviolet (UV) diode array detector (18-66) (Table II). HPLC coupled with UV

Table I. FDA-approved tyrosine kinase inhibitors.

| Drug         | Commercial name | FDA approval (year) | Primary target             | Disease  | Major enzyme involved in its pharmacokinetics | Ref.      |
|--------------|-----------------|---------------------|----------------------------|--|---|-----------|
| Afatinib     | Gilotrif®       | 2013                | EGFR, HER2                 | NSCLC  | None  | (35)      |
| Axitinib     | Inlyta®         | 2012                | VEGFR, PDGFR, c-Kit        | Advanced RCC   | ABCB1, CYP3A4/5, CYP1A2, CYP2C19, UGT1A1      | (6)       |
| Bosutinib    | Bosulif®        | 2012                | BCR-ABL, Src               | Ph <sup>+</sup> CML  | CYP3A4  | (35)      |
| Cabozantinib | Cometriq®       | 2012                | VEGFR                      | MTC  | CYP3A4  | (35)      |
| Crizotinib   | Xalkori®        | 2011                | ALK, HGFR                  | ALK <sup>+</sup> advanced or metastatic NSCLC  | ABCB1, CYP3A4                                 | (6,35)    |
| Dasatinib    | Sprycel®        | 2006                | BCR-ABL, c-Kit, PDGFR, Src | Ph <sup>+</sup> CML, ALL   | ABCB1, ABCG2, CYP3A4, UGT                     | (6,35,45) |
| Erlotinib    | Tarceva®        | 2005                | EGFR                       | NSCLC, pancreatic cancer   | ABCG1, ABCG2, CYP3A4/5, CYP2D6, CYP1A2        | (6,35,45) |
| Gefitinib    | Iressa®         | 2009                | EGFR                       | NSCLC  | ABCG2, CYP3A4/5, CYP2D6                       | (6,35,45) |
| Imatinib     | Gleevec®        | 2001                | BCR-ABL, c-Kit, PDGFR      | Ph <sup>+</sup> CML, ALL, GIST, myelodysplastic syndrome/ myeloproliferative disorders | ABCB1, ABCG2, ABCC4 CYP3A4/5, CYP2C8          | (6,35,45) |
| Lapatinib    | Tyverb®         | 2008                | EGFR, HER2                 | Metastatic breast cancer   | ABCB1, ABCG2, CYP3A4/5                        | (6,35,45) |
| Nilotinib    | Tasigna®        | 2007                | BCR-ABL                    | Ph <sup>+</sup> CML, ALL   | ABCG1, ABCG2, CYP3A4                          | (35,45)   |
| Pazopanib    | Votrient®       | 2010                | VEGFR                      | Advanced RCC, soft tissue sarcoma  | ABCB1, ABCG2, CYP3A4                          | (6,35)    |
| Regorafenib  | Stivarga®       | 2012                | VEGFR                      | Metastatic colorectal cancer   | CYP3A4, UGT1A9                                | (35)      |
| Ruxolitinib  | Jakavi®         | 2011                | JAK                        | Myelofibrosis  | CYP3A4  | (6)       |
| Sorafenib    | Nexavar®        | 2006                | VEGFR, B-Raf, PDGFR        | Advanced RCC, hepatocellular carcinoma   | CYP3A4, UGT1A9                                | (6,35,45) |
| Sunitinib    | Sutent®         | 2006                | PDGFR, VEGFR, c-Kit        | GIST, metastatic RCC, pancreatic neuroendocrine tumor                                  | ABCB1, ABCG2, CYP3A4                          | (6,35,45) |
| Vandetanib   | Caprelsa®       | 2011                | VEGFR, EGFR                | MTC  | CYP3A4  | (6,35)    |
| Vemurafenib  | Zelboraf®       | 2011                | B-Raf                      | Melanoma with B-Raf mutation   | ABCB1, ABCG2                                  | (6)       |

FDA, Food and Drug Administration; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; NSCLC, non-small cell lung cancer; VEGFR, vascular growth factor receptor; PDGFR, platelet-derived growth factor receptor; RCC, renal cell carcinoma; ABC, ATP-binding cassette; CYP, cytochrome P450; BCR-ABL, breakpoint cluster region-Abelson oncogene; Src, sarcoma oncoprotein; Ph, Philadelphia chromosome; CML, chronic myeloid leukaemia; ALK, anaplastic lymphoma kinase; HGFR, hepatocyte growth factor receptor; ALL, acute lymphoblastic leukaemia; UGT, uridine 5'-diphospho-glucuronosyltransferase; GIST, gastrointestinal stromal tumor; JAK, Janus kinase; Raf, rapidly accelerated fibrosarcoma; MTC, medullary thyroid cancer; UGT, UDP glucuronosyltransferase.

