The NIB-cohort study

In the oral small molecule targeted anti-cancer agent-cohort study we will be able to monitor patients and to reach study objectives as formulated in this proposal.

Coordinating investigator
Neeltje Steeghs, MD, PhD
Dept. of Internal Medicine, Antoni van Leeuwenhoek Hospital/The Netherlands Cancer Institute
Amsterdam, The Netherlands

Investigators

Alwin Huitema, PharmD, PhD
Dept. of Pharmacology, Slotervaart Hospital
Amsterdam, The Netherlands

Jos Beijnen, PharmD, PhD
Dept. of Pharmacology, Slotervaart Hospital
Faculty of Pharmaceutical Sciences, Utrecht University
The Netherlands

Hans-Martin Otten, MD, PhD
Dept. of Internal Medicine, Slotervaart Hospital
Amsterdam, The Netherlands

Marcel Soesan, MD
Dept. of Internal Medicine, Slotervaart Hospital
Amsterdam, The Netherlands

Klaas Bolt, MD
Dept. of Internal Medicine, Slotervaart Hospital
Amsterdam, The Netherlands

Jan Schellens, MD, PhD
Dept. of Internal Medicine, Antoni van Leeuwenhoek Hospital/The Netherlands Cancer Institute
Amsterdam, The Netherlands

Sjoerd Rodenhuis, MD, PhD
Dept. of Internal Medicine, Antoni van Leeuwenhoek Hospital/The Netherlands Cancer Institute
Amsterdam, The Netherlands

Neeltje Steeghs, MD, PhD
Dept. of Internal Medicine, Antoni van Leeuwenhoek Hospital/The Netherlands Cancer Institute
Amsterdam, The Netherlands

Contact

Neeltje Steeghs, MD, PhD
Dept. of Internal Medicine, Antoni van Leeuwenhoek Hospital/The Netherlands Cancer Institute
Amsterdam, The Netherlands

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Therapeutic Drug Monitoring of Tyrosine Kinase Inhibitors

Abstract

Title of the study
Therapeutic drug monitoring of oral small molecule targeted anti-cancer agents.

Background of the study
Interpatient (and intrapatient) variability in pharmacokinetics may be a major determinant in therapy outcome of oral small molecule targeted anti-cancer agents since this may lead to unpredictable efficacy and safety. Oral small molecule targeted anti-cancer agents include numerous tyrosine kinase inhibitors (TKIs or 'nibs'), 'musses' like everolimus and other new types of agents. In case of imatinib, there is already emerging evidence for a relationship between plasma levels and clinical efficacy/occurrence of side effects. Knowledge of this PK-PD relationship can be used to optimize therapy in order to prevent failure of TKIs by monitoring of drug resistance and reducing drug toxicity. Since the burden of TKI therapy is severe and TKI therapy is very expensive (> € 2000 / month) monitoring of plasma drug levels in order to optimise therapy is likely to be cost effective. Since all oral small molecule targeted anti-cancer agents have large similarities in mechanism of action and target proteins, similar PK-PD relations are to be expected for all oral small molecule targeted anti-cancer agents. This underlines the rationale for a structured prospective follow up of patients using any oral small molecule targeted anti-cancer agent.

Objective of the study
Primary: To determine the pharmacokinetics of several clinically used oral small molecule targeted anti-cancer agents
Secondary: To examine the relationship between treatment response/toxicity and plasma (or intracellular) drug levels.
To evaluate the specific influence of different parameters on variability in pharmacokinetics and –dynamics

Methodology
A longitudinal follow up cohort study of all patients of the Slotervaart Hospital, Antoni van Leeuwenhoek (AvL), Academic Medical Center Amsterdam (AMC), University Medical Center Groningen (UMCG), University Medical Center Nijmegen (UMCN st Radboud) and Leiden University Medical Center (LUMC) using oral small molecule targeted anti-cancer agents for treatment of cancer.

Patient number
To meet protocol objectives we expect to include approximately 150 patients a year in the participating centres. Several oral anticancer drugs have a limited number of indications and users. Therefore we expect to need a total inclusion period of 5 years

Inclusion criteria
- All patients using a (currently approved or new) oral small molecule targeted anti-cancer agent for treatment of cancer
- Patients from whom it is possible to collect blood samples
- Informed consent is given

Exclusion criteria
The study objective is to monitor the oral small molecule targeted anti-cancer agent therapy in a 'real life' cohort of patients. Therefore no strict exclusion criteria will be used.

Study design
Patients will be treated with the oral small molecule targeted anti-cancer agent on a dose according to the prescription of the physician. No further intervention is needed. The patients will be sampled during routine follow up.

Primary study parameters/outcome of the study

- Pharmacokinetics

Secundary study parameters/outcome of the study

- Treatment outcome (response, suboptimal response, treatment failure)
- Toxicity (graded on basis of the National Cancer Institute Common Toxicity grading Criteria for adverse events (CTC))
- Genotype of drug metabolising enzymes and drug transporters
- Mutations in TK domain
- Drug-drug interactions

Nature and extend of the burden and risks associated with participation
The sampling scheme of the Slotervaart NIB-cohort study will be minimally invasive. In the NIB-cohort all sampling is carried out during routine follow up for oral small molecule targeted anti-cancer agent therapy and does not require additional vena punctures. The benefit for the participating patients exists of individual treatment optimisation by routinely applied TDM using the knowledge concerning PK-PD relations of oral small molecule targeted anti-cancer agents acquired from the NIB-cohort study.

Samenvatting

Titel onderzoek
Therapeutische drug monitoring van orale doelgerichte antikankermiddelen.

Achtergrond van het onderzoek
Interpatiënt (en intrapatiënt) variabiliteit in farmacokinetiek kan een groot probleem vormen bij therapie met orale doelgerichte antikankermiddelen, omdat dit kan leiden tot onvoorspelbare effectiviteit en veiligheid van de therapie. In het geval van imatinib is er reeds groeiend bewijs voor een relatie tussen plasmaspiegels en klinische effectiviteit/toxiciteit. Kennis over deze PK-PD relaties kan worden toegepast om therapie te optimaliseren en zo het falen van TKI's door resistentieontwikkeling te voorkomen en bijwerkingen te verminderen. TKI therapie is kostbaar (> € 2000 per maand) en belastend voor de patiënt, waardoor de kosteneffectiviteit van het monitoren van plasmaspiegels voor therapie optimalisatie waarschijnlijk is. Omdat alle orale doelgerichte antikankermiddelen grote overeenkomsten vertonen in werkingsmechanisme en doeleiwitten, worden overeenkomstige PK-PD relaties verwacht voor alle orale doelgerichte antikankermiddelen. Dit onderstreept de rationale voor een gestructureerde follow-up van patiënten die orale doelgerichte antikankermiddelen gebruiken.

Doel van het onderzoek:
Primair: Bepalen van de farmacokinetische parameters van verschillende klinisch toegepast orale doelgerichte antikankermiddelen
Secundair: Bestuderen van de relatie tussen effectiviteit/toxiciteit en plasmaspiegels
Evalueren van de specifieke effecten van verschillende parameters op de variabiliteit in farmacokinetiek en farmacodynamiek van orale doelgerichte antikankermiddelen

Methodologie
Een longitudinale follow-up cohort onderzoek van alle patiënten in het Slotervaartziekenhuis, Antoni van Leeuwenhoek (AvL),Academisch Medisch Centrum Amsterdam (AMC), Universitair Medisch Centrum Groningen (UMCG), Universitair Medisch Centrum Nijmegen (UMCN st Radboud) en Leiden Universitair Medisch Centrum (LUMC), die een oraal doelgerichte antikankermiddel gebruiken voor behandeling van kanker.

Patiëntenaantal
We verwachten ongeveer 150 patiënten per jaar te includeren in de deelnemende centra. Sommige orale antikankermiddelen worden niet zeer frequent voorgeschreven en derhalve verwachten wij een totale inclusieperiode van 5 jaar.