Table II. Summary of publications about quantification of tyrosine kinase inhibitors in biological matrices, with a lower LOQ and preclinical and clinical applications.

| Molecules and techniques                      | LOQ, ng/ml (ref.)   | Applications (ref.)  |
|---|---|--|
| Capillary electrophoresis                     |   |  |
| Imatinib                                      | 125 (21)  | Human plasma   |
| ELISA   |   |  |
| Imatinib                                      | 0.04 (19)   | Mouse plasma   |
| HPLC-UV                                       |   |  |
| Imatinib                                      | 80 (23), 150 (24), 50 (25), 25 (26)   | Human plasma (23-25) and rat plasma (26)                         |
| Nilotinib                                     | 5 (27), 10 (28), 250 (29)   | Human plasma (27-29), human urine and cells line in culture (29) |
| Imatinib, norimatinib and nilotinib           | 12 (30)   | Human plasma   |
| Imatinib, dasatinib and nilotinib             | 5 (18)  | Human plasma   |
| Gefitinib and erlotinib                       | 20 and 80 (31)  | Human plasma   |
| Sorafenib                                     | 80 (32), 500 (33)   | Mouse serum (32) and human plasma (33)                           |
| Sunitinib                                     | 20 (34)   | Human plasma   |
| Vemurafenib and erlotinib                     | 1,250 and 50 (35)   | Human plasma   |
| HPLC-MS                                       |   |  |
| Gefitinib                                     | 0.5 (36)  | Human plasma   |
| Imatinib                                      | 1 (37)  | Human serum  |
| Imatinib, dasatinib and nilotinib             | Imatinib, 78.1; dasatinib and nilotinib, 62.5 (38)  | Human plasma   |
| HPLC-MS/MS                                    |   |  |
| Axitinib                                      | 0.2 (39)  | Human plasma   |
| Dasatinib and two metabolites                 | 1 (40)  | Human plasma   |
| Erlotinib and desmethyl erlotinib             | Erlotinib, 10 (41,42), 1.6 (43);<br>desmethyl erlotinib, 1 (41), 1.8 (42), 5 (43)               | Human plasma (41,42) and rat plasma (43)                         |
| Gefitinib and desmethyl gefitinib             | 5 (44)  | Human plasma   |
| Imatinib                                      | 10 (45,46), 20 (47)   | Human plasma (45,46) and rat tissues (47)                        |
| Imatinib and norimatinib                      | Imatinib, 1 (48), 4 (49), 30 (50), 500 (22);<br>norimatinib, 2 (48), 10 (49), 30 (50), 500 (45) | Monkey plasma (48), human plasma (49,50) and human serum (22)    |
| Lapatinib                                     | 100 (51)  | Human plasma   |
| Pazopanib                                     | 3.9 (52)  | Mouse brain and plasma   |
| Sunitinib                                     | 0.2 (53), 1.5 (54), 2 (55)  | Human plasma (53), mouse plasma and brain (54,55)                |
| Regorafenib                                   | 25 (56)   | Mouse plasma   |
| Vemurafenib                                   | 100 (57), 1,000 (58)  | Human plasma   |
| Erlotinib, sunitinib, gefitinib and sorafenib | 1 (59)  | Human plasma, serum and whole blood                              |

Table II. Continued.