Inclusie criteria
- Alle patiënten die een oraal doelgerichte antikankermiddel (reeds geregistreerd of in de toekomst geregistreerd) gebruiken voor de behandeling van kanker
- Patiënten bij wie het mogelijk is om bloedmonsters af te nemen
- Patiënten die een informed consent hebben ondertekend.

Exclusie criteria
Het doel van de studie is om de therapie met orale doelgerichte antikankermiddelen te monitoren in een 'real life' cohort van patienten. Om deze reden zullen geen strikte exclusie criteria worden gehanteerd.

Onderzoekspopulatie
Patiënten die een (reeds geregistreerde of een nieuwe) oraal doelgerichte antikankermiddel gebruiken voor de behandeling van kanker.

Onderzoeksopzet
Patiënten zullen worden behandeld met het orale doelgerichte antikankermiddel (en in de dosering) die is voorgeschreven door de behandelend arts. Er is geen sprake van interventie. Bloedafnames zullen tegelijkertijd plaatsvinden met routine controles tijdens de bezoeken aan de kliniek.

Primaire uitkomstmaten
- Farmacokinetiek

Secundaire uitkomstmaten
- Behandelingsresultaat (respons, suboptimale respons, therapiefalen)
- Toxiciteit (gradatie op basis van NCI-CTC)
- Genotype van geneesmiddel metaboliserende enzymen en geneesmiddeltransporters
- Mutaties in het TK domein
- Geneesmiddel-geneesmiddel interacties

Omschrijving en inschatting van belasting en risico
De monstername in de Slotervaart NIB cohort studie is minimaal invasief, omdat monstername plaatsvindt tijdens routine follow-up voor orale doelgerichte antikankermiddel therapie en hiervoor dan ook geen extra venapuncties nodig zijn. Het voordeel voor deelnemende patiënten bestaat uit optimalisatie van de individuele behandeling door routinematige toepassing van TDM. Hierbij zal namelijk meteen de kennis over PK-PD relaties van orale doelgerichte antikankermiddelen, verkregen uit de NIB cohort studie, worden toegepast.

Background

The introduction of oral small molecule targeted anti-cancer agents for treatment of various malignancies has significant impact on the management of these diseases. One form of the oral small molecule targeted anti-cancer agents are the selective tyrosine kinase inhibitors (TKIs). TKIs are directed against tyrosine kinase receptors (TKs), which play an essential role in the transduction of growth signals in the cells. Application of these agents in patients with malignant diseases has shown to successfully induce clinical responses in several malignancies[1].

Besides favourable patient outcomes, these drugs also have advantage above conventional therapies with regard to toxicity. Because of specific toxicity for tumour cells this therapy is often less toxic than conventional cytotoxic therapy. However, there may still be some effects in normal tissues which leads to unwanted effects that are difficult to eliminate e.g. severe acneiform rash (CTC grade III/IV) [2].

The ease of oral administration enables patients to get their drug regime in an outpatient setting, which is more patient friendly. However, oral administration also entails the possibility of variable drug exposure due to patient non-compliance, drug interactions with co-medication and variability in oral drug availability.

Despite proven efficacy, cases of treatment failure and suboptimal response have been reported in oral small molecule targeted anti-cancer agent therapy [3]. The failure of oral small molecule targeted anti-cancer agent most likely arises from a combination of tumour and host related factors that contribute to pharmacokinetic variability and/or induction of resistance to these agents [4-6].

For the widely used oral small molecule targeted anti-cancer agent imatinib the mechanisms of developing resistance are well documented. Primary resistance, defined as the failure to achieve an initial response to the therapy, has an incidence of only 5% in patients in the chronic phase of CML. Diminished drug bioavailability due to variability in drug absorption, expression of drug transporters, hepatic metabolism and plasma protein binding is a possible cause for primary resistance [7,8]. Secondary resistance, defined as a loss of initial response during therapy, is seen in 10-15% of all patients on imatinib therapy [9]. Development of resistance, pharmacokinetic variability, non-adherence and drug toxicity can be seen as the major contributors to treatment failure. In order to optimise treatment and prevent early treatment failure or treatment change, it is important to have insight in the role of these factors for treatment outcome. However, it is extremely difficult to assess these factors in controlled clinical trials due to strict in- and exclusion criteria and structured follow-up. Therefore, a

study in a “real life” cohort of patient may be very useful. The aim of this study is to prospectively collect patient data in order to establish relationships between treatment outcome (toxicity and clinical response), tumor and host related factors an pharmacokinetics.

TDM data

Relations between treatment outcome (adverse effects and/or treatment failure) and plasma concentrations have been described for several oral small molecule targeted anti-cancer agents [10-12]. Pharmacokinetic variability (both interpatient and intrapatient) may, therefore, be an important factor for treatment outcome. High variability in imatinib plasma levels between patients was found [13-16]. This suggests that plasma levels may be more predictive than absolute dose in predicting treatment response and adverse effects [17,18]. For imatinib there is emerging evidence for a relationship between exposure to the drug and the clinical efficacy [19,20]. Additionally, the occurrence of side effects is more frequent at higher imatinib plasma levels [21]. For the other, currently used and future, oral small molecule targeted anti-cancer agents data on pharmacokinetic-pharmacodynamic (PK-PD) relationships are inconclusive or unavailable at present. However, based on the similarities in mechanism of action and target proteins, similar PK-PD relationships can be expected for these oral small molecule targeted anti-cancer agents. Therefore, therapeutic drug monitoring (TDM) is proposed to prevent failure of oral small molecule targeted anti-cancer agents by reducing drug toxicity, reducing drug resistance and achieving a good level of adherence with a higher likelihood of treatment response. This may be even more important in special circumstances, as for example organ dysfunction, the use of co-medication leading to potential drug-drug interactions, suspected non-compliance and occurrence of side effects. TDM may represent a practical tool to improve the outcome of patients receiving oral small molecule targeted anti-cancer agents leading to therapy optimization on a patient-by-patient basis. The current limitation of routine clinical use of TDM is the lack of data confirming PK-PD relations of oral small molecule targeted anti-cancer agents and of data demonstrating improved outcome of patients when TDM is routinely applied. Rational quantification of oral small molecule targeted anti-cancer agent plasma levels can provide a better understanding of treatment failure or suboptimal response in patients receiving oral small molecule targeted anti-cancer agents [22].

Drug levels in Dried Blood Spots (DBS)

Recently, in a large meta-analysis of pharmacokinetic and pharmacodynamic data of patients treated with sunitinib a dose-efficacy relation for sunitinib treatment has been established [23]. The relationship between sunitinib exposure and efficacy and tolerability was not only observed for sunitinib exposure but also for its active metabolite SU12662. Additionally, a significant relationship between exposure and probability of partial response or complete response in patients with metastasized renal cell carcinoma indicates that patients should be dosed as high as possible. Target plasma concentrations of sunitinib plus metabolite (N-desethyl sunitinib) are in the range of 50 to 100 ng/mL, as deduced from pharmacokinetic/pharmacodynamic preclinical data [24-28]. Using this target concentrations, therapeutic drug monitoring (TDM) may represent a practical tool to improve the outcome of patients receiving sunitinib leading to therapy optimization on a patient-by-patient basis.

Current clinical practice for TDM is to measure drug concentrations in plasma. Drug concentrations are measured in plasma after routine visits to the outpatient clinic. This way of performing TDM has several pitfalls. For example measuring trough levels is not feasible at the outpatient clinic, since most patients on a sunitinib regime take their medication early in the morning and/or late in the evening due to once and twice daily dosing.

We are currently able to determine sunitinib and N-desethyl sunitinib levels in DBS. This enables sample collection by means of a simple fingerprick, as was previously shown for antiretroviral drugs [29]. This patient-friendly technique has several advantages over the classic way of performing TDM:

1. It allows pharmacokinetic studies in non-hospital based settings, allowing self-sampling of trough levels at home at consecutive occasions
2. It allows determination of drug levels in populations where intensive venous sampling is unethical or impossible as for example in children, neonates and intravenous drug users with veneral damage
3. There is no need for use of anticoagulant containing sampling tubes, plasma separation or the necessity of cold sample storage. Therefore, the logistics of DBS samples are much less complicated [30].