| Molecules and techniques  | LOQ, ng/ml (ref.)  | Applications           | Ref. |
|---|--|------------------------|------|
| Imatinib, nilotinib, dasatinib, sunitinib, sorafenib and lapatinib  | Sorafenib, 100; lapatinib, 5; others, 1  | Human plasma           | (17) |
| Dasatinib, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, sorafenib and sunitinib                        | Dasatinib and sunitinib, 5,000; others, 20,000   | Human plasma           | (60) |
| Erlotinib, imatinib, lapatinib, nilotinib, sorafenib and sunitinib  | Sunitinib, 10; others, 50  | Human plasma           | (20) |
| Imatinib, norimatinib, dasatinib, nilotinib, erlotinib, gefitinib, lapatinib, sorafenib and sunitinib           | Norimatinib and gefitinib, 10; dasatinib and sunitinib, 120; others, 400   | Human plasma and serum | (61) |
| Ruxolitinib and nilotinib   | 0.16 and 0.86  | Mouse plasma           | (62) |
| UPLC-MS/MS  |  |                        |      |
| Sunitinib and desmethyl sunitinib   | 0.2  | Human plasma           | (63) |
| Vemurafenib   | 100  | Human plasma           | (64) |
| Sunitinib, gefitinib, imatinib, norimatinib, dasatinib, erlotinib, axitinib, nilotinib, lapatinib and sorafenib | Dasatinib and axitinib, 0.1; sunitinib and gefitinib 0.4; others, 10   | Human plasma           | (65) |
| Imatinib, norimatinib, sunitinib, desethyl sunitinib, nilotinib, dasatinib, pazopanib and regorafenib           | Imatinib, norimatinib and nilotinib, 100; sunitinib and desethyl sunitinib, 2; dasatinib, 5, 1,000; pazopanib, 1,000; regorafenib, 0.1 | Human plasma           | (66) |

HPLC, high-performance liquid chromatography; UV, ultraviolet; MS, mass spectrometry; UPLC, ultra-performance liquid chromatography; LOQ, limit of quantification; ELISA, enzyme-linked immunosorbent assay.



(HPLC-UV) or with other fluorescence detectors can be used as an alternative in laboratories not equipped with LC-MS/MS machinery (18). In clinical studies of pharmacokinetics and TDM of TKIs, the assays were carried out with LC-MS/MS, which is specific, sensitive and reproducible (19).

HPLC coupled to MS (HPLC-MS) has become the reference method for the quantification of large volumes of drugs in biological fluids (20). The coupling of these two techniques combines the advantages of chromatography (high separation selectivity and efficiency) with the advantages of MS (obtaining information on the structure, increase in mass and selectivity) (67). Consequently, the combination of TKIs has been used as a strategy to prevent the development of resistance against these molecules. In addition, it induces a rapid and effective response (68). It is therefore important to apply an analytical method for the quantification of several molecules simultaneously, while maintaining a reduced sample volume, such as HPLC-MS, which is a robust technique also used for the separation and quantification of a molecule and its metabolite(s) (imatinib and norimatinib, sunitinib and N-desmethylnsunitinib), which is not necessarily possible with other assay methods (20). For certain TKIs such as dasatinib, the therapeutic target is relatively low (2.5 ng/ml for dasatinib vs. 1,000 ng/ml for imatinib) (7). In these cases, LC-MS/MS is well suited, since it enables the determination of low plasmatic concentrations of drugs (7). The main disadvantage of LC-MS/MS is its cost, which is considerably higher than that of other methods (7). In addition to the high cost of this technique, it is also necessary to have specific and technical knowledge about LC-MS/MS in order to develop and validate quantitative methods in a laboratory.

### 3. Pharmacokinetics of TKIs

**Adherence.** Oral therapy can potentially be administered for a long period of time. It has been shown that adherence is often better in patients treated for acute diseases, as opposed to chronic ones, and often adherence stops after the first 6 months of treatment interruption (69). Patients may not understand the point of continuing their treatment following the disappearance of the symptoms, as it may be the case in CML (18); however, treatment termination can lead to a reduced efficacy. In 87 patients with CML in chronic phase treated with imatinib (400 mg/day), the high rate of adherence was significantly associated with a major molecular response ( $P < 0.001$ ) (70).