No relationship between DBS and plasma concentrations has been established for sunitinib and N-desethyl sunitinib and other tyrosine kinase inhibitors yet. Due to binding of the drugs to components in blood that are not present in plasma or due to differences in venous and capillary blood, the concentrations of drugs and metabolites in DBS and plasma are probably not equal [30]. Since the target concentrations for TDM of sunitinib are defined in plasma samples, there is need to establish the relationship between DBS and plasma concentrations. Therefore, the DBS to plasma ratio of sunitinib and its metabolite has to be defined in simultaneously drawn DBS and plasma samples of patients using sunitinib.
By quantification of the levels of oral small molecule targeted anti-cancer agents in DBS and plasma samples, we will investigate the relationship between DBS and plasma concentrations of these drugs.

Intracellular drug levels

Like the plasma concentration also the intracellular concentration of imatinib may show considerable interpatient variability. Variability in intracellular drug concentrations may account for differences in treatment outcome and is most likely due to differences in expression of influx and efflux transporters [31,32]. Therefore, intracellular drug levels in peripheral blood mononuclear cells (PBMCs) may more accurately reflect drug effects of the TKIs indicated for haematological malignancies. The determination of intracellular drug levels is technically demanding. However, for imatinib, determination of intracellular drug levels in PBMCs has been done before using a method previously developed for intracellular measurement of HIV-antiviral drugs [33,34]. Therefore, samples for the determination of intracellular drug levels will be collected for the establishment of relationships between treatment effects and drug levels.

Genotypic resistance

Akin to development of resistance to antibiotic and antiretroviral agents, mutations due to selection pressure can lead to development of resistance in tumour cells during oral small molecule targeted anti-cancer agent therapy. For the widely used Bcr-Abl kinase inhibitor, imatinib, the mechanisms of resistance are well documented [35]. Point mutations in TK domain, due to selection pressure during imatinib therapy, are the most common cause of secondary resistance. These mutations can be seen in 10-90% of the patients with CML depending on the stage of the disease [36]. More than 50 mutants have been described already, but there are seven mutations (M244V, G250E, Y253F/H, E255K/V, T351I, M351T and

F359V) that account for 85% of the frequency of all mutations and thereby for 66% of the reported cases of secondary resistance to imatinib [37-39]. Some mutations are associated with clinical relevant loss of sensitivity to imatinib, but only T351I is known to cause absolute insensitivity [40]. Second generation TKIs like dasatinib and nilotinib have shown to be effective against imatinib resistant BCR-ABL forms. However, neither drug is effective in case of the T351I mutation [41,42]. Additionally, a mutation encoding for resistance to dasatinib has already been documented [43]. Also for EGFR kinase inhibitor erlotinib mutations in TK domain are reported, of which T790M is the most abundant. Moreover, structural similarities between the TKs make it reasonable that mutations in TK domains can occur during therapy with all types of TKIs [44].

Although monitoring for resistance to oral small molecule targeted anti-cancer agents by mutational testing is not universally applied at this moment, it can be considered in patients with treatment failure or suboptimal therapeutic responses. Recently Khorashad et al found that identification of a TK domain mutation during imatinib treatment in CML is highly predictive for loss of response and for disease progression[45]. Identification of TK mutations, therefore, can be used for subsequent decisions in optimization of treatment strategy by for example dose escalation or implementation of therapy with an alternative TKI. Resistance testing may also be helpful in selecting initial therapy [46].