**Absorption and distribution.** The absorption of TKIs occurs a few hours after dose administration, with a bioavailability of 60-80%, depending on the molecules (6). Concomitant food intake tends to increase these values. Following absorption, >90% of TKIs are bound to plasma proteins (albumin and  $\alpha$ -1-acid glycoprotein); however, only free drugs are in an active pharmacological form (6,71). Numerous transport systems including ATP-binding cassette transporters, contribute to the distribution of TKIs (72). Limited data have been published on the impact of polymorphism of ATP-binding cassette transporters on TKI distribution.

**Metabolism.** TKIs are metabolized mainly by cytochrome P450 3A4 (CYP3A4) (Table I), sometimes to an active metabolite, such in the case of imatinib, dasatinib and sunitinib (9). The activity of CYP3A4 is regulated by various factors, including drug interactions, genetic variability or age, and comorbidities (72). Furthermore, the enzymatic activity of CYP3A4 can be induced or inhibited by co-medication (72). In the case of prodrugs, which require metabolic activation, the inhibition of biotransformation enzymes leads to an increased drug concentration and higher toxicity frequency, or may result in reduced drug effect (73). In addition, several CYP isoforms are polymorphic, which can modify the enzyme activity. Increased or decreased exposure, due to an alteration in CYP activity, may lead to clinically relevant toxic effects or altered efficiency of the treatment with TKIs (21). Following the administration of a given dose of the molecule, a large inter-individual variability of blood levels can be observed among patients (21).

### 4. Variability in pharmacokinetic parameters

The marked variability among individuals results in variable circulating blood levels of TKIs, influenced by physiological and pathophysiological changes in each patient (7). At a given dose, plasma concentrations may vary among different patients and lead to an over- or under-exposure, resulting in treatment failure or occurrence of adverse effects (20), as observed for example with imatinib (7). Monitoring the concentration of TKIs in plasma can help to evaluate treatment efficiency, patient compliance and potential interactions with other molecules that influence their pharmacokinetic parameters. Certain studies have already reported a wide variability in the plasma levels of imatinib among different patients, which suggests that adequate plasma levels are important for an optimal clinical response (74-76). The measurement and monitoring of residual plasma levels of imatinib are already being used to evaluate the course of patients treated for CML (21); however, this monitoring is yet to be applied to other TKIs.

### 5. Correlation between TKIs' plasma concentration and therapeutic effect

**Imatinib.** Previous studies have reported a correlation between trough plasma concentrations of imatinib and the clinical response of patients with CML (74-78). Larson *et al* (74) studied this correlation in 351 patients treated with 400 mg imatinib. Blood samples were obtained from the patients after 29 days of treatment, the period corresponding to the equilibrium state. The plasma concentrations of imatinib and norimatinib were determined using HPLC-MS/MS, and were  $979 \pm 530$  ng/ml and  $106 \pm 106$  ng/ml, respectively. The residual concentration of imatinib was <647 ng/ml in 87 patients, 647-1,170 ng/ml in 178 patients, >1,170 ng/ml in 86 patients and >2,000 ng/ml in 19 patients. The residual concentration of imatinib in patients who achieved a complete cytogenetic response was significantly higher than that of patients who did not achieved such response ( $P = 0.004$ ), whose mean value was  $1,099 \pm 544$  ng/ml. Maintaining the imatinib rate above the threshold of 1,000 ng/ml appears to be important for achieving a complete cytogenetic response. This threshold is consistent

with the one reported in the study by Picard *et al* (76), which involved 68 patients treated with imatinib for  $\geq 1$  year at a dose of 400 or 600 mg/day. The trough plasma concentrations of imatinib were significantly higher in patients with a major molecular response,  $1,452 \pm 649$  ng/ml vs.  $869 \pm 427$  ng/ml ( $P < 0.001$ ), or a complete cytogenetic response.

Certain studies have demonstrated that there is an association between the plasma levels of imatinib and the clinical response of patients treated for GIST (1,100 ng/ml); however, studies on this disease are considerably rare (79-81).