Genotype of drug transporters and metabolizing enzymes

Drug levels of oral small molecule targeted anti-cancer agents may be strongly influenced by the genotype of drug transporters (e.g. P-glycoprotein, organic cation transporter (OCT-1, ABCG1) and/or the activity of drug metabolising enzymes (e.g. CYP3A4, 1A2). The drug transporters are also important for intracellular drug accumulation. These effects may cause treatment failure or may increase toxicity. Certain (rare) genotypes may e.g. result in reduced systemic absorption, reduced plasma clearance or reduced intracellular penetration. For several drug metabolising enzymes and drug transporters variant alleles have been identified. However, for many of these polymorphic forms, no relationship with enzyme or transporter activity has been demonstrated yet. Decreased cellular drug influx due to low OCT-1 activity has been correlated to suboptimal responses to imatinib [47-49]. Otherwise, second generation TKI nilotinib, is not dependent of OCT-1 for cellular uptake [50]. Drug efflux due to higher expression of multi-drug resistance efflux pump ABCB1 has been shown to contribute to imatinib resistance. Also efflux pump ABCG2 may play a role in imatinib resistance [51-53]. Although data on influx and efflux of other oral small molecule targeted anti-cancer agents

are not available at present [52,54], we aim to collect a single whole blood sample from the patients in the NIB-cohort study, for the determination of the genotype of relevant drug metabolising enzymes and drug transporters. Using this sample the relationship between the (intracellular) pharmacokinetics and genotype and (possibly) the relationship between treatment outcome and genotype can be assessed. The blood samples will only be used for this purpose and no other genotypic determinations, which are outside the scope of these aims, will be carried out.

Drug levels in sweat

The most clinically relevant side effect of sunitinib and other TKIs is the development of Hand Foot Syndrome (HFS). HFS (all grades) occurred in 19% of patients in a pooled analysis of published clinical trials of sunitinib of which 5% experienced sever HFS (grade 3 or 4) [55]. This skin toxicity severely impacts the quality of life of patients treated with TKIs and impairs activities of daily living. Moreover, it can lead to unavoidable dose modifications or dose interruptions, which can negatively affect treatment efficacy [56,57].

The exact pathogenesis of HFS is unknown, but some hypotheses exist concerning the mechanism of which HFS is caused. The primarily affected sites in HFS, the palmoplantar surfaces, have a high density of eccrine glands which continuously excrete sweat [58]. Since eccrine glands exhibit expression of tyrosine kinase receptors as c-Kit and PDGFR, it is assumed that secretion of the TKIs into the eccrine glands results in direct toxicity of the MKI to the skin. Additionally, in some cases of HFS structural and cytotoxic changes of the eccrine glands have been observed in patients receiving sunitinib. However, secretion of TKI by the eccrine glands in sweat has not been studied yet [59,60].

Sweat can contain virtually any substance present in blood [61]. With conventional antineoplastic agents, such as doxorubicine, previous studies have indicated a possible relationship between hyperhydrosis on the palms and plantae and the development of skin toxicity [62]. This indicates that sweat functions as a carrier of drug to the skin surface. After excretion on the skin surface, sweat containing the drug may penetrate into the stratum corneum. In therapy with doxorubicin the skin reactions could be avoided by the prevention of hyperhydrosis [63].

In our outpatient clinic we observed that patients on sunitinib treatment suffered more from severe HFS in summer than in winter. This observation supports the assumption that the development of HSF during sunitinib treatment is comparable to the development of skin

toxicities in case of hyperhydrosis during treatment with conventional antineoplastic agents, like doxorubicin. Moreover, occurrence of skin toxicity of sunitinib could not be associated with toxicities in other organ systems. Therefore, it seems unlikely that the skin toxicity is only correlated with toxic sunitinib plasma levels. We hypothesized that aggravation of skin toxicities is caused by the secretion of sunitinib in sweat.

By quantification of the levels of oral small molecule targeted anti-cancer agents in sweat and plasma samples, we will investigate the relationship between cumulative oral small molecule targeted anti-cancer agent sweat secretion and the severity of HFS. Furthermore, we would like to study the relationship between cumulative oral small molecule targeted anti-cancer agent sweat secretion and plasma levels, and the seasonal influence on the secretion.

Objectives

In summary, it can be concluded that interpatient (and intrapatient) variability in pharmacokinetics may be a major determinant in oral small molecule targeted anti-cancer agent therapy outcome since this may lead to unpredictable efficacy and safety. In case of imatinib, there is already emerging evidence for a relationship between plasma levels and clinical efficacy/occurrence of side effects. Knowledge of this PK-PD relationship can be used to optimize therapy in order to prevent failure of oral small molecule targeted anti-cancer agents by monitoring of drug resistance and reducing drug toxicity. Since the burden of oral small molecule targeted anti-cancer agent therapy is severe and oral small molecule targeted anti-cancer agent therapy is very expensive (> € 2000 / month) monitoring of plasma drug levels in order to optimise therapy is likely to be cost effective. Since all oral small molecule targeted anti-cancer agents have large similarities in mechanism of action and target proteins, similar PK-PD relations are to be expected for all oral small molecule targeted anti-cancer agents. This underlines the rationale for a structured prospective follow up of patients using any oral small molecule targeted anti-cancer agent.

In the ongoing HIV cohort study in the Slotervaart Hospital, a structured follow up of patients has already been applied successfully to study the variability in pharmacokinetics of antiviral agents. The NIB-cohort study, as described in this proposal, will be carried out analogous to the Slotervaart HIV cohort study. Besides the Slotervaart Hospital, also the Antoni van Leeuwenhoek Hospital/The Netherlands Cancer Institute will take part in the NIB-cohort study to ensure the inclusion of a sufficient number of patients.

The primary objectives of the NIB-cohort study are (1) to determine the pharmacokinetics of several clinically used oral small molecule targeted anti-cancer agents, and (2) to study the relationship between treatment response/toxicity and plasma (or intracellular and sweat and sweat) drug levels. Secondary objectives are: the specific influence of different parameters on variability in pharmacokinetics and –dynamics, and the effect of genotype of drug metabolising enzymes and drug transporters, mutations in TK domain and drug-drug interactions on pharmacokinetics and pharmacodynamics. Eventually, the knowledge about PK-PD relations of oral small molecule targeted anti-cancer agentz acquired from the NIB-cohort study will be used to optimize treatment of individual patients by routinely applied TDM.

Endpoints

1. Pharmacokinetics
2. Treatment outcome (response, suboptimal response, treatment failure)
3. Toxicity (graded on basis of the National Cancer Institute Common Toxicity grading Criteria for adverse events (CTC)[64])
4. Genotype of drug metabolising enzymes and drug transporters
5. Mutations in TK domain
6. Drug-drug interactions

Study design

A longitudinal follow up cohort study of all patients treated with an oral small molecule targeted anti-cancer agent for treatment of cancer. After informed consent is given, patients are included in the Slotervaart NIB-cohort study. Since the therapeutic use of oral small molecule targeted anti-cancer agents is an emerging field at present, the place of these agents in the management of malignancies is not fully established yet. Besides, new oral small molecule targeted anti-cancer agents are already in development. For these reasons it is difficult to make an estimation of the expected patient numbers to be included in this cohort study. However, based on the current use of oral small molecule targeted anti-cancer agents in both participating centres a number of 150 patients is expected to be recruited in the 3 year inclusion period of this study.

Study duration

The total study take 6 years, which exists of an inclusion period of 5 years and a follow-up after inclusion of 1 year.

Inclusion criteria

- All patients using a (currently approved or new) oral small molecule targeted anti-cancer agent for treatment of cancer
- Patients from whom it is possible to collect blood samples
- Informed consent is given

Visit schedule and evaluations

Patients will have a regular visit to the outpatient clinic every 4 weeks to 3 months depending on disease and therapy. Blood samples will be taken with regular laboratory investigations as noted in table 2. Blood sample collection will be intensified if an event occurs. An event is defined as a change of oral small molecule targeted anti-cancer agent, TDM outside the normal range, failure of treatment or occurrence of severe toxicity (CTC grade > II). Then blood samples will be drawn 1 week and 3 months after the event. Whenever possible and/or indicated two 8 ml PCT tubes for intracellular drug levels are drawn at control visits at the outpatient clinic. Whenever indicated sweat samples will be collected by application of a sweat patch during 7 consecutive days. Most of the laboratory investigations are routinely performed and the material is routinely stored at -70ºC. The intracellular drug levels and the determination of genotypic metabolic enzymes and drug transporters are performed at the laboratory of the hospital pharmacy. The material is also stored there.