With regard to imatinib, studies evaluating the feasibility and efficacy of TDM in clinical practice are almost complete, and are based on the assumption that drug plasma concentration monitoring can increase treatment efficacy and/or reduce its toxicity (74,76). The threshold target of trough plasma concentrations of imatinib for patients with CML is set arbitrarily at a value of 1,000 ng/ml. Drug plasma concentration monitoring is currently recommended by the European Society for Medical Oncology only for imatinib, and only in cases of treatment failure or occurrence of adverse events on patients with CML treated with imatinib (6,82). Dose adjustment of imatinib, based on the drug concentration in the plasma, was applied in a clinical study (83), which involved 56 patients randomized in an intervention 'routine TDM' arm and a control 'rescue TDM' arm. The patients allocated in the 'routine TDM' arm were administered a dose targeting a C0 of 1,000 ng/ml. Due to the small number of patients and the poor adherence of prescribers to the dosage recommendation (13/28), the trial could not definitively demonstrate the benefit of a 'routine TDM' of imatinib; however, the study suggested that TDM can be useful in patient management during imatinib treatment, particularly during the first 3 months (83).

TDM is useful for imatinib in particular, since its inter-individual variability interferes considerably with its efficiency, but also due to the fact that there is a marked association between dose and response (7). TDM also is important in the evaluation of compliance to daily oral therapy (18); however, for other TKIs, it is important to establish the plasma target values through clinical studies (2).

**Other TKIs.** It has been reported that 15-20% of patients treated with imatinib exhibit clinical resistance, which is characterized by a reduced affinity of imatinib to its targets (84). In such cases, imatinib should be substituted with a different TKI such as nilotinib or dasatinib (16). The threshold of effectiveness has been defined for imatinib; however, this is not the case for other TKIs, despite the publication of few studies on the pharmacokinetics of nilotinib (85). With regard to dasatinib, no correlation has been observed between trough concentration and therapeutic response in patients with CML or Ph<sup>+</sup> ALL (13); however, these results are controversial, as the population pharmacokinetic model established from the data of a phase II study revealed a significant correlation between the trough plasma concentration of dasatinib (2.5 ng/ml) and a major cytogenetic response ( $P < 0.01$ ) (86). With regard to nilotinib, no significant correlation was observed between the drug plasma concentration and major molecular response at 12 months (87). Further research is required to define the role of blood level testing in cases of non-adherence or potential drug interactions (88).

In patients with advanced NSCLC treated with gefitinib at 250 mg/day, the ratio of the minimum gefitinib concentration was calculated on day 8 (D8) and D3. A high D8/D3 ratio was significantly associated with a better progression-free survival ( $P = 0.0129$ ) (88). Another study confirmed these results by revealing that a threshold of 200 ng/ml was associated with a better median survival (4.70 vs. 14.60 months,  $P = 0.0009$ ) (89).

With regard to sunitinib, an estimation of threshold value could be 50 ng/ml for patients with advanced solid malignancy (90), but no pharmacokinetic studies have been performed. No threshold values have been determined for erlotinib and lapatinib.

The evaluation of TKI plasma concentrations may aid to obtain optimal therapeutic results, particularly in patients who experience adverse effects and for whom the dosage should be adjusted (20). Several recent publications have reported the validity of this analytical method for the quantification of imatinib levels in blood using chromatographic techniques coupled with UV detection or MS (6,17,18,23,30,37,45,61).

## 6. Conclusion

TKIs are characterized by large inter-individual variations such as genetic polymorphisms and drug-drug interactions. Furthermore, TKIs are administered orally and can be given over an extended period of time, which may occasionally lead to poor patient adherence. All these factors affecting variability can also affect the pharmacokinetics of TKIs and cause an inadequate exposure system. In the majority of clinical studies, pharmacokinetic analysis is carried out using LC coupled to MS. This method is associated with a considerably high sensitivity and specificity, and comprises the reference method for simultaneous quantification of several TKIs in the plasma. LC coupled to MS can be easily used in TDM. The marked variability of TKIs requires regular monitoring to ensure an optimal response and reduction of potential adverse effects. In cases of treatment failure, unusually severe side-effects or suspected drug interactions, measuring the trough plasma concentration of the drug may assist the clinician in the decision-making process of keeping the current treatment by adjusting the drug dosage or changing the type of drug molecule administered to the patient. The correlation between therapeutic efficacy and trough plasma concentration of drugs has been widely studied, and, in the case of imatinib, has been reported. Different thresholds have been identified for other TKIs, but the existing studies on them are limited and sometimes contradictory, thus rendering further research on therapeutic monitoring required.

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