Table 2. Laboratory investigations

Parameter	Baseline	Routine laboratory control at outpatient clinic	Intensification of laboratory investigation if an event* occurs	Needed material
Disease evaluation**	+	+	+	-
TDM in plasma	+	+	+	Natrium-EDTA tube, 5 mL (plasma)
TDM in dried blood spots	-***	-***	-***	Finger prick on dried blood spot paper 200 µL (capillary blood)
Intracellular drug levels	-***	-***	+	CPT tube, 16 mL
Sweat drug levels	-***	-***	-***	Sweat patch, 7 days
Genotypic resistance testing	+	$	$	Natrium-EDTA tube, 5 mL (whole blood)
Determination of genotype metabolic enzymes and drug transporters	+	-	-	Natrium-EDTA tube, 5 mL (whole blood)
Investigation of pharmacokinetics during dose interval	-	-	#	Natrium-EDTA tube, 10 x 5 mL (plasma)

* An event is defined as a change of oral small molecule targeted anti-cancer agent, TDM outside the normal range, failure of treatment or occurrence of toxicity

** Type of test is depending on indication for which the oral small molecule targeted anti-cancer agent is used and corresponding treatment goals. No specific additional laboratory investigations are needed. Insight in medical dossier will be sufficient.

*** Only performed whenever possible and/or indicated

$ Only tested again after treatment failure

Only performed in case of hospitalization for other purposes.

Logistics

For pharmacokinetic investigations Na-EDTA plasma is needed. Patient whole blood samples will be drawn in Na-EDTA tubes. These tubes can be sent to the laboratory of the hospital pharmacy. Here, the tubes will be centrifuged to obtain plasma. Whenever needed also some whole blood will be stored for pharmacogenetic investigations.
When indicated (haematological malignancies) two CPT tubes will be drawn for measurement of the intracellular drug concentration. These tubes can be sent to the laboratory of the hospital pharmacy (floor 2B). Here, the CPT tubes will be further processed to obtain plasma and PBMC samples.
When indicated sweat samples will be collected by application of a sweat patch during 7 consecutive days. The sweat patches can be sent to the laboratory of the hospital pharmacy in an accessory plastic envelope. Here, the patches will be further processed to extract the oral small molecule targeted anti-cancer agents out of the patches.

Ethics

The sampling scheme of the Slotervaart NIB-cohort study will be minimally invasive. In the NIB-cohort all sampling is carried out during routine follow up for oral small molecule targeted anti-cancer agent therapy and does not require additional vena punctures. However, in case of hospitalization for other purposes, this opportunity will be used to investigate pharmacokinetics during dose interval. Hereby patient circumstances and properties of the used oral small molecule targeted anti-cancer agent will be taken into account in the sampling scheme in order to minimize patient inconvenience.

All study samples will be coded in a way in which the origin of a sample is traceable only by the involved researcher according to the standard operating procedures of the bioanalytical laboratory of the Slotervaart Hospital.

Insight in the patients' medical file is essential to gain information about the pharmacodynamic study endpoints such as treatment outcome, toxicity, drug-drug interactions and pharmacokinetic parameters. Only the information needed to fulfill the study objectives will be extracted from the medical dossier and will be documented on a case report form (CRF).

The whole blood samples will only be used for genotypic determination of drug metabolising enzymes, drug transporters and mutations involved in drug resistance. No other genotypic determinations, which are outside the scope of these aims, will be carried out. The patient will in principal not be informed about determined mutations. When the genotypic determination reveals an mutation that is relevant for the treatment of a patient, the treating physician will be informed.

It can be expected that new oral small molecule targeted anti-cancer agents may be approved or discovered in near future and these will, whenever possible, also be assessed in the NIB-cohort study.

Statistical considerations

For the concentration-time data and baseline patient characteristics descriptive statistics will be performed. The PK data will be analysed according to a nonlinear mixed effects ("population") approach using the NONMEM program. Furthermore, correlation tests will be performed to examine the influence of different covariates on pharmacokinetics. For each analysis a separate statistical analysis plan will be prepared.

